ARTEMIA

RESEARCH AND ITS APPLICATIONS

volume 3

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RESEARCH AND ITS APPLICATIONS

VOLUME 3

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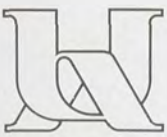
Editors

Proceedings of the
Second International Symposium on the
brine shrimp *Artemia*, organised under the
patronage of His Majesty the King of Belgium



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Welcome address

Prof. Walter Decleir
Co-organizer, Dean of the Faculty of Sciences, Antwerp State University Center (RUCA).

Mr. Chairman of the University of Antwerp, Representatives of the Ministries of Foreign Development and Cooperation, Agriculture, and Education, of the European Economic Community, and the Universities of Antwerp, Ghent and Brussels, Your Excellencies representing the Countries of Brazil, India, Israel, Malaysia, Panama, Peru, Spain, Thailand, and the United States of America, dear Colleagues, Ladies and Gentlemen,

It is both a great honour and a pleasure to welcome you to the University and City of Antwerp to participate in the Second International Symposium on the Brine Shrimp Artemia. This symposium has been honoured by the patronage of His Majesty the King of the Belgians. During a recent visit to Professor Persoone's Laboratory for Mariculture in Ghent and to the associated Artemia Reference Center, of which Dr. Sorgeloos is the Director, the King has already shown his keen interest in the research into and the applications of the brine shrimp.

As you may know, the first International Symposium on Artemia was held in 1979 in Corpus Christi, Texas, USA, and we are very happy to be able to organize this second symposium on the same subject here in Antwerp. This was made possible thanks to the generous help of many institutions. Therefore I want to thank the Antwerp State University Center (RUCA) and the Antwerp University Institution (UIA), the Belgian National Science Foundation, the Ministry of Education, the Belgian Administration for Development Cooperation, the Belgian Center for Oceanography, the Artemia Reference Center and the Institute for Marine Scientific Research (IZWO). The assistance of colleagues who kindly accepted to serve as Chairmen, Moderators, and Rapporteurs has been greatly appreciated, as has the help of all those members of the staff who have contributed to the organization of this symposium. Last but not least I wish to thank the Ambassadors and Consuls-General of Canada, France, Germany, Malaysia, Spain, and Thailand, who kindly contributed to the banquet by offering drinks typical of their country.

I am greatly indebted to the Antwerp State University Center (RUCA) for the use of the university's premises for this symposium and to the UIA, the second of the three bodies which together constitute the University of Antwerp, for making its facilities available in order to organize a workshop.

There were three reasons why we decided to organize this second symposium on the brine shrimp in Belgium. The first is the generous support of the Belgian National Science Foundation, which four years ago agreed to sponsor a research program in which various departments of the Universities of Ghent, Antwerp, and Leuven have participated. The organization of this symposium seemed to be the appropriate climax to this initiative. The second reason is that for many years the Artemia Reference Center of the State University of Ghent has played a central role in the coordination of research into *Artemia* and has created the 'International Study on Artemia' group, in which research laboratories from Italy, Spain, Great Britain, the USA, and Belgium are

participating. And thirdly, Belgium through its Administration for Development Cooperation is actively promoting Belgian know-how with regard to *Artemia* applications in third world countries. This is carried out by supporting research, sponsoring training courses, and setting up demonstration projects in various countries in Africa, Asia, and South America. It is not only Belgian governmental organizations which support *Artemia* research and development. The private sector too has recently shown an interest and has set up the Belgian joint venture company 'Artemia Systems'.

All this is sufficient proof of the key role which Belgium has played so far and is still continuing to fulfil in *Artemia* research. The organization of this symposium with 250 participants from 38 countries is an excellent illustration of this.

I should like to finish this short welcome address by expressing my sincere hope that you will enjoy your stay in our City of Antwerp and at our University. Furthermore, I hope that your participation in this symposium will be very fruitful and that this meeting will be a milestone on the way towards a growing knowledge about that tiny crustacean which we call *Artemia* and which seems to be predestined to play such an important role in future world food production.

Opening address

Mr. J. P. Goyens
Director-General of the Belgian Administration for Development Cooperation.

Mr. Chairman, Excellencies, Ladies and Gentlemen,

At a time when industrialized nations combine efforts to boost food productions in developing countries, when in 1985 alone an estimated amount of 3 billion US dollars will be spent on emergency food to only some 20 of the most effected countries, it is encouraging to realize that the scientific community contributes to these efforts by developing more pressing strategies and also inventing new resources.

Aquaculture is one of the fields where opportunities to increase food production in a relatively short time and at reasonable cost, seem very promising. Developing countries which are willing and able to exploit intensively their salt lakes and saltworks can obtain very profitable results by taking *Artemia* developing initiatives and introducing new techniques. In the first place, this food source creates a potential for improving local aquaculture production, and in most cases and at the same time, a better quality of the salt produced by solar evaporation. Secondly, in a number of third world countries, climatological and geological conditions prevail which favor mass production of *Artemia* cysts. Export of high quality cysts can become an important source of income. The promising results of past research initiated by the Belgian Artemia Reference Center, and implemented in collaboration with numerous national and international research and development organizations, have incited the Belgian Government to include in its national development cooperation programme a chapter on *Artemia* which aims at rendering the acquired know-how in the field of selection and reproduction of *Artemia* strains accessible to third world countries.

Each year, the Belgian Administration for Development Cooperation offers a number of fellowships to enable citizens of developing countries, interested in *Artemia* production, to participate in a special training course organized by Belgian universities. Since two years the same administration provides the necessary funds for a project of applied research in view of optimizing the use of *Artemia*, standardizing inoculation techniques, and selecting strains appropriate for inoculation in saltponds. More recently, two *Artemia* projects were started in the field. One aims at the inoculation of *Artemia* in saltponds in Malindi near Mombasa in Kenya, in view of intensive production in a region where *Artemia* are not present under natural conditions. A second project should help Thailand to master and improve the brine shrimp production techniques and coordinate its inoculation programmes. At the present moment, it is too early to evaluate these development cooperation activities in all their aspects. One conclusion we can draw already is that these *Artemia* projects, directed to practical applications in well-chosen environments, do not need important investments, as they are based for the major

part on results obtained by relatively simple techniques. We can also conclude that the exchange and most of all the pooling of numerous and different experiments on this micro-shrimp can only lead to new progress in the knowledge of *Artemia.*

Mr. Chairman, Excellencies, Ladies and Gentlemen, on behalf of the Belgian Administration for Development Cooperation, I wish the participants of this second international Symposium on *Artemia* fertile discussions and work, in view of improving this new resource for the benefit of the third world countries which ultimately is to the interest of the industrialized nations.

Speakers at the opening session (from left to right) : Dr. Patrick Sorgeloos, Prof. Dr. Walter Decleir (Symposium Organizers) and Mr. J. P. Goyens (Director-General of the Belgian Administration for Development Cooperation).

Group picture of participants

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Ecology

In this section, Lenz's review of *Artemia* ecological studies is immediately followed by the important comments of Geddes and Williams regarding uncontrolled transplantations of *Artemia* for aquaculture. The reader is directed to the last paragraph of the latter paper for the text of the only resolution considered and passed by the Symposium attendees, to wit, *Artemia* genetic resources must be conserved.

A detailed review of cryptobiosis in *Artemia* is presented by Lavens and Sorgeloos (3).

The next four papers (4-7) report studies of life-history or reproductive aspects of *Artemia*. The rigorous and exhaustive study of Wear and Haslett (8) is then followed by eight papers on primary ecological characterizations of *Artemia* populations around the world (9-16).

(1) P. H. Lenz.
Ecological studies on *Artemia* : a review.

(2) M. C. Geddes and W. D. Williams.
Comments on *Artemia* introductions and the need for conservation.

(3) P. Lavens and P. Sorgeloos.
The cryptobiotic state of *Artemia* cysts, its diapause deactivation and hatching : a review.

(4) M. A. Thun and G. L. Starrett.
The effect of cold, hydrated dormancy and salinity on the hatching of *Artemia* cysts from Mono Lake, California, USA.

(5) W. W. Sawchyn.
Ecological factors controlling the hatchability of *Artemia* cysts in inland saline lakes in Canada.

(6) Y. Nimura.
A probable reason why *Artemia* is confined to isolated saline waters.

(7) P. H. Lenz and G. Dana.
Life-cycle studies in *Artemia* : a comparison between a sub-tropical and a temperate population.

(8) R. G. Wear and S. J. Haslett.
Studies on the biology and ecology of *Artemia* from Lake Grassmere, New Zealand.

(9) S. C. Bhargava, G. R. Jakher, M. M. Saxena, and R. K. Sinha.
Ecology of *Artemia* in Didwana Salt Lake (India).

(10) N. Ramanathan and P. Natarajan.
Ecology of *Artemia* along the southeastern coast of India.

(11) J. A. Basil, D. R. D. Premkumar, A. P. Lipton, and M. P. Marian.
Artemia in the salt pans of Vedaranyam, southern India.

(12) M. R. Ahmadi.
First report of *Artemia* occurrence in Shurabil Lake (Iran).

(13) A. Majić and I. Vukadin.
Preliminary report on the brine shrimp (*Artemia*) from Yugoslav saltworks.

(14) G. Mura, A. Filauri, and G. B. Palmegiano.
A survey of *Artemia* and *Branchinella* populations in coastal lagoons and salt pans of Sardinia (Italy).

(15) T. Castro, L. Sanchez, and R. De Lara.
Natural sources of brine shrimp (*Artemia*) in Mexico.

(16) F. Conte and P. A. Conte.
Ecology of Oregon's Great Salt Lake : Lake Abert.

Artemia *Research and its Applications. 1987. Vol. 3. Ecology, Culturing, Use in aquaculture*
P. Sorgeloos, D. A. Bengtson, W. Decleir, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 556 p.

Ecological studies on *Artemia*: a review

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Content

Introduction

Compared to other aspects of the brine shrimp's biology, ecological studies on *Artemia* are still few in number (Persoone and Sorgeloos, 1980). *Artemia* inhabit hypersaline lakes and ponds, characterized by low species diversity. Because these ecosystems are relatively simple they lend themselves particularly well to quantitative ecological analyses. At the same time the large range of habitat characteristics under which *Artemia* live provides an unusual variety of ecological responses that can be studied within a single genus. Furthermore, a better knowledge of its ecology will be of value in optimizing the production of *Artemia* for aquacultural use. This review is intended to complement a previous one by Persoone and Sorgeloos (1980). Focus will be on population dynamics, life-history strategies, productivity, and biological interactions. Each topic will be discussed first with reference to Mono Lake, California, USA, and Great Salt Lake, Utah, USA. Although not representative of all natural *Artemia* habitats, studies on these two lakes have included a greater diversity of ecological aspects than most. Next, these studies will be compared with those on other habitats. The emphasis will be on ecological responses to various biotic and

abiotic environmental factors. Discussions of habitat characteristics *per se* are treated elsewhere (*e.g.* Cole and Brown, 1967 ; Persoone and Sorgeloos, 1980 ; Bowen *et al.*, 1984).

Population dynamics

The population dynamics (*e.g.* seasonal cycles, abundance patterns and age structure) of zooplankton are affected both by the abiotic environment and by biological interactions. The latter are limited in hypersaline communities owing to low species diversity. Therefore, the abiotic factors, especially those regulating seasonal characteristics, have particularly important impact. Two critical factors determine the *Artemia* population dynamics : a) whether lake conditions are such that animals can survive throughout the year ; and b) whether the seasonality of the environment is predictable or not.

In large temperate lakes such as Great Salt Lake and Mono Lake, annual salinity changes are small and seasonality is determined primarily by the temperature cycle. The population dynamics in these two lakes have been detailed by Mason (1967), Wirick (1972), and Lenz (1980, 1982, 1984). The cold temperatures during the winter (around 2 °C) preclude the survival of the *Artemia*, and the annual cycle begins in spring with the hatching of diapause cysts. This first generation reaches maturity in May/June and a second generation is produced ovoviviparously. Two major generations have been reported per year in both lakes (Gillespie and Stephens, 1977 have estimated up to five in Great Salt Lake). In June/July reproductive mode switches and becomes primarily oviparous. During the fall the population steadily declines due to lack of recruitment, increased predation (Cooper *et al.*, 1984), and thermal death. Similar temperature regulated annual cycles can be expected in other relatively deep (mean depth greater than 2 m) temperate lakes such as Abert Lake, Oregon, USA and some of the inland Russian lakes (Spitchak, 1980). In some lakes, such as Lake Shurabil, Iran (Ahmadi, 1987), seasonal fluctuations in salinity can be substantial, but if the salinity ranges are within the tolerance limits for *Artemia*, one might expect seasonality to be primarily determined by temperature, with adults disappearing during the cold winters.

In many habitats salinity fluctuations are extreme. High salinity or desiccation kill *Artemia* and hence can be the primary factor driving seasonality. In shallow (mean depth less than 1 m) temperate ponds the annual temperature cycle is often complemented by large changes in salinity. Dana (1981, 1984) described such a physico-chemically driven cycle in Fallon Ponds, Nevada, USA. These ponds fill during winter precipitation and dry up during the summer. An *Artemia* population made up of nauplii was present in March and Dana's data suggest two or three generations during an approximate growth period of 5 months. Environmental stress increased throughout the summer as the ponds became increasingly saline, and the *Artemia* population disappeared a few weeks before complete desiccation.

Shallow tropical and sub-tropical hypersaline ponds, which may have a smaller annual temperature range, can still become uninhabitable for *Artemia* during part of the year due to salinity fluctuations. Although, as in ephemeral temperate ponds, tropical lakes may desiccate, studies on Caribbean and Indian lakes have shown that the disappearance of the *Artemia* may also be due to extreme dilution (Curaçao and Bonaire, Antilles : Kristensen and Hulscher-Emeis, 1972 ; Veppalodai and Tuticorin, South India : Ramamoorthi and Thangaraj, 1980 ; Didwana Lake, Rajasthan, India : Bhargava *et al.*, 1987). It is not clear how these ponds become resettled by *Artemia* as the salinity increases again.

The ecological situation changes markedly if there is no interruption in the growth period, so that the population is no longer dependent on a cyst hatch for the annual re-seeding of the pond. A well-documented example occurs in Lake Grassmere, New Zealand (Wear and Haslett, 1987). This lake has an insular, temperate climate with water temperatures ranging from 5 to 23 °C (Wear and Haslett, 1987). During the winter, *Artemia* abundances are lowest and females suspend reproduction, but viable individuals survive. In the spring, population numbers increase through ovoviviparous reproduction rather than cyst hatching, and ovoviviparity continues throughout the reproductive season. This results in many overlapping generations in a year (up to eight as estimated by Wear and Haslett, 1987). Oviparous reproduction is uncommon among the Lake Grassmere females. The reproductive emphasis appears to have shifted with the difference in habitat characteristics.

Similar year-round favorable conditions probably occur in several sub-tropical and tropical habitats. Solar Lake, Sinai, Egypt is characterized by a relatively constant environment and the parthenogenetic strain that inhabits this lake reproduces primarily by ovoviviparity (Por, 1969 ; Cohen *et al.*, 1977a ; Dimentman and Spira, 1982). Although complete annual population studies are lacking, uninterrupted growth seasons may also be expected at Laysan Lagoon, Hawaii, USA (Lenz and Dana, 1987), Galapagos Islands, Ecuador (Bowen, pers. commun.), and Canary Islands, Spain (Amat, 1982). Like the *Artemia* in Solar Lake and in Lake Grassmere, the females from these habitats appear to have lost or nearly lost the ability to produce cysts. Because *Artemia* are long-lived compared to their generation time, and females produce multiple broods, these tropical and sub-tropical populations, like that of Lake Grassmere, probably have a large number of overlapping generations per year with relatively small fluctuations in population densities.

Large temperate lakes like Mono Lake and Great Salt Lake have a predictable cycle, though a strongly seasonal one, with relatively little variation between years. These lakes do, of course, undergo longterm changes due to climatic fluctuations and human intervention (Mason, 1967 ; Winkler, 1977). However, the relative predictability of the environment is reflected in the reproducible dynamics of the *Artemia* population (Lenz, 1984). Permanent sub-tropical and tropical lakes are less seasonal, but may or may not be more predictable. Solar Lake, at least, has been described as a constant and predictable environment (Dimentman and Spira, 1982). By comparison the small temporary ponds, like Fallon ponds (Nevada, USA), are probably very unpredictable. Because of the small volume of these habitats, they can be expected to respond very quickly to even short periods of a particular weather condition.

Artemia life histories

Theoretical and empirical studies on life-history strategies have attempted to relate reproductive and lifespan traits to environmental characteristics (Stearns, 1977). Because of the diversity of *Artemia* habitats, it is not surprising that life-history traits vary among strains (Browne, 1980 ; Amat, 1982 ; Browne *et al.*, 1984). Although comparative life-history data have been collected for a large number of *Artemia* populations under laboratory conditions (*e.g.* Browne *et al.*, 1984), little is known about how these correlate with specific environmental conditions. *Artemia* is a good subject for the study of life-history strategy, because predictions can be tested directly through experimentation owing to ease of culture and short generation time,

and by comparing populations from the wide range of habitats available. Further investigations on life-history strategies in *Artemia* could thus contribute significantly to evolutionary theory.

Parthenogenetic *versus* bisexual strains

Unlike Cladocera, which are capable of both unisexual and bisexual reproduction, individual *Artemia* strains are either bisexual or parthenogenetic. The selective advantages and costs of parthenogenesis and sex are discussed extensively in the literature (*e.g.* Williams, 1975 ; Daly and Wilson, 1978 ; Lloyd, 1980 ; Browne and MacDonald, 1982). Although parthenogenetic reproduction confers an immediate advantage since it is more efficient, it also tends to promote low genetic variability (Abreu-Grobois and Beardmore, 1980). Bisexual reproduction maintains higher genetic variability among individuals, which would be advantageous in dispersal to differing habitats (Williams, 1975) and would presumably allow a more rapid evolutionary response to environmental change (Daly and Wilson, 1978).

Parthenogenesis in *Artemia* predominates among Old World strains, whereas in the New World only sexual reproduction has been reported (Abreu-Grobois and Beardmore, 1980 ; Bowen *et al.*, 1980 ; Persoone and Sorgeloos, 1980 ; Browne and MacDonald, 1982). Parthenogenetic *Artemia* may be absent from the New World, because it may not have arisen more than once (Abreu-Grobois and Beardmore, 1980 ; Browne and MacDonald, 1982) and parthenogens may not have dispersed to the New World. *A. parthenogenetica* has been introduced in Australia, where it is doing well (Geddes, 1980).

In the Old World where bisexual and parthenogenetic populations overlap in their distribution, the bisexual strains tend to occur in inland lakes, and the parthenogenetic *Artemia* are found in coastal salterns (Browne and MacDonald, 1982). Amat (1983) reported the co-occurrence of a bisexual and a parthenogenetic strain in Cadiz, Spain, and found that the bisexual strain dominated during the winter and spring at lower salinities and temperatures. The parthenogenetic strain occurred during the summer and fall at higher temperatures and salinities. Browne *et al.* (1984) found that in addition to mode of reproduction, the Old World bisexual and the parthenogenetic strains differed consistently with respect to other reproductive characteristics. The field data from Cadiz and the laboratory data suggest that in the Old World the two types of *Artemia* occupy somewhat different niches. How this correlates with dispersal characteristics or habitat unpredictability is still uncertain. It seems somewhat contradictory that the Old World bisexual strains have few offspring (see section on reproductive output), since adaptations for dispersal usually include high fecundity. Further studies on the ecology of parthenogenetic and bisexual strains are needed before the selective advantages and disadvantages of the two types of reproduction can be understood.

Ovoviviparity *versus* oviparity

Artemia females of most strains can reproduce both ovoviviparously and oviparously. Nauplius production allows a rapid population growth, whereas the production of diapause cysts ensures the survival of a population through unfavorable conditions (Persoone and Sorgeloos, 1980 ; Lenz and Dana, 1987). Upon maturation under constant laboratory conditions, *Artemia* females tend to reproduce ovoviviparously at first and then switch to oviparity (Amat, 1982 ; Dana and Lenz, 1986). Switching reproductive mode in the natural environment can be expected to vary

depending on the environmental conditions. A female should continue to reproduce ovoviviparously as long as there is a good probability that her offspring will reproduce themselves. If, however, conditions are such that offspring survival is unlikely, then females should invest in oviparous reproduction, in the expectation that these cysts will hatch under more favorable conditions.

Artemia females differ in their genetic tendency to reproduce either ovoviviparously or oviparously depending on strain origin (Amat, 1982 ; Browne *et al.*, 1984). The preference for either reproductive mode appears to be related to the length of the inhabitable period of their environment. In both Mono Lake and Great Salt Lake, females reproduce at first ovoviviparously, and then switch to cyst production for the remaining summer and fall (Wirick, 1972 ; Lenz, 1984). Second generation females only reproduce oviparously. In these habitats it appears that after the initial population increase the reproductive effort is focused on providing seeds for the following year. Similarly, *Artemia* inhabiting unpredictable ponds or ponds with a short growth season would be expected to emphasize oviparity, to the near exclusion of ovoviviparity. Although most other Anostraca are only capable of oviparous reproduction, no *Artemia* strains have been reported to lack the ability to produce nauplii. Browne *et al.* (1984) found that oviparity was the preferred mode of reproduction in Old World sexual *Artemia*, and they therefore concluded these strains occur in uncertain habitats. In contrast, loss or near loss of oviparity has occurred in females inhabiting lakes with favorable conditions year-round (Lenz and Dana, 1987 ; Wear and Haslett, 1987). Browne *et al.* (1984) suggested that the greater preference for ovoviviparity in parthenogenetic strains indicated an adaptation to more certain habitats. This prediction awaits field verification. Thus, *Artemia* in different habitats differ markedly in this important parameter of reproductive strategy. The data are suggestive, though not conclusive, that this is mainly due to length of growth period.

Reproductive output and lifespan

All *Artemia* females are iteroparous, *i.e.* they produce multiple broods during their lifespan. The reproductive output, however, varies greatly among strains (Browne, 1980 ; Amat, 1982 ; Browne *et al.*, 1984). In general, a large number of offspring is advantageous in rapidly growing populations, whereas a smaller reproductive output can be expected in populations that are near carrying capacity and only maintaining population size. Females with a short reproductive period will tend to concentrate their reproductive efforts in a small number of large and closely spaced broods. Longer lived ones will distribute their reproductive effort. In theory high fecundity would also be expected in populations that have high mortality rates, which in *Artemia* would include those strains inhabiting unpredictable or temporary environments.

Under constant laboratory conditions Browne *et al.* (1984) found that lifetime production of offspring per female ranged from 100 to 1 600 offspring depending on strain. The total reproductive output reflects a combination of brood size, brood interval, and length of reproductive period. The lowest output occurred among Old World bisexual *Artemia*, which were characterized by small broods (20 to 30 offspring/brood), short reproductive periods (20 to 40 days) and relatively short brood intervals (3.5 to 4.8 days). Parthenogenetic females from Turkey and India, and *Artemia franciscana* females, both had high reproductive outputs (600 to 1 600 offspring) with brood sizes ranging from 50 to 110 offspring. These more fecund *Artemia* strains were also characterized by long reproductive periods (40 to almost 110 days), and for

the parthenogenetic females by longer brood intervals (4.6 to 6 days). Browne *et al.* (1984) ascribed the dominance of *A. parthenogenetica* in the Old World to their higher fecundity.

The laboratory data may not be representative of fecundity in the field. Genetic differences in reproductive characteristics are further modified in the natural habitat in response to environmental factors. Brood size and interval, and longevity change as a function of food level (Browne, 1982), temperature (Von Hentig, 1971 ; Wear and Haslett, 1987) and salinity (Dana and Lenz, 1986 ; Wear and Haslett, 1987). In many *Artemia* habitats at least two of these three factors undergo large seasonal fluctuations. Brood sizes determined in the field vary temporally within a habitat, as well as among habitats (Lenz and Dana, 1987). Small brood sizes in Boca Chica Salt Lake, Venezuela (mean broods of five to six offspring ; Scelzo and Voglar, 1980) and Laysan Lagoon (mean broods of two to three offspring ; Lenz and Dana, 1987) suggest that the populations in these habitats were near their carrying capacity. In contrast, the larger brood sizes in Mono Lake (Lenz and Dana, 1987) and Great Salt Lake (Wirick, 1972) may be necessary for a rapid population increase in the spring, and the large investment in cysts may offset high mortality rates. Brood intervals and lifespan parameters are less well studied in the field. The first generations in Mono Lake and Great Salt Lake are slow growing, taking approximately 2 months to reach sexual maturity (Wirick, 1972 ; Lenz, 1980, 1984). Length analyses on adults in Mono Lake suggest that some first generation animals live until September (Lenz, 1984). This would indicate a longevity of 6 to 7 months with a potential of 10 broods and over 500 offspring, which is lower than Browne *et al.* (1984)'s laboratory results for *A. franciscana.*

Further knowledge is needed before we can understand the key factors that govern life-history traits in *Artemia.* Comparative data from laboratory experiments have underscored the variation among strains. The genetic variation undoubtedly reflects adaptations to habitats with different selective pressures. Field data, however, are still limited, and it is difficult to relate the available information to present theory.

Production in *Artemia* habitats

Primary production, which is the total amount of new organic matter produced through photosynthesis, is regulated by both abiotic (*e.g.* nutrients and temperature) and biotic (*e.g.* grazing) factors. Although secondary production is also influenced by abiotic factors, it ultimately seems to be limited by the primary production. Por (1980) suggested that in saline lakes, overall production decreases with increasing salinity, in part due to the rise in physiologically stressful conditions. Although this scheme might apply to saline lakes overall, it understates the variation among *Artemia* habitats and the factors controlling productivity in these environments. Density estimates for *Artemia* are available for a number of habitats, but secondary production estimates are almost non-existent. Nutrient concentrations and primary production have been measured in a few hypersaline lakes, and these studies are reviewed below.

Nutrient concentrations

The availability of nutrients, in particular nitrogen and phosphorus, is a major factor affecting primary production in aquatic systems. Freshwater lakes are usually phosphate limited, in contrast to marine environments, where nitrogen limitation is more usual. In Great Salt Lake, nutrient concentrations are high during the summer (Table I ; Stephens and Gillespie, 1976),

These authors found phosphate levels to be above 32 µmol/l in all samples. However, ammonium and nitrate concentrations did not increase until after April and enrichment experiments suggest nitrogen limitation before then (Stephens and Gillespie, 1976). Mono Lake is similar to Great Salt Lake : phosphate is abundant throughout the year, whereas ammonium and nitrate concentrations in the epilimnion are low in the spring and increase through the summer (R. Jellison, pers. commun.).

The accumulation of nutrients during the summer may be in part the result of *Artemia* excretion. Moffett and Fisher (1978) measured ammonium production by *Artemia* in laboratory experiments. From their data it can be estimated that at 20 °C, and adult *Artemia* densities between 1 and 10 ind./l, ammonium production would range from 0.2 to 2.4 µmol/l/day ; at 25 °C this estimate rises to 0.4 to 4.1 µmol/l/day. These estimates for ammonium regeneration are high compared to other aquatic environments (*e.g.* Ganf and Blazka, 1974 ; Liao and Lean, 1978). Excretion rates in zooplankton change as a function of the physiological and nutritional state of the animal (*e.g.* Blazka *et al.*, 1982), and actual *Artemia* excretion rates in the natural environment may be different. Nutrient recycling by *Artemia* may be particularly important in Great Salt Lake and recently in Mono Lake, since both of these lakes are meromictic and lack an annual turnover, so the hypolimnion is in effect a nutrient sink.

Nutrient concentrations for other *Artemia* habitats are presented in Table I. Nutrient levels are reported high for habitats which have an inland location (*e.g.* Mono Lake and Great Salt Lake). Concentrations are lower in near-ocean lakes and salterns, as would be expected from the low nutrient levels of the seawater which supplies these habitats. Such differences in nutrient levels might be expected to be reflected in the primary and secondary production levels of inland *versus* near-ocean habitats.

Primary production

Primary production in Great Salt Lake and Mono Lake is high. Mono Lake has en estimated annual production of 1 000 gC/m^2/year (Mason, 1967). During the summer, algal biomass is low, probably as a result of grazing by the *Artemia*, and primary production is low. From July to September, 1976, mean daily primary production was about 100 mgC/m^2/day (Winkler, 1977). In Great Salt Lake annual primary production was estimated at 160-220 gC/m^2/year, which is well below Mason's estimate for Mono Lake, but still moderately high compared to other lakes (Wetzel, 1975). Little Manitou Lake, Saskatchewan, Canada, another inland lake, seemed to be less productive and Haynes and Hammer (1978) estimated an annual primary production of only 70 gC/m^2/year. None of these estimates includes benthic production, which may be substantial, in particular in the shallow lakes such as Great Salt Lake and Little Manitou Lake. More primary production measurements are needed before the relationship between production and abiotic factors can be established for *Artemia* habitats.

Low primary production has been measured in coastal salterns such as Lake Grassmere (Wear and Haslett, 1987) and the ESSA salterns in Mexico (Javor, 1983). Similar to the shallow inland lakes, the contribution of the benthos to saltern production is probably important (Javor, 1983).

Meromixis, which occurs in a number of *Artemia* habitats (*e.g.* Solar Lake, Sinai, Egypt ; Zuni Salt Lake, New Mexico, USA ; Great Salt Lake, Utah, USA), has a major effect on primary production. First of all the chemical stratification serves as a barrier for nutrients between the hypolimnion and the epilimnion. Secondly, photosynthetic bacteria tend to grow in abundance

TABLE I

Nutrient concentrations in *Artemia* habitats

Artemia habitat	Source	Month	Nutrient concentration (μmol/l)			
			PO_4	NH_4	NO_3	NO_2
Alviso Salt Pond #6*, California, USA	Carpelan (1957)	March	0.24	–	0.89	–
		October	0.42	1.21	0.12	–
Boca Chico Salt Lake*, Venezuela	Scelzo and Voglar (1980)	June	0.14	1.7	0.08	0.007
			0.08-0.34	1.2-3.2	0.06-0.09	0.003-0.018
Didwana Lake**, India	Bhargava *et al.* (1987)	January-December	0.89	–	13.6	0.13
			0.20-2.39	–	6.8-24.5	0.01-0.28
ESSA saltern*, Mexico	Javor (1983)	January-December	<1	<5	<2	–
Lake Grassmere*, New Zealand	Wear and Haslett (1987)	January-December	0-0.01	0.002-0.11	0-0.08	–
Great Salt Lake**, Utah, USA	Stephens and Gillespie (1976)	April-November	⩾32.3	37.5-50	2.1-12.9	–
Little Manitou**, Saskatchewan, Canada	Hammer (1978)	March-September	10	4300	20	–
			2-30	1400-7100	0-110	

* Coastal

** Inland

– No data.

in the metalimnion (Cohen *et al.*, 1977ab). Algal and bacterial production has been studied in detail in Solar Lake, Sinai (Cohen *et al.*, 1977ab). It was determined that the bacteria plates in the metalimnion contribute 92 % of total water column primary productivity. In the epilimnion primary production is low, possibly because of *Artemia* grazing pressure, which is very high (Cohen *et al.*, 1977b).

Secondary production

It is difficult to estimate secondary production of a zooplankton population without a detailed understanding of its dynamics and life-history characteristics. This is further complicated by continuous changes in the abiotic environment (*e.g.* seasonal temperature fluctuations) and temporal changes and spatial patchiness in the food supply, all of which affect growth, reproduction and mortality. It is therefore not surprising that production estimates for natural *Artemia* populations are uncommon. Gillespie and Stephens (1977) estimate an annual *Artemia* production in Great Salt Lake at 100 to 200 g dry weight/m^2/year. This estimate is based on preliminary data and additional information is needed before *Artemia* production in natural habitats can be judged more accurately. Under experimental, high food conditions *Artemia* production is very high (*e.g.* 208 g dry weight/m^3/day, Sorgeloos and Persoone, 1975).

Biological interactions

Biological interactions can be as important as abiotic factors in affecting life-history strategies and population dynamics. The two types of interactions, which are usually studied in greatest detail, are predator-prey and competition. In hypersaline communities the *Artemia*-phytoplankton interaction is one of the major ones. However, in some *Artemia* habitats predation and competition can be important.

Grazing

Herbivorous zooplankton have been implicated in controlling phytoplankton standing stock and influencing natural phytoplankton assemblages through selective grazing (Wetzel, 1975). *Artemia* can attain high densities in their natural habitats (*e.g.* Bradbury, 1971 ; Wirick, 1972 ; Gillespie and Stephens, 1977 ; Ramamoorthi and Tangaraj, 1980 ; Scelzo and Voglar, 1980 ; Lenz, 1984) and negative correlations between their densities and phytoplankton abundances have been attributed to grazing by the *Artemia* (Anderson, 1958 ; Mason, 1967 ; Wirick, 1972). As in many other zooplankton (*e.g.* Frost, 1972), grazing rates in *Artemia* are a function of algal densities (Reeve, 1963 ; Lenz, 1982). Peak filtering rates measured in the laboratory are between 150 and 250 ml/adult/day (Reeve, 1963 ; Lenz, 1982). At this feeding rate, densities of 4 to 7 adults/l would clear the water column once per day. In Mono Lake, with mean epilimnetic densities frequently above 4/l, grazing by *Artemia* exceeded phytoplankton growth during the summer (Lenz, 1982). *Artemia* may thus be food limited in this situation and might well be in many others. Although food limitation in *Artemia* has not been demonstrated, small summer brood sizes in Mono Lake (Lenz and Dana, 1987), Great Salt Lake (Wirick, 1972), Boca Chica Salt Lake (Scelzo and Voglar, 1980) and Laysan Lagoon (Lenz and Dana, 1987) support this hypothesis.

Artemia is usually described as an indiscriminate filter-feeder (Persoone and Sorgeloos, 1980) and no studies have indicated selective feeding. However, Gibor (1956) has demonstrated that *Stichococcus* cannot be digested by *Artemia* and thus passes unharmed through the gut. This may explain the high densities of this alga in the high salinity pools in the Alviso salt ponds (San Francisco Bay, California, USA), and the virtual absence of *Dunaliella salina*, which could grow readily under the existing physico-chemical conditions (Carpelan, 1957).

Predation

In spite of its tolerance of salinities below that of seawater, *Artemia* occurs only in hypersaline lakes. Edmondson (1966) suggested that predation and competition limited *Artemia* to the higher salinities. The absence of *Artemia* from an otherwise suitable habitat is often explained by this zooplankter's inability to withstand fish predation (Persoone and Sorgeloos, 1980). However, fish predation on *Artemia* occurs to a limited extent in certain natural habitats. Usually this area involves a salinity gradient where predation occurs in a limited area of overlap (*e.g.* Scelzo and Voglar, 1980). Insect predation has been observed in several Indian lakes (Ramamoorthi and Tangaraj, 1980 ; Bhargava *et al.*, 1987), Solar Lake (Dimentman and Spira, 1982), and Laysan Lagoon (W. Gagne, pers. commun.). Bhargava *et al.* (1987) hypothesize that invertebrate predation may contribute to the disappearance of *Artemia* during low salinity periods. Kristensen and Hulscher-Emeis (1972) report that in Curaçao and Bonaire, Antilles, *Artemia* is excluded from low salinities through predation by a cyclopoid copepod.

As discussed in the review by Persoone and Sorgeloos (1980) the primary predators on *Artemia* are waterfowl. More specifically, reported predators include gulls (Isenmann, 1975 ; Winkler, 1977 ; Wear and Haslett, 1987), flamingos (MacDonald, 1980 ; Bhargava *et al.*, 1987), avocets (Carpelan, 1957), stilts (Carpelan, 1957 ; Bradbury, 1971 ; Bhargava *et al.*, 1987), grebes (Bradbury, 1971 ; Cooper *et al.*, 1984), ducks (Bradbury, 1971) and other shorebirds (Bradbury, 1971 ; Carpelan, 1957).

Few studies have quantified the impact waterfowl predation has on *Artemia* populations. At Mono Lake, migrating grebes consumed between 8 000 and 70 000 *Artemia*/grebe/day, and monthly grebe predation accounted for 8 to 83 % of *Artemia* mortality during the fall when predator densities were high (Cooper *et al.*, 1984). Wear and Haslett (1987) report daily consumption rates for gulls at Lake Grassmere of 50 g wet weight/gull, which is approximately equivalent to 6 000 adults/gull/day. Although waterfowl can ingest large numbers of brine shrimp per day, their densities are usually too low to severely impact the *Artemia* populations.

Competition

In general, competitive ability appears to decrease with increasing salinity (Por, 1980) and *Artemia*, which is the most salt tolerant crustacean, has been described as a fugitive species (Edmondson, 1963). In Great Salt Lake, *Artemia* is reported to be the sole zooplankter (Wirick, 1972). In Mono Lake, Mason (1967) reported the occurrence of two rotifer species in addition to *Artemia*. However, recently no rotifers have been found in the lake (Lenz, 1982). Even in the 1960's, rotifers and *Artemia* were segregated temporally, rotifers predominating during the winter. Temporal or spatial separation between *Artemia* and other zooplankters has been found in other habitats (Anderson, 1958 ; Broch, 1969 ; Javor, 1983). Geddes (1980) observed

temporal separation along a salinity gradient for *Parartemia* and *Artemia* in Australia. This separation appeared to be due to competitive exclusion, since their salinity tolerances overlap.

Conclusion

The genus *Artemia* has diversified to inhabit lakes ranging from permanent to highly temporary, from seasonal to aseasonal, from predictable to unpredictable. In seasonal habitats, such as Mono Lake and Great Salt Lake, animals are adapted for a rapid repopulation of an "empty" lake at the beginning of the season, followed by a large production of cysts to assure the survival during the uninhabitable period. The life-history strategy of these *Artemia* include high fecundity with a short initial period of ovoviviparity followed by oviparity. Permanent and relatively aseasonal habitats on the other extreme, promote ovoviviparity (to the near exclusion of oviparity) and a low but steady reproductive output, resulting in a multiplicity of asynchronous generations each year.

Inland habitats may be more productive than coastal salterns and ponds, as is suggested by differences in nutrient concentrations and primary production. Thus commercial harvesting of *Artemia* from saltworks may be inefficient, unless nutrient levels are raised. Little is known about secondary production in salt lakes. However, *Artemia* population and grazing studies are suggestive that this zooplankter may be food limited in some habitats. Additional data on salt lake productivity and the factors regulating it will permit more effective management of *Artemia* production ponds.

Artemia offers an unusual opportunity for the study of the relationships between habitat characteristics and population biology within a single genus. With the marked increase in ecological data since the last Symposium, the complexities of *Artemia* ecology have become more apparent.

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Comments on *Artemia* introductions and the need for conservation

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Abstract

Although *Artemia* has a range of very specific adaptations, more ecological field studies are required to make effective use of these. Only then will aquaculturalists be able to select the strain of *Artemia* best suited for a given characteristic or local environment. Furthermore, there are hazards attached to the introduction of *Artemia* into regions where natural populations of brine shrimp occur. This point is illustrated by reference to the Australian brine shrimp, *Parartemia*, an especially interesting halobiontic anostracan. An important hazard is the possible loss of genetic diversity, and thus of useful features. The importance of conservation is emphasized.

Introduction

In response to the increasing requirements for *Artemia* cysts, and as part of solar pond management techniques, *Artemia* has been introduced worldwide into a large number of salterns (artificial coastal saline ponds) and salt lakes. These introductions have involved the transfer of *Artemia* to parts of the world where it does not occur naturally, as well as to parts where it does. The result is that few regions of the world with non-marine saline water-bodies now lack *Artemia*, providing suitable environmental conditions prevail. Persoone and Sorgeloos (1980) have given an account of the present distribution.

When *Artemia* was first being spread by man, there seemed no reason why the process should be regarded as other than advantageous. Salt lakes were seen as having little biogeographical variability, a situation summarized succinctly by Macan (1963) who, writing about natural salt lakes, noted that "... the similarities between these lakes, scattered over America, Africa and Asia ... are startling. *Artemia salina*, Rotifera, Cladocera, Copepoda, Corixidae, and Ephydridae constitue the fauna". *Artemia* was thought to be a single species, cosmopolitan, and a species typical of non-marine salt waters.

We now know that views of this sort are far from reality. Not only are there differences in the fauna of salt lakes between continents, there are also regional differences within a continent, and even local differences according to patterns of salinity, water permanence, climatic predictability, and other factors. Such differences are certainly evident for the fauna of Australian salt lakes (*e.g.* Williams, 1984). As for *Artemia*, after the recognition of sibling species (Halfer-Cervini *et al.*, 1968), it soon became clear that a very large number of reproductively isolated taxa are involved within the genus (Bowen *et al.*, 1980 ; Browne and MacDonald, 1982). In the Iberian peninsula alone some 36 different strains have been recognised (Amat Domenech, 1980).

Reflecting geographical differentiation, the fauna of natural salt lakes, including of course *Artemia* when present, is adapted to the local environmental conditions. This means that a considerable diversity of biological material is available for use by man. More specifically, natural populations of *Artemia* possess a wide and genetically based range of adaptational abilities. We believe that this point is frequently lost sight of when *Artemia* populations are artificially transferred between localities. We also believe that the hazards associated with the apparently serendipitous manner in which many transferences of *Artemia* are effected is even more frequently lost sight of. Several authors (*e.g.* Persoone and Sorgeloos, 1980) have already drawn attention to these matters. The extent to which the Australian fauna and flora may be at risk is, however, of special concern to us, and the purpose of the present paper is to underscore further the hazards associated with uncontrolled introductions.

The paper has not been prepared as one designed unnecessarily to constrain the use of *Artemia*; rather, it is offered as a constructive contribution to the sensible and most efficacious use and management of a resource (salterns and inland salt lakes) at a time when increasing recognition is being accorded to their value as localities of both scientific and economic significance (Williams, 1981). Recognition of the important role played by conservation in the use of *Artemia* is seen as a significant management criterion.

The range of adaptations

Reflecting local adaptation, particular strains of species of *Artemia* possess distinctive abilities with regard to a variety of features. Recognizing this, *Artemia* aquaculturalists are becoming increasingly aware that it is better to select a given strain of *Artemia* for a given set of environmental conditions, than to attempt to alter local environments to suit. Adaptation to local temperatures seems to be a particular problem when, for example, San Francisco Bay *Artemia* are introduced into tropical areas (de los Santos *et al.*, 1980).

Recognizing this, a few studies have already outlined procedures designed to select strains of *Artemia* for a particular habitat (Tobias *et al.*, 1980; Vanhaecke *et al.*, 1984). Such procedures may be useful for inoculations that are of a temporary nature (De los Santos *et al.*, 1980), but are much less so for large scale introductions into permanent salt pond systems. Even so, it is very difficult to conduct experiments on the comparative value of different strains in field conditions. Predicting the likely success of various strains would be possible if information were available on their ecology. Unfortunately, with some notable exceptions (and these often involving somewhat unusual *Artemia* habitats), **field** studies of *Artemia* are few indeed. It is not easy, therefore, to match desirable biological characteristics, or known environmental features, with particular strains or features of their environment. Even for what would appear to be the most obvious features of interest to aquaculturalists, namely, rates of growth, reproduction and biomass production, information on natural populations is extremely scarce. Moreover, when information is available for one population, it is often difficult to compare it with information for another. Considering only biomass data as a case in point, difficulties derive from various sources, *e.g.* the use by different authors of different units in the reporting of results, the great variation between and within sites (itself a reflection of contagiously distributed animals), and, not least, an apparent confusion by workers in the field of what constitutes **biomass** (or density) and what constitutes **production** (or productivity) (*cf.* Persoone and Sorgeloos, 1980).

As a general comment on this matter, then, we note that of all the questions relating to *Artemia* in its natural habitat, those concerning population dynamics are the most critical. There is no doubt, of course, that laboratory investigations can add considerably to our knowledge of *Artemia* in many areas of its ecology ; but the extent to which laboratory derived data can be extrapolated to field situations is problematical, and complementary field studies will always be needed. There is an obvious dearth of such field studies, and until more have been undertaken we are a long way from any full appreciation of the range of adaptation possessed by *Artemia*, and of the value, therefore, of individual strains or species.

The hazards of introductions

There are always certain hazards involved when species are spread beyond natural boundaries. Deleterious effects are most obvious when the species involved is large and of particular concern to man. Less obvious, of course, are the biological effects of exotic introductions involving small animals of relatively little concern to man ; but we may be sure that size and human significance are not features which preempt such effects! So far as *Artemia* is concerned in this connection, almost no information is available.

What, then, are the possible effects of *Artemia* introductions on other organisms inhabiting natural salt water-bodies? An obvious one is that competition with local strains or species of *Artemia* may occur. It has been shown that under experimental conditions co-occurrence of two *Artemia* strains may lead to the extinction of one, and it has been suggested that sexual strains will outcompete parthenogenetic strains (Browne, 1980). According to Browne and MacDonald (1982), *Artemia* introductions have been somewhat restricted to date, with no recorded introductions of non-native strains to North America, Eurasia or the Indian subcontinent. However, San Francisco Bay *Artemia* have been established in South America (Brazil) and Australia, two continents where natural halobiont faunas exist.

The introduction of *Artemia* is of particular concern to Australian biologists because *Artemia* appears to be an exotic introduction into Australia (Geddes, 1979) and because Australian salt lakes are populated by a large number of endemic species of *Parartemia*, a genus of brine shrimp in a quite separate family to that containing *Artemia* (Fig. 1). Australia, in fact, represents a special case with respect to the introduction of *Artemia* ; it is the only continent where a distinctly different halobiont anostracan has evolved. There is an urgent need to ensure conservation of *Parartemia* which is the only other model of a truly halobiont strongly hypo-osmotic anostracan. Furthermore, *Parartemia* comprises several morphologically distinct species with localized distributions.

Studies on the biology of *Parartemia* are in their early stages, but we already recognize that it copes with high salinity by mechanisms of osmoregulation similar to those of *Artemia* (Geddes, 1975abc), and that nauplii utilize a larval dorsal salt organ similar to that of *Artemia* (Conte and Geddes, in press). On the other hand, there are major differences between the two genera in respiratory physiology, feeding and reproduction ; *Parartemia* does not use haemoglobin as a respiratory pigment (Mitchell and Geddes, 1977 ; Manwell, 1978), *Parartemia* appears to be a sediment feeder rather than an open water filterer (Marchant and Williams, 1977), and preliminary work suggests that the factors controlling the determination of oviparity and ovoviviparity may be different in *Parartemia* than *Artemia*. Much basic biology remains to be done on *Parartemia* ; for example, there is the possibility that particular species of *Parartemia*

may have a unique intermediary respiratory metabolism that allows survival in acid salt lakes (Conte and Geddes, in press).

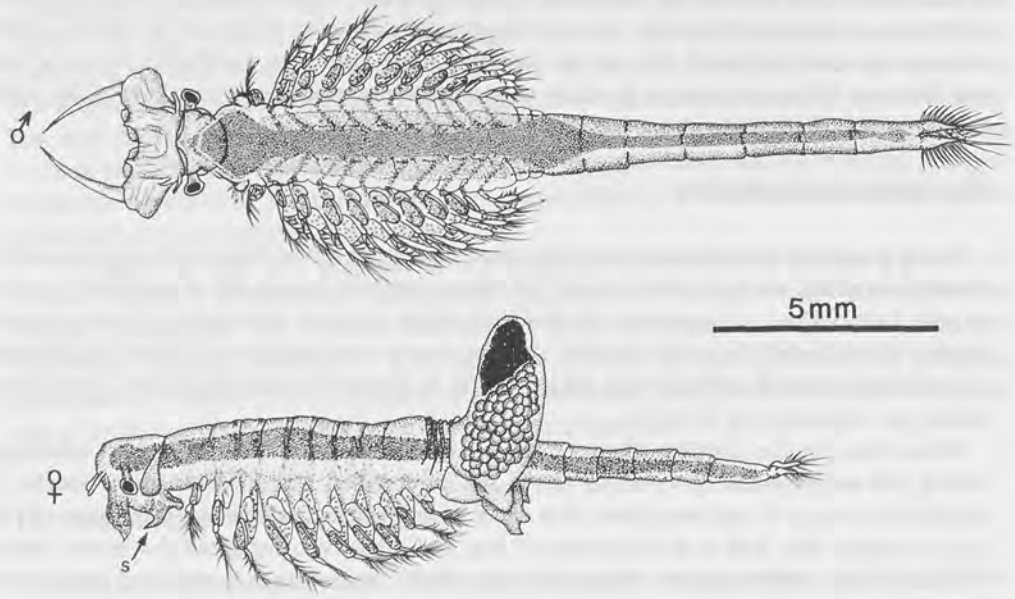

FIG. 1. *Parartemia zietziana*, one of many species of brine shrimp endemic to Australia. s = spine on labrum (after Geddes, 1981).

No work has been done on applied aspects of *Parartemia* biology, such as its suitability for aquaculture. It appears that because it produces resting eggs that sink and hence are difficult to collect, and because it does not use a respiratory pigment and so is less well adapted to low O_2 levels, it has an inferior aquacultural prospect than *Artemia*. However, species of *Parartemia* attain much larger size than *Artemia*, with maximum length of 22 mm and dry weight of 4 mg for males and in some natural lakes they attain very high biomass (up to approximately 5 g dry weight/m^2) and production (up to 11.3 g dry weight/m^2/year) (Marchant and Williams, 1977). It is difficult to make comparisons with *Artemia* because of the lack of reliable production studies on that genus, as previously indicated, but it may be that where production of brine shrimp biomass is the aquaculture goal, *Parartemia* may be a useful species.

Given the high conservation value of *Parartemia*, is there any suggestion that *Parartemia* species or populations are at risk from *Artemia* in Australia? Earlier work has suggested that *Artemia* was restricted to coastal saltworks (Geddes, 1981) and not found in natural salt lakes. However, in recent years a few sporadic records have been made of *Artemia* in natural or semi-natural environments, *i.e.* from a natural salt lake south of Perth, from samphire swamp at Port Adelaide, from a hyperhaline lake at Port Augusta and, most disturbingly, from a saline pool in Cooper's Creek, central Australia (Fig. 2). These are all small and isolated sites ; it is not known whether dispersal was by natural means or by man, nor is it known whether the

populations will persist in these sites. These recent records require that further consideration be given to the possibility of *Artemia* becoming established in natural lakes in Australia.

One consideration required when discussing whether *Artemia* might become established in Australia is the wide range of environments offered by Australian salt lakes. It has been suggested that the ephemeral salt lakes that have been studied in south-east and south-west Australia are unsuitable for *Artemia* because they fill in the winter when water temperatures are low and they have low algal productivity (Geddes, 1980). However, in central and northern Australia, water temperatures are high and *Artemia* may be well suited. Endemic species of *Parartemia* occur in these areas including *P. minuta* in Lake Buchanan in Queensland and an undescribed species of *Parartemia* from Lake Eyre (Williams and Kokkinn, in press). Salt lakes in central and northern Australia are little studied and further new species of *Parartemia* may occur.

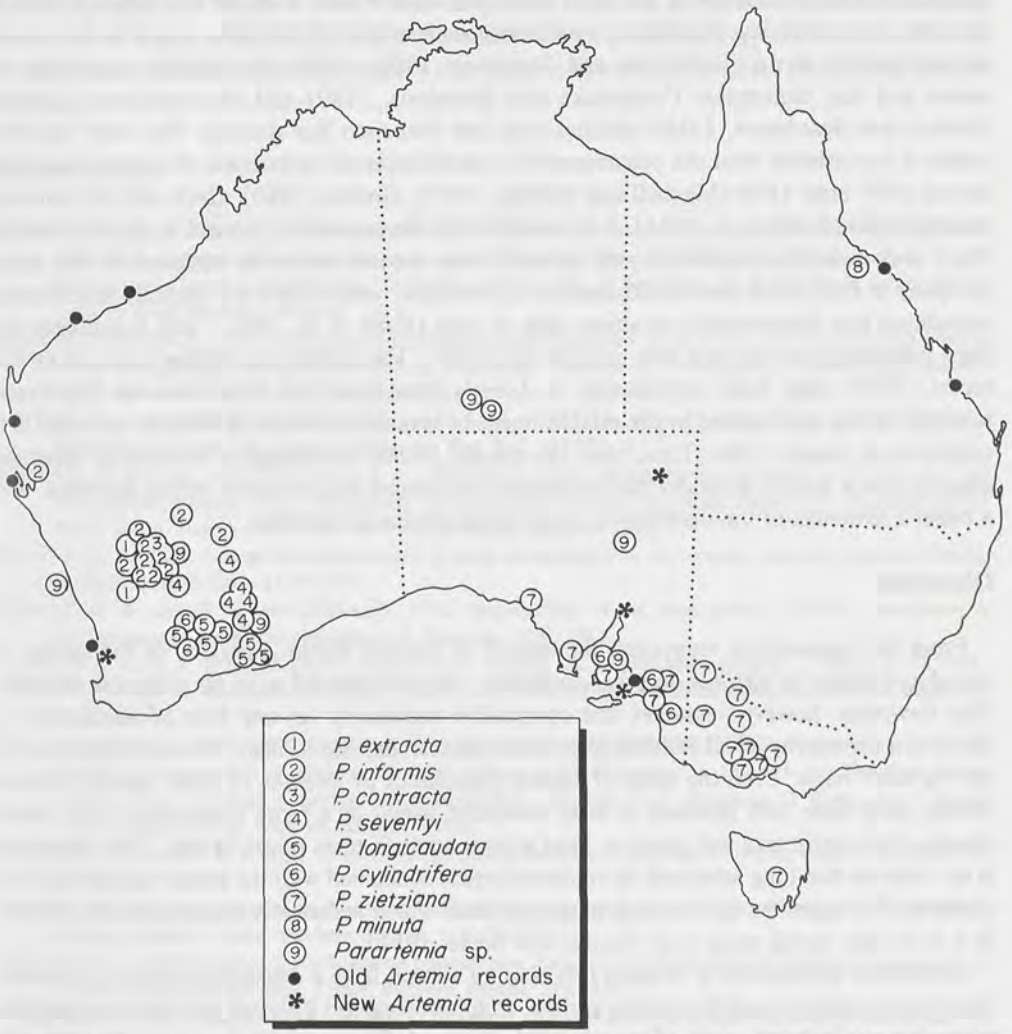

FIG. 2. Distribution of *Parartemia* and *Artemia* in Australia. This figure updates the information given by Geddes (1981).

A second consideration is that because the various strains and species of *Artemia* have different environmental preferences, there is a strong likelihood that some can effect successful establishment in Australia. Already, it appears that two forms of *Artemia* occur in Australia. A parthenogenetic form similar to others in India, France, Russia and Japan occurs on Rottnest Island and at Port Hedland in Western Australia (Bowen *et al.*, 1978) and other, probably similar, parthenogenetic populations occur at Shark Bay, Lake McLeod and at the ICI Dry Creek Saltfields near Adelaide (Geddes, 1980). A sexual form that is cross-fertile with San Francisco Bay *Artemia* (Clark and Bowen, 1976) and probably represents a recent introduction is recorded from Rockhampton, Queensland. Another sexual population probably of San Francisco Bay origin occurs at Bowen in Queensland.

More recently, two collections of Australian *Artemia* material have been included within the International Study on *Artemia*, one from Shark Bay and the other from the Dry Creek Saltfields, Adelaide. The Shark Bay population is parthenogenetic and is biometrically similar to the Indian parthenogenetic strain (Vanhaecke and Sorgeloos, 1980), while the Adelaide population is sexual and has biometrical (Vanhaecke and Sorgeloos, 1980) and electrophoretic (Abreu-Grobois and Beardmore, 1980) affinities with San Francisco Bay *Artemia*. This latter identification is inconsistent with the occurrence of a parthenogenetic population of *Artemia* over the period 1975 until 1978 (Mitchell and Geddes, 1977 ; Geddes, 1980) which did not produce resistant cysts (Jones *et al.*, 1981). It is probable that the population thought to derive from the Dry Creek Saltfields, Adelaide, were actually from another saltworks operated by the same company at Port Alma near Rockhampton, Queensland, where there is a sexually reproducing population that intermittently produces rafts of cysts (Jones *et al.*, 1981). This is probably the same population studied by Clark and Bowen (1976). This situation is further confused by the recent (1980) large scale introduction of *Artemia* cysts from Port Alma into the Dry Creek saltfields which has resulted in the establishment of sexually reproducing *Artemia* recorded in a collection in August 1985. Thus, even the present limited knowledge of *Artemia* in Australia suggests that a variety of strains may have been introduced and relocated within Australia, and a better knowledge of the distribution of particular strains is required.

Discussion

From the aquacultural viewpoint, the spread of *Artemia* within Australia, or the spread of introduced strains of *Artemia* on other continents, may be regarded as of no particular moment. This viewpoint, however, assumes that competitive superiority (as one form of interaction) is allied to a superiority in all features of possible interest and use to man. This assumption is, to say the least, naive. Until the value of natural populations of *Artemia*, or other species of brine shrimp, have been fully assessed, a wiser viewpoint would be a more conservative one, which assumes that native taxa will prove to have at least some features of use to man. This viewpoint is the same as that long advanced by conservationists concerned with the preservation of genetic diversity. The argument has a strong pragmatic basis which is certainly as applicable to *Artemia* as it is to, say, cereal crops (*e.g.* Frankel and Soulé, 1981).

Introduced populations of *Artemia* may also, of course, have a deleterious effect on elements of saline ecosystems apart from brine shrimp. In this connection it should be borne in mind that increasing use is being made of, or recognition accorded to many other species found in salt lakes, *e.g. Dunaliella* as a source of glycerol and β-carotene, and *Spirulina* as a source of protein.

Modern techniques of recombinant genetics will undoubtedly greatly extend the present list of useful organisms.

Persoone and Sorgeloos (1980), amongst others, have pleaded for the conservation of all remaining natural *Artemia* habitats. To pleas of this sort we add that *all* salt lakes, irrespective of the presence or absence of *Artemia*, are of high conservation value. Threats to them are many and varied ; they include drainage, diversion of influents, and, not least, the introduction of exotic *Artemia*. With a particularly distinctive halobiont fauna, including *Parartemia*, special consideration needs to be accorded to this matter in so far as Australia is concerned.

In so far as *Artemia* itself is concerned, we wholeheartedly support the resolution put forward at the end of this Symposium with regard to the need to conserve *Artemia* ; *i.e.* "... the 2nd International Symposium on *Artemia*, meeting in Antwerp in September 1985, resolves that all possible measures be taken to ensure that the genetic resources of natural *Artemia* populations are conserved ; such measures include the establishment of gene-banks (cysts), close monitoring of inoculation policies, and where possible the use of indigenous *Artemia* for inoculating *Artemia* free waters".

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The cryptobiotic state of *Artemia* cysts, its diapause deactivation and hatching : a review

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Content

Introduction

The ability of an organism to create a cryptobiotic state during its lifespan is a major survival mechanism in populations inhabiting biotopes characterized by unstable environmental conditions which may preclude survival of the individual. As a representative of such a group *Artemia* ensures its survival during periods of *e.g.* desiccation, extreme temperatures, etc., by the production of dormant embryos. These so-called cysts can resist extremely unfavorable circumstances and will be the origin of a new population when abiotic and biotic parameters in the habitat become again favorable.

Although the formation of a dormant state during the reproductive part of the life cycle is not unique in crustaceans, brine shrimp seem to have developed a sort of flexibility towards the variety of 'unstable' habitats they inhabit. Unlike related phyllopods, *Artemia* females easily switch from cyst production (oviparity) to live nauplii birth (ovoviviparity), resulting in a fast increase of the population when environmental conditions are optimal. Moreover, there is no 'sexual' control over these modes of reproduction as in most cladocerans or rotifers where only fertilized, mictic females produce resting eggs. This ensures a fast response to fluctuating

circumstances which may prevail in a period of severe environmental conditions. In fact, this flexible life-history strategy may also explain why some strains inhabiting relatively stable biotopes appear to have lost this ability to produce dormant cysts, *i.e.* this second mode of reproduction has no adaptive value anymore (D'Agostino and Provasoli, 1968 ; Mitchell and Geddes, 1977 ; Dimentman and Spira, 1982 ; Lenz, 1987 ; Lenz and Dana, 1987).

The basic biochemical mechanisms involved in the switch of the reproduction mode in *Artemia* are not yet fully understood. Laboratory experiments showed that cyst production can be induced by applying oxygen stresses when Fe-EDTA is present in the medium (Versichele and Sorgeloos, 1980 ; Lavens and Sorgeloos, 1984). Under these conditions a specific haemoglobin is produced which may play a role in the formation of the outer cyst shell (Lavens and Sorgeloos, 1984). The triggering mechanism(s) for the induction of the state of diapause is (are), however, not yet known.

The unraveling of the processes involved in dormancy will help to better understand the ecological adaptations of this crustacean to its specific biotopes ; it will furthermore result in a more optimal use of *Artemia* as a study-object in many disciplines of biological sciences and as a live food source in fish and crustacean rearing : *i.e.* for the aquarium hobbyist as well as the aquaculturist the cysts are of great importance since they can be stored for years in a dry form to resume their development and produce free-swimming nauplii within a 24-h incubation period in seawater. However, not all sources of cysts yield maximal hatching outputs even when the cysts were harvested, processed, and dried following standardized techniques. Such 'inferior' products are not suitable for application in research, and, from an economical point of view, cannot be optimally used in larval rearing of aquatic organisms.

This review aims to characterize the state of dormancy, identify its role in *Artemia* embryos, and elucidate the impact of different environmental and genetical factors on this process. Specific treatments which may deactivate the diapause process resulting in improved hatchability of the cysts will be discussed, including strain-specific adaptations which may be related to the ecology of the natural biotope. Topics on fundamental and biochemical processes involved with the cryptobiotic state (*e.g.* anhydrobiosis), and its depressed metabolism are covered *in extenso* by Crowe *et al.* (1987).

Finally, we hope that this review will create a better basis for more directed research related to dormancy and its termination.

Definitions, occurrence, and functional role of cryptobiosis in the animal kingdom

Because of the confusion in the literature on cryptobiosis it is essential to first define the different states of arrested metabolism. The fact that different cryptobiotic life stages (eggs, larvae) have been described for widely different groups of plants and animals, *e.g.* protozoa, rotifers, tardigrades, nematodes, crustaceans, and insects (Lees, 1961 ; Crowe and Clegg, 1973, 1978 ; Crowe and Madin, 1974 ; Gilbert, 1974 ; Belk and Cole, 1975), is probably at the origin of the confusion in terminology. Furthermore, all definitions related to this subject (Keilin, 1959 ; Hinton, 1960 ; Sussman and Halvorson, 1966 ; Gilbert, 1974 ; Belk and Cole, 1975 ; Clegg, 1978a ; Grice and Marcus, 1981 ; Wommersley, 1981) deal with two characteristics which may be subjected to different interpretations, *i.e.* the degree of depressed metabolic activity of the resting stage, and the influence of the environment on this process.

The following terminology has been adapted in this review for the precise description of the different states of arrested or retarded development in cysts of *Artemia* and of other animals (species list in Table III, see further).

Cryptobiosis is a general term which, as indicated by Keilin (1959), comprises those states of an organism which show no visible sign of life and of which the metabolic activity becomes hardly measurable, eventually reaching a reversible standstill.

Dormancy is a specific form of cryptobiosis with an endogenous control of metabolism and development ; *i.e. Artemia* gastrulae enter the dormant state within the uterus independently of the prevailing environmental circumstances.

Quiescence, on the other hand, refers to the environmental (exogenous) control of metabolism and development, *e.g.* extremes of temperature, oxygen, desiccation, etc., inducing a state of retarded development ; further embryogenesis will only resume when the environmental conditions become favorable. Depending on the adverse factor, different types of quiescence can be considered, *i.e.* **anhydrobiosis** (lack of sufficient amounts of water), **cryobiosis** (low temperatures), **anoxybiosis** (lack of oxygen), etc. (Crowe *et al.*, 1987).

The denomination diapause is frequently used in relation to *Artemia* and indicates the state of dormancy where an arrest of development is initiated by internal factors before the environment has become unfavorable (Fig. 1). In the oviparous mode of reproduction embryonic development in *Artemia* is arrested at the gastrula stage within the uterus. This onset of dormancy is rigidly programmed into the development of *Artemia*, independent of geographical origin or environmental conditions (Olson and Clegg, 1976). The dormant embryos, composed of a partial syncytium of about 4 000 nuclei (Nakanishi *et al.*, 1962) surrounded by complex shells (Morris and Afzelius, 1967 ; Anderson *et al.*, 1970 ; Khalaf *et al.*, 1978) are released from the ovisac into the biotope. There is no measurable metabolic activity (Dutrieu, 1960 ; Finamore and Clegg, 1969 ; Clegg, 1974) and the cysts will not hatch, even when climatic conditions are favorable, *i.e.* they remain in a dormant state. The term diapause is correctly applied here since the induction for an ovigerous reproduction may have been initiated before the climatic circumstances turned unfavorable (Lenz, 1987 ; Lenz and Dana, 1987), or even without adverse environmental conditions occurring. Further development to a quiescent phase can only be achieved when the endogenous mechanism(s) responsible for the induction of diapause is (are) deactivated. In most cases this is realized by dehydration of the cysts, *i.e.* by air drying of the cysts which accumulate on the shore or by osmotic water removal in the highly saline waters where they are normally released. Once this dormancy has been terminated, the metabolism and embryonic development is controlled by external factors, and as soon as optimal conditions are restored (*e.g.* rehydration) cysts will eventually hatch. The cellular and developmental biology/biochemistry involved with these processes have been covered *in extenso* by Bagshaw (1980), and Clegg and Conte (1980).

To be complete we should also cite the reports of non-dormant *Artemia* cysts (also called 'subitaneous' or 'summer eggs') which hatch immediately after deposition (Mathias, 1937 ; Lochhead, 1941 ; Dutrieu, 1960 ; Versichele, 1983 ; Amat *et al.*, 1987). This rather uncommon phenomenon might be a malfunctioning of the diapause-inducing mechanism, resulting in the direct production of quiescent cysts or even embryos, *i.e.* when no thick shells are formed, and might only occur when the female brine shrimp switches its mode of reproduction.

Besides *Artemia* and other anostracans dormant cysts are also produced by some notostracan, conchostracan, cladoceran, and calanoid copepod species, as well as some rotifers (Monogononta) (Table III, see further).

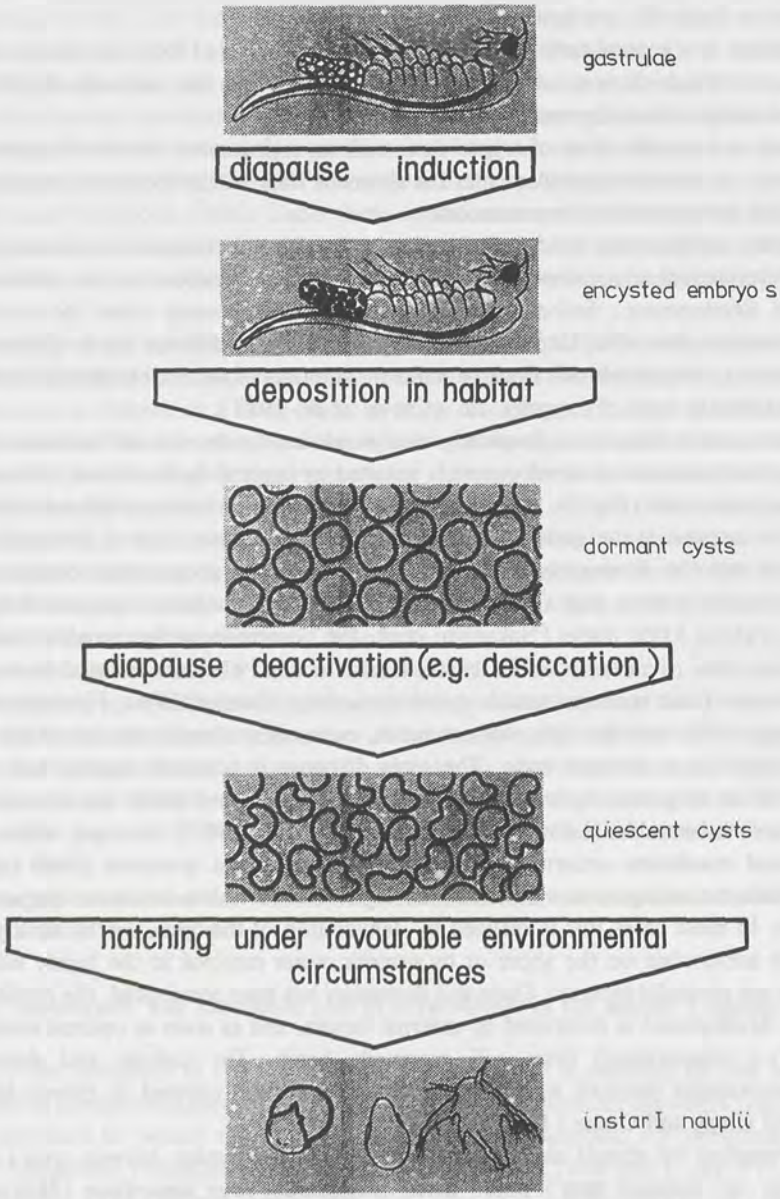

FIG. 1. Schematic diagram explaining the specific terminology used in relation with dormancy of *Artemia* embryos.

As already mentioned the major functional role of cryptobiosis is most likely the survival of the population under temporarily adverse conditions. From an ecological point of view, the most appropriate strategy in biotopes with more or less predictable cyclic environmental stresses would be dormancy, whereas quiescence would be optimal in non-cyclic circumstances (Belk and Cole, 1975). By the production of diapausing eggs the organisms may anticipate sub-optimal biotope conditions. Dormancy as a cryptobiotic process may also be relevant as a life-history strategy, *i.e.* by synchronising the life cycles to the variations that occur in the habitat. Endogenous control over metabolism and development ensures a synchronous hatching, resulting in a fast start and consequent development of the population shortly after the re-establishment of favorable environmental conditions. It is this synchrony that allows effective colonization in temporal biotopes. Last but not least dormancy also provides indirect ways to effectively overcome dispersion difficulties. The properties of the cysts to resist severe conditions without losing their viability make them ideal both for passive and active transportation by wind, waterfowl, and men into new suitable biotopes (Horne, 1966 ; MacDonald, 1980 ; Persoone and Sorgeloos, 1980).

Mechanisms for the induction and termination of diapause

The following hypotheses have been formulated on diapause mechanisms in freshly-released cysts of *Artemia*.

Anderson *et al.* (1970) suggested that the degree of permeability of the cyst shell regulates diapause. According to these authors this permeability may be controlled by the thickness of the shell, or by density, microstructure or chemical composition of particular layers in the shell. In this regard Morris and Afzelius (1967) considered an outer membrane to be the barrier for penetration ; *i.e.* they claimed that this 'sealing envelope' is built up after dehydration of the cysts in the uterus, and that resumption of development will only take place after destruction or removal of this membrane. Our observations on cysts freshly released from a controlled cyst-production system (Lavens and Sorgeloos, 1984) revealed, however, that cysts are not always anhydrobiotic nor that they have an intact outer membrane, but still remain in diapause. Even the complete removal of the tertiary envelope (*i.e.* decapsulation) of the latter cysts did not result in the resumption of their embryonic metabolism.

Dutrieu (1960) assumed that diapause is interrupted by the splitting of carotenoproteins into free carotenoids and proteins, as a result of which the cysts become quiescent. The splitting mechanism, however, has not been elucidated. The recent discovery of egg-specific *cis*-canthaxanthins (Nelis *et al.*, 1984ab, 1987a) might, however, increase the speculation of a possible function of these carotenoids in the cryptobiotic process in cysts, *i.e.* until now pigments with the unusual *cis*-configuration have only been isolated in *Artemia* and some related cyst-producing crustaceans (Nelis *et al.*, 1987a). Especially the divergence in relative abundance of *cis*- and *trans*-canthaxanthins in hydrated *versus* dehydrated cysts is remarkable (Nelis *et al.*, 1987ab). It is, however, not yet clear if the role of these egg-specific carotenoids is related to the basic diapause mechanism(s) or to the biochemical processes which protect the embryo's viability during *e.g.* anhydrobiosis by *e.g.* stabilizing the membrane structures (see also Crowe *et al.*, 1987).

Finally, the team of Crowe and co-workers detected intimate changes in the intra cyst pH of dormant and quiescent (anoxybiotic) cysts (Busa and Crowe, 1983 ; Drinkwater and Crowe, 1986 ; Crowe *et al.*, 1987), and revealed that depression of the internal pH (pH_i) can lead to

the breaking of dormancy. Since the pH_i affects respiration, metabolism, and development in anoxybiotic cysts, Crowe *et al.* (1987) assumed that dormancy might be induced by an internal pH which exceeds the physiological range ($pH_i > 7.9$). However, other data collected by the same team suggest that there may actually be two compartments in the dormant cysts that are separated in pH_i by about 0.5 pH units. The function of these compartments has still to be elucidated. As mentioned by Crowe *et al.* (1987), terminating dormancy "appears to be considerably more complex than acidification of a basic cytoplasm".

Factors affecting the hatchability of cysts

Fundamental research on potential mechanisms and processes involved in diapause induction and/or inhibition is seriously hampered by potential interferences, *e.g.* various mechanisms which may mask possible effects of eventual diapause deactivation mechanisms. We have tried to group the factors which operate at succeeding stages of the cyst-to-nauplius process into five distinct clusters (Fig. 2). Overlapping or interactions, however, may occur, especially with regard to the diapause terminating mechanisms (see further).

As a consequence, a high degree of interference exists between the different sets of parameters which are involved in cyst hatching, eventually resulting in misinterpretation of the experimental results. Since this might explain much of the confusion in the literature we have extended the present review on diapause to the other groups of factors which are involved in cyst hatching.

Genotypic factors

It is very likely that the hatching capability of *Artemia* cysts differs in function of the sibling species or the geographical origin. Genotypical differences may be the basis of varying hatching characteristics (hatching percentage and/or hatching rate) among brine shrimp populations (Vanhaecke and Sorgeloos, 1982 ; Tackaert *et al.*, 1987), as was found in rotifers (Gilbert, 1974). However, hitherto literature has not provided the decisive evidence. Browne *et al.* (1984) compared reproductive and life-span characteristics in 12 *Artemia* strains and found that hatching was strongly correlated with the environment and not with the genotype. Some indications of strain-specific adaptations with regard to diapause are given in Lavens *et al.* (1986b) ; (see further under diapause termination techniques).

Culture conditions

The recent development of laboratory systems for the controlled production of brine shrimp cysts (Versichele and Sorgeloos, 1980 ; Lavens and Sorgeloos, 1984) has provided the experimental tool to study the influence of abiotic and/or biotic factors on the hatchability of *Artemia* cysts. Indeed, the variation in hatching characteristics of cyst batches from the same origin (Vanhaecke and Sorgeloos, 1982) could never be fully explained due to lack of essential data on the antecedents of these dormant cysts produced by wild populations. Cunningham and Grosch (1978), Sarasquete Reiriz (1979), and Bohra (1980) claimed the existence of hatching differences depending upon the season in which the cysts were produced. Pond production tests in conditions which facilitated a better control over the environmental production parameters and over the harvesting conditions, revealed some indications that the biotope may have interfered

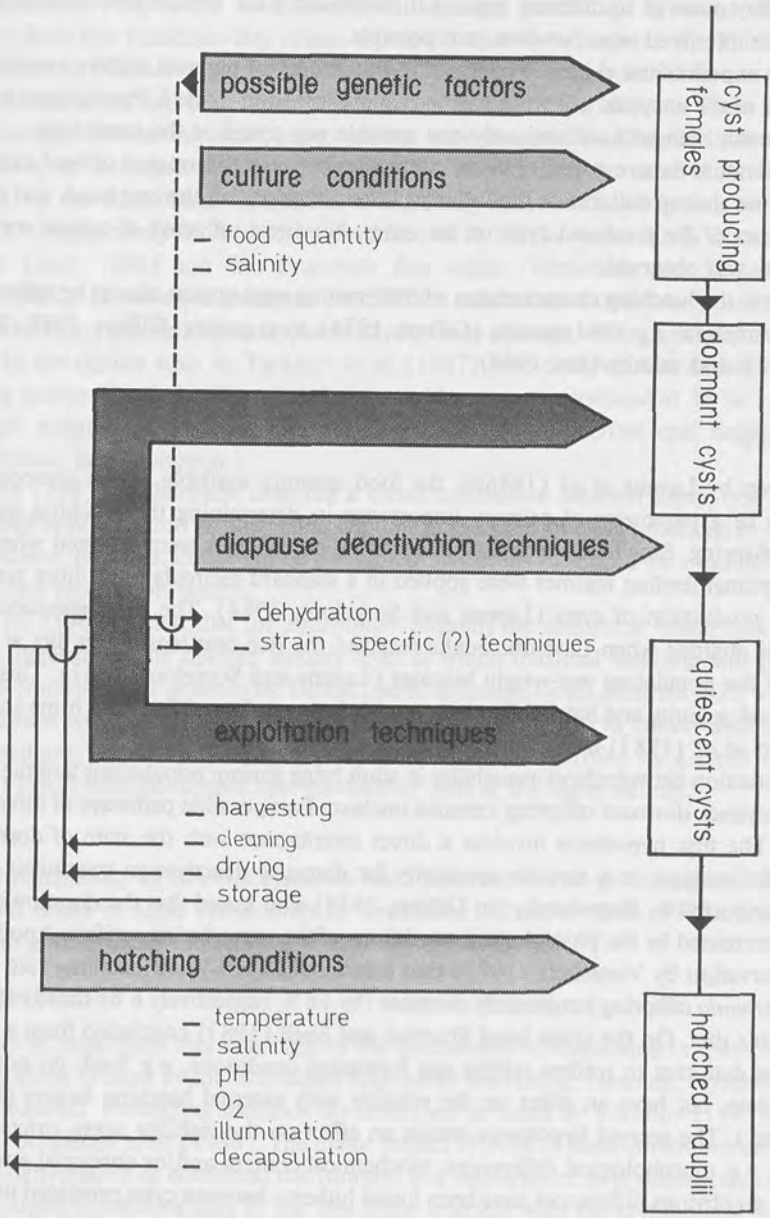

FIG. 2. Schematic diagram showing the (combined) interaction of various clusters of factors on the hatchability of *Artemia* cysts.

with the hatching quality. Vos *et al.* (1984) demonstrated varying hatching efficiencies (from 214 000 up to 339 200 nauplii/g of cysts) for cysts produced after inoculation with San Francisco Bay material in different regions in Southeast Asia. Precise detection of the parameter(s) which interfered was, however, not possible.

Decisive experimental evidence concerning the impact of parental culture conditions on the hatchability of the encysted offspring has been summarized in Table I. Production circumstances were maximally standardized and only one variable was tested at the same time.

These literature data reveal an obvious interaction between the amount of food available to the parental brine shrimp cultures or the salinity of their biotopes on the one hand, and the hatching characteristics of the produced cysts on the other. No direct influence of culture temperature or food quality was observed.

In rotifers, the hatching characteristics of their resting eggs appear also to be influenced by the culture parameters : *e.g.* food quantity (Gilbert, 1974), food quality (Gilbert, 1980 ; Pourriot and Snell, 1983), and salinity (Ito, 1960).

Food quantity

As proven by Lavens *et al.* (1986b), the food quantity available to the reproducing adults appears to be a parameter of primary importance in determining the hatching quality of the encysted offspring. Significant increases in hatching percentages were detected when two types of (sub-)optimal feeding regimes were applied in a standard recirculating culture system for the controlled production of cysts (Lavens and Sorgeloos, 1984). The most spectacular increase (27 %) was attained when Lavalduc adults received the rice bran/corn bran diet at a daily rate of 15 % of the population wet-weight biomass (Lavens and Sorgeloos, 1987). This correlation between food quantity and hatchability has recently been confirmed for other brine shrimp strains by Tackaert *et al.* (1987) using live algae as food source.

The interaction between food availability in adult brine shrimp populations and the hatchability of their encysted, dormant offspring remains unclear. Two possible pathways of interaction seem plausible. The first hypothesis involves a direct interference with the state of dormancy itself, resulting for example in a variable sensitivity for diapause deactivation treatments. This hypothesis is supported by Bogoslavsky (in Gilbert, 1974) who stated that the duration of dormancy may be determined by the physiological conditions of the reproducing rotifers. Another example is the observation by Versichele (1983) that hatchability (H %) and hatching rate (T_{90}) of the encysted *Artemia* offspring temporarily decrease (by 18 %, respectively 6 h) shortly after a change of the adult's diet. On the other hand Pourriot and Snell (1983) concluded from a compilation of hatching data that in rotifers resting egg formation conditions, *e.g.* food, do not modify the dormant state, but have an effect on the relation with external hatching factors (temperature, salinity, etc.). The second hypothesis entails an effect on the viability *sensu stricto* of the cysts produced, *e.g.* morphological differences, biochemical content and/or abnormal embryogenesis. However, no obvious differences have been found hitherto between cysts produced under optimal *versus* sub-optimal conditions. Even preliminary analysis of carbohydrate levels – which are known to be critical for an optimal functioning of the trehalose-glycerol hyperosmotic regulatory system (Conte *et al.*, 1977) – revealed no correlation between food availability for the parental brine shrimp, trehalose or glycerol concentrations in the cysts produced under laboratory conditions, and their hatchability (Lavens and Crowe, unpubl.).

Salinity

According to Versichele and Sorgeloos (1980) cysts produced at low salinity appear to have a low hatchability ; *e.g.* a 50 % difference in hatching percentage is noted in laboratory populations from San Francisco Bay origin kept in 35 and 90 ‰ respectively. This has recently been confirmed by Naessens (pers. commun.) who harvested cysts from 70 ‰ and over 100 ‰ saltponds in Kenya that had been inoculated with San Francisco Bay *Artemia*. These observations might be strain specific as only small improvements were observed for Chaplin Lake and Tientsin cysts produced at increased salinities (35 *versus* 90 ‰ ; Tackaert *et al.*, 1987). High salinity (> 150 ‰), on the other hand, also seems to negatively affect hatchability : *e.g.* hatching levels drop by 50 %, respectively 35 % in cysts produced at > 150 ‰ *versus* 90 ‰ from Mono Lake (Dana and Lenz, 1986) and San Francisco Bay origin (Versichele and Sorgeloos, 1980 ; Versichele, 1983). However, to interpret the different results one should take possible variations in food availability (qualitatively and quantitatively) into account. This parameter was carefully optimized in the culture tests by Tackaert *et al.* (1987) who observed the slightest interaction on hatching quality. Similarly, Great Salt Lake and Macau cysts produced at 35 ‰ S in intensive flow-through systems gave hatching levels comparable to commercial cyst batches (Lavens, unpubl. ; Tobias, pers. commun.).

Versichele (1983) furthermore detected a direct correlation between lower cyst hatchability and decreased concentration of haematine content in the shell of cysts produced at low salinity. This observation, however, could not be confirmed by Lavens (unpubl.) for cysts collected from a standard cyst-production system.

Finally, Ito (1960) stated that for *Brachionus plicatilis* the salinity at which cyst production took place, determines the optimal salinity level at which maximal hatching will prevail. This finding was confirmed for *Artemia* by Tobias (pers. commun.) who detected a 20 % increase in hatching success when incubating cysts produced at low salinity (35 ‰ culture system) in a 5 ‰ hatching medium (Table I). However, this could not be confirmed by Lavens (1981) and Versichele (1983) who performed cyst-production tests at the same salinity.

Other factors

Reduced hatchability of *Artemia* cysts has been reported in cyst-producing cultures exposed to non-lethal doses of toxic compounds or irradiation (Grosch, 1966, 1973 ; Squire, 1970 ; Cunningham and Grosch, 1978).

EXPLOITATION FACTORS

A third set of conditions that may control the hatchability of diapausing or quiescent *Artemia* embryos are those created by the techniques applied for harvesting, cleaning, drying, and storing of the cyst material. Processing methods are described in detail by Voronov (1973), Rakowicz (1975), and Sorgeloos *et al.* (1986). The major impact of most of these conditions can be related to effects of dehydration or combined dehydration and rehydration, as is summarized in Table II. As a result, these conditions may in the first place interfere with the cryptobiotic state and act as true diapause deactivation processes if the cyst material is dormant. For anhydrobiotic, quiescent cysts, on the other hand, insufficient dehydration levels, too long periods of hydration before a next dehydration, or too many hydration/dehydration cycles result in a serious drop in the viability of brine shrimp cysts (Morris, 1971 ; Rakowicz, 1975 ; Sorgeloos *et al.*, 1976 ; Benijts *et al.*, 1977 ; Vanhaecke and Sorgeloos, 1982 ; Sorgeloos *et al.*, 1986) (Fig. 3).

TABLE I

Experimental results on the influence of abiotic and biotic conditions on the hatchability of cysts, produced under controlled conditions, and processed under standard circumstances

Type of production system	Strain origin	Experimental abiotic and biotic parameter(s)	Hatching percentage	Standard deviation	Reference
Laboratory system : batch culture	San Francisco Bay (USA)	*Spirulina* - 20 °C - 35 ‰	28	–	Versichele and Sorgeloos (1980)
		Spirulina - 28 °C - 35 ‰	26	–	Versichele (1983)
		Spirulina - 20 °C - 90 ‰	76	–	
		Spirulina - 28 °C - 90 ‰	72	–	
		Spirulina - 20 °C - 180 ‰	38	–	
		Rice bran - 20 °C - 35 ‰	19	–	
		Rice bran - 28 °C - 35 ‰	22	–	
		Rice bran - 20 °C - 90 ‰	66	–	
		Rice bran - 28 °C - 90 ‰	78	–	
		Rice bran - 20 °C - 180 ‰	40	–	
		Spirulina - Baker's yeast - 25 °C - 90 ‰	83	–	
		Scenedesmus - 25 °C - 90 ‰	45	–	
		live *Dunaliella* - 25 °C - 90 ‰	85	–	
Laboratory system : closed flow-through culture (90 ‰ ; inert diets)	Lavalduc (France)	optimal feeding[1]	20.1	4.7	Lavens *et al.* (1986b)
		sub-optimal feeding[1]	14.5	2.8	
	San Francisco Bay (USA)	optimal feeding[1]	53.0	4.4	
		sub-optimal feeding[1]	42.4	5.4	
		optimal feeding regime[2]	63.7	7.8	
		sub-optimal feeding regime[2]	36.6	6.5	
Laboratory system : closed flow-through culture (inert diet)	Great Salt Lake (USA)	25 °C - 35 ‰	68.9	5.6	Lavens (unpubl.)

TABLE I. Continued

Type of production system	Strain origin	Experimental abiotic and biotic parameter(s)	Hatching percentage	Standard deviation	Reference
Laboratory system : open flow-through culture (live *Chaetoceros*)	Macau (Brazil)	25 to 28 °C - 35 ‰	61 (35 ‰) 84 (5 ‰)	8 –	Tobias (pers. commun.)
Laboratory system : batch culture (live *Dunaliella*)	Great Salt Lake (USA)	35 ‰ - optimal feeding	11.1	–	Tackaert *et al.* (1987)
		90 ‰ - optimal fedding	35.1	–	
	Chaplin Lake (Canada)	35 ‰ - optimal feeding	14.5	–	
		35 ‰ - suboptimal feeding	7.1	–	
		90 ‰ - optimal feeding	19.2	–	
	Tientsin (PR China)	35 ‰ - suboptimal feeding	30.1	–	
		35 ‰ - optimal feeding	51.8	–	
		90 ‰ - optimal feeding	60.2	–	
Outdoor culture ponds	San Francisco Bay (USA)	85 ‰	27.0	5.1	Naessens (pers. commun.)
		155 ‰	69.5	7.1	
Laboratory system : culture in vials (inert diet mixed with *Spirulina* powder)	Mono Lake (USA)	76 ‰	62	–	Dana and Lenz (1986)
		88 ‰	66	–	
		97 ‰	58	–	
		118 ‰	66	–	
		133 ‰	64	–	
		159 ‰	15	–	

[1] Feeding regimes were kept constant, and densities varied.

[2] Different feeding regimes : optimal and suboptimal refer to amounts of food respectively 10 and 15 % of biomass (wet weight).

TABLE II

Possible effects of exploitation factors on hatching quality of *Artemia* cysts

Exploitation step/technique		Possible effects
Harvesting	From pond-water surface	
	+ at regular intervals	Dormant (or quiescent) cysts ; their dehydration levels are determined by pond salinity
	+ at irregular intervals	Risks for exposure to dehydration/hydration cycles as a result of rainfall/salinity changes in the pond Risks for mixture with cysts temporarily accumulated on-shore
	From the shore	(Complete) dehydration Risks for rehydration (rainfall, air, humidity) and for rehydration/dehydration cycles Risks for exposure to high temperatures (> 40 °C) during sun drying Risks for exposure to UV-radiation
Storage before processing	In pond water	No effect when salinity does not change ; longterm effects not known
	In brine (saturated NaCl solution)	Dehydration to ± 20 % H_2O content
Cleaning	In brine (saturated NaCl solution)	Dehydration to ± 20 % H_2O content
	In tapwater	Rehydration (60 % H_2O content after 15 min at 20 °C)
	Eventual different cycles	Dehydration-rehydration cycles
Drying	Several techniques	Varying dehydration rates ; varying levels of final water content Possible exposure to high temperatures, possible exposure to UV radiation (for sun drying)
Stocking	Several techniques	Varying hatching quality when insufficiently dehydrated or stored in presence of oxygen

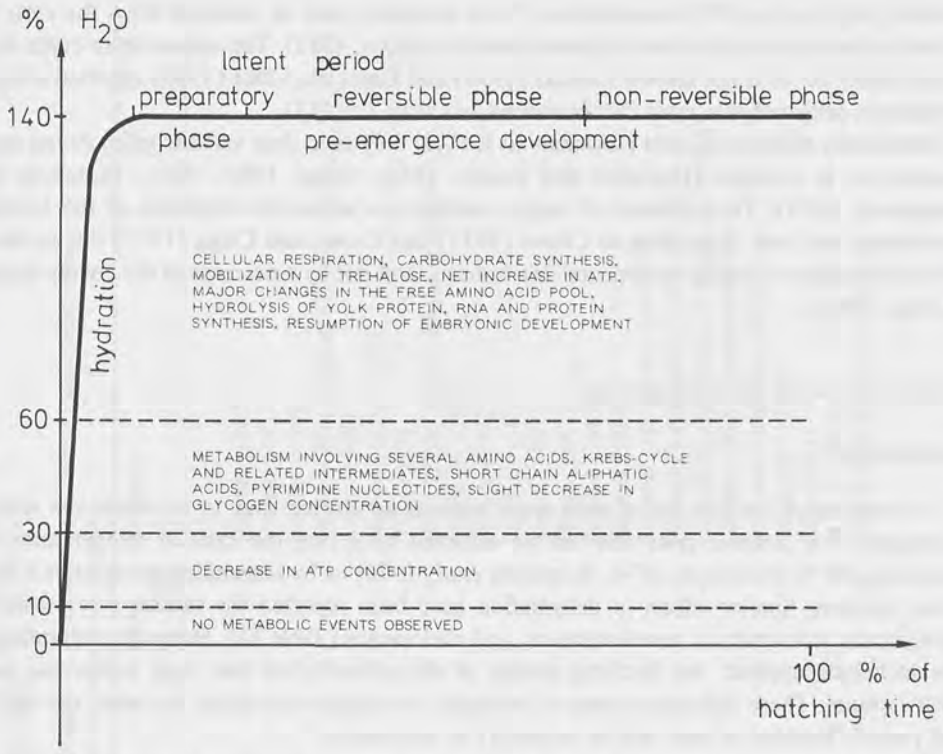

FIG. 3. Hydration dependence of cellular metabolism in *Artemia* cysts. (For supporting evidence see Morris, 1971 ; Clegg, 1974, 1976a-d, 1977, 1978ab ; Clegg and Cavagnaro, 1976 ; Clegg and Lovallo, 1977 ; Olson, 1979).

Cysts exposed for too long a period to water levels exceeding 65 % will have completed their pre-emergence embryonic development. Subsequent dehydration of these cysts will not result in an otherwise reversible interruption of the hatching metabolism (Morris, 1971 ; Olson, 1979) but in the killing of the differentiated embryos. Additional negative effects on hatchability are noted when hydrated cyst material is exposed to prolonged UV-irradiation or to temperatures above 40 °C (Nimura, 1968 ; Voronov, 1974).

Insufficient dehydration or rehydration resulting in water levels in the range 30-65 % H_2O will initiate metabolic activities which may critically reduce the energy contents down to levels which are insufficient to reach the state of emergence when incubated in seawater under optimal hatching conditions.

A depletion of the energy reserves is furthermore attained when the cysts undergo subsequent hydration/dehydration cycles. Long-term storage of such material results in the loss of the cumulative effect of hydration on development and hatching percentages decrease substantially (Morris, 1971 ; Vanhaecke and Sorgeloos, 1982).

Hatching quality in stored cysts is slowly decreasing when the cysts contain water levels from 10 to 35 % H_2O. Clegg and Cavagnaro (1976) detected indications of enzyme activity and a

serious drop in the ATP concentration. These processes may be retarded when the cysts are stored at freezing temperatures (Vanhaecke and Sorgeloos, 1982). The optimal intra-cystic water level (under 10 %) is not known. Iwasaki (1958) and Engel and Fluke (1962) reported a higher irradiation sensitivity for cysts that contained less than 1 % H_2O.

Sufficiently dehydrated cysts (less than 10 % H_2O) only keep their viability when stored under vacuum or in nitrogen (Dempster and Hanna, 1956 ; Clegg, 1962, 1967 ; Vanhaecke and Sorgeloos, 1982). The presence of oxygen results in a substantial depletion of the hatching percentage and rate. According to Crowe (1971) and Crowe and Clegg (1973) this is caused by the formation of highly detrimental free radicals, and not by a decrease of the energy content (Clegg, 1962).

Diapause deactivation conditions

Dehydration

In many cases the removal of intra-cystic water is an efficient way to terminate the state of dormancy. For *Artemia* cysts this can be achieved by drying the cysts at temperatures not exceeding 40 °C (Voronov, 1974 ; Sorgeloos *et al.*, 1976) or by suspending the cysts in a NaCl brine solution. Similar effects of dehydration have been reported for various cyst producing anostracans, notostracans, conchostracans, and cladocerans (Table III). However, depending on the technique applied, the hatching quality of the treated cysts may vary within the same (sub-)species. These differences seem to be highly correlated with either the level, the rate, or the period (moment of start and/or duration) of desiccation.

The relative humidity at which the drying cysts are kept seriously affects hatching (Hall, 1961 ; Hempel-Zawitkowska, 1971a ; Khalaf *et al.*, 1977 ; Scott and Grigarick, 1979). More pronounced desiccation may result in europlasticity, *i.e.* the resistance of *Triops* cysts against large variations in temperature becomes much higher (Carlisle, 1968 ; Hempel-Zawitkowska and Klekowski, 1968 ; Hempel-Zawitkowska, 1971b).

The influence of the length of the dehydration period on the hatchability of diapausing eggs of *Streptocephalus* (Moore, 1967 ; Bernice, 1972), *Chirocephalus* (Hall, 1953, 1961 ; Khalaf *et al.*, 1977), *Limnadia* (Bishop, 1968), and *Triops* (Takahashi, 1977) is very significant. Bernice (1972) illustrated the need for a drying period of more than 15 days to obtain 80 % hatching of *Streptocephalus* cysts. Too long desiccating periods, on the other hand, result in lower hatching percentages or hatching rates. Analogous findings for *Triops* are reported by Takahashi (1977).

Only minor hatching is recorded when drying is started immediately after deposition of the eggs of *Streptocephalus* or *Triops* (Prophet, 1963 ; Hempel-Zawitkowska, 1967, respectively). In the latter case the insertion of a rest period of at least 5 days before dehydration is started results in increased hatching levels up to 90 %.

Similar tendencies have been observed in *Artemia*, however, only scarce data are available on true diapausing cysts. Most information is indeed on cyst material that was already (partially) dehydrated : *e.g.* collected from highly saline waters or from the shore, or processed. Some of the effects noted in these cases are therefore not due to the dehydration techniques applied but to *e.g.* dehydration/hydration cycles (Vanhaecke and Sorgeloos, 1982) and will therefore not be discussed here.

TABLE III

Literature review on diapause terminating techniques for crustacean (non-*Artemia*) and rotifer cysts
(effect : + = positive, 0 = none, − = negative)

Diapause-inhibiting method		Species	Effect H %	Effect HR	Reference
	CRUSTACEA				
Desiccation	Anostraca	*Branchinecta lindahli*	+		Prophet (1963)
		Branchipus stagnalis	+		Mathias (1937)
		Eubranchipus serratus	+		Prophet (1963)
		vernalis	+		Castle (1938) ; Weaver (1943)
			−		Avery (1939)
		Chirocephalus diaphanus	0	−	Mathias (1926, 1929)
			+/−	−	Hall (1953)
			0	+	Hall (1959a, 1961) ; Khalaf *et al.* (1977)
			0	−	Nourisson (1964)
		Streptocephalus dichotomus	+		Bernice (1972)
		seali	+/−		Prophet (1963)
			0		Moore (1957)
			−		Moore (1967)
		texanus	+		Prophet (1963)
		Thamnocephalus platyurus	+		Prophet (1963)
	Notostraca	*Lepidurus apus*	+		Braswell (1967)
		Triops cancriformis	+	+	Hempel-Zawitkowska (1967, 1971b)
				−	Hempel-Zawitkowska and Klekowski (1968)
		granarius	+		Carlisle (1968), Takahashi (1977)
		longicaudatus	−		Scott and Grigarick (1979)
			+		Takahashi (1977)
	Conchostraca	*Eulimnadia antlei*	+		Belk (1972)
		Limnadia stanleyana	+		Bishop (1968)
		Caenestheriella gynecia	0	−	Mattox and Velardo (1950)

TABLE III. Continued

Diapause-inhibiting method		Species	Effect H %	Effect HR	Reference
	Cladocera	*Daphnia magna*	−		Van De Vel (1984)
			+		Doma (1979)
		pulex	−	−	Pancella and Stross (1963)
Dehydration/ hydration	Notostraca	*Triops cancriformis*	+		Fox (1949), Hempel-Zawitkowska (1967)
		longicaudatus	+		Takahashi (1977)
Low temperature	Anostraca	*Eubranchipus vernalis*	+		Weaver (1943)
		Chirocephalus stagnalis	+		Nourisson (1961)
	Notostraca	*Lepidurus apus*	+		Braswell (1967)
		Triops cancriformis	0/+		Hempel-Zawitkowska (1971b)
		granarius	0/+		Takahashi (1977)
		longicaudatus	0		Takahashi (1977)
	Copepoda (Calanoida)	*Pontella meadi*	+		Grice and Gibson (1977)
		Labidocera aestiva	+	+	Marcus (1979, 1980, 1984), Grice and Marcus (1981)
		Tortanus forcipatus	0		Kasahara and Uye (1979)
Light/UV	Notostraca	*Triops cancriformis*	0/+	+	Hempel-Zawitkowska (1970), Takahashi (1975, 1977)
		longicaudatus and *granarius*	+		Takahashi (1975, 1977)
	Conchostraca	*Eulimnadia antlei*	+		Belk (1972)
		Limnadia stanleyana	+		Bishop (1967)
	Cladocera	*Pleuroxus denticulatus*	+		Shan (1970)
	Copepoda (Calanoida)	*Labidocera aestiva*	+		Marcus (1982)
Osmotic shock	Anostraca	*Branchipus stagnalis*	+		Mathias and Bouat (1934)

TABLE III. Continued

Diapause-inhibiting method		Species	Effect H %	Effect HR	Reference
Decapsulation	Notostraca	*Triops granarius*	+		Takahashi (1977)
	Cladocera	*Daphnia magna*	+	+	Van de Vel (1984)
		pulex	+	+	Pancella and Stross (1963)
Organic chemicals	Anostraca	*Branchipus stagnalis*	+		Mathias and Bouat (1934)
Organic detritus	Anostraca	*Chirocephalus bundyi*	+		Broch (1965)
Anaerobiosis	Anostraca	*Chirocephalus bundyi*	+		Broch (1965)
	Notostraca	*Triops cancriformis*	+		Hempel-Zawitkowska (1971b)
Freshwater	Anostraca	*Chirocephalus diaphanus*	+		Hall (1959a)
Water depth	Anostraca	*Chirocephalus bundyi*	0		Broch (1965)
		diaphanus	–	–	Hall (1959a, 1959b)
		Streptocephalus dichotomus and *seali*	0		Moore (1967), Bernice (1972)
	ROTIFERA				
Desiccation	Monogononta	*Brachionus plicatilis*	+		Lubzens *et al.* (1980)
Hibernation		*Asplanchna* spp.	+	+	Nipkow (1961), Birky (1964)
		Brachionus plicatilis		+	Blanchot and Pourriot (1982b)
			–		Lubzens *et al.* (1980)
			+		Minkoff *et al.* (1983)
		rubens	+		Blanchot and Pourriot (1982a)
Light		*Brachionus plicatilis*	+		Minkoff *et al.* (1983), Hagiwara *et al.* (1985)
			–		Ito (1960)
		rubens	+		Blanchot and Pourriot (1982a)
Osmotic shock		*Brachionus plicatilis*	+		Hagiwara *et al.* (1985)

Experimental results that may help to interpret the possible roles of specific dehydration methods in the termination of diapause are summarized in Table IV. According to Versichele and Sorgeloos (1980) a minimum level of dehydration is required to obtain maximal hatchability, *i.e.* 80 % hatching at a water content below 20 %, *e.g.* when the cysts are suspended in saturated NaCl brine for at least 24 h (Clegg, 1978b). Further water removal to less than 10 % did not improve the hatching output (Godeluck, 1980 ; Versichele and Sorgeloos, 1980 ; Lavens *et al.*, 1986b ; Naessens, pers. commun.). On the other hand the inferior results recorded with the 48 h $CaCl_2$ treatment might be related to the extremely low water content achieved in these cysts, which seems to make them far more sensitive to radiation effects (Iwasaki, 1958 ; Engel and Fluke, 1962).

It furthermore appears from Table IV that the type of drying technique interferes with diapause inhibition, *e.g.* very significant increases in the hatching percentage are noted when San Francisco Bay cysts are oven dried at 40 °C instead of at 30 °C (Versichele and Sorgeloos, 1980) or when cysts from Lavalduc are dried in a fluidized bed dryer *versus* an oven (Godeluck, 1980). Apparently a faster water removal seems to improve hatchability. The small differences reported by Lavens (unpubl.) and Lavens *et al.* (1986b) can be interpreted in the same way. Cyst drying was, however, most probably an insufficient deactivation method and more specific diapause inhibitors were required.

Considering the criterion hatching rate, Versichele (1983) detected no correlation between the dehydration technique applied and the hatching rate of lab produced SFB cysts : both oven dried and brine-stored cysts yielded hatching curves analogous to those of the parental material.

Repeated dehydration/hydration cycles

Fox (1949), Hempel-Zawitkowska (1967), and Takahashi (1977) revealed that most of the dried cysts of *Triops*, which failed to hatch at their first incubation, did develop after one (or two) successive dehydration/hydration (D/H) cycles. Analogous observations were reported for *Artemia* by Barigozzi (1939), Morris (1971), and Metalli and Ballardin (1972). Up to 70 % hatching was recorded for these so-called 'delayed hatching cysts' which were distinguishable from the other cysts by structural differences : "cysts examined had the space between the shell and embryo filled in most regions with rosette-like particles assumed to be glycogen" (Morris, 1971). More recently, Browne (1980), Versichele (1983), Browne *et al.* (1984), and Lavens (unpubl.) studied the effect of up to 5 D/H cycles. They found high variations between the different sibling species or even within the same species (Table V). Generally speaking *Artemia parthenogenetica* and *A. franciscana* gave the best hatching output after 2 D/H cycles, while *A. persimilis* and *A. tunisiana* needed up to 5 D/H cycles. The fact that the hatching levels often did not reach 50 % indicates once more that this diapause treatment will only be effective in specific cases (see further). An overview of the effect of several dehydration/hydration cycles on the hatchability of *Artemia* cysts is given in Table V.

Several dehydrations may also have a negative effect on the hatching rate when the treated cysts are stored for a long time. Cysts exposed to 2 D/H cycles initially hatch faster – confirming the cumulative development theory of Morris (1971) – but after several weeks storage need 2 h extra incubation to reach hatching (Sorgeloos *et al.*, 1976 ; Vanhaecke and Sorgeloos, 1982). This delay may be due to the drying technique applied, *i.e.* the more slowly water is removed, the more the hatching rate is delayed after storage.

TABLE IV

Effect of various dehydration techniques on the hatching performance of *Artemia* cysts

Strain	Specific characteristics of the non-processed cyst material	Dehydration technique used	Hatching %	Reference
SFB	Lab produced cysts (90 ‰ S)	Saturated NaCl-brine for 1 h	6	Versichele and Sorgeloos (1980)
		Saturated NaCl-brine for 4 h	17	
		Saturated NaCl-brine for 6 h	27	
		Saturated NaCl-brine for 12 h	49	
		Saturated NaCl-brine for 24 h	76	
		Saturated NaCl-brine for 48 h	79	
		Oven drying at 30 °C for 24 h	20	
		Oven drying at 30 °C for 48 h	60	
		Oven drying at 40 °C for 48 h	81	
		Drying over $CaCl_2$ for 24 h	72	
		Drying over $CaCl_2$ for 48 h	23	
	Produced in extensive ponds (85 ‰ or 155 ‰*) in Kenya ; harvested from the water	Saturated NaCl-brine	12/56*	Naessens (pers. commun.)
		Oven drying at 35 °C for 6 h	15/59*	
GSL	Lab produced cysts (90 ‰) batch 210584	NaCl-brine	4	Lavens *et al.* (1986b)
		MgCl-brine	3	
		Oven drying at 35 °C for 24 h	11	
	batch 0483	$NaCl_2$-brine	46	Lavens (unpubl.)
		Oven drying at 35 °C for 24 h	69	
RAC	Lab produced cysts (90 ‰) batch 210584	NaCl-brine	35	Lavens (unpubl.)
		Oven drying at 35 °C for 24 h	40	
LVD	Harvested from the lake water (180 ‰)	NaCl-brine	37	Godeluck (1980)
		Oven drying at 35° C	41	
		Fluidized bed drying at 35 °C	90	
ML	Harvested from the lake water (90 ‰)	No treatment	17	Dana (1981)
		NaCl-brine	40	

TABLE V

Effect of several dehydration/hydration cycles on the hatchability of *Artemia* cysts produced under controlled conditions

Strain	Hatching % after 1 to 5 dehydration/hydration cycles (non-cumulative hatching data)					Cumulative hatch (%)	Reference
	1	2	3	4	5		
San Francisco Bay, USA	19	50	0	0	0	69	Browne (1980), Browne *et al.* (1984)
Puerto Rico, USA	6	9	8	0	0	23	
Hidalgo, Argentina	19	12	8	12	2	53	
Chott Ariana, Tunisia	2	5	2	12	2	23	
Larnaca, Cyprus	14	1	2	3	7	27	
Santa Pola, Spain	17	4	0	0	12	33	
Tuticorin, India (cited as Madras)	7	29	8	1	0	45	
Kutch, India	0	17	1	0	0	18	
Cadiz, Spain	1	12	1	0	12	26	
Izmir, Turkey	21	0	0	0	1	22	
Macau, Brazil	40	44	–	–	–	88	Versichele (1983)
Great Salt Lake, USA	2	3	0	–	–	5	Lavens *et al.*(1986b)
Lavalduc, France	30	20	–	–	–	50	Lavens (unpubl.)

Hibernation

The need for a cold preincubation treatment in other crustaceans and rotifers is documented in Table III. Weaver (1943) indicated that non-dried *Eubranchipus vernalis* cysts only hatched (33 %) after slow freezing and thawing. Another anostracan, *Chirocephalus stagnalis*, showed analogous effects when preincubated at 4 °C for daily cycles of 3 up to 18 h (Nourisson, 1961). Braswell (1967) noted the need for a hibernation period for the notostracan *Lepidurus apus*, that was, however, not essential for *Triops* (Hempel-Zawitkowska, 1971b ; Takahashi, 1977). Remarkable for the latter organism is that freezing can restore the negative effect of an inefficient drying. Marcus (1979, 1980) demonstrated for the copepod *Labidocera aestiva* that resting eggs which are chilled at 5 °C for a minimal period of 30 days, will hatch better and more synchronously. This seems also to be valid for *Pontella meadi* (Grice and Gibson, 1977). A far better synchronous hatching occurred also with cysts of the rotifer *Brachionus plicatilis* after hibernation for 3 months at 5 °C ; only 2 days instead of 3 months were required to obtain a 50 % hatching (Blanchot and Pourriot, 1982b).

Substantial evidence on the effect of a cold treatment as diapause inhibitor in brine shrimp cysts has been revealed by several authors. For Great Salt Lake (GSL) *Artemia* Rackowicz (1975) reported the need of a wintering period of at least 7 months, while Karmiol (1981) and Van der Haegen (1981) detected a hatching increase of 10 % when dehydrated cysts were frozen at -20 °C. Lavens *et al.* (1986b) used laboratory-produced GSL cysts to prove that the hibernation effect can be quantified : *i.e.* 8 weeks at 4 °C or -25 °C improved hatchability by 5 %, respectively 50 %, and a 70 % value was reached after 32 weeks storage at -25 °C. Two freezing periods interrupted by an acclimation period did not result in a cumulative deactivation : only 39 % hatching instead of 50 % was obtained after a 2×4 weeks preincubation at -25 °C. They also confirmed the earlier observation of Kinne (1977) that following hibernation, the cysts should be acclimated at room temperature for at least 1 week prior to incubation, otherwise poor hatching will prevail.

Experiments with cysts from Mono Lake (California, USA), harvested from 90 ‰ S lake water, revealed an analogous time-temperature dependent hibernation effect (Dana, 1981, 1982 ; Dana and Lenz, 1986 ; Thun and Starrett, 1987). As can be seen from Fig. 4 the preincubation period not only affected hatchability but also hatching rate : best results (> 80 % in less than 6 days) were obtained after storing the cysts at 4 °C for more than 100 days. The diapause inhibition was less effective at higher storage temperatures : only 12 % hatched when treated at 10 °C (Dana, 1981). Nonetheless Mason (1967) reported a good hatching after a longer preincubation period of 150 days at 15 °C.

In Lake Lavalduc (France) *Artemia* maximal hatchability (90 %) is ensured after 2 months storage of the dormant embryos at about -30 °C (Sorgeloos, 1979 ; Godeluck, 1980). Hibernation is also an efficient diapause inhibitor for brine shrimp cysts from the Soviet Union — salt lakes in Kazakstan and Siberia (own data, unpubl.) and the Shivash-saltworks along the Azov Sea (Voronov, 1973), and for *Artemia* from Chaplin Lake (Canada) (Sorgeloos, 1979 ; Van der Haegen, 1981 ; Sawchyn, 1985).

Peroxide or other chemicals

Mathias (1937) reported the positive effect (50 % hatching) of hydrogen peroxide on *Artemia* cysts that normally did not hatch. This was confirmed by Bogatova and Shmakova (1980) and Bogatova and Erofeeva (1985) for *Artemia* from the Soviet lakes near the Black Sea and the Altai.

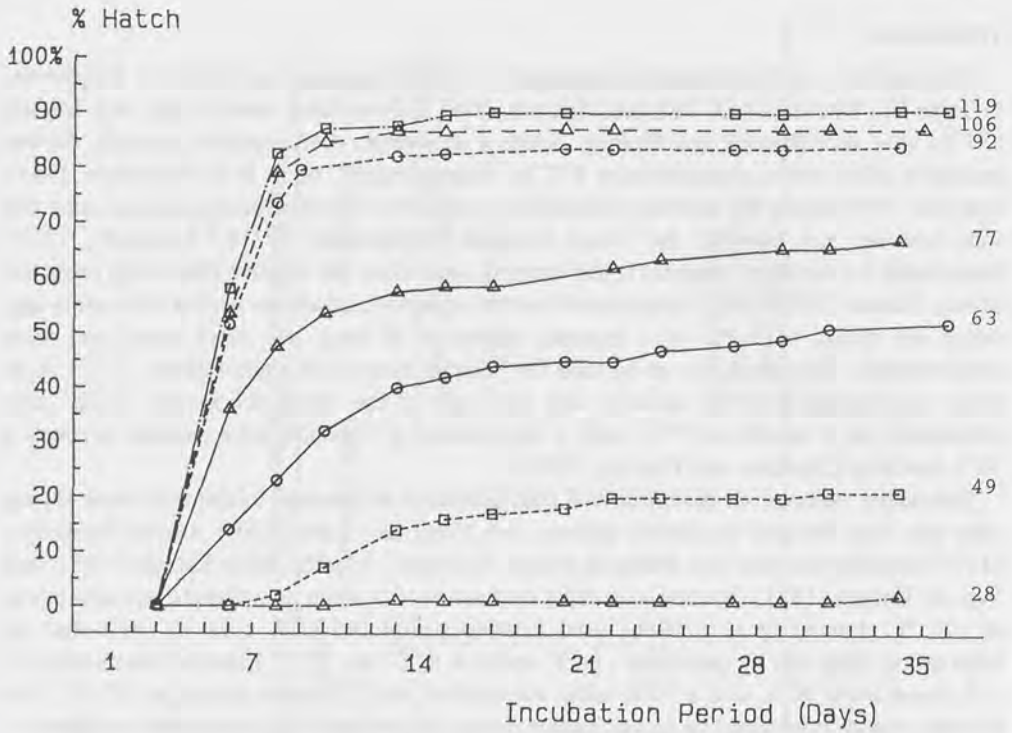

FIG. 4. Hatching rate and percentage of Mono Lake *Artemia* cysts incubated at 10 °C and 90 ‰ S ; this in function of various preincubation periods (28, 49, 63, 77, 92, 106, 119 days) at 4 °C (from Thun and Starrett, 1987).

Hatching yields increased from 5 to 90 % hatch and more by soaking the dried cysts for 15 min in a 3 % H_2O_2 solution prior to incubation, or when 30 up to 230 ppm H_2O_2 was added to the hatching medium. Small but significant variations were reported when different peroxide concentrations were used ; at higher peroxide doses the hatching rates also increased. Vu Do Quynh *et al.* (1987) studied Great Salt Lake *Artemia* cysts harvested from pond cultures in Vietnam ; the hatchability increased 10 to 20 % after treatment with a 1.5 %, respectively 2 % H_2O_2 solution in freshwater. Lavens *et al.* (1986b) reported an incubation-time effect : for Great Salt Lake cysts maximal larval outputs of 80 % were obtained after 30 min preincubation, whereas at longer periods hatching levels slightly decreased. For Lavalduc cysts the highest number of nauplii were hatched (52 %) after a 1 h pretreatment with 3 % H_2O_2 (Lavens, 1981).

More recent tests at our laboratory with Great Salt Lake cysts produced in inoculated ponds in Vietnam (Vung Tau, UN-Mekong Project, courtesy W. Tackaert) revealed an obvious dose-time effect (Table VI). High concentrations of H_2O_2 (5-10 %) have their optimal effect at short incubation periods (5, respectively 1 min) while lower doses need more exposure time (15-60 min) to reach comparable hatches. Diapause inhibition by peroxide treatment can be reversible, *i.e.* Bogatova and Shmakova (1980) detected a loss of the H_2O_2-effect when the cyst material was dried and stored ; we observed constant hatching levels after redrying and storage for 4 months under vacuum or in air, but storage in brine or exposure of the dry cysts to freezing temperatures seemed to affect hatchability (Lavens, unpubl.).

TABLE VI

Dose-time effect of H_2O_2 preincubation treatment
on the hatchability of *Artemia* cysts from Vung Tau (Vietnam).
Data are expressed as percentage of hatching results obtained at 2 %/15 min treatment (74 % hatch)
(Lavens, unpubl.)

Time (min.)	Doses (%)					
	0.5	1	2	3	5	10
1					46	105
2					94	
5			54	69	102	32
10	47		90	81	88	
15		46	100	76		
20			91	94	52	
30		91	95			
60	56	85		6	1	
120		15				
180	47					

The effect of the peroxide treatment on brine shrimp hatching is explained by Bogatova and Shmakova (1980) as a catalyzation, via the reactive oxygen atoms, of the oxidation-reduction process whereby trehalose is converted into glycerol and glycogen during embryogenesis. In this regard the observations of Van der Linden (pers. commun.) that the threshold for light sensitivity in hydrated *Artemia* cysts decreases in those cysts that have been preincubated in peroxide, may be of interest, especially since the illumination energy normally serves to catalyze oxidation-reduction processes in the cysts via captation by haempigments (Van der Linden *et al.*, 1986).

Other chemicals reported to be effective in increasing the hatchability of brine shrimp cysts are aceton, n-butanol, ethylether, xylene (Tazawa and Iwanami, 1974), and hypochlorite (Bruggeman *et al.*, 1980 ; Vanhaecke and Sorgeloos, 1983). The latter product is used for so-called cyst decapsulation, *i.e.* to dissolve the cyst envelope. It is not clear if decapsulation acts as a diapause inhibitor or if the positive effect is related to a beneficial role during the hatching metabolism. The breaking stage may be reached more easily if the thick shell barrier has been removed, or if the resistant outer cuticular membrane is affected. Evidence for the latter hypothesis might be found in *Daphnia* where hypochlorite treatment of the ephippia results in the release of the individual embryos from the protective envelope and in an increase of their hatching percentage and rate (Pancella and Stross, 1963 ; Van De Vel, 1984). On the contrary, preliminary experiments comparing the effect of peroxide and hypochlorite treatment on *Artemia* cysts support the first hypothesis, *i.e.* in both cases hatching percentages increased very significantly from about 5 to over 60 % hatch (Lavens, unpubl.).

Other diapause-terminating techniques

The following other diapause-terminating techniques exist :

- ionizing irradiation (Metalli and Ballardin, 1972) ;
- permanent magnetic fields (2 000 Oersted) for less than 24 h (Dolgopol'skaya *et al.*, 1969, 1970 ; Taneyeva and Dolgopol'skaya, 1973) ;

– illumination of the cysts in hydrated and aerobic conditions. Dana (1982) demonstrated that light is not needed when Mono Lake cysts were previously hibernated. Since Belk (1972) revealed that a light stimulus is not essential for *Eulimnadia antlei* cysts that have been properly dried, Vanhaecke *et al.* (1981) hypothesized that light could act as a diapause inhibitor rather than a trigger of the hatching metabolism. Since no further evidence has become available that proves that light can replace other diapause inhibitors (*e.g.* dehydration) we have transferred the detailed discussion on light effects to the section "hatching conditions".

Is diapause termination strain-specific ?

Dormancy plays a decisive role in the persistence of species under temporarily adverse conditions, and is a life-cycle strategy to synchronize population developments to the variations of their specific biotope. In this regard it is very likely that the process of diapause and its termination are adapted to the local environment. Such climatic adaptations might have contributed to strain-specific differences, *e.g.* inter-population differences in diapause deactivation sensitivity. Proofs of inter-population variations in cyst hatchability were provided by Browne *et al.* (1984), Lavens *et al.* (1986b), and Tackaert *et al.* (1987) who performed experiments with *Artemia* cysts from different geographical origin, produced under analogous conditions in the laboratory, and by Vu Do Quynh *et al.* (1987) with field tests in identical ponds but inoculated with different strains.

Two hypotheses may explain the inter-population differences : either the basic biochemical process involved in the induction of diapause is strain-specific, or different levels of diapause-deactivation sensitivity exist. The first hypothesis might have but limited value as it is most likely that the causal mechanism in establishing dormancy in the early embryo stages is universal within the genus *Artemia*, and maybe even within the phyllopods. Extra support for the hypothesis on differences in diapause-deactivation sensitivity are provided by the following observations. Firstly diapause deactivation is influenced by environmental conditions of the biotope, *e.g.* food quantity and salinity. This means that within the same population the possibility exists to adapt the diapause-deactivation sensitivity to a higher threshold value. Secondly, specific diapause terminating methods are not just on/off-reactions but have a quantitative effect : *e.g.* temperature-time dependence in hibernation and dose-time interaction in the peroxide treatment.

Strain-specificity in diapause termination is most probably explained by differences in the minimal threshold value for diapause deactivation. For example within the *A. franciscana* species San Francisco Bay *Artemia* cysts seem to have a low threshold value since a simple dehydration in brine for 24 h already results in a maximal hatching (> 90 %), whereas cysts from Great Salt Lake may have a high threshold value : dehydration is insufficient and more specific diapause deactivation techniques, *e.g.* hibernation and peroxide treatment, are needed to finally break dormancy. Specific levels of the intracellular pH – as suggested by Drinkwater and Crowe (1986) for Mono Lake cysts – may explain how differences in diapause-deactivation sensitivity may occur.

Differences between *Artemia* strains in diapause-deactivation sensitivity may furthermore be related to specific variations in habitat conditions and in this way may have an ecological significance. When considering the low temperature effect, it is striking that especially *Artemia* strains which occur in temperate regions need a more or less prolonged hibernation as diapause

terminator, *e.g.* Great Salt Lake, Lavalduc Lake, Mono Lake, Chaplin Lake, Azov Sea saltworks, and Kazakstan lakes. Their encysted offspring produced during summer will not develop when hatching conditions might become acceptable with the autumn rains because this might lead to the elimination of the future "seed-stock" which will be vital to generate a new population after the winter. The hibernation effect will ensure that in the spring all cysts are quiescent, resulting in a synchronous hatching and consequently a fast build-up of the new population. A similar adaptation has been reported by Marcus (1979, 1980) for the calanoid copepod *Labidocera aestiva.* Furthermore, this relation with the habitat explains why dehydration/hydration cycles do not affect *e.g.* Great Salt Lake cysts but do terminate dormancy in (sub-)tropical or coastal populations, *e.g.* Macau, Tuticorin, Hidalgo, San Francisco Bay, etc. (Table V).

A practical example of the ecological significance of specific dormancy terminating processes may be given by Vu Do Quynh *et al.* (1987) who suggested that the disappearance of the Macau population and the persistence of Great Salt Lake *Artemia* after the rainy season in inoculated salt ponds in Vietnam, was caused by differences in their cysts' diapause termination characteristics.

Differential adaptation of geographically separated populations has been documented in various other crustaceans, *e.g. Triops* (Table III). Until now, however, there is no evidence that differences in dormancy termination between various geographical brine shrimp strains are correlated with different phenotypes or genotypes ; almost all experiments mentioned in this review were dealing with cysts produced under controlled conditions only during one generation (laboratory systems) or a few generations (temporal inoculations in extensive systems).

Factors affecting cyst hatching in *Artemia*

Quiescent *Artemia* cysts resume their embryonic metabolism as soon as the environmental parameters become favorable, *e.g.* when sufficient water has been taken up (more than 65 % of the cyst dry weight). The carbohydrate metabolism is activated under aerobic conditions and trehalose is converted into glycogen (as an energy supply for respiration) and glycerol. The accumulation of the hygroscopic glycerol within the outer cuticular membrane leads to increased intra-cystic osmotic pressures eventually resulting in the breaking of the shell. The embryo now differentiates into an instar I nauplius within its hatching membrane which it can leave, head first, once a hatching enzyme is secreted supposedly in the head region of the nauplius. More details on the hatching metabolism can be found in the paper by Clegg and Conte (1980). As a consequence of differential, 'strain-specific' adaptations to their local environment, the interactions of the parameters may be slightly different for the various strains resulting in variable optimal ranges for hatching.

Salinity

The qualitative ionic composition of the incubation medium can interfere either with the embryo's toxicity tolerance, *e.g.* K^+, Ca^{2+} and Zn^{2+} (Sato, 1966b ; Bagshaw *et al.*, 1986 ; Rafiee *et al.*, 1986), the osmotic capacity of the emerging embryo (Conte *et al.*, 1977), or with specific activities, *e.g.* of the hatching enzyme (Sato, 1966ab, 1967). With regard to the latter hatching enzyme, its inhibition by Fe^{2+} or Cu^{2+} can be prevented by the addition in the hatching medium of $NaHCO_3$ or chelating agents. Ca^{2+} has an activating effect on the hatching enzyme provided

it is released from its heavy metals. When K^+ and/or Mg^{2+} are absent no effect on the rate of excystment has been observed (Sato, 1966a).

Quantitative effects of the incubation salinity on cyst hatching are related in the first place with the hydration-level that can be reached in the cysts. Above a threshold salinity insufficient quantities of water can be taken up to support the embryo's metabolism. This threshold value varies from strain to strain and is well documented in the literature, *i.e.* approximately 85-90 ‰ is the maximum salinity for most *Artemia* strains : GSL (D'Agostino, 1965 ; Von Hentig, 1971), SFB (Sorgeloos, 1975), Bonaire (Kristensen and Hulscher-Emeis, 1972), Lavalduc (Sorgeloos, 1979), Kiatuthlana Green and Red ponds (Cole and Whiteside, 1965). Lake Ontario and Mono Lake cysts hatch even at salinities higher than 95 ‰ (Ivanovskii *et al.*, 1981), respectively 125 or 190 ‰ (Dana, 1981 ; Thun and Starrett, 1987). On the contrary, a lower value was detected for Tuticorin (75 ‰) (Royan, 1975), Penley Lake (58 ‰) and Jesse Lake (32 ‰) (Collins, 1977).

In the second place the incubation salinity will interfere with the amount of glycerol that needs to be built up to reach the critical intra-cystic osmotic pressure. The fastest hatching rates will thus be noted at the lowest salinity levels since it will take less time to reach breaking. When considering high salinities, it is is very likely that cysts from a certain geographical origin contain insufficient quantities of carbohydrates to meet their varying hyperosmotic requirements (Vanhaecke, 1983). As a result optimal seawater salinity for cyst hatching varies from strain to strain in *Artemia* : *e.g.* 15-70 ‰ for GSL cysts (Von Hentig, 1971), 5-80 ‰ for SFB cysts (Sorgeloos, 1975), 40 ‰ or less for Mono Lake cysts (Dana, 1981), 5 ‰ for Buenos Aires cysts (Vanhaecke and Sorgeloos, 1983), 5-15 ‰ for Chaplin Lake cysts (Kurata, 1967 ; Smith, 1969 ; Provenzano and Goy, 1976 ; Vanhaecke and Sorgeloos, 1983). Differences found in salinity optima for this latter sulphate lake strain when incubated in sulphate or chlorine hatching media (Provenzano and Goy, 1976 ; Vanhaecke, 1983 ; Sawchyn, 1985) are attributed to the same phenomenon of osmotic pressure and not to qualitative differences in osmotic composition (Vanhaecke, 1983). The osmotic pressure of a 35 ‰ Na_2SO_4 water is far lower than that of a 35 ‰ NaCl medium (Weast, 1973), as a result of which Chaplin Lake cystst do hatch in the first medium with a normal salinity (35 ‰) and only after dilution of the second medium to 5-15 ‰.

Temperature

Hydrated *Artemia* embryos do not resist temperatures below the freezing point -10 °C (Hempel-Zawitkowska, 1971a) or above 40 °C (Voronov, 1974) (Fig. 5). Their metabolism is also arrested below 4 °C (Iwasaki, 1964 ; Iwasaki and Nakanishi, 1966), and above 33-37 °C (Sorgeloos, 1975 ; Vanhaecke, 1983). The latter reversible onset and arrest of the early metabolism seems to be controlled by the molecular sensor cytochrome oxidase which activation/deactivation pattern is analogously influenced at the same temperatures (Vallejo *et al.*, 1980). According to Von Hentig (1971) maximal hatching is ensured between 15 and 30 °C. Similar but more narrow ranges were confirmed : 20-30 °C by Jones (1972), 20-28 °C by Sorgeloos (1975), and 26-30 °C by Ivanovskii *et al.* (1981). Experiments carried out by Vanhaecke (1983) on cysts from 17 different strains revealed maximal hatchabilities in the temperature range 25-30 °C, except for Larnaca and Chaplin Lake cysts which yielded 65 % respectively 30 % less nauplii at 30 °C and for Tuticorin *Artemia* with a 30 % improvement at 30 °C *versus* 25 °C (confirming earlier findings of Royan, 1975).

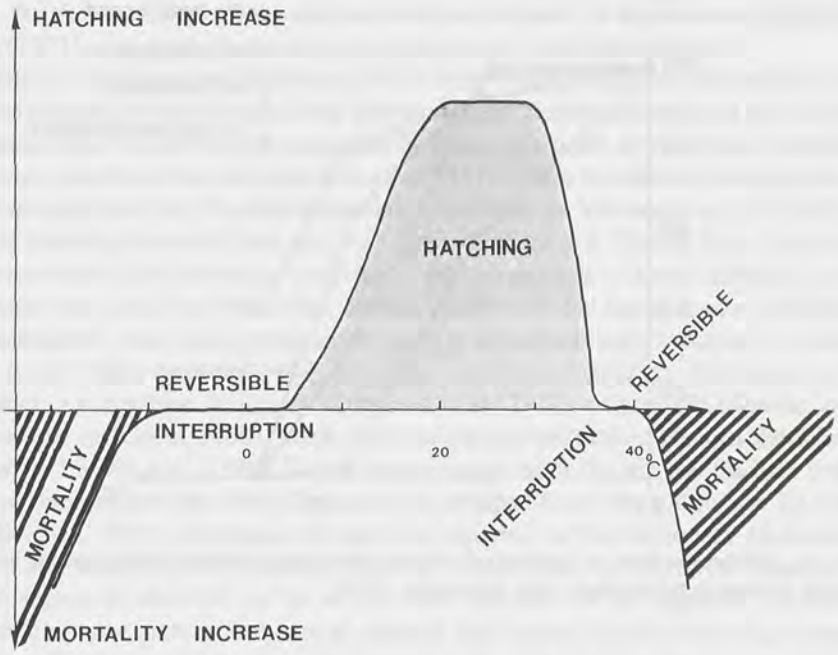

FIG. 5. General schematic diagram of the relationship between incubation temperature and hatching characteristics of *Artemia* cysts. Note that the Y-axis is not scaled with only relative hatching unit values used in this general model.

Vanhaecke (1983) furthermore showed that the hatching tolerance for 35 °C is specific for each sibling species, *i.e.* the proportional hatching decrease is limited for *Artemia franciscana* and *A. persimilis* whereas drastic decreases have been recorded for *A. parthenogenetica* and *A. tunisiana* (Fig. 6). No correlation could be detected between the temperature regime of the brine shrimp biotopes and their temperature tolerance for cyst hatching.

pH

Whereas the hatching rate is not affected by pH, a maximal hatching efficiency can be reached at alkaline pH's in the range 8-8.5 (Jennings and Whitaker, 1941 ; Nimura, 1968 ; Jones, 1972 ; Metalli and Ballardin, 1972). Sato (1967) correlated this finding with the optimal pH activity range for the hatching-enzyme which digests the inner cuticular membrane, eventually facilitating the release of the free-swimming nauplius. This may also explain why the addition of $NaHCO_3$, up to 2 g/l, to artificial or diluted seawaters, or to dense suspensions of cysts does result in improved hatching outputs. Under those circumstances increased buffer capacities are required to avoid a dropping of the pH of the hatching medium (Jones, 1972 ; Rogers and Johnston, 1977 ; Sorgeloos *et al.*, 1983 ; Lavens *et al.*, 1986a).

Oxygen concentration

Although it is well known that an essential requirement for the (aerobic) resumption of development is molecular oxygen at an adequate partial pressure, only few data are found in the

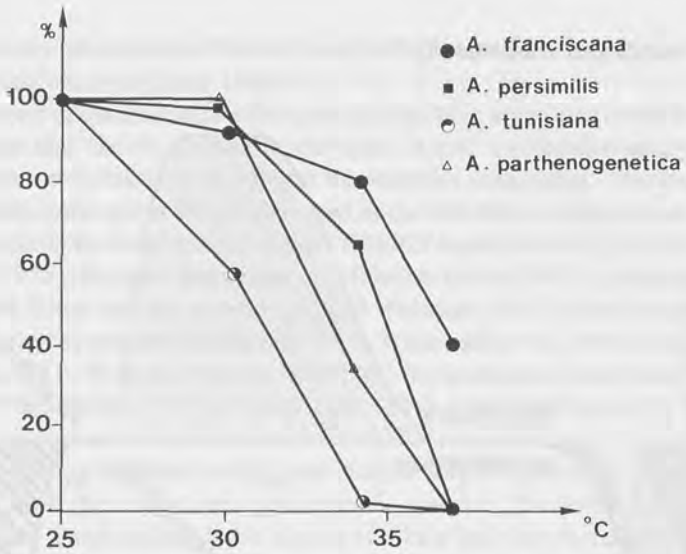

FIG. 6. Average decrease in hatching of *Artemia* cysts from different sibling species in relation to increasing incubation temperature (after Vanhaecke, 1983).

literature. Kurata (1967) described a linear effect on hatching percentage ; Nimura (1968) and Sorgeloos and Persoone (1975) detected an increasing hatching efficiency between the 0.6 ppm minimum O_2 level and 2 ppm O_2, and maximal larval hatching above this concentration. An analogous minimum oxygen concentration is required for the embryonic metabolism of the anostracan *Chirocephalus seali* (Moore, 1963, 1967) and the conchostracan *Limnadia stanleyana* (Bishop, 1967). To avoid oxygen gradients during hatching it is obvious that a good, homogenous mixing of the cysts in the incubation medium is required.

Cyst density

The cyst density may interfere with the other abiotic conditions which are essential for hatching, *i.e.* pH, O_2, illumination. Provided that the other prerequisites are fulfilled, the hatching process will not be affected by high cyst densities up to 17 g/l (Sorgeloos, 1975 ; Kurata, 1967).

Illumination

The effect of light on the hatching process was first described in 1973 by Sorgeloos. Compared to controls incubated in darkness, hatching increases up to 50 % were demonstrated for San Francisco Bay, Great Salt Lake, and Bulgarian *Artemia* cysts hatched in light (Sorgeloos, 1973). This photoquiescence phenomenon has been confirmed for the same as well as for other strains : San Francisco Bay (Meade, 1975 ; Spektorova and Syomik, 1979 ; Van der Haegen 1981), Tuticorin (Royan, 1976), Larnaca (Person-Le Ruyet and Salaun, 1977), Caspian and Crimean *Artemia* (Spektorova and Syomik, 1979). Fuchs (1976) and Van der Haegen (1981), however, detected no light sensitivity in cysts from San Francisco Bay and Lavalduc, respectively Great

Salt Lake. The fact that (part of) the encysted embryos do hatch in darkness was explained by Sorgeloos (1973) as a response to anteriorly captated and stored light stimuli.

According to Sorgeloos and Persoone (1975) brine shrimp cysts are susceptible to light triggering as soon as they have reached full hydration under aerobic conditions. They also found that a minimal dose of light energy is needed to trigger the onset of embryonic metabolism. Dose-response data from Van der Linden *et al.* (1985) confirm the relation between the light intensity/exposure time and the hatching effect. According to Vanhaecke *et al.* (1981), the critical light intensity threshold does vary from strain to strain, *e.g.* Chaplin Lake cysts require at least a continuous light intensity of more than 1 000 lux in order to hatch optimally. In other strains (Great Salt Lake, San Pablo Bay, Buenos Aires) only the hatching rate is affected by various illumination levels since progressively more accumulation time is needed to reach the triggering dose. These variations between strains may be attributed to differences in shell characteristics, *e.g.* envelope thickness (Vanhaecke *et al.*, 1981) or haematin pigment concentration (Gilchrist and Green, 1960), which can delay the light infiltration (Hempel-Zawitkowska, 1970 ; Van der Linden *et al.*, 1986). This is further supported by the fact that the light intensity threshold of decapsulated San Pablo Bay cysts has dropped from 100-500 lux, to 20-100 lux (Vanhaecke *et al.*, 1981). Analogous findings were reported for the cladoceran *Daphnia pulex* by Pancella and Stross (1963). Recently Van der Linden *et al.* (1985, 1986) identified the wavelength region of 400-600 nm to be the most effective one in triggering the onset of metabolism. The same authors furthermore reported that in total 21 600 $\mu E/m^2$ light energy is needed to ensure maximal hatching in San Francisco Bay cysts. It is assumed that a photoreceptor, which may be a haempigment, mediates in the light-induced hatching.

Conclusions and recommendations

Innumerable research efforts have been contributed to the elucidation of the biological, biochemical, ecological, and other aspects of *Artemia* cyst hatching. To a large extent this interest has been inspired by economic motives, *i.e.* hatching quality is of primary importance to aquaculturists and aquariologists. Further contributions were generated by its scientific relevance, *i.e.* its extensive use as a practical research object, and the human fascination for the 'state of suspended animation' as an excellent adaptation to survive extreme habitat conditions. However, this review shows that all these efforts have hitherto not resulted in a complete understanding of all processes involved. It is obvious that the major cause for this should be sought in the numerous factors which interact with the cyst's hatchability, some of which have often been disregarded. Consequently, experimental data have been misinterpreted sometimes resulting in confusion or contradictory statements in the literature.

Therefore we would like to make a strong plea for the use of standard cyst material, the antecedents of which are well known, to further study biochemical, biological or physiological effects related to the cryptobiotic state. In this regard we hope that the laboratory technique for controlled cyst production of Lavens and Sorgeloos (1984) can soon be scaled-up to produce sufficient amounts of these 'standard *Artemia* cysts'. Meanwhile, productions from more or less controllable extensive *Artemia* operations may be helpful. We also recommend more interdisciplinary cooperation to elucidate the mechanisms involved in diapause.

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The effect of cold, hydrated dormancy and salinity on the hatching of *Artemia* cysts from Mono Lake, California, USA

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Abstract

The effect of varying the period of cold, hydrated dormancy on the hatching of *Artemia* cysts from Mono Lake was examined. Unlike other populations, *Artemia* cysts at Mono Lake sink and remain hydrated throughout their dormancy period. Prolonged cold hydration, which mimics the natural overwintering condition at this alpine, saline lake, has been shown to be an effective method of resuming development. Maximal hatching was achieved after 90 days of cold, anoxic dormancy.

The effect of salinity on hatching was examined because gradually increasing lake salinity is one factor that could eventually affect the hatching of these *Artemia* cysts. Hatching percentages at 90, 115, 125, and 140 ‰ Mono Lake water were sequentially reduced from 87 % to 4 %. Increasing salinity delayed the onset of hatching and reduced the overall hatching success.

Introduction

The seasonal cycle of *Artemia* production in Mono Lake, a permanent saline lake in Northern California (USA), involves ovoviviparous and oviparous bisexual reproduction. The cysts of Mono Lake *Artemia* are unique among brine shrimp in their ability to sink. As a result, the cysts undergo an obligate cold, hydrated dormancy period prior to hatching (Dana, 1981). In contrast, non-Mono Lake populations of *Artemia* cysts typically require a period of desiccation and subsequent rehydration for successful hatching. Moreover, Mono Lake *Artemia* cysts inhabit a deep, temperate alpine lake and have an optimal hatching temperature range which is much lower than other *Artemia* populations (Vallejo *et al.*, 1980 ; Dana, 1981). The low temperature criterion allows for hatching in early spring, when the lake's temperature ranges between 2 and 8 °C.

The purpose of this study was to gain an understanding of some of the environmental factors, such as salinity fluctuations and natural temperature alterations, that may influence the hatching of Mono Lake *Artemia*. A knowledge of the environmental parameters that regulate hatching success will clarify yearly differences in the timing and magnitude of the spring hatch which ultimately determines the seasonal dynamics of the population.

Materials and methods

Adult *Artemia* were collected by 20 m vertical net hauls from three representative stations at Mono Lake in the late summer of 1982. Vertical hauls were taken with a Kalhsico plankton net (0.3 m diameter by 1.0 m length) with a nitex mesh size of 120 μm.

Gravid females carrying cysts were separated out, pooled and placed in a large 20 l Nalgene tank filled with unfiltered, aerated Mono Lake water (*ca.* 25 °C). Cysts deposited by the females were collected from the bottom of the tank with a pipet.

Cysts were preincubated in 60 ml BOD glass bottles filled with 0.45 μm filtered Mono Lake water, following a 15 min purge with N_2. During the dormancy periods, a Forma Scientific refrigerated incubator maintained the cysts at a temperature of 4.0 °C±1.0 °C. Cysts were preincubated at salinities of 90, 115, 125, and 140 ‰. The control salinity was 90 ‰ which represents 1982 lake salinity concentrations. These preincubation salinities were also used in the subsequent incubation and hatching studies. Mono Lake water was evaporated to the appropriate test salinity and then filtered with a Whatman GF/C glass-fiber filter. Salinities were measured with an American Optical hand-held temperature compensated refractometer.

To convert the values reported in this study from parts per thousand to g/l, specific gravity numbers were generated from a regression analysis of concentrated Mono Lake brine solutions. Equivalent units for 90, 115, 125, and 140 ‰ salinity values are 97, 126, 138 and 157 g/l, respectively.

Three 2.0 ml subsamples of cysts were removed at seven different intervals from 28 days to 119 days after the initiation of dormancy. The dormancy periods were 28, 49, 63, 77, 92, 106, and 119 days. After each subsampling, all vials, whether sub-sampled or not, were repurged with N_2 for 15 min. At the three shortest dormancy periods, only cysts preincubated at 90 ‰ were tested. For all other dormancy periods, all four salinities were tested.

