THE BRINE SHRIMP
ARTEMIA

volume 3

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p. sorgeloos
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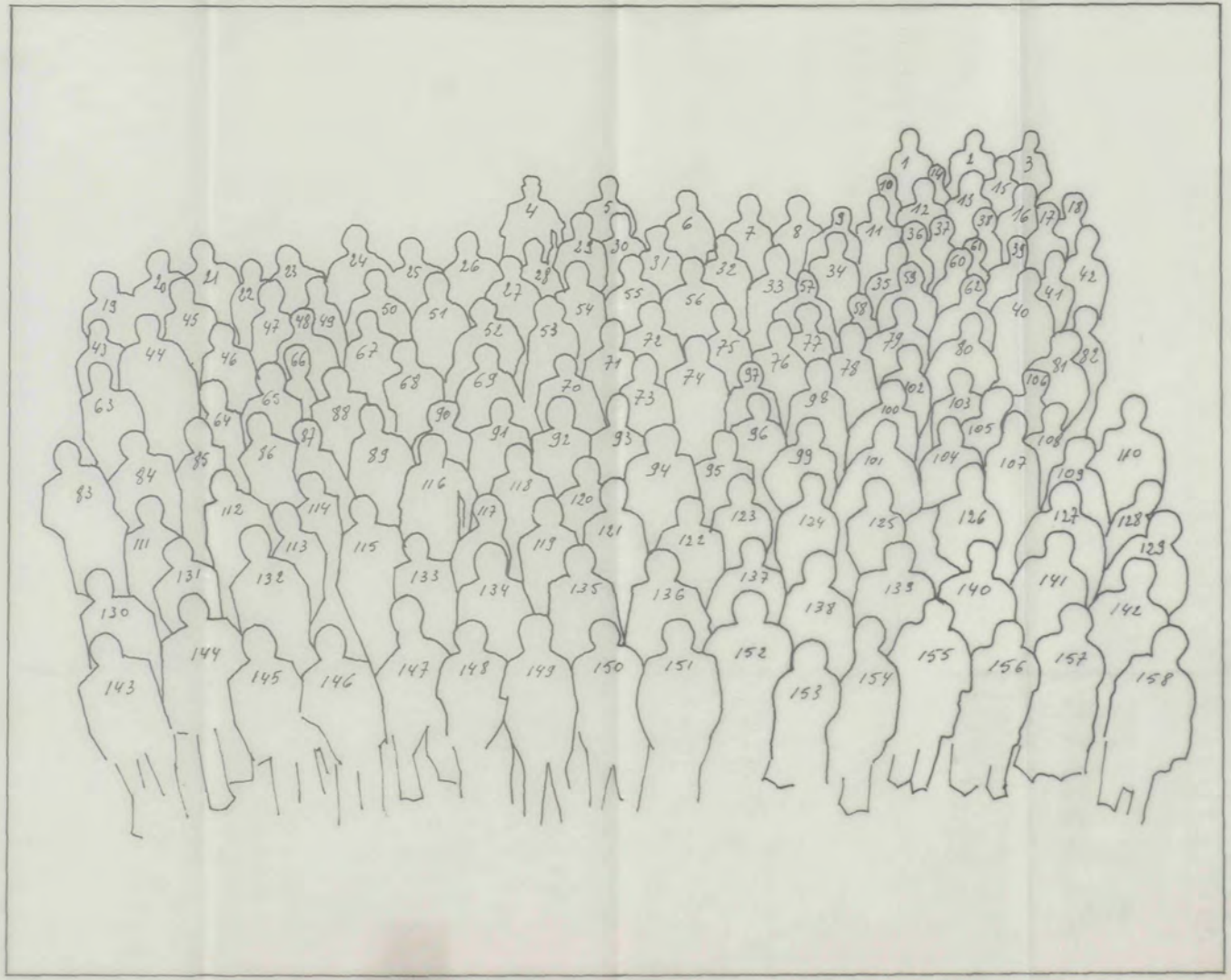

THE BRINE SHRIMP
ARTEMIA

Guido Persoone, Patrick Sorgeloos,
Oswald Roels and Edmonde Jaspers

Editors

Proceedings of the International Symposium on the brine shrimp *Artemia salina*.
Corpus Christi, Texas, USA, August 20-23, 1979.

State University of Ghent
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B-9000 Ghent, Belgium

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VOLUME 3

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Foreword

The "International Symposium on the brine shrimp Artemia salina*" was convened at the La Quinta Royale Motor Inn in Corpus Christi (Texas, USA) in August 1979 and was attended by approximately 200 participants from 26 countries of the five continents.*

This symposium was entirely devoted to all research and application aspects of an aquatic invertebrate which is used worldwide as a most suitable study object for fundamental research in practically all biological disciplines, and which moreover constitutes an indispensable source of live food for larval crustaceans and fishes mass cultured for commercial purposes.

The purpose of the Convention was three-fold :

- *To bring together all those working or interested in* Artemia *to exchange their findings on this unique crustacean ;*
- *To promote contacts among specialists in different* Artemia *research areas and to stimulate interdisciplinary research on brine shrimp ;*
- *To publish the reviews, the contributed papers and the syntheses and recommendations of the workshops as a reference book giving the state of the art of the present knowledge on* Artemia.

Because the response to the call for papers was overwhelming, the program was divided in concurrent sessions with the following topics :

Morphology – Radiobiology – Genetics
Physiology – Toxicology
Biochemistry – Molecular Biology
Ecology – Culturing – Use in Aquaculture

During these sessions seven reviews covering the major areas of Artemia *research were presented by invited authorities and complemented by approximately 100 contributed papers.*

In order to make a synopsis of the progresses reported at the Symposium and to define particular areas of Artemia *research deserving urgent attention, the last day of the Symposium was devoted to four workshops on the following themes :*

- *Characterization of* Artemia *strains for application in aquaculture ;*
- *Commercial aspects of* Artemia *exploitation ;*
- *Species characterization in* Artemia ;
- *Proposal for an intercalibration exercise for a standard* Artemia *toxicity test.*

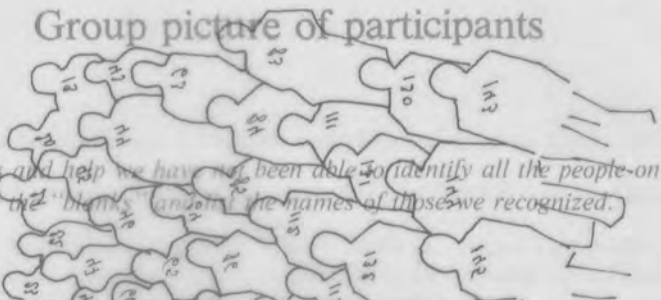
According to most participants, this interbreeding of "fundamentalists" with "applicants" and of "researchers" with "commercially oriented people" has been extremely stimulating. Everybody went home convinced of the following facts :

1) Artemia *and* Artemia *are two ;*
2) Despite the tremendous amount of literature on Artemia *(nearly 3 000 references) there are large gaps in our knowledge of this unique crustacean ;*
3) The advantages of Artemia *as a study object for fundamental research are not yet fully exploited and the potential of brine shrimp as a direct or indirect source of food for man becomes more and more obvious ;*
4) Interdisciplinary research on Artemia *which was embryonic so far is gradually developing and deserves full attention and stimulation.*

The Editors


Welcome
International Symposium on Brine Shrimp
"ARTEMIA SALINA"

Group picture of participants



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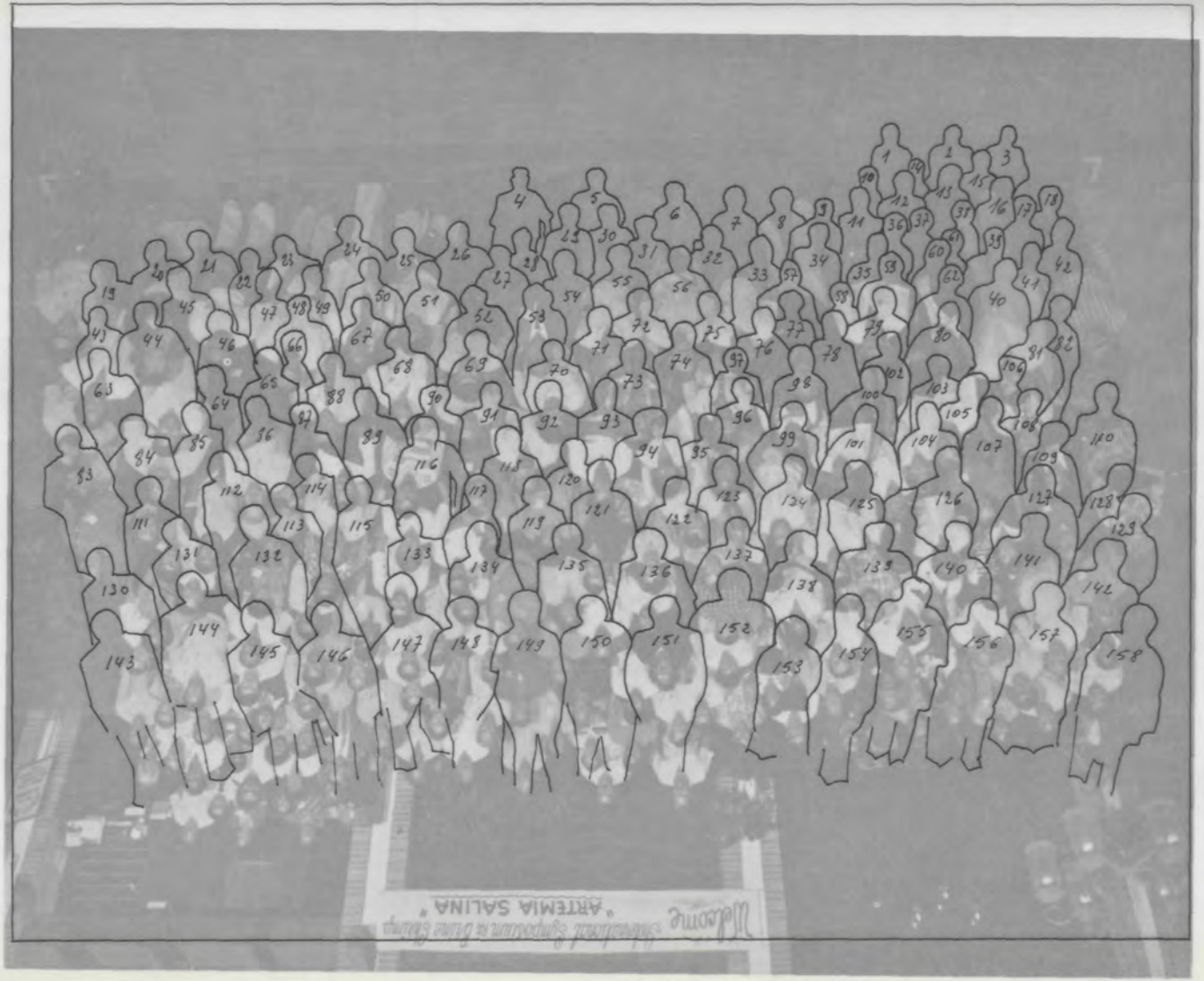
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Editorial note on the taxonomy of *Artemia*

The binomen Artemia salina *L. is taxonomically no longer valid (Bowen and Sterling, 1978).*

Crossing experiments of different Artemia *populations revealed reproductive isolation of several groups of populations (Halfer-Cervini* et al., *1968 ; Barigozzi, 1972, 1974 ; Clark and Bowen, 1976) and led to the recognition of sibling species to which different names have been given according to the International Conventions of Taxonomical Nomenclature (Bowen and Sterling, 1978). So far 20 bisexual strains have been classified into five sibling species (Bowen* et al., *1978).*

Theoretically the conventional name Artemia salina *L. can only be used for the original material from salt ponds in Lymington, England employed by Schlosser in 1755 to make the first drawing and by Linnaeus in 1758 to make the first description of the species (Kuenen and Baas-Becking, 1938). Because these salt ponds have disappeared, and* Artemia *no longer occurs in England, the species name* salina *should no longer be used.*

In view of the important genetical differences that exist between parthenogenetical strains of brine shrimp (Abreu-Grobois and Beardmore, 1980) species definition in the genus Artemia *has become confusing.*

It is suggested that unless the exact sibling species of a bisexual Artemia *strain can be identified* (cf. *Bowen* et al., *1978, 1980) and until speciation in brine shrimp is more clearly understood, only the genus designation* Artemia *should be used.*

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Life history of the brine shrimp *Artemia*

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The first written record of the existence of the brine shrimp dates back to 1755 (Schlosser in Kuenen and Baas-Becking, 1938). Nonetheless this "filtering animal" was known since much longer times by different ethnic groups who attributed a better salt production in brine pools to the presence of Artemia *; hence its popular names such as brineworm, Salztierchen, verme de sale, sóféreg, Bahar el dud, Fezzanwurm,* etc.

Despite the primitive optical equipment available at that time, Schlosser's drawings were very detailed (Fig. 1) and rightly gave the adult animal 11 pairs of thoracopods. Several other scientists, including Linnaeus (1758), later described adult Artemia *with only 10 thoracopods. This controversy lasted until 1836 when finally Audouin confirmed the observations of Schlosser.*

From the second half of the 19th century on, several studies were published dealing with the morphology and taxonomy of this Anostracan crustacean. Soon Artemia *was used as a most suitable test-object in the most diverse disciplines of biological sciences : histology, genetics, radiobiology, toxicology, biochemistry, molecular biology, ecology,* etc.

Salt lakes and brine ponds with Artemia *populations are found worldwide. The ecological conditions in these biotopes are extreme* (e.g. *the salinity can exceed 300 g salts/l water), and as a result only a small number of bacterial and algal species can survive. As a consequence of the often occurring blooms of monocultures of specific algal species, these waters are colored red, blue or green. One of the very few invertebrates that could adapt to such an extreme habitat is the brine shrimp* Artemia. *Favored by the absence of predators and food competitors,* Artemia *mostly develops into very dense populations in the salinas.*

At certain moments of the year, enormous quantities of minuscule brown particles (200-300 µm in diameter) are floating at the lake's surface and are finally thrown ashore by wind and waves (Fig. 2. 1). These apparently inert particles are in fact the inactive dry cysts of the brine shrimp which remain in diapause as long as they are kept dry or under anaerobic conditions. Upon immersion in seawater, the cysts hydrate, become spherical and within their shell the metabolism of the embryo is activated. A number of hours later, the outer membranes of the cyst burst (= "breaking" or E-1 stage) and the embryo appears, surrounded by the hatching membrane. The only structural feature which can be observed is the nauplius eye (Fig. 2. 2). During the following hours the embryo leaves the cyst's shell (E-2 stage ; Fig. 2. 3). Inside the hatching membrane, the newly differentiated antennae and mandibles start moving ; within a short period of time the hatching membrane is ruptured and the free-swimming nauplius is

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born. This first instar larva which is colored brownish-orange due to the presence of yolk, has three pairs of appendages : the antennae which have a locomotory function, the sensorial antennulae and the rudimentary mandibles (Fig. 2. 3). An unpaired red ocellus is situated in the head region between the antennulae. The ventral side of the animal is covered by a large labrum.

The larva grows and differentiates through about 15 molts : the trunk and abdomen are elongating ; the digestive tract becomes functional ; food particles are collected from the medium by the setae of the antennae ; paired lobular appendages which will differentiate into the thoracopods are budding in the thrunk-region ; lateral complex eyes are developing on both sides of the ocellus; etc. (Fig. 2. 4 and 5).

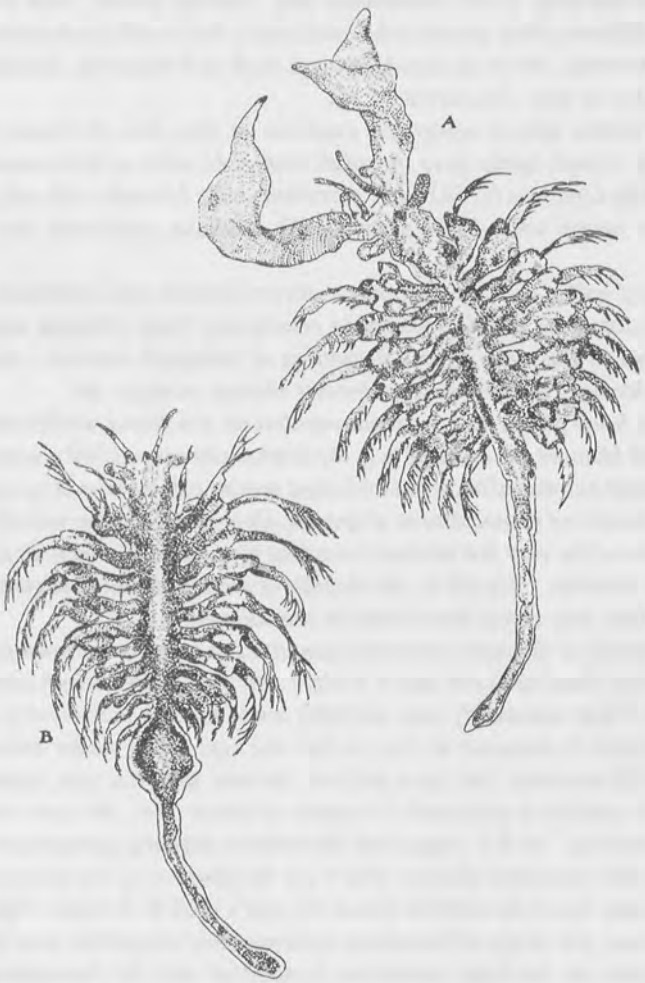

FIG. 1. Schlosser's drawing of a male (A) and a female (B) brine shrimp (From Kuenen and Baas-Becking, 1938).

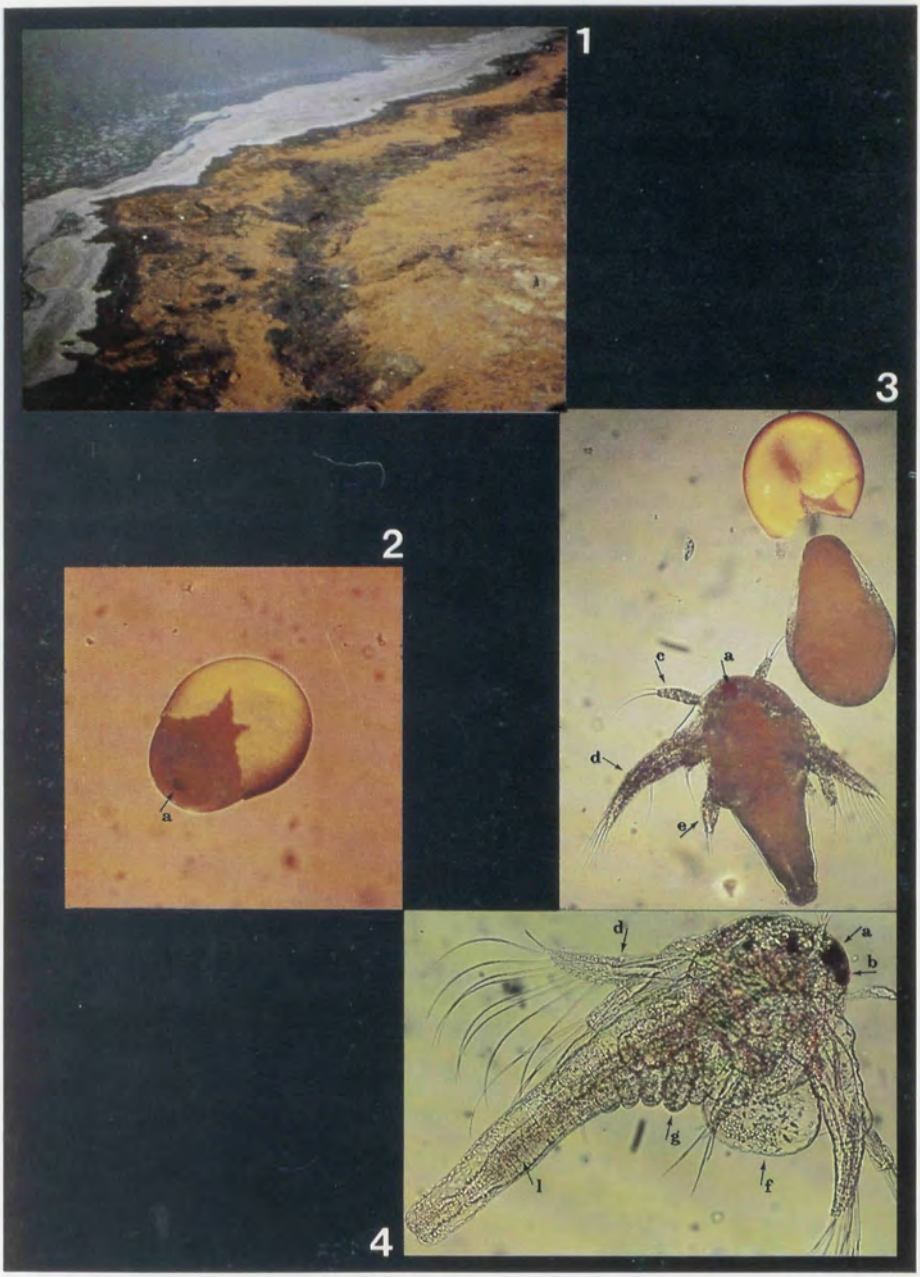

FIG. 2. 1. Brown layer of brine shrimp cysts accumulated on the shore of a salina ; 2. Pre-nauplius in E-1 stage ; 3. Pre-nauplius in E-2 stage and freshly hatched instar I nauplius ; 4. Instar V larva. a. nauplius eye ; b. lateral complex eye ; c. antennula ; d. antenna ; e. mandible ; f. labrum ; g. budding of thoracopods ; h. digestive tract.

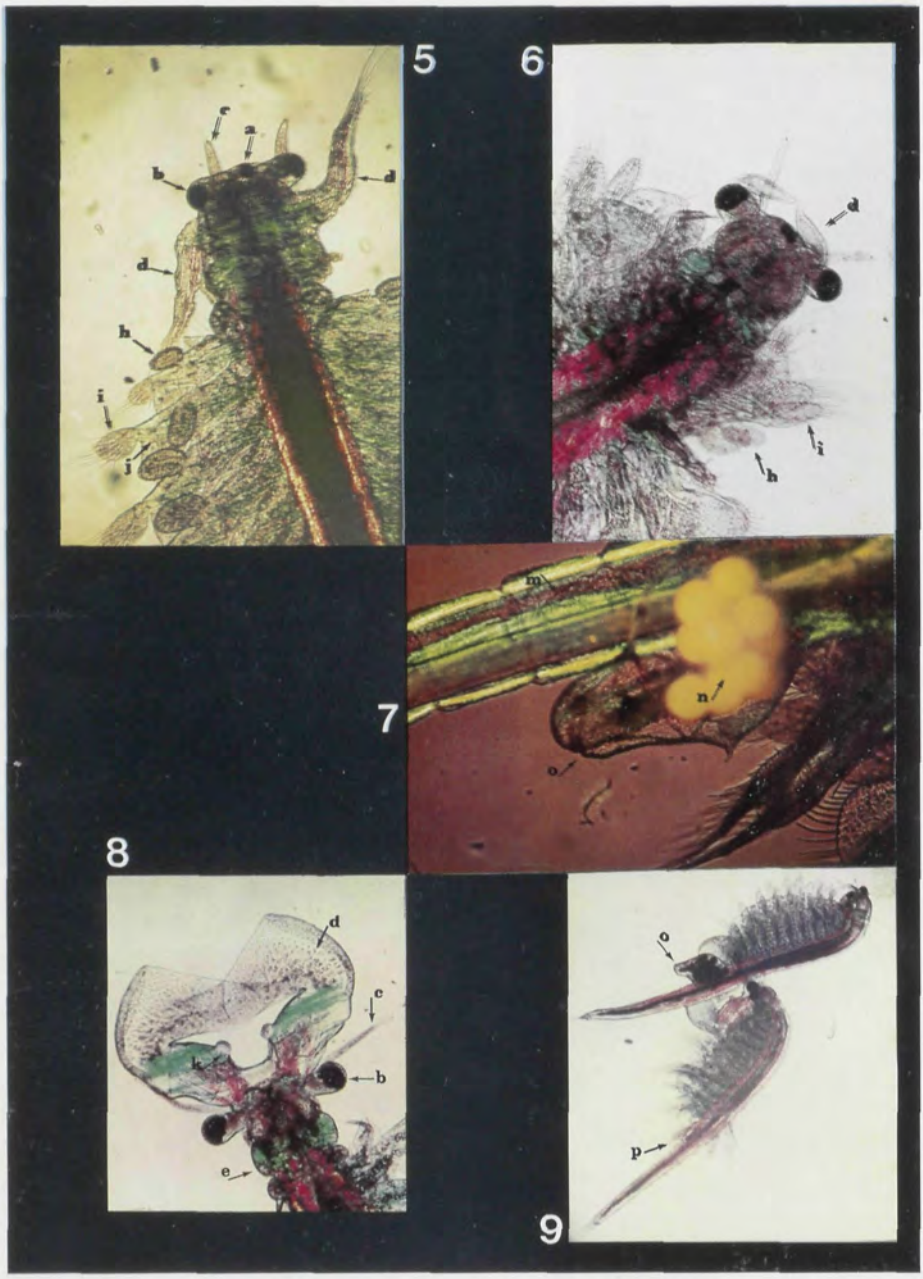

FIG. 3. 5. Head and anterior thoracic region of instar XII ; 6. Head and anterior thoracic region of young male (instar XV) ; 7. Posterior thoracic region and uterus of fertile female ; 8. Head of adult male ; 9. *Artemia*-couple in riding position. a. nauplius eye ; b. lateral complex eye ; c. antennula ; d. antenna ; e. mandible ; h. exopodite ; i. telopodite ; j. endopodite ; k. frontal knob ; m. inactive ovary ; n. ripe eggs in oviduct ; o. uterus ; p. penis.

From the 10th instar on, important morphological changes are taking place : the antennae loose their primitive locomotory function ; i.e. *they loose their long setae and undergo sexual differentiation. In the future males they develop into hooked graspers, while in the females the antennae degenerate into sensorial appendages (Fig. 3, 5, 6, and 8). The thoracopods are now differentiated into three functional parts : the telopodites acting as a filter, the oarlike endopodites having a locomotory activity, and the membranous exopodites functioning as gills (Fig. 3, 5 and 6).*

The adult animal 8-10 mm long, is characterized by the stalked lateral (complex) eye, the sensorial antennulae, the linear digestive tract, and the 11 pairs of functional thoracopods (Fig. 3, 6 and 9). In the male Artemia *the antennae are transformed into muscular graspers which have a frontal knob at their inner side (Fig. 3, 8). In the posterior part of the trunk region a paired penis can be observed (Fig. 3, 9).*

Female Artemia *have very primitive antennae with sensorial function ; their paired ovaries are situated on both sides of the digestive tract behind the thoracopods. The ripe oocytes are transported from the ovaries into the unpaired brood pouch or uterus via two oviducts (Fig. 3, 7).*

Precopulation in adult brine shrimp is initiated by the male in grasping the female with its antennae between the uterus and the last pair of thoracopods. In this "riding position" the couples can swim around for long periods (Fig. 3, 9).

Copulation itself is a very fast reflex : the male abdomen is bent forward and one penis is introduced into the uterus aperture. The fertilized eggs develop into either free-swimming nauplii (ovoviviparous reproduction) which are set free by the mother, or when reaching the gastrula stage, they are surrounded by a thick shell and are deposited as cysts, which are in diapause (oviparous reproduction).

[Morphology of Artemia *after Heath (1924), Nakanishi* et al. *(1962), Anderson (1967), Benesch (1969), and Wolfe (1973)].*

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General aspects of the ecology and biogeography of *Artemia*

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Content

Introduction

The present knowledge on brine shrimp in the natural environment is extremely poor ; the number of people involved in ecological *Artemia* research is very limited and as a consequence the number of scientific papers published on *Artemia* ecology is very restricted. Among the

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2 700 papers of the recently updated *Artemia* bibliography (Sorgeloos *et al.*, 1980) it is hard to find more than 50 articles which are strictly ecologically oriented. Collins (1977) is right when he points out that "... considering *Artemia*'s commercial importance as a tropical fish food, its possible utility in aquaculture and the voluminous literature on its physiology, development and behavior in the laboratory, why do we not have dozens of field studies on *Artemia* ?"

From the many branches of science for which *Artemia* has become a most useful study object, ecology is without any doubt the least practiced. This is the more amazing when one considers that further progress of *Artemia* research in physiology, genetics, biochemistry, radiobiology, and not the least in the practical use of brine shrimp in aquaculture, will be dependent to a large extent on our knowledge of the ecology of brine shrimp populations in their natural habitats.

In this review, we shall try to make a synthesis of the present knowledge on *Artemia* as related to its natural environment. We will comment on the many factors (and the complexity of their interactions) which limit and control the presence, the thriving or the disappearance of *Artemia* populations.

Geographical distribution

Decades ago *Artemia* has been recorded from over 80 saline habitats in many countries on the five continents (Abonyi, 1915 ; Artom, 1922 ; Stella, 1933 ; Mathias, 1937). However, many of the ancient salt pans, salt lakes and salt works where brine shrimp were reported to occur, have been destroyed or abandoned ; *e.g.* brine shrimp are not found any more in Germany, Great Britain, and Yugoslavia.

With the aid of the available literature and through personal contacts, we have tried to make up the list of all the recent *Artemia* find-spots (Table I through VII). However impressive this listings might appear, they are but provisional and tentative. In most countries no specific *Artemia* survey work has been undertaken yet. The few distribution studies on brine shrimp in the Saskatchewan province in Canada and in Spain resulted in long lists of brine shrimp habitats for these countries (Saskatchewan Fisheries Laboratory, Dr. Atton, personal communication respectively Amat Domenech, 1980).

The recent find-spots of *Artemia* are scattered throughout the tropical, subtropical, and temperate climatic zones, along coastlines as well as inland, sometimes at hundreds of miles from the sea. Although it would seem logical to characterize the coastal brine shrimp biotopes as thalassohaline and the inland ones as athalassohaline, this terminology in fact refers to the chemical composition of the water of the *Artemia* habitat.

Thalassohaline waters are concentrated seawaters (so-called chloride waters) with NaCl as major salt. They make up most, if not all, of the coastal *Artemia* habitats where brines are formed by evaporation of seawater in land-locked bays or lagoons, well-known under the common name of salt pans. During the dry season extensive parts of the shallow lagoons turn into salt flats, many of which in ancient times were transformed by man into solar salt works for salt production. There are, however, also *Artemia* habitats of the thalassohaline type which are located inland, the best known example of this type being the Great Salt Lake in Utah, USA.

TABLE I

Recent *Artemia* find-spots in Africa

Country	Locality
Algeria	Chegga Oasis
	Chott Djeloud
	Chott Quargla
	Dayet Morselli
	Gharabas Lake
	Sebket Djendli
	Sebket Ez Zemouk
	Sebket Oran
Egypt	Wadi Natrun
Kenia	Elmenteita
Libya	Mandara
	Ramba-Az-Zallaf, Fezzan
	Oum el Mâ
	Trouna
	Gabr Aoûn
Madagascar	Salins de Diego
Morocco	Larache
	Moulaya estuary
	Oued Ammafatma estuary
	Oued Chebeica estuary
	Sebket Bon Areg
	Sebket Zima
Mossambica	Nhamaiane
Nigeria	Teguidda-In-Tessoun
Senegal	Dakkar
	Lake Kayur
	Lake Retba
Tunisia	Chott Ariana
	Chott el Djerid
	Sebket Kourzia
	Sebket Sidi el Hani
South Africa	Coega Salt Flats
	Swartkops

TABLE II

Recent *Artemia* find-spots in Australia

Country	Locality
Central Queensland	Port Alma
	Bowen
	Rockhampton
New Zealand	Lake Grassmere
Southern Australia	Dry Creek Saltfields, Adelaide
Western Australia	Dampier
	Lake McLeod
	Port Hedland
	Rottnest Island
	Shark Bay

TABLE III

Recent *Artemia* find-spots in North America

Country	Locality
Canada	Akerlund Lake
	Alsask Lake
	Aroma Lake
	Berry Lake
	Boat Lake
	Burn Lake
	Ceylon Lake
	Chain Lake
	Chaplin Lake
	Coral Lake
	Drybore Lake
	Enis Lake
	Frederick Lake
	Fusilier Lake
	Grandora Lake
	Gull Lake
	Hatton Lake
	Horizon South
	Ingerbright North
	Landis Lake
	Little Manitou Lake
	Lydden Lake
	Mawer Lake
	Meacham Lake
	Muskiki Lake
	Neola Lake
	Oban lake
	Penley Lake
	Richmond Lake
	Shoe (Horseshoe) Lake
	Snakehole Lake
	Sybouts Lake – East
	Sybouts Lake – West
	Verlo East
	Vincent Lake
	Wheatstone South
	Whiteshore Lake
USA Arizona	Long H. Lake
	Kiatuthlana Red Pond
	Kiatuthlana Green Pond
California	Carpinteria Slough
	Elkhorn Slough
	Mono Lake
	Moss Landing
	San Diego
	San Francisco Bay
	San Pablo Bay
	Vallejo West Pond

Country	Locality
USA Hawaii	Laysan Atoll
Nebraska	Alkali Lake # 2
	Ashenburger Lake
	Cook Lake
	East Valley Lake
	Grubny Lake
	Homestead Lake
	Jesse Lake
	Johnson Lake
	Lilly Lake
	Reno Lake
	Richardson Lake
	Ryan Lake
	Sheridan County Lakes
Nevada	Big Soda Lake
North Dakota	Miller Lake
	Stink (Williams) Lake
New Mexico	Quemado
	Zuni Salt Lake
Texas	Playa Tahoka
Utah	Great Salt Lake
Washington	Hot Lake
	Omak Plateau

TABLE IV

Recent *Artemia* find-spots in Central America

Country	Locality
Bahamas	Great Inagua
	Long Island
Martinique	
Mexico	Baja California
	Pichilingue Island
	San Jose Island
	Yavaros
Netherlands Antilles	Aruba
	Bonaire Gotomeer
	Pekelmeer
	Slagbaai
	Curaçao
Puerto Rico	Bahia Salinas
	Bogueron
	La Parguera
	Tallaboa
Santo Domingo	
St. Martin	

TABLE V

Recent *Artemia* find-spots in South America

Country	Locality
Argentina	Bahia Blanca
	Buenos Aires
	Carahue
	Hidalgo
	Mar Chiquita
	La Pampa
Bolivia	
Brazil	Cabo Frio
	Macau
Chili	
Colombia	Galera Zamba
	Manaure
Ecuador	
Peru	Callao
	Caucato, Pisco
	Chilca, Lima
Venezuela	Coro Coastline
	Los Roques
	Boca Chica Salt Lake
	Port Araya
	Salinas Grandes de Hidalgo
	Tucacas

TABLE VI

Recent *Artemia* find-spots in Asia

Country	Locality
China	Tientsin
	Tsjingtao
India	Bhayander, Bombay
	Jamnagar
	Karsewar Island
	Kutch
	Sambhar Lake
	Tuticorin
	Vadala, Bombay
Iraq	Abu-Graib, Baghdad
Iran	Lake Rezaiyeh
	Schor-gol
Israel	Eilat North
	Eilat South
	Kalia potash works (Dead Sea)
	Solar Lake
Japan	Chang dao
	Yamaguchi, Seto Naikai
Turkey	Çamalti saltern, Izmir

TABLE VII

Recent *Artemia* find-spots in Europe

Country	Locality
Bulgaria	Burgas Salt Works
	Pomorije Salt Works
Cyprus	Akrotiri Salt Lake
	Larnaca Lake
France	Aigues Mortes
	Carnac – Trinité sur Mer
	Guérande – Le Croisic – La Boule
	La Palme
	Mesquer – Assérac
	Salin du Giraud
	Sète
	Lavalduc
Italy	Comacchio
	Margherita di Savoia
	Sicily
Portugal	Alcochete
	Tejo estuary
	Sado estuary
	Ria de Aveiro
	Ria de Faro
Roumania	Lake Techirghiol
Sardinia	Cagliari
	San Bartholomeo
	Santa Gilla
Spain	Armalla
	Ayamonte
	Barbarena
	Cabo de Gata
	Cadiz – San Felix
	– San Fernando
	Calpe
	Campos del Puerto, Mallorca
	Delta del Ebro
	Gerri de la Sal
	Imon
	Isla Cristina
	Janubio, Lanzarote
	Laguna de la Playa Bujaraloz
	Laguna de Quero
	Lepe
	Lerin
	Medinaceli
	Molina del Segura
	Peralta de la Sal
	Poza de la Sal
	Rienda
	Roquetas
	Saelices

Country	Locality
Spain	Salinera Catalana
	Salinera Espanola, Formentera
	Salinera Espanola, Ibiza
	Salinera Punta Galera
	Salinera San Antonio
	Salinera San Felix
	San Juan del Puerto
	Sanlucar de Barrameda
	San Pedro del Pinatar
	Santa Pola – Bonmati
	– Bras de Port
	– Salinera Española
	Siguenza
	Villena
USSR	Bol'shoe Otar – Mojnaskoe
	Burlinskoe
	Dzharylgach
	Ghenicheskoyo Lake (Herson)
	Kuchukskoe
	Kujalnic estuary
	Odessa
	Petukhouskoe
	Popovskoe Lake
	Sakskoe
	Sasyk Lake (Sivash)
	Sasykul Lake (Pamir)
	Tabichigskoe Lake
	Tambukan
	Toberchicskoe Lake

Athalassohaline *Artemia* biotopes are all located inland and are characterized by an ionic composition that differs very much of that of natural seawater. There are sulphate waters (*e.g.* Chaplin Lake in Saskatchewan, Canada ; Hammer *et al.*, 1975), high carbonate waters (*e.g.* Mono Lake in California, USA ; Mason, 1967), and potassium-rich waters (several of which are located in Nebraska, USA ; Cole and Brown, 1967).

As far as the size of *Artemia* biotopes is concerned, brine shrimp occur as well in very large biotopes (Lake Rezaiyeh, formerly lake Urmia, in Iran, with a surface of approximately 6 000 km^2 ; Löffler, 1961) as in small salt ponds such as Solar Lake in Israel, that has a surface of only a few hundreds of m^2 (Por, 1968).

Most of the coastal *Artemia* biotopes are very shallow, with minimal physical, chemical or biological stratification. Some of the inland *Artemia* habitats on the contrary, are relatively deep and stratified, such as Mono Lake in California, USA (Dana *et al.*, 1977).

Notwithstanding the cosmopolitan character of the occurrence of *Artemia*, it appears, when taking a closer look at the regional level, that its distribution is discontinuous in many places of the world. In other words, *Artemia* does not occur in every existing body of saltwater. The main reason for this is that *Artemia* cannot migrate from one saline biotope to another via the

seas, because it does not have any anatomical defense structure against predation by carnivorous aquatic organisms, *e.g.* larger crustaceans and fish. The principal dispersion mechanism of *Artemia* is transportation of cysts by wind and by waterfowl, as well as deliberate human inoculation in solar salt works. The fact that in most cases the cysts float at the surface of the water lays at the basis of transportation both by wind and waterfowl. The cysts either adhere to the feet and the feathers of the birds which come down on the water or they are washed ashore where they dry and are carried away by the wind.

Flamingos and some species of seagulls and ducks contribute to the geographical distribution of *Artemia* strains not only by external transportation but also via the ingestion of food. Some of these birds feed indeed on live brine shrimp (which may have cysts in their uterus) or on cysts washed ashore. It has been demonstrated, (Horne, 1966 ; MacDonald, 1980) that part of the cysts ingested withstand digestive enzymes and are excreted without having lost their viability. Löffler (1964) has shown experimentally that *Artemia* cysts can remain intact for 3 days in the digestive tract of birds and during this period some types of birds (*e.g.* flamingos) can cover more than 1 000 km. An example of possible long-distance transportation can tentatively be extrapolated from the findings of Bowen *et al.* (1978). These authors discovered that the brine shrimp in the salterns of Kutch in north India and those of Madras[4] in south India are genetically identical. Most probably this is the result of transportation of this specific strain by flamingos which migrate between the salt pans of both areas.

The absence of a migration route of birds is probably the reason why the very large salinas along the northeast coast of Brazil are not inhabited by brine shrimp, with the exception of the Macau salt works where *Artemia* was inoculated by man in one saltern just a few years ago (see further).

The deliberate inoculation of *Artemia* in solar salt works by man has been a current practice in the past. The presence of brine shrimp in salterns indeed seems to have a positive influence on the production of seasalt (Davis, 1977). Geddes (1980) mentions that all *Artemia* populations recorded in Australia have probably been imported by man. The strains occurring in Shark Bay in Western Australia and those in Rockhampton in Queensland are indeed very similar to the San Francisco Bay (California, USA) strain (Bowen *et al.*, 1978 ; Abreu-Grobois and Beardmore, 1980).

Ecological characteristics

Tolerance levels

Temperature

Most geographical strains do not seem to survive at temperatures below 6 °C unless of course under the form of cysts. The maximum temperature that *Artemia* populations tolerate has repeatedly been reported to be close to 35 °C, a temperature which is often attained in the shallow tropical salterns that constitute a large part of the *Artemia* habitats. This tolerance

[4] According to Royan (personal communication) the Madras *Artemia* are in fact populations from the Tuticorin salterns in India.

treshold is, however, strain-dependent. Recent inoculation tests in Thailand revealed that, after a certain adaptation period, brine shrimp from Macau (Brazil) survived for weeks at temperatures around 40 °C (Vos and Tansutapanit, 1979). As far as the optimum temperature is concerned there are probably as many temperature optima as there are *Artemia* habitats. The growth of animals in nature is indeed influenced by the entire set of abiotic and biotic factors of the environment in which it lives. On the basis of all data available it is, however, probably no heresy to claim that the optimum for *Artemia* must be situated in the range from 25 °C to 30 °C.