Subsamples of cysts were microscopically examined and only indented cysts were selected, since our preliminary hatching research (unpubl.) conducted in the spring of 1982, revealed a significantly higher viability among Mono Lake *Artemia* cysts that were indented when compared to their spherical counterparts. This was further substantiated by Dana and Lenz (1982). Replicates of 50 cysts from each dormancy period and at each test salinity were placed in covered polycarbonate weighing bottles (44×38 mm), each containing 20 ml of Mono Lake water. The tops of the bottles were further sealed with parafilm to retard evaporation. Only 25 cysts per replicate were used at 90 ‰ for the 28, 49, and 63 day dormancy periods.

Cysts were hatched at 10 °C±0.3 °C in a Forma Scientific incubator. The hatching medium was filtered, oxygenated Mono Lake water of the same salinity as the preincubation medium. Vials were examined three times a week to monitor the emergence process.

Statistical analyses

The effects of salinity and dormancy on hatching success and synchrony were tested using the analysis of covariance with two types of results reported. First, the Type I results addressed the effect of the treatments without consideration of the effect of time on hatching, the variable quantified as percent cumulative hatch. Type III results were adjusted for the effect of the covariate. When this effect is removed, it can be determined if salinity and dormancy still control hatching. In the special circumstance when treatment effects on hatching rate are considered,

Type I and Type III results are equivalent, and only Type I results are presented. Results are reported in probability levels only.

The variable, percent cumulative hatch, met the criterion of homoscedasticity. Scheffe's multiple range test was used with analysis of covariance if significant differences between treatment levels were observed. All results from the Scheffe's test were based on a 0.05 probability level.

Hatching rates were determined from a linear regression of percent cumulative hatch *vs* time. To assure analysis of the actual hatching period, only percent cumulative hatch values less than 95 % of final hatch were analyzed.

Results

Effect of dormancy

Control salinity

Seven dormancy periods ranging from 28 to 119 days were tested at a salinity of 90 ‰. Dormancy had a significant effect on hatching (Table I ; Type I and Type III, $P<0.0001$). Increasing the length of dormancy increased hatching rate as well as hatching success (Fig. 1 and 2 ; Type I, $P<0.01$). Scheffe's test indicates that hatching success was significantly greater at each incremental increase of dormancy from 28 days to 92 days. Hatching was negligible (1 %) at the 28 day dormancy period. When the dormancy period was extended to 3 months, hatching success improved to 84 %. However, increasing dormancy longer than 3 months (from 92 days to 119 days) did not significantly alter hatching success.

Table I

Percent cyst hatching success at 90 ‰ S
(standard errors are included with values for hatching percent)

	Dormancy period (in days)						
Hatching percent (±s.e.)	28 1±2	49 21±14	63 51±9	77 67±6	92 84±7	106 90±3	119 87±7

A more synchronous hatch was also observed at the longer dormancy periods (Fig. 1). Hatching rates at periods below optimum (<92 days) ranged from 0.5 to 2 %/day while hatching rates of 9-11 %/day were observed at the longer dormancy intervals (92, 106, 119 days) in the 90 ‰ medium.

Test salinities

All four salinities were tested at the four longest dormancy periods (Fig. 3). The effects of dormancy on hatching were similar even at the higher salinities, *i.e.* hatching success and hatch rate substantially improved with longer dormancy periods (for hatching success : Type I, $P<0.0001$ and Type III, $P<0.01$; for hatch rate : Type I, $P<0.01$). When all salinities were considered, maximal hatching success was greatest at the 106 and 119 day periods. There was no significant difference between hatching at 106 days and 119 days. For example, at 115 ‰

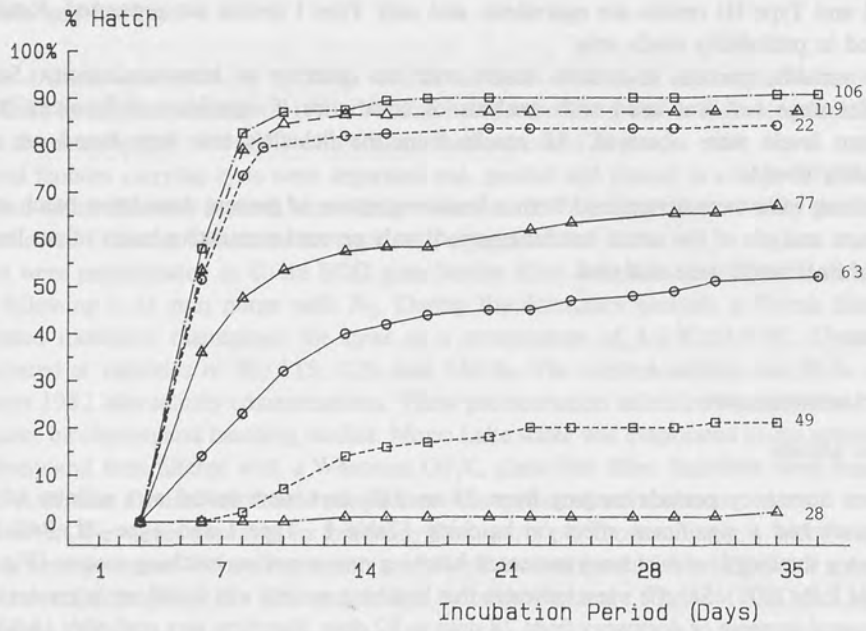

FIG. 1. Hatching rate at 90 ‰ S of cysts preincubated for various time-periods (in days). Standard deviations are given in Table I.

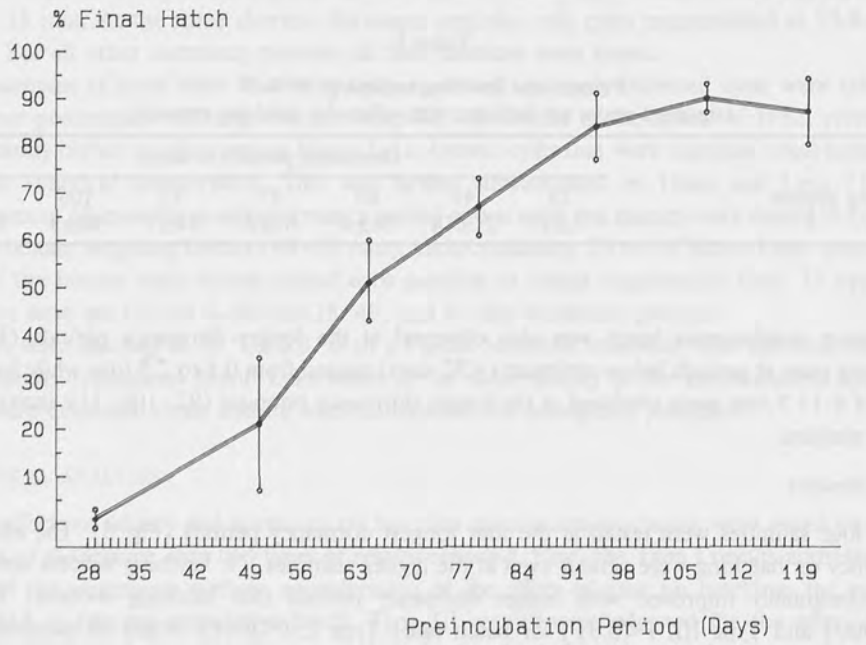

FIG. 2. Maximal hatching percentage at 90 ‰ S of cysts preincubated for various time-periods. Incubation times ranged from 31 to 36 days.

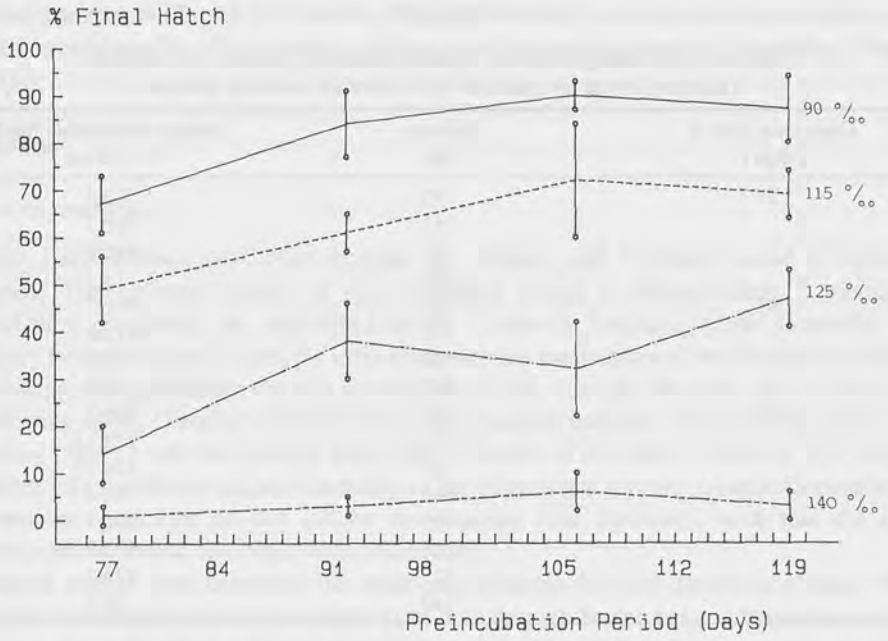

FIG. 3. Maximal hatching percentage at four different salinities of cysts preincubated for various time-periods. Incubation times ranged from 34 to 36 days.

hatching success after 77 days of dormancy was 49 %±7 % and at 92 days hatching increased to 61 %±4 %. At the two longest dormancy periods final hatch ranged from 69 to 72 %. Similarly, at 125 ‰, final hatch was only 14 %±6 % at 77 days but increased to 32-47 % as dormancy was extended. Hatching of cysts at 140 ‰ remained low (2-6 %) regardless of the dormancy period.

EFFECT OF SALINITY

Hatching success was substantially reduced with increasing salinity (Type I and Type III, ($P<0.0001$). When the length of dormancy was 92 days or longer, hatching success at 90 ‰ ranged from 84 to 90 %. Increasing salinity to 115 ‰ and 125 ‰ reduced hatching success to approximately 65 % and 40 %, respectively. Hatching was substantially reduced from 125 ‰ to 140 ‰. At 140 ‰, hatching was only 5 %.

Salinity also retarded the hatch rate (Fig. 4A-D, Type I, $P<0.0001$). At optimum dormancy, hatch rate at 90 ‰ was 9-11 %/day. A less synchronous hatch with a rate of 3-5 %/day, was observed at 115 ‰. A hatch rate of 1-2 %/day was observed at 125 ‰. Negligible hatching occurred at 140 ‰.

INTERACTION OF SALINITY AND DORMANCY

There was a significant synergism between dormancy and salinity. An increase in salinity with a simultaneous reduction in dormancy affected cyst hatching more than the additive effects of

TABLE II

Percent cyst hatching success at different dormancy periods and salinities (standard errors are included with values for hatching percent)

Dormancy period (days)	Salinity (‰)	Percent cumulative hatch (in %±s.e.)
77	90	67±6
	115	49±7
	125	14±6
	140	1±2
92	90	84±7
	115	61±4
	125	38±8
	140	3±2
106	90	90±3
	115	72±12
	125	32±10
	140	6±4
119	90	87±7
	115	69±5
	125	47±6
	140	3±3

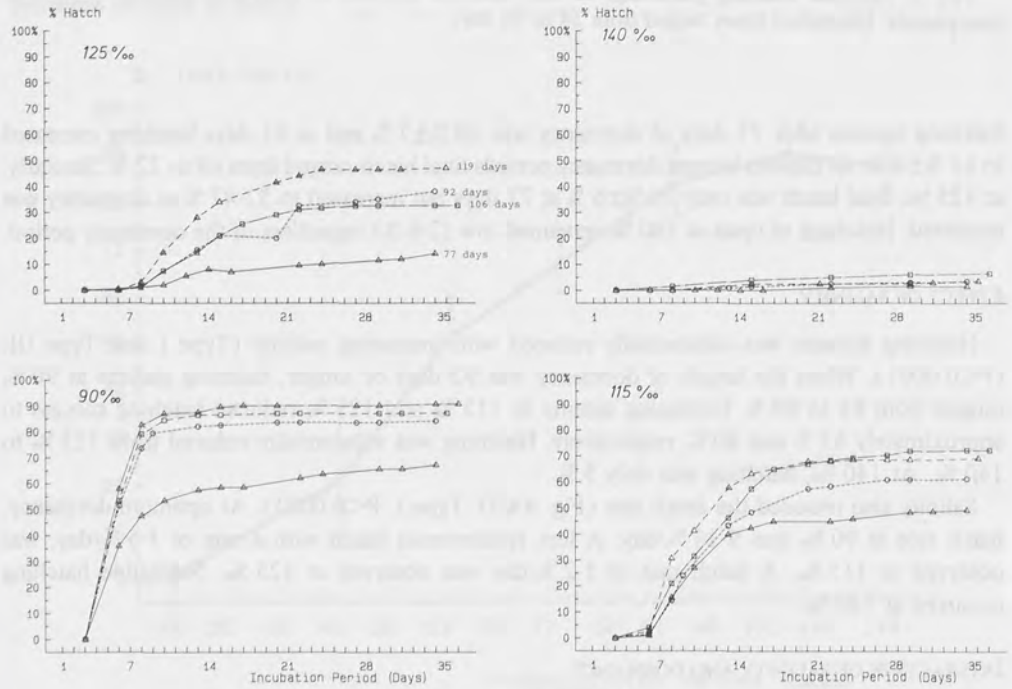

FIG. 4. Hatching rates at four different salinities of cysts preincubated for various time-periods. Standard deviations are given in Table II.

these two parameters (Type I, $P<0.0001$; Type III, $P<0.001$). As well, the rate of hatching was retarded synergistically with increasing salinity and decreasing length of dormancy (Type I, $P<0.01$).

Discussion

Effect of dormancy

Mono Lake *Artemia* cysts must undergo an obligate cold hydrated period to terminate dormancy. The optimum length of this dormancy period is approximately 3 months. If preincubation conditions are interrupted before 3 months, hatching is less successful. This obligatory period explains in part the cysts overwintering mechanism. Cysts which are produced and subsequently deposited in the lake sediment from June through November do not hatch until the following spring (February through May). This research indicates that cysts deposited in the late spring (June) could theoretically hatch after 3 months of dormancy. However, it is probable that either lake conditions are not conducive to hatching in late summer (August/September) or that summer conditions do not initiate the requisite cold dormancy, such that the actual dormancy period would not begin until much later.

Previous studies have examined the cysts' requirements for cold dormancy (Dana, 1981). Dana collected cysts from the lake bottom from June through September and found that hatching success was not appreciably affected by collection date. The results of the present study, which utilized cysts collected directly from females concurs with Dana's work. No appreciable hatching was observed within the first 30 days of laboratory dormancy. In Dana's study, hatching was not optimized until cysts had undergone 4 months of dormancy. This suggests that lake conditions during the summer, when cysts were deposited in the sediment, did not simulate the requisite cold conditions. Since Dana collected these cysts at an approximate depth of 0.5 m, the cysts were subjected to temperatures much higher than 5 °C from June to September. Therefore, cysts deposited in the shallow periphery of the lake probably do not receive dormancy conditions until late fall and would not be ready to hatch until early winter. In the past 6 years, hatching has never been observed before February. This could be the result of cold temperatures and/or anoxic conditions which retard or deter the hatching process.

A longer dormancy also appears to increase the synchrony of the Mono Lake population's hatching as well as its hatching success. Other anostracan and crustacean populations also display hatching which is synchronous with environmental conditions conducive to survival (Broch, 1965 ; Marcus, 1979). In the case of Mono Lake *Artemia*, an obligate dormancy period of 90 days deters hatching during the late fall and early winter when low temperatures (0-1 °C) severely retard developmental rates (Dana and Lenz, 1982). Shrimp hatched in March, a full month before lake warming is usually observed, appear to have slower development rates and higher mortality in some years than the April and May "hatchers" whose emergence is coincident with lake warming. Other environmental factors, such as temperature and salinity, have been shown to control hatching synchrony (Dana, 1981 ; Dana and Lenz, 1982).

Effect of salinity

Increased salinity significantly affects hatching success and the rate of hatching. Hatching success declined from the 84-90 % hatch at 90 ‰ to 2-5 % at 140 ‰. This pattern agrees with

the observations from our preliminary hatching experiments using cysts from the lake sediment in which hatching success was also reduced to a 1-5 % hatch at 140 ‰. At lower salinities, cysts collected directly from females had a slightly higher viability than cysts collected from lake sediment. For example, at 115 ‰, cysts collected from females showed a 61-72 % hatch while cysts collected from the sediment had only a 35-54 % hatch. In both cases, cysts showed negligible hatching at 140 ‰.

It is probable that at a salinity of 140 ‰ the cyst is unable to maintain adequate amounts of internal water to run the necessary metabolic pathways. These pathways are crucial to hatching and cyst viability. One of these metabolic processes, involving the breakdown of a macromolecule (trehalose), allows the cyst to imbibe enough water to break the shell and hatch (Clegg, 1978). At salinities between 90 and 140 ‰, it is probable that this metabolic process is retarded, resulting in a lag period prior to the initiation of hatching and a slower hatch rate. Clegg (1964) verified this response in non-Mono Lake *Artemia* by demonstrating that as salinity was increased, oxygen consumption and the breakdown of trehalose was retarded.

Interaction of salinity and dormancy

A synergistic effect was observed between the effects of salinity and dormancy on hatching. However, it seems unlikely that a substantial reduction in hatching success, due to a reduction in dormancy period, would occur because physical alterations of the lake that could reduce the dormancy period are improbable.

However, climatic variability can significantly alter the conditions during the cysts' overwintering period. Variability in heavy freshwater inflow to Mono Lake during 1982 and 1983 due to El Niño substantially increased the lake's chemical stratification. This has resulted in the persistence of thermal as well as chemical stratification through the fall and winter, when the lake is usually isothermal and well-mixed. This is based on field data collected since 1974. As a result of the chemical stratification, which is still evident in 1985, important hatching criteria (temperature, salinity and oxygen) vary with depth. Changes in these hatching stimuli were coincident with an unprecedented large spring hatch in 1984 and 1985. The unknown interactions of these hatching stimuli may explain this hatching success which was unexpected from our understanding of the contribution of individual parameters to hatching success.

Conclusion

It can be concluded from this study that cysts require a dormancy period of at least 90 days for optimal hatching. In the shallow periphery of the lake this dormancy is probably not initiated until lake temperatures cool in the fall. The deeper areas remain cold throughout the year and dormancy may begin as soon as cysts are deposited.

It is also clear that salinity has a significant effect on the success of hatching. Increasing salinity was inversely proportional to hatching success. The synergism that exists between the effects of salinity and dormancy would only have important biological implications if the physical dynamics of the lake were severely altered.

This study was initiated to gain a better understanding of some of the environmental factors that may influence hatching in Mono Lake *Artemia* and ultimately the seasonal dynamics of the population.

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We are indebted to J. H. Crowe for his critique of our research and for enlightening discussions on cryptobiosis ; to G. Dana and P. Lenz for many fruitful discussions ; to C. Foley and R. Lawson for their assistance in the preparation of the manuscript ; and to E. Horst for his encouragement.

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Ecological factors controlling the hatchability of *Artemia* cysts in inland saline lakes in Canada

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Abstract

Attempts to hatch *Artemia* cysts from Canada using standard hatching-techniques have generally met with poor success. Our studies, seeking to explain the poor hatchability, have shown that Canadian cysts hatch best at brine concentrations of 1.010 specific gravity (10 to 12 % salinity). The *Artemia* are adapted to Na_2SO_4 and $MgSO_4$ brines characteristic of the inland lakes and respond poorly to seawater and sodium-chloride media. *Artemia* cysts from saline lakes with varying brine compositions hatch well in sodium-citrate brine.

Canadian *Artemia* produce cysts that undergo a developmental arrest which protects the species during the severe winter conditions. The developmental arrest is overcome at low temperatures when environmental conditions prevent hatching. Synchronized hatching occurs at the time of snow melt in the spring. Attempts to hatch the cysts in the wrong medium, at brine concentrations outside the optimal range or before the developmental arrest has been completely overcome, will yield low numbers of nauplii.

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A probable reason why *Artemia* is confined to isolated saline waters

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Abstract

A demographic approach was made to discover the reason why *Artemia* is confined to isolated saline waters in contrast to other zooplankton which colonized open waters where predators lived including Rotifera, Cladocera, and Copepoda. The demographic parameters considered were age at maturity, age of maximum reproduction, life span, daily birth rate, and total offspring per adult.

Although the daily birth rate (number of young/day/adult) is the largest in *Artemia* and the smallest in the rotifer, the population growth rate is usually the highest in the latter and the lowest in the former. This relationship is thought to be mainly due to the difference in the age at maturity, which is clearly confirmed by the Lewontin equation.

Introduction

Artemia is able to adapt physiologically to very wide ranges of environmental conditions. For example, they can tolerate salinities from a half to over ten times of that of seawater, and temperatures from nearly 0 to over 30 °C. Their daily maintenance ration is about 20 % of the body mass even at 28 °C (Nimura, 1967). It is thought that they have the ability to ingest this ration from seawater containing particulate organic carbon (POC) in the order of 35-350 mg/m^3 (Nimura, 1980), which is commonly found in sea biotopes (Finenko and Zaika, 1970). However, they naturally live in isolated saline waters only. Previous papers concerning this subject even state that *Artemia* biotopes are waters not colonized by predators (Gilbert, 1890 ; Vorhies, 1917 ; Whitaker, 1940 ; Carpelan, 1957). It is very difficult, however, to understand the reason for this, since there are many species living with predators such as Rotifera, Cladocera, Copepoda and others. The latter case is quite common in nature, otherwise the predator itself cannot maintain its own life. Although an attempt to explain the phenomenon was made previously (Nimura, 1985), a little more developed approach is given here.

This paper describes the approach to the problem by comparing demographic factors for *Artemia* with those of the above mentioned animals. It was difficult to select the demographic parameters properly because of the wide variety of species and of living conditions. The data were compiled by Allan (1976) except those on *Artemia*. Experimental results on *Artemia* were obtained in a rearing experiment on San Francisco Bay brine shrimp (Nimura, 1967). Using these data, two types of approach were made. One is the hypothetical case study to simulate the processes in the establishment of a newly colonizing population. Freshly-hatched larvae are

released to a new habitat under various predation pressures and are simulated. The other is the analysis of already colonized populations by the Lewontin equation (Lewontin, 1965). In this way the relation among the parameters of the population which grows exponentially and which has a stable age distribution is described. The latter approach clearly shows the influence of the demographic parameters on the instantaneous growth rate of the population, when comparing the partial derivative of the growth rate with respect to each parameter.

Materials and methods

Biological data

The demographic data used in this article, except those on *Artemia*, were chosen mainly from those summarized by Allan (1976) and are thought to be obtained at the optimal temperatures. The data on bisexual *Artemia* were based on rearing experiments on San Francisco Bay brine shrimp (Nimura, 1967). They were furthermore simply extended to the parthenogenetic strains, since culture tests on one of the strains (Nimura, unpubl.) supported the above estimation. All these data are summarized in Table I. Some additional data considered were those on rotifers reported by Hirayama and Kusano (1972).

Table I

Parameters used for calculations

	A Age to mature (days[a])	T Peak of reproduction (days)	W Life span (days)	S Offspring per adult	B Daily birth rate[b]
Artemia[c]					
Bisexual	16	23	60-80	660- 960	15
Unisexual	16	23	60-80	1320-1920	30
Copepoda	7-8	17.5	40	500- 750	15.6-22.7
Cladocera	5.5-7[d]	13.5-15[d]	40-52[e]	180[d]- 700	4[d]-20.3
Rotifera	1-2	2	5	15- 25	4 - 6.7

Data at about 25 °C mainly from Allan (1976) or otherwise stated.

[a] Rounded to the wider every half day.

[b] S = B(W-A), see Appendix.

[c] Bisexual data from Nimura (1967) and unisexual ones estimated from the former.

[d] At 20 °C from Frank *et al.* (1957).

[e] At 28 °C from MacArthur and Baillie (1929).

Hypothetical case study on the colonizing process

The case considered here is as follows: a group of newly-hatched larvae is released or transplanted only once, in conditions of sufficient food availability and without dispersion difficulties which might disturb the sexual reproduction. This population is subject to various degrees of predation.

TABLE II

Matrix P(I,J), with for example A=4

Age group	Days after release, I											
J	0	1	2	3	A	5	–	–	–	I	–	N
0	y	y	y	y	M	M	M	M	M	M	M	M
1												
2												
3												
A					y	y	y	y	M	M	M	M
5						y	y	y	y	M	M	M
–							y	y	y	y	M	M
J								y	y	y	y	M
–									y	y	y	y
–										y	y	y
–											y	y
N												y
P(I)	P(0)	–	–	–	–	–	–	–	–	P(I)	–	P(N)

P(I,J) Size of J day-born group on day I.

$P(I) = \sum_{J=0}^{J} P(I,J)$, size of whole population.

(A) Age of maturity.

(M) Mature one.

(y) Young.

Filling the matrix as shown in Table II, P(I) or the whole population size on day I is computed as follows :

$$P(I) = \sum_{J=0}^{I} P(I,J) \qquad (1),$$

where P(I,J) is the size of the J day-born group on day I. Before sexual maturity (y), the size of cohort P(I,J) is obtained by the following equation.

$$dP(I,J)/dI = -(m+p)\ P(I,J) \qquad (2),$$

where (m) is the instantaneous daily natural mortality and (p) the instantaneous daily predation rate. From this equation, the finite daily survival rate (s) is computed as shown in the equations (6 and 7) and used to fill the matrix as follows :

$$P(I+1,J) = s\ P(I,J) \qquad (3).$$

After sexual maturity (M), they can contribute to increase the population size directly. P(I,I) the size of new-born young on day I is obtained by the following equation :

$$P(I,I) = B \sum_{J=0}^{I-A} P(I,J) \qquad (4),$$

where (B) is the finite daily birth rate as shown in Table I. However, as shown in Table II, when $0 < J < A$ or $I < J$,

$$P(I,J) = 0 \quad (5).$$

For the given parameters shown in Table I, the population size P(I) was calculated for a period or more than ten times of the age of maturity. Until that time the population size becomes steady as shown in Fig. 3. In this way it is possible to know whether the population size will be stationary or not.

In these treatments the life span (W) was assumed to be the length of time from hatching to 1 % of survivorship, and the calculation step was a 1-day unit. Although the instantaneous predation rate (p) was assumed to be constant throughout the life span, the survivorship curves employed belonged to two types :

a) The instantaneous daily natural mortality (m) is assumed to be constant throughout life span. This assumption is commonly used, although the animals seemed to have a much lower mortality during the first stages.

$$s = \exp(-\ln 100/W - p) \quad (6).$$

b) The age-specific daily natural mortality (m) is assumed to be proportional to the 6th power of age x (=I-J). Though this assumption is not so common, animals cultured in good conditions often show the lower natural mortality in the younger stages as shown in Fig. 1.

$$s = \exp[-\ln 100/W^7 \{(x + 1)^7 - x^7\} - p] \quad (7),$$

where $x = I-J$.

Study on the status of an already established colony

This study was made by employing the Lewontin equation (Lewontin, 1965). The equation describes the relationship among demographic parameters of a population which was successful in colonizing a new habitat. The equation was based on the Lotka equation which assumes a stable age composition and exponential growth of the population. He further assumed a simple triangular form of V-function (Vx), the product of survivorship (Lx) and fecundity (Mx) at age (x), where (r) is the instantaneous growth rate (Fig. 2).

The Lotka equation is shown in Fig. 2. Letting (A) denote the age of maturity, (T) the age of maximum reproduction as expressed by (Vx) (the reproductive value at age x), (W) the age when the animal stops reproducing (or the life span), and (S) the number of young produced during the mean life time of an adult, and defining $E(X) = \exp(-rX)$, the Lewontin equation (8) is as follows :

$$\frac{E(A) - E(T)}{T - A} + \frac{E(W) - E(T)}{W - T} - \frac{(W - A)r^2}{2S} = 0 \quad (8).$$

To know the effect of demographic parameters on (r), the partial derivatives of (r) with respect to any of the parameters are obtained from the above equation :

$$\frac{\partial r}{\partial A} = \frac{r^2}{2DS} + \frac{E(A)\{1 + R(A-T)\} - E(T)}{D(A-T)^2} \quad (9),$$

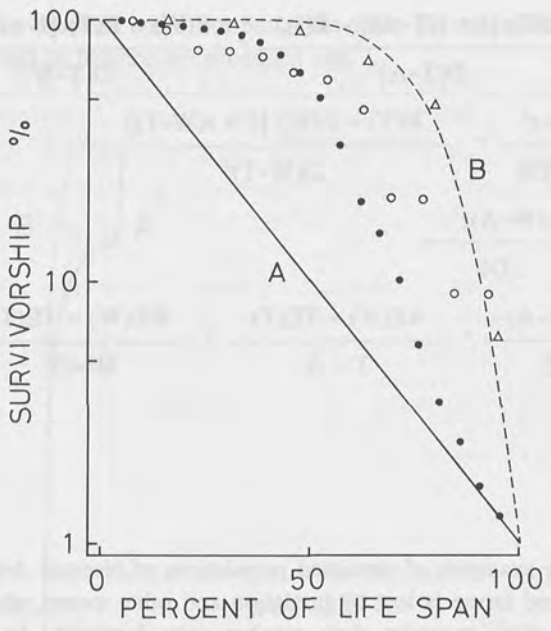

FIG. 1. Survival curves used and some examples. (A) (solid line) is used in case study A, and (B) (broken line) in study B. Open triangles and circles are for rotifers (King, 1967 ; Hirayama and Kusano, 1972 respectively). Solid circles for Cladocera (Frank *et al.*, 1957).

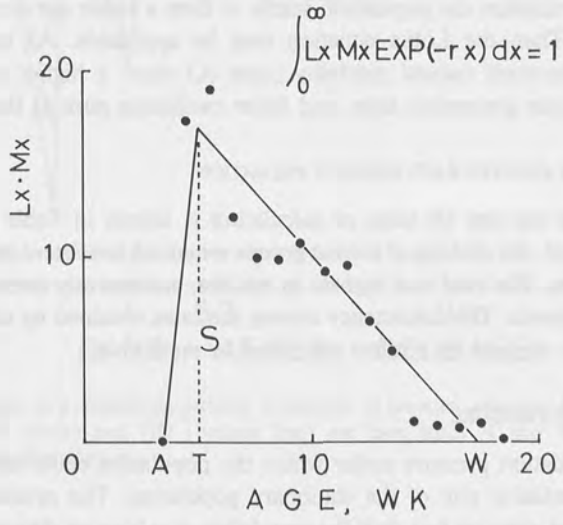

FIG. 2. Explanation of the Lewontin equation. The equation in the figure is the Lotka equation. (Lx) age-specific survivorship at age (x) ; (Mx) age-specific fecundity ; (r) instantaneous growth rate ; (A) age of maturity ; (T) age of maximum reproduction ; (W) life span (cessation of reproduction) ; (S) total young produced by one adult (shown by the area of the triangle). Solid circles are observed (LxMx) figures for a beetle *Calandra oryzae* (Birch, 1948, cited in Lewontin, 1965).

$$\frac{\partial r}{\partial T} = \frac{E(T)\{1 + r(T-A)\} - E(A)}{D(T-A)^2} + \frac{E(W) - E(T)\{1 + r(T-W)\}}{D(T-W)^2} \quad (10),$$

$$\frac{\partial r}{\partial W} = \frac{-r^2}{2DS} + \frac{E(T) - E(W)\{1 + r(W-T)\}}{D(W-T)^2} \quad (11),$$

$$\frac{\partial r}{\partial S} = \frac{(W-A)r^2}{2DS} \quad (12),$$

where $$D = \frac{r(W-A)}{S} + \frac{AE(A) - TE(T)}{T - A} + \frac{WE(W) - TE(T)}{W - T} \quad (13).$$

Results

Colonizing process

Fig. 3 shows some examples of simulated populations of bisexual *Artemia*. As expected, a group of newly-hatched larvae is lost by predation and other causes when released to a new habitat. Before they reach maturity, their number only decreases. At maturity the second generation contributes to increase the number as shown in Fig. 3, but the age composition varies for a while. When the growth rate of the population without predation balances the predation rate, the population size becomes stationary after several periods of oscillation. The duration of oscillation varies with the age-specific mortality which also influences the age composition. After establishment of colonization the population seems to have a stable age composition and might grow exponentially. Then the Lotka equation may be applicable. As shown in Fig. 3, the populations having constant natural mortality (case A) show a higher mortality rate in the younger stages, a shorter generation time, and fewer oscillation periods than those of case B.

Instantaneous daily growth rate without predation

The growth rate at the last 10 steps of calculation is shown in Table III. Although some difference was observed, the ranking of animal groups remained consistent in spite of the different methods of estimation. The rate was highest in rotifers, successively decreasing in Cladocera, Copepoda, and in *Artemia*. The consistency among the rates obtained by different methods was quite good except for the rate for rotifers estimated by method A.

Tolerable predation pressure

The maximum predation pressure under which the population could exist was estimated by the instantaneous predation rate of the stationary population. The pressure was obtained by changing the predation pressure (p) until the population size became stationary. The calculation error was estimated at less than 0.02, which was also the changing level for the (p) rate. The ranking of the animal groups was consistent among the different methods of estimation and with the instantaneous growth rate as described above (Table III). Only minor differences among both methods were observed : the rates obtained by method B were a little lower than the others.

Although the Lewontin equation could not be solved when (r) was exactly zero, the inherent growth rate was assumed to balance the predation rate.

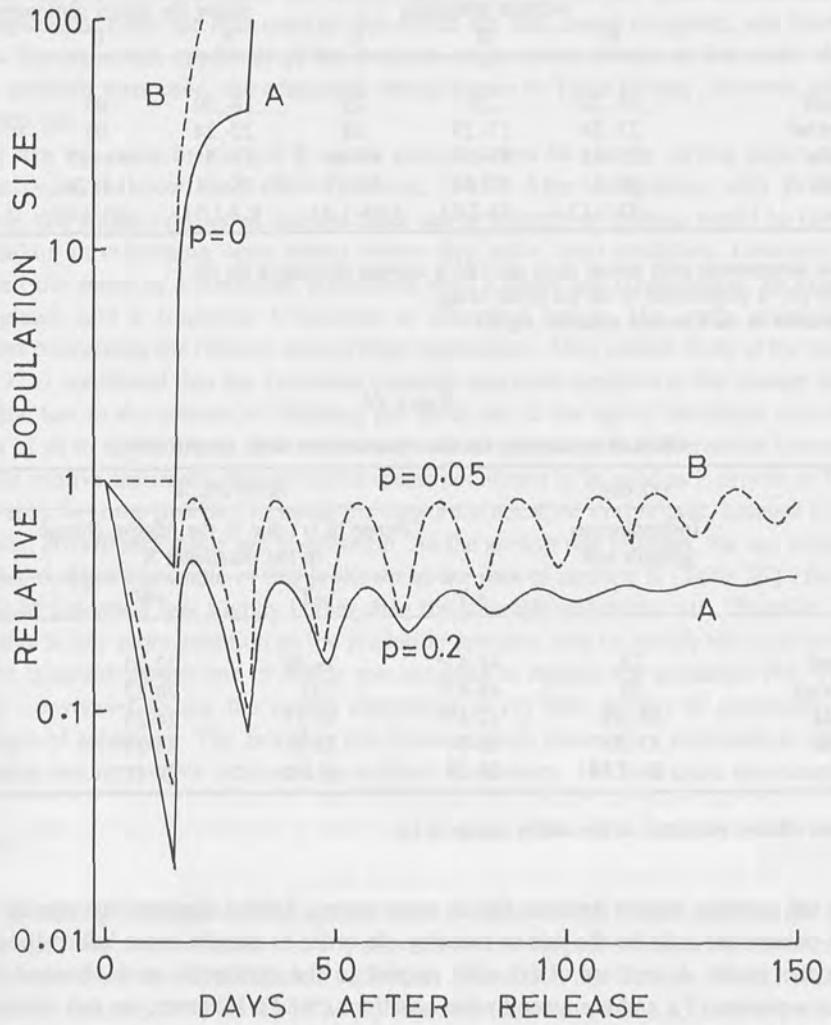

FIG. 3. Population size in a colonizing process. Examples of bisexual *Artemia* (A=16, W=80, daily birth rate B=15). (A) (solid circle) and (B) (broken line) are case study A and B, respectively and (p) instantaneous daily rate of predation.

EFFECT OF DEMOGRAPHIC PARAMETERS ON THE GROWTH RATE

It is likely to say that a younger age at maturity, more offspring per adult and a higher daily birth rate will favour population growth. However, it is difficult to know how the peak of reproduction and life span will work on the growth. Considering these tendencies and also the

TABLE III

Instantaneous daily growth and predation rate

	Instantaneous growth rate without predation			Instantaneous predation rate under the nearly stationary state		
	A	B	L	A	B	L
Artemia						
Bisexual	.18-.20	.26	.25	.18-.20	.05	.25
Unisexual	.22-.24	.27-.29	.28	.22-.24	.06	.28
Copepoda	.35-.45	.47-.57	.38-.44	.35-.45	.16-.22	.38-.44
Cladocera	.28-.51	.37-.63	.32-.53	.28-.51	.13-.28	.32-.53
Rotifera	.07-1.12	.93-2.03	1.01-1.34	0.7-1.11	.84-2.03	1.01-1.34

(A) The instantaneous daily natural death rate (m) is constant throughout the life.
(B) The (m) is proportional to the 6th power of age.
(L) Obtained by the Lewontin equation (Eq. 8).

TABLE IV

Effect of parameters on the instantaneous daily growth rate

	(r)/day	$X\partial r/r\partial X$, %			
	Instantaneous growth rate	Change in (r) due to the relative change in the parameter X			
		-A	-T	-W	S
Artemia					
Bisexual	.25	41-43*	35-36	22-23	18
Unisexual	.28	44-45*	35	20-22	16
Copepoda	.38-.44	42-45*	31-33	24-25	20
Cladocera	.32-.53	36-44*	32-36	24-29	21-25
Rotifera	1.01-1.34	26-29	31-34	38-41*	35-41

* The most effective parameter on the relative change in (r).

fact that the animals, except *Artemia*, live in open waters, Table I suggests that one of the most effective parameters may be the age at maturity. In order to receive more information on this problem the partial derivatives of (r) with respect to the parameter were obtained from the Lewontin equation (8), and are given by the equations (9-13). However, we feel uncomfortable to compare them directly since the parameter itself varies greatly. Therefore the derivative was modified to give a relative change in (r) due to the relative change in the parameter : if the parameters is (X), the relative change in (r) can be given by $X\partial r/r\partial X$ as shown in Table IV.

The most effective parameter on the growth rate indeed was the age at maturity, except in rotifers whose age at maturaty was the youngest of all ; *e.g.* it is only 1 to 2 days while for the others more than 5 days are needed (Table I). In contrast the length of life span was most effective in rotifers which have the shortest life span. The number of young produced by a female adult, which was highly correlated to the daily birth rate, was least affected for all organisms considered except rotifers.

Discussion

The population growth is influenced by many factors other than those considered above. Moreover, the processes on which the factors may have influence are quite various and specific to the organisms. Even the data used in this article are not always complete, and have a wide variance. Therefore this approach of the problem might seem strange at first sight. Although different methods were used, the consistent results shown in Table III may, however, encourage one to step out.

A first step was made by method B : many growth curves for slightly varying parameters were drawn in order to know their effect (Nimura, 1985). After comparison with Rotifera and Cladocera, this author concluded that the older age at maturity in *Artemia* would be responsible for the failure in colonizing open waters where they suffer from predation. Lewontin (1965) considered the status of a colonized population with a stable age composition, an exponential growth phase, and a triangular V-function as described before. His study revealed several indications concerning the relation among these parameters. After careful study of the same data, Allan (1976) concluded that the Lewontin equation was most sensitive to the change in the age at maturity, less to the amount of offspring per adult and to the age of maximum reproduction, and least of all to the life span. However, when considering the partial derivatives themselves as well as the relative sensitivity, this comment seems not always to be valid as is proven in Table IV.

There may be some problem in using the Lewontin equation in this case, because it assumes exponential growth and stable age distribution. As the growth rate changes, the age composition also varies. A typical example of this is shown in the data of method B (Table III) : the growth rate without predation was slightly higher than the tolerable predation rate. Therefore it might be desirable to pay more attention to the predation pressure, and to modify the equation. In this article the inherent growth rate or ability was assumed to balance the predation rate. However, it is very convenient to use the partial derivatives of (r) with respect to parameters for the comparison of sensitivity. The fact that the most sensitive parameters estimated as above was equal to the one as roughly estimated by method B (Nimura, 1985) is quite encouraging.

Appendix

After presenting the article at the symposium, I realized that (S) total offspring per adult per mean life span was overestimated : both I and Allan (1976), presumably from his comment on Copepoda and from checking his cited references (Anderson and Jenkins, 1942 ; Hall, 1964 ; King, 1967), estimated (S) as $S = B(W-A)$ from the data on (W), (A) and (B) which were usually obtained as the number of female produced per brood per period between spawning. In this estimation the females which died during the experiment were completely neglected. Since $S = (W-A)V_T/2$, as given by Lewontin (1965), and since $B \leqslant V_T$, as mentioned above, $S \leqslant B(W-A)/2$ should be used in the Lewontin equation.

The survivorship at age (T) (L_T) is estimated 14-31 % or 99-100 % on the assumption used in case study A or B respectively, when using the ratios of T/W (Table I). These data actually obtained may be still underestimated as already stated by Allan (1976). Therefore, for the simplicity, I assumed $B = V_T$ which was the product of (B) and survivorship at age (T) and used $S = B(W-A)/2$ in the following comparison (Table V).

TABLE V

Effect of parameters on the instantaneous daily growth rate

	(r),/day	$X\partial r/r\partial X$, %			
	Instantaneous growth rate	Change in (r) due to the relative change in the parameter X			
		-A	-T	-W	S
Artemia					
Bisexual	.22	39-40*	35-36	24-26	20
a)	.36-.37	38-39*	36	25-26	23-24
Unisexual	.25	41-43*	36	25-26	23-24
a)	.42-.43	42-43*	35	23-24	21
Copepoda	.33-.38	38-41*	33-34	26-28	21-23
Cladocera	.26-.45	31-40*	34-37*	26-33	24-30
Rotifera	.71-1.08	21-30	28-34	40-47	42-55*

* The most effective parameter on the relative change in (r).

a) A=7 and T=14 as reported by Vu Do Quynh and Nguyen Ngoc Lam (1987).

Vu Do Quynh and Nguyen Ngoe Lam (1987) stated that a certain strain starts spawning already on the 7-8th day after hatching. Though I do not think it is common among *Artemia* populations, such a strain is also included in the comparison. Table IV is, therefore, to be modified as Table V. In fact, the conclusions from Table V did not change dramatically. The instantaneous daily growth rate for Cladocera was more than 0.39, if only the data cited by Allan (1976) were used.

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Life-cycle studies in *Artemia*: a comparison between a sub-tropical and a temperate population

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Abstract

Life histories of *Artemia* from Mono Lake and Laysan Lagoon were studied. Mono Lake (California, USA) is a large (150 km^2) temperate lake. Laysan Lagoon (Hawaii, USA) is small (1.3 km^2) and subtropical. In Mono Lake *Artemia* disappear during the winter. During the growing season (March to November) the population has a continuously changing instar distribution. In March the population is composed of nauplii only, in June both adults and nauplii are abundant, and after September adult *Artemia* predominate. The Laysan population had a stable instar distribution from April to July. In Mono Lake ovoviviparous reproduction peaks in June, rapidly declining thereafter. Subsequently females produce diapause cysts, which overwinter on the bottom of the lake and contribute to the following spring population. The Laysan females reproduce ovoviviparously and no diapause cysts were found in this population. In Laysan Lagoon the broods were small (1 to 4 offspring/brood) as compared to Mono Lake (mean brood sizes range between 30 and over 130 offspring).

Introduction

Theoretical predictions have been made on the expected impact of the environment in the selection of life-history patterns such as clutch size (Cody, 1966), iteroparity *versus* semelparity (Murphy, 1968), and length of reproductive lifespan (Schaffer, 1974). Such theoretical concepts can be tested empirically via two general approaches : inter-population comparisons and laboratory experimentation (Stearns, 1977). Natural *Artemia* populations are good subjects for life history studies, because both approaches can be used easily (Browne *et al.*, 1984). The genus *Artemia* is made up of a complex of closely related species (Barigozzi, 1974 ; Clark and Bowen, 1976 ; Abreu-Grobois and Beardmore, 1980 ; Bowen *et al.*, 1980). *Artemia* populations are found from tropical to temperate regions, and from temporary ponds to large deep salt lakes (Persoone and Sorgeloos, 1980 ; Melack, 1983 ; Dana, 1984), and are thus adapted to a broad range of habitats. In this paper we compare life-history patterns of two natural *Artemia* populations occurring in two very different habitats, Mono Lake and Laysan Lagoon. We discuss the data in the context of life-history strategies in *Artemia*.

Study site descriptions

Mono Lake (38°N, 119°W ; California, USA) is ca. 150 km^2 in area with a mean depth of 17 m (Fig. 1). Because of its large size and depth, annual salinity fluctuations are usually less than 5 ‰ and rarely exceed 10 ‰. Water exports from the Mono Basin have caused a decline in lake level, and the salinity increased from 48 to 93 ‰ between 1941 and 1982 (Vorster, 1985). Salinity in the mixolimnion in the fall of 1983 was 76 ‰ (R. Jellison, pers. commun.). Until 1982 a thermocline and an anoxic hypolimnion developed each spring and lasted through the summer until turnover occurred in October/November. During the winter the lake circulated freely and the whole watercolumn was oxygenated (Melack, 1983). In 1983 unusually heavy precipitation caused the lake to rise almost 2 m and Mono Lake became chemically stratified. Meromixis has persisted (Melack *et al.*, 1985), preventing winter mixing and the lake has been anoxic below 15 m since then. Epilimnetic temperatures range between 2 and 25 °C throughout the year. The limnology of Mono Lake has been described in detail in Mason (1967), Winkler (1977), Lenz (1980, 1982, 1984), and Melack (1983). The Mono Lake *Artemia* belong to the *A. franciscana* complex. However, they appear to be reproductively isolated from the other *A. franciscana* populations, and a separate species denomination has been proposed (Bowen *et al.*, 1980, 1985).

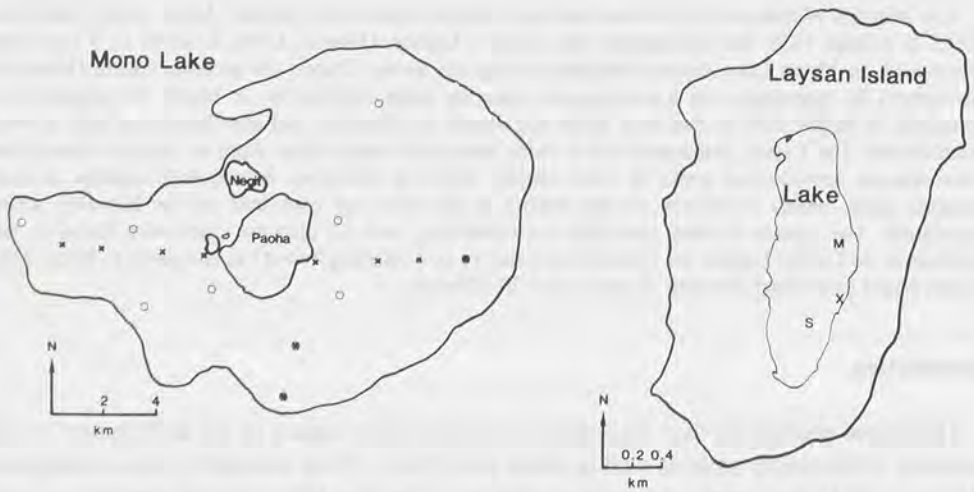

FIG. 1. Maps of study sites with *Artemia* sampling stations indicated. For Mono Lake, crosses (×) denote stations used for 1982 (and earlier) samples and open circles (○) those used in 1983-84 (● denotes a station used all years) ; the shoreline indicated is for a lake elevation of 1 942 m (Lenz, 1980). For Laysan Lake, three sampling stations are indicated : X near-shore, M with depth 6 m, and S with depth 6.5 m (map drawn from a May 1981 aerial photograph by the US Fish and Wildlife Service, Honolulu, Hawaii).

Laysan Island (25°46′N, 171°44′W), the largest of the Northwestern Hawaiian Islands (USA) has an approximate area of 3.7 km^2 (Ely and Clapp, 1973). Laysan Lagoon (Fig. 1) is a small (1.3 km^2) hypersaline lake, which fluctuates seasonally in level and salinity (Ely and Clapp,

1973 ; Maciolek, 1982). Maximum lake depth is between 6 and 7 m. The lake level is highest during the winter and early spring, and it reaches its lowest point (1 m or less below peak) in August or September. The lake has not been reported to ever dry completely. The lake's salinity was measured at 62 ‰ (Tsuda, 1965) and 130 ‰ (Caspers, 1981), however, there is no mention of the respective lake levels at the time of the measurements. Mean summer water temperatures approach 30 °C and in the winter drop to *ca.* 18 °C. The mean annual air temperature at the nearest recording station, Midway Island has a 9 °C range, with the lowest monthly temperature mode over a 10 year period at 15 °C (Clapp and Wirtz, 1975). Laysan Island, which is further south than Midway Island, has a similar temperature regime, hence our estimate for winter lake temperatures. Usually the watercolumn mixes daily, although weak thermal stratification may persist over several days under unusually calm weather conditions (Lenz, unpubl.). The presence of brine shrimp in Laysan Lagoon was first recorded by Sars in 1903 from samples collected in 1896 (Ely and Clapp, 1973). The origin for the Laysan *Artemia* has not been determined, however, the early descriptions of their occurrence suggest that this population is endemic. Migratory shorebird routes between Laysan and the west coast of North America support the hypothesis that this population originated from the North American continent and, therefore, belongs to the *A. franciscana* complex. There are few limnological observations on Laysan Lagoon and its biota (but see Tsuda, 1965 ; Ely and Clapp, 1973 ; Caspers, 1981 ; Maciolek, 1982).

Materials and methods

At Mono Lake, *Artemia* were sampled quantitatively for 3 years at 10 stations (Fig. 1) with a plankton net (30 cm diameter, 120 µm mesh) towed vertically through the watercolumn from either the bottom, or 15, or 20 m depth (Lenz, 1984). Duplicate or triplicate samples were taken at each station at bi-weekly intervals from March to September and thereafter at monthly intervals until November. Samples were preserved in 5 % formalin and *Artemia* were counted under a dissecting microscope. Samples with over 200 individuals were subsampled with a Folsom plankton splitter. Sample counts were corrected for a 70 % net efficiency (Lenz, 1982). *Artemia* were classified into three size classes : instars 1-7, juveniles (instars 8-11) and adults (instars 12 and greater). For each date instars 1 through 7 were classified separately at three stations. Adults were sexed and females were classified according to their reproductive state (ovigerous or non-ovigerous) and mode (ovoviviparous, oviparous or unclassifiable). Mean abundances and standard errors were calculated from all samples.

For Laysan Lagoon, qualitative *Artemia* samples were taken by inverting a 500 ml jar at a depth of 10 cm at the nearshore station (station X, Fig. 1) at approximately weekly intervals from April to July. *Artemia* were samples quantitatively at station M with a 2 l Van Dorn bottle in April (three depths) and July (five depths). Three replicate samples were taken at each depth. Abundances in individuals/m^2 were calculated by integrating mean densities for each depth (Lenz, 1980). Coefficients of variation (Snedecor and Cochran, 1967) were calculated for each depth and averaged for an overall estimate of variation. In April vertical net tows at stations M and S were taken from near the bottom to the surface. Samples were preserved, counted and classified as described above.

Spring development rates for Mono Lake *Artemia* were estimated from three separate *in situ* experiments in 1982. For each experiment samples of lake water containing *Artemia* were

incubated for 7 or 8 days at 1 m depth in 4.75 l Nalgene polycarbonate containers (three or four replicates) fitted with screened (120 μm Nitex) mesh windows. Upon retrieval, animals were preserved and their instar distribution compared to the initial one. Centroids were calculated for each instar distribution ($c=\Sigma i.d/\Sigma d$ with c=centroid in instar, i=instar number [1-12], and d=number of *Artemia* in each size category ; Dana and Lenz, 1982). Mean instar development rates were calculated as the difference between mean initial and final centroids divided by the interval in weeks.

Brood sizes in Mono Lake in 1983 were determined by isolating females and individually preserving them in 5 % formalin. Eggs/cysts both in the brood pouches and in the preservative were counted. This method was also used at Laysan in 1985. In 1984 brood sizes were determined for females kept in captivity for 7 days.

Laysan Lagoon sediments were examined for the presence of diapause cysts in July 1984. Sediments from five downwind locations were scanned under a dissecting microscope. Sediment cores (2 cc) were collected along eight four-station transects extending from the water's edge to the vegetation. These 32 samples were incubated in seawater for 7 days and checked daily for the presence of nauplii.

Results

Artemia abundance and instar distribution

The *Artemia* population in Mono Lake has a marked seasonal cycle with a single peak in abundance during the summer (Fig. 2). *Artemia* were absent during the winter months. Nauplii hatched from overwintering cysts in early spring. Samples taken in early March always contained first instar *Artemia.* Mean March and April densities ranged between 2 000 and 25 000 ind./m^2, with the lowest densities occurring in 1982. Throughout March and April the population was composed exclusively of instars 1-7 (Fig. 2 and 3). *Artemia* development rates in mid April were very low (0.043 instar/day, Table I), but by early May had increased to 0.24 instar/day. Water temperature rose from 8 °C to almost 11 °C during this 3 week period (Table I).

Table I

Artemia development rates in Mono Lake in 1982

Initial date	Incubation period (days)	Temperature* (°C)	Instar centroids		Development rate (instars/day)
			Initial (instar)	Final (instar)	
April 15	7	8.2	1.1	1.4	0.043
April 22	7	8.6	1.5	2.4	0.129
May 2	8	10.9	1.5	3.5	0.243

* Based on averages of measurements taken at 1 and 2 m depths ; a mean temperature for the incubation period was calculated from linear interpolations of measurements taken on April 13, 20, 27 ; May 5 and 20.

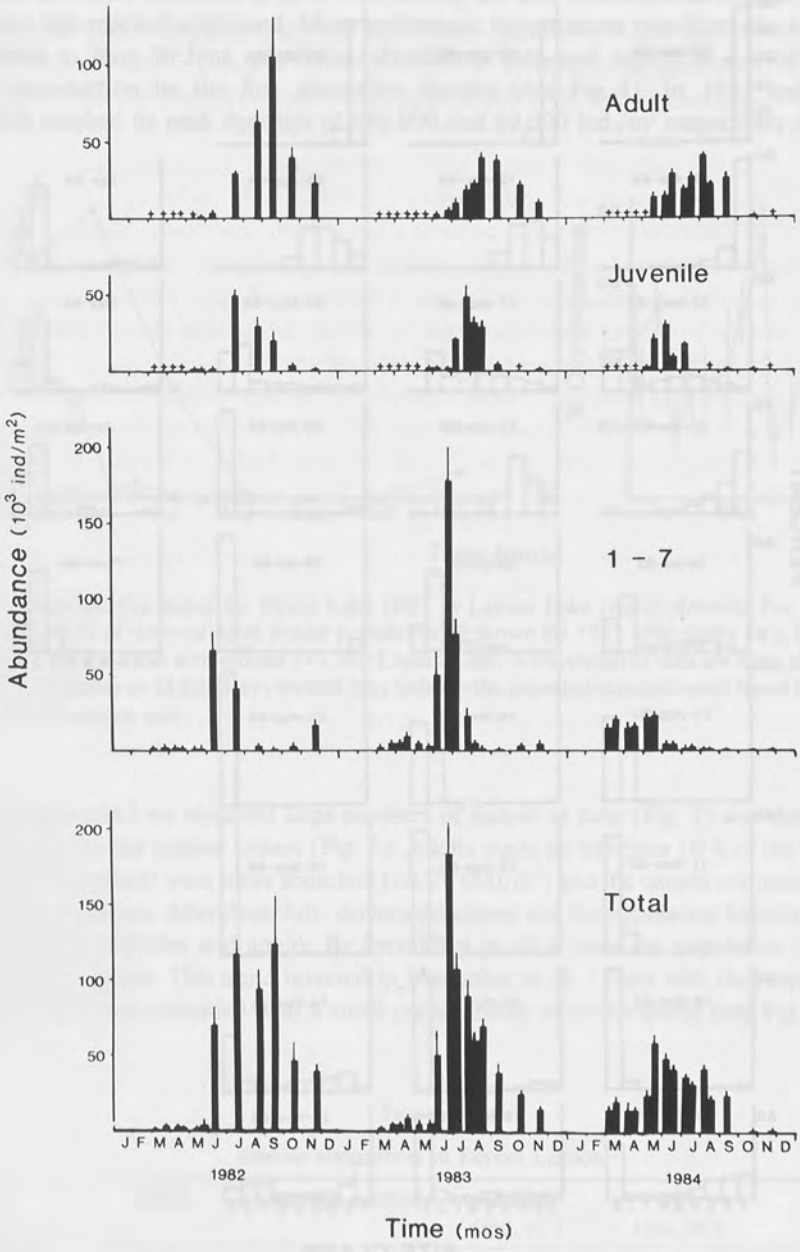

FIG. 2. Mean *Artemia* abundances and standard errors (thin bars) for 3 years at Mono Lake. Top graphs : animals classified into three developmental categories, bottom : total abundance of all stages (sum of top 3). Cross (+) indicates absence of stage from sample.

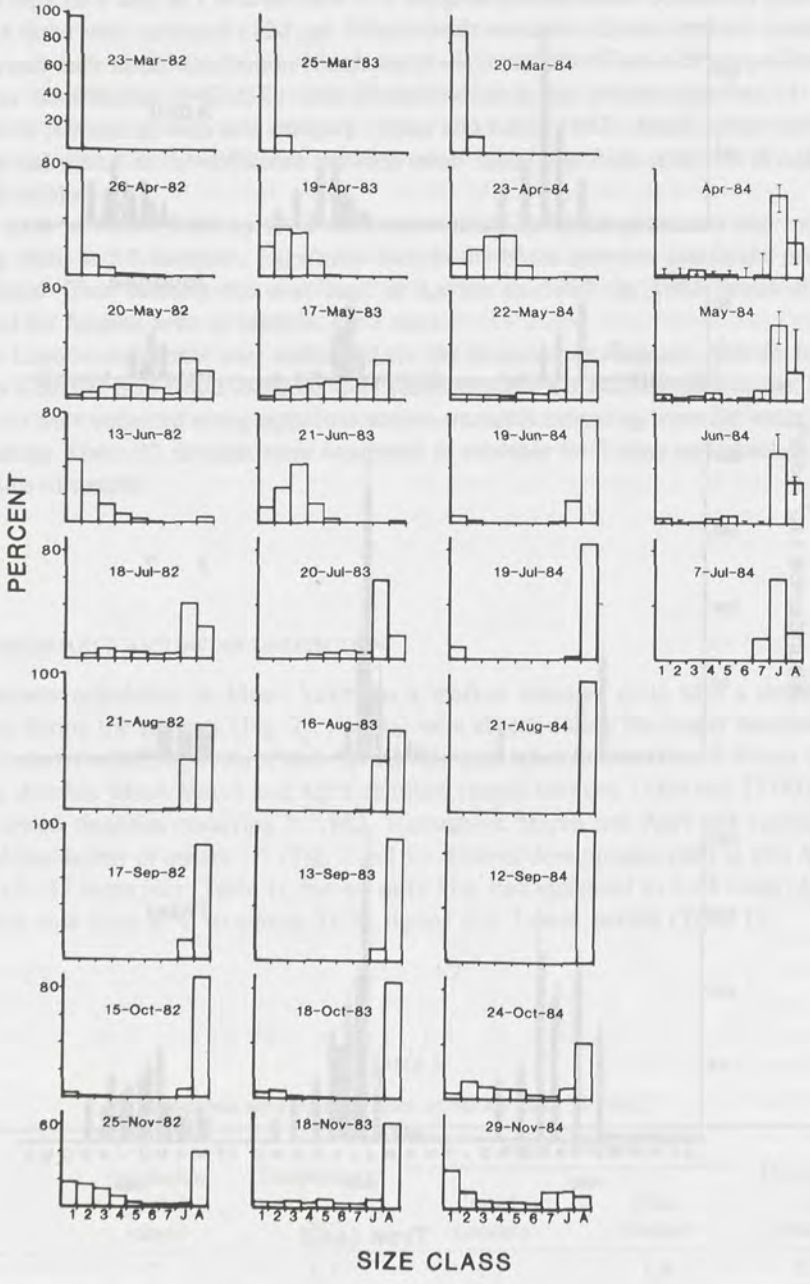

FIG. 3. *Artemia* instar distribution patterns for 3 years at Mono Lake (left three columns) and 4 months at Laysan Lake (right column). Mono Lake data pooled from three stations. Laysan Lake data from single qualitative samples at station "X" (Fig. 1). Brackets with each bar for April-June indicate range for up to four such weekly samples in the month indicated.

The first juveniles appeared in early May and by the end of the month at least 20 % of the population had reached adulthood. Mean epilimnetic temperatures rose from about 4 to 15 °C from March to June. In June, population abundances increased rapidly as a result of ovoviviparous reproduction by the first generation females (see Fig. 4). In 1983 and 1984 the population reached its peak densities of 180 000 and 60 000 ind./m^2 respectively at this time.

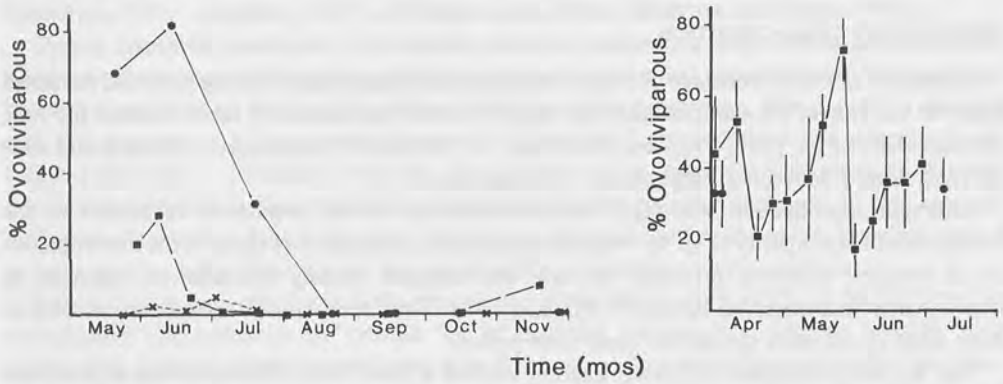

FIG. 4. Reproductive status for Mono Lake (left) *vs* Laysan Lake (right) *Artemia*. For Mono Lake, ovoviviparity (as % of the total adult female population) is shown for 1982 with circles (●), for 1983 with squares (■), and for 1984 with crosses (×). For Laysan Lake, % ovoviviparity data are from single samples at station X (squares) or M (circles) ; vertical bars indicate the expected standard error based on a binomial model for each sample size.

In 1982 and 1983 we observed large numbers of nauplii in June (Fig. 2) and the population was dominated by the smaller instars (Fig. 3). Adults made up less than 10 % of the population. In June 1984 the adults were more abundant (*ca.* 20 000/m^2) and the nauplii comprised less than 10 % of the population. After June/July, densities declined and the population became increasingly dominated by juveniles and adults. By September in all 3 years the population consisted of more than 90 % adults. This trend reversed in November in all 3 years with the reappearance of nauplii. In 1983 this coincided with a small reappearance of ovoviviparity (see Fig. 4).

TABLE II

Artemia abundances in Laysan Lagoon

Date	Station	Sampling method	Density (ind./m^2)	SE* (ind./m^2)	CV**
April 2, 1984	S,M	Vertical net tow	68400	16900	0.61
April 4, 1984	M	Van Dorn series	85200		0.21
July 7, 1984	M	Van Dorn series	58800		0.25

* Standard error.

** Coefficient of variation.

In contrast to Mono Lake, the *Artemia* population in Laysan was consistently dominated by juvenile and adult stages throughout the observation interval (April-July 1984 ; Fig. 3). Population density measurements averaged 70 000 ind./m^2 (Table II). Mean lake temperatures increased from 24 to 30 °C and the lake level dropped 30 cm between the beginning of April and mid July.

Reproductive characteristics

In Mono Lake the percentage of ovigerous females increased rapidly during June and remained high for the rest of the summer and fall. Mean percent reproductively active females for June through October for 1982, 1983 and 1984 were 76, 89 and 69 % respectively. Mean brood sizes in 1983 ranged between 30 and almost 140 eggs/brood.

Although reproduction was high, direct recruitment to the population is limited to the incidence of ovoviviparity (Fig. 4). Nauplius production, calculated as the percent ovoviviparous of all females, occurred primarily in June and dropped rapidly thereafter. A decrease in ovoviviparity was observed between 1982 and 1984. This trend coincided with an increase in June adult abundances during the same time period.

The *Artemia* population in Laysan Lagoon showed a lower level of reproductive activity than that in Mono Lake. Incidence of ovigerous (=% ovoviviparous) females ranged between 17 and 73 % (Fig. 4) and averaged 37 %. Brood sizes of females collected on July 7, 1984 and kept in captivity for 7 days averaged three offspring/brood with a range from 1 to 4. In June 1985 mean brood sizes were two with a range from one to four. None of the females produced cysts. No cysts were found in shoreline sediment samples and no nauplii hatched from any of the sediments incubated in seawater. Cysts were always present in sediments collected from Mono Lake and nauplii hatched readily in incubation experiments (Dana and Lenz, 1982).

Discussion

Instar distribution

Ecological studies on the *Artemia* in Mono Lake have shown that this population for the last 7 years has had only two major generations per year, and the timing varies little among years (Lenz, 1980, 1984, present study). A similar two generation pattern was described by Wirick (1972) for Great Salt Lake (Utah, USA). *Artemia* in Fallon ponds (Nevada, USA) have a similar instar distribution pattern, albeit a shorter period of occurrence, since the ponds dry in late July (Dana, 1984).

The instar distribution for the Laysan Lagoon population, in contrast, varied little over the 4-month study period, and was always dominated by juveniles. Stable-age distributions are indicative of a near steady-state situation and the reproductive activity of the Laysan females suggests a continuous, though low, recruitment to the population. Further data are needed to determine whether this stable instar distribution persists throughout the year. However, given the environmental characteristics of Laysan Island, we have little reason to expect much deviation from the pattern we observed.

Oviparity *versus* ovoviviparity

The production of resistant diapause cysts by *Artemia* is a major survival mechanism in populations exposed to conditions which preclude the survival of the individual. A dependence on oviparity occurs in populations with a strong seasonal cycle of either temperature (*e.g.* Mono Lake : Lenz, 1980 ; Great Salt Lake, Utah, USA : Wirick, 1972) or salinity, including the possibility of desiccation (*e.g.* Fallon, Nevada, USA : Dana, 1984 ; San Francisco Bay salterns, California, USA : Carpelan, 1957 ; Didwana Lake, India : Bhargava and Alam, 1980).

During favorable conditions, ovoviviparity gives an immediate reproductive advantage (for review see Persoone and Sorgeloos, 1980). In Mono Lake the first generation produces naupliar broods (usually two or less) in June before switching to cyst production. Wirick (1972) reported that first generation *A. franciscana* females in Great Salt Lake produced one naupliar brood before switching to oviparity. However, the benefits of an ovoviviparous phase in a cyclic population decrease as the probabilty of mortality for the progeny increases. In less seasonal environments, such as Laysan Lagoon, the relative advantages of ovoviviparity increase, even if stresses such as a low food supply and intraspecific competition may be present.

Recent studies have emphasized the importance of the genetic component in determining the reproductive characteristics in *Artemia.* Under constant laboratory conditions females from several bisexual and parthenogenetic strains tended to reproduce ovoviviparously at first and then switch to oviparity (Amat, 1982), which suggests that switching does not depend exclusively on environmental cues but is also under genetic control. Furthermore, Amat (1982) and Browne *et al.* (1984) have demonstrated a wide range of variation in the incidence of ovoviviparity among *Artemia* strains under controlled environmental conditions. In addition, Browne (1983) found that the incidence of ovoviviparity had decreased in a laboratory *A. franciscana* culture which had been kept in a hydration : dehydration cycle of 4:8 months for 25 years. The author concluded that this decrease had resulted from selection against ovoviviparity. The genetic variation in reproductive mode among *Artemia* strains may therefore be the result of natural selection.

Brood size

A. franciscana had smaller brood sizes when kept on a low food regime than at high food (Browne, 1982). This relationship appears to occur in Mono Lake. The larger brood sizes, including oviparous broods (mean size for cyst broods in June exceeded 130 eggs), occurred in early summer while phytoplankton abundances were high, whereas the small brood sizes correlated with lower late-summer food abundances (Lenz, 1982 ; Dana and Lenz, 1984). Ludskanova (1974) reported a mean brood size of 66 eggs/brood in the Bulgarian salterns of Pomerije and Burgas. In Great Salt Lake, Wirick (1972) reported clutch sizes of 30 to 50 for ovoviviparous and less than 20 for oviparous broods. Brood sizes of one to four in Laysan females are among the lowest ever recorded in natural *Artemia* populations. Similar low brood sizes (means of 5.5 and 6.5 eggs/brood) were reported by Scelzo and Voglar (1980) in Boca Chica Salt Lake, Venezuela.

Besides environmental factors, in particular food availability, brood sizes in *Artemia* are also under genetic control (Amat, 1982 ; Browne *et al.*, 1984). Browne *et al.* (1984) found mean brood sizes from different *Artemia* populations to range from 21 to 111 eggs/brood under identical laboratory conditions. The largest differences were found between parthenogenetic and Old World bisexual strains.

ARTEMIA ECOLOGY

Because *Artemia* is a cosmopolitan genus occurring in a large variety of habitats, they are subjected to many different ecological regimes, and hence their population dynamics and reproductive strategies can be expected to differ. Even within the *A. franciscana* complex (to which the Mono and Laysan *Artemia* appear to belong) one can expect genetic variation in life-history characteristics reflecting adaptations to the specific environments. Based on our comparative data from Mono Lake and Laysan Lagoon and other published information (*e.g.* Wirick, 1972 ; Persoone and Sorgeloos, 1980 ; Scelzo and Voglar, 1980 ; Dana, 1984), we propose two general predictions on how *Artemia* life-history might vary with habitat characteristics :

- The relative incidence of oviparity *versus* ovoviviparity is a function of the length of the inhabitable period. We predict that *Artemia* living in highly temporary habitats will have a genetic tendency to oviparous reproduction. In contrast, *Artemia* with ovoviviparity dominating will occur in lakes with prolonged periods of favorable conditions.
- Diapause cysts have two important functions : they secure the survival of the population through unfavorable conditions ; and they are effective dispersal agents (MacDonald, 1980 ; Persoone and Sorgeloos, 1980). On Laysan Island cyst dispersal would be counterproductive since there are no other salt lakes nearby, nor is there evidence for an interruption in the growth season. Reduction[1] of oviparity may therefore be expected in *Artemia* strains inhabiting lakes where neither of these factors is important. *Artemia* populations from New Zealand (Wear and Haslett, 1987) and the Canary Islands (Amat, 1982) appear to support this.

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[1] Some cyst production has been observed in laboratory cultures of Laysan *Artemia*.

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Studies on the biology and ecology of *Artemia* from Lake Grassmere, New Zealand

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Abstract

During 1981-83 the biology of *Artemia franciscana* from the solar salt ponds of Lake Grassmere (Marlborough, New Zealand) was studied in the laboratory, and a field sampling programme to estimate biomass was carried out over 18 months including two southern summers. *Artemia* densities were highest in 13 final concentrating ponds 238.5 ha in total area, and were contagiously distributed over two strata, *i.e.* the 50 m wide edge stratum averaged 2.5 times the biomass concentration of the central stratum. A statistically unbiased estimate of biomass was developed for each bimonthly sampling, giving asymmetric 95 % confidence bound estimates, based on the bootstrap method, each within 25 % of the estimated total *Artemia* biomass in sampled ponds. Maximum estimated total biomass for any sampling time over 238.5 ha was 12 tonnes dry weight, and was highest during late spring and summer. In one of the 13 ponds, estimated biomass concentration from November 1981 to April 1982 averaged 4.9 g/m^3 dry weight. Between November 1983 and May 1984, 4.2 g/m^3 dry weight *Artemia* were recovered by total filtration from the adjacent pond.

Harvestable biomass over the pond system was conservatively estimated at about 35 tonnes dry weight per year and could be extracted without affecting standing biomass, regenerative capability, or salt production. This estimate took into account field nutrient levels, pond algal densities and algal growth rates achieved in the laboratory, as well as laboratory studies on *Artemia* growth, mortality, longevity, generation times and fecundity carried out in 24 replicated combinations of temperature (8-32 °C) and salinity (80-260 ‰). The two factors, temperature and salinity, accounted for 81.3 % and 87.9 % of the total sums of squares for growth and percentage juvenile survivorship respectively, with temperature the major determinant in both cases. *Artemia* achieved fastest growth at 20-28 °C in 100-170 ‰ salinity. Over this range at least 90 % of the nauplii survived to maturity, which was achieved in 10-20 days ; generation times were 14-20 days ; and individuals lived for up to 6 months. The average number of generations per year was estimated to be seven. Females produced non-resistant eggs rather than cysts both in the laboratory and in the field, suggesting that the strain is adapted for sustained high biomass production in conditions which are rarely as harsh as in salinas fringing continental areas.

Introduction

This paper summarises and synthesises the results of an intensive research programme on *Artemia* from Lake Grassmere, Marlborough, New Zealand (41°43'S ; 174°10'E) completed during 1981-83. A major aspect of the study was commercial interest in harvesting *Artemia* on a sustainable basis without placing at risk the current levels of high quality salt production.

Lake Grassmere is a 1 782 ha area of former salt marsh reclaimed for solar salt production and developed as two large preliminary concentration ponds totalling 324 ha in area, a series of eight smaller preliminary concentration ponds (P3-P10) averaging 22 ha in area and about 0.5 m deep, and a final concentrating series (F1-F5) of more or less triangular ponds of similar depth, averaging 12.5 ha in area, arranged in a circle about a centrally located pumphouse servicing all F-series ponds (Fig. 1).

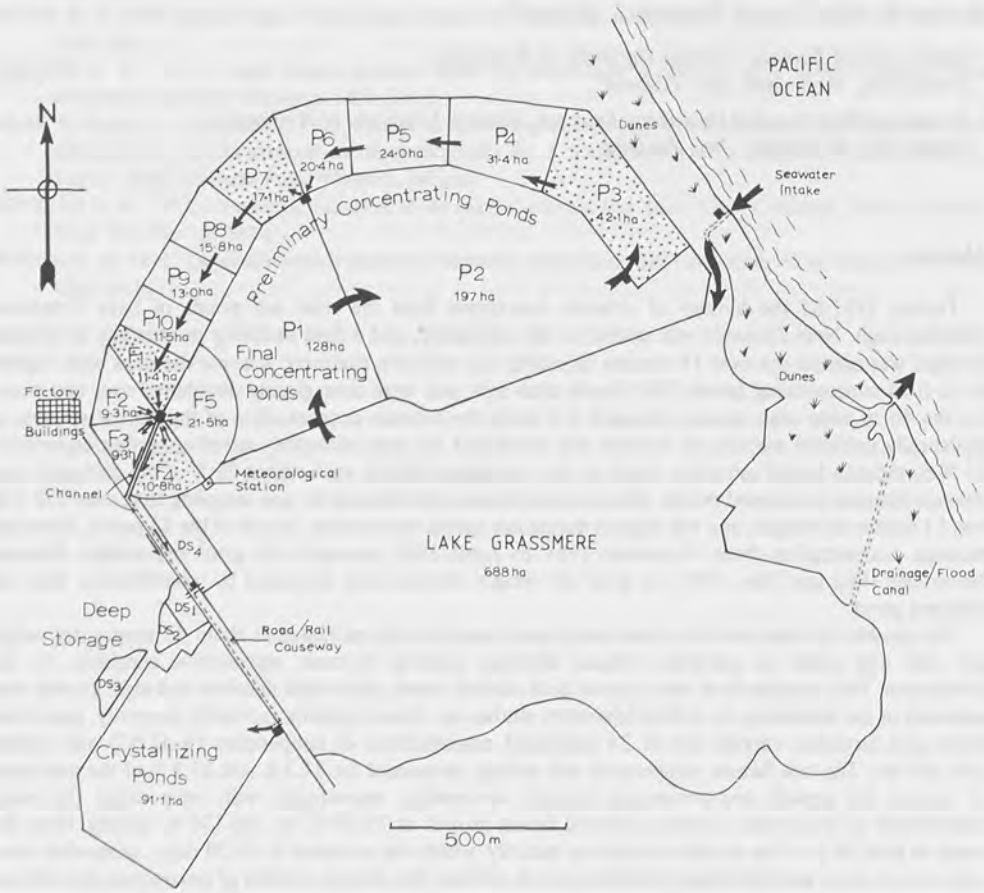

FIG. 1. Map of Lake Grassmere, showing layout of solar salt concentration pond system. Arrows indicate direction of brine flow during months of net evaporation. Sampled ponds are stippled. Relevant pump houses are indicated by solid blocks (after Haslett and Wear, 1985).

The general techniques of solar salt production are outlined by See (1960). In Lake Grassmere, the method has been modified to take account of the fact that the climatic conditions of 41 °S in a temperate and essentially insular regime are marginal for salt production.

Modifications include extensive development of deep storage facilities to accommodate overwintering brines, and continuous water transfers from pond to pond up the salinity gradient during the months of net evaporation (October to April inclusive). Following salt harvest (usually mid-March to mid-April), saturated brines remaining in the F pond series are pumped to deep storage. Over the following 2 weeks all ponds are topped up for the winter by flushing the entire pond system forwards. Water overwintering in the F pond series may thus originate from as far back down the series as P2 (Fig. 1). During September, back flushing takes place in preparation for the following months of net evaporation. This process is essentially a reversal of forward flushing, and involves all ponds once more – ponds F2 to F5 are refilled from deep storage, dilute water flows from the main lake to the sea, and the main lake is refilled with new seawater to top up brines backflushed from P1. From late September until salt harvest, water flows or is pumped forwards up the series commensurate with the rate of evaporation, and a salinity gradient exists at all times. Pumping is required from the sea to the main lake, from the main lake to P1, between P2 and P3, P6 and P7, from F1 to F2, F3, F4 or F5, and thence along a deep channel to the crystallizing ponds. All remaining flows between ponds are gravitational.

Artemia was presumed to be absent during the early days of commercial salt production beginning 1954, but was introduced either naturally or accidentally by man at a later date. The *Artemia* now present is a locally adapted bisexual strain belonging to the "*franciscana*" sibling species complex (P. Sorgeloos, pers. commun.). Our field studies were limited to the preliminary and final concentration ponds totalling 238.5 ha in which the *Artemia* concentrations are highest. *Artemia* were observed in the remainder of the system (P1, P2, crystallizing ponds), but in numbers too low to justify sampling and analysis, or commercial harvesting.

Commercial salt production records suggested that *Artemia* cyst production is sporadic and usually insignificant, presumably because climatic conditions are not harsh compared with salinas in lower latitudes and those fringing continental areas. However, biomass levels are always particularly high in the P and F pond series, and females ovoviviparously produce non-resistant eggs or nauplii, presumably to maximize success in the strictly managed Lake Grassmere biotope in which salinity perturbations are minimized in the interests of commercial salt production.

The study programme was thus designed to estimate *Artemia* biomass over a full year of salt production, within minimum error bounds given resource constraints, and to further determine what proportion of this biomass could be extracted from the pond system without deleteriously affecting *Artemia* standing crop and reproductive potential, or salt production. The role of *Artemia* in maintaining a healthy biological system in salt production ponds is well documented (Davis, 1980 ; Sorgeloos, 1983).

The extent to which biomass is renewable at Lake Grassmere was determined by analyses of field nutrients and algal concentrations, and by laboratory studies on *Artemia* growth, mortality, fecundity and generation times over the range of temperature/salinity combinations occurring in the ponds. A further consideration was that the 1st International Symposium on the brine shrimp *Artemia* (Corpus Christi, Texas, USA, August 1979) provided abundant evidence of the economic importance of *Artemia* and of its value in schools and universities as a teaching aid and as a vehicle for scientific research. The central problems of ecology and population dynamics in natural habitats (Pielou, 1981) have been frequently stated, but seldom researched for *Artemia* (Persoone and Sorgeloos, 1980).

Materials and methods

Laboratory

The unicellular halophilic phytoflagellates *Dunaliella euchlora* and *D. salina* are the principal natural food of *Artemia* in Lake Grassmere. These algae always co-occurred in the ponds, but in differing proportions depending on salinity (Knight, 1974). Their laboratory growth in mixed batch culture was studied using five temperatures (8, 14, 20, 26, and 32 °C) in combination with three salinities (120, 190, and 260 ‰) and three light intensities (180, 126 and 54 $\mu e/m^2/s$) (Chang *et al.*, 1986). Conditions were chosen to simulate those occurring in the Lake Grassmere salinas over an annual cycle. This study was intended to predict maximum cell densities, to determine cropping intervals appropriate for a regulated laboratory supply, and also to gain some insight into algal growth rates achievable in the absence of *Artemia* grazing pressure should over-exploitation of *Artemia* in the ponds occur.

Laboratory studies on *Artemia* growth, mortality or survivorship, maturation rates, fecundity and generation times were carried out in 24 replicated combinations of temperature (8, 14, 17, 20, 26, and 32 °C) with salinity (80, 140, 200, and 260 ‰ salinity) (Wear and Haslett, 1986 ; Wear *et al.*, 1986). All experiments were subject to a 12 h day/night cycle and run in 5 l clear perspex containers or in 1- or 2 l covered glass jars all containing 10 or 50 *Artemia*/l. Each replicate was stocked with nauplii 12 to 24 h old (hatched in each temperature/salinity combination) and grown at an initial density of 10/l in 25 µm filtered brine from Lake Grassmere which was progressively replaced with seawater/stack-salt brine during each twice weekly feeding, observation and processing schedule. Full details of all experimental procedures are given in Wear and Haslett (1986).

A logistic growth curve $Y=A/(1+Be^{-Kt})$ was fitted to the manifestly non-linear time series data over the first 100 days where A, B and K are appropriate parameters separately determined for each temperature/salinity combination, t=time in days and Y=length in mm. Separate unbalanced analyses of variance for growth time to size at first maturity (6.0 mm) and for mortality up to that time were calculated. Polynomial prediction equations or response surfaces for growth time to 6.0 mm and for percentage pre-maturation mortality were separately completed. Note that because pre-maturation mortality and pre-maturation survival are connected by a deterministic relationship, mortality percent being 100 less survival percent, the non-polynomial ANOVA tables for mortality and survival are therefore identical, and the polynomial coefficients for survival are the negative of those for mortality with the exception of the constant term.

Relative maturation success was determined as the first day on which at least 50 % of cultured *Artemia* were mature in all 24 different temperature/salinity combinations, and a polynomial response surface was fitted from the experimental data. Fecundity was estimated from the culture vessels at each twice weekly sampling of progeny arising from spring-acclimated broodstock and was also determined by way of detailed counts of offspring from individual replicated pair cultures stocked ex summer-acclimated broodstock from Lake Grassmere. Procedures and methods are detailed in Wear *et al.* (1986). The gestation period was scored from the time of first pairing until the first brood parturition day ; the length of reproductive life was timed from the first brood parturition day to the last. The generation time was determined as the number of days between hatching of nauplii originally stocked, and the first appearance of F1 generation nauplii.

FIELD

Artemia were sampled in the field using a clear polycarbonate box with a sliding base which filtered an 18.0 l sample through 180 μm plankton mesh on uplift, and condensed it to 0.5 l (Fig. 2). Laboratory processing involved washing and centrifugal separation from algae and detritus followed by filtration and drying to constant weight at 60 °C. Full details are given in Haslett and Wear (1985).

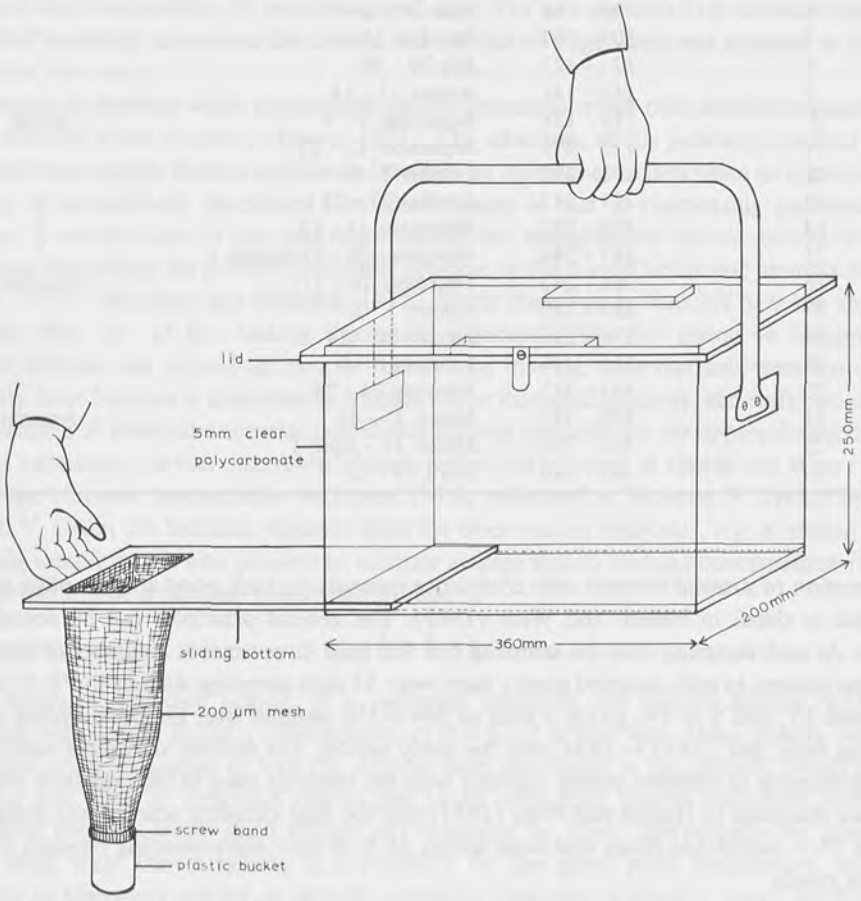

FIG. 2. Scale diagram of transparent polycarbonate box used for *Artemia* sampling (after Haslett and Wear, 1985).

Ponds P3, P7, F1 and F4 were sampled for *Artemia* on 23 occasions throughout an annual cycle (Table I) thereby including the extremes of low (P3) and high (F4) salinities along the solar salt concentration series at any given time, with each pair of sampled ponds separated by at most three unsampled ponds (Fig. 1).

TABLE I

Sample numbers and sampling times at Lake Grassmere during 1981 and 1982

Sample no.	Sample days	Dates	Season
1	0 - 2	March 30 - April 1	Autumn
2	14 - 16	April 13 - 15	"
3	35 - 37	May 4 - 6	"
4	51 - 52	May 20 - 21	"
5	70 - 71	June 8 - 9	Winter
6	86 - 88	June 24 - 26	"
7	105 - 107	July 13 - 15	"
8	121 - 122	July 29 - 30	"
9	140 - 141	August 17 - 18	"
10	156 - 158	September 2 - 4	Spring
11	175 - 176	September 21 - 22	"
12	191 - 192	October 7 - 8	"
13	211 - 212	October 27 - 28	"
14	226 - 227	November 11 - 12	"
15	245 - 246	November 30 - December 1	"
16	261 - 262	December 16 - 17	Summer
17	281 - 282	January 5 - 6	"
18	296 - 297	January 20 - 21	"
19	315 - 316	February 8 - 9	"
20	331 - 332	February 24 - 25	"
21	350 - 351	March 15 - 16	Autumn
22	366 - 367	March 31 - April 1	"
23	385 - 386	April 19 - 20	"

Estimation of *Artemia* biomass with confidence intervals for each pond and sampling time are discussed in detail in Haslett and Wear (1985). The general principles can be described as follows. At each sampling time the sampling box was used three times at a number of designated sampling stations in each sampled pond ; there were 33 such sampling stations in P3, 22 in each of P7 and F1, and 9 in F4, giving a total of 86×3=258 samples over the pond system at each sampling time, and 258×23= 5934 over the study period. The number of sample stations and their positioning in sampled ponds, together with the methods used in the necessary feasibility study are discussed in Haslett and Wear (1985) and the final sampling scheme was designed to provide 95 % confidence limits that were within 25 % of their corresponding biomass levels in sampled ponds.

The estimated biomass of *Artemia* ($\hat{B}$) in kilograms salt free dry weight in a sampled pond at a particular sampling time was estimated with the formula :

$$\hat{B} = (V / 1\,000v) \sum_{j=1}^{S} \{ (V_j / V) [1/3 \sum_{i=1}^{3} b_{ji}] \}$$

where V = the volume of the particular pond in m^3 ;
v = the volume of the sample box in m^3 (0.018 m^3) ;
V_j/V = the ratio of volume represented by station j to the total volume of the pond ;

s = the number of sampling stations in that pond ;

b_{ji} = the *Artemia* biomass in g measured by the ith replicate at the jth sampling station ;

(subscripts denoting pond and sampling time have been suppressed for clarity).

For each pond $\hat{B}$ is an appropriately weighted arithmetic sum of the biomass measurements at sample stations for that particular sampling time. Although it is not obvious from the formula for $\hat{B}$, each biomass estimate implicitly involves stratification, through the volume ratios V_j/V. There were two strata in each pond, edge and center. Sample stations were assigned to the edge stratum if they were within 50 m of the pond edge. For any position in a sampled pond, the associated sampling station was the closest, except that no edge water was assigned to a center stratum or *vice versa.*

Estimates of biomass which intrinsically involve geometric rather than arithmetic means have been proposed in the literature (Elliott, 1977). The advantage of the geometric method is that it provides appropriate skewed confidence intervals for biomass estimates when an organism, *e.g. Artemia,* is contagiously distributed ; its disadvantage is that it consistently underestimates biomass. A modification to this procedure applies the multiplicative factors derived from the confidence bounds for the geometric biomass estimate, to the correct arithmetic biomass estimate (Elliott, 1977 ; Marchant and Williams, 1977). While these two procedures have the appeal of simplicity they are *ad hoc,* lacking theoretical foundation, the first giving an inappropriate biomass estimate, the second an incorrect confidence interval. Such methods were not used in this study since biomass is undoubtedly additive rather than multiplicative, and sufficient samples were available to determine accurate confidence interval estimates for the appropriate arithmetic biomass estimators. Further discussion of these points can be found in Haslett and Wear (1985).

Average biomass concentration estimates ($\hat{b}$) is measured as biomass $\hat{B}$ divided by pond volume, V. Given the biomass obtained from the three station replicates, *e.g.* at station j for a particular sampling, it is also possible to estimate average station biomass concentration ($\hat{b}_j$) and hence to determine a biomass concentration ratio :

$$\hat{r}_j = \hat{b}_j / \hat{b}$$

which measures, for each station, at a particular sampling, the relative density of *Artemia* compared with overall biomass concentration in that pond at that time. These relative densities, which give some indication of how the *Artemia* were distributed in the sampled ponds at each sampling time, have an important role to play in the confidence interval estimation method.

Biomass over the entire pond system was estimated by linear interpolation for unsampled ponds using their known salinity as a predictor for any given pond temperature, and with reference to laboratory studies on growth, mortality, fecundity, generation times and longevity (Wear and Haslett, 1986 ; Wear *et al.*, 1986).

A programme designed to monitor population structure in sampled ponds was initiated on October 29, 1981. At weekly intervals (until April 20, 1982) one 18 l sample was taken from the center station and *Artemia* were categorized as mature adults, juveniles (sexually immature but with functional natatory thoracopods), and nauplii (including metanauplii), and then counted. When total numbers greatly exceeded 100, the sample was divided using a Folsom plankton splitter. Numbers in each cohort were expressed as a percentage with average sample size of 186 (minimum 100).

Nutrients NO_3/NO_2-nitrogen, NH_3-nitrogen, and reactive dissolved phosphate were measured in sampled ponds (analyses following Smith *et al.*, 1982 except that samples for NH_3 analysis were diluted five times to avoid Mg and Ca precipitation, Chang *et al.*, 1986). Algal samples were taken from all sampled ponds on each bimonthly sampling occasion from mid-winter (sample no. 8) to the end of the programme, and counted using standard haemocytometer techniques.

Meterological data and hydrological conditions monitored daily or on each sampling occasion were temperature, salinity, rainfall, sunshine hours, wind direction and strength, evaporation rates, pond volumes and brine flows. Although not presented in detail here, relevant data were considered by Haslett and Wear (1985) and full data records are held by Dominion Salt Ltd. Ecological observations, including predation levels by the red-billed gull (*Larus novaehollandiae*) were also made.

Results

Laboratory results

Dunaliella salina achieved a maximum growth rate of 1.50 doublings/day at 26 °C, 190 ‰ salinity and at a light intensity of 126 $\mu e/m^2/s$. *D. euchlora* showed a maximum growth rate of 1.16 doublings/day at 20 °C, 120 ‰ salinity, in the highest light intensity of 180 $\mu e/m^2/s$. Predicted maximum values and associated 95 % confidence intervals of 1.41 ± 0.38 respectively 1.14 ± 0.25 doublings/day were calculated from all 45 replicated treatment combinations. The range of experimental variables clearly spanned optimum conditions for exponential growth of *D. salina*, but did not span the optimum salinity for *D. euchlora* which may have achieved equivalent or faster experimental growth below 120 ‰ in similar conditions of light and temperature. Optimal conditions for growth in both species were not sharply defined, but occurred over a considerable range of temperature, salinity and light conditions. ANOVA results for *D. euchlora* showed the three-factor interaction (temperature × salinity × light), all two-factor interactions, and all main effects to be significant at the 5 % level or less ; for *D. salina* the three-factor interaction, both the two-factor interactions involving temperature, and the two main effects temperature and salinity were significant at the 5 % level or less. Of the total sums of squares for both the ANOVAs, 70 % or more of the total are accounted for by the sums of squares for temperature and salinity alone. Of the three variables tested, temperature is related to the greatest variation in exponential growth followed respectively by salinity and light intensity. Detailed results and analyses are given in Chang *et al.* (1986).

Using the above results as an indicator, *D. euchlora* and *D. salina* were cultured in the laboratory as a bi-algal mixture in a number of 20 l vessels each containing enriched and filtered seawater brine, and cropped serially at about 7 day intervals as food for the *Artemia* cultures. Cell counts up to 2×10^6/ml were achieved in salinities up to 200 ‰ and to 6×10^5/ml in 260 ‰. Ratios of *D. euchlora* to *D. salina* were about 100:1 in 140 ‰, 10:1 in 200 ‰, and 8:1 in 260 ‰.

Results of laboratory work on *Artemia* showed that growth was fastest at 20 to 28 °C in salinities between 100 and 170 ‰. Over this range growth time to 6.0 mm (approximate length at first maturity) was 10 days or less. Females grew to a mean length of 12.0 mm between 17 and 26 °C in salinities of 140 to 200 ‰. Males measured about 11.0 mm. In 260 ‰ *Artemia* grew to no more than 8.0 mm in any temperature with males reaching 7.5 mm. No growth was recorded at 8 °C/260 ‰ or in 32 °C/260 ‰ in which all nauplii died within 24 h. Logistic

growth curves are given in Wear and Haslett (1986). Results of separate unbalanced ANOVA calculations for growth time to 6.0 mm showed the effects of temperature and salinity together to account for 83.1 % of the total sums of squares, with temperature being the major determinant.

More than 90 % of the nauplii survived to maturity between 20 and 28 °C in combination with salinities 100 to 170 ‰ (Fig. 3). Results of ANOVA calculations for pre-maturation mortality are given in Wear and Haslett (1986). The time lapse to 50 % survival generally exceeded 100 days in all salinities at temperatures 17 °C and below, and was greatest in 8 °C/140 ‰ (180 days). At 26 °C this value varied between 70 and 90 days, at 32 °C 56 days or less. Individual *Artemia* survived for up to 5 months at 17 and 26 °C in 140 to 260 ‰ and up to 6 months in 8 °C/140 ‰. Maximum longevity at 32 °C was between 2 and 3 months other than in 260 ‰ where all nauplii and adults stocked died within 2 days. Male *Artemia* generally lived longer than females. Full details and analyses of results are given in Wear and Haslett (1986).

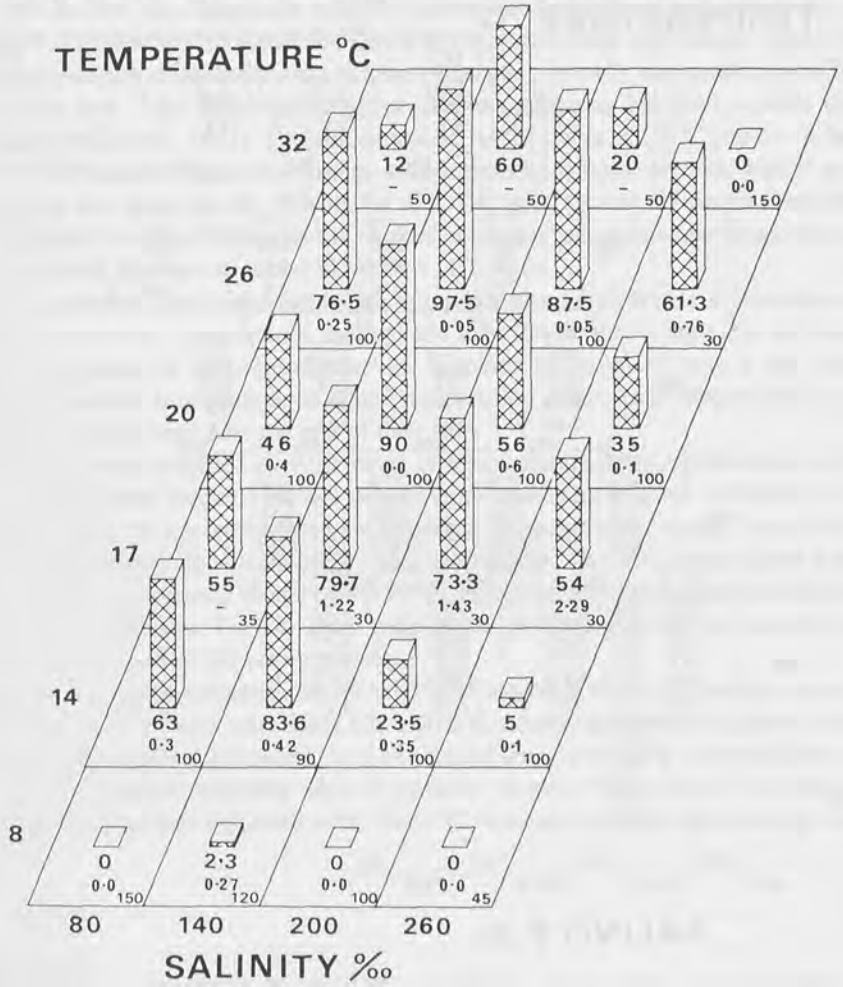

FIG. 3. Average survivorship to maturity determined in 24 combinations of temperature and salinity (each block value is supported beneath by the estimated standard error, and at right the number of individuals).

Experimentally achieved times to 50 % maturity are given in Fig. 4 as a block chart supported beneath by the estimated standard error, and the number of replicate females. A polynomial response surface derived from these data is given in Wear *et al.* (1986). Results for the non-polynomial ANOVA showed temperature to have the major effect, with the model explaining 99.9 % of the variance.

Data for fecundity counts from individual replicated pair cultures (INDFEC) and for fecundity estimated from mass culture vessels (AGGFEC) are fully analysed and figured in Wear *et al.* (1986). In approximate terms, each female at 26 °C produced about 1 000 offspring in the salinity range 80 to 200 ‰, but only about 300 in 260 ‰ ; at 20 and 17 °C between 400 and 1 000 were produced in all salinities except 20 °C/260 ‰ which yielded only 200 offspring ; at 14 °C between 300 and 400 were produced in 80 to 200 ‰, but <100 in 260 ‰. At the two

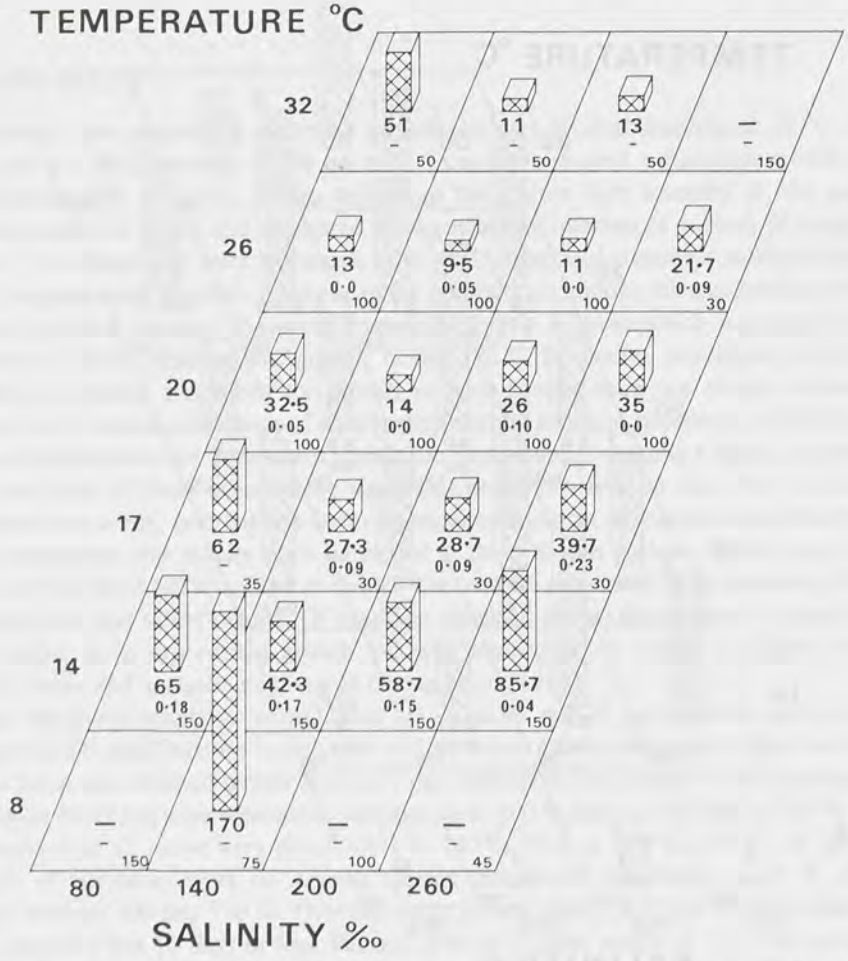

FIG. 4. Time in days to 50 % maturity determined in 24 combinations of temperature and salinity (each block value is supported beneath by the estimated standard error, and at right the number of replicate females).

temperature extremes, reproduction did not occur at 8 °C, and at 32 °C fecundity was lowest (about 200 offspring in 140 ‰, between 20 and 150 in 80 ‰, and 0 in 260 ‰). ANOVA results for INDFEC showed that temperature and salinity separately have significant effects.

The favoured mode of reproduction was to produce nauplii either by ovoviviparity or from oviparously produced, non-resistant cysts which hatched within 2 or 3 days. Detailed counts of offspring from 69 pairs cultures (n offspring = 31 299) included only 2 144 cysts (<7 %) with no consistent pattern of cyst production. Individual females sometimes alternated their reproductive mode between nauplii and cysts, and seven composite batches containing both nauplii and cysts were recorded (n batches = 428). During AGGFEC observations nauplii and cysts occurred together on four occasions, indicating that conditions favouring ovoviviparous reproduction in some females induced cyst production in others.

Batches of progeny were largest in 26 °C/140 ‰ ($\bar{x}$ = 125 offspring) and smallest in 32 °C/260 ‰ ($\bar{x} < 45$). Results for ANOVA showed the factors temperature, salinity, and their interaction to have separately significant effects on batch size (Wear and Haslett, 1986). For most temperature/salinity combinations the number of nauplii per batch was smaller at the beginning and near the end of the female reproductive lifespan, and larger but more variable during the mid-stages (Alexander, 1982). Females produced most batches at 26 °C (up to 26 batches in 26 °C/80 ‰ over 104 days) decreasing with increasing salinity or with higher and lower temperatures at a given salinity. Results for ANOVA again showed temperature and salinity to have significant individual effects on the number of batches, but in this case temperature was the more important. Data are tabulated in Wear *et al.* (1986).

Data for gestation time and mean length of reproductive life in female *Artemia* are presented in Wear *et al.* (1986). Temperature, salinity, and their interaction all appear to have significant effects. The period of post-reproductive life amounts to no more than a few days in all temperature/salinity combinations in which reproduction occurs, and females tend to continue producing offspring until near the end of their lives.

Generation times recorded over the range of temperature/salinity combinations are given in Fig. 5. A polynomial response surface derived from these data is given in Wear *et al.* (1986). Significant effects on generation time are attributed to temperature, salinity, and their interactions, but temperature alone is the most important variable. The error mean square is extremely small in the non-polynomial model (R^2=0.9992), as generation times varied by little more than 1 day between replicates. For any given temperature, generation times were usually longest in 260 ‰ ; at 8 °C *Artemia* did not reproduce.

Experimental results indicated that for the Lake Grassmere strain of *Artemia*, temperatures of 20 to 28 °C combined with salinities of 100 to 170 ‰ are most favourable for growth and survival to maturity, but longevity is extended in lower temperatures, especially in intermediate salinities. Reproductive variables, including time to maturity, length of reproductive life, fecundity and generation times are also enhanced at 20 to 28 °C, but over a slightly higher salinity range, 120 to 200 ‰.

Field results

Nutrient levels

Nutrient concentrations (Table II) were variable but very rarely could they not be detected. The nutrients NO_3-N and NH_3-N were always present (except for NO_3-N when on two occasions

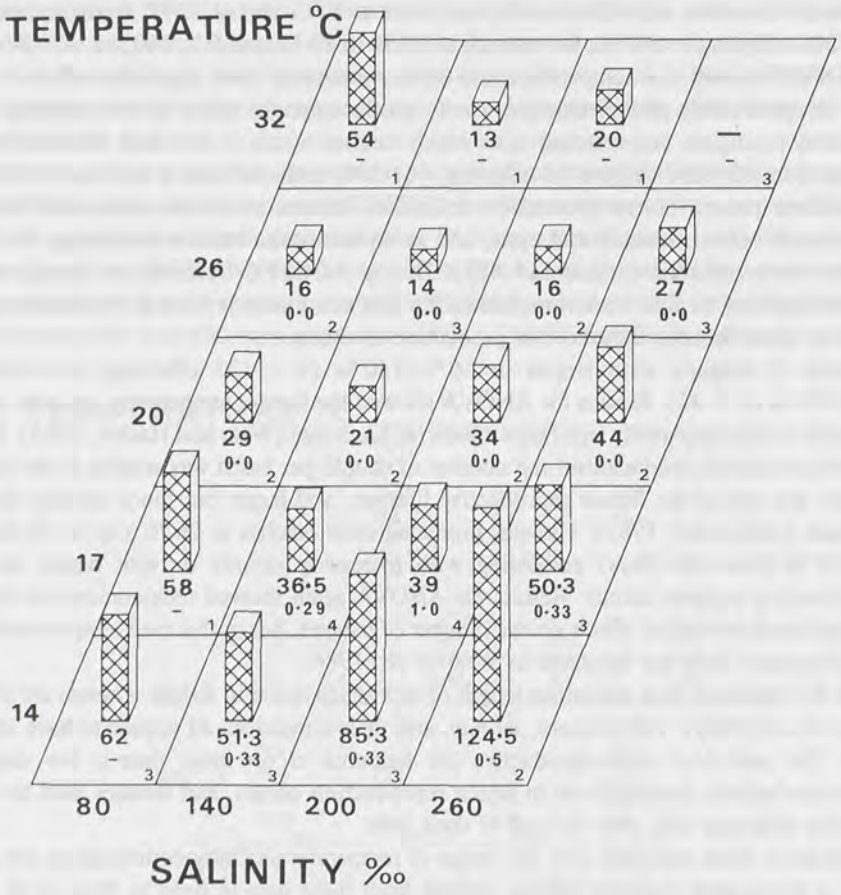

FIG. 5. Generation time in days determined in 20 combinations of temperature and salinity (each block value is supported beneath by the estimated standard error, and at right the number of replicates, most of which contained five or more mature females).

pond F4 was almost completely drained), with mean values lying within the usual range for seawater (Parsons and Tagahashi, 1975). Mean values for reactive dissolved phosphate were relatively high (N:P ratio = ~2.5:1) reflecting runoff from the agricultural catchment which is aerially dressed with phosphate fertilizers (Haslett and Wear, 1985). Nutrients were not therefore considered limiting for algal growth. Values for pH were also within the usual seawater range, with slightly lower values recorded in the higher salinity ponds F1 and F4. For each sampled pond, pH was negatively correlated with *Artemia* concentration, $r = -0.62$ to -0.85 ($P<0.01$). Correlations between nutrient levels and algal concentrations, or with any other recorded variable estimated for each pond were not consistently significant due to complex interactions within the ponds, and probable time lags.

TABLE II

Analyses of pH and nutrient levels (recorded in g/m^3) for sampled ponds P3, P7, F1 and F4

		P3	P7	F1	F4
pH	Min.	7.50	7.50	7.10	7.60
	Max.	9.00	8.70	8.50	8.50
	Mean	8.26	8.06	7.96	7.99
	S.D.*	0.39	0.38	0.39	0.35
NO_3-N	Min.	0.003	0.001	0.001	0.000
	Max.	0.150	1.110	0.076	0.046
	Mean	0.025	0.015	0.017	0.014
	S.D.	0.032	0.016	0.016	0.013
NH_3-N	Min.	0.030	0.045	0.040	0.035
	Max.	1.600	1.107	0.300	0.600
	Mean	0.307	0.167	0.119	0.136
	S.D.	0.396	0.228	0.072	0.118
Reactive dissolved phosphate	Min.	0.009	0.002	0.003	0.005
	Max.	0.083	0.290	0.310	0.211
	Mean	0.038	0.035	0.047	0.045
	S.D.	0.022	0.060	0.065	0.047

* Standard deviation.

Algal and Artemia *concentrations*

Results of algal counts from all ponds starting from sample no. 8 (mid-winter) are graphed in Fig. 6a. Total cell numbers/ml were generally low in winter, rose in early spring, but declined in late spring. A second peak occurred by mid-summer, and in all ponds algal concentration fell to low levels by late autumn. This general trend was evident in all ponds, but differed in amplitude and precise timing between ponds. Algal concentrations in P3 ($\bar{x}$=430 000, S.D. 369 000 cells/ml) and in P7 ($\bar{x}$=173 000, S.D. 156 000 cells/ml) were generally higher than in F1 or F4 ($\bar{x}$=86 000, S.D. 52 000 ; 99 000, S.D. 73 000 cells/ml), respectively.

Results for *Artemia* concentration for all 23 sampling occasions are illustrated in Fig. 6b. For pond F4 the data were smoothed across samples 11, 19, 21, 23 during which the pond was nearly empty. The annual cycle was clearly divided into two phases : a time of low biomass concentration during winter and early spring, and a period of rapid increase to peak levels in February and early March, followed by a sharp decline through autumn. *Artemia* concentrations were lowest in P3 (averaging 1.37 g/m^3 dry weight) and increased with salinity (P7, F1 and F4 averaging 3.19, 4.11, and 5.01 g/m^3 respectively). More complete results, showing biomass concentrations with confidence intervals for each sampled pond are published in Wear and Haslett (1985).

Field growth and development of Artemia

The population structure of *Artemia* samples from all sampled ponds taken over the period of highest biomass concentration is given in Fig. 7. Field data suggest that ponds P3 and P7 serve

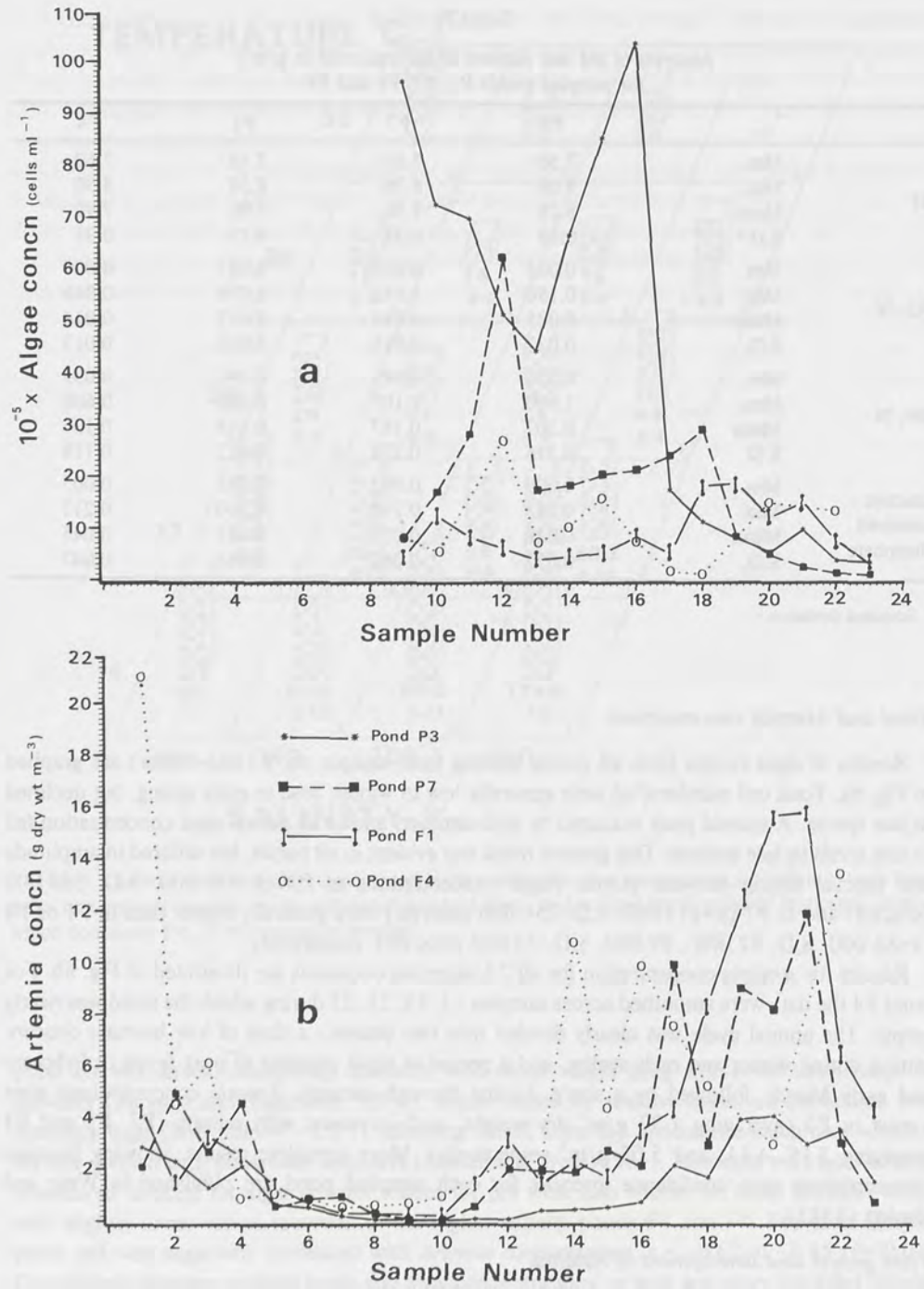

FIG. 6. a. Concentrations of *Dunaliella euchlora* and *D. salina* (in aggregate cell numbers/ml) recorded from sampled ponds. b. Average *Artemia* concentration (in g/m^3 salt free dry weight) for sampled ponds.

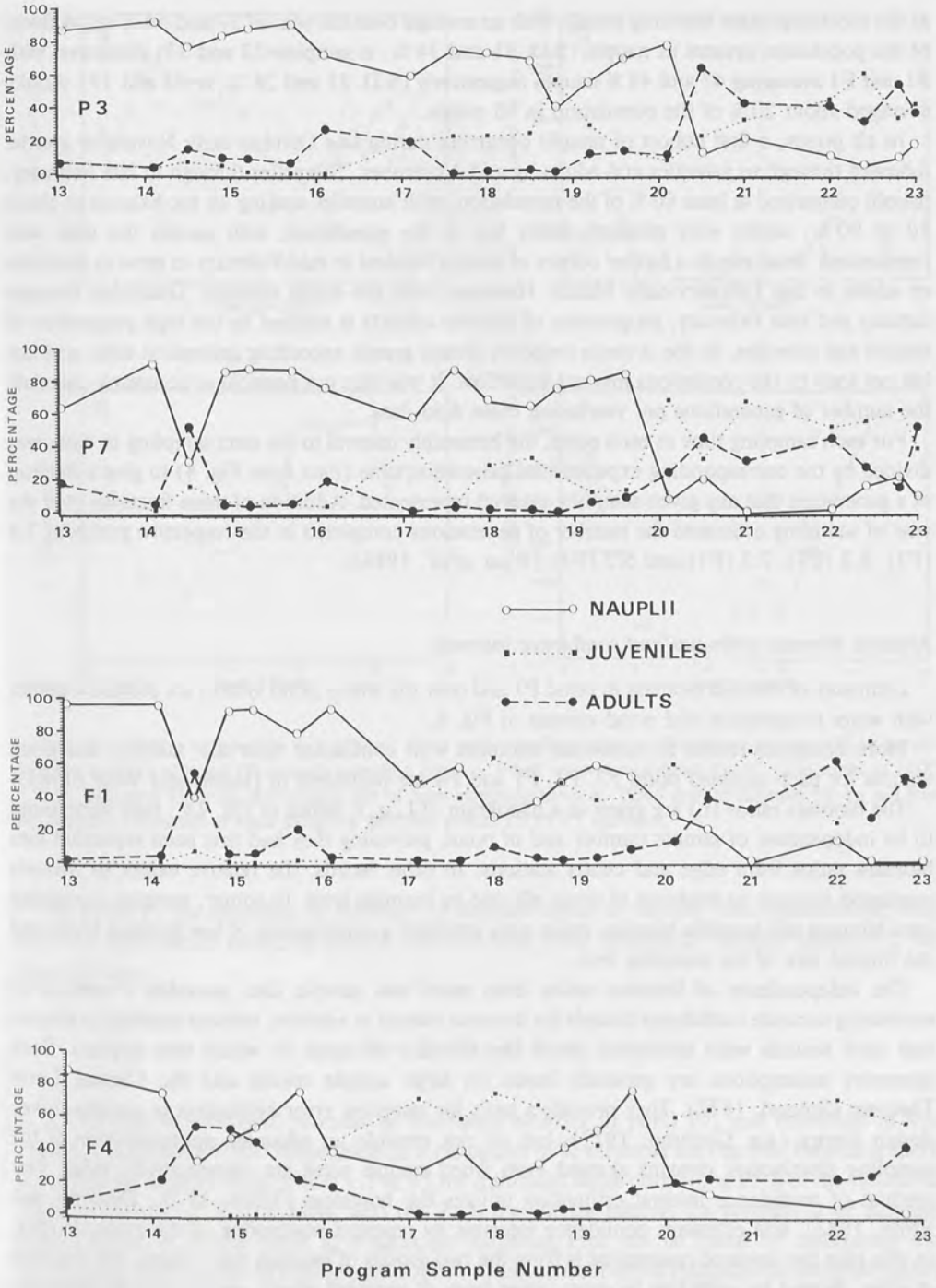

FIG. 7. *Artemia* population structure of sampled ponds at weekly intervals (nauplii, juveniles and adults are recorded as a percentage of the whole sample).

as the most important breeding ponds, with an average over the year of 57 and 54 % respectively of the population present as nauplii (S.D. 31 and 34 % ; n samples=23 and 23) compared with F1 and F4 averaging 47 and 40 % nauplii respectively (S.D. 35 and 26 % ; n=23 and 19). Adults averaged about 20 % of the population in all ponds.

In all ponds, a first cohort of nauplii occurring during late October-early November can be followed through to juveniles and adults by mid-November. Thereafter through to late February, nauplii comprised at least 60 % of the population, with juveniles making up the balance to about 80 or 90 % ; adults were proportionately few in the population, with nauplii the least well represented. In all ponds a further cohort of nauplii hatched in mid-February to grow to juveniles or adults in late February-early March. However, over the entire summer, December through January and into February, progression of discrete cohorts is masked by the high proportion of nauplii and juveniles, by the *Artemia* longevity always greatly exceeding generation time, and last but not least by the continuous forward waterflow. It was thus not possible to accurately calculate the number of generations per year using these field data.

For each sampling time in each pond, the bimonthly interval to the next sampling in days, was divided by the corresponding experimental generation time (data from Fig. 4) to give a fraction of a generation that any given sampling interval represented. Addition of these fractions over the year of sampling estimated the number of generations completed in the respective ponds as 7.4 (P3), 8.3 (P7), 7.3 (F1) and 5.2 (F4) (Wear *et al.*, 1986).

Artemia *biomass estimates and confidence intervals*

Estimates of *Artemia* biomass in pond P7 and over the whole pond system are plotted together with water temperature and pond volume in Fig. 8.

More complete results for estimated biomass with confidence intervals, salinity, and pond volume for each sampled pond P3, P7, F1 and F4 are published in Haslett and Wear (1985).

The biomass ratios ($\hat{r}_j$) are given as a histogram of $\text{Log}_e\, \hat{r}_j$ values in Fig. 9A ; they were found to be independent of sample number and of pond, providing they had first been separated into biomass ratios from edge and center stations. In other words, the relative extent of *Artemia* contagion showed no evidence of being affected by biomass level. In winter, samples containing zero biomass and unstable biomass ratios were probably a consequence of low biomass levels and the limited size of the sampling box.

The independence of biomass ratios from pond and sample date provided a method of estimating accurate confidence bounds for biomass treated as additive, without needing to assume that such bounds were symmetric about the biomass estimates to which they applied. Such symmetry assumptions are generally based on large sample results and the Central Limit Theorem (Spiegel, 1972). They provide a basis for sampling error estimation in sample survey design theory (*e.g.* Cochran, 1977), but do not provide an adequate approximation if the sampling distribution remains skewed even when sample sizes are comparatively large. Our method of confidence interval estimation utilizes the bootstrap (Efron, 1979 ; Diaconis and Efron, 1983), and estimates confidence intervals by repeated resampling of the available data. In this case the repeated resampling is from the two groups of biomass ratio values, one for each stratum, formed by including biomass ratios from all sampled ponds and non-winter sampling times in the appropriate group ; each group contained more than 2 500 biomass ratio values.

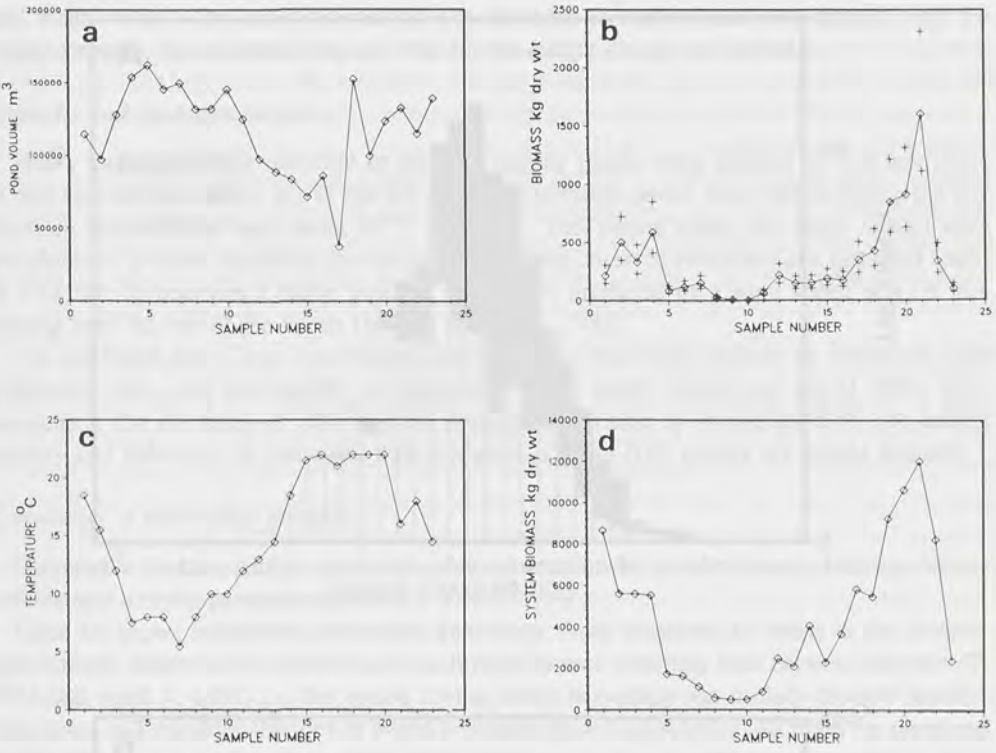

FIG. 8. a. Water volume (in m^3) of pond P7 at each sampling time. b. Estimated biomass ($\hat{B}$, in kg salt free dry weight) of pond P7 at each sampling time. c. Pond water temperature (in °C) measured at 1100 h N.Z. Standard Time on the second day of each sampling time. d. Pond system biomass (in kg salt free dry weight) estimated at each sampling time for ponds P3 to F5 inclusive.

For pond P7 for example, by drawing 22 biomass ratios at random with replacement, each from the appropriate sampling stratum, and denoting these resampled biomass ratios by $\hat{r}_j$, we may estimate :

$$\hat{R} = \sum_{j=1}^{22} (V_j / V) \hat{r}_j$$

$\hat{R}$ is the ratio of simulated biomass to estimated biomass in pond P7, and repetition of this sampling procedure 3 000 times leads to a histogram of $\hat{R}$ values as for Fig. 9B. Providing there is no bias the average value of $\hat{R}$ is 1. For P7 the minimum range containing 95 % of the simulated values is $0.6919 < \hat{R} < 1.4404$, and the 95 % confidence interval for biomass $\hat{B}$ or biomass concentration $\hat{b}$, in this pond at a particular sampling time can be found by multiplying the appropriate $\hat{B}$ or $\hat{b}$ value by 0.6919 and 1.4404. Confidence interval estimates for sampled ponds found by this method were all asymmetric. Further general discussion of the appropriateness of, and estimates for, the various confidence interval estimation procedures can be found in Haslett

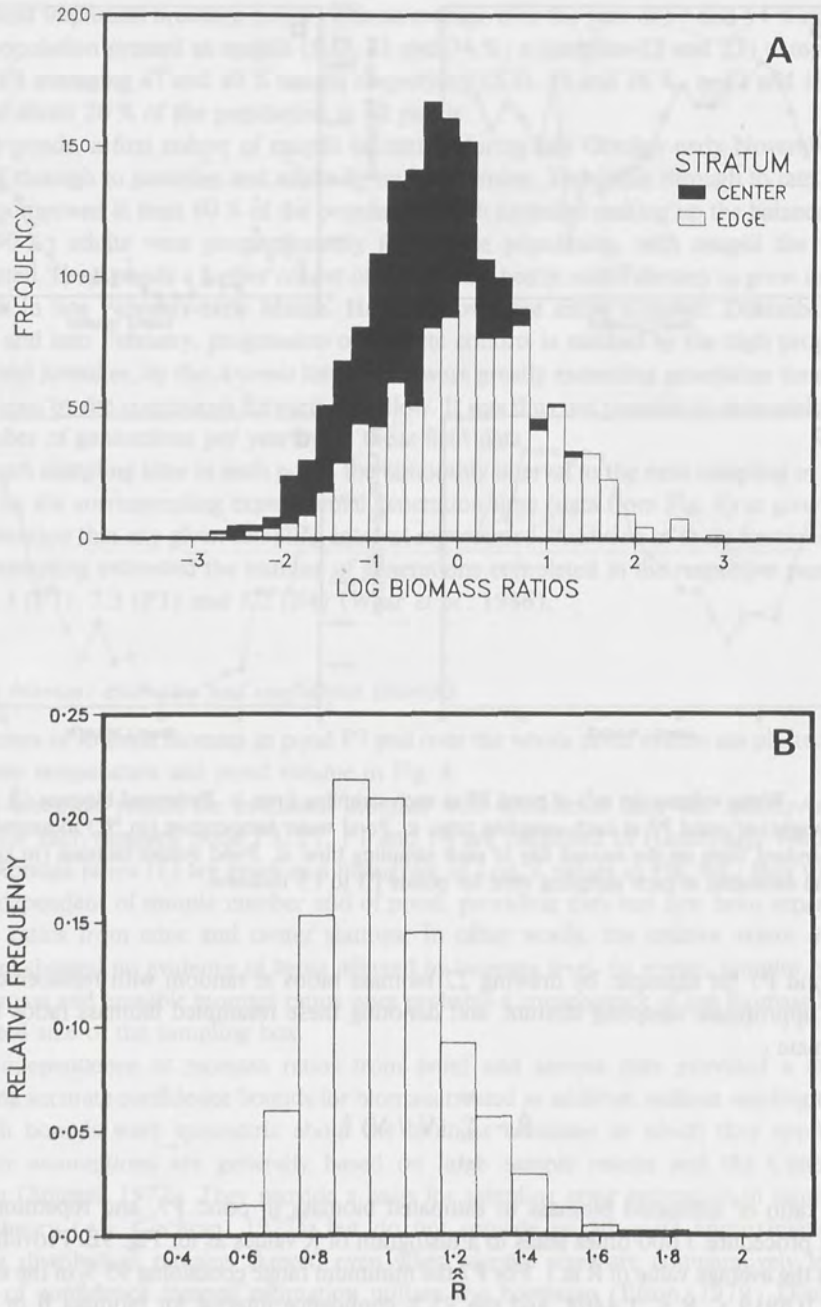

FIG. 9. A. Histogram for log biomass ratios amalgamated over both time and pond. B. Histogram for values of $\hat{R}$ (the simulated proportion of biomass, or biomass concentration) for pond P7 at any sampling time (achieved via the bootstrap method and used for confidence interval determination).

and Wear (1985). The bootstrap method was also used to approximate error bounds even for winter samples, since biomass was too low for harvesting during this period.

Mortality and predation factors

Heavy mortalities were observed in the high salinity ponds when drained to very low levels in mid-summer, and when any of the five final concentration ponds were refilled during the day when air temperatures were about 28 °C or higher. Very strong winds and heavy rainfall were not observed to cause significant mortality. *Artemia* were observed swimming at a sustained speed of 0.13 m/s to maintain a stable position in currents produced by a wind speed of 12.9 m/s passing over the top of the bunds (Haslett and Wear, 1985).

The red-billed gull (*Larus novaehollandiae*) is the only important predator on *Artemia* at Lake Grassmere, with each bird capable of ingesting 50 g live weight *Artemia* per day (J. Mills, pers. commun.). On the basis of 200 resident nesting birds rising by immigration to 700 during January and February, we estimated that predation is about 0.05 tonnes dry weight annually.

Calculation of harvestable biomass

Harvestable biomass can be estimated using information on pond volumes, *Artemia* concentrations and *Artemia* generation times.

Table III shows laboratory determined generation times measured in weeks at the temperature/salinity combinations prevailing in each pond at each sampling time between October 27, 1981 and April 1, 1982, *i.e.* the period during which harvesting was initially thought feasible. Also noted in Table III is the time in weeks between consecutive samples. All times are measured to the nearest 0.5 weeks. Generation times were not estimated with greater precision since laboratory estimates were based on fixed water temperature. Pond water temperature fluctuates on a diurnal cycle especially during spring and autumn, *i.e.* the maximum diurnal variation measured between April 1981 and April 1982 was ±4.5 °C.

TABLE III

Experimental generation times in harvestable ponds (P6-F5) during harvestable months (November to March) (calculated to the nearest 1 week)

Sample no.:	13	14	15	16	17	18	19	20	21	22
Time interval (weeks):	2	3	2.5	3	2	2.5	2.5	3	2.5	
Pond										
P6	5	4	3	3	2	2	2	2	5	6
P7	5	4	3	3	2	2	2	2	5	6
P8	7	5	4	3	3	2	2	2	5	6
P9	7	5	4	3	3	2	2	2	5	6
P10	7	5	4	3	3	2	2	2	5	6
F1	7	5	4	4	3	2.5	2.5	2.5	5	5
F2	7	6	5	4	4	4	4	4	4	5
F3	7	6	5	4	4	4	4	4	4	5
F4	7	6	5	4	4	4	4	4	4	5
F5	7	6	5	4	4	4	4	4	4	5

Given the known time between samples together with these estimated generation times, it was possible to calculate the number of generations of *Artemia* produced in each pond between consecutive sampling times by dividing the interval between samples by the appropriate generation time. This fraction exceeded one on those occasions where generation time was less than the time between samples.

Given known pond volumes in all ponds, together with biomass concentration estimates for sampled ponds (P3, P7, F1, F4) and the interpolation procedure discussed previously for estimating *Artemia* concentration in unsampled ponds (P4-P6, P8-P10, F2, F3, F5), biomass was estimated for all ponds at each sampling time. Ponds of interest for harvesting were restricted to P6 and F5 due to siting of existing pumphouse facilities. Multiplication of these standing biomass figures by the corresponding sampling interval/generation time fraction yielded an estimate of potentially harvestable biomass for each pond between consecutive sampling times, without including standing biomass in harvestable biomass estimates. These subtotals are detailed in Table IV, and were added to give a total estimated harvestable biomass for ponds P6 to F5 between October 27, 1981 and March 31, 1982 of 35.1 tonnes salt free dry weight. Such a figure assumes that ponds are able to be filtered sufficiently quickly as to harvest all the available *Artemia*, but is otherwise a conservative figure because standing biomass estimates in unsampled ponds are themselves conservative estimates ; furthermore multiple generations, consequent compounding of generations and consequent high fecundity are not considered.

TABLE IV

Harvestable biomass in ponds P6 to F5 on a fully replacable basis over the period November 1981 - March 1982 inclusive (in tonnes salt free dry weight)

Sample no. :	13	14	15	16	17	18	19	20	21	Totals/ single ponds/ total time
Time interval :	2	3	2.5	3	2	2.5	2.5	3	2.5	
Pond										
P6	0.07	0.08	0.13	0.12	0.33	0.30	0.61	0.98	0.32	2.94
P7	0.06	0.10	0.14	0.17	0.35	0.55	0.96	1.30	0.73	4.36
P8	0.05	0.11	0.12	0.28	0.35	0.43	1.03	0.98	0.49	3.84
P9	0.05	0.11	0.13	0.20	0.32	0.51	1.06	1.81	0.43	4.62
P10	0.04	0.11	0.10	0.15	0.19	0.46	0.71	1.58	0.39	3.73
F1	0.03	0.17	0.10	0.23	0.23	1.14	0.73	1.52	0.44	4.59
F2	0.02	0.13	0.06	0.19	0.13	0.10	0.32	0.37	0.20	1.52
F3	0.03	0.22	0.08	0.24	0	0.13	0.33	0.40	0.27	1.70
F4	0.06	0.48	0.18	0.57	0.45	0.36	0	1.26	0	3.36
F5	0.10	0.64	0.10	0.64	0.67	0.53	0.96	0	0.81	4.45
Totals/all ponds/time intervals	0.51	2.15	1.14	2.79	3.02	4.51	6.71	10.20	4.08	35.11

During a trial harvesting programme, undertaken by the DSIR – Industrial Processing Division, an *Artemia* recovery process was developed and run through the 1983-84 season (Janata *et al.*, 1987). The average *Artemia* concentration in brine filtered during pump transfer

between P6 and P7 was 4.2 g/m^3 dry weight for the period November 28, 1983 to May 17, 1984. While biomass is probably subject to variation between annual cycles, these values are very similar to our own for estimated average biomass concentration over the period of sampling, November 11, 1981 to April 20, 1982, on the same part of the pond system, *i.e.* 4.87 g/m^3 with 95 % confidence bounds estimated via the bootstrap method of 4.14 to 5.73 g/m^3. The higher estimates for our average biomass concentration are in part due to the inclusion in the DSIR values of an additional one-month autumn period of low biomass concentration. Estimated harvestable biomass in the sampled pond P7 for the period October 27, 1981 to March 31, 1982 was 4.4 tonnes salt free dry weight.

Discussion

The major objective of this study was to estimate *Artemia* standing biomass in the Lake Grassmere solar salt pond system, and to determine how much of this was harvestable on a sustainable basis. The complex biological and statistical considerations involved, not the least being the high degree of contagion in *Artemia* distribution, demanded a well organised interdisciplinary approach and careful analysis of a large number of interconnected data sets. As this is the first such study on the ecology of *Artemia*, no procedural framework was established for comparison. As a result, several of our areas of investigation may be rather more detailed than would be necessary if the project were to be repeated elsewhere. We have nevertheless achieved progress on the general question by evolving a method for conservatively estimating harvestable *Artemia* biomass without making restrictive mathematical assumptions or placing the overriding consideration of high quality salt production at risk. We have thus provided an incentive for the development of technology to harvest *Artemia* biomass as a marketable product (Janata *et al.*, 1987). With site-specific modifications in respect of locality, pond size, water flows, pond productivity, and *Artemia* strain characteristics, this technology package would be transferable.

If large scale harvesting of *Artemia* biomass is to be contemplated in commercial salt pond systems, it must always be remembered that productive salinas maintain their viability only if the biological system remains in healthy balance in which *Artemia* plays a major role (Davis, 1980 ; Sorgeloos, 1983). Harvesting *Artemia* populations to a level approaching that of their estimated total biomass, during periods when fecundity is low or generation times long, may well endanger their capacity to reproduce rapidly enough to graze phytoflagellate blooms in the ponds of intermediate salinity, to the detriment of good quality salt production. This is particularly likely to occur when harvesting from any pond occurs at intervals appreciably less than the generation time relating to the particular salinity/temperature combination existing during the interval between successive harvests. In some salinas, such significant reduction of *Artemia* standing crop may lead to a *Coccochloris* dominated biotope and the production of slimes in the crystallizing ponds (Davis, 1980).

No cognisance need be taken of brine flows up the salinity gradient at Lake Grassmere when estimating harvestable biomass, since the flows themselves provide the practical mechanism by which a particular pond can be filtered for *Artemia*. For harvestable biomass over the pond system most of the flows are internal, being flows between adjacent ponds within the P and F series. Those external flows which do occur (*e.g.* into P3 and out of the F series during summer) require approximately 5 months to empty the pond system. Given the length of the summer salt production period is approximately 4 months, it is clear that one year's brines substantially

provide next year's salt harvest, and that flows between ponds for Lake Grassmere are not sufficiently rapid to be of major consequence when assessing potentially harvestable biomass, especially since water sent out of the pond system to the crystalizing ponds would also be filtered for *Artemia*. *Artemia* biomass estimates at a particular time, either over the pond system or in a particular pond, are obviously not affected by flows between ponds.

Artemia biomass estimates given in earlier work (see Persoone and Sorgeloos, 1980) should not be considered as true production as they have no temporal basis, and take no account of *Artemia* fecundity, generation times, growth and mortality, *i.e.* renewable biomass and hence total allowable catch in the sense of fisheries management. Neither are such estimates truly quantitative for an entire biotope, as they are based on numerically insufficient samples taken over just a few months, often in patches of *Artemia*. Also, few such existing estimates are comparable, as different authors have used different standards to express their results. These vary between numbers of adults and/or nauplii/l, wet weight or dry weight/l or /m^3, and kg wet weight/ha harvested or estimated, with water depth or pond volume unspecified (Persoone and Sorgeloos, 1980). It is also clear that comparable units vary considerably from one site to another, and even within the same biotope for different samplings. This latter observation serves to illustrate the high degree of contagion in *Artemia* distribution patterns.

The unit of expression preferred by us is *Artemia* concentration in salt-free g dry weight/m^3. Since most production salinas keep reasonably accurate records of their pond volumes, this unit of concentration can be readily and meaningfully extrapolated on a total volume basis, providing sufficient samples are taken. Without information on *Artemia* developmental stages and associated salt free dry weight per individual, standards based on *Artemia* numbers per unit volume are of little value. Wet weight figures are also disadvantaged in that they have a variable numerical equivalent depending on salinity, size of individuals, and the extent to which the samples are dried out before weighing. We would therefore enter a plea for all future work estimating *Artemia* biomass to be properly standardized and quantified with an adequate and statistically acceptable data base.

The methods used here for estimating *Artemia* biomass extend procedures established in the literature (*e.g.* Elliott, 1977) and have wider application than in *Artemia* ecology alone. The methods used in this study, and more particularly the bootstrap method of confidence interval estimation, may well provide a general approach to some of the more difficult distributional problems in ecology (Haslett and Wear, 1985). The advantage of this biomass estimation procedure is that it treats biomass as additive, which it undoubtedly is, and that it calculates, via the bootstrap, appropriate skewed confidence intervals for such estimates.