The ametabolic dehydrated cysts resist to a much wider temperature range, which is never occurring in nature ; *i.e.* the minimum being the absolute zero (-273 °C ; Skoultchi and Morowitz, 1964) and the maximum close to 100 °C (Hinton, 1954).

Ionic composition of the medium

Artemia can withstand environments in which the ratio of the major anions and cations is not only totally different from that in seawater but reaches extreme values (inferior as well as superior) in comparison to natural seawater. The Na^+ to K^+ ratio which is 28 in seawater attains 8 respectively 173 in some *Artemia* habitats ; that of Cl^- to CO_3^{2-}, which is 137 in seawater may decrease to 101 and reach 810 at the other extreme ; the Cl^- to SO_4^{2-} ratio which is 7 in seawater has been reported to be 0.5 respectively 90 in certain *Artemia* biotopes (Cole and Brown, 1967 ; Bowen *et al.*, 1978). This striking physiological adaptation to such extreme chemical habitats, already described by several biologists at the beginning of this century, made Cole and Brown (1967) conclude that "... the ionic composition of the waters inhabited by *Artemia* varies more than that of any other aquatic metazoan".

Salinity

As far as the upper limit of salinity is concerned, brine shrimp have been found alive in supersaturated brines at salinities as high as 340 ‰ (Post and Youssef, 1977). It is, however, quite understandable that under these extreme conditions the animals barely manage to survive and do no longer assume most of their normal physiological and metabolic functions.

The lower salinity limit in which *Artemia* is found in nature, is in most cases function of the presence of predating animals. Brine shrimp are indeed very seldom found in waters with a salinity lower than 45 ‰, although physiologically they thrive in seawater and even in brackish waters. As a general rule, we may say that the lowest salinity at which *Artemia* is found in nature varies from place to place and is determined by the upper salinity tolerance-level of the local predator(s). Hedgpeth (1959) mentioned that for several species of marine fish and invertebrates this level can be as high as 80 to 100 ‰ ; some fish species even seem to survive in salinities above 100 ‰ and even up to 130 ‰.

As is the cause for temperature, there is no well-defined optimum for salinity ; for physiological reasons, this optimum must, however, be situated towards the lower end of the salinity range. Indeed the higher the salinity, the more energy *Artemia* must spend for its osmoregulation.

An important aspect of salinity in the life cycle of brine shrimp is the effect of this physico-chemical factor on the metabolism of the cysts. *Artemia* cysts will start to develop when the salinity of the medium drops below a certain treshold value, which is strain dependent (*e.g.*

85 ‰ for the San Francisco Bay strain). At salinities above this treshold *Artemia* cysts will never hatch because they cannot hydrate enough, which is one of the perequisites for the onset of the hatching metabolism.

Oxygen

Artemia is a typical euroxybiont since it has been reported to survive in environments with less than 1 ppm dissolved oxygen and, at the other extreme, in situations where algal blooms increase the oxygen level beyond 150% saturation. The optimal oxygen concentration, though unknown, must logically be close to the saturation level.

pH

Although in nature brine shrimp are found in neutral to alkaline waters, very little is known about the influence of the pH on juveniles or adults. For the cysts it is important to note that the hatching efficiency decreases when the pH drops below 8 (Sato, 1967).

PHYSIOLOGICAL ADAPTATION MECHANISMS

As already said, brine shrimp do not possess any anatomical defense mechanism against predation and *Artemia* populations are always in danger at salinities which are tolerated by carnivorous species. Brine shrimp, however, have developed a very efficient ecological defense mechanism by their physiological adaptation to media with very high salinity. As such they can escape from their predators thanks to this salinity barrier.

Although brine shrimp possess the best osmoregulation system known in the animal kingdom, the fact that they thrive in media with a high salinity means that at the same time they have to live in environments with low oxygen levels (the saturation value for oxygen indeed being inversely proportional to the salinity level). At the exception of short periods of oversaturation of eutrophic waters during the day, *Artemia* usually has to cope with low oxygen concentrations. Brine shrimp are weapened against this unfavorable environmental condition by their capability of synthesizing very efficient respiratory pigments. The concentration of these haemoglobins increases with increasing salinities, thus with decreasing dissolved oxygen levels.

The third ecological adaptation mechanism is the ability of *Artemia* to assure the survival of the species by formation of encysted, ametabolic embryos or cysts which resist better to extreme environmental conditions than do the juveniles and the adults. There are many theories about the exact mechanisms which control the onset of cyst formation in brine shrimp. The latest information points to the major role which oxygen fluctuations or more exactly fluctuations in the redox-potential of the water seem to play in this mechanism (Versichele and Sorgeloos, 1980).

Artemia cysts will stay in diapauze as long as the salinity of the medium remains above the hatching treshold. Decreases of the salinity in brine shrimp habitats mostly occur on a cyclic or seasonal basis by rainfall or runoff of freshwater in the biotope. As said before, whenever the salinity drops to a value below the hatching treshold a new brine shrimp population can develop. It should be emphasized that situations of a temporary low salinity often occur in a salt lake, *e.g.* the restricted area of inflowing freshwater, or after rainfall when for a while a freshwater layer remains on top of the heavier salt water.

Another proof that brine shrimp have an extreme adaptability to salinity stresses is that nauplii which hatch out of cysts in a water layer of very low salinity (down to 5 ‰) survive very well when this water layer is mixed by wind action or currents with waters of very high salinity. This phenomenon was probably overlooked by Royan *et al.* (1978) when they extrapolated from salinity readings in nature that cysts had hatched in media of 130 to 160 ‰ salinity.

It seems appropriate to make here a small digression about the great advantage which brine shrimp have conserved during their developmental history in comparison to freshwater anostracan crustaceans, such as *Chirocephalus* and *Streptocephalus*. Freshwater anostracans have lost the capability of producing live offspring. This can be seen as an adaptation to their natural habitat (ephemeral ponds, *i.e.* temporal biotopes) characterized by cyclic successions of drying out completely and being refilled after a certain time by rainfall. Since the aquatic phase of the cycle is in most cases rather short (from a few weeks to a few months) the animals, after hatching, have merely the time to grow out into one generation of adults which form cysts in order to resist to the dry period. As a result, in nature one seldom encounters dense populations of freshwater anostracans.

The *Artemia* cycle is quite different. When the conditions required for hatching are fulfilled, the cysts hatch into nauplii which grow in a few weeks to adults. Since most *Artemia* habitats are perennial, a dual mode of reproduction has an adaptive value : through ovoviviparity a small number of adults give rise to a fast population explosion leading to very high densities of animals, very typical for *Artemia* biotopes. It is only when the environmental conditions arrive at a certain critical treshold that ovoviviparity shifts to oviparity with formation of cysts.

Feeding characteristics

Brine shrimp are typical filter-feeders, ingesting particulate material of a size range which laboratory experiments have shown to extend from a few micrometer up to approximately 50 micrometer. Since the continuous beating of the thoracopods carried out by the animal for respiration serve at the same time to collect food particles, *Artemia* does not have any choice but to feed continuously.

The food consumed by *Artemia* in nature is made up of varying percentages of inert particulate material of biological origin (organic detritus) and living organisms of the appropriate size-range (mostly microscopic algae and bacteria).

In many *Artemia* biotopes the presence of high numbers of brine shrimp often coïncides with blooms of microscopic algae (green algae, blue green algae, diatoms, *etc.*). The richness in dissolved or particulate organic matter of these blooming waters in turn promotes the development of large numbers of heterotrophic bacteria.

The presence of algal material in the gut or the intestine of brine shrimp should not be considered as an evidence of its nutritional value nor of its digestibility for *Artemia* ; experiments performed by Reeve (1963) and Dobbeleir *et al.* (1980) have indeed shown that *Artemia* even ingests sand grains or glass microspheres.

As far as competition for food is concerned, *Artemia* does not seem to have competitors in the high salinity waters. The brine fly *Ephydra*, often encountered in large numbers in *Artemia* biotopes, is more a benthic feeder and does not interfere with the *Artemia* food chain.

At the lower end of the salinity range brine shrimp, however, must suffer the presence and competition for food of several groups of invertebrates, such as rotifers, ciliates, and other crustaceans (anostracans and copepods).

Predation

As already mentioned, *Artemia* populations are subject to serious predation in all situations where the predator can withstand the salinity of the medium. The list of *Artemia* predators thus includes per definition all species feeding on zooplankton that populate natural seawaters. Since this list comprises as well all tropical fish, prawn, shrimp, lobster and fish species which one now endeavors to mass culture on a controlled basis, we find here the major reason for the great interest which aquariologists and aquaculture people show for *Artemia*. In typical *Artemia* habitats (thus at higher salinities) several categories of insects regularly predate on brine shrimp : Odonata larvae, aquatic Hemiptera and Coleoptera ; some of the hemipteran families Corixidae and Notonectidae can withstand very high salinities. Mullet, milkfish, and *Tilapia* predate heavily on brine shrimp in salt pans and salt lakes ; some of these fish species can indeed withstand salinities up to 120 ‰. Predators to which *Artemia* cannot escape through the high salinity barrier are of course the birds. For several species of waterfowl *Artemia* constitutes an important part of their diet. Isenmann (1975) reported that in the salt pans of the Camargue (France) little gulls (*Larus minutus*) feed almost exclusively on brine shrimp from March to October. Besides gulls and avocets, flamingos are the group of birds most often quoted to feed on *Artemia* in saltwater bodies (Rooth, 1965). Later we shall comment on the relation *Artemia*-waterfowl and its ecological importance.

Parasites and diseases

Not much is know about parasites and diseases of *Artemia* in their natural habitats. Scattered throughout the scientific literature it is reported that brine shrimp can be contaminated by viruses, endosymbiontic procaryots, bacteria (spirochaetes), fungi, and flatworms of the group of the Cestodes, but no information is given as to what extent these contaminants affect the populations.

Strain characteristics

Since *Artemia* biotopes are geographically isolated from one another, each habitat can theoretically be populated by a different geographical strain. The study of these strains, especially from the genetic point of view, has already resulted in several most important findings.

The first and not the least important is that the genus *Artemia* consists in fact of several sibling species which are isolated from the reproductive point of view (Kuenen, 1939 ; Barigozzi and Tosi, 1959 ; Clark and Bowen, 1976). As a result we are no longer entitled to refer to *Artemia salina* ; instead we are dealing with *Artemia monica*, *A. tunisiana*, *A. urmiana*, *A. persimilis* or *A. franciscana*[5].

[5] See editoral note on the taxonomy of *Artemia* in this book.

Since the characterization of *Artemia* strains is treated in detail in many papers of these Proceedings, we only mention here that there are bisexual or zygogenetic strains (with males and females) and parthenogenetic strains (only females). Strains also differ in their chromosome number : there are diploid, triploid, tetraploid, pentaploid, and even dekaploid strains.

The large variety among *Artemia* strains in genotypical and phenotypical differences is reflected in a number of features which can be morphological, biometrical, biochemical or physiological. This subject is now investigated in detail by several teams of scientists participating in the International Study on *Artemia* (Sorgeloos, 1980a).

Nutritional differences between geographical strains of *Artemia* are related in the first place to the biochemical composition of the animals which can vary to a large extent between strains growing in different biotopes under the influence of different abiotic as well as biotic factors. For the abiotic factors, all hydrographic and climatological parameters play a role, since they will either have a direct or an indirect influence on the physiology of the brine shrimp. The water temperature for example, which is different from one place to another as a result of the configuration of the biotope as well as of the prevailing climate, is known to influence, at least in part, the biochemical composition of *Artemia* (Hines *et al.*, 1980). The indirect influence which environmental factors exert on the biochemical composition of brine shrimp acts through the food chain. Depending on the local conditions of climate and nutrients, a specific type of food will dominate in each *Artemia* habitat ; this in turn will influence the biochemical composition and thus the nutritional value not only of the adult brine shrimp but also of the embryos and thus of the nauplii which will hatch out of the cysts. In addition, a factor which unfortunately has already proved to have an adverse influence on the nutritional value of brine shrimp is contamination of some *Artemia* habitats with persistent pesticides (Olney *et al.*, 1980).

Productivity of *Artemia* biotopes

Production of nauplii, juveniles, and adults

Collecting information on the quantity of brine shrimp present in a saltwater body, either as number of individuals or as biomass per unit of water or per surface area, is not a very easy task. As a consequence it is not surprising that there are few quantitative data (even approximate ones) in the literature. The origin of this difficulty lays in the fact that *Artemia* is an organism which not only may show a strong phototactic behavior but which as a true planktont cannot overcome water currents created by winds. As a consequence brine shrimp are often swept in patches of very high density from one spot to another. Baker (1966) gave the following pertinent comment on her failure to quantify brine shrimp in salt ponds "... I have visited the ponds, filtered gallons of pond brine, walked around the pond and decided that the population was very small because no shrimp were visible, only to return the next day and find the brine 'boiling' with *Artemia*".

Tentatively we gathered all quantitative estimates on the maximum densities of brine shrimp in different sites, which we could find in scientific papers (Table VIII). No doubt commercial harvesters of brine shrimp could add more precise information.

TABLE VIII

Literature data on the productivity of *Artemia* habitats

Site	Country	Maximum production	Period	Author
Lake Rezaiyeh	Iran	1.2 adults/l		Parker (1900)
Sivash Salt Lakes	USSR	400/l		Gun'ko (1962)
Slagbaai	Bonaire, Netherlands Antilles	200-360/l	Oct.-June	Rooth (1965)
Mono Lake	California, USA	4 adults/l		
		12 nauplii/l	June-Sept.	Mason (1967)
		400/l	Aug.-Sept.	Lenz (1980)
Great Salt Lake	Utah, USA	10/l		Wirick (1972)
Salin de Giraud	Camargue, France	10-100/l	March-Oct.	Isenmann (1975)
		0.02-0.2 g/l wet weight		
Long Island salina	Bahamas	25-100/l	May-Sept.	Davis (1978)
Alviso Salt Ponds	California, USA	13 g/m^3 dry weight	summer	Carpelan (1957)
San Francisco Bay Salt Ponds	California, USA	5 kg/ha wet weight (harvest)	per week	Baker (1966)
Crimea Salt Lakes	USSR	250 kg/ha	October	Voronov (1973)
		3 000 kg/ha	June	
Burgas-Pomorije Salt Works	Bulgaria	2.75 g/l adults wet weight		
		0.93 g/l juveniles	June-Sept.	Lüdskanova (1974)
		0.05 g/l nauplii		

A closer look at the *Artemia* biomass-data reveals that :

1) different authors use different standards to express their results ; intercomparison is extremely difficult if not impossible ;
2) the productions which are expressed in identical units vary very much from one site to another and even within the same biotope between different samplings ;
3) some authors report extremely high productivities : the 3 000 kg/ha (probably wet weight figure) given by Voronov (1973) for the Crimea Salt Lakes in the USSR during June, means a production of 300 g/m^2 which most probably is a hazardous extrapolation to the entire biotope of a few samplings in a patch of *Artemia*.

It should be reminded that the distribution of brine shrimp over the entire habitat is seldom homogenous and that as a consequence it is extremely difficult to calculate exact productions. The best method of approximation should always be to sample at different moments, in as many places as possible, by vertical plankton hauls, and then calculate the average for the entire biotope.

Whatever the data presented here are worth, the *Artemia* productivity is definitely associated with the primary productivity and/or with the richness in particulate organic matter.

During the 4th World Symposium on Salt Production, Davis (1977) reported on the positive influence which micro-organisms (algae or bacteria) exert on the production of salt

and concluded that the most productive salinas (from the point of view of salt production) are those where the inflowing water is rich in the essential nutrients nitrogen and phosphorus. According to this author salt works should best be located as close as possible to "mineral rich" areas, such as population centers, river mouths, deep water and ocean upwellings. From his statement we can deduce that the most productive salt works will also be the most productive *Artemia* biotopes. With regard to this, it is interesting to mention the reciprocal benefit of the predator-prey relationship between *Artemia* and waterfowl, especially flamingos. As said earlier, saline biotopes are much visited by waterfowl which feed on brine shrimp, but these birds in turn fertilize the biotope with their guano, contributing in this way to the productivity of the ecosystem by a feedback mechanism.

We would like to add from our own experience, a fifth category to the list of most productive salinas (thus most productive *Artemia* biotopes) proposed by Davis (1977), namely the solar salt works which receive their intake waters from a mangrove area. A good example of this category are the salt works of Macau in Brazil. Since their recent inoculation with a small quantity of brine shrimp, these salt works in northeastern Brazil became one of the most productive *Artemia* biotopes in the whole world.

Production of cysts

Most *Artemia* strains produce cysts that float. In Mono Lake (California, USA), however, the local sibling species *Artemia monica* produces cysts that sink. As a result the classic dispersion mechanism does not take place and the latter strain is, from the biogeographical point of view, much more isolated than brine shrimp strains which produce floating cysts.

We have tried to make up a table of existing data on the production of cysts in *Artemia* habitats (Table IX). A thorough literature search revealed only four papers with data on this matter. Since we know from wholesalers that the quantity of cysts which are sold annually must now be close to 100 tons, we can but wonder about the scant scientific information on cyst production in different countries. From the table one can of course not conclude very much ; according to our own estimations a good *Artemia* biotope produces some 10 to 20 kg of cysts per hectare per season.

Table IX

Literature data on the production of cysts in *Artemia* habitats

Site	Surface	Country	Harvest	Author
Marina-Salina	1 ha	California, USA	50 kg/year	Boone and Baas-Becking (1931)
Crimea Salt Lakes	70 km²	USSR	32 400/l/year	Voronov (1973)
San Francisco Bay	> 1 000 ha	California, USA	18 kg/ha (4 months per year)	Rakowicz (in Helfrich 1973)
Burgas Pomorije Salt works	550 ha	Bulgaria	from 326 g/m³ to max. 838 g/m³	Ludskanova (1974)

Exploitation of *Artemia*

The key position that brine shrimp are presently occupying in aquaculture and in aquariology both under the form of cysts and of adults is well-known (Sorgeloos, 1980a).

Adult *Artemia* are mainly collected from shallow salt ponds with conical nets mounted in front of a very small raft or boat equipped with an outboard motor. With this relatively simple technique, Rakowicz (quoted in Baker, 1966) reported a daily harvest of up to 4 tons of fresh weight *Artemia*. The best catches are made on a cloudy morning after a calm night. In such conditions the dissolved oxygen concentration in the highly eutrophic San Francisco Bay salt ponds is so low that the animals concentrate in very dense "blow-ups" to perform surface respiration. These accumulations are so spectacular that they can be seen from small planes flying over the salt ponds.

Another type of harvesting technology takes advantage of the positive phototactic behavior of the brine shrimp, the intensity of which, is, however, strain and temperature dependent.

Apparently no attention has ever been paid to the influence which massive *Artemia* catches may have on the ecosystem of a particular saltwater body. To saveguard the maximum productivity of the *Artemia* habitat from the point of view of production of both adults as well as cysts it is imperative that, in analogy to fisheries, the "maximum sustainable yield" be determined.

To date *Artemia* cysts are harvested at many places in the world (Sorgeloos, 1979, 1980b). They are collected either directly in the water or after being thrown ashore where they accumulate in reddish-brownish layers, several cm thick and many meters in length. The best catches are usually made in sites where the direction of the dominant winds is relatively constant and the cysts are always thrown ashore at the same spot. With unstable wind regimes cysts move around the salt ponds and before they can be harvested they risk to be hydrated and hatch out (*e.g.* in the surface water layer of lower salinity after a period of rainfall). The same can also happen with cysts accumulated on shore. For this reason, and also because dry cysts can be blown away by winds, commercial harvesters should always collect cysts as regularly as possible. From the practical point of view of *Artemia* cysts exploitation, advantage can be taken of the positive influence of the winds for the accumulation of the cysts. Ponds for cyst production should be built very long and quite narrow, with the length axis in the direction of the dominating winds ; the cysts produced will then accumulate at one end of the pond where they can be easily collected (more details on cyst harvesting and processing can be found in Sorgeloos, 1978).

Artemia inoculation in saltwater bodies

Aside from failure of dispersion, adverse climatological conditions, especially in monsoon-climates, are probably also limiting the presence of *Artemia* in saltwater bodies which at first glance look suited for brine shrimp. In South East Asia for example (Thailand, Philippines, Indonesia, *etc.*), where thousands of hectares of salt pans can be found during the dry season, *Artemia* is not naturally occurring. This can be explained by the fact that neither the cysts nor the live brine shrimp could survive throughout the rainy season ; *i.e.* at the low salinities cysts would hatch and all live animals would be eliminated by predating fish and crustaceans.

Considering the important role of *Artemia* in aquaculture today, the question can be raised if we cannot utilize our present knowledge on the biology of the brine shrimp and on the ecology (even if the latter is still relatively poor) to develop *Artemia* farming in natural saltwater bodies.