Laboratory studies indicate that the Lake Grassmere strain of *Artemia* is best adapted (in terms of reproductive activity) to the temperature range 20 to 28 °C combined with salinities 120 to 200 ‰, with growth and survivorship favoured over a slightly lower salinity range, 100 to 170 ‰. There is little change in response over these temperature/salinity ranges (Wear and Haslett, 1986 ; Wear *et al.*, 1986). These results compare well with those for >90 % survivorship over 9 days in three *Artemia franciscana* strains in which the response surface is relatively flat over a similar temperature range up to 120 ‰, and probably beyond (Vanhaecke *et al.*, 1984). However, survivorship in the Lake Grassmere strain is reduced in salinities below 100 ‰ (Wear and Haslett, 1986). Reproductive variables maturation, fecundity, and generation times follow the same general pattern (Wear *et al.*, 1986).

Laboratory studies also confirm field observations that the majority of offspring are produced ovoviviparously or by way of rapidly-hatching, non-resistant cysts. It is considered a selective advantage to reproduce by ovoviviparity to maximize reproductive potential in the essentially stabilized environment of the solar salt pond system in Lake Grassmere where sufficient algal food exists year round (Haslett and Wear, 1985). Any delay in population growth as created by encystment would be a disadvantage in such circumstances (Browne, 1980). Highest fecundity occurs over the temperature/salinity range in which pre-maturation mortality is least (Wear and Haslett, 1986) indicating that the more offspring produced, the greater the chance of any given nauplius surviving to maturity. The reverse trend occurs for oviparous cyst production indexed over five strains (Browne, 1980). Our estimate of up to eight generations per year based on laboratory results compares well with similar estimates by Carpelan (1957) for the San Francisco Bay strain.

Production and population dynamics of the Australian endemic brine shrimp *Parartemia zietziana* have been elucidated using conventional ecological methods (Geddes, 1976 ; Marchant and Williams, 1977). Females of *P. zietziana* matured after 11 to 21 days, produced only one clutch of eggs, and rarely survived for more than 1 month ; there were three generations based on field length frequency data ; production was estimated from life-history, growth and mortality data conveniently calculated by integrating Allen curves (Marchant and Williams, 1977). However, analysis of the population dynamics of *Artemia* is a more complex issue at Lake Grassmere, made difficult by high fecundity, variable generation times and survivorship, long reproductive lifespan and compounding of successive generations.

Results of this integrated study provide some insight into the population dynamics and production of *Artemia*, and the ecology of the Lake Grassmere salt concentration pond system over an annual cycle. Brine flows between individual ponds are greatest during summer months, with average flows for P3, P7, and F1 equivalent to complete emptying every 12 to 14 days (about one *Artemia* generation time). Since brine flows in and out of the ponds simultaneously at opposite ends of the ponds, complete brine replacement occurs over a very much longer time span than 14 days. Slowly inflowing brine is of only slightly lower salinity than that already in a given pond. This unidirectional and relatively constant flow pattern involving small salinity gradients effectively moderates conditions within a given pond, thus facilitating use of laboratory data on *Artemia* growth, survival and generation times to investigate population dynamics of the Lake Grassmere *Artemia* strain. Pond F4 is rather less predictable in these respects due to periodic almost complete draining during the summer.

Analysis of laboratory data show that of the two factors temperature and salinity, temperature is the more important individual factor explaining variations in experimental growth of algae (Chang *et al.*, 1986) and in many biological responses of the *Artemia* population (Haslett and Wear, 1986 ; Wear *et al.*, 1986). In the ponds, apart from the seasonal effect relating to temperature, there is an obvious interaction between *Artemia* and *Dunaliella euchlora* and *D. salina* as food. Natural predators are absent with the exception of birds which have little effect on *Artemia* production and probably benefit the system as nutrient recycling agents ; in fact, nutrients appear adequate for algal growth at all times. The general features of the relationship between algae and *Artemia*, and of the seasonal cycle are given below, but precise temporal relationships and lag factors are complex, and the amplitude and timing of the trends outlined below may vary from year to year.

In winter algal cell numbers and *Artemia* biomass concentrations are low (Fig. 6). However, algal cell concentrations are relatively high in the lowest salinity ponds in which *D. euchlora* dominates. *Artemia* overwinter as juveniles and adults rather than as cysts, as suggested by their ovoviviparous mode of reproduction and by laboratory survivorship data in winter temperature/salinity combinations. Little reproductive activity occurs in winter. As temperatures rise during early spring, algal cell concentrations increase, with *D. euchlora* growth favoured in the lower salinity ponds ; above 14 °C cell numbers potentially double at least every second day (Chang *et al.*, 1986). From early spring (*i.e.* temperatures of about 15 °C) *Artemia* biomass concentrations rise slowly, but from December through to April this rise accelerates (Fig. 6b), caused by rapid growth, and by generation times being much less than longevity. From spring onward, ponds P3 to P7 appear to be the most important breeding ponds.

During summer, nauplii and juveniles are numerically prevalent, and individual cohorts cannot be traced in a given pond after water temperatures reach 18 °C in early November (Fig. 7 and 8c). To some extent cohorts are further masked by flows between ponds. Over the summer period the effects of grazing pressure on algal cell concentrations is evident (Fig. 6). Conditions in summer are otherwise suitable for very rapid algal growth, with *D. salina* growing best in the high salinity ponds and growth of *D. euchlora* potentially enhanced in the lower salinity ponds (Knight, 1974 ; Chang *et al.*, 1986). Towards the end of summer into early autumn (February to early March), biomass concentration in individual ponds and total system biomass reaches its maximum (Fig. 6b and 8d). Trends evident in other sampled ponds are not precisely followed in pond F4 because of periodic draining to the crystallizing ponds and coincident high temperature/high salinity combinations deleteriously affecting growth, reproductive activity, or causing mortalities among all life-history stages.

As pond water temperatures fall from 18 to 15 °C from late February to early March (autumn), a further cohort of nauplii can be traced as they develop to juveniles and adults (Fig. 7). Their progression is no longer completely masked by short generation times and rapid growth and maturation. During autumn through to early winter pond biomass concentration and pond system biomass falls (Fig. 6b and 8d) with adults and juveniles numerically dominant over nauplii (Fig. 7).

As a result of these investigations we have recommended to Dominion Salt Ltd. an optimal harvesting scheme for the ponds P6 to F5 over the 5 month period November to March, which in 1981-82 would have yielded 35.1 tonnes salt-free dry weight *Artemia* without reducing standing biomass in the ponds (details of these recommendations are proprietary information of Dominion Salt Ltd.).

Summary

1. This paper summarises and synthesises the results of intensive research on *Artemia* from Lake Grassmere, New Zealand, completed during 1981-83. The major aim was to estimate field biomass over an annual cycle and the proportion of this biomass harvestable on a renewable basis without deleteriously affecting standing biomass or salt production.

2. Concurrent laboratory studies established strain characteristics over the range of temperature/salinity combinations occurring in the ponds. Temperatures 20-28 °C combined with salinities 100-170 ‰ were most favourable for growth and survival to maturity, but reproductive

variables including time to maturity, length of reproductive life, fecundity and generation times were also enhanced at 20-28 °C, in the slightly higher salinity range 120-200 ‰.

3. The reproductive mode is to produce nauplii either by ovoviviparity or by way of oviparously produced, non-resistant cysts which hatched within 2-3 days ; cysts accounted for only 7 % of reproductive output with no consistent pattern evident.

4. In the field, *Artemia* biomass was sampled at twice monthly intervals on 23 separate occasions from four ponds spaced among the 13 concentration ponds so as to span the entire salinity gradient. On each sampling occasion 86 samples were taken in triplicate. *Artemia* were contagiously distributed and also stratified throughout the annual cycle, with a 50 m wide pond edge stratum averaging 2.5 times the biomass concentration of the pond center.

5. An unbiased estimate of biomass in each sampled pond at each samping time was calculated using stratification and the arithmetic mean, together with appropriately skewed confidence bound estimates within 25 % of the estimated biomass using the bootstrap method. Biomass concentration was expressed in salt-free g dry weight/m^3 and estimated over the entire pond system using linear interpolation for unsampled ponds based on the laboratory data and known pond salinities and temperature. Our estimation methods extend published procedures, and may have wider application for some of the more difficult distributional problems in ecology.

6. *Artemia* biomass was highest during late spring and summer, and lowest during autumn and winter. Maximum estimated biomass over the 238.5 ha pond system was 12 000 kg salt-free dry weight during January 1982.

7. Harvestable biomass was conservatively estimated as 35.1 tonnes dry weight over a 5 month period (November to March). This was calculated by multiplying the standing biomass figures in each pond by the relevant sampling interval/generation time fraction and summing these products over the final 10 ponds in the concentration series.

8. *Artemia* can complete up to eight generations per year in Lake Grassmere. Individual cohorts are evident only during mid-spring and autumn, but are masked during summer by the compounding effects of generation times as short as 2-3 weeks, long reproductive lifespan, high fecundity, and flows between adjacent ponds.

9. The population dynamics of *Artemia* over an annual cycle, and their role in the ecology of Lake Grassmere were clarified by considering the laboratory data together with the effect of flows in moderating the environment. During summer *Artemia* grazing causes suppression of potentially exponential algal growth. Algal levels are however sufficient at all times for *Artemia* growth and development.

10. Our study has provided considerable information on the ecology of the Lake Grassmere brine shrimp. Furthermore, quantitative information of sufficient accuracy were gained to warrant a pilot study for *Artemia* harvesting from the ponds. Results of this successful pilot study (Janata *et al.*, 1987) confirmed the relevant biomass estimates derived from our investigations.

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Ecology of *Artemia* in Didwana Salt Lake (India)

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Abstract

The ecology of *Artemia* in Didwana Salt Lake (Rajasthan), the second largest inland salt-lake of India, was studied from December 1983 to January 1985. The lake water was analyzed for abiotic parameters, its plankton composition, chlorophyll concentration, and phytoplankton primary productivity.

Overwintering cysts began to hatch in late February, attaining maximal hatching in mid-March (salinity about 90 ‰, water temperature ranging from 20 to 25 °C). The population maximum of this parthenogenetic strain of *Artemia* (1 308/l) comprising 14.7 % adults, 11.9 % juveniles, and 73.2 % nauplii occurred in March, in salinities of 90 to 150 ‰. All other zooplankters were absent above 75 ‰ salinity and a fall in the brine shrimp population was observed at salinity 200 ‰.

Introduction

In India, *Artemia* has been recorded from coastal saltpans near Bombay (Kulkarni, 1953), Tuticorin (Royan *et al.*, 1970 ; Ramamoorthi and Thangaraj, 1980), Veppalodai (Royan *et al.*, 1978) and the Gulf of Kutch (Royan, 1979). The first record of its occurrence in inland saline waters was made by Baid (1958) in Sambhar Salt Lake and lately by Bhargava and Alam (1980) in Didwana Salt Lake in the same region. Recent studies on these two salt lakes of Rajasthan (Alam, 1980 ; Bhargava, 1984) revealed no *Artemia* in Sambhar Salt Lake, probably as a consequence of recent severe floods. The present communication deals with the ecological study of *Artemia* in Didwana Salt Lake carried out from 1983 to 1985.

Didwana Salt Lake is situated at 27° 3 N and 74° 5′ E in the Nagaur district of Rajasthan State. It is about 16 km^2 in area, its maximum depth seldom exceeds 1 m, and it is surrounded by a number of earthen salt pans which are periodically filled with lake brine for solar salt production. The catchment area of the lake supports xerophytic plants, but the lake itself is devoid of macrophytes and nektonic fauna. Flocks of flamingos (*Phoenicopterus roseus*) and solitary Indian black winged stilts (*Himantopus himantopus himantopus*) are seen wading.

Materials and methods

Observations were made from December 1983 to January 1985 at fortnightly intervals at three stations (Fig. 1). Zooplankton, including *Artemia*, were collected by filtering water through a 0.3 mm mesh-sized zooplankton net. For phytoplankton, 500 ml of water was collected in

polyethylene bottles and the cells were fixed and preserved with lugol's solution and 3 % formaldehyde. Plankton identification and counting were done under an inverted microscope. The primary productivity of the lake was measured with the light and dark bottle method (Gaarder and Gran, 1927) and the chlorophyll content determined according to Strickland and Parsons (1972). Chemical water analyses were done according to APHA (1975).

The term "salinity" herein used at large refers to one derived from chlorinity value, whereas the ionic fractions in millequivalent percentage (m Eq %) were as follows. Cations : Na 98.61, K 0.21, Ca 0.40, Mg 0.76. Anions : CO_3 0.39, HCO_3 0.30, Cl 98.90, and SO_4 0.39.

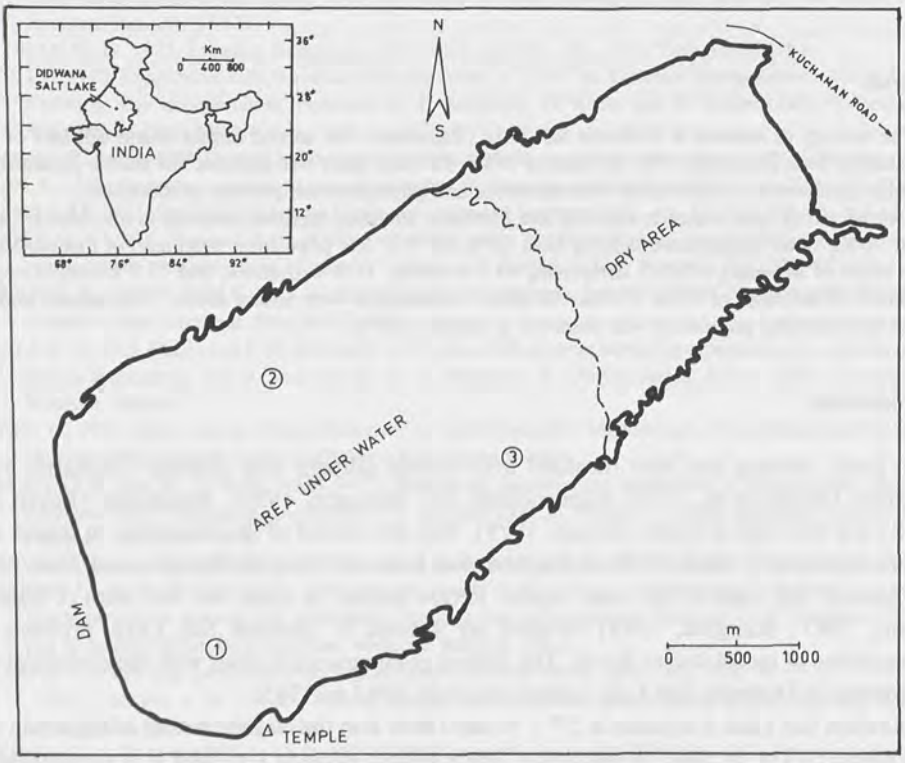

FIG. 1. Map showing location of Didwana Salt Lake and sampling stations.

Results and discussion

THE PHYSICAL AND CHEMICAL ENVIRONMENT

Table I provides the mean monthly values of water temperature, pH, dissolved oxygen, salinity, carbonate, bicarbonate, total alkalinity, nitrate, nitrite, inorganic phosphate, and silica. No data could be recorded from May to August as the lake was dry during this period.

TABLE I

Mean monthly values of various abiotic parameters at three stations in Didwana Salt Lake, from December 1983 to January 1985. Lake remained dry from May to August

Parameters	Months									
	D	J	F	M	A	S	O	N	D	J
Water temperature (°C)	16.0	13.0	15.3	20.0	25.0	24.0	24.0	18.5	17.7	19.0
pH	9.0	9.1	8.8	8.6	8.2	9.0	8.8	8.8	8.5	8.0
Dissolved oxygen (mg/l)	7.12	5.00	0.57	0.64	0.60	4.79	6.10	4.41	1.87	nil
Salinity (‰)	46.30	64.85	82.65	120.40	237.56	15.73	34.77	70.45	203.00	336.01
CO_3 (ppm)	146.0	177.0	209.5	290.5	498.5	48.5	145.5	150.5	226.7	310.1
HCO_3 (ppm)	372.5	450.0	626.0	1064.5	1574.0	158.5	180.0	275.0	374.2	391.5
Total alkalinity (ppm)	518.5	627.0	835.5	1355.0	2072.5	207.0	325.5	425.5	600.9	701.6
Nitrate (μg/l)		420.0	470.0	795.0	874.0	770.0	571.0	1520.0	1190.0	1000.0
Nitrite (μg/l)		2.2	1.1	3.3	13.0	0.5	0.8	1.9	2.6	1.8
Inorganic phosphate (μg/l)		74.0	64.0	88.0	41.0	19.0	34.0	123.0	227.0	93.0
Silica (mg/l)		0.49	0.41	1.66	4.02	0.30	1.11	3.55	2.59	5.02

Water temperature (recorded at 0800 h) ranged from 13 °C to 25 °C, probably with large diurnal fluctuations. With increasing salinity, the phytoplankton population decreased which in turn caused the depletion of dissolved oxygen. Because of the high alkalinity and salinity the lake can be classified as "highly alkaline and saline".

The salinity levels in December 84 and January 85 were very high as compared to those measured in December 83 and January 84. This was due to poor rains in 1984 during monsoon months (July-October) resulting in low water levels and in turn high evaporation rates during the post monsoon period.

THE BIOTIC ENVIRONMENT

Fig. 2 provides data on phytoplankton population, chlorophyll, and gross primary productivity in relation to salinity.

The maximal phytoplankton population (3 851 × 10^3 cells/l) was observed at a salinity of 34.7 ‰. Blue green algae (Cyanophyceae) formed the dominant group during the major part of the study period. However, at a salinity of 203 ‰ most of the phytoplankton disappeared leaving only a small population of diatoms. Cyanophyceae contributed the maximum number of genera, *i.e. Anabaena, Aphanocapsa, Spirulina, Nodularia, Nostoc, Merismopedia,* and *Microcystis.* Diatoms were represented by *Nitzschia* and *Synedra* and the Chlorophyceae only by *Closterium.*

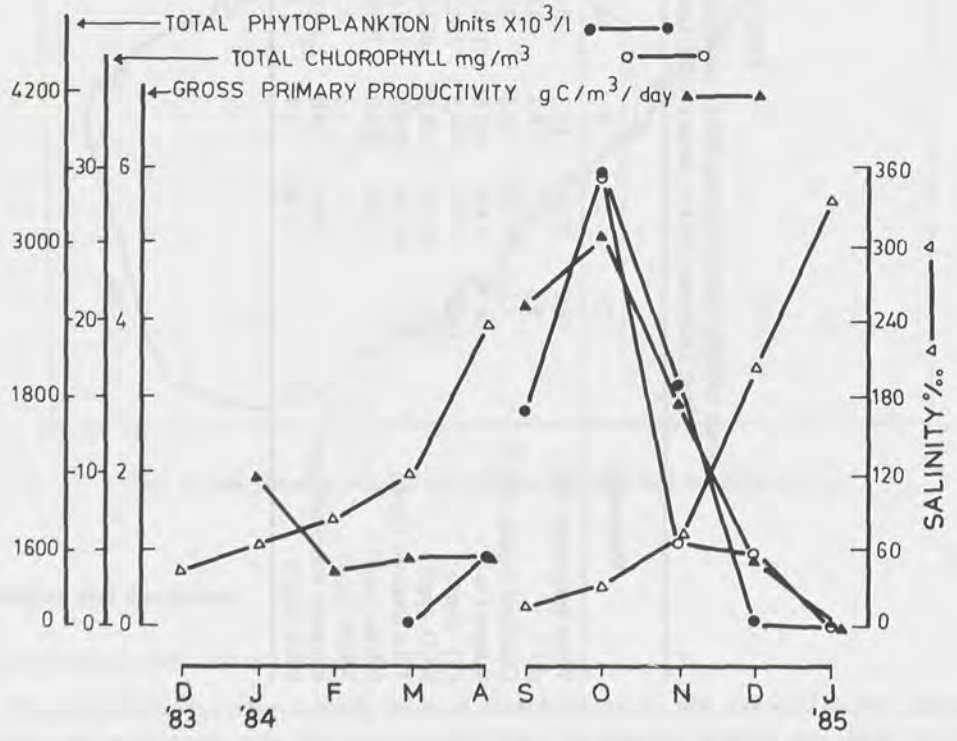

FIG. 2. Fluctuations in certain biotic parameters in relation to salinity in Didwana Salt Lake.

The primary productivity of the lake was low. Maximal gross primary production (5.1 $gC/m^3/day$) was recorded during October coinciding with the highest phytoplankton population ($3\,851 \times 10^3$ cells/l) and chlorophyll concentration (29.33 mg/m^3).

Parthenogenetic *Artemia*, which occurred in salinities of 15-203 ‰ was the most dominant zooplankter in the lake (Fig. 3). The size of the adult *Artemia* ranged between 10 and 14.5 mm.

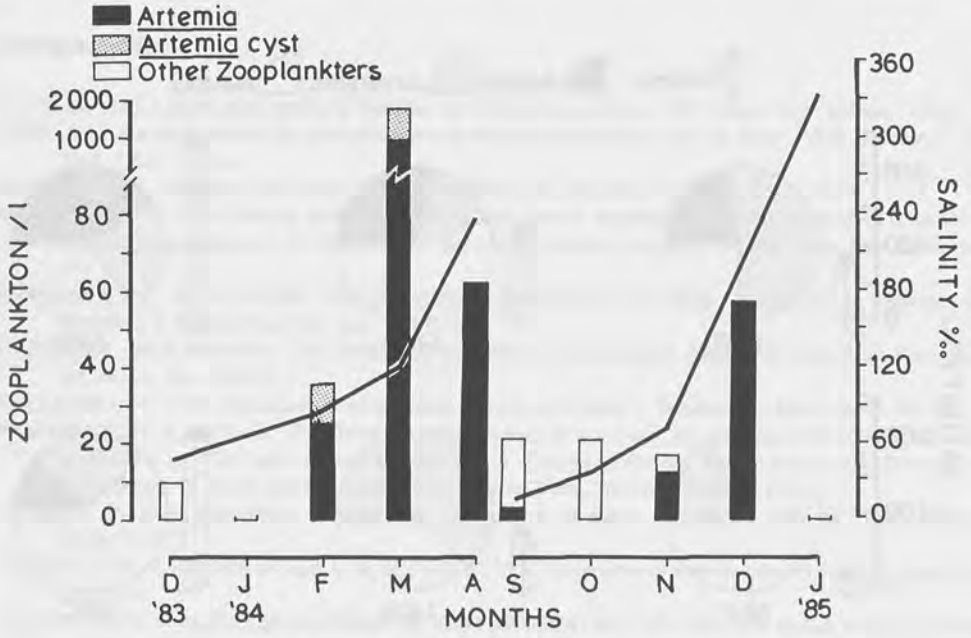

FIG. 3. Zooplankton population of Didwana Salt Lake in relation to salinity.

Salinity and temperature were related to the occurrence, abundance, and growth of *Artemia*. When the water temperature was low in January (13±5 °C) no hatching of cysts was observed. Cysts began to hatch in late February, attaining maximal hatching by mid-March (salinity 90 ‰, water temperature 20-25 °C). The resulting population (1 308/l) consisted of 14.7 % adults, 11.9 % juveniles, and 73.2 % nauplii (Fig. 4). A decline in the brine shrimp population was observed when the salinity reached 200 ‰. At 270 ‰ no more adults and juveniles could be observed. At this stage, dense accumulations of cysts (drifted by winds) were seen along the shore.

After a prolonged dry period of 4 months, the young stages of brine shrimp appeared soon after the first rain in September when the salinity was only 15.73 ‰. At this low salinity other zooplanktonic forms (*Moina* sp., *Cyclops* sp., *Cypris* sp., *Brachionus* sp. and insect larvae) reappeared and took over from *Artemia* which could no longer survive at these low salinity levels. However, with the rise in salinity during November (77.68 ‰), the other zooplankters vanished and were succeeded by *Artemia* which remained present up to a salinity of 203 ‰.

The salinity range of 90 to 150 ‰ and water temperature of 20 to 25 °C seem to be the most suitable ones for breeding and growth of *Artemia* in Didwana Salt Lake, as the highest proportion of juveniles were collected in these ranges (Fig. 4). In this lake Bhargava and Alam (1980) found a maximal population of *Artemia* larvae at 170 ‰. Breeding populations have been reported at higher salinities by Royan *et al.* (1978) (130-160 ‰) and Scelzo and Voglar (1980) (214-274 ‰).

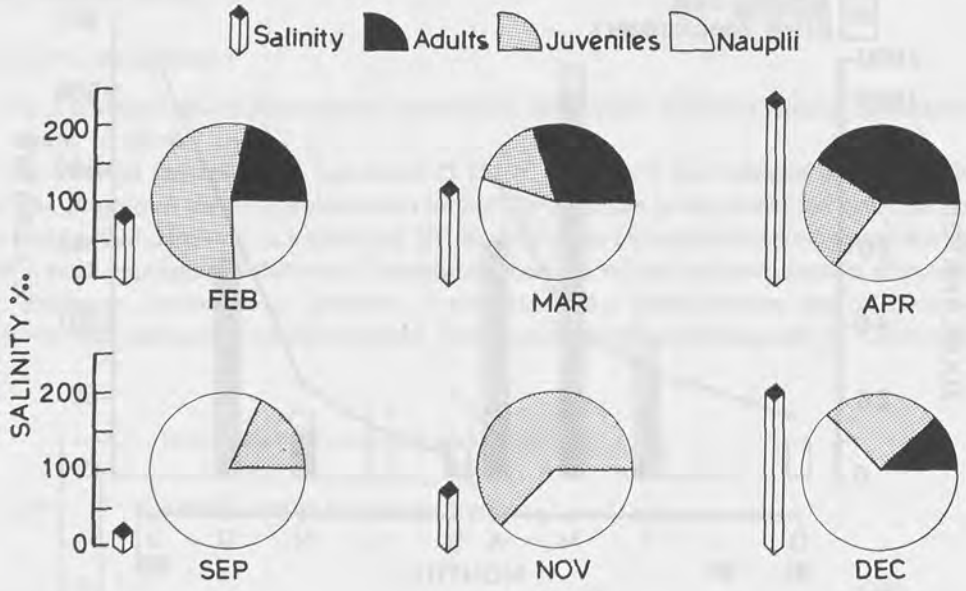

FIG. 4. Proportions of different developmental stages in *Artemia* population in relation to salinity.

We observed *Artemia* to be more densely aggregated around the shores than in the middle of the lake. Interestingly, at noon, very large numbers of *Artemia* were found to harbour in iron pipes (30 cm diameter) used to transfer the lake brine. This perhaps shows their tendency to evade intense sunlight. Chemical limnology and temperature of this microenvironment were, however, similar to that of the lake.

Conclusions

Didwana Salt Lake is the only existing natural inland biotope of *Artemia* in India.

Parthenogenetic *Artemia* is found to occur in densities up to 1 308/l in a salinity range of 15 to 203 ‰.

Salinity ranges of 90 to 150 ‰ and water temperatures between 20 and 25 °C are recorded to be the most suitable for breeding and growth of *Artemia* in Didwana Salt Lake.

Acknowledgements

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Ecology of *Artemia* along the southeastern coast of India

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Abstract

This paper outlines the distribution of *Artemia* in the salt pans near Veppalodai, Thirespuram, Karapad, Spicnagar, and Pullavalai along the southeastern coast of India. Data are provided for the different habitats with regard to salinity, temperature, dissolved oxygen concentration, pH, and the phytoplankton biomass. The study reveals that *Artemia* occurs in thalassohaline salt pans, but is absent from athalassohaline habitats.

Introduction

In India the distribution of *Artemia* has been reported from Bombay (Kulkarni, 1953), Sambhar lake (Baid, 1958), and Tuticorin area (Royan *et al.*, 1970 ; Achari, 1971 ; Ramamoorthi and Thangaraj, 1980). Except these references not much is known on the factors which influence the distribution of the brine shrimp. In the present study information was gathered on the distribution of *Artemia* along 45 km of the southeastern coast of India, and on selected environmental parameters over a period of 12 months from March 1984 to February 1985.

Materials and methods

All stations are salt pans of about 0.5 ha with an average depth of 0.5 m (Fig. 1). The bottom is composed of clay covered with *Chaetomorpha brachygonia.* Above 100 ‰ S the algal bed is gradually replaced by gypsum.

Monthly collections of *Artemia* and phytoplankton were made at all the stations. The following physico-chemical parameters were studied : salinity, temperature, dissolved oxygen concentration, and pH. Salinity and dissolved oxygen concentration were estimated following the procedures of Strickland and Parsons (1972). The pH was determined with an Elico Model LI-10 pH meter. Phytoplankton determinations were done following the method of Ramamoorthi and Thangaraj (1980). The plankton biomass was expressed in cells/l.

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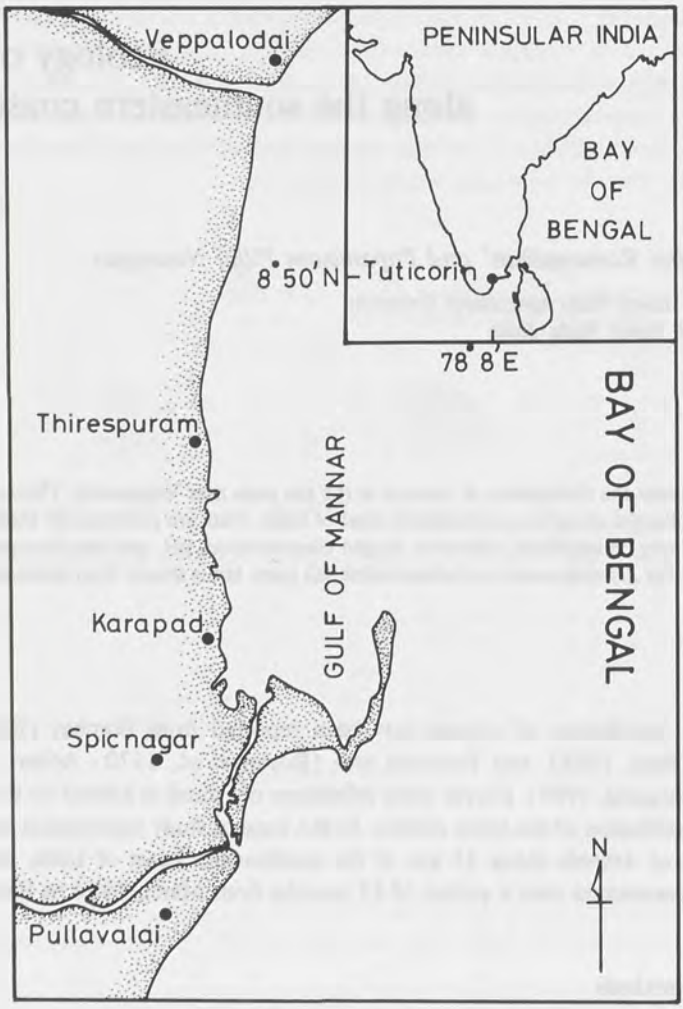

FIG. 1. Locations of study sites : Veppalodai (station I), Thirespuram (station II), Karapad (station III), Spicnagar (station IV), Pullavalai (station V).

Results

The values of salinity, temperature, dissolved oxygen concentration, and pH for all five sites are presented in Fig. 2.

The quantitative occurrence of phytoplankton and *Artemia* are given in Fig. 3. The major phytoplankton species during the monsoon season (November to December) are *Anabaena* sp., *Oscillatoria* sp., and *Spirogyra* sp. During other seasons *Navicula* sp., *Coscinodiscus* sp., and *Pleurosigma* sp. were observed. The *Artemia* population was present in stations I to III, from March to October, 1984 and in January 1985, while it was completely absent in stations IV and V (Spicnagar and Pullavalai).

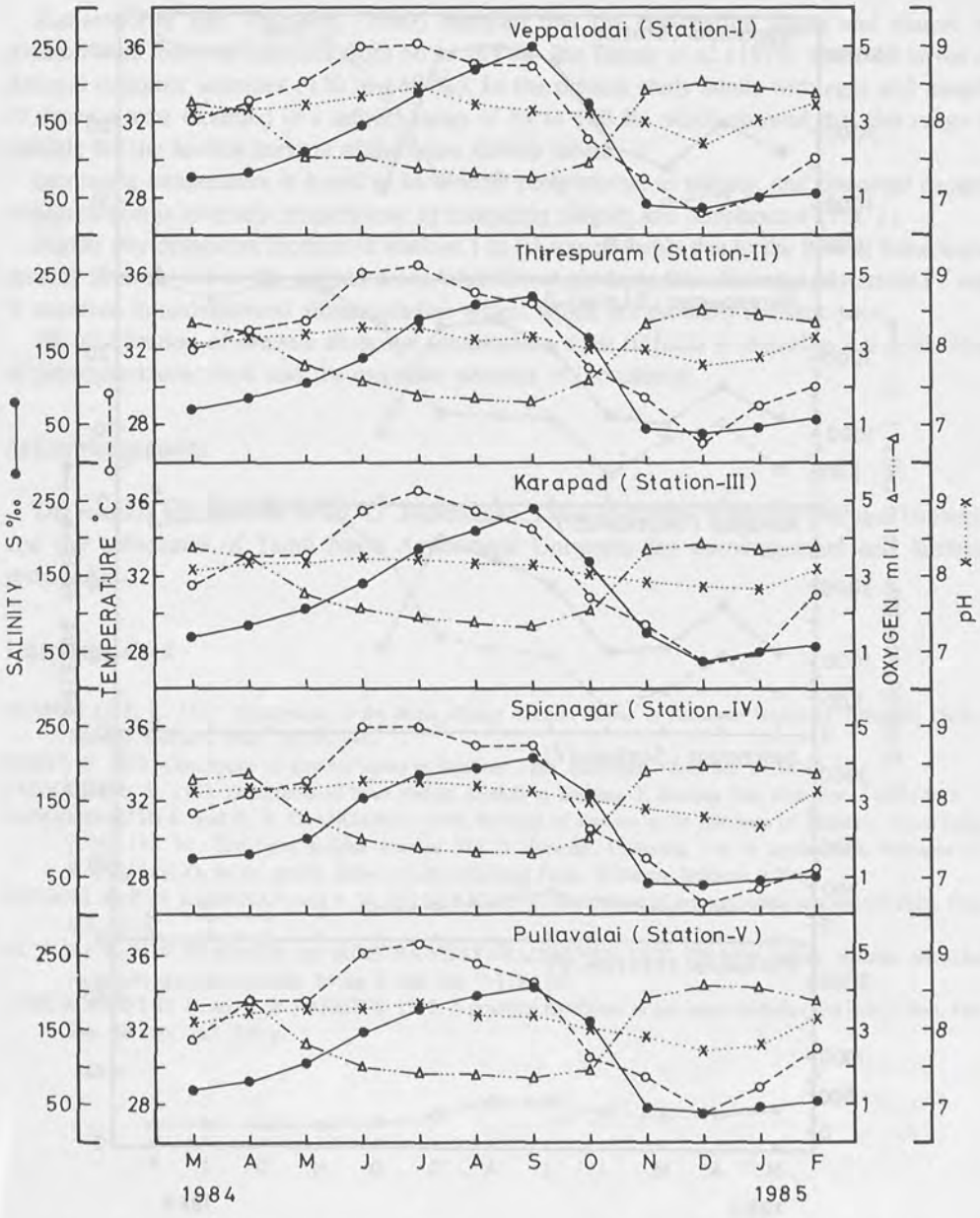

FIG. 2. Seasonal variation of salinity, temperature, dissolved oxygen concentration, and pH.

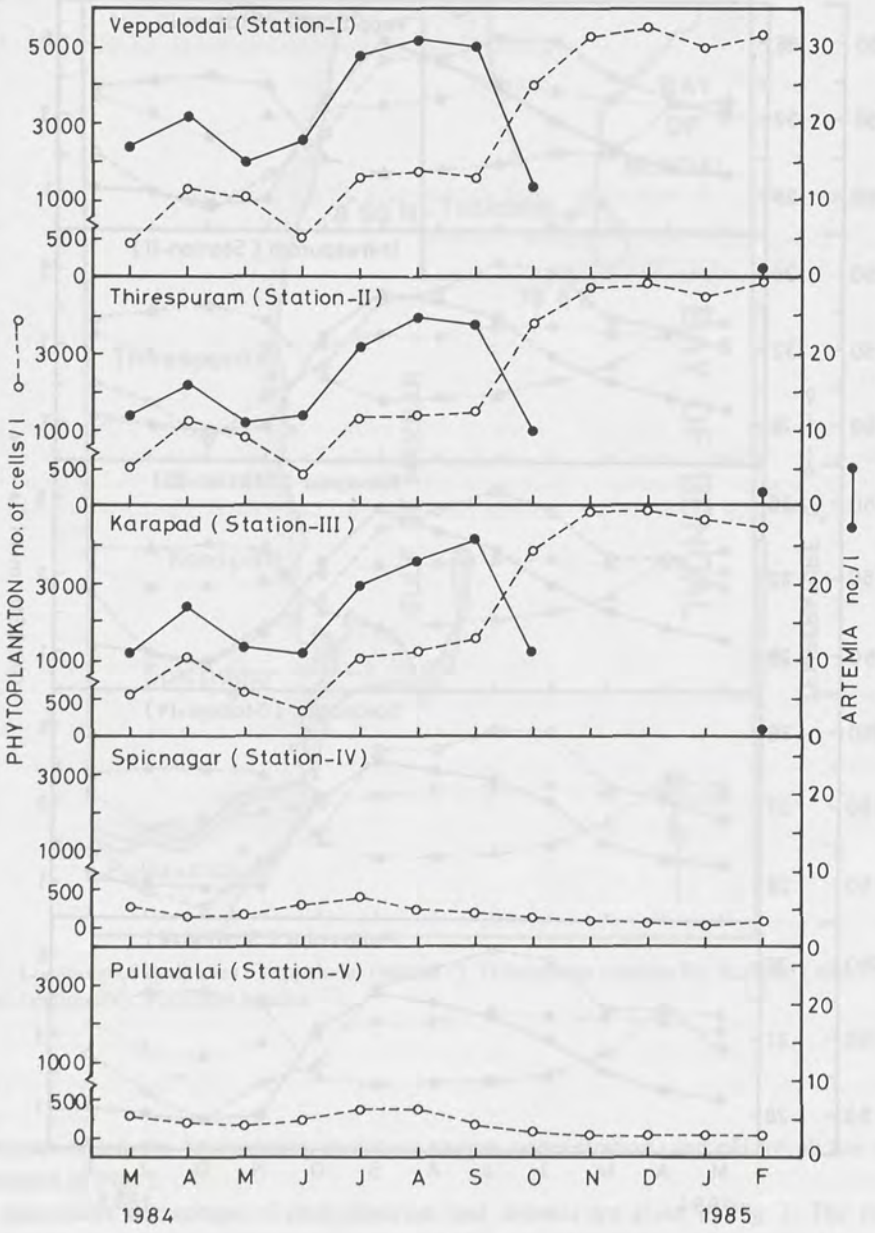

FIG. 3. Seasonal variation of phytoplankton and *Artemia*.

Discussion

Ramamoorthi and Thangaraj (1980) reported that the egg-bearing adults and nauplii of *Artemia* were found in salinities from 56 to 101 ‰. But Royan *et al.* (1978) observed larvae of *Artemia* at higher salinities (130 to 160 ‰). In the present study adults with eggs and nauplii of *Artemia* were recorded in a salinity range of 55 to 160 ‰, which showed that this range is suitable for the healthy survival of the brine shrimp larvae.

Increasing temperature is found to be directly proportional to salinity, and dissolved oxygen concentration is inversely proportional to increasing salinity and temperature (Fig. 2).

Higher phytoplankton biomass in stations I to III was probably due to the flow of brine water directly from the sea to the salt pan areas, while lower phytoplankton biomass in stations IV and V occurred in underground athalassohaline waters which are probably nutrient poor.

The distribution of *Artemia* along the southeastern coast of India is related to the availability of phytoplanktonic food and the unknown patterns of inoculation.

Acknowledgements

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Artemia in the salt pans of Vedaranyam, southern India

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Summary

In 1983, the occurrence of brine shrimp *Artemia* in three salt pans near Vederanyam (10° 1′ N, 79° 50′ E) was recorded as an additional new area in southern India.

The physico-chemical parameters of the ponds were recorded when the *Artemia* were found in greatest abundance (Table I). Details on the population composition are given in Table II.

TABLE I

Physico-chemical parameters recorded in three salt pans near Vederanyam during 1983

Parameters	Salt pans		
	A	B	C
Average depth (cm)	7.5	11.3	8.8
Water temperature (°C)	34	37	36
Salinity (‰)	155	204	162
Oxygen (mm Hg)	47	63	83
pH	7.6	7.5	7.4

TABLE II

Population structure of *Artemia* recorded in three salt pans near Vederanyam (average data ± s.d.)

Artemia population	Salt pans		
	A	B	C
Total number of population (numbers/l)	167±28	183±47	238±39
Mean size of *Artemia* (mm)	10.5±0.81	10.2±0.82	9.5±0.75
Mean weight of *Artemia* (mg)	6.4±0.49	5.5±0.44	4.9±0.39
Biomass of *Artemia* (g/l)	1.07±0.14	1.01±0.30	1.17±0.21

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First report of *Artemia* occurrence in Shurabil Lake (Iran)

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Summary

Shurabil Lake is an alkaline saline lake (latitude 48°17'N, longitude 38°15'E) located in the Azarbaijahn Province near the city of Ardabil at an altitude of 1 260 m above sea level. This temperate lake covers a surface area of about 145-155 ha (dry respectively wet season) and has an average water-depth of 2 m (maximum 3.5 m near the center of the lake). The lake freezes completely during winter (ice thickness of 50-70 cm). NW winds prevent thermocline formation during the rest of the year. The climate is mesomediterranean with dry summers and cold winters. Rainfall is limited to 320-370 mm/yr. The lake has no apparent outlet and main water inflow is through precipitation and springs in the lake basin. Salt precipitation occurs during summer at the shallow part of the lake in the NW region.

The zooplankton community is limited to *Artemia* (probably a parthenogenetic strain as only very few males were observed), the rotifers *Brachionus plicatilis, Hexarthra* sp. and Rotifera sp., and several protozoans. In the summer *Artemia* is dominant comprising most of the biomass. Physicochemical and biological data of Lake Shurabil are summarized in Table I.

The bottom sediment is composed of a mixture of greyish-black clay and organic materials with a strong H_2S smell. The alga *Chaetomorpha* sp. is growing in layers on top of this substratum. Sediments can contain up to 123 700 cysts/425 cm^3.

In the littoral area large numbers of the brine fly *Ephydra* sp. are observed together with *Notonecta* sp. and *Coleoptera* sp.

TABLE I

Physicochemical and biological parameters of Lake Shurabil in the period June-October 1984

Date and hour	Water temperature (°C)	pH	Oxygen content (mg/l)	Conductivity (µm/cm at 20 °C)	Secchi disk turbidity (m)	Alkalinity (meq/l)	Rotifer density (ind./l)	*Artemia* density (ind./l)	
								Juveniles and/or adults	Cysts
une 29-0800	21.0	8.3	5.5	62 000	2.3	7.9	182	–	–
uly 27-0800	23.0	8.4	4.1	73 200	2.5	10.1	475	65	–
August 24-0900	19.5	8.4	5.0	73 200	2.0	10.1	5 920	1.8	20
Sept. 27-0900	19.0	8.5	3.6	87 000	1.5	10.1	4 350	2.0	9.1
Oct. 26-1130	10.0	8.5	5.0	90 500	1.7	10.2	6 773	5.9	12.4

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Preliminary report on the brine shrimp (*Artemia*) from Yugoslav saltworks

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Abstract

Parthenogenetic brine shrimp were found in saltworks in operation in Strunjan and Ulcinj in 1983, and in Portorož in 1984. The temperature ranged from 21 to 28 °C and salinity from 70 to 234 ‰. The dissolved oxygen concentration varied between 1.37 and 8.87 ml/l and the pH between 7.7 and 8.6. The major plytoplankton genera in the *Artemia* biotopes were *Dunaliella* and *Chlamydomonas*.

Artemia from Portorož ranged from 5 to 14 mm in total length in June 1984. Differences in furca allometry were observed in *Artemia* from the three saltworks.

Introduction

According to Floris (1933) the first investigations on brine shrimp in Yugoslavia were in Capo d'Istria by Brauer and Siebold in 1893 and Artom in 1906. Parthenogenetic brine shrimp were recorded from saltworks of Capo d'Istria by Steuer (1910). Barigozzi (1946) reported that these *Artemia* were parthenogenetic tetraploid, and those in Portorož triploid and tetraploid. The *Artemia* from Piran are triploid and tetraploid (Barigozzi, 1974).

No data on the occurrence of *Artemia* in Ulcinj were found in the available literature.

During 1983 and 1984, staff of the Institute of Oceanography and Fisheries (IOR, Split) visited all active and passive saltworks in Yugoslavia. *Artemia* adults were recorded from the saltworks in Strunjan and Ulcinj in 1983 and cysts were recorded in Portorož in 1984 (Fig. 1).

Comments by a local resident of Pag suggested that *Artemia* were present there 10-15 years ago, but none were found during this survey.

Material and methods

Hydrographic parameters and nutrients were determined by standard oceanographic methods (Strickland and Parsons, 1972). Phytoplankton samples were collected from operational saltworks and preserved in 2.5 % formalin previously neutralized with sodium borate. Samples were counted under an inverted Utermöhl microscope after 24 h. Preadult and adult *Artemia* collected from the field in June 1984, were narcotized in chloroform-saturated water and measured under a dissection microscope (Amat Domenech, 1980). Sites in the salinas were selected to represent a wide range of hydrological parameters in the *Artemia* biotope.

FIG. 1. Study area where brine shrimp were recorded (+) or not recorded (-).

Results

Hydrographic parameters are given in Table I, II, and III.

Phytoplankton communities contained diatoms, dinoflagellates, unidentified coccolithophorids and microflagellates. The genera *Dunaliella* and *Chlamydomonas* made up more than 95 % of the total Portorož phytoplankton in October 1984. The mean density of these two genera was 551×10^3 cells/l. Other phytoplankton groups were *Navicula, Pleurosigma, Nitzschia,* and *Prorocentrum micans.* Many benthic diatoms were present but were not identified.

TABLE I

Hydrographic parameters and nutrient concentrations at saltworks where brine shrimp were not recorded

Place	Date	T (°C)	S (‰)	O_2 (ml/l)	pH	SiO_2-Si (µg-at/l)	NO_2-N (µg-at/l)	NO_3-N (µg-at/l)	NH_3-N (µg-at/l)	PO_4-P (µg-at/l)
Pag (A)	9.8.83	26	241.8	1.61	7.70	100.4	0.65	4.7	21.4	0.51
Pag (B)	9.8.83	26	142.3	2.44	7.88	77.0	2.75	3.0	13.8	0.47
Nin	9.8.83	25	94.6	6.66	7.86	205.8	0.95	4.7	137.0	0.60
Ston	16.8.83	27	146.7	5.80	7.30	60.7	0.65	4.7	6.8	0.51

TABLE II

Hydrographic parameters and nutrient concentrations in Portorož where brine shrimp were recorded

Date	T (°C)	S (‰)	O_2 (ml/l)	pH	SiO_2-Si (µg-at/l)	NO_2-N (µg-at/l)	NO_3-N (µg-at/l)	NH_3-N (µg-at/l)	PO_4-P (µg-at/l)
13. 6.84	22.0	179.8	3.66	8.0	48.2	0.440	0.38	1.29	1.190
23.10.84	16.2	150.2	3.93	7.8	27.6	0.400	1.14	3.07	0.342
24.10.84	15.0	150.2	3.08	7.8	34.1	0.620	1.43	3.48	0.708

TABLE III

Hydrographic parameters and nutrient concentrations at saltworks where brine shrimp were recorded.
Those recorded at Ulcinj (A) on 18.8.83 were dead

Place	Date	T (°C)	S (‰)	O_2 (ml/l)	pH	SiO_2-Si (µg-at/l)	NO_2-N (µg-at/l)	NO_3-N (µg-at/l)	NH_3-N (µg-at/l)	PO_4-P (µg-at/l)
Ulcinj (A)	18. 8.83	27	346	1.23	7.23	50.7	1.19	5.5	21.4	0.43
Ulcinj (B)	18. 8.83	25	234.9	2.10	7.80	128.6	0.71	5.6	2.6	0.51
Strunjan (A)	1.10.83	23	157.7	1.37	7.67	63.2	0.47	4.9	8.2	0.81
Portorož salt pond I	13. 6.84	21	98.6	4.54	8.20	71.0	0.155	0.81	1.20	0.20
Portorož (A)	13. 6.84	24	78.3	5.56	8.40	60.7	0.089	0.96	2.22	0.85
Portorož (B)	13. 6.84	22	132.2	4.07	8.40	50.7	0.167	0.46	1.86	0.07
Portorož (C)	14. 6.84	25	99.7	5.60	8.40	154.3	0.101	0.90	1.10	0.65
Strunjan (B)	15. 6.84	28	96.4	8.87	8.60	122.2	0.005	0.96	0.82	0.11

Artemia from Portorož ranged from 5 to 14 mm in total length during June, 1984. The mean length was 10.71 mm. The number of eggs varied from 0 to 97 in the ovisac of the females. The egg diameter ranged from 15.3 to 28.9 μm, with a mean value of 24 μm. Biometric data are given in Table IV.

TABLE IV

Biometric data on the population of *Artemia* from Portorož, June 1984 (salinity 132.3 ‰ ; T 22 °C)

	Minimum value (μm)	Maximum value (μm)	Mean value (μm)
Total length	5 950	14 110	10 713
Abdomen length	3 230	7 055	5 711
Maximal width of brood pouch	1 505	2 580	2 066
Width of 3rd abdominal segment	340	817	606
Furca length	170	425	279
Width of head	589	1 207	876
Length of 1st antenna	578	1 224	992
Maximal diameter of complex eye	170	357	292
Distance between complex eyes	765	1 751	1 513
The number of setae on each furcal branch	1	1	1
The number of eggs	0	97	
Egg diameter	15.3	28.9	24.1

Differences in furca allometry were observed in *Artemia* from three saltworks. In Portorož at salinities of 70-98.6 ‰ individuals were recorded with 2-5 setae on each furcal branch and at 150-179.8 ‰ with only one seta. The individuals found in Strunjan had 6-9 setae on each branch of furca at salinities 69.4 and 157 ‰. In Ulcinj, at a salinity of 234 ‰ individuals were recorded with one and sometimes two setae on the furcal branches. In Ulcinj at 346 ‰ salinity, a temperature of 27 °C and 1.28 ml/l O_2, dead individuals were recorded. The number of setae per furcal branch was reduced in individuals found at higher salinities. Allee and Schmidt (1960) attributed the variability of setae numbers on furca branches to the salinity of a biotope, *i.e.* increasing salinity reduced the number of setae per furcal branch and the furca length. Cysts were recorded only in Portorož, at a temperature of 23 °C and a salinity 180 ‰.

The fish *Cyprinodon calaritanus* S.V. (*Aphanius fasciatus*) were observed in shallow salt ponds 20-25 cm deep and in channels in Pag and Ston at a salinity of 100-146 ‰.

Acknowledgements

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Artemia from Portorož ranged from 5 to 14 mm in total length during June, 1980. The mean length was 10.71 mm. The number of eggs varied from 0 to 97 in the ovisac of the females. The

Artemia *Research and its Applications. 1987. Vol. 3. Ecology, Culturing, Use in aquaculture*
P. Sorgeloos, D. A. Bengtson, W. Decleir, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 556 p.

A survey of *Artemia* and *Branchinella* populations in coastal lagoons and salt pans of Sardinia (Italy)

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Abstract

Artemia is recorded in most salt ponds as well as in numerous ponds and lagoons along the Sardinian coasts.

An interesting co-occurrence of *Artemia* and *Branchinella* has been recorded. The brackish water *Branchinella* is dominant during wintertime ; when salinity levels increase *Artemia* becomes more numerous and in hypersaline waters *Branchinella* disappears.

Because Sardinian salt pans are the main production source of Italian salt (*i.e.* 0.25×10^6 tonnes or 28 % of the total Italian salt production in 1979, and 0.3×10^6 tonnes or 37 % of the total production in 1983) and in view of the important role of *Artemia* in salt production (quality and quantity), a plan for the introduction and proper management of *Artemia* is proposed to the "Monopoli di Stato", the public owner of most of the 2 000 ha of salt ponds in Sardinia.

Next to *Artemia*, *Branchinella* could also be considered as a potential food source to be used in aquaculture.

A survey of *Artemia* and *Branchinella* populations in coastal lagoons and salt pans of Sardinia (Italy)

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Abstract

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Natural sources of brine shrimp (*Artemia*) in Mexico

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Abstract

This paper presents descriptions and preliminary data on *Artemia* populations from eight sites in Mexico. The data include geographical location, climate, mean temperatures, precipitation, and soil type. Listings of the fauna and flora are given for each habitat. In six habitats cysts could be collected for biometrical analysis.

Introduction

Artemia has been utilized in Mexico as a food for fish and crustaceans in experimental and pilot cultures. Mexico has imported *Artemia* cysts especially from the USA. Aquaculture is increasing in Mexico particularly with shrimps (*Penaeus*) and freshwater prawns (*Macrobrachium*). The government, the fishery cooperatives, and private companies are all involved.

In Mexico, approximately 107 salt works have been registered (data from Asociación Mexicana de Productores de Sal, AC, 1981), *i.e.* 44 are located along the Pacific coastline, 44 on the Gulf of Mexico, and 19 in inland waters. In these factories, salt (NaCl) is the only product, exception made for the Texcoco Sosa Company in the State of Mexico that produces also : sodium carbonate ($NaCO_3$), calcium carbonate ($CaCO_3$), caustic soda (NaOH), and *Spirulina* (Texcoco Sosa Company, pers. commun.).

In 1979, the Fisheries Department and the Universidad Autónoma Metropolitana - Xochimilco started a project to assess the potential for *Artemia* production in Mexico. Studies were carried out on the environmental conditions and some biological characteristics. Since 1984, eight populations have been characterized (Fig. 1, Table I) located in eight States. The reasons why these habitats were chosen are : 1) information about the biotopes was received from various sources ; and 2) these places were easily accessible, although some of them not on paved roads.

Materials and methods

IELD COLLECTIONS

For climatological, geographical, and type of soil characteristics for each site, we consulted the charts[1] from the Dirección General de Estudios del Territorio Nacional and water registers[2] from the Secretaría de Recursos Hidráulicos.

[1] Data from Secretaría de Programación y Presupuesto, 1985.
[2] Atlas del agua (1911-1976), Secretaría de Recursos Hidráulicos.

TABLE I

Artemia populations in Mexico

State	Locality
Baja California Sur	Guerrero Negro
	* Pichilingue (San Juan Nepomuceno)
	* Isla del Carmen
Sonora	* Yavaros
Sinaloa	* Bahía de Ceuta
Oaxaca	Salina Cruz
Chiapas	Coastal lagoons :
	* Laguna del Mar Muerto
	La Joya, Buenavista, Los Palos, Solo Dios, Carretas, Pereyra, Chanchuto, Panzacola
San Luis Potosí	* Las Salinas de Hidalgo
México	* Ecatepec (Texcoco)
Yucatán	* San Crisanto

* Populations studied.

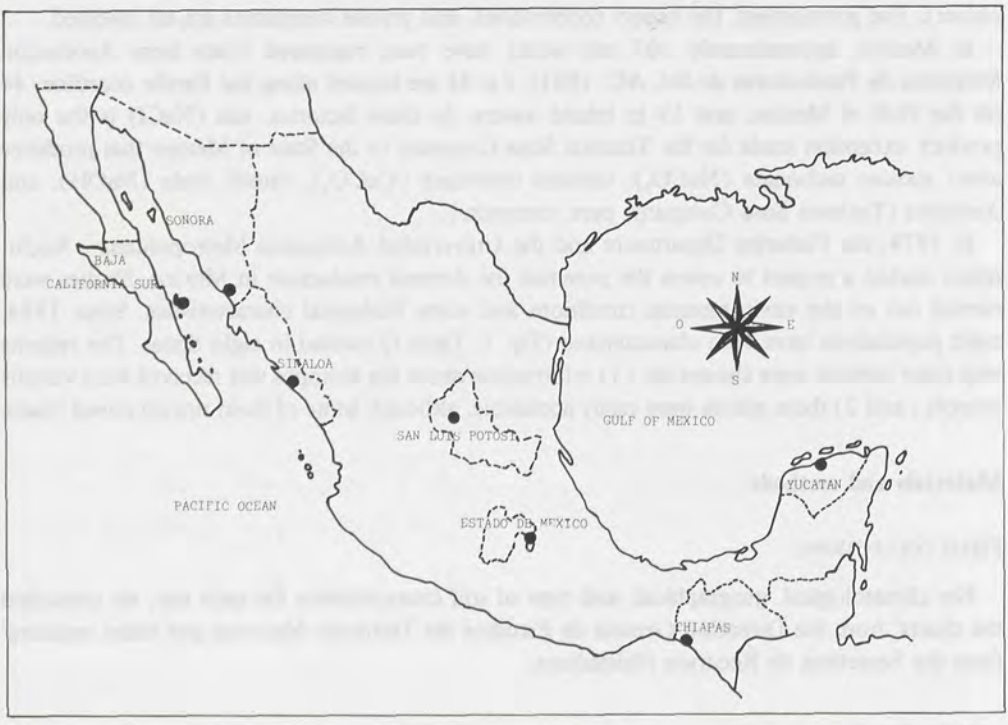

FIG. 1. Map of Mexico with location (●) of the *Artemia* populations studied.

Cyst measurements and hatching

Ten samples of 200 cysts were taken and weighed on an analytical balance (Sartorius Mod. 2842). Then, an extrapolation was made of the total weight of 10 samples to calculate the average and to determine the number of cysts in 1 g.

A stereoscopic microscope (American Optical), with a micrometer eyepiece calibrated with a stage micrometer (Carl Zeiss), was used to measure 100 cysts. To determine hatching, a sample of 0.1 g cysts was incubated in 800 ml seawater (35 ‰ S) at 22 °C under constant illumination and aeration. After 48 h 10 samples of 2 ml each were taken with a pipette and the nauplii counted.

Vegetation and fauna samples

The vegetation collected in each site was dried and identified in the botany laboratory. Phytoplankton was collected using a net with a 0.333 µm mesh, diameter 20 cm and length 50 cm. It was then preserved with formol acetic acid fixative and identified in the laboratory using a microscope at 400 × magnification.

At the sites, the birds were identified only by direct observation, other groups such as insects, fishes, crustaceans, and amphibians were collected and identified in the laboratory.

Results

In Tables II and III physical characteristics and data on cyst presence and some ecological descriptions of eight sites where *Artemia* has been found are shown. The Texcoco population (Ecatepec, Estado de México) was introduced from San Francisco Bay, California (USA) in 1975 (Castro *et al.*, 1984).

It should be noted that in some habitats more information was obtained than in others, because we had the opportunity to carry out more collections at various seasons of the year, *e.g.* Ecatepec (Texcoco) and Bahía de Ceuta (Table III). In Bahía de Ceuta, shrimps and fishes are found only in some months, when the climatological conditions and the dynamics of the bay make their presence possible. The halophytic vegetation is perennial as is *Ephydra* but the abundance changes with the seasons.

In Ecatepec (Texcoco) the land vegetation is seasonal, but phytoplankton is constant throughout the year. Insects also show differences of abundance during the year, depending on the climate. Crustaceans are also seasonal and amphibians and fishes have diminished during the last 2 years.

In the other habitats we can say that the halophytic vegetation is perennial. In many of the habitats we mentioned here, *Ephydra* is present.

The largest cysts were those from Ecatepec (Texcoco) and Las Salinas, Hidalgo (Table IV). It is an interesting coincidence that they both belong to the inland saltworks. The average diameter of the cysts of Yavaros, Bahía de Ceuta, and San Crisanto is almost the same. The first two sites are located near one another on the Pacific coastline and the third is located in the Yucatán peninsula on the Gulf of Mexico. San Crisanto and Isla del Carmen cysts have a wider standard deviation compared to those of the other locations.

Cysts from Ecatepec, Ceuta, and Yavaros exhibited higher hatching percentage than did those from the other three sites (Table IV).

TABLE II

Physical characteristics of eight sites where *Artemia* has been found

Sites	Natural and artificial ponds	Geographical location		Climate	Annual mean		Type of sediment
					Temperature (°C)	Precipitation (mm)	
Isla del Carmen, BCS (Gulf of California)	Earthen salt ponds	lat. long.	26° 0′ N 111° 40′ W	Very dry or desertic	22	5-10.2	Slime
Pichilingue (San Juan Nepomuceno), BCS (Gulf of California)	Man-made salterns	lat. long.	24° 16′ N 110° 20′ W	Dry to desertic	22	5-10	Slime
Yavaros, Sonora (Pacific Ocean)	Coastal bay	lat. long.	26° 46′ N 109° 35′ W	Dry	22	250	Slime
Bahia de Ceuta, Sinaloa (Pacific Ocean)	Coastal bay	lat. long.	23° 50′ to 24° 20′ N 106° 30′ to 107° 30′ W	Dry	22	650	Sand, slime, clay
Coastal lagoons in Chiapas (Pacific Ocean)	Coastal lagoons	lat. long.	16° 0′ to 16° 30′ N 94° 0′ to 95° 0′ W	Warm, humid	27	1800-3500	Sand, slime
San Crisanto, Yucatán (Gulf of México)	Man-made salterns	lat. long.	21° 15′ N 89° 10′ W	Dry	26	700	Calcareous sand
Las Salinas de Hidalgo, San Luis Potosí (Inland State)	Cement tanks	lat. long.	22° 39′ N 101° 43′ W	Semi-arid	12	300-400	Slime
Ecatepec, Estado de México (Inland State)	Cement tanks	lat. long.	19° 23′ N 99° 0′ W	Semi-arid to temperate	15-16	400-600	Clay

TABLE III

Cysts presence and some ecological descriptions of *Artemia* habitats in Mexico

Sites	*Artemia* present	Ecological observations
Isla del Carmen, BCS (Gulf of California)	With cysts	This island is 15 km from the littoral of the Peninsula of Baja California. The salt water comes from underground wells. Halophytic vegetation. Phytoplankton mainly diatoms : *Pleurosigma, Navicula, Pinnularia.* Also numerous *Chlorella* and *Dunaliella.* Zooplankton : only *Artemia.* Aquatic vegetation : *Batis maritima* L., *Salicornia pacifica* Stendal.
Pichilingue, San Juan Nepomuceno, BCS (Gulf of California)	With cysts	Pichilingue is a harbor for ferries 18 km from La Paz City. The saltwork is called "San Juan Nepomuceno", and is surrounded by dunes. Halophytic vegetation, bacteria and *Artemia* in the ponds. Shore birds, preying on fish.
Yavaros, Sonora (Pacific Ocean)	Without cysts	The salt ponds are protected from the Pacific Ocean by two peninsulas that make a bay. Halophytic vegetation. Shore birds, bacteria and *Artemia* in the salt works.
Bahia de Ceuta, Sinaloa (Pacific Ocean)	With cysts	This bay is separated from the Pacific Ocean by a peninsula 26 km long. The surrounding vegetation is diverse. *Avicenia germinants* ; *Allenrofea occidentalis* ; *Sesuvium portulacastrum* and *Monantochloe litoralis.* Insects : the most important genus, *Ephydra.* Close to the bay entrance abundant fisheries of *Penaeus, Mugil, Centropomus, Pleuronichthys,* etc. Turtles : *Chelonia* and *Lepidochelis.* Birds : pelican and heron (Family Ardeidae).
Coastal lagoons in Chiapas (Pacific Ocean)	Without cysts	This is a large lagoon system with seven zones where *Artemia* is present. In these areas the vegetation is halophytic predominated by *Sesuvium portulacastrum.* The phytoplankton is abundant during April to July. Zooplankton : dominance of copepods and shrimp larvae. Insects : great variety, predominant *Ephydra.* Birds : heron and ducks.
San Crisanto, Yucatán (Gulf of Mexico)	With cysts	Along the Yucatán coast (from Progreso to Islám de Bravo) there are small saltworks : Uaymitun, Telchac, San Crisanto, Sinanche, and Islám de Bravo. Halophytic vegetation. Bacteria and *Artemia* are found in the ponds. Cysts were collected in San Crisanto salt works.
Las Salinas de Hidalgo, San Luis Potosi (Inland State)	With cysts	Close to the salt work is a lagoon of freshwater with some windmills. The saltwater comes from the underground. Insects : a few unidentified beatles. Birds : swallows dominate. *Artemia* and bacteria were found in the tanks.
Ecatepec, Estado de México (Inland State)	With cysts	The former Texcoco Lake is located near Mexico City (35 km). The vegetation is halophytic, submerged during part of the year. The plants belong to the families Poaceae (Graneae), Aizoaceae, Chenopodiaceae, Amarantaceae, and Borraguinaceae. Phytoplankton : *Spirulina, Oscillatoria, Chlamydomonas, Nostoc,* and *Ctenocladus.* Insects : mainly *Ephydra* and *Corixa.* Crustacea : *Gammarus, Cambarellus, Moina,* and *Daphnia.* Amphibia : *Hyla, Rana,* and *Boto. Chirostoma* predominates among the fishes.

TABLE IV

Some characteristics of cysts from six sites in Mexico

Sites	Cyst diameter ($\bar{x} \pm$ S.D.) (μm)	Number of cysts/g	Hatching %	Time of collection
Isla del Carmen, BCS	179.6 ± 19.05	384 615	33	July 1983
Yavaros, Sonora	195.4 ± 14.21	369 102	60	December 1980
Bahia de Ceuta, Sinaloa	195.6 ± 13.68	380 000	64	March 1980
San Crisanto, Yucatán	195.4 ± 29.76	377 869	50	April 1982
Las Salinas Hidalgo, SLP	201.0 ± 11.32	358 000	25	December 1984
Ecatepec (Texcoco), Edo. de México	212.0 ± 15.41	355 622	60-70	November 1983

Discussion

Persoone and Sorgeloos (1980) reported the presence of *Artemia* at 101 sites on the American continent. For the Mexican *Artemia* populations they mentioned four locations and we would like to add the following clarifications : Baja California is not a locality but a State of Mexico, to which Pichilingue belongs ; this is not an Island but it is a place near the city of La Paz on the Baja California peninsula.

The population mentioned by Persoone and Sorgeloos (1980) as Pichilingue corresponds to what in this paper is named as San Juan Nepomuceno, which is a saltwork located in Pichilingue, Baja California Sur. The Yavaros population mentioned by Persoone and Sorgeloos is confirmed in this paper.

During our survey of Baja California Sur, we also found brine shrimps in Isla del Carmen, but have no information about San José Island. We tried to compare the Texcoco *Artemia* with the strain of San Francisco Bay, California (USA) but it has been only possible with regard to diameter of the cysts, those from Texcoco being smaller (212 μm) than those from San Francisco Bay (223.9 *vs* 235.6 μm) reported by Vanhaecke and Sorgeloos (1980). It is important to study these populations simultaneously to discover if the population introduced in the Mexican plateau has undergone important changes.

From the localities visited up to now we identified some sites suitable for *Artemia* culture in Mexico. The most important ones are : Yavaros, Sonora ; Bahia de Ceuta, Sinaloa ; Coastal lagoons of Chiapas ; and Ecatepec (Texcoco), State of Mexico. The first one, Yavaros, is situated in an area where rains are scarce which helps to prolong the period during which *Artemia* can be cultured. In this area shrimp and freshwater prawn cultures are being developed.

Nowadays, an experimental *Artemia* culture in Texcoco (Ecatepec) has been set up. This culture is important in the supply of *Artemia* cysts and to fulfill the commercial demands of aquarium keepers and aquaculturists near Mexico City.

In May 1985, the Universidad Autónoma Metropolitana-Xochimilco and the Fisheries Department held a meeting on crustacean production[3]. On that occasion several Mexican specialists met and estimated the demand for *Artemia* cysts at 500 kg/year for the larval prawn cultures that are presently operating. It was also mentioned that the estimated potential of the

[3] National meeting of interchange of Scientific and Technological Information in the Production of Crustaceans.

annual cyst requirement is near to 3 000 kg for aquaculture in the Pacific zone and 700 kg for the coastline of the Gulf of Mexico. For this reason the search for new localities for *Artemia* production is increasing even without considering the cyst inoculations in appropriate habitats such as the saltworks, saltponds, saltmarshes, and inland waters.

Acknowledgements

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Ecology of Oregon's Great Salt Lake : Lake Abert

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Abstract

Lake Abert (199 km^2), a remnant of pleistocene Lake Chewaucan, is a large body of saline water located in south central Oregon (USA) between the semi-desert and volcanic terrain lying near the fault scarp ridge known as Abert Rim. Its chloro-carbonate water is alkaline (pH 9.8) and contains 40 to 80 g/l total dissolved solids.

Abundance of brine shrimp population, as well as horizontal and vertical spatial distributions, were determined through the use of several types of zooplankton sampling systems.

Capture analyses in 1981, primarily in the eastern side of Abert Lake, showed densities ranging between 1 650 and 3 600 adults/m^3. In 1982, the capture analyses emphasized lakewide sampling and began earlier in the growing season. Peak abundance occurred in early July with brine shrimp populations reaching levels ranging from 5 260 to 7 360 adults/m^3. Estimated total lakewide abundance of brine shrimp derived from 13 collecting stations has been calculated to be 3.38×10^{11} adults with a total biomass of 6.62×10^{6} kg or nearly 7 000 tonnes.

Temperature profiles in the summer months ranged from 22±1 °C at the surface layer to a bottom temperature of 20±1 °C. Redistribution patterns in both spatial and temporal distribution were observed in both the 1981 and 1982 growth seasons indicating that large-scale patchiness occurred in the brine shrimp population.

Secchi-disk readings and chlorophyll *a* analysis of phytoplankton densities confirmed a paucity of planktonic algal biomass consisting of the filamentous green alga (*Ctenocladus circinnatus*) and various species of diatoms.

Culturing

The years 1975-1976 produced the first *Artemia* cyst shortage as aquaculture demand began outstripping supply. The success story of *Artemia* inoculation in northeast Brazil relieved that shortage at the time of the first International Symposium on *Artemia*. Although Brazil's success was short-lived, the impetus had been provided for feasibility studies and attempted inoculations around the world. This section includes many papers resulting from those studies and operations.

The first two papers from a sort of transition from the previous section on Ecology, with a laboratory study (1) and a mesocosm study (2) of reproductive aspects. The sub-section on Extensive Culturing covers first commercial operations (3-7), then development of a commercial operation integrated with livestock poultry and aquaculture (8-9), followed by feasibility studies in Asia (10-12) and Central and South America (13-15).

The final sub-section on Intensive Culturing looks primarily at feasibility of various diets for *Artemia* culture (17-21), then at system design (22-23), and finally gives production data from two case studies (24-25).

General studies on reproduction

(1) N. J. Berthélémy-Okazaki and D. Hedgecock.
Effect of environmental factors on cyst formation in the brine shrimp *Artemia*.

(2) F. Amat, F. Hontoria, and J. C. Navarro.
Life history of an experimental Great Salt Lake *Artemia* population kept in outdoor culture.

Extensive Culturing

(3) M. R. Camara and R. de Medeiros Rocha.
Artemia culture in Brazil : an overview.

(4) W. Tarnchalanukit and L. Wongrat.
Artemia culture in Thailand.

(5) J. M. Perez Rodriguez.
Cyst production of *Artemia* in salt ponds in southeastern Spain.

(6) R. G. Wear and S. J. Haslett.
A minimal strategy for assessing *Artemia* biomass harvestable from production salinas.

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The DSIR-*Artemia* harvesting programme.

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Commercial production of *Artemia* in the Philippines.

(9) N. A. Jumalon and D. M. Ogburn.
Nutrient flow and physicochemical profile studies of an integrated poultry-salt-*Artemia*-milkfish-sea bass-shrimp pond production system.

(10) Vu Do Quynh and Nguyen Ngoc Lam.
Inoculation of *Artemia* in experimental ponds in central Vietnam : an ecological approach and a comparison of three geographical strains.

(11) S. C. Bhargava, G. R. Jakher, M. M. Saxena, and R. K. Sinha.
Rearing *Artemia* in a salt pan near Sambhar Lake (India).

(12) J. A. Basil, D. R. D. Premkumar, A. P. Lipton, and M. P. Marian.
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(13) L. C. A. Naegel.
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Preliminary trials of extensive culture of *Artemia* in Peru
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Intensive culturing

(17) Ph. Douillet.
Effect of bacteria on the nutrition of the brine shrimp *Artemia* fed on dried diets.
(18) P. Lavens, A. De Meulemeester, and P. Sorgeloos.
Evaluation of mono- and mixed diets as food for intensive *Artemia* culture.
(19) T. Castro, G. Castro, and G. De Lara.
Experimental production of an introduced *Artemia* strain in alkaline waters in the State of Mexico.
(20) N. Vieira.
Culture of *Artemia* from Aveiro (Portugal) fed with wheat bran and seaweed.
(21) C. Balasundaram and A. K. Kumaraguru.
Laboratory studies on growth and reproduction of *Artemia* (Tuticorin strain).
(22) P. Lavens and P. Sorgeloos.
Design, operation, and potential of a culture system for the continuous production of *Artemia* nauplii.
(23) R. R. Platon and J. Zahradnik.
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(24) Ch. M. James, T. S. Abu-Rezeq, and P. Dias.
Production of *Artemia* biomass for feeding marine fish larvae.
(25) B. Vishnu Bhat and R. Ganapathy.
A simplified technique for mass production of *Artemia* in India.

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Effect of environmental factors on cyst formation in the brine shrimp *Artemia*

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Abstract

Cyst formation (oviparity) by the brine shrimp *Artemia* in nature is affected by environmental conditions which introduce uncertainty in the production of this important commodity for the aquaculture industry. Since development of strains or culture systems that continuously produce cysts would remove this uncertainty, we have examined the effects of several physical and biological factors on the mode of reproduction in *Artemia* from San Francisco Bay. Maternal age, photoperiod, temperature and salinity are found to be major factors controlling the mode of reproduction. At low (16 °C) or medium (20-22 °C) temperature, females are 68-99 % oviparous under photoperiods with 12 h or less of light, but are only 10 % oviparous under long days or constant light. At higher temperatures (25 °C), photoperiod has little or no effect with 50 % cyst broods produced under short and long days. Few females are oviparous for the first brood (10 %) but nearly 100 % produce cysts by the third brood. Under laboratory conditions, salinities above 120 ‰ inhibit cyst formation at least for the first three broods. Brine shrimp density and hypoxia tend to promote oviparity but variable results are found for hypoxia over different experiments. No one environmental factor is clearly responsible for controlling reproductive mode, moreover, interactions among several factors appear to be significant. A more promising approach to understanding the control of reproductive mode in *Artemia* may lie in investigations into the common physiological pathway whereby diverse environmental factors induce a switch from oviparity. A new working hypothesis focusing on stress induced metabolism is offered.

Introduction

The brine shrimp *Artemia* exhibits two modes of reproduction : ovoviviparity, the production of live nauplii within the uterus, and oviparity, the production of dormant cysts (encysted gastrulae), which after release must undergo desiccation and rehydration before hatching. With few exceptions, these modes of reproduction are mutually exclusive, for any particular brood.

Because dormant eggs are easily collected, canned and shipped, later rehydrated in seawater, and the resulting larva and adults fed to a wide variety of aquatic species, *Artemia* cysts have become an important commodity in aquaculture. Traditionally, cysts have been collected from natural habitat or commercial saltworks, but increasing demands for cysts in the 1970's prompted the introduction of *Artemia franciscana* into coastal salterns in Brazil (Camara and De Medeiros Rocha, 1987). While these populations initially produced cysts, in recent years, cyst production has fallen. Possible reasons for this are changes in environmental conditions that shifted the reproductive mode toward ovoviviparity and unwitting selection for oviparity as cyst harvesting removed genotypes predisposed towards oviparity. In order to revive cyst production in salterns,

a highly oviparous strain should be restored and environmental factors that affect cyst production should be identified and appropriately manipulated. An understanding of the physiological basis underlying oviparity determination in the female brine shrimp might be of even greater help in controlling cyst production.

Environmental stress, particularly hypoxia, is thought to induce the production of cysts in *Artemia*. Gilchrist (1954) and Gilchrist and Green (1960) showed that levels of hemoglobin and its metabolic end-product hematin rise with hypoxic stress. Dutrieu (1960) then suggested that under low oxygen concentrations, excretion of hematin via the shell gland situated in the ovisac induced the dormancy of the cysts contained in the uterus. Such a hypothesis appears to fit well with the appearance in nature of cysts in summer when ponds are drying out. At that time of the year, days are long, at least at temperate latitudes, oxygen levels drop with desiccation and higher salinities, temperatures increase and halophilic algal and bacterial populations bloom. Yet, the effects of these several factors on *Artemia*'s mode of reproduction have been studied by various researchers with contradictory results. Barigozzi (1939) found a slight preponderance of oviparity at salinities less than 80 ‰ but Ballardin and Metalli (1963) later found no correlation between oviparity and high salinities, temperature, photoperiod, food type, or densities in an Italian strain. They attributed oviparity to stress in general. Dutrieu (1960) showed that in addition to hypoxia, algae were necessary for cyst formation since in her cultures yeast-fed *Artemia* were predominantly ovoviviparous. Iron was demonstrated by Bowen *et al.* (1969) to be the algal component necessary for cyst formation. Provasoli and Pintner (1980) noticed that in amphigonous *Artemia* from the Great Salt Lake, Utah, USA, and in a parthenogenetic strain from Sète, France, a long daylight cycle favored ovoviviparity and that the photoperiod effect was stronger when applied 10-12 days before offspring deposition, while the eggs were still in the ovaries. They also found that dimmer light favors oviparity. Finally, Versichele and Sorgeloos (1980) reported the importance of hypoxic stress in maximizing cyst formation in an intensive mass culture system.

In our laboratory, study of cyst induction was started based on the widely accepted hypothesis that hypoxic stress is the major environmental factor inducing oviparity in *Artemia*. However, since nearly 100 % of females produced cysts by the third brood, even in the absence of hypoxia, we began systematic study of several physical and biological factors that might affect the mode of reproduction. First, oviparity was correlated with brood number in order to describe the normal ontogeny of reproduction under standard laboratory conditions. Second, a comparison of reproductive mode for females held in mass culture *versus* those held in isolation was made in order to validate the experimental protocol. Finally, density, food availability and type, salinity, hypoxic stress, photoperiod and temperature were correlated with the mode of reproduction.

Materials and methods

Stocks and general husbandry

All *Artemia* used were hatched from cysts obtained from the Metaframe Corporation, San Francisco Bay Brand, Stock # 65034. One gram of dried cysts was mixed with seawater at a salinity of 30 ‰. Cysts hatched within 36 to 48 h at 20 to 24 °C. Larvae were then raised in 1 l plastic boxes, on an algal diet described below, and the culture media were completely replaced every 2-3 days. Seawater filtered to 1 μm, UV-sterilized, and diluted to 30 ‰ with distilled water,

was used for algal and *Artemia* cultures. At 4-7 days, larvae were spread among several boxes in order to reduce densities to 700-1 000/l.

A new batch of cysts was hatched and raised for each experiment described below. Depending on the experiment, 60 to 200 juvenile brine shrimps (4-7 mm long, 7-15 days old) were raised to maturity in 1 l plastic boxes on the following diets : the flagellate *Tetraselmis* in Experiments 1 to 7, the diatom *Actin* in Experiment 8 and the flagellate *Dunaliella* in Experiments 9 and 10. Media were changed every 2 to 3 days at which time females were checked for maturity, maturity being defined as the first occurrence of eggs in the uterus. Mature females were taken out of mass culture and placed individually in 60×15 mm petri dishes (25 ml capacity) with an adult male. Dishes were labelled so that the time of offspring deposition and the type of brood could be recorded for each female. After a brood had been produced, care was taken to eliminate all cysts or nauplii from the petri dish.

Experiment 1. Cyst formation as a function of brood number

In preliminary culture experiments in which ovoviviparity had been expected, nearly 100 % of females became oviparous by the third brood. Thus, it was necessary to describe first the mode of reproduction as a function of brood number under ambient laboratory conditions. Unless specified, 1 l cultures of 200 juveniles each were aerated and exposed to dim daylight or fluorescent lighting during normal working hours (0800-1700 h). Temperature varied between 19 and 23 °C. As females matured, couples were isolated in covered 25 ml petri dishes and kept under similar photoperiod and temperature conditions until the seventh brood was recorded or until the female had died.

Experiment 2. Effect of culture vessel transfer

Increase in cyst production in later broods could be caused by the transfer of females to the small 25 ml petri dish. An experiment was designed to verify if females staying in the 1 l boxes had a percentage of oviparity comparable to that of females transferred to petri dishes after maturation of the first brood in mass culture. As in Experiment 1, 200 juvenile *Artemia* were placed in three replicate 1 l boxes, A, B and C. Boxes A and B were treated as in the first experiment ; females were transferred to petri dishes at first maturity. After 7 days, when most of the females isolated from Boxes A and B had already produced their first brood, females from Box C were placed in small petri dishes and their type of reproduction recorded.

Experiment 3. Effect of brine shrimp density

Juveniles were reared to maturity at three densities, 50, 100 and 200 ind./l, with three replicates per treatment, following by observation of reproductive mode as previously described. In this experiment, as well as in the following ones, the first three broods were scored but only the results from the 1st brood are given here since most of the second and third broods were encysted.

Experiment 4. Effect of algal concentration and yeast

Fifty juveniles *Artemia* were cultured at four concentrations of *Tetraselmis* algal diet. Density of algal cells in a stock *Tetraselmis* culture was first estimated with a hemocytometer ; four algal

densities were then made by using the stock culture directly and by diluting the stock to 1/2, 1/4, and 1/8 of its initial concentration with 30 ‰ filtered seawater. Algal cells were also counted in the used medium (just before changing the brine shrimp culture) in order to estimate how much of the initial amount of cells had been eaten. A fifth diet treatment, 500 mg/l of brewer's yeast, was added in order to compare the effect of different diets on oviparity. Each treatment had three replicates.

Experiment 5. Effect of hypoxic stress

Hypoxic stress was induced by bubbling a mixture of nitrogen gas and air (ratio 1:1) into the 1 l culture boxes, thereby reducing the oxygen level from the normal 20 % to 10 % of the atmospheric pressure (8 to 4 mg/ml). Controls were aerated normally with bubblers. Oxygen level was verified before media changes with a Yellow Springs Instrument Model 54A oxygen meter. Control and stress conditions were replicated thrice. For each box, 60 juvenile brine shrimps were subjected to the treatment until maturity and then removed to the small petri dishes. Water changes, female maturity checks, and data recording were done as above.

Experiment 6. Effect of salinity

Salinities ranging from 15 to 180 ‰ were tested in two series because of limitations in the availability of algae, space, and time. Salinities of 15, 30, 45, and 80 ‰ were tested in the first series. The salinity of 15 ‰ was attained by diluting the algal medium by half with distilled water. To reach salinities of 45 and 80 ‰, 15 and 50 g of NaCl, respectively, were added to the algal media. In the second series, the *Artemia* were cultured at 30, 80, 120, 150, and 180 ‰ with gradual acclimations to higher salinities. For gradual acclimation to higher salinities, 50 g of NaCl (S:9625-Lot 41-F-0510. Sigma Company) were added to all boxes except the three to be kept at the salinity of 30 ‰. After 2 days, media were changed and 50 g of NaCl were added to each of the three 80 ‰ boxes while 90 g were added to the 120, 150, and 180 ‰ boxes. After 2 days, the same procedure was repeated except that 50 and 90 g of NaCl were added to the 80 and 120 ‰ boxes, respectively, and 120 g were added to the 150 and 180 ‰ boxes. Finally, 3 days later, 50, 90, 120, and 150 g of NaCl were added to the 80, 120, 150, and 180 ‰ boxes respectively.

Experiment 7. Effect of photoperiod

The photoperiods tested were LD 0:24, LD 6:18, LD 12:12, LD 18:6 and LD 24:0, all a room temperatures (20-24 °C). Cardboard boxes covered with black plastic sheets were used a light chambers. Each of them except the LD 0:24 had a slit on the top to allow the light from a cool-white lamp (General Electric F20/T12-CW) to illuminate the box. Each light was covered with a sheet of black plastic, taped to the box to avoid leakage of room light into the light chamber. The lights were connected to timers set at the proper photoperiod. One hundred juveniles were placed in each box at the start of the experiment. Isolation of females and data collection were as described above. Once in small petri dishes, mature females were not submitted to experimental photoperiod but to room conditions (natural daylight cycle about LD 12:12).

Experiment 8. Effect of photoperiod, temperature, hypoxia, and ferric EDTA

In this series, juveniles subjected to each treatment, except the hypoxic condition, were younger than those used in the experiments described above ; they measured 2-3 mm compared to 5-7 mm as previously. A matrix of two photoperiods, LD 24:0 and LD 12:12, and three temperatures, 16, 21-24 (average 22 °C), and 24-27 °C (average 25 °C), was used to analyze interactions between these two factors. In addition, treatments to test for the effects of hypoxic stress and ferric EDTA were added to the design. Hypoxia was tested by acclimating the juveniles to 4 mg/ml oxygen concentration for 4 days before decreasing to levels of 1.8 mg/ml of oxygen. This severe hypoxic stress was applied to 5 to 7 mm juveniles in order to avoid the slow growth and high mortality that occur at very low oxygen levels. Ferric EDTA (30 mg) was added to three replicates after each water change. Eighty juveniles were used in all low oxygen and ferric EDTA boxes which were exposed to a photoperiod of LD 24:0 at room temperatures around 22 °C.

Experiment 9. Interaction between salinity and temperature

The interaction between two salinities (30 ‰ and 120 ‰) and two temperatures (20 °C and 28 °C) was tested. For each treatment which was replicated thrice 200 5 mm long juveniles were used. The photoperiod for all treatments was LD 24:0.

Experiment 10. Interaction between photoperiod and salinity

Two photoperiods (LD 24:0 and 12:12) combined with two salinities (30 ‰ and 120 ‰) were tested. Each treatment was replicated thrice and 200 5 mm long juveniles were placed in each 1 l box. The temperature for all treatments was 16 °C.

Statistical analysis

RxC tests of independence using the *G* statistic (Sokal and Rohlf, 1981) and analysis of cross-classified categorical data (ACCCD) were used to test results in the form of absolute numbers of cyst *versus* larval broods in each treatment and replicate (Fienberg, 1981 ; Sokal and Rohlf, 1981). These methods were chosen over standard ANOVA because the data are not normally distributed with equal variance even after angular transformation of percentages. Arcsine square-root transformation was performed on percentages when these were averaged for the presentation of treatment means.

Results

Cyst formation as a function of brood number

The percentage of oviparity increases from an overall average of 33 % in the first brood to 65-85 % in succeeding broods (Fig. 1). Statistical analysis shows the effect of brood number on oviparity to be highly significant (G=169.69, df=2, $p \ll 0.005$). Consequently, maternal age influences the mode of reproduction. By analyzing the proportion of oviparity by date of first maturity, it is also clear that females maturing early tend to be more ovoviviparous than slower maturing ones. Less than 15 % of females maturing by 18 days are oviparous compared to at least 50 % oviparity among females maturing between 20 and 27 days (Fig. 2).

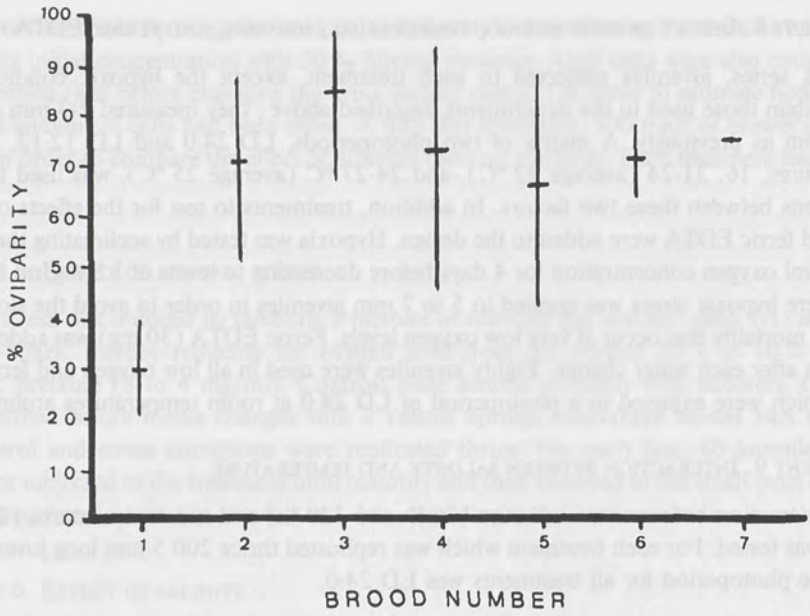

FIG 1. Percentage of oviparous female *Artemia* in relation to brood sequence.

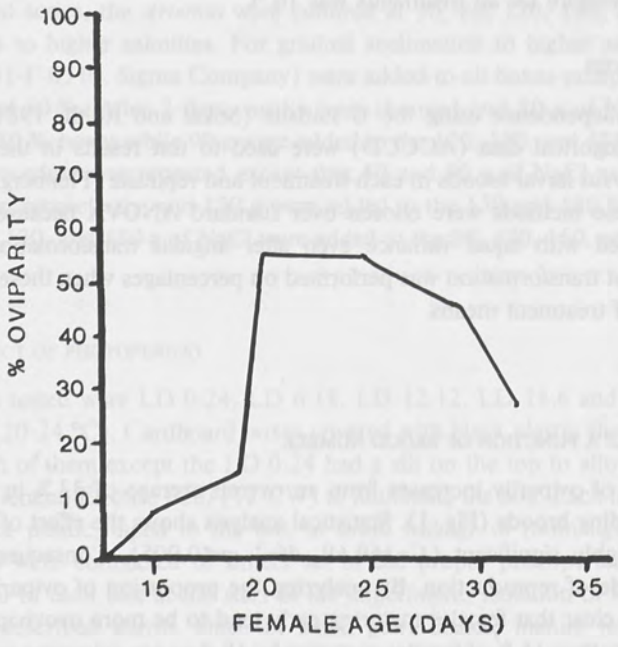

FIG. 2. Percentage of oviparous female *Artemia* in relation to time of maturity.

EFFECT OF VESSEL TRANSFER ON CYST FORMATION

Increased oviparity in the second and later broods is apparently not triggered by transfer of females to small petri dishes. The percentage of oviparity for females that remained in mass culture until the second brood of eggs had matured in the uterus (85 %) is nearly identical to the percentage of second brood oviparity in females transferred to small petri dishes before hatching of the first brood (83 % ; G=5.10, df=2, $0.1<p<0.05$). In keeping with the results of Experiment 1, only 46 % of first brood from females isolated at first maturity (Boxes A and B) are encysted (Fig. 3). Differences in proportion of oviparity among the first scored broods in treatments A and B and the first scored (second) broods in treatment C are highly significant (G=40.75, df=2, $p \ll 0.005$) as expected.

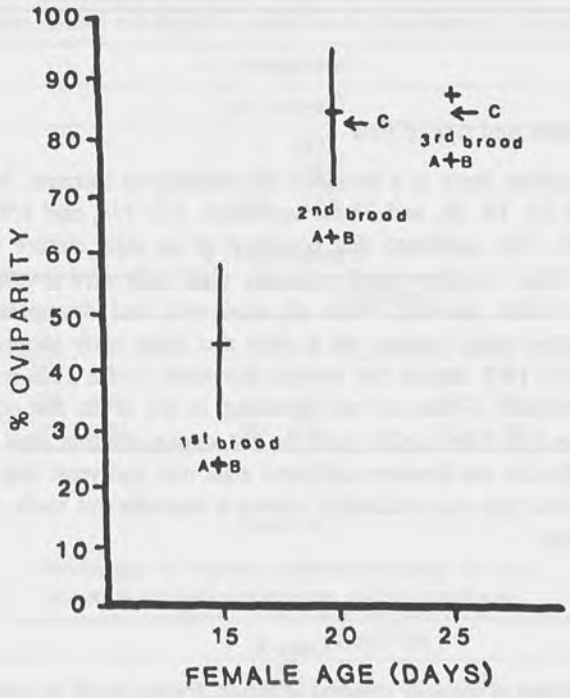

FIG. 3. Comparison of percentages of oviparous female *Artemia* held in 1 l boxes (A+B) with those transferred to 25 ml petri dishes (C) in relation to brood sequence.

EFFECT OF VARIOUS ENVIRONMENTAL FACTORS ON CYST FORMATION

Effect of density

Mean percentages of oviparity are 29, 21, and 41 % for the 50, 100, and 200/l *Artemia* densities, respectively (Table I). The increase in oviparity is not proportional to the increase in

density, but the high value at the lowest density could be due to smaller sample sizes. Overall, density has a significant effect on reproductive mode (G=15.17, df=2, $p \ll 0.005$ for the pooled replicates).

TABLE I

Percentages of oviparity observed in female *Artemia* reared to maturity under three density conditions (number of sampled females in parentheses)

Density (ind./l)	Replicates			
	1	2	3	Total
200	41 (80)	43 (69)	40 (96)	41 (245)
100	38 (48)	15 (41)	6 (32)	21 (121)
50	37 (19)	29 (17)	17 (12)	29 (48)

Effect of food availability and type of food

As food becomes scarce, there is a tendency for oviparity to increase. Mean percentages of oviparous females are 23, 19, 28, and 33 for undiluted, 1/2, 1/4, and 1/8 diluted algal diets, respectively (Table II). The undiluted diet consisted of an algal culture of about 1 000 000 *Tetraselmis* cells/ml. Prior to culture media changes, algal cells were always present in the first two treatments (20-50 000 cells/ml) while all algal cells had disappeared in the two last treatments. On the other hand, females fed a yeast diet since early nauplius stage are mostly ovoviviparous with only 13 % having cyst broods compared to the 23 % in the undiluted algal diet treatment. Nevertheless, G-tests are not significant in any of the diet comparisons. Pooling replicates, G values are 2.56 (df=3, $p<0.1$) for the test among different algal concentrations, and 1.09 (df=1, $p<0.5$) for the test between undiluted algal diet and yeast diet, respectively. Thus, the hypothesis that food type and availability influence reproductive mode is rejected for these experimental conditions.

TABLE II

Percentages of oviparity observed in female *Artemia* reared to maturity on four concentrations of algal diet and one yeast diet

Diet	Replicates			
	1	2	3	Total
Algal conc.				
1/1	30 (20)	10 (20)	31 (13)	23 (53)
1/2	39 (18)	14 (22)	6 (17)	19 (57)
1/4	16 (25)	43 (23)	24 (25)	27 (73)
1/8	20 (15)	37 (19)	38 (13)	32 (47)
Yeast conc.	21 (33)	13 (16)	4 (26)	13 (75)

Effect of hypoxia and ferric EDTA

Hypoxic conditions do not induce cyst formation in Experiment 5 as slightly fewer oviparous first broods are produced in this hypoxic treatment (11 %) compared to the control (15 %, Table III). Results from a second comparison of hypoxic and normal air treatments made in Experiment 8 are also presented in Table III. Compared to the appropriate normal air control (the control in Table IIIB is the same as line 1 from Table IV), the hypoxic treatment does show a significantly larger proportion of cyst broods, 65 % *versus* 48 % (G=6.87, df=1, $p<0.01$). Ferric EDTA also induces a larger proportion of cyst broods (74 %) compared to the same normal air control (48 %) (G=16.17, df=1, $p \ll 0.005$).

TABLE III

Percentages of oviparity observed in female *Artemia* reared under normal and hypoxic conditions in Experiments 5 (A) and 8 (B)

A : Experiment 5

Brood	Air (normal)	Air/N_2
1	15 (47)	11 (53)
2	71 (28)	95 (20)
3	100 (5)	100 (8)

B : Experiment 8

Treatment	Replicates			
	1	2	3	Total
Control	35 (26)	58 (36)	47 (45)	48 (107)
10 % O_2	63 (40)	47 (34)	84 (38)	65 (112)
Fe EDTA	73 (37)	68 (38)	82 (38)	74 (113)

TABLE IV

Percentages of oviparity observed in female *Artemia* reared to maturity under seven salinity conditions

Salinity (‰)	Replicates			
	1	2	3	Total
Series 1				
15	17 (6)	50 (4)	67 (6)	44 (16)
30	25 (8)	50 (6)	100 (1)	40 (15)
45	36 (14)	13 (8)	13 (8)	23 (30)
80	0 (11)	0 (12)	0 (12)	0 (35)
Series 2				
30	12 (26)	17 (29)	52 (31)	25 (76)
80	17 (18)	46 (13)	17 (24)	24 (55)
120	8 (39)	10 (30)	3 (29)	7 (98)
150	4 (23)	7 (15)	7 (28)	6 (66)
180	7 (15)	4 (27)	0 (11)	4 (53)

Effect of salinity

In the first series of salinities in Experiment 6, oviparity decreases from 44 to 40 to 23 to 0 % as salinity increases, respectively, from 15 to 30 to 45 to 80 ‰ (Table IV). In the second series a similar trend of decreasing oviparity appears with percentages of cyst broods ranging from a high of 25 down to 4 % for increasing salinities from 30 to 180 ‰ (Table IV). In both series of experiments, G values show a highly significant effect of salinity upon reproductive mode (G=22.48, df=3, $p \ll 0.005$ and G=24.73, df=4, $p \ll 0.005$ for series 1 and 2, respectively).

Effect of photoperiod

High levels of oviparity are triggered by photoperiods with 12 h or less of light in Experiment 7. From 70 to 93 % cyst broods were produced at these shorter photoperiods compared to about 31 % at longer photoperiods (LD 18:6 and 20:4, Table V). These treatment effects are highly significant (G=261.77, df=4, $p \ll 0.005$).

TABLE V

Percentages of oviparity in female *Artemia* reared to maturity under five photoperiod regimes

Photoperiod	Replicates			
	1	2	3	Total
LD 0:24	65 (20)	74 (34)	67 (15)	70 (69)
LD 6:18	98 (47)	100 (40)	100 (31)	99 (118)
LD 12:12	93 (44)	97 (33)	91 (44)	93 (121)
LD 18:6	47 (30)	22 (45)	34 (35)	30 (120)
LD 24:0	30 (30)	33 (39)	32 (28)	32 (97)

Effect of photoperiod and temperature

Responses to photoperiod and temperature are not independent in Experiment 8 (Table VI, G=100.16, df=7, $p \ll 0.005$). At low temperature (16 °C), the effect of the photoperiod is strong (G=74.15, df=1, $p \ll 0.005$) ; females are mostly oviparous under LD 12:12 (87 %) while they are mostly ovoviviparous under LD 24:0 (13 %). At medium temperature (22 °C), this effect is still present but it is not as pronounced 68 % *vs* 48 % ; G=8.83, df=1, $p \ll 0.005$). At higher temperature (25 °C) the trend is reversed, with the LD 12:12 treatment producing an overall 42 % cyst broods while the LD 24:0 treatment yields 49 % cyst broods, but the difference is not significant (G=0.68, df=1, $p < 0.1$). The effect of temperature is stronger at LD 12:12 than at LD 24:0 (G=64.93, df=2, $p \ll 0.005$ and G=21.83, df=2, $p \ll 0.005$, respectively). Primarily because of the smaller sample size in the 16 °C, LD 24:0 treatment, none of the eight models tested by ACCCD could be fitted to the overall observed frequency matrix.

Effect of salinity and temperature

Oviparity is again promoted by lower salinity in Experiment 9. At least one third of the *Artemia* females maturing at a salinity of 30 ‰ are oviparous while only 7-8 % of encysted broods are produced at a salinity of 120 ‰ (G=91.36, df=4, $p \ll 0.005$). Temperature has a slight effect at

TABLE VI

Percentages of oviparity observed in female *Artemia* reared to maturity under all combinations of two photoperiods and three temperatures

Temp. (°C)	Photop.	Replicates 1	2	3	Total
16	LD 24:0	5 (20)	21 (14)	15 (13)	13 (47)
	LD 12:12	76 (17)	88 (32)	92 (34)	87 (83)
22	LD 24:0	35 (26)	58 (36)	47 (45)	48 (107)
	LD 12:12	70 (37)	71 (31)	63 (32)	68 (100)
25	LD 24:0	68 (25)	44 (27)	33 (24)	49 (76)
	LD 12:12	33 (33)	42 (31)	51 (35)	42 (99)

the lower salinity (44 % *vs* 33 % cyst broods produced at 20 °C and 28 °C respectively ; Table VII) but no effect at higher salinity (8 *vs* 7 % cyst broods produced at 20 °C and 28 °C). ACCCD allows rejection of all models in which the three factors, reproductive mode, temperature and salinity are each independent from each other or from pairwise interactions involving the other two factors ; best fit is to a model of pairwise interactions among all these three factors.

TABLE VII

Percentages of oviparity observed in *Artemia* females reared to maturity under the following combinations of temperatures and salinities

Treatment		Replicates 1	2	3	Total
20 °C	30 ‰	53.8 (80)	18.2 (55)	54.4 (57)	43.8 (192)
	120 ‰	7.7 (78)	9.9 (71)	6.9 (29)	8.4 (178)
28 °C	30 ‰	19.0 (42)	38.9 (36)	41.7 (36)	32.5 (114)
	120 ‰	7.7 (26)	5.4 (37)	8.3 (48)	7.2 (111)

Effect of salinity and photoperiod

The rate of oviparity is very low in all treatments of Experiment 10, below 11 % (Table VIII). Average percentages of oviparous broods are 10 % and 5 % at salinities of 30 and 120 ‰, respectively, and the photoperiod LD 12:12 induces more cyst broods (8 %) than constant light (3 %) at 120 ‰ salinity. However, reproductive mode, salinity and photoperiod are completely independent in the ACCCD.

TABLE VIII

Percentages of oviparity observed in *Artemia* females reared to maturation under the following combinations of salinity and photoperiod

Treatment		Replicates 1	2	3	Total
LD 24:0	30 ‰	15.2 (33)	2.7 (37)	10.7 (28)	9.2 (98)
	120 ‰	3.6 (84)	5.0 (60)	0 (49)	3.1 (193)
LD 12:12	30 ‰	7.5 (40)	9.4 (32)	15.2 (46)	11.0 (118)
	120 ‰	10.3 (78)	5.1 (79)	8.5 (47)	7.8 (204)

Discussion

From this study of physical and biological factors affecting the mode of reproduction in the brine shrimp *Artemia* four factors are found to strongly influence oviparity : brood number, photoperiod, salinity, and temperature. Density and oxygen levels have lesser effects while vessel transfer and food availability and type have no demonstrable effects. All of these factors have previously been considered by various authors to influence the mode of reproduction.

The factor previously thought to have a major influence in cyst induction, hypoxia, gave contrary results in Experiments 5 and 8. In the first experiment, lack of response might have been due to an insufficient time of exposure to the hypoxic stress or to application of the stress after a developmentally critical time (stage ?) for oviparity determination. Other experiments (Berthélémy-Okazaki, 1986) suggest that this critical time is during the late juvenile stage, just before the ovaries become visible under a dissecting microscope, when the females are about 6-7 mm long. Dutrieu (1960) linked hypoxia, hemoglobin synthesis, and hematin excretion by the dark shell gland in cyst producing females and concluded that eggs are induced into dormancy when they are in the uterus. In her experiments, hypoxia was not the only factor involved as she noticed that females fed yeast were ovoviviparous even under hypoxic conditions. Baker (1966) saw no effect of hypoxia. Sorgeloos *et al.* (1975) found an effect of hypoxia in females adapted to oxygen concentration of 2 mg/l. However, as discussed below, hypoxia might trigger oviparity through metabolic changes rather than by a direct effect.

Since cysts are adapted to the desiccation brought about by solar drying of natural habitats, increasing salinity brought about by evaporation might act upon the adult as a signal for oviparous reproduction (Sorgeloos *et al.*, 1975). Nevertheless, we find the reverse of what one expects, females are strongly ovoviviparous in brine. Also, as mentioned already, high salinity is the only factor able to maintain ovoviviparity after the first brood. No previous experimental study of the effect of salinity on *Artemia*'s mode of reproduction is known to us, but several authors report increased cyst production at lower salinities in natural habitats. *Artemia* females from San Francisco Bay transplanted into Lake Grassmere, New Zealand, were predominantly ovoviviparous at salinities ranging from 80 to 260 ‰ (Wear, pers. commun.). Amat (1982) found increased ovoviviparity with rising salinities in *Artemia* from Great Salt Lake, Utah, USA. In contrast, Perez-Rodriguez (pers. commun.) correlated cyst formation in *Artemia* from Spain with high salinities (130 ‰ to 175 ‰) and Ahmadi (pers. commun.) noticed increased oviparity associated with high salinities in *Artemia* populations from Lake Urmia (Iran).

Temperature, another factor present during the summer desiccation might also trigger oviparity. In this study, its influence was strongly modified by salinity and photoperiod. No other experimental data concerning the role of this factor are known to us.

Dutrieu (1960) first mentioned the effect of food type on cyst formation. In her cultures, *Artemia* raised on a yeast diet were mostly ovoviviparous while others fed algae had cyst broods. In our study, females fed on a yeast diet also tended to produce larval broods but not significantly more often than algal-fed females. Moreover, in another experiment, *Artemia* raised solely on yeast since early juvenile stages and placed under photoperiods of LD 12:12 and 24:0 had respectively 80 and 5 % cyst broods (unpubl. data). Thus, a yeast diet can support oviparity under some circumstances. Iron (ferric EDTA) in the diet, on the other hand, does stimulate cyst formation as previously described by Bowen *et al.* (1969). This would appear to support Dutrieu's (1960) hypothesis that excretion of hematin by the shall gland induces dormancy in

ovisac eggs, but better experimental controls are needed before the ferric EDTA effect can be attributed solely to the ferric iron. Food scarcity does not significantly increase oviparity in our study. Although Vu Do Quynh (pers. commun.) suggests that starvation caused a sudden increase in oviparity in a Macau-Brazilian population, crowding may have been the underlying cause in this case. Our study shows that significantly more cyst broods are produced in crowded conditions. In natural habitats, density is likely to be a confounding factor determining mode of reproduction.

The effect of photoperiod is very strong in Experiments 7 and 8 ; at lower temperatures below 25 °C, short daylight cycles induce oviparity. Provasoli and Pintner (1980) also describe a similar photoperiod effect in *Artemia*. In Experiment 9, however, LD 12:12 does not induce significantly more oviparity than LD 24:0, the only known differences in protocol between Experiments 7 and 8 and Experiment 9 were the algal diets, *Tetraselmis* and *Actin* in 7 and 8 and *Dunaliella* in 9, respectively. Whether diet, like temperature, mediates the effect of photoperiod awaits further study. Photoperiod or interaction of photoperiod with other environmental factors like temperature and diet influences mode of reproduction and diapause in other microcrustaceans. Stross and Hill (1968) demonstrated a relationship between short photoperiod and production of ephippial eggs in *Daphnia pulex*. Marcus (1982) found that in the copepod *Labidocera aestiva*, short photoperiod is a primary stimulus to diapause. In *D. magna*, an interaction between photoperiod, water quality, temperature, food availability and density influence diapause, but photoperiod is not a predominant factor (Bunner and Halcrow, 1977).

Browne (1980) reported an effect of brood number on cyst formation, with oviparity rising from about 10 to 60 % between the third and the 15th broods depending on the geographic origin of strains. In his study, the San Francisco Bay population reaches a peak of 50 % at the seventh brood while the Puerto Rican population reaches 55 % oviparity at the third brood, making its profile the most similar to the one found in this study. The difference in response between Browne's San Francisco population and the one studied here may be due to slight genetic differences, owing to differences in sampling time and location, or more probably to differences in rearing procedures. The experimental procedure of transferring females from mass culture to 25 ml petri dishes apparently does not provide this trigger since females remaining in the larger container also switch predominantly to oviparity at the second brood (see Fig. 3). Such a persistent ontogenic pattern in the mode of reproduction suggests that oviparity is triggered with greater ease at a certain stage of development by environmental factors. When the percentage of oviparity in the first brood is plotted against time (Fig. 2), females maturing later tend to be more oviparous than fast maturing ones. Females genetically predisposed to grow and mature faster might also be predisposed to ovoviviparity.

Having reviewed the results of laboratory studies of factors affecting the mode of reproduction in *Artemia*, can we now explain reproductive patterns in natural or man-made brine shrimp habitats ? Perhaps the primary lesson of the experimental studies is that no single physical or biological factor controls reproductive mode. It seems untenable, for example, to maintain that hypoxia alone induces oviparity in the field when maternal age, salinity, temperature, photoperiod, and their interactions have greater and more consistent effects on the mode of reproduction in the laboratory. At the same time, inextricable confounding of the numerous factors and interactions among factors shown by laboratory studies to influence reproductive mode makes prediction of reproductive patterns in the field virtually impossible.

Contradictions between laboratory results and field observations are perhaps irresolvable in the absence of more precise field ecological data. For example, the photoperiod-temperature interaction observed in this study suggests that cyst production ought to be occurring year-round in San Francisco Bay. High oviparity rates in the laboratory are associated with cool temperatures and daylight cycles shorter than 12 h (fall, winter and spring) and with warm temperature and long daylight cycles (summer). High salinities, however, ought to inhibit oviparity regardless of photoperiod-temperature conditions. To the extent that hypoxia occurs seasonally in natural or man-made brine ponds, these expectations may contrast with the predictions of the Dutrieu-Sorgeloos hypothesis that hypoxia alone is sufficient to induce cyst production. Spot checks of adult *Artemia* purchased twice weekly by our laboratory from a San Francisco supplier show that, contrary to our expectation, most females carry larval broods during fall, winter, and spring and about 25 to 35 % are oviparous during summer. We cannot evaluate the reason for this discrepancy without data on pond temperatures, salinities, oxygen concentrations, and brine shrimp densities and demographics. Interestingly, Swarth *et al.* (1982) did observe year-round occurrence of cysts in San Francisco Bay ponds, although this result is also unexpected since salinities were reported to range from 64 to 140 ‰, conditions under which most of the females are ovoviviparous in the laboratory. In the related Mono Lake population of *Artemia* benthic cysts are produced in the fall (short days, cool temperature) for overwintering (Winkler, 1977 ; Dana, 1981).

A more promising alternative to field study of reproductive ecology as an approach to understanding the control of reproductive mode in *Artemia* may be studies that can synthesize, at the physiological or biochemical level, the often contradictory data on the diverse factors affecting mode of reproduction. An often-voiced theme in the *Artemia* literature is that oviparity is somehow related to stress in the female brine shrimp. The main effects of hypoxia, temperature and density on rate of oviparity fit this intuitive notion. The inverse correlation of salinity with oviparity appears problematic, but brine shrimp may have evolved physiological adaptations to higher salinities in order to escape predators, competition and disease organisms prevalent in normal seawater. Maternal age, photoperiod and certain interactions amongst factors are not at present obviously related to physiological stress. Of course it may be that sudden change in environment is a more significant source of stress than the steady state condition of any of the particular factors thus far considered to influence reproductive mode (Ballardin and Metalli, 1963).

If stress, regardless of its cause, does initiate a common physiological pathway to the induction of oviparity, then we should look for physiological or biochemical correlates of environmental stress. Metabolic acidosis might be such a candidate. Sudden hypoxic shock causes accumulation of large quantities of lactic acid in *Artemia* (Vos *et al.*, 1979) which probably lowers the pH of the hemolymph. Sudden salinity increases also trigger the synthesis of large quantities of acids in the nauplius ; the pH in the external medium can drop by more than one unit due to lactic acid secretion (Conte *et al.*, 1980). Some foods have a known acidifying effect (*e.g.* vitamine C) and certain types of algae rich, for example, in fatty acids, could induce transient acidosis ; the unexplained failure to observe short day induction of oviparity in Experiment 9 may have been due to the use of a *Dunaliella* diet rather than the *Tetraselmis* diet in the previous experiments. The density effect could be due to the accumulation of metabolic wastes which acidify the external medium. Heavy metabolic demands, made on the females during vitellogenesis of the first brood, might also cause changes in pH which could in turn trigger the encystment of the following

broods. More studies are needed to test this hypothesis and to understand the role of factors like photoperiod that are not seemingly related to stress. Indeed, the photoperiod effect suggests the role of a hormone in cyst induction in addition to a stress-related mechanism.

Control of reproductive mode has been accomplished in intensive aquaculture systems (Lavens and Sorgeloos, 1984), but cyst production in large ponds is still not assured. Salinity is the only factor that might be manipulated in a pond habitat, and our results suggest that maintaining salinity near to that of normal seawater (30 ‰) might increase cyst production. Knowledge of the physiology of cyst induction might provide the means to trigger oviparity reliably in extensive pond culture systems.

Conclusion

The primary result of this experimental study of the effect of environmental factors on the mode of reproduction in *Artemia* is that no single factor such as hypoxia controls the switch from ovoviviparity to oviparity. In all but highly saline conditions, female brine shrimps tend to be ovoviviparous for the first brood and then to switch to oviparity by the third brood. High salinities, above 120 ‰, inhibit cyst production. Photoperiod has a strong effect (short days induce oviparity) but its influence diminishes with increasing temperature and salinity. Brine shrimp density and in some but not all cases, hypoxia, promote oviparity, but neither isolation of maturing females from mass culture nor food availability and type has a demonstrable effect. The failure of laboratory results to explain satisfactorily the patterns of reproduction in the field is perhaps not surprising given the number of factors involved and their confounding in poorly described natural or man-made habitats. Study of the physiological basis of the induction of oviparity, particularly analysis of stress-related metabolism, may provide greater insight into the control of reproduction mode in this important species.

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Life history of an experimental Great Salt Lake *Artemia* population kept in outdoor culture

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Abstract

Aiming to verify several aspects of *Artemia* biology according to common variations occurring in natural hypersaline environments, an experimental brine shrimp population was cultured in a 5 m^3 tank outdoors. A Great Salt Lake, Utah (USA) bisexual strain was chosen in order to compare the present results with former ones obtained with Mediterranean autochtonous strains.

The influences of salinity, temperature, and microalgal food-availability on the population's evolution were studied for 1 year. Induction and holding of both reproductive patterns : oviparity and ovoviviparity were especially focused, using *Tetraselmis* and *Phaeodactylum* as food, as well as introducing mass cultured halo-tolerant algae such as *Asteromonas* isolated from natural brines.

The possibility of collecting cysts during a long period of time and under various environmental conditions affecting the experimental population, allowed to study the effect of the environment on cyst hatchability.

Introduction

Although brine shrimp *Artemia* has been one of the main test organisms for a wide variety of biological laboratory experiments in physiology, genetics, biochemistry, toxicology, radiobiology, etc., the scarce information available on the relationships between *Artemia* and its natural environment is astonishing. Ecological research and field work on hypersaline ecosystems are not abundant, resulting in a poor knowledge of *Artemia* responses to environmental changes.

To improve this situation two former projects were further developed with Mediterranean strains involving bisexual and parthenogenetic diploid ones. Their inter-relationships and the influence of environmental parameters were examined, initially through the study of laboratory populations. These populations started with nauplii hatched from cysts collected in salterns for 2 years during different seasons. Once grown to adults, population compositions were determined for both strains (Amat, 1983). The latter work dealt with the composition variations displayed by broader populations kept in an outdoor tank for 2 years. This allowed exposure to the intense influence of climatic conditions as happens with wild populations (Amat, in prep.).

The results obtained from both experiments allowed to draw fair explanations of cyclical changes, involving competition and coexistence between bisexual and parthenogenetic populations, assumed to occur in the wild ; their relationship with the reproductive patterns of oviparity and ovoviviparity developed by these strains in a given moment ; and the influence of environ-

mental factors such as temperature, salinity, food availability, etc. Lateron the results and conclusions were verified in experimental tanks arranged in salterns, where populations from both strains were introduced.

In order to obtain similar information on another *Artemia* species, different from the Mediterranean ones, it was decided to perform the same experiments with the Great Salt Lake *Artemia* because lately much research has focused on this strain, from elementary biometrics to the evaluation of the nutritional qualities of nauplii used in aquaculture.

This test demonstrates the feasibility of holding a Great Salt Lake *Artemia* population during an entire year under Mediterranean climatic conditions. The population displayed both reproductive patterns according to the environmental changes, which allowed to draw some conclusions on the triggering and persistence of these basic mechanisms.

Materials and methods

The experimental population was kept in a round 5 m^3 tank located outdoors. Phytoplankton blooms of *Tetraselmis* and *Phaeodactylum* were enhanced and sustained through periodical checks of the temperature, salinity, cell density, N/P ratio, and oxygen, as previously described (Amat, in prep.).

The initial population was held from the end of September 1983 until November 1984, although the difficulties arisen during May 1984 forced us to end the first trial and to start a second one. The first period, autumn 1983-spring 1984, began after inoculation performed on September 30, 1983, and lasted until May 21, 1984. Then the tank was emptied, cleaned, and prepared for the next period, summer-autumn 1984, starting with inoculation on June 4, 1984 and ending on November 23, 1984.

Nauplii were hatched from a commercial sample (Sandtech) originating from the Great Salt Lake, and released in the tank at an initial density of 10 nauplii/l. After 15 days, at a density of 6 individuals/l, most animals of the population had developed to adults. Of the females 30 % displayed ovulation at a mean size of 7.70 mm. This fast development was favoured by suitable environmental conditions, namely a temperature between 23 and 25 °C, a maximal salinity of about 42 ‰, and plenty of microalgal food available.

From then on, the population density and composition were checked weekly. The individuals samples were sorted by sex, and the females sorted again according to their reproductive pattern (oviparity or ovoviviparity). Fecundity rates were calculated after counting the offspring contained in the uterus of anaesthesized females. Nauplii and metanauplii present in the sample were also estimated.

Cysts were collected periodically from the moment they accumulated on the tank wall. They were processed by the method of differential buoyancy in strong brine and distilled water (Amat, 1985b) in order to separate full cysts and cyst shell fractions. Once sundried and oven-dried (40 °C) they were stored until used in hatching efficiency tests (Amat, 1985b).

The phytoplankton culture enhanced in the experimental tank could be well maintained during the first period, but it later failed, owing to the extensive settlement of dipteran larvae (Ephydridae and Chironomidae) on the bottom, and the uncontrollable proliferation of the macroalga *Enteromorpha* on the walls.

Because of temperature and salinity increases during the second period, it was impossible to keep adequate phytoplankton levels of *Tetraselmis* and *Phaeodactylum*. Therefore a mixture of the halo-tolerant species *Dunaliella* and *Asteromonas* was added to the tank every 3 to 4 days. These algal cultures were set up separately from the experimental *Artemia* tank but were supplied with water pumped from the *Artemia* tank after proper filtration.

Results and discussion

The population evolution is described in the histograms of Fig. 1 and 2 for the first period autumn 1983-spring 1984, and in Fig. 3 for the period summer-autumn 1984. The narrow columns show the population composition, the black portion referring to partial percentage of adult and juvenile individuals, and the white portion to nauplii and metanauplii. The wide columns show the composition of the adult stage with the white portion representing the ovoviviparous females, the dotted portion the oviparous ones, and the striped portion the males.

The numbers next to the arrows indicate the date and amount of harvested biomass (wet weight). The numbers on the top of the columns in Fig. 1 refer to the total biomass present in the tank. Under the x-axis the values of the main environmental parameters are given : temperature, salinity, and phytoplankton availability. Systematic data on oxygen concentration are not given but the oxygen levels always exceeded 80 % saturation, except during August, 1984, when between 58 and 70 % saturation was recorded.

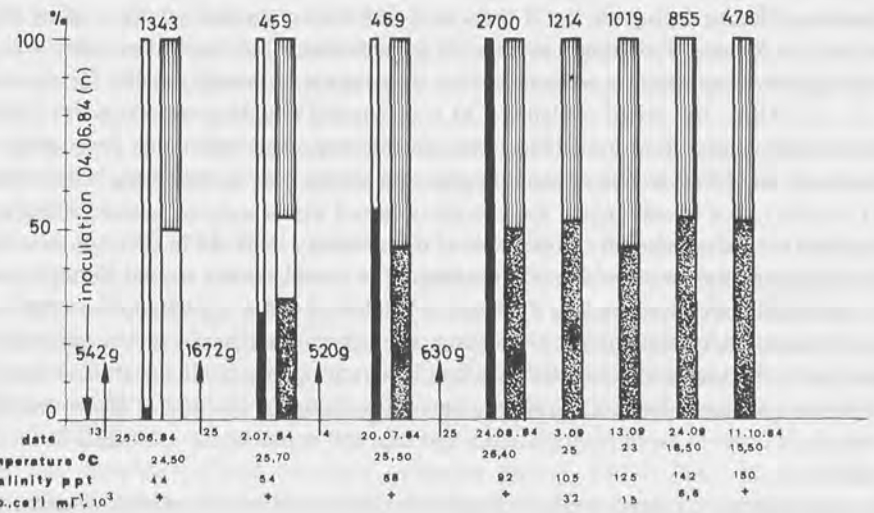

FIG. 1. Histogram showing the first period (October 1983-May 1984) evolution of the Great Salt Lake *Artemia* population (first part). The narrow columns show the general population composition : black part adults and subadults, white part % nauplii and metanauplii. The wide columns show the adult population composition : white part % of viviparous females, dotted part % of oviparous females, striped part % of males. Numbers on arrows : biomass harvests (g wet weight). Number on top of columns : biomass present in the tank (g wet weight).

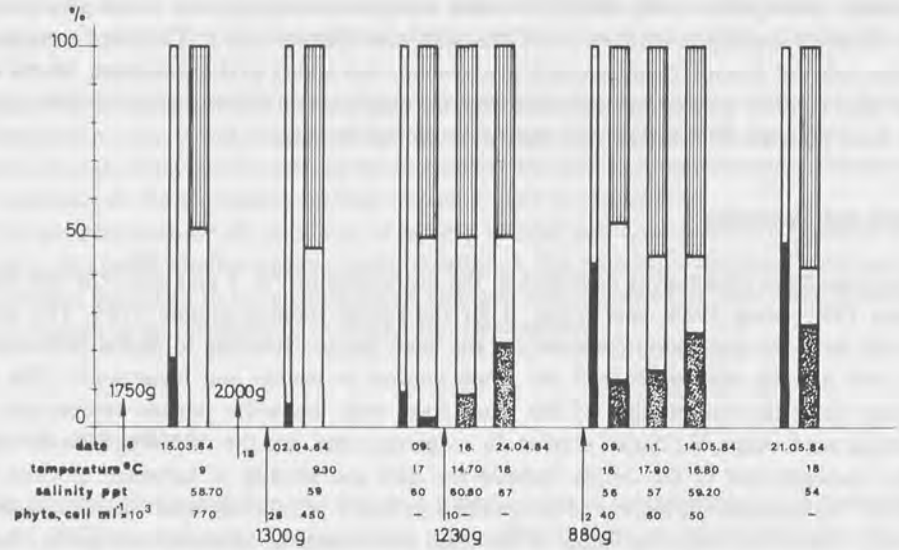

FIG. 2. Histogram showing the first period (October 1983-May 1984) evolution of the Great Salt Lake *Artemia* population (second part) (same indications as in Fig. 1).

Population checks during the last 3 months of 1983 show surprisingly high values (62-70 % of oviparity in females. Prevailing oviparism in females which just reached maturity is not usual even though environmental conditions in this experiment appeared suitable for ovoviviparity (Fig. 1, 25.10.83), the actual ovulating (30 % of females ovulating on 13.10.83) could have coincided with scarce food availability, because this happened when the *Tetraselmis* culture lessened and the *Phaeodactylum* bloom began, in a similar way as described before (Amat, in prep.). A short time of suboptimal food levels occurred which induced marked oviparity.

The effect on the population composition of this oviparity is shown in the high levels of adult individuals during the entire month of November. The weather in the second fortnight was quite rainy, the salinity decreased causing the cysts to hatch in the tank („subitaneous" cysts ?) which increased nauplii recruitment in the population. The population composition changed drastically in December, with low levels of adult individuals and a strong increase in nauplii and metanauplii. The biomass increased markedly making a harvest necessary at the end of December (2 800 g wet weight) in order to avoid phytoplankton shortage and consequently a decrease in the *Artemia* population.

The coexistence of oviparity and ovoviviparity under the same environmental conditions (64 % and 36 % of females respectively, on 09.12.83) during the last months in 1983 enabled their quantification. Again higher fecundity rates in ovoviviparity than in oviparity (Fig. 4) were recorded. Despite the former biomass harvest, it was impossible to avoid phytoplankton depletion what, together with low temperatures during the first days of January, caused some mortality and lessened the population markedly. A new mass inoculation of *Phaeodactylum* entailed a new bloom, resulting in an optimal algal availability until April 1984.

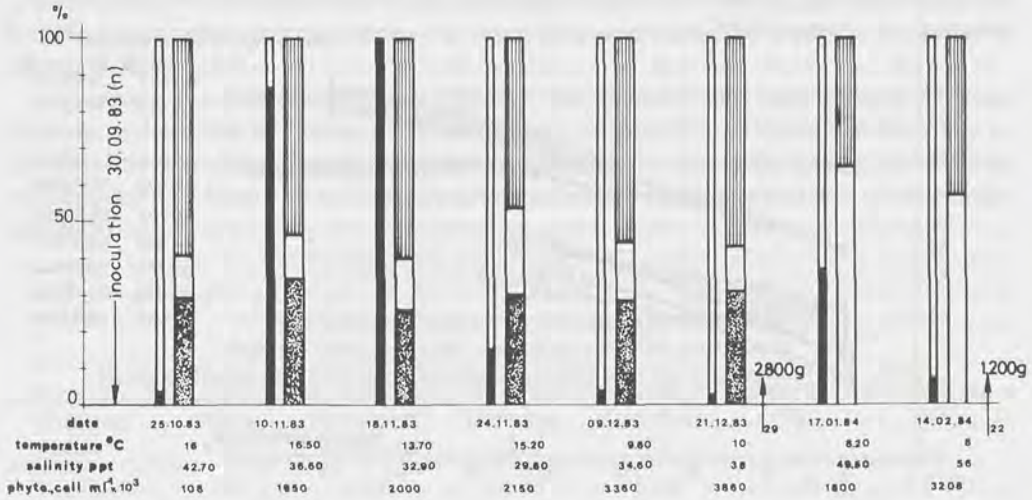

FIG. 3. Histogram showing the second period (June-November 1984) evolution of the Great Salt Lake *Artemia* population (same indications as in Fig. 1).

Fig. 1 and 2 show that the reproductive pattern changed entirely after the population weakened, with a prevalence of ovoviviparity in females during this low temperature and high food availability period. This gave relatively low adult levels and new naupliar recruitment through ovoviviparous offspring. The estimation of this ovoviviparity is also shown in Fig. 4 (control on 14.02.84). Its fecundity rate deserves to be compared with the one formerly obtained and with all subsequent ones (Fig. 4).

From January to April the steady ovoviviparity brought important biomass increases which obliged periodical harvests in order to keep a healthy population. The total harvest amounted to 7 kg wet weight.

At the end of April 1984, under salinities exceeding 60 ‰, the phytoplankton levels were reduced to submit *Artemia* population to continued suboptimal levels of food availability. This led to a progressive oviparity in adult females, attaining 44 % in 2 weeks, and not exceeding 65 % during May. The spring of 1984 (April and May) was, however, abnormally rainy, the salinity did not exceed 60 ‰ which induced the cysts to hatch immediately after being laid by the females. In this season it was impossible to collect viable cysts, only empty shells were harvested (Table I sample of 20.03.84).

During this experimental and provoked oviparous period, 100 to 200 l of mass cultured *Phaeodactylum* were added to the tank every other day, in order to avoid the *Artemia* population to collapse. The troubles caused by the settlement of dipteran larvae and proliferation of *Enteromorpha* necessitated interrupting this experimental period. The tank was emptied and prepared for a population to be developed during the next summer and autumn.

On June 4th, under a suitable *Phaeodactylum* availability, the tank was inoculated with 50 Great Salt Lake nauplii/l. The suitable conditions favoured a fast growth. In the first population check 9 days later, a total biomass of about 816 g (w.wt) mainly consisting of subadult individuals

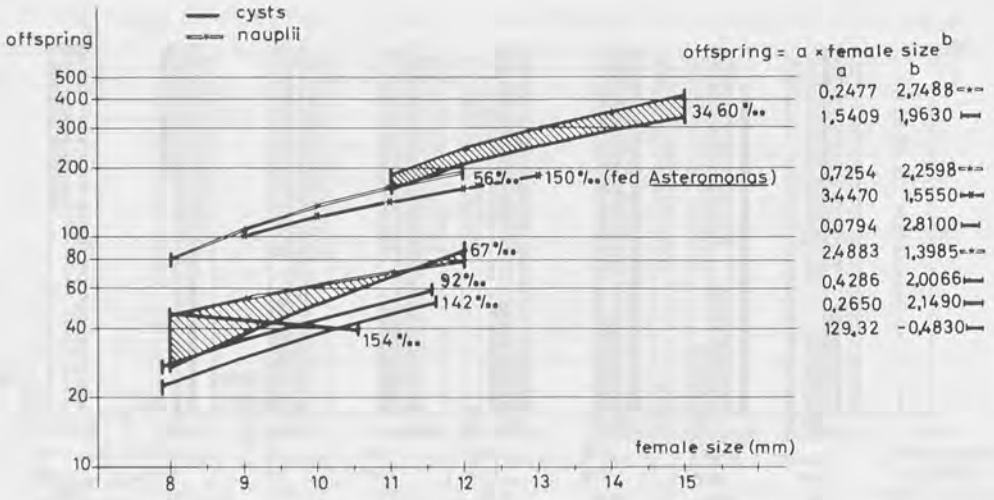

FIG. 4. Graphical and mathematical expression of fecundity rates, viviparity and oviparity in the females of the Great Salt Lake *Artemia* population, size of females (mm), salinity, and food availability.

actively mating was recorded. This allowed to perform a first harvest (542 g). On 25.06.84 (Fig. 3) a population with 3 % adult individuals and 97 % nauplii and metanauplii, meaning 83 % and 7 % wet weight biomass, respectively occurred. All the females were ovoviviparous and the biomass increase was high, and a second harvest of 1 672 g (25.06.84) was carried out.

It is to be emphasized that in this experiment all the females developed ovoviviparity once reproductive maturity was attained, contrary to what happened initially on October 1983. The only environmental difference between both periods was a low food availability when former females ovulated and a higher one in the more recent experiment. The data given under the columns of the histogram in Fig. 1 to 3 may therefore bring confusion. Environmental parameters such as temperature, pH, salinity, and oxygen content during both ovulating periods were very similar.

At the beginning of July, with minimal temperatures over 25 °C and salinities higher than 60 ‰, it was impossible to keep *Tetraselmis* and *Phaeodactylum* cultures in the tank. The food availability became scarce, promoting immediately oviparity. On 11.07.84 oviparous females reached 60 %, and 100 % on 20.07.84. The initial coexistence of both reproductive patterns allowed a new comparative quantification under the prevailing conditions (salinity about 67 ‰ on 12.07.84) (Fig. 4). It was again possible to note a higher fecundity rate for ovoviviparity, which changed in favour of oviparity in females of larger size.

Mass culture of halo-tolerant algae *Dunaliella* and *Asteromonas* was possible under the water conditions in the *Artemia* tank. This allowed us to introduce this algal food once every 2 to 3 days and to attain about 50 000-20 000 cells/ml during the first and second day, respectively. These phytoplankton levels permitted to keep a steady oviparity from then on (Fig. 3). This decreased the biomass production due to scarce nauplii recruitment, and a partial increase in adult individuals, attaining 40 % at the end of August until the end of the experiment.

Cysts began to accumulate on the tank wall and were collected weekly. After processing and a check for full cysts and cyst shells, they were dried and stored to determine the hatching efficiency (Table I).

Considering the persistence of total oviparity, the fecundity rates were checked monthly, allowing to determine its inverse relation to salinity increase. Fig. 4 shows that from July to October, this fecundity evolution proceeded according to the size of the female, the salinities increased from 67 ‰ to 150 ‰, and a progressive decrease in the maximal size of the females was noted.

TABLE I

Main characteristics of the cyst samples collected
from the Great Salt Lake *Artemia* experimental populations.
Hatching efficiencies (HE) were checked 14 months after the first harvest (May 1985)

Collecting date	Salinity (‰)	Dry weight of cysts (g)	Full cysts (%)	Cyst shells (%)	HE_a[1]	HE_b[2]
01.84	50	0.50	64.60	35.40	3.66	273 229
20.03.84	58-60	0.30	–	100	–	–
14-27.07.84	67-76	1.14	74.20	25.80	75	13 333
27.08.84	96	3.42	86.80	13.20	90	11 111
30.08.84	118	0.55	85.30	14.70	39.47	25 333
06-11.09.84	126	12.75	97.30	2.70	99	10 101
21-27.09.84	130-142	9.41	95.80	4.20	59.66	16 761
01-05.10.84	151-153	6.05	94.60	5.40	36.61	27 317
08-10.10.84	163-166	8.10	97.50	2.50	35.87	27 881
18.10.84	174	7.66	100	–	13.38	74.765
31.10.84	186	4.56	100	–	14.76	67 771
06.11.84	181	4.53	94.90	5.10	15.42	64 840
23.11.84	*150	4.11	100	–	6.52	153 282
12.84-01.85	*150				5.77	173 291

[1] HE_a = cyst weight (g)/10^6 nauplii.
[2] HE_b = nauplii/g cysts.
* *Artemia* fed dense *Asteromonas* culture.

The unexpected results obtained on oviparity persistence – with low levels of halo-tolerant microalgae as food – and under high temperature and salinity conditions, could be maintained by the continuous presence of about 1 000 cysts/l in the whole water mass during September. This suspected relationship between oviparity and halo-tolerant algae needed further investigation. So, at the beginning of October, a 0.5 m^3 experimental plastic container was set up with an *Asteromonas* mass culture attaining 1.3 to 1.5 × 10^5 cells/ml, under salinities increasing from 110 ‰ to 150 ‰. Adult and oviparous *Artemia* individuals coming from the experimental tank (154 ‰) were inoculated in this container. These initial individuals attained a mean biomass (w.wt) of about 9.16 mg for females and 8.53 mg for males. At the end of October the population developed in the container – at a temperature of about 17 °C, a salinity of 150 ‰ and an algal density of around 135 000 cells/ml – displayed an exceptional state summarized as follows (Fig. 4) :

– Mean individual size increased markedly compared to the initial one. Females reached reproductive maturity at a larger size, similarly to what happens under low salinity, and attained also a larger maximal size. Under these conditions the individual mean biomass had increased to 16.20 mg for females and 11.90 mg for males (w.wt).

– Oviparous fecundity rates increased markedly (Fig. 4) attaining quantitative values similar to those displayed for ovoviviparity under low salinity (56 ‰ on 14.02.84).

– These fecundity and biomass controls allowed to calculate the ratio biomass wet weight/cyst dry weight. From the particular environmental conditions it was possible to deduce that 60 g biomass (w.wt) were necessary to obtain 1 g dry cysts in a period of 9-10 days. In a bisexual population with a 1:1 sex ratio, and the mean individual biomass given above, this represents about 2 135 *Artemia* couples.

The prolonged oviparous reproduction in this parallel population kept under high *Asteromonas* availability allowed cyst sampling from November until the end of 1984. These cysts were handled as the ones before.

Table I gives information on the cyst samples collected, their total dry weight and relative composition (%) of estimated full cysts and cyst shells. These data clearly show the importance of salinity in attaining high levels of viable cysts. From the end of August 1984 85 % of the cysts harvested in the experimental tank (salinities higher than 90 ‰) were assumed to be viable. Salinities below 80 ‰ are not successful to obtain absolute cryptobiosis, so, under these circumstances, cysts can behave as „subitaneous cysts". Table I also shows the hatching efficiencies for these cysts, expressed according to two widely accepted criteria (Amat, 1983, 1985b ; Vanhaecke and Sorgeloos, 1983) : 1) dry weight of cysts necessary to obtain 1×10^6 nauplii ; and 2) the number of nauplii obtained after hatching 1 g of cysts (dry weight).

The data in Table I show two different responses. High hatching efficiencies were obtained for the January cysts collected in the experimental tank, as well as for the November-December cysts collected in the parallel experimental container with a dense culture of halo-tolerant algae. On the contrary, very low efficiencies were noted for the cysts sampled in the experimental tank between July and November 1984.

From Fig. 1, and the characteristics of the parallel container culture mentioned before, it can be deduced, that the only similarity between both conditions was a high availability of phytoplanktonic food : 1.8×10^6 cells/ml for *Phaeodactylum* and 1.3×10^5 cells/ml for *Asteromonas*.

During the entire summer of 1984 cysts with a low hatching-efficiency were collected in the tank when microalgal availability was low and suboptimal. The slight efficiency increase is, however, linked to the higher salinity of the environment.

The influence of food availability and salinity on the cyst quality and on their hatching efficiency, are not exclusive. Another factor may also be responsible in the induction of cryptobiosis and, mainly in deactivation and starting the prenaupliar development. Among cyst dealers (Sanders Cy and Biomarine Cy) of the Great Salt Lake (GSL) strain it is a generalized belief that a prudential overwintering time between the harvest and their use in aquaculture is imperative in order to obtain optimal hatching (Sorgeloos, pers. commun.).

Because hatching of the cysts collected in 1984 was checked in April 1985, the sample subjected to the longest ageing period (15 months) was the one collected on January 1984. This sample displayed the best hatching efficiency, this fact has, nevertheless, also been previously linked to a better food availability for the *Artemia* population that generated these cysts. The other cyst samples collected in the experimental tank, with an ageing period shorter than 10 months

showed very low efficiencies. Later and consecutive checks of these samples, after longer overwintering periods, will provide data allowing to confirm or reject the validity of these findings.

Conclusions

As stated elsewhere (Amat, in prep.) *Artemia* females originating from Mediterranean populations and from GSL, displayed oviparity under similar circumstances. Once attaining reproductive maturity, their ovulation always coincided with food scarcity in the environment. This was also mentioned by D'Agostino and Provasoli (1968), Mc Dermott (1974), D'Agostino (1980), and Lenz (1980) for several American strains. In our experiments ovoviviparous reproduction was, however, never excluded as is shown in the data obtained during the last months of 1983 (Fig. 1). Maybe because of the low salinities, the embryos inside these cysts did not attain cryptobiosis completely, but hatched all the time, providing a constant nauplii recruitment similar to an exclusively ovoviviparous reproduction.

The cysts collected in the beginning of January 1984 (Table I) – accumulating at the tank surface and on the walls during the last days of 1983 – displayed a cyst shell percentage of about 35.40 %, while the poor samples obtained in March 1984 – with cysts probably laid by females of later generations and supplied with plenty of food – consisted of only empty shells. It is suspected that low temperatures and salinities favour ovoviviparity.

The coexistence of both oviparity and ovoviviparity allowed to quantify their relative importance in the generic mechanism of *Artemia* reproduction. The curves and regression formulas in Fig. 4 provide evidence of a higher fecundity rate in ovoviviparity as in oviparity. In other words, oviparity is always more expensive in terms of energy, than ovoviviparity. Browne (1980a) reports that oviparous offspring usually attains 74 % of the ovoviviparous one, while Clegg (1962) and Von Hentig (1971) stated that 22 % of dry cyst material correspond to the shell or chorion, which explains the smaller size of oviparous offspring. It is possible to draw the same conclusion with regard to the role played by salinity.

On 09.12.83, the population kept below 34.60 ‰ salinity, displayed oviparity attaining between 78 % and 94 % of respondent ovoviviparity, increasing inversely with the size of the females. The population checked on 12.07.84, kept at 67 ‰ salinity, displayed values of oviparity between 59 % and 82 %, and this time showing a direct report to the increasing size of the females. One has also to take the influence of food availability into account because the population had been supplied with a dense *Phaeodactylum* culture and later with a suboptimal level of *Asteromonas*.

A closer look at the formulas used to quantify fecundity rates allows to evaluate the importance of salinity increase. It does not seem to be a direct cause effect response. The fecundity decrease seems to be closely linked to the general shortening of the individual's size as a consequence of the salinity increase (Amat, 1980, 1982). The curves in Fig. 4 show that under low salinities the females do not perform their reproductive task until larger sizes are attained. At the same time that salinity increases a forwardness in reproductive maturity occurs, while the maximal sizes attained are smaller and the fecundity decreases accordingly.

This trend is displayed in oviparity as well as in ovoviviparity and the data show that more drastical decreases in fecundity appear during the interval of salinity increase from 35 ‰ to nearly 75-80 ‰. For both reproductive patterns, female fecundity under 67 ‰ ranges between 34 % and

43 % as regard to original 35 ‰ salinity. For higher salinities, like 92 ‰ and 142 ‰, the fecundity attained ranges between 31 % and 27 % as compared to the control.

The results obtained in the parallel culture in the plastic container, under 150 ‰ salinity and high *Asteromonas* availability, however, question the former statements or, at least, suggest a way to provoke or to maintain a prolonged oviparous mechanism. This should be linked to particular conditions entailed by high salinity because *Asteromonas*, can only be mass cultured successfully in salinities higher than 100 ‰.

Although more data are required, it seems that Great Salt Lake cysts need an overwintering period or ageing time (longer than 12 months ?) in order to obtain optimal hatchability. Among the best hatching rates obtained in this experiment (Table I), only the cysts from the first batch (01.84) could be influenced by an ageing period, and not those from the last batches. In both instances, however, an influence of the high phytoplankton availability to females producing these offsprings is suspected. The influence of increasing salinities on obtaining viable or full cysts is unquestionable.

In the experimental system used and under the biotic and abiotic conditions described the largest biomass production, with a total amount of 7 kg (w.wt), were attained between January and April 1984. In this period the water temperature ranged between 10 and 20 °C (with a 6 °C minimum in January), the salinities between 40 and 70 ‰, and the algal food availability was high. The best cyst harvests occurred during September and October 1984, with a total amount of 50 g dry weight, at temperatures ranging between 17 and 25 °C, salinities between 105 ‰ and 186 ‰, and suboptimal or scarce availability of halo-tolerant *Asteromonas* algae.

These experiments also allow to conclude the feasibility of developing and keeping a population of *Artemia* originating from the American continent, under Mediterranean climatic conditions. It is impossible to say if this would be possible with any other American strain coming from a different latitude. Anyhow, the results obtained with the Great Salt Lake strain, originating from an ecosystem endowed with water temperatures of about - 5 °C in winter and 45 °C in shallow areas in the summer, which promote the disappearance of *Artemia* in the northern part of the lake in November, when temperatures become lower than 6 °C (Post, 1977), allow to suspect that its accidental or purposeful introduction in any Mediterranean hypersaline environment, should bring about competition with autochtonous strains. Most probably the latter would be outcompeted.

This assumption was confirmed by the data on comparative fecundity obtained for autochtonous and San Francisco Bay (California, USA) strains (Amat, 1982). Browne (1980b) also reported that when environmental conditions are suitable, with regard to food supply, bisexual American strains outcompete the Eurasian parthenogenetic ones. This has been confirmed repeatedly in the laboratory when experimentally cultured autochtonous populations were accidentally inoculated with *Artemia franciscana*, usually from the Great Salt Lake or from the San Francisco Bay.

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Artemia culture in Brazil : an overview

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Abstract

After inoculation in 1977 with *Artemia* cysts from San Francisco Bay (California, USA) in solar-salt ponds near Macau (Brazil) this crustacean has been dispersed to all neighboring saltwork areas. Following a period of high yields, a continual decline in *Artemia* cyst production over the last 3 years has occurred.

Historical information on *Artemia* in Brazil and possible causes of decreasing cyst production are discussed. Recent experiments indicate the potential for semi-intensive production of *Artemia* cysts in salinas that have been abandoned.

Historical review

The occurrence of *Artemia* in Brazil was recorded for the first time in the salinas of Cabo Frio, Rio de Janeiro, in 1972 (Da Costa, 1972). However, the origin of this *Artemia* in the southeastern coast of Brazil is unknown. It was only in the northeastern coast of Brazil a few years later that this crustacean was to play a unique role in the development of local aquaculture as inoculations made with cysts from San Francisco Bay (California, USA) in Macau, Rio Grande do Norte, in April 1977, have dispersed this crustacean in all neighboring saltwork areas (Persoone and Sorgeloos, 1980 ; Camara and De Castro, 1983).

Through human, bird, and wind dissemination, *Artemia* dispersed in many salinas in the States of Rio Grande do Norte (RN) and Ceara (CE), and we were soon able to find this crustacean in many spots on Brazil's salt-producing areas, mainly around the cities of Macau, Mossoro (RN), and Aracati (CE).

The ecological conditions found in these salinas favoured a good *Artemia* production and several tonnes of cysts and biomass have been collected since that time. However, after an initial period of high yields, when the total yearly production in the Macau saltwork area exceeded 10 tonnes of harvested cysts (dry weight), present production is low and barely reached 1 240 kg in 1984.

Large initial populations of *Artemia* in these salinas, which are supplied by mangrove waters, later became reduced as a function of food availability. These salt ponds started to receive less nutrients due to a prolonged drought affecting all Brazil's northeast from 1979 to 1983. As a consequence, algae were not present in large quantities and the ponds were clear or colored by suspended sediments only. As a result the cyst and biomass production gradually decreased.

Although it seems unlikely that a single environmental factor functions as a trigger for cyst production (Versichele and Sorgeloos, 1980), it is clear that the availability of proper nutritional materials has played an important role in the physiology of cyst-producing populations in Macau. It may be assumed that the difference between proper and improper nutritional materials (qualitatively and/or quantitatively) depends upon whether algae found in salt ponds and ingested by *Artemia* stimulate hemoglobin synthesis, thus increasing the production of cysts. Similar correlations between cyst production and food quality or quantity have also been noted in previous observations (Baker, 1966 ; D'Agostino and Provasoli, 1968). This inference seems to be confirmed by the fact that contrary to cyst (in high salinity ponds), the biomass production (in low salinity ponds) has matched the current food availability which has been continuously observed in Brazilian salt ponds.

In addition to the above, it is likely that the dial fluctuations in dissolved oxygen in response to the presence of algae in the ponds may be also linked to *Artemia* cyst production in Macau. In fact, cyclic oxygen stress in combination with Fe-EDTA addition to the culture medium have been used as an efficient induction mechanism for oviparous reproduction in laboratory experiments (Lavens and Sorgeloos, 1984).

Nowadays, though *Artemia* production is mostly extensive and carried out in large salt ponds as a by-product, incipient semi-intensive production is being accomplished on a pilot-scale *Artemia* farm.

Salinas

After this successful introduction and initial *Artemia* production, little or no modification has been made in existing salt evaporation ponds in Brazilian salinas. Meanwhile, several processing plants have been installed based exclusively on these extensive production methods. However, current cyst production represents less than 5 % of the 1979 figures (Table I). It seems clear that the return to high cyst production in Brazil requires a different approach.

TABLE I

Production of *Artemia* cysts in Brazil in the period 1977-1984 (in kg of dry cysts)

Year	Cyst harvest
1977	6 000
1978	24 600
1979	30 800
1980	18 050
1981	10 730
1982	5 400
1983	1 567
1984	1 240

We think that *Artemia* cyst production in our country could be normalized and probably increased by the alternative use of abandoned salinas and, in some situations, by the change from current extensive methods being applied in large mechanized salinas to semi-intensive production techniques in specific *Artemia* production areas. The feasibility of similar projects has been

proven in countries such as the Philippines, India, Thailand, Costa Rica, and Indonesia (Sorgeloos, 1986).

In Brazil, due to the mechanization of the large salinas in the late sixties, with a considerable reduction of their operational costs, medium to small size artisanal salinas have been severely affected. On the other hand, economic recession has inhibited the salt industry as a whole, and the number of small salinas in full operation is becoming smaller (Table II).

TABLE II

Saltwork areas in the States of Ceara (CE) and Rio Grande do Norte (RN) in NE-Brazil (in ha)

Location		Total area	Abandoned[1]	In operation
CE				
Aracati		1 354	571	783
Beberibe/Cascabel/Aquiraz		431	315	116
Trairi/Acarau		687	497	190
Camocim/Granja		1 135	645	490
Chaval		1 100	330	770
Fortaleza/Caucaia		719	576	143
	Sub-total	5 426	2 934	2 492
RN				
Mossoro		10 515	4 206	6 309
Macau		14 420	5 768	8 652
Guamare/Galinhos		1 905	953	952
Natal		600	600	–
Tibau do Sul		320	304	16
Canguaretama		1 792	1 752	40
	Sub-total	29 552	13 583	15 969
	Total	34 978	16 517	18 461

[1] Or under restricted artisanal operation.

From a total salt pond area of 34 978 ha in the States of Rio Grande do Norte and Ceara, 18 461 ha are now abandoned or under restricted artisanal operation (Camara and De Castro, 1983 ; Machado, 1984). Evaporation ponds in some of these areas could be converted into semi-intensive *Artemia* farms with small dike modifications, and financial and technical aid from private and government institutions.

Pilot project

In the State of Ceara, an abandoned salina has been modified into a pilot-scale *Artemia* farm, with six experimental ponds. Mangrove water was pumped to a 20 ha evaporation pond and by gravity flow taken into the production area. The ponds were inoculated with cysts from Macau (Brazil) at the rate of 3 nauplii/l of pond water. The salinity was kept between 100 and 120 ‰, the temperature never exceeded 38 °C in 35-40 cm water depths, and the transparency was

around 30 cm (Secchi dish reading). Weekly fertilization with chicken manure (500 kg/ha), urea (50 kg/ha), and triple-superphosphate (15 kg/ha) was used. The productivity ranged from 1 to 5 kg of dry cysts/ha in a 1 month period. The unexpected flood of the ponds, due to heavy rainfall in early January 1985, terminated the experiments. The biomass was collected at the rate of 400 to 500 kg (wet weight)/ha in the same period.

We would like to point out that this unusually rainy season has severly affected all saltwork areas in NE-Brazil, and delayed the salt production itself. It is also likely that in some salinas *Artemia* will have to be re-inoculated. We suggested that cysts from the same Macau strain should be used.

We also expect that with the normalization of climatological conditions, and the probable natural eutrophication of estuarine waters provided to these salinas, an increase in cyst and biomass production might be achieved, provided that the *Artemia* populations are properly restored.

Conclusions

Artemia productivity in Brazil has varied widely since this crustacean was introduced in the saltworks of NE-Brazil, probably in response to the food quality and quantity available in the course of time.

Although today the potential for *Artemia* in Brazil is evident, normalization and the probable increase of the present production depend on the following actions to be taken :

- re-inoculation of *Artemia*, Macau strain, in flood-affected salinas ;
- continuation of studies regarding the role played by food availability on the mode of reproduction of *Artemia* ;
- alternative use of small to medium size abandoned salinas as semi-intensive *Artemia* farms ;
- change from extensive to semi-intensive production methods where very large evaporation ponds is not a limiting factor ;
- further research on present *Artemia* cyst production in Brazil, reduced by the dominance of ovoviviparous populations, and also on the potential valorization of adult biomass (live, frozen, and freeze-dried).

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Artemia culture in Thailand

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Abstract

Successful farming of giant freshwater prawn larvae initiated interest in *Artemia* cyst-production in Thailand. Since 1980 several salt farms started commercial production of *Artemia*. The culture methods vary from one farm to another but the harvesting and processing techniques are very uniform. The number of farms varies from year to year depending on the salt prices and the demand for fish fry and shrimp. In 1985 three farms operated about 13 ha of *Artemia* ponds for biomass and cyst production. Recently biomass production is preferred because of higher profits and easier harvesting.

The potential of *Artemia* production and industry in Thailand should be carefully considered because the biomass production might exceed the local market demands while the cyst production is insufficient.

There is also a great need of technical inputs to improve the present methods of *Artemia* production and processing. These tasks are being taken care of by the National Artemia Reference Center of the Kasetsart University, through a joint Thailand-Belgium cooperation project.

Introduction

The brine shrimp (*Artemia*) is an unnatural occurrence in the coastal area of Thailand (Persoone and Sorgeloos, 1980). In Thailand governmental fish hatcheries and private fish-seed producing farms prefer to use *Artemia* cysts as a more convenient food source compared to other zooplankton, in spite of its high price. Prices per kg range from US $ 47 to 85 depending on the quality of the product. The annual cyst consumption in Thailand was estimated to be more than 3 tonnes in 1979 (Vos and Tunsutapanich, 1979), and was projected as high as 23.8 tonnes in 1985 (Dep. Fisheries, 1980).

The purpose of this paper is to provide information on the utilization of *Artemia* cysts and biomass ; methods of culture, production, collection and processing ; and future potential of *Artemia* culture in Thailand.

Historical background

The success in rearing giant freshwater prawn larvae using *Artemia* nauplii as their essential feed initiated interest in *Artemia* cyst-production in Thailand (Gatesanchai and Suksacheep, 1970). Following successful inoculations of *Artemia* for cyst-production purposes in Brazil and the Phillippines, the technical feasibility of integrated salt and *Artemia* production in Thailand was highly recommended (Sorgeloos, 1978). Consequently, the Department of Fisheries

conducted a small-scale culture test in a 0.25 ha salt pond in Chonburi province. Fifty g of cysts composed of 50 % San Francisco Bay (California, USA) strain and 50 % of Macau (Brazil) strain were simultaneously hatched and inoculated in the culture pond filled with 70 ‰ seawater. The culture pond was modified from the existing salt pond which was trenched and the dikes raised. In a 45-day period, over 10 kg dry weight of cysts were harvested. The experimentally produced cysts were reported of high hatching efficiency (Tunsutapanich, 1979a, 1982 ; Vos and Tunsutapanich, 1979). In the same period experiments were conducted at Rayong Fishery Station on the indoor culture of *Artemia* using rice bran (Hutasingh *et al.*, 1978) and at Samut Sakorn Fishery Station on the large-scale production of cysts (Yashiro *et al.*, 1978). In 1980, approximately 11 farms consented to inoculate *Artemia* in their salt ponds. By August 1980, 4 months of intermittant production on approximately 5.6 ha yielded 600 kg wet weight of cysts, which were processed to 300 kg dry weight (Dep. Fisheries, 1980).

At the moment 10 farms distributed over five provinces are producing *Artemia* biomass. Among these, three farms operate about 13 ha of *Artemia* ponds for biomass- and cyst-production on a year-round basis. The produced cysts are harvested and kept for the farmers' own use to maintain their biomass production. In 1984 the estimated total biomass production was 86.4 tonnes wet weight which valued about US $ 319 495.

Cyst and biomass utilization

In Thailand *Artemia* cysts are used both in fish and crustacean hatcheries for mass seed-production purposes. Since its unnatural occurrence, most cysts were imported from various origins, and their prices varied depending on the strains of cysts (Table I). According to the Department of Fisheries (1980) seven types of cysts are currently in use. These seven may represent labelled brands. The generic sources of these cysts are limited to five locations : Thailand, California, Utah, Canada, and Brazil. Most of the hatchery managers ordered cysts directly from the foreign producers/dealers. However, some of them obtained the cysts through local dealers and consequently pay 30 % to 50 % more for the same product.

TABLE I

Origin, strain, quantity, and price of *Artemia* used in Thailand aquaculture (modified from Vanhaecke, 1983)

Origin	Strain	Quantity (%)	Price (US $/kg)
China (PR)	Tientsin	45	47-48
USA	San Francisco Bay	38	65
Thailand	Locally produced cysts : obtained directly from producers	2	65-70
Others	Probably Tientsin under several brands : Vita Rich, Salina Gold, Aqua Marine, etc.	15	55-85

Approximately 98 % of the *Artemia* cysts was imported at a yearly expense of about US $ 480 000 (Vanhaecke, 1983). The cysts are mainly used as primary food for four major species : giant freshwater prawn (*Macrobrachium rosenbergii*), giant tiger prawn (*Penaeus monodon*),

white prawn (*Penaeus merguiensis*), and sea bass (*Lates calcarifer*) and to some extent of grouper (*Epinephelus tauvina*). The expansion of fish and prawn hatcheries results in rapidly increasing demands for cysts (Table II). The minor part of cysts and biomass is used for common marine fish in aquaria, laboratory study, and shrimp broodstock in culture. Biomass is also reported to be tested as food for human and its taste is considered good depending on the recipes and personal favor (Mot, 1984). Because of its high price, the use of either cysts or biomass is still limited to aquaculture purposes. To reduce the cost, some hatcheries have been culturing biomass for their own uses.

TABLE II

Estimated *Artemia* cyst requirement for Thailand, 1980-1985 (Dep. Fisheries, 1980)

Year	Required (metric tonnes)
1980	1.5
1981	2.6
1982	4.5
1983	7.8
1984	13.5
1985	23.8

In view of strain selection, locally produced cysts are preferred for freshness and their good hatching efficiencies (Table III). Among various strains of cysts imported, San Francisco Bay origin is preferred for cyst and biomass production. Tientsin is often used in hatcheries as primary food for fish larvae and shrimp nauplii. As for inoculation purposes, Tientsin is good for biomass production.

TABLE III

Characteristics of cysts and nauplii from parental cysts (San Francisco Bay Brand SFB 1728) and from local cysts harvested from inoculated ponds (Bangpakong - BP 1979) (modified from Vos *et al.*, 1980)

Characters	Parental cysts-SFB 1728 (San Francisco Bay Brand Cy batch 1728)	Local cysts-BP 1979 (Bangpakong)
Hatching efficiency (nauplii/g)	100 800	304 000
Hatching rate characteristics[1]		
T_0	24.5	14.5
T_{50}	32.8	18.8
T_{90}	39.2	25.6
Color of cysts	pale	dark
Diameter of cysts (µm)	225.8	232.2
Standard deviation	17.3	11.8
Nauplius survival at day 7 (%)	94	96
Nauplius growth at day 7[2]	100	106

[1] Values refer to time-lapses (in hours) incubation until appearance of the first nauplii (T_0) or the moment by which 50 % (T_{50}) and 90 % of the hatching efficiency has been reached.

[2] Expressed as % recorded for *Artemia* reference strain, San Francisco Bay Brand Cy batch 288-2596.

There is an indication that the locally produced cysts possess a greater hatching efficiency and that the nauplii have a higher nutritional quality as compared to their parental products, *i.e.* San Francisco Bay strain. The cysts' color changed from pale of parental cysts to dark in the local cysts (Table III). The nutritional value evaluation of cysts was limited to fatty acid analysis of the nauplii and to a feeding test with the marine mysid *Mysidiopsis bahia*. Higher survival rates and growth of the mysid larvae were observed when they were fed on the local product. The good nutritional value of the nauplii from the local cysts for *Mysidiopsis bahia* also correlated with a higher content of the essential fatty acid 20:5ω5 (Vos *et al.*, 1984).

Presently, it is proven that pre-adult *Artemia* have a higher nutritive value than freshly-hatched nauplii (Sorgeloos, 1980), *i.e.* adults contain 60 % protein, they are rich in all essential amino acids, contain a large amount of fatty acids, and have an ash content of 10 % only. This encourages some hatcheries in Thailand to use live *Artemia* biomass as supplementary feeding of sea bass and shrimps giving better growth and production. One hatchery-nursery farmer is administering adult *Artemia*, known to be rich in hormones, to trigger maturation of shrimp (*P. monodon*) broodstock. Various experiments on biomass processing are in progress, *i.e.* dried *Artemia* as cheap substitute for fish meal in the formulation of pellets to feed shrimp in grow-out pond, frozen biomass targets for tropical fish feed, particularly large aquarium as major consumer.

Culture methods

Experimental cyst production in Thailand has been primarily carried out through trial and error according to methods found in the literature combined with ingenuity. Experimentation was conducted on a practical level, in ponds previously used for salt production (Dep. Fisheries, 1980). Consequently, the culture of cyst and biomass has been developed and the production increased. Based on the aquatic animal culture-system, the cyst and biomass production in Thailand can be devided into the two following systems :

Integrated system

Artemia-*salt-fish production*

Artemia ponds, salt ponds, and fish/shrimp ponds are completely separate but located in the same farm area. Production of each item is supplied by the same farm water system (Fig. 1). Seawater is brought in a series of six salt ponds using a windmill. Ponds 1-5 are used for evaporation to obtain sufficiently high salinities. The last pond is used for crystallization. Water from ponds 3-5 is alternatively taken in to supply the *Artemia* biomass production ponds. To use water from a particular pond chiefly depends on the prevailing salinity in the selected biomass pond to maintain a suitable salinity range.

Prior to stocking, the pond bottom, dikes, and trench are renovated and undesirable species are removed or killed. Then the pond is exposed to sunlight for about 1 week. Green water with a salinity of 30-50 ‰ from a fish/shrimp pond is pumped in and then the water from ponds 2-5 (to be properly selected) is added to obtain the adequate depth (30 cm at pond bottom and 60 cm at trench) and salinity (90-110 ‰).

During the culture period, high salinity water from the evaporation ponds is occasionally added. The rich green water from the fish/shrimp pond is also pumped in to feed the *Artemia*.

Water used for the biomass culture pond is occasionally released back into the evaporation ponds. Brine water of high salinities (more than 250 ‰) is sold to shrimp and prawn hatcheries for their seed production. Organic fertilizer (chicken manure) is added in subsequent doses depending on the water quality.

In fish/shrimp rearing ponds, water is pumped in from another canal located at the opposite side of the canal supplying water to the salt pond series. A number of sea bass, Tilapia, giant tiger prawn, and white prawn including miscellaneous species come in with the water mass. In this type of pond, the presence of green water results from fertilization and partly from fish/shrimp wastes. The water used in fish/shrimp ponds is released into the *Artemia* biomass culture ponds as water supply. The water used in the *Artemia* pond itself is therefore identified as a static system and considered as an integrated water system in terms of the whole farm.

Artemia-*salt production*

Seawater is pumped into the salt pond series (6 ponds). The ponds 1-5 are used for evaporation and the last one is used for crystallization (Fig. 2).

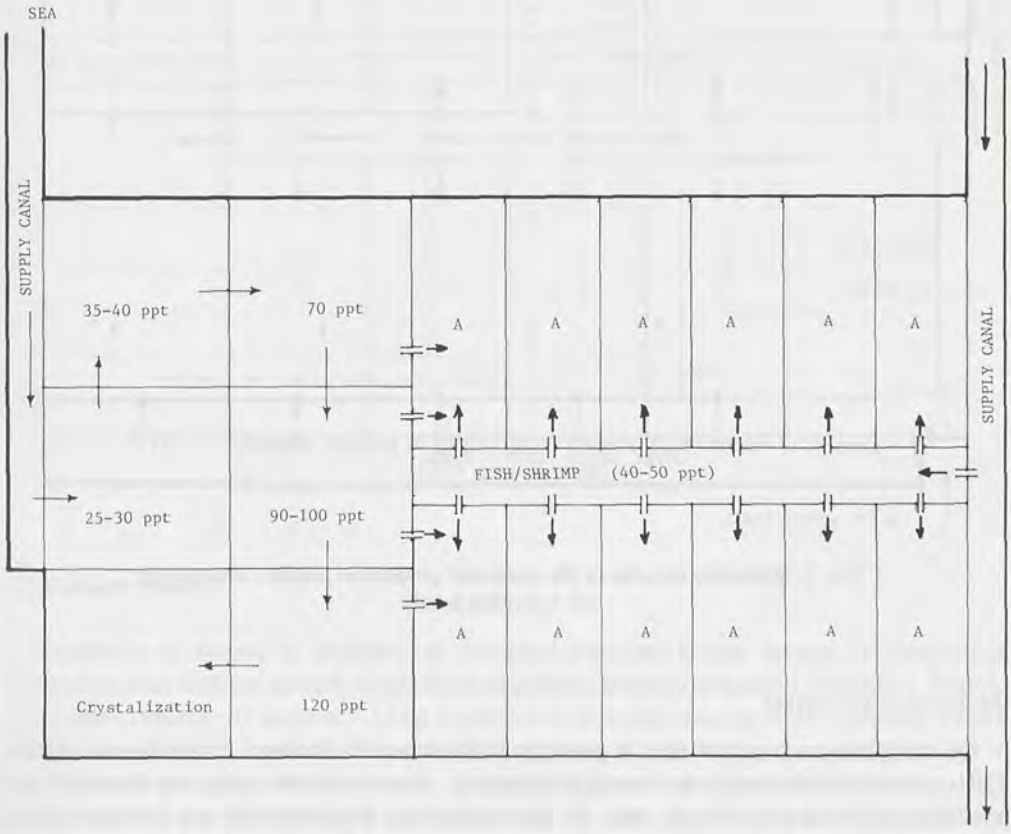

FIG. 1. Schematic drawing of the integrated production system : *Artemia*-salt-fish/shrimp. (A = *Artemia* pond).

Water with salt concentration water from ponds 3-5 is alternatively selected to be released into the biomass culture pond. The water from water supply canal is also pumped in to obtain the desirable salinity range. Ponds are initially fertilized with chicken manure at the rate of 1 250 kg/ha prior to inoculation of the *Artemia* nauplii. Later the ponds are fertilized once a week. During culture operation, water from either the evaporation pond or the water supply canal is pumped into the biomass ponds to maintain an adequate salinity range. No waste water is discarded from the rearing ponds. The water system used in this culture practice is also a static one.

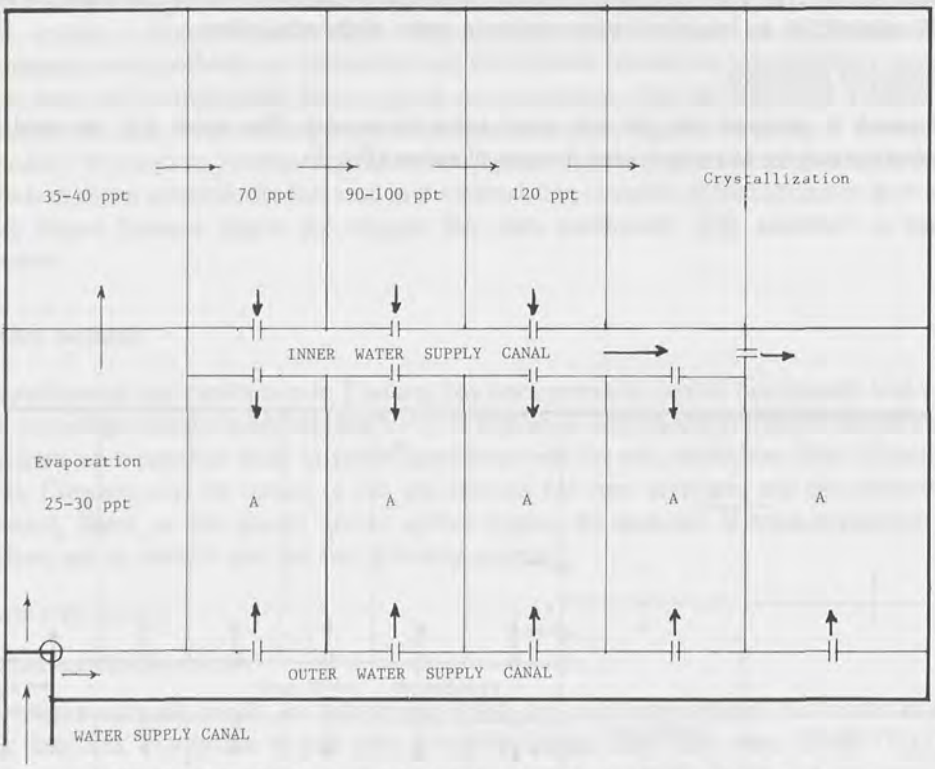

FIG. 2. Schematic drawing of the integrated production system : *Artemia*-salt. (A = *Artemia* pond).

MONOCULTURE SYSTEM

One single *Artemia* culture farm is presently in operation in Thailand. In this type of culture, salt and *Artemia* production are managed separately. *Artemia* culture ponds are converted and modified from ponds previously used for salt production. Culture ponds and a water holding canal are constructed (Fig. 3). The culture operation begins with filling the culture ponds and water holding canal with seawater by using water from the evaporation pond containing brine of 90-120 ‰. The water holding canal is first fertilized with chicken manure at the rate of

1 250 kg/ha. When water becomes green, *Artemia* nauplii are inoculated in the culture ponds, and water is recirculated by pumping a few days later. This system is called re-used or recirculating system. Later the ponds are fertilized once a week. Seawater is occasionally added to replace the amount evaporated thereby maintaining the salinity range in the culture pond for an optimal production.

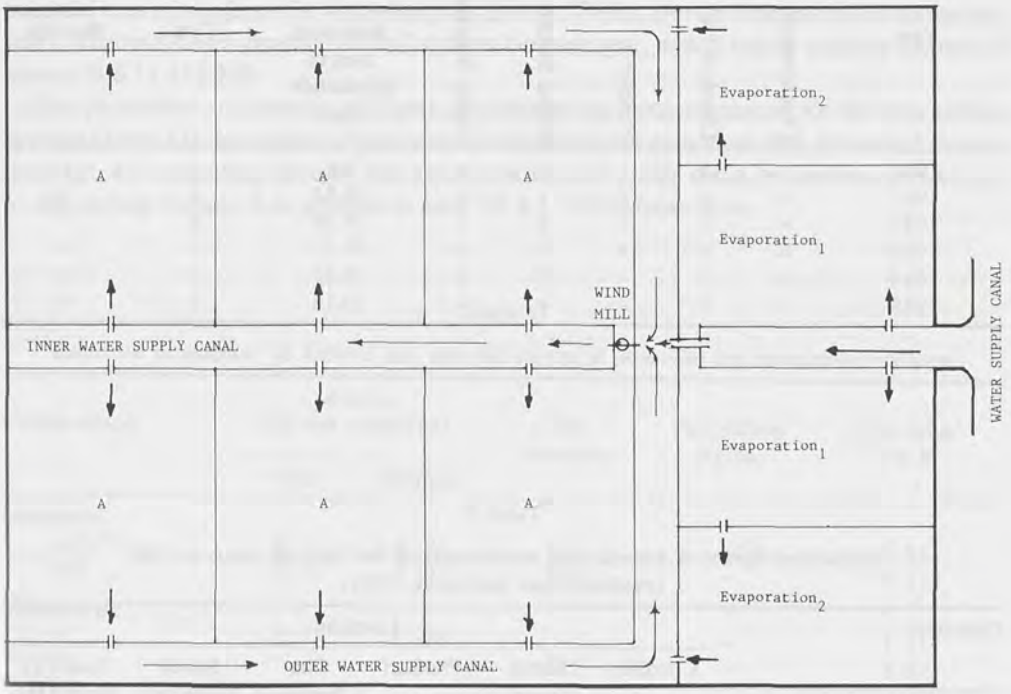

FIG. 3. Schematic drawing of single culture (monoculture) system of *Artemia*. (A = *Artemia* pond).

Production results

Production of *Artemia* in Thailand has fluctuated from year to year because of variations in production area and the number of salt farms operating *Artemia* production (Table IV). Prior to 1980 approximately 20 farms of 7.52 ha consented to inoculate *Artemia* (Dep. Fisheries, 1980). During the following 2 years (1980 and 1981) the area of salt farming increased from 8.0 to 15.84 ha and cysts were the main harvest. A year later, the area increased slightly, then in 1983 it dropped and was followed by a gradual increase till now. In 1982 the production of cysts equaled about that of biomass. The situation reversed recently, because *Artemia* farmers prefer to produce biomass to cysts giving higher profits and being easier to harvest. With good pond management biomass can be harvested year-round.

TABLE IV

Locations, areas, and production categories of *Artemia* culture in Thailand during 1980-1985

Year	Location					Estimated area in production (ha)	Production categories	
	Chachoengsao	Chonburi	Phetburi	Samut Sakorn	Samut Songkram		Cyst	Biomass
1980	×	×	-	-	×	8.0	×	-
1981	×	×	×	-	×	15.84	×	-
1982	×	×	×	×	×	19.36	×	×
1983	×	×	×	×	×	15.2	×	×
1984	×	×	-	×	×	19.52	×	×
1985	×	×	-	×	×	20.16	×	×

TABLE V

Production figures of *Artemia* cysts and biomass of five Thai salt farms in 1983 (modified from Vanhaecke, 1983)

Parameter	Location					
	Chonburi	Samut Sakorn	Phetburi	Cha-Choengsao	Samut Songkram	Total (T) Mean (M)
Area (ha)	3.2	0.3	1.4	1.4	1	T = 7.3
Quantity of cysts produced (kg wet weight)	900	20	50	300	180	T = 1 450
Cysts produced/season/ha (kg wet weight)	278	62	34	204	185	M = 153
Length of season (month)	9(12)[1]	6	5	6(12)[1]	6	
Cysts produced/season/ha (kg dry weight)	139	31	17	105	92	M = 76.6
Biomass harvested/season (kg wet weight)	3 600	±120[2]	±200[2]	±250[2]	±300[2]	T = ±4 470[2]
Biomass harvested/season/ha (kg wet weight)	1 110	370	136	173	309	M = 420

[1] Biomass production.

[2] At best estimation by the salt farmers.

The cyst production in Thailand was reasonably high. During 1980 the cyst production averaged 25 kg wet weight/ha/month (Dep. Fisheries, 1980). Later it increased slightly and an average biomass of 350 kg/ha/month was recorded (Vanhaecke, 1983). Table V shows production figures of *Artemia* cysts and biomass of five salt farms in 1983. It indicates that *Artemia* farmers tended to harvest more biomass. Based on the data of 1984, the amount of biomass produced was approximately 86.4 tonnes with a value of US $ 319 495. Presently only three farms operate about 13 ha for year-round biomass and cyst production. In view of the total potential area of salt farms for *Artemia* production (4 374 ha, average biomass of 4.4 tonnes/ha/year), Thailand could produce 19 247 tonnes biomass/year, which means a yearly income of about US $ 71 213 900.

The production of *Artemia*, salt, and fish/shrimp has been compared for the two culture systems (Table VI). In a monoculture system a salt farmer can earn more than his current income from salt. By integrating salt and *Artemia* production the profits could be doubled. In addition to fish/shrimp culture, it is possible to earn US $ 1 720/ha/year more.

TABLE VI

Estimated production[1] of *Artemia*, salt, and fish/shrimp in integrated and monoculture systems

Culture system	*Artemia* (kg wet weight/ha)		Salt (tonne/ha)	Fish/shrimp (kg/ha)	Total value[3] US $
	Cyst	Biomass			
Integration					
A-S-F[4]	22.7	8 295	62.5	1 125[2]	40 832
A-S	22.7	8 295	62.5	–	39 112
Monoculture					
A	22.7	8 295	–	–	31 112
S	–	–	62.5	–	8 000

[1] Production calculations are taken from the *Artemia* farm in Chonburi (Tambon Klong Tam-ru).
[2] Includes approximately sea bass 120 kg, shrimps 300 kg, Tilapia 600 kg, and miscellaneous 105 kg.
[3] Prices in US $/kg : cyst 18.5, biomass 3.7, sea bass 2.4, shrimps 4.4, Tilapia 0.1, and miscellaneous 0.3. Salt price 128 US $/tonne. (US $ 1 = 27 Bhat).
[4] A = *Artemia* ; S = salt ; and F = fish/shrimps.

Collection and processing

The cysts and biomass are harvested according to the methods of Sorgeloos *et al.* (1978), Tunsutapanich (1979b), and Vos and de la Rosa (1980).

The cysts are collected as regularly as possible preferably daily to avoid exposure to rain as the cysts might hydrate. The cysts gathered by wind action at a corner of the culture pond can easily be collected with fine mesh dipnets (150 µm mesh openings). Harvested cysts are collected in a cloth sack of 60 µm and submerged in a vat of saturated brine until they are ready for processing. Generally, the storage vats are kept covered or sheltered to protect them from rain as described by Tunsutapanich (1979b). The drained cysts are rinsed through a sieve (300 µm

mesh openings), the finer waste particles and cysts are collected by a second sieve (150 μm mesh openings). The preliminary separating and washing release dissolved soluble wastes. A second density separation is performed using saturated brine. Then the pre-washed cysts are placed into an inverted 20 l plastic water bottle which is modified to extract the product and waste through the inverted neck of the bottle. An additional 10 l of saturated brine solution is added and the solution is aerated for several minutes. Aeration is stopped, the cysts and light debris are allowed to float to the top while the heavy debris settles on the bottom and is discharged. The cysts are drained into a cloth sack and washed with freshwater for 4-5 min to dehydrate them. After hand squeezing the sack to get rid of excess water, the cysts are spread out 2-3 cm thick on a paper sheet and sun dried. The product is turned 2-3 times a day till dry. During the rainy period, intermittent solar drying may take 2 days to reach a batch moisture level of 10 %. When the level is estimated to be attained, the cysts are packed in 425 g lots in vacuum-sealed plastic bags or cans and sent to the users or private firms. The private firms can repack them in tin cans for further sale.

The biomass is mostly harvested in the afternoon using fine dipnets. Live biomass is temporarily stocked in 1.5 × 2.0 × 0.9 m nylon cages suspended in the culture ponds. The *Artemia* biomass is packed in water-filled plastic bags with oxygen at atmospheric pressure. A foam box of 30 × 45 × 60 cm can be used as an alternative container. Two plastic bags containing live adult *Artemia* in 90 ‰ salt water (5.75 kg each) are placed in the foam box together with five bags (500 g each) of ice. This packing method is suitable for long distance transport.

Potential development and problems

Artemia farming seems to be expanding only ver slowly due to the lack of information on cultivation techniques. There is a big gap between supply and demand for *Artemia* cysts in the country which may encourage the salt farmers to engage more in *Artemia* culture in the near future. The fluctuating price of salt makes salt farming an unreliable business which may be replaced by *Artemia* culture.

Presently, there are in Thailand over 1 000 salt farms totaling about 12 500 ha, distributed over seven provinces (Table VII). The salt production in these areas averages 45 tonnes/ha. The salt price has been decreasing continuously since 1977 (Sahavacharin, 1981). In 1981, however, the price increased enormously as a result of an increased demand. The current mean salt price is about US $ 45/tonne. Recently, salt can be produced more cheaply from the modern mining of rock salt and the development of salt mining could eventually remove the economic basis for solar salt farming. One may expect the sea-salt price to drop as a result of the expansion of rock-salt mining in the northeastern part of the country (Samut Sakorn Land Settlement Co-op, Ltd., pers. commun.).

Observations were made in the different salt farms on cost/benefit data by Vanhaecke (1983). The following average cost/benefit is believed to be possible for integrated *Artemia* production in Thailand. The total cost/ha (investment and operation) was US $ 1 210. The production of 180 kg wet weight/ha of cysts and of 500 kg wet weight/ha of biomass gives an income of US $ 5 080, with an average benefit of US $ 3 040/ha.

The salt farms in Thailand are very suited for the integrated production of salt plus *Artemia*, because of the long dry season (November-April), the impermeable soil with high clay content,

the supply of water from nutrient rich mangrove creeks, and minimal needs for pond modification. These conditions allow a year-round pond usage, resulting in an increase in the farmers' income, and offering the advantage to fully exploit Thailand's natural resources. The importation of *Artemia* will be reduced and possibly eliminated provided Thailand is capable to evolve towards self-sufficiency.

It is clear, that the production of *Artemia* in solar salt farms is a profitable business. The total income through *Artemia* production is almost triple that of the salt. If there is a shortage of cysts on the world market, and the current pricing plus marketing elements are correctly aligned, Thailand may earn foreign currencies through the export of *Artemia* cysts instead of importing cysts. Additionally, over 1 000 tonnes of *Artemia* biomass can be harvested from the local salt farms. Though the industry in Thailand is favored by some powerful economic incentives for intensive development, the farmers are confronted with some problems. One of the most pressing ones is the low price of *Artemia* when there is a production surplus.

The limited knowledge on *Artemia* culture systems and related aspects, results in a slow process of gathering information needed by the *Artemia* farmers. Despite these problems, an expansion in the number of *Artemia* ponds can be noticed in the salt farm areas where profits obtained from salt production decrease. Consequently, the number of fish and prawn hatcheries increases though they rely on traditional techniques rather than on improved methods.

TABLE VII

The geographical distribution of salt production in Thailand and its potential for *Artemia* production

Province	Salt farm		Expected for *Artemia* farm[1]		
	Area (ha)	Production (tonne)	Area[2] (ha)	Production (kg wet weight)	
				Cyst	Biomass
Samut Sakorn	6 437	305 820	2 253	344 701	946 050
Samut Songkram	2 917	140 040	1 021	156 205	428 799
Pattani	1 702	50 000	596	91 142	250 194
Phetburi	1 135	50 000	397	60 779	166 845
Samut Prakarn	41	2 250	14	2 196	6 027
Chachoengsao	200	6 866	70	10 710	29 400
Chonburi	66	3 240	23	3 534	9 702
Total	12 498	558 216	4 374	669 267	1 837 017

[1] Average productions are from Vanhaecke (1983) : production per season per ha ; cyst 153/kg ; biomass 420/kg.
[2] Assuming that approximately 35 % of salt farm areas will be used for *Artemia* culture.

Recommendations

The future of *Artemia* culture in Thailand looks bright but lower cost systems must be designed. The potential of *Artemia* production and industry in Thailand should be carefully considered because the biomass production might exceed the local market demands while the cyst production is insufficient.

There is also a great need of technical input to improve the present methods of *Artemia* production and processing. These tasks are being taken care of by the National Artemia Reference Center (NARC) of the Faculty of Fisheries, Kasetsart University, through a joint Thailand-Belgium cooperation project. This project is aiming at research activities with regard to the optimization in the production of *Artemia* cysts and biomass in solar salt operations, the quality control of the *Artemia* products obtained in salt farms, and the optimization of the use of *Artemia* biomass in aquaculture hatcheries. The main purpose of the above project is that it minimizes Thailand's dependence on imported *Artemia* cysts. Information and extension services to the salt farmers are also part of the program. Upon completion of this project, further work should be pursued, *i.e.* further technical cooperation with various international organizations (EEC, UNDP, IDRC, FAO, etc.) or the establishment of a Regional Artemia Reference Center in Thailand which will be responsible for the Southeast Asian countries.

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Cyst production of *Artemia* in salt ponds in southeastern Spain

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Abstract

Large *Artemia* populations are found in solar saltworks in the southern Province of Cadiz (Spain) near Ribera Gaditana in the Bay of Cadiz, and near Sanlucar de Barrameda along the estuary of the Guadalquivir River.

The *Artemia* presence is discontinuous from salinity levels of 50 to 60 ‰ up to 300 ‰.

In Ribera Gaditana, bisexual and parthenogenetic populations alternate in presence depending upon the water temperature and the salinity. In Sanlucar de Barrameda diploid as well as tetraploid parthenogenetic *Artemia* are found the year round. Since 2 years bisexual *Artemia* has been observed in one saltwork of the latter region.

Cyst production mainly occurs in operational saltworks at salinity levels of 130 to 175 ‰. A direct correlation with cyst production was found for the following parameters : water depth, salinity, and rainfall.

Average yields amount to 10 kg/ha/year, 60 % of which is produced in the period April-July and the rest from October through March.

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A minimal strategy for assessing *Artemia* biomass harvestable from production salinas

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Abstract

A simplified procedure is given for estimating *Artemia* biomass harvestable from commercial solar salt pond systems on a fully sustainable basis. Recommended procedures minimize the possibility of salt production being deleteriously affected.

Introduction

It is likely that *Artemia* biomass will in future years become increasingly important as a feed or feed supplement in aquaculture systems (Webber and Sorgeloos, 1980). However, our knowledge of the ecology and population dynamics of *Artemia* in natural habitats is incomplete, and any meaningful study requires very detailed consideration of the many complex interactions involved (Haslett and Wear, 1985 ; Wear and Haslett, 1986, 1987 ; Wear *et al.*, 1986). Assessing *Artemia* biomass harvestable on a renewable basis from solar salt production ponds, or determining total allowable catch of *Artemia* in the context of fisheries management, does not require such sophistication. Biotopes which should be seriously considered as potential producers of harvestable *Artemia* biomass are those in which ovoviviparity is the preferred reproductive mode, rather than oviparous production of cysts. Since *Artemia* plays an important role in maintaining a healthy biological balance in commercial salinas (Davis, 1979, 1980 ; Sorgeloos, 1983), any over-exploitation may reduce grazing pressure on unicellular algal blooms and may also in turn deleteriously affect the quantity and quality of solar salt produced. The principles outlined below provide a minimal program necessary to plan and maintain an economic harvest of *Artemia* biomass without placing salt production at risk. Site-specific modifications may be appropriate.

Methods

Harvesting procedures

– Design or obtain *Artemia* harvesting equipment, preferably as a scale model of the intended production unit. Janata *et al.* (1987) provide a method for removing algae from brines, and for harvesting high quality salt-free adult *Artemia* biomass.

- Install the pilot system in a position(s) where brine can be filtered or screened coincident with normal periodic pumped transfer or flow between concentration ponds. The harvesting equipment should be static and the brine moved, rather than the reverse (*e.g.* tow nets).
- Pilot harvesting should be run over an annual cycle, or that part of the year during which *Artemia* is potentially harvestable (*i.e.* the period of net *Artemia* production). This will be *Artemia* strain dependent. Biomass harvested (wet weight) should be monitored daily at each pilot harvesting site over the period(s) that brine is filtered. Biomass concentration is calculated from the volume filtered.

Field data

Basic equipment required is as follows : field thermometer ; salinity refractometer ; water sample bottles (for later algae and nutrient analyses) ; haemocytometer ; binocular microscope ; digital balance (top-loading).

- Monitor the volume of the harvested pond(s) as closely as possible. Most production salinas keep reasonably accurate records of pond volumes.
- Monitor water temperature and salinity daily in the harvested pond(s) ; take algal counts at least once each week, and record the nutrient levels NO_3-N, NH_3-N and reactive dissolved phosphate weekly if feasible. These data are necessary to ensure that algal concentrations remain sufficiently high for sustained *Artemia* growth, and to monitor the overall biological balance in the pond(s). If possible, compare algal concentrations and nutrient levels with data from an unharvested pond as a control.
- Collect a water sample each week from the harvested pond(s) and subsample until it contains about 300-500 *Artemia.* Count *all* individuals under a binocular microscope and classify into mature adults, juveniles and nauplii according to Wear and Haslett (1987). Make frequent observations along the shoreline for the incidence of cysts. These data will together provide essential information on the *Artemia* population structure, on whether reproductive activity is high or low, and if reproduction is tending towards ovoviviparity, or oviparity (cysts).
- Monitor record water temperatures and salinities about once each week over the harvestable period in potentially harvestable ponds which are not part of the pilot scheme.

Laboratory data

For this aspect, temperature and salinity control is essential. General methods are published by Wear and Haslett (1986) and Wear *et al.* (1986).

- Determine generation times of the local strain over the range of temperature and salinity combinations recorded in harvestable ponds over an annual cycle. The approximate range may already be known if routine recordings are made during the course of salt production. Experiments can be carried out on a moderately small scale with a minimum of 10 *Artemia* nauplii stocked in about 500 ml brine. Other biological parameters such as growth and maturation rates will be reflected in the generation time and need not be separately recorded. Fecundity is likely to follow a similar pattern (Wear and Haslett, 1987) and will be adequate to ensure net population growth over the harvestable period when generation times are relatively short. If any doubt exists, fecundity can be determined experimentally on a very small

scale using individual pair cultures. As variability will probably be high, at least five replicates will be required for each combination.

– Analyse data and produce a block diagram for generation times (and fecundity) as in Wear and Haslett (1987). If a computer is available, generate a polynomial response surface as a simple method for interpolating temperatures and salinities intermediate between those used in the laboratory studies (see Wear and Haslett, 1986 ; Wear *et al.*, 1986).

Interpretation

The above information is used to assess whether more than the sustainable yield of *Artemia* is being harvested while the pilot harvesting scheme is running. In the pilot study this is unlikely, as harvesting will occur in only part of a much wider and essentially confluent system, but the data will also be adequate to gauge maximum, full scale, sustainable yield of *Artemia* biomass providing annual cycles are similar from year to year.

Artemia biomass being generated per day in a given harvested pond is biomass concentration multiplied by the pond volume, divided by the generation time in days relevant for the temperature/salinity conditions prevailing at the particular time. The maximum biomass actually harvested should be some fraction less than one of generated biomass. The closer the fraction to unity, the nearer the harvested biomass approaches the maximum sustainable yield and the more detailed the monitoring program needs to be. If harvesting exceeds the maximum sustainable yield, salt production may be deleteriously affected by uncontrollable algal blooms and possible production of slimes in the crystallizing ponds.

High fecundity with nauplii and juveniles numerically more than about 80 % of the population, and ovoviviparity (as opposed to cyst production) dominating over appropriate temperature/salinity ranges, ensure that biomass is being renewed. If more than about 50 % of the population is adult, or if a bloom of cysts occurs, harvesting should cease unless brine is moving directly to crystallizing ponds following removal of *Artemia*. If pond nutrient levels become undetectable or fall to levels below the normal seawater range (Parsons and Tagahashi, 1975), or if pond algal concentrations fall below about 10 000 cells/ml and become limiting for *Artemia* growth and development, controlled pond fertilization may be considered. This figure of 10 000 cells/ml is an approximation only, as growth and assimilation efficiencies vary with differing algal feeds in a single strain (Sick, 1976). The lower density level at which food becomes limiting will thus change with the algal species in dominance, and may also be *Artemia* strain dependent.

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The DSIR-*Artemia* harvesting programme*

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Abstract

A process for the recovery of *Artemia* from the salt ponds at Lake Grassmere (New Zealand) has been successfully developed. This process involves the separation of the shrimp from other material by air flotation followed by the recovery of the shrimps on a screen. The product is then washed and either dried or frozen depending on the intended market. Pilot scale trials of this process have been carried out and the results are included in this paper along with information for the design of a commercial scale plant.

Introduction

Intensive studies undertaken by Wear and Haslett (1987) indicated that the harvesting of *Artemia* at Lake Grassmere may be a profitable operation. An annual sustained harvest of about 300 tonnes of wet *Artemia* biomass had been predicted, which is equivalent to about 40 tonnes dry weight. We were able to confirm this figure during our experimental session. However, the potential of *Artemia* biomass has been constrained by the difficulties in recovering *Artemia* in an uncontaminated form from brine ponds. A programme of work to overcome this problem was carried out by DSIR for Dominion Salt Ltd. This resulted in the development of a new technology for the harvesting of *Artemia* biomass (Janata *et al.*, 1984 ; Janata and Bell, 1984).

This paper describes the technology and discusses some of the important results of pilot plant operation at Lake Grassmere over a period of approximately 6 months. Scale-up information has been obtained to enable the design of a commercial scale plant, processing up to 300 l of brine per second.

Process flow

The flow diagram shown in Fig. 1 provides a description of the harvesting process : brine containing *Artemia*, algae and debris is pumped from the pond through a flotation unit (1) which separates most of the algae and debris from the brine shrimp. The *Artemia* and brine then pass a screen (2) which selectively separates the adult brine shrimp. Juvenile *Artemia*, cysts and unicellular algae wash through with the brine and are returned to the pond. The recovered adults are transferred to a processing facility where they are water washed (3) and dried (4).

* Under provisional patent status.

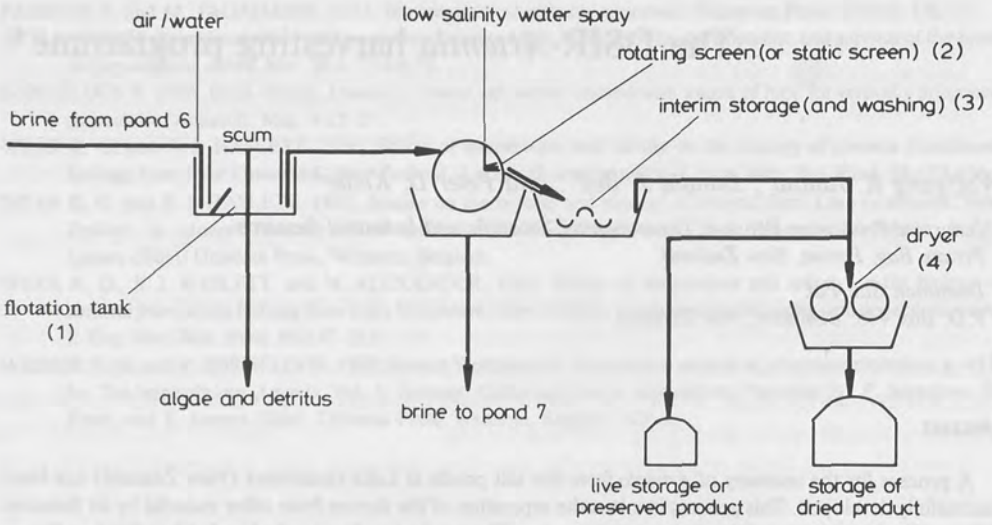

FIG. 1. *Artemia* harvesting programme : process flow diagram.

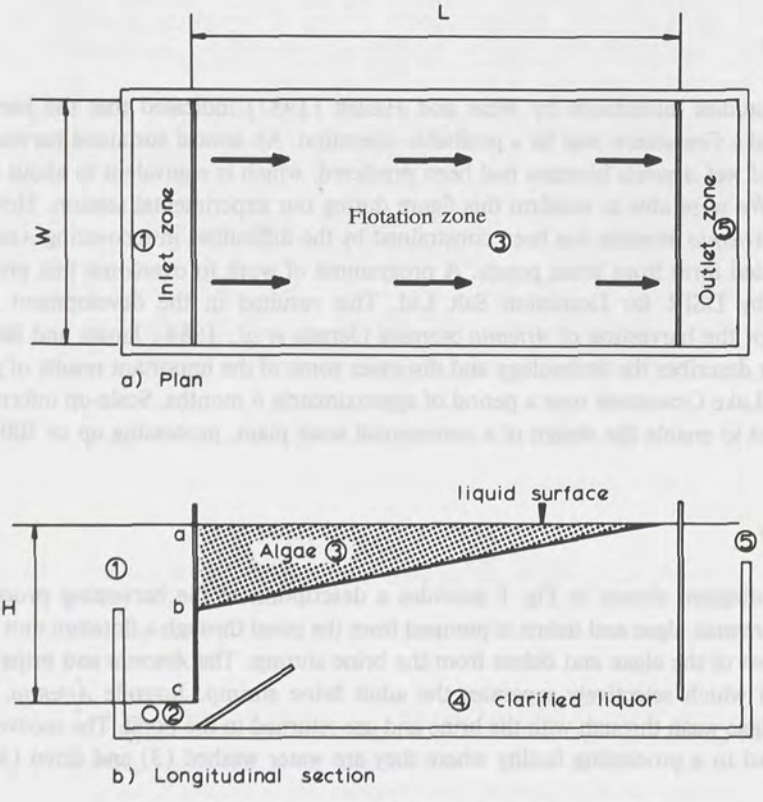

FIG. 2. Flow characteristics of a rectangular flotation chamber.

By means of the drawing of the flotation chamber in Fig. 2 the separation of algae from the brine shrimp can be explained as follows : the brine containing the algae and brine shrimp enters the tank (1), and is mixed with an air-supersaturated recycle stream. The suspended algae are floated to the brine surface (3) by attaching microscopic air bubbles to the individual particles. The *Artemia*, while moving, free themselves from the minute air-bubbles and mainly stay within the clarified liquor at the bottom of the tank (4), and are carried through by the main brine stream (5).

In a commercial plant the top algae is removed as necessary and can be processed separately or returned as feed to the pond up- or downstream of this harvesting point.

Fig. 3 shows that flow conditions in the flotation cell are crucial for an effective design. Here the design-coefficient denotes the degree of disturbance, present in the pilot-plant chamber and it shows clearly that algae removal has been most effective at and below $DC_f < 2\,000$.

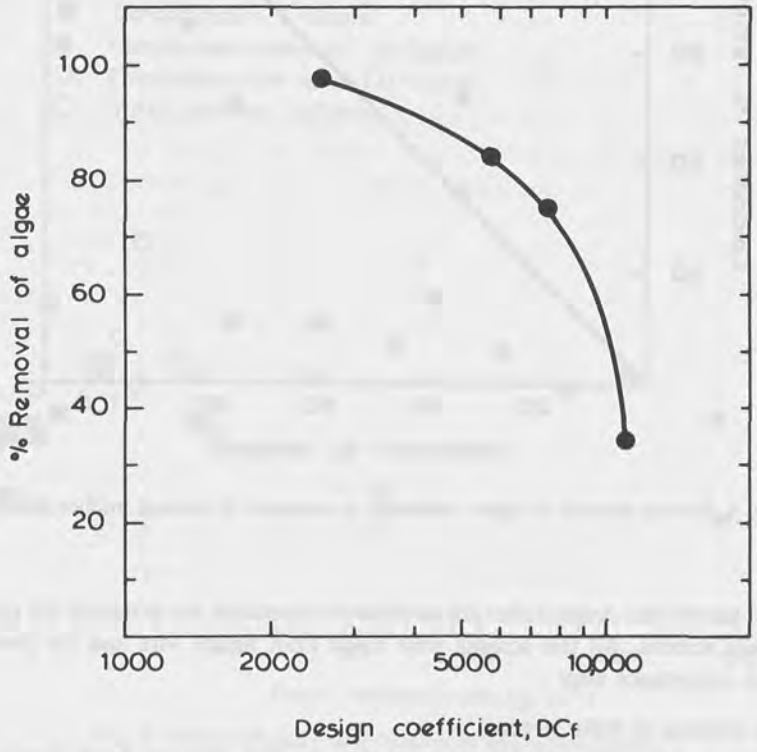

FIG. 3. Pilot plant removal of algae as a function of design coefficient.

In practice however, this design criteria cannot be used directly, because it conflicts with the rapid movement of the *Artemia* of 12 mm/s directed against the main stream in the flotation chamber. Consequently suitable flow conditions have to be selected and the tank dimensions calculated to allow for high removal efficiency of algae with little effect on the *Artemia* population

itself. A cross-check on the validity of this design-method is given by means of the pilot plant data shown in Fig. 4. Since typical bubble sizes range from approximately 45 to 60 μm, values of 50 and 60 μm were used to calculate the percent removal. As can be seen the calculated percent removals correspond well with the removals measured for the pilot plant. The design-method is therefore recommended for use in scale-up.

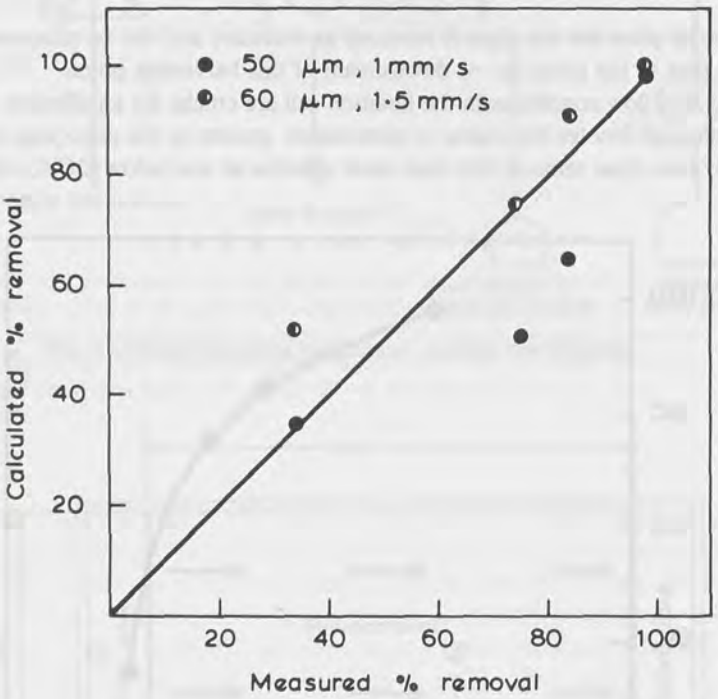

FIG. 4. Percent removal of algae : calculated *vs* measured % removal in pilot plant.

In order to harvest the *Artemia* after the air-flotation operation we evaluated the performance of five different screens. All test screens were made from wedge wire and the parameters of constructional importance were :

- use of a rotating or static screen ;
- the direction of rotation with regard to product loading onto and brine flow through the screen ;
- rotational speed and gapsize ;
- product take off and collection efficiency.

Fig. 5 summarizes all the data as a function of feed concentration. From there it can be seen that a Contrashear screen, run without guide vanes and in direction of main water flow proved to be the most effective device with 60 % screen efficiency. However, even though this configuration gave the best spot efficiencies problems occurred in removal of product from the

Contrashear screens. The high attrition rate, due to the continuous tumbling action of the product and its shear against the sharp edges of the wedgewire did not allow any sensible mass balance to be obtained. On the other hand the other screen results could be mass balanced with >90 % accuracy over all the running periods. Overall, and this includes operation under worst and best conditions, a 40-50 % total screening efficiency can be predicted for the use of such a device, the actual configuration not being a predominant factor. However, these efficiencies are based on mass balances which include shrimp, cysts and some algae. If the levels of non-shrimp related material are accounted for the actual screen efficiencies for shrimp, removals are about 20 % higher, as evidenced in Fig. 6.

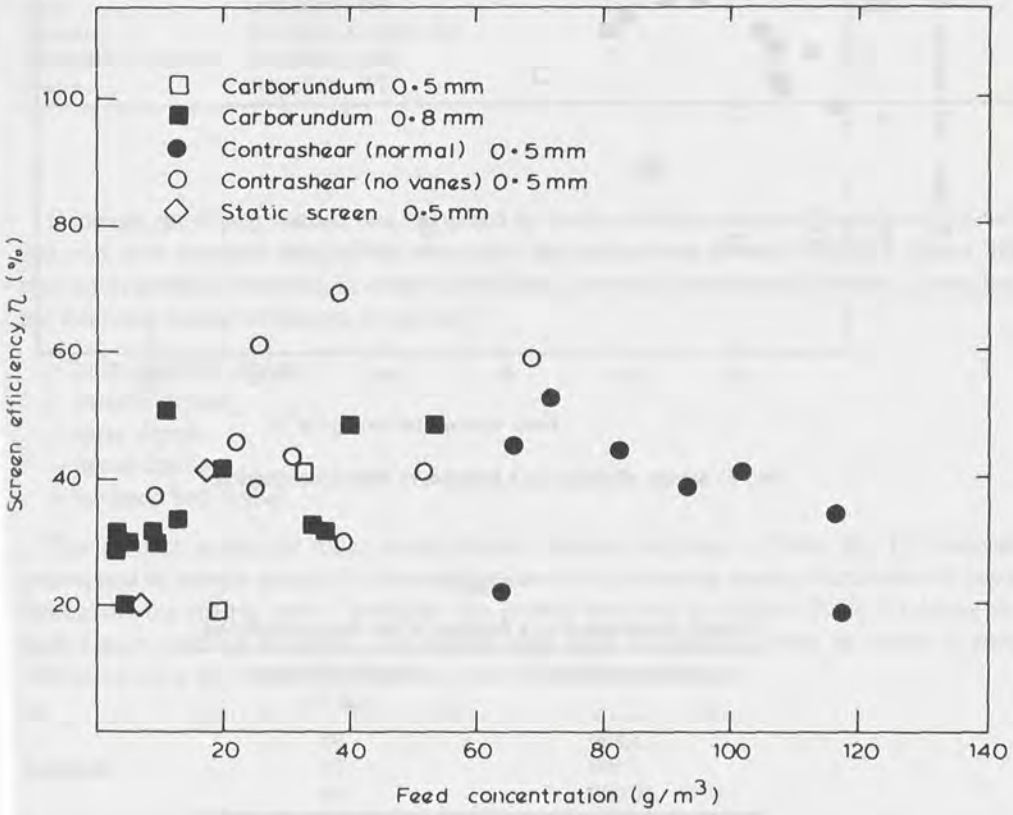

FIG. 5. Screen efficiency as a function of feed concentration.

The overall process efficiency of our pilot plant tests up to this point and based on product purity is given in Table I. The design-coefficient again refers to the conditions of flow in the flotation chamber and, as mentioned above, the running of the flotation tank at high design-coefficients, but still obtaining efficiences of >90 %, is possible by using an appropriately sized chamber. It also appears that the use of floodlights and night harvesting can be used to either increase harvest or to reduce required pumping time to catch a certain amount of *Artemia*. The results show a significant improvement of up to 560 % in product yield.

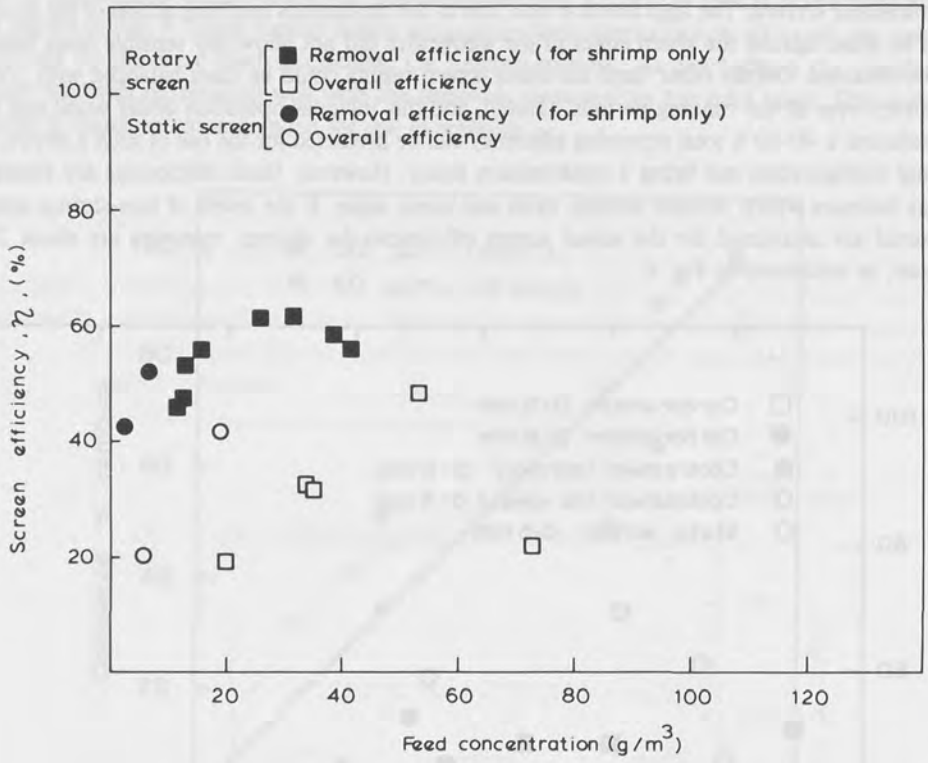

FIG. 6. Screen efficiency as a function of feed concentration.

TABLE I

Overall performance as a function of the design-coefficient

Design-coefficient	Process efficiency (in %)
5,800	95
7,500	75
10,000	69

During our trials only little work has been undertaken in the area of preservation of *Artemia* biomass. However, the *Artemia* product has been successfully preserved in its fresh state for up to 4 days by the introduction of sodium-metabisulphite (1 % on wet weight basis), and for up to 4 months by freezing this preserved product.

A second purpose of this investigation was to find the most suitable means of drying and use of the *Artemia* product. Product quality and marketability were the deciding measure.

Table II shows the types of dryers which were evaluated and includes information on relative cost indicators of a commercial 500 kg/h unit.

TABLE II

Dryer evaluation data

Type of dryer	Location of dryer and experiments conducted by	Manufacturing cost comparison	Total fixed cost comparison	Operating cost comparison
Forced air (60°C)	Grassmere, DS	1	1	1
Forced air (150°C)	Gracefield, IPD			
Fluid bed	Gracefield, IPD	1	1.7	1
Drum	Palmerston, DRI & IPD	1.4	1.7	1
Drum	Grassmere, DS & IPD			
Spray	Gracefield, IPD	1.2	2.3	1
Vacuum	Blenheim, Cudden Ind.	–	–	–
Continuous vacuum	Gracefield, IPD	3	10	3
Freeze	Gracefield, IPD	6	19	6

Generally the drying method was hampered by the low solids content of the original product and only slow moisture transfer was observed if the product was allowed to form a leather-like skin on its surface. Therefore, in order of preference, we would recommend for ease of handling the following drying techniques to be used :

- roller or drum drying ;
- vacuum drying ;
- spray drying ;
- freeze drying ;
- fluidized bed drying.

The product quality of roller dried *Artemia* biomass is given in Table III. The analyses correspond to a single sample of *Artemia* taken late in the harvesting season. Variations will occur throughout the season, and in particular the protein level will be higher (50-60 %) during the peak season (January to April). The relative high value for arsenic is seen as normal if cross referenced on a dry weight basis against other food of marine origin.

Outlook

A limited market research study has been undertaken with the following results : at present there is no specific market for an annual extra output of some hundreds of tonnes of brine-shrimp biomass ; however, a considerable commercial demand could arise for various *Artemia* products ; *e.g.*

- brine shrimp meal as a complement to or substitute of fish meal and/or as a supplement in commercial foods (both land based and in aquaculture) ;
- *Artemia* protein concentrate ;
- *Artemia* as raw material to extract interesting biochemical products ;
- brine shrimp preparations for human nutrition.

TABLE III

Chemical analyses of roller dried *Artemia* biomass

Bulk analysis (in %)		Fatty acid analysis (in %)		Amino acid analysis (% amino acids in air-dry product)	
Protein (Kjeldahl)	42	C14:0	1.4	Asp	3.39
Ash	20	C14:1	0.9	Thr	1.66
Total lipid	6	C15:0	0.5	Ser	1.83
Moisture	1	C16:0	16.4	Glu	4.66
		C16:1	8.7	Gly	1.76
		C16:2	1.1	Ala	1.97
		C18:0	5.3	Val	1.74
		C18:1	35	Met	0.79
		C18:2	5.4	Iso	1.55
		C18:3	13	Leu	2.59
		C18:4	1.3	Tyr	1.41
		C20:2	0.5	Phen	1.46
		C20:5	2.8	His	0.99
				Lys	2.16
				Arg	2.32

Ash analysis

% composition of ash

CaO	Na_2O	K_2O	MgO	SiO_2	SO_3	P_2O_5	Cl	Al_2O_3	Fe_2O_3
3.7	27.0	4.5	4.0	12.9	4.6	7.1	14.7	3.2	1.6

% composition of sample

Ca	Na	K	Mg	Si	SO_4	PO_4	Cl	Al	Fe
0.5	4.0	0.7	0.5	1.2	1.1	1.9	3.0	0.3	0.2

As	Hg	Cd
0.0025	<0.0008	<0.0003

Samples of drum-dried *Artemia* product were distributed to a number of interested groups and their comments are summarized below :

- in relation to the Japanese market,
  - * carotenoids, which are concentrated in *Artemia*, are used as colouring material in chicken feed preparations to enhance the yellow to orange colour of the egg-yolk ;
  - * *Artemia* flake may find a market as "pet food" for the ornamental fish market, where some species are traded for up to NZ$ 1 000 ;
- a number of samples have been given to members of the Wellington Buddhist Society. Their response was that an immediate use is possible if the product is marketed in a more "concentrated" form as a fish-stock. *Artemia* flake as a condiment type food preparation is not common in Asian cooking. The taste and appearance however was acknowledged generally quite favourable ;
- a positive response with the possibility of a processed cheese production trial was received.

From this one can be quite enthusiastic about the prospect of commercial *Artemia* harvesting. All the more, because a rigorous economic analysis (including a rate of return assessment and a product sensitivity analysis) looks promising indeed (Dominion Salt Ltd., proprietary information).

Acknowledgements

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Commercial production of *Artemia* in the Philippines

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Abstract

This paper summarizes experiences and findings in the development of a tidal saltpond system, integrated for commercial *Artemia* production. The basic rules of successful pond production of fish and shrimp, which are the exclusion of predators and competitors, the maintenance of good water-quality, the provision of sufficient food and the proper harvesting method, also govern *Artemia* culture.

In attempting to achieve successful *Artemia* culture, an integrated flow-through system was developed. The necessity for a detailed plan of the ponds with well-determined elevations, advantageous positioning of gates and canals, correct pond orientations, and well-constructed dikes is considered crucial to integration.

Procedures for proper *Artemia* pond preparation are discussed. The relatively high rainfall (1 200-2 000 mm/year) in the Philippines necessitates reliable screen designs to exclude potential fish predators and overflow devices for freshwater runoff, to extend the duration of *Artemia* seasons.

Installation of tidal plane gauges and an auxiliary high volume pump in the salt farm permits the manipulation of water levels to ensure adequate water exchange or supply to the system, high evaporation : salt bed ratios, and optimal depths of high salinity water. The daily water requirements for the system were determined and its relationship to salt production examined.

The integration of organic fertilization and the utilization of drainage from semi-intensive prawn and shrimp ponds enabled to achieve high standing crops of *Artemia* (up to 7 tonnes wet weight/ha) in high-salinity ponds that are unsuited for fish or prawn culture. Under optimal conditions, the *Artemia* cyst production reaches 20 kg dry weight/ha/month.

Introduction

The brine shrimp *Artemia* has long been recognized as an excellent live food for fish and crustaceans. Since no natural population of *Artemia* are found locally, shrimp and sea bass hatcheries in the Philippines are highly dependent on expensive imported cysts. *Artemia* is known to account for as high as 50-70% of the hatcheries' operational costs. The great demand for brine shrimp in the world market further increased the difficulty of obtaining consistent supply of good-quality cysts.

The feasibility of locally producing *Artemia* has been shown by De los Santos *et al.* (1980) and by our previous studies (Jumalon *et al.*, 1983). Although other reports of man-made inoculations have been released locally and abroad (Vos and Tansutapanit, 1979 ; Royan, 1981 ; Vos, 1981), *Artemia* production in the Philippines has remained on a pilot-scale, largely because

of lack of systematic and repeatable methods of producing *Artemia* in ponds. To solve this, we conducted various experiments and production trials at the SEAFDEC Aquaculture Department Leganes Research Station in Iloilo and some selected saltfarms to define optimum pond conditions for *Artemia* culture. A commercial-scale *Artemia* production venture was finally set. This paper summarizes our experiences and findings in the development of a tidal salt pond system, integrated for commercial *Artemia* production.

Background information

The series of experiments and production trials conducted from 1980 to 1982 at the SEAFDEC Aquaculture Department Leganes Research Station in Iloilo, Philippines (Jumalon and Robles, 1983 ; Jumalon *et al.*, 1983) were extended in 1983 to include pilot-scale *Artemia* production in various saltfarms around the country, *i.e.* in Bohol, Cebu, Negros Oriental and Bulacan. Vos (1981) started *Artemia* inoculation in some of these areas through a FAO/UNDP project, but brine shrimp production was not established because the project was discontinued.

With the increasing demand and high cost of *Artemia* in the Philippines, a commercial scale *Artemia* production venture was started in 1984 at the Sycip Plantation, Inc. (SPI) salt farm in Manjuyod, Negros Oriental.

The relatively short and wet summer of 1984 and 1985, the abundance of *Tilapia* and other predators in the Sycip farms and the limited supply of organic fertilizer or high cost of imported inorganic fertilizers made it necessary to develop a modified system for *Artemia* pond culture.

Description of culture system

Design of the integrated *Artemia* production system

Past *Artemia* production trials in the Philippines involved inoculation of the brine shrimp in one or more evaporation compartments of the saltfarm and then operating the culture either independently or as part of the salt pond system, acting as saltbed-water source (De los Santos *et al.*, 1980 ; Primavera *et al.*, 1980 ; Vos, 1981). The major changes in the design created for the SPI commercial salt farm in Negros Oriental include : construction of a poultry over the main inlet/reservoir, culture of sea bass (*Lates calcarifer*) in supply canals and in reservoir ponds receiving seawater directly, and operation of the farm as an integrated flow-through system. A detailed description of the model 20 ha farm (map in Fig. 1) is given in Jumalon and Ogburn (1987).

The necessity for a detailed plan of the ponds with well-determined elevations, advantageous positioning of gates and canals, correct pond orientation, and well-constructed dikes is considered crucial to integration of *Artemia* culture with poultry-salt-fish and prawn/shrimp production. The pond elevation controls the allowable water depth and flow of water in ponds, while pond orientation generally affects harvesting, especially of *Artemia* cysts. In selection of evaporation compartments for development to *Artemia* ponds, one must take into account the presence of a canal or water intake/drainage structures by which it can be drained or filled with high salinity or fresh seawater any time the need arises. This is important for effectively managing the pond to bring in or replenish natural food and to manipulate salinity to induce cyst or nauplii production. In the model farm (Fig. 1), this is served by the brine supply/storage canal (BSC),

the main supply canal (MNS), the gates between pond AB and the chicken pond (CKP), and the standpipe between the penaeid shrimp pond (ISP) and pond AC.

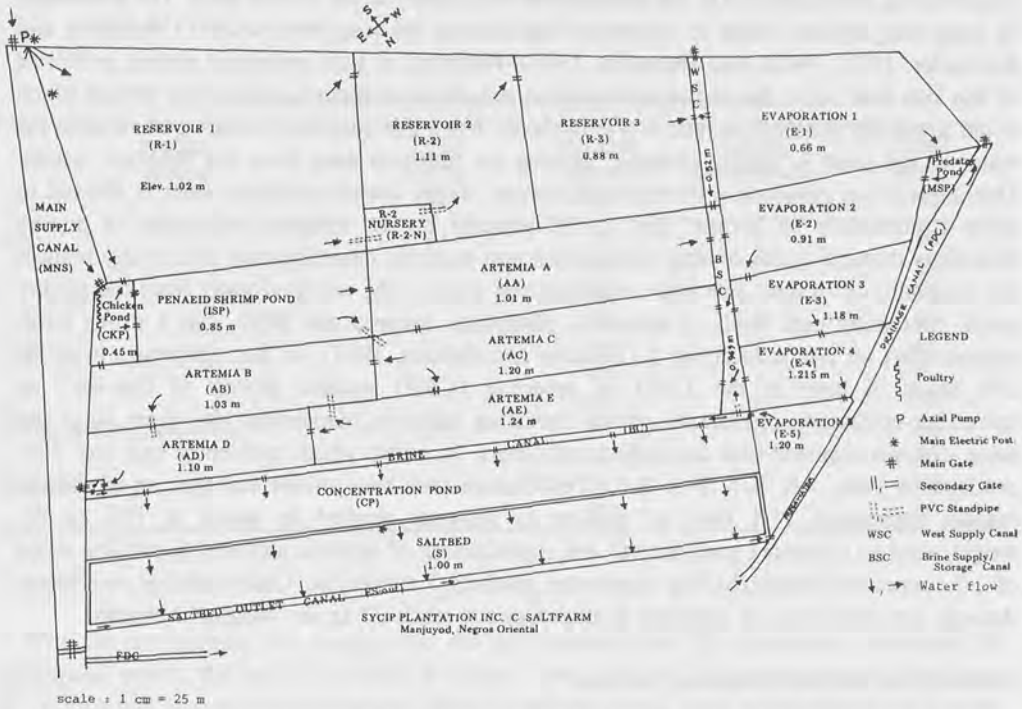

FIG. 1. Lay-out of the integrated flow-through pond production system (from Jumalon and Ogburn, 1987).

Orientation of *Artemia* ponds must take into consideration the prevailing wind direction during the dry season (mainly NE) so that floating cysts can accumulate at the windward corner of the pond, thereby facilitating cyst collection (Jumalon *et al.*, 1983). Gates and canals are best positioned to enhance pond mixing of incoming water before flowing through to the next compartment. Proper screening of gates is necessary to prevent entry of potential predators. Bag screens made from 0.5-1.0 mm mesh nylon shaped by rattan hoops and mounted on rectangular frames that slide into grooves on the gate are found more effective than the usual semicircular screen which are just pegged to the dikes and pond bottom. Constant water movement washes out some of the dike soil, thus leaks easily occur with the latter design.

The presence of burrowing crabs was observed to be a big problem in maintaining dike stability. Leaks resulting from poorly constructed dikes are also detrimental since they allow entry of predators to *Artemia* or competitors to prawn and milkfish ponds. Construction of puddle trenches solves the problem only temporarily. Planting of vegetables and grass along the side of the dikes helps prevent soil erosion. The use of concrete dikes is uneconomical in the Philippines, thus studies are now being made to look for alternative cheap materials that can be utilized as impervious barriers in pond dikes.

Poultry enrichment effects in a flow-through pond system

The increasing cost of imported inorganic fertilizers and poultry products (transported from neighbouring provinces) led to the construction of a poultry at the SPI salt farm. The advantages of integrating animal wastes to freshwater fish-farming has long been studied (Wohlfarth and Schroeder, 1979 ; Pullin and Shehadeh, 1980). However, in such integrated system, problems of fish kills may occur due to the accumulation and decay of manure at the pond bottom which could lower the dissolved oxygen to a lethal level. When this condition occurs, part or all of the water in the pond is usually changed, flushing out nutrients away from the fishpond system. Operation of the ponds in a flow-through system, where manure enriched water is allowed to move continuously by bottom flow to the adjacent ponds, enhances utilization of poultry droppings through cycles of algal assimilation and bacterial mineralization and greatly reduces the possibility of oxygen deficiency in subsequent ponds. The enriched water from the poultry pond, containing high levels of ammonia, phosphate, bacteria and BOD_5, has a strong fertilization effect on ponds receiving it (Jumalon and Ogburn, 1987). In fact, droppings from the 200 heads of layer at the 1 300 m^2 reservoir (CKP) enabled growth of "lab-lab", an epibenthic-epilimnion production system consisting mainly of filamentous blue-green algae and some diatoms together with associated consumers, in ponds which previously had very poor productivity (AA, AB, R-1, R-2, R-3). Fertilization tests have shown that the use of chicken manure suspension (1:1 ratio of manure to seawater applied to ponds at 100 kg dry weight/ha/day) promotes good growth and reproduction of *Artemia*, supporting standing crops of 2-3 tonnes wet weight/ha and sometimes reaching 7 tonnes/ha. Under optimal conditions, *Artemia* cyst production in intensely fertilized ponds reach 20 kg dry weight/ha/month.

Sea bass culture for predator control

The relatively high rainfall (1 200-2 000 mm/year) in the Philippines makes it difficult to exclude fish predators from ponds by salinity manipulation. In Negros Oriental province, the seasons are not very pronounced and dry season is short, lasting only 1-3 months/year (Yambot, 1975), thus proper pond preparation is necessary for *Artemia* production. The introduction of sea bass into supply canals in the integrated system greatly helped in controlling the entry of predators to culture ponds, which in previous years had uncontrollable populations of *Tilapia* (*Sarotherodon mossambicus*) and gobies. This approach to sea bass culture is also advantageous in that no additional feed inputs, like trash fish or live *Tilapia*, are required, because the water coming into the supply canals and reservoirs is usually rich in small shrimps (*e.g. Acetes*) and fish.

Artemia pond prepartion

At the start of pond preparation, the ponds are dried completely to help eliminate potential predators.

Fish left in small mud holes, where *Tilapia* previously nested, are killed by the use of non-residual poisons such as rotenone extracted from *Derris* roots, or calcium oxide plus ammonium sulphate. Raking or plowing the upper 2-5 cm layer of the pond bottom when the ponds are dry is recommended, especially where black soil resulting from accumulation and decay of organic matter is observed. This improves soil mineralization and subsequent release of nutrients.

High salinity water of about 90-100 ‰ is best used to fill *Artemia* ponds during the first flooding. This is an effective means of killing any remaining predators. Table I shows the lethal salinity levels for commonly found *Artemia* predators.

TABLE I

Tolerance of *Artemia* predators to increasing salinity levels

Predator species	Initial salinity (‰)	Stocking salinity (‰)	Salinity increment/day (%)	Lethal salinity (‰)
Goby	35	40	5	90
		60	5	80
		80	5	85
		90	0	90
Tilapia	35	40	5	110
		75	5	90
		100	0	100
Diving beetle (Dytiscidae)	35	35	5	60
		60	0	60
Mosquito larvae	35	35	5	60
		60	0	60

With the construction of a poultry over the inlet reservoir and the continuous enrichment of subsequent ponds, the use of inorganic fertilizers (urea or 46-0-0, monoammonium phosphate or 16-20-0 and others) to promote good plankton growth during pond preparation is minimized or eliminated.

The extremely low tide levels occurring during the dry seasons often make it difficult to get sufficient quantity of water to serve both *Artemia* and salt bed requirements. The 2.2 ha crystallization pond of the model integrated farm requires 500 m^3 water daily, for bed cleaning and salt production. Evaporation and seepage rates average about 40 m^3/day, thus the system requires 540 m^3/day to maintain constant depth. Maintenance of deeper *Artemia* ponds of at least 25-30 cm make it necessary for the system to operate at tide levels as high as 1.50 m, thus lowering salt production if water cannot move to the crystallization pond during low tides. To ensure adequate water exchange or supply to the system, high evaporation : saltbed ratios, and optimal depths of high salinity water, tidal plane gauges were installed on the gates of some ponds (B-1, CKP, ISP, BSC, E-1, AB and AD), and an axial pump (610 mm diameter) with an average flow rate of 37 850 l/min, was constructed at the gate area of the main supply canal. The pump also serves another adjacent 10 ha saltfarm.

MAINTENANCE OF *ARTEMIA* PONDS

From past studies (Jumalon and Robles, 1983), it has been shown that *Artemia* is best inoculated at a density of 50 nauplii/l. Recent inoculations at the farm followed this rate, using San Francisco Bay strain (batch number 373), and were done when good growth of microplants (10 000-12 000 cells/l), especially *Tetraselmis*, was observed. *Artemia* pond depth should not

go below the minimum recommended depths of 25-30 cm to prevent lethal high water temperatures (40-42 °C) occurring. The lack of wind movement during very calm days, a common occurrence at the peak of summer, also causes temperature stratification, even when the water is bottom-flowed from pond to pond. Mass mortalities in *Artemia* ponds can be prevented by mixing the water either through the use of paddle wheel circulators or by rowing around the pond in a small boat. The installation of coconut fronds (De los Santos *et al.*, 1980), though not solving the high water temperature problem, was considered beneficial because *Artemia* tend to hide under these shades on sunny days.

To maintain higher salinities in *Artemia* ponds even during rainy days, overflow devices were installed. These include PVC standpipes and well-regulated gates, which allow easy flow of the top layer of rain water. Although De los Santos *et al.* (1980) state that *Artemia* inoculation in Southeast Asia will be of temporary nature due to elimination of the population by predation at the onset of the rainy season, our results using overflow system combined with proper screening showed otherwise. Large brine shrimp biomass (2-7 tonnes wet weight/ha standing crop) can be maintained in the farm at salinities of 60-80 ‰, even after heavy rains. Below 60 ‰, *Artemia* predators and competitors, like copepods, mosquito larvae and the carnivorous dytiscid beetle, may start to inhabit the pond water. The fungi, *Lagenidium*, is also observed to infest *Artemia* after successive rains cause deterioration of water quality. Mortality is prevented by water change or increase in salinity to 60 ‰.

Salinity manipulation for cyst production is possible only during the dry season when sufficient store of very high saline-water (above 90 ‰) is available. Continuous biomass production, however, is possible during the rainy season since nauplii production is triggered by sudden salinity drops of 20-30 ‰ (Jumalon *et al.*, 1983).

Continuous flow of enriched water to the *Artemia* ponds during summer is observed to promote good plankton blooms (*Tetraselmis* and centric diatoms) and minimizes "lab-lab" production. This is particularly ideal for *Artemia* cyst production because good food is provided while higher salinities are maintained. Also, cyst harvest will not be hindered by excessive "lab-lab" growth.

During the rainy period, the flow-through management has to be stopped or modified so that water flow ends at the *Artemia* ponds or is diverted directly to the saltbeds through other ponds in the system. This is necessary to prevent eventual lowering of salinity to that of a normal seawater. In this case, additional food inputs are required for *Artemia* since the more stagnant water does not promote good plankton production. Pure chicken or cow manure suspensions (1:1 ratio of manure to seawater) or combined chicken and cow manure suspensions applied in small, frequent doses (up to four times per day) at a total rate of 1.0-3.5 tonnes dry weight/ha/month is found very effective. This not only promotes good *Artemia* standing crops of 2-7 tonnes/ha (equivalent to 220-270 kg dry weight/ha), but also results in large accumulation of floating, easily harvestable "lab-lab", reaching 1.2-5.5 tonnes dry weight/ha/month (Ogburn *et al.*, 1986). These findings indicate that "lab-lab" and *Artemia* could co-exist in ponds without detrimental effects and that *Artemia* ponds can also be converted as "lab-lab kitchen" pond after the salt/cyst season. The clearing effect of the *Artemia*'s continuously filter-feeding movement enables better penetration of sunlight to the pond bottom, thus increasing photosynthesis and algal production. Daily harvests of 15-30 kg wet brine shrimp/ha (1.6-3.3 kg dry weight/ha) and 0.5-3.0 tonnes wet "lab-lab"/ha (0.1-0.5 tonnes dry weight/ha) has been done at the model farm.

The *Artemia* that may be trapped in the "lab-lab" mats increase the nutritive value of the latter when it is fed to fish and shrimps. From the 0.87 and 0.66 ha *Artemia* ponds presently operating, enough "lab-lab" is harvested to supplement the milkfish/shrimp culture in the model farm, as well as those of another 10 ha salt farm. The daily harvest of *Artemia* could be increased further, when better processing techniques, *e.g.* incorporation in pellets, are locally developed. Frequent thinning of the *Artemia* population is necessary to prevent large mortalities due to overpopulation. During regular "nauplii blooms" which occur every 2-4 weeks, especially after heavy rains, over a liter of *Artemia* nauplii (equivalent to 1 kg dry cysts) is easily collected per ha/day.

The utilization of enriched water draining off from semi-intensively stocked shrimp ponds ($3/m^2$) within the integrated pond system was observed to have similar effects as the application of animal manures in maintaining good production of *Artemia.*

The problems of large-scale *Artemia* harvest processing are now being studied for better pond management and utilization.

With the modified integrated flow-through system, annual production of salt in the farm increased four times over the past year when *Artemia* was just introduced and there was competition for high salinity water. *Artemia* could also eventually increase salt production by promoting growth of desirable organisms in the salt pond (Sorgeloos, 1983).

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Nutrient flow and physicochemical profile studies of an integrated poultry-salt-*Artemia*-milkfish-sea bass-shrimp pond production system

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Abstract

The physicochemical and biological parameters of an integrated flow-through poultry-salt-*Artemia*-milkfish-sea bass-shrimp pond production system were monitored for 6 months to determine the pattern of nutrient changes and associated plankton communities as water passed through the system. A 20 ha salt farm in Negros Oriental, Philippines, was used as a model of the integrated system. The construction of a poultry farm at the inlet reservoir provides continuous fertilization and allows considerable control of nutrient inputs to the pond system. Nutrients increase markedly in the chicken pond and in subsequent ponds undergo cycles of biological assimilation and bacterial mineralization.

Principal component analysis ordinates temporal and spatial changes in 33 variables that were monitored. Using scatter diagrams of the principal components enabled separation of ponds adjacent to the crystallization bed and ponds high in fish biomass from the rest of the system. Variables that served to numerically delineate the ponds in this manner were salinity, phosphate, ammonia, alkalinity, turbidity, acidity, microplants, pico- and nannoplankton. The implications of this ordination in relation to pond management techniques is discussed.

Introduction

Production of the brine shrimp *Artemia* in the Philippines is largely based on integration with existing salt pond systems (De los Santos *et al.*, 1980). These salt farms are traditionally operated as milkfish/shrimp ponds in the wet season (July-December), while during the dry season, milkfish is cultured in the reservoir and first evaporation compartments, with salt harvested from the crystallization beds as the main product. *Artemia* is introduced into the normally unstocked highly-saline evaporation ponds. *Artemia* cysts or biomass that are harvested thus provide additional income for the salt farmers.

In an attempt to model optimal production criteria, we have undertaken various studies involving experimental and pilot-scale production of *Artemia* (Jumalon and Robles, 1983; Jumalon *et al.*, 1983). Due to the great demand of *Artemia*, resulting from the increased number of shrimp hatcheries and the recent development of the sea bass (*Lates calcarifer*) hatchery technology in the country, a commercial-scale *Artemia* production venture was started. This led to the development of a modified design integrating *Artemia* culture with poultry, salt, milkfish,

sea bass and shrimp production in a flow-through pond system. The physical and chemical parameters of the system were characterized to determine the pattern of nutrient utilization and consequent biological pond productivity as water passed through the system. This study is important for proper management of the new pond system.

Materials and methods

Development of an integrated flow-through poultry-salt-*Artemia*-milkfish-sea bass-shrimp pond production system was started in 1984 in the 20 ha salt farm of the Sycip Plantation, Inc. in Manjuyod, Negros Oriental, Philippines. Fig. 1 shows a map of the ponds and the direction of water flow around the system. The southwest side of R-1, R-2, R-3 and E-1 is thickly lined with mangroves (*Rhizophora* sp.) which serve as boundary to a big tidal flat. The poultry was constructed primarily because of the lack of organic fertilizers in the vicinity and to minimize the use of costly imported inorganic fertilizers. Waste from a cattle-fattening shed also enters the system through a trench running parallel to the main supply canal (MNS), and serves as additional ferrtilizer source. The pond preparations made and the utilization of ponds as culture areas for various species (aside from provision of continuous water supply to the saltbeds) are described in Table I.

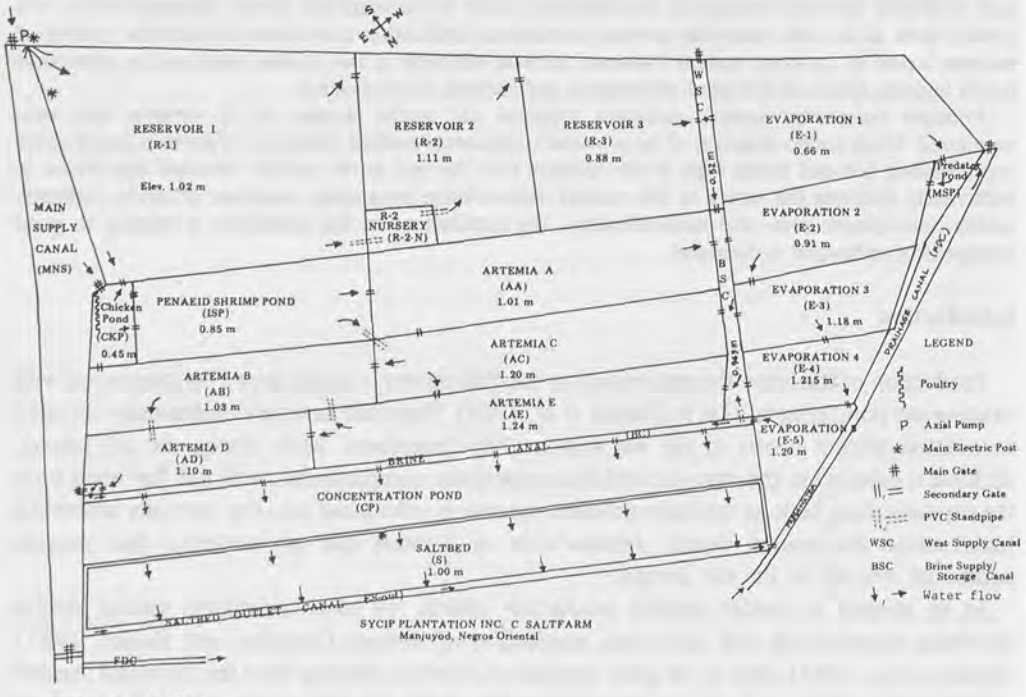

FIG. 1. Lay-out of the integrated flow-through pond production system.

TABLE I

Pond preparation and utilization of ponds as culture areas during the monitoring period

Ponds	Species cultured	Culture type and stocking density	Pond preparation/inputs
R-1	Milkfish (*Chanos chanos* Forskal)	Grow-out 0.2/m^2	100 kg lime ($CaCO_3$) 1 000 kg cow manure (CMN) 1 000 kg chicken manure (CK)
R-2	Milkfish	Grow-out/transition 0.2/m^2	100 kg $CaCO_3$ 1 000 kg CMN 1 000 kg CK
R-3	Milkfish	Grow-out 0.2/m^2	Same as R-1
WSC	Sea bass (*Lates calcarifer*)	Nursery 1/m^2	0
BSC	Sea bass	Grow-out/maturation 0.02/m^2	0
E-1	Tiger shrimp (*Penaeus monodon*) White shrimp (*Penaeus indicus*)	Grow-out 2/m^2 Grow-out 1/m^2	1 000 kg $CaCO_3$ 1 000 kg CK
E-2	Milkfish	Grow-out/transition 0.5-2/m^2	50 kg $CaCO_3$ 200 kg CK 100 kg CMN
E-3	Milkfish	Grow-out/nursery 0.5-3/m^2	25 kg $CaCO_3$ 100 kg CMN 250 kg CK 10 kg biozome
E-4	Milkfish	Same as E-3	0
MSP	Milkfish	Maturation 0.42/m^2	0
CKP	Sea bass	Grow-out/maturation 0.02/m^2	Droppings from 200 layers (600 chicks later added)
ISP	White shrimp Tiger shrimp	Grow-out 2/m^2 Grow-out 1/m^2	50 kg $CaCO_3$
AA	White shrimp Tiger shrimp	Grow-out 0.5/m^2 Grow-out 0.5/m^2	100 kg $CaCO_3$
AB	*Artemia*	Biomass production 50 nauplii/l	50 kg $CaCO_3$ 10 kg urea
AC	Milkfish	Grow-out 0.5/m^2	10 kg CaO 10 kg urea 50 kg $CaCO_3$ 10 kg biozome
AD	*Artemia*	Biomass/cyst production 50 nauplii	50 kg $CaCO_3$ 10 kg CaO 10 kg urea
AE	Milkfish	Grow-out 0.5/m^2	10 kg CaO 10 kg urea 50 kg $CaCO_3$ 10 kg biozome

Monitoring of environmental and biological parameters was done from January to July, 1985. Ponds representing major parts of the integrated flow-through system were selected for monitoring purposes. These include R-1, CKP, ISP, BSC, E-1, MSP, CP, S-out, AB and AD. The physicochemical variables studied and the references/materials used are listed in Table II. The plankton community was classified (modified from Sieburth, 1979) into the following size groups :

picoplankton	0.2 - 2 μm
nannoplankton	3 - 10 μm
microplantkon	11 - 60 μm
meioplankton	61 - 120 μm
macroplankton	>120 μm

Picoplankton and nannoplankton were obtained from 500 ml water samples collected at the center of each pond. Cell counts were made immediately from fresh, unfixed samples using a Neubauer haemocytometer. Microplankton counts were made from 40 μl subsamples (of the collected 500 ml pond water) fixed in lugol's solution and placed in an improvised Sedgwick-Rafter counting chamber. Meio- and macroplankton were taken from 2 000 ml water samples concentrated through a 50 μm sieve and backwashed with a known volume of plankton-free seawater before fixing in lugol's solution. As with microplankton, 40 μl subsamples were analyzed using a Sedgwick-Rafter counting chamber. Plankton counts were made under a compound Olympus microscope (model BHTU) using phase contrast. Identification of genera were made, whenever possible, based on the works of Umezaki (1961), Shirota (1966), and Sieburth (1979).

TABLE II

Physicochemical parameters monitored and references/materials used

Variable	Reference/materials
Water depth	Depth gauge
Salinity – top and bottom water	AO Goldberg T/C refractometer
Water temperature – min. and max.	Taylor (Sybron) minimum-maxium thermometer
Turbidity – Secchi reading	Improvised Secchi disk
Turbidity – 545 and 880 nm	Brinkmann PC/600 colorimeter
Particulate dry matter	Millipore filtration (Whatman glass microfibre filter GF/A on top of Whatman 42)
Alkalinity – phenolphthalein and total	APHA (1971)
Water acidity	Boyd (1979)
pH	Fisher Accumet pH meter Model 140
Phosphate	Grasshoff (1976)
Ammonia	Boyd (1979)
Ammonia + amino acids	Grasshoff (1976)
Nitrite + nitrate	Grasshoff (1976)
Silicate	Grasshoff (1976)
Dissolved oxygen : 1800, 2400, 0500 and 0700 h	Winkler titration (Grasshoff, 1976)
Total rainfall	Victor rain gauge
Relative humidity	Bacharach Sling psychrometer

All parameters were monitored once or twice weekly, except for water depth, salinity, temperature, rainfall and relative humidity which were read daily.

Mean and standard deviations of variables were calculated for each ponds' observations. Each variable was then standardized over the entire samples to present a data matrix for R type factor analysis (Rummel, 1970). This method of collapsing a data cube results in a variance that defines interrelationships between changes in variables across the 10 ponds sampled. Factor analysis and varimax rotation algorithms were taken from Cooley and Lohnes (1962).

Results and discussion

Average values and standard deviations of each variable for each pond are given in Table III and IV.

Large differences in some physicochemical variable means were found between ponds. As expected, salinity tended to increase and depth decreased as water flowed from the inlet (R-1) to the saltbeds. Salinity of R-1, which is similar to incoming seawater, averaged 36 ‰. *Artemia* ponds (AB and AD) averaged 75 ‰. Water entering the crystallization beds varied between 70-85 ‰.

Concentration of nutrients showed a distinct rise as water passed from R-1 through the poultry pond (CKP). Phosphate increased approximately 20 % and ammonia increased almost 100 % on average. These nutrients decreased as they flowed to subsequent ponds, probably as a result of biological assimilation. Ponds adjacent to the crystallization bed had relatively high nutrient contents (silica, ammonia, and phosphate), possibly caused by evaporation of water. Water acidity (free CO_2) was notably much lower in this area. Nitrate (NO_3) was in significant concentrations only near the entrance point of new seawater (R-1 and CKP).

Mean diurnal oxygen values did not show clear trends except that early morning oxygen rise in CKP was very low, probably a result of shading by the poultry house. Storage of water under the poultry for more than a week resulted in very low early morning dissolved oxygen values.

Rainfall was above the seasonal average, with a total of 563 mm for the period January-June, as compared to a total of 503 mm for the same months in previous years (unpublished rainfall data for 1964-1983 at the Sycip Plantation Inc.).

Pico- and nannoplankton components are bacteria and yeasts, and a few protozoans. Microplants are composed mainly of phytoflagellates (especially *Tetraselmis*) and centric diatoms (*e.g. Chaetoceros*). Meioplants and macroplants are mostly filamentous blue-green algae (*Lyngbya* and *Oscillatoria*). Some pennate diatoms were also identified in the meioplants. Micro- and meioanimals are predominantly flagellates (including various dinoflagellates) and spirotrich ciliates. Crustaceans, the rotifer *Brachionus* and some big ciliates were found in the macro-animal group.

Mean values of pico- and nannoplankton concentration (Table IV) increased progressively as the water passed through the pond system, particularly ponds with high fish biomass (*e.g.* MSP) until it reached *Artemia* ponds adjacent to the saltbed where counts dropped dramatically.

Micro- and meioplants had highest mean concentration at and immediately after the poultry, and prior to the saltbeds. No other major trends in the biological community examined were discernible. However, the application of manure from either the chicken pond or cattle fattening shed resulted in a much higher production of benthic "lab-lab", a biological complex of

TABLE III

Means and standard deviations of physicochemical parameters in selected ponds at the SPI salt farm

Parameter	Ponds (number of observations)				
	R-1 (24)	CKP (25)	ISP (23)	BSC (25)	E-1 (25)
Pond area (m^2)	28 700	1 300	9 000	1 500	14 800
Water depth (cm)	42.6 ± 6.63	99.8 ± 6.96	58.2 ± 6.06	51.7 ± 23.2	72.9 ± 8.79
Salinity – top (‰)	35.6 ± 3.81	37.1 ± 3.72	39.9 ± 3.97	39.5 ± 6.38	37.0 ± 5.79
Salinity – bottom (‰)	36.2 ± 3.47	37.8 ± 3.67	40.7 ± 3.63	40.2 ± 6.43	37.7 ± 5.32
Temperature – minimum (°C)	25.9 ± 1.21	26.5 ± 1.60	25.9 ± 1.49	26.5 ± 1.53	27.1 ± 1.37
Temperature – maximum (°C)	33.7 ± 2.05	33.4 ± 2.02	33.6 ± 1.87	32.4 ± 2.21	34.6 ± 1.49
Turbidity – Secchi (cm)	37.8 ± 8.84	39.9 ± 14.3	41.4 ± 5.82	32.6 ± 5.30	47.0 ± 9.27
Turbidity – 545 nm	.024± .020	.026± .020	.011± .010	.037± .030	.026± .010
Turbidity – 880 nm	.013± .10	.012± .010	.015± .020	.037± .040	.022± .030
Particulate dry matter (g/l)	.523± .280	.646± .320	.813± .760	1.07 ± 1.40	.743± 1.06
Alkalinity – phenophthalein (ppm)	3.7 ± 5.83	5.1 ± 8.09	3.5 ± 5.62	4.3 ± 6.39	4.3 ± 6.32
Alkalinity – total (ppm)	106 ± 39.0	112 ± 46.4	88 ± 20.9	85 ± 23.4	83.8 ± 31.2
Water acidity (ppm)	2.5 ± 2.67	5.2 ± 5.60	2.5 ± 3.86	3.4 ± 3.83	6.5 ± 18.9
pH	7.6 ± .38	7.6 ± .38	7.8 ± .33	7.6 ± .43	7.6 ± .45
Orthophosphate (ppm)	.172± .160	.207± .190	.211± .210	.137± .130	.120± .110
Ammonia (ppm)	.174± .160	.325± .440	.162± .240	.219± .310	.130± .160
Ammonia + amino acids (ppm)	.599± .430	.668± .340	.736± .380	.522± .340	.538± .370
Nitrite + nitrate (ppm)	.117± .480	.052± .240	.006± .020	.012± .040	.010± .020
Silicate (ppm)	.790± .640	.580± .310	.520± .310	.820± .960	.720± .570
Dissolved oxygen – 1800 h (ppm)	6.21 ± 1.77	7.09 ± 2.00	7.01 ± 1.85	7.20 ± 2.04	7.14 ± 1.94
Dissolved oxygen – 2400 h (ppm)	4.98 ± 1.40	5.78 ± 1.38	5.00 ± 1.66	5.40 ± 1.79	5.28 ± 1.55
Dissolved oxygen – 0500 h (ppm)	3.89 ± 1.04	4.28 ± 1.41	4.35 ± 1.17	3.70 ± 1.33	4.90 ± 1.80
Dissolved oxygen – 0700 h (ppm)	4.37 ± 1.28	4.27 ± 1.13	4.73 ± 1.99	4.08 ± 1.30	4.94 ± 1.26
Total rainfall (mm/sampling)	20.8 ± 52.1	19.8 ± 50.8	22.9 ± 52.8	20.3 ± 50.0	20.3 ± 50.0
Relative humidity (%)	82.6 ± 10.6	83.7 ± 9.9	85.6 ± 8.65	82.6 ± 10.2	82.6 ± 10.2

TABLE III. Continued

Parameter	Ponds (number of observations)				
	MSP (26)	CP (20)	S-out (13)	AB (17)	AD (20)
Pond area (m^2)	1 200	18 400	500	8 700	6 600
Water depth (cm)	47.6 ± 10.6	17.9 ± 8.8	4.3 ± 2.0	26.7 ± 8.5	32.3 ± 8.8
Salinity – top (‰)	37.3 ± 6.39	46.2 ± 19.6	27.7 ± 9.6	75.3 ± 12.6	74.0 ± 12.0
Salinity – bottom (‰)	37.8 ± 5.99	48.1 ± 19.1	38.8 ± 11.2	77.8 ± 11.8	77.3 ± 10.2
Temperature – minimum (°C)	26.3 ± 1.62	25.6 ± 0.91	26.6 ± 0.50	26.6 ± 1.20	26.9 ± 1.48
Temperature – maximum (°C)	34.5 ± 1.99	36.8 ± 2.17	31.4 ± 1.30	35.4 ± 1.80	34.7 ± 2.35
Turbidity – Secchi (cm)	29.9 ± 5.95	17.7 ± 10.2	4.3 ± 2.00	25.6 ± 7.8	30.9 ± 8.5
Turbidity – 545 nm	.092± .090	.028± .040	.178± .500	.017± .020	.012± .010
Turbidity – 880 nm	.039± .030	.014± .020	.114± .330	.007± .010	.006± .010
Particulate dry matter (g/l)	.562± .390	.695± .470	.638± .450	1.34 ± .530	1.23 ± .520
Alkalinity – phenophthalein (ppm)	1.3 ± 4.54	22.4 ± 18.7	21.7 ± 18.2	27.8 ± 16.9	37.4 ± 19.8
Alkalinity – total (ppm)	78.6 ± 23.5	150 ±120	237 ±114	100 ± 37.0	124 ± 38.0
Water acidity (ppm)	4.9 ± 3.0	0.4 ± 1.17	4.4 ± 10.3	1.6 ± 3.4	0.2 ± 0.6
pH	7.5 ± 0.43	7.7 ± 0.54	7.9 ± 0.50	8.1 ± 0.30	8.2 ± 0.29
Orthophosphate (ppm)	.176± .170	.352± .360	.493± .300	.269± .170	.210± .210
Ammonia (ppm)	.156± .150	.446± .780	.715± .570	.186± .280	.230± .330
Ammonia + amino acids (ppm)	.538± .420	.767± 1.65	2.73 ± .910	.809± .690	.691± .370
Nitrite + nitrate (ppm)	.004± .010	.027± .080	.070± .130	.068± .100	.001± .020
Silicate (ppm)	1.12 ± 1.00	1.72 ± 1.76	2.55 ± 2.37	0.97 ± 1.53	0.52 ± 0.28
Dissolved oxygen – 1800 h (ppm)	6.88 ± 1.61	7.58 ± 2.60	7.06 ± 3.47	9.11 ± 2.38	7.98 ± 1.75
Dissolved oxygen – 2400 h (ppm)	5.15 ± 1.70	3.80 ± 2.02	3.88 ± 2.47	5.09 ± 2.08	5.57 ± 1.51
Dissolved oxygen – 0500 h (ppm)	3.36 ± 1.23	3.08 ± 2.23	2.80 ± 1.37	3.72 ± 1.44	4.39 ± 1.40
Dissolved oxygen – 0700 h (ppm)	5.33 ± 2.44	3.56 ± 1.62	5.35 ± 1.56	4.28 ± 1.63	5.14 ± 2.40
Total rainfall (mm/sampling)	21.6 ± 50.0	20.3 ± 54.4	22.9 ± 54.4	25.4 ± 53.3	30.5 ± 58.2
Relative humidity (%)	82.6 ± 10.2	81.2 ± 10.8	83.7 ± 10.3	88.5 ± 3.5	89.1 ± 3.4

TABLE IV

Average plankton production (individuals/l) in selected ponds at the SPI saltfarm

Plankton classification	Ponds (number of observations)									
	R-1 (24)	CKP (25)	ISP (23)	BSC (25)	E-1 (25)	MSP (26)	CP (20)	S-out (13)	AB (17)	AD (20)
Picoplankton (0.2-2 μm)	658 043 ±477 304	1 025 625 ±1 325 288	843 182 ±942 867	1 460 283 ±1 274 747	1 908 269 ±3 356 050	2 571 400 ±2 298 964	598 611 ±406 430	1 911 250 ±2 034 835	251 563 ±249 604	671 667 ±1 652 045
Nannoplankton (3-10 μm)	149 130 ±149 780	253 958 ±296 799	255 227 ±280 513	342 217 ±353 708	481 154 ±824 625	654 800 ±729 447	135 556 ±152 597	886 250 ±748 560	39 375 ±43 870	61 111 ±60 128
Microplants (11-60 μm)	590.5 ±1 103.6	3 324.9 ±6 452.8	3 804.1 ±6 504.0	775.1 ±1 285.3	789.0 ±1 230.0	550.1 ±939.8	2 803.8 ±4 725.2	651.7 ±1 559.4	5 571.5 ±7 192.7	5 559.0
Microanimals	3 030.8 ±5 370.5	3 232.8 ±5 289.2	4 914.4 ±5 318.8	4 606.5 ±7 389.1	4 296.1 ±8 873.3	5 827.1 ±9 379.1	3 166.9 ±5 765.8	14 597.3 ±42 730.3	4 796.5 ±4 617.2	5 339.1 ±7 570.6
Meioplants (61-120 μm)	1.12 ±1.87	2.32 ±4.22	13.99 ±58.53	8.13 ±20.06	4.90 ±7.82	11.36 ±23.65	91.24 ±232.01	29.16 46.41	159.25 ±277.26	136.59 ±186.93
Meioanimals	44.41 ±41.66	36.28 ±37.53	42.41 ±31.52	61.73 ±44.16	78.92 ±78.93	63.09 ±44.03	53.30 ±36.69	32.65 ±12.68	47.89 ±47.19	59.33 ±70.44
Macroplants (above 120 μm)	1.98 ±2.17	30.76 ±130.92	7.81 ±15.06	29.30 ±51.62	42.63 ±95.99	27.26 ±37.41	24.60 ±35.12	10.33 ±12.43	11.49 ±26.97	9.59 ±13.43
Macroanimals	20.08 ±40.89	25.86 ±63.91	13.92 ±17.37	38.22 ±58.65	71.97 ±103.16	82.27 ±134.39	25.72 ±46.19	14.41 ±34.12	33.81 ±80.76	21.44 ±30.02

filamentous blue-green algae and diatoms together with associated consumers (Jumalon, 1983). Unfortunately, no precise quantification of the produced "lab-lab" was made.

Factor analysis of the physicochemical and plankton matrix (Table V and Fig. 2) gave a number of significant results. After Varimax rotation, the major principal components were PC1, PC4, PC6, PC3 and PC5. Total variance of the 33 variable matrix accounted for in the first 10 principal components (eigenvalues >1.0) was 67.5 ‰.

PC1, 4, 8 and 9 differentiated ponds close to the crystallization bed from the rest of the system, as shown by the plot of factor scores.

The first principal component is oriented with the flow of water through the system (a positive correlation for water depth and a negative correlation for salinity). The plot of factor scores in Fig. 2 indicates that water increases in carbonate (phenolphthalein) alkalinity, particulate matter, pH, and meioplants as it flows towards the saltbed.

The second component relates meio- and macroplankton counts. This factor appears to indicate the effects of fish biomass on the pond water quality. As biomass increased during the season, ponds stocked with milkfish, and to a lesser extent shrimps, tended to have increased numbers of meioanimals, macroplants and macroanimals in the water column. This may be due to the continuous disturbance of the pond bottom by the fish and shrimps. This is supported by the third factor which has negative loadings for turbidity and microanimals. Ponds with high fish/shrimp biomass seemed to be more turbid and also have higher numbers of microanimals in the water column. *Artemia* ponds have relatively high scores (low turbidity, zooplankton and macroplants) on the second and third factors.

Nutrients and alkalinity load negatively on the fourth component ; pond depth, secchi depth and dawn oxygen values are positive. A very strong pattern, as indicated in the plot of factor scores (Fig. 2), can be discerned as water flows toward the saltbeds. Nutrients tend to increase and early morning dissolved oxygen decreases.

The fifth principal component is not related to water flow. It shows the positive relationship between dissolved oxygen values indicating that high oxygen values at any one time during the night-post dawn period would have correspondingly high dissolved oxygen at other periods. This relationship is strongest for midnight and dawn oxygen values. No clear pattern can be seen as the water moves through the system.

Seasonal trends are indicated in the sixth vector. Minimum water temperature and humidity increased during the season.

Maximum temperature and rainfall are negatively loaded on the seventh vector, indicating that water temperature increased during rain. Certainly, this is the case when ponds become stratified after heavy rains. Bottom water temperature could easily reach 42 °C. This condition is detrimental to *Artemia* ; hence, it is necessary to provide an overflow system to immediately drain off the surface freshwater or to mix the water either by rowing in a small boat or by the use of pond circulators.

The *Artemia* ponds can be distinguished in PC8 by their consistent negative scores. Pico- and nannoplankton (high positive loadings) were low in *Artemia* ponds and were most probably ingested by the *Artemia*. In contrast ponds with high fish biomass (*e.g.* MSP) had high pico- and nannoplankton counts.

The ninth factor has a negative loading for acidity (free CO_2) and positive for microplants (mostly *Tetraselmis*), the major food component of *Artemia* adults. This component correlates highly ($P<0.001$) with PC1 (positive) and PC4 (negative), separating the ponds adjacent to the

TABLE V

Factor loadings (PC) of pond data principal components.
Significant values with a component, and factors with eigenvalues >1 are shown (N=240)

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Eigenvalues	4.97	3.77	3.35	2.02	1.89	1.61	1.36	1.24	1.05	1.04
Variance	11.20	4.50	9.00	9.40	6.90	9.30	4.50	5.40	3.80	3.60
Sum of variance	11.20	15.60	24.60	34.00	40.90	50.20	54.70	60.10	63.90	67.50
Minimum water temperature						0.69				
Maximum water temperature							-0.74			
Depth	-0.37			0.64						
Turbidity (540 nm)			-0.95							
Turbidity (880 nm)			-0.95							
Phenolphthalein alkalinity	0.66			-0.38						
Total alkalinity				-0.63						
Acidity									-0.76	
Orthophosphate				-0.54				0.32		
Indophenol ammonia				-0.47						0.32
Ammonia + amino acids				-0.64						
Salinity (top)	0.91									
Salinity (bottom)	0.89									
Silicate				-0.58						
Nitrate										-0.79
Millipore dry weight	0.47						0.50			
pH	0.49					0.44	0.31			
Dissolved oxygen (1800 hr)					0.59	0.37				
Dissolved oxygen (2400 hr)					0.81					
Dissolved oxygen (0500 hr)				0.30	0.73					
Dissolved oxygen (0700 hr)					0.68					
Secchi depth				0.76				0.30		
Rainfall						0.36	-0.40			0.32
Humidity						0.85		-0.34		
Microplants									0.55	
Microanimals			-0.90							
Meioplants	0.68									
Meioanimals		-0.57								
Macroplants		-0.80								
Macroanimals		-0.55				0.43				
Picoplankton								0.70		
Nannoplankton								0.73		
Time						0.76				

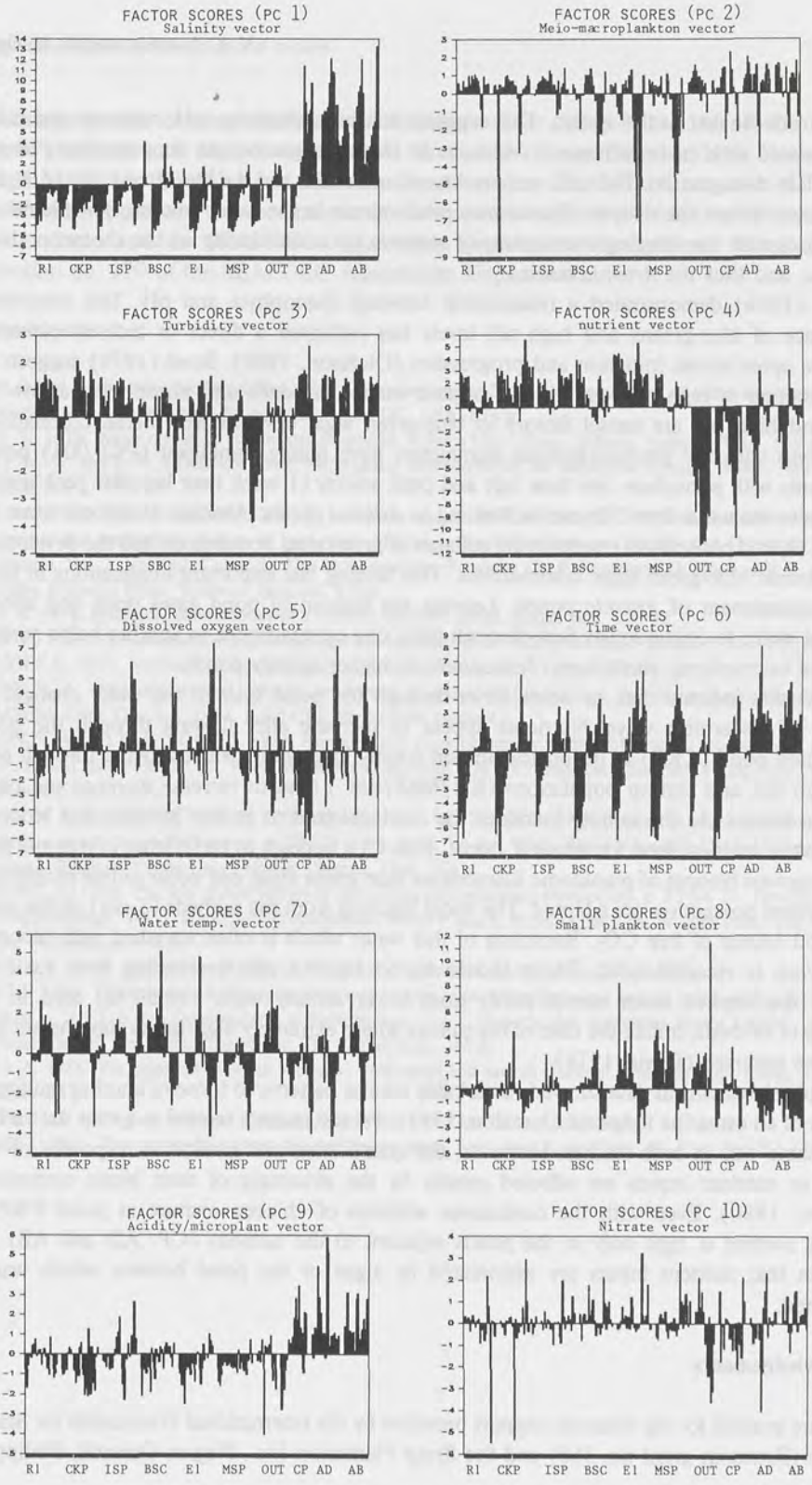

FIG. 2. Factor scores based on pond data principal components.

saltbed from the rest of the system. This suggests that high alkalinity, pH, nutrients and salinity are associated with the occurrence of microplants. However, green algae are particularly sensitive to the effect of stagnation. The cells settle on the pond bottom and the blue-green alga (*Lyngbya*), and to some extent the diatom *Chaetoceros*, predominate in the water column. The filaments of *Lyngbya* disrupt the filtering mechanism of *Artemia* by accumulating in the thoracopods and antennae, and thus the *Artemia* starve.

Fogg (1956) demonstrated a relationship between cyanophyta and pH. The coincidental occurrence of blue-greens and high pH levels has indicated a direct or indirect pH-related influence upon bloom initiation and progression (Olofsson, 1980). Boyd (1979) suggests that a combination of high concentration of organic matter, nitrogen and phosphorus at low CO_2 levels and high pH are causal factors in blue-green algal development. Serial correlation of *Tetraselmis* with the physicochemical parameters gave highly significant ($P<0.001$) positive coefficients with phosphate (no time lag) and peak acidity (1 week time lag after peak acidity). Our results show that free CO_2 can be limiting in *Artemia* ponds. Absence of influent water high in free CO_2 and phosphates results in the collapse of green algal populations and the development of planktonic blue-green algae communities. This finding has important implications in proper water management of *Artemia* ponds. Leaving the bottom of pond gates open and allowing enriched water to continuously flow-through from one compartment to another helps stimulate bloom of microplants, particularly *Tetraselmis*, in higher salinity ponds.

The results indicate that, as water flows through the pond system, the water changes in a number of discernible ways. Nutrients appear to increase after flowing through the poultry fertilization pond (CKP) as do phytoplankton counts in general. Water moving through ponds with high fish and shrimp populations (E-1/MSP/BSC) tend to increase in pico- and nannoplankton counts. As the salinity increases the nutrients tend to further increase and blooms of microplants, an ideal food for *Artemia*, occur. Free CO_2 appears to be limiting in *Artemia* ponds and dangerous blooms of planktonic filamentous blue-green algae can occur unless proper water management procedures are adapted. The water leaching from the saltbeds (S-out) in this system is a good source of free CO_2. Recycling of this water which is often enriched with blooms of *Tetraselmis* is recommended. There should be no harmful effects resulting from toxic salts because the leached water comes partly from lower salinity water (50-80 ‰) used in daily cleaning of saltbeds, unlike the case of big salinas where extremely high-saline supernatant liquid has to be removed (Davis, 1978).

The physicochemical data showed remarkably similar patterns to those of another multivariate analysis of an estuarine fishpond (Jumalon, 1983). Factor analysis tended to group the variables in the same way in both studies. However, the quantitative and qualitative responses of water bodies to nutrient inputs are affected greatly by the structures of their biotic communities (Shapiro, 1980). Even with the continuous addition of chicken manure at pond CKP, the nutrient content is high only in the ponds adjacent to the saltbeds (CP, AD and AB). This indicates that nutrient inputs are assimilated by algae or the pond benthos which was not monitored.

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Inoculation of *Artemia* in experimental ponds in central Vietnam : an ecological approach and a comparison of three geographical strains

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Abstract

Artemia from Macau (Brazil), Great Salt Lake (GSL, Utah, USA) and China were inoculated in four experimental ponds of 312 m^2 each. Growth and population dynamics, with special regard to the reproductive biology of *Artemia*, have been investigated in relation to the main environmental factors. Potentialities of the three strains for cyst production were studied, and different inoculation densities were tested for the Macau strain.

With an inoculation density < 20 nauplii/l, the three *Artemia* strains exhibited rapid growth and maturation ; the life cycle was completed in 10-14 days. The Chinese parthenogenetic strain showed a slower maturation rate and a lower mean fecundity (44 eggs/female) than the two bisexual strains (19 and 104 eggs/female for Macau and GSL, respectively). At higher inoculation densities, mean size and fecundity of adult *Artemia* (Macau strain) were significantly reduced. The first brood was mainly composed of nauplii, but *Artemia* rapidly switched to oviparous reproduction and cysts began to accumulate 2 weeks after the inoculation date.

The disruption of the trophic balance, through the dramatic increase of the population density, and resulting starvation condition, seemed to be sufficient to stimulate the oviparous mode. However, the food shortage, in the absence of proper fertilization, also resulted in a rapid decrease of the animal density and in an unstable cyst harvest. The mean yield ranged from 1.4 to 6.8 kg dry weight cysts/ha/month.

Since March 1985, a flow-through type management improved the yield to 8.6 kg cysts/ha/month, without fertilization of the water. Suitable climatic and environmental conditions have resulted in the establishment of a "natural" *Artemia* population in the ponds adjacent to the experimental area since 1983.

Introduction

For nearly a decade, many improvements in establishing sources of *Artemia* cysts for use in aquaculture have been achieved, such as the successful transplantation in the Brazilian Macau salterns (Camara and De Castro, 1983). In southeast Asia, the absence of any natural *Artemia* populations (Persoone and Sorgeloos, 1980) is a serious handicap to the development of regional aquaculture activities because high-priced cysts have to be imported to cover the increasing demand from hatcheries and nurseries, especially those for prawns.

However, during these past years, *Artemia* inoculations in salt fields during the dry season have been successfully tested in the Philippines (De los Santos *et al.*, 1980) and have even resulted in some artisanal semi-intensive cyst production in Thailand (Anonymous, 1980 ; Tunsutapa-

nich, 1982). Manuals describing the elementary steps to follow for cyst production in salt ponds, have also been published (Sorgeloos, 1978 ; Vos and De la Rosa, 1980). From these preceding experiments, we assumed that *Artemia* inoculation in salt farms in Vietnam would likely be successful. This, however, had to be demonstrated before any large-scale production could be planned. Moreover, most of our knowledge on *Artemia* inoculations are still on a "trial and error" basis and scarce literature is available on this subject as well as on the ecology of *Artemia* in natural habitats.

The San Francisco Bay and the Macau *Artemia* have already been successfully tested in southeast Asian salterns. Because two other strains, namely from Great Salt Lake, Utah, USA (GSL) and China, were available, their performances were compared with regard to cyst production. In particular, the China strain, despite a lower individual fecundity level, inherent to most parthenogenetic strains (Amat, 1982), may give good yields, as the entire population is composed of females. The optimal density for an inoculation had also to be determined. Provided that enough food is present, the higher the inoculation density, the faster the population will reach a high density, thus saving time in the production.

The first trial was carried out from March to May 1983 (Vu Do Quynh and Vo Thi Dieu Duyen, 1983). One pond, 2 800 m^2 in surface area and located in a salt-producing unit, was inoculated with about 1.2×10^6 Macau nauplii. Difficulties in controlling the water level prevented good cyst production, although the stocked *Artemia* showed very rapid growth. Later, a near-by salt-pond unit was used, finally independent experimental ponds, were built and new trials could be started in 1984. This paper presents comparative results from a small-scale inoculation, using three geographical *Artemia* strains. Much attention was paid to the population dynamics, the reproductive biology of *Artemia*, and the potential of cyst production.

Description of the site

The salt farm is located in the Cam Ranh Bay, 60 km south from Nha Trang town (Fig. 1) ; its total surface area is about 100 ha and it is divided into production units of 3-5 ha each, which are operated manually. The annual precipitation amount is among the lowest for the Phu Khanh Province ; the number of rainy days usually does not exceed 60/year. The total salt production is about 10-14 000 tonnes/year.

Water is pumped from reservoir ponds surrounded by a small mangrove ; the greenish turbid waters, rich in organic matter, have an initial salinity of 40-45 ‰ and are circulated by gravity through a series of evaporation ponds to the salt crystallizers. As each unit is small, the water is kept in each pond until the salinity increases to a determined level and is then allowed to flow into the following pond (static system). The water level is very shallow, usually from 20 to 30 cm in the low salinity ponds, and down to 5 cm in the salt crystallisers. In the high season, salt is harvested every 10-20 days, depending on climatic conditions, to prevent any losses due to potential rainfalls. The gypsum ($CaSO_4$), which is precipitated in specially prepared ponds, is harvested at the end of the dry season.

Materials and methods

Four abandoned salt crystallizers, each of 312 m^2 surface area (8 m × 39 m), were transformed into experimental *Artemia* ponds by digging a perimetric ditch (2 m wide × 0.15 m deep) inside,

and by heightening the walls as recommended by Vos and Tunsutapanich (1979) and Vos and De la Rosa (1980). One pond was also divided in two, one of 64 m^2 (pond 5) and one on 248 m^2 (pond 3) for testing different inoculation densities. Wooden sluice gates, equipped with screens to prevent predator intrusion, were installed for each pond. The water (40-45 ‰ S) was taken into each pond from the pumping canal and the salinity was allowed to increase up to 75-80 ‰ before starting the inoculation.

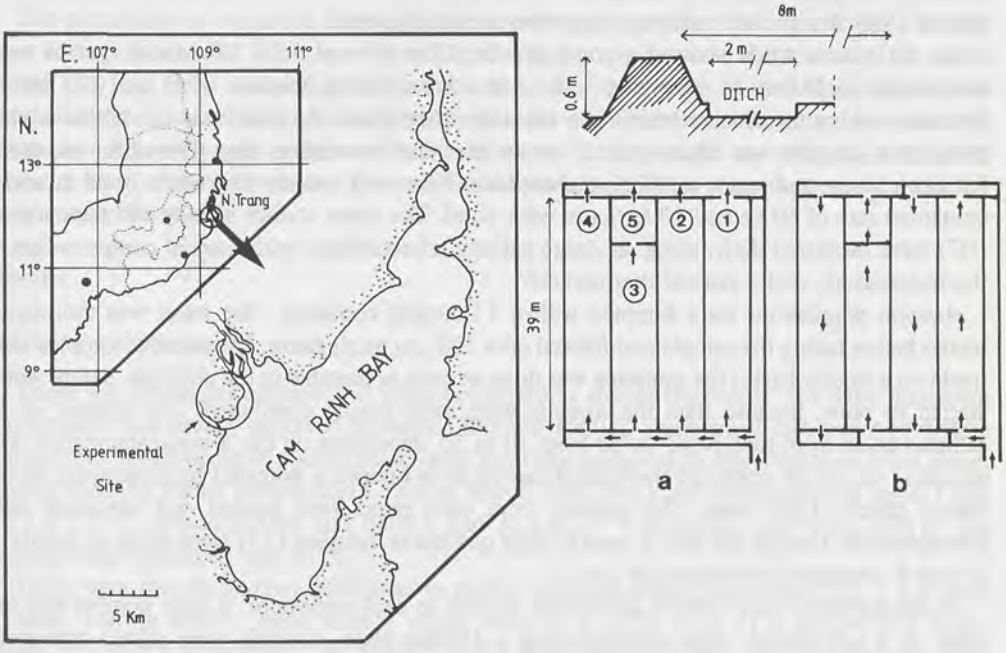

FIG. 1. Location of the experimental site and schematic lay-out of the experimental earthen ponds for *Artemia* inoculations in the Cam Ranh salt farm. The arrows indicate flow directions of the intake water (40-45 ‰ S) from the pumping canal. Two types of water management were consecutively tried :
– from March to April 1984, intake water was provided to each pond and salinity conditions were identical ;
– from May to September 1984, intake water was only provided to pond 1 (circulation between each pond was free) and a salinity gradient was established from pond 1 (70-80 ‰) to pond 4 (140 ‰).

Ponds 1, 2, and 4 were fertilized with chicken manure, 1 week before stocking *Artemia* ; because the locally available chicken manure was mixed with rice hulls, used as litter in the chicken cages, it had to be put into sacks and soaked in the water of the pond.

Cysts from Macau (Brazil), Great Salt Lake (USA) (Aquafauna Biomarine Inc.) and P.R. China (Greatwall Brand, National Cereals, Oils and Foodstuffs Import and Export Corporation, Tientsin Branch) were utilized for inoculation experiments. The hatching time and the quantity of cysts to be hatched was determined after a preliminary estimation of the hatching percentage

and hatching rate. After hatching, three 5 ml samples were taken from each hatching container and fixed for a further estimation of the total number of nauplii hatching. The *Artemia* nauplii were immediately transported to the near-by ponds for stocking. Unfortunately, as the counting of hatched nauplii was only done after the inoculation had taken place, it was impossible to adjust the real stocking density to the adequate level. Therefore, on March 2 at 2200 h, ponds 1, 2, and 4 were inoculated with 1.3×10^6 Macau nauplii, 0.5×10^6 GSL nauplii, and 0.7×10^6 China nauplii, resulting in corresponding inoculation densities of 17 ind./l, and 7/l, respectively. After stocking, simultaneously with taking in water, the ponds were fertilized at weekly intervals at a rate of 13 kg dry chicken manure (impurities included)/pond.

As the Macau strain showed a good growth, 2.0×10^6 and 3.2×10^6 Macau nauplii were inoculated, on March 14, in ponds 3 and 5 at corresponding densities of 61 and 175 ind./l. Because soaking the chicken manure in the water throughout the pond was too labour-consuming, this practice was abandoned 2 weeks after the inoculation day. Therefore, inorganic fertilizers, urea, and mono-ammoniumphosphate, were used, mainly for ponds 3 and 5, and a combined rate of 60 kg and 10 kg/ha in each pond. The water surface salinity and temperature (°C) were measured daily, using an Atago salinity refractometer, with manual compensation of the temperature, and a normal thermometer.

Artemia populations were sampled with a 1 l conical container ; the water was thoroughly mixed before taking the sample and filtered on a 100 µm mesh gauze. Quantitative samples were made on a weekly basis ; the sampling was done as early as possible in the morning, before winds started to blow, because then the *Artemia* were more evenly distributed. The number of 1 l samples taken from each pond varied from 10 to 30, depending on the *Artemia* abundance. The sampling sites were scattered throughout the pond to minimize potential heterogeneous distribution effects. Each week, the samples from each pond were pooled and preserved with formaldehyde. During the first 2 weeks, daily qualitative samples (5 l) were taken in ponds 1, 2, and 4, to assess the maturation rate.

In the laboratory, all *Artemia* individuals present in each sample or, if their number was too high, in a sub-sample, were counted using a Dollfus plate. Animals were sorted into three groups : juveniles, males, and females. We separated the females into immature and mature individuals on the basis of the presence or absence of any ovarian development. The number of females with developed ovaries, full oviducts or full uterus, was recorded. The total length of *Artemia*, from the anterior part of the head to the end of the furca, were measured with a dissecting microscope equipped with an ocular micrometer, at a 20 × magnification. *Artemia* females with full uterus were dissected and the number of embryos counted (for simplification, we also refer to "eggs" for designating, in a general way, the embryos in the female uterus). Individuals that had visibly lost a part of their brood, through sampling and fixation, were discarded before computing fecundity estimates. All mean values ± the standard error were recorded. The sex-ratio was calculated as the number of males present in the sample, divided by the number of females.

As it became increasingly evident that mutual contamination by *Artemia* from other ponds occurred in each pond (*e.g.* the presence of males in pond 4 inoculated with the parthenogenetical strain), it was necessary to distinguish the individuals from each strain. Fortunately, some morphological differences, such as the relative length of the abdomen *versus* the total length, the length of the first pair of antennae, and the number of furca setae, were sufficient to allow separation. Amat Domenech (1980) also reported such differences between strains.

To determine the type of reproduction, the embryonic developmental stages were codified on the following basis :

a) eggs more or less soft, often sticking together and irregular in shape ; easily smashed ;
b) embryos spherical and well dissociated from each other ;
c) embryos surrounded by a slightly opaque brownish membrane, or embryos surrounded by a translucent bright membrane ;
d) opaque brown shell surrounding the embryos = cyst, or embryos triangular in shape = nauplius.

The percentage of oviparous females in the sample was determined from the females bearing eggs in stage 3 onwards. The cysts were collected with a scoop-net from the corners of the ponds where they had been accumulated by wind action. The cysts were washed and cleaned with saturated brine and freshwater, as described by Sorgeloos (1978). Before weighing, the cysts were pressed on filtering paper to remove the excess water. The dry weight was roughly extrapolated as being 50 % of the wet weight (Vos and Tunsutapanich, 1979) to express the yield in kg dry weight cysts/ha/month.

Results

Physical factors

There was almost no difference in water temperatures among the ponds. The daily maximum temperature was usually observed between 1200 and 1500 h. Some small differences in surface temperature could occur in a given pond, with the areas protected by the walls from wind action usually showing higher temperatures. During March 1984, the mean temperature in pond 1 was 25.7 ± 1.5 °C, between 0700 and 0800 h and 31.9 ± 2.7 °C between 1300 and 1500 h ; the highest value measured was 36 °C.

There were also no marked differences in surface salinities among the ponds (Fig. 2 for pond 1 data). During March, mean salinity values were 93 ± 10 ‰, 95 ± 11 % and 95 ± 9 ‰ for ponds 1, 2 and 4 respectively. As ponds 3 and 5 were inoculated later, the mean values, from March 15 to April 18, were slightly higher, namely 101 ± 13 ‰ and 106 ± 15 ‰, respectively. The heavy rainfall which occurred on March 28 (about 70 mm in 1 day) diluted the water at the surface down to 24 ‰ S ; low surface salinities persisted 2 days before the wind action homogenized the water column.

The water level was different from pond to pond ; ponds 1 and 2 were the shallowest and pond 5 was the deepest. The minimum water level, in pond 1, was around 25 cm in the ditch (Fig. 2). During April, we had to increase the salinity to eliminate some *Tilapia*, which occurred in pond 1.

Artemia density

The measurements of *Artemia* density in ponds 1, 2, and 4 indicated that no mortality seemed to occur after the inoculation of *Artemia* (Fig. 3). Quantitative estimates, on March 10, were roughly similar to the inoculation densities. After reproduction began, the population density dramatically increased 12 days after the inoculation and reached a peak around 400 ind./l in ponds 1 and 2 a few days later. For the China strain (pond 4), this increase took place later than for the two bisexual strains and the peak value was lower, around 300/l.

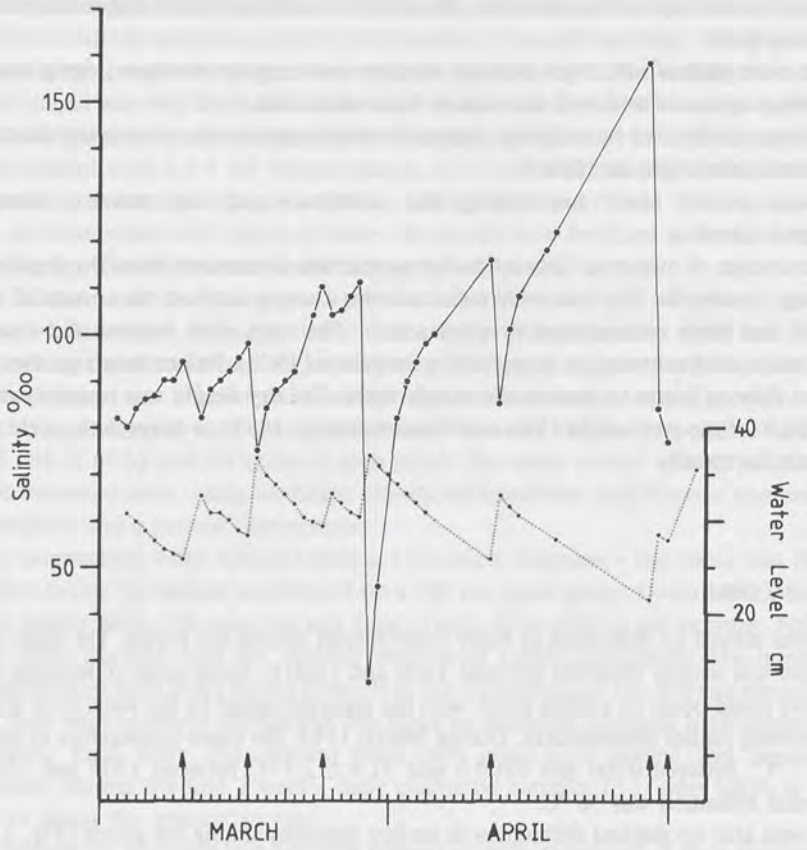

FIG. 2. Changes in surface salinity (solid line) and fluctuations in the water level (dotted line) for pond 1, during March and April, 1984. Arrows indicate times of water intake.

The *Artemia* density began to increase 3 weeks after the inoculation in the three ponds and was around 100 ind./l at the end of March. The density of the parental population decreased about 2 weeks after the inoculation. This phenomenon was particularly clear for the Macau strain, as its estimated density was only 1-2 ind./l by the end of March. In early April, almost all the parental *Artemia* had disappeared and the population was composed mainly of juveniles (> 90 %).