A promising step in this direction is the artificial inoculation of *Artemia*. The benefits which can be derived from inoculating brine shrimp in salinas where these crustaceans do not occur naturally are obvious : as a result of the high reproductive capacity of brine shrimp, a small inoculum of nauplii in highly productive salt ponds at salinities around 120 ‰ and water temperatures in the range from 25 to 35 °C, will lead to a fast population explosion. In salinas managed for salt production animals will be drained from one evaporation pond to another and cysts will be produced in the higher salinity ranges ; in man-managed *Artemia* ponds, salinity conditions can be controlled at will as to favor the production of either *Artemia* biomass or cysts.

A distinction should be made here between definitive and temporary inoculations. Definitive inoculations are those where one single inoculation will lead to the permanent establishment of an *Artemia* population. A first attempt in colonizing natural saltwater bodies with *Artemia* has been tried out in the early seventies in hypersaline lagoons on Christmas Island in the Central Pacific (Helfrich, 1973). This inoculation has not been very successful for two major ecological reasons :

1) the salinity is too low in most of the ponds to protect the developing brine shrimp populations against predation by several fish species ;
2) although one had hoped to be able to enrich the intake waters in order to permit the development of a substantial phytoplankton biomass, this has not been the case. Helfrich (1973) indeed concluded that "... without enriching the Christmas Island waters there is presently insufficient phytoplankton productivity potential to support the proposed *Artemia* culture scheme".

Artificial nutrient enrichment with fertilizers, which could have been a practical solution for the second obstacle, was unfortunately not feasible because the transport of inorganic nitrogen and phosphor salts to this isolated and desert atoll island in the Pacific was economically prohibitive.

More recent experimental inoculations have been carried out in Macau (Brazil) and in Cuba. The seeding of one salt pond in a very large salt work in Macau (Rio Grande do Norte, Brazil) with nauplii hatched out of 250 g of San Francisco Bay (California, USA) cysts has been extremely successful. All prerequisites for a mass development of *Artemia* were fulfilled and brine shrimp spread out over the 3 000 ha solar salt works. The first kg of cysts was harvested after only a few months after inoculation. Present harvests exceed 20 metric tons per year (for a harvesting period from September through March, Van Tilburg, personal communication). It is interesting to note that neighboring salt works have been inoculated with *Artemia* through dispersion from Macau by local waterfowl.

As far as temporary inoculations are concerned, successful trials have been made during the dry season in the Philippines (de los Santos *et al.*, 1980), in Thailand (Vos and Tansutapanit, 1979) and in India (Royan, personal communication). Water levels in these salt ponds were increased from a traditional level of less than 10 cm to 25 cm or more in order to keep temperatures around or below 35 °C during the hotest moment of the day. Extrapolated

from the production figures obtained so far, average cyst harvests amounted to approximately 20 kg/ha and per season. Experiments are now in progress in Thailand to test the possibility of increasing *Artemia* productions by application of organic manure, *e.g.* duck and chicken dung (Tansutapanit, personal communication).

Temporary inoculations offer a number of biological advantages in comparison to definitive inoculations. The former permit to experiment with different *Artemia* strains in order to find the strain that is best adapted to the local conditions. A bad choice is in this case less of a disaster than with a definitive inoculation, since the *Artemia* population is only established temporarily. Furthermore temporary inoculations offer unique possibilities for fundamental experimentation in natural salt ponds, *e.g.* with regard to genetical stability and phenotypical characteristics of the numerous geographical strains of brine shrimp from which cyst material is now available.

Perspectives of brine shrimp production in nature

To conclude this review we would like to turn to the future and look at the tremendous potential of controlled *Artemia* production in natural sites found at many places around the world ; *i.e.* thousands of hectares of abandoned salterns as well as the large flats along estuaries and mangrove areas. Part of the latter sites can easily be transformed by the construction of dikes into evaporation ponds ; when properly managed from the point of view of temperature and salinity, these ponds can produce thousands of tons of *Artemia* biomass either on a continuous or on a cyclic basis.

Finally we want to make a strong plea for the preservation and saveguarding of all existing natural *Artemia* habitats. Salt lakes and salt ponds are unique and well-balanced ecosystems of which man can easily destroy the very particular food chain including *Artemia* and migrating birds. We should be aware, that if we destroy the original *Artemia* gene pools we condemn at the same time our basic potential of genetic improvement and cross-breeding of *Artemia* strains. And this, exactly as in any type of farming and husbandry, would be the biggest drawback for all the hopes which we are placing today in the advancement of aquaculture and the mass culturing of *Artemia* as a most wellcome addition to the production of animal protein.

Acknowledgements

We are very indebted to the many persons who provided us with information on the geographical distribution of brine shrimp, especially Drs. Atton and Morgan for their unpublished list of Canadian respectively North African find-spots.

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The use of the brine shrimp *Artemia* in aquaculture

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Content

Introduction

One of the major differences between aquaculture and cattle breeding is that the larvae of most aquatic animal species of commercial interest, which are grown in intensive hatchery systems, have to be offered a live food whereas cattle accept inert diets throughout their live cycle (Kinne and Rosenthal, 1977).

Culturing of the zooplankton that normally constitutes the natural food of fish and shrimp larvae, is either commercially unfeasible or technically hard to realize (Girin and Person-Le Ruyet, 1977). As a result "... the efforts of early pioneers to rear marine fish were hampered by inadequate and unsuitable larval food supplies" (Shelbourne, 1968).

A very significant pogress in hatchery aquaculture was made with the discovery by Seale (1933) in the USA and Rollefsen (1939) in Norway, that the 0.4 mm nauplius larva of *Artemia* constitutes an excellent food source for newborn fish larvae.

[1] "Bevoegdverklaard Navorser" at the Belgian National Foundation for Scientific Research (NFWO).

Technically speaking the advantage of using *Artemia* is that one starts from an apparently inert product, namely the dry cysts. These cysts which are in fact inactive embryos are commercially available, can be stored for years and only have to be incubated for 24 hr in seawater to produce free-swimming larvae. Furthermore, brine shrimp are very well accepted as a food source. It is not exactly known if this is due to their biochemical composition, their very thin carapace, the fact that they are a moving prey (swimming) or a combination of all these factors.

Artemia has been found to be a suitable food for the most diversified groups of organisms of the animal kingdom, *e.g.* foraminifers, coelenterates, flatworms, polychaetes, squids, insects, chaetognaths, and of course a wide variety of both marine and freshwater crustaceans and fishes. In his treatise on "Cultivation of marine organisms", Kinne (1977) pertinently indicated that "... more than 85 % of the marine animals cultivated thus far have been offered *Artemia salina* as food source, either together with other foods or, more often, as a sole diet".

It has been proved many times that a diet of live *Artemia* gives better results than any preparation of dead brine shrimp (Serfling *et al.*, 1974 ; Carlberg and Van Olst, 1975 ; Beck, 1979 ; Schauer *et al.*, 1979). The recent finding by Fluchter (1980) that "... whitefish larvae get through metamorphosis equally well whether they are given *Artemia* that is shock-frozen in liquid nitrogen (- 196 °C) or living *Artemia*", but not when given slow-frozen nauplii, point to the fact that essential substance(s) is (are) lost during freezing and freeze-drying. In a few cases, it has been demonstrated that dried brine shrimp can be successfully used as protein source in pelletized diets for fish and shrimp (Deshimaru and Shigueno, 1972 ; Gabaudan *et al.*, 1980).

In most cases brine shrimp are used as freshly-hatched nauplii. Although outgrown *Artemia* larvae are reported to be a better food than nauplii for many predators (Kelly *et al.*, 1977 ; Purdom and Preston, 1977), the fact that they have to be cultured for a few days has limited this type of application in many aquaculture hatcheries (Brouillet, 1977). Adult brine shrimp are harvested from saline biotopes as food for the larvae of lobsters (Shleser and Gallagher, 1974) and the freshwater prawn *Macrobrachium rosenbergii* (Anonymous, 1978).

Historical aspects of the "supply and demand" of cysts

Initially the commercial supply of cysts, first from saltponds in the San Francisco Bay area (California, USA) and later also from Great Salt Lake (Utah, USA) and Little Manitou Lake (Saskatchewan, Canada), seemed to be unlimited. The exponentially increasing demand of brine shrimp cysts by aquarium hobbyists and aquaculture hatcheries, however, soon exceeded by far the yearly harvest of approximately 30 to 50 metric tons. From the late sixties on, the dramatic impact of the aggravating cyst shortage on the expansion of aquaculture was repeatedly underlined at international conferences (Provasoli, 1969 ; FAO, 1972, 1976 ; ASEAN, 1976, 1977). Resolutions, such as the one taken by FAO that "... a fuller exploration and exploitation of the world's resources of *Artemia* for aquaculture purposes were considered to be of special importance", all pointed to the urgency of the problem. The situation did not improve however ; prices continued to soar, and the hatching quality of the product delivered became less and less reliable. When one was lucky enough to receive a brand of good quality, only 4 g of cyst-material was needed to produce 1 million nauplii ; in the worst case, however, it took up to 50 g, signifying a 90 % difference in output (Sorgeloos

et al., 1978). As a result commercial aquaculture has been impeded very seriously. This is especially true for the farming of *Macrobrachium* and *Penaeus* which are dependent upon an *Artemia* diet during a long period in their larval development (Bledsoe *et al.*, 1978 ; Glude, 1978ab ; Smith *et al.*, 1978). Third world countries could hardly afford to import the very expensive cysts. At a regional workshop in Indonesia in 1977 it was concluded that "... the inadequate supply of brine shrimp for feeding shrimp larvae remains as the major constraint in the mass propagation of penaeids in Thailand as in the other countries" (ASEAN, 1977).

Although we had announced at the Kyoto 1976 FAO Technical Conference on Aquaculture (Sorgeloos, 1979a) that the cyst shortage was a technical and only a temporal problem, many people remained sceptical. It was not before the end of 1978 that a change in the situation became visible, firstly by the exploitation of several new natural sources of *Artemia* in Europa, Asia, North and South America, and Australia (Sorgeloos, 1979b) and secondly by the successful inoculation of *Artemia* in North-East Brazil (Sorgeloos *et al.*, 1979). According to the latest data available, cyst provisions now exceed 100 metric tons per year.

The increased availability of cysts resulted in competition among dealers and a substantial decrease in prices to about US $ 35.00 to 40.00 per kilogram (FOB-prices). Thanks to the application of new harvesting techniques, the hatching quality of the cysts put on the market improved and became more reliable. The classic method of harvesting cysts from the shore required an air-classified treatment as a final purification step in order to remove small dirt particles included in the harvest (Helfrich, 1973). During their stay on the shore the cysts are also often subjected to repeated hydration-dehydration cycles which affect the energetic content of the embryos and eventually lead the embryonic development to the breaking stage. In this latter situation many so called "light" cysts are harvested which are in fact empty cyst shells (Sorgeloos *et al.*, 1976 ; Benijts *et al.*, 1977). A very pure cyst-product which does not need air-classifying processing can be obtained by harvesting the cysts directly from the water surface. Accumulation of cysts on the shore by wind and wave action can be prevented by the construction of dikes or the installation of floating barriers (Sorgeloos, 1978). Since cysts are mainly produced at high salinities, they remain ametabolic even during light rains, provided that the water is sufficiently turbulent to prevent salinity stratification ["... fresh rainfall on a calm lake provides a lower salinity surface layer where eggs could hatch" (Post, 1977)].

Conditions for maximal hatching output

The production of *Artemia* nauplii by incubation of cysts in seawater is a very simple procedure. However, when working on a larger scale and with high densities of cysts (which is mostly the case in aquaculture enterprises) the use of appropriate techniques is imperative to obtain maximal hatching efficiencies and to minimize the quantity of cysts needed to produce a specific weight or number of *Artemia* nauplii.

During the last years we have had the opportunity to study in detail the effect of various abiotic parameters on the hatching process (review by Sorgeloos, 1979c). Although the quantitative data vary from one geographical strain to another, the qualitative effect of each individual parameter is similar for all strains studied.

Temperature

The various effects of water temperature on the hatching metabolism of *Artemia* cysts are summarized in Fig. 1. The fastest hatching rate and the maximal hatching efficiency are attained around 30 °C. It is interesting to note that as long as the cysts have not reached the breaking stage, an increase of the water temperature within the range of about 33 to 40 °C causes a reversible interruption of the cyst metabolism (Sorgeloos, 1975). When hours or even days later, the water temperature is adjusted to the optimal level for hatching, the cyst metabolism is resumed and the nauplii are born. In the meantime, however, the hatching rate decreased as a function of the duration of exposure to the temperatures above the optimum (Sorgeloos *et al.*, 1976 ; Benijts *et al.*, 1977). Molecular biological studies have recently been initiated to study this phenomenon in more detail (Vallejo *et al.*, 1980). In practice, it can be deduced from this observation that cysts which have been exposed by accident (*e.g.* a technical failure of a heating device) for a short period of time to temperatures above 30 °C (but below 40 °C), are not necessarily useless but can be saved by decreasing the temperature of the medium, and can still produce nauplii.

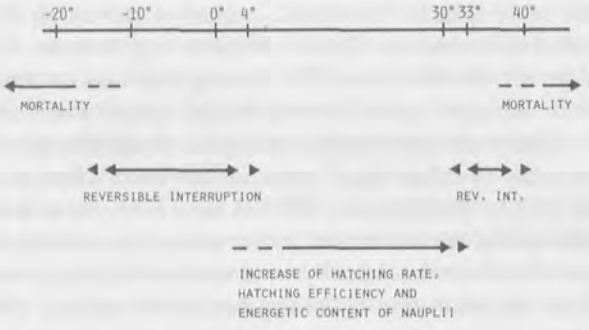

Fig. 1. Schematic diagram of the effect of water temperatures from below -20 °C to over 40 °C, on the cyst metabolism in *Artemia*.

Salinity

For reasons of practical convenience natural seawater is mainly used to hatch cysts. Recently, however, it has been demonstrated that at lower salinities the hatching rate increases, that the nauplii have a higher energy content, and that in many cases even higher percentages are scored for the hatching efficiency (Vanhaecke *et al.*, 1980a).

From the "trehalose-glycerol hyperosmotic regulatory system" – theory of Clegg (1964) and Conte *et al.* (1977) we deduced that the increased energy content of the nauplii hatched at 5 ‰ can be explained by the lower levels of glycerol which have to be built up at this salinity to reach the breaking stage (Fig. 2). Since less energy is consumed in hatching, more is left in the nauplius resulting in a higher energy content per unit food available to the predator. In those cases where an increased hatching efficiency was noted, the energetic content of the encysted

embryos might be so close to the critical level needed for hatching that, depending on the level of glycerol that has to be build up, these embryos can reach the breaking stage in water of 5 ‰ salinity but not in natural seawater.

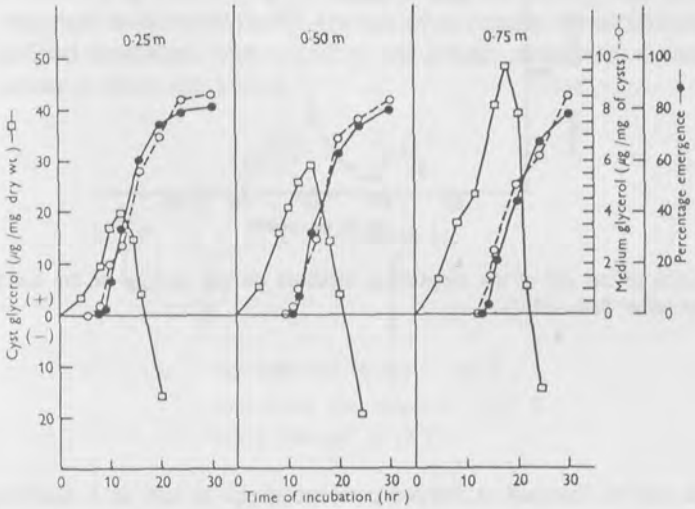

FIG. 2. Relationship between the concentration of glycerol in the cysts (—□—), the glycerol level in the medium (—○—), the percentage of cysts in "breaking" (— ● —) and the time of incubation of *Artemia* cysts with 3 different concentrations of NaCl (0.25 m NaCl = 14.6 ‰ salinity) (after Clegg, 1964).

The 5 ‰ level has been chosen arbitrarily. At this salinity neither the survival of the hatched nauplii nor their tolerance to salinity stresses is affected. The larvae may indeed be transferred directly and without any harm to seawater of up to 150 ‰ salinity. The critical minimal salinity for survival has not yet been defined, though it is well-known that in freshwater the physical process of breaking is reached but that the embryos die at the E-1 stage.

pH

One of the key factors for successfull hatching at low salinity is the pH of the medium. Sato (1967) demonstrated that hatching at the E-2 larval stage is triggered by a hatching enzyme that has a maximal activity in the pH-range 8 to 9 (Fig. 3). In diluted seawater media and especially at high cyst densities the buffer capacity of the medium must be increased to keep the pH above 8. This can be achieved by the addition of Na_2CO_3 [1 ml of a 0.5 M solution/l medium (Jones, 1972)] or CaO (65 mg/l medium). The poor hatching results reported by many authors using artificial seawater or diluted seawater are probably to a large extent related to this pH-effect.

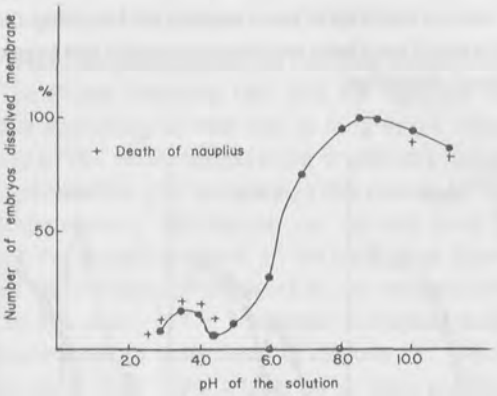

FIG. 3. Influence of the pH of the incubation medium on the activity of the hatching enzyme in *Artemia* embryos (after Sato, 1967).

OXYGEN

Artemia cysts can be hatched at oxygen concentrations as low as 1 mg/l (Sorgeloos and Persoone, 1975). At lower levels the metabolism is reversibly interrupted. In order to obtain a maximal hatching efficiency, oxygen levels close to saturation are recommended, and most important, all cysts should be kept in suspension. Indeed accumulation of cysts on the bottom of the hatching container creates anaerobic zones which interrupt the cyst's metabolism.

Optimal hatching can be achieved with various types of funnel-shaped containers that are aerated from the bottom. We found a very handy solution in using heat-sealed plastic bags made from polyethylene sheets.

CYST DENSITY

As demonstrated by Kurata (1967), who incubated up to 17 g cysts/l medium, the hatching process is not affected by cyst density, provided, however, that the other perequisites are fulfilled. In view of the technical problems encountered in maintaining high oxygen levels without inducing foaming or mechanical injury of the hatched nauplii, it is recommended not to exceed densities of 10 g/l, especially when working with large quantities.

ILLUMINATION

When hatching is performed in darkness the hatching success is only half of what it would be if the operation was carried out in light conditions (Sorgeloos, 1973). More recent experiments indicate that not only the hatching efficiency but also the hatching rate are affected by the light intensity (Vanhaecke *et al.*, 1980b). Considering the differences which are observed among strains, a continuous illumination of about 1 000 lux assures a maximal hatching output. This light intensity is attained when the hatching container is placed at about 20 cm from a fluorescent light tube of 60 W.

In order to obtain a maximal energetic output, it does not suffice to incubate the cysts in optimal hatching conditions ; one also has to harvest the nauplii at the right moment (Benijts *et al.*, 1976). Upon hatching, the instar I nauplii are not yet able to take up food and completely thrive on their energy reserves. Within a few hours they molt into the 2nd and 3rd larval stage. By this time they have already lost over 20% of their energy reserves (Fig. 4). Consequently, the most economical use of *Artemia* cysts implies the incubation of the cysts under strictly defined conditions with regard to the abiotic parameters outlied above, and harvest of the larvae in the instar I stage.

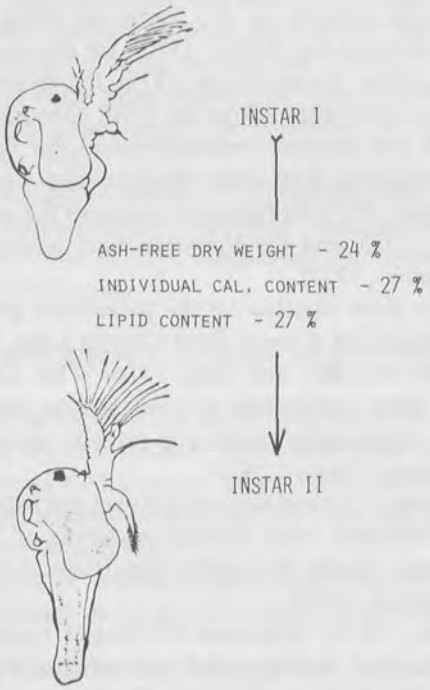

FIG. 4. Changes in energetic content of *Artemia* nauplii upon molting from instar I into instar II-III nauplii.