In ponds 3 and 5, where the inoculatuion was made later with the Macau strain, the situation was more complex because some *Artemia* had already come in from adjacent ponds. The estimated density of these "contaminants" (mostly larval stages) was about 24-28/l. One week after inoculation, densities of 162 and 202/l, were reached, consisting of 74 % and 95 % of juveniles, in ponds 3 and 5 respectively. One month after the inoculation, the densities in these two ponds were 31 and 23/l, comprising 66 % and 91 % of juveniles.

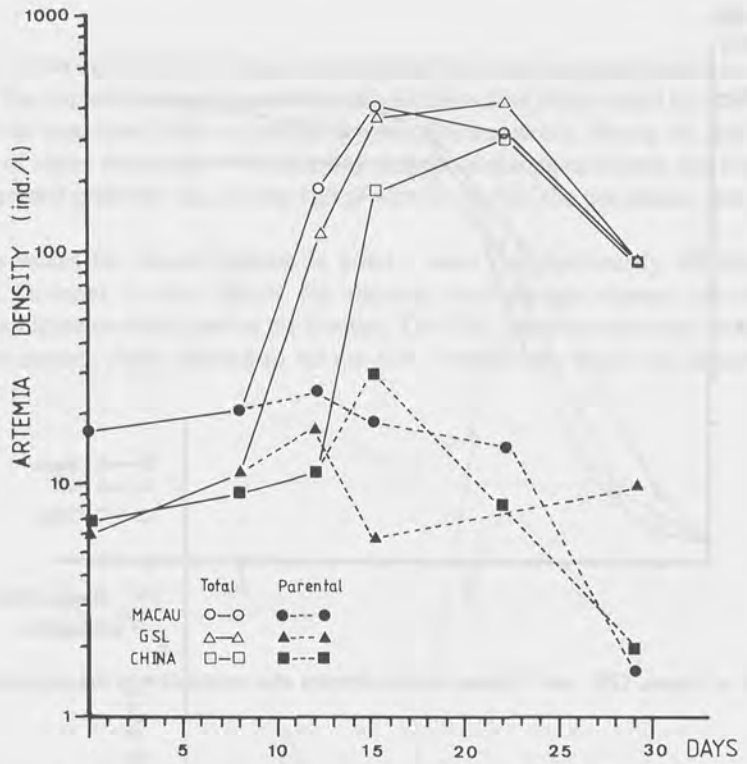

FIG. 3. Changes in density (ind./l) of three *Artemia* populations inoculated in ponds 1, 2, and 4. The changes in the parental populations (solid symbols) were deduced from the observed densities of actively reproducing females on each sampling date, multiplied by 2 for the bisexual strains.

GROWTH

The growth pattern of *Artemia* females in ponds 1, 2, and 4 was slightly sigmoidal (Fig. 4). The growth rate increased from days 2-12 and then leveled off. GSL *Artemia* had a faster growth rate than the two other strains and China *Artemia* seemed to have an initial low growth rate, but it increased sharply between 8 and 10 days of age.

GSL and China *Artemia* females had nearly the same adult size and were much larger than the Macau females ; the largest female measured was 11.70, 11.35, and 10.15 mm total length for the GSL, China and Macau strains, respectively. Adult males of the two bisexual strains were smaller than the females (Fig. 5) : at 12-days-old, the mean sizes were respectively 7.06 ± 0.67 mm and 7.45 ± 0.71 mm for Macau and GSL *Artemia* males *versus* 8.44 ± 0.52 and 9.22 ± 1.13 mm for the females of the corresponding strains.

In pond 3, the growth of Macau *Artemia* was less than in pond 1 ; the mean lengths at 16 and 30-days-old were respectively 6.82 ± 0.54 mm and 7.37 ± 0.30 mm for the egg-bearing females. In pond 5, no data were obtained as adult *Artemia* were scarcely found. Most of the individuals present were larval stages or juveniles and their growth was very slow.

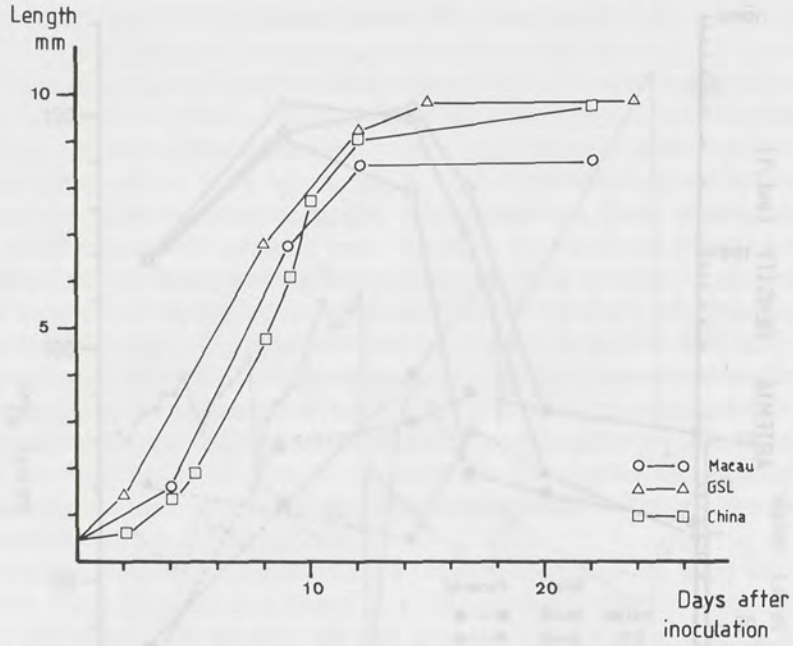

FIG. 4. Growth of Macau, GSL, and Chinese *Artemia* females after inoculation in the experimental ponds.

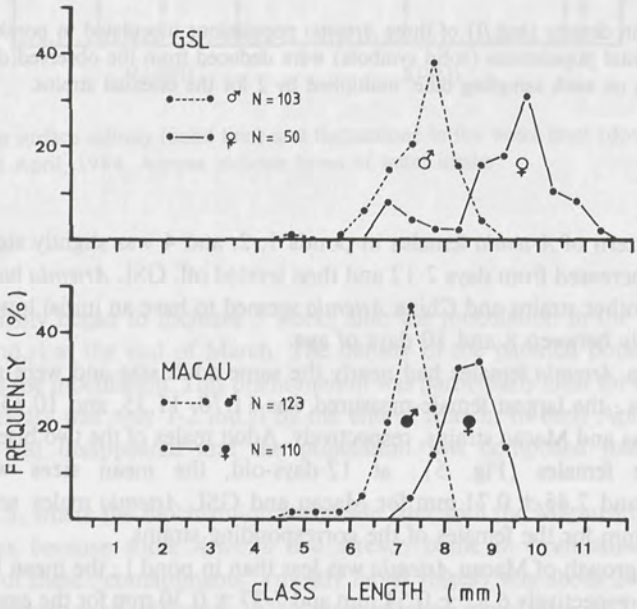

FIG. 5. Frequency distribution of male and female *Artemia* from Macau and GSL on March 14, 12 days after the inoculation.

SEX RATIO

Complex patterns of sex-ratio values were observed for *Artemia* populations in ponds 1, 2, and 4 (Fig. 6). The contamination of pond 4 by animals from other ponds could be estimated because that pond was inoculated with the parthenogenetic Chinese strain. During the first 2 weeks, the percentage of males was around 4 %, meaning that the contamination level was less than 10 % ; then, it increased gradually and, by the end of March, 50 % of the population was composed of males.

Sex-ratio values for Macau *Artemia* in pond 1 were not significantly different from unity ($P = 0.05$). However, in late March, the sex-ratio was strongly skewed toward the males, suggesting a higher mortality rate in the females. The GSL *Artemia* population exhibited a more complicated pattern, males dominated for the first 3 weeks after which the females took over.

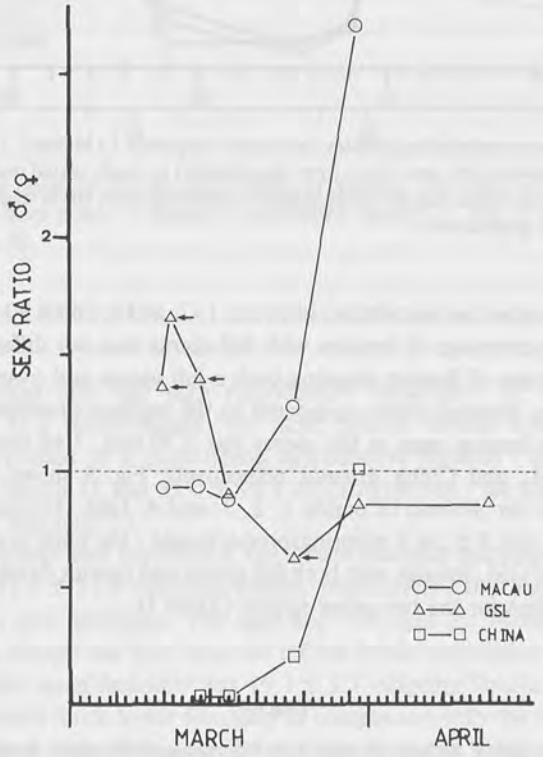

FIG. 6. Sex-ratio values observed in the samples in ponds 1 (Macau), 2 (GSL), and 4 (China). Small arrows indicate the values significantly different from 1 at the $P < 0.05$ level for the GSL strain.

REPRODUCTIVE BIOLOGY

Artemia matures in a very short time ; in ponds 1 and 2, some riding couples were already observed on March 9, 1 week after inoculation. At that same time, no China *Artemia* female was showing any signs of ovarian development (Fig. 7) and sexual maturity was only attained a few

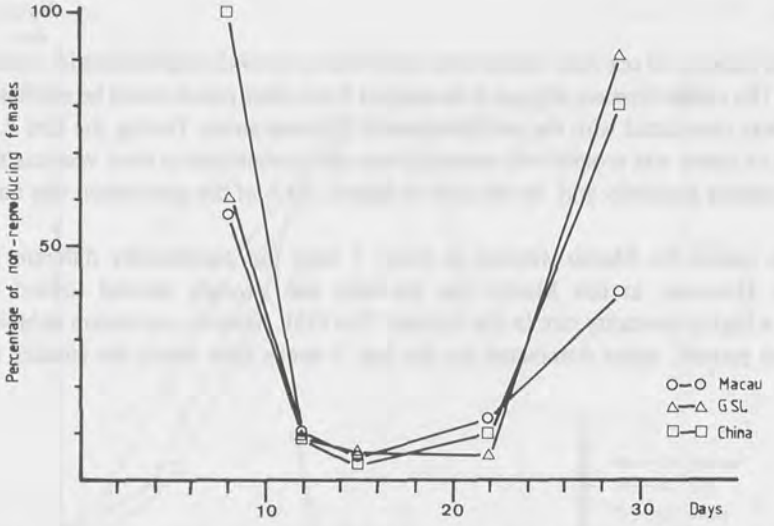

FIG. 7. Percentage of non-reproducing females (immature) in ponds 1 (Macau), 2 (GSL), and 4 (China). The curve is U-shaped, showing the time (days from inoculation) to reach sexual maturity for the inoculated populations, and the time to obtain sexual differentiation (transition from larval stages to immature females) for the new-born *Artemia* generations.

days later. Twelve days after the inoculation, in ponds 1, 2, and 4, about 90 % of the females were in reproduction. The percentage of females with full uterus was not different among the three strains, but the percentage of females showing both a full uterus and ovarian development was already high in the two bisexual strains compared to the parthenogenetical strain (Table I).

The smallest female bearing eggs in the uterus was 5.70 mm, 7.40 mm, and 8.00 mm total length for Macau, GSL, and China *Artemia*, respectively. Fig. 8 shows the mean number of offspring/brood/female for *Artemia* in ponds 1, 2, 3, and 4. GSL *Artemia* showed the highest reproductive capacity, 103.8 ± 24.4 offspring/brood/female ; the most prolific female bore 156 eggs. The percentage of GSL females with both full uterus and ovaries developing a second brood was generally higher than for the two other strains (Table I).

TABLE I

Percentage of *Artemia* females with full uterus and ovaries developed
(N is the number of egg-bearing females)

Number of days after inoculation	Pond 1, Macau		Pond 3, Macau		Pond 2, GSL		Pond 4, China	
	N	%	N	%	N	%	N	%
10		26.1				44.0		
12	86	37.2			47	54.4	65	1.0
15	176	14.1	36	35.4	171	63.9		
22	51	14.6			98	47.1	80	37.7
30			14	27.5				

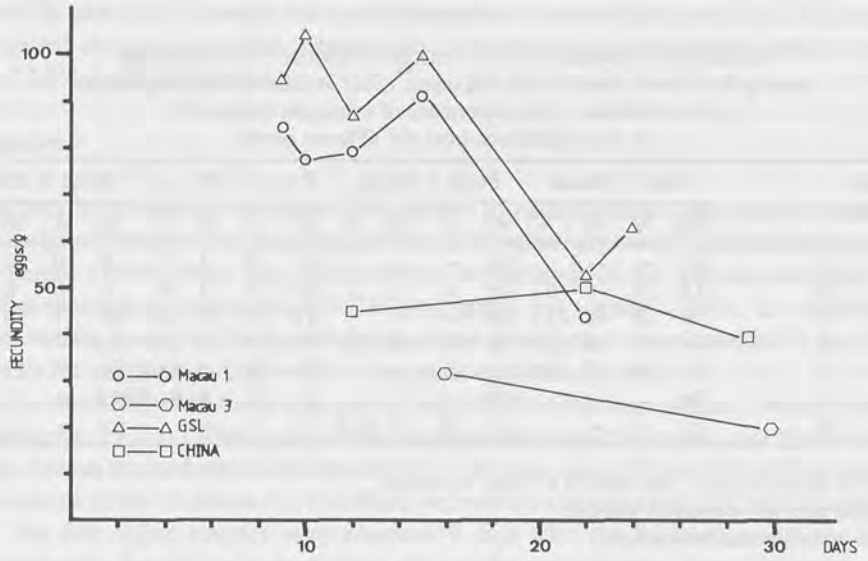

FIG. 8. Mean fecundity observed for the three strains of *Artemia*. The effect of crowding conditions is illustrated by the results from pond 1 (Macau 1, inoculation density 17 ind./l) and pond 3 (Macau 3, inoculation density 6 ind./l).

Macau *Artemia* females also had high reproductive capacities ; *i.e.* the maximum figure observed was 90.5 ± 32.5 brood/female, the most prolific female bore 149 eggs. Higher inoculation densities resulted in a diminishing reproductive capacity ; in pond 3, the mean fecundity observed on March 31 was 31.0 ± 11.4 offspring/female ; the highest number of eggs counted in one female was only 53.

In ponds 1 and 2, 3 weeks after inoculation, the female fecundity had decreased to 43.3 ± 31.2 offspring/female and 51.6 ± 21.4 offspring/female, respectively ; the highest number of eggs counted in one female also decreased, 109 eggs and 106 eggs for Macau and GSL *Artemia*, respectively. The same change was also observed for the female population in pond 3 ; 1 month after the inoculation, the mean fecundity was 19.1 ± 5.3 offspring/female.

China *Artemia* exhibited much lower fecundity in comparison with the other two strains ; the most prolific female bore only 80 eggs and the mean fecundity value was 49.7 ± 16.1 offspring/female. The reproductive capacity, however, did not obviously decrease as it did for Macau and GSL *Artemia*.

About 10 days were necessary for the new-born *Artemia* generations to attain sexual differentiation (Fig. 7), thereafter the recruitment of new immature females increased, starting the appearance of the first new generation.

Twelve days after inoculation, about 1/4 of Macau and GSL *Artemia* had switched to oviparity (Table II). This percentage increased with time and, 3 weeks after inoculation, 60-80 % of the females were oviparous ; it even reached 90 % in an *Artemia* sample from pond 2.

TABLE II

Proportions of oviparous (♀c) *versus* ovoviviparous (♀n) individuals among the *Artemia* females with full uterus (♀ut) at different sampling times, with estimation of the percentage of oviparous females (%) in the populations from the different ponds

Days after inoculation	Pond 1, Macau				Pond 3, Macau				Pond 2, GSL (a)				Pond 4, China			
	♀ut	♀c	♀n	%	♀ut	♀c	♀n	%	♀ut	♀c	♀n	%	♀ut	♀c	♀n	%
9	(b)	–	–		(b)	–	–		21	0	4	0	0	0	0	
10	18	0	5	0	(b)	–	–		20	0	13	0	0	0	0	
12	79	16	48	25.0	(b)	–	–		41	11	25	30.6	62	1	50	1.9
15	44	17	9	65.4	40	14	14	50.0	31	19	10	65.5	(b)	–	–	
22	54	28	13	68.3	(b)	–	–		28	21	5	80.8	66	34	13	72.3
24	(b)	–	–		(b)	–	–		64	56	5	91.8	(b)	–	–	
30	(c)	–	–		21	10	6	62.5	(c)	–	–		(c)	–	–	

[a] Sample, at day 24 in pond 2, was made in a "cloud" of *Artemia*.
[b] No sample taken or incompletely analyzed.
[c] Collapse of the parental *Artemia* stock.

CYST HARVEST

As a consequence of the oviparous reproduction, cysts were floating at the water surface in ponds 1, 2, and 4 on March 18, *i.e.* 16 days after the inoculation. We started to collect cysts 1 week later. Pond 1 gave the best yield (Table III) ; however, cyst harvest could only be performed once. Afterwards, the collapse of the parental stock and the retarded maturation of new *Artemia* generations prevented any further accumulation of cysts.

TABLE III

Cyst harvest in the different ponds and extrapolated yield for each pond. Ponds 1, 3, and 5 were inoculated at 17, 61, 175 ind./l respectively. Cyst collection was made on March 22-24 for ponds 1, 2, 4, and from April 5 onwards in pond 3. The interval (in days) separating two successive collections is indicated in parentheses

	Pond 1, Macau	Pond 3, Macau	Pond 5, Macau	Pond 2, GSL	Pond 4, China
Actual harvest (in g wet weight)	128 (9 days)	20.5 (6) 51.4 (6) 19.4 (7) 11.0 (10) 25.0 (2)	4.6 (18)	41.1 (9)	48.2 (12)
Extrapolated yield (in kg dry weight/ha/month)	6.8	2.6	0.6	2.2	1.9

The extrapolated yield, based on that 10-day period, was 6.8 kg/ha/month. On the other hand, in pond 3 the harvest continued for a whole month ; but the quantity collected was nearly the same as in pond 1. Thus, the yield was only about 2 kg/ha/month. In pond 5, the amount of cysts harvested was negligible.

For GSL and China *Artemia*, the same phenomenon occurred as in pond 1. Cysts could only be collected once and the yields obtained were similar to those of the Macau strain in pond 3, about 2 kg dry cysts/ha/month.

Discussion

The very heterogeneous distribution of *Artemia* in natural habitats has been pointed out by many authors (Persoone and Sorgeloos, 1980). In a preliminary study Vu Do Quynh and Vo Thi Dieu Duyen (1983) found that the coefficient of variation of the *Artemia* abundance, from stratified samples in one rectangular 2 800 m^2 pond, was usually > 50 %. In this study, the smaller surface area and the regular design of the ponds might have resulted in much lesser variability, at least low enough to allow comparisons between ponds.

Under laboratory conditions, *Artemia* can grow to the adult stage within 2 weeks (Sorgeloos and Persoone, 1975). Tobias *et al.*, (1980) observed that, under laboratory conditions, GSL and Macau *Artemia* attained sexual maturity within 13-14 days *versus* 19 days for a China strain. The three *Artemia* strains inoculated in Cam Ranh were able to complete their life cycle in a very short time. The first riding couples were observed 7 days after the inoculation and the Chinese parthenogenetic females began to develop ovaries after only 10 days. Amat Domenech (1980) reported that *Artemia* become sexually mature earlier in the natural environment than in laboratory cultures, but at about 22 days old (San Francisco Bay strain, SFB), which is a longer time than observed here. On the other hand, Tunsutapanich (1982) also reported that riding couples could be observed 9 days after the inoculation of SFB *Artemia* in Thai salt-ponds. In our study, GSL and Macau *Artemia* had nearly the same maturation time and it was higher than in the China strain ; confirming the data of Tobias *et al.* (1980). Differences in size were also observed between males and females in the two bisexual strains as reported by Baker (1966) and Jensen (1918) for *Artemia* from the SFB and GSL strains, respectively.

The growth and the reproduction of *Artemia* are governed by several factors such as the amount and quality of available food, the salinity, and the water temperature (D'Agostino, 1980). From the short maturation time observed, we may deduce that satisfactory trophic conditions prevailed at the stocking time ; however, the maximum adult sizes observed were somewhat smaller than those usually reported in the literature. Amat Domenech (1980) stated that *Artemia* living in natural environments usually have smaller sizes than those cultured in the laboratory.

In his experiments, Browne (1980, 1982) found fecundity *versus* age curves were bell-shaped for several *Artemia* strains. Amat Domenech (1980) also found an increase of the reproductive capacity within successive broods, starting from $\pm$ 50 offspring/brood/female for the first brood, and reaching very high figures of 300-350/brood/female for SFB *Artemia*. The relatively high number of offspring recorded for the first brood in the two bisexual strains inoculated in Cam Ranh, also supports the hypothesis of initial good trophic conditions. On the other hand, we found that under laboratory conditions, Macau *Artemia* showed higher figures at the first brood than in the field, *i.e.* 238 offspring *versus* 149. The same phenomenon was registered for the Chinese strain, since individuals coming from an indoor mass-culture (250 l "green water" cultures) had a higher mean fecundity value, *i.e.* 82.2 ± 50.9 eggs/brood/female ($N = 19$) than observed in the field. The highest number of offspring/female was 171 *versus* 80. However, as there is a positive relationship between fecundity and size (Amat, 1982), the larger size (13.1 ± 1.9 mm) of the laboratory-cultured animals may explain these differences. It is possible

that the initial trophic conditions (at inoculation time) were satisfactory enough to ensure a fast maturation, but were limiting for the reproductive performances.

Interactions between food availability, *e.g.* at different inoculation densities, and the growth and reproduction of *Artemia*, are also obvious from the data for pond 3. Both growth and reproduction were reduced in more crowded conditions, unless the food availability was improved, *e.g.* through a higher fertilization rate. In pond 5, the gap between the carrying capacity of the medium and the density of the inoculated population probably resulted in starving conditions and trophic deficiencies. Growth was very poor and most of the animals died.

A striking event was the short lifespan of the parental population, *i.e.* around 1 month. Vanhaecke *et al.* (1984) have studied the combined effects of salinity and temperature on the survival of various geographical strains of *Artemia*. From their results, it is clear that the salinities observed in our study remained in the range for an optimal survival rate. On the other hand, the diurnal temperatures were usually above 30-32 °C and may have caused mortalities as the limit for 90 % survival is below 30 °C for the Macau and Chinese strains.

Another possible cause for the observed mortality are the extremely unbalanced trophic conditions after the dramatic increase of the *Artemia* density. Two weeks after inoculation, it was difficult to sustain the labor involved in fertilization procedures and we had to switch from organic fertilizers to inorganic ones.

Many aspects of the fertilization were not satisfactory. Firstly, the unknown amount of impurities (gravel) introduced uncertainty about the qualitative value of the chicken manure available. Secondly, the chicken manure could have been directly utilized by *Artemia* provided that it had been ground into minute particles before distribution into the ponds, but this was not the case. Thirdly, the use of inorganic fertilizers may have been inefficient as the permanent grazing pressure by *Artemia* could have resulted in a very poor phytoplankton population. The density of females with full uterus decreased sharply during the 4th week in ponds 1, 2, and 4. The high value for the sex-ratio in pond 1 (Fig. 6) indicates that mortality affected females more than males. Actively-reproducing females might not have been able to divert enough of their energy to surviving because of their involvement in reproductive metabolism, and may have faced sudden adverse trophic conditions. Browne (1982) stated that the nutritional resources play a critical role in determining whether reproductive costs will affect the female's life-span. He also found that under low food conditions, there is a negative relationship between reproductive output and female life-span. Our observations provide much evidence for such a trend, in fact, the life-span of Macau *Artemia* was longer in pond 3 than in pond 1, with corresponding reduced adult size and fecundity in the former.

Considering the type of reproduction, cysts appeared very soon and harvests could be made 3 weeks after the inoculation. The salinity was rather low, about 100 ‰, when oviparous females occurred in the population. The major factors stimulating the oviparous mode of reproduction in *Artemia* are rather well known today. Low levels or large and frequent fluctuations in the dissolved oxygen concentrations of the medium (Versichele and Sorgeloos, 1980) stimulate hemoglobin synthesis and the consecutive secretion by the shell glands of haematin, the main component of the cyst shell (Dutrieu, 1960 ; Fautrez and Fautrez-Firlefijn, 1971).

We have not measured the dissolved oxygen ; thus, we cannot assess its role in the reproductive behaviour of *Artemia* in our experiments. However, as the salinity conditions were more or less constant during the experimental period, they are probably not responsible for any

substantial decrease in the dissolved oxygen content of the water. The high diurnal temperatures, mostly in the afternoon, are more likely to have caused oxygen stresses through a decrease of the gas solubility in the medium. Some differences in pigmentation between males and females were observed in the ponds where cysts had been produced. The males were translucent, unpigmented or sometimes greenish and the females were pink or fleshy colored, probably due to a higher hemoglobin concentration in their blood. Such pigmentation differences between males and females were not observed in other ponds where the reproduction pattern was ovoviviparous. Indeed, we think that the consequences following the drastic increase in *Artemia* density, 2 weeks after the inoculation, have been sufficient to stimulate the appearance of oviparity through a decrease in the dissolved oxygen.

If we consider the cyst production data, the figure obtained in pond 1, with the Macau strain, is the most interesting. The best yield was obtained there, as we had a combination of high reproductive performances and of high female population density. However, the rapid collapse of the parental population hampered collecting reliable data.

The yield of cysts from pond 1 is still far below the results obtained in Thailand, due to low adult density and to the improper fertilization procedure. Our data, based on very short periods of time, make extrapolations and scaling-ups very hazardous. In terms of inoculation strategy, the less cysts are used for the inoculation, the better it is from an economic point of view. The optimal inoculation density, for a given environment, will depend on the determined carrying capacity of the medium and on the use of fertilizers to increase this carrying capacity. Our results indicate that an inoculation density of about 10-20 ind./l lays in the optimal range for the Cam Ranh salterns, as a very fast increase in population density was obtained within 3 weeks. It is high desirable to obtain high population densities for production purposes. However, proper fertilization must be accomplished to prevent collapse of the population. Thus, more costs and labor are required, but they should result in higher yields.

Since the experiments reported here, the design of the experimental pond system has been altered to establish a salinity gradient from pond 1 to pond 4. Again 1.5×10^6 Macau *Artemia* nauplii were inoculated in pond 1 and the system was let to "stabilize" by itself; the only intervention was the intake of new water. Cysts were found to occur only in pond 2 and onwards, and the extrapolated yields confirm the data obtained during the first experiment, *i.e.* without fertilization cyst production yields a minimum of about 2 kg/ha/month. As the dry season is quite long in the Cam Ranh region, we could expect around 10 kg dry cysts/ha/season (considering a minimum of 5 months), which figure falls in the range of good productive natural habitats (Persoone and Sorgeloos, 1980).

The last observation was that *Artemia* individuals, which escaped from the experimental ponds, were found to survive in the surrounding evaporation ponds as also happened after our first trial in 1983. Besides the occurrence of a "natural" population there at the onset of the 1984 dry season, in January 1985, salt-workers reported some cyst accumulation in a few ponds.

An *Artemia* population also established naturally in early March 1985 after filling the experimental system with water. Cysts were collected every 5 days during a 43-day period, giving a yield corresponding to 8.6 kg/ha/month. These new data confirm the high potential for cyst production in the region. The establishment of a "natural" *Artemia* population in the salterns of Cam Ranh is opening large and unexpected perspectives for future *Artemia* field research in Vietnam.

Acknowledgments

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Rearing *Artemia* in a salt pan near Sambhar Lake (India)

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Abstract

Attempts were made to reintroduce *Artemia* into the Sambhar Salt Lake, the largest inland salt lake of India. The lake, once a natural biotope of brine shrimp, is now totally devoid of *Artemia* because heavy floods during the recent past resulted in considerable ecological changes.

Rearing trials with laboratory-hatched *Artemia* nauplii from Didwana Lake (India) were made in a 2 ha salt pan filled with brine from Sambhar Lake. Prior to and after inoculation, the water of the pan was analyzed for the main abiotic and biotic parameters. The inoculated *Artemia* did not survive in Sambhar Lake, but did survive when cultured in the laboratory in aerated Sambhar Lake water. Low levels of dissolved oxygen probably prohibit *Artemia* from living in Sambhar Lake.

Introduction

The ecological history of Indian inland saline lakes reveals that they have been subjected to extreme environmental conditions varying from immense floods to long dry periods. Sambhar Salt Lake which once harboured a rich *Artemia* population (Baid, 1958) is now totally devoid of it (Alam, 1980 ; Bhargava, 1984). This has been a consequence of repeated floods in the region during the last decade, resulting in the introduction of a variety of freshwater biota including predatory fishes through a riverine system. The salinity of the lake has been reported to drop from 160 ‰ to 4.3 ‰ during flood conditions. Adverse climatological conditions, apart from considerations of *Artemia* dispersion, are considered as limiting for the presence of *Artemia* in salt-water bodies which seem to be suitable for brine shrimp (Persoone and Sorgeloos, 1980).

Since the salinity of Sambhar Salt Lake has regained a moderately high level and no predators occur, an endeavour has been made to reintroduce the brine shrimp in this lake environment. To begin with, rearing trials with the *Artemia* from Didwana Salt Lake (India) have been carried out in a salt pan near Sambhar Salt Lake. The present communication deals with the results of two inoculation experiments conducted in a salt pan under different weather conditions, and also of the rearing of *Artemia* of Didwana Salt Lake origin in the brine of Sambhar Salt Lake under controlled laboratory conditions.

Materials and methods

Didwana Salt Lake (27° 3′ N and 74° 5′ E) is, presently, the only inland natural biotope of *Artemia* in India. Cysts collected from this lake during March-April 1984 were used for the experiments.

Sambhar Salt Lake (26° 58′ N and 75° 55′ E) is the largest inland alkaline saline lake in India, located in the semi-arid region of the State of Rajasthan. The lake is shallow, with a maximum depth of 3.75 m but is widely spread covering an area of about 190 km^2. In the close vicinity of the lake are large numbers of salt pans which are used for extraction of edible salt from the lake. One of the salt pans (2 ha area ; 0.25 m depth) was chosen for the inoculation experiments.

Cysts were hatched in the field laboratory at the lake site. Freshly-hatched nauplii in instar I stage were separated and immediately released in the pan during the cooler hours of the day at various points in the direction of the wind so as to ensure their quick dispersal. The stocking density was 12 nauplii/l. Prior to and after the inoculation the water was analyzed for the main abiotic and biotic parameters : minimum and maximum temperature, pH, dissolved oxygen, salinity, phytoplankton, and zooplankton. The experiments were carried out during October 1984 and March 1985.

Results and discussion

At the initiation of the October 1984 experiment, the salinity of the pan was 63.22 ‰, the dissolved oxygen level 8 mg/l, and pH 9. A zooplankton population (105 individuals/l) comprising *Cyclops* sp. and *Brachionus* sp., and unialgal phytoplankton population of *Spirulina* sp. (425×10^3 cells/l) formed the main biota of the pan.

It was surprising to note that after inoculation no nauplii survived after 12 h in the pan while populations of other zooplankters also declined to less than half of the number (45 ind./l). Competition for food among zooplankton does not seem to be the cause of this mass mortality of nauplii as at this stage they do not require food. As the pan was free from predators, the question of predation is also ruled out. It was presumed that possibly the low water temperature (around 15 °C) and drop in dissolved oxygen concentration during the night resulted in the mortality of the nauplii and other zooplankters.

In order to verify the above presumption the experiment was repeated during March 1985 when the water temperature was moderate (20-31 °C). The salinity of the pan during this period was 90 ‰ and the pH 9.4. The pan was free from zooplankters, while a phytoplankton population of 700×10^3 cells/l comprising *Spirulina* sp., *Aphanocapsa* sp., and *Nitzschia* sp. was present. The dissolved oxygen was low (2.14 mg/l) even during sunshine. Nauplii inoculated during this period also showed a total mortality within 12 h.

The ionic composition of Didwana Salt Lake, to which the *Artemia* cysts originally belonged, was compared with that of the salt pan in which the population was inoculated (Table I). Although the water of the salt pan was relatively harder, there was not much difference in the milliequivalent percentage (mEq %) of major cations and anions.

In order to ascertain any specific role of ionic composition in the mortality of the *Artemia* population, laboratory culture in brine from the same salt pan was tried. The temperature was maintained at 30±2 °C. The brine was aerated through air pumps. No supplementary food was

TABLE I

Ionic composition (in mEq %)
of the inoculated salt pan (near Sambhar Salt Lake) and Didwana Salt Lake

Ions	Salt pan	Didwana Salt Lake
Cations		
Na^+	97.64	98.61
K^+	0.13	0.21
Ca^{++}	1.08	0.40
Mg^{++}	1.12	0.76
Anions		
CO_3^-	5.80	0.39
HCO_3^-	2.01	0.30
Cl^-	91.03	98.90
SO_4^-	1.14	0.39

provided for the first 2 days of culture ; however, thereafter the manuring was done as described by Dwivedi *et al.* (1980). Interestingly, the brine shrimp population thrived well. In another culture in which no artificial aeration was provided, the nauplii died similarly to those in the experiments in the salt pan. In the aerated culture the brine shrimp attained the adult stage 15 days after inoculation. The length of the adults varied from 10 to 14.5 mm. The adults commenced to breed on the 48th day.

The study reveals that a major bottleneck in *Artemia* culture in salt pans of Indian arid or semi-arid regions is the high rate of evaporation causing a rapid increase in the salinity (6 ‰/day) which in turn results in low levels of dissolved oxygen. This is probably due to : the mass mortality of phytoplankton and an increase in the decomposition rate of the dead algae.

During their study on *Artemia* rearing in earthen salt ponds in the Philippines, Primavera *et al.* (1980) observed a similar phenomenon of total collapse of *Artemia* population when solar salt was added to a pond to increase its salinity. They attributed it to two major factors : resultant high concentration of $CaSO_4$ which may have been toxic to *Artemia* (Vos, 1979), and a salinity increase (44 to 53 ‰) stimulating excessive lablab growth which may have led to unfavourable environmental conditions (low oxygen concentration, a high BOD, high concentration of ammonia and/or sulphide, etc.). Lablab was described as a microbenthic complex of bacteria, diatoms, blue green algae, protozoans, and other microorganisms by Rabanal (1966, cited from Primavera *et al.*, 1980). In their study on brine shrimp in temporary saline ponds of Iraq, Khalaf *et al.* (1977) also pointed out the effect of low oxygen on the disappearance of *Artemia* population.

The rearing of *Artemia* in such salt pans is not recommended.

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Preliminary studies on the culture of *Artemia* using renewable organic wastes

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Summary

Although rice/wheat bran is being widely used as a feed in *Artemia* culture, many developing countries which are unable to offer rice bran as feed, are trying alternative cheap sources of feed items. In India, Dwivedi *et al.* (1980) initiated work on the utilization of organic wastes, especially pig dung, in combination with ingredients such as super-phosphate, commercial yeast, and ground nut oil cake. The feasibility of utilization of organic wastes in *Artemia* culture has prompted us to undertake the present investigations on the use of agricultural as well as organic domestic wastes. Three organic wastes, cabbage leaves, cow dung, and poultry manure, were used because they are cheap and favour the growth of bacteria and algae on which the *Artemia* thrive. This represents a low input technology which suits the rural conditions in India and helps commercial prawn farming.

Artemia cysts were collected from Tuticorin, hatched under laboratory conditions and nauplii were reared up to adults. Initially several experiments were conducted by exposing the nauplii to different salinities (40, 60, 80, and 100‰) and organic wastes (cabbage, poultry manure, and cow dung) to select the suitable medium/media for maximal survival. Marian and co-workers (unpubl. data) revealed that fresh cow dung and 20-day-treated poultry manure ensured maximal survival and growth in the culture of fish-feed organisms. Hence, in the present study fresh cow dung, 20-day-treated poultry manure, and waste cabbage leaves were used.

In each of the media *Artemia* culture was undertaken in cement tanks (65 cm diameter, 100 cm height, 159 l capacity). Sun-dried soil was added up to 10 cm thickness. The tanks were filled with 50 l water. The salinity was maintained between 60-80‰. A week after the introduction of the manure, the tanks were inoculated with *Artemia* nauplii and the cultures were maintained for a month.

The organic wastes tested and their NPK values are given in Table 1. Survival tests were conducted by maintaining the *Artemia* for about 7 days. Survival was low in the cabbage leaf medium and poultry manure medium, moderate in the cow dung medium, and maximal in the medium containing a mixture of the above three organic wastes. The highest survival rates were obtained at 60-80‰ salinity in the mixed medium (Fig. 1). The medium played a more dominant role in the survival of *Artemia* than did salinity (Anova ; $P < 0.001$).

The growth pattern of individual *Artemia* was sigmoid with an initial slow growth phase up to the 4th day followed by a rapid logarithmic phase (from 4th to 16th day). Thereafter, the weight stabilized around 6.5 mg and further energy input was channelled to reproduction rather than somatic growth (Fig. 2).

Eggs started to develop when the females reached 3.8 mg, *i.e.* between the 10th and 12th day. Almost all the females became gravid within 14 to 16 days and had on the average 73 eggs per brood sac.

The first batch of nauplii was released on the 15th day and successive batches were released in 34 to 44 days (Fig. 3). Starting from the first batch, *i.e.* in a period of 15 days, the maximum number of nauplii released by the *Artemia* increased to 188.

TABLE I

Nature of organic wastes used as food

Organic wastes	N	P	K
	(% dry wt)		
Cow dung	0.5	0.09	0.36
Poultry manure	0.5	0.50	0.45
Cabbage wastes	3.5	0.5	0.5

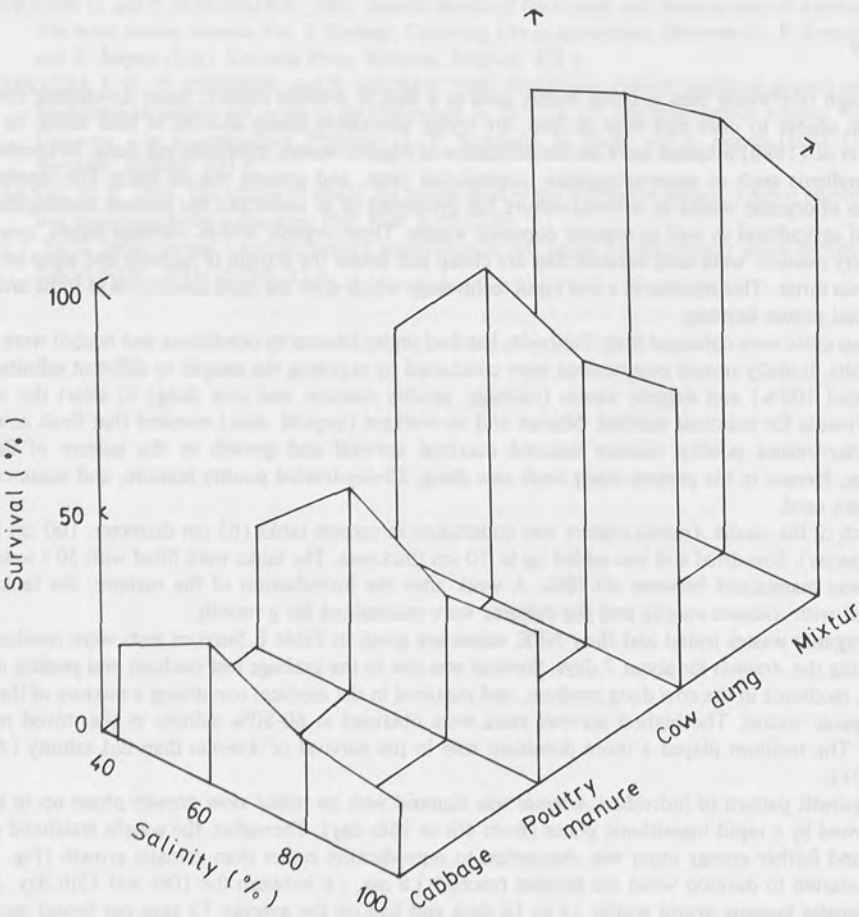

FIG. 1. Survival of *Artemia* in different culture media at four salinities.

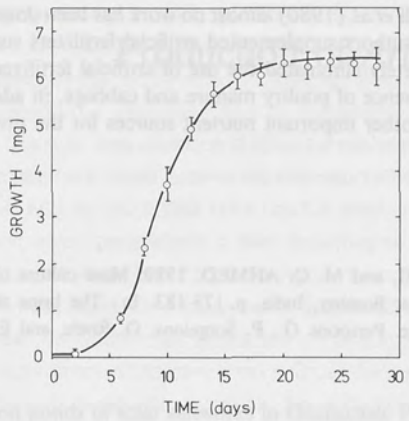

FIG. 2. Growth pattern of individual *Artemia* as a function of time.

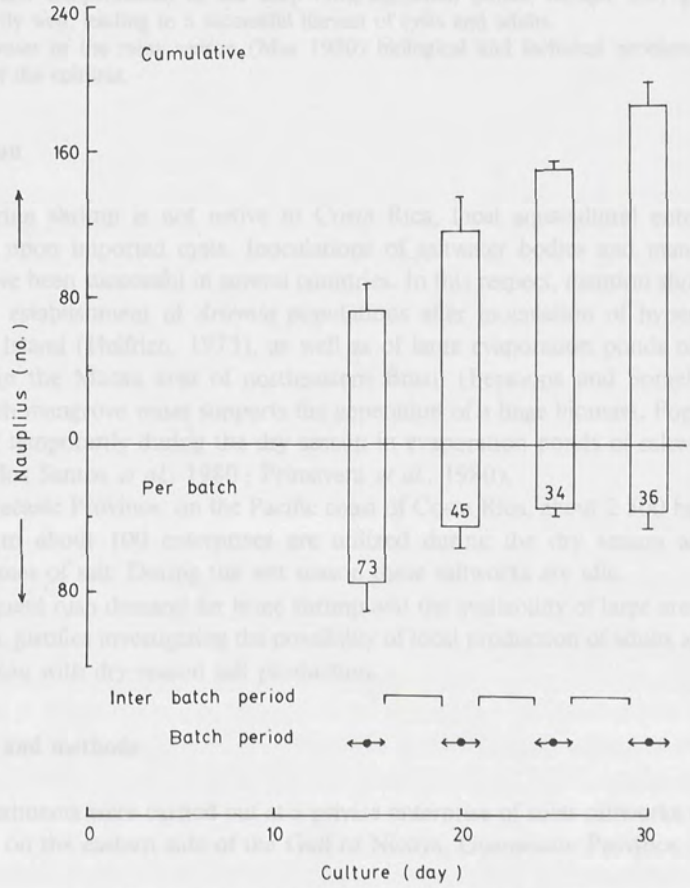

FIG. 3. Nauplius production in cultured *Artemia*.

Other than the work of Dwivedi *et al.* (1980) almost no work has been done on low-cost feed technology. However, in their system, these authors supplemented artificial fertilizers such as superphosphate. In our system, although we have completely eliminated the use of artificial fertilizers, we obtained a comparable production of *Artemia* in the presence of poultry manure and cabbage, in addition to the cow dung which contributed to the nitrogen and other important nutrient sources for the production of feed organisms of *Artemia*.

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Production of *Artemia* in Costa Rica : a pilot project

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Abstract

Several small evaporation ponds of solar saltworks in Guanacaste Province, Costa Rica, were inoculated with cysts and nauplii of *Artemia* during the dry season (December 1979 to April 1980). *Artemia* is not native to Costa Rica.

Brine shrimp did not do well in the shallow evaporation ponds, due to heavy predation by fish and insects and high water temperatures. In the deep brine-collection ponds, though, they grew and propagated extraordinarily well, leading to a successful harvest of cysts and adults.

At the onset of the rainy season (May 1980) biological and technical problems led to a complete extinction of the cultures.

Introduction

Since brine shrimp is not native to Costa Rica, local aquacultural enterprises are wholly dependent upon imported cysts. Inoculations of saltwater bodies and man-made ponds with *Artemia* have been successful in several countries. In this respect, mention should be made of the permanent establishment of *Artemia* populations after inoculation of hypersaline lagoons on Christmas Island (Helfrich, 1973), as well as of large evaporation ponds of commercial solar saltworks in the Macau area of northeastern Brazil (Persoone and Sorgeloos, 1980) where nutrient-rich mangrove water supports the generation of a huge biomass. Populations have been established temporarily during the dry season in evaporation ponds of saltworks in the Philippines (De los Santos *et al.*, 1980 ; Primavera *et al.*, 1980).

In Guanacaste Province, on the Pacific coast of Costa Rica, about 2 500 ha of solar saltworks belonging to about 100 enterprises are utilized during the dry season and produce about 20 000 tonnes of salt. During the wet season these saltworks are idle.

The frequent rush demand for brine shrimp and the availability of large areas of ponds for salt production, justifies investigating the possibility of local production of adults and cysts of *Artemia* in connection with dry-season salt production.

Materials and methods

All experiments were carried out at a private enterprise of solar saltworks close to Colorado/ Abangares on the eastern side of the Gulf of Nicoya, Guanacaste Province, Costa Rica.

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Due to the heavy and frequent rainfalls during the rainy season (May to November) salt production in Costa Rica is only feasible during the dry season, which lasts from December to the end of April. The solar saltworks comprise about 13 ha of reservoir-, evaporation- and salt crystallization ponds. For the construction of this saltworks a mangrove area has been cleared and dikes of mud have been thrown up to build the ponds and to ward off the tide. At high tide a gate at the outer reservoir pond is opened and water from the mangrove area (salinity about 30 ‰) fills the pond to a depth of about 0.8 m. After filling, the gate is closed. From this reservoir (surface area about 1 ha) water is pumped with a stationary diesel-powered pump to the first shallow solar evaporation pond (water depth about 15 cm, surface area about 5 000 m^2, salinity about 40 ‰). From there, water flows and is pumped with small mobile diesel-powered pumps to a series of smaller evaporation ponds (surface area 500-5 000 m^2, water dept 5-15 cm) where the salinity increases gradually to 240 ‰. The concentrated brine is pumped to a small and deep collection pond (surface area about 200 m^2, water depth about 1 m) from which it is pumped either to solar crystallization beds or to cooking vats.

On 22 January, 1980 the first evaporation pond (salinity about 50 ‰) was inoculated with brine shrimp nauplii. Cysts of this culture ("Living World", San Francisco Bay Brand Co., batch no. 56034) hatched under controlled conditions in seawater (salinity 32 ‰, temperature 28 °C) in 1 000 and 2 000 l tanks, respectively, under aeration and illumination at the installations of Maricultura S.A. at Chomes, about a 1-hour drive from the salt ponds. During the following weeks further evaporation ponds of different salinities were inoculated. Prior adaptation of nauplii to higher salinities was not necessary.

Results

Problems with predatory invertebrates, fishes and birds are experienced wherever brine shrimp are cultivated or occur in nature (Kristensen and Hulscher-Emeis, 1972 ; Crear, 1980 ; Persoone and Sorgeloos, 1980 ; Scelzo and Vogler, 1980). In our experiments it was mainly in the evaporation ponds with salinities below 120 ‰ that predation was a major problem. Even when filtering the influx water with netting material (stretched mesh size 2 mm) it was impossible to exclude all predators. The first inoculation tests failed for this reason.

In Costa Rica, evaporation ponds with higher salinities are usually very shallow (water depth 5-15 cm), leading to noontime temperatures at or above 42 °C. Because this temperature is lethal to *Artemia*, several inoculations failed.

Since all water from the solar evaporation ponds is finally collected in a small, deep collection pond, it was not surprising to find a high density of *Artemia* there. As this pond was not inoculated, brine shrimp must have come with the influx water. Only bacteria, a few species of phytoplankton and brine shrimp are able to survive at very high salinities (up to 240 ‰) (Davis, 1980). This accounts for the absence of predators in the Dead Sea and also in deep brine-collection wells.

Constant water circulation from upwelling resulted in water temperatures between 28 and 35 °C. Animals propagated well, even without aeration, fertilization or supplemental feeding. Thanks to the frequent inflow of water from the evaporation ponds, sufficient natural food was available. Continuous filtering of the organic matter by *Artemia* simultaneously improved the purity of the salt produced. Thus, *Artemia* plays an important part also in salt production in solar salt ponds (Davis, 1980).

To prevent loss of *Artemia* when pumping from the brine-collection pond to the crystallization beds or cooking vats, the suction pipe of the pump was covered with a nylon monofilament netting of 900 µm mesh opening. Frequent cleaning of the netting was necessary to prevent clogging with salt crystals.

About 4 weeks after the first inoculations *Artemia* cysts were found in the brine-collection pond. In this pond a few grams of cysts were produced daily and driven by the wind to the edges of the pond where they could be harvested. Harvesting with a fine scoop net was very difficult due to the irregular structure of the earthen dikes. In a preliminary test harvested cysts showed a high hatching rate. Adult brine shrimp could be easily harvested at night with scoop nets and fluorescent light. The collected adults were frozen and later on used by Maricultura S.A. as supplementary feed in the maturation program for adult shrimps (*Penaeus vannamei* and *P. stylirostris*). At the onset of the wet season unexpected technical problems arose. After heavy rainfall a layer of freshwater built up over the heavy brine, drastically reducing the water circulation and thus causing a lethal rise in water temperature. As the salinity in the brine-collection pond decreased, the number of predators of *Artemia* increased. About 1 month into the wet season, the brine shrimp were extinct.

Discussion

It could be demonstrated that the production of adults and cysts of *Artemia* in man-made salt ponds is feasible both from a biological and a technical point of view. In Costa Rica, as in Southeast Asia, production without technical and engineering intervention is, however, only possible during the dry season. In order to know whether the combined production of salt and *Artemia* is economically feasible, the following factors must be considered :

a) minimum water depth in the evaporation ponds, the resulting water circulation, and the water temperature ;
b) water management regime ;
c) impact on salt production of increased depth of evaporation ponds and a water management regime modified for *Artemia* production ;
d) investment costs for modification of the saltworks, and expected income from the sale of adults and cysts.

The additional use of modified evaporation ponds during the wet season for the production of fish or shrimp, as successfully done in Southeast Asia, would help to justify high investment costs (De los Santos *et al.*, 1980).

Experiments on the combined production of salt and *Artemia* in Southeast Asia show that about 5 kg of cysts ha/month can be produced during the dry season (De los Santos *et al.*, 1980). Experiments in Brazil demonstrate that from an abandoned salt pond of 800 m^2 during a period of 60 days, 900 g of cysts can be collected, this is 5.75 kg/ha/month (Da Costa and Vergara, 1979). According to estimates from Persoone and Sorgeloos (1980) a good *Artemia* biotope can produce some 10-20 kg of cysts/ha/season.

On the assumption that the above-mentioned studies provide positive results and the here described technical procedures can be improved with regard to inoculation, predator control, supplementary feeding and/or fertilization, and harvesting, it might be possible to produce in

Costa Rica during the 4-months dry season as much as 1 to 2 tonnes of cysts in about 100 ha of solar evaporation ponds. This local production would help to alleviate the import-dependence for this product and offer the "salineros" an additional income. Increased benefits could be obtained by utlizing the modified solar evaporation ponds during the wet season for fish or shrimp farming.

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Preliminary production results of *Artemia* to be used in local shrimp farming in La Paz (Mexico)

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Abstract

Large *Artemia* populations are present in several saltworks as well as in abandoned saltpans of the Mexican State of Baja California Sur (BCS). Cyst production rates are, however, low. Biomass production-trials have been carried out in earthen ponds. Details of production yields obtained with cheap organic and inorganic fertilizers are presented. The adult *Artemia* were used as shrimp feed. The integrated use of different *Artemia* products in shrimp culture in the Baja California Sur is coordinated through institutional research projects as well as through new developing enterprises.

Introduction

The Centro de Estudios Tecnologicos (CET) del Mar in La Paz, Mexico, started operating shrimp culture facilities this year, including a hatchery, which is the second one in the country. This shrimp program is oriented towards : 1) the feed needed for the different developmental stages of the organisms Pacific brown shrimp (*Penaeus californiensis*), and blue shrimp (*P. stylirostris*) ; and 2) the comparative evaluation of intensive, semi-intensive, and extensive culture systems (Flores, 1984).

With regard to the *Artemia* needs encountered by the hatchery, it is important to mention that 10 natural populations have been reported in Mexico, most of them along the Pacific coast. Out of these populations, three are found in the State of Baja California Sur, where La Paz is located (Castro *et al.*, 1985, 1987).

Although the largest salinas of the world (Guerrero Negro) are found in this state, the occurrence of cysts has never been reported. In most of the existing saltworks of the BCS State, there is a high potential for biomass production/harvesting, because *Artemia* is found almost year-round, in considerable amounts. Significant cyst production has, however, never been reported (Javor, 1983). In view of its prevailing climatic conditions, its large coastal areas with proper soil characteristics, and its existing salt operations, Mexico can be considered to have a high potential for *Artemia* production.

Aquaculture is also offering perspectives for many people in the BCS State who, having their communities and land in coastal areas, are not able to use them, neither for agriculture nor for cattle breeding, because of the high salt levels in these areas and the scarcity of freshwater.

Consequently, integrated aquaculture activities are very attractive as important alternatives for social development.

The integrated use of *Artemia* in aquaculture has been reported in the literature, such as : direct feed for juveniles of shrimps in the nursery stage, protein biomass, an attractant ingredient for shrimp pellets, and the maturation triggering effect of *Artemia* in adult females of brown shrimp, although this last point requires much further research and experimentation (Persoone and Sorgeloos, 1982 ; Sorgeloos, 1983, 1986).

Materials and methods

Studies on the possibilities of using *Artemia* at different stages in the shrimp program, started with biomass production in small earthen ponds filled through underground infiltration, resulting from excavating a soil with a high salt content. Two experimental ponds, measuring 2.0 m × 5.0 m were used, and two smaller ponds (1.0 m × 1.0 m) as controls. The salinity remained quite constant at 65±5 ‰ in all the ponds throughout the experiments, except in the second experimental run, when rainfall decreased the salinity to 45 ‰. The water depth showed very light variations, 60±5 cm, due to the location of the ponds at about 100 m from the seashore. The temperature fluctuated from 25 to 29 °C, respectively during the night and day.

Experimental work in these ponds consisted in testing different locally available fertilizers, both organic and inorganic, to grow *Artemia* starting from freshly-hatched nauplii (San Francisco Bay Brand) up to the adult stage, and then harvesting the biomass, with nets. Fertilization was provided, if needed, according to cell counts and water transparency.

The *Artemia* biomass was then used in small-scale feeding experiments with postlarval shrimp. The tests were carried out in plastic aquaria containing 10 l water. In each aquarium 100 young postlarval shrimp were placed, and fed from 30 to 100 % biomass/day, on a wet weight/wet weight relationship.

Different experiments were run, using adult frozen *Artemia* as food as well as pellets and a mixture of fresh fish and clams. The *Artemia* used in these experiments were frozen at -30 °C in thin layers (2-3 cm), after removal of excess water by sieving through a fine mesh cloth.

Artemia biomass was also used as protein source and added at different levels in pelleted feeds, ranging from 5 to 25 % inclusion (wet weight). Brown shrimp maturation experiments were conducted in 1 500 l round fiberglass tanks. The photoperiod and water temperature were kept constant. No eyestalk ablation was applied (Primavera, 1985).

Another source of *Artemia* biomass was used from nearby salt operations where it is routinely frozen and stored using the process described above.

Results and discussion

Fig. 1 and 2 show the results obtained in the biomass-production trials.

The ponds were harvested when the organisms reached the adult stage, and the females were in the brood-carrying stage ; this occurred when the mean length was about 7.0 mm. After harvest, some organisms remaining in the ponds grew up to 15 mm in 7 more days, producing large amounts of nauplii.

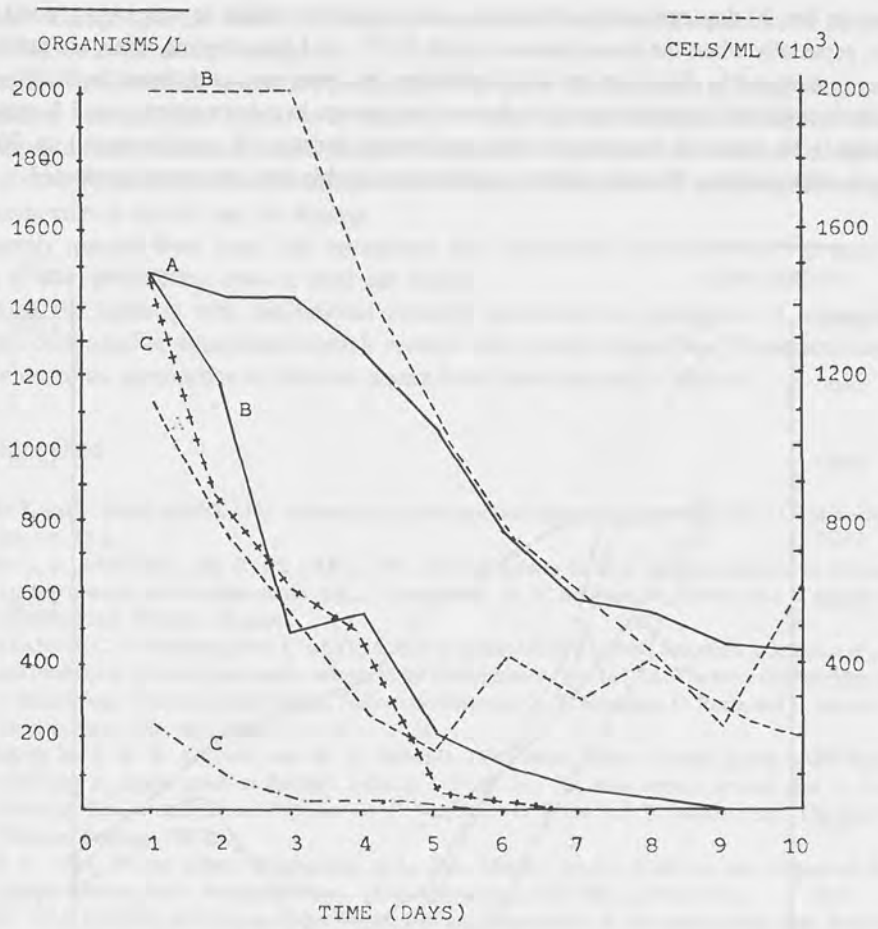

FIG. 1. Biomass-production trial I (fertilizers : chicken manure + urea + superphosphate ; in kg). Tank A : 80+4+2 (refertilization day 5). Tank B : 120+6+3 (refertilization day 8). C control (no fertilization). Water temperature 27.5-30 °C (average 28.1 °C) ; salinity 65-69 ‰ (average 67.2 ‰).

De los Santos *et al.* (1980) reported similar growth rates at higher salinities, and at similar temperatures. Dwivedi *et al.* (1980) observed maturity in *Artemia* of 12 days fed on pigdung, superphosphate, oil cake, and yeast. These authors also reported cyst production which in our experiments did not occur.

To estimate the food density available to *Artemia*, the inert particles present in the water were counted ; live cells included *Navicula, Nitzschia, Chaetoceros,* dinoflagellates and other unidentified oval cells. Presently further research on the food availability is being carried out.

Although the optimal amount of *Artemia* biomass to be fed to postlarval shrimp could not been determined in the small scale experiments, up to 100 % biomass (wet weight)/day has been used with good growth and survival at the higher levels, namely of up to 0.65 mm/day and 80 %

survival in the 20-day experiment. However, care should be taken to maintain a good water quality, especially when the temperatures exceed 28 °C, and high feeding levels are used.

Artemia included in the pelletized feed for shrimp has been used at different levels. The effect varies from improved acceptability of the feed to the shrimps in culture systems (at 5 % inclusion, dry weight) to observed maturation effect on brown shrimp (*P. californiensis*) at 20-25 % inclusion (dry weight). Presently further experiments in this area are being conducted.

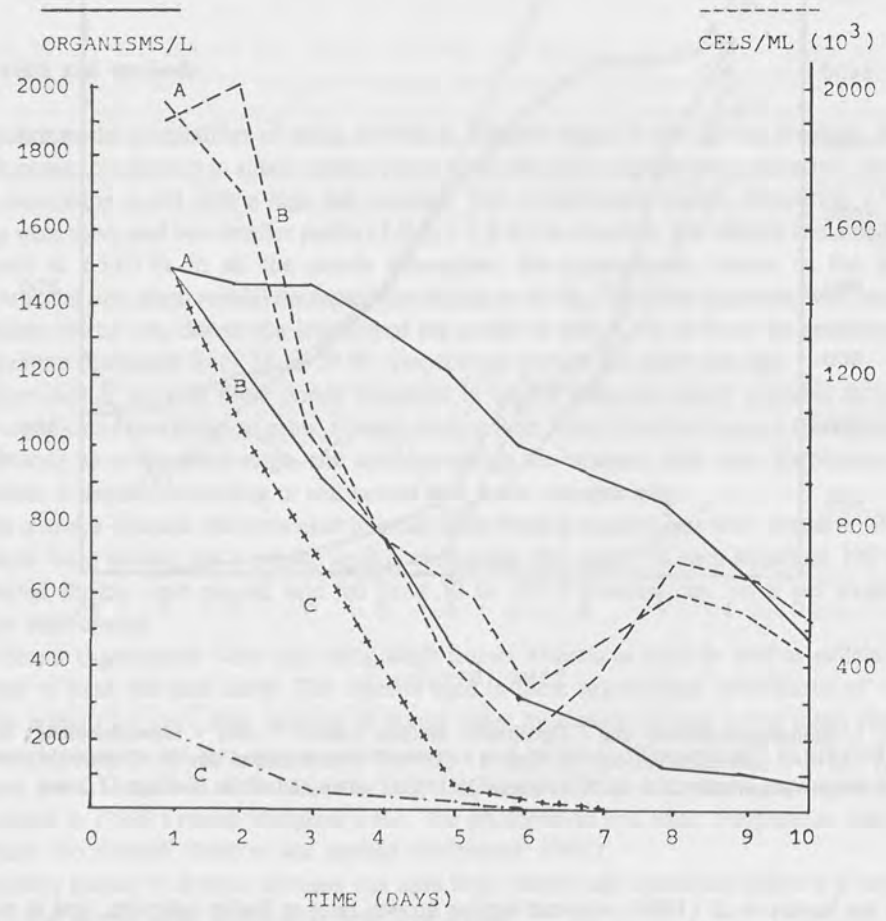

FIG. 2. Biomass-production trial II (fertilizers : chicken manure + urea + superphosphate ; in kg). Tank A : 80+4+2 (refertilization day 6). Tank B : 100+5+2.5 (refertilization day 6). C control (no fertilization). Water temperature 24.6-29 °C (average 27.2 °C) ; salinity 59-67 ‰ (average 64.2 ‰).

Conclusions

The biomass-production trials, as well as the experiments regarding its use in shrimp culture have been run only on a small demonstration scale. Important information is, however, being

developed locally, and the interest of using *Artemia* in the fast growing aquacultural industry of the BCS State is increasing rapidly.

Although the presented results are preliminar, they show the feasibility of integrating *Artemia* – a natural sub-exploited resource in Mexico – with shrimp culture, an activity which is considered to have paramount importance in Mexico, as well from the economic as the social point of view. A shrimp culture cooperative was recently formed and will initiate different aspects of *Artemia* culture and its use for shrimp.

Presently, people from local salt operations who considered *Artemia* as a "salt-eater" are willing to start production trials in their salt fields.

Linking the industry with institutional research promotes the integration of technological activities developed at education/research centers with private enterprises. These activities can be considered as approaches to enhance aquaculture development in Mexico.

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Preliminary trials of extensive culture of *Artemia* in Peru

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Summary

Although *Artemia* is naturally occurring in Peru, commercial exploitation of its local resources is not feasible because of low and/or erratic production. However, in view of favorable climatological conditions, presence of land not suitable for traditional agriculture (salt-affected alluvial soils) and abundant availability of cheap organic fertilizer (marine bird manure), the northern coastal area of Peru is offering very interesting opportunities for man-managed pond production of *Artemia*.

In order to demonstrate the technical feasibility of *Artemia* pond production in northern Peru, two inoculation experiments (under different seasonal conditions) have been carried out in a man-made earthen-pond facility near Virrila, consisting of a reservoir (4 528 m^2) and evaporator (3 348 m^2) receiving high salinity water (55-90 ‰) from an estuary.

In the first experiment, performed in the summer (December-March), the evaporator was inoculated with *Artemia* from Hierba Blanca, Sechura (Peru). The waterdepth of the pond was maintained at 40 cm by regular pumping of high salinity water from the estuary and reservoir. Throughout the experiment, salinity and temperature ranged from 162 to 200 ‰, respectively 23.4 to 28.7 °C, while the pH remained around 7.0. Marine bird manure was applied at a rate of 1 000 kg/ha/month. The inoculated nauplii grew into reproductive active adults in less than 3 weeks. Initially the population reproduced ovoviviparously ; however, after 2 weeks cyst production became dominant. Over a period of 13 days 5.6 kg wet-weight cysts have been harvested (*i.e.* 1.3 kg/ha/day). Their quality analysis revealed a hatching efficiency of 250 000 nauplii/g. The *Artemia* biomass (standing crop), averaged over a production period of 48 days, was estimated at 60 g w wt/m^3.

The second inoculation experiment was carried out in the reservoir (salinity 96 ‰) under wintertime conditions (March-October) and without fertilization. The water depth was maintained at 40 cm. Average daily temperatures ranged from 18.6 to 24.5 °C, while the salinity gradually increased from 96 to 164 ‰. Over a period of 105 days, 4.9 kg cysts were harvested (hatching efficiency 293 000 nauplii/g).

The present *Artemia* culture trials demonstrate that high quality cysts can be produced in man-made earthen ponds in northern Peru and that fertilization with bird manure can result in high cyst yields. Furthermore our experiments indicate that *Artemia* production in northern Peru is feasible on a year-round basis.

In view of these promising results more work should be carried out to establish *Artemia* pond-production technology as a new economic activity using abandoned lands in the northern coastal areas of Peru.

metabolites, then one can appreciate the chemical and physical impact exerted on the pond systems through organic means. This organic contribution to the evaporation process can therefor be expected to influence the production of the salts derived from these ponds.

As several papers on ecology have suggested, these ecosystems can be managed for better control of the organisms. This hydrobiological management can be developed to provide a previously neglected but critical control for enhancing salt production. What role *Artemia* plays in the hydrobiology of solar salt ponds was the central topic of this workshop.

The discussion was organized around four thematic questions :

1. What role do *Artemia* play in the hydrobiology of a salina ?
   The beneficial role of *Artemia* in cleaning the brine through their grazing activity was discussed. It was suggested that *Artemia* (in interaction with algae) might contribute to the heat budget of the salina, although the most important factor influencing evaporation is the relative humidity at the water surface, which is mostly affected by wind, especially in coastal lagoons. In general most participants of the symposium regretted that there is a lack of quantitative data giving evidence of real *Artemia* contribution to the hydrobiology of the salina. Furthermore one should not isolate *Artemia* as the one (and only) component playing a role in the salt making process ; studies including the overall ecology of the salina were highly recommended.
2. How can the *Artemia* population in a salina be managed ?
   Discussions focused on various aspects dealing with maintenance of a healthy algal population, which is considered to be the key to success in extensive *Artemia* production. It was suggested that in those salinas associated to a mangrove area one could take more advantage of the nutrients, by pumping only at low tide. The relation between depth of the ponds and food presence for *Artemia* received much attention : apparently the most productive saltworks in terms of salt and *Artemia* are those with deep ponds, *i.e.* at high water depths, nutrients are converted into phytoplankton (available to *Artemia*) which through shading inhibit development of phytobenthos (not totally available to *Artemia* and sometimes reducing evaporation when starting to float). This observation was confirmed by participants working in Italy, Vietnam, and especially the Phillipines and Thailand where pond construction is modified by increasing the depth in order to ensure higher phytoplankton production. The highest *Artemia* yields have indeed been reported for Southeast Asian *Artemia* ponds where water depths range from 30 to 100 cm. It is only under food-limiting conditions that *Artemia* start to graze on algal mats. In this situation representing a two-dimensional feeding habitat, *Artemia* densities are much lower than in the three-dimensional system, when *Artemia* thrives on phytoplankton.
3. What parameters should be monitored in evaluating the ecosystem of a salina ?
   Next to the biotic parameters which had been discussed previously (*i.e. Artemia* density, algal species and densities), more attention should be paid to bacterial productivity as it might represent an important food source for *Artemia*. As to chemistry of the water, the following parameters are considered to be important : nitrates, phosphates, organic contribution to the system, specific gravity or salinity, temperature differences, pH, and ionic composition of the brine in the successive ponds. One should be very careful, however, when using the standard chemical methods for analysis of brine waters. It was suggested to always run a series of spike tests to determine the percent recovery in the high saline environments. Another important parameter, which sofar has been mostly neglected,

is the hydrology of the salina, *e.g.* the retention time of the systems (*i.e.* almost static in large saltworks, quick flow-through in artisanal salterns) is expected to have a major effect on the ecosystem in general and on the population dynamics of the *Artemia* in particular.

4. Can selection of various *Artemia* strains be critical to proper hydrobiological management ? Contrary to the general assumption, various "natural" *Artemia* populations might not be adapted to their environment because of (unreported) artificial introduction or because the local habitat has been modified. A typical example for the latter situation is the southern Italian Margherita di Savoia population which is not adapted to the high temperatures prevailing in the saltwork during summertime. Decades ago the former natural salt lake ("Lago Salpi") has been converted into a modern solar salt operation with shallow ponds, as a result the natural (parthenogenetic) strain was exposed to a new situation (*e.g.* higher water temperature) to which it was not adapted. In this regard, strain selection when knowing the natural conditions, has become very important. In those cases where *Artemia* has to be introduced in an unknown habitat, it might be more desirable to choose from strains with a high genetic variability, and with good characteristics in their natural environment, allowing for greater buffering and better selection of the (pre) adapted genotypes. In this regard it was recommended that genetic experiments should be undertaken to see whether desirable characteristics can be selected. In conclusion the moderator stressed the good future potential of solar salt production because of its minimal energy requirements for a highly pure product. In view of its beneficial role in the hydrobiological management of the salina it is expected that in the future *Artemia* will be further developed as a very valuable byproduct of solar salt production.

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Effect of bacteria on the nutrition of the brine shrimp *Artemia* fed on dried diets

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Abstract

Large-scale brine shrimp production for aquaculture requires nutritionally suitable, inexpensive foods. The use of dried algae and natural by-products would seem to solve this problem, but the extent to which microbes in the culture system modify the nutritional quality of these diets is unknown. The objective of this study was to determine the effect of bacteria on these diets when fed to *Artemia* nauplii.

Six dried foods, *Spirulina*, Cerophyl, yeast, defatted rice bran, soybean and lactoserum, were tested under xenic and axenic culture conditions where the concentration of food, salinity, temperature and dissolved oxygen had been optimized for the growth of *Artemia*. None of the diets used under axenic condition met the nutritional requirements of *Artemia*. Inoculation of the axenic cultures with bacteria ("the selected microflora") derived from xenic cultures showing maximal development permitted growth. This indicates that the microflora was essential to the nutrition of *Artemia*. Furthermore, axenic cultures of *Spirulina*, lactoserum, and soybean provided greater improvement in growth of *Artemia* than rice bran, Cerophyl and yeast following inoculation with "selected microflora". *Spirulina* gave the best survival and growth of brine shrimp when inoculated with "selected microflora". Comparison of the chemical composition of these diets suggests that the nutrient content of *Spirulina*, lactoserum and soybean may be more likely to enhance colonization by adventitious microorganisms. For this reason these diets perform better with preinoculation of the "selected microflora", but under other circumstances, contamination with unfavorable bacteria would lead to deterioration of the cultural conditions and, therefore, to poor *Artemia* growth.

Introduction

The brine shrimp *Artemia* has been widely used as food for diverse groups of organisms reared for aquaculture or research purposes. The nutritional value of the brine shrimp varies as the nauplii mature. If the brine shrimp are not fed, their dry weight decreases by 20 % and their caloric value by 27 % from instar 1 to instar 3 (Benijts *et al.*, 1976) ; thus, if they are not used at instar 1, they must be fed in order to maintain their nutritional value.

Recently, attention has been given to the adult brine shrimp as a possible source of food for post-larval stages of invertebrates and vertebrates in aquaculture. The adult has a very high nutritional value ; 60 % of its dry weight consists of protein rich in all essential amino acids and it contains significant concentrations of vitamins and carotenoids (Gallagher and Brown, 1975 ; Soejima *et al.*, 1980). In crustacean and fish farming, adult brine shrimp are considered to be

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a better reference diet than formulated feeds (Barahona-Fernandes and Girin, 1976 ; San Feliu *et al.*, 1976). In optimal culture conditions brine shrimp can grow from larvae to adults in less than 2 weeks, increasing in length by a factor of 20, and in biomass by a factor of 500 (Reeve, 1963a).

Since this animal is a non-selective filter-feeder (Lochhead, 1941 ; Reeve, 1963b ; Dobbeleir *et al.*, 1980), a wide variety of very inexpensive natural foods have been considered for the possible mass culture of *Artemia*. For large-scale production of brine shrimp adults, natural dried by-products are less expensive than live algae (Dobbeleir *et al.*, 1980) and, unlike algae, they can be stored for long periods of time.

Most of the studies on the nutrition of *Artemia* have been carried out under xenic conditions, where the fortuitous occurrence of bacteria and protists of one kind or another could not be controlled. Other studies in axenic systems (Gibor, 1956 ; Provasoli *et al.*, 1959 ; Provasoli and Shiraishi, 1959 ; D'Agostino and Provasoli, 1968 ; Sick, 1976) have provided information on the nutritional value of the algal species in the absence of bacteria. Xenic and axenic cultures of food organisms have different nutritive values (Gibor, 1956). Provasoli *et al.* (1959) showed that bacteria and other algae can successfully supplement species of algae that alone cannot support the growth of brine shrimp.

None of the studies in which dried natural products were used as food were conducted under axenic conditions. Therefore the extent to which a bacterial flora may have modified the nutritional quality of the natural products remains unknown. The purpose of the present work is to determine if, under axenic conditions, different dried, powdered foods fulfill the nutritional requirements of *Artemia* as completely as they do under xenic conditions, and to determine which one of these foods yields best results for the production of adult brine shrimp.

Materials and methods

Initiation of experiments

All four experiments were performed with the bisexual San Francisco strain (San Francisco Bay Brand) cultured in filtered (55 µm) seawater from Long Island Sound (New York, USA).

The nauplii used in Experiments I and II were hatched in a separatory funnel filled with seawater (30 ‰ S) recirculated by gentle aeration. Bacteria-free larvae were used in Experiments III and IV. These were obtained by disinfection of the eggs with an aqueous solution of Merthiolate 1:1000 (Gibor, 1956 ; Provasoli *et al.*, 1959). The eggs, rinsed free of the germicide, were incubated and hatched in capped test tubes filled with sterile seawater at 30 ‰. In all the experiments transfer of larvae to the culture tubes was done with Pasteur pipettes 24 h after hatching. For Experiments III and IV, this manipulation was done with sterile Pasteur pipettes under a transfer hood to avoid bacterial contamination. Screw cap glass tubes (25×200 mm) containing 10 ml of media were used for the culture of five nauplii per tube for a period of 8 days. Day one of development was 24 h after hatching (day of transfer to culture tubes). The cultures were maintained in an incubator which controlled temperature between 24 and 28 °C.

The different dried foods were kindly provided by Dr. D'Agostino who obtained them from the Artemia Reference Center (State University of Ghent, Belgium). The dried foods (Cerophyl, lactoserum, defatted rice bran, soybean, *Spirulina* and yeast (Fleischmann's) were dry-sieved (44 µm) to remove large particles not consumable by *Artemia* (Dobbeleir *et al.*, 1980). Yeast

and Cerophyl were ground fine with a mortar and pestle before being sieved. Because food particles precipitate in the stagnant conditions of the media, the tubes were shaken twice a day to maintain the particles in suspension.

Experiment I

The objective of the first experiment was to determine the concentration of food yielding the highest survival of *Artemia* on different diets under xenic conditions. Three concentrations of each diet (2.5, 5.0, 10.0 mg/10 ml) were prepared by dilution and homogenization of specific weights of the sieved dried food in seawater at 30 ‰ S. The survival of unfed larvae in pure seawater was determined as a control. The experiment was run five times with four replicates for each treatment.

Experiment II

The second experiment determined the effects of salinity on the survival of *Artemia* fed the different diets at the food concentration determined to be optimal in Experiment I. Four salinities were tested (30, 60, 90, 120 ‰) under xenic conditions. The range of salinities was obtained by adding "Instant Ocean Salt" (Aquarium Systems) to seawater. The experiment was run twice with six replicates for each treatment.

Experiment III

The third set of experiments was conducted to determine whether the different foods satisfy the nutritional requirements for the growth and development of *Artemia* in the absence of bacteria. Two series of tubes were prepared with the different diets at the optimal food concentration and salinity as determined in Experiments I and II. One of the series of tubes was sterilized in an autoclave at 121 °C for 20 min, the other was allowed to remain xenic. Bacteria-free larvae were transferred from their hatching tubes to both series of culture tubes. The sterilized tubes were prepared 2 days before larvae were to be transferred and were stored in a sterile hood in order that gas exchange might take place through the loosely sealed caps. To avoid excessive bacteria growth, the xenic culture tubes were prepared on the same day the larvae were transferred.

Agar 2216 was used to test bacterial contamination of larvae and food solutions (each tube of media) at the beginning and end of the experiments. The experiment was run twice with ten replicates for each culture treatment.

Experiment IV

The last experiment was conducted to determine the effect of the type of bacteria on the survival and growth of *Artemia*. Bacteria from two sources were used. "Fortuitous" bacteria developed naturally in experimental xenic tubes. "Selected microflora" for a given diet were taken from established cultures of brine shrimp fed the respective diet.

Two series of tubes were prepared with the different diets at the same optimal conditions of food concentration and salinity as in Experiment III. One series of tubes was sterilized (121 °C for 20 min), left in a sterile hood 48 h for gas exchange and then inoculated with three drops of media from an established xenic culture of *Artemia* ("selected microflora") fed the respective

diets. Inoculation samples were taken from successful cultures after they were shaken to mix the medium. Bacteria-free larvae were then transferred to both series of culture tubes. The experiment was run twice with eight replicates per treatment.

Data collection and analysis

For all the experiments, survival per tube was recorded. Total length of the brine shrimp from the anterior margin of the head in front of the ocellus to the base of the caudal furca was determined using a dissecting microscope fitted with an ocular micrometer. The results were analysed using one-, two- and three-way analysis of variance and multiple range tests using subprograms of SPSS (Nie *et al.*, 1975).

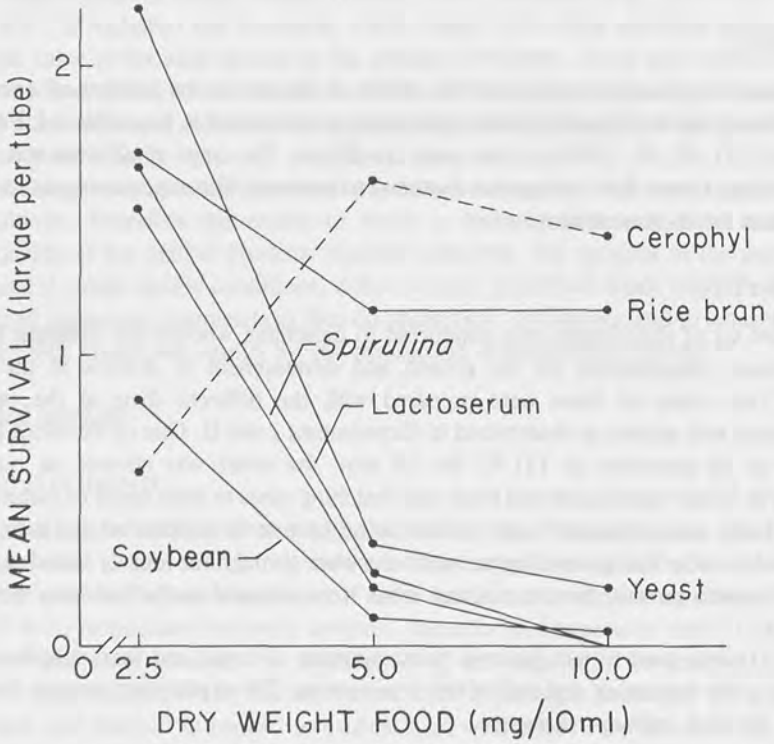

Fig. 1. Survival of *Artemia* after 8 days of culture on six different diets, at three concentrations. Each point is based on 20 replications (five trials with four replicates each). Significant difference among trials on Cerophyl ($P<0.05$) represented by a non-continuous line. Error bars omitted for clarity.

Results

Experiment I was run five times because of variability in the results. Differences in survival of *Artemia* among the five trials was significant only with Cerophyl ($P<0.05$), but statistical analysis of separate trials demonstrated no effect of food concentration with this diet. The effect of food

concentration on survival was analysed for the other diets using the combined results of the five trials. The best food concentration was 2.5 mg/10 ml for *Spirulina*, lactoserum, yeast and soybean ($P<0.01$) (Fig. 1). No differences among the three food concentrations were found for rice bran. Unfed controls did not survive to the 4th day of culture. No relationship between length of the brine shrimp and food concentration could be established.

In Experiment II, no difference in survival was found between the trials for any of the diets. When the data of both trials were combined, the effect of salinity on the survival of *Artemia* was significant for Cerophyl, lactoserum, yeast, rice bran and soybean ($P<0.01$), and not significant with *Spirulina* because of the extremely low overall survival obtained with this diet. Highest survival was obtained at 60 ‰ for all diets (Fig. 2). The multiple range test showed 60 ‰ to be the best salinity for survival of *Artemia* fed rice bran and lactoserum ($P<0.01$). Significant differences in the length of *Artemia* between trials were found with *Spirulina*, lactoserum ($P<0.05$), and Cerophyl ($P<0.01$). Therefore, the effect of salinity on growth of *Artemia* on

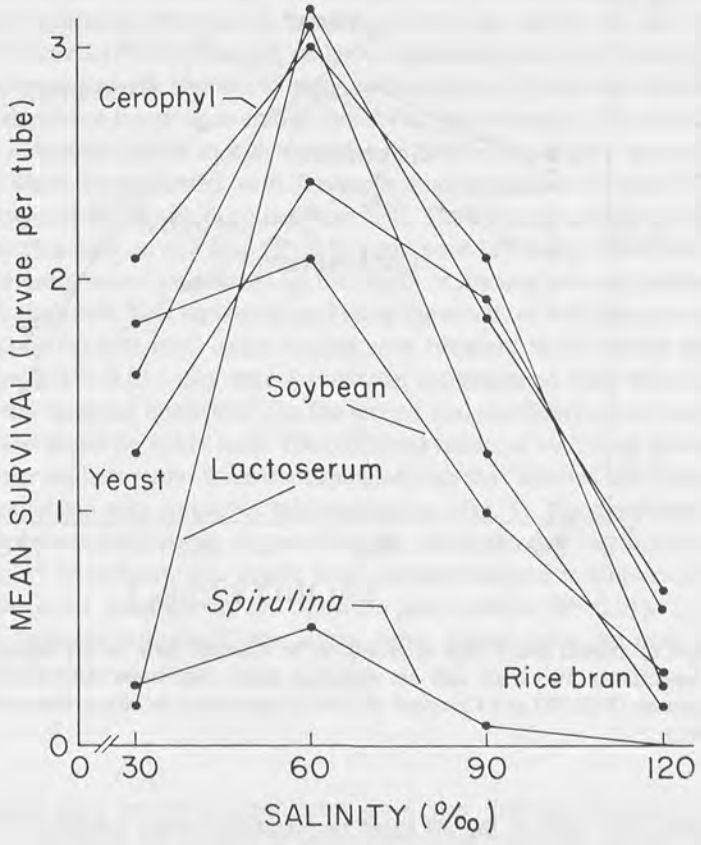

FIG. 2. Survival of *Artemia* after 8 days of culture on six different diets, at four salinities. Each point is based on 12 replications (two trials with six replicates each). Error bars omitted for clarity.

these three diets was analysed for each trial separately. The salinity of 60 ‰ resulted in the best growth, or was among the group of salinities which resulted in the best growth, in all trials with all diets (Fig. 3). No significant differences in length were found at the three salinities for *Artemia* fed *Spirulina*. Nevertheless, brine shrimp fed *Spirulina* at 60 ‰ reached a greater length than did those fed the other diets at this salinity ($P<0.01$).

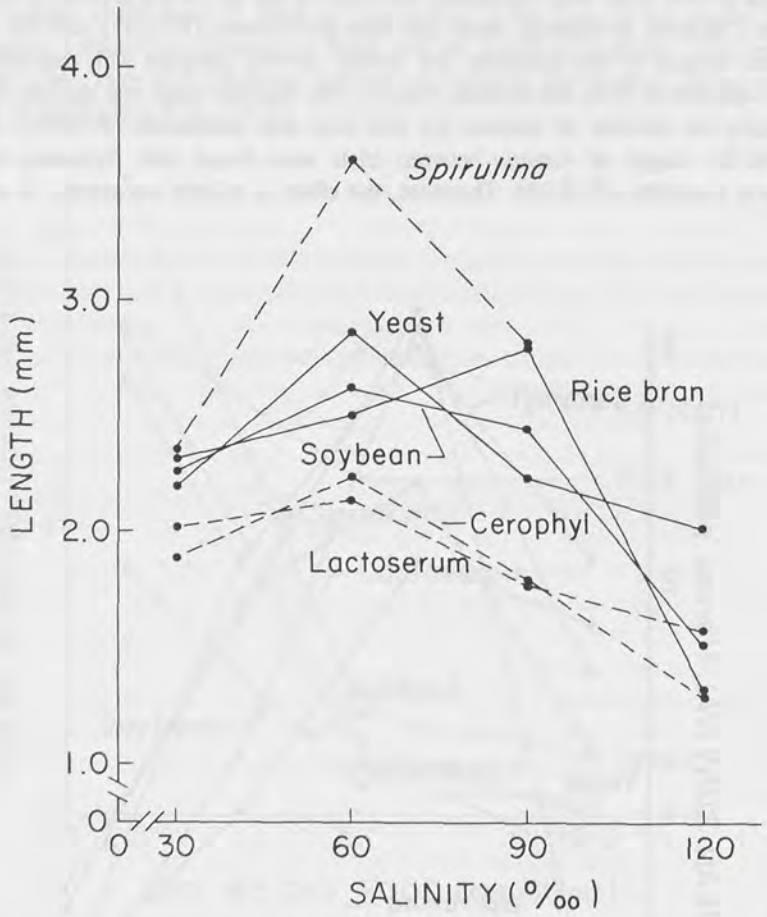

FIG. 3. Length of *Artemia* after 8 days of growth on six different diets, at four salinities. Each point is based on 12 replications (two trials with six replicates each). Significant difference between trials on *Spirulina*, lactoserum ($P<0.05$) and Cerophyl ($P<0.01$) represented by non-continuous lines. Error bars omitted for clarity.

In Experiment III, eight of the 10 tubes per treatment were considered in the statistical analysis. The two others were chosen at random and maintained in the incubator for a longer period of time as controls. No larvae survived to the 10th day of culture on any one of the axenic diets. After 8 days of culture no differences in survival of *Artemia* between trials was detected in

any of the cultures. The results of both trials were combined for each food type. Under xenic and axenic conditions, significant differences in survival were found among the diets ($P<0.01$). The multiple range test showed that the *Spirulina* diet yielded lower survival of *Artemia* than did the other diets under xenic conditions. Under axenic conditions, highest survival, which was very low compared with that obtained in xenic cultures, was achieved in cultures of *Artemia* fed rice bran. In the xenic cultures, survival of brine shrimp was significantly greater than in the axenic cultures of lactoserum, yeast, soybean, Cerophyl ($P<0.01$), and *Spirulina* ($P<0.05$) (Fig. 4). The difference was nearly significant for rice bran, as well ($P=0.052$). The sizes of *Artemia* fed the various diets were similar in both trials, except for those fed rice bran ($P<0.05$). The statistical analysis of length data for *Artemia* fed rice bran was carried out for each trial separately. The combined results of both trials were used for the other five foods. The presence of bacteria in the culture media was required for the growth of the larvae on *Spirulina*, rice bran, yeast, soybean ($P<0.01$), and lactoserum ($P<0.05$) (Fig. 4). Because *Artemia* did not survive when fed Cerophyl under axenic conditions, no statistical analysis of length could be performed for this diet.

In Experiment IV, no variation in survival between trials was determined for any one of the diets. There were significant differences in survival of the larvae fed the six diets contaminated with "fortuitous" bacteria ($P<0.01$) as well as those contaminated with the "selected microflora" ($P<0.01$). However, it was not possible to separate the groups of foods with the multiple range tests because of significant interactions among them. The culture media of *Spirulina*, lactoserum, and soybean contaminated with inoculated bacteria resulted in higher survival of *Artemia* ($P<0.01$) than when contaminated with fortuitous microorganisms (Table I ; Fig. 5). No statistical difference was found with the other three diets. The lengths were different between trials for the brine shrimp grown on rice bran ($P<0.01$) and yeast ($P<0.05$). Therefore, the effect of type of bacteria (fortuitous or inoculated) on the length of *Artemia* was analysed using the data of each trial with these two diets separately, and using the results of both trials combined for the four other diets. In the first trial, larger shrimp were obtained from cultures with rice bran ($P<0.01$) and yeast ($P<0.05$) that were fortuitously contaminated than from those cultures inoculated with the "selected microflora". In the second trial, no difference in length due to the type of bacteria was found for either food. The combined results of both trials demonstrated that *Artemia* grew better on lactoserum when contaminated with the "selected microflora" ($P<0.01$) than when contaminated with fortuitous microorganisms (Fig. 5). No significant difference in length of the brine shrimp as a result of type of bacteria contamination was found on media with *Spirulina*, Cerophyl or soybean. The largest brine shrimp obtained in cultures with fortuitous contamination were the ones grown on *Spirulina* and soybean ($P<0.01$). In the cultures inoculated with "selected microflora" the largest brine shrimp were the ones fed *Spirulina* ($P<0.01$).

Discussion

The dried foods used in these experiments have very different chemical compositions (Douillet, 1984). Therefore, the quantity of food required for maximum growth and survival of *Artemia* varied from one type of food to another. If *Spirulina*, yeast, soybean (rich in protein), and lactoserum (rich in carbohydrates, mainly lactose) enhanced bacterial growth, this could

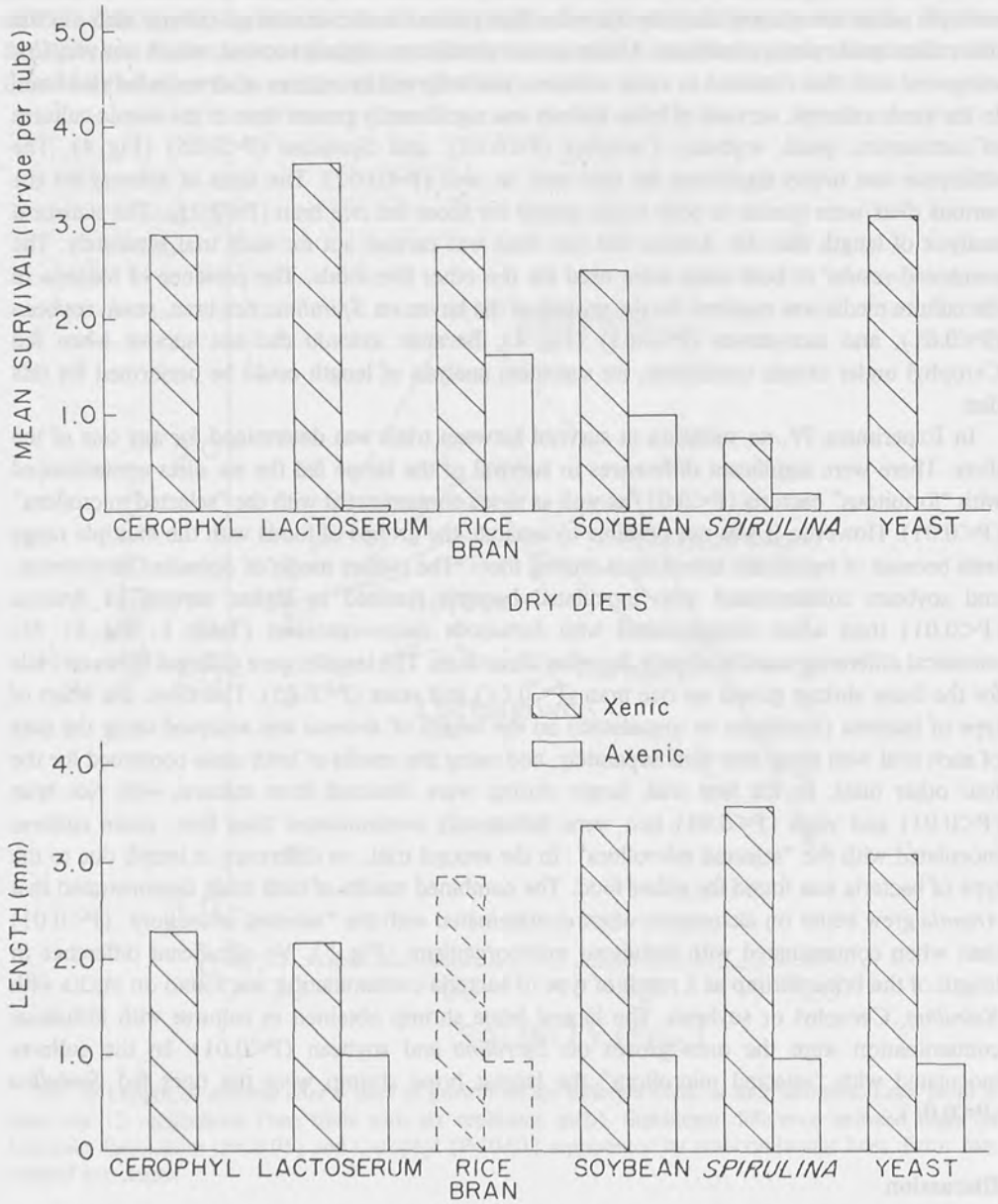

FIG. 4. Survival and length of *Artemia* after 8 days of culture on six different diets, under axenic and xenic conditions. Each column is based on 16 replications (two trials with eight replicates each). Dashed column indicates a significant difference between trials for rice bran ($P<0.05$).

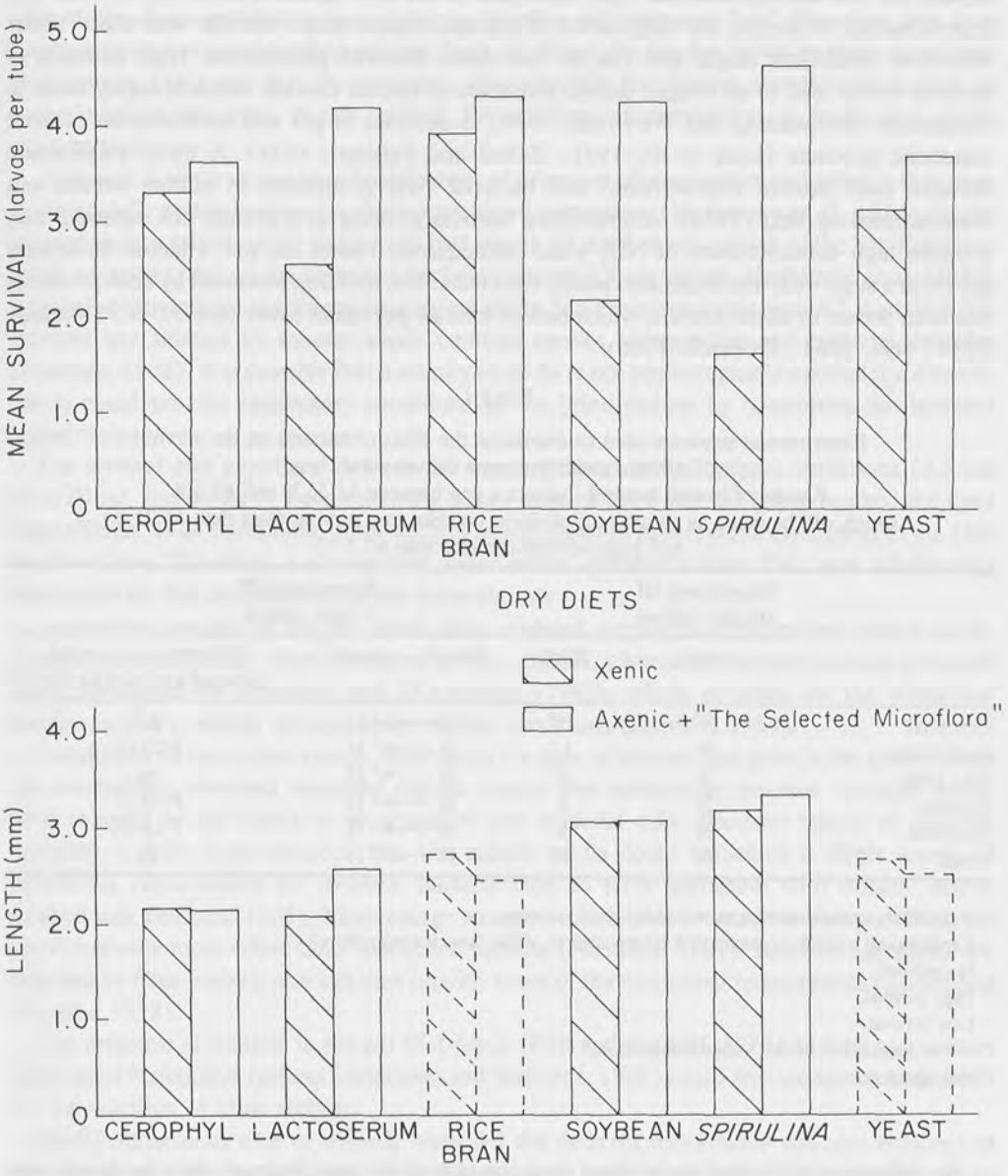

FIG. 5. Survival and length of *Artemia* after 8 days of culture on six different diets contaminated with bacteria from two different sources. Xenic conditions denote the "fortuitous" bacteria developed naturally on non-sterile culture media. Axenic culture media were contaminated by inoculation of "selected microflora" taken from established cultures of brine shrimp fed the respective diet. Each column is based on 16 replications (two trials with eight replicates each). Dashed columns indicate significant differences between trials for rice bran ($P<0.01$) and yeast ($P<0.05$).

explain the low survival obtained with these diets at the two highest food concentrations. The internal surface of the gut, the body surface of *Artemia*, organic matter and the "wall effect" under laboratory conditions might give rise to very dense bacterial populations. High densities of bacteria would lead to an oxygen deficit, formation of carbon dioxide which is highly toxic to crustaceans (Wolvekamp and Waterman, 1960), a decrease in pH and accumulation of toxic metabolic products (Luck *et al.*, 1931 ; Zobell and Feltham, 1938). A direct relationship between food protein concentration and bacterial NH_4^+ production in culture vessels was demonstrated by Seki (1964). Ammonifying bacteria growing in a protein-rich substrate may produce high concentrations of NH_4^+ which subsequently lowers the pH. Glucose fermenters grown in a sugar-rich diet might also acidify the media. The pH drop produced by both processes has been shown to affect *Artemia*, which cannot tolerate pH values lower than 5.0-6.5 (Mathias, 1934 ; Seki, 1964 ; Provasoli, 1966).

TABLE I

Experimental sequence used to determine the effect of bacteria on the survival of *Artemia* under the same culture conditions. Results of overall survival indicate a gap between 51.25 % and 62.5 % which will be taken as an arbitrary division between low survival (less than 51.25 %) and high survival (greater than 62.5 %)

	Experiment III 10 days culture	Experiment IV 8 days culture			
	Axenic	Xenic	Xenic[1]	Axenic[2]	Difference in survival between xenic and axenic
Cerophyl	N[3]	H[4]	H ⟶[6] H		ns[7]
Lactoserum	N	L[5]	H ⟶[6] H		P<0.01
Rice bran	N	H	H ⟶[6] H		ns
Soybean	N	L	H ⟶[6] H		P<0.01
Spirulina	N	L	H ⟶[6] H		P<0.01
Yeast	N	H	H ⟶[6] H		ns

[1] Successfully established non experimental stock cultures.
[2] Experimental cultures contaminated by inoculation of the "selected microflora".
[3] No survival.
[4] High survival.
[5] Low survival.
[6] ⟶ inoculation of the "selected microflora".
[7] Not significant.

No difference in survival at the three food concentrations was obtained when the larvae were fed with rice bran and Cerophyl, because the rich carbohydrate fraction of these foods were mainly formed by polysaccharides. Several bacterial enzymes which hydrolyze oligosaccharides are not absolutely specific (Mehler, 1957), thus oligosaccharides are widely used as carbon source by microorganisms (Rose, 1976). The breakdown of polysaccharides requires specific enzymes (Mehler, 1957) ; therefore, the fact that only a limited number of microbial species could colonize these polysaccharide-rich substrates might have reduced the probability for unfavorable bacterial contamination.

The food concentration of 2.5 mg/10 ml was favorable for the survival and growth of *Artemia* with all the diets, and after 8 days of culture all tubes still contained food. Therefore, this food concentration was considered to provide food *ad libitum* for the five larvae in these short-term experiments. Although this concentration did not yield the highest growth and survival of *Artemia* in all the diets, it was selected for purposes of comparison of foods at a single concentration.

Although *Artemia* strains have broad ranges of tolerance of temperature and salinity, they have strain-specific optimal values for these interrelated parameters (Vanhaecke *et al.*, 1984). In the present study a salinity of 60 ‰ gave the best results for survival and growth of the San Francisco strain of *Artemia* fed all the diets at a concentration of 2.5 mg/10 ml. Vanhaecke *et al.* (1984) found that the optimal conditions for survival of the San Francisco strain were 62 ‰ at 20.6 °C. Bacteria are affected by salinity which controls species composition and bacterial densities (Kushner, 1978). It is possible that a salinity of 60 ‰ is not physiologically optimal for *Artemia*, but it could provide satisfactory conditions to the brine shrimp by controlling the bacterial population.

The survival and growth of *Artemia* on axenic cultures at the optimal conditions (2.5 mg food/10 ml, and 60 ‰ S) were extremely poor : none of the 8 day old larvae reached the third stage (Heath, 1924), and none of the control larvae reared in axenic cultures survived to the 10th day of culture. Therefore, it is clear that under axenic conditions these diets were nutritionally inadequate for the development of the brine shrimp.

Qualitative analysis of the six dried diets revealed nutritional deficiencies (amino acids, vitamins, nucleic acids) when compared to the artificial, chemically defined medium (medium 100), developed by Provasoli and D'Agostino (1969), which provides all the nutritional requirements for rearing *Artemia* under aseptic conditions (Douillet, 1984).

Conditions in the culture vessels determined the type of bacteria that grew in the tubes. These microorganisms absorbed dissolved organic matter and synthesized essential nutrients which were released to the media or incorporated into bacterial cells. Bacterial release of essential nutrients in high concentrations into the culture media could constitute a direct supply of nutritional requirements for *Artemia*. Bacteria tend to form aggregates with organic matter (Zobell and Feltham, 1938). More energy sources are available to attached bacteria which are physiologically more active than unattached bacteria (Nienhuis, 1981). Bacterial aggregates are ingested by filter-feeders, and can thus provide some of the nutritional requirements (Zobell and Feltham, 1938).

The presence of bacteria in the gut of *Artemia* (Post and Youssef, 1977) and the assimilatory function of exoskeletal epiflora (Anderson and Stephens, 1969) could provide symbiotic benefits for the nutrition of brine shrimp.

Highly variable survival of *Artemia* when fed the different dried foods was also obtained in several attempts to rear the brine shrimp in beakers. Success was more often obtained when Cerophyl or rice bran were used. An excessive dose of food provided to recently established cultures led to deterioration of the media and mortality of the brine shrimp. This effect appeared to be less drastic in the cultures with Cerophyl and rice bran. When an excessive dose of any of the diets was provided to old and successfully established cultures, no deterioration of the media or mortalities were apparent. This led to the conclusion that it was neither the concentration of food nor the food type that caused the deterioration of the media, but rather the type of microorganisms growing in the beakers. Experiment IV was conducted to determine the effect

of the type of bacteria (fortuitous or inoculated) on the cultures of *Artemia* fed with the six diets. This bacterial "selection" improved survival and growth of *Artemia* on three otherwise nutritionally rich diets. *Spirulina* and soybean were richest in protein, while lactoserum was rich in lactose. Under xenic conditions, these substrates for bacterial growth might have enhanced a rapid rise in bacterial population density of both favorable and unfavorable microorganisms. This might have increased the possibility of unfavorable bacterial colonization resulting in massive mortalities. If the culture was stable over a long period of time it was probably due to a selection and balance among microbial species. This microbial species composition and balance was transferred by inoculations from stable cultures. This was demonstrated by changes in mean survival values. The lowest mean survival in all the foods contaminated with fortuitous bacteria was obtained with *Spirulina* (32.5 %), but when these foods were inoculated with "selected microflora", the brine shrimp grown on *Spirulina* achieved the highest mean survival (92.5 %).

For large-scale production of brine shrimp adults, cost must be considered. The least expensive of all the diets tested is rice bran (Sorgeloos *et al.*, 1980). Because of its chemical composition the control of water quality is much easier than with other dried foods, and a favorable bacterial environment for *Artemia* is more readily obtained. The nutritionally rich diets have the disadvantage that in large-scale production it is impossible to maintain a bacterial equilibrium. It is probable that unfavorable bacterial colonization would occur, leading to high mortalities of brine shrimp.

Further research is needed to identify the bacterial clones growing on these dried foods which may provide the nutritional requirements for *Artemia*, without affecting the culture media, the brine shrimp or the cultured species to which *Artemia* is fed. It is also important to consider the chemical composition of *Artemia* which depends on the composition of the diet (Claus *et al.*, 1979). Therefore, the chemical analysis of the brine shrimp grown on these bacterial/dried food complexes will determine if they fulfill the nutritional requirements of the invertebrates and finfish being raised on brine shrimp adults.

Summary

1. A salinity of 60 ‰ was optimal for the San Francisco Bay strain of *Artemia* larvae fed six different dried diets under xenic conditions.
2. A food concentration of 2.5 mg/10 ml was favorable for the culture of *Artemia* on six different dried diets tested under xenic conditions.
3. Six dried diets tested under axenic conditions at the food concentration and salinity determined to be optimal under xenic conditions did not meet the nutritional requirements of *Artemia*.
4. Axenic cultures inoculated with bacteria ("selected microflora") derived from established cultures of *Artemia* fed the same diets did meet the nutritional requirements of the crustacean. Therefore, bacteria are essential for the culture of *Artemia* on these dried diets.
5. Cultures of *Artemia* contaminated by the "selected microflora" provided better conditions for survival and growth of *Artemia* when fed *Spirulina*, soybean and lactoserum, than when these same cultures were contaminated by fortuitous bacteria.
6. Establishment of favorable, fortuitous bacteria was seldom obtained on cultures of *Artemia* fed *Spirulina*. Xenic cultures of *Artemia* fed *Spirulina* inoculated with "selected microflora" provided better mean survival and growth of the brine shrimp than any other diet with any

type of contamination. Therefore, under controlled bacterial conditions, *Spirulina* was the best diet of the six dried foods tested.

7. Establishment of favorable, fortuitous bacteria was more often obtained on cultures of *Artemia* fed rice bran and Cerophyl. Thus, these diets are most likely to provide the best results for large-scale production of brine shrimp adults under conditions where bacterial contamination cannot be controlled.

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Evaluation of mono- and mixed diets as food for intensive *Artemia* culture

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Abstract

In order to alleviate the problems encountered when using live algae or rice bran as *Artemia* food in intensive culturing, different alternative types of brine shrimp diets have been evaluated. Using a high-density flow-through recirculation culture system, a Single Cell Protein yeast and mixed diets, consisting of this yeast and micronized waste products from agricultural crops, were selected as suitable *Artemia* feeds.

Production yields after 2 weeks culturing varied from 2 to 5 kg live weight brine shrimp biomass per culture tank of 300 l. The mixed diets corn/soybean, corn/wheat husks, and corn/yeast were found to be suitable or even better alternatives for the rice bran diet which is commonly used for feeding *Artemia*.

Introduction

Although the cheapest source of brine shrimp biomass is from semi-natural biotopes or man-managed ponds, *Artemia* produced in intensive culture systems is becoming more attractive, especially in climates that are unsuitable for outdoor production and when quality control is critical (Lavens *et al.*, 1985). Since *Artemia* is a non-selective, obligate particle feeder at least with respect to the fulfillment of its requirements for carbohydrates and proteins (Barker-Jörgensen, 1966 ; D'Agostino, 1980), most of the high-density culturing techniques rely on cheap agricultural by-products instead of live algae in order to cut feed costs. Critical in the selection of a suitable diet for intensive culturing are its particle size which should be less than 50 µm (Dobbeleir *et al.*, 1980), its nutritional value, and its digestibility which may be influenced by the colonizing microflora (D'Agostino, 1980 ; Douillet, 1987), and its solubility which should be minimal in order to avoid quality deterioration of the culture water.

Rice bran has been reported to be a cheap and suitable feed source for intensive *Artemia* culture (Sorgeloos *et al.*, 1979 ; Brisset *et al.*, 1982 ; Lavens and Sorgeloos, 1984 ; Platon and Zahradnik, 1987). However, culture success depends upon the batch of rice bran used : *i.e.* the composition of the product may fluctuate according to origin, harvest, processing, etc., and, moreover, may be contaminated with pesticides which are used as a storage treatment (Dobbeleir *et al.*, 1980).

Therefore this study has been conducted to evaluate possible alternatives which are more consistent and reliable in composition, have a better micronization capability, and finally yield

high biomass production of preadult or adult brine shrimp. Special attention has been attributed to the potential use of Single Cell Proteins (SCP) because they have a far more complete nutritional composition, are relatively inexpensive, do not require additional grinding, and ensure a more optimal physical performance of the particles in the culture medium.

Materials and methods

General culturing conditions

Great Salt Lake *Artemia franciscana* (Sanders Brine Shrimp Co., lot 185-O) were hatched under standard conditions (Sorgeloos *et al.*, 1983) for 24 h at 25 °C. After separation and washing from cyst residues the instar I nauplii were counted and inoculated into the culture tanks at a density of 10 000 larvae/l. In the second set of experiments the SCP mono-diet was evaluated at different densities, respectively 5 000, 10 000, and 15 000 nauplii/l.

The 300 l culture tanks are part of a flow-through recirculating system which has been described by Lavens *et al.* (1985) (Fig. 1). Natural seawater originating from the Scheldt-estuary and to which crude seasalt and 1 g/l $NaHCO_3$ is added to obtain a salinity of 50 ‰ and a pH of 8 flows continuously into the culture vessels at varying flow rates ; *i.e.* depending on the growth stage of the brine shrimp water retention times are adjusted from 4 h to 1 h (Table I). Aeration by means of a central aeration collar is adjusted regularly to keep oxygen levels around 4 ppm.

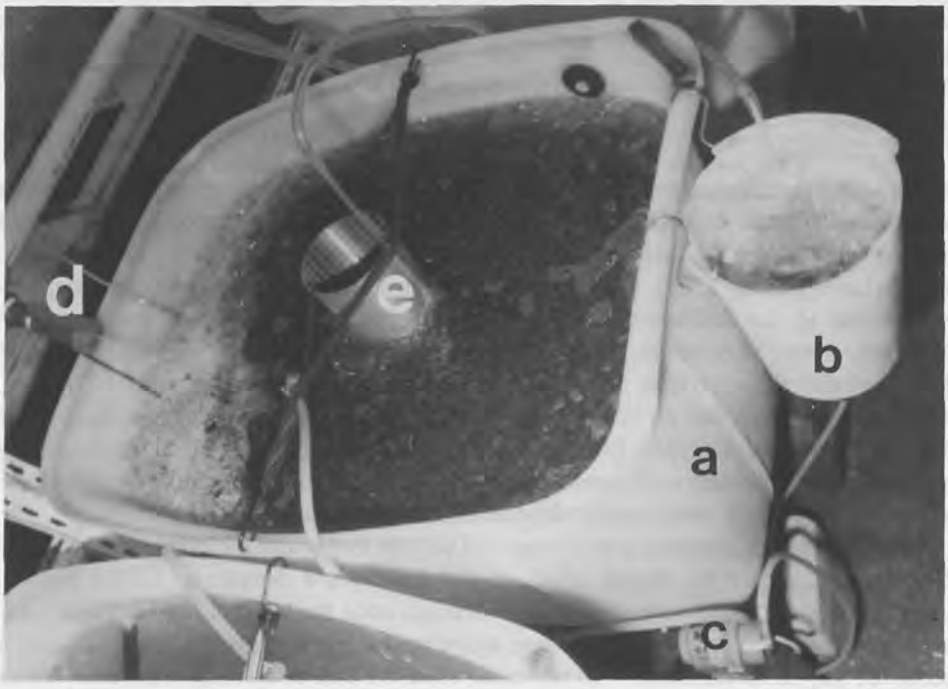

FIG. 1. Overview of the *Artemia* culture unit of the closed flow-through system for brine shrimp biomass production. (a) 300 l culture tank ; (b) feed tank ; (c) feed pump ; (d) water intake ; (e) *Artemia* retaining filter, and water outlet.

TABLE I

Culture water renewal in relation to the growth stage of *Artemia* and the slit opening of the welded-wedge screen filter, as used under optimal culturing conditions

Culture day	Mesh size of filter (µm)	Flow rate (l/h)	Retention time in culture tank (h)
1 - 2	150	80	4
3 - 4	200	100	3
5 - 7	250	150	2
8 - 10	300	150	2
10 - 12	350	200	1.5
12 - 14	450	300	1

FOOD

The different feed components and their chemical composition used in this study are listed in Table II. All agricultural by-products were micronized and supplied by Artemia Systems NV, Belgium ; the SCP-yeast needed no extra processing. Only the yeast has been evaluated as mono-diet. The five mixed diets tested here all contain the corn waste in equal ratios, except for the soybean diet that consists of only 20 % corn waste (Table III).

FOOD PREPARATION AND DISTRIBUTION

In order to avoid bacterial degradation, fresh diet suspensions were made up daily by mixing the suspension of dry feed and saturated NaCl brine in a kitchen blender. Optimal feeding conditions were created by adding food suspension on a semi-continuous basis, *i.e.* food pumps were controlled by electronic time clocks which were adjusted daily to maintain a culture medium transparency of 20-25 cm. Only in the second set of experiments different transparency levels were applied (Table IV).

DATA COLLECTION

Individual length and survival rates were recorded daily by sub-sampling the culture tanks : *i.e.* three samples of 25 ml were taken and fixated with lugol solution. The average length of 30 brine shrimp measured from the top of the head to the base of the caudal furca was determined using a dissecting microscope equipped with a drawing mirror. Biomass production was estimated by wet weight analysis of the *Artemia* collected on a small sieve from a 1 l sample. Food conversion efficiency was calculated by dividing the total amount of feed added until that day by the wet-weight biomass amount of that moment.

Results

In the first set of experiments a high *Artemia* biomass production was achieved after 14 days culturing with the diets partially consisting of corn and mixed with soybean product (diet A/E), wheat product (A/D), or yeast (A/F) (Table III, Fig. 2). If the yeast series would not have faced a high mortality starting from day 10 onwards (Fig. 3), much higher biomass production-levels

TABLE II

Average chemical composition of the various feed components used in the *Artemia* diets

Feed origin (code)	Description of feed	Crude protein (%)	Crude fat (%)	Carbohydrates (%)	Ash (%)
Corn (A)	Mixture of waste products from corn industry	62	6	20-27 + 3-10 % fibers	2
Rice (B)	Waste product from polishing rice	11-12	11-13	25-33 + 3-13 % fibers	8-12
Wheat (C)	Wheat germ product	25	8	25 + 3 % fibers	4
Wheat (D)	By-product from wheat industry ; contains mostly husks	14	3	20 + 11 % fibers	6
Soybean (E)	Waste product from soybean industry ; contains small amounts of soybeans	9	1	86 (mainly fibers from cellulosic type)	4
SCP : yeast (F)	Commercially available dry *Kluyveromyces* spp. yeast	> 48	6-8	28-33 (mainly mannanes and glucanes)	7-9

would have been obtained (*i.e.* more than 20 kg). This can be extrapolated from the large biomass increase during the 1st week which was twice as good as with the other feeds. The wheat diet (A/C) and the yeast mono-diet (F) did not perform well in terms of biomass yields after 14 days culturing. As compared to the other diets, the latter two happen to contain high protein levels (> 40 %) and only small amounts of fibers (Table II). Although good survival and relatively good length increase were obtained on diet A/C, poor biomass production prevailed. This indicates that the average length is not always a good estimate of the brine shrimp's growth.

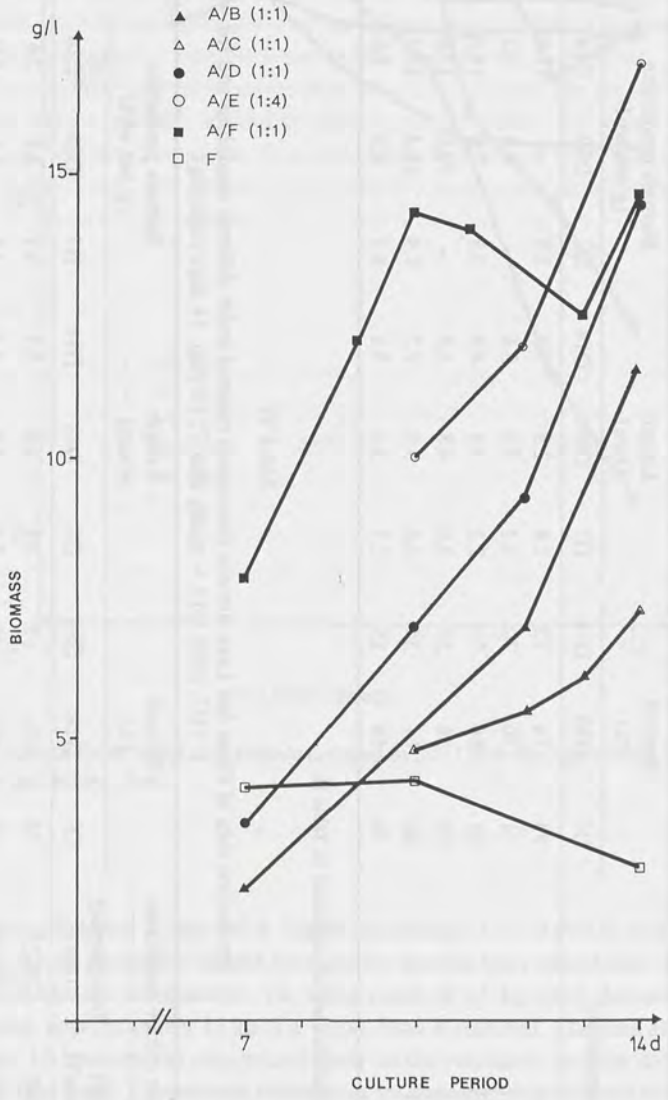

FIG. 2. Biomass production of Great Salt Lake *Artemia* in a flow-through recirculation system, using different mono- and mixed diets as food.

TABLE III

Production data of Great Salt Lake *Artemia franciscana* cultured on various diets during 14 days in 300 l culture tanks (D7, D10, D14 = results after 7, 10 resp. 14 days culturing)

Diet composition[1]	Survival (%)			Length (mm)			Biomass production (g wet wt/l)			Conversion efficiency (g dry wt/g wet wt)		
	D7	D10	D14	D7	D10	D14	D7	D10	D14	D7	D10	D14
A/B 1:1	93	74	73	1.8	2.9	3.5	2.3	–	11.6	1.60	–	0.65
A/C 1:1	71	70	70	2.2	3.5	5.6	–	4.8	7.2	–	0.84	1.04
A/D 1:1	85	86	71	2.2	3.8	4.9	3.5	7.0	14.5	1.30	1.10	0.80
A/E 1:4	65	58	57	3.0	4.8	5.8	–	10.0	17.0	–	0.80	0.75
A/F 1:1	80	61	35	3.6	5.9	7.7	7.9	14.3	14.7	0.51	0.50	0.82
F	76	56	15	2.1	3.6	5.1	4.1	4.3	2.6	0.48	0.58	2.05

[1] Abbreviations used are explained in Table II.

TABLE IV

Production data of Great Salt Lake *Artemia franciscana* cultured under different conditions and fed with yeast (D7, D10, D14 = results after 7, 10 resp. 14 days culturing)

Specific culture conditions		Survival (%)			Length (mm)			Biomass production (g wet wt/l)			Conversion efficiency (g dry wt/g wet wt)		
Density (*Artemia*/l)	Transparency (cm)	D7	D10	D14	D7	D10	D14	D7	D10	D14	D7	D10	D14
10 000	20-25	76	56	15	2.1	3.6	5.1	4.1	4.3	2.6	0.48	0.58	2.05
10 000	30-35	63	69	19	2.8	3.8	4.3	3.8	7.7	5.0	0.52	0.60	0.98
15 000	40-45	75	65	38	2.7	3.6	3.8	3.5	6.5	6.4	0.46	0.54	0.83
5 000	40-45	87	87	63	2.9	4.3	4.3	2.0	3.7	6.0	0.55	0.57	0.52

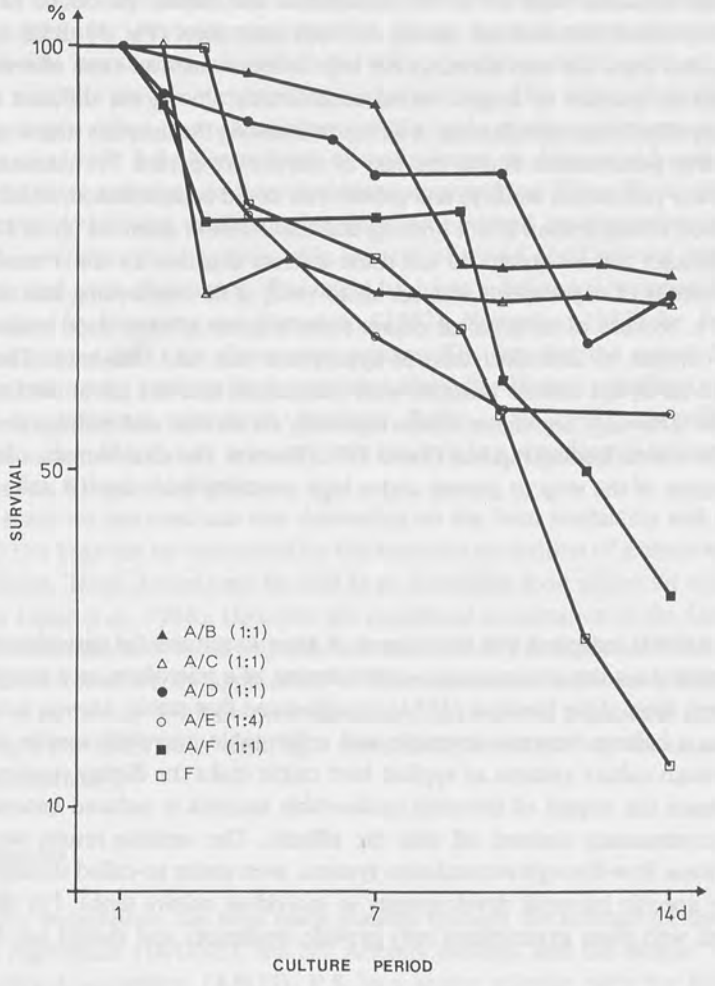

FIG. 3. Survival rates of Great Salt Lake *Artemia* cultured in 300 l flow-through culture tanks for 14 days on different mono- and mixed diets.

Food conversion efficiency at day 14 is highly correlated ($r = -0.9003$) with final biomass production ; however, an exception should be made for the rice bran mixed-diet. This means that under optimal production circumstances, *i.e.* when yields of 15 kg adult *Artemia*/m^3 of culture medium are attained, approximately 12 kg of a 'good' food is required. The data also show a large difference until day 10 between the micronized diets on the one hand, and the mono- and mixed SCP diets on the other hand. Conversion efficiencies for the latter experiments were already high during early *Artemia* development (± 0.5) while for the other diets only conversions above 1.0 were obtained during the first part of the culture period.

Survival rates remained high (> 60 %) throughout the culture period of 14 days for all micronized diets tested, but declined rapidly for both yeast diets (Fig. 3). Both A/E and A/C showed mortalities from the start onwards but kept constant survival rates afterwards. Growth rates, expressed as increase in length, varied considerably among the different trials (1.8 to 3.6 mm) already after 7 days of culturing. A fast growth during the naupliar stages resulted always in a good growth performance during the rest of the culture period. No relationship between mortality (= lower population density) and growth rate could be established, which supports the idea that the food concentration is not limiting and that *Artemia* densities up to 10 000/l in the present flow-through culture system do not cause a stress situation for the *Artemia*.

The second series of experiments was set up to verify if no overfeeding had occurred when using yeast ; *i.e.* because of its physical nature yeast ensures a better food availability for the *Artemia* as it consists of individual cells of appropriate size and buoyancy. Therefore, lower transparency levels of the culture medium were maintained and the larval density varied from 5 000 to 15 000 *Artemia*/l. Significant effects especially on survival and biomass production were recorded for the lowest feeding regimes (Table IV). However, the total biomass output remained rather low because of the stop in growth and a high mortality from day 10 onwards.

Discussion

D'Agostino (1980) indicated that the success of *Artemia* cultures fed agricultural by-products is strictly dependent on the instantaneous establishment of a microflora as a supplement to the nutrient-deficient diets. Also Douillet (1987) hypothesized that stable *Artemia* cultures are only sustained when a balance between favorable and unfavorable microbial species is established. Using flow-through culture systems as applied here might make the dietary requirements less or more critical since the impact of favorable/unfavorable bacteria is reduced because part of the microflora is continuously drained off with the effluent. The variable results which are often obtained with these flow-through recirculation systems, even under so-called standard conditions, may be due to specific bacterial developments in individual culture tanks. For this reason the results obtained with these experiments only provide tendencies and should not be statistically interpreted.

Present literature on the nutritional requirements of *Artemia* is very scarce. D'Agostino (1980) suggested that brine shrimp require more carbohydrates than proteins and little if any fatty acids. According to Hanaoka (1973) and Sick (1976) growth rates of *Artemia* are positively correlated with the amount of crude protein in the diet. Levels above 28 % protein will, however, negatively affect growth by deteriorating the water quality of the culture medium (Hanaoka, 1973). Our results suggest that protein content as a sole factor is not of major importance in obtaining high yields of adult *Artemia* biomass. The diets A/E and A/F contain 34 %, respectively 55 % crude protein and both gave maximal yields, *i.e.* 15 g wet wt/l. Carbohydrates of fibrous nature (such as the soybean waste) apparently ensure good growth (results with diet A/E *versus* diets A/C and F), either as a dietary ingredient or as a bacterial substrate.

The physical characteristics of potential *Artemia* feeds need also to be taken into consideration, *e.g.* micronized products have the tendency to easily form aggregates in water, and therefore can no longer be ingested by the *Artemia* and may clogg the *Artemia* retaining filter. Diets, on the contrary, that enhance fast *Artemia* growth especially in the early juvenile stages, will allow a

faster change to larger mesh filters and to work at shorter retention times. In this respect, Single Cell Proteins might offer interesting opportunities. Yeasts for example are available as individual cells of appropriate size that do not pollute the water. As their nutritive substances are isolated in a rigid cell wall, the growth of microflora is also reduced. This better physical availability of a yeast diet probably allows to work at lower particle loads or water transparencies (Table IV).

Shimaya *et al.* (1967) fed different types of marine yeast to *Artemia* and recorded a growth to 9 mm in 14 days culturing, and survival rates varying from 50 to 90 %. Nimmannit and Assawamunkong (1985) also used a marine yeast but obtained good growth only until day 4. Using the *Kluyveromyces* yeast as mono-diet we also observed good survival and growth rates only during the first week of culturing. This might be due to a deficiency or imbalance of nutrients as is hypothesized by Hirayama and Funamoto (1983), Hirayama (1987) for *Brachionus*, and Urban and Langdon (1984) for *Crassostrea* cultures. The fact that the mixed diet yeast/corn waste gave the best results (only up to the pre-adult stage after 10 days culturing) provides further evidence for the deficiency hypothesis. Similarly, Robin *et al.* (1981) showed that a 50 % substitution of a *Spirulina* diet by brewers' yeast resulted in a significantly better survival of the SFB *Artemia* after 6 days of culturing.

From this study we can conclude that depending on the local availability and prices, various substitutes for rice bran can be considered for the intensive production of *Artemia* in flow-through culture conditions. These *Artemia* can be used as an acceptable food source for various predators (see review by Léger *et al.*, 1986). However, the nutritional composition of the *Artemia* especially with regard to essential fatty acids (*e.g.* 20:5ω3 and 22:6ω3) is expected to be very low. This deficiency can be remedied by application of bioencapsulation techniques (Sakamoto *et al.*, 1982 ; Léger *et al.*, 1987). With the new enrichment procedures, nutritional compositions can be modified in a few hours time, ensuring a nutritionally excellent prey for aquaculture species (Léger, pers. commun.).

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Experimental production of an introduced *Artemia* strain in alkaline waters in the State of Mexico

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Abstract

The present work was carried out in the former lake of Texcoco, Mexico State. Three different diets for *Artemia* were compared : rice bran, fresh *Spirulina*, and algae grown with chicken manure as a fertilizer. The most effective one was *Spirulina*, which gave the best *Artemia* growth (9.7 mm), the best survival (70-80 %), and a high percentage of fertile females (63.6 %) in a period of 30 days. *Artemia* was able to feed on fresh *Spirulina* at all stages of its development.

Introduction

The culture work referred to in this paper was performed with water of the alkaline former Texcoco Lake, located near Mexico City. This underground deposit of salt water is used by the Texcoco Sosa Company to produce sodium carbonate (Na_2CO_3), calcium carbonate ($CaCO_3$), sodium chloride (NaCl), caustic soda (NaOH) and *Spirulina* (information from Texcoco Sosa Company, 1985).

Parallel to the *Spirulina* culture, some *Artemia* culture tests were done in December 1975. *Artemia* cysts (strain from San Francisco Bay, California, USA) were inoculated into tanks filled with water from wells located in the Texcoco Sosa Company area (Pontes *et al.*, 1977). Semi-controlled *Artemia* culture was begun and reached a production of 150 kg of wet biomass and 10 kg of cysts in a period of 18 months (C. Santillan, pers. commun.). In spite of the promising results this culture was abandoned, because of company policy. However, the *Artemia* population had adapted to the prevailing environmental conditions in the former Texcoco Lake and has continued to live there.

In 1982, the Universidad Autónoma Metropolitana established an agreement with the Texcoco Sosa Company and started to work with *Artemia* in its installations (Castro *et al.*, 1984). There are great possibilities for the commercial development of *Artemia* production in this area, to meet the demand of the aquarium market and aquaculture, in the form of live or dry food, or as an ingredient of formulated diets for carnivorous species of commercial value.

Materials and methods

Artemia cysts

The cysts used during all the tests were collected in November 1983 from the tanks with an *Artemia* culture left by Sosa Texcoco Company.

The hatching percentage was determined after 48 h incubation at 25 °C of 100 cysts in artificial seawater (made with the salt produced by the Texcoco Sosa Company) in petri dishes.

To determine the hatching efficiency, 1 g cysts were placed in 500 ml of artificial seawater in a glass bottle with a funnel shaped bottom. Continuous aeration from the bottom of the bottle kept the cysts in suspension for a period of 48 h at 25 °C.

Diets

The diets used in these experiments were : untreated rice bran, fresh *Spirulina*, and algae grown with chicken manure as fertilizer.

Rice bran was obtained from a commercial company (San José) in Cuautla, State of Morelos ; chicken manure from a chicken farm in Iztapalapa, Mexico City ; and fresh *Spirulina* was collected from the processing factory of Texcoco Company.

The rice bran diet was prepared following the recommendations of Sorgeloos as outlined in the 1982 *Artemia* course held in Mexico (Castro and Gallardo, 1985) : a mixture of 200 g rice bran in 1 l 35 ‰ seawater. This mixture was homogenized in a domestic blender and poured through a sieve of 60 µm to obtain a suspension with small particles. Every 3rd day, 4 l of this stock suspension was given to each tank during the 28 days of the experiment.

The fresh *Spirulina* was collected from the conveyor belts in the factory of Sosa Texcoco. The *Spirulina* jelly was suspended in 35 ‰ seawater at a rate of 1 kg wet dry *Spirulina* in 9 l water. Every 3rd day during the 28 day culture period, this suspension was added to the tanks until a transparency of 15 cm as measured with a Secchi disk.

The chicken manure was added to the tanks at a concentration of 0.25 g/l and was left to oxidize for 15 days prior to adding nauplii to the tanks. No more manure was added during the *Artemia* culture experiment.

The experimental system

The experiments were carried out in four cement tanks of 1 m^3 each.

The chemical composition of the water and salt utilized in these experiments are shown in Tables I and II.

Measurements of the physico-chemical parameters of the water (Table III) were made with the following equipment : handheld refractometer (American Optical), scale 0-160 ‰, for salinity ; thermometer, scale -10° to 100 °C for temperature ; oxygen meter (114WA1000 Kahlsico), scale 0-15 mg/l, for dissolved oxygen ; pH meter (Corning Model 5), scale 0 to 14 units. The transparency of the water was determined with a Secchi disk.

The experiment

Each experiment lasted 28 days. At the beginning of each experiment 375 l of water was poured into each tank (salinity 35 ‰, pH 8, and dissolved oxygen 6 mg/l).

TABLE I

Physical and chemical analysis of the water used for the *Artemia* culture texts

Chlorine (Cl)	1 438 mg/l
Bicarbonate (HCO_3)	610 mg/l
Carbonate (CO_3)	48 mg/l
Calcium (Ca)	322 mg/l
Sulphate (SO_4)	26 mg/l
Silicate (SiO_2)	77 mg/l
Total hardness ($CaCO_3$)	1 840 mg/l
Conductivity	3 900 µS/cm
pH	7.6
Temperature	26 °C

TABLE II

Chemical analysis and humidity percentage of the salt produced by the Texcoco Sosa Company and used in the *Artemia* culture tests

	Percent composition
Sodium chloride (NaCl)	98.97
Carbonate (CO_3)	0.2625
Bicardonate (HCO_3)	0.0254
Potassium (K)	1.3720
Sulphate (SO_4)	0.0266
Calcium (Ca)	0.0101
Phosphate (PO_4)	0.0008
Silicate (SiO_2)	0.0543
Magnesium (Mg)	0.0014
Aluminium (Al)	0.0013
Iron (Fe)	0.0017
Humidity maximum	19.79
average	7.58
minimum	1.15

TABLE III

Dates and environmental conditions for each experiment

Environmental condition	Diet		
	Rice bran	*Spirulina*	Chicken manure
Dates (1984)	June 8-July 6	July 27-Aug. 24	Nov. 9-Dec. 26
Air temperature (°C)	17-18	16.1-17.3	8.5-12.7
Water temperature (°C)	16-22	15-19	13-15
Salinity (‰)	24-32	32-42	35-45
pH (units)	7.7-9.1	8.6-9.7	8.0-9.2

Constant aeration was maintained for the entire test period. Nauplii (48 h old) were inoculated in each tank at a density of about 1 organism/ml.

The measuring of the physico-chemical parameters and sampling of the population in the experiments with rice bran and *Spirulina* were made at the following times : the first day, when the nauplii were put in the tanks, then on days 2, 4, 7, 10, 14, 17, 21, 24, and 28. Measurements and sampling in the experiment with chicken manure were different : the first day, then on days 3, 7, 14, 17, 19, 26, and 28.

For sampling purposes the water in each tank was geometrically divided into nine equal squares. A 50 ml sample was taken from each square (450 ml total). All the organisms in each sample were counted. In addition 30 organisms were measured from the anterior tip of the ocellus to the base of the caudal furca. At the end of each experiment, the total population was harvested from each tank to obtain the total biomass. Thirty organisms were also taken at random in order to estimate the male:female ratio. In addition, 100 females were examined to determine the percentage of females with a totally or partially full ovisac.

Results

Cysts

The hatching percentage was 45 and the hatching efficiency was 172 000 nauplii/g.

Survival

A high mortality rate (70-80 %) was observed in the first 14 days for *Artemia* fed on rice bran or *Spirulina*. Nevertheless, the diet which resulted in the survival of the largest number of organisms was *Spirulina* (Fig. 1).

Growth

The comparative growth of *Artemia* raised on each diet was similar until about day 10 (Fig. 2). After that, *Artemia* fed on rice bran or *Spirulina* grew more quickly than those fed on chicken manure/algae. From day 15 onward, the *Spirulina* diet appeared to give the best growth. The final average size of *Artemia* raised on *Spirulina* was 9.7 mm ; those reared on rice bran, 6.6 mm ; and those raised on algae produced with chicken manure, 3.8 mm.

The biomass obtained at the end of the experiment with rice bran was 350 g with approximately 122 000 organisms. For *Spirulina* the final biomass was 660 g with approximately 184 000 organisms and for algae with chicken manure, the biomass was 32 g with 166 540 organisms.

Reproduction

When fed rice bran, the first reproduction was noted on day 25.

The sex ratio was 1:1, the reproduction was ovoviviparous and about 32 % of the females were reproducing. Similar results were noted for *Artemia* fed *Spirulina*, except that the percentage of reproductive females was higher, *i.e.* 63.6 %. In the 28 days of the experiment with chicken manure, the stage of sexual differentiation was not reached.

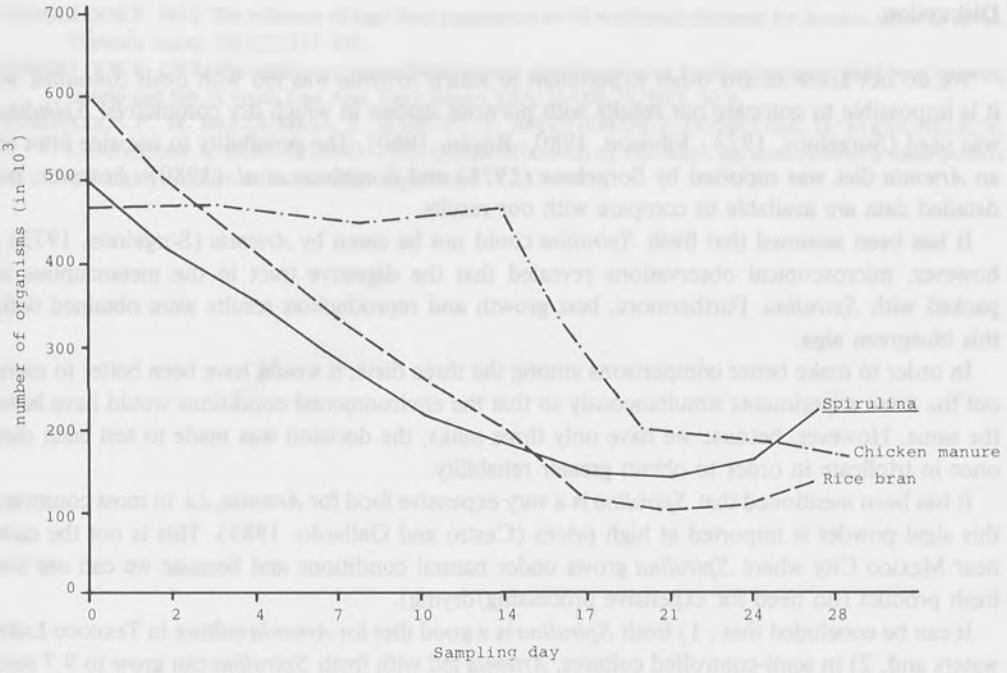

FIG. 1. Survival rates in culture experiments with the three diets.

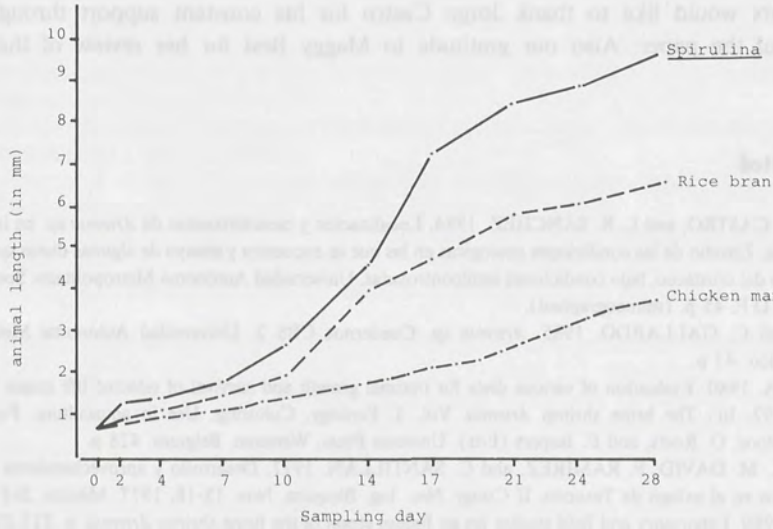

FIG. 2. Comparative growth in culture experiments with three diets.

Discussion

We do not know of any other experiment in which *Artemia* was fed with fresh *Spirulina*, so it is impossible to compare our results with previous studies in which dry commercial *Spirulina* was used (Sorgeloos, 1973 ; Johnson, 1980 ; Royan, 1980). The possibility to use rice bran as an *Artemia* diet was reported by Sorgeloos (1978) and Sorgeloos *et al.* (1980) ; however, no detailed data are available to compare with our results.

It has been assumed that fresh *Spirulina* could not be eaten by *Artemia* (Sorgeloos, 1973) ; however, microscopical observations revealed that the digestive tract in the metanauplius is packed with *Spirulina*. Furthermore, best growth and reproduction results were obtained with this bluegreen alga.

In order to make better comparisons among the three diets, it would have been better to carry out the three experiments simultaneously so that the environmental conditions would have been the same. However, because we have only three tanks, the decision was made to test each diet once in triplicate in order to obtain greater reliability.

It has been mentioned that *Spirulina* is a very expensive food for *Artemia*, *i.e.* in most countries this algal powder is imported at high prices (Castro and Gallardo, 1985). This is not the case near Mexico City where *Spirulina* grows under natural conditions and because we can use the fresh product (no need for expensive processing/drying).

It can be concluded that : 1) fresh *Spirulina* is a good diet for *Artemia* culture in Texcoco Lake waters and, 2) in semi-controlled cultures, *Artemia* fed with fresh *Spirulina* can grow to 9.7 mm length after 28 days culturing, they reach sexual maturity in 23 days and by day 28 the percentage of females that are reproductively active averages 63.6 %.

Acknowledgements

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Culture of *Artemia* from Aveiro (Portugal) fed with wheat bran and seaweed

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Summary

The brine shrimp of Aveiro is an autochtonous strain, used to feed fish and crustacean larvae in the rapidly growing Portuguese aquaculture industry. The aim of this study was to measure growth and survival of Aveiro *Artemia* reared on two food types, in order to determine the most cost-effective diet.

Sorgeloos (1974) and Dobbeleir *et al.* (1980) showed that many species of algae were excellent food for *Artemia* in the different phases of their development. Inert food as well as dried and micronized algae have also been used, with rice bran proving to be the most practical one (Sorgeloos *et al.*, 1980).

We tested two food types, wheat bran and *Ulva* (a green macroalga), both of which are inexpensive and available in great quantity in Portugal. Both foods were dried at 40 °C and micronized in a ball-mill to 50 µm in particle size. Each food-type treatment consisted of replicate 2 l flasks placed in a water batch at 24.8±1.4 °C with continuous light at 5 000 lux. Each flask contained 2 l of seawater (34.5±1.4 ‰ salinity ; pH = 7) inoculated with newly-hatched *Artemia* nauplii at a concentration of one nauplius/ml. The different food-types were added at a rate of 0.05 mg dry wt/ml/day until day 3, and 0.2 mg dry wt/ml/day thereafter, based on the results of Aguiar Pinto (1982).

The *Artemia* were counted, measured (Wild microscope with ocular micrometer lens), and weighed (Cahn balance) every 2 days for 18 days.

The caloric value of the foods was determined with a Phillipson micro-bomb calorimeter previously calibrated with benzoic acid (Phillipson, 1964 ; Grodzinski *et al.*, 1975).

The results obtained in the first 3 days confirmed that rapid growth occurs with a small quantity of exogenous food (Fig. 1 and 2), in agreement with the findings of Johnson (1980). The yolk reserves are exhausted by the third day and the animals must then feed exogenously by filtration of particles (Johnson, 1980).

Ulva provided better survival, growth, and food conversion efficiency than did wheat bran, 72 % *versus* 60 % (Fig. 1), 11.1 mm *versus* 8.4 mm (Fig. 2), and 1.9 *versus* 2.2, respectively, by the end of the experiment. The *Ulva* had a higher caloric value than did wheat bran (3.4 cal/mg *versus* 3.0 cal/mg, respectively) and that difference may account for part of the better results obtained with *Ulva*. However, the wheat bran may also have lacked essential fatty acids for *Artemia* and its predators that were present in the *Ulva*.

In future studies, it will be necessary to obtain more information on the biochemistry of the food types and on the *Artemia* fed these items. We intend to determine which food is optimal to produce large *Artemia* biomasses with adequate biochemical composition, at the lowest possible cost.

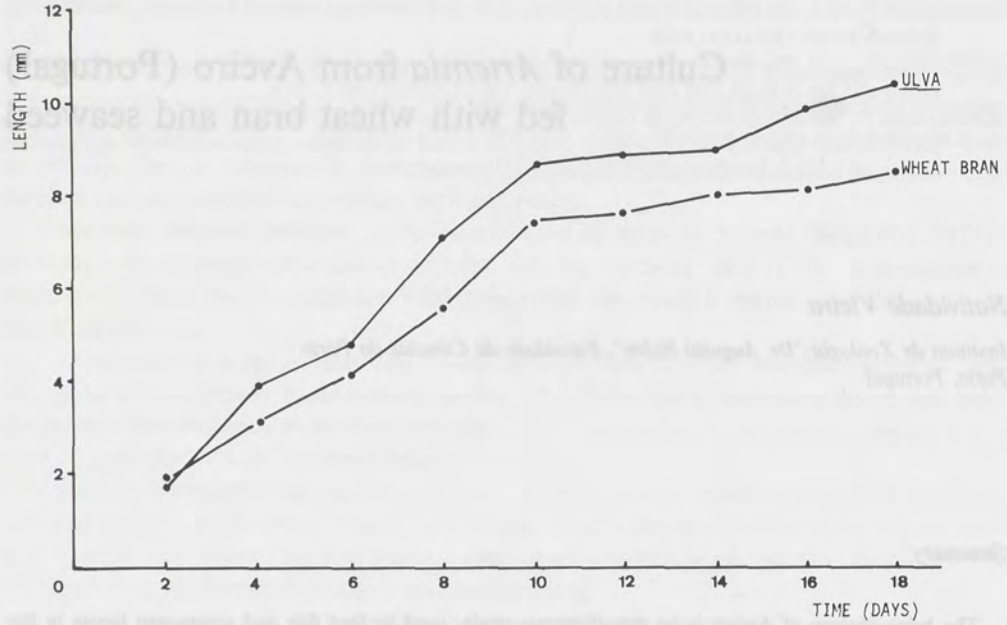

FIG. 1. Length of *Artemia* (Aveiro strain) grown on two different diets.

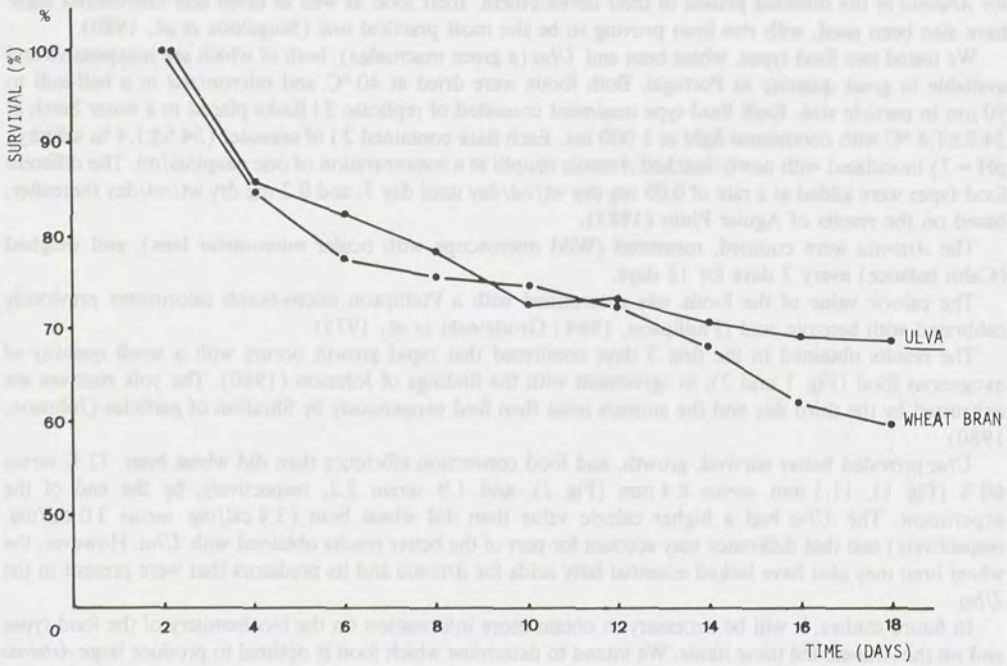

FIG. 2. Survival of *Artemia* (Aveiro strain) grown on two different diets.

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FIG. 1. Length of *Artemia* (Aveiro strain) grown on two different diets.

FIG. 2. Survival of *Artemia* (Aveiro strain) grown on two different diets.

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Laboratory studies on growth and reproduction of *Artemia* (Tuticorin strain)

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Abstract

The growth and reproduction rate of *Artemia* from Tuticorin (India) were studied at densities of 25 individuals/l seawater of 50 ‰ S using six different types of food. Of the diets tested, the composite diet made up of a mixture of rice bran, yeast, algae from saltpans, and decomposed cabbage yielded the best growth. It took 9 days for the ovisac to appear in animals fed with the composite diet, whereas it took 20±2 days in animals fed with other diets. The egg number/brood did, however, not vary significantly ($P<0.05$).

The effect of salinity on the growth and fecundity of animals fed the composite diet, was studied at 20, 30, 50, 75, 100, 125, and 150 ‰. Nauplii reared at 20‰ suffered total mortality on the second day of rearing. The growth was greatest in salinities ranging from 50 to 100 ‰, but was significantly less at 30, 125 and 150 ‰ ($P<0.05$). *Artemia* reared at 50 ‰ achieved the maximal growth in 18 days (10.24 length and 4.0 mg weight).

Although ovoviviparous reproduction was dominant at all the tested salinities, the cyst/nauplii ratio revealed an increased oviparity in salinities exceeding 50 ‰. At all salinities the cyst production increased when ferric EDTA was added at concentrations of 5, 10 or 15 mg/l.

Introduction

The importance of *Artemia* is increasing mainly because it can be used as a convenient food organism in aquaculture. The other advantages are that it has the ability to tolerate a wide range of salinities and can grow to adult stage and reproduce within a period of 2-3 weeks (Sorgeloos, 1980 ; Persoone and Sorgeloos, 1980). This has, in recent years, created enormous interest among biologists to study the possibility of successfully culturing this animal under laboratory conditions (Bossuyt and Sorgeloos, 1980 ; Dwivedi *et al.*, 1980). Many have tried to improve the growth efficiency of these animals by providing them with different types of diets (Sorgeloos, 1974 ; Sick, 1976 ; Dobbeleir *et al.*, 1980 ; Johnson, 1980 ; Royan, 1980).

Effects of salinity on survival (Royan, 1980 ; Dwivedi *et al.*, 1980) and growth (Dwivedi *et al.*, 1980) have been reported for some Indian strains. Although Tuticorin strain ranks as one of the best in terms of biomass production (Tobias *et al.*, 1980) there is a paucity of information on its growth and reproduction as a function of salinity. Besides, the influence of Fe-EDTA on its reproductive efficiency (Versichele and Sorgeloos, 1980) has not been studied in this strain. Hence the present study was undertaken to investigate the growth and reproductive performance of *Artemia* (Tuticorin strain) in relation to salinity, diet and Fe-EDTA.

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Materials and methods

Diet experiment

Artemia cysts were collected from the salt pans of Tuticorin (latitude 8°50′N ; longitude 78°8′E), washed, dried and stored in a dessicator. Benthic algae from the salt pans were also collected and brought to the laboratory in aerated containers. These algae were immediately introduced into artificial salt pans of 1.0×2.5×0.15 m dimensions. The bottoms of these pans were lined with high density polyethylene sheets over which clean sand was strewn. The pans contained 75 ‰ S filtered saline water made from commercial salt. Within a week the algae established well and grew profusely. From these pans algae were harvested periodically and used in formulating the diet for *Artemia.* Commercial salt of known weight was dissolved in tap water, filtered to remove the suspended impurities, and used to prepare solutions of required salinity. All the experiments were conducted at room temperature (28±2 °C) and normal photoperiod (14L : 10D).

Batches of the stored cysts were hatched in glass hatchers (model I : Sorgeloos and Persoone, 1972) in 10 ‰ saline solution. The freshly hatched nauplii (0.55±0.03 mm) were carefully collected and introduced, at a density of 50 nauplii per container, into a series of 2 l containers containing 50 ‰ saline water. Five different types of diets were tested to study the growth of these individuals. Each diet was fed to *Artemia* in at least 3 replicate containers. The compositions of the diets were as follows :

Algae

Algae collected from the artificial salt pans were filtered through plankton net (mesh 80 µm). An examination of a sample under the microscope revealed the presence of *Anabaena, Chaetomorpha, Navicula, Pleurosigma, Rhizosolenia, Coscinodiscus,* etc. Algal solution (250 g/l) at a rate of 5 ml per container was fed to the shrimp twice a day.

Bacteria

Spirullum sp. was isolated from the salt pan sediments, cultured (Kannan, 1982) and used in the present study. 5 ml of bacterial culture were added to each container twice a day.

Rice bran

Rice bran purchased from commercial dealers was processed and prepared following the methods of Sorgeloos *et al.* (1980).

Yeast

Commercial yeast tablets (500 mg) were purchased and used as a diet, after dissolution in 50 ‰ saline water (10 g/l).

Cabbages

About 250 g of cabbage leaves were soaked in 1 l of 50 ‰ salt water for 15 days and allowed to decompose. Then it was filtered through 80 µm sieve. The filtrate was used as a diet.

Mixed diet

This consisted of all of the above diets mixed in equal quantities by volume.

5 ml of diet were added twice a day to each container. The ration was gradually increased to 10 ml twice a day toward the end of the experiment. The lengths of the *Artemia* were measured on the 3rd, 7th, 11th, 15th, 18th and 21st days. The length increment data were tested using analysis of variance (ANOVA) to determine the relationship between growth and the various diets (Sokal and Rohlf, 1969).

SALINITY AND EDTA EXPERIMENTS

In order to test the effect of different salinities on the growth of *Artemia*, freshly hatched nauplii were reared in 20, 30, 50, 75, 100, 125 and 150 ‰ salinities, and growth was monitored for 21 days. All treatments received the mixed diet during this period. Animals reared up to 21 days (eggs formed) were then given Fe-EDTA (Baker, 1966) at a concentration of 0, 5, 10 or 15 mg/l. The containers were checked daily for the presence of cysts and nauplii which were carefully collected and counted. The cyst/nauplii ratio was analysed using ANOVA (Sokal and Rohlf, 1969 ; Steel and Torrie, 1980) to find out the effect of chosen salinities and Fe-EDTA on the pattern of reproduction. Water was changed every 3 days to avoid animals resorting to surface respiration (Baker, 1966 ; Moore and Burn, 1968 ; Horne, 1971 ; Versichele and Sorgeloos, 1980).

Results

DIET EXPERIMENT

Irrespective of the type of diet a positive linear relationship was observed between growth and time (Table I). *Artemia* growth on the different diets differed significantly, so to identify the best diet Student Newmen-Keuls Test (SNK) was performed and the growth means of the chosen diets were ranked (Table II). Of all the 6 diets, mixed diet gave the best growth. The least growth was found in animals fed with *Spirullum*. Animals fed with the mixed diet also attained maturity faster (9 days) than those fed with other diets (rice bran : 16, algae : 15, cabbage : 20; bacteria : 14 ; yeast : 19 days). However, in all the experimental animals, once the ovisac appeared, eggs were formed in the next 2 or 3 days irrespective of the diet.

TABLE I

Regression equations for growth of *Artemia* on different types of food, where X=time in days and Y=length in mm.
* indicates statistical significance at the $P<0.005$ level

Food	Equation	r
Mixed diet	Y = 0.55 X -0.12	0.93*
Rice bran	Y = 0.51 X -0.72	0.97*
Bacteria	Y = 0.45 X -0.33	0.97*
Cabbage	Y = 0.47 X -1.08	0.99*
Algae	Y = 0.46 X -0.52	0.99*
Yeast	Y = 0.47 X -0.45	0.99*

TABLE IIA

Analysis of variance for growth (mm) in relation to food and time (days).
** indicates statistical significance at the P<0.005 level

Source of variation	SS	df	MS	F
Total	362.49	35	10.36	
Between foods	12.79	5	2.56	9.14**
Between days	342.68	5	68.54	244.80**
Error	7.02	25	0.28	

TABLE IIB

Results of SNK test to determine the significant differences
among the growth means ranked as a function of food.
Treatments joined by underlining are not significantly different from each other

Cabbage	Algae	Bacteria	Yeast	Rice bran	Mixed
4.73	5.27	5.31	5.43	5.66	6.76

SALINITY AND EDTA EXPERIMENT

Effect of salinity on growth

Nauplii reared at 20 ‰ salinity suffered total mortality on the 2nd day of rearing. Growth was found to be linear under all the other tested salinities (Table III) and the relationship was found to be statistically significant. The relationship between the growth means at the tested salinity levels was also found to be significant ($P<0.05$) ; therefore, the individual treatment means were tested for significant differences by the SNK procedure. The growth means at 50, 75 and 100 ‰ salinity did not vary significantly and ranged from 6.2 to 6.7 mm. At salinities of 30, 125 and 150 ‰, growth was found to be significantly less (growth means : 4.3, 4.55 and 4.56 mm) and not different from each other (Table IV). Thus in terms of growth, the 50-100 ‰ salinity range has been found to be the optimum.

TABLE III

Regression equations for growth of *Artemia* in different salinities,
where X=time in days and Y=length in mm.
* indicates statistical significance at the P<0.005 level

Salinity (‰)	Equation	r
30	Y = 0.39 + 0.114 X	0.98*
50	Y = 0.57 + 0.47 X	0.98*
75	Y = 0.55 + 0.57 X	0.99*
100	Y = 0.55 + 0.72 X	0.99*
125	Y = 0.47 - 1.32 X	0.97*
150	Y = 0.45 - 1.1 X	0.99*

TABLE IVA

Analysis of variance for growth (mm) in relation to salinity and time (days).
** indicates statistical significance at the P<0.005 level

Source of variations	SS	df	MS	F
Total	402.23	35		
Between salinities	35.17	5	7.034	19.98**
Between days	358.27	5	71.654	203.66**
Error	8.80	25	0.352	

TABLE IVB

Results of SNK test to determine the significant differences
among the growth means ranked as a function of salinity (‰).
Treatments joined by underlining are not significantly different from each other

30 ‰	125 ‰	150 ‰	100 ‰	75 ‰	50 ‰
4.3	4.55	4.56	6.20	6.35	6.70

Effect of salinity and EDTA on reproduction

Under all the experimental conditions, the first brood was always ovoviviparous and the subsequent broods were either oviparous or ovoviviparous. The cyst/nauplii ratio was found to increase as a function of salinity. Both the tested salinity and the Fe-EDTA levels have been found to influence the cyst/nauplii ratio significantly (Table V). There was also significant interaction effects of these two variables on cyst/nauplii ratio ($P<0.05$). Regarding salinity, the ratio has been found to significantly increase at 125 and 150 ‰ salinity, the means being 2.62 and 2.88, compared to the ratios at 50, 75 and 100 ‰ salinity (Table V). Fe-EDTA also influenced the cyst/nauplii ratio. When compared to the control group the cyst/nauplii ratio increased significantly at 5 mg Fe-EDTA concentration (5.19 and 7.89 at 0 and 5 mg). At 10 and 15 mg Fe-EDTA concentration cyst/nauplii ratio was still significantly greater (16.26 and 14.8, respectively).

Discussion

Various diets ranging from algae, yeast, bacteria, micronized inert diets, etc., have been successfully used to grow *Artemia* since it is a non-selective, obligatory filter-feeder (Dobbeleir *et al.*, 1980 ; Johnson, 1980 ; Dwivedi *et al.*, 1980 ; Royan, 1980). In the present study, a mixed diet consisting of algae, rice bran, bacteria, decomposed cabbage, and yeast was found to promote maximum growth. With this diet, the animals could attain a maximum length of 10.24 mm in 18 days, similar to that obtained by Royan (1980) for the same strain. However, egg pouch and egg formation occurred 2 days earlier in the present study (*i.e.*, 9th and 12th day, respectively). The number of days taken for sexual maturity to occur in different strains of *Artemia* varies considerably and ranges from 11 to 21 days (Tobias *et al.*, 1980). Although the time taken to attain sexual maturity for the Tuticorin strain has been reported as 14 days by Tobias *et al.* (1980), under our culture conditions in the present study it was less.

TABLE VA

Analysis of variance for cyst/nauplii ratio in relation to salinity (‰) and Fe-EDTA concentration (mg/l). ** indicates statistical significance at the $P<0.005$ level

Source of variation	SS	df	MS	F
Subgroups	363.32	19		
Between salinities	201.05	4	50.26	46.97**
Between concentrations	107.04	3	35.68	33.35**
Interaction	55.23	12	4.60	4.30**
Error	64.34	60	1.07	
Total	427.66	79		

TABLE VB

Results of *a priori* test (Sokal and Rohlf, 1969) to compare the cyst/nauplii ratio means at different EDTA concentrations with that of control (0 mg concentration). Underlined means are not significantly different from each other

Control	5 mg	10 mg	15 mg
5.19	7.89	16.26	14.8

TABLE VC

Results of SNK test to compare the cyst/nauplii ratio means as a function of salinity (‰). Underlined means are not significantly different from each other.

75 ‰	100 ‰	50 ‰	125 ‰	150 ‰
0.28	0.34	.365	2.62	2.88

Wide fluctuation in salinity, either due to evaporation caused by solar radiation or dilution due to sudden rains, plays a major role in the life history of *Artemia*. For instance, in the salt pans of Tuticorin the salinity ranges from 15.9 ‰ in December to 211 ‰ in July (Ramamoorthi and Thangaraj, 1980). The results of our study indicate that 50-100 ‰ is optimal for growth of Tuticorin strain *Artemia*. Indeed, the salt pans of Tuticorin also supported maximum *Artemia* population when the salinity was 100 ‰. For Bombay strain, Dwivedi *et al.* (1980) reported optimal growth between 40 and 75 ‰.

Artemia (Tuticorin strain) is a parthenogenetic species. It resorts to ovoviviparity under favourable environmental conditions and oviparity when these factors become adverse (Ivleva, 1969). In this study, the salinity effects on the pattern of reproduction revealed that with increasing salinity the cyst production also increased. In terms of cyst-nauplii (C/N) ratio the salinity effect could be grouped as 50, 75 and 100 ‰ in one group and 125 and 150 ‰ in another group. It appears that C/N ratio becomes stabilized at 125 and 150 ‰ and does not differ significantly. The effect of Fe-EDTA, which is known to stimulate haemoglobin synthesis and cyst production (Baker, 1966), has also been determined at three concentrations. At 10 and 15 mg concentration the cyst production increased significantly when compared to the control and 5 mg concentration. Such a positive relation between Fe-EDTA concentration and cyst production has also been reported for other strains (Baker, 1966 ; Chow, 1968 ; Versichele and Sorgeloos, 1980). For *Artemia* (Tuticorin strain) 10 mg/l is optimal to induce ovoviviparous reproduction.

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Design, operation, and potential of a culture system for the continuous production of *Artemia* nauplii[1]

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Abstract

A flow-through culture system is described for the controlled production of *Artemia* nauplii. High densities of adult brine shrimp are induced to ovoviviparity through diet manipulation, optimal culture water exchange, and high constant oxygen levels.

The nauplii produced are separated from the culture tank effluents in a special recuperation filter system. The culture water is recirculated over a rotating biodisc, a cross-flow sieve, and a plate separator.

The potential of this nauplii production system for application in aquaculture hatcheries is discussed.

Introduction

The application in aquaculture of ongrown *Artemia* as a food source is steadily increasing (Lai and Lavens, 1987). Research efforts of the Artemia Reference Center, Belgium, have resulted in improved techniques for the production of brine shrimp biomass (Brisset *et al.*, 1982 ; Lavens *et al.*, 1985). The same techniques which have been developed for intensive *Artemia* rearing from nauplius to adult under flow-through conditions can be used to maintain cultures of reproductively active animals. By applying cyclic oxygen stresses Lavens and Sorgeloos (1984) managed to induce and to maintain the oviparous reproduction mechanism. This paper reports how an exclusive nauplii-production can be achieved by minimizing stresses or fluctuations in culturing conditions. A recuperation system for the continuous harvesting of liveborn nauplii is also described.

Description and operation of the system (Fig. 1, 2)

The nauplii production system is integrated in the biomass culture unit of an existing flow-through recirculating installation, which has been described by Lavens *et al.* (1985).

[1] Technique protected by patent no. 83201335-3.

Artificial seawater of 25 °C and 50 ‰ salinity flows continuously into the 100 l culture tanks at a flow rate of 100 l/h, *i.e.* a retention time of 1 h as to remove as quickly as possible all soluble and particulate waste (eventually including freshly-born nauplii) via the cylindrical welded-wedge screen filter with a slit opening of 550 µm. Oxygen levels are kept around 4 ppm by the air-bubbling from the aeration collar surrounding the bottom part of the central filter. Following the culture procedures outlined in Lavens *et al.* (1985, 1987a) Great Salt Lake *Artemia franciscana* are fed on a micronized corn byproduct and cultured in the biomass production unit until 2-weeks-old. After harvesting at day 14, the adults are transferred to the 100 l tanks at densities of 5 000 brine shrimp/l.

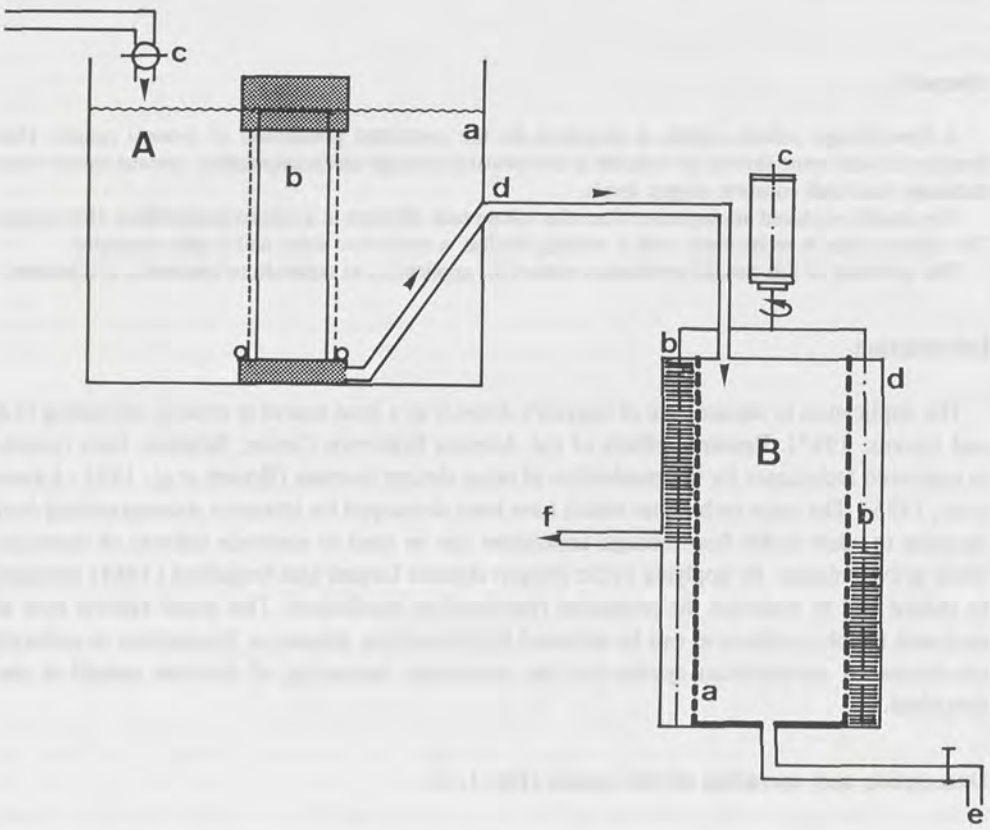

FIG. 1. Schematic drawing of the *Artemia* nauplii production system (not drawn to scale). A. Culture unit with 100 l tank (a), *Artemia* retaining filter (b), and in/outflow (c, d). B. Nauplii recuperation filter with welded-wedge filter (a), cleaning brushes (b) driven by rotor (c), cylindrical holding tank (d), collector drain (e), and effluent drain to recirculation systems (f).

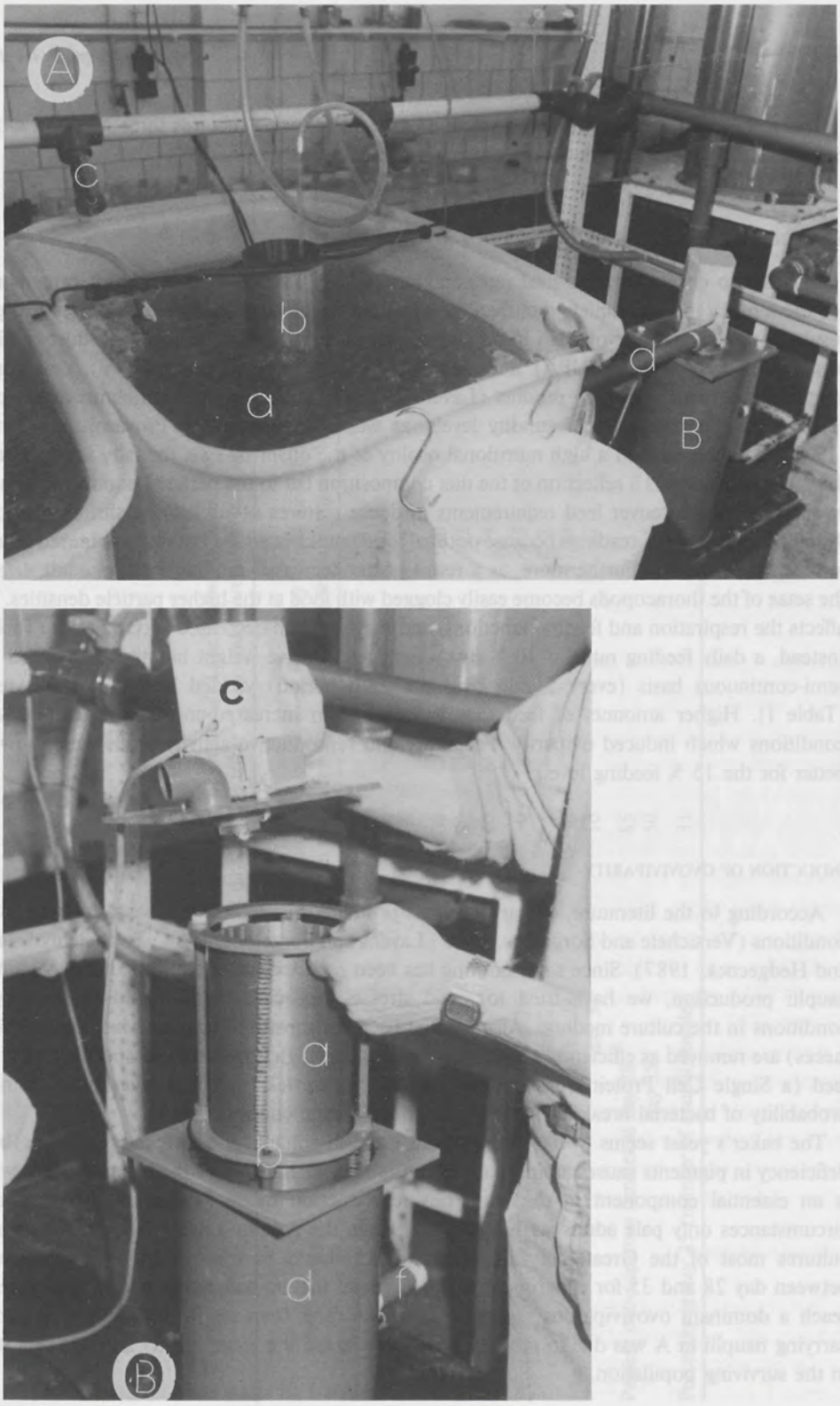

FIG. 2 A,B. Overview of the culture system for continuous production and separation of *Artemia* nauplii (for legend to abbreviations see Fig. 1).

The technique for controlled production of *Artemia* offspring requires three essential modifications of the biomass production technique :

Specific diet and feeding strategy for adults

In order to ensure high survival rates and maximal reproductive activity adult brine shrimp require a much more complete diet than the one used for larval growth. We have obtained good results with a mixed diet of fresh baker's yeast and an emulsified enrichment product of highly unsaturated fatty acids (HUFA) and vitamins (Selco, Artemia Systems NV), occasionally supplemented with a corn by-product (Lavens *et al.*, 1987a). The lipid enrichment (especially HUFA) not only enhanced fecundity levels, as was also reported by Provasoli and Pintner (1980), but also ensured a high nutritional quality of the offspring ; *i.e.* the fatty acid pattern of the produced larvae is a reflection of the diet composition fed to the parental population (Lavens *et al.*, 1987b). Moreover feed requirements in dense cultures of adult brine shrimp cannot be dosed by transparency readings because optimal food uptake in adults is already achieved at much lower concentrations. Furthermore, as a result of the decreased molting rates in adult *Artemia*, the setae of the thoracopods become easily clogged with food at the higher particle densities. This affects the respiration and feeding functions, and may result in decreased survival rates (Table I). Instead, a daily feeding ratio of 10 % dry weight feed to live weight biomass distributed on a semi-continuous basis (every 5 min over the 24-h period) yielded best production results (Table I). Higher amounts of feed (15 %) resulted in increased mortality rates and stress conditions which induced oviparity. Fecundity and reproductive activity levels were, however, better for the 15 % feeding level.

Induction of ovoviviparity

According to the literature, oviparity or cyst production is basically induced by cyclic stress conditions (Versichele and Sorgeloos, 1980 ; Lavens and Sorgeloos, 1984 ; Berthélémy-Okazaki and Hedgecock, 1987). Since sofar nothing has been reported about controlled ovoviviparity or nauplii production, we have tried to avoid stresses, especially with regard to the oxygen conditions in the culture medium. All potential bacterial substrates (*e.g.* exuviae, uneaten food, faeces) are removed as efficiently as possible by the use of high flow-rates and specific filters. The feed (a Single Cell Protein) has optimal physical properties (maximal ingestibility, minimal probability of bacterial breakdown) and is distributed semi-continuously.

The baker's yeast seems to have a beneficial effect in enhancing ovoviviparity, *i.e.* maybe its deficiency in pigments causes an incapability in the *Artemia* to produce the haem-pigment which is an essential component in the oviparous reproduction mode (under these experimental circumstances only pale adults were observed). From the data in Table I it appears that in all cultures most of the Great Salt Lake females reproduced by ovoviviparity. The differences between day 28 and 35 for experiment B and C reveal that an adaptation period is required to reach a dominant ovoviviparous reproduction mode. The decrease in the number of females carrying nauplii in A was due to mortalities which affected the water quality and initiated stress in the surviving population.

TABLE I

Population characteristics of Great Salt Lake *Artemia* cultures
set up at an animal's age of 14 days and fed baker's yeast under three different feeding regimes

	A In relation to culture medium transparency	B Ratio dry weight food to live weight *Artemia* biomass = 10 %	C Ratio dry weight food to live weight *Artemia* biomass = 15 %
Animal length (in mm) at day 14	4.0	6.1	6.1
day 28	7.1	7.0	7.4
day 35	–	7.3	7.8
Percent survival at day 14	100	100	100
day 28	80	92	84
day 35	40	74	62
Percent of reproductive active females at day 14	0	< 5	< 5
day 21	< 5	8	17
day 28	30	44	66
day 35	10	67	88
Percent ovoviviparous females at day 28	85	–	60
day 35	50	95	90
Fecundity at day 35 (number of nauplii/female)	31	64	76

RECUPERATION TECHNIQUE FOR CONTINUOUS HARVESTING OF THE NAUPLII

The system consists of an inversed type of a welded-wedge screen cylinder (150 µm slit opening) precisely fitted into a cylindrical PVC holding tank (Fig. 1, 2B). The half-submerged filter retains all produced nauplii and particles larger than 150 µm from the culture effluents and drains water and small wastes via the holding tank back into the recirculating unit. Although this 150 µm filter has a high filtering capacity, a mechanical cleaning system is needed if a 24 h autonomy is required : *i.e.* two brushes driven by an electrical rotor (12 rpm) rubb the outer surface of the welded-wedge screen. The nauplii are harvested once or twice a day and separated from the waste materials by taking advantage of the phototactic behavior of the larvae. For this, use is made of a cylindro-conical tank (100 l) with a central overflow tube above which a hole is cut in the cover and a light bulb is installed. The nauplii suspension is poured into the separator-tank and a water flow is adjusted as to let all particles sediment and evacuate the nauplii attracted by the light with the overflowing water.

This harvesting technique seems to operate very efficiently. Preliminary production trials with this new set-up yielded 30 g wet weight nauplii/day/100 l culture tank, suggesting a daily reproduction rate of 10 nauplii/female. Moreover, only early larval stages were collected, suggesting an efficient recuperation from the culture tanks.

Potential of an *Artemia* nauplii production system

The technique for the controlled production of ovoviviparous nauplii offers interesting prospects for application in aquaculture hatcheries. It may not only create an independency from the international cyst market with its fluctuating prices, available quantities, and quality but it also provides a far better control of the quality of this live food product, with even the possibility to produce offspring of a far superior quality (Lavens *et al.*, 1987b).

Such a technique also allows a proper integration of *Artemia* in the aquaculture plant : the produced offspring can be used either directly as food source in the hatchery, or for stocking culture tanks which produce juvenile and reproductive active *Artemia* to be fed respectively to nursery and maturation stages of the predator. This vertical integration may, as a result of its beneficial impact on fish or crustacean outputs, greatly improve the cost effectiveness (Lavens *et al.*, 1985), resulting in economically feasible intensive *Artemia* production plants (Lai and Lavens, 1987).

Besides industrial applications, a controlled nauplii-production technique furthermore offers opportunities for biological research with regard to comparative studies (*e.g.* morphological, physiological, and biochemical characteristics) between oviparous and ovoviviparous offspring, produced under identical circumstances ; and the selection of obligate ovoviviparous *Artemia* populations which have a genotypical adaptation towards ovoviviparous reproduction, *e.g.* Laysan Lake *Artemia* (Lenz and Dana, 1987).

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Scale-up studies on the culture of brine shrimp (*Artemia*) fed with rice bran

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Abstract

The effects of water movement or agitation on the biological performance of planktonic organisms under intensive culture have rarely been studied quantitatively. Stagnation or minimal values are considered important in the problem of scaling up based on optimal conditions. Near stagnation or inadequate water movement brings about undesirable effects, *e.g.* accumulation of metabolites, uneven distribution of feed, and decrease in dissolved oxygen concentration. An important mechanism associated with water movement under these conditions is the oxygenation process which defines the oxygen transfer-rate from the gas to the liquid phase.

Experiments were conducted using potable water to determine the overall oxygen mass-transfer coefficient in two types of container designs : a cylindri-conical tank and an oblong-shaped center-partitioned raceway. For each type of container, three geometrically similar sizes were investigated with scale ratios of approximately 1:2:3.5. Agitation was induced by the introduction of air into the system. General correlations for both tank designs were obtained from experiment data and were expressed in terms of the operating and geometric parameters. The correlations are in the form of dimensionless groups (Froude and Reynolds numbers) making them appropriate for scale-up estimates.

The general correlations for the overall oxygen mass-transfer coefficient were subsequently used to provide the scaling equations to define the operating parameters in different sizes of containers, for the culture of brine shrimp in seawater fed with rice bran. The high correlation coefficient obtained for the relationship between total brine shrimp biomass-production and the overall mass-transfer coefficient indicates that the overall mass-transfer coefficient is an effective scale-up criterion in brine shrimp culture.

Scale-up studies on the culture of brine shrimp (*Artemia*) fed with rice bran

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Abstract

The effects of water movement or agitation on the biological performance of planktonic organisms under intensive culture have rarely been studied quantitatively. Stagnation or minimal values are considered important in the problem of scaling up based on optimal conditions. Near stagnation or inadequate water movement brings about undesirable effects, e.g. accumulation of metabolites, uneven distribution of feed, and decrease in dissolved oxygen concentration. An important mechanism associated with water movement under these conditions is the oxygenation process which defines the oxygen transfer rate from the gas to the liquid phase.

Experiments were conducted using potable water to determine the overall oxygen mass-transfer coefficient in two types of container designs: a Cylindri-conical tank and an oblong-shaped cement partitioned raceway. For each type of container, three geometrically similar sizes were investigated with scale ratios of approximately 1:2:3.5. Agitation was induced by the introduction of air into the system. General correlations for both tank designs were obtained from experiment data and were expressed in terms of the operating and geometric parameters. The correlations are in the form of dimensionless groups (Froude and Reynolds numbers) matting them appropriate for scale-up estimates.

The general correlations for the overall oxygen mass-transfer coefficient were subsequently used to provide the scaling equations to define the operating parameters in different sizes of containers, for the culture of brine shrimp in seawater fed with rice bran. The high correlation coefficient obtained for the relationship between total brine shrimp biomass-production and the overall mass-transfer coefficient indicates that the overall mass-transfer coefficient is an effective scale-up criterion in brine shrimp culture.

Artemia *Research and its Applications. 1987. Vol. 3. Ecology, Culturing, Use in aquaculture*
P. Sorgeloos, D. A. Bengtson, W. Decleir, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 556 p.

Production of *Artemia* biomass for feeding marine fish larvae

Charles M. James, T. S. Abu-Rezeq, and P. Dias

Mariculture and Fisheries Department, Kuwait Institute for Scientific Research
P.O. Box 1638, Salmiya, Kuwait

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Batch mass-culture of *Artemia* from nauplius to adult stage using 5 m^3 and 10 m^3 culture tanks without water renewal is described. Using Great Salt Lake (Utah, USA) *Artemia* cysts, it was possible to produce up to 7 kg/m^3 of wet *Artemia* biomass in 2 weeks. The marine yeast *Candida* was used as food source at 1.25 kg dry weight *Candida*/m^3 culture medium/2 weeks-culture batch.

The amino acid and fatty acid contents of *Artemia* in such production systems have been analysed and are discussed in detail.

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A simplified technique for mass production of *Artemia* in India

Baje Vishnu Bhat and Ramur Ganapathy

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Abstract

The Marine Products Export Development Authority (MPEDA) is establishing a model prawn hatchery in Vallarpadom Island situated in the Cochin backwaters. As part of the project, experiments were conducted to develop a simplified indigenous continuous-culture technique for the mass production of *Artemia*. Cysts from the salt pans of Gujarat State procured through a private company were successfully hatched in saltwater (35±2 ‰) and reared initially in 25 l plastic bins containing old brackish water of increased salinity. Later these larvae were inoculated (1 000 larvae/l) to larger 200 l and 1 tonne fiber-glass tanks containing 150 l and 400 l water respectively. Of the four types of feeds (clam-meat suspension, *Squilla* powder, rice-bran juice, ground nut-oil cake juice) fed twice a day, the first one gave very high survival (85 %) and good growth (14 mm).

The biomass- and cyst-production experiments were carried out in two separate systems. In one set, where the salinity was kept at 60 to 65 ‰, 12 to 14 days old adults, showing bright red coloration, started producing nauplii at an average rate of 100 to 125/individual. In another experiment carried out to obtain cyst production, the salinity was increased initially to 70 to 75 ‰. When it was further raised to 100 ‰, the adults started releasing batches of cysts.

A simplified technique for mass production of *Artemia* in India

Raja Pillai and Ramu Ganapathy

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Use in aquaculture

Since the first *Artemia* symposium, the importance of essential fatty acids has been recognized as the major factor in *Artemia* nutritional value for marine predators. The enrichment of *Artemia* to augment these fatty acids has been a major theme of the past few years and enrichment studies are well represented in the following papers.

Léger *et al.* (1) review the present knowledge of *Artemia* nutritional value for use in aquaculture worldwide and Watanabe (2) then reviews *Artemia* usage and nutritional value in Japan. The next four papers (3-6) discuss the methods and results of evaluating enriched *Artemia.* Papers (7-11) compare *Artemia* as a diet for marine predators with other potential diets, and the subsequent two papers (12-13) describe large-scale feeding of *Artemia* in commercial operations. The final two papers discuss research on the importance of pesticide levels in *Artemia* fed to a predator (14) and the possibility of *Artemia* usage as a feed for mammalian species (15).

The two workshops of this session, which included very vigorous discussions, are summarized in the last two reports (16-17).

(1) Ph. Léger, D. A. Bengtson, P. Sorgeloos, K. L. Simpson, and A. D. Beck.
The nutritional value of *Artemia* : a review.

(2) T. Watanabe.
The use of *Artemia* in fish and crustacean farming in Japan.

(3) Ph. Léger, M. D. Johns, and P. Sorgeloos.
Description of a standard bioassay with the marine crustacean *Mysidopsis bahia* (M.) for the evaluation of the nutritional effectiveness of *Artemia* nauplii and metanauplii.

(4) Ph. Léger, E. Naessens-Foucquaert, and P. Sorgeloos.
International Study on *Artemia.* XXXV. Techniques to manipulate the fatty-acid profile in *Artemia* nauplii, and the effect on its nutritional effectiveness for the marine crustacean *Mysidopsis bahia* (M.).

(5) F. Amat, F. Hontoria, and J. C. Navarra.
International Study on *Artemia.* XLIV. Preliminary nutritional evaluation of different *Artemia* nauplii as food for marine fish and prawn larvae.

(6) J. H. Robin, C. Le Milinaire, and G. Stephan.
Production of *Artemia* using mixed diets : control of fatty acid content for marine fish larvae culture.

(7) R. Yashiro.
The effect of *Artemia* fed with different diets on the growth and survival of *Penaeus monodon* Fabricius postlarvae.

(8) P. Trotta, P. Villani, G. B. Palmegiano, G. Forneris, and C. Sarra.
Laboratory-grown *Artemia* as reference food for weaning fish fry and shrimp postlarvae.

(9) R. F. Bombeo.
Growth and survival of *Penaeus monodon* and *Chanos chanos* fry fed with *Artemia* singly or in combination with an artificial diet.

(10) G. A. Takami.
The use of *Artemia* from Ormia Lake (Iran) as food for sturgeon fry.

(11) H. E. Leibovitz, D. A. Bengtson, P. D. Maugle, and K. L. Simpson.
Effects of dietary *Artemia* lipid fractions on growth and survival of larval inland silversides, *Menidia beryllina.*

(12) J. I. Guimarães and F. Lira do Rego.
The use of freeze-dried *Artemia* as food for penaeid shrimp larvae.

(13) V. Franičević.
Large-scale *Artemia* cyst hatching at the CENMAR fish hatchery in Yugoslavia.

(14) S. McLean, Ch. E. Olney and K. L. Simpson.
International Study on *Artemia.* XLV. The effects of *cis*-chlordane and dieldrin on the food chain *Artemia* to winter flounder.

(15) Ph. C. Ronsivalli and K. L. Simpson.
The brine shrimp *Artemia* as a protein source for humans.

(16) K. L. Simpson and Ph. Léger.
Workshop report : The use of *Artemia* as food in aquaculture.

(17) L. Lai and P. Lavens.
Workshop report : *Artemia* as a business perspective.

Artemia *Research and its Applications. 1987. Vol. 3. Ecology, Culturing, Use in aquaculture*
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The nutritional value of *Artemia*: a review

Philippe Léger[1], *David A. Bengtson*[2], *Patrick Sorgeloos*[1], *Kenneth L. Simpson*[2], *and Allan D. Beck*[3]

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Content

Introduction

Successful rearing of larval stages of aquatic organisms is a challenge for aquarium hobbyists, an aim and tool for aquatic ecologists and ecotoxicologists, and a necessity for the success of the aquaculturist. All these people will agree that the primary problem in any type of larval rearing is that of food. Ideally, one would prefer to feed larvae their natural diet, which is characterized by a wide diversity of nutritious live organisms. Although not a "natural" food, *Artemia* have been successfully used by many as food for larval organisms. It is perhaps surprising that such success could be attained with a food from such an unusual (*i.e.* hypersaline) environment. Some recent experiences suggest that the use of *Artemia* does not absolutely guarantee success (Sorgeloos, 1980 ; Simpson *et al.*, 1983). Explanations for and remedies to this variable success will be covered in this review through an analysis of the larval organism's requirements for food (Fig. 1). A more complete review on the nutritional value of *Artemia* was presented by Léger *et al.* (1986).

REQUIREMENTS OF A FOOD FOR LARVAL ORGANISMS

FOR THE CULTURIST	FOR THE PREDATOR
consistent availability	physical requirements :
	– clean
simple production procedures	– no alien materials
	– no diseases
euryplasticity and versatility :	– acceptable
– salinity/temperature tolerance	– perceptible
– handling	– catchable
– disinfection	– palatable
– different sizes and forms	– ingestible
– use as a carrier	
	nutritional requirements :
	– digestible
	– nutrient requirements

FIG. 1. Summary of the requirements a food must meet to be a suitable diet for larval organisms.

Practical requirements for the culturist

A food organism must first meet the nutritional needs of the predator. In addition, other practical requirements have to be met to satisfy the culturist. The consistent availability of food organisms is of utmost importance for continuous cultures. In this respect, *Artemia* outvies all other food organisms since it is available as an off-the-shelf food in the form of dormant cysts. From those cysts, nauplii are obtained through simple hatching procedures (Sorgeloos *et al.*, 1983). Ideally, a food organism should also be hardy and easily cultured. *Artemia* nauplii fulfill this last requirement quite well, since they are very tolerant to various culture environments, they resist even rough handling, and may be disinfected resulting in a biologically uncontaminated life food (Sorgeloos *et al.*, 1983).

The wide size range of *Artemia* and their different physical forms (Fig. 2) make them very versatile in the use. Since they are easily cultured, *Artemia* nauplii and later stages may be fed according to the growth and development of the predator. Also a smaller food particle may be used in the form of decapsulated cysts, which are some 50 % smaller than freshly-hatched nauplii and have several other advantages : 1) they are disinfected and separated from the cyst shells during the decapsulation process (Sorgeloos *et al.*, 1977) ; 2) the hatchability of the embryos is improved (Bruggeman *et al.*, 1980), so that otherwise unhatchable cysts can be valorized ; 3) the energy content is higher (Vanhaecke *et al.*, 1983), resulting in a higher naupliar biomass production per gram of cysts and a smaller, more energy-rich food particle for the larval organism.

A last example of the versatility of *Artemia* as a food consists in the possibility of using *Artemia* (nauplii or adults) as a carrier for components which are otherwise difficult to administer to fish and crustacean larvae. Indeed, essential nutrients, pigments, prophylactics, and therapeutics may be bioencapsulated in *Artemia* and introduced into the consumer organism (Fig. 3) (Léger *et al.*, 1987).

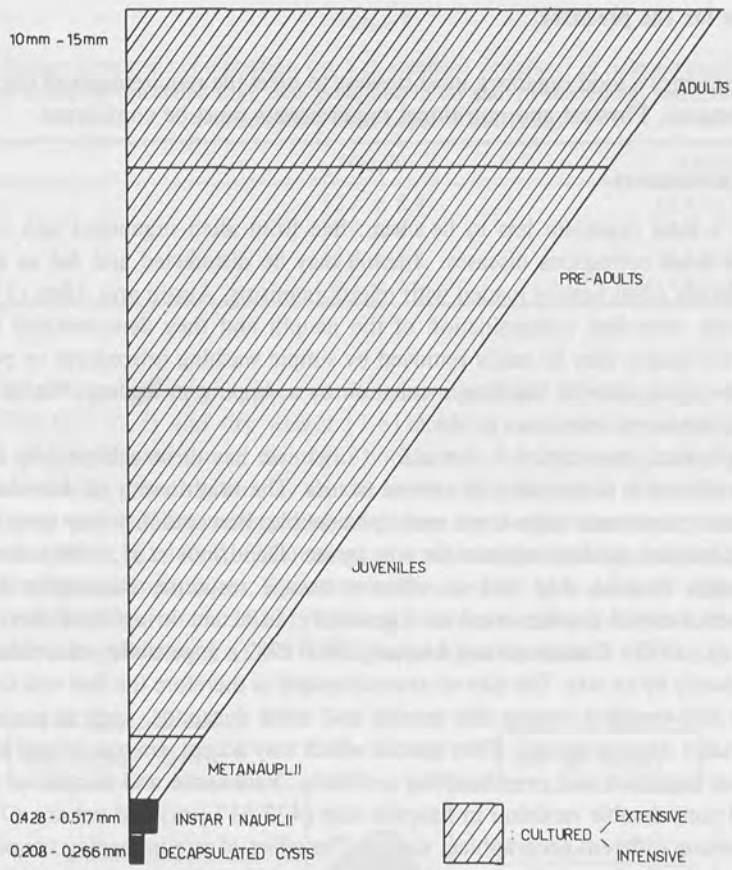

FIG. 2. The size ranges of various *Artemia* life stages.

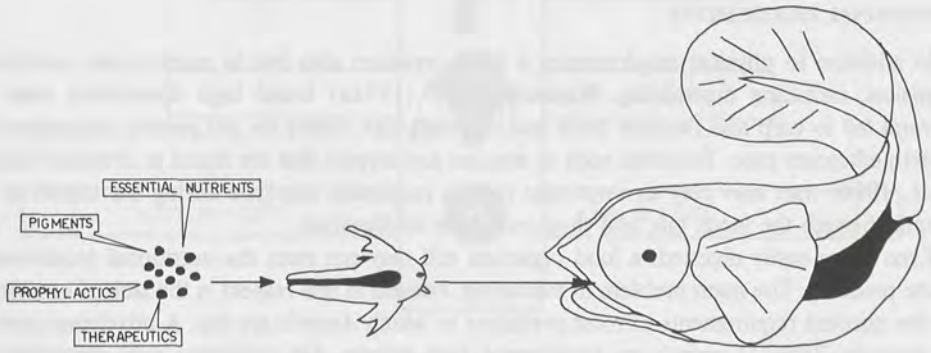

FIG. 3. Schematic outline of the technique of using *Artemia* as a carrier for various nutritional, prophylactic, and therapeutic components.

Requirements for the predator

The characteristics a food organism must possess to meet the requirements of the predator are of great importance. Physical and nutritional requirements must be considered.

PHYSICAL REQUIREMENTS

Physically, a food organism has to be clean, free from alien organisms and materials and especially free from contagious diseases. *Artemia* may be disinfected and fed as a clean food. Although cysts are often heavily loaded with microorganisms, Austin and Allen (1981) did not find an intimate microbial contamination of the nauplii and they demonstrated that bacteria surrounding the nauplii may be easily removed by simple washing procedures or even better by disinfecting the cysts prior to hatching incubation by a dipping procedure. No direct evidence exists for *Artemia*-borne infections in larvae.

A second physical requirement is that a food organism has to be accepted by the predator. Acceptability of food is determined by several factors. The bright color of *Artemia* nauplii and their continuous movement make them easily perceptible. Perceptibility may even be enhanced by staining techniques, as demonstrated for sole larvae (Dendrinos *et al.*, 1984). *Artemia* nauplii are easily caught because they lack an effective escape response. Palatability is apparently adequate, since *Artemia* is often used as a gustatory attractant in artificial diets (Barahona-Fernandes *et al.*, 1977 ; Gatesoupe and Luquet, 1981/1982). Ingestibility of a palatable food is governed primarily by its size. The size of *Artemia* nauplii is therefore the first real consideration. Indeed, most first-breeding marine fish species and some decapods, such as penaeids, cannot ingest (or handle) *Artemia* nauplii. Even species which may accept *Artemia* nauplii as a first food sometimes face ingestion and prey-handling problems. Vanhaecke and Sorgeloos (1980) have demonstrated considerable variation in naupliar size (422-517 μm) and volume (7 638-13 604 $\times 10^6$ μm^3) among different geographical strains. The effect of size in feeding nauplii to fish has been described by Beck and Bengtson (1982). They fed freshly-hatched nauplii from eight different *Artemia* strains to Atlantic silverside (*Menidia menidia*) larvae. The correlation between the size of nauplii and mortality of the fish larvae indicated that 20 % mortality or more could be expected when nauplii larger than 480 μm were fed as a first food.

NUTRITIONAL REQUIREMENTS

In addition to physical requirements, a food organism also has to meet certain nutritional requisites, including digestibility. Watanabe *et al.* (1978a) found high digestibility rates for *Artemia* fed to carp and rainbow trout and reported high values for net protein utilization and protein efficiency ratio. Enzymes such as amylase and trypsin that are found in *Artemia* (Samain *et al.*, 1980) may also play an important role in enzymatic autolysis during the transit of the nauplii through the larval gut, and thus contribute to digestion.

Even when easily digested, a food organism still may not meet the nutritional requirements of the predator. The main problem in evaluating *Artemia* in this respect is the lack of knowledge on the nutrient requirements of most predators to which *Artemia* are fed. A proximate analysis of *Artemia* (Table I) reveals an equilibrated high-protein diet indicating that macronutrient requirements are probably satisfied for most predators. However, several investigators reported considerable variation in larval culture success (Léger *et al.*, 1986).

TABLE I

Average proximate composition (in % ± standard deviation) of *Artemia* nauplii and adults as calculated from data presented in 26 and 15 references, respectively (data compiled from Léger *et al.*, 1986)

	Nauplii	Adults
Protein	52.2 ± 8.8	56.4 ± 5.6
Lipid	18.9 ± 4.5	11.8 ± 5.0
Carbohydrate	14.8 ± 4.8	12.1 ± 4.4
Ash	9.7 ± 4.6	17.4 ± 6.3

Variation in larval growth rate has been attributed to significant differences in individual energy content (0.0366-0.0725 J) and dry weight (1.61-3.33 µg) of *Artemia* nauplii from different geographical origin (Vanhaecke *et al.*, 1983). Selection of high-energy strains is therefore recommended. Varying growth rates may also be attributed to the use of older unfed instar stages which contain up to 39 % less energy and 34 % less dry weight than freshly-hatched nauplii (Fig. 4) (Vanhaecke *et al.*, 1983). This energy and dry weight loss can be avoided by storing instar I nauplii at lower temperatures, since they survive well for 24 h at 2-4 °C without significant losses in dry weight (Léger *et al.*, 1983). This cold storage technique further allows a complete automation of feeding, permitting a 24 h feeding of energy-rich instar I. Starved nauplii not only

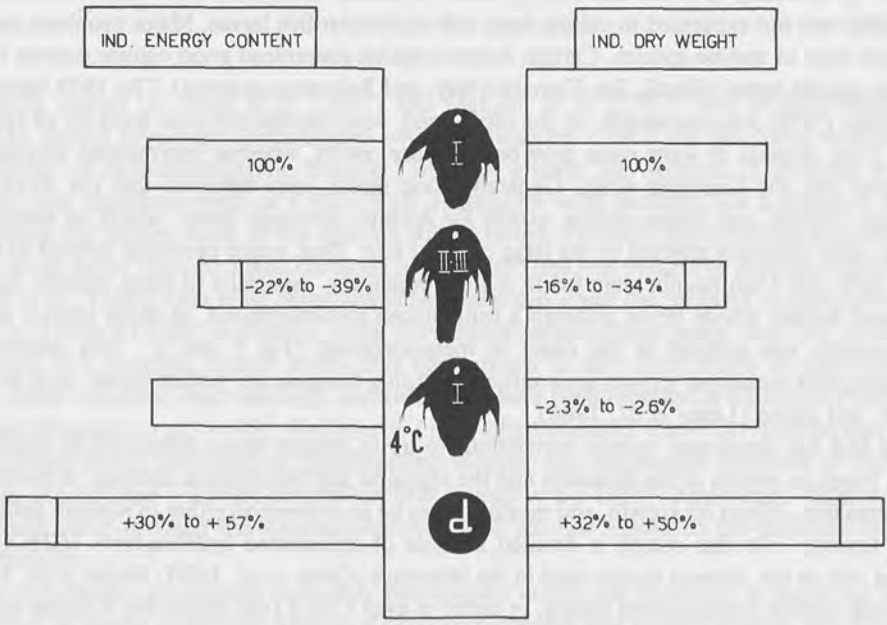

FIG. 4. Change in energy content and dry weight of different forms of *Artemia* (newly-hatched instar I nauplii are considered to have 100 % values for those variables). The percent decrease or increase from 100 % is shown for successively instar II-III metanauplii, cold stored instar I nauplii, and decapsulated cysts.

contain less energy and dry weight, making them less suited to meet the requirements of the predator, but they are also less visible, larger, and faster swimming, and therefore less acceptable. Starved nauplii have a lower free amino acid content (Dabrowski and Rusiecki, 1983), which may reduce their digestibility. All these negative factors may be reflected in poor growth of the larval predator. Decapsulated cysts, on the other hand, constitute the highest energy form of *Artemia* and are preferably used except when a predator feeds only on moving prey.

Besides variable growth rates, other problems have been attributed to the use of *Artemia*. With the availability of different geographical strains of *Artemia*, a relationship has been found between the use of particular strains and the appearance of various symptoms in fish and crustacean larvae, such as lethargy, lack of coordination, abnormal development, problems at metamorphosis, abnormal pigmentation, and even mortality (Wickins, 1972 ; Campillo, 1975 ; Beck *et al.*, 1980 ; Johns *et al.*, 1980 ; Klein-MacPhee *et al.*, 1980, 1982). Several authors have tried to explain these observations and have formulated diverging and sometimes contradictory explanations. For example, Bookhout and Costlow (1970) suspected that high levels of DDT caused the problems, but Wickins' (1972) research suggested that a nutritional deficiency was involved.

International Study on *Artemia*

The elucidation of the nutritional variability of *Artemia* was one of the major concerns of the participants in the International Study on *Artemia* (ISA) (Sorgeloos, 1980). Nutritional bioassays with several larval fish and crustacean species fed various *Artemia* strains confirmed previous reports of variability in food value among different strains of *Artemia* (Table II). However, this variability was not expressed in culture tests with freshwater fish larvae. Major problems seemed to occur only in marine species. Certain *Artemia* strains guaranteed good culture success for all marine species tested (Brazil, San Francisco Bay, and Reference *Artemia*). The 1978 batch San Pablo Bay (SPB) *Artemia* nauplii, on the other hand, were consistently poor food for all species. Also, Utah *Artemia* in some cases gave poor culture results, whereas intermediate success was obtained with the Canadian strain. Generally good results were obtained with the Australian, Chinese, French, and Italian strains, except for Atlantic silverside larvae, which, as mentioned before, were adversely affected by the large naupliar size. Thus, major problems seemed to occur when SPB and Utah nauplii were fed to marine organisms, especially to those species, such as crab and flatfish, whose larvae undergo a pronounced metamorphosis. In those species almost all mortality was suffered at the onset of metamorphosis (Fig. 5 and 6). This information coincides with numerous reports from authors culturing decapod and flatfish larvae, such as crab, turbot, and plaice (Léger *et al.*, 1986).

The ISA has developed various correlations to try to explain strain differences in nutritional value, based on results of the bioassays and the chemical and biochemical analyses of the strains. Abnormalities, effects on growth, and mortality may be an expression either of nutrient deficiency or of toxicity. For this reason a detailed analysis of chlorinated hydrocarbons (CHCs) was carried out on the *Artemia* strains used in the bioassays (Olney *et al.*, 1980 ; Seidel *et al.*, 1982). The most heavily contaminated strains, in terms of total CHCs (the Italian and Chinese strains) gave, however, excellent results in the bioassay. On the other hand, Utah ranked among the cleanest strains. The only similarity between SPB and Utah samples was their higher level of dieldrin. Furthermore, SPB had the highest level of chlordane and high molecular PCBs.

TABLE II

Summary of nutritional bioassay results for several categories of aquatic organisms fed 10 geographical strains of *Artemia*

			Artemia geographical strain									
			Australia	Brazil	Canada	China	France	Italy	Utah	San Pablo Bay	San Francisco Bay	RAC
Marine	Crustaceans	Metamorphosis	+	+	±	+	+	+	-	-	+	+
		----------	+	+	±	+	+	+	+	-	+	+
	Fish	Metamorphosis	+	+	+	+	+	+	-	-		+
		----------	±	+	±	±	±	-	±	-	+	+
Freshwater fish		----------	+	+	+	+	+	+	+	+	+	

Follow-up studies by Johns *et al.* (1981) and McLean *et al.* (1987) have demonstrated that Brazilian nauplii purposely contaminated with the suspected CHCs did not cause mortality in crab larvae or post-metamorphic flounder, although growth was reduced in flounder fed on *Artemia* with moderate levels of CHCs. CHCs therefore were probably not a principal factor controlling the dietary value of the *Artemia* strains tested. Probably heavy metals were also an insignificant factor since no metal common to SPB and Utah was present in dramatic high levels (Olney *et al.*, 1980). One must nevertheless be concerned about the problem of toxic materials in *Artemia* because CHC and heavy metal contamination is anthropogenic and thus subject to variations. For example, copper levels have been shown to vary considerably in Utah *Artemia* (Blust, pers. commun.) and very high pesticide levels have been reported in a batch of Philippine *Artemia* (Simpson *et al.*, 1983). Shelbourne (1968) hypothesized that Utah *Artemia* may have accumulated toxins produced in dinoflagellate blooms, but measurable amounts of paralytic shellfish poison have not been detected in SPB (Olney *et al.*, 1980). Apparently, differences in nutritional value between the *Artemia* sources tested are not related to the presence of toxic contaminants.

The hypothesis that nutrient deficiency may explain nutritional variability is borne out by the difference in results of feeding *Artemia* strains to freshwater and marine species, which indeed have different dietary requirements. In-depth biochemical profiles of the different strains tested showed first that differences in amino acid profile could not explain differences in culture results (Seidel *et al.*, 1980). All strains were found to meet the essential amino acid requirements for

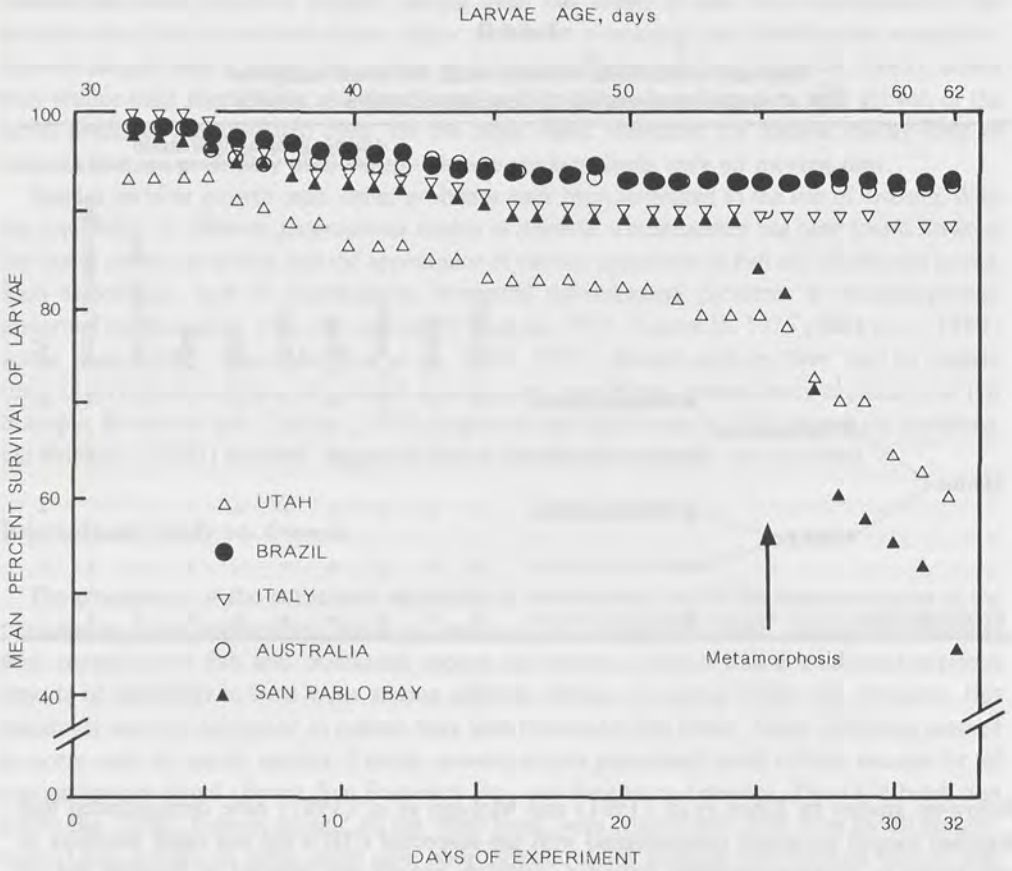

FIG. 5. Percent survival of winter flounder larvae fed on five geographical strains of *Artemia* (from Klein-MacPhee *et al.*, 1980).

chinook salmon, though methionine appeared to be the first limiting amino acid. Similarly, the varying culture success could not be explained by differences in carotenoid (Soejima *et al.*, 1980), mineral (Watanabe *et al.*, 1978a), caloric or lipid content (Schauer *et al.*, 1980). However, pronounced differences were found in fatty acid profiles. As compared to other strains, Utah and especially SPB *Artemia* contained high levels of 18:3ω3 and particularly low levels of 20:5ω3 (Schauer *et al.*, 1980 ; Seidel *et al.*, 1982). The relative lack of 20:5ω3 may explain the poor results in feeding the Utah and SPB strains to marine organisms. The highly unsaturated fatty acid (HUFA) 20:5ω3 is known to be essential for marine fish and crustacean larvae (Teshima, 1978 ; Yone, 1978 ; Kanazawa *et al.*, 1979). Canadian nauplii, in spite of high levels of 20:5ω3, provided intermediate results in the bioassays, which indicates that for this strain other factors, possibly energetic ones, may be involved.

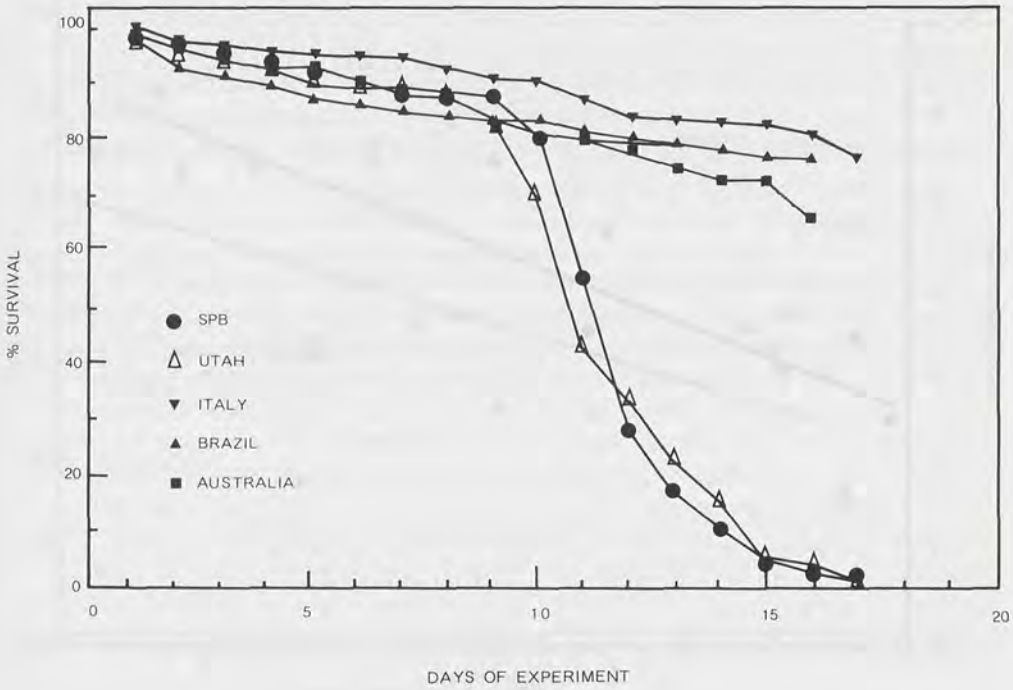

FIG. 6. Percent survival of mud crab larvae fed on five geographical strains of *Artemia* (from Johns *et al.*, 1980).

Importance of essential fatty acids

Léger *et al.* (1985b) further studied the relationship between 20:5ω3 level and nutritional value of *Artemia* by evaluating different batches of the San Francisco Bay strain together with Reference *Artemia* and SPB as, respectively, positive and negative controls. Levels of 20:5ω3 varied considerably among different batches within the same strain. Batches with high 20:5ω3 levels yielded high biomass production in culture tests with mysids, while batches with low 20:5ω3 levels consistently yielded less biomass (Fig. 7). CHC analyses were also performed on these different batches and again considerable differences were found, but could not be correlated with the biomass figures (Fig. 8). From these studies we may conclude that the content of the essential fatty acid 20:5ω3 seems to be the most important factor determining the nutritional value of *Artemia* nauplii to marine organisms. This is supported by the observation that Utah and San Pablo Bay nauplii provided good survival of freshwater fish which do not require highly unsaturated fatty acids such as 20:5ω3 in their diet.

Watanabe *et al.* (1978b) classified *Artemia* strains into marine type *Artemia*, which contain high levels of 20:5ω3, and freshwater type *Artemia*, which contain low levels of 20:5ω3. They obtained good survival of red seabream larvae fed with the marine type nauplii and poor survival of those fed the freshwater type. However, when the freshwater type nauplii were fed on

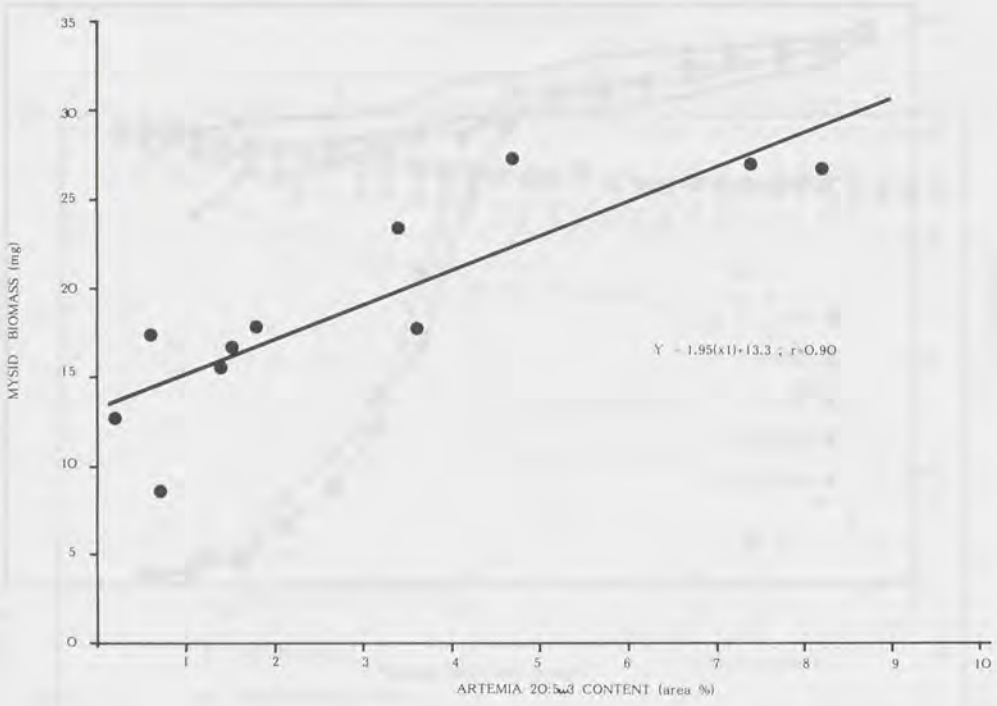

FIG. 7. Linear relationship between the 20:5ω3 content of several *Artemia* collections from San Francisco Bay origin and the biomass of mysids to which the *Artemia* were fed (data from Léger *et al.*, 1987).

20:5ω3-rich diets, such as marine *Chlorella* or ω-yeast, before they were fed to the fish, superior survival rates of the seabream larvae were achieved. Watanabe *et al.* (1982) have demonstrated that 20:5ω3 could be incorporated into *Artemia* by feeding them for 24 h on 20:5ω3-rich diets. Nauplii prefed ω-yeast can also contain significant levels of 22:6ω3 and red seabream larvae did best on these nauplii. Similarly, Léger *et al.* (1985a) have shown that feeding a HUFA-enrichment diet to San Pablo Bay 1628 *Artemia* increased its levels of 20:5ω3 and 22:6ω3 and markedly enhanced its nutritional value for penaeid shrimp larvae. The nutritional improvement of fatty-acid-enriched Utah *Artemia* has also been demonstrated for mysids, two penaeid species, and seabass larvae (Van Ballaer *et al.*, 1985 ; Amat *et al.*, 1987 ; Léger *et al.*, 1987).

If the abundance of certain essential fatty acids governs the nutritional value of *Artemia* nauplii, what exactly determines their respective levels in *Artemia*? Schauer and Simpson (1985) have demonstrated that *Artemia* have a limited need to produce their own 20:5ω3 and Millamena *et al.* (1985) reported that *Artemia* HUFA levels strongly resembled those of the algal diets on which they were fed. In a recent experiment, Lavens (pers. commun.) has cultured *Artemia* nauplii containing about 5 % 20:5ω3 in a controlled cyst production unit (Lavens and Sorgeloos, 1984). Two different diets were used, one containing 6.7 % and the other one only 0.7 % 20:5ω3. The cysts produced by adults grown on the 20:5ω3-rich diet contained high levels of 20:5ω3 while the others contained very low levels of this fatty acid. This experiment clearly demonstrated that *Artemia* cysts reflect 20:5ω3 levels of the diet available for the parental population.

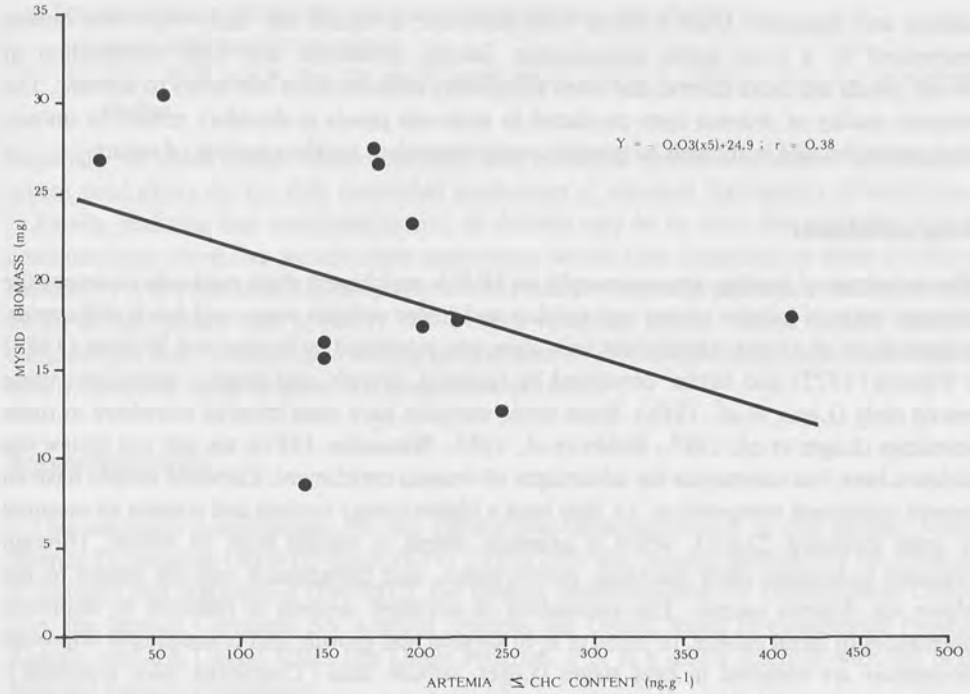

FIG. 8. Linear relationship between the chlorinated hydrocarbon content of several *Artemia* collections from San Francisco Bay origin and the biomass of mysids to which the *Artemia* were fed (data from Léger *et al.*, 1987).

If these results can be extrapolated to wild populations one can deduce that different food conditions in *Artemia* ponds and lakes probably explain differences in fatty acid profile between *Artemia* strains – and even within the same strain. Compilation of data from literature and our own analyses (Table III) (Léger *et al.*, 1986) show that 20:5ω3 levels may indeed vary considerably among and within strains. Variability is particularly great in strains produced in solar saltworks, *e.g.* San Francisco Bay, Brazilian, and Chinese *Artemia.* Variability is small in

TABLE III

Intra-strain variability of levels of the essential fatty acid 20:5ω3 in *Artemia.*
Data are given as percentage of total fatty acid methyl esters and represent analyses of samples taken over several seasons or years (data compiled from Léger *et al.*, 1986)

Artemia geographical strain	20:5ω3 content (area %)
San Francisco Bay	0.3 - 13.3
Brazil	3.5 - 10.6
China	1.3 - 15.4
Canada	5.2 - 9.5
Utah – southern arm	2.7 - 3.6
– northern arm	0.3 - 0.4

Canadian and especially Utah *Artemia*, both produced in inland salt lakes which are mostly characterized by a more stable environment. Salinity conditions and food composition in solar-salt ponds are more diverse and often completely different from one pond to another. The nutritional quality of *Artemia* cysts produced in solar-salt ponds is therefore subject to uncontrolled variability and is difficult to predict, being dependent on the caprices of nature.

Artemia enrichment

The technique of feeding *Artemia* nauplii on HUFA enrichment diets markedly increases the nutritional value of inferior strains and batches and hence reduces strain and batch differences. The application of *Artemia* enrichment with algae was pioneered by Forster and Wickins (1967) and Wickins (1972) and further developed by Japanese, French, and Belgian researchers using prepared diets (Léger *et al.*, 1986). Since those methods have been covered elsewhere in these proceedings (Léger *et al.*, 1987 ; Robin *et al.*, 1987 ; Watanabe, 1987), we will not review the techniques here, but summarize the advantages of *Artemia* enrichment. Enriched nauplii have an improved nutritional composition, *i.e.* they have a higher energy content and contain all essential fatty acids including 22:6ω3, which is generally absent in nauplii from all strains. Through enrichment techniques other nutrients, prophylactics, and therapeutics may be passed to the predator via *Artemia* nauplii. The application of enriched *Artemia* is reflected in improved performances in larval culture, in terms of both survival and growth, and consequently improved performances are obtained in later stages (Léger, unpubl. data ; Chamorro, pers. commun.). Larvae fed on enriched *Artemia* are indeed healthier and more resistant to stressful conditions, such as infections, weaning of fish, or transfer of fry/postlarvae from hatchery tanks to nursery ponds. The only disadvantage of using enriched *Artemia* is their larger size, which may be a problem for the early larval stages of the predator. If size is indeed a problem, freshly-hatched high-quality instar I nauplii may be fed for the first few days, followed by a gradual switch to enriched metanauplii as soon as the predator's size permits ingestion of larger particles. Optimized enrichment procedures may also reduce the disadvantage of size by obtaining similar enrichment levels in less time (Léger *et al.*, 1987). Similar enrichment techniques may also be applied for juvenile and adult *Artemia* which may be used as a carrier for essential nutrients and other components to be administered to postlarval shrimp, juvenile fish, and lobster larvae.

Conclusions and recommendations

To summarize, *Artemia* is an excellent food for a wide variety of cultured marine and freshwater organisms. The major constraint of *Artemia* as a food organism for marine predators is its variable nutritional quality. However, we recommend some measures to remedy this problem :

- for the problem of size and variable energetic content between strains and instar stages, one should :
  a) select suitable strains ;
  b) use freshly-hatched first instar nauplii (*i.e.*, through application of optimized hatching procedures, cold storage of nauplii, and optimized feeding strategies) ;
  c) when possible, use decapsulated cysts.

– for the problem of variable nutritional composition, one should :
  a) apply enrichment techniques ;
  b) select high quality lots for early larval stages and as a reference material in ecological studies.

Especially for those studies where reproducibility of culture results is of utmost importance, an urgent need exists for the fully controlled production of standard high-quality *Artemia* cysts.

Finally, realizing that nutritional quality of *Artemia* may be so poor that mortality of certain predators may result, we wonder how mariculture would have developed if Seale (1933) and Rollefsen (1939) had used a poor quality *Artemia* source when they pioneered the use of *Artemia* for larval fish culture back in the 1930's. Despite variability in size, caloric content, nutritional composition, and contaminants, among geographical strains, *Artemia* has proven to be the most widely used and successful diet for aquaculture purposes.

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The use of *Artemia* in fish and crustacean farming in Japan

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Content

Abstract

At present more than 20 fish and crustacean species are mass-produced in governmental fish farming centers and private hatcheries in all the prefectures in Japan. The farmed fry are either released into coastal waters or used for cultivation to commercial size. With the advance of rearing techniques and methods to mass produce live foods, the number of cultured species is increasing every year.

In the early 1970's the brine shrimp *Artemia* has been used most extensively, especially for the mass propagation of Kuruma prawn *Penaeus japonicus*. Later on, when the rotifer *Brachionus plicatilis* was also found to be very suitable as live food for larval marine organisms, a gradual decline in the use of brine shrimp occurred. Since the end of the 1970's very reproducible techniques in the fry production of various fish species were developed and the use of *Artemia* has increased again and is till increasing every year. Recently, the total amount of *Artemia* cysts imported yearly from various countries reached about 38 tonnes. These are imported mainly from China, the USA, and Brazil.

The dietary value of *Artemia* nauplii to fish and shrimp larvae differs from strain to strain and depends upon the geographical origin. The dietary value of the nauplii has been demonstrated to be mainly governed by their fatty acid composition which is also affected by the season at cyst harvest. The class of essential fatty acids (EFA) contained in *Artemia* is the principal factor determining the variation in its food value for fish. Similar findings were also demonstrated for rotifers. The EFA composition of *Artemia* nauplii reflects their dietary history in terms of algal blooms to which they have been exposed. Recently it has been shown, however, that the dietary value of brine shrimp nauplii can be effectively manipulated. This was accomplished by using two methods. First, the direct method, which is essentially based on the addition of an emulsion of lipids rich in ω3-HUFA, to the rearing environment of the brine shrimp nauplii. Second, the indirect enrichment, which is based on the addition of ω-yeast to the brine shrimp diets.

The decreased reliance upon *Artemia* as a sole larval food in aquaculture seems to be dependent upon the development of formulated microdiets. This is being gradually accomplished in Japan, *e.g.* artificial microdiets have recently become available at semi-practical hatchery level with very encouraging results with regard to larval growth and survival of marine organisms.

Introduction

In recent years, the total production of Japanese aquaculture from both freshwater and seawater aquaculture has exceeded 1.0 million tonnes, being only 10 % of the total production of Japanese fisheries, but about 20 % of the total sales. In Japan, aquaculture and especially mariculture has been expanding very rapidly during the last decade. The recent expansion of mariculture is based on newly developed technologies such as the so-called "sea ranching". Its principle is to effectively utilize the natural productivity of the sea by replenishing diminished resources of fish and shellfish of high commercial value by the release of seedlings into coastal regions. The Japanese government promoted this project because of its predicted impact on the reestablishment of coastal fisheries. Since the implementation of this program, coastal fisheries managed to counterbalance the big gap between supply and demand for fisheries products. This became especially important after the worldwide establishment of the 200 miles fishing zone. Twelve National Fish Farming Centers together with about 40 Prefectural Fish Farming Centers have been set up in the coastal area of Japan. The success of sea ranching in Japan is a direct result of the technological developments in these centers for the mass-propagation of seed of various species of fish, shellfish, and crustaceans. The principal technological developments in these centers are in the areas of rearing techniques, methods for mass production of live foods, improvement of their nutritional quality and facilities. At present more than 20 fish and crustacean species are mass-produced in governmental fish farming centers and private hatcheries in all the prefectures of Japan for the purpose of release into coastal waters or for cage or pond rearing to commercial size.

The mass propagation of marine species has been supported by marked technological advancements in such areas as induced and natural spawning, larval rearing, and methods for mass-culture of living organisms. The suitability of the rotifer *Brachionus plicatilis* as the initial live food for hatched larvae was discovered in 1965. The establishment of mass-culture technique of rotifers in the 1970's, made fish larval production possible and as a result mass seedling techniques developed very rapidly. Among the various live foods used in the mass propagation of juvenile fish, *Artemia* nauplii appeared to be essential for rearing larval fish and especially for Kuruma prawns *Penaeus japonicus*. More recently, however, rotifers were found to be very suitable as shrimp larval food and became in many cases a good replacement for *Artemia*. With the improvements in the mass rearing techniques and the increase in the number of species which are mass produced in Japan, the use of *Artemia* nauplii has been gradually increasing again in the 1980's.

This paper deals mainly with the present situation and problems in the use of *Artemia* in Japanese aquaculture.

Maricultured species and their food schedule in Japan

At present more than 20 fish and shrimp species are mass-produced by almost completely reproducible systems in governmental fish farming centers and private hatcheries. The number

of species exceeds 60 if one includes the species reared on a laboratory scale, and this number is still growing every year. The main species of fish and the amount of seedlings produced in the governmental fish farming centers are listed in Table I.

TABLE I

Number of fry of main fish species produced in 18 prefectures in southern Japan in 1982

Species		Number of seed produced
Common name	Latin name	
Red sea bream	*Pagrus major*	19 300 000
Black sea bream	*Acanthopagrus schlegeli*	5 820 000
Ayu fish	*Plecoglossus altivelis*	4 520 000
Japanese flounder	*Paralichthys olivaceus*	6 060 000
Mud dab	*Limanda yokohamae*	1 100 000
Puffer	*Takifugu rubripes*	16 310 000
Rockfish	*Sebastes schlegeli*	140 000
Marbled rockfish	*Sebastiscus marmoratus*	120 000
Japanese sea bass	*Lateolabrax japonicus*	120 000
Rabbit fish	*Siganus fuscescens*	100 000
Yellowtail	*Seriola quinqueradiata*	60 000

KURUMA PRAWN

Among the marine species cultured in Japan, Kuruma prawn *Penaeus japonicus* production scores the highest proportion with more than 2.5 billion seedlings per year. Kuruma prawn culturing was initiated already 20 years ago, *i.e.* the larvae are usually produced in 200 tonnes tanks, stocked with 1-2 million larvae. In the course of one season (from April to September), it is possible to produce 3-4 batches of prawn seedlings. The spawners are usually obtained from the sea. As a result one of the most important problems in the production of Kuruma prawn is the provision of cultured broodstock.

Mass-cultured diatoms are given to hatched larvae as the initial diet, followed by rotifers, *Artemia* and formulated feeds according to the larval stage until postlarvae. *Artemia* nauplii have been used most extensively as food for larvae until rotifers were found to be a suitable substitute. In recent small-scale experiments, Kanazawa (1985) demonstrated that Kuruma prawn larvae can be reared to post-larvae solely on formulated microdiets with a survival of 75 %. The micro-alga *Tetraselmis tetrahele* has recently been introduced to Japan as a food suitable for the larvae and could eventually replace diatoms (Fukusho, pers. commun.).

SEA BREAM

Similarly to the previous species, red sea bream *Pagrus major* is one of the most valuable species in Japan. Its mass production was first tried in the 1920's, leading to full-scale research in the 1950's. Although seed production of marine fish has a history of only 20 years in Japan, red sea bream is now the second biggest in the total amount of larvae produced, *i.e.* about

25 million juvenile fish in 1982. Cultured broodstock of both male and female are used as spawners. In the food schedule for larval rearing of red sea bream, rotifers have been used most extensively and are very important as the initial live food. When fish reach 7 mm or more in body length, rotifers become too small as food and marine copepods (*e.g. Tigriopus, Acartia, Oithona* and *Paracalanus*) are fed to the larvae. When there is a shortage of marine copepods, brine shrimp *Artemia* are used as food for the larvae. Others *Sparidae*, such as black sea bream *Acanthopagrus schlegeli* and Japanese parrot fish *Oplegnathus fasciatus*, are also mass produced according to the same food schedule as red sea bream. Formulated microdiets when fed to larvae together with rotifers have to some extent proven to be acceptable.

Flatfish

Recently the production of juvenile flatfish has increased markedly. Mud dab *Limanda yokohamae* and Japanese flounder *Paralichthys olivaceus* are the main species. Cultured and natural broodstock are used as spawners. In 1982 more than 7 million juvenile flatfish, ranging from 1 to 10 cm in body length, were released into coastal waters or used for cultivation to commercial size. Rotifers are used as the initial food ; when the larvae reach about 5 mm in body length rotifers are supplemented with *Artemia* nauplii. As a result of marked increase of the total production of juvenile flatfish the use of *Artemia* in mass propagation of flatfish has been gradually increasing every year. However, serious problems with abnormal flatfish body coloration seems to be closely associated with the use of *Artemia* of some geographical strains (see further).

Yellowtail

The total production of yellowtail *Seriola quinqueradiata* in captivity recently attained near 180 000 tonnes, being about five times higher than those supplied by the fisheries. Hatchery production of yellowtail fry is still very limited as wild juveniles are used for net cage culture. As a result live foods such as rotifers and *Artemia* are not much required at present for yellowtail culture.

Trends in *Artemia* cyst imports in Japan

Recently, the total amount of *Artemia* cysts imported from various countries reached about 40 tonnes, *i.e.* about 40 % of the total world supply (Sorgeloos, 1980). As shown in Fig. 1 imports are mainly from China, USA and Brazil ; consumption of *Artemia* cysts in aquaculture has markedly increased during recent years, being about four times higher in 1984 than in 1980. This is a reflection of the rapid increase of seedling production of maricultured species. In Japan, the import of *Artemia* cysts from Brazil and the USA has gradually decreased since 1981, respectively 1983. On the other hand imports from China have increased since 1980 ; *i.e.* today about 76 % of the total amount of *Artemia* used in seed production are from China. The reduced import to Japan of the two former *Artemia* strains is probably due to the rapid expansion of mariculture in the world.

The evolution of the prices for imported *Artemia* cysts is shown in Fig. 2. The increased requirements for cysts in mariculture resulted in a yearly increase in price, which is highest for *Artemia* from Brazil and lowest for those from China. However, the average value of the total imported *Artemia* has been gradually decreasing from about 40 US$/kg in 1980 to 25 US$/kg

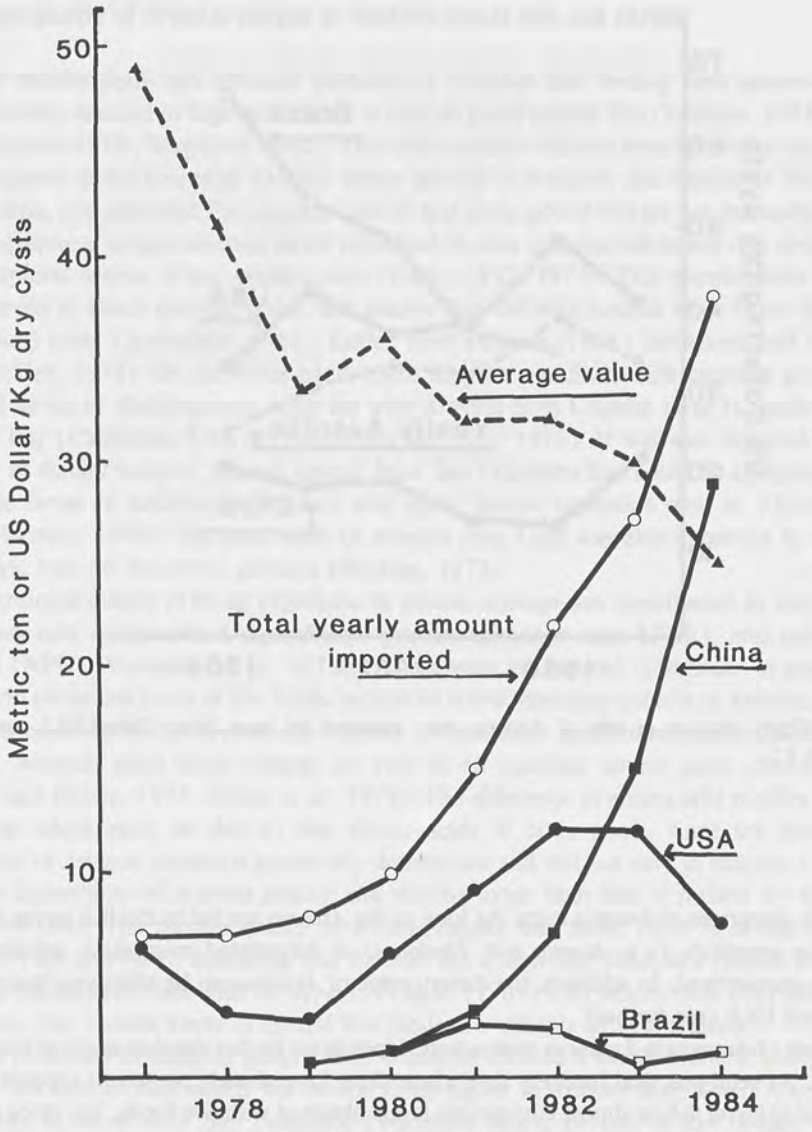

FIG 1. Recent trends in *Artemia* cyst imports in Japan.

in 1984 as a result of competition among various geographical sources. Once they reach the local market, the price of cysts increases markedly and hatcheries often pay about 60 US$/kg. The cheapest cysts imported in 1984 were about 110 kg from Argentina, being sold at 11 US$/kg. The big difference in prices among geographical strains seems to be governed by demand and supply in terms of the available amount, and the quality. Cyst hatchability has improved and has become more reliable by the application of new harvesting techniques. At present the nutritional quality of nauplii in terms of the class and content of essential fatty acids (EFA) seems to have

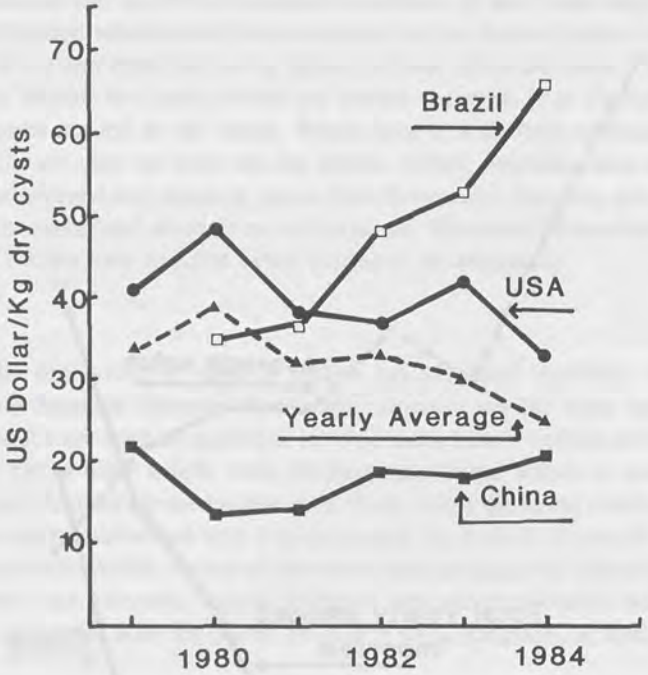

FIG. 2. Yearly changes in cost of *Artemia* cysts imported in Japan from China, USA, and Brazil (1 US$=250 ¥).

no effect on the prices of *Artemia* cysts. As long as the *Artemia* are fed to the fish larvae together with marine copepods (*e.g. Acartia* and *Tigriopus*) or formulated microdiets, no nutritional problem is encountered. In addition, the dietary value of *Artemia* can be effectively improved by feeding them EFA (see further).

Future use of *Artemia* in Japanese mariculture depends on further developments of formulated microdiets. At semi-practical hatchery level these diets have already proven to support growth and survival of larval fish at almost comparable levels obtained with live foods. The price of larval diets, live or inert, is probably the most important factor in the mass propagation of juvenile fish. The cost for the production of 1 kg of dry biomass of rotifers was estimated at US$ 2 000 (Gatesoupe and Luquet, 1981) including facilities, heating, manpower, etc.). On the contrary, the present price of commercially available artificial microdiets in Japan is only US$ 80-175 kg. When compared with live or inert feeds, *Artemia* nauplii obtained from cysts are comparable in price to formulated microdiets, easier in availability than rotifers and by far better than microdiets in terms of their effect on water quality in the rearing tanks. However, live foods are clearly inadequate in terms of available quantity to supply the present and future needs in mariculture at the world level. In this regard, the development of formulated microdiets to replace live foods is an urgent need for further expansion of aquaculture.

Nutritional quality of *Artemia* nauplii as food for larval fish and shrimp

Already several years ago different publications revealed that feeding with *Artemia* nauplii alone frequently resulted in high mortalities in various larval marine fish (Fushimi, 1971 ; Fujita, 1973 ; Kitajima, 1978 ; Sorgeloos, 1980). This phenomenon differed from species to species and was also related to the source of *Artemia.* Some species of flounder, one species of mullet *Liza haemotocheila,* one salmonid *Plecoglossus altivelis* and some gobiid fish are not markedly affected by the geographical origin, whereas larval yellowtail *Seriola quinqueradiata* are very sensitive for the geographical source of the *Artemia* used (Fujita, 1972, 1973). This phenomenon has also been observed in larval prawns, crabs, and marine fish fed with nauplii from Great Salt Lake (Utah, USA) cysts (Slobodkin, 1968 ; Little, 1969 ; Reeve, 1969 ; Bookhout and Costlow, 1970 ; Wickins, 1972). On the other hand, there were no significant differences in growth and survival of larvae of *Palaemonetes pugio* fed with *Artemia* from Chaplin Lake (Canada) or San Francisco Bay (California, USA) (Provenzano and Goy, 1976). It was also reported that the deficiency in dietary value of *Artemia* nauplii from San Francisco Bay could be compensated by feeding the larvae in addition to *Artemia* with some marine copepods such as *Tigriopus* and *Acartia* (Fukusho, 1974). The food value of *Artemia* from Utah was also improved by allowing them to first feed on *Isochrysis galbana* (Wickins, 1972).

The nutritional quality of living organisms as protein sources was investigated by determining their amino acid composition, digestibility, protein efficiency ratio (PER), and net protein utilization (NPU) (Watanabe *et al.*, 1978b). There were no marked differences in amino acid composition of various kinds of live foods, except for a low threonine content in *Artemia. Artemia* nauplii are reported to be low in the content of histidine, methionine, phenylalanine, and threonine, whereas adult brine shrimp are rich in all essential amino acids (Stults, 1974 ; Gallagher and Brown, 1975 ; Claus *et al.*, 1979). The difference in amino acid profiles between nauplii and adults must be due to free amino acids in body pools, since the amino acid composition of *Artemia* protein is genetically determined and will not vary in function of the life stage. The digestibility of *Artemia* protein was slightly lower than that of rotifers for both carp and rainbow trout. The protein quality of *Artemia* nauplii was about 70-80 % of that of casein in terms of PER and NPU, indicating that *Artemia* has a high feed value as a protein source for fish. These results agree well with the report of Ogino (1963) who judged from their amino acid composition that various kinds of natural live foods are valuable protein sources.

Artemia produced in different geographical locations showed a similar mineral composition, except for the iron content which was several times higher in *Artemia* from South America and Canada than in those from San Francisco (Watanabe *et al.*, 1978a). A low dietary value of *Artemia* was reported to be due to its low iron content (Roeder and Roeder, 1966). However, although it differs from lot to lot or from location to location, the iron content of *Artemia* was found to be high enough to satisfy the iron requirements of fish (Watanabe *et al.*, 1983).

As already described earlier, feeding with some *Artemia* strains resulted in high mortalities in a few species, but in other species the source of *Artemia* had no effect on survival nor on growth performance. The big difference in dietary value of *Artemia* from various geographical origin is mainly related to the difference in EFA requirements of fish.

As shown in Table II, recent studies have demonstrated species-specificity in EFA requirement of freshwater and marine fish (Watanabe, 1982). Freshwater species mainly require 18:2ω6 or 18:3ω3 or both as EFA (Castell *et al.*, 1972 ; Watanabe *et al.*, 1974, 1975a ; Takeuchi and

TABLE II

Essential fatty acid requirement of various fish species

Fish species	Requirement (% of total lipids)	Reference
Rainbow trout	18:3ω3 1 %	Castell *et al.* (1972)
	18:3ω3 0.8 %	Watanabe *et al.* (1974)
	18:3ω3 20 %	Takeuchi and Watanabe (1976)
	ω3-HUFA 10 %	Takeuchi and Watanabe (1977b)
Carp	18:2ω6 1 % and 18:3ω3 1 %	Watanabe *et al.* (1975a) ; Takeuchi and Watanabe (1977a)
Eel	18:2ω6 0.5 % and 18:3ω3 0.5 %	Takeuchi *et al.* (1980)
Chum salmon	18:2ω6 1 % and 18:3ω3 1 %	Takeuchi *et al.* (1979, 1980)
	ω3-HUFA 0.5 %	Takeuchi *et al.* (1980)
Coho salmon	Tri-18:3ω3 1-2.5 %	Yu and Sinnhuber (1979)
Ayu	18:3ω3 1 % or 20:5ω3 1 %	Kanazawa *et al.* (1982)
Tilapia zillii	18:2ω6 1 % or 20:4ω6 1 %	Kanazawa *et al.* (1980)
Tilapia nilotica	18:2ω6 0.5 %	Takeuchi *et al.* (1983)
Red sea bream	ω3-HUFA 0.5 % or 20:5ω3 0.5 %	Yone (1978)
Turbot	ω3-HUFA 0.8 %	Gatesoupe *et al.* (1977)
Yellowtail	ω3-HUFA 2 %	Deshimaru (1984)

Watanabe, 1977a ; Takeuchi *et al.*, 1979, 1980). These fatty acids are not effective for marine fish which require for their normal growth ω3 highly unsaturated fatty acids (ω3-HUFA) such as 20:5ω3 and 22:6ω3 (Yone, 1978 ; Yone and Fujii, 1975ab). However, it is very important to keep in mind that ω3-HUFA, the EFA for marine fish, are also very effective for most of the freshwater fish (Watanabe and Takeuchi, 1976 ; Takeuchi and Watanabe, 1976, 1977b), with *Tilapia* being an exception since it requires 18:2ω6 for its maximal growth (Kanazawa *et al.*, 1980 ; Takeuchi *et al.*, 1983). Furthermore, in rainbow trout ω3-HUFA (20:5ω3 and 22:6ω3) have a biological or EFA efficiency twice as high as that of 18:3ω3. This is similar to the observed efficiency of 20:4ω6 as compared to 18:2ω6 as EFA in mammals.

A reason for the difference observed in the essential role of 18:3ω3 between marine and freshwater fishes might be related to their ability to elongate 18:3ω3 supplemented to the diet. Long term feeding experiments in freshwater fishes, carp, trout, eel, Ayu fish, showed that the concentration of 20:5ω3 and 22:6ω3 in body lipids increased as a result of feeding 18:3ω3, but this was not observed in plaice *Pleuronectes platessa* (Owen *et al.*, 1972) and red sea bream (Fujii *et al.*, 1976). From these findings, it was presumed that marine fishes, when compared with freshwater fishes, possess lower abilities to convert 18:3ω3 to ω3-HUFA. In practice, this species-specificity for EFA must be taken into consideration in the choice of *Artemia* nauplii for larval rearing.

Improvement of nutritional quality of *Artemia*

Artemia from different geographical locations can be classified into two types by the fatty acid composition ; *i.e.* the "freshwater type", with a high proportion of 18:3ω3, the EFA for freshwater fish ; and the "marine type", with a high content of 20:5ω3, the EFA for marine fish (Watanabe *et al.*, 1978e). Similar differences have been shown by Benijts *et al.* (1976), Claus *et al.* (1977, 1979), and Schauer *et al.* (1980).

As shown in Fig. 3, *Artemia* cysts from San Francisco Bay (California, USA) differed quite markedly from year to year or lot to lot (the exact origin of the different batches and their place of production were unknown). A similar variation was found in the fatty acid composition of cysts collected within the same year at a similar location (Schauer *et al.*, 1980), *i.e.* they were found to consist mainly of the types high in 18:3ω3 (together with 18:4ω3) or high in 20:5ω3 ; however, some lots were low in both 18:3ω3 and 20:5ω3.

In both the freshwater and marine types the proportion of 18:2ω6 was relatively high and in addition 20:4ω6 was high in the marine types. *Artemia* which do not belong to these two types were low in the percentage of 18:2ω6 and high in the content of 16:0.

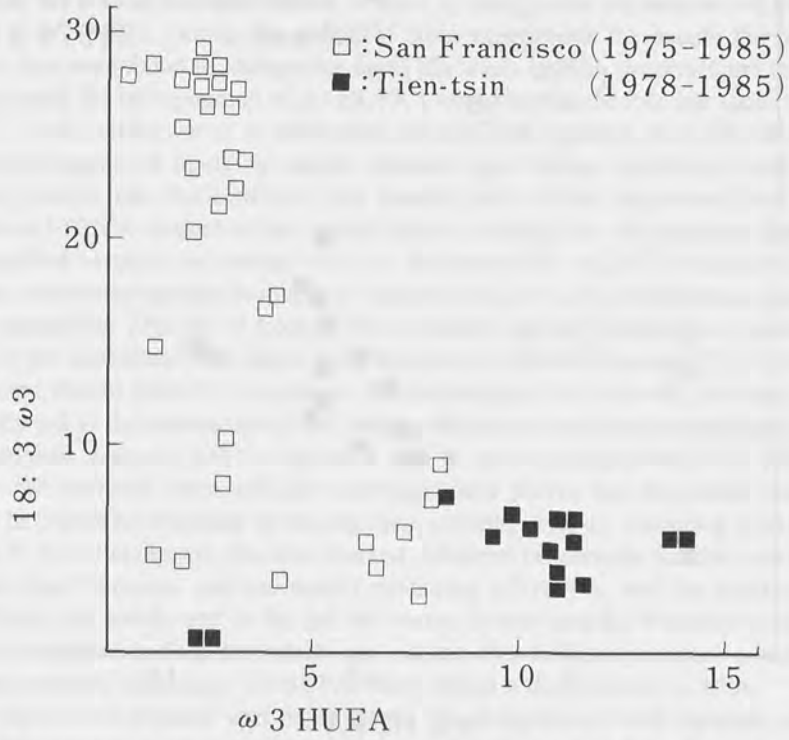

FIG. 3. Classification of *Artemia* cysts from San Francisco Bay (California, USA) and from Tientsin (China) into "marine" and "freswhater" types based on the proportions of their 18:3ω3 and ω3-HUFA (expressed as % of total fatty acids).

Artemia cysts from Tientsin (China) in general belong to the marine type ; some batches, however, did not belong to any type, being low in both ω3 and ω6 fatty acids and high in 16:0. *Artemia* from Australia and Italy seem to belong to the marine type (Schauer *et al.*, 1980 ; Watanabe *et al.*, 1982).

As can be seen in Fig. 4 Brazilian cysts were characteristically low in the content of 18:3ω3 and 18:4ω3, but high in the proportion of ω6 fatty acids such as 18:2ω6 and 20:4ω6. Some were high in both 20:5ω3 and 18:2ω6, and some scored the highest percentage of 22:6ω3 ever observed in *Artemia*.

The difference in fatty acid composition among the geographical strains probably reflects variation in algal blooms which are ingested by the *Artemia* prior to cyst production. Changes in the composition of algal blooms due to varying environmental conditions may result in seasonal variation in fatty acid composition of *Artemia* cysts.

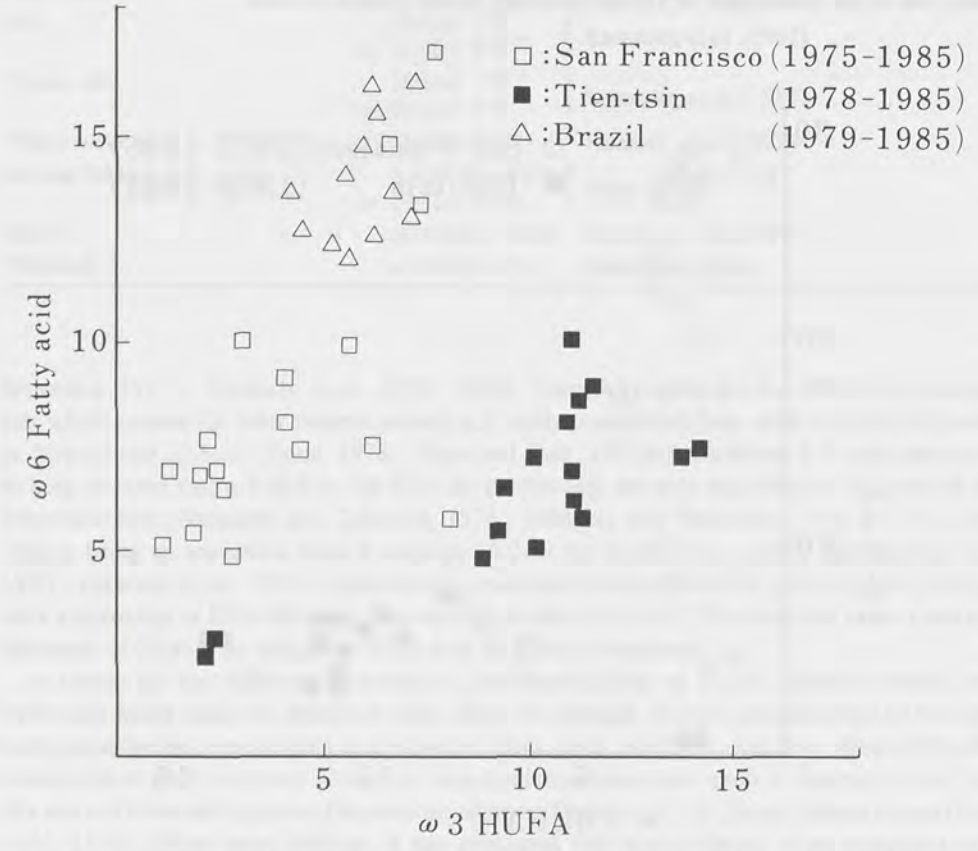

FIG. 4. Classification of *Artemia* cysts from San Francisco Bay (California, USA), Tientsin (China) and Macau (Brazil) into "marine" and "freshwater" types based on the proportions of their ω6 fatty acids and ω3-HUFA (expressed as % of total fatty acids).

Artemia of the marine type were found to be satisfactory as a food for juvenile red sea bream, although it depends also on the total concentration of ω3-HUFA in the nauplii (Watanabe *et al.*, 1978e, 1980). Various trials have been conducted in order to improve the dietary value for marine fish juveniles of *Artemia* nauplii of the freshwater type, by allowing them to feed on ω3-HUFA.

Attempts to improve the dietary value of *Artemia* nauplii in Japan were performed by so-called direct and indirect methods. In the former method lipids containing ω3-HUFA were given directly to *Artemia* nauplii as an emulsion which was made up by homogenizing lipid with a small amount of raw egg yolk and water or emulsifier, together with baker's yeast *Saccharomyces cerevisiae*. In the indirect method the nauplii were fed on ω-yeast produced by adding fish oil or cuttlefish liver oil as a supplement to the culture medium of the baker's yeast, resulting in a high content of lipid and ω3-HUFA, the EFA for marine fish (Watanabe *et al.*, 1978e, 1980 ; Imada *et al.*, 1979). *Artemia* nauplii can also be enriched by culturing them in a medium containing dry microparticulated diets with suitable nutrients for larval fish (Gatesoupe and Luquet, 1981), microencapsulated diets containing various kinds of lipids (Sakamoto *et al.*, 1982) or live microalgae rich in ω3-HUFA such as marine *Chlorella* (Watanabe *et al.*, 1978e) and *Isochrysis galbana* (Wickins, 1972 ; Tandler, pers. commun.).

The dietary values of the newly-hatched nauplii compared with those fed on the emulsified cuttlefish liver oil, pollock liver oil, corn oil, ω-yeast or baker's yeast are summarized in Table III (Watanabe *et al.*, 1982). During the period of these experiments the nauplii fed on various enrichment diets were found to incorporate lipids effectively by both the direct and the indirect method, although the incorporation of ω3-HUFA's varied among the four test series (*e.g.* from 0.31 to 0.77 % of total fatty acids in the nauplii enriched with cuttlefish liver oil). This suggests that the incorporation of lipids by nauplii depends upon culture conditions such as water temperature, density and the activity of the nauplii used. In the experiment with Japanese flounder the ω3-HUFA content of the nauplii fed on cuttlefish liver oil increased from 0.05 % in freshly hatched nauplii to an average of 0.4 % ; and was as low as 0.05 % in those fed on corn oil. Feeding with newly-hatched nauplii, and those fed on corn oil both resulted in heavy losses of the fish around the 13th day of feeding. The cumulative percent mortality of flounder larvae on day 19 of the experiment was higher when fed corn oil enriched *Artemia* (72.9 %) than with freshly hatched nauplii (64.4 %). In addition the percentage of fish showing abnormal behavior in the activity test at the termination of the feeding experiment was significantly higher with the corn oil enriched *Artemia* (100 %) than with freshly hatched nauplii (86.7 %). The marked decrease in fish mortality when enriched *Artemia* is used proves that the dietary value of the nauplii can be improved effectively by feeding them cuttlefish liver oil containing a high amount of ω3-HUFA. In red sea bream, the most marked difference between the fish fed newly-hatched nauplii from San Francisco, and the nauplii containing ω3-HUFA, was the shock syndrome observed during the activity test in the fish fed on the former nauplii (Watanabe *et al.*, 1980). This was not observed in flounder, which may indicate that flounder possesses a better ability than red sea bream to synthesize ω3-HUFA when offered a diet deficient in EFA.

Similar results were obtained with other species. In an experiment with Japanese parrot fish the ω3-HUFA content in the naupliid fed on ω-yeast and cuttlefish liver oil was increased to about 0.3 % as compared to 0.1 % in freshly hatched and baker's yeast enriched nauplii. Similar results in growth, survival and activity test were observed as in flounder fed with *Artemia* high

TABLE III

Improvement of dietary value of *Artemia* nauplii for fish larvae by both the direct and indirect method of ω3-HUFA enrichment

Feed given to *Artemia*	ω3-HUFA in *Artemia* (in % of total fatty acids)	Total body length (in mm)		Total body weight (in mg)		Survival rate (%)	Normal fish in activity test[1] (%)
		Initial	Final	Initial	Final		
Red sea bream (9 days feeding)							
Baker's yeast	0.12	14.7	22.0	35.2	151.9	58.9	23.0
Corn oil	0.03	14.7	22.6	35.2	158.0	52.3	31.5
Pollock liver oil	0.21	14.7	23.7	35.2	188.9	76.3	86.5
Cuttlefish liver oil	0.77	14.7	23.6	35.2	182.5	83.1	99.6
ω3-HUFA concentrate	0.71	14.7	23.4	35.2	178.7	72.0	99.3
Red seam bream (7 days feeding)							
Baker's yeast	0.09	8.9	12.5			32.0	21.4
Corn oil	0.07	8.9	12.2			35.7	15.2
Pollock liver oil	0.15	8.9	13.4			39.7	56.7
Pollock and cuttlefish liver oil (1:1)	0.30	8.9	13.1			53.4	67.9
Cuttlefish liver oil	0.33	8.9	13.3			63.1	98.2
ω3-HUFA concentrate	1.01	8.9	14.1			52.0	96.7
Rock sea bream (10 days feeding)							
ω-yeast	0.30	9.7	20.4	9.0	145.1	78.3	86.7
Cuttlefish liver oil	0.31	9.7	20.3	9.0	142.9	81.4	100
Baker's yeast	0.08	9.7	19.3	9.0	117.2	41.4	3.4
Control[2]	0.10	9.7	19.5	9.0	124.8	59.2	10.0
Japanese flounder (19 days feeding)							
Cuttlefish liver oil	0.40	7.4	12.4		13.7	67.6	80.0
Corn oil	0.05	7.2	9.9		5.8	27.1	0
Control[2]	0.05	7.3	11.2		9.8	35.6	13.3

[1] 30-50 fish were taken out of the culture tank with a scoop net, held dry for 5 seconds, and transferred to a 30 l tank for a check of fish activity 24 h later.

[2] Nauplii just after hatching (48 h).

in ω3-HUFA ; on the other hand, in the fish fed on the newly hatched nauplii or those fed on baker's yeast (both low in the ω3-HUFA content), the rate of growth and survival was low and the percentage of normal fish in the activity test was as low as 10.0 % and 3.4 %, respectively.

In conclusion, the dietary value of *Artemia* of the freshwater type is found to be effectively improved by elevating the ω3-HUFA content in the nauplii indicating that, as was already demonstrated previously in rotifers (Watanabe *et al.*, 1978d, 1979), the class of EFA contained in *Artemia* is the principal factor in determining its nutritional quality to fish larvae.

Similar to the application with rotifers (Watanabe *et al.*, 1983), it is possible to further improve the dietary value of nauplii by allowing them to feed on fat-soluble vitamins A, D and E or lipid materials such as phospholipids and cholesterol together with other lipids (Fig. 5, 6 and 7).

In conclusion, *Artemia* nauplii and rotifers (Watanabe, 1983 ; Watanabe and Seikai, 1983) containing ω3-HUFA levels higher than 0.3 % (*e.g. Artemia* nauplii of the marine type) may be satisfactory as a single feed for most fish (Fig. 8). In addition the quality of *Artemia* can be effectively improved by feeding it any type of lipid containing ω3-HUFA by either the direct or the indirect method. The enrichment of *Artemia* is very important since the lipid content and,

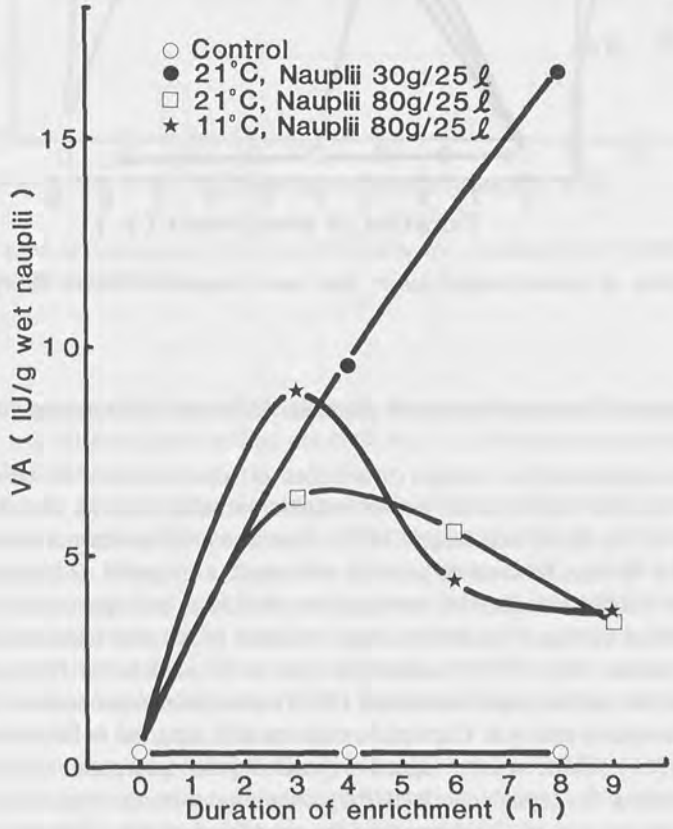

FIG. 5. The effect of *Artemia* nauplii density (30 respectively 80 g wet weight/25 l) and water temperature on the incorporation of vitamin A (VA) in international units (IU)/g wet weight nauplii.

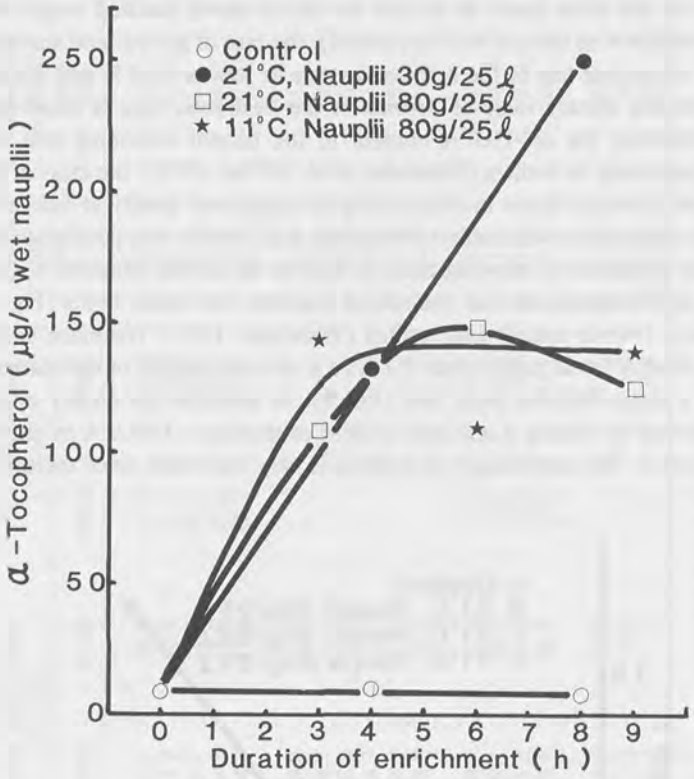

FIG. 6. The effect of *Artemia* nauplii density and water temperature on the incorporation of α-tocopherol.

as a result, the energy content of the nauplii gradually decreases after hatching (Wathanabe *et al.*, 1978e).

A remarkable characteristic of *Artemia* cysts is their extreme resistance to various environmental conditions, *e.g.* the exposure to various organic solvents (Tazawa and Iwanami, 1974 ; Iwanami *et al.*, 1975 ; Smith and Siegel, 1975). Cyst hatchability was not affected when cysts were exposed for 30 days to absolute acetone, n-butanol, n-propanol, isopropanol, ethyl ether and xylene. Cyst viability is reduced to some extent when cysts are exposed to absolute ethanol ; exposure to absolute methanol resulted in a high mortality of the cysts (Smith and Siegel, 1975). Furthermore, Iwanami *et al.* (1975) soaked dry cysts in ^{14}C-acetone for 10 days and suggested acetone penetration. Susheela and Jayaraman (1976) also claimed penetration of ^{32}P inorganic phosphate into encysted embryos. Carbon dioxide was also reported to be able to penetrate the cysts (Clegg, 1966). These results suggest a possibility to incorporate ω3-HUFA into the embryos, by soaking dry cysts in ω3-HUFA containing solvents. Various trials have been conducted by Yasunaga *et al.* (1981) to alter the nutritional quality of *Artemia* by soaking dry cysts in a wide variety of organic solvents (acetone, chloroform, benzene, ethanol, methanol, chloroform-methanol mixture) containing different levels (0-30 %) of cuttlefish liver oil or

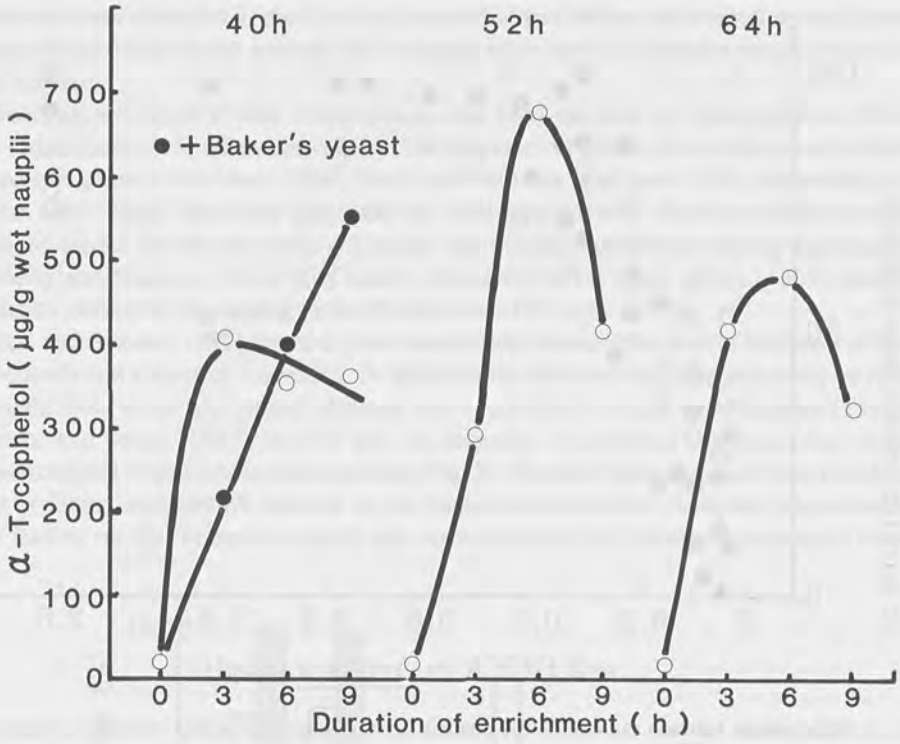

FIG. 7. The effect of *Artemia* age (40, 52 and 64 h after cyst incubation) and duration of enrichment on the incorporation of α-tocopherol.

ω3-HUFA concentrate (80 % purity). These trials were performed at different temperatures (-23 to 30 °C) for 1 to 50 days, or by boiling dry cysts for 5 to 180 minutes in acetone or chloroform containing ω3-HUFA.

These treatments gave no significant effect on the viability of the cysts, except for those kept in absolute ethanol, methanol and a mixture of methanol and chloroform (1:2). The hatchability of the cysts soaked in ethanol for more than 5 days was markedly reduced. The *Artemia* cysts did not hatch when they were soaked in methanol or the methanol-chloroform mixture for 3-7 days. During soaking in the mixed solvents lipids seemed to be extracted from the cysts. These results suggest that the viability of dry cysts is strongly affected by their exposure to polar organic solvents. Similar observations were reported by Smith and Siegel (1975) who found that absolute methanol rapidly killed the cysts. Preservation of dry cysts at a temperature of more than 25 °C in organic solvents containing lipids markedly reduced their hatchability as compared with those kept in the same solvents without the supplementation of lipids. No marked influences on viability were observed due to boiling for 5-180 minutes in acetone or chloroform containing 30 % of cuttlefish liver oil. The hatching assays suggested that the time required for hatching was shortened after the boiling treatments.

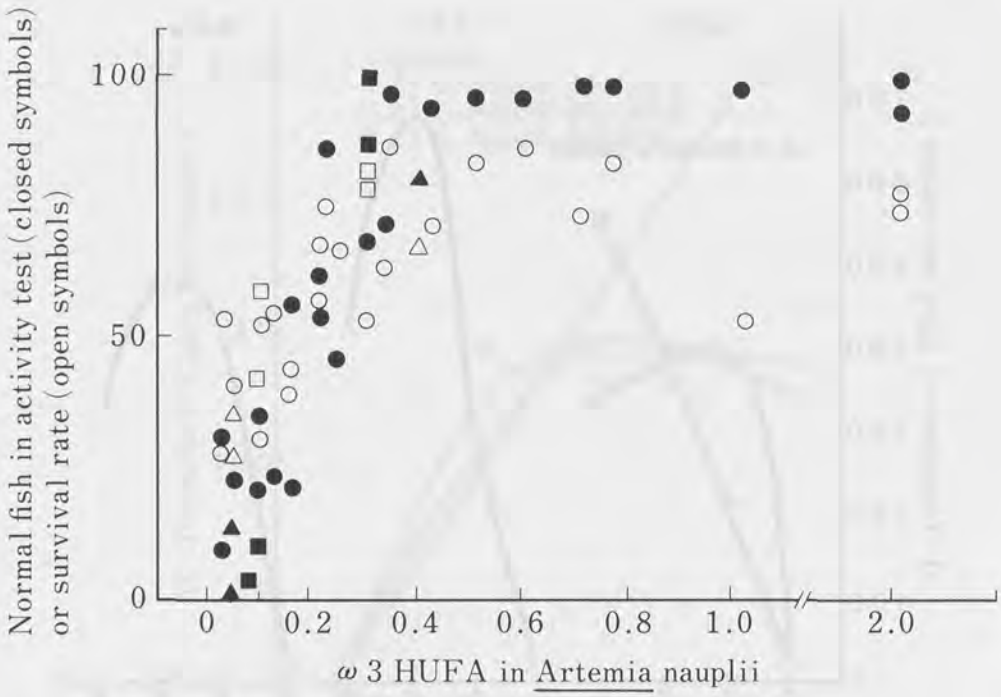

FIG. 8. Relationship between the ω3-HUFA content (in % of total fatty acids) of *Artemia* nauplii and fich activity (in %) and survival rate (in %). The data are from red sea bream (○, ●), rock sea bream (□, ■), and Japanese flounder (△, ▲).

The concentration of 18:3ω3 and monoethylenic fatty acids (16:1 and 18:1) decreased in the nauplii hatched from *Artemia* cysts kept for 10-50 days in chloroform in a refrigerator, and that of ω3-HUFA slightly increased regardless of lipid content in the solvent. When cysts were kept in acetone containing 30 % cuttlefish liver oil for 7 days at room temperature (23-30 °C), the concentration of ω3-HUFA in the nauplii increased from the initial value of 3.0 % to 11.0 % while that of 18:3ω3 decreased. A similar result was obtained when the dry cysts were soaked in the chloroform containing 30 % of the ω3-HUFA concentrate for 30 days. No marked changes were recognized in fatty acid spectra in the nauplii due to boiling the cysts in acetone with or without supplement of cuttlefish liver oil for 5-180 minutes. Hence the results seem to suggest that lipid or ω3-HUFA did penetrate encysted embryos together with acetone or chloroform as observed in the case of ^{14}C-acetone by Iwanami *et al.* (1975). However, detailed fatty acid analyses on both the nauplii and the cyst shells after each treatment have clearly shown that the ω3-HUFA detected in the nauplii was due to contamination with cyst shells on which lipids adsorbed. Thus the dry cysts seem to be essentially impermeable, as demonstrated by various authors for lead phosphate, radioactive ions, amino acids, sugars, nucleotides and glycerol (Clegg, 1966, 1967 ; Morris and Afzelius, 1967 ; Finamore and Clegg, 1969 ; Conte *et al.*, 1977).

Recently serious problems have been encountered in Japan with abnormal coloration in juvenile flounder. As a result of the advancement of hatchery techniques, millions of juvenile

flounder are now produced as seed for both cultivation and release into coastal waters. However, discolored individuals are in general low in market value, and it is doubtful whether they survive when released.

Abnormal coloration in wild *Heterosomata* has been reported by many authors (Norman, 1934 ; Matsubara, 1955 ; Dawsen, 1962). The frequency of albino and ambicolored wild plaice *Pleuronectes platessa* (de Veen, 1969) from the North Sea is at most 2.0 %, respectively 4.0 %. On the other hand, abnormal pigmentation in hatchery-reared *Heterosomata* is very high. Suggested causes of the abnormal coloration are the light conditions during egg incubation (Dannevig and Hanson, 1952), feed quality during the larval stages (Riley, 1966), and larval population density in the rearing tanks (Shelbourne, 1974).

Seikai and Sinoda (1981) found a close relationship between the time of initiation of feeding with *Artemia* and abnormal coloration. In addition, the abnormality could effectively be reduced by feeding these larvae with natural plankton rich in ω3-HUFA. Seikai and Watanabe (1983) and Watanabe and Seikai (1983) showed that the frequency of abnormal coloration was related to the geographical origin of the *Artemia* used (Fig. 9). Feeding larval flounder with nauplii from Brazil or China cysts always resulted in the highest proportion of abnormal coloration, while larval feeding on San Francisco nauplii was associated with the lowest occurrence of discolor-

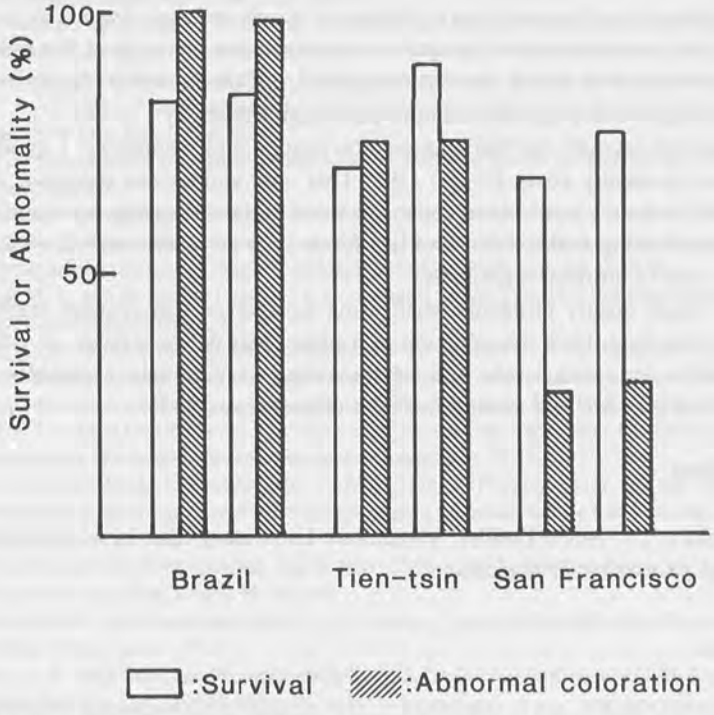

FIG. 9. Effect of feeding larval flounder with *Artemia* nauplii from different geographical origin on the occurrence of abnormal coloration (larval flounders were kept in 100 l tanks at 1 000 fish/tank ; they were fed *Artemia* nauplii together with rotifers from the 10th day after hatching for 31 days under 1 000 lux at water temperature of 17.5 °C).

ation. The *Artemia* strains from Brazil and China belong to the marine type, high in the content of 20:5ω3, while the San Francisco strain is high in 18:3ω3. This suggests that the problem of discoloration is independent of the fatty acid composition and the positive effect of the natural plankton may be associated with its abundance in ω3-HUFA. Moreover, the incorporation of 18:3ω3 into the Brazil and China *Artemia* nauplii by the direct method did not improve the abnormality. Differences in the concentrations of heavy metals and pesticides that may be responsible for the anomaly have not been detected among these geographical strains, *i.e.* their mineral distributions were not so markedly different from each other, although the concentration of Mn was higher in the nauplii from San Francisco (Watanabe *et al.*, 1978b). Detection of difference in chemical compositions is now focused on the vitamin contents, especially vitamin D which may concern melanophore formation.

Concluding remarks

Artemia nauplii are a very useful live food in aquaculture because they can be produced by simple and cheap hatching of commercially available dry cysts which can be stored for many years. Long time storage, however, was found to reduce the rate of hatching. In contrast to *Artemia* production, the success of rotifer mass-production depends on costly equipment and on weather conditions.

The present situation of aquaculture in the world clearly requires *Artemia* as larval food and it may be said that *Artemia* should no longer be considered as a luxury food, but rather as a cheap and high quality source of animal protein (Sorgeloos, 1980). However, further use of *Artemia* in aquaculture seems to depend on its future price and availability.

When taking into account the hatching quality, heating and manpower, 1 kg of live *Artemia* nauplii in Japan presently costs 80-100 US$. This cost approaches the price of formulated microdiets which are now available in Japan. In terms of larval rearing management and water quality control in rearing tanks, the use of live foods such as rotifers and *Artemia* nauplii is by far better than that of artificial microdiets.

Despite the water quality problems when using microdiets, fish growers prefer a complete replacement of live food, from the initiation of feeding. This desire is based on economical and practical reasons. As a result, the use of microdiets is gradually expanding in the mass propagation of various fish and shrimp in Japan (Kanazawa, 1985).

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Description of a standard bioassay with the marine crustacean *Mysidopsis bahia* (M.) for the evaluation of the nutritional effectiveness of *Artemia* nauplii and metanauplii

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Abstract

A standardized bioassay is described for the nutritional evaluation of *Artemia* nauplii and metanauplii using juvenile *Mysidopsis bahia*. This test is particularly suited to evaluate the dietary value of different strains and batches of *Artemia*. One day starved, fed or enriched *Artemia* metanauplii can also be tested.

For this test, a homogeneous population of freshly-released juveniles (≤ 24 h-old) were produced in a mysid generator-separator. The test animals were transferred into an experimental apparatus, cultured under standard conditions and offered *Artemia ad libitum*. After 12 days, data on survival, length, individual dry weight, and reproductive characteristics were recorded and treated statistically.

Introduction

Successful rearing of larval stages of aquatic animals is of primary importance for the aquaculturist, the ecologist, and the ecotoxicologist. Reproducible high survival and growth will determine the profit of the first and contribute to the credibility of the results of the latter two. The main factors affecting larval culture success include technological, environmental, and nutritional parameters. Technological and environmental parameters are relatively easy to study and to adapt while so far nutritional factors are poorly understood. Recently, several authors have stressed the importance of the nutritional quality of feeds used in marine larval rearing as an important factor affecting culture success (review in Léger *et al.*, 1986).