In spite of the various methods described to facilitate and maximize the separation of nauplii from the hatching debris (Sorgeloos and Persoone, 1975 ; Sorgeloos, 1979a ; Dye, 1980), many limitations still exist. This is particularly true for the separation based on light attraction because nauplii of some strains are negatively phototactic [Venezuela and Spain (Claus *et al.*, 1977)]. In this regard the cyst decapsulation treatment proposed by Sorgeloos *et al.* (1977) with recent improvements by Bruggeman *et al.* (1979, 1980), can be considered as a very important advance in the use of *Artemia* cysts in aquaculture hatcheries. The use of decapsulated cysts indeed not only eliminates all separation problems but has several other advantages, *e.g.* desinfection of the cysts, maximized hatchability, and increased naupliar energetic content. In addition this process opens up the possibility of feeding the cultured species directly on decapsulated cysts (Bruggeman *et al.*, 1980).

Nutritional value of *Artemia* nauplii as food source in aquaculture hatcheries

With the outlook for increasing *Artemia* cyst provisions better than ever before, the next aspect that deserves urgent attention is the food value of *Artemia* nauplii for various cultured species. Although at the end of 1978 cysts from about a dozen geographical strains of brine shrimp were, or will soon be commercially available[2], data on the nutritional value of the freshly-hatched nauplii for various cultured species are extremely scarce. We tentatively report the following information :

- Good production results are reported for two freshwater fishes fed nauplii from Burgas-Pomorije, Bulgaria (Lüdskanova and Joshev, 1972), for *Dicentrarchus labrax* fed nauplii from the Çamalti saltern-Izmir, Turkey (Uçal, 1979), for various marine and freshwater fishes fed on several brine shrimp strains from the USSR (Oleynikova and Pleskachevskaya, 1979 ; Spektorova, 1979 and personal communication), for *Palaemon* sp. and *Penaeus japonicus* fed *Artemia* originating from Little Manitou Lake, Canada (respectively Kurata, 1967 ; Fujinaga and Kittaka, 1967), for *Penaeus kerathurus* fed nauplii from San Fernando-Cadiz, Spain (Rodriguez, 1975) and for *Macrobrachium americanum* fed *Artemia* from Manaure, Colombia (Cantillo, 1978).
- No significant differences were reported for the parameters growth and survival of the larvae of *Palaemonetes pugio* fed *Artemia* from Chaplin Lake, Canada or San Francisco Bay, California, USA (Provenzano and Goy, 1976). This Canadian strain, however, performed better than other unspecified *Artemia* strains when offered to *Panulirus interruptus* ; "... There is considerable variation in growth and survival of phyllosomes in regards to source of *Artemia*" (Dexter, 1972).
- Fuchs and Person-Le Ruyet (1976) and Person-Le Ruyet and Salaun (1977) did not observe significant production differences when feeding the larvae of *Solea*, *Dicentrarchus*, and *Scophthalmus* with *Artemia* nauplii from either Sète (France), Larnaca Lake (Cyprus) or San Francisco Bay (California, USA).
- Watanabe *et al.* (1978abc, 1979), Watanabe (1979) and Fujita *et al.* (1980) compared *Artemia* nauplii (freshly hatched, starved or fed with enriched diet up to 3 days long) from Canada, San Francisco Bay (California, USA), South America and Tientsin (People's Republic of China) as a food source for red sea bream larvae *Pagrus major*. From their chemical analyses and feeding tests with the red sea bream it appears that high fish mortalities are induced by low levels of the essential fatty acids (EFA) 20:5ω3 and 22:6ω3 in the *Artemia* nauplii. Canadian, Chinese, and San Francisco Bay *Artemia* (two batches of the latter) contained high amounts of EFA, whereas nauplii from South America and from four other San Francisco Bay batches were deficient in EFA.
- High mortalities have been observed for various cultured species fed Great Salt Lake (GSL, Utah, USA) nauplii as sole food source[3] :

[2] Buenos Aires (Argentina), Shark Bay (Australia), Macau-area (Brazil), Chaplin Lake (Canada), Lavalduc (France), Gujarat-area (India), Tientsin (People's Republic of China), Barotac Nuevo (Philippines), Cadiz-area (Spain), San Francisco Bay (California, USA) and Great Salt Lake (Utah, USA).

[3] Here we do not consider the poor performance results with GSL-nauplii that are related to the large size of the nauplius and the resulting inability of the predator to ingest the prey (Smith, 1976).

. after 3 weeks of feeding on this diet, sole and plaice larvae refused to further ingest these nauplii, did not undergo metamorphosis and died (Shelbourne, 1968) ; GSL-nauplii were reported by Slobodkin (1968, in Kinne, 1977) to be toxic to plaice larvae ;
. Little (1969), Reed (1969), and Reeve (1969) observed high mortalities in their decapod cultures fed with GSL-*Artemia* ;
. *Palaemon serratus* larvae fed on GSL nauplii died upon metamorphosis (Forster and Wickins, 1967 ; Wickins, 1972) ;
. Bookhout and Costlow (1970) fed Great Salt Lake-nauplii to the larvae of four crab species and reported high mortalities and abnormal developments in the megalopa and first crab stage. Similar observations were published by Roberts (1971, 1974) ;
. the total length of the period of larval development in *Palaemonetes pugio* is unaffected by the geographical origin of the brine shrimp diet ; much higher mortalities are, however, noted with Great Salt Lake nauplii as compared to San Francisco Bay-*Artemia* (Provenzano and Goy, 1976) ;
- Matsuoka (1975, in Murai and Andrews, 1978) reported that Chinese *Artemia* nauplii are toxic for the larvae of *Macrobrachium rosenbergii*. Pesticide analyses revealed 5 times and 10 times higher concentrations of DDT, respectively chlorinated hydrocarbons in Chinese *Artemia* compared to SFB-*Artemia*.

Various theories have been advanced to explain the poor performances of the Great Salt Lake *Artemia*-diet :
- residual pesticides from the surrounding agricultural lands are drained into the Great Salt Lake and accumulate in the GSL-*Artemia* (Slobodkin, 1968 in Kinne, 1977) ;
- through the past centuries GSL-*Artemia* might have developed immunity for a toxic alkaloid secreted by algal blooms in the Great Salt Lake and concentrated in the *Artemia* cysts (Shelbourne, in Provasoli, 1969) ;
- the Great Salt Lake being of athalassohaline origin, Oppenheimer (in Provasoli, 1969) considered mineral deficiency as the possible source of problems.

The few analytical data that have been published with regard to the chemical composition of GSL-*Artemia* are very confusing :
- Bookhout and Costlow (1970) detected 3 times more DDT in GSL-*Artemia* than in cysts from the San Francisco Bay ;
- pesticides, heavy metals, carotenoids, sterols, and fatty acids were analyzed in both SFB and GSL-*Artemia* by Wickins (1972), "... some differences were found but none of them could be confidently labeled as the cause of the poor food value of the Utah *Artemia* nauplii" ;
- Helfrich and Shigueno (in Helfrich, 1973) found high levels of DDT in both SFB and GSL-nauplii ;
- the observation of Wickins (1972) that GSL-nauplii, when fed during 2 days with live algae, become a good food for *Palaemon*-larvae, incited Claus *et al.* (1979) to perform a detailed biochemical analysis of fed and unfed *Artemia* larvae from Great Salt Lake and San Francisco Bay origin. Their results, however, were not conclusive in explaining earlier reports of poor performances of freshly hatched GSL-brine shrimp as a food source in aquaculture hatcheries.

Aside from the specific knowledge with regard to red sea bream culturing in Japan (see above), it is presently impossible to define the chemical and/or nutritional parameters which determine the biological effectiveness of a specific batch or strain of *Artemia* as a good diet for particular cultured species. The analytical methods varied from one study to another, the cyst batches used were never the same, the culturing tests were performed with fish and crustacean larvae which probably show interspecific differences in nutritional requirements and/or sensitiveness.

As a result there is great need for a thorough characterization study of *Artemia* strains which should be undertaken on an interdisciplinary level. Guidelines for the selection of *Artemia* strains for specific uses in the aquaculture hatcheries are urgently needed since at present the choices of new sources of brine shrimp with regard to their potential suitability for the cultured species are arbitrary and as such not without risks.

Such an interdisciplinary research program was initiated in 1978 through the Artemia Reference Center at the State University of Ghent in Belgium under the title of "International Study on *Artemia*" (ISA) based on the collaboration of five laboratories from different countries. The participants in this study and their specific research tasks are the following :

- Artemia Reference Center, State University of Ghent, Belgium (Coordinator : P. Sorgeloos).
  Biometrical analyses ; hatching, growth, and reproductive characteristics in function of different temperature-salinity combinations ; hybrid tests ; preparation and standardization of research material for the participating laboratories ;
- Department of Food Science and Technology, Nutrition and Dietetics, University of Rhode Island, USA (Coordinator : K. L. Simpson).
  Chemical and biochemical analyses of cysts, nauplii and adults : amino acids, fatty acids, lipids, carotenoids, chlorinated hydrocarbons and heavy metals ;
- Environmental Research Laboratory, Environmental Protection Agency at Narragansett, Rhode Island, USA (Coordinator : A. D. Beck).
  Biological effectiveness of brine shrimp for the fishes *Menidia menidia* and *Pseudopleuronectes americanus*, and the crustaceans *Menippe mercenaria*, *Mysidopsis bahia*, and *Rhithropanopeus harrisii* ; naupliar swimming behavior ;
- Center of Mariculture Research, Port Aransas Marine Laboratory of the University of Texas Marine Science Institute, USA (Coordinator : O. A. Roels).
  Biological effectiveness of brine shrimp for the fish *Cynoscion nebulosus* and the crustacean *Penaeus vanamei* ;
- St. Croix Marine Station, University of Texas Marine Science Institute, US Virgin Islands (Coordinator : O. A. Roels).
  Production performances of *Artemia* in the local Artificial Upwelling Mariculture System ; production of nauplii, cysts and/or adults as testmaterial for the other participating laboratories ;
- Department of Genetics, University College of Swansea, UK (Coordinator : J. A. Beardmore).
  Genotype characterization : inheritance of specific quantitative characteristics ; temperature and salinity adaptation studies.

Five strains were selected for an initial characterization study :

- Great Salt Lake (Utah, USA) harvest 1977 made available by "Sander's Brine Shrimp Cy" ;
- Macau (Rio Grande do Norte, Brazil) harvest 1978 made available by "Companhia Industrial do Rio Grande do Norte" (CIRNE) ;
- Margherita di Savoia (Italy) harvest 1977 made available by P. Trotta ("Laboratorio per lo Sfruttamento Biologico delle Lagune", Lessina, Italy) ;
- San Francisco Bay (California, USA) batch Living World 1628 purchased from "San Francisco Bay Brand Cy" ;
- Shark Bay (W. Australia) batch 114 made available by "World Ocean Pty".

These strains were selected on the basis of the following criteria : availability ; their use in aquaculture hatcheries (all except Margherita di Savoia) ; same genotype but produced in different habitats [the Macau salt ponds were inoculated with San Francisco Bay *Artemia* in 1977 (Sorgeloos *et al.*, 1979)] ; geographical isolation free from contamination by urban, industrial and/or agricultural waste waters (Macau and Shark Bay) and genetic differences (Margherita di Savoia and Shark Bay are parthenogenetic strains, the others zygogenetic).

During the course of this study we have been informed that Living World batch 1628 cysts were not harvested from San Francisco Bay saltworks (as stated on the label of the commercial product) but from San Pablo Bay salt ponds in the Nappa Valley, north of San Francisco (A. Schmidt, personal communication). Although these two *Artemia* habitats are situated within a few hundred kilometers from each other, the San Pablo Bay drains much more agricultural run-off waters to the ocean than does the San Francisco Bay. In expectation of further data on the exact origin of San Francisco Bay *Artemia sensu lato*, San Pablo Bay *Artemia* ("Living World, San Francisco Bay Brand Cy" batch 1628) are considered as distinct from San Francisco Bay *Artemia* (cysts sold under the label "San Francisco Bay Brand Cy").

The results of the detailed characterization study of the five selected strains of *Artemia* are reported by Johns and Walton (1979), Beck *et al.* (1980), Johns *et al.* (1980), Klein-MacPhee *et al.* (1980), Olney *et al.* (1980), Schauer *et al.* (1980) and Seidel *et al.* (1980). A wider range of strains was studied for their genetic similarities (Abreu-Grobois and Beardmore, 1980), their biometrical characteristics (Vanhaecke and Sorgeloos, 1980a), their growth and production performances on live algae in a batch culturing system (Vanhaecke and Sorgeloos, 1980b) and in a flow-through system (Tobias *et al.*, 1980), their carotenoid composition and metabolism (Soejima *et al.*, 1980), and their naupliar locomotory rates, patterns and photoresponses (Miller *et al.*, 1979).

From this ISA-study it appears that for most parameters studied, considerable variability exists between *Artemia* strains. These initial data already provide pertinent information for the selection and practical use of brine shrimp nauplii in aquaculture : *e.g.* the difference in nutritional value of particular strains for specific predators, the size, biochemical composition, and energetic content of the freshly hatched nauplii, etc. It is clear that this comparative ISA-program should be further extended not only to more cyst samples but also to more test-organisms, in order to further unravel the parameters that define the "suitability" of *Artemia* nauplii as food source in aquaculture hatcheries. In this regard the following *Artemia* strains have been selected for the 1979-1980 ISA-program : *Artemia* Reference Cysts (see Report Workshop I "Characterization of *Artemia* strains for application in aquaculture", this symposium), Chaplin Lake (Canada), Lavalduc (France) and Tientsin (People's Republic of

China). Very valuable research material will furthermore result from the production of *Artemia* cysts in standardized culturing tests with feeding of the brine shrimp with formulated diets containing various amounts of EFA and pesticides.

Through application of the latest developments in quantitative genetics, the ISA-program aims, on a long term basis, to develop new strains of brine shrimp for the benefit of aquaculture ; *e.g.* availability of minute *Artemia* nauplii, smaller than 150 μm in length, could eliminate the need for expensive and cumbersome rotifer production, necessary to culture fishes with very small larvae (milkfish, mullet, turbot, *etc.*) and crustaceans such as shrimp and crab.

The use of adult *Artemia* as food source

Although for technical reasons the use of *Artemia* is limited in most cases to freshly hatched nauplii, adult brine shrimp definitely deserve more attention for many reasons :

- adult *Artemia* are 20 times larger and weigh 500 times more than freshly hatched nauplii (Reeve, 1963) ; their nutritional value changes considerably during growth : the fat content decreases from $\pm 20\%$ to less than 10 % of the dry weight and the protein content increases from $\pm 42\%$ to over 60% (Von Hentig, 1971 ; Helfrich, 1973) ; whereas nauplii are deficient in histidine, methionine, phenylalanine, and threonine, adult brine shrimp are rich in all essential amino acids (Stults, 1974 ; Gallagher and Brown, 1975 ; Watanabe *et al.*, 1978a ; Claus *et al.*, 1979) ;
- *Artemia* is a euryhaline and eurythermal crustacean and a non-selective particle filter-feeder ; contrary to many other crustaceans its food requirements do not change during growth ; it has a high food conversion efficiency and can be cultured in very high densities (Helfrich, 1973 ; Sorgeloos and Persoone, 1975) ;
- *Artemia* has a short generation time (minimum of about 2 weeks), a high fecundity rate (up to over 100 offsprings every 4 days) and a long lifespan (up to more than 6 months) (Nimura, 1967 ; Ivleva, 1969) ;
- the exoskeleton of the adult is only 1 μm thick which allows consumption of the whole animal without previous processing ; for many aquaculture organisms pre-adult or adult *Artemia* are known to be a much better reference diet than formulated feeds : *e.g.* for *Homarus americanus* (Hughes *et al.*, 1974 ; Gallagher *et al.*, 1976), *Macrobrachium rosenbergii* (Aquacop, 1977), *Penaeus kerathurus* (San Feliu *et al.*, 1976), *Penaeus aztecus* (Venkataramiah *et al.*, 1975), *Callinectes sapidus* (Milliken *et al.*, 1980), *Solea solea* and *Scophthalmus maximus* (Aronovich and Spektorova, 1971), *Sparus auratus* (Alessio, 1974) and *Dicentrarchus labrax* (Barahona-Fernandes and Girin, 1976).

In view of its high price (wholesale price up to US $ 20.00 per kilogram fresh weight), live as well as frozen *Artemia* adults are presently used as a luxury food source in the pet market and, to some extent, for research work in lobster and prawn farming (Anonymous, 1978). Although natural brine shrimp populations are still the most important source of commercially available *Artemia*, they are only exploited in a few areas in Canada, France, and the USA with a total yearly output of approximately 1 000 metric tons. The future output from nature where *Artemia* has to date been recorded from more than 150 habitats (Persoone

and Sorgeloos, 1980) will probably increase considerably. However, new exploitations should be carefully planned, taking into account maximum sustainable yields (in order not to affect cyst production) and the potential local role of *Artemia* as energy source for migrating and breeding waterbirds (Rooth, 1965 ; Herbst and Dana, 1980). New suitable areas for the production of substantial tonnages of *Artemia* biomass (and eventually cysts) can furthermore be considered, without any serious risks for ecological disturbances, by converting thousands of hectares of hypersaline lagoons and abandoned saltponds, which can be found allover the world (Serene and Webber, in Hempel, 1970 ; Rabanal and Beuschel, 1978), into *Artemia*-biotopes ; this implies of course well-defined inoculation and production projects.

Lately, another interesting source of *Artemia* production has come into perspective. Tertiary treatment plants for industrial effluents of high salinity are capable of producing substantial amounts of adult *Artemia*. Attention shall, however, be paid to the eventual bio-accumulation of toxic substances (Milligan *et al.*, 1980).

The present output of brine shrimp from controlled intensive culturing systems is still limited. However, in view of the very important progresses made in this field (see hereunder) the interest in this type of *Artemia* production is increasing considerably. When it comes to a choice, cultured *Artemia* are always to be preferred over brine shrimp harvested from nature. The latter animals indeed often carry parasites or suffer from bacterial and fungal diseases (Persoone and Sorgeloos, 1980) ; furthermore they have mostly been starved for days before being shipped to their final destination.

Since it has been shown that "... progressively larger *Artemia* ... were required by the fish as they grew themselves" (Purdom and Preston, 1977) an adequate and simple technology for cheap production, in the aquaculture hatcheries, of brine shrimp larvae of intermediate size will receive more and more attention (Barahona-Fernandes *et al.*, 1977).

Progress in controlled intensive *Artemia* culturing

Most of the techniques which have been described in the past for high-density culturing of *Artemia* in batch systems have only limited application. This is due to either the complexity of the technique and/or the limited availability or the high price of the food used (reviews by Bossuyt and Sorgeloos, 1980 ; Dobbeleir *et al.*, 1980).

A major innovation in the technology of *Artemia* batch-culturing with potential for world-wide application is the air-water-lift raceway, originally developed for the intensive culture of post-larval penaeid shrimp (Mock *et al.*, 1973) but modified for brine shrimp culturing at the Artemia Reference Center (Sorgeloos *et al.*, 1977). Details on design and construction as well as the description of simple food distribution systems are given by Bossuyt and Sorgeloos (1980). Food dosing in this raceway method is based on readings of water turbidity, which allow automatization of the food distribution by use of turbidimetric devices (Versichele *et al.*, 1979). A cheap and worldwide available food source was found in rice bran (Sorgeloos, 1978 ; Sorgeloos *et al.*, 1980). It now appears that many other types of agricultural waste products, such as whey powder can also be used successfully as a monodiet to culture brine shrimp (Dobbeleir *et al.*, 1980).

Presently 10 g of cysts can be converted into 2 kg pre-adult *Artemia* within 2 weeks in a raceway of 1 m^3. The protein content and amino acid composition of *Artemia* fed ricebran do

not differ significantly from those of brine shrimp harvested from nature (Sorgeloos *et al.*, 1980). However, in view of the differences in fatty acid composition, further studies are needed to evaluate the nutritional value of brine shrimp raised on waste products for various cultured organisms and, if necessary, to consider diet formulations and/or alterations (Dobbeleir *et al.*, 1980).

Whereas batch culturing in air-water-lift raceways has a potential worldwide application in aquaculture hatcheries to produce brine shrimp of various sizes, a much more intensified mass production can be achieved in flow-through systems. This is, however, only possible in a very restricted number of situations. In a joint collaboration effort, the St. Croix Marine Station of the University of Texas Marine Science Institute and the Artemia Reference Center developed a technique for flow-through culturing of brine shrimp in very high densities (Tobias *et al.*, 1979). The keys to success with this particular technology are the circular screen cylinder (which must be changed regularly) and the cleaning effect of an aeration collar fixed at the bottom of the filter cylinder to keep the screen free from clogging.

The flow-through tests carried out in St. Croix were run with the effluent of the two algal ponds of the local Artificial Upwelling Project (Roels *et al.*, 1976). By extrapolation from repeated 100 l trials (Tobias, personal communication) it has been calculated that 30 g of cysts can be converted in a 1 m^3 tank into 25 kg adult biomass within 2 weeks ! The maximal productivity potential of flow-through culturing has not yet been reached in St. Croix. Water temperatures during the period of the experiments were rather low (22-25 °C), and as a consequence of the limited cell densities in the algal cultures (5.10^4 *Chaetoceros curvisetus* cells/ml) the maximum *Artemia* density had to be restricted to 12 000 ind./l. Laboratory tests indicate, however, that densities in flow-through cultures may reach 40 000 *Artemia*/l (Nimura, 1967).

The successes obtained so far in replacing live algae by inert diets such as whey powder or rice bran, will now be studied further. At the same time endeavors will be made to further automize the flow-through culturing technique.

ISA-studies on the production potential of various *Artemia* races [*e.g.* differences in growth rate (Tobias *et al.*, 1980 ; Vanhaecke and Sorgeloos, 1980b) ; temperature optimum ; food conversion efficiency ; protein content ; *etc.*] indicate that it will become possible to select strains with improved characteristics for intensive culturing.

As pointed out above, the potential sites where *Artemia* flow-through production is possible are much more limited than for batch culturing in closed raceways, especially with regard to the need for large volumes of seawater at a temperature within the range of 20 to 30 °C. As potential sources of alternative energy, Ocean Thermal Energy Conversion systems (OTEC) as well as geothermal projects are gaining more and more interest (Anonymous, 1979 ; Bardach, 1979). In an OTEC-plant *Artemia* could be grown in the effluent on an inert diet or on phytoplankton cultures produced in an artifical upwelling system of the St. Croix-type (Roels *et al.*, 1976) connected to the same OTEC-plant. Since the salinities of geothermal water range from brackish up to 100 ‰ brine, *Artemia* is the only invertebrate that can be cultured in such a wide salinity range. For the same reason flow-through culturing of brine shrimp can be considered in the warm brine effluent of desalination plants.

Semi-industrial production of *Artemia* should now be started in pilot-plants in order to assess the economical feasibility of a potential annual output of thousands of tons of animal protein from these new bio-industries.

Potential use of *Artemia* as protein source

From the foregoing it appears that the yearly production of brine shrimp may increase substantially during the next decade. Besides an improved perspective for the use of *Artemia* in the aquaculture hatcheries, it becomes obvious that other applications show very high potential even including direct use in human nutrition. Although the acceptability of brine shrimp as food for man might seem to be speculative or restricted to a few areas in the world, it is certainly worthwhile to be considered, not the least for third world countries. From an energetic point of view brine shrimp production is a much more efficient way to produce animal protein than to culture carnivorous fish and crustaceans with *Artemia* and fish meal as diet ingredients !

Direct consumption of brine shrimp by man has been and is still practised by primitive tribes in America respectively in Africa : "... Indians inhabiting this region used to collect large quantities of this crustacean which they dried and used as food" (Jensen, 1918). The Dawada-people in Libya consume dried *Artemia* flakes as "... a superb source of protein rich in β-carotene and riboflavin" (Ghannudi and Tufail, 1978) and market these "... pains d'*Artemia*" (*Artemia* bread) as a nutritious delicacy over a wide area (Oudney, 1828, in Bovill, 1968 ; Delga *et al.*, 1960 ; Monod, 1969). Taste panel tests on *Artemia* conducted in Hawaii should be further extended since "... the response to an experimental shrimp tempura prepared from frozen brine shrimp was quite favorable" (Davidson, 1974 ; Helfrich, 1973).

If not consumed directly as human food, *Artemia* meal can be used as a rich source of animal protein in livestock diets (Anonymous, 1978). In this regard dried brine shrimp may be used as a valuable alternative to fish meal, especially in those countries that are entirely dependent on fish meal import.

Concluding remarks

In conclusion it may be said that *Artemia* should no longer be considered as a luxury food in aquaculture but rather as a cheap and high quality source of animal protein. Now more than ever, many aspects dealing with the use of *Artemia* in aquaculture need to be studied further in order to reach this goal. A first promising step in this direction is the Aquaculture Planning Program for Hawaii (Anonymous, 1978) in which brine shrimp has been considered as a first priority species.

In his mathematical evaluation of the overall value and market potential of 24 of the most important aquaculture candidate species or groups of species, Nash (1974, in Kinne and Rosenthal, 1977) ranks brine shrimp second after salmon (Table I). The recent finding of cheap inert diets for brine shrimp lifts this species to the top place in rank of importance in the field of aquaculture.

TABLE I

Overall state of the art and market potential
of 24 of the most important aquaculture candidate species or groups of species
[1 : no ; 5 : yes ; 2 to 4 : in-between scorings (after Nash, 1974, in Kinne and Rosenthal, 1977)]

Parameter	Score																							
Controlled spawning possible	5	5	5	4	4	2	1	3	1	**5**	5	4	4	1	1	1	2	4	1	3	1	2	4	1
Simple larval development achieved	5	5	5	5	5	4	5	5	2	**5**	5	5	2	1	1	1	3	5	3	3	5	3	4	1
Mass-produced in hatchery	5	5	5	4	3	1	4	4	1	**5**	5	5	1	1	1	1	1	1	1	2	1	1	4	1
Fast growth rate potential	5	5	4	4	4	3	4	4	4	**5**	5	4	4	4	5	5	5	4	4	5	5	4	3	3
Satisfactory feeds known	5	4	4	3	3	1	3	3	3	**5**	5	3	3	1	2	1	5	3	3	5	5	3	3	2
Commercial feeds available	1	1	1	1	1	1	2	1	1	**1**	5	1	1	1	1	1	3	1	1	2	1	1	1	1
High conversion efficiency	2	2	2	2	2	3	3	3	3	**4**	5	4	3	2	4	4	4	4	3	3	5	3	2	1
Hardy in captivity	5	5	3	3	3	5	3	3	3	**5**	5	5	5	3	3	3	5	2	3	5	5	2	3	3
High disease resistance	4	4	4	4	4	4	3	4	3	**5**	4	4	4	3	3	3	4	2	3	4	4	3	2	4
High density potential	5	5	5	5	4	5	3	3	5	**5**	4	4	4	3	4	4	4	5	5	5	4	3	3	5
Farming systems developed	5	5	3	3	3	1	4	2	1	**5**	4	4	4	1	1	3	5	1	1	5	5	1	3	4
High price range	5	2	4	4	4	1	4	5	1	**5**	5	3	2	1	4	4	5	2	2	5	1	4	5	3
High market potential U.S.	5	1	5	5	5	2	5	5	1	**5**	5	3	1	1	5	5	4	3	3	2	1	3	5	5
High market potential foreign	5	5	5	5	5	3	5	5	3	**5**	5	4	4	4	5	4	5	4	4	5	5	5	5	3
Matrix total	62	54	55	52	51	36	49	50	32	**65**	67	53	42	27	40	40	55	40	37	56	48	38	47	37
	Oysters	Mussels	Clams	Scallops	Abalone	Crabs	Shrimps	Lobster	Krill	*Artemia*	Salmon	Flatfish	Mullet	Rabbitfish	Dolphinfish	Pompano	Yellow-tail	Anchovy	Herrings	Eels	Milkfish	Octopus	Turtles	Bloodworm

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Experiences with *Artemia* at solar saltworks

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Abstract

A biological system important to salt production dwells in the ponding system of a solar saltworks. *Artemia* is essential to the proper operation of the biological system. Reviewed are three experiences at solar saltworks involving the resident biological system and *Artemia*.

Introduction

A solar saltworks is a series of interconnected ponds in which seawater (or salt water from another source) evaporates to produce sodium chloride. Seawater enters the first pond of the saltworks where after increasing slightly in salinity by solar evaporation, flows to the next pond of the series. This continues until the water becomes saturated with sodium chloride : the brine is then pumped into "crystallizer" ponds where sodium chloride precipitates. Details on salt manufacture by the solar process are reviewed by Tressler and Lemon (1951), and See (1960).

In addition to the physical processes just mentioned, biological events of considerable importance to salt production occur in a solar saltworks. These biological phenomena are derived from the resident biological system. This report will review in general terms certain aspects of the biological system and it will relate some of the author's experiences associated with *Artemia* during a 12 year worldwide consultancy period at solar saltworks.

For convenience, a solar saltworks can be divided into ponds of low, intermediate and high salinities. Each set of ponds has a unique biota of plants, animals and bacteria organized into a planktonic community and a bottom community. The ponds of low salinity (3.5 to 9 % salts) possess numerous species of algae, protozoa, bacteria, molluscs, vascular plants and fish. Biological primary productivity is high, but dissolved organic matter is low. Part of the planktonic and bottom community flows downstream. Ponds of intermediate salinity (8-18 % salts) have few species, low biological primary productivity and relatively high amounts of dissolved organic material in the water. The chief planktonic organisms are the blue-green algae and brine shrimp. Ponds of high salinity (19-29 % salts) have but five or six species, the living organisms in these ponds consume more organic materials than they produce, and dissolved organic matter is very high. The chief planktonic organisms are the red halophilic bacteria of the genus *Halobacterium*. The latter is present in concentrations sufficient to color

the water bright red. By coloring the water, the planktonic component of each pond serves to increase solar absorption and thus aid evaporation. Existing as mats and deposits of bacteria and algae, the bottom community helps prevent leakage of water from the ponds. Davis (1979) presents further details on the biota of solar saltworks.

The communities of all ponds constitute a biological system that can be managed to aid salt production and to prevent biological catastrophies (Davis, 1979). *Artemia* plays an important part in the biological system and in the management of the system.

Role of *Artemia* in the brine biological system

Brine shrimp can be found in ponds with salinities ranging from 8 to 26 % salts, but it is in the ponds of intermediate salinities that the animals grow, reproduce and occur in the highest concentrations. *Artemia's* lower salinity occurrence coincides with salt concentrations just excluding fish. Ponds of intermediate salinity receive from the upstream ponds planktonic organisms, organic debris, fecal pellet fragments and carbonate crystals. Funnelled from the ponds of highest biological productivity, this heavy particulate load is largely ingested by *Artemia*. In its locomotion, reproduction and metabolism, *Artemia* consumes (oxidizes) significant portions of the ingested organic material. Undigested organic matter, metabolic wastes and inorganic substances are excreted in membrane-bound fecal pellets that sink to the bottom of the pond. On the pond bottom, the fecal pellet's organic and inorganic materials promote the production of mats. The brine-cleaning activities of the the brine shrimp enable light to penetrate to the pond bottom to maintain the resident algae-bacteria mat. Baas-Becking (1931) cited saltworks officials claiming the activities of the "clearer worm" essential to salt production.

In the intermediate salinity range, the unicellular blue-green algae, *Coccochloris elabens* grows best (Brock, 1976), and may predominate the biota. In the ponds of intermediate salinity, a contest of considerable importance to salt manufacture exists between *Coccochloris* and *Artemia*. When conditions allow *Coccochloris* to dominate the plankton, nearly all other organisms of the planktonic and bottom communities are suppressed. *Artemia* cannot survive a diet solely of *Coccochloris* (Gibor, 1956; Provasoli *et al.*, 1959). If not corrected, *Coccochloris* can become a monoculture in the ponds, reaching hundreds of millions of cells per ml, excluding the other organisms, creating nearly anerobic conditions at night, and producing a viscous mucilage. The latter thickens and layers the brine, reduces evaporation, and deleteriously effects physical and biological processes in the downstream ponds. When conditions permit brine shrimp to thrive, much planktonic *Coccochloris* is ingested by the animals and deposited in fecal pellets on the bottom. A healthy *Artemia* population can effectively control high concentrations of *Coccochloris*.

Brine shrimp eventually flow into the ponds of high salinity where they die and disappear from view. Organic matter derived from *Artemia* combined with that from the upstream ponds results in high concentrations of organic carbon in the highly saline ponds. At a number of solar saltworks the author has related abnormally high concentrations of organic matter, particularly excessive *Coccochloris* mucilage, to decreased sodium chloride crystal size and to colored inclusions in the crystals.

Halobacterium, the predominant organism in the high salinity ponds, requires a wide range of amino acids for its nutrition (Holt, 1977); carbohydrates are poorly utilized. Dissolved

Artemia from upstream ponds must supply an important part of the amino acid requirement for the bacteria. With adequate amino acids, part of the carbohydrate-rich mucilage of *Coccochloris* (Jones and Yopp, 1979) might also be utilized by *Halobacterium*. A not uncommon observation at solar saltworks relates bright red colors in high salinity ponds to high concentrations of *Artemia* during the previous year. Thus, high concentrations of *Halobacterium* aid evaporation, and they help to prevent organic material from reaching abnormally high values. Food utilization by *Halobacterium* is accelerated by light and oxygen (Odum *et al.*, 1977). If large, clear salt crystals of design capacity are desired by the management officials of a saltworks, *Artemia* must win the contest in the ponds of intermediate salinity.

Reviewed below are three experiences with the biological system in solar saltworks in which *Artemia* had an important role.

A *Coccochloris*-dominated system

A solar saltworks fed with underground brine supplemented by ocean water to give a total salt concentration of 9% at the intake, had changed during a few years from a productive installation with a high quality product to one with a reduced output of low quality salt (very small crystals containing colored organic matter).

From conservations with saltworks officials, it seemed clear that during the first years of operation, the ponds had contained a variety of organisms, including a brine shrimp population. When I arrived, every pond of the saltworks was colored deep green, and dominated with *Coccochloris*, at concentrations of several hundred million organisms per ml. The cells of the *Coccochloris* monoculture had secreted sufficient mucilage to make the brine quite viscous. In ponds whose salt concentrations were 9-18%, the *Coccochloris* cells were bright green, thriving and reproducing. Above the 18% concentration, the cells became light green, and the water was black with decomposing mucilage. The mucilage prevented adequate brine circulation, for the uppermost 3-5 cm of brine was uncomfortably hot, and below that, temperatures felt quite cool. In the crystallizer ponds, the brine was gray to dull red, containing several thousand bright green algal cells per ml of the genus *Dunaliella*. *Halobacterium* was present, but at concentrations less than 1000 cells/ml. In one of the crystallizer ponds where machinery was harvesting the salt, some 12 to 15 layers of black mucilage occurred throughout the 10 cm deposit of salt. The individual salt crystals were all similar in size to table salt; microscopic examination revealed brown or black inclusions in each crystal. When washed by a rather vigorous process, and allowed to "bleach" in the sun, the harvested salt retained the inclusions and remained colored.

This saltworks was restored by allowing the supplementary seawater to pass through two new ponds and evaporate to produce a salt concentration of near 9% before it joined the pond receiving underground brine.

When examined after 10 months, the new ponds contained a biota and communities typical of ponds of low salinity. The pond receiving the combined output of the second new pond and the underground brines contained nearly clear brine in which thrived a large *Artemia* population. The microscopic plankton therein was a diverse assemblage of species of algae, protozoa and bacteria. The bottom community was a thin mat of blue-green algae, bacteria and *Artemia* fecal pellets. The other ponds of intermediate salinity were biologically

similar to the pond just mentioned. *Coccochloris* was present in the plankton and on the bottom, but this alga did not dominate either community.

Downstream from the ponds of intermediate salinity, the black mucilage had disappeared, and a biota typical of high salinity ponds was present. Crystallizer ponds contained salt crystals free of colored inclusions, and crystal size had increased. The *Halobacterium* population had increased dramatically from its former size.

Noteworthy to contrast with the saltworks just described was a nearby similar installation whose intake water was also from underground brines supplemented with seawater. However, the combined concentration of salts entering the first pond was about 6 %. The first pond of the system had a diverse biota that nourished a large *Artemia* population in the intermediate salinity ponds. *Coccochloris* was nearly absent from the plankton. Ponds of high salinity possessed a biota typical of such ponds, and crystallizer ponds produced clear salt crystals of adequate size.

Use of fertilizers to establish a biological system

Fertilizers have been used to establish or improve the biological system at several solar saltworks. The first use of fertilizers was in a saltworks whose ponds initially were not able to retain water. Most areas of the pond bottoms were without mats or deposits and the water in all ponds was clear. Fertilizers were first applied to the downstream-most ponds (later the ponds of high salinity). When evidence revealed the ponds had developed a bottom community sufficient to seal against leakage, fertilization was decreased and the process was repeated in the middle series of ponds until these became sealed. In this way the entire system of ponds became sealed after three to four years. In addition to promoting the development of bottom mats, fertilization also encouraged the production of a planktonic community in each pond. When salinities stabilized in the ponds of the saltworks, an *Artemia* population appeared in the ponds of intermediate salinity. Approximately 2 years later, *Halobacterium* appeared in the ponds of high salinity and crystallizers in sufficient numbers to color the brine red (Davis, 1978).

Toward the end of the fertilization period when only the ponds of low salinity were still receiving fertilizer, certain ponds of intermediate salinity were inadvertently fertilized. An abnormally high *Coccochloris* concentration then occurred, and the *Artemia* population declined. Brine in the downstream ponds and crystallizers thickened with the mucilage produced by *Coccochloris*. During the salt harvest procedure, the mucilage-laden salt retained considerable water that lessened harvest efficiency and damaged the roads as it drained from the vehicles carrying the salt.

The last experience to be mentioned occurred at a saltworks whose intake was seawater, and whose ponds of intermediate salinity were dominated with *Coccochloris*. Brine shrimp were not present in these ponds. Black mucilage from *Coccochloris* covered the bottom of the ponds, and floating black ropy strands of *Coccochloris* mucilage were flowing downstream to the ponds of high salinity. Evidence from several sources indicated the amount of mucilage was increasing in the system. Engineering changes to the ponds in order to create a more favorable habitat for *Artemia* were not possible. A large quantity of *Artemia* from a neighboring saltworks was introduced into the ponds of intermediate salinity. As the *Artemia* population slowly became established throughout the ponds of intermediate salinity, excessive

mucilage production decreased and was no longer a threat to the downstream ponds and to salt production.

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The brine shrimps *Artemia* and *Parartemia* in Australia

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Abstract

Two genera of brine shrimp, *Artemia* and *Parartemia*, occur in Australia. Their biogeography and ecology is reviewed. *Artemia* occurs only in saltworks and seems to have been introduced into Australia on at least two separate occasions : gonochoristic populations in Queensland from North America and parthenogenetic population in Western Australia from Europe or Asia. Several species of *Parartemia* occur in natural salt lakes. Speciation in this genus may be promoted by low dispersal ability. In a South Australian saltworks *Artemia* and *Parartemia* co-occur, *Artemia* occupying only the ponds of highest salinity. Both physicochemical conditions and biological interactions control the distribution of *Artemia*. It seems that the absence of *Artemia* from natural salt lakes in Australia is related to biogeographic, physiological and ecological factors.

Introduction

Two genera of brine shrimp, *Artemia* and *Parartemia*, occur in Australia. *Artemia* is in the monogeneric family Artemiidae, while *Parartemia* belongs to the Branchipodidae and seems morphologically close to *Branchipodopsis* which is distributed in arid regions of Africa and Asia (Linder, 1941). *Parartemia* is endemic to Australia and is ecologically and physiologically similar to *Artemia*. The most studied species, *P. zietziana* Sayce, has powers of osmoregulation similar to *Artemia* (Geddes, 1975abc) and occurs in the field at salinities between 40 and 300 ‰ (Geddes, 1976 ; Marchant and Williams, 1977a). Because of its halobiont character it seems reasonable to consider this species as a "brine shrimp". It is only one of eight described species of *Parartemia* and although little is known of the biology of the other species most seem to have been collected only from highly saline environments. Although there are many similarities between the two genera there are differences too. *Parartemia* differs at least in the following : 1) although *Parartemia* is capable of synthesizing haemoglobin it does not use it for oxygen transport (Maxwell, 1978) ; 2) although it can filter feed (Lea, 1978) it is predominantly a benthic feeder (Marchant and Williams, 1977b) ; 3) whereas *Artemia* populations are morphologically similar throughout the world, *Parartemia* has evolved several morphologically distinct species (Linder, 1941) ; and 4) whereas some *Artemia* populations are parthenogenetic all *Parartemia* populations studied are sexually reproducing.

There have been several studies on the distribution, ecology and physiology of *P. zietziana* but there is little information available on *Artemia* in Australia. Mitchell and Geddes (1977) reported on the distribution of *Artemia* within a saltworks in South Australia and Geddes

(1979) has listed other localities where *Artemia* occurs in Australia. A series of papers on the genetics and reproductive isolation of *Artemia* has provided information on populations from Rockhampton, Queensland, and Rottnest Is. and Port Hedland, Western Australia (Clark and Bowen, 1976 ; Bowen and Sterling, 1978 ; Bowen *et al.*, 1978). This present paper aims to use published and unpublished information to review the biogeography of brine shrimps in Australia and to investigate the co-occurrence of *Artemia* and *Parartemia* in a South Australian saltfield. It then may be possible to reach conclusions about the significance of *Artemia* in Australia and to predict the probable outcome of its further introductions.