The nauplii of the brine shrimp *Artemia* have been widely used as a food source for the larval stages of aquatic laboratory animals and most commercial species so far cultured. However, the availability of different commercial sources of *Artemia* of varying nutritional quality can affect both hatchery production success and results of bioassays (review in Léger *et al.*, 1986).

In order to assess the nutritional quality of *Artemia* nauplii, a standardized bioassay has been developed with the marine crustacean *Mysidopsis bahia* Molenock. This mysid is particularly suited as a test organism because of its sensitivity to toxicants, its ease of culture and handling,

its short life cycle, its small size, its direct larval development, and its ecological importance in estuarine and marine food chains (Nimmo *et al.*, 1977 ; Nimmo and Hamaker, 1982). For these reasons *Mysidopsis bahia* has been accepted and is widely used in acute and chronic aquatic ecotoxicological testing (Bahner *et al.*, 1977 ; Anonymous, 1978 ; Nimmo and Hamaker, 1982). *Mysidopsis bahia* and other mysid species have also been used in energetic studies (Clutter and Theilacker, 1971 ; Reitsema, 1981) and in nutritional research (Johns *et al.*, 1981 ; Léger and Sorgeloos, 1984).

This paper describes a short-term bioassay using newly-released juveniles of *M. bahia* to assess the nutritional value of freshly-hatched and 24 h-old fed and starved *Artemia* nauplii.

Materials and methods

Mysidopsis bahia

Mysidopsis bahia (M.) is a semitropical crustacean (Mysidacea) first described by Molenock (1969). This species has been reported in West Bay, Galveston, Texas (Molenock, 1969) and South Florida (Odum and Heald, 1972). It is an estuarine organism living near the substratum, positively oriented towards the current (Nimmo *et al.*, 1977).

Mysidacea are often called 'opossum shrimp' since they carry their brood from the egg to the juvenile stage in a broodpouch (marsupium, Fig. 1b). *M. bahia* is sexually mature in 10-12 days post-hatch and three spawns may be released by a single female within 20 days of hatching (Nimmo *et al.*, 1977). The adult size varies from 4.4 mm to 9.8 mm (Molenock, 1969) while newly-released juveniles measure about 1.5 mm. After their release, the young are planktonic during the first 24 h (Nimmo *et al.*, 1977) ; thereafter they orient to the current and chase their prey. Not much is known about the feding habits of *Mysidopsis bahia*, however, Molenock (1969) describes the presence of dentated mandibulae and laciniae which allow *M. bahia* to macerate larger food particles.

In 1979 a few dozen adult *M. bahia* were transfered from the EPA Environmental Research Laboratory Narragansett (Rhode Island, USA) to the Artemia Reference Center, Gent (Belgium). Since then a reproducing mysid population has been successfully maintained in a culturing system as desribed by Léger and Sorgeloos (1982) (Fig. 2). At present, the culturing system consists of eleven 100 l and one 300 l aquaria and holds a standing stock of several thousand mysids.

For the bioassay, newly-released juveniles ($\leq$ 24 h) were used. These were harvested in a juvenile generator-separator (Fig. 3) as described in Léger and Sorgeloos (1982). To collect juveniles, gravid females from the stock cultures were transferred to a filter (1 200 µm)-basket (1) submersed in compartment A. The temperature was gradually increased by 2-4 °C, and the system was shaded from the light. Newly-released juveniles ($\leq$ 24 h old) were collected one day later, on the screen (4) in compartment B. These juveniles were then transferred to the experimental apparatus (see below).

Artemia

The bioassay described here was developed for evaluating the nutritional quality of freshly-hatched and 24 h-old *Artemia* nauplii. For comparative tests on different strains or batches of *Artemia* nauplii, all nauplii used as a food source were harvested at the instar I stage. The T_{90}

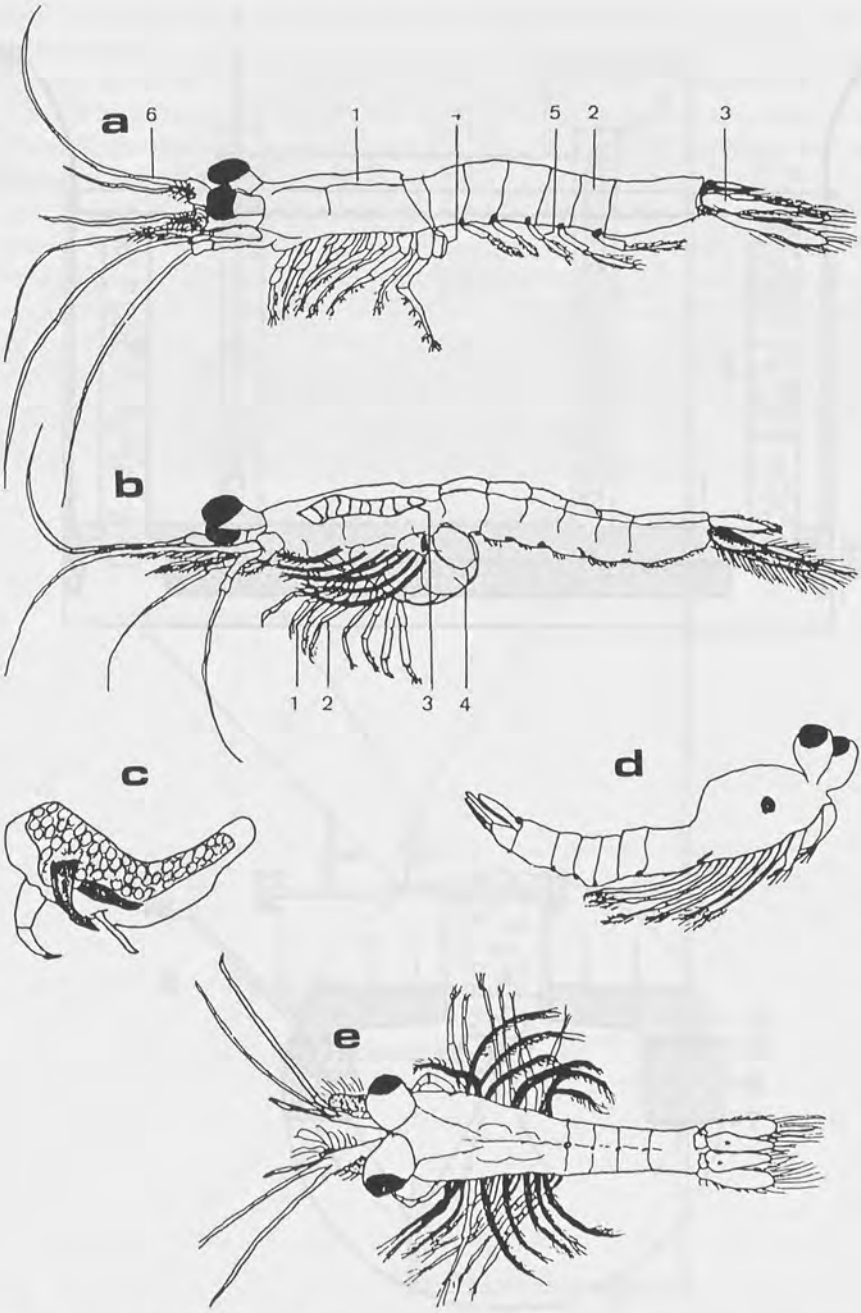

FIG. 1. *Mysidopsis bahia* Molenock. (a) adult male : (1) cephalothorax, (2) abdomen, (3) ciropodes : exo- and endopodite, (4) testes, (5) pleopods, (6) antennae ; (b) adult female : (1) thoracopods : exopodite, (2) thoracopods : endopodite, (3) ovaria, (4) marsupium filled with eggs ; (c) non-eyed juvenile removed from marsupium ; (d) eyed juvenile removed from marsupium ; (e) freshly-released juvenile.

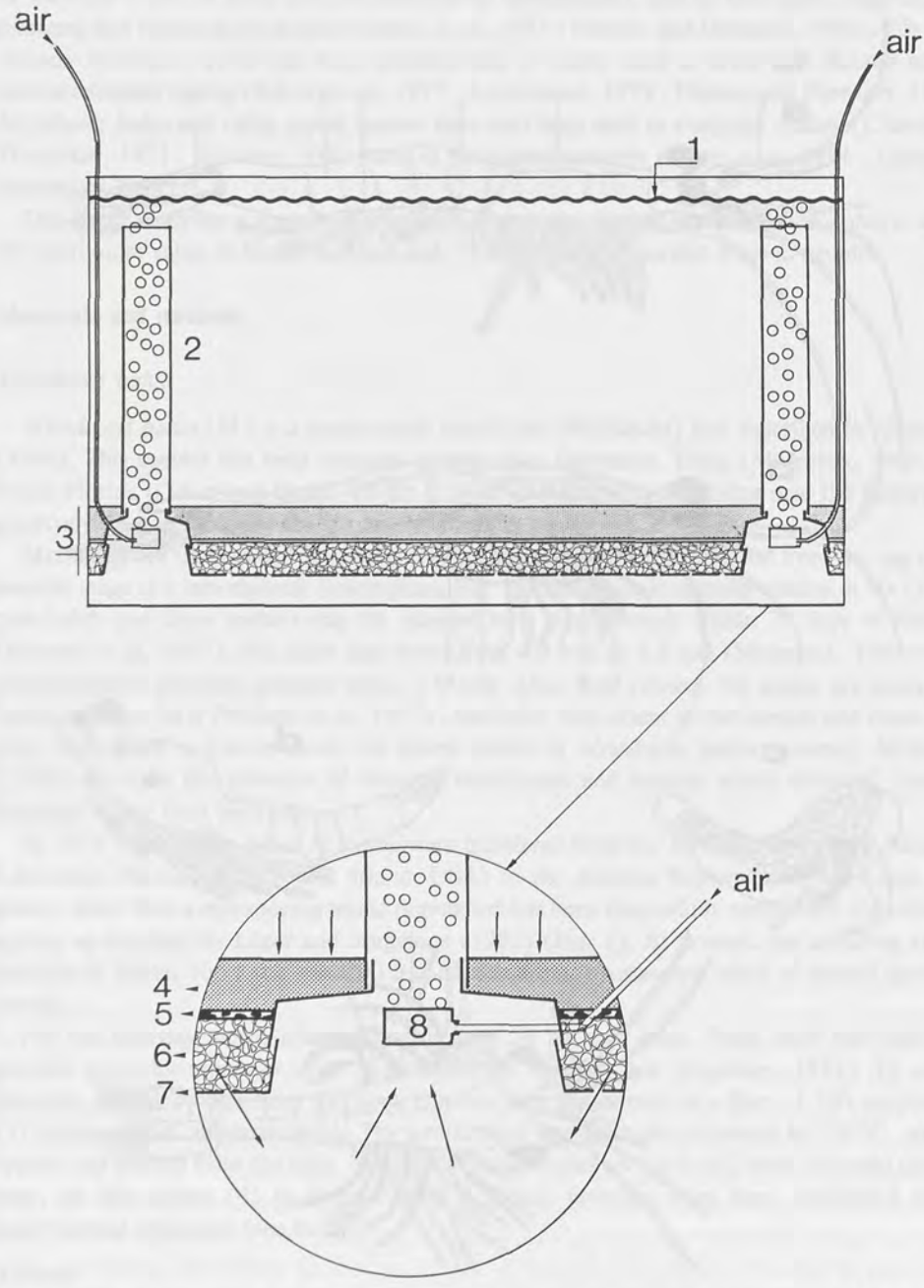

FIG. 2. Schematic diagram of stock-culture aquarium for *Mysidopsis bahia* (M). (1) automatic distribution of cold stored freshly-hatched *Artemia* nauplii ; (2) air-water lift ; (3) sub-gravel bottom filter system ; (4) fine sand ; (5) crushed sea shells ; (6) silex grains ; (7) perforated plastic bottom plate ; (8) air diffuser. (modified from Léger and Sorgeloos, 1982).

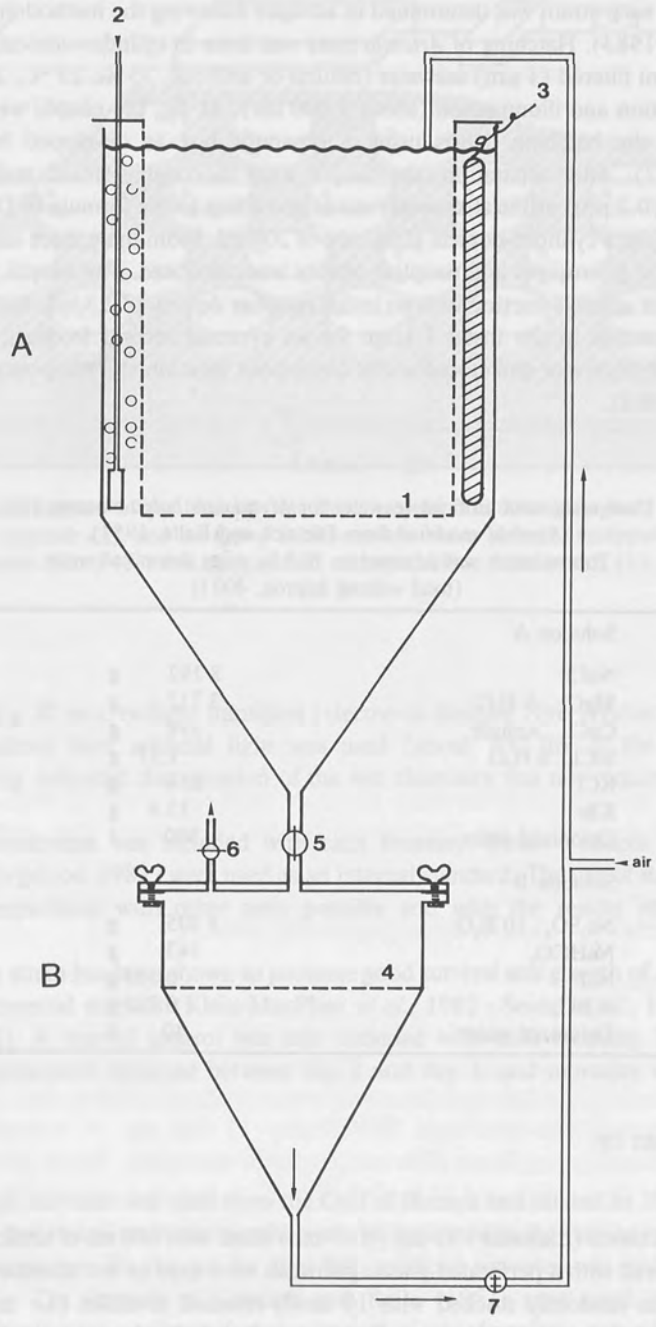

FIG. 3. Schematic diagram of the mysid juvenile generator-separator. (A) incubator for adult mysids retained in a filter basket (1), airline with diffuser (2), thermostat-heater (3) ; (B) separator box equipped with a sieve (400 µm) for retaining the juveniles (4), wide bore stopcock (5), purge valve (6), and air-water-lift with stopcock (7). (modified from Léger and Sorgeloos, 1982).

harvest time for each strain was determined in advance following the methodology of Vanhaecke and Sorgeloos (1983). Hatching of *Artemia* cysts was done in cylindro-conical glass tubes (1 l) filled with 800 ml filtered (1 µm) seawater (natural or artificial, 35 ‰, 25 °C, 2 g cysts/l) under continuous aeration and illumination (about 2 000 lux). At T_{90}, the nauplii were harvested and separated from the hatching debris using a separator box as developed by Persoone and Sorgeloos (1972). After separation, the nauplii were thoroughly rinsed and resuspended in aerated filtered (0.2 µm) artificial seawater made according to the formula of Dietrich and Kalle (1957) (Table I) in a cylindro-conical glass tube of 200 ml. From there three samples were taken with an automatic micropipet and naupliar density was calculated. The nauplii were then fed *ad libitum* to the test animals starting with an initial naupliar density of 2.5/ml. In order to maintain the remaining nauplii in the instar I stage for an eventual second feeding, the nauplii were transferred to a refrigerator and stored under continuous aeration at a temperature of about 5 °C (Léger *et al.*, 1983).

TABLE I

Composition of artificial seawater for *Mysidopsis bahia* bioassay test. (formula modified from Dietrich and Kalle, 1957). This solution was adjusted to 30.0 ‰ using deionized water (total volume approx. 400 l)

Solution A		
NaCl	8 192	g
$MgCl_2 . 6 H_2O$	3 712	g
$CaCl_2$. Anhydr.	394	g
$SrCl_2 . 6 H_2O$	1.37	g
KCl	234	g
KBr	33.9	g
Deionized water	300	l
Solution B		
$Na_2SO_4 . 10 H_2O$	3 105	g
$NaHCO_3$	143	g
NaF	0.103	g
H_3BO_3	0.925	g
Deionized water	40	l

EXPERIMENTAL SET UP

Test chambers

Circular glass bowls (diameter : 15 cm ; h : 7 cm) filled with 600 ml of artificial seawater (see below) and covered with a perforated plastic petridish were used as test chambers (Fig. 4). Each test chamber was randomly stocked with 10 newly-released juveniles (*i.e.* ± 17 cm^2 per test organism) produced as mentioned above. For this, each test chamber consecutively received two juveniles, or five times two in total. Nine replicate bowls were used per treatment (see below). All treatments were randomly assigned to the individual test chambers. The test chambers were placed in a thermostatic water bath at 25 °C ± 0.1 °C. A 14 h light : 10 h dark light regime

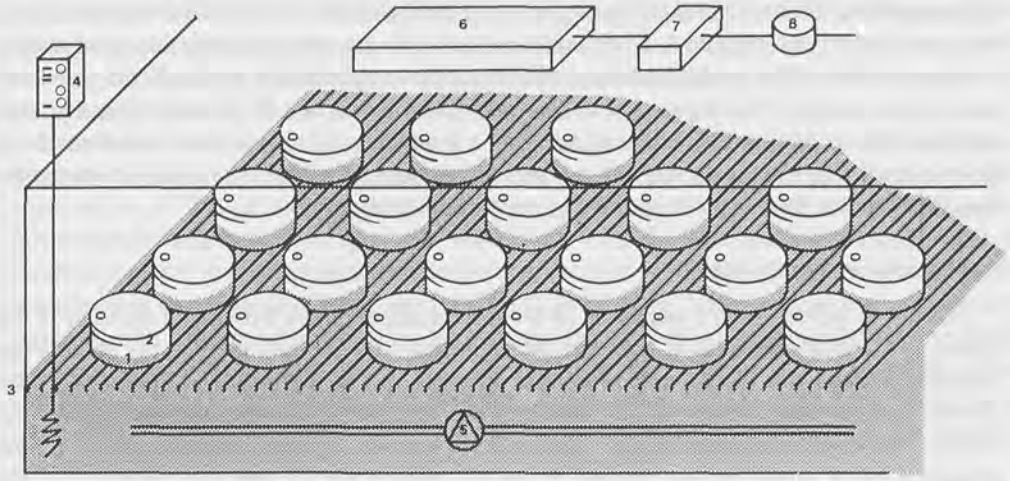

FIG. 4. Schematic diagram of the bioassay test chamber setup : circular glass bowl (1) covered with perforated plastic petridish (2), and incubated in thermostatic water bath (3) equipped with thermostat-heater (4), circulation pump (5), and photoperiod control : diffuse light source (6), dimmer (7), and timer (8).

was applied with a 30 min. twilight transition (electronic dimmer Niro Avidum 5805 equipped with timer). Indirect faint artificial light was used (about 100 lux at the water surface). Preliminary testing indicated that aeration of the test chambers was not required for successful culturing.

One control treatment was included with each bioassay. Instar I nauplii from Reference *Artemia* cysts (Sorgeloos, 1981) were used as an internal standard. The use of Reference *Artemia* cysts makes comparisons with other tests possible and with the results obtained in other laboratories.

The Reference strain has been shown to promote good survival and growth of *Mysidopsis bahia* and other experimental animals (Klein-MacPhee *et al.*, 1982 ; Seidel *et al.*, 1982 ; Léger and Sorgeloos, 1984). A starved control was also included with each bioassay. Mortality in the starved control treatment occurred between day 2 and day 3, and mortality was complete by day 5.

Seawater

Initially, natural seawater was used from the Gulf of Biscaya and diluted to 30 ‰. Preliminary testing indicated that variations in test results could be attributed to the varying quality of different batches of this seawater. To correct for this fact, artificial seawater was tested for culturing *Mysidopsis bahia*. The formula of Dietrich and Kalle (1957) gave good results when the experimental animals were acclimated over a period of two days from natural seawater to the artificial formula. All stock aquaria were switched from natural to artificial seawater. We noticed, however, that in the test chambers the pH of this artificial seawater dropped considerably in the course of a test. We therefore increased the alkalinity of the seawater by increasing the $NaHCO_3$

concentration a 20-fold (Table I). This stabilized the pH between 7.7 and 8.3 during the course of a test. Comparative culture tests with this medium guaranteed reproducible and good results.

Between 400-1 500 l artificial seawater was prepared with chemically pure salts and deionized water before testing. Two separate solutions were made up (A and B, in Table I) and poured together (B into A) under vigorous agitation. The resulting solution was then aerated for 24 h, after which it was filtered through a 1 µm and 0.2 µm cartridge filter. The required volume for one bioassay was kept at 25 °C and oxygenated using airstones.

Feeding and water exchange

In order to harvest instar I nauplii or 24 h-old metanauplii on the morning of each day of the bioassay, T_{90} values were taken into account to incubate the *Artemia* cysts at the appropriate moment. *Artemia* was fed *ad libitum* at least once per day in the morning. Preliminary tests showed that an initial density of 2.5 nauplii/ml was required to provide sufficient food for the mysids. The density for guaranteeing *ad libitum* feeding was adjusted daily and usually a final density of 10-15 nauplii/ml was reached. If needed a second feeding was administered from the chilled *Artemia* suspension (see above).

The calculated volume of the *Artemia* suspension to be fed was filtered over a 110 µm sieve. The nauplii were rinsed and transferred with filtered seawater to the test chambers. Before the first feeding in the morning the remaining *Artemia* (live and dead) were carefully siphoned off along with other debris (dead mysids, faeces, molts) ; special care was taken not to damage the experimental animals. For this a wide-bore glass tube or pipette equipped with a 400 µm screen on one side and a flexible tube on the other side was used. A complete water exchange was carried out on the morning of the 4th and 8th day of the bioassay. Preliminary testing had indicated that more frequent water exchanges (daily or every other day) did not result in a better culture success. To accomplish the water exchange, all surviving mysids from one test chamber were transferred to another test chamber filled with oxygenated, fresh artificial seawater at 25 °C ± 0.1 °C. Mysid transfer was accomplished using a wide-bore pipet or glass tube with an attached rubber bulb or syringe. Transferring the experimental animals was done as carefully and as quickly as possible in order to avoid unnecessary stress.

Duration of the bioassay

In preliminary tests, the results of mysid survival, growth, and reproductive characteristics were compared after 10, 12, and 14 days culture. Differences in survival were not significant. However, growth in terms of final length and dry weight was significantly higher after 12 days than after 10 days. The growth rate attenuated between day 12 and day 14 indicating a decreasing probability for detecting significant differences between treatments. Sexual differentiation of the mysids was about 90 %, 96 %, and 98 % after 10 d, 12 d, and 14 d, respectively. The percentage of females carrying offspring (eggs, non-eyed, and eyed juveniles) (see below) in the broodpouch was respectively 4.5 %, 36 %, and 61.5 %. Average broodsize was about eight (eggs or juveniles) in the three groups. Based on these data a 12-d test period was selected for testing.

Criteria

Survival, growth, and reproductive characteristics of the experimental animals were used to evaluate the culture success.

Survival was followed by recording daily mortality (one check in the morning and one in the afternoon) and survival at the end of the experiment. These data were represented in a graph showing the change in survivorship over time. The final survival value (%) was used for statistical analysis (after arcsine transformation, see below).

Growth was determined by measuring the individual length and dry weight at the end of the experiment. The individual length was determined by transferring the animals from each test chamber to a 7.71 ‰ ammonium formate solution, which is isotonic to 30 ‰ seawater. Through this treatment salts and impurities were washed out from the anaesthetized animals which is essential for later dry weight analysis (ammonium formate is volatile). Since the animals show a sigmoid body curvature, length measurements were made using a dissecting microscope equipped with a drawing mirror. Lines were drawn which follow an imaginary lateral line going from the base of the eyestalk to the tip of the exopodite of the uropodes, excluding the setae (Mauchline, 1973) (Fig. 5). For each replicate all lines were drawn on one page, including a standard (*e.g.* 2 mm micrometer slide). The lines were measured using a curve-meter or digitizer and the individual length was calculated for each treatment.

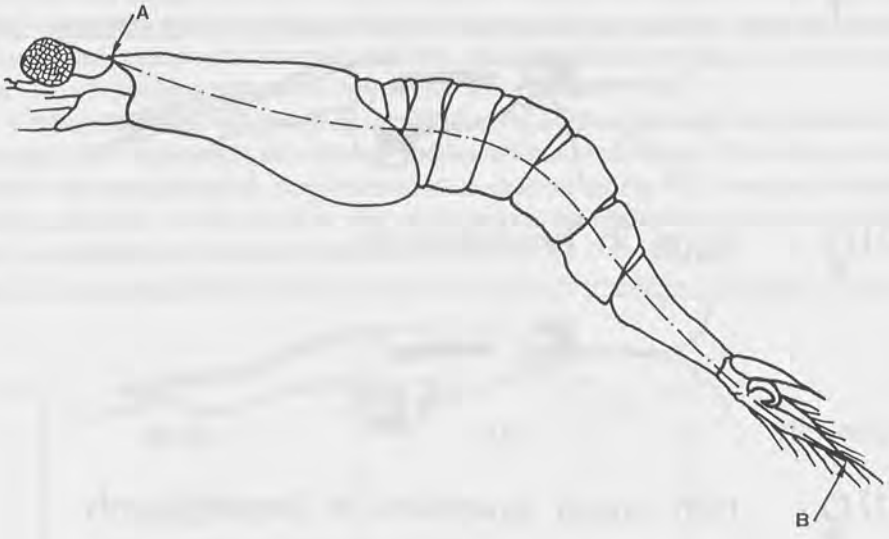

FIG. 5. For length measurements of *M. bahia* a lateral line A-B (dotted) is drawn, following the body curvature from the base of the eyestalk to the tip of the exopodite of the uropodes, excluding the setae.

Once the length measurements were completed, the individual dry weight was determined by transferring the experimental animals from one replicate into a small pre-dried and pre-weighed aluminum cup. Drying was done in a thermostatic oven at 60 °C over a period of 24 h. After cooling in a desiccator, weighing (to the nearest 1 μg) was done on an analytical balance and the individual dry weight was calculated.

The following reproductive characteristics were determined by microscopic observation at the end of the experiment when preparing the animals for length meassurement : sexual differentiation, maturity, and developmental stages of the brood carried by the females.

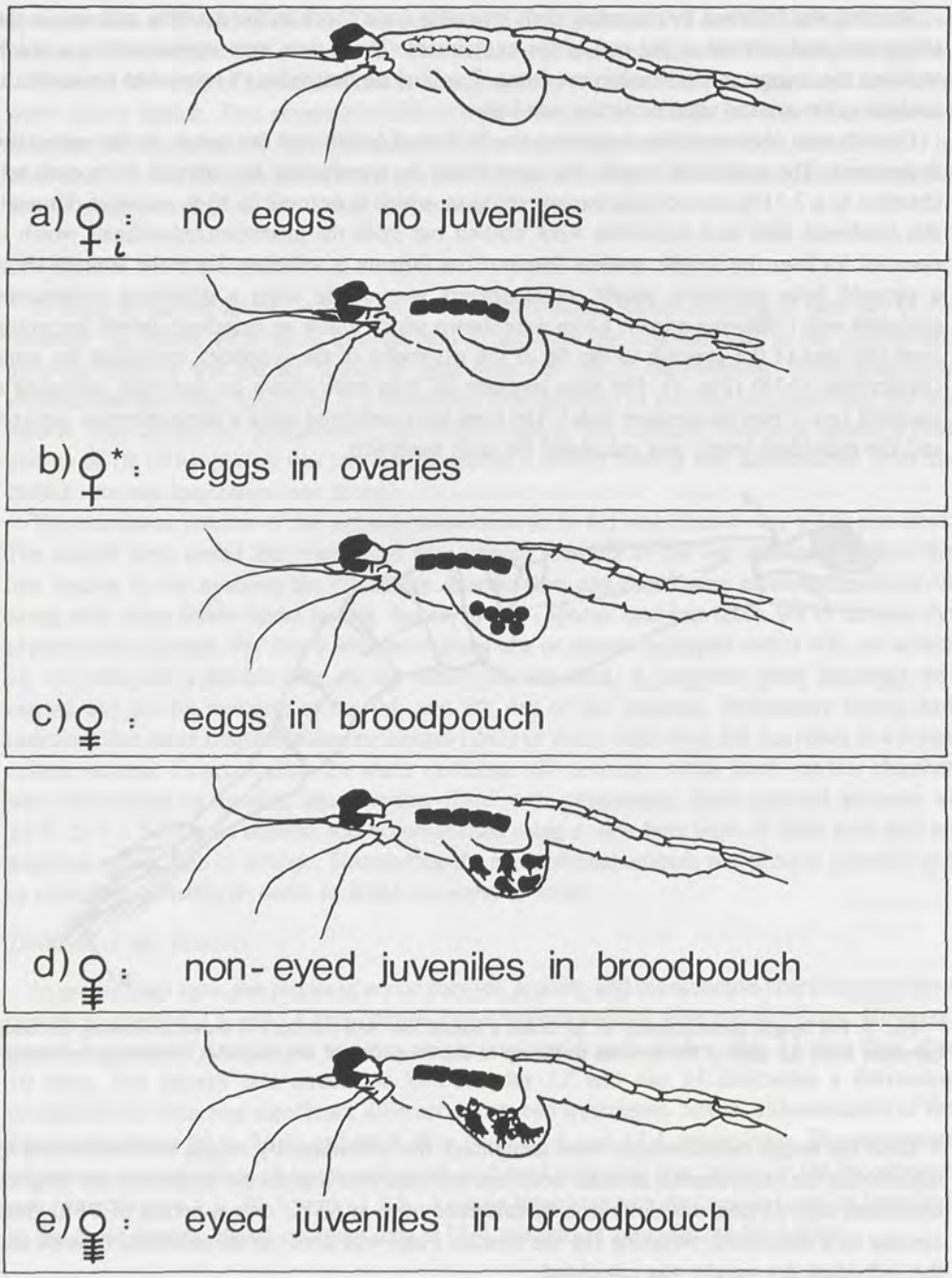

FIG. 6. Reproductive stages in *Mysidopsis bahia* (M.) females : (a) females carrying neither eggs nor juveniles ; (b) females carrying eggs in ovaries ; (c) females carrying eggs in marsupium ; (d) females carrying non-eyed juveniles in marsupium ; (e) females carrying eyed juveniles in marsupium.

Sexual differentiation as a first characteristic divides the surviving animals in two groups (expressed in %) : animals that have developed sex organs (testes, penis, ovaries, broodpouch) and those that have not. The sexually differentiated males and females are then further classified as being mature or immature. Mature males (Fig. 1a) were distinguished by the presence of : 1) two clearly developed testes near the last pair of thoracopods (transition thorax-abdomen) ; 2) presence of penis (difficult to distinguish from pleopods) ; 3) strongly-developed pleopods ; and 4) long antennal flagellae. Mature females (Fig. 1b) were distinguished by the presence of : 1) two functional ovaries and oviducts which appear as tubular crenated organs situated under the thorax above the gastrointestinal organs ; 2) a fully-formed marsupium appearing as a rounded pouch situated at the transition thorax – abdomen ; 3) poorly-developed pleopods ; and 4) antennal flagellae not as long as in mature males. The number of mature and immature animals was expressed as a percentage of the total number of surviving sexually-differentiated animals of the same sex.

Since mysids are transparent, the development of the brood from egg to juvenile can be followed in the female without dissection. The several developmental stages can be distinguished into the following categories (Fig. 6) : a) ovaries and marsupium empty ; b) ovaries filled with eggs and marsupium empty ; c) eggs present in marsupium (see also Fig. 1b) ; d) non-eyed juveniles present in marsupium (see also Fig. 1c) ; e) eyed juveniles present in marsupium (see also Fig. 1d) ; f) juveniles released by female (see also Fig. 1e).

The number of females with brood in the different developmental stages was expressed as a percentage of the total number of surviving sexually-differentiated females. The different classes of females may be represented in a histogram (see example in Fig. 7). Since the number of experimental animals was too small for statistical analysis, the reproductive characteristics were used as a supplementary evaluation criterion.

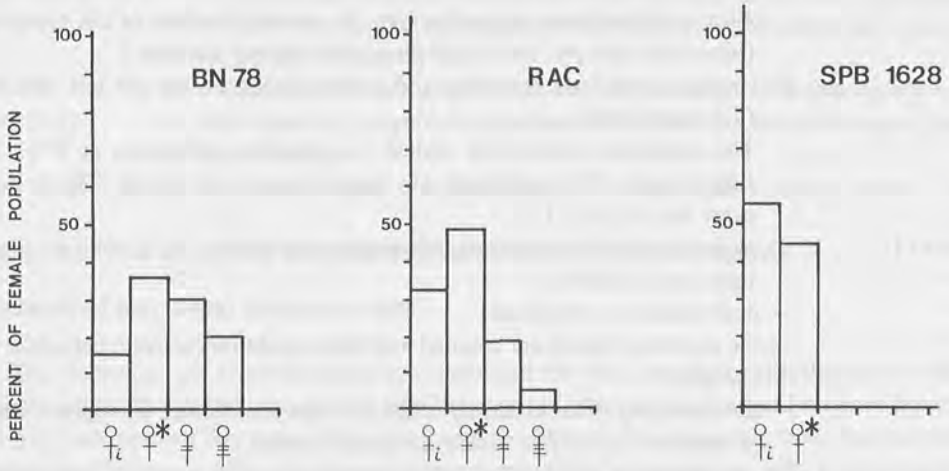

FIG. 7. Histographic presentation of results for reproductive characteristics in female *Mysidopsis bahia* fed three different strains of *Artemia* : Barotac Nuevo, Philippines (BN 78), Reference *Artemia* cysts (RAC), and San Pablo Bay, USA (SPB 1628). Legend to symbols : see Fig. 6.

Data analysis

Data for survival, individual length, and dry weight were analyzed by one-way analysis of variance. Percent survival data were normalized through arcsine transformation (arcsin $\sqrt{p}$, Snedecor and Cochran, 1967). Prior to statistical analysis, the Duncan Multiple Range Test was applied to detect significant differences among means (Goodnight, 1979).

From preliminary tests it was determined that six replicates per treatment enabled us to detect significant differences between means in a multiple range test (*e.g.* Duncan's test in Goodnight, 1979) at a level of $\alpha = 0.05$. For most tests, however, we used nine replicates and were able to detect differences at the $\alpha = 0.01$ level.

Description of testing sequence

day (- 3) : artificial seawater is prepared and aerated overnight ;

day (- 2) : artificial seawater is filtered (0.2 µm), oxygenated (airstones), and kept at a temperature of 25 °C ;

day (- 1) :
a) gravid females from stock cultures are transferred to the juvenile generator-separator. To be sure of obtaining enough juveniles to conduct a test, one gravid female should be incubated for each juvenile to be produced within the following 24 h (see also Léger and Sorgeloos, 1982).
b) daily, from day (- 1) till day (11), enough cysts are incubated for the following day's food requirements. When evaluating 24 h post-hatch (fed or unfed) *Artemia* cysts should be incubated daily, starting day (- 2) ;
c) the experimental set is prepared :
   – test chambers, should be thoroughly cleaned (*e.g.* dichromate – sulfuric acid and repeatedly rinsed with deionized water) ;
   – thermostatic bath is set at 25 °C ± 0.1 °C ;

day (0) :
a) the newly-released juveniles are harvested from the juvenile generator-separator and distributed at random into the required number of test chambers (nine replicates per treatment) filled with 600 ml seawater ;
b) *Artemia* nauplii are harvested and 1 500 nauplii are fed per test chamber (2.5 nauplii/ml)
The remaining *Artemia* are chilled (*e.g.* kept in refrigerator at 5 °C, see Léger *et al.*, 1983) and eventually used for a second feeding. This is done every day till day (12) ;

days (1-3) :
a) each test chamber is checked individually on a viewer (*e.g.* ground glass with light source below) :
– dead mysids are removed ;
– the surviving mysids are counted and their condition (activity, behaviour) is checked ;
– the remaining *Artemia* density is checked and one decides if a higher density is desirable in order to assure *ad libitum* feeding ;
b) the remaining *Artemia* (live and dead) and other debris are siphoned off and the mysids fed with fresh *Artemia*. The water level in the test chambers should be maintained at 600 ml. These manipulations should be done very cautiously in order not to disturb the experimental animals ;

c) a second check of the *Artemia* density is done in the late afternoon and a second feeding is given when needed ;

day (4) : a) same as day (1-3), a,

b) all surviving mysids are individually transferred to cleaned test chambers filled with fresh seawater and counted. Depending on the individual needs for food (*Artemia* density check, see above) test chambers are fed with *Artemia* nauplii.

c) same as day (1-3), c ;

days (5-7) : same as day (1-3), abc ;

day (8) : same as day (4) ;

days (9-11) : same as day (1-3), abc ;

day (12) : a) mysids in all test chambers are fed with the required number of *Artemia* nauplii ;

b) the bioassay is terminated by performing the following on each test chamber taken out of the thermostatic bath :

- dead mysids are removed and counted ;
- the water level is lowered and ammonium formate (7.71 g/l) is added in order to anaesthetize the surviving experimental animals in the shortest time ;
- mysids in each test chamber are then individually examined for survival, growth, and reproductive characteristics. To accomplish this, (surviving) mysids from one test chamber are transferred to a small Petri dish in an ammonium formate solution (7.71 g/l) and counted. The animals are individually checked under a binocular dissecting microscope. Reproductive characteristics are recorded and the individual lengths are determined (see above).

  All animals from one test chamber are then transferred with a fine forceps into a small predried and tared aluminum cup and placed for 24 h in a drying oven at 60 °C.
- the experimental set up is cleaned and prepared for a new bioassay ;

day (13) : a) individual dry weights are calculated after weighing the dried cups containing mysids ;

b) individual lengths are determined by measuring the curves drawn at day (12) ;

c) all data are treated statistically and a report is prepared.

Example of nutritional bioassay results

The following two *Artemia* strains were evaluated for their nutritional effectiveness for *Mysidopsis bahia* (M.) : a Philippine strain (BN7B) obtained after inoculating San Francisco Bay cysts in local salt ponds (Vos *et al.*, 1984) and a batch (SPB 1628) originating from the San Pablo Bay area (California, USA). Reference *Artemia* cysts (RAC) (Sorgeloos, 1981) were used as a control treatment while another series was starved.

From these results presented in Table II, Fig. 7 and 8, it is clear that the SPB 1628 *Artemia*, as compared to the RAC-*Artemia*, is a significantly inferior food source for *Mysidopsis bahia*. The

Philippine strain, on the other hand, provides excellent survival and results in the best growth (significant at $\alpha = 0.01$ level for individual dry weight). The reproductive characteristics of the test animals fed the three *Artemia* sources confirm the results obtained from determining survival and growth.

TABLE II

Results of a bioassay comparing the nutritional effectiveness of three *Artemia* strains as food for *Mysidopsis bahia*

	RAC	BN78	SPB1628
survival (%)	98.3	96.6	57.1
arcsin $\sqrt{p} \pm \sigma$	$*86.9 \pm 7.5^{aA}$	85.6 ± 10.8^{aA}	49.3 ± 10.2^{bB}
Individual length ($\mu m \pm \sigma$)	$4\,804 \pm 243^{aA}$	$4\,885 \pm 213^{aA}$	$4\,032 \pm 81^{bB}$
Individual dry weight ($\mu g \pm \sigma$)	343.2 ± 76.0^{bB}	438.3 ± 82.9^{aA}	281.4 ± 31.8^{cB}
Reproductive characteristics** (%)			
– Sexual differentiation	98.3	100	57.1
– ♂$_i$	0	0	3.5
– ♀$_i$	31.1	15.4	55.6
– ♀*	47.8	34.6	44.4
– ♀	17.4	30.8	0
– ♀	4.4	19.2	0
– ♀	0	0	0
– Average brood size	5.2	7.1	0

* Means with different superscript are significantly different at the $\alpha = 0.05$ (small letters) or $\alpha = 0.01$ (capitals) level.

** (♂$_i$, ♀$_i$) immature males respectively females ;

(♀*) females carrying eggs in ovaries ;

(♀) females carrying eggs in broodpouch ;

(♀) females carrying non-eyed juveniles in broodpouch ;

(♀) females carrying eyed juveniles in broodpouch.

Conclusions

The bioassay with *Mysidopsis bahia* (M.) is a short, simple, and inexpensive test which can be run on a year-round basis at any location in the world (*i.e.* use of artificial seawater and thermostatic bath at 25 °C). Besides a few aquaria for maintaining stock cultures, only some glassware, a dissecting microscope equipped with a drawing mirror, an analytical balance, a drying oven and some auxiliaries are required to conduct the tests. Both stock culture maintenance and bioassays can be done by one technician. This bioassay has been designed to detect differences in nutritional value among different batches and different strains of *Artemia* nauplii. Following the same procedure, 24 h old fed or unfed metanauplii can be evaluated.

Comparative culture tests with *Penaeus stylirostris* and P. *vannamei* have further shown that the results obtained in the mysid bioassay for *Artemia* nauplii and 24 h enriched metanauplii from San Francisco Bay, San Pablo Bay, and Great Salt Lake may be extrapolated to these commercially important species (Léger *et al.*, 1985, 1987).

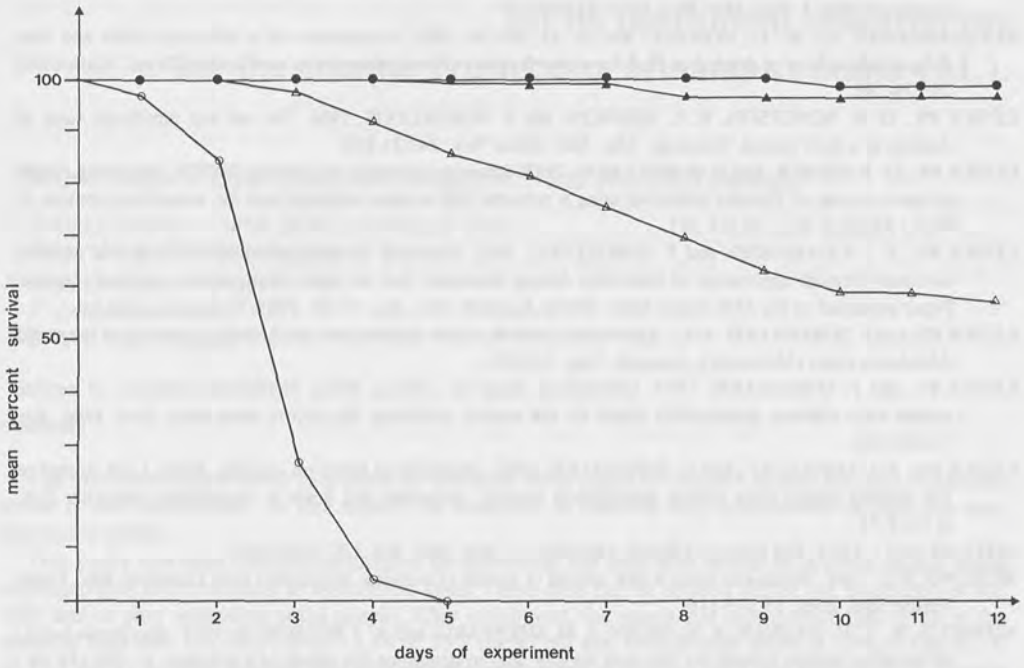

FIG. 8. Evolution of survivorship of *Mysidopsis bahia* (M.) juveniles when starved (○) or fed three different strains of *Artemia*: Reference *Artemia* (●), Barotac Nuevo, Philippines (▲), San Pablo Bay 1628 (CA, USA) (△).

Acknowledgements

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International Study on *Artemia*[1] XXXV. Techniques to manipulate the fatty acid profile in *Artemia* nauplii, and the effect on its nutritional effectiveness for the marine crustacean *Mysidopsis bahia* (M.).

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Abstract

The importance of essential fatty acids for the early larval stages of cultured marine fish and crustacean larvae is well documented. In this regard, the variability in essential fatty acid content of their live prey *Artemia* is critical.

This paper describes different techniques for improving the fatty acid profile in *Artemia* nauplii using microparticles and emulsions as enrichment diets. These diets can be applied during the incubation of the cysts and/or after separation of the nauplii. After enrichment, the nauplii not only contain high levels of the essential fatty acid 20 : 5ω3 (up to 13.5% or 35.2 mg/g) but also considerable levels of 22:6ω3 (up to 7% or 18.1 mg/g), another essential fatty acid which is rarely found in *Artemia*. Fatty acid enrichment of *Artemia* nauplii therefore not only minimizes differences in nutritional quality between strains (*e.g.* in 20:5ω3 content), but it also converts the nauplii into a high-quality food (*e.g.* 22:6ω3).

The beneficial effect of using essential fatty acid enriched *Artemia* as a larval diet is demonstrated in a bioassay test with *Mysidopsis bahia* (M.).

Introduction

In the last decade, the nutritional value of *Artemia* as a food source for larval fish and crustaceans has been very much on the foreground. Indeed, using different sources of *Artemia* or even different batches from the same source, an important variation in nutritional effectiveness for marine larval organisms has been experienced (reviews in Léger *et al.*, 1986, 1987). This geographical and temporal variation has generated intensive research in determining which factors govern the nutritional value of *Artemia*. Besides differences in naupliar dimensions, one *Artemia* factor has unambiguously been related to the culture success in marine larviculture : *i.e.* the presence of essential fatty acids, especially the long chain highly unsaturated fatty acids (HUFA) such as eicosapentaenoic acid (20:5ω3).

[1] International Interdisciplinary Study on *Artemia* strains coordinated by the Artemia Reference Center, State University of Ghent, Belgium.

When comparing various strains and batches of *Artemia* (Table I) a considerable variability in 20:5ω3 is noticed. Striking is the wide range in 20:5ω3 content in San Francisco Bay, Brazil, and China *Artemia* and the narrow range in Utah *Artemia*. Ecological differences between evaporation ponds (*e.g.* salinity, nutrient composition) in a solar saltworks (*e.g.* San Francisco Bay, Brazil, China) are indeed translated in differences in food composition (*e.g.* phytoplankton) *i.e.* in a varying fatty acid profile of the *Artemia* diet, which in turn influences the fatty acid quality of the offspring (Lavens *et al.*, 1987). Ecologically stable environments such as hypersaline lakes (*e.g.* Great Salt Lake and Chaplin Lake) will therefore produce cysts with a more predictable fatty acid profile (Léger *et al.*, 1986). Docosahexaenoic acid (22:6ω3), another essential fatty acid of the marine type (Yone, 1978 ; Holland and Jones, 1981 ; Léger and Frémont, 1981 ; Léger *et al.*, 1985 ; Bell *et al.*, 1985 ; Jones *et al.*, in press) has been detected in insignificant levels only in *Artemia* nauplii (Léger *et al.*, 1986). The unpredictability of essential fatty acid levels and the fact that these essential nutrients may even be completely lacking in *Artemia* are a major constraint for the successful use of *Artemia* as a nutritionally suitable food source in marine larviculture.

TABLE I

Intra-strain variability of 20:5ω3 content in *Artemia*
Data represent the range (area percent) and coefficient
of variation of data as compiled by Léger *et al.*, 1986, 1987

Artemia geographical strain	20:5ω3 range (area %)	coefficient of variation (%)
USA – California : San Francisco Bay	0.3-13.3	78.6
USA – Utah Great Salt Lake (south arm)	2.7- 3.6	11.8
USA – Utah Great Salt Lake (north arm)	0.3- 0.4	21.2
Canada-Chaplin Lake	5.2- 9.5	18.3
Brazil-Macau	3.5-10.6	43.2
PR China-Tientsin	1.3-15.4	50.5

Recently, however, several authors have elaborated techniques for the nutritional enhancement of *Artemia* nauplii (review in Léger *et al.*, 1986). Several types of enrichment diets have been used such as microalgae (Morris, 1956 ; Forster and Wickins, 1967 ; Wickins, 1976 ; Kelly *et al.*, 1977 ; Bromley, 1978 ; Howell, 1979 ; Howell *et al.*, 1981), ω-yeast (Watanabe *et al.* 1978, 1980, 1982, 1983), microparticles and emulsions (Robin, 1982 ; Robin *et al.*, 1981, 1984 ; Gatesoupe, 1982 ; Watanabe *et al.*, 1982 ; Léger *et al.*, 1985). The use of algae for *Artemia* enrichment is not to be recommended since they have to be cultured and contain variable levels of 20:5ω3 and 22:6ω3, the latter being rarely present (Moal *et al.*, 1978 ; Scott and Middleton, 1979 ; Enright, 1984). The use of ω-yeast – baker's yeast enriched with fish-oil, may eliminate the problem of inconsistent levels of the essential fatty acids but has the disadvantage not to be commercially available since the ω-yeast should be used in a living condition (Watanabe, pers. commun). The application of formulated and emulsified diets based on oils containing high levels of HUFA's or HUFA-concentrates provides better opportunities for effective large scale *Artemia* enrichment. Efficient enrichment using such diets will, however, largely depend on diet stability, availability in the water column and on enrichment procedures.

In this paper we describe the use of microparticulate and emulsified HUFA-boosters for *Artemia* nauplii. Culture tests were run with the mysid shrimp *Mysidopsis bahia* in order to evaluate the effect of enrichment on the nutritional value of the *Artemia*. The experiments with microparticles are discussed separately since they were the epochal precursor of the experiments using emulsified diets.

Materials and methods

ARTEMIA HATCHING

The *Artemia* used for these studies originated from San Pablo Bay (CA-USA batch 1628 ; abbreviated SPB), San Francisco Bay (CA-USA batch 236-2016 ; abbreviated SFB), Great Salt Lake (UT-USA northern arm ; abbreviated GSL) and Tientsin (PR China ; abbreviated TTS). Hatching was carried out in filtered (5 µm) artificial seawater (formula of Owens, in Sorgeloos *et al.*, 1983) at 25 °C or 28 °C, 35 ‰ S, 2 000 lux under continuous aeration. The nauplii were harvested after 24 h (SPB, SFB, GSL) or 48 h (TTS), separated from hatching debris, and thoroughly rinsed before being transferred to the enrichment medium.

ARTEMIA ENRICHMENT

With microparticles

In a series of experiments carried out in 1981 three experimental microparticulate diets were used : cod liver oil coated ricebran (CLORB), rice oil coated ricebran (RORB), and AA18, an experimental microparticulate diet manufactured by Artemia Systems NV-SA, Gent, Belgium. The diets differed in content of ω3-HUFA (Table II) and were used to study the manipulation of the fatty acid profile in *Artemia* and its effect on the nutritional value for *Mysidopsis bahia*. For this the freshly-hatched SPB (24 h, 25 °C) *Artemia* nauplii were transferred (naupliar density 25/ml) into an aerated suspension of freshly-prepared enrichment diet (0.6 g/l) in filtered (0.2 µm) artificial seawater (35 ‰ S, 25 °C). A turbidity of 15 cm was installed (± 0.6 g diet/l) and maintained. After 24 h the enriched metanauplii were harvested, rinsed, and fed to the mysid larvae or used for analytical examination.

With emulsions

These enrichment procedures described above were adapted for large scale application of *Artemia* enrichment in fish and shrimp farming (*i.e.* selection of widely available and cheap cysts, of Great Salt Lake, Utah-USA origin, *Artemia* densities up to 300 nauplii/ml during enrichment). Enrichment was performed with the commercial product Selco (Artemia Systems NV-SA, Gent, Belgium), *i.e.* a stable (chemically and physically) enrichment diet under the form of a HUFA enrichment concentrate ; this diet is a self dispersing liquid producing finely dispersed globules (approximately 2 µm) which are readily ingested by *Artemia* nauplii.

Three enrichment procedures were tested :

1. Enrichment of freshly-hatched nauplii (separated from their hatching debris after 24 h, 28 °C) incubated at a naupliar density of 300/ml in a prepared emulsion of 0.6 g Selco/l artificial

seawater. The enrichment diet was administered in one, two or three rations. The required weight of Selco was vigorously shaken in water and diluted in the required volume of seawater. The enriched metanauplii were harvested after 12 h, 24 h, or 48 h incubation at 28 °C, and were thoroughly rinsed before feeding or analysis.

2. Enrichment in the hatching medium without separation of the nauplii. A series of trials was set up adding Selco after 24 h hatching incubation at 28 °C without separating the nauplii from the empty cyst shells. For this, disinfected cysts (20 min incubation in 200 ppm active chlorine) were incubated at a density of 2 g/l which yielded approximately 300 nauplii/ml. Enriched metanauplii were harvested and separated from the hatching debris after 12 h, 24 h, or 48 h incubation and thoroughly rinsed before feeding or analysis.

3. Enrichment during hatching incubation using the commercial formulation Supar (Artemia Systems, NV-SA, Gent, Belgium), consisting of *Artemia* cysts treated with a special self emulsifying ω3-HUFA concentrate. Supar cysts were incubated at 2 g/l during 36 h at 28 °C in filtered (0.2 µm) artificial seawater after which the enriched metanauplii were harvested, separated from their hatching debris, and thoroughly rinsed before feeding or analysis.

The effect of temperature (25 °C and 30 °C) and light on ω3-HUFA accumulation in *Artemia* was also investigated in a separate experiment using Selco as enrichment diet.

All enrichment trials were carried out in triplicate in 100 l polyethylene cylindroconical tanks provided with three aeration lines (one open tube and two equipped with air stones) as to maintain 4-5 ppm oxygen during enrichment. One trial with Chinese cysts was carried out in a 2 000 l cylindroconical polyester tank.

Mysidopsis bahia culture test

The nutritional value of freshly-hatched and enriched *Artemia* nauplii was assessed in a bioassay test with juvenile *Mysidopsis bahia* (Molenock). The testing procedure used, is outlined by Léger *et al.* (1987).

Fatty acid analysis

Fatty acid profiles were determined by capillary gas chromatography. For this, samples were first homogenized with an ultrasonic homogenizer (Sonifier B12), total lipids were extracted according to the method of Bligh and Dyer (1959), and saponification and esterification was done according to the procedure described by Schauer and Simpson (1978). Fatty acid methyl esters were injected on a capillary column (25 m fused silica inner diameter i.d. : 0.32 mm, liquid phase : Silar 10C, film thickness : 0.3 µm) installed in a Carlo Erba Mega 2350 gas chromatograph.

Operating conditions were as follows : on-column injector, carrier gas : hydrogen, flow rate : 2 ml/min, FID detection, oven temperature programme : 105 °C to 150 °C at 10 °C/min and 150 °C to 200 °C at 5 °C/min. Peak identification and quantification was done with a calibrated plotter integrator (HP3390A) and reference standards. Fatty acid composition was expressed in area percent and mg fatty acid methyl ester per g dry weight sample applying the internal standard method using 20:2ω6 as a standard.

Results

Microparticulate diets

It appears from Table II that the ω3-HUFA content in SPB *Artemia* nauplii is considerably increased when the nauplii are fed during 24 h with a ω3-HUFA rich diet (*e.g.* CLORB and AA18). Only a slight increase is noticed when a ω3-HUFA lacking diet (RORB) is used for enrichment or when the nauplii are starved for 24 h. This suggests that *Artemia* nauplii are able to elongate the chain and desaturate lower fatty acids, be it not very efficiently. This bioconversion

Table II

ω3-HUFA content (area percent and mg fatty acid per g dry weight) of enrichment diets and *Artemia* preparations. (-) not detected ; (tr) trace ; (Σω3-HUFA) sum ω3-HUFA > 20:3ω3 ; (24 hE) 24 h enriched ; (AS) after separation of nauplii ; (NS) no separation

	20:5ω3		22:6ω3		Σω3-HUFA	
	Area %	mg/g	Area %	mg/g	Area %	mg/g
Enrichment diets						
• CLORB	8.0	6.3	10.0	5.2	20.9	14.2
• RORB	–	–	–	–	tr	tr
• AA18	8.5	6.4	9.9	7.5	20.8	15.9
• Selco	11.2	78.2	13.2	91.8	30.4	212.6
• Coconut	–	–	–	–	–	–
Artemia preparations						
• SFB – freshly-hatched	9.3	11.8	0.2	0.3	11.5	14.6
• SPB – freshly-hatched	0.5	0.5	–	–	0.7	0.8
• SPB – 24 h starved	1.4	1.1	0.6	0.4	3.5	3.0
• SPB – CLORB, 24 hE	6.3	7.3	1.5	1.9	8.9	10.1
• SPB – RORB, 24 hE	0.9	0.8	–	–	1.9	1.9
• SPB – AA18, 24 hE	8.2	9.9	1.5	2.4	10.6	13.9
• GSL – freshly-hatched	0.3	0.5	–	–	1.2	1.9
• GSL – AS, 0.6 g coconut, 24 hE	0.9	0.8	0.4	0.4	3.4	3.0
• GSL – AS, 0.6 g Selco, 12 hE	5.2	7.9	2.9	4.4	9.8	14.4
• GSL – AS, 0.6 g Selco, 24 hE	9.9	21.3	5.9	12.7	17.8	37.4
• GSL – AS, 0.6 g Selco, 48 hE	13.5	35.2	7.0	18.1	23.0	58.6
• GSL – AS, 2 × 0.3 g Selco, 24 hE	11.7	25.8	4.6	10.2	18.6	37.7
• GSL – AS, 3 × 0.2 g Selco, 48 hE	12.2	33.8	4.8	13.2	19.9	53.5
• GSL – NS, 0.6 g Selco, 12 hE	4.5	6.4	2.4	3.3	8.2	11.2
• GSL – NS, 2 × 0.3 g Selco, 24 hE	7.0	12.1	4.4	7.5	13.3	22.1
• GSL – NS, 3 × 0.2 g Selco, 48 hE	12.0	22.3	6.4	11.9	21.0	38.3
• SUPAR	4.6	8.0	4.0	6.8	9.7	16.7
• TTS *	13.0	13.6	–	–	15.6	16.3
• TTS, decaps., NS, 2 × 0.3 g Selco, 19 hE *	12.3	17.9	3.6	5.2	20.9	30.3
• TTS, decaps., NS, 2 × 0.3 g Selco, 24 hE *	12.6	26.8	4.1	8.9	20.5	45.2

* Data from Lisac *et al.* (1986).

ability was shown before in ongrown *Artemia* (Kayama *et al.*, 1963 ; Jezyck and Penicnak, 1966 ; Hinchcliffe and Riley, 1972).

The effect of ω3-HUFA enrichment on the nutritional value of *Artemia* nauplii is clearly illustrated in the culture test with *Mysidopsis bahia* (Table III) : a significant improvement in both survival and growth is obtained when ω3-HUFA-fortified nauplii were fed. Survival and growth (in terms of individual length) in *M. bahia* fed AA18-enriched *Artemia* is not significantly different from the treatment fed SFB nauplii.

Emulsified diets

Table II and Fig. 1, 2, and 3 produce results of ω3-HUFA levels in GSL *Artemia* nauplii submitted to different enrichment techniques using the prepared emulsion Selco. When adding only one fraction of 0.6 g Selco/l, the ω3-HUFA concentration in separated nauplii increases from 1.9 mg/g after hatching to 14.4 mg/g after 12 h enrichment, to 37.4 mg/g after 24 h, and to 58.6 mg/g after 48 h enrichment. Splitting up 0.6 g Selco in two or three fractions does not yield increased levels. When enrichment is done without separating the nauplii from the hatching debris, *i.e.* by adding the emulsion directly to the hatching medium, a rationing is necessary for a 24 h and 48 h enrichment. One ration of 0.6 g Selco/l during 12 h enrichment gives similar ω3-HUFA levels as when separating the nauplii. However, the same application for a 24 h or 48 h enrichment resulted in a high naupliar mortality. Even when rationing the Selco in two or three fractions, the ω3-HUFA accumulation is always lower than enrichments with separated nauplii.

Enrichment during hatching incubation (Supar formulation) yields a significant increase in ω3-HUFA after about 30 h incubation (which coincides with about 6 h enrichment after the first Selco addition in previous procedures). The levels obtained after 36 h incubation are slightly higher than those after 12 h enrichment in the previous procedures. Survival of the nauplii, stocked at 300 individuals/ml was always above 90 % provided that the oxygen levels were maintained above 4 mg/l. In the procedure adding one portion of emulsion in the hatching medium for 24 h and 48 h enrichment oxygen depletion was the cause of high mortality.

When using separated nauplii and applying one or more rations of emulsion, no differences in survival nor enrichment success were noticed. Nonetheless for large scale application which involves more difficulties to maintain high oxygen levels, it is advisable to apply the emulsion in two fractions. Enrichment in the hatching medium requires disinfection of the cysts prior to incubation as to suppress bacterial blooming and oxygen demand. Good enrichment results have also been obtained in the hatching medium when using decapsulated cysts (Table II, Chinese cysts). Freshly-decapsulated cysts were incubated at 28 °C in 1 500 l seawater ; at maximal hatch (48 h) 0.3 g Selco/l was added and a second ration of 0.3 g/l was administered about 12 h later ; a sample of enriched *Artemia* was taken after 19 h and 24 h enrichment. Results show good ω3-HUFA accumulation when using this method.

The experiment on temperature and light effects on enrichment reveals that only temperature interacts (Table IV), *i.e.* ω3-HUFA buildup is significantly faster at 30 °C than at 25 °C. The effect of GSL *Artemia* enrichment with emulsified diets on their food value for mysid larvae is documented in Table V. Both survival and growth are significantly improved when feeding a ω3-HUFA rich diet. *Artemia* enrichment with a coconut oil emulsion does not improve survival in mysids although a significant effect on growth is noticed. This could be due to the higher

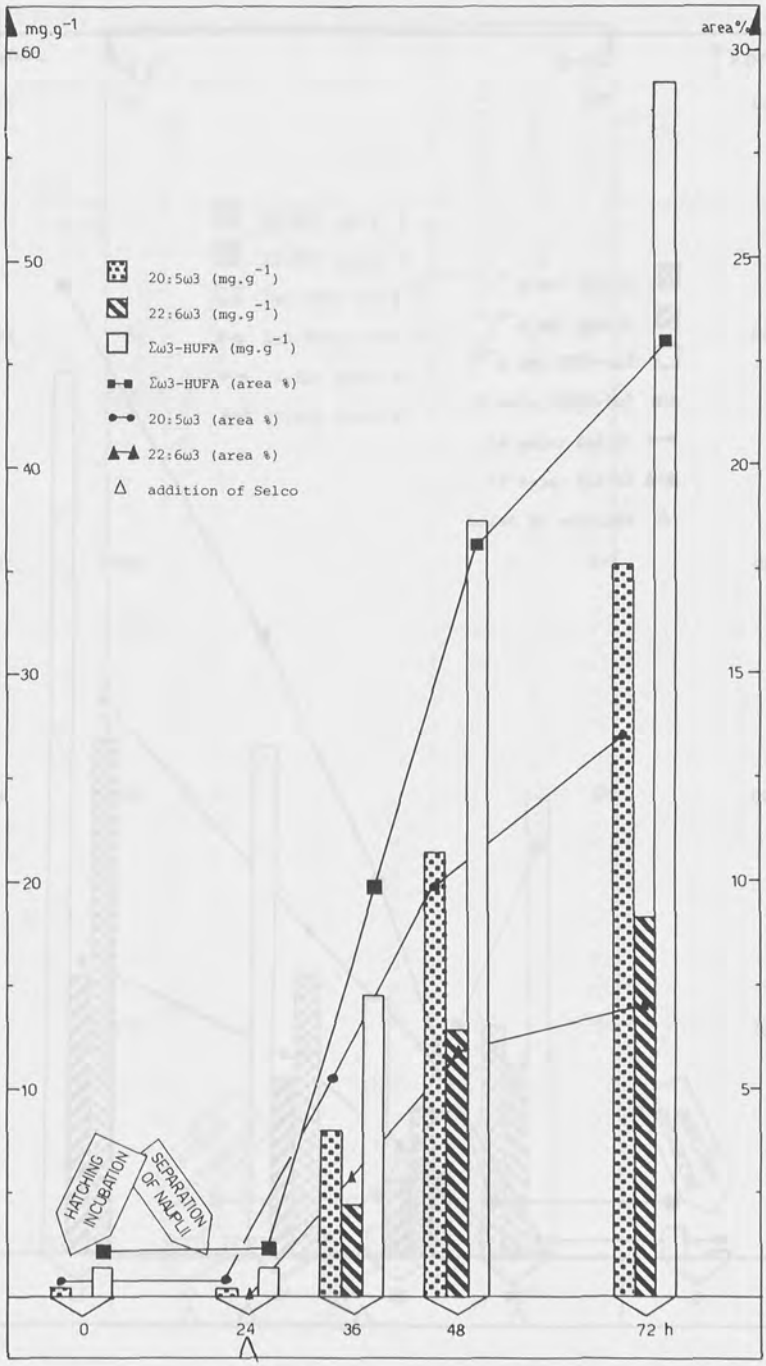

FIG. 1. Histographic presentation of ω3-HUFA accumulation (area % and mg/g values) in GSL *Artemia* nauplii enriched with a prepared emulsion (Selco), after separation from their hatching debris.

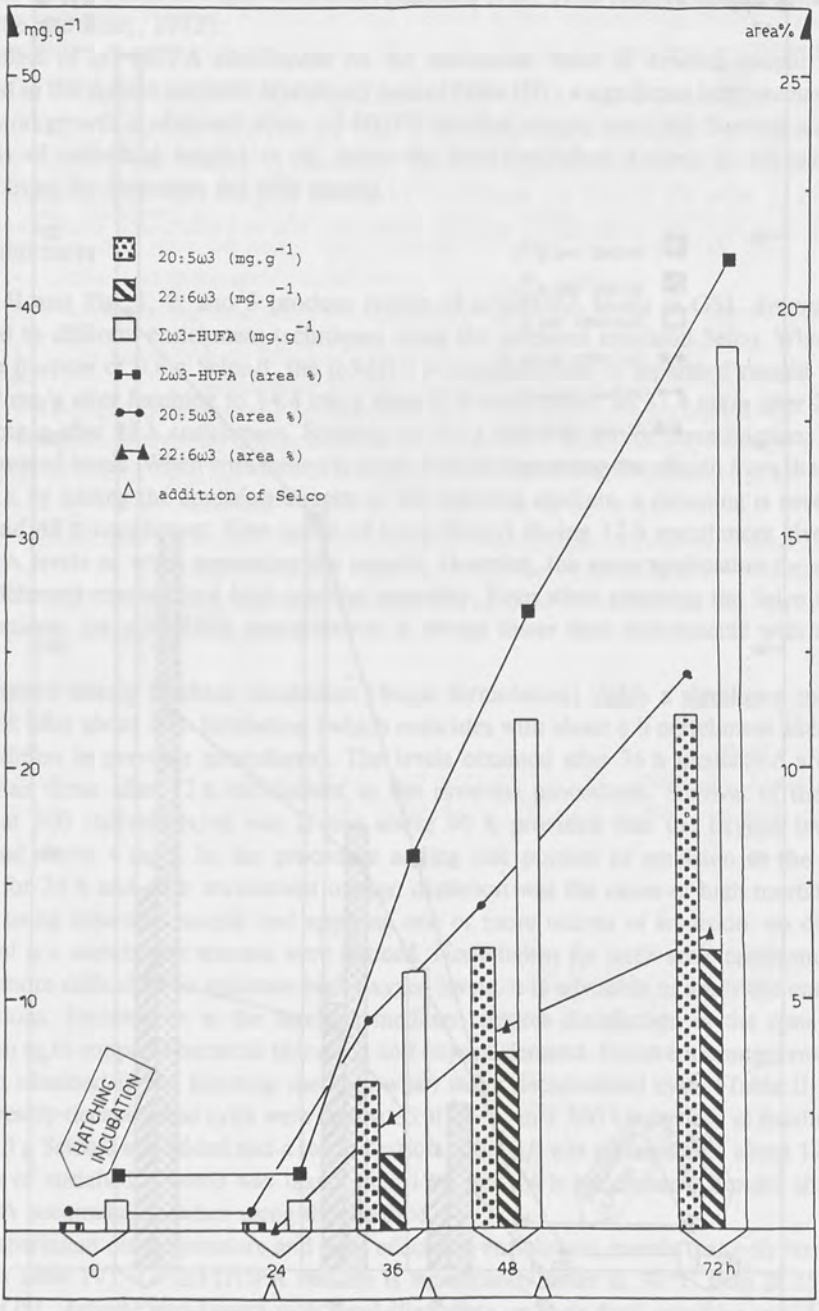

FIG. 2. Histographic presentation of ω3-HUFA accumulation (area % and mg/g values) in GSL *Artemia* nauplii enriched with a prepared emulsion (Selco) in the hatching medium.

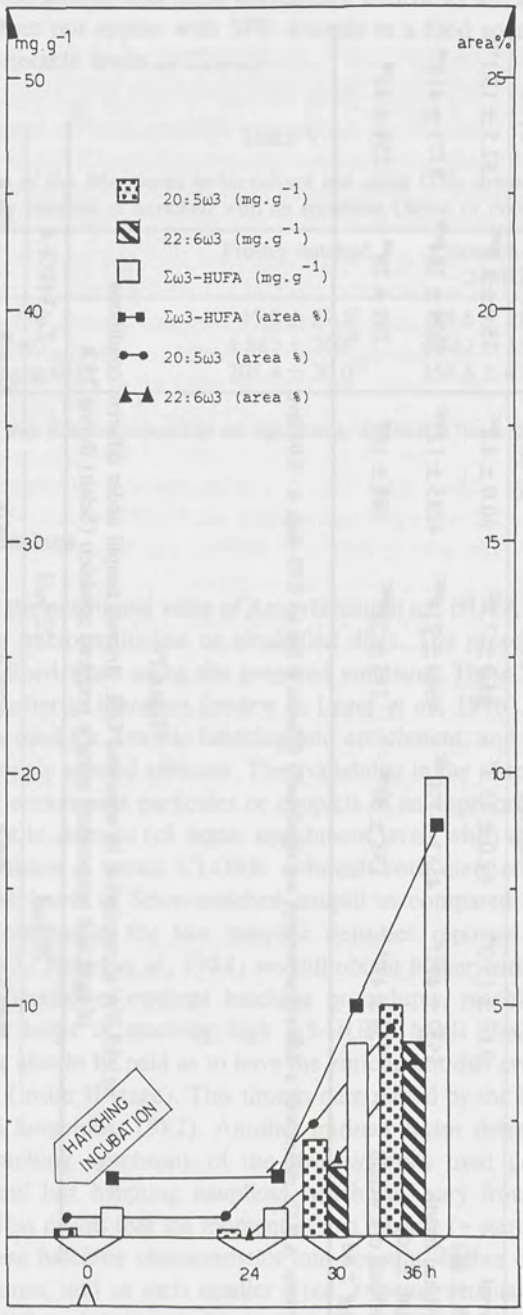

FIG. 3. Histographic presentation of ω3-HUFA accumulation (area % and mg/g values) in *Artemia* nauplii produced from treated cysts (Supar formulation), during hatching incubation.

TABLE III

Results of the *Mysidopsis bahia* culture test using *Artemia* nauplii freshly-hatched or enriched with microparticulate diets. (legend to symbols : see text)

Culture results	SFB	SPB				
	Newly hatched	Newly hatched	Starved 24 h	RORB 24 hE	CLORB 24 hE	AA18 24 hE
Survival (% ± sd)	93.3 ± 15.0^{aA}	62.0 ± 15.3^{cC}	55.0 ± 7.5^{cC}	60.0 ± 4.8^{cC}	75.0 ± 8.9^{bBC}	92.5 ± 13.1^{aAB}
Individual length (µm ± sd)	5532 ± 471^{aA}	4587 ± 270^{bBC}	4504 ± 214^{bBC}	4285 ± 135^{bC}	5029 ± 284^{aAB}	5375 ± 415^{aA}
Individual dry weight (µg ± sd)	354 ± 47^{aA}	198 ± 30^{cBC}	216 ± 28^{bcBC}	188 ± 16^{cC}	259 ± 29^{bB}	259 ± 33^{bB}

a,b,c resp. A,B,C : means with a different superscript are significantly different at the α : 0.05 resp. α : 0.01 level.

TABLE IV

ω3-HUFA accumulation in separated GSL *Artemia* nauplii under different conditions of light and temperature using a prepared emulsion (Selco) during 24 h

	20:5ω3		22:6ω3		Σω3-HUFA	
	Area %	mg/g	Area %	mg/g	Area %	mg/g
Light, 25 °C	7.4 ± 0.2^{b}	10.9 ± 0.7^{b}	4.0 ± 0.7^{a}	5.9 ± 1.1^{b}	16.3 ± 0.3^{ab}	23.9 ± 0.9^{b}
Light, 30 °C	8.7 ± 0.4^{a}	15.5 ± 0.6^{a}	4.3 ± 0.2^{a}	7.7 ± 0.3^{a}	18.7 ± 0.1^{a}	33.3 ± 0.1^{a}
Dark, 25 °C	7.5 ± 0.8^{ab}	11.7 ± 1.6^{b}	3.9 ± 0.7^{a}	6.0 ± 1.3^{b}	15.4 ± 1.7^{b}	23.9 ± 3.1^{b}

a,b : means with a different superscript are significantly different at the α : 0.05 level.

energetic content of the nauplii after lipid enrichment and/or to the slightly increased level of 22:6ω3. This effect does not appear with SPB *Artemia* as a food source where starved nauplii also contain low but notable levels of 22:6ω3.

TABLE V

Results of the *Mysidopsis bahia* culture test using GSL *Artemia* nauplii freshly hatched or enriched with an emulsion (Selco or coconut oil)

Culture results	Freshly-hatched	Coconut oil 24hE	Selco 24hE
Survival (% ± sd)	45.8 ± 27.5^{b}	45.6 ± 12.8^{b}	78.1 ± 13.5^{a}
Individual length (µm ± sd)	$4\,087 \pm 200^{cB}$	$4\,482 \pm 337^{bB}$	$5\,108 \pm 135^{aA}$
Individual dry weight (µg ± sd)	205.4 ± 20.0^{bB}	256.8 ± 47.4^{bB}	325.3 ± 14^{aA}

a,b,c resp. A,B,C : means with different superscript are significantly different at the α :0.05 resp. α : 0.01 level.

Discussion and conclusions

Aiming to enhance the nutritional value of *Artemia* nauplii ω3-HUFA enrichment may be done in various ways using microparticulate or emulsified diets. The present study reveals that the highest levels are obtained when using the prepared emulsion. These levels are notably higher than those reported earlier in literature (review in Léger *et al.*, 1986). This may be due to the optimized procedures used for *Artemia* hatching and enrichment, and also to the high stability of the emulsion in strongly aerated seawater. The availability in the water column of a sufficiently high concentration of enrichment particules or droplets of an appropriate size is indeed critical for optimal enrichment in *Artemia* (cf. better enrichment levels when using AA18 – with better granulometric composition – versus CLORB, although both diets contain similar ω3-HUFA concentrations ; higher levels in Selco-enriched nauplii as compared to literature results with other emulsions). Contrary to the low naupliar densities reported by other authors (*e.g.* 10-15/ml, Robin, 1982 ; Robin *et al.*, 1984) we still obtain higher enrichment levels with up to 300 nauplii/ml. Application of optimal hatching procedures, reaching maximal hatch in a minimal time, further helps in reaching high ω3-HUFA levels after only short enrichment periods. Attention has also to be paid as to have the enrichment diet available at the time of first feeding of the nauplii (instar II-stage). This time is determined by the hatching rate of the cysts used (Vanhaecke and Sorgeloos, 1982). Another intrinsic factor determining maximal enrichment rates is the hatching synchrony of the *Artemia* cysts used (*e.g.* time lapse between appearance of first and last hatching nauplius) which can vary from 5 h to 17 h at 25 °C (Vanhaecke, 1983). This means that the moment of first feeding (= start of enrichment) will also be spread. Taking these hatching characteristics into account, higher enrichment levels can be achieved in shorter times, and as such smaller sized *Artemia* metanauplii are yielded. Indeed, 12 h to 48 h enriched *Artemia* metanauplii, produced according to the procedures described in this study measure 660 µm (12 h enrichment) to 790 µm (48 h enrichment) instead of over 900 µm when other methods are applied. The literature provides numerous examples of prey size being an important parameter in larval culture success, since the larger it is, (*e.g.* enriched

Artemia) the more delayed its earliest introduction in the feeding regime (Léger *et al.*, 1986) will be.

The culture test with *Mysidopsis bahia* illustrates the beneficial effect on survival and growth of feeding ω3-HUFA enriched *Artemia*. When feeding enriched SPB *Artemia*, survival and growth (in terms of length) of *M. bahia* are not significantly different from the treatment fed SFB nauplii. Growth in terms of individual dry weight, however, is best in mysids fed SFB *Artemia*. This might confirm that SPB *Artemia* are not only deficient in ω3-HUFA but that they may contain particular antinutrients as suggested by Olney *et al.* (1980). These observations could also indicate that, for *M. bahia*, the sum of ω3-HUFA is more important than the contribution of particular fatty acids, *e.g.* 22:6ω3. On the other hand the effect of ω3-HUFA enrichment in GSL *Artemia* as a food source for *M. bahia* is very clear on both survival and growth. When growth in terms of individual dry weight is compared with the SFB-treatment (previous test), we see that the ω3-HUFA enriched GSL *Artemia* are still inferior. This is, however, not the case with other predators such as shrimp larvae *Penaeus stylirostris* (Léger *et al.*, 1985) and *Penaeus vannamei* (Léger *et al.*, 1987), and fish larvae *Dicentrarchus labrax* (Franičević *et al.*, 1987) and *Sparus aurata* (Lisac *et al.*, 1986) where SPB, GSL, or other *Artemia* nauplii enriched with the same ω3-HUFA diet always performed significantly better than a SFB-control. For these predators the presence of the 22:6ω3 fatty acid in enriched *Artemia* was believed to be an essential factor, confirming the role of 22:6ω3 as an essential component in the larval nutrition of marine fish and crustaceans (Yone, 1978 ; Holland and Jones, 1981 ; Léger and Frémont, 1981 ; Léger *et al.*, 1985 ; Bell *et al.*, 1985). Since this essential fatty acid is mostly absent in *Artemia* and the 20:5ω3 content varies considerably it is advised to apply ω3-HUFA enrichment techniques as a routine procedure in the larval culture of marine fish and shrimp species. The enrichment techniques described in this paper may also be applied for incorporating other essential nutrients (*e.g.* vitamines, amino acids, etc.) and other active components (pigments, therapeutics, etc.) in *Artemia*. This will be presented in other studies.

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International Study on *Artemia*[1] XLIV. Preliminary nutritional evaluation of different *Artemia* nauplii as food for marine fish and prawn larvae

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Abstract

Artemia nauplii with different contents of ω3-HUFA were tested in order to determine their nutritional value as food for some marine fish and prawn species : *Dicentrarchus labrax, Sparus aurata, Penaeus kerathurus, Palaemon serratus* and *Palaemon adspersus.* An increase in long chain ω3-HUFA was accomplished with different methods of *Artemia* enrichment. Survival, length, weight, and stage of larval development were often significantly increased when ω3-HUFA-rich treatments were used. Generally, the best results in survival and growth were achieved with enriched nauplii and nauplii naturally high in ω3-HUFAs.

Introduction

Artemia nauplii have long been widely used as food for cultured fish and crustacean larvae. Recently, experimental research has been carried out in order to establish the nutritional value of *Artemia* nauplii. Shelbourne (1968) reported deficiencies in flatfish larvae fed with Great Salt Lake nauplii. Likewise Reeve (1969), Wickins (1972), and Bookhout and Costlow (1970) reported bad results in marine crustacean and fish cultures, and Provenzano and Goy (1976) and Matsuoka (1975) in freshwater species such as *Palaemonetes* and *Macrobrachium.* However, not only that strain was responsible for high mortality rates and bad results, but other strains from Canada, California, China, and South America (Watanabe, 1979 ; Fujita *et al.*, 1980 ; Watanabe *et al.*, 1980) as well.

Different authors suggested various hypotheses to explain the poor results. For Beck and Bengtson (1982) an important factor was the size of the nauplii. Bookhout and Costlow (1970) reported that deformations and mortalities might be due to the presence of toxics and pesticides in the cysts. Fujita *et al.* (1980), Schauer *et al.* (1980), Watanabe *et al.* (1980), and Léger *et al.* (1984) gave more importance to the lack of essential fatty acids in some nauplii. Finally,

[1] International Interdisciplinary Study on *Artemia* strains coordinated by the Artemia Reference Center, State University of Ghent, Belgium.

Klein-MacPhee *et al.* (1980), Olney *et al.* (1980), and Schauer *et al.* (1980) pointed out that a synergistic effect between pesticides and low HUFA levels could be the cause. We feel that Watanabe *et al.* (1978), Fujita *et al.* (1980), and Léger *et al.* (1984) have shown that the amount and class of the essential fatty acids contained in the nauplii are decisive in determining their nutritional value.

This study was designed to test the nutritional value of commercially-available *Artemia* nauplii with and without enrichment with ω3-HUFAs. Experiments were conducted with two fish species, *Dicentrarchus labrax* and *Sparus aurata*, and three crustacean species, *Penaeus kerathurus, Palaemon serratus* and *Palaemon adspersus,* all of which were chosen because of their availability in our culture facility.

Materials and methods

Experimental system

Tests were carried out in three 150 l tanks with the water level controlled by a siphon/standpipe coming from the base. Eight test chambers made of sanitary PVC pipe with one end closed by a 160 µm plankton net were suspended in each of the tanks.

An individualized drip flow system renewed the medium. The refill flow was estimated at 2 l/h. Water used for renewing was stored in another 150 l tank and had been previously filtered to 10 µm.

A system of heaters, under thermostatic control, assured constant temperature with fluctuations of ±1 °C. Gentle aeration was provided in each tank.

Diets

Two *Artemia* strains, whose freshly hatched instar I nauplii contained low levels of long chain ω3-HUFA, were used : San Francisco Bay Brand lot no. 1628 from San Pablo Bay (SPB) (California, USA), and Great Salt Lake (GSL) (Utah, USA) from the northern arm of the lake.

These strains were submitted to enrichment processes constituting various treatments. Two experimental enrichment products were tested : AA18 (Artemia Systems NV, Gent, Belgium) and SEC-HAT (Artemia Systems NV, Gent, Belgium). AA18 is a micronized dry powder (average particle size 5 µm) which was mixed with the dry cysts at a ratio 1:5 in the hatching medium. SEC-HAT is an emulsified enrichment product which is added to the medium twice, during the instar I stage and 2 h before the final harvesting during the instar II stage.

The control diet consisted of instar I nauplii of Reference *Artemia* (RAC) (Sorgeloos, 1981), but after the *D. labrax* and *P. kerathurus* experiments and due to unknown causes, these cysts gave a very bad hatch. We therefore substituted another strain from Macau (Brazil) as a control diet. Previous fatty acid analysis had shown these *Artemia* (referred to as RAC') to be very similar to those of RAC.

In the *S. aurata* experiment another treatment was also applied, namely instar I nauplii of the Chinese strain from Tientsin, because of the irregular results in its industrial use in some Spanish hatcheries.

In all the experiments, the cysts were hatched in 5 µm filtered, 28 ‰ S seawater, under constant aeration at a temperature of 28 °C and an illumination of 1 500 lux at the water surface. The cyst hatching-density ranged from 1 to 2 g cysts/l according to the daily amount of nauplii needed.

After the appropriate hours (T_{90}) of incubation (Vanhaecke and Sorgeloos, 1982) freshly-hatched instar I nauplii were separated from the hatching debris and thoroughly rinsed with tapwater and given to larvae, or submitted to enrichment (SEC-HAT). When AA18 enrichment was used, the nauplii were harvested after 48 h (T_{90}+enrichment in the same hatching medium).

The levels of essential fatty acids in the diets tested are presented in Table I.

Culture conditions and initial ages and sizes of experimental animals are given in Table II.

Both fish and crustacean larvae were fed daily *ad libitum*. When animal sizes allowed it, the bottoms of the recipients were cleaned and, in all cases, removal of nauplii excess was attempted. A starved control treatment was used only in the fish experiments. When working with *P. kerathurus*, the small size of the larvae and their fragility did not allow bottom cleaning causing high mortalities because of debris and metabolites.

The experiments were terminated when mortality suddenly increased in any of the treatments used or, in *Palaemon* spp. tests, when an advanced larval stage was achieved. Lengths and weights of surviving individuals were recorded (except in the *P. kerathurus* experiment). In the crustacean tests, the larval stage was also determined.

Treatments and abbreviations used are :

– freshly hatched instar I nauplii (T_{90}) :
  San Pablo Bay (California, USA) : SPBi
  Great Salt Lake (Utah, USA) : GSLi
  Tientsin (China) : CHi
  Reference *Artemia* (Artemia Reference Center) : RAC
  " " (Macau, Brazil) : RAC'
– enriched nauplii (T_{90}+enrichment time) :
  San Pablo Bay (California, USA), AA18 enrichment : SPBe
  Great Salt Lake (Utah, USA), AA18 enrichment : GSLe
  Great Salt Lake (Utah, USA), SEC-HAT enrichment : GSLe'.

TABLE I

Results of qualitative analysis of fatty acid methyl esters (FAME) in freshly hatched and enriched *Artemia* nauplii (expressed in area %). Analysis carried out by Artemia Reference Center (State University of Ghent, Belgium). RAC' analysis was made by the Departamento Interfacultativo de Fisiologia Animal (Facultad de Ciencias, Universidad de Granada, Spain)

FAME	Diet treatment					
	RAC	RAC'	GSLi	SPBi	GSLe	SPBe
18:2ω6	9.0	5.8	8.7	6.4	6.8	5.5
18:3ω3	2.0	3.5*	24.3	28.6	20.1	25.2
20:5ω3	7.3	7.2	0.3	0.5	2.1	1.8
20:6ω3	–	0.1	–	–	1.4	2.5

* Not separated from 20:1.

PEROXIDE VALUES

Peroxide values were calculated according to the Official Methods of Analysis of the Association of Official Analytical Chemists (Horwith, 1980).

TABLE II

Species	Age (days)	Mean size (mm)	Larval stage	Feeding before experiment start	Culture density (larvae/l)	Starting nauplii amount (nauplii/larvae)	Temperature (°C)	Length of experiment (days)
D. labrax	37	8.78	–	*Brachionus plicatilis*	10	100	18-23	19
S. aurata	35	6.82	–	*Brachionus plicatilis*	15	50	20	24
P. kerathurus	5-7	–	Mysis	*Skeletonema costatum* *Brachionus plicatilis*	50	10	26-28	12
P. serratus	2-3	3.78	Zoea	*Brachionus plicatilis*	25	20	20	22
P. adspersus	2-3	–	Zoea	*Brachionus plicatilis*	25	20	20	18

DATA ANALYSIS

All experiments were carried out in four replicates. Biometric parameters used in the analysis of the results were : length, wet weight, survival and, in crustacean experiments, larval stage. Data were treated statistically in a one-way analysis of variance. Survival data were normalized by an arc-sine square-root transformation (Snedecor and Cochran, 1967). A Duncan's multiple range test was used to determine significant differences among treatments means (Goodnight, 1979).

Results and discussion

FISH

Fish fed reference and enriched strains had significantly greater survival than those fed instar I nauplii of the other strains (Table III).

TABLE III

Growth and survival results for fish experiments.
Means in each row that are followed by the same letter are not significantly different (α=0.05)

Variable	Diet treatment					
Species	SPBi	GSLi	SPBe	GSLe	RAC'*	CHi
Length						
D. labrax	18.2 A	#	19.6 A	18.2 A	19.4 A	–
S. aurata	9.0 A	#	9.2 A	8.7 A	11.5 B	11.7 B
Wet weight						
D. labrax	43.8 A	#	60.5 A	50.0 A	58.0 A	–
S. aurata	5.3 A	#	10.1 BC	5.6 AB	11.8 C	12.3 C
Survival						
D. labrax	15.6 A	1.4 A	66.5 B	93.0 C	92.3 C	–
S. aurata	9.8 A	0.9 A	5.2 A	6.1 A	83.0 B	79.3 B

The shortage of data caused us to exclude this treatment from statistical analysis.
– Not used in this experiment.
* With *D. labrax* RAC was employed.

Confirming previous reports (Yone, 1975 ; Watanabe *et al.*, 1978, 1980, 1982, 1983 ; Fujita *et al.*, 1980), greater survival was obtained when employing those products rich in HUFAs. High mortalities occurred from the 11th to 16th days of the experiments (Fig. 1 and 2).

Low survival was also obtained when SPBe and GSLe nauplii were fed to *S. aurata* larvae. That fact led us to suspect a rancidity problem. It is well known that rancid lipids are toxid *per se*. They react with protein to lower its biological value and have a deleterious effect on those vitamins which are not themselves antioxidants (Roberts, 1978). Peroxide values determined after the experiment gave levels exceeding 30 mg/l (GSLe=112.22 mg/l ; SPBe=32.25 mg/l) confirming that the lipids had become rancid (Wolff, 1981).

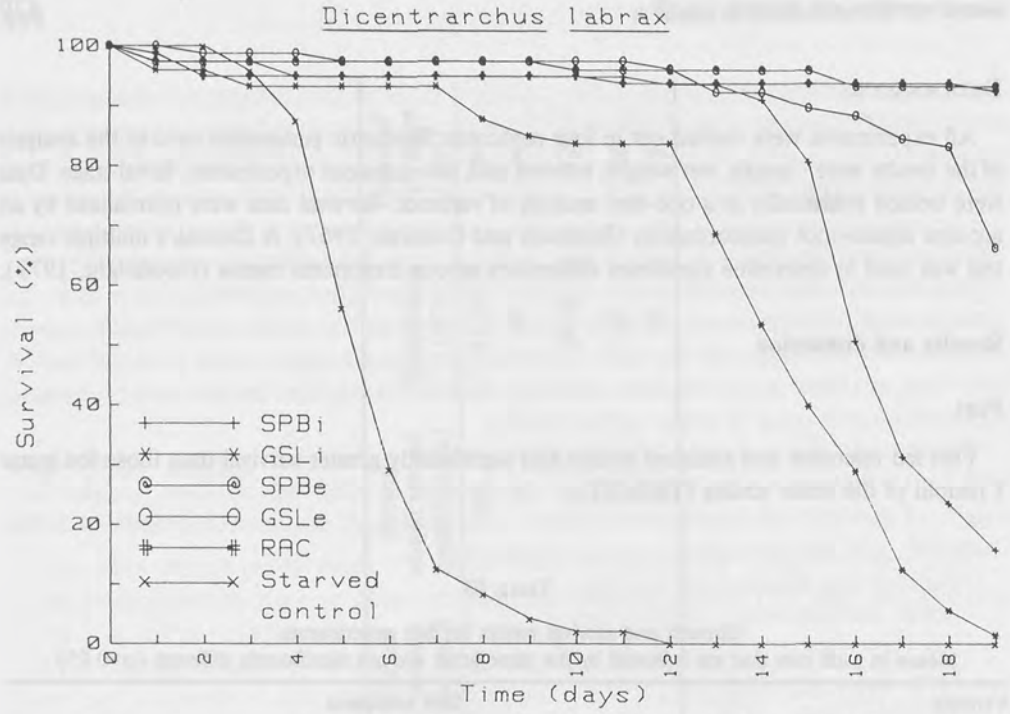

FIG. 1. Survival curve of *Dicentrarchus labrax* fed with different *Artemia* nauplii.

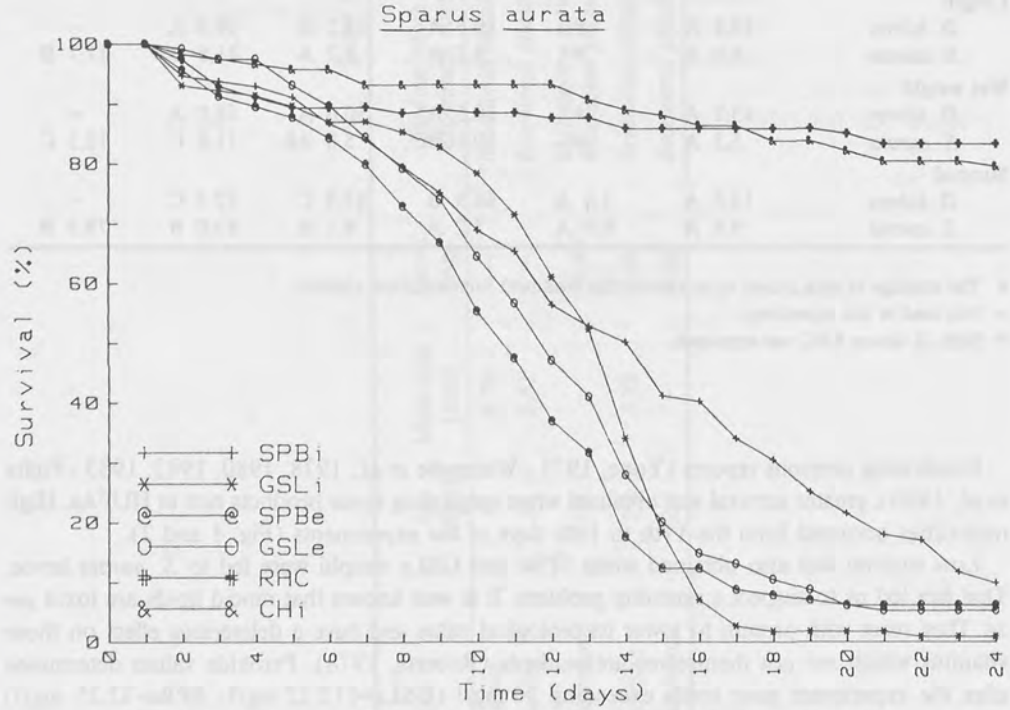

FIG. 2. Survival curve of *Sparus aurata* larvae fed with different *Artemia* nauplii.

Contrary to what had been reported by Robin (1982) for *D. labrax* but in the same way that Beck and Bengtson (1982) reported for *Menidia menidia* and Klein-MacPhee *et al.* (1980, 1982) for *Pseudopleuronectes americanus*, the weight and length of *D. labrax* did not show significant differences. This could be explained in several ways : the growth of sea bass is not affected by the lack of long chain HUFA (20:5ω3, 22:6ω3), the surviving animals were the largest and therefore masked the results, or the enrichment levels were insufficient. Differences were more marked for *S. aurata*, although there was a general trend favoring survival of the largest individuals.

Crustaceans

The best growth and survival of shrimps were obtained with those *Artemia* strains endowed with the highest long chain HUFA levels (Table IV, Fig. 3, 4 and 5), although, in certain cases, no significant differences occurred. Usually, differences among crustaceans were less pronounced than those among fish.

Given that the experiment with *P. serratus* was carried out just before the *S. aurata* one, the abnormal results obtained with enriched products with the former are probably also due to the rancidity problem.

Table IV

Growth and survival results for crustacean experiments.
Means in each row that are followed by the same letter are not significantly different (α=0.05)

Variable	Diet treatment					
Species	SPBi	GSLi	SPBe	GSLe	RAC'*	GSLe'
Length						
P. kerathurus	–	–	–	–	–	∠
P. serratus	7.0 B	6.6 A	6.6 A	6.6 A	7.9 C	∠
P. adspersus	6.5 B	6.7 C	6.2 A	6.4 B	6.9 D	6.8 C
Wet weight						
P. kerathurus	–	–	–	–	–	∠
P. serratus	5.7 B	5.6 B	6.7 C	4.8 A	8.7 D	∠
P. adspersus	3.2 B	3.6 C	2.9 A	3.0 AB	3.1 B	3.5 C
Survival						
P. kerathurus	2.0 A	7.7 BC	6.0 AB	10.3 BC	15.7 C	∠
P. serratus	48.7 A	47.3 A	53.3 AB	60.7 B	61.3 B	∠
P. adspersus	76.7 AB	80.6 B	49.3 A	82.0 B	90.0 B	85.3 B
% of advanced larvae						
P. kerathurus (% Pl. V)	0 A	0 A	0 A	3.2 B	2.1 B	∠
P. serratus (% Pl. VIII)	2.0 A	1.5 A	5.2 A	2.7 A	19.8 B	∠
P. adspersus (% Pl. VIII)	31.3 B	63.6 C	8.1 A	35.8 B	84.4 D	82.8 CD

– Not determined.
* With *P. kerathurus* RAC was employed.
∠ Not used in this experiment.

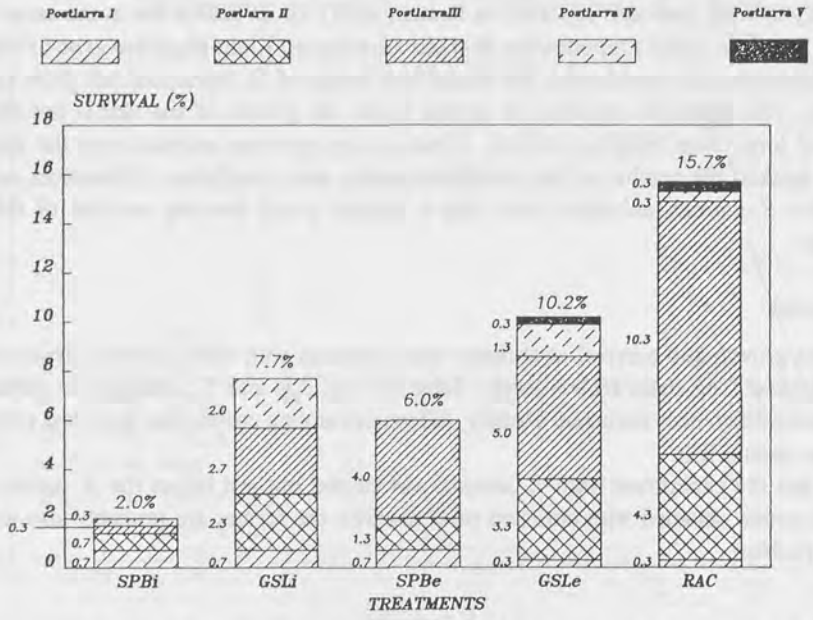

FIG. 3. Survival and larval stage achieved by *Penaeus kerathurus* after 12 days feeding with different *Artemia* nauplii.

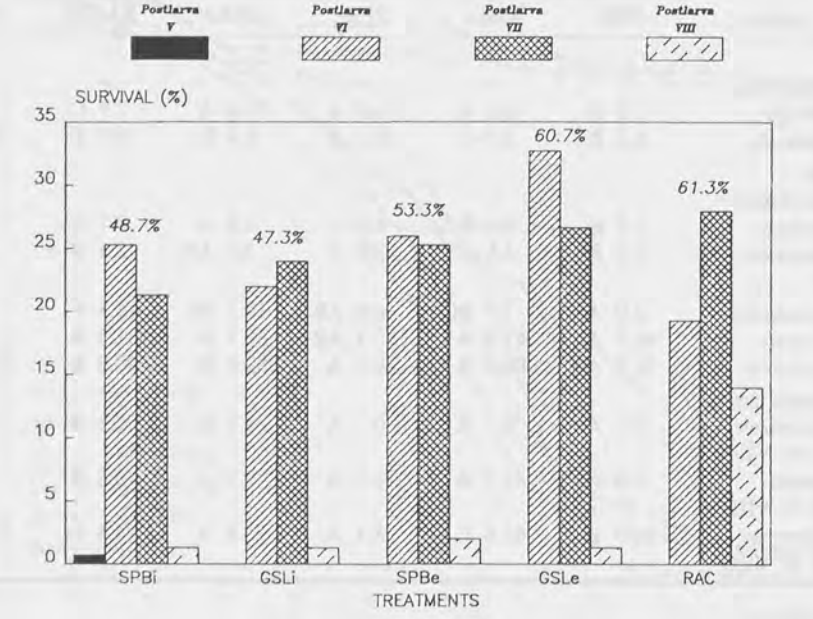

FIG. 4. Survival and larval stage achieved by *Palaemon serratus* after 22 days feeding with different *Artemia* nauplii.

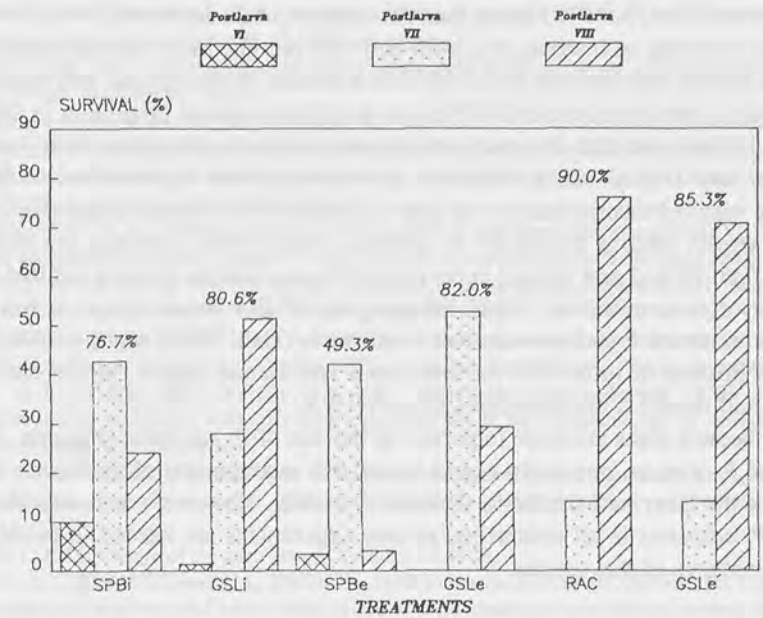

FIG. 5. Survival and larval stage achieved by *Palaemon adspersus* after 18 days feeding with different *Artemia* nauplii.

There is also a clear difference among prawn species with regard to the weight attained, *e.g.* the discrepant results obtained with *P. serratus* and the other species when fed the reference strain nauplii. It is quite surprising that *P. adspersus* showed no differences among treatments with high or low HUFA levels, and achieved the best weight results when fed a treatment without enrichment.

Significant differences in survival were not observed with *P. adspersus*. Only SPBe caused a lower survival level, and the best quantitative results were obtained with the reference strain nauplii and GSLe'. These results could be explained because the enrichment product (AA18) was the same one previously used and suspected of rancidity. Survival was best in *P. serratus*, *P. adpsersus* and *P. kerathurus* fed the reference strain, although the experiment with *P. kerathurus* larvae displayed a general low survival due to imperfections in the experimental system.

The more advanced larval stages were attained with reference strains and with those provided non-rancid enrichment (Table IV).

Generally speaking, the experimental results with crustaceans were more similar among treatments than were those with fish, suggesting that HUFAs must be more important for the latter. However, an additional problem may have been that the enriched *Artemia* were too large for the youngest shrimp larvae to catch or ingest. *Artemia* must be raised to the instar II stage to allow enrichment and are therefore considerably larger than newly-hatched instar I nauplii.

Kanazawa *et al.* (1978, 1979) found that the addition of 22:6ω3 and 20:5ω3 to the diet of *P. japonicus*, promotes an increase in growth and survival. The same authors pointed out that, although the linoleic acid pathway to ω3-HUFAs is present in this species, this metabolic route does not attain a maximum level of HUFAs, so enrichment should be present in the diet.

Martin (1980) showed that *P. serratus* should be capable of synthesizing 20:5ω3 and 22:6ω3 from 18:2ω6 and 18:3ω3 but, nevertheless, growth rates were improved when 20:5ω3 and 22:6ω3 were supplied incorporated in the diet. Guary *et al.* (1976) also stated that 22:6ω3 and 20:5ω3 are growth limiting factors for *P. japonicus*. Read (1981) reported the importance of 18:3ω3, 18:2ω6, 20:5ω3 and 22:6ω3 in the diet of *Penaeus indicus*, pointing out that the feeding habits (carnivorous-omnivorous) could influence the HUFA requirements. It has also been reported that in nature *P. indicus* was more omnivorous (Hall, 1962) and therefore more likely to contain a mixture of those HUFAs from plant and animal origin. On the other hand, *P. japonicus* would require a preponderance of ω3-HUFA.

Similarly, because algae are more important in the diet of *P. adspersus* (Figueras, 1984) than in the diet of *P. serratus*, one might expect the HUFA requirements of the former to be lower than those of the latter and similar to those of *P. indicus*. However, our results showed good survival of *P. adspersus* in all treatments, so new experiments are needed to better assess the HUFA requirements of this species.

Conclusions

The present study generally showed that *Artemia* strains furnished with higher ω3-HUFA levels gave better results. When differences among treatments did exist, they were more marked for fish than for crustaceans, and may be related to the capacity of the latter to synthesize ω3-HUFAs from 18-carbon lipids. Because of technical problems in culture and enrichment procedures in this study, further research is needed to accurately assess the HUFA requirements of these species.

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Production of *Artemia* using mixed diets : control of fatty acid content for marine fish larvae culture

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Abstract

Different techniques of production or enrichment of *Artemia* were tested in order to control their content in ω3-highly unsaturated fatty acids (ω3-HUFA) and therefore their feeding value for marine fish larvae.

The mixed diets used to grow *Artemia* were made of dry single-cell proteins supplemented with vitamins, methionine, and cod liver oil. After feeding 48 h with a diet containing 4 % cod liver oil, ω3-HUFA levels increased at least in strains low in these nutrients. Higher values can be obtained either by using an enrichment technique or 24 h feeding on emulsified oil or 48 h feeding with diets supplemented with 10 or 20 % oil. Since *Artemia* growth depends on animal density, the effect of dietary oil on *Artemia* composition was studied at 10 or 20 animals/ml. *Artemia* cultured at 20 animals/ml had lower growth. At this density with 10 % and even more with 20 % dietary oil, ω3-HUFA content was higher than in *Artemia* cultured at 10 animals/ml.

For the culture of fish larvae, with low ω3-HUFA requirement (sea bass) *Artemia* fed on 4 % cod liver oil diet have a level of ω3-HUFA sufficient to meet the requirement. This level is similar to that of nauplii of a good quality strain. However, for a species such as turbot, the ω3-HUFA requirement of which is very high, sufficient levels of ω3-HUFA can be obtained only with the enrichment technique or with feeding with a high level of oil in the diet.

Introduction

The food quality of the brine shrimp (*Artemia*) is of prime importance for aquaculture. Experiments done with various geographical strains of *Artemia* showed differences in their food values (Beck *et al.*, 1980 ; Johns *et al.*, 1981). Watanabe *et al.* (1978) related nutritional quality of *Artemia* to their content of highly unsaturated fatty acids of the ω3 series (ω3-HUFA). They found an influence of feeding period and prey food composition on this content (Watanabe *et al.*, 1980). Inert food being cheaper than live algae, Person-Le Ruyet (1976) grew *Artemia* on spray-dried *Spirulina.* Later, mixed diets based on dried single-cell protein supplemented with cod liver oil and other additives were tried. This kind of food, originally developed for rotifers (Gatesoupe and Robin, 1981), can also be used to feed *Artemia.* The nutritional value of *Artemia* fed with these diets was tested on sea bass larvae (Robin *et al.*, 1983). In the case of turbot larvae,

which have a very high ω3-HUFA requirement (Le Milinaire *et al.*, 1983), rotifers are used as the first prey, but prey of larger size, such as *Artemia*, are needed later. Unfortunately, no strain of *Artemia*, contains enough ω3-HUFA to meet the requirements of these larvae.

The data presented in this paper concern analyses of *Artemia* from various origins fed or enriched with different methods in order to influence their ω3-HUFA content. The effect of increasing dietary oil level, and that of other techniques which can be used to improve ω3-HUFA, such as enrichment (Gatesoupe, 1982) or feeding emulsified oil (adapted from Watanabe *et al.*, 1982) were tested. The influence of *Artemia* ω3-HUFA level on fish larvae is illustrated by two previously published examples, the sea bass (*Dicentrarchus labrax*) Robin (1982), and the turbot (*Psetta maxima*) (Le Milinaire, 1984).

Materials and methods

Artemia

Cysts of *Artemia* obtained from San Francisco Bay, California, USA (SFB lot 2557), Brazil (CIRNE, Macau) and Great Salt Lake, Utah, USA were hatched in vigorously aerated seawater in 150 l tanks held at 24 °C ± 1 °C. Those obtained from People's Republic China (Tientsin) and San Francisco Bay (lot 694) were hatched in the same tanks at 30 °C ± 1 °C. After 2 days at 24 °C or 1 day at 30 °C the nauplii were separated from unhatched cysts and washed in clean seawater.

Compound diets

Composition of diets is shown in Table I. All dry powders (yeast or autolysate, vitamins, methionine) were first mixed until homogenization, then oil was added and the ingredients were mixed again. Diets prepared for short periods were sealed and stored at low temperature ; more recently (experiments using SFB 694 strain) 2 ‰ of tocopherol was added to the oil to prevent oxidation. Only *Artemia* from the Brazilian strain had been fed on the SYA diet. Food or enrichment mix were distributed after homogenization in a small amount of water.

Table I

Composition of food and enrichment diets for *Artemia* (in weight percent)

	SYA	Af4	Af10	Af20	Ea
Fish autolysate					73
Brewer's yeast	49.4	89.4	83.4	73.4	
Spray-dried *Spirulina*	40				
Dl-methionine	2	2	2	2	2
Choline chloride	1	1	1	1	4
Vitamin premix [a]	3.6	3.6	3.6	3.6	11
Cod liver oil	4	4	10	20	10

[a] In g or I.U./100 g of premix : Vitamin A, 0.004 I.U. ; Vitamin D_3, 0.07 I.U. ; Vitamin E, 8 g ; Vitamin K, 0.3 g ; Thiamine chlorhydrate (B1), 0.3 g ; Riboflavin (B2), 0.7 g ; Pyrodixine chlorhydrate (B6), 0.4 g ; Ascorbic acid (C), 30 g ; Folic acid, 0.3 g ; Vitamin B12 concentrate, 4 g ; Inositol, 40 g ; Biotin, 6 g ; Ca pantothenate, 1.5 g ; Niacin, 5 g ; BHT, 0.6 g ; $CaHPO_4$, 2.79 g.

ARTEMIA FEEDING AND ENRICHMENT TECHNIQUES

Artemia nauplii (10 to 20 × 10^6) were cultured for 48 h in a 1 m^3 tank containing vigorously aerated seawater of 24 °C ± 1 °C.

The daily ration was 40 and 60 (day 0), 60 and 80 (day 1) g of diet. At day 2 *Artemia* were filtered on a 150 µm mesh, then washed in clear seawater. The enrichment technique described by Gatesoupe (1982) consisted of dipping 1 million *Artemia* for 30 min in a well-aerated bath containing 5 g diet Ea before delivering them to the fish.

EFFECT OF ANIMAL CONCENTRATION UPON *ARTEMIA* GROWTH

Three sets of experiments were conducted in 10 l glass bottles (25 °C ± 0.4 °C seawater). Food was distributed in the same rate per volume unit as in typical productions. Initial density of newly-hatched nauplii from San Francisco Bay (lot 694) varied from 7 to 24 *Artemia*/ml. After 48 h feeding, the animals were washed, concentrated in 1 l seawater and dry matter was weighed after 24 h at 105 °C.

ANALYSES

Animals were rinsed in tap water and frozen ; after lyophilization their lipids and fatty acids were determined by the methods described by Le Milinaire *et al.* (1982).

Results

FEEDING AND ENRICHMENT EFFECT ON COMPOSITION OF *ARTEMIA* FROM DIFFERENT LOCATIONS

The content in ω3-HUFA of *Artemia* from different origin at hatching, after feeding or after enrichment is given in Table II. A marked variability was found among the nauplii. The percentage of ω3-HUFA increased after feeding the Af4 diet and this increase was more important in the strains of which the nauplii have a low content of ω3-HUFA. The use of the enrichment technique or emulsified oil also increases this level in treated *Artemia* ; however, our results with emulsified oil are not as good as those obtained by Watanabe *et al.* (1982). One cause of this discrepancy could be the nature of the oil used. Higher level of cod liver oil in the diet also increased HUFA in the San Francisco Bay strain.

When the previous results are expressed on a dry matter basis (Table III) quite different tendencies appear ; only strains with nauplii low in HUFA have a better value with diet Af4. Enrichment with foods high in cod liver oil led to increased lipid level and induced higher ω3-HUFA content expressed on a dry matter basis. The different fatty acid series in four strains are presented in Fig. 1.

Watanabe *et al.* (1978) have classified *Artemia* strains from the point of view of the ω3 series fatty acids. They defined two *Artemia* types : the marine type which has mainly eicosapentaenoic acid and the freshwater type which has mainly linolenic acid. In our trial Brazilian and Chinese strains were of the marine type, San Francisco Bay, and Utah strains of the freshwater type. Diet Af4 increased the HUFA level of the freshwater type strains but not that of the marine types. In the San Francisco Bay strain the linolenic acid decreased during feeding while eicosapentaenoic acid increased and 2-day old *Artemia* resembled nauplii of the marine type. This change was the same for the two SFB and 2-day old *Artemia* were more similar in fatty acid profile than the

TABLE II

ω3-HUFA content (expressed in % of total fatty acids) in *Artemia* of various origin and enriched with different diets

Strains	Na[a]	A_1[b]	A_2[c]	A_2	A_2	A_2
		Diet and enrichment				
		Ea	Af4	Af4 + Ea	Af10	Af20
Brazil	6.8		9.1	10.4		
China	11.7		12.9			
France	6.9		8.5			
San Francisco Bay						
– lot 2557	5.7		12.7	14.9	10.1	
– lot 694	4.0	7.5	12.2		14.4	16.6
Great Salt Lake	4.0		8.9	10.9		

[a] Newly-hatched nauplii.
[b] 24 h-fed *Artemia*.
[c] 48 h-fed *Artemia*.

TABLE III

ω3-HUFA content on dry matter basis for *Artemia* nauplii (Na) *versus* enriched *Artemia* (A1 and A2)

Strains	Na	A_1	A_2	A_2	A_2	A_2
		Diet and enrichment				
		Ea	Af4	Af4 + Ea	Af10	Af20
Brazil	0.92		0.71	0.86		
China	1.1		1.0			
France	0.76		0.54			
San Francisco Bay						
– lot 2557	0.86		0.84	1.59	1.02	
– lot 694	0.58	1.30	0.90		1.40	2.28
Great Salt Lake	0.52		0.90	1.23		

corresponding newly-hatched nauplii. However, the variability in fatty acid content of different lots of cysts of a given origin may be high as shown by Watanabe *et al.* (1980). With only two lots tested it is difficult to assert that the feeding effect will always be the same for a given strain.

GROWTH OF *ARTEMIA* AND EFFECT ON BIOCHEMICAL COMPOSITION

Since the initial density of *Artemia* in our typical production units varied between 10 and 20 animals/ml, a set of experiments was conducted to study the influence of density on *Artemia* growth. Results of three experiments are summarized in Fig. 2. Growth was best at 10 *Artemia*/ml and decreased with increasing density. A significant ($P < 0.01$) regression line can be drawn :

$$DW = 4.885 - 0.0987 \times d$$

in which $r = 0.82$;
DW = mean dry weight in µg ;
d = initial density in *Artemia*/ml.

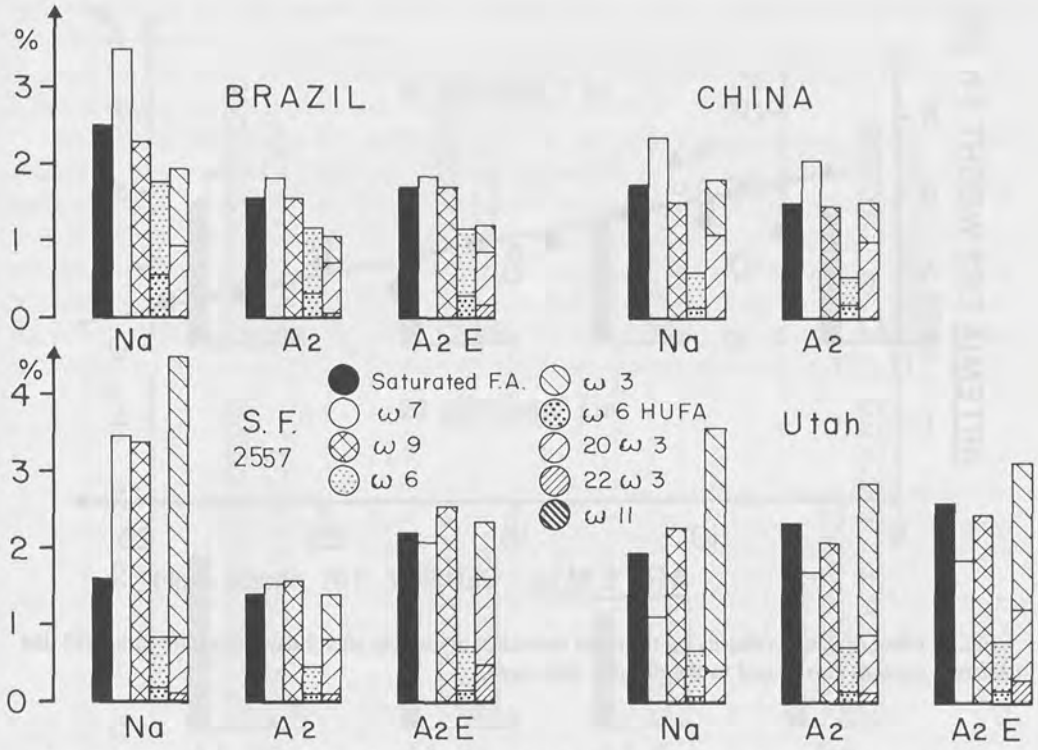

FIG. 1. Fatty acid pattern (in % of animal dry weight) in *Artemia* from various origins : newly-hatched (Na), after 2 days of feeding on a diet with 4 % cod liver oil (A2), same fed *Artemia* after enrichment with Ea mixture (A_2E).

In order to test the possible effect of growth rate on the composition of *Artemia*, *Artemia* were cultured under typical production conditions with two concentrations (10 or 20 *Artemia*/ml) three diets (4 %, 10 %, or 20 % cod liver oil) (Table IV).

TABLE IV

Mean dry weight and lipid levels in *Artemia* fed on three diets at densities of 10 or 20 animals/ml (diet compositions as in Table I)

	Af20		Af10		Af4	
	20	10	20	10	20	10
Individual dry weight (in μg)	2.86	3.12	3.27	3.4	2.77	2.97
Total lipids[a]	18.9	16.3	14.7	15.7	11.6	12.8
Σ20C n-3[b]	12.5	11.8	11.6	11.0	10.8	10.3
Σ22C n-3[b]	5.1	4.1	3.3	1.9	0.8	0.8
n-3 HUFA[a]	2.5	1.9	1.6	1.4	0.9	0.9

[a] In % of dry weight.

[b] In % of total fatty acids.

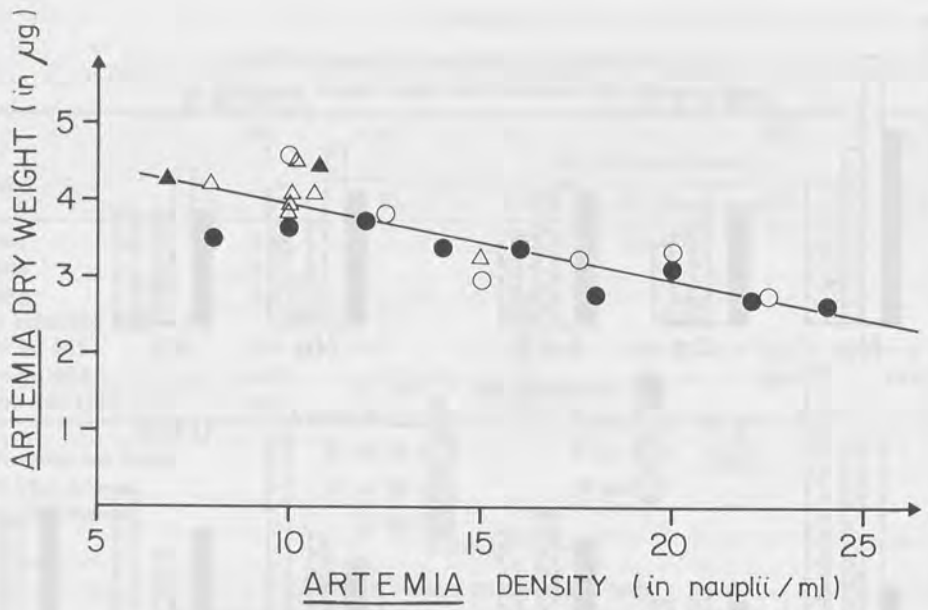

FIG. 2. Effect of *Artemia* density on the mean individual dry weight after 2 days of culture with Af10 diet (different symbols correspond to the different trial runs).

With Af10 and especially Af20 diets, the *Artemia* had a better HUFA value. This is particularly clear if 22:5ω3 and 22:6ω3 are considered. Moreover, with 20 % cod liver oil in the diet, the *Artemia* lipid content was higher in the more densely concentrated animals. This can be interpreted as a better fat deposition in *Artemia* raised under restrictive conditions. Fatty acid profiles of these animals were compared to newly-hatched nauplii and cod liver oil (Fig. 3). The fatty acid composition of fed *Artemia* cannot be considered only as an intermediate between nauplii and food lipids. Even if the fatty acid pattern of Af20-fed *Artemia* was closest to that of cod liver oil, *Artemia* fed on Af4 have a particular fatty acid profile quite different from that of nauplii and cod liver oil. This result is more evident if the change in each series in percent of total fatty acids is considered (Fig. 4) ; there is a high level of ω7, a reduced content of C18ω3, with an increase of 20 carbon fatty acids in the ω3 and ω6 series.

Discussion

The effects of *Artemia* enrichment and feeding technique have been tested with sea bass and turbot larvae as predators. Robin (1982) fed sea bass larvae on *Artemia* that had been fed and/or enriched on each of the following diets : yeast, yeast + Ea, Af4, Af4 + Ea. The *Artemia* ω3-HUFA levels (% dry weight) after feeding on each of those diets were, respectively : 0.3, 0.4, 0.8, and 1.5. Survival and growth of the sea bass larvae were significantly improved in the yeast + Ea treatment compared to the yeast treatment ; however, the best growth was obtained in the Af4 and Af4 + Ea treatments, which were not significantly different from each other. Robin (1982)

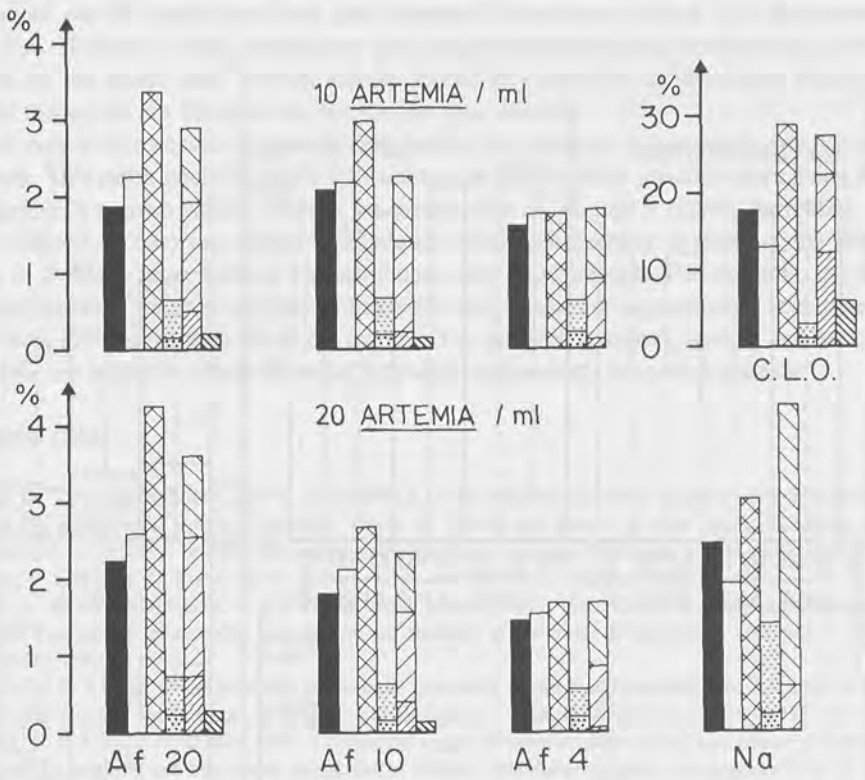

FIG. 3. Fatty acid profiles (in % of animal dry weight) for *Artemia* fed on three levels of cod liver oil in the diet and at two culture densities, compared with newly-hatched nauplii and cod liver oil (in % of total fatty acids) (same symbols as in Fig. 1).

concluded that the Af4-fed *Artemia*'s ω3-HUFA level of 0.8 % must therefore be adequate for sea bass larvae and that Ea enrichment did not further improve the *Artemia*.

Le Milinaire (1984) fed turbot larvae on *Artemia* that had been enriched with Ea or Eω (ω3-HUFA concentrate), resulting in *Artemia* ω3-HUFA levels of 1.2 and 1.7 (% dry weight), respectively. Weight of turbot larvae in the *Artemia*-Eω treatment was significantly greater than that of larvae in the *Artemia*-Ea treatment. Therefore, turbot larvae must have a higher ω3-HUFA requirement than sea bass larvae (at least 1.7 % *versus* 0.8 %, respectively) for optimal growth. The ω3-HUFA levels in newly-hatched *Artemia* nauplii (Table III) are occasionally adequate for sea bass larvae, but never adequate for turbot larvae. Bell *et al.* (1985) have shown that turbot require docosahexanoic acid, which is lacking in nauplii and must be provided through *Artemia* enrichment techniques.

The use of enrichment is a way to control fatty acid level and pattern in *Artemia*. For fishes like sea bass, newly-hatched nauplii of good quality can be sufficient. However, other species like turbot need higher levels of ω3-HUFA which can only be obtained by improving *Artemia* quality. These techniques provide also docosahexanoic acid, which is generally lacking in *Artemia* cysts. With diets high in cod liver oil or with enrichment mix containing ω3 concentrate, freshwater

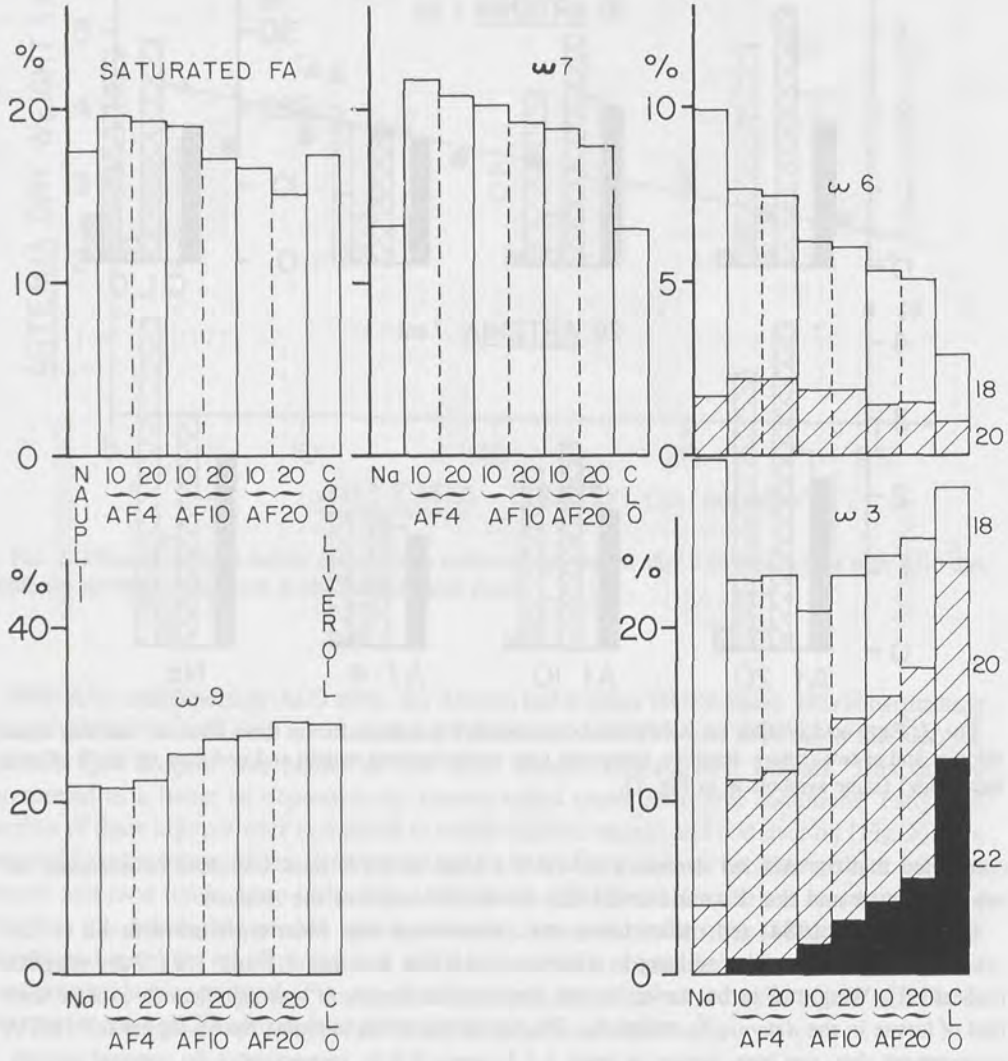

FIG. 4. Fatty acid profiles (in percent of total fatty acids) for 2-day old *Artemia* fed with three diets (containing 4, 10, and 20 % cod liver oil) at two larval densities (10 and 20 *Artemia*/ml), compared with newly-hatched nauplii (Na) and cod liver oil (CLO); the ω6 series is split up in C18 and C20; the ω3 series in C18, C20, and C22 (same symbols as in Fig. 3).

type nauplii can be transformed into prey adequate for predators having high requirements in ω3-HUFA. However, when *Artemia* are fed on low-lipid-level diets, retention of ω3-HUFA depends on the strain used. Further studies should be conducted to investigate whether high levels of dietary oil are effective also on marine type *Artemia.*

The increase of essential fatty acids observed in the densely concentrated *Artemia* is quite surprising. This result could be taken into account in hatcheries to produce more living food of good quality in a given volume, even if the growth rate of *Artemia* is slightly decreased.

It is difficult to compare different techniques which can be used to improve the fatty acid pattern of *Artemia.* Each method has advantages and disadvantages. For example, the feeding technique allows production of prey of large size and reduces consumption of expensive cysts, but requires more tanks than the direct method. For research purposes, feeding and enrichment techniques are helpful to study essential fatty acid requirements of marine animals.

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The effect of *Artemia* fed with different diets on the growth and survival of *Penaeus monodon* Fabricius postlarvae

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Abstract

Penaeus monodon postlarvae (PmI) were fed to excess on a diet of either finely ground cooked mussel meat or live preadult *Artemia* (fed with either wheat flour, rice bran, or milled rice extracts) for 20 days. The postlarvae fed with cooked mussel meat had significantly lower weight gain ($P < 0.05$) than those fed with *Artemia*. No significant differences were found among the weight gains of shrimp fed with the various *Artemia*, although slightly higher weight gains were observed with the postlarvae that received *Artemia* fed with milled rice extract. The survival rate was highest for shrimp fed with cooked mussel meat. This was significantly higher than those for shrimp fed with the various *Artemia* except that of shrimp fed with *Artemia* that received milled rice extract. The total body lipid content of the post-experimental shrimp was higher than that of the pre-experimental shrimp. Fatty acid composition of shrimp was generally similar to the fatty acid composition of the diets. Shrimp fed with cooked mussel meat had a fatty acid profile similar to that of the pre-experimental shrimp. The ratio of $\omega 3/\omega 6$ fatty acids was highest (2.17) in shrimp fed cooked mussel meat, which had a ratio of 4.06. The other ratios were 1.17, 0.99, and 0.60 for shrimp fed *Artemia* that received milled rice, wheat flour and rice bran extracts, respectively.

Introduction

Penaeus monodon Fabricius has a great potential for culture in Southeast Asia. However, seed supply is one of the limiting factors in its culture and in the aquaculture industry, in general. Therefore, the search for a suitable and high quality food for the postlarval stages is one of the important areas of study in seed production.

Artemia is known worldwide as an excellent food source for early larval stages of many crustaceans and fishes. It has great practical value because of its availability as dry cysts. However, since the demand is perennially great, it is always in short supply and thus very expensive. *Artemia* can not only be used alone, but also mixed with artificial diets as a source of protein, as an attractant, or simply as a supplementary feed along with artificial diets (Sick and Andrews, 1973 ; Sick and Beaty, 1975 ; New, 1976 ; Murai and Andrews, 1978 ; Sorgeloos, 1980).

During recent years new sources of natural cysts have been discovered (Sorgeloos, 1980). Moreover, intensive culture under controlled conditions with commercially available cheap diets has been tested (Jahnig, 1977 ; Dobbeleir *et al.*, 1980 ; Sorgeloos, 1980). However, the

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nutritional value of *Artemia* from different environments, strains, and stages has been shown to differ with some distinct effects on growth and survival of fry and larvae. According to Watanabe *et al.* (1978) and Schauer *et al.* (1980), the quality and quantity of fatty acids in *Artemia* are the principal factors that determine nutritional value of *Artemia.* Dobbeleir *et al.* (1980) pointed out that it is possible to improve the nutritional suitability of *Artemia* through manipulation of its diets. Sakamoto *et al.* (1982) incorporated the ω3 highly unsaturated fatty acids (HUFA), 20:5ω3 and 22:6ω3, which are essential for marine fish and crustacean larvae, in micro-encapsulated diets fed to *Artemia* and demonstrated the subsequent appearance of those HUFAs in *Artemia* tissue. Successful production of *P. monodon* postlarvae by feeding newly-hatched nauplii of *Artemia* during mysis and postlarval stages is generally known, but because of high price and unstable supply of cysts, other live food and artificial diets have been tested as a substitute or supplement to *Artemia.* However, neither good growth nor high survival has been obtained without *Artemia. Artemia* is one of the most effective foods, not only for the postlarval stage but also for juvenile shrimp. Furthermore, *Artemia* acts as a booster for the growth of penaeid shrimp when used as a supplement with other foods (Kittaka, 1975).

Consequently, this study aimed to determine the effect of *Artemia,* cultured on different diets, on the growth and survival of *P. monodon* postlarvae grown under controlled conditions and to show the fatty acid pattern of *P. monodon* postlarvae fed with different diets.

Materials and methods

Culture of *Artemia*

Experimental facilities and cysts.

The experiment was set up at SEAFDEC Aquaculture Department, Iloilo City, Philippines, during the summer of 1982. Three 1 500 l oval-shaped fiber glass thanks were used, each equipped with a wooden plate separator and an air-lift system (Sorgeloos *et al.*, 1977), and a hanging feeding bottle of 1 l capacity. At the bottom of each tank, two plastic sheets (one blue and one yellow) with a dimension of 2.5 cm × 10 cm were provided to facilitate the determination of daily feeding rate. Each tank was filled with approximately 1 000 l of filtered seawater.

Artemia cysts (Great Salt Lake, Utah strain) were hatched in a 25 l conical plexiglass container filled with about 15 l of 30 ‰ S seawater. Strong aeration was provided, and the cysts were incubated at room temperature (28 °C-30 °C) for a period of approximately 36 h. Nauplii were separated from the empty cysts and debris, and were transferred to clean filtered seawater in a 15 l shallow basin with aeration. They were sampled and counted three times with a 1 ml medicine dropper for estimation of density. They were then randomly selected and stocked into 1 500 l fiber glass tanks described above, at a density of 5 000 nauplii/l.

Feeds and their preparation

Extracts of wheat flour (Pillsbury), rice bran, and milled rice were used as feeds for *Artemia.* Each feed was mixed with crude sea salt at a ratio of 2:1. Filtered seawater was added and the mixture was blended for 3 to 5 min. with a kitchen blender. The solution was then extracted by squeezing through a 50 μm mesh nylon filter bag. The extracts were kept refrigerated at 10 °C. A quantity sufficient for 3 to 5 days feeding was prepared each time for each feed.

Management

Feeding of *Artemia* was done four times daily by transferring the extract to the 1 l feeding bottle and the solution was allowed to drip continuously at a rate of 5-10 ml/min until the bottle was emptied. The feeding rate was determined through observation of the transparency of the culture medium, enough to see the yellow and blue plastic sheets described above, at the depth of 30 cm. No water change was done throughout the culture period. The plate separator was cleaned thoroughly once every 2 days to remove the excess feed and feces. On the third day of culture, a piece of 25 mm mesh nylon gill net (30 cm × 40 cm) equipped with sinkers was placed vertically inside each tank. The net, which served as accumulation site for excess feed and feces, was removed and cleaned once or twice daily. Water temperature, salinity, and pH were measured twice daily at around 0800 h and 1600 h. However, when shrimp culture was started, the quantity of preadult *Artemia* was insufficient to meet the requirement for feeding of *P. monodon* postlarvae throughout the experiment. Therefore, another set of *Artemia* culture was started on the same day using three 250 l conical fiber glass tanks. Each tank was provided with an aeration system using a 10 cm long air stone. The *Artemia* strain, stocking rate, feed and feeding scheme, and culture management used were the same as previously described.

CULTURE OF *P. MONODON* POSTLARVAE

Experimental animals and facilities

P. monodon larvae were obtained from a wild spawner caught along the shore of Tigbauan. The larvae were reared up to early mysis stage, then used in this experiment. A total of 30 000 larvae were randomly selected and stocked in the experimental tanks and the remaining larvae were kept in extra tanks for replacement purposes.

Twelve 250 l conical fiberglass tanks were each equipped with an aeration system using a 10 cm long air stone and filled with 150 l of filtered seawater. The larvae were acclimatized in the tanks for 2 days until they reached the first day of postlarval stage. During this period they were fed with Brazilian strain *Artemia* nauplii twice daily at a concentration of 1 to 2 nauplii/ml of culture medium. Dead larvae were replaced during this period. At the end of the acclimatization period ten postlarvae were randomly selected from each of the 12 tanks for initial weight measurement. Stocking density was 1 500 larvae/tank (10 larvae/l). Weight measurement was done in mass with a Mettler analytical balance.

Black, 1 mm mesh nylon net was used to cover each tank in order to reduce light intensity and to prevent the shrimp from jumping out. After the fourth day of culture a bundle of synthetic straw (1 m long), tied to a PVC pipe with a diameter of 6.25 cm and length of 5 cm, was placed in each tank to serve as shelter for shrimp larvae.

Feeds and feeding

Feeds for the shrimp larvae consisted of brown mussel meat (*Mytilus* sp.) and the *Artemia* fed with extracts of wheat flour, rice bran, or milled rice, and the freshly-hatched *Artemia* nauplii of the Brazilian strain. The mussel meat was removed from the shell, steamed for about 15 min., allowed to cool to room temperature and homogenized with a kitchen blender for 3 min. An amount sufficient for 4 to 5 days of feeding was prepared each time, stored in a closed bottle and kept refrigerated at 4 °C.

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Management

This feeding experiment was conducted for 20 days. The postlarvae were fed twice daily (at around 0900 h and 1700 h) every day. At PL-I to PL-V, they were fed with *Artemia* which passed through the nylon screen of 350 µm mesh size at a concentration of 0.5 nauplii/ml of water. From PL-VI to PL-XX *Artemia* which passed through a 450 µm mesh, but retained by 350 µm mesh were used. The feeding concentrations were 0.7, 0.8, and 1.0 ind./ml for PL-VI to PL-X,PL-XI to PL-XV, and PL-XVI to PL-XX, respectively.

For postlarvae which received mussel meat as feed, at PL-I to PL-V they were fed a daily rate of 400 % (wet weight basis) of their body weight along with 2-3 *Artemia* nauplii/ml of water. After these stages they were fed only mussel meat at rates of 300 %, 200 %, and 300 % of biomass for PL-VI to PL-X, PL-XI to PL-XV, and PL-XVI to PL-XX, respectively. Before the daily feeding, the shrimp in all tanks were observed for mortality and dead shrimp were removed. About one-third of the water volume was changed daily in the morning by siphoning to remove excess feeds and feces. Water temperature, salinity, and pH were determined twice daily around 0800 h and 1600 h.

Experimental design and statistical analyses of data

The experiment consisted of four treatments with three replications per treatment : treatment A, shrimp received *Artemia* fed with wheat flour extract ; treatment B, shrimp received *Artemia* fed with rice bran extract ; treatment C, shrimp received *Artemia* fed with milled rice extract, and treatment D, shrimp received *Artemia* nauplii and cooked mussel meat. The treatments were assigned randomly using a completely randomized design (CRD). Analyses of the data were done at 5 % probability level using the one way analysis of variance (Steel and Torrie, 1960). When significant differences were found among the treatments, Duncan's Multiple Range Test was used to test differences between treatment means. The survival rates (%) were subjected to arc-sine transformation before analysis of variance.