Biogeography of brine shrimps in Australia

The distribution of *Artemia* and *Parartemia* in Australia is shown in Fig. 1. Early records of *Artemia* [recorded as *A. proxima* by King (1856), and *A. australis* and *A. westraliensis* by Sayce (1903)] are not included, as Geddes (1979) has suggested that they may represent misidentifications of *Parartemia*.

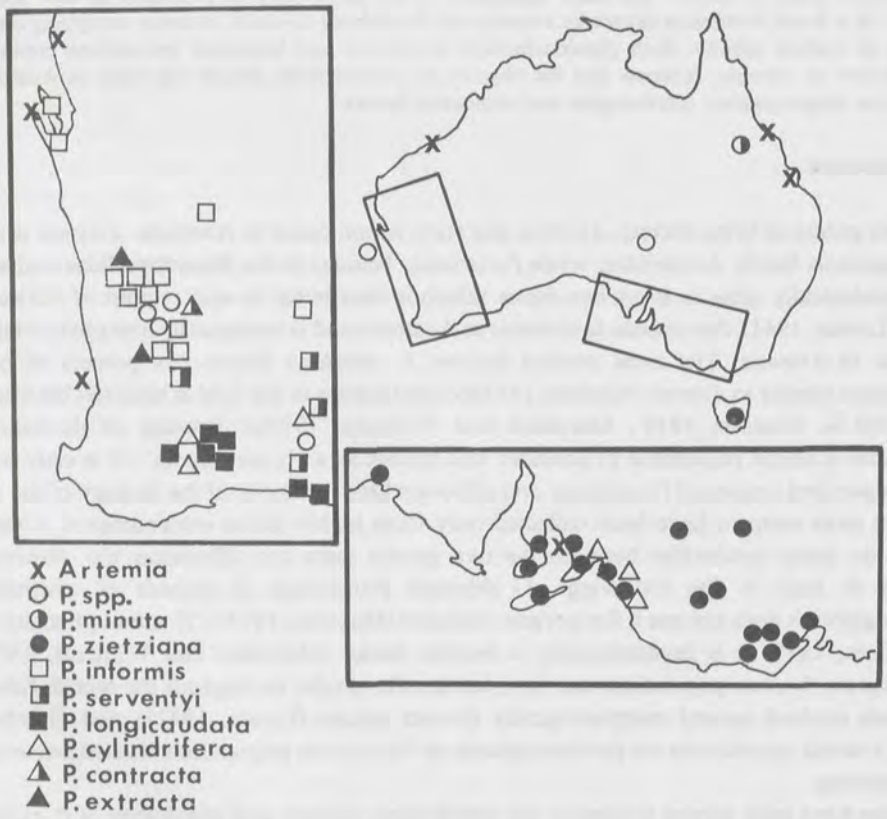

FIG. 1. The distribution of *Artemia* and the various species of *Parartemia* in Australia. For exact location of sites, see papers of Geddes (1973, 1976, 1979).

At present *Artemia* occurs in two localities in Queensland, Bowen and Rockhampton ; it is present in at least four localities in Western Australia, Port Hedland, L. McLeod, Shark Bay and Rottnest Is. ; and it has been found near Adelaide in South Australia. All localities are coastal and all except those on Rottnest Is., are used for salt production ; the Rottnest Is. lakes were used for salt production in the mid 19th and early 20th century. Most localities are man made ponds where seawater is evaporated but the lakes on Rottnest Is. and L. McLeod are natural and separated from the sea. The populations from Queensland are gonochoristic while those from Western Australia and South Australia are parthenogenetic. Clark and Bowen (1976) have shown that the population from Rockhampton is crossfertile with *Artemia* from San Francisco and therefore is *A. franciscana* Kellogg. They suggested that the Rockhampton population was probably introduced as brine shrimp eggs from Great Salt Lake or San Francisco Bay. The same is probably true for the Bowen population.

The parthenogenetic populations from Rottnest Is., and Port Hedland are identical and are genetically similar to parthenogenetic populations in India, France, Russia, and Japan although they do have a slightly different Hb-I relative mobility (Bowen *et al.*, 1978). Shark Bay and L. McLeod lie between Rottnest Is. and Port Hedland and so it is probable that these four populations are monophyletic. The origin of the Western Australian populations is not clear. It is possible that *Artemia* was introduced into the Rottnest Is. system when the saltworks were established last century and that the brine shrimps since have spread or been transplanted along the West Australian coast. It is also possible that this parthenogenetic form of *Artemia* dispersed from Asia to Australia and occupied a few suitable habitats on the west coast. The lakes on Rottnest Is. and L. McLeod may have been suitable and there may have been hypermarine salt lagoons at other localities including Shark Bay and Port Hedland. Some support for the former alternative may be provided by the fact that *Artemia* is not recorded from any natural salt lake in Western Australia or in any other state, and this suggests that the genus has not been in Western Australia for long and that its distribution is related to the activities of man. Recent introduction of eggs of *Artemia* from San Francisco Bay to the saltworks at Shark Bay and from there to the large new salt ponds at Dampier, Western Australia (Dr J. S. Davis, personal communication), represent what will most likely be continuing introductions of *Artemia* into Australian saltworks.

The population near Adelaide in South Australia is parthenogenetic, although a few male specimens have been found. The existence of a very small number of males in an otherwise parthenogenetic population was also reported by Bowen *et al.* (1978). This isolated population is more than 2 000 km from congeners in the east and the west and its origins are obscure. Keunen (1938) found only *P. zietziana* in saltworks in Victoria at Geelong and Altona. The absence of *Artemia* from those localities suggests that the population at Adelaide has been introduced.

The distributions of eight species of *Parartemia* are shown in Fig. 1. All species are gonochoristic and are quite distinct morphologically (Linder, 1941 ; Geddes, 1973). To date no studies on cross fertilization among them have been made. The major taxonomic character of the species of *Parartemia* is the ornamentation of the last thoracic segments of the female, although most species can also be identified from the male clypeus. In most species of the Anostraca the second antennae and frontal appendage of the male are the primary specific characters. However it might be expected that ornamentation of that part of the female where

contact by the male is first made would be as effective an isolating mechanism as would be variation in the male appendages which make that contact.

The species of *Parartemia* are probably a monophyletic group descended from an ancestor which colonized inland saline water. For speciation to proceed, gene flow among populations must have been low and this may have resulted from limited powers of dispersal. Most species of *Parartemia* are of rather local distribution ; *P. minuta* and *P. contracta*, for example are known from only one locality and *P. longicaudata* and *P. extracta* are extremely limited in distribution. The most widespread are *P. cylindrifera* in the south and west, *P. zietziana* in the south east and *P. informis* in the west. One feature of the biology of *Parartemia* that might limit its powers of dispersal is that the resistant eggs of this genus sink after being laid and so become bound in the mud and salt crust of the lakes. This contrasts with the situation in *Artemia* where, except for *A. monica*, the eggs of all other strains known float and are blown to the edge of lakes in windrows. It might be expected that the probability of *Parartemia* eggs being dispersed by wind or birds is low compared to that for *Artemia*.

Co-occurrence of *Artemia* and *Parartemia* in a saltworks in South Australia

The only ecological information on *Artemia* in Australia is on the population at the Dry Creek Saltworks, near Adelaide, South Australia, where *Artemia* and *P. zietziana* co-occur (Mitchell and Geddes, 1977). Observations on the distributions of the two species have been made from 1975 to 1979. In February-March (late summer) each year, except 1977, a survey has been made of the distribution of the two species over 15 ponds which ranged in salinity from 109 to 325 ‰ (Fig. 2). Samples are taken with a plankton net mounted on sleds and the proportions of adult *Artemia* and *Parartemia* were counted. In 1978 a quantitative study of the occurrence of the two species in one pond (pond 6) was made by Lea (1978). A 30 l Schindler plankton trap was used and ten samples are taken on each sampling day and counted separately. The standard error was usually from 10-15 % of the mean.

The distribution of adult brine shrimp at the end of the summers of 1975 to 1979 is shown in Fig. 3. The distribution remained unchanged from 1975 to 1976. In 1978, however, *Artemia* was found in all ponds and *Parartemia* was restricted to ponds 1 to 6. Although salinities of all ponds were not measured in 1978 it is apparent from the salinity of pond 6 (200 ‰ *cf.* 176 and 157 ‰ in 1975 and 1976) that the series was more saline that year. New ponds in the low salinity end of the saltworks had been constructed and they were probably responsible for the new salinity regime. Although salinities in 1979 were similar to those of 1978, the distribution of *Artemia* had become more restricted, covering only ponds 7 to 15, while *Parartemia* was present in ponds 1 to 9 and 11.

The seasonal changes in populations of *Artemia* and *Parartemia* from pond 6 in 1978 are shown in Fig. 4b. Total population and adult population numbers are shown for both species. Seasonal changes in midday water temperature and salinity are shown in Fig. 4a. *Artemia* maintained a large population with all stages represented over the late summer months. In early May the numbers fell drastically and adults disappeared completely. Although *Artemia* eggs continued to hatch throughout the winter, none grew to adulthood. In fact most died soon after hatching. In late summer the small *Parartemia* population consisted mostly of late juveniles and adults. In early May large numbers of nauplii hatched and the population remained large and continued to reproduce throughout the winter. The major change in the

population of *Artemia* and *Parartemia* in early May coincided with a drop in salinity from 207 to 177 ‰ and of midday water-temperature from 21 to 13 °C between April 18 and May 12. The mean water temperature in May was about 12 °C. Throughout the winter, salinity and temperature continued to fall.

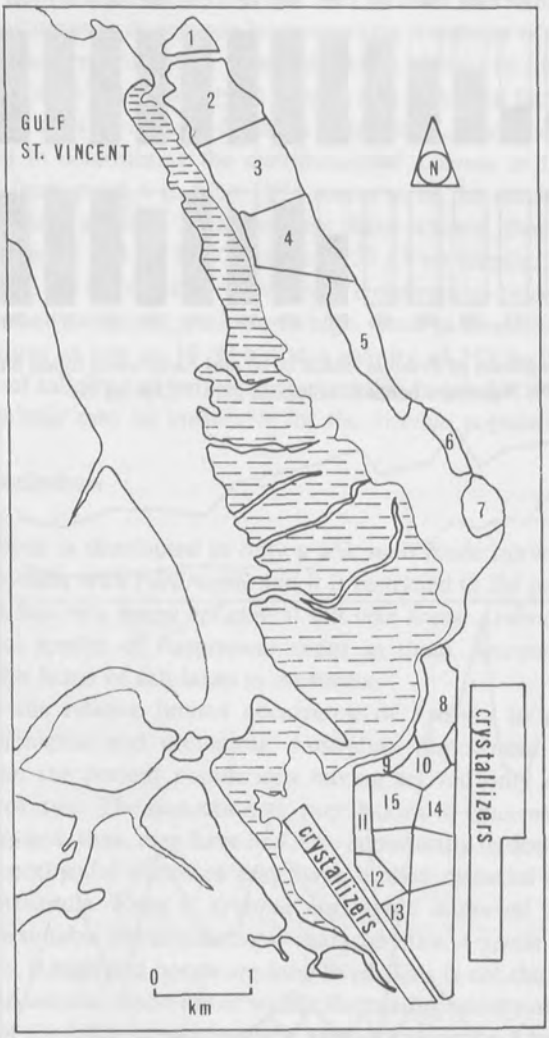

FIG. 2. Part of the Dry Creek Saltworks, South Australia, showing the ponds where brine shrimp occur. Salinity of ponds increase with numbers.

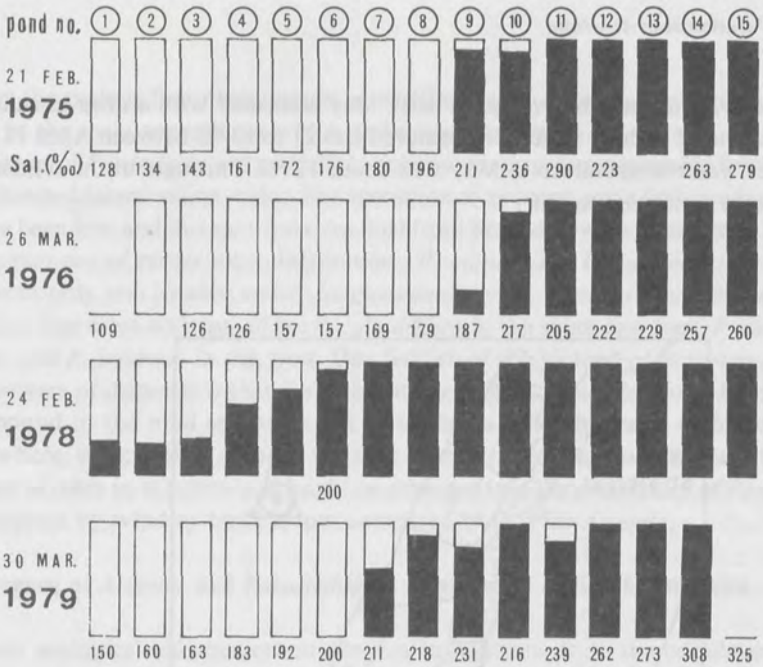

FIG. 3. The relative proportions of *Artemia* (black bars) and *Parartemia* (open bars) in ponds 1 to 15 over the years 1975 to 1979. Numbers beneath columns give T.D.S. in ‰.

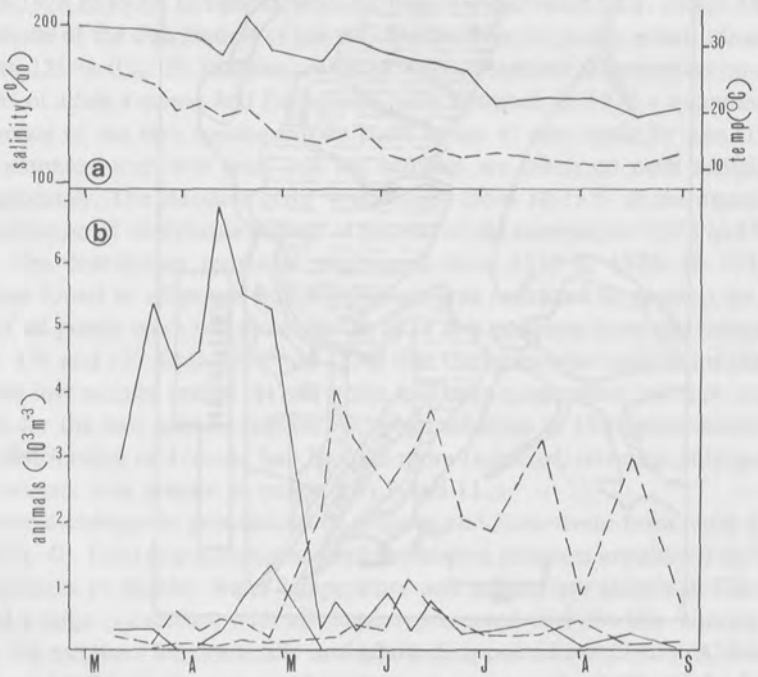

FIG. 4. (a) Fluctuations in salinity (solid line) and temperature (broken line) in pond 6 from March to September 1978. (b) Fluctuations in the total population of *Artemia* and in *Artemia* adults (solid lines) and the total population of *Parartemia* and in *Parartemia* adults (broken lines) in pond 6 from March to September 1978.

Parartemia does not occupy the most saline ponds and possible reasons for its absence are discussed by Mitchell and Geddes (1977). It is interesting that *Artemia* does not occupy the less saline ponds even though they have salinities well in excess of 100 ‰. The lowest record for an *Artemia* habitat is 187 ‰, although in February 1978 pond number 1 would have been approximately 150 ‰. This contrasts with the situation in the Alviso Salt Ponds of California where a large population of *Artemia* was present in waters where midpond salinity varied from 80 to 105 ‰ (Carpelan, 1957). Cole and Brown (1968) reported *Artemia* at salinities form 61 to 258 ‰ with one very low record of 31.3 ‰ from Iran. Although comparison with other *Artemia* populations is dangerous because of the existence of many sibling species, it would appear that the *Artemia* at Dry Creek, Adelaide, should be physiologically capable of occupying the ponds with lower salinity. It way be that biological factors such as food availability and interaction with *Parartemia* limits its distribution. Physicochemical factors also are probably important in determining the distribution of *Artemia* at Dry Creek. The sudden demise of *Artemia* from pond 6 in May 1978 seems to be the result of significant drops in water temperature and salinity. Many studies have shown that *Artemia* does best at temperatures between 20 and 30 °C (Carpelan 1957 ; Von Hentig, 1971 ; Sorgeloos *et al.*, 1976). Although there are records of *Artemia* from temperatures below this, it may be able to persist there only when salinity is particularly high. Thus in Great Salt Lake, Utah, *Artemia* occurs at temperatures as low as 10 °C but at a salinity of 259 ‰ (Stephens and Gillespie, 1976). Interactions of salinity and temperature operating in pond 6 and the other low salinity ponds during the winter may be unsuitable for the *Artemia* population.

Discussion and conclusions

In Australia *Artemia* is distributed in only a few man made saltworks. In at least one of these it shares the system with *Parartemia* but it is restricted to the ponds of highest salinity. Although Australia has very many ephemeral salt lake areas, *Artemia* is not recorded from any. Rather, several species of *Parartemia* occur in them. *Artemia* is, therefore, not an important part of the fauna of salt lakes in Australia.

The reasons for the relative limited occurrence of *Artemia* in Australia may be biogeographical, physiological and ecological. Australia's remoteness has been a barrier to dispersal of *Artemia*, the present populations having arrived only recently, and probably though the agency of man. The gonochoristic populations in Queensland have been present for less than 20 years and, thus, they have had little opportunity to disperse to other areas. The considerable intra-continental distances may have limited dispersal of the parthenogenetic form in Western Australia. Even if *Artemia* eggs were dispersed it might be that many habitats do not have suitable physicochemical characteristics. *Artemia* prefers warm environments where salinity is high and ponds are long lived. This is not the normal pattern for the natural salt lakes of Australia ; most are of widely fluctuating salinity and relatively ephemeral and astatic. Many of the lakes in southeastern and southwestern Australia exist only in the winter months when temperatures may be too low for the survival of *Artemia*. Finally, Australian salt lakes are occupied by species of *Parartemia* and it appears that there may be biological interaction between the two genera. The several species of *Parartemia*, having evolved in Australia, may be so well adapted to the salt lakes they occupy that they outcomplete the alien *Artemia* whenever it is introduced.

It should be noted that, although there are several sibling species of *Artemia* (Bowen *et al.*, 1978), only one parthenogenetic form seems to have been in Australia for any considerable period. It is possible that other strains, races or species may differ physiologically and ecologically, and might therefore, have quite different impacts if they were introduced to Australian salt lakes.

Acknowledgements

I am grateful to I.C.I. Australia for granting access to their saltworks at Dry Creek, Adelaide ; to a group of undergraduate students in the Zoology Department of the University of Adelaide for collecting samples in 1979 ; and to Dr. G. A. Cole, Arizona State University, for critically reading the manuscript.

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Artemia nauplii as a food source for cyclopoids : extrapolation of experimental measurements to the metabolic activities of copepods in Lake Kinneret, Israel

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Abstract

Freshly hatched *Artemia* nauplii have been very widely used as food for predatory cyclopoids both in the freshwater and in the marine environment. It was found that the freshwater cyclopoid, *Mesocyclops leuckarti*, preyed upon a similar biomass of *Artemia* nauplii as it does on *Ceriodaphnia reticulata*. Rates of consumption, respiration, and productivity of adult *M. leuckarti* fed with *Artemia* nauplii were extrapolated to the metabolic activities of natural copepod populations in Lake Kinneret and the results are discussed.

Introduction

The metabolic activity of natural populations of predatory zooplankters has been estimated using methods which can be classified in two major groups. The first group is based on *in situ* experiments, performed in enclosures, with measuring of the rate of ingestion of a labeled material or counting of the number of preyed or grazed organisms. The second group extrapolates the results of feeding experiments on various food sources to natural populations and natural conditions (Nauwerck, 1963 ; Sorokin, 1968 ; Sorokin and Panov, 1966 ; Rigler, 1971 ; Haney, 1974 ; Porter, 1973).

Among the different food sources utilized for such experiments *Artemia* nauplii play an important role ; they indeed offer many advantages such as : 1) permanent availability : dried eggs can be purchased all over the world and hatched with simple techniques ; 2) homogenous chemical composition, bioenergetic qualities and size of the organisms ; 3) slow swimming behavior ; 4) relative softness of body tissues ; 5) many data available on their biological and biochemical features. Moreover *Artemia* nauplii can also be used as prey in freshwater since they are able to survive a few hours in such an environment (Smyly, 1970 ; Gophen, 1976).

These properties of *Artemia* nauplii explain why many authors have utilized them in feeding experiments ; Smyly (1970) on *Acanthocyclops viridis*, Anraku and Omori (1963) in their study of the feeding behavior of the marine copepods *Calanus finmarchicus*, *Centropages*

typicus, *Centropages hamatus*, *Acartia tonsa*, *Labidocera*, and *Tortanus discaudatus* ; Haq (1976) in his study of nutritional physiology of *Metridia lucens* and *Metridia longa* ; Robertson and Frost (1977) in the study on the feeding of the marine copepod *Aetideus divergens* and others. In these studies similarities were found between the predation rates of copepods fed with *Artemia* nauplii and natural food and the investigators have suggested that *Artemia* nauplii can be used routinely as experimental prey for predators and omnivorous zooplankters in freshwater and marine environments.

Feeding experiments with *Mesocyclops leuckarti*, the most common cyclopoid in Lake Kinneret (Gophen, 1976) were conducted with *Artemia* nauplii as a food source. Since *Artemia* is not present in the lake we ran parallel feeding experiments with *Ceriodaphnia reticulata* which accounts for a large part of the natural food of *Mesocyclops leuckarti* in the lake (Gophen, 1977). Since we found that the rates of predation and respiration of *Mesocyclops leuckarti* were very similar when this copepod was fed *Ceriodaphnia* or *Artemia* nauplii the experimental data were used to calculate the metabolic activity of *in situ* populations.

In the present study we report on both the experimental feeding data and the long term variations of metabolic activities of natural populations of adult copepods in Lake Kinneret during the period 1972-1977.

Materials and methods

The experimental procedures to measure feeding rates were described by Gophen (1976). The Californian strain of *Artemia* eggs was used. The cysts were hatched at 25 °C in a 2.5 % NaCl solution with air bubbling ; the nauplii were harvested after 24 hr and used as prey for the predatory cyclopoids.

In the feeding experiments, metabolic parameters as described by Gophen (1976) at three temperatures 15°, 22°, and 27 °C simulating the most common temperatures observed in the lake epilimnion (Gophen, 1978b, Serruya, 1978). These temperatures correspond to four periods in the lake namely : 1) the turnover period (January-March) 15°-20 °C ; 2) the development of the stratification period (April-June) 20°-24 °C ; 3) the stable stratification period, (July-October) 24°-28 °C ; 4) the destratification period (November-December) 24-20 °C. Monthly rates of metabolic activities were calculated by multiplying the experimental values by the monthly averages of standing crop biomass and are expressed in $gC/m^2/month$. The physiology, development and distribution in Lake Kinneret of adult males and females of *Mesocyclops leuckarti* have been described earlier (Gophen, 1976, 1977, 1978ab, 1979). Carbon was chosen as a common basis for all calculations and estimated to represent 5 % of the wet weights of adult males and females of *M. leuckarti* and *Artemia* nauplii (Burgis, 1971 ; Cummins and Wuycheck, 1971). The carbon equivalent of respired oxygen was calculated by assuming a respiratory quotient of 0.8. Defaecation rates were calculated by using the formula : $F = C - (R + P)$; (C = predation rate ; R = respiration rate ; P = productivity ; F = defaecation rate including excreted ammonia). It was found that ammonia excreted by adult males and females of *Mesocyclops leuckarti* varied between 0.1-0.2 % of the total faeces (Gophen, 1976) ; it was therefore ignored in our calculations.

TABLE I

Number of freshly hatched *Artemia* nauplii that were preyed by cultured adult males and females of *M. leuckarti* in 8 days at three different temperatures. Averages ($\bar{X}$) and standard deviation ($\pm$ S.D.) are given

	15 °C		22 °C		27 °C	
	Females	Males	Females	Males	Females	Males
	8.0	3.5	23	14.5	26.5	20
	6.0	3.0	23.5	12.5	25	20.5
	6.0	3.0	25.5	17.5	26.5	20
	5.5	3.0	25	18.0	26.5	20.5
	4.5	3.0	22.5	14.5	27	21
	5.0	2.5	25	14.5	26	22
	5.5	2.5	24	14	27	20.5
	7.0	2.5	23.5	16.0	27	23
	5.0	2.5	24	15.5	24.5	20.5
	5.0	2.0	23.5	14.5	24.5	20
	4.0	3.0	24	15.5	26	20.5
	6.0	3.0	22	16	26.5	21
	6.0	3.5	27	15	25.5	20.5
	5.5	2.5	24	15.5	28.5	18
	5.5	3.0	22	14	26.5	20
	5.0	3.0	23.5	14	28	19.5
	4.5	3.5	23	14	27.5	20.5
	5.0	3.5	22	17.5	26.5	20.5
	5.5	3.5	22	15	28	20.5
	5.5	3.0	22.5	15	26.5	20.5
$\bar{X}$	5.5	3.0	22.5	15.0	26.5	20.5
(S.D. $\pm$)	(1.0)	(0.5)	(1.5)	(1.5)	(1.0)	(1.0)

Results and discussion

The results of an 8 days feeding experiment of adult males and females of *M. leuckarti* on *Artemia* nauplii are presented in Table I. The predation rates expressed as number of nauplii preyed per individual are lower for males than for females at all temperatures. There is a net increase in predation rates with temperature. When the results were calculated in terms of ingested biomass per unit body weight, the experimental values were found to be similar for males and females at 15 °C (0.3 $\mu g_{(ww)}/\mu g_{(ww)}$/day). Conversely at 22 °C and 27 °C the results were higher for males (1.6 and 2.0 $\mu g_{(ww)}/\mu g_{(ww)}$/day respectively) (Gophen, 1977). The number of half preyed *Artemia* nauplii (as observed under the microscope) increased with temperature. When fed with fresh *Ceriodaphnia reticulata* the adult females of *M. leuckarti* prey 0.63, 1.07 and 1.13$\mu g_{(ww)}/\mu g_{(ww)}$/day at 15°, 22°, and 27 °C respectively (Gophen, 1977). In experiments where adult males and females were fed 8 days with *Artemia* nauplii and then starved, females survived 18 days ($\pm$ S.D. 7 days) and males 14 days ($\pm$ S.D. 3 days). Similar results were obtained in experiments conducted at 15 °C when males and

females were isolated as copepodid stage V from the lake population, fed with *Artemia* nauplii for 1-2 days until they moulted to the adult stage and then starved (Gophen, 1977). At both 22° and 27°, however, males survived 6 days and females 12 days. These experiments show that the survival time of organisms prefed 8 days with *Artemia* nauplii was similar to the survival time of organisms taken from the lake and fed mostly on natural food sources.

TABLE II

Metabolic parameters of adult *M. leuckarti* fed *Artemia* nauplii at three different temperatures[1]

Parameter	Temperature (°C)		
	15°	22°	27°
Predation rate[2]	14.1	65.0	76.3
Respiration rate[2]	6.5	11.5	20.0
Defaecation rate[2]	6.6	50.5	51.8
Productivity[2]	1.0	3.0	4.5
Monthly P/B ratio	1.5	2.6	4.5

[1] See Gophen (1976) and Gophen and Landau (1977).

[2] mg $C/g_{(ww)}/day$.

The experimental rates of food consumption (C), defaecation (F), respiration (R) and monthly P/B ratios for organisms cultured at three temperatures are given in Table II. The metabolic activity of the lake population during the period 1972-1977 was calculated from these data and the biomass data. The rates of food consumption, respiration and defaecation are presented in Fig. 1 and 2. In all these years winter metabolic activity was low and was related to the low temperatures in the lake. Metabolic activity in summer was higher with distinct decreases observed during September-October (except for 1973). Since the monthly average epilimnic temperatures did not change significantly during the whole period of investigation (Gophen, in preparation) and since the biomass of adult copepods was higher in 1972, 1976 and 1977 than in 1973-1975 (Gophen, 1972-1977 ; Gophen and Landau, 1977) it follows that the overall activity of the adult copepod population was mainly related to the variation in copepod biomass. The fluctuations in the biomass of adult copepods were strongly affected by the variations observed in the sardine population (Gophen and Landau, 1977). The higher than usual number of sardines observed in 1973-1975 increased the predation pressure not only on Cladocera but also on adult copepods. It is clear that during 1973-1975 biomass was relatively low and the metabolic activity of adult copepods was lower and accompanied by large biomass fluctuations (1-8 g/m^2). In contrast, in 1972, 1976, and 1977, the biomass and metabolic activity were higher followed by a smaller range of biomass fluctuations (3-7 g/m^2).

The most prominent increase of metabolic parameters in summer is mostly due to an increase of defaecation (Fig. 1 and 2). The zooplankton faeces play an important role in the Kinneret ecosystem during the summer (Serruya, *et al.*, 1979). During the summer months

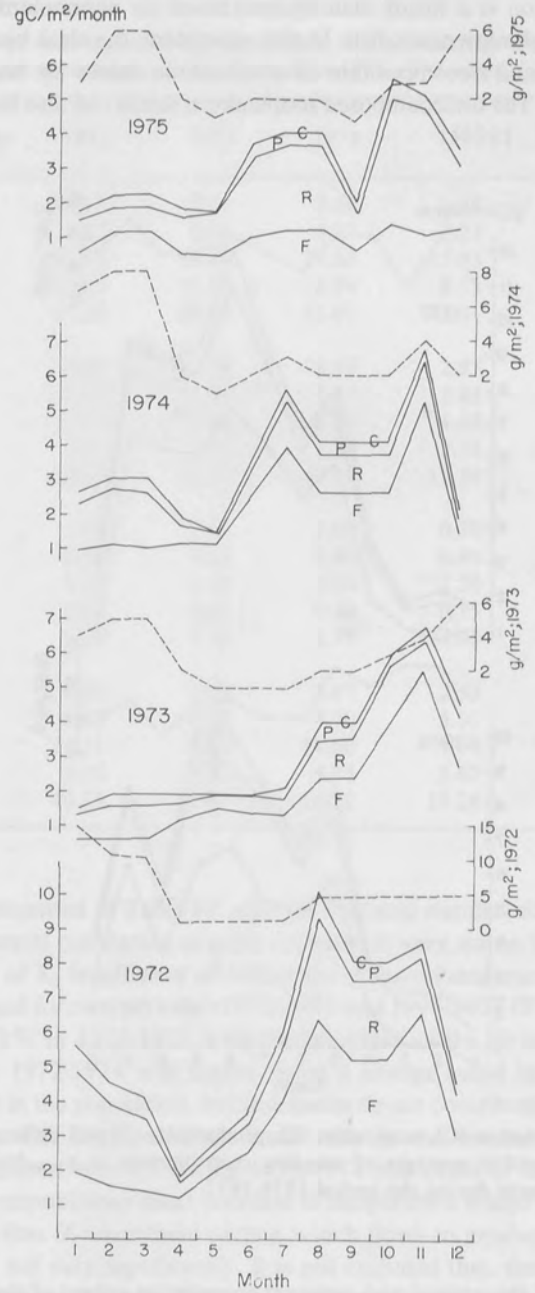

FIG 1. Food consumption (C), respiration (R), productivity (P) and defaecation (F) rates (——) in gC/m^2/month and monthly averages of standing crop biomass in $g_{(ww)}/m^2$ (– – – –) of predatory copepods in Lake Kinneret during the period 1972-1975.

the Kinneret epilimnion is a steady state system based on nannoplanktonic algae, bacteria, zooplankton and zooplanktivorous fish. In this ecosystem, the algal biomass is controlled by zooplankton grazing and decomposition of zooplankton faeces by bacteria which supplies nutrients to the algae. The undecomposed zooplankton faeces can also be grazed upon by filter feeders (Serruya *et al.*, 1979).

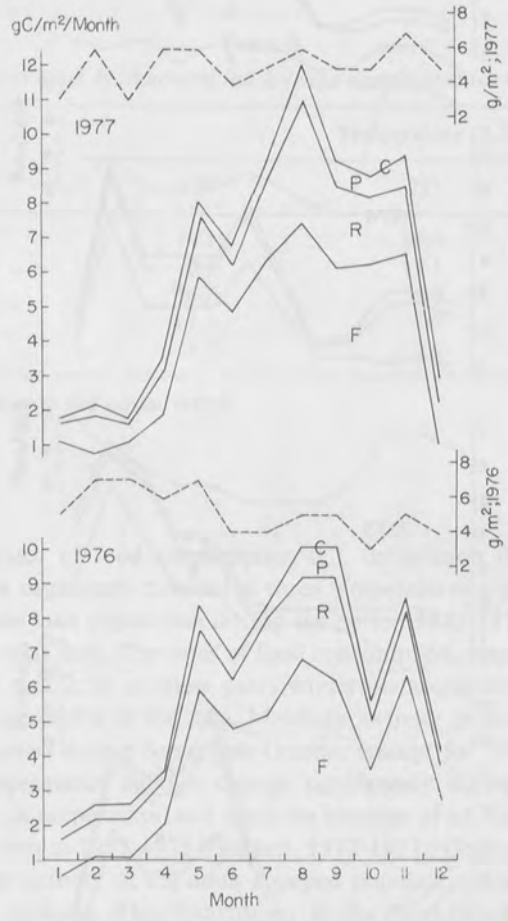

FIG. 2. Food consumption (C), respiration (R), productivity (P) and defaecation (F) rates (——) in $gC/m^2/month$ and monthly averages of standing crop biomass in $g_{(ww)}/m^2$ (– – – –) of predatory copepods in Lake Kinneret during the period 1976-1977.

In Tables III to IV the annual and seasonal cumulative values of the metabolic activity of the lake populations are presented. The high cumulative values of C, R, P, and F (Table III) especially during the summers of 1972, 1976, 1977 compared to those of 1973-1975 are very prominent. The cumulative values of U^{-1} (coefficient of assimilation efficiency $= [R + P]/C$), K_1, (coefficient of utilization of energy for growth $= P/C$) and F/C, all expressed

TABLE III

Cumulative values of food ingestion (C), respiration (R), productivity (P) and defaecation (F) for natural populations during the period 1972-1977 in g C/m²/period

Period (month)	Parameter	1972	1973	1974	1975	1976	1977
I-III	(C)	14.13	5.21	8.56	6.26	7.08	5.82
IV-VI		8.22	5.58	6.87	7.23	18.84	18.54
VII-X		32.16	16.14	17.64	15.63	33.72	39.51
XI-XII		13.05	11.34	8.94	8.73	12.75	12.45
I-XII		67.56	38.27	42.01	42.01	72.39	76.32
I-III	(R)	7.41	2.74	4.49	2.97	3.66	2.43
IV-VI		2.07	1.35	1.77	1.82	4.53	4.62
VII-X		9.60	4.80	5.04	4.44	9.78	10.32
XI-XII		3.00	2.91	1.89	2.31	2.76	3.30
I-XII		22.08	11.80	13.19	11.54	20.73	20.67
I-III	(P)	1.90	0.65	1.00	0.70	1.04	0.48
IV-XI		0.50	0.15	0.40	0.40	1.62	1.24
VII-X		1.85	1.40	1.00	1.20	2.78	2.80
XI-XII		0.50	0.50	0.30	0.75	0.85	1.43
I-XII		4.75	2.70	2.70	3.05	6.29	5.95
I-III	(F)	4.82	1.82	3.07	2.59	2.38	2.91
IV-VI		5.65	4.08	4.70	5.01	12.69	12.68
VII-X		20.71	9.94	11.60	9.99	21.16	26.41
XI-XII		9.55	7.93	6.75	5.67	9.14	7.72
I-XII		40.73	23.77	26.12	23.26	45.37	49.72

in percentages and presented in Table IV, did not fluctuate significantly. It is likely that the metabolism of the natural population of adult copepods is very stable. Nevertheless there is a significant difference of K_2 (coefficient of utilization of assimilated energy for growth, = P/[R + P]) values averaged for two periods : 1972-1974 and 1975-1977 (Table IV). These values were higher by 11-68% in 1975-1977 with the exception of the turnover period when the average K_2 value for 1972-1974 was higher. Such a change could be generated by a high proportion of females in the population, because males do not contribute to the productivity of the adult population. The average female/male ratio in the natural populations of adult copepods in Lake Kinneret was 0.5 during 1972-1974 and equal or higher than 1.0 during 1975-1977 (Gophen, unpublished data). Increase of temperature is also a possible explanation for the higher proportion of assimilated carbon which flows to productivity (Gophen, 1976) but temperatures did not vary significantly. It is not excluded that, simultaneously, the food that was consumed during this period, had a higher nutritional value.

The comparison of cumulative K_1, K_2, U^{-1} and F/C values of adult predatory copepods and herbivorous zooplankters (Gophen, in preparation) (Table V) allows the following observations : Coefficients of assimilation (U^{-1}) of predators and herbivores are similar, except at low temperatures where the U^{-1} value of predators is much higher than that of herbivorous

TABLE IV

Cumulative values of U^{-1}, K_2, F/C and K_1 expressed as percentages during the period 1972-1977

Period (month)	Parameter	1972	1973	1974	1975	1976	1977
I-III	(U^{-1})	66	65	64	59	66	50
IV-VI		31	27	32	25	33	32
VII-X		36	38	34	36	37	33
XI-XII		27	30	25	35	28	38
I-XII		40	38	38	39	37	35
I-III	(K_2)	28	19	18	19	22	16
IV-VI		19	10	18	18	26	21
VII-X		16	23	17	21	22	21
XI-XII		14	15	14	25	24	30
I-XII		16	19	17	21	23	22
I-III	(F/C)	34	35	36	41	34	50
IV-VI		69	73	68	69	67	68
VII-X		64	62	66	64	63	67
XI-XII		73	70	76	65	72	62
I-XII		60	62	62	61	63	65
I-III	(K_1)	13	12	12	11	15	8
IV-VI		6	3	6	5	9	7
VII-X		6	9	6	8	8	7
XI-XII		4	4	3	9	7	11
I-XII		7	7	6	8	9	8

TABLE V

Cumulative values of U^{-1}, K_2, F/C and K_1 expressed as percentages (±S.D.), averaged for the period 1972-1977, for predatory (P) and herbivorous (H) zooplankton in Lake Kinneret

Parameter	K_2		K_1		U^{-1}		F/C	
Period	P	H	P	H	P	H	P	H
I-III	20 (4)	47 (3)	12 (2)	22 (1)	62 (6)	46 (2)	38 (6)	54 (2)
IV-VI	19 (5)	44 (4)	6 (2)	11 (2)	30 (3)	26 (3)	69 (2)	74 (3)
VII-X	20 (3)	27 (2)	7 (1)	10 (1)	36 (2)	37 (1)	64 (2)	63 (1)
XI-XII	20 (7)	38 (5)	6 (3)	12 (2)	31 (5)	33 (4)	70 (5)	67 (4)
I-XII	20 (3)	37 (3)	8 (1)	13 (1)	38 (2)	34 (2)	62 (2)	66 (2)

species. In contrast algae feeders defaecated more than predators. The K_1 and K_2 values of predators were found to be lower than those of algae feeders. This rather unexpected result calls for the following comments : 1) the Kinneret nannoplanktonic algae which contribute the bulk of the herbivorous food have a high nutritive value as shown by the increase of the K_2 value of herbivorous zooplankton during the years when the nannoplankton was dominant ; 2) the predatory copepods ingest a high amount of undigestible material such as setae and other chitinous fragments (Fryer, 1957 ; Gophen, 1977) which increase the amount of faeces.

Acknowledgements

I wish to give my warm thanks to Drs. C. Serruya and U. Pollingher for their helpful encouragement and cooperation ; Dr. D. Wynne for reviewing the manuscript, M. Schiper for technical assistance and R. Herschovitch for typing the manuscript.

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G. Persoone, P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 456 p.

Size and sex composition of *Artemia* from the salt water springs of Tuticorin, South India

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Abstract

The brine shrimp *Artemia* was reported in India from the salt pans of Vadala on the outskirts of Bombay, from the Sambhar lake in Rajasthan, and from Tuticorin. The *Artemia* population found in the Tuticorin area is restricted to the salt water springs of Tuticorin, the adjacent high saline back-waters, and the salt pans.

The present observation is based on the collection of *Artemia* from the salt water springs of Tuticorin. A nylon net with a mesh size of 0.2 mm was used for the catches.

The total length of the *Artemia* collected ranged from 1 to 10 mm. Of all the individuals 87.2% were in the size range 7.0 to 10.0 mm. Respectively 1.1% and 4.2% were 1.0 to 2.0 mm and 3.0 to 4.0 mm in length.

The sex ratio was 1:5 males to females. From a total number of 839 specimens 79% were males in the 5.0-6.0 mm length-category *versus* 63.9% and 96.8% females in the 7.0-8.0 and 9.0-10.0 mm categories respectively. The sex ratio varied with the increase in size of the *Artemia*. Sex differentiation was not possible in the 1.0 to 4.0 mm length categories and 20% of the *Artemia* in the 5.0 to 8.0 mm length groups were still of undeterminable sex. Above 8.0 mm all the animals observed were mature. It was observed that *Artemia* start maturing when it attains 7 mm. In the 7.0 to 8.0 mm length group, 26.1% were found to be mature *versus* 57.6% in the 9.0 to 10.0 mm category. When the gonads are ripe the color of the brine shrimp changes to a red tinge and the egg pouches becomes reddish.

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G. Persoone, P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 456 p.

Ecology of an alkali-adapted variety of *Artemia* from Mono Lake, California, U.S.A.

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Abstract

The demographic characteristics and spatial distribution of the *Artemia* in Mono Lake (California) were