Chemical analyses

Proximate analyses

Cooked mussel meat and *Artemia* fed with different feeds were collected, stored in sealed plastic bags and kept frozen at about - 8 °C for subsequent analyses. The samples were oven-dried at 105 °C for 24 h, ground finely in a mortar and kept in a dessicator at room temperature. The sub-samples were taken from each sample for complete proximate analyses using the method given by AOAC (1975). Ground feeds and their extracts fed to the *Artemia* were also analyzed for their proximate composition.

Fatty acid analyses

Samples of *Artemia* fed with different feeds were collected and stored frozen at - 8 °C for 2 to 3 days before lipid extraction. Lipid extraction of the homogenized mussel meat was done without prior storage.

Prior to the start of the experiment, *P. monodon* postlarvae (PL-1) were sampled and lipid extraction was done immediately. At the end of the feeding experiment, the shrimp postlarvae

(PL-XX) from various replicates of the same treatment were collected, pooled, stored in sealed plastic bags and kept frozen at - 8 °C for 5 days prior to the extraction of the lipids.

Extraction of the lipid was carried out by the chloroform-methanol-water method described by Bligh and Dyer (1959). The extracted lipids were then kept in a well-closed screw-capped glass bottle and stored at - 20 °C for about a week. Thereafter, the lipids were evaporated in a water bath at 35-40 °C and methylated with boron trifluoride methanol (BF_3 methanol) according to the method given by Metcalfe *et al.* (1966). The fatty acid composition was determined by gas-liquid chromatography (GLC). The GLC was performed with a Shimadzu GC-3BPF equipped with a flame ionization detector and column (3 mm i.d. × 3 m long), packed with Schinchome E-71 (5 °b) on Shimolik (AW) (201) Chromosorb (80-100 mesh), supported with a 90 ml/min flow of nitrogen as the carrier gas, at 230 °C. The temperature of the injector and detector was the same (350 °C) and the injector pressure was 2.2 kg/cm^2. Peaks were identified by the use of commercially available standards. Peak areas were quantified by formula : $A = HW_{1/2H}$ where : A = area of peak, H = height of peak, and $W_{1/2H}$ = width of peak at one half height. Results were calculated and expressed as weight percentage of total lipids.

Fat extraction and preparation of the fatty acid methyl esters (FAME) ready for injection into the GLC were done at the SEAFDEC laboratory, Tigbauan Research Station. The samples of FAME were packed in 1 ml glass vials covered with rubber cap and hand-carried to Japan. The fatty acid analyses were done at Kagoshima University.

Results and discussion

Growth of shrimp

The growth responses of *P. monodon* postlarvae fed with various diets are presented in Table I. The postlarvae in treatment C gained an average of 5.46 mg which was numerically higher but not significantly different from the shrimp in treatments A and B (4.40 mg and 4.56 mg), respectively. Shrimp fed with mussel meat in treatment D had the lowest weight gain (2.61 mg) and was significantly lower than those fed with *Artemia.*

Table I

Average weight gain and survival rate of *P. monodon* postlarvae. Numbers in each column with the same superscript are not significantly different ($P < 0.05$)

Treatment	Weight gain (mg)	Survival rate (%)
A (fed wheat flour *Artemia*)	4.40 ± 0.66^a	35.2 ± 4.2^a
B (fed rice bran *Artemia*)	4.65 ± 0.40^a	35.5 ± 1.9^a
C (fed milled rice *Artemia*)	5.46 ± 0.63^a	$41.7 \pm 5.1^{a,b}$
D (fed cooked mussel meat)	2.61 ± 0.55^b	47.0 ± 6.0^b

This indicates that *Artemia* is a better food to grow *P. monodon* postlarvae than cooked mussel meat which is in agreement with the generally recognized fact that *Artemia* is an excellent food source for the early larval stages of most aquatic species. Sorgeloos (1980) noted that *Artemia* is a very efficient protein converter. Thus, even if the feeds contained low levels of plant protein, the preadult *Artemia* had crude protein values that varied from 41.56 % to 56.48 % (Table II).

Both quality and quantity of protein is known to have a significant effect on the growth of *P. monodon.* Proximate analyses of the *Artemia* fed with different feeds and of cooked mussel meat are given in Table II. These values are within the range required for good growth of penaeid shrimp. Alava (1979) found that 40-45 % protein is optimal for the growth of *P. monodon* juveniles. Furthermore, Bages and Sloane (1981) reported 55 % protein level was required for the early larval stages of *P. monodon.* The significantly lower weight gain of the shrimp fed with mussel meat may be attributed to the quality of the protein.

TABLE II

Proximate chemical composition of *Artemia* feeds, *Artemia* itself and cooked mussel meat

Sample	Nutrient (%)[1]			
	Crude protein	Crude fat	Crude fiber	Ash
Wheat flour	20.27	2.88	2.53	1.61
Rice bran	13.29	14.85	11.14	10.30
Milled rice	9.04	0.83	1.62	0.71
Wheat flour extract	7.45	0.33	1.50	43.29
Rice bran extract	7.27	6.22	1.83	64.01
Milled rice extract	4.31	0.25	0.78	48.05
Artemia fed with wheat flour extract	54.00	7.55	3.24	21.07
Artemia fed with rice bran extract	56.48	9.54	3.47	20.18
Artemia fed with milled rice extract	41.56	6.62	4.40	22.63
Cooked mussel meat	55.79	15.62	0.16	9.89

[1] Values are means from two replicates and expressed on dry matter basis.

Artemia fed with milled rice extract had the lowest protein content, but provided better weight gain of shrimp than any other diets. This might be due to the better quality of protein of *Artemia* fed with milled rice extract. Therefore, it is possible that the amino acid pattern of *Artemia* in treatment C was better than the other treatment (amino acid analyses were not carried out in this experiment). The biological value of rice protein was found to be better than that of wheat protein when fed to rats (Jones *et al.*, 1948, cited in Piedad, 1956).

The high level of total lipid in cooked mussel meat (15.62 %) could have also caused a low weight gain. Lim *et al.* (1979) reported that fresh brown mussel meat was inferior to squid meat for the growth of *P. monodon* postlarvae. Mendoza (1982) found that the dietary level of lipid of around 12 % was optimal for the growth of *P. monodon* juveniles. Increasing the level of lipid beyond this value slightly decreased growth.

The composition of essential fatty acids and their ratio are also important factors in determining the nutritional value of the feed. Watanabe *et al.* (1978) and Fujita *et al.* (1980) stated that the class of essential fatty acids (ω3) contained in *Artemia* is one of the principal factors in

the food value of *Artemia* to fish. For shrimp, the ω3 polyunsaturated fatty acids such as 18:3ω3, 20:5ω3 and 22:6ω3 are more essential and more efficient for promoting growth than the ω6 fatty acids (Kanazawa *et al.*, 1977 ; Kayama *et al.*, 1980). Based on this finding, the highest growth rate of shrimp fed *Artemia* in treatment C could have been due to the higher amounts of total ω3 and lower amounts of total ω6 fatty acids compared to the other *Artemia* used (Table III). Sick and Andrews (1973) reported that low levels of ω3 and high levels of ω6 fatty acids inhibited the growth of *P. duorarum* ; 20:5ω3 and 22:6ω3 acids were higher in *Artemia* fed milled rice extract (treatment C) than in the other *Artemia*. Furthermore, the ratio of ω3/ω6 was also higher in *Artemia* treatment C. Colvin (1976) reported that in shrimp diets, proper levels and ratio of ω3 and ω6 fatty acids may be necessary for efficient lipid metabolism. It is possible that shrimp fed with cooked mussel meat had the lowest weight gain because there is a relatively higher ratio of ω3/ω6 fatty acids and less total essential fatty acids of the ω3 series (18:3ω3, 20:5ω3, and 22:6ω3) in the cooked mussel meat (17.5) than in *Artemia* fed with milled rice extract (20.31).

Survival of shrimp

The survival rate obtained from this feeding experiment ranged from 35.2 % to 47.0 % (Table I). The highest survival rate was obtained in shrimp fed with cooked mussel meat. This was significantly higher than those of the other treatments except for treatment C. Shrimp in treatments A and B had similar survival rates (35.2 % and 35.7 %, respectively) but these were not significantly different from that of the shrimp in treatment C.

The lower level of total ω3 fatty acids of *Artemia* from treatment A and B compared to those *Artemia* in treatment C or the cooked mussel meat in treatment D could also have contributed to the low survival of the shrimp fed with these *Artemia* (A and B). Kanazawa *et al.* (1977, 1979) found that the linolenic series (ω3) fatty acids are more essential for growth and survival of shrimp than are the linoleic series (ω6) fatty acids and that the HUFAs, 20:5ω3 and 22:6ω3, are more effective than linoleic and linolenic acids. *Artemia* fed wheat flour and rice bran extracts (A and B) had lower 20:5ω3 and 22:6ω3 ω3/ω6 ratio than *Artemia* fed milled rice extract (C) and cooked mussel meat (D) (Table III). The ratio of ω3/ω6 for *Artemia* C was higher than other *Artemia* and close to the ratio of ω3/ω6 in cooked mussel meat. Therefore, it is not surprising that survival rate was similar for shrimp in treatment C and D. The highest survival in shrimp treatment D may also be the result of feeding *Artemia* nauplii along with cooked mussel meat at stages PL-I to PL-V, because *Artemia* may act as a booster for growth and survival of *P. monodon* as Kittaka (1975) found for other penaeids.

Aside from the nutritional value of the feeds, other factors could have caused the differences in survival rate of the shrimp. Relatively low survival of shrimp in treatments fed with *Artemia* could be due to overfeeding. Gopalakrishnan (1976) found that excessive feeding with *Artemia* caused severe fouling and stress of *P. merginatus* larvae, which could be the same for *P. monodon* larvae. Another cause of mortality was the "jumping" behavior of the shrimp, which resulted in escape or being trapped on the sides of the tanks. The highest mortality from this cause (9.24 % of initial stocking) was observed in treatment C which had the largest larvae.

Physicochemical conditions of the water are also important factors affecting the survival of shrimp. The average morning and afternoon salinity was the same and ranged from 32 to 33 ‰. This value if higher than the optimum range required by *P. monodon* postlarvae which was

TABLE III

Fatty acid composition of *Artemia* fed with different diets and of cooked mussel meat (*Mytilus* sp.)

Fatty acid	*Artemia*			Cooked mussel meat
	Treatment A	Treatment B	Treatment C	
14:0	1.51	0.76	0.54	5.13
16:0	9.61	12.44	12.84	17.66
16:1ω7	6.92	4.93	4.03	15.54
16:3ω3	0.41	0.45	trace	2.26
18:0	6.64	4.71	9.82	6.50
18:1ω9	28.90	34.36	23.40	6.71
18:2ω6	22.80	26.14	10.09	1.51
18:3ω3	7.94	4.48	11.16	1.65
18:4ω3	1.15	0.26	trace	1.13
20:1ω9	0.82	0.60	0.94	6.24
?	–	–	–	1.41
20:3ω3	0.72	0.35	0.67	0.24
20:4ω6	0.92	0.71	4.44	4.10
20:5ω3	2.34	2.18	7.67	12.06
22:0	0.20	–	0.34	0.09
22:1ω9	1.08	0.41	2.42	–
22:3ω3	–	–	–	3.25
22:5ω3	0.06	0.30	0.27	0.85
22:4ω3	0.36	0.15	0.94	0.42
22:4ω6	0.23	0.07	0.40	0.71
22:4ω3	0.41	0.30	1.48	3.97
24:0	0.15	–	0.27	0.47
Total ω3	13.39	8.47	22.19	25.65
Total ω6	23.95	26.92	14.91	6.32
Ratio ω3/ω6	0.56	0.32	1.49	4.06
Total lipid (% of wet weight)	1.24	1.26	1.63	3.83

reported to be 18-20 ‰ (MSU-IFRD, 1975a). The average water temperature (26.5-28.7 °C) and average pH (8.1-8.3) were well in the optimum ranges for good growth and survival of *P. monodon* postlarvae (Catedral *et al.*, 1977 ; MSU-IFRD, 1975b).

LIPID CONTENT AND FATTY ACID COMPOSITION OF SHRIMP

The percentage of lipid of shrimp before starting the experiment was 1.86 %. After 20 days of feeding, shrimp fed with *Artemia* C had a percentage lipid of 3.32 % followed by shrimp fed *Artemia* B, 3.14 % and A, 2.19 %. Shrimp in treatment D contained 2.59 % lipid. Mendoza (1982) found that the percentage of crude body fat of *P. monodon* juveniles was directly related to the level of crude lipid in the diets. Results from this study seemed to indicate that there was a direct relationship between the levels of lipid in the *Artemia* fed to the shrimp and the animal

body lipid levels. However, the shrimp in treatment D fed with cooked mussel meat which contained the highest lipid (3.83 %) had a lower lipid content compared to those shrimp fed with *Artemia*. The total lipid in cooked mussel meat contained the highest level of ω3 polyunsaturated fatty acids and the lowest level of ω6, resulting in a ratio of 4.06, which was also highest of the four treatments (Table III). Thus, the ratio of fatty acids in the diets may have played an important role in the amount of fat deposition. Shewbart and Mies (1973) stated that, since postlarvae and juveniles are still in the stages of rapid growth, the higher unsaturated fatty acids may be retained selectively to meet the demand for the biosynthesis of phospholipids required for metabolic processes and formation of cellular membranes.

The major fatty acids of the pre-experimental shrimp were 16:0, 16:1ω7, 18:0, 18:1ω9, 20:4ω6, 20:5ω6, and 22:6ω3 (Table IV) which is the typical pattern of penaeid shrimp fatty acids, as Ward *et al.* (1979) reported for *P. setiferus* postlarvae and Colvin (1976) reported for *P. indicus* juveniles.

TABLE IV

Fatty acid composition (in %) of *P. monodon* postlarvae before (PL-I) and after being fed with various diets for a 20-day period (PL-XX)

Fatty acid	PL-I	PL-XX			
		Treatment A	Treatment B	Treatment C	Treatment D
14:0	3.21	0.46	0.47	2.40	1.33
16:0	18.87	13.63	16.42	18.47	17.94
16:1ω7	7.40	4.98	–	9.02	6.11
16:3ω3	1.73	0.38	–	1.12	0.66
18:0	9.37	9.57	9.01	7.48	12.62
18:1ω9	17.27	23.28	27.20	24.02	14.62
18:2ω6	4.07	14.30	20.17	9.94	3.72
18:3ω3	0.62	6.84	4.57	7.12	1.99
18:4ω3	0.04	trace	trace	0.37	trace
20:1ω9	0.99	0.71	0.87	0.67	1.46
?	–	–	–	–	0.53
20:3ω3	0.25	1.25	1.48	0.40	0.26
20:4ω6	5.43	4.08	4.83	1.67	7.97
20:5ω3	14.06	7.91	7.43	4.07	13.55
22:0	–	trace	trace	0.48	0.13
22:1ω9	3.70	1.84	1.31	2.38	1.73
22:5ω3	1.48	–	–	trace	0.80
22:4ω3	1.36	0.82	0.49	1.23	1.20
22:4ω6	1.60	0.23	–	0.59	1.06
22:6ω3	6.66	1.15	0.96	trace	9.17
24:0	–	0.15	0.09	–	1.06
Total ω3	26.20	18.35	14.93	14.31	27.63
Total ω6	11.10	18.61	25.00	12.20	12.75
Ratio ω3/ω6	2.36	0.99	0.60	1.17	2.17
Total lipid (percent of wet weight)	1.86	2.19	3.14	3.32	2.59

The postlarvae fed cooked mussel meat had a fatty acid pattern similar to that of the pre-experimental shrimp while the fatty acid patterns of the postlarvae fed with *Artemia* differed markedly. Shrimp fed with *Artemia* had substantially high levels of 18:1ω9, 18:2ω6, and 18:3ω3 ; and low levels of 20:5ω3 and 22:6ω3. These patterns were similar to the fatty acid patterns of the *Artemia* used (Table III). According to Kanazawa *et al.* (1977), Ward *et al.* (1979) and Clarke and Wickins (1980), the fatty acid composition of shrimp is a reflection of dietary fatty acid. However, no explanation can be given for the much lower value of 20:5ω3 (4.07 %) in the treatment C postlarvae than in their feed. It is also interesting to note that the ratio of ω3/ω6 of PL-XX in treatment C (1.17) is similar to *Artemia* itself.

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Laboratory-grown *Artemia* as reference food for weaning fish fry and shrimp postlarvae

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Abstract

Margherita di Savoia (Italy) brine shrimp have been mass-cultured on the microalgae *Tetraselmis suecica* Butcher and *Chaetoceros simplex* Ostenfeld. Frozen adult *Artemia* were tested along with ground fresh sand-smelt meat and commercial pellets as weaning diets for sea bass (*Dicentrarchus labrax* L.) fry. In weaning tests with *Penaeus japonicus* Bate post larvae, diets of adult *Artemia* collected from the Margherita di Savoia saltworks and laboratory-grown adult *Artemia* (both frozen) were compared.

Frozen *Artemia* diet fed to fish fry gave the best survival rate whereas commercial diet gave the best fish biomass increase. Shrimp postlarvae fed wild *versus* laboratory-grown *Artemia* had nearly equal survival and growth.

Laboratory-grown *Artemia* can probably be used as a reference diet for nutritional studies, provided that standard methods for culturing microalgae and *Artemia* are used.

Introduction

The availability of cultured fry of fish and shrimp for growout purposes is restricted by *inter alia*, postlarval survival during weaning stages. Proper diet formulation based on both fresh and dry feed implies experiments to determine the nutritional requirements of the animals tested. Feeds currently used for weaning postlarvae (bivalve meat, fishery by-products, commercial pellets) do not ensure constant nutritional constituents.

Excellent studies have recently been undertaken on the essential nutrients during larval and postlarval stages, as well as fattening, of crustaceans and fish through both living prey (rotifers, crustacean zooplankters) (Imada *et al.*, 1979 ; Teshima *et al.*, 1979 ; Kitajima *et al.*, 1980 ; Watanabe *et al.*, 1983ab) and artificial micro-capsules (Colvin, 1976 ; Kanazawa *et al.*, 1982ab ; Sakamoto *et al.*, 1982 ; Teshima and Kanazawa, 1983 ; Teshima *et al.*, 1983). The purpose of this study was to ascertain whether brine shrimp grown in controlled conditions (Hinchcliffe and Riley, 1972) could be a reference food for weaning fish fry and shrimp postlarvae.

Materials and methods

Two types of frozen adult *Artemia* have been considered : those of the Margherita di Savoia (MdS) parthenogenetic strain collected from the saltwork ponds and those grown, in 1.5 m^3

raceway tank (starting from MdS cysts), on selected microalgae, *Tetraselmis suecica* Butcher and *Chaetoceros simplex* Ostenfeld (Trotta, 1983). The microalgae were grown in a greenhouse under controlled conditions, centrifuged, then frozen and used periodically for the *Artemia* production cycles.

The experimentation with sea bass (*Dicentrarchus labrax* L.) fry was done three times, each time with four replicates per treatment. The same diet treatments used each time were : laboratory-grown *Artemia*, ground sand-smelt meat, and commercial micro-pellets. Experiments were carried out at 20 °C with an initial density of 2 fish/l. The shrimp (*Penaeus japonicus* Bate) postlarvae were tested in two experiments, each of which consisted of two treatments : laboratory-grown *Artemia* and wild *Artemia*. Experiments were run at 25 °C with initial densities of 80 and 50 shrimp/m^2, respectively. Both fish and shrimp were obtained through artificial fertilization in the laboratory (Lumare, 1981 ; Villani and Anagnopoulos, 1983). Further information on lengths of experiments and initial weight of test organisms is given in Table I and II.

Tests were carried out in a system of aquaria with recirculating seawater and 15 % daily water renewal (Palmegiano and Trotta, 1983). Each treatment had four replicates. The light regime was fixed at 12 L/12 D. The first test of fish feeding was effected manually three times a day, the second and the third automatically with timer feeding devices working during the light phase.

Proximate compositions of the diets are listed in Table III and the amino acid and fatty acid composition of laboratory-grown *Artemia* are given in Table IV. Daily food rations, based on dry weight/g of animal tested, were 15 % for fish and 30 % for shrimp.

Results and discussion

In the experiments with sea bass fry, laboratory-grown *Artemia* gave the best survival, but the commercial diet gave the best growth, as well as a good survival rate (Table I).

TABLE I

Experimental results for sea bass grown on three diets.
Data are means (± SE) for three experiments, each with four replicates

	Diet		
	Artemia	Pellets[a]	Sand-smelt
Number of animals	100	100	100
Length of trial (days)	40	38	40
Final weight (A)	790 ± 31	1030 ± 88	583 ± 45
Initial weight (B)	172 ± 10	180 ± 13	172 ± 10
Weight gain in mg (A-B)	618 ± 26	850 ± 75	411 ± 45
Survival (%)	72.2 ± 4.6	70.2 ± 5.7	54.2 ± 4.5
Biomass increase (g/m^2)	47.5 ± 3.5	57.0 ± 3.5	21.1 ± 2.2
Daily growth rate (%)	4.0 ± 0.1	4.6 ± 0.2	3.1 ± 0.2
Feeding ratio[b]	27.5 ± 1.8	1.9 ± 0.1	25.0 ± 4.5

[a] Average of two experiments.
[b] g of food/g of fish.

TABLE II

Experimental results for shrimp grown on laboratory-cultured *Artemia* or wild *Artemia*. Each data point is the mean of four replicates

	Experiment A		Experiment B	
	Cultured *Artemia*	Wild *Artemia*	Cultured *Artemia*	Wild *Artemia*
Number of animals	20	20	12	12
Length of trial (days)	44	44	32	32
Final weight (A)	1.75	1.81	0.47	0.77
Initial weight (B)	0.87	0.87	0.29	0.33
Weight gain in G (A-B)	0.88	0.94	0.18	0.44
Survival (%)	87.5	91.2	83.5	93.8
Biomass increase (g/m^2)	60.9	68.6	4.9	18.85
Daily growth rate (%)	1.6	1.7	1.7	2.7

TABLE III

Proximate analyses of the diets used in this study. Figures are given as % of dry matter

Component	Diet			
	Wild *Artemia*	Cultured *Artemia*	Sand-smelt	Pellets
Crude protein	41.92	55.43	78.13	60.56
Total lipids	3.48	4.02	7.09	6.43
Ash	–*	20.57	12.67	9.22
Carbohydrates	–*	19.98	2.11	23.79

* Data not available.

In the experiments with shrimp, a diet of wild *Artemia* provided only slightly better survival and growth than did laboratory-grown *Artemia* (Table II).

Both the *Artemia* and micro-pellets were of suitable shape and size to be caught by the fish, whereas ground sand-smelt meat, even when reduced to thin particle sizes, tended to become compact again and sink to the aquarium bottom before being caught. Micro-pellets used during a previous trial (not reported here) were not distributed gradually by automatic feeder and gave drastic mortality. The gradual administration of micro-pellets effected through the feeder device during the second and the third trial, reported here, allowed us to feed fish almost continuously. This could help to explain why the micro-pellets gave the best growth.

Sea bass fry fed with both *Artemia* and micro-pellet diets produced a striking quantity of feces, probably made up of exoskeleton and fiber, respectively.

TABLE IV

Composition of cultured *Artemia*
(amino acids as % of protein, fatty acids as % of the total fatty acid methyl esters Fame)

Amino acid	%	Fatty acid	%
ASP	9.42	14:0 3	3.18
THR	4.69	16:0 3	18.84
SER	4.51	16:1 3	13.02
GLU	13.83	17:0 3	0.24
PRO	7.00	17:1 3	0.39
GLY	5.23	18:0 3	6.84
ALA	6.13	18:1 3	32.55
VAL	5.79	18:2 3	4.36
CYS	2.20	18:3 3	0.29
MET	1.34	20:1 3	5.71
ILEU	3.84	18:4 3	1.13
LEU	7.72	20:3 3	0.59
TYR	4.07	20:4ω3	0.16
PHE	6.44	20:5ω3	3.57
LYS	7.75	22:5ω3	0.36
HIS	2.27	22:6ω3	1.18
ARG	7.79		

The use of *Artemia* grown on selected microalgae represents a convenient methodological approach to study the nutritional requirements of the tested animals with regard to its intrinsic nutritive value, as well as the bioaccumulation of essential biochemical components in *Artemia* by the manipulation of the food fed to it. It must be pointed out that standard methods are necessary for microalgae production, so that its biochemical composition is standard and, therefore, the *Artemia* nutritional value will also be as constant as possible.

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The use of *Artemia* grown on selected microalgae represents a convenient methodological approach to study the nutritional requirements of the larval animals with regard to its intrinsic nutritive value, as well as the bioaccumulation of essential biochemical components in *Artemia* by the manipulation of the food fed to it. It must be pointed out that standard methods are necessary for microalgae production, so that its biochemical composition is standard and, therefore, the *Artemia* nutritional value will also be as constant as possible.

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Growth and survival of *Penaeus monodon* and *Chanos chanos* fry fed with *Artemia* singly or in combination with an artificial diet[1]

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Abstract

An experiment was conducted to compare the growth and survival of prawn and milkfish fry fed with *Artemia*, an artificial diet, and the combination of *Artemia* and artificial diet in a 1:1 ratio. *Penaeus monodon* postlarvae and *Chanos chanos* fry were reared for 30 days at a stocking density of 15 ind./l in aquaria and white basins respectively. The artificial diet was presented in both pelletized and moist form to *P. monodon* postlarvae and in both pulverized and moist form to *C. chanos* fry.

There was no significant difference in growth of prawn postlarvae fed the various diets except that postlarvae fed moist diet alone grew significantly less. Postlarvae fed with *Artemia* + moist diet had the highest average length (25.71 mm), which was not significantly different from the average length of postlarvae fed with *Artemia* + pellet or *Artemia* alone. Postlarvae fed with pelletized diet had a feed conversion ratio of 1.52 which is significantly less than the feed conversion of postlarvae fed with *Artemia* but not less than that of postlarvae fed with other diets. The protein efficiency ratio of postlarvae fed with *Artemia* + pellet (4.09) was significantly higher than that obtained with all other diets. The survival of postlarvae was significantly higher when they were fed with pellets (64 %) or *Artemia* + pellets (54 %) than when they were fed other diets.

Milkfish fry fed *Artemia* showed the greatest weight (0.14 g) and length (26.91 mm), significantly greater than when they were fed the other diets. The best feed conversion of 9.52 was obtained with fry fed the pulverized diet which is significantly different only from the feed conversion of fry fed with moist diet. Protein efficiency ratio of milkfish fry fed with *Artemia* is significantly higher than the other treatments. Survival was highest for fry fed with *Artemia* (48 %), but this is not significantly higher than survival of fry fed with the other diets.

[1] Contribution number 222, SEAFDEC Aquaculture Department.

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The use of *Artemia* from Ormia Lake (Iran) as food for sturgeon fry

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Summary

Since 1970 farming of sturgeon was started in the northern part of Iran near the Caspian Sea. Initially white worm *Enchytraeus albidus* and the Cladoceran *Daphnia* sp. have been used as live food sources for the fry. As of now, about half of the live food used in sturgeon fry rearing consists of nauplii and adult *Artemia urmiana.*

Artemia cysts are collected all along the shore of Ormia Lake, a 6 000 km^2 sodium chloride lake about 6 m deep, located at an altitude of 1 500 m in NW Iran. Data on the chemical composition of Ormia Lake water, sampled at different stations in November 1978 and August 1981 are given in Table I.

TABLE I

Physicochemical analysis of Lake Ormia water sampled on November 28, 1978 and/or August 18, 1981 (*) (range of values for six sampling stations all along the shore of the lake)

Temperature : 5-9.5 °C/23-25 °C (*)
Conductivity : 234 000-300 000 µm
Total alkalinity : 206-312 mg $CaCO_3$/l
Total hardness : 23 000-28 400 mg $CaCO_3$/l
Refractive index : 1.3670-1.3700
Salinity : 151-167 ‰
Total dissolved solids (180 °C) : 200 650-223 047 mg/l (*)
Dissolved oxygen : 2.3-2.6 mg/l (*)
Calcium : 200-640 mg/l (*)
Magnesium : 2 496-2 668 mg/l (*)
Sodium : 55 000-75 000 mg/l (*)
Potassium : 1 400-2 150 mg/l (*)
Total kations : 2 664-3 569 mg/l (*)
Bicarbonate : 293-451 mg/l (*)
Chloride : 93 820-125 315 mg/l (*)
Sulphate : 588-884 mg/l (*)
Total anions : 2 656-3 555 mg/l (*)
Nitrate-nitrogen : 13.7-58 mg/l (*)
Nitrate-nitrogen : 0.03-0.9 mg/l (*)
Ammonia-nitrogen : 6-14.5 mg/l (*)

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Adult *Artemia* are collected from the lake or grown in 50 m^3 concrete tanks (90 cm water depth) fertilized with the following products (data given are ratios applied per m^3 water) : 10 kg fertile garden soil, 1 kg ammonium sulphate, 0.5 kg superphosphate, 0.5 kg potassium chloride, 0.5 kg pigeon manure (in a submerged cloth sac, hanging near the water surface). Three days after fertilizer application cysts (with a 10-15 % hatching rate) are added at a rate of 30 g/m^3. Every 3-5 days 20 g hydrolized yeast is added to the culture tank as extra food.

Generally sturgeon fry starts feeding after absorbing half of its yolk sac. *Daphnia* and *Artemia* nauplii are offered during the first 2-3 days after which adult *Artemia* can be eaten. After 7-10 days in the nursery ponds, the fry have grown up to 120 mg individual weight, after which they are transferred to earthen growing ponds (2 ha each). After 20-30 days an individual weight of 2-3 g is reached and the fry are released in the river for migration to the Caspian Sea.

Feeding rates and weight increases of *Acipenser stellatus* larvae fed with *Artemia* are summarized in Table II. It appears from Table III that growth and survival of *Acipenser güldenstädti* and *A. stellatus* are best when given a pure diet of *Artemia.*

The use of nauplii and adult *Artemia* as food for sturgeon fry has several advantages, *i.e.* cheaper production cost of this food source than for *Daphnia* and *Enchytraeus*, better growth and survival of the fish fry, higher haemoglobin content in their blood, and a lessened sensitivity of the fish to infectious diseases.

TABLE II

Feeding rate and weight increase in *Acipenser stellatus* fed *Artemia* (nauplii and adults) and *Enchytraeus*

Age of fry (days)	Daily growth of fry (mg)	Amount of food (g) offered per 1 000 fry	
		Artemia	*Enchytraeus*
1	6	30.0	–
2	8	35.0	1.6
3	10	40.0	4.0
4	12	42.0	7.2
5	15	45.0	12.0
6	19	47.0	19.0
7	23	57.5	23.0
8	27	67.5	27.0

TABLE III

Comparative growth and survival in *Acipenser güldenstädti* and *A. stellatus* fed different live food

Live food source	Species of sturgeon	Duration of culture test (days)	Average fish weight (g)		Mortality
			Start	End	
Daphnia	*A. güldenstädti*	18	0.35	1.5	25
	A. stellatus	18	0.22	1.2	30
Daphia + *Enchytraeus*	*A. güldenstädti*	18	0.35	1.6	20
	A. stellatus	18	0.22	1.25	24
Artemia	*A. güldenstädti*	16	0.35	3.2	17
	A. stellatus	16	0.22	2.3	19

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Effects of dietary *Artemia* lipid fractions on growth and survival of larval inland silversides, *Menidia beryllina*

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Abstract

Lipids were extracted from freeze-dried *Artemia* nauplii and fractionated. *Artemia* total lipids, neutral lipids or polar lipids were each added separately to a basal, artificial diet. Larvae of the estuarine fish, *Menidia beryllina*, were fed the basal diet, basal diet plus the appropriate *Artemia* lipid fraction, or live *Artemia* nauplii. The non-living diets were microencapsulated. The best growth and survival of fish were obtained on the live nauplii diet. Addition of *Artemia* lipids to the basal diet did not significantly improve growth or survival of fish compared to the basal diet alone.

Because of the poor growth of the fish in the experiment, a second experiment was designed to test the value of freeze-dried *Artemia* (which contains the total lipids) in microencapsulated and unencapsulated forms against a diet of live nauplii. Live nauplii again provided the best survival and growth of fish larvae. Freeze-dried *Artemia* in either form yielded better growth of fish than did the basal diet plus *Artemia* lipid in the first experiment, although the microencapsulated, freeze-dried *Artemia* gave better growth of the fish than did unencapsulated. Apparently, the quality of the basal diet in Experiment I was so poor that addition of *Artemia* lipids was not sufficient to overcome the deficiencies.

Introduction

Live *Artemia* nauplii are a primary food used to rear larval marine fish and crustaceans. Many nutritional factors have been proposed to explain the success of *Artemia* as a live food. Significant qualitative differences have been found in fatty acid compositions between various strains. The quality of *Artemia* for marine larvae depends largely on essential fatty acid levels (Watanabe *et al.*, 1978, 1980 ; Beck *et al.*, 1980 ; Fujita *et al.*, 1980 ; Schauer *et al.*, 1980 ; Seidel *et al.*, 1982).

Artificial diets that produce survival and growth of larval marine fish equal to that obtained with live *Artemia* have yet to be developed. Beck and Bengtson (1979) found that supplementing artificial diets with live *Artemia* only once every 8 days significantly increased survival, indicating that some nutritional factor in live *Artemia* is necessary for normal growth and survival. Flüchter (1982) reported that addition of acetone extracts of *Artemia* to an artificial diet for whitefish larvae greatly increased their survival through metamorphosis. Deshimaru (1981) fractionated total clam lipids and found that the addition of phospholipid and sterol components of clam lipid to an artificial diet for shrimp resulted in growth and survival equal to that on a live clam diet. Allahpichay and Shimizu (1984ab) discovered fish-growth-promoting fractions in methanol

extracts of non-muscle krill meal, indicating that a polar lipid component may be necessary to provide normal growth and survival.

The purpose of this study was to add lipids from freeze-dried *Artemia* nauplii to microencapsulated artificial diets for larvae of the inland silverside, *Menidia beryllina*. We compared growth and survival of larvae to which total, neutral, or polar lipids were added with that of larvae fed live *Artemia* nauplii. Based on the results of this initial experiment, a second experiment was conducted to compare freeze-dried *Artemia*, which contains the total lipids, and microencapsulated freeze-dried *Artemia* as food for *M. beryllina*.

Materials and methods

Lipid fractionation

Nauplii of Reference *Artemia* Cysts (RAC II, Artemia Reference Center, Gent, Belgium) were collected 24 h after cyst incubation and freeze-dried. Cysts were supplied by the Artemia Reference Center (Gent, Belgium). Total lipids were extracted from the dry *Artemia* nauplii in ethyl ether at 40 °C for 12 h. Total lipids were separated into neutral and polar lipids using a modification of a method developed by Deshimaru (1981). Instead of four fractions, only two fractions were collected. Deshimaru's fractions 1, 2 and 3 were combined into one neutral fraction and the fourth fraction was the polar fraction. Separation of both fractions and identification of their lipid components were determined by thin layer chromatography on Silica Gel-G plates (20×20) (Supelco, Inc.) which had been activated at 110 °C overnight. Lipid classes were identified in the neutral fraction using Non-polar Lipid Mix A and B (Supelco, Inc.) as references in a solvent system containing petroleum ether : ethyl ether : acetic acid (90:10:1 by volume). Lipid classes in the polar fraction were identified using Polar Lipid Mix (Supelco, Inc.) as a reference in a solvent system containing chloroform : methanol : water (65:25:4 by volume).

Phospholipid components in the polar lipid fraction were identified using two dimensional thin layer chromatography on Redicoat 2-D plates (Supelco, Inc.). The first dimension solvent system was chloroform : methanol : ammonium hydroxide (65:25:5 by volume) and the second dimension solvent system was chloroform : acetone : methanol : acetic acid : water (3:4:1:1:0.5 by volume). Serum Lipid Mixture (Supelco, Inc.) was the standard reference for the identification of phospholipids.

Fatty acid analysis of each fraction was examined using methods described by Schauer *et al.* (1980).

Diets

A basal diet was formulated (Table I) for the first experiment. Four microencapsulated diets (MED) were produced by adding 10 % herring oil, 10 % total *Artemia* lipid, 10 % neutral *Artemia* lipid or 10 % polar *Artemia* lipid to the basal diet containing 10 % lipid, for a total lipid level of 20 %.

In the second experiment three freeze-dried *Artemia* diets were used, *i.e.* unencapsulated, microencapsulated and microencapsulated with 1.0 % FDC Yellow 6 coloring added. Coloring was added to mimic the color of live 24 h RAC II nauplii.

The diets were microencapsulated by mixing 50 g of diet with 500 ml of 1.5 % sodium-alginate solution. The aqueous phase (diet mixture) was added 1:3 to an organic phase consisting of chloroform and cyclohexane (1:1). A polytron homogenizer (PT 10/35) was used to break and mix the aqueous phase with the organic phase. Once the aqueous phase was evenly mixed, it was hardened by addition of 20 g of $CaCl_2$ dissolved in 50 ml methanol. The organic phase was strained out and the MED was washed and resuspended by stirring in 20 % $CaCl_2$ in H_2O for further hardening. The $CaCl_2$ wash was repeated with fresh solution, strained thoroughly and washed with cold tap water. The MEDs were spread in pans, vacuum-dried in a freeze-drier and sieved to separate and measure sizes. Dry MEDs ranged in size from 100-150 μm.

TABLE I

Experimental basal diet for Experiment I

Ingredients	Composition (%)
Fish protein isolate	17.5
Herring meal	23.5
White fish meal	23.3
Corn gluten meal	6.0
Wheat gluten flour	6.0
Condensed fish solubles	5.0
Meat and bone meal	3.7
Whey	3.0
Vitamin premix*	2.0
Added experimental lipid	10.0

* US Biochemical Corporation 23431.

FISH CULTURE AND EXPERIMENTATION

Menidia beryllina adults were collected from the Pettaquamscutt River estuary and allowed to spawn naturally in the laboratory. The resulting embryos were removed from the spawning tanks and incubated in 37 l aquaria at 25 °C and 30 ‰ salinity. Upon hatching, the *M. beryllina* were fed rotifers (*Brachionus plicatilis*) for the first 4 days of life and then a combination of rotifers and RAC II nauplii until the beginning of the experiment. At the age of 5 days (0.4 mg wet weight) (Experiment I) or 7 days (0.7 mg wet weight) (Experiment II), the larvae were distributed to the experimental chambers at a density of 25 larvae/chamber. The chambers were 6 l hatching jars used previously for *Menidia* diet studies and described fully by Bengtson *et al.* (1978) and Beck *et al.* (1980). Water flowed through the chambers at a rate of 50 ml/min. Introduction of water at the bottom of the chamber and light aeration from an airstone provided a general upwelling of the water. Temperature of the water in the chambers was controlled at 25±1 °C and ambient salinity during the experiments was 30±2 ‰.

Experiment I consisted of six treatments and Experiment II of five treatments (Table II). Each treatment contained three replicates, except that the unfed control in each experiment had only one replicate. The unfed treatment was considered a control to determine if material entering the system via the filtered water could provide any growth or survival of the fish. The live *Artemia* nauplii treatment in each experiment was also a control to determine good survival and growth of the fish.

TABLE II

Experimental treatments

Experiment I
1. Live *Artemia* nauplii
2. Basal diet + herring oil
3. Basal diet + *Artemia* total lipid
4. Basal diet + *Artemia* neutral lipid
5. Basal diet + *Artemia* polar lipid
6. Unfed
Experiment II
1. Live *Artemia* nauplii
2. Freeze-dried *Artemia*
3. Microencapsulated freeze-dried *Artemia*
4. Microencapsulated freeze-dried *Artemia* with 1.0 % FDC 6 food coloring
5. Unfed

On each day of the experiment, debris was removed from the bottom of each chamber during the morning and any dead larvae were removed and counted. The daily ration of the appropriate diet for fish in each chamber was then weighed and divided in thirds for the three daily feedings at approximately 1000, 1300 and 1700 h.

After 13 days, fish were removed from the chambers, anesthetized, measured to the nearest 0.1 mm total length and weighed to the nearest 0.1 mg blotted wet weight. Survival data (arc-sine transformed) and growth data for the fish in each experiment were statistically analyzed by analysis of variance and Student-Newman-Keuls comparison of treatment means (Sokal and Rohlf, 1969). Differences were considered significant at the α=0.05 level.

Results

EXPERIMENT I

The addition of *Artemia* lipid fractions to the basal diet did not significantly improve survival or growth of the fish larvae in this study (Table III, Fig. 1). Fish fed live *Artemia* nauplii in Experiment I had the best survival and growth (86 % and 5.7 mg), although their survival was not significantly better than that of fish fed basal diet plus any *Artemia* lipid fraction. Fish fed the basal diet with herring oil added had survival that was also not significantly different from that of fish fed basal diet plus any *Artemia* lipid fraction. The only significant difference in survival was between fish fed live *Artemia* and those fed basal diet plus herring oil. The fish larvae fed live nauplii showed increases in wet weight about 14-fold during the experiment, whereas those fed the other diets only doubled their weight.

The components of the neutral and polar fractions of *Artemia* lipids and the fatty acids profiles for all the added lipids are given in Table IV and V. It seemed that the composition of the added lipids should be adequate for acceptable growth and survival of this species. The fact that the diets with added *Artemia* lipids increased survival to some degree, but not growth, over that of the basal diet plus herring oil led us to question both the manner in which the diet was formulated and its availability to the fish. Most mortality occurred early in the experiment at about the time that

TABLE III

Survival and final wet weight of *Menidia beryllina* larvae in Experiment I.
The initial weight of the larvae was 0.4 mg.
Means followed by the same letter are not significantly different

Treatment	Survival (%)	Final wet weight (mg)
Live *Artemia*	86 ± 13 a	5.7 ± 1.5 a
Basal diet + polar lipids	76 ± 8 a,b	0.8 ± 0.3 b
Basal diet + neutral lipids	73 ± 9 a,b	0.9 ± 0.4 b
Basal diet + total lipids	62 ± 10 a,b	0.7 ± 0.4 b
Basal diet + herring oil	55 ± 9 b	0.8 ± 0.3 b
Unfed	0	–

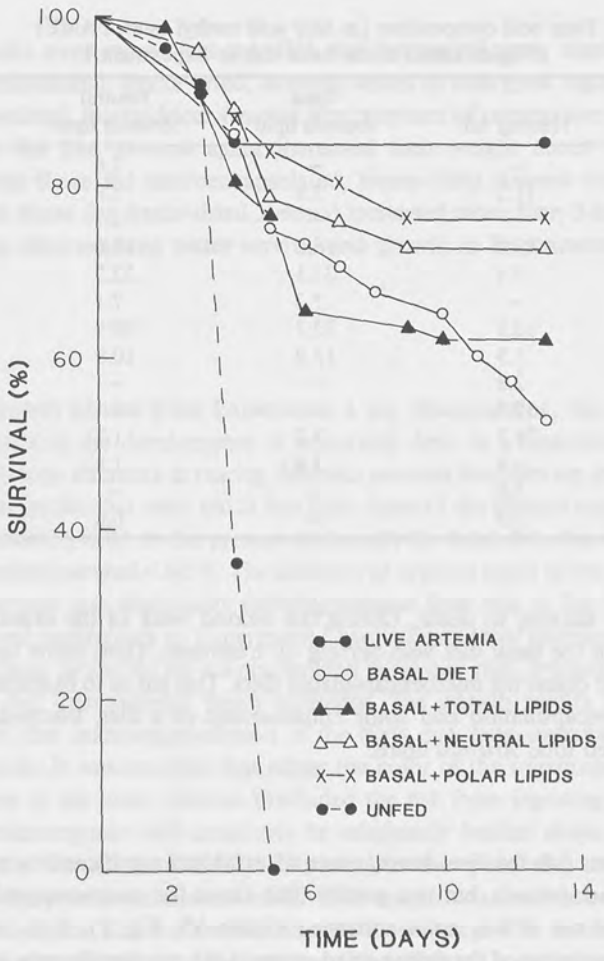

FIG. 1. Survival of *Menidia beryllina* larvae during Experiment I.

TABLE IV

Lipid components in neutral and polar fractions of *Artemia*

Neutral lipid	Polar lipid
Triglycerides	Phosphatidylcholine
Diglycerides	Phosphatidylethanolamine
Monoglycerides	Phosphatidylserine
Cholesterol	Phosphatidylglycerol
Cholesterol esters	Phosphatidylinositol
Free fatty acids	Lysophosphatidylcholine
Canthaxanthin	Diphosphatidylglycerol
	Sphingomyelin

TABLE V

Fatty acid composition (as fatty acid methyl ester, FAME) of lipids added to the basal diet in Experiment I

FAME	Herring oil	Total *Artemia* lipid	Neutral *Artemia* lipid	Polar *Artemia* lipid
10:0	–	–	3.6	–
14:0	11.5	3.8	1.9	–
14:1	–	–	2.7	–
16:0	13.0	16.0	16.6	7.7
16:1ω9	7.1	21.1	22.2	13.2
18:0	–	7.3	7.1	6.6
18:1ω9	10.9	32.3	30.9	43.6
18:2ω6	1.9	12.9	10.9	18.6
18:3ω3	2.8	–	–	–
20:1ω9	16.3	–	–	–
20:4ω6	26.7	3.2	1.7	7.5
20:5ω3	3.9	3.4	1.8	2.8
22:6ω3	3.6	–	–	–
Unknown	2.3	–	0.6	–

the unfed fish were starving to death. During the second week of the experiment, mortalities occurred primarily in the basal diet with herring oil treatment. Thus, some larvae may not have been able to ingest or digest the microencapsulated diets. This led us to examine in Experiment II the effect of microencapsulation and color enhancement of a diet, freeze-dried *Artemia*, that intrinsically contained total *Artemia* lipids.

EXPERIMENT II

In this experiment, fish fed live *Artemia* nauplii exhibited significantly greater survival than those fed freeze-dried *Artemia*, but not greater than those fed microencapsulated, freeze-dried *Artemia*, whether or not it was color-enhanced (Table VI, Fig. 2). It is important to note, however, that encapsulation of the freeze-dried *Artemia* did not significantly increase survival of the larvae.

TABLE VI

Survival and final wet weight of *Menidia beryllina* larvae in Experiment II.
The initial weight of the larvae was 0.7 mg.
Means followed by the same letter are not significantly different

Treatment	Survival (%)	Final wet weight (mg)
Live *Artemia*	89 ± 3 a	10.0 ± 2.0 a
Freeze-dried microencapsulated *Artemia* with coloring	84 ± 8 a,b	3.7 ± 1.1 b
Freeze-dried microencapsulated *Artemia*	79 ± 8 a,b	3.6 ± 0.9 b
Freeze-dried *Artemia*	70 ± 4 b	2.5 ± 0.9 c
Unfed	0	–

The growth results were more clear-cut. Fish fed live nauplii grew significantly better than those fed microencapsulated, freeze-dried, *Artemia* which in turn grew significantly better than those fed unencapsulated, freeze-dried *Artemia*. For purposes of comparison with Experiment I, we note that fish fed live *Artemia* again increased their weight about 14-fold during the experiment, whereas those fed microencapsulated, freeze-dried *Artemia* showed a more than 5-fold increase and those fed freeze-dried *Artemia* increased more than 3-fold. In general, fish fed non-living diets demonstrated better survival and growth in Experiment II than in Experiment I.

Discussion

Although the growth results from Experiment I are disappointing, this study nevertheless represents an advance in the development of non-living diets as a replacement for *Artemia* for *Menidia* larvae. Previous attempts at rearing *Menidia menidia* juveniles on artificial diets yielded survival and growth results that were much less than those of the present study (Bengtson *et al.*, 1978; Beck and Poston, 1980). In the present study, only the basal diet plus herring oil treatment in Experiment I yielded survival <60 %. The addition of *Artemia* lipids to the basal diet improved it such that fish survival was statistically indistinguishable from that of fish fed live nauplii.

A pilot experiment preliminary to Experiment I had indicated that microencapsulation of diets was necessary, because larvae fed an unencapsulated basal diet plus *Artemia* lipids survived very well for 10 days, but then suffered heavy mortality. The growth results from Experiment I, however, suggested that microencapsulation of the diets may have overshadowed the effect of adding *Artemia* lipids. It was possible that either the color of the microcapsules (very pale) or their residence time in the water column precluded the fish from ingesting a sufficient amount of them, or the microcapsule wall could not be adequately broken down by the fish so that complete digestion of nutrients could not take place. Another possibility was that the basal diet formula based on the proximate analysis of RAC II nauplii was inadequate in such a way that addition of *Artemia* lipids did not ameliorate that inadequacy. The results of Experiment II indicated that microencapsulation indeed had a positive effect on both survival and growth of the fish, but growth was still far short of that obtained with live nauplii.

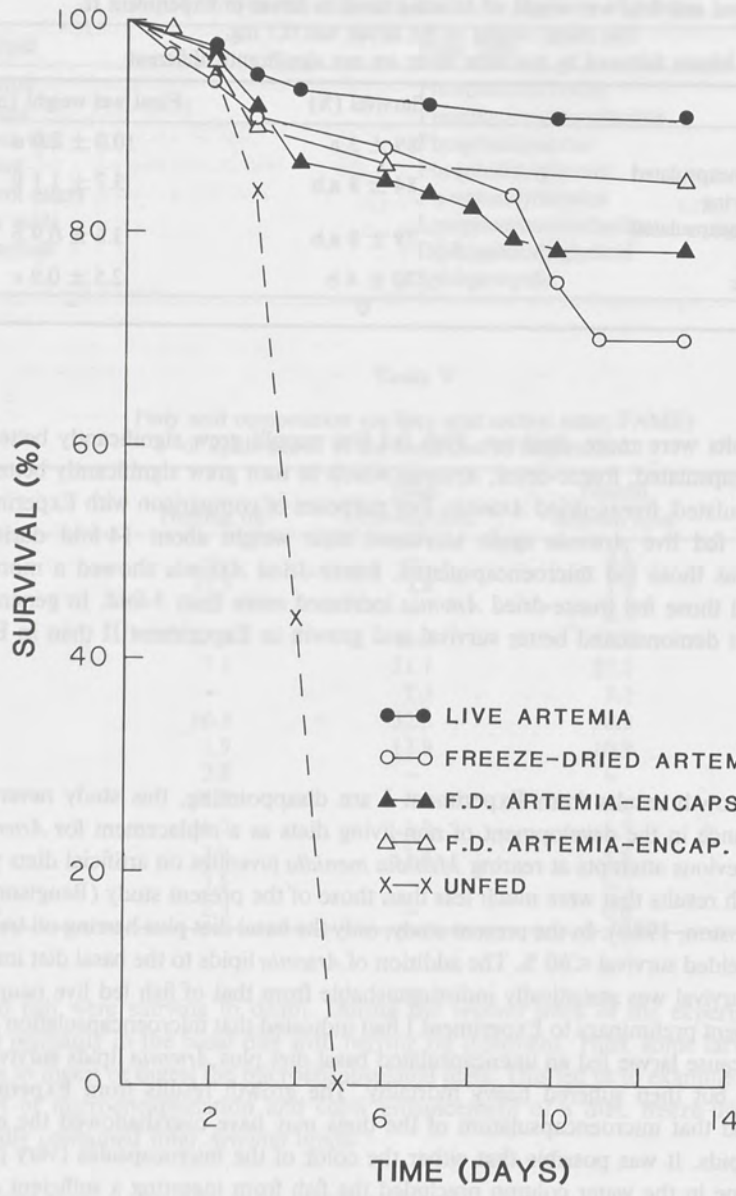

FIG. 2. Survival of *Menidia beryllina* larvae during Experiment II.

The improvement in growth of fish fed non-living diets in Experiment II, compared with those in Experiment I, may be due to multiple factors. In Experiment II, all the non-living diets were colored more reddish-brown than were those in Experiment I and may have been more visible to the fish. Also, the freeze-dried *Artemia* may have better satisfied the nutritional needs of the larvae, perhaps with regard to non-lipid components, than did the basal diet of Experiment I. Thus, the nutritional factors that Beck and Bengtson (1979) thought to be present in live *Artemia* probably depend on many factors and the search for them will be more complex than just adding lipid fractions to a basal diet. The basal diet in Experiment I was formulated based on the proximate composition of *Artemia* nauplii, but did not completely mimic the chemical composition of *Artemia* and was probably not nutritionally adequate for these fish larvae. The relatively good growth and survival of fish fed freeze-dried *Artemia* is encouraging because it clearly indicates that a non-living food can be adequate for the larvae, and is the subject of further investigation at the University of Rhode Island.

It is difficult to compare our results with those of others who have added lipids to artificial diets for marine fish larvae (Kanazawa *et al.*, 1981, 1983ab ; Allahpichay and Shimizu, 1984ab), because of differences in species used, basal diets, length of experiments, etc. In general, the other studies have shown that addition of lipids to artificial diets improves them, but not to the point of providing growth and survival equal to that on a diet of live food. In our study, addition of lipids to the basal diet slightly improved survival, but not growth.

The continuation of our research involves improvement of the basal diet, improvement of the microcapsules and the addition of *Artemia* enzymes, along with lipid fractions, to a refined basal diet. An explanation for the high quality of living *Artemia* as food for larval fish will probably be discovered in a series of small steps, rather than in one simple, giant stride.

Acknowledgements

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The use of freeze-dried *Artemia* as food for penaeid shrimp larvae

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Summary

Artemia cyst-production in Macau, Brazil was tremendously successful in the late 1970's, but began to collapse in the early 1980's. Today, the *Artemia*'s reproduction in the salt-evaporation ponds is ovoviviparous and large quantities of *Artemia* biomass are available. Since 1981, the Companhia Brasiliera de Aquicultura (CBA) has experimented with frozen and freeze-dried adult *Artemia* as a food for larval shrimp in its commercial hatchery which has a yearly capacity of 70 million postlarvae (Table I).

The *Artemia* live in the evaporation ponds of 90-120 ‰ salinity. The biomass is harvested at the pumping stations with a funnel-shaped, 1 mm mesh, nylon net, of which the reinforced opening is positioned in the current created by the pumping station. Every 2 h, the net bag is emptied into plastic containers. The average daily harvest of adult *Artemia* is about 100 kg wet biomass. After harvest, the *Artemia* are rinsed thoroughly with fresh water, centrifuged, and frozen or freeze-dried.

The freeze-dried *Artemia* appears as a cake of small particles. This cake is ground and sieved to particle sizes : < 0.5 mm (Type A) and 0.5-1.0 mm (Type B). Type A is fed to larval shrimp stages zoea I through mysis III and Type B is administered from zoea III onwards. Both *Penaeus japonicus* and *Penaeus aztecus* are fed with the *Artemia*.

TABLE I

Hatchery production of postlarval shrimp at CBA, using freeze-dried *Artemia* diets

Year	No. of nauplii	No. of postlarvae	Survival rate (%)
1982	67 961 300	46 230 250	68
1983	98 176 000	53 647 280	54
1984	84 651 000	46 026 650	54
1985	20 703 000	14 187 600	68
	Total : 271 491 300	160 091 780	59

The water temperature varies from 28 to 30 °C, the salinity is 35 ‰ in the dry season and 30 ‰ in the rainy season. The seawater is filtered to 1 µm. The larval production is started in 8 m^3 of water, 50 % of which is exchanged when the larvae reach the nauplius IV stage. The first food is added at the nauplius V stage, namely *Tetraselmis chuii* at 1 000-7 500 cells/ml and bakers' yeast, *Saccharomyces cerevisiae*, between 15 000 and 30 000 cells/ml. The food concentrations are checked and eventually adjusted at 3 h intervals. Ten hours after the larvae reach the zoea I stage, the Type A diet is administered at 0.65 g/m^3 every 3 h. The water volume is increased to 10 m^3 after the larvae reach the zoea II and increased again to 12 m^3 when the larvae reach the mysis I stage. At zoea III, the Type A ration is increased to 1.0-1.3 g/m^3 and Type B

is introduced at 0.65 g/m^3. From then until reaching the mysis III stage, the Type A ration is gradually decreased to about 0.5 g/m^3 and the Type B ration gradually increased to about 1.5-2.0 g/m^3. Beginning with the postlarva I stage, fresh *Artemia* are fed (homogenized in a liquifier and sieved over a 0.2, 0.5, or 1.0 mm filter). On the third day of the postlarval stage feeding with freeze-dried *Artemia* is discontinued. A daily exchange of 40 % of the water volume is started at mysis III. Postlarvae VIII/X are then transferred to the nursery ponds. Three examples of daily logs of a hatchery tank are given in Appendix I, II, and III.

The advantages of using freeze-dried *Artemia* in a commercial hatchery are : good nutritional value, long shelf-life, and easy dispersion in water without pollution of the larval rearing tanks. As can be seen in Table I our results from 1982 to 1985 indicate that an average penaeid survival rate of 59 % can be obtained with this method in our facility.

APPENDIX I

June 1984. Daily log of hatchery tank no. 2.
Penaeus japonicus, nauplii (N), zoea (Z), mysis (M), postlarva (PL), *Tetraselmis chuii* (T), yeast *Saccharomyces cerevisiae* (F)

Day	Hour	Larval stage	Cells/ml		*Artemia* Meal (g/m³)		*Artemia* Fresh (ml)			Tank volume (l)	Water exchange (%)	Temperature (°C)	Salinity (‰)	Remarks
			Residual	Feeding	A	B	0.2 mm	0.5 mm	1.0 mm					
10	2000	Spawning								8 000		29.5	35	17 gravid females from maturation ponds; 1 million nauplii. Water enriched with 80 g EDTA.
11	0800	egg, N												
12	1600	N-V		5 000 T							40	29.2		
13	0800	Z-I	2 500 T 15 000 F	2 500 T 10 000 F	0.81									
14	0800	Z-II	5 000 T		1.3					10 000			35	
15	0800	Z-III	6 250 T		1.3	0.65					40			
16	0800	M-I	5 000 T		1.08	1.08				12 000				
17	1000	M-II			1.08	1.62						29.0		
18	0800	M-III			1.08	1.08	50							
19	0800	PL-I			0.46	0.92	50			14 000	40		35	
20	1000	PL-II					100	150						
21	1000	PL-III						200	50		40			
22	0800	PL-IV						200	100					
23	1000	PL-V						100	300		40			
24	0800	PL-VI							350		40			
25	1000	PL-VII							400					661 200 PL's harvested; survival 66.1 %.

APPENDIX II

December 1984. Daily log of hatchery tank no. 15.
Penaeus japonicus, nauplii (N), zoea (Z), mysis (M), postlarva (PL), *Tetraselmis chuii* (T), yeast *Saccharomyces cerevisiae* (F)

Day	Hour	Larval stage	Cells/ml		*Artemia* Meal (g/m³)		*Artemia* Fresh (ml)			Tank volume (l)	Water exchange (%)	Temperature (°C)	Salinity (‰)	Remarks
			Residual	Feeding	A	B	0.2 mm	0.5 mm	1.0 mm					
12	2000	Spawning								8 000		29.5	35	12 gravid females from production pond V-8 ; 900 000 nauplii. Water enriched with 80 g EDTA.
13	0800	egg-N												
14	2000	N-V		5 000 T 30 000 F							40			
15	0800	Z-I	3 750 T 25 000 F		0.81									
16	0800	Z-II	2 500 T	2 500 T	1.30					10 000		29.0	35	
17	0800	Z-III	6 250 T		1.30	1.30					40			
18	1000	M-I	5 000 T		1.30	1.30								
19	0800	M-II			1.08	1.62				12 000		29.1		
20	1000	M-III			0.46	1.85				14 000			35	
21	0900	PL-I			0.46	0.92	100				40			
22	1000	PL-II			0.40		100	100		16 000	40			
23	0800	PL-III						200	100		40			
24	1000	PL-IV						100	200		40			
25	1100	PL-V							400		40		35	
26	0800	PL-VI							400		40			720 000 PL's harvested ; survival 80 %.

APPENDIX III

June 1985. Daily log of hatchery tank no. 3.
Penaeus aztecus, nauplii (N), zoea (Z), mysis (M), postlarva (PL), *Tetraselmis chuii* (T), yeast *Saccharomyces cerevisiae* (F)

Day	Hour	Larval stage	Cells/ml		*Artemia* Meal (g/m³)		*Artemia* Fresh (ml)			Tank volume (l)	Water exchange (%)	Temperature (°C)	Salinity (‰)	Remarks
			Residual	Feeding	A	B	0.2 mm	0.5 mm	1.0 mm					
2	2200	N-V		20 000 F 2 500 T						8 000		29.1	35	Wild gravid *Penaeus aztecus* catched near Parnaiba, PI, Brazil ; 860 000 nauplii. Water enriched with 80 g EDTA.
3	1600	Z-I	5 000 T 11 500 F	6 000 F	0.81									
4	2300	Z-II	8 750 T		0.65					10 000		29.0		
6	2200	Z-III	6 250 T		1.04									
7	1100	M-I			1.08	0.50				12 000				
8	1100	M-II			1.08	1.08					40		35	
9	0500	M-III			1.08	1.08						28.5		
10	2300	PL-I			0.50	1.08	50				40		35	
11	2200	PL-II					50	100		14 000	40	29.5		
12	2000	PL-III						100	100		40			
13	2100	PL-IV							300		40		35	
14	2000	PL-V							400		40			
15	2000	PL-VI							400	16 000	40		35	
16	2100	PL-VII							450		40			
17	2000	PL-VIII							450		40			
18	2000	PL-IX							450		40		35	
19	1000	PL-IX												472 000 PL's harvested ; survival 54.9 %.

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Large-scale *Artemia* cyst hatching at the CENMAR fish hatchery in Yugoslavia

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Summary

The yearly production of sea bass (*Dicentrarchus labrax*) at the marine fish hatchery CENMAR has increased from 1.1 million fry in the first year of production (1983/84) up to 1.93 million fry in the following season (1984/85). After an initial feeding period with rotifers (*Brachionus plicatilis*), *Artemia* nauplii are being fed for a period of approximately 30 days. With daily needs of about 2.5 billion brine shrimp nauplii, the cyst consumptions approximated 430 kg in the first season and 1 040 kg in the second one. Details on the monthly consumptions of *Artemia* nauplii during the two production seasons are given in Fig. 1 and 2.

The decapsulation technique of Bruggeman *et al.* (1980) has been adapted to treat up to 5 kg cysts at a time. After 1 h hydration in seawater, the cysts are transferred into a 65 l cylindrical container made of copper which is placed in a fiber glass tank functioning as cooling system, *i.e.* the continuous flow of tapwater in the cooling tank maintains a temperature below 30 °C within the decapsulation container. According to the methods of Sorgeloos *et al.* (1977) and Bruggeman *et al.* (1980) the cysts are decapsulated in a medium of seawater, NaOCl, and NaOH for about 10 min and thoroughly washed before being transferred to the 2 000 l conically shaped hatching tanks.

Incubation is performed under optimal conditions (Sorgeloos *et al.*, 1983), *i.e.* 34-39 ‰ salinity, 26-28 °C, cyst densities of 4 g/l, and continuous aeration and illumination. After 24 to 48 h the hatched larvae are separated and concentrated on a 125 µm sieve. Then they are stocked for maximally 30 min into 150 l tanks containing phytoplankton, and fed to the sea bass larvae.

The surplus of brine shrimp nauplii is kept during 24 h in analogous constructions and fed with adequate amounts of phytoplankton. Stocking densities never exceed 0.5×10^6/l and the temperature remains at 20-22 °C. The average survival during this stocking period is about 90 %. These 24 h fed nauplii are mainly used for feeding older larvae and fry or, exceptionally, as a supplement for the younger stages when hatching did not meet the daily requirements. In order to avoid the loss of this expensive live food during the constant change of water which is applied at a rate of 60 %/h, the tank outlets are equipped with filter nets to retain the nauplii.

The production of *Artemia* nauplii is further improved by selecting cyst batches with good hatching characteristics. Indeed, hatching tests with various batches of San Francisco Bay, USA, and Chinese or Yugoslavian *Artemia* revealed significant differences, *i.e.* hatching percentages ranging from 25 up to 56 % (Table I, II). Hatching efficiencies even varied from 5 to 13 g of cysts needed to obtain 1 million nauplii. Yugoslavian brine shrimp showed an extremely low hatching efficiency with 33 g being needed to produce 1 million nauplii. This is most probably due to inappropriate techniques used for collecting, processing, and preserving the cyst material.

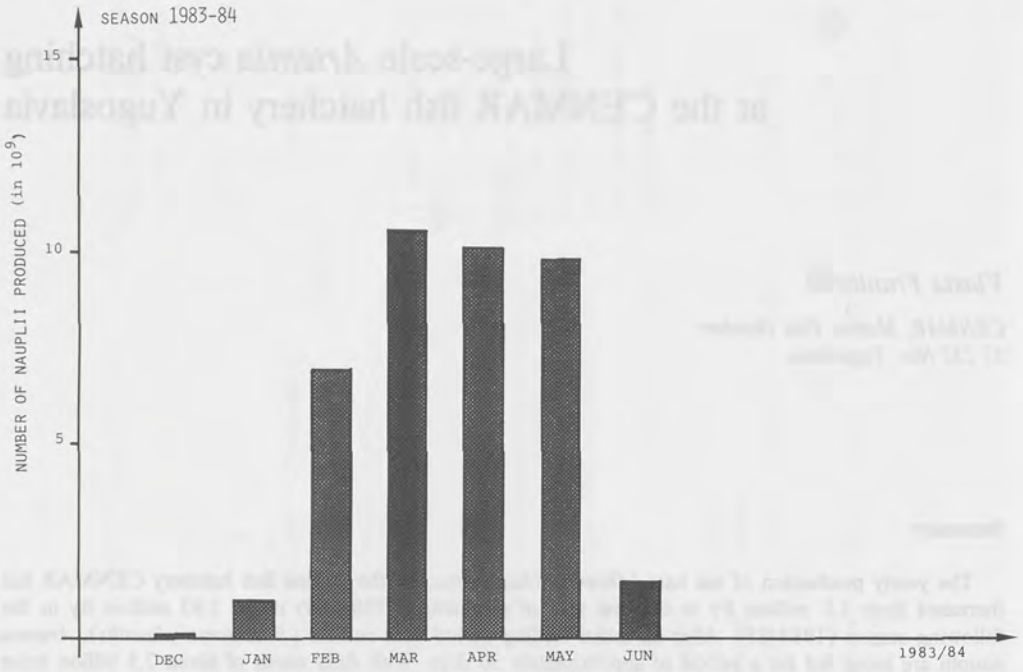

FIG. 1. Monthly production of *Artemia* nauplii during the season 1983/84.

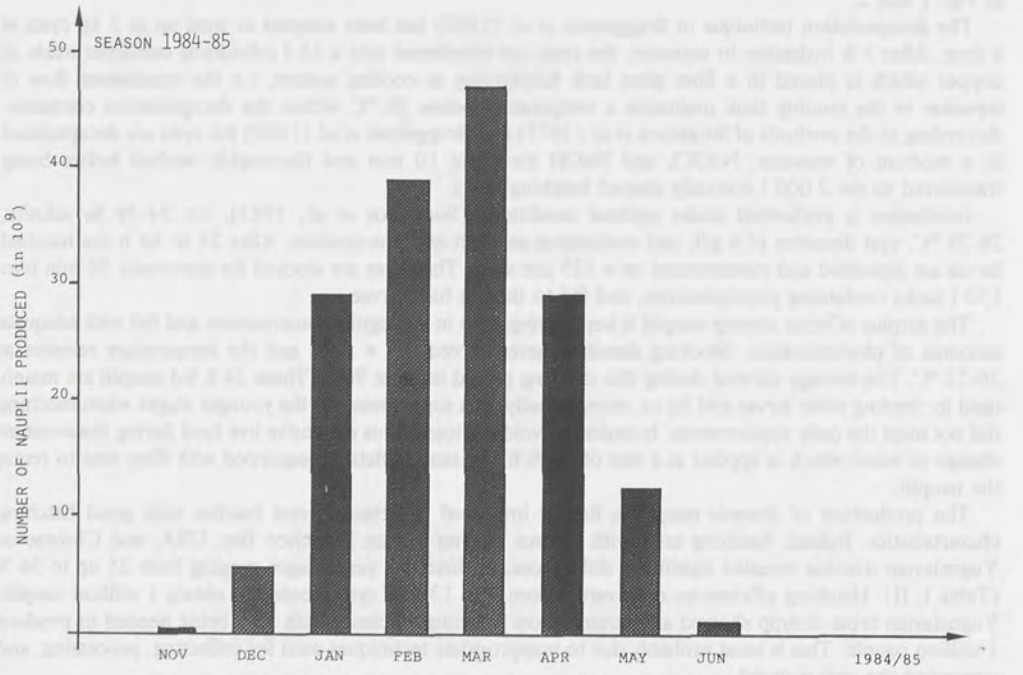

FIG. 2. Monthly production of *Artemia* nauplii during the season 1984/85.

TABLE I

Hatching rates of various commercial batches of *Artemia* cysts from San Francisco Bay, USA (SFB) ; People's Republic of China, and Yugoslavia

Cysts	Quantity used (kg)	Number of nauplii hatched ($\times 10^6$)	Hatching percentage[1]	Hatching efficiency[2]
SFB 2783	104.61	12 953	41.3	8.0
SFB 0124	89.65	6 784	25.6	13.0
SFB 3462	0.33	31	32.0	10.4
SFB 0264	192.81	18 610	53.6	10.3
SFB 0234	14.92	2 013	45.0	7.4
SFB 1233	292.55	33 105	39.1	8.5
SFB 2443	52.75	4 174	26.4	12.6
SFB 0694	279.00	48 578	58.0	5.7
SFB 0394	26.50	2 881	36.3	9.1
SFB 3044	216.05	46 849	49.1	4.6
China	192.50	25 898	55.9	7.4
Yugoslavia	0.015	0.45	29.2	33.3

[1] Assuming that 1 g contains 300 000 cysts except for SFB 0264, SFB 3044, China and Yugoslavia for which exact data are given in Table II.

[2] Number of g cysts needed to obtain 1 million nauplii.

TABLE II

Number of *Artemia* cysts in 1 g product of batches from various origin

Batch	Number of cysts/g	
	Mean	Standard deviation
SFB 0264	163 000	30 600
SFB 3044	423 000	64 700
China	241 500	31 000
Yugoslavia	139 800	25 500

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FIG. 1. Monthly production of *Artemia* nauplii during the season 1983/84.

FIG. 2. Monthly production of *Artemia* nauplii during the season 1984/85.

Artemia *Research and its Applications. 1987. Vol. 3. Ecology, Culturing, Use in aquaculture*
P. Sorgeloos, D. A. Bengtson, W. Decleir, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 556 p.

International Study on *Artemia*[1] XLV. The effects of *cis*-chlordane and dieldrin on the food chain *Artemia* to winter flounder

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Abstract

Live *Artemia* nauplii were contaminated with the pesticides *cis*-chlordane and dieldrin singly and in combination at two concentrations. The bioconcentration factors were 88.29 (±13.09) and 128.03 (±24.78) for *cis*-chlordane and dieldrin, respectively. Larvae of the winter flounder, *Pseudopleuronectes americanus*, fed the contaminated nauplii accumulated the pesticides. The resulting bioaccumulation factors were 0.67 (±0.33) for *cis*-chlordane, and 0.38 (±0.16) for dieldrin. Metabolism of *cis*-chlordane by *Artemia* is indicated by the presence of oxychlordane. However, metabolism of dieldrin in *Artemia* is not clearly implied. *Cis*-chlordane was only metabolized in the winter flounder when high levels of dieldrin were present. The rate of dieldrin metabolism in the winter flounder increased with an increase in its body concentrations.

The increase in length of the fish was less in the flounder fed the combined pesticides treatment *Artemia* than in the control or single contaminated *Artemia*, suggesting that *cis*-chlordane and dieldrin acted in combination to reduce growth.

No mortalities occurred among the winter flounder during the experimental period. This demonstrates that dieldrin and *cis*-chlordane probably were not solely responsible for mortalities observed in test animals fed San Pablo Bay and Utah *Artemia* in previous studies (Beck *et al.*, 1980 ; Johns *et al.*, 1980 ; Klein-MacPhee *et al.*, 1980 ; Klein-MacPhee, unpubl. data).

Introduction

Biological performance studies utilizing several geographically different strains of brine shrimp have been carried out at the Environmental Protection Agency's Environmental Research Laboratory in Narragansett, Rhode Island, USA (EPA-ERL). Differences in growth and survival of test organisms was attributed to the strain of *Artemia* used.

Larvae of two species of crab, *Rhithropanopeus harrisii* and *Cancer irroratus*, proceeded through normal zoeal development and metamorphosed to first crab stage successfully when reared on nauplii hatched from cysts from Macau (Brazil), Shark Bay (Australia), Margherita di Savoia (Italy), and San Francisco Bay, California (USA) (Johns *et al.*, 1980). However, when the crabs were fed *Artemia* hatched from cysts from Great Salt Lake, Utah (USA), or San Pablo

[1] International Interdisciplinary Study on *Artemia* strains coordinated by the Artemia Reference Center, State University of Ghent, Belgium.

Bay, California (USA) (SPB), abnormal development occurred, usually ending in mortality during metamorphosis to the first crab stage. Klein-MacPhee *et al.* (1980) showed that larvae of the winter flounder, *Pseudopleuronectes americanus* cultured with *Artemia* from Utah and SPB also had significantly greater mortality than winter flounder grown on the other strains. In another experiment, *P. americanus* larvae were fed Brazil *Artemia* nauplii through metamorphosis and then the fish were changed to SPB nauplii. Mortality in the experimental fish was first observed on the 13th day of being fed the SPB diet and peaked on day 20, when the study was terminated (Klein-MacPhee, unpubl. data). Likewise Atlantic silversides, *Menidia menidia*, exhibited increased mortality when fed the Utah and SPB brine shrimp nauplii (Beck *et al.*, 1980).

Biochemical analyses of the same *Artemia* strains were performed in the laboratories of the Department of Food Science and Nutrition at the University of Rhode Island.

Analysis of protein and amino acid quality by Seidel *et al.* (1980) on the five strains of *Artemia* showed no obvious cause for the observed mortalities. Soejima *et al.* (1980) found no obvious difference in the carotenoid content, but did detect the presence of chlorophyll in the SPB *Artemia*.

Analysis of the lipid fraction in various strains of *Artemia* by Schauer *et al.* (1980) showed that both strains which caused mortalities have lower levels of specific, essential fatty acids, than the other strains. They suggested that the deficiency in the fatty acids may not have been sufficient to cause the observed mortalities but may have been capable of producing a nutritional stress which could have been aggravated by some toxic compound or other stressful condition.

Olney *et al.* (1980), using multi-residue pesticide analysis, were able to demonstrate that DDT probably was not the causal agent of mortality. The Italian strain, showed excellent growth and survival although it had the highest level of DDT (395 ppb) of the five strains analyzed. This finding is contrary to that of Bookhout and Costlow (1970), who reported DDT concentrations of 7 050 ppb in Utah nauplii, and suggested that this insecticide was the causal agent of mortality observed in the crab larvae fed this *Artemia*. Olney *et al.* (1980) did not detect this level of DDT in the Utah *Artemia* and therefore could not support the conclusions of Bookhout and Costlow (1970).

Cis-chlordane and dieldrin residues, both considered toxic to fish, were shown to be present in SPB and Utah *Artemia* (Olney *et al.*, 1980). Although the concentrations are relatively low, it is felt that either through the action of bioaccumulation or in concert with the suggested fatty acid deficiency, these two pesticides might exert a toxic stress on the test organism.

To test the bioaccumulation hypothesis, *Artemia* nauplii were contaminated in the laboratory with *cis*-chlordane and dieldrin. Because good correlation has been shown to exist between water solubility of a compound and the bioconcentration factor of that compound (Belluck and Felsot, 1981; McLean, unpubl. data), it was decided to utilize a bioconcentration technique for contaminating the *Artemia*, which were then fed to winter flounder larvae. The results of this research are presented in this paper.

Materials and methods

Culture and contamination of *Artemia*

Seawater was prepared at the EPA-ERL by filtration through a 0.45 μm filter and irradiation with UV-light.

All organic solvents used in this experiment were pesticide analytical grade reagents.

The pesticides, *cis*-chlordane and dieldrin, were provided by the EPA Pesticide and Toxic Substances Repository at Research Triangle Park, North Carolina, USA. Solutions of 1 000 ng/ml (ppb) were made in acetone. One serial dilution was made for each pesticide to 100 ng/ml.

Artemia cysts from Macau-Brazil were provided by the Artemia Reference Center (Gent, Belgium) and hatched in a 4 l separatory funnel containing 3 l of filtered seawater and 15 ml of *Artemia* cysts. The hatching containers were vigorously aerated and maintained at 25 °C for 28 h. After interruption of the aeration, stage I nauplii sank to the bottom of the funnel from where they were drained into a sieve and rinsed with distilled water ; the excess water was blotted from the sieve.

Details on the ten treatments used in the contamination experiment are given in Table I. One g lots of stage I nauplii were transferred to each of the contamination Erlenmeyer flasks containing 500 ml seawater. The flasks were aerated to provide mixing of their contents.

Contamination was terminated after 24 h. The contents of each flask were drained through a sieve, the *Artemia* rinsed with distilled water and blotted dry. Enough *Artemia* to feed the winter flounder were weighed and placed in a 20 ml screw-cap vial which was filled with seawater. The vials were transported to the EPA-ERL where the flounder culture facilities were located.

The contamination procedure was repeated every 2 days to provide live, contaminated nauplii to the winter flounder every other day. The contaminated nauplii in excess of those used to feed the fish were accumulated in composite samples of each treatment group for later biochemical analysis.

TABLE I

Artemia contamination treatments, amounts of contamination, contamination concentrations and treatment designations

Treatment number	Treatment	Contamination (in acetone)	Concentration (ppb)	Designation
1	Biological control	None	0	Cont.
2	Acetone control	1 ml acetone	0	Ace. cont.
3	Low *cis*-chlordane	0.5 ml 100 ppb *cis*-chlordane 0.5 ml acetone	0.1	LC
4	High *cis*-chlordane	0.5 ml 1000 ppb *cis*-chlordane 0.5 ml acetone	1.0	HC
5	Low dieldrin	0.5 ml 100 ppb dieldrin 0.5 ml acetone	0.1	LD
6	High dieldrin	0.5 ml 1000 ppb dieldrin 0.5 ml acetone	1.0	HD
7	Low *cis*-chlordane	0.5 ml 100 ppb *cis*-chlordane 0.5 ml 100 ppb dieldrin	0.1	LC/LD
8	Low *cis*-chlordane High dieldrin	0.5 ml 100 ppb *cis*-chlordane 0.5 ml 1000 ppb dieldrin	0.1 1.0	LC/HD
9	High *cis*-chlordane Low dieldrin	0.5 ml 1000 ppb *cis*-chlordane 0.5 ml 100 ppb dieldrin	1.0 0.1	HC/LD
10	High *cis*-chlordane High dieldrin	0.5 ml 1000 ppb *cis*-chlordane 0.5 ml 1000 ppb dieldrin	1.0 1.0	HC/HD

Winter flounder culture

Winter flounder, *Pseudopleuronectes americanus*, larvae were provided by Dr. Klein-MacPhee of the EPA-ERL. They had been spawned on February 7, 1980 and cultured as described by Klein-MacPhee *et al.* (1980). When large enough to ingest *Artemia* nauplii, the fish were fed uncontaminated, 24 h old Macau *Artemia*. As the flounder grew, 4-10 day old *Artemia* cultured at the EPA-ERL were fed to them (Klein-MacPhee, pers. commun.).

Winter flounder, 107 days old, were provided from the above culture for the pesticide study. At this age the fish were post-metamorphosed flatfish and many were developing pigmentation.

The experimental fish were selected randomly from the common culture tanks. Each fish was measured for standard length, from tip of the lower jaw to base of the caudal fin rays. No attempt was made to secure wet weights since previous experience had shown that high mortalities would ensure. Twenty fish were stocked in each of the ten treatment tanks. 17 more fish were sacrificed immediately and the wet weights, dry weights and lengths were measured.

The treatment tanks were black polyethylene dish pans (34.0×29.5×15.0 cm) containing 6 l of seawater as described by Klein-MacPhee *et al.* (1980). The tanks were maintained in a running seawater table at ambient ocean temperatures (16.3 to 19.6 °C). A 12:12 h photoperiod was maintained.

The winter flounder were acclimated for 5 days to a diet of live nauplii (Macau, Brazil). A feeding rate of 0.6 g nauplii per tank added every 2 days was chosen initially. This rate allowed for the complete consumption of all the *Artemia*.

Upon initiation of the experiment, each tank was fed with the appropriate *Artemia* contamination group at a rate of 0.6 g nauplii/tank every 2 days. The tanks were siphoned daily to remove fecal detritus. The lost water was replaced with new seawater.

On experimental day 11, the fish, which were usually docile and lying on the tank bottom, were observed swimming in the water column and gulping air through the air-water interface. The dissolved oxygen was measured in the tanks and it was found to be below optimum for the fish (Klein-MacPhee, pers. commun.). Thereafter, the tanks were aerated and one third of the water was changed every 2 days.

The feeding rate of the first four feedings was 0.6 g nauplii/tank every 2 days. The subsequent eight feedings were at a rate of 0.8 g nauplii/tank every 2 days when it was found the fish would consume more nauplii. The total weight of nauplii fed to each tank was 8.80 g. The last feeding occurred on day 24 of the experiment.

The feeding experiment was terminated on day 25. The length and wet weight of each fish was measured and each fish was individually freeze-dried for 24 h in a Virtis Unitrap freeze-drier. Dry weights were then determined, and the fish pooled into treatment groups for later analysis.

Length *versus* dry weight graphs were plotted for the initial and treatment group fish. Change in length was determined by matching the longest final length with the longest initial length and taking the difference.

Pesticide analysis

All biochemical analyses were performed in duplicate on the composite *Artemia* samples and pooled fish samples.

A schematic flow diagram for the chlorinated hydrocarbon analysis is presented in Fig. 1.

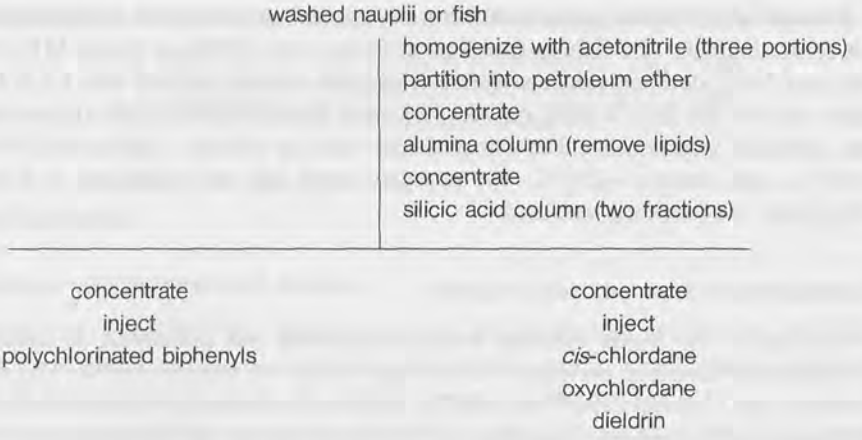

FIG. 1. Flow chart of pesticide analysis.

Chlorinated hydrocarbons were extracted using the Enos micro-method as modified by Wilson (in Warlen, 1974). A preweighed sample not exceeding 1.5 g was ground three times with 10 ml portions of acetonitrile in a 20 ml Potter Elvehjem tissue homogenizer. After each grinding the liquid and tissue were separated by filtration.

The 30 ml of acetonitrile was quantitatively transferred to a 250 ml separatory funnel and 25 ml of petroleum ether (PE) was added together with 100 ml of distilled water. The separatory funnel was inverted several times and the phases were allowed to separate. Sodium chloride was added if an emulsion formed. The aqueous (lower) phase was drained and discarded. The PE phase was washed twice with 100 ml distilled water which was also discarded. The PE was decanted to a 25 ml Kuderna-Danish (KD) concentrator tube with micro-snyder condenser and evaporated to approximately 1 ml.

For every five samples that were anlyzed, one reagent blank was processed to determine the contamination introduced by the procedure.

Lipid and other co-extractants partitioned with the chlorinated hydrocarbons into the PE were removed from the sample using the modified micro-alumina chromatography techniques described by Holden and Marsden (1969). The sample was chromatographed with 3 g of Woelm alumina, activity grade 3, packed in a glass column (1×20 cm). The eluting solvent was 2 % dichloromethane (DCM) in PE. One 20 ml fraction was collected and it was evaporated as described above. Two ml hexane was added and the sample was evaporated to near dryness. This insured the complete removal of DCM.

Silicic acid micro-columns were used to chromatographically separate the PCB interference from the pesticides. Glass columns (1×20 cm) were packed with 2.5 g of silicic acid (deactivated with water, 4 % by weight) and washed with DCM as described by Bidleman *et al.* (1978). The concentrated samples were chromatographed on these columns. The PCBs were eluted first with 35 ml PE and the pesticides were then eluted with 20 ml DCM. The fractions were concentrated as before.

Pesticide identification and quantification were performed using dual column electron capture gas chromatography (ECGC) as described by Olney *et al.* (1980). A Tracor MT-220 equipped with two Ni-63 detectors and two 180×0.4 cm glass columns packed with 1.5 % OV-17/1.95 QF-1 and 4 % SE-30/6 % QF-1 on 100/120 mesh Supelcon AW-DCMS (Olney *et al.*, 1980) was operated isothermally. The temperatures were as follows : injector =250 °C, column =200 °C, and detector =350 °C. The nitrogen carrier gas was maintained at a flow rate of 60 ml/min. No purge gas was used.

Experimental design and date analysis

One run of the winter flounder feeding experiment was performed. It consisted of the 10 treatments described previously. A two-way analysis of variance (ANOV) as described by Snedecor and Cochran (1967) was used to separate the analytical variation from the variation attributable to the pesticide contamination. However, because the individual fish were so small (approximately 0.02 g/fish dry weight), pooled samples were used for all biochemical analyses. Therefore biological variation could not be separated from the analytical or treatment variation. Furthermore, because only one experiment was performed, experimental variation could not be tested.

The variable tested by ANOV was increased in length. The variable was tested in both the *Artemia* and winter flounder.

Statistical analyses were performed by the University of Rhode Island Academic Computer Center utilizing the SAS '79 statistical package. Variables with statistically significant variation beyond that attributable to replication had their means ranked in increasing order and the means were then separated with Duncan's Multiple Range Test.

The difference in the change in length of the winter flounder was statistically significant between treatments at the 0.05 confidence level.

The bioconcentration factors (BCF) were calculated from the following equation :

$$BCF = \frac{C_A}{C_W}$$

where : C_A = concentration (ng/g) of pesticide in the *Artemia*
C_W = concentration (ng/ml) of pesticide in the water

The bioaccumulation factors (BAFs) were calculated from the following equation :

$$BCF = \frac{C_{WF}}{C_A}$$

where : C_{WF} : concentration (ng/g) of pesticide in the flounder
C_A = concentration (ng/ml) of pesticide in the *Artemia.*

The % uptake values for *Artemia* were calculated according to the following equation :

$$\% \text{ uptake} = \frac{\begin{array}{c}\text{total ng of pesticide} \\ \text{in } \textit{Artemia}\end{array}}{\begin{array}{c}\text{total ng of pesticide} \\ \text{presented in the water} \\ \text{to 1 g of } \textit{Artemia}\end{array}} \times 100$$

The % uptake values for the winter flounder were calculated using the following equation :

$$\% \text{ uptake} = \frac{\text{total ng of pesticide in all fish of one treatment}}{\text{total ng of pesticide in 8.80 g of } \textit{Artemia}} \times 100$$

Results and discussion

Bioconcentration of pesticides in *Artemia*

Cis-chlordane, oxychlordane and dieldrin were the only chlorinated hydrocarbon pesticides detected in the experimental *Artemia* and they were found only in those groups specifically contaminated. Polychlorinated biphenyls (PCBs), primarily of the Aroclor 1242 type, occurred in all treatment groups at similar levels (approximately 5 ppb). The source of the PCBs was not determined, however several possible sources do exist. It has been demonstrated that by pumping PCB contaminated air through water, the water will become contaminated in a level sufficient to allow bioconcentration to occur in plankton (Scura and Theilacker, 1977). PCB could also be introduced in the water secured at the EPA laboratory.

The pesticide concentrations of *cis*-chlordane and dieldrin found in the *Artemia*, the exposure concentrations, the bioconcentration factors (a) and the percent uptake of the pesticides are presented in Table II.

The dieldrin BCFs were significantly larger ($\bar{x}$=128.0, s=36.68) than the *cis*-chlordane values ($\bar{x}$=88.26, S=16.33). Veith *et al.* (1979) suggest that a polar function, such as the epoxide function on dieldrin, would reduce the BCF of the compound. This was not the case here, *cis*-chlordane, which has no polar function, did not concentrate as greatly as dieldrin.

The variation of the estimates for the dieldrin and *cis*-chlordane BCFs indicate that for these experimental conditions, the values are relatively accurate. They are somewhat less than expected when compared to the values found in the literature. Veith *et al.* (1979) suggest that generally the BCFs of halogenated compounds should be greater than 5 000. Epifanio (1973), on the other hand, found that *Artemia* nauplii hatched and maintained in water containing 0.5 ppb dieldrin for 36 h had a BCF of 426.

Belluck and Felsot (1981) demonstrated that caddisfly egg masses required 72 h of contamination to attain a maximum stable level of pesticide. We made no attempt to bring the *Artemia* nauplii to equilibrium which could explain the BCF results that are lower than those found in the literature.

Oxychlordane was detected at approximately 3 % of the *cis*-chlordane levels in those *Artemia* treated with high levels of *cis*-chlordane. Oxychlordane is a metabolic degradation product usually associated with *trans*-chlordane in animals (Brown, 1978 ; Feroz and Khan, 1979) ; however it has been formed from *cis*-chlordane in the laboratory (Schwemmer *et al.*, 1970). Its presence indicates that metabolism of *cis*-chlordane was occurring in the HC treatment groups. It was not detected in the low *cis*-chlordane *Artemia*, but its concentration may have been below the limit of detection for the procedure. Metabolism of *cis*-chlordane in *Artemia* may be important because higher animals do not appear to have this ability (Brown, 1978). Ecologically, an invertebrate's ability to metabolize *cis*-chlordane could provide a biological means for reducing the environmental levels of this persistent compound.

TABLE II

Pesticide concentrations (ng/g), bioconcentration factors (BCFs) and % uptake of the pesticides in the *Artemia* treatment groups

Treatment no.	1 2	3	4	5	6	7	8	9	10
Treatment designation	Cont. Ace. cont.	LC	HC	LD	HD	LC/LD	LC/HD	HC/LD	HC/HD
Pesticide concentration in water (ng/ml)									
cis-chlordane	0	0.1	1.0	0	0	0.1	0.1	1.0	1.0
dieldrin	0	0	0	0.1	1.0	0.1	1.0	0.1	1.0
ng exposure									
cis-chlordane	0	50	500	0	0	50	50	500	500
dieldrin	0	0	0	50	500	50	500	50	500
Pesticide concentration in *Artemia* (ng/g)									
cis-chlordane	0	10.8[1]	92.7	0	0	9.65	7.44	78.2	79.6
		2.45[2]	6.80			1.72	0.91	7.47	11.9
dieldrin	0	0	0	12.0	170.0	13.4	115.0	9.70	131.0
				0.74	63.5	1.99	52.1	0.50	37.9
BCFs of *Artemia*									
cis-chlordane	0	108.0	92.7	0	0	96.5	74.4	78.2	79.6
dieldrin	0	0	0	120.0	171.0	134.0	115.0	96.9	131.0
% uptake									
cis-chlordane	0	21.7	18.5	0	0	19.3	14.9	15.6	15.9
dieldrin	0	0	0	24.1	34.2	26.8	23.1	19.4	26.2

[1] Means of the two analytical replicates.
[2] Standard deviation of the means.

Cis-chlordane BCFs are significantly lower in the combined treatment groups 8 through 10 ($\bar{x}$=77.4, s=7.84) than in groups 3, 4 or 7 ($\bar{x}$=99.1, s=15.51). Either *cis*-chlordane assimilation from the environment was lower in these groups, or metabolism and subsequent depuration was greater. The previous observation of oxychlordane production supports the metabolism viewpoint. If the lower *cis*-chlordane BCFs in the combined treatment groups are indicative of a higher level of metabolism, this could mean that the mixed-function oxidase (MFO) activity has been synergistically induced to higher levels in the combined treatment groups. *Cis*-chlordane metabolism, as shown by oxychlordane production alone, is evidence of MFO activity. Increased MFO activity in the combined groups shows the effect of dieldrin, a known inducer of the MFO in mice and rats (Triolo and Coon, 1966 ; Chadwick *et al.*, 1975), on the MFO of *Artemia.* It should be noted that a high concentration of dieldrin and/or *cis*-chlordane is required to produce this synergistic effect. This suggests that a threshold concentration of pesticide may be necessary to induce the *Artemia* MFO and that groups 8-10 have surpassed that threshold.

The bioconcentration of pesticides in the *Artemia* from the aqueous environment produced higher body concentrations than did accumulation of the pesticides from the diet. In a preliminary experiment, *Artemia* were fed a defatted rice bran diet contaminated with 0.1 and

1.0 ppb *cis*-chlordane and dieldrin for 14 days. The *Artemia* produced had much lower body concentrations of the pesticides. The bioaccumulation factors (BAFs) were consistently less than one. This finding is in agreement with the general belief that contamination from the aqueous environment is more significant than contamination via the food web (Epifanio, 1973 ; Macek *et al.*, 1979 ; Veith *et al.*, 1979). Veith *et al.* (1979) go on to say that only if the food contaminant concentration is very great and the water levels very small will the food make a large and significant contribution to the overall contaminant concentration of the organism.

The *Artemia* were produced prior to the feeding experiment and frozen until needed because of the greater amount of time and logistical support (*i.e.* daily feeding and cleaning of the cultures) needed for contamination. The winter flounder, when fed the thawed product, did not eat them. Thus the diet was not only difficult to produce, but it was also undesirable to the fish.

Bioaccumulation of pesticides in winter flounder

Chlorinated hydrocarbons, other than those already mentioned, were not detected in any of the winter flounder treatment groups. *Cis*-chlordane was found in all the treatment groups, including the controls and the single contaminant dieldrin groups. In those cases where *cis*-chlordane was not specifically fed, it was detected at an average level of 2.76 ppb. This value was used when correcting the *cis*-chlordane concentrations prior to calculation of the bioaccumulation factors (BAFs) and the % uptake. Table III contains the results showing the pesticide concentrations in the fish, BAFs and % uptake.

The BAFs for all the winter flounder groups are less than 1 except group four (HC). The fact that the BAFs are so small is significant because biomagnification, defined by Macek *et al.* (1979) as the increase of contaminant concentration from one trophic level to the next higher trophic level, does not appear to be occurring in this experiment.

The dieldrin BAFs and % uptake values are inversely proportional to the dieldrin diet concentrations. This suggests that the fish MFO may have been induced more by the high dieldrin concentrations than the low, resulting in increased metabolism and depuration of dieldrin and lower apparent accumulation factors. These results also suggest that a threshold level of dieldrin may be required to stimulate the MFO. Dieldrin depuration has been shown to occur in the channel catfish, *Ictalurus punctatus* (Argyle *et al.*, 1975) and lake trout, *Salvelinus namaycush* (Reinert *et al.*, 1974).

Although *cis*-chlordane did accumulate in the winter flounder, no distinct pattern was evident concerning its uptake. The BAF for the HC group (1.28) is nearly twice as large as the next largest value (HC/LD=0.74) and almost four times larger than the smallest value (0.24 of the LC group). No reason for this accumulation pattern has been developed.

The residual level of *cis*-chlordane detected in all the experimental fish had either been accumulated from the feed used prior to the experiment or it had been concentrated from the water. No determination of its source was attempted however.

Oxychlordane was detected in all the HC treatment groups, at approximately 3 % of the *cis*-chlordane concentrations. Its presence can be explained in two ways, *i.e.* either oxychlordane was accumulated from the *Artemia* which have previously been shown to be contaminated with it, or it is a metabolic degradation product being formed from *cis*-chlordane present. Feroz and Khan (1979) have showed that *cis*-chlordane is particularly stable in the goldfish, *Carassius auratus*. Therefore, oxychlordane was probably accumulated from the diet.

TABLE III

Pesticide concentrations (ng/g), bioaccumulation factors (BAFs) and % uptake of the pesticides in the winter flounder treatment groups

Treatment no.	1 2	3	4	5	6	7	8	9	10
Treatment designation	Cont. Ace. cont.	LC	HC	LD	HD	LC/LD	LC/HD	HC/LD	HC/HD
Pesticide concentration in *Artemia* (ng/g)									
cis-chlordane	0	10.3	92.7	0	0	9.65	7.44	78.2	79.6
dieldrin	0	0	0	12.0	171.0	13.4	115.	9.70	131.0
Pesticide concentration in fish (ng/g)									
cis-chlordane	3.11[1]	6.66	121.	2.41	2.75	8.30	5.27	62.1	53.2
	1.66[2]	2.32	16.3	1.08	0.57	1.62	4.06	37.7	6.39
dieldrin	0	0	0	7.73	57.2	5.29	24.4	4.27	30.2
				1.90	0.22	1.98	8.27	3.12	10.3
BAFSs									
cis-chlordane	0	0.24	1.28	0	0	0.57	0.37	0.74	0.63
dieldrin	0	0	0	0.65	0.36	0.39	0.25	0.43	0.23
% uptake									
cis-chlordane	0	10.4	35.0	0	0	18.6	10.3	19.0	18.4
dieldrin	0	0	0	20.6	8.50	11.8	5.88	11.0	6.66

[1] Means of the two analytical replicates.
[2] Standard deviation of the means.

GROWTH AND SURVIVAL OF WINTER FLOUNDER

No mortalities occurred in any winter flounder treatment group during the experimental period. The amounts of *cis*-chlordane and dieldrin in the low concentration *Artemia* were within one order of magnitude of the strains, SPB and Utah, which were being simulated by this experiment. The levels of contamination in the high concentration *Artemia* were an order of magnitude greater when compared in this context. The winter flounder did accumulate some of the pesticides which demonstrates that assimilation did occur. Because SPB *Artemia* were not fed simultaneously in this experiment, these results cannot be correlated to the previous biological studies performed at the EPA-ERL. However, it can be said conclusively that the pesticides, *cis*-chlordane and dieldrin, when fed in the diet to larval winter flounder at apparently background levels and at levels elevated above background levels, do not cause mortalities. Epifanio (1973) showed that 213 ppb of dieldrin in a diet did not cause mortalities in crabs fed the diet.

The change in length of the fish from the beginning to the end of the experimental period was found to be significantly different at the 5 % confidence limit between the treatment groups by ANOV. Ranking of these data confirmed that there was an effect due to the pesticides (Table IV). The Duncan's Multiple Range Test showed that the combined high treatment group (HC/HD) caused the smallest increase in length and that this treatment was statistically different from the remaining groups. The other combined treatment groups were clustered together as having the

TABLE IV

Effect of feeding *Artemia* contaminated with dieldrin and chlordane on the larval length of winter flounder

Treatment no.	Treatment	Increase in length (cm)
10	HC/HD[1]	0.74[a,2]
7	LC/LD[1]	1.40[b]
9	HC/LD	1.43[b]
8	LC/HD	1.50[b]
6	HD	2.02[c]
2	Acetone control	2.83[d]
4	HC	3.31[d,e]
3	LC	3.45[d,e]
5	LD	3.70[e]
1	Control	4.11[e]

[1] High/low – dieldrin/chlordane.

[2] Values with the same leters in the superscript are similar, using the Duncan's Multiple Range Test.

next smallest increase in length. The single contaminant group, HD, stood alone, followed by the remainder of the treatment groups, all of which were ranked together.

These results suggest that *cis*-chlordane and dieldrin act in combination to reduce the increase in length of the young winter flounder. Dieldrin, when fed in the diet, has been shown to reduce the growth in the channel catfish (Argyle *et al.*, 1975). These results demonstrate that combining *cis*-chlordane with dieldrin produces a greater reduction in change in length than dieldrin alone, while *cis*-chlordane alone does not affect the change in length. Low levels of dieldrin alone do not appear to affect the change in length. Acetone, however, did reduce the increase in length somewhat.

The reduction in growth by nearly 75 % (treatment 1 *vs* treatment 10) has ecological significance. With the growth rate reduced, the fish may be removed from its predator-prey niche. The food it relies on to grow may not be available to it in the new time frame produced by the new growth rate. O'Connors *et al.* (1978), after showing that PCBs and dieldrin both suppressed the growth of certain algae, suggest that this could cause a shift in the food web dynamics. Smaller algae might bring in a different primary consumer which would change the entire structure of the food web. A slower growing flounder may find itself with a different food supply. The most obvious effect a change in growth rate will have on the fish is to change its survivability as a prey of other species, either by increasing the number of predators or by increasing the length of time a specific predator has to prey on it.

Conclusion

The *Artemia* bioconcentrated the pesticides to levels somewhat lower than previous research would lead one to expect. Oxychlordane was detected in the high *cis*-chlordane treatment *Artemia* suggesting a degree of *cis*-chlordane metabolism. There appears to be a threshold concentration required to induce dieldrin metabolism. A slight combined effect of the pesticides on the induction of the MFO is suggested.

The winter flounder bioaccumulate the pesticides from the diet. The evidence suggests that biomagnification is not occurring. Some dieldrin metabolism seems to exist. The results are conflicting concerning the metabolism of *cis*-chlordane.

Cis-chlordane and dieldrin did not cause mortalities in the winter flounder when they were fed in the diet. Growth did decrease in the winter flounder of the combined treatment groups over the single treatment and control groups as a result of the pesticide contamination.

These experiments were performed to simulate the conditions of previous growth studies at the EPA-ERL using *Artemia* nauplii as the food. The conditions were not strictly the same as the winter flounder were older in this experiment than those previously reported (Klein-MacPhee *et al.*, 1980 ; Klein-MacPhee, unpubl. data). The *Artemia* also were 24 h older. In retrospect a negative control consisting of SPB *Artemia* nauplii should have been maintained with the rest of the treatments. However, the research did show that the pesticides *cis*-chlordane and dieldrin probably were not the causal agents of mortality in the previous growth and survival studies. It has also been demonstrated that live *Artemia* nauplii can be contaminated to desired concentrations easily and quickly for use in toxicological assays where a contaminated food for larval and juvenile stages of aquatic animals might be desired.

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The brine shrimp *Artemia* as a protein source for humans

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Abstract

Artemia nauplii were cultured in raceway systems for 7 or 15 days and were grown on either rice bran powder or whey powder as the sole diet for the entire culture period. Following each culture period, *Artemia* were rinsed with deionized water, drained, and freeze-dried. A commercially obtained adult population of San Francisco Bay (SFB) *Artemia* was also studied.

Individual dry-weight analysis (done prior to freeze-drying) and subsequent proximate analysis showed that *Artemia* is a highly efficient feed converter (up to 40 % efficiency) and contained as much as six times more protein than its culture feed. The essential/total (E/T) amino acid ratio and chemical score exceeded recommended values for infants, children, and adults. Chemical scores for SFB *Artemia* and the rice bran-fed 15-day-old group were above a value of 74 (soy bean) and therefore of high nutritional value. The SFB group was also tested against casein in a protein efficiency ratio (PER) study and found to be slightly superior in quality.

The measures of protein quality used in this study all show *Artemia* to be of high nutritive value. Previous work has indicated that man has already used *Artemia* as food and that modern taste-panel tests on *Artemia* gave quite favorable results.

Therefore, high protein content and quality, ease of production, and good acceptability by consumers makes *Artemia* very attractive as a potential protein source for man as well as the well studied aquacultural species.

Introduction

Geographical collections of *Artemia* have been made from many salines around the world. While there is variation in size, reproductive mode, and biochemical composition, etc., the protein content is consistently very high at 42-60 % of the animals dry weight (Benijts *et al.*, 1975 ; Sorgeloos *et al.*, 1980 ; Tobias *et al.*, 1980).

The relative ease with which *Artemia* can be cultured, along with its high content of animal protein suggest that it might provide a potential source of high quality protein for humans. According to Sorgeloos (1983), millions of hectares of non-arable land exist in the tropical belt, much of which, if properly managed, would be favorable to *Artemia* production. *Artemia* farming in these areas could potentially improve the quality of local diets and also serve as the basis for other industries (*i.e.* solar salt production, fish hatcheries, etc.) to develop, thus aiding local eonomies as well. *Artemia* has been cultured in the laboratory on waste products such as whey powder and micronized rice bran (Dobbeleir *et al.*, 1980 ; Sorgeloos *et al.*, 1980). It was the intent of this research to investigate the value of *Artemia* raised on these materials as a potential human food supplement.

Materials and methods

The *Artemia* used in this study were hatched from Brazilian cysts (Macau) obtained commercially from Aquarium Products (lot no. 10, 1980). The cysts were hatched at 22 °C in 4 l separatory funnels filled with 0.45 µm-filtered, UV-treated seawater from Narrangansett Bay, Rhode Island (30 ‰ S). Approximately 10 cc (4.26 g) cysts/l were aerated for exactly 48 h. Aeration was then discontinued and the hatched nauplii separated from unhatched cysts and other particulates on the basis of their positive phototactic behavior (Persoone and Sorgeloos, 1972).

Culturing of *Artemia*

Preliminary experiments demonstrated that rice bran powder, given at an initial concentration of 0.005 mg/*Artemia* and increased by 0.01 mg/*Artemia*/day, yielded good growth and survival of *Artemia*, provided that 1/3 of the culture medium was replaced with fresh seawater every second day. Whey powder could be given at only 0.025 mg/*Artemia*/day with an increase of just 0.005 mg/*Artemia*/day. The culture medium also had to be changed daily, in order to obtain good growth and survival over the 15-day culture period.

Under these feeding conditions, 48-h nauplii were batch-cultured in 430 l air-water-lift (AWL) operated raceway systems (Bossuyt and Sorgeloos, 1980). In the two raceway systems used, *Artemia* were raised at a population density of one animal/ml of medium under diffuse light. Water temperature (28 °C) was maintained by thermostatically controlled heaters and feed was given twice daily by weighing the material into 300 ml plastic screw capped jars, mixing with some medium and then pouring the contents into the tank.

Starting with 48-h nauplii, *Artemia* were cultured until either the 7th or 15th day, at which time the tank contents were filtered through a 250 µm sieve. The *Artemia* collected on the sieve were then washed in deionized water and kept frozen (-20 °C) under N_2 gas until the time of analysis.

When all culturing was complete, a small sample from each group was taken from the freezer and allowed to thaw. A known number of *Artemia* was then placed onto pre-weighed aluminum pans and put in a drying oven at 60 °C for 24 h. Each pan was weighed to the nearest 1 µg and average individual dry weight (IDW) were calculated.

Calculations

The average individual dry weight (IDW) was used as the basis for extrapolating other growth data in the table. By multiplying the IDW for each group times the population of nauplii inoculated into the AWL-raceways (430 000), the "total theoretical yield" (assuming 100 % survival of the *Artemia* in culture) was determined. By subtracting the total yield of 48-h nauplii from that of each cultured group, an estimate of *Artemia* production or "biomass produced" was obtained. This value was then multiplied by the corresponding percent protein for that group in order to derive the "total protein produced".

Feed conversion efficiency (FCE) was calculated by dividing the biomass produced by the total feed consumed, then multiplying by 100 (Reeve, 1963). Similarly, the protein conversion efficiency was taken as the total protein produced, divided by the total available feed protein, times 100.

ANALYSES OF *ARTEMIA* AND DIETS

All frozen *Artemia* were lyophilized in a Virtis Unitrap 11 freeze-drier for 96 h prior to analysis. For comparison, a commercial sample of adult *Artemia* harvested from San Francisco Bay salterns (SFB) was also lyophilized and analyzed. These were later used to determine the protein efficiency ratio (PER). Whey powder and rice bran powder which were also analyzed, were not freeze-dried as they were already in dry form.

Moisture analysis was performed by placing a known amount of sample into dry, pre-weighed Alundum crucibles, lined with Whatman no. 2 filter paper. The samples were then put in a drying oven at 60 °C for 24 h, cooled to room temperature in a desiccator and reweighed. They were then placed in pre-weighed extraction flasks containing 25 ml ethyl ether and connected to a Baily-Walker lipid extractor. Extraction was continued for 16 h, at which time the Alundum crucibles were replaced in the flasks by glass crucibles and set aside to be used in determining the ash content of the samples. Further distillation allowed recovery of the ether. After evaporating any residual solvent, the crude lipid was fried for 1/2 h, cooled in a desiccator, and weighed.

Fatty acid content was determined on separate samples by extracting the lipid materials using the Bligh and Dyer (1959) technique, as modified by Kates (1972), and methylating the resulting fatty acids with 13 % Boron Trifluoride-Methanol (w/v) (Morrison and Smith, 1964).

Fatty acid methyl esters (FAME) were then injected into a single column Varian Aerograph 1200 gas-liquid chromatography unit operated isothermally at 195 °C and equipped with a flame ionization detector. The temperatures of the injector and the detector were 262 °C and 272 °C, respectively.

Identification of the FAME was made on a 15 % diethylene glycol succinate polyester (DGSP) column, 2.1 m long × 3.2 mm O.D., on a 100-120 mesh Gas Chromosorb W-HP support with a 37.5 ml/min flow of nitrogen as the carrier gas. Identification and quantification of the FAME were made with a Hewlett-Packard 3380 A electronic integrator, programmed with relative retention times of authentic standards. Results are represented as FAME weight percent of the total lipid.

Ashing was determined quantitatively by removing filter paper and samples from the Alundum crucibles used in the moisture and crude lipid determinations, placing them in pre-weighed porcelain ashing dishes in a muffle furnace at 550 °C for 5 h. The samples were then allowed to cool overnight in a desiccator and reweighed.

Protein content was determined by a modified microkjeldahl method of Hiller *et al.* (1948) using an Orion 901 Ionalyzer equipped with an ammonia electrode to detect dissolved NH_3 in the samples. The values obtained (in ppm) were converted to % nitrogen and then to % protein, using the general calculation factor 6.25 for all samples analyzed. Carbohydrate content was then calculated by % difference.

Amino acid analysis was performed by obtaining enough of each material to yield about 10-12 mg of protein which was then acid hydrolized, using the technique reported by Seidel *et al.* (1980). The exchange column used in the present study was packed with Dionex DC1-A (8 % cross-linked) resin.

Since tryptophan is destroyed by this procedure, another method of hydrolysis had to be employed. An alkaline hydrolytic technique which appears to be applicable to intact materials such as those tested here has been developed (Hugli and Moore, 1972), using 4.2 N NaOH. A

modification of this procedure involves the *in vacuo* hydrolysis of about 10-12 mg of protein in 0.3 ml of 4.2 N NaOH and 0.5 ml of pH 4.25 of sodium citrate buffer (the latter is used in place of hydrolyzed starch in order to prevent gel formation in the hydrolysis mixture) at 110 °C for 98 h. The hydrolysis was carried out in the same heavy-walled tubes used in the acid procedure with the exception that Nalgene polypropylene centrifuge tubes (10.9 × 77 mm) were used to line the glass in order to prevent silicate formation during hydrolysis (Oelschlegel *et al.*, 1970).

Following cooling to room temperature, the alkaline hydrolysates were brought to volume with pH 4.25 buffer in 5 ml volumetric flasks containing 420 µl of 6 N HCl which were chilled in an ice bath. The solutions were then run through a Millipore filter (0.45 µm) and analyzed immediately on the amino acid analyzer. Tryptophan peaks were hand integrated and compared to the corresponding peak of the authentic standard amino acid mixture, mentioned earlier in the acid hydrolysis procedure, to which 50 nmoles of tryptophan had been added. The absolute values for tryptophan were then calculated from this comparison. Quantitative amounts of each amino acid were determined by combining the absolute values for tryptophan with those of all other amino acids obtained from the acid hydrolysate of each corresponding sample and are expressed as g of amino acid/100 g of protein. A known amount of tryptophan (Nutritional Biochemicals Corp., Cleveland, Ohio) was also hydrolyzed and analyzed. The amount detected by the amino acid analyzer was then used to obtain a value for the efficiency of the technique which was found to be slightly above 91 %.

A protein efficiency ration (PER) test was performed with 21-day-old male weaning rats obtained from the Charles River Breeding Laboratories Inc. (Wilmington, Massachusetts, USA). The control group was fed a diet containing 10 % protein supplied by casein, while the test group was fed a similar diet except that protein was supplied by *Artemia* (SFB).

Results and discussion

All production data, including biomass production, feed conversion efficiencies, and protein conversion efficiencies, are given in Table I. For whey-fed 7- and 15-day-olds (W-7, W-15) and rice bran-fed 7- and 15-day-olds (RB-7, RB-15), feed conversion efficiency fell within the range of previous studies (Mason, 1963 ; Reeve, 1963).

These results show some very interesting differences with regard to the effect of feed used and the age of the cultured *Artemia* on growth parameters. For example, the IDW and FCE values point out an apparent discrepancy in that whey power appears to be a superior food to rice bran for younger *Artemia*, however, the opposite seems true for older animals. One possible explanation for this lies in the relationship between energy requirements and food availability. Food levels established during the preliminary feeding experiments were based on the maximum amount of either feed which could be cleared from the culture medium in 24 h, as determined by the lack of turbidity. This was then expanded to determine the daily increase which would promote growth without producing some toxic effect during the 15-day period. However, no consideration was given to possible changes in optimum feed concentration as the *Artemia* advanced from one developmental stage to the next. It has been shown that growth increases sharply after the first few days of life as the *Artemia* progress toward adulthood (Mason, 1963 ; Reeve, 1963 ; Johnson, 1980). Therefore, while food levels appear to be adequate for the W-7 group, increases in the feed concentration may not have kept pace with the additional metabolic

TABLE I

Production data for *Artemia* raised on rice bran powder (RB) and whey powder (W) for 7 (W-7, RB-7) or 15 (W-15, RB-15) days and initial data for unfed nauplii (48 h old)

Artemia	Individual dry weight[1]	Total yield[2]	Biomass produced[3]	Feed consumed[4]	Total available protein[5]	Total protein produced[6]	Feed conversion efficiency[7]	Protein conversion efficiency[8]
W-7	0.0417	17.9	17.24	75.2	7.70	10.47	22.9	136.0
W-15	0.148	63.6	62.95	307.4	31.45	38.64	20.5	123.0
RB-7	0.0352	15.1	14.44	150.5	20.60	8.83	9.6	42.9
RB-15	0.512	220.1	219.47	541.8	74.17	110.02	40.5	148.3
48 h	0.0016	0.688	–	–	–	–	–	–

[1] Individual dry weight (g) = $\frac{\text{total weight of } \textit{Artemia} \text{ in Sturdier pan}}{\text{number of } \textit{Artemia} \text{ in pan}}$

[2] Total yield (g) = individual dry wt (in mg of cultured *Artemia*) × 430

[3] Biomass produced (g) = total yield (cultured *Artemia*) - total yield (nauplii)

[4] Feed consumed (g) = total feed consumed in 7- or 15-day period

[5] Total available protein (g) = % protein in feed × feed consumed

[6] Total protein produced (g) = % protein (cultured *Artemia*) × biomass produced

[7] Feed conversion efficiency (%) = $\frac{\text{biomass produced}}{\text{feed consumed}} \times 100$

[8] Protein conversion efficiency (%) = $\frac{\text{total protein produced}}{\text{total available protein}} \times 100$

needs of rapid growth and maintenance later on and became limiting. Conversely, the higher initial levels of rice bran powder (and daily increases) may have been near the saturation level of young larvae. This might cause food particles to move too rapidly through the gut for adequate digestion and absorption to occur and might therefore depress initial growth rates and the FCE. However, as the *Artemia* began to grow more rapidly and feed more effectively, the higher concentration of rice bran powder was probably beneficial in promoting growth and higher feed conversion. Proper feed concentration is a very important consideration because this ensures a sufficient food level, while avoiding excess. Another important food-related consideration is that filter-feeding *Artemia* are not able to effectively ingest particles which settle out of suspension, therefore, adequate circulation of the culture medium is also imperative.

There are several other possible contributors to the peculiar growth pattern shown in Table I. For example, when *Artemia* embryos hatch, the resulting nauplii do not have an effective filter-feeding capability and must depend on their endogenous yolk supply for nourishment. The higher solubility of whey powder in seawater might, in this case, be useful in providing nutrition to the young nauplius (Dobbeleir *et al.*, 1980). Therefore, if ingestion of solubles such as whey powder is more effective than ingestion of particles such as rice bran powder during early development, it follows that whey-raised *Artemia* might grow more quickly in the early stages. This difference in solubility seems to have the opposite effect in later stages when *Artemia*'s feeding mechanism becomes more effective at removing suspended particles from the culture medium. Furthermore, Dobbeleir *et al.* (1980) stated that "except for the first few larval stages, soluble products cannot be efficiently ingested and as such do not support growth in *Artemia*." As the *Artemia* develops then, whey powder probably declines in food value due to its higher solubility, so this could also contribute to the apparent shift in superiority of the two feeds for young and older *Artemia*.

The higher solubility of whey powder is also responsible for supporting microbial growth in the culture medium. The blooms which occurred apparently had a bearing on survival of *Artemia* (with W-15, this was sometimes a problem) and also seemed to be a likely cause for over-estimation of protein conversion and feed conversion efficiency. No attempt was made to sterilize either feed, nor was any antimicrobial agent added to the culture media. There were noticeable differences in gross appearance and growth characteristics of the microbial colonies. The one(s) associated with the rice bran cultures appeared after 8 days of culturing and were detectable as one or two red areas on the tank bottom. These spots seemed to enlarge in a roughly geometric fashion and tended to restrict themselves to their original location until day 14 or 15. This growth in no way seemed to adversely affect the *Artemia*.

With the whey cultures, the situation was quite different. The microorganisms which were roughly the same color as whey, began to appear after only about 4 to 5 days of culturing. They also seemed to disperse in the AWL-raceway and even though the culture medium was replaced with fresh, UV-treated seawater on a daily basis, they grew at such a rate that there was substantial foaming of the culture medium by about day 13. At this time the culture medium probably became toxic to *Artemia*, since a strong odor of ammonia began to emanate from the W-15 tanks and it was at this point that most culture failures occurred. The negative effect of excessive ammonia levels may have been further aggravated by culturing *Artemia* at elevated temperature, where metabolic production has been found to increase (Moffet and Fisher, 1978). The amount and type of microorganisms existing in the four cultured groups therefore bore a relationship to growth and survival of the cultured *Artemia* (Dobbeleir *et al.*, 1980).

In addition to these factors, whey powder is lower in protein and especially in lipid than rice bran. Higher levels of these materials in the latter may have had a positive effect on growth of the RB-15 group since it has been reported that in later *Artemia* development, lipids and proteins are the prime energy source for the animal. Thus, feed type probably contributed to differences in growth performance between feed groups (Von Hentig, 1971 in Johnson, 1980).

Table II shows the proximate composition of all *Artemia* groups and the two culture feeds. These data generally do not show the kinds of differences due to feed consumption or developmental stage that was expected. Presumably, this was due to the brevity of the culture period and that W-15 animals were under stress, therefore being of limited comparative value. There are some interesting differences, however, which may be related to the above parameters. For example, the ash content of all groups (excluding nauplii) and the wild San Francisco Bay (SFB) population are quite similar. Since older *Artemia* contain less lipid, it seems that lipid level in *Artemia* is age (possibly stage) dependent. This trend appears to hold true within and between feed groups but may be more exaggerated in the older whey-fed animals due to lower feed concentration (possibly limiting), decreased suitability as an *Artemia* feed, low dietary lipid content, or environmental stress.

TABLE II

Proximate composition of *Artemia* and the monodiets used in culturing (all values are expressed as % of the dried samples ; abbreviations as in Table I ; SFB = San Francisco Bay adults)

Artemia or diet	Ash	Moisture	Lipid	Protein	Carbohydrates
W-7	11.08	2.85	10.92	60.73	14.42
W-15	9.16	4.81	7.45	61.38	17.20
RB-7	10.01	2.63	11.65	61.14	14.57
RB-15	9.93	6.37	9.47	50.13	24.10
48 h	7.17	6.96	19.40	58.70	7.77
SFB	11.16	4.58	3.37	53.25	27.64
Whey	7.68	4.93	1.30	10.23	75.86
Rice bran	10.77	8.30	6.54	13.69	60.70

Dietary factors do not seem to play any direct role in influencing protein or carbohydrate levels in cultured *Artemia*. In fact, the data seem to point out that stage of development is again quite influential with respect to these two components, especially when comparing RB-15 and SFB adults with other *Artemia* groups. The W-7, W-15, and RB-7 are thought to be at different developmental stages (due to differences in their weights), however, the difference may not be large enough to be reflected biochemically. The RB-15 and SFB groups, on the other hand, are obviously different from the others in terms of morphological characteristics and also protein content. The trend seems to be, at least when comparing adults with sub-adults, that there is a noticeable drop in protein with increasing development in *Artemia*.

Carbohydrate levels likewise show that there is little difference among the W-7, W-15, and RB-7 *Artemia*. When these three groups were again compared to RB-15 and SFB, the developmental effect on carbohydrate levels becomes more readily apparent. From the data in Table II, it is clear that rice bran-raised adults (RB-15) resemble the natural population (SFB) in most respects.

Table III shows the fatty acid patterns for *Artemia* and their feeds. SFB adults and 48-h nauplii illustrate the patterns of a wild adult population and that of the experimental groups prior to batch-culturing in the AWL-raceways. There is some agreement with previous results in that *Artemia* fatty acid levels may be influenced by their dietary intake (Claus *et al.*, 1979 ; Dobbeleir *et al.*, 1980). In some instances (*i.e.* 16:1ω7, 18:0), higher levels in either of the feeds are reflected in the observed *Artemia* patterns ; however, this simple association does not always apply. Several fatty acids, for example 14:1, 22:1, 20:5ω3, appear in the cultured animals without being contained in their feeds. Rice bran contains more lipid than whey power and has a slightly higher percentage of fatty acids which are C_{16} or longer. Furthermore, rice bran also contains higher levels of ω6 and ω3 polyunsaturated fatty acids (PUFA). These fatty acids are important for good growth and maintenance in a variety of marine organisms. If this also applies to *Artemia*, dietary lipid content and quality are probably affecting growth characteristics (Table I) by providing (or not providing) sufficient materials to meet growth and maintenance costs in the developing animal.

Both linolenic and linoleic acid, which have human essential fatty acid (EFA) activity, appear in cultured *Artemia*. The latter fatty acid is most important since it can be converted, in humans, to another EFA, arachidonic acid (Krause and Mahan, 1979). Linoleic acid is contained by all *Artemia* in substantial amounts, especially in the rice bran-raised *Artemia*.

TABLE III

Fatty acid patterns of *Artemia* and their feeds
(abbreviations as in Table I and II)

Fatty acid methyl ester	Feeds		*Artemia*					
	RB	W	48 h	RB-7	RB-15	W-7	W-15	SFB
14:0	8.21	14.94	–	–	–	–	–	–
14:1	–	–	10.28	3.40	7.56	13.60	9.25	–
15:0	–	3.08	–	–	–	–	–	–
15:1	–	–	–	1.33	0.77	–	–	–
16:0	22.98	32.51	12.94	10.57	17.16	16.33	16.12	22.72
16:1ω7	–	2.95	14.38	6.32	7.66	16.67	14.69	9.42
18:0	4.08	11.20	9.01	5.82	5.34	10.05	8.47	7.02
18:1ω9	33.23	28.66	26.07	47.10	48.21	29.42	44.35	45.05
18:2ω6	29.17	6.23	11.08	23.03	8.90	3.51	3.44	2.14
18:3ω3 / 20:1	2.30	0.45	10.30	–	1.19	1.35	0.89	9.74
18:4ω3	–	–	–	0.75	1.92	1.12	–	3.91
22:1	–	–	9.21	1.22	0.24	3.97	1.21	–
20:5ω3	–	–	7.01	0.44	1.03	3.96	1.58	–

Table IV illustrates the amino acid patterns of all samples and shows that there is only slight disagreement between values for the cultured groups. An attempt to connect this to differences in the patterns of the two feeds proved inconclusive, as expected. W-7 contained a substantial amount of tryptophan, as did W-15 though only a trace amount of this amino acid was present in whey powder. W-15 also contained a small amount of 1/2 cystine, which was absent in whey powder. Since *Artemia* has been previously reported to be a highly efficient protein converter at the qualitative level (Sorgeloos, 1980), the above observations are probably the result of *Artemia* having ability to synthesize these amino acids or obtain them from bacterial synthesis. In

TABLE IV

Amino acid patterns in cultured *Artemia* and their feeds (in g/100 g protein, abbreviations as in Table I and II)

Amino acid	Feeds		*Artemia*					
	RB	W	48 h	RB-7	RB-15	W-7	W-15	SFB
ASP	10.26	10.80	9.75	9.07	8.90	9.82	8.68	10.61
THR	4.64	6.77	4.29	4.03	4.01	3.93	3.97	3.57
SER	5.70	5.61	4.73	3.70	4.14	3.89	3.85	5.42
GLU	14.77	18.25	11.42	11.76	14.47	12.79	11.24	12.66
PRO	5.78	6.30	4.14	5.63	2.94	6.29	5.66	5.80
GLY	6.39	2.24	5.26	5.61	5.61	4.26	5.82	3.40
ALA	7.51	5.16	6.14	5.87	6.60	7.60	5.63	8.47
CYS[a]	0.66	–	–	–	–	–	0.72	0.49
VAL	5.90	7.47	5.39	5.18	5.31	5.09	5.08	5.82
MET	2.74	2.16	2.00	1.70	2.60	1.73	1.59	2.32
ISO	3.97	5.23	5.03	4.73	4.25	4.67	4.60	4.45
LEU	8.52	11.61	6.80	6.96	9.34	7.69	6.93	8.17
TYR	3.49	2.54	3.89	3.66	4.63	3.74	3.73	3.87
PHE	5.35	3.74	4.51	4.23	5.65	4.45	4.25	5.01
HIS	2.64	2.31	0.17	2.34	3.69	2.89	2.34	2.62
LYS	3.72	7.91	7.47	6.54	9.43	7.40	6.61	7.85
TRY	1.24	0.86	11.00	4.91	4.42	2.99	2.83	4.47
ARG	8.27	3.39	7.34	6.93	9.13	6.82	7.23	7.83

[a] CYS was actually read as 1/2 CYS.

attempting to correlate amino acid composition with age, values within the two feed groups were compared. In general, the data do not indicate stage-related differences.

The amino acid values were the basis of the calculated essential/total (E/T) amino acid ratio and chemical score based on reference values (FAO/WHO, 1973) (Table V).

E/T ratios included histidine as part of the overall essential amino acid value for each sample tested. This was done to compare all samples with infant requirements which are higher than those of children or adults (43, 36, and 19 respectively) (Krause and Mahan, 1979). The results reported here indicate that all samples meet the E/T requirement for infants with the exception of rice bran powder which does, however, meet the requirement of both children and adults.

TABLE V

Calculated essential/total (E/T) amino acid ratio and chemical score of *Artemia* and their feeds and protein efficiency ratio (PER) of the San Francisco Bay (SFB) group

	Feeds		*Artemia*					
	RB	W	48 h	RB-7	RB-15	W-7	W-15	SFB
E/T	40.67	49.32	50.89	47.69	50.73	46.41	46.99	47.30
Chemical score	67.64	61.71	57.14	48.47	74.29	49.43	66.00	80.29
PER	SFB	3.2						
	Casein	3.0						

When determining the chemical score (limiting amino value) of *Artemia* proteins, amino acid requirements of the human infant were not considered. This is because of the conclusion by the joint FAO/WHO committee that these values should be excluded from any guide to protein scoring that might be developed for older children and adults. Nevertheless, a comparison of the various *Artemia* groups was made with the suggested amino acid pattern for infants (values are presented).

The chemical score in all cultured and SFB-*Artemia* is the one for total sulfur-containing amino acids. It is possible that the scores would be increased if the sulfur amino acids were estimated by the method employing performic acid oxidation as a pre-hydrolysis treatment step (Schram *et al.*, 1954, as modified by Moore, 1963) prior to acid hydrolysis, as described earlier. However, that procedure was not used in this study. It was found that in most cases, the identity of the limiting amino acid did not change as a consequence of comparing observed values with the suggested pattern for infants. However, the chemical score does rise because the suggested value for cystine and methionine in infants is lower than in the FAO/WHO reference pattern. The limiting amino acid does change in the SFB group (threonine) because these animals showed the highest value of all for total sulfur-containing amino acids and the lowest value for threonine, which is required in greater amounts by the infant. The protein evaluation techniques such as those mentioned thus far, are valuable in estimating the worth of test materials like *Artemia* for humans ; however, PER goes further in that it gives some indication of a protein's digestibility. Results of the PER conducted with SFB *Artemia* as the test protein show that it is comparable to casein for digestibility and utilization in rats (3.2 and 3.0, respectively). Other estimations of the biological value of this protein, such as net-protein utilization (NPU) and slope-ratio analysis will clarify still further the ability of *Artemia* to serve as a dietary protein source for humans.

Earlier work has shown that various biotic and abiotic parameters may affect the biochemical composition of marine organisms (Conover, 1978). With *Artemia* it has been reported that biochemical characteristics can be influenced by factors such as geographical origin (Schauer *et al.*, 1980 ; Seidel *et al.*, 1980 ; Tobias *et al.*, 1980), food type (Sick, 1976 ; Claus *et al.*, 1979), food concentration (Mason, 1963 ; Reeve, 1963), and environmental characteristics such as temperature, salinity, or oxygen levels (Morris, 1971 ; Benijts *et al.*, 1975 ; Boulton and Huggins, 1977). For these reasons and others (*i.e.* methods of analysis, stage of development, etc.) strict comparison of data generated by separate investigations can be difficult and somewhat speculative. Within the *Artemia* groups cultured for this research, some of the above factors may be interacting to produce the observed results.

In this study *Artemia* were able to convert rice bran powder to *Artemia* biomass with about a 40 % (2.47:1) efficiency. Reported efficiencies for beef cattle are much lower.

These data show that *Artemia* may potentially be very competitive with common food animals in economy of production for human consumption. Production of *Artemia* biomass and cysts is expected to increase as a result of current projections of aquacultural needs.

There is evidence that *Artemia* has been used as food by man in the past. For example, Indians inhabiting the Great Salt Lake area used to collect and dry *Artemia* to be used as food (Jensen, 1918). The Dawada people of Libya have also consumed dried *Artemia* (Ghannudi and Tufail, 1978 in Sorgeloos, 1980) and sold them to obtain necessary items for their tribe (Bovill, 1968). In addition, modern taste panel tests have been conducted on oriental tempura using whole *Artemia* in the preparation and the results were quite favorable (Helfrich, 1973). In view of the data presented by this and other investigations, it seems possible that in the future the brine

shrimp *Artemia* can be produced as a protein source for humans using cheap feeds (low in protein) and in ponds using fertilizers.

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Workshop report : The use of *Artemia* as food in aquaculture

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Major changes have occurred in the assessment of the nutritional quality of *Artemia* since the first International Symposium on *Artemia*. At that 1979 symposium, a series of papers from the International Study on *Artemia* (ISA) warned that different geographical strains of *Artemia* differed in quality as a food for marine organisms. The ISA also suggested some possible causative factors. Since then, the amount of ω3-highly unsaturated fatty acids (ω3-HUFA) has been shown to be the single most important determinant of *Artemia* nutritional quality. Based on that information, techniques have been developed to augment or enrich the levels of ω3-HUFA in *Artemia* nauplii shortly after hatching. Thus, a single chemical analysis can now predict *Artemia* quality and remedial steps can be taken if the quality is found lacking.

The ISA studies were based on experiments with only one lot or batch from each geographical strain and prematurely concluded that each strain was consistently good or consistently bad. Subsequent analyses of hundreds of lots from many strains now indicates that no strain is uniformly good for marine organisms. Each lot needs to be analyzed for fatty acids (and hopefully for pollutants as well) because the *Artemia* quality depends on the pond conditions (water quality, phytoplankton bloom). If such analyses are done by the commercial suppliers to guarantee quality, the cost of the cysts will be increased.

More research is needed to produce cysts of higher quality. Controlled, intensive small-scale production might meet the demands of ecotoxicologists for small quantities of high-quality "research-grade" cysts. However, ecological research is required in small, manageable pond systems to learn more about the water quality and phytoplankton conditions required for high-quality cysts. If the production systems are too large or otherwise unmanageable, we may at least have advance knowledge of the cyst quality, by monitoring the conditions.

In the discussion of the posters it was noted that many of the contributors had fed or otherwise enriched the *Artemia* before feeding them to fish or crustaceans. Many people simply raise

Artemia on algae for a few days either to increase the nutritional value or to obtain a larger *Artemia* to present to the predator.

A great discrepancy exists between reported amounts of ω3-HUFA obtained after enrichment by Japanese scientists and European scientists. An extended discussion of respective enrichment techniques and expression of results in terms of wet weight *versus* dry weight failed to resolve the discrepancy. A subgroup of interested parties was invited to convene after the workshop to make further attempts to solve the problem. It was suggested that an interlaboratory calibration exercise be conducted to determine the degree to which the discrepancy is due to differences in analytical methods.

A written question was submitted to the workshop for discussion of alternatives to *Artemia*, especially how the demand for cysts would be affected by alternative products. It was pointed out that there are a range of artificial diets available for various kinds and sizes of fish and crustacean predators. The cost of the product will obviously be a very critical factor in choosing *Artemia versus* some alternate product. At some point in the future, inert feeds will probably replace *Artemia*, but the question remains whether they are cheaper today. The analysis of costs of using *Artemia versus* artificial diets in a penaeid shrimp hatchery is quite complex. Expensive, high ω3-HUFA, newly-hatched *Artemia* nauplii have not to be used during the entire postlarval production ; cheaper enriched or cultured *Artemia* can be used for later stages. One company that sells microencapsulated diets produces relatively small amounts of product that sell at a seemingly high price, but their production capability will increase tremendously after construction of a new plant and that could affect the unit price. The user's community (hatchery managers) have a variety of rearing philosophies, a variety of tank designs (some impractical for artificial feeds), and probably require a variety of foods to meet the needs of a non-standardized industry.

Finally, it was noted that one of the recommendations of the 1979 Symposium, that commercial suppliers should list the geographical origin of the *Artemia* cysts on each can, was not being followed. The geneticists, biochemists, and molecular biologists consider this information essential for their research. The marine fish farmers would like to see both origin and results of chemical analyses on the cans. One of the commercial suppliers explained that *Artemia* cysts are primarily for sale in pet shops and that one should not expect research-grade cysts if one buys those cysts that are commercially available. Cysts from different sources may be mixed together to make up such a product. If scientists have specific requirements for cysts used in their research, those requirements should be made known to the commercial supplier, who will then divulge the cyst origin.

Artemia *Research and its Applications. 1987. Vol. 3. Ecology, Culturing, Use in aquaculture*
P. Sorgeloos, D. A. Bengtson, W. Decleir, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 556 p.

Workshop report : *Artemia* as a business perspective

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It is generally believed that the young *Artemia* industry (first commercialization occurred only in the early 50's with the exploitation of Great Salt Lake cysts) will greatly expand in the near future. Higher sales are expected as a result of the fast development of shrimp aquaculture industry, especially in Southeast Asia and South America, and, also, by a further diversification of the *Artemia* products.

Cysts remain a very important product, *i.e.* in 1985 about 80 to 90 tonnes of cysts, harvested from only a few biotopes, will be marketed ; before the end of the century sales are expected to increase up to 150-170 tonnes annually. With present stocks estimated at over 200 tonnes, there is no shortage of cysts today, except perhaps, for quality product since only 50 % of the available cysts have a hatching quality of more than 50 % hatching (of which only 30-35 % with more than 70 % hatching). In view of these quality differences, which are often beyond the control of the supplier, *Artemia* users strongly urged the suppliers/distributors to provide each batch of cysts with a quality label. Moreover, as nutritional composition is often function of the geographical origin of the cysts, specific identification of the strain appeared to be of primary importance to the aquaculture producer as it may provide further quality information, *e.g.* specific strain characteristics, possibilities for pesticide contamination, etc. Hatcheries of shrimps and marine fish could improve their outputs when provided with details on the fatty acid content (HUFA) of commercial cyst batches.

Today more than 3 500 tonnes of *Artemia* biomass (adults) are harvested on an annual basis from natural systems and commercialized as a live or frozen product mostly for the tropical and ornamental pet fish industry. In view of the fast expanding aquaculture industry for which *Artemia* biomass has proven to be a very valuable food, it is predicted that the demand for biomass will increase very significantly in the years to come. The (freeze-) dried product appears to have the highest potential although its production cost is much higher than that of frozen *Artemia* (*i.e.* an extra of US$ 14 per kg dry product). This eventual preference over the frozen form is related

to the reduction in transport and storage costs, its availability as an off the shelf product, and its constant quality.

The *Artemia* producers/suppliers are convinced that the natural production systems will be able to fulfill without a problem all future demands for *Artemia* biomass by the developing aquaculture industry.

Intensive biomass production plants are only economically feasible when set up close to metropolitan areas, *i.e.* near the (pet) market for live *Artemia* biomass, or when integrated into aquaculture plants for the production of juvenile and reproductively active brine shrimp and, eventually, of ovoviviparous nauplii with a high nutritional quality.

It is also stated that the development of small-scale *Artemia* business in many developing countries will become more and more important, *e.g.* in Southeast Asia several salt farmers have already changed their activities into more profitable (salt +) *Artemia* production. Local independency in some cases has almost become a reality by proper integration of extensive *Artemia* farming into the aquaculture plant, which makes this brine shrimp application very promising. This type of artisanal *Artemia* production, eventually integrated in existing pond production of shrimp and/or fish, will gain more interest as it contributes to local independency of costly and vital imports of *Artemia*.

Concluding remarks

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Concluding remarks for Symposium Session III : Ecology, Culturing, Use in aquaculture

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As compared to the first *Artemia* conference much more information has been provided on *Artemia* ecology. Several reports were presented on *Artemia* resources in India, Mexico, Portugal, Australia, Italy, Yugoslavia, the USA, and New Zealand. However, our ecological knowledge of brine shrimp is still very limited.

As discussed during the workshop, more attention should be paid to quantitative ecological work with *Artemia*. New sampling techniques have been proposed. Also in the field of statistical analyses new methods have been tried and found useful. We heared about developments with regard to harvesting and processing *Artemia* biomass using artisanal methods or with new machinery. Harvesting and processing costs will determine which new markets can be developed. When integrated in local aquaculture projects the use of adult biomass appears to be a very attractive new application. Contrary to what had been reported in earlier years, solar salt production and *Artemia* production can go hand in hand. This activity provides some unique opportunities for integration especially in artisanal saltworks where it can have a positive socio-economic impact.

We have noticed much progress since the symposium in Corpus Christi (1979), in the field of pond production of *Artemia*. Nonetheless in most cases it is still an art rather than a blue-print production system. Although very high yields of biomass and cysts have been reported, productions are still at the demonstration level. Further optimizations can be expected from applied research on the role of bacteria, algae, cyst induction conditions, genetic control of production, etc.

Intensive culturing of *Artemia* biomass and nauplii has experienced further progress through the selection of a wider range of cheap feeds (mostly agricultural byproducts) and by the use of more efficient filter systems. It is expected that indoor *Artemia* culturing will steadily gain in interest as the use of ongrown *Artemia* reveals its high food value in nursery feeding of fish and shrimp larvae.

The new information on diapause regulation in brine shrimp cysts clarifies some of the (formerly unexplained) variations in hatching quality reported in earlier publications. Better understanding of these phenomena will allow the development of improved processing techniques yielding more consistent and higher hatching quality cyst products.

As a result of the various contributions on the use of *Artemia* nauplii as a larval food source it becomes obvious that brine shrimp are more a practical than a highly nutritious source of live food. The need for *Artemia* enrichment has been well documented. However, some problems in comparing/evaluating the quantitative effects of the different enrichment techniques applied

(using different products with different predator species) in Japan *versus* Europe, necessitate the development of standardized techniques to quantify the fatty acid levels in mg/g dry *Artemia* product. Arrangements have been made to perform an intercalibration exercize for fatty acid analysis and enrichment procedures among laboratories from Europe, Asia, and the America's. It has been suggested that the technique for HUFA-enrichment of *Artemia* could be applied for other *Artemia* bio-encapsulation purposes, e.g. administration of pigments, hormones, medicine, etc.

The critical effect of pesticides found in *Artemia* cysts, on their nutritional quality still is largely speculative. Maybe more sensitive (physiological) life processes have to be analyzed for diet quality evaluation, rather than the present criteria larval survival and growth. In this regard inspiration might be found in the activity (or stress) test as applied by Japanese scientists with red sea bream larvae.

During the workshops the audience expressed a strong interest in a better characterization of the origin of *Artemia* cysts by the commercial distributors. It also became clear that cyst stocks are increasing and that as a result of large differences in product quality, cyst prices fluctuate from as low as US$ 30/kg up to US$ 80 and more. The vivid workshop discussions have resulted in a better understanding between the commercial producers of *Artemia* cysts and their academic and industrial clients. The private sector expressed the willingness to better cooperate and make a distinction between the product for scientific utilization and that for large-scale use in the aquaculture industry.

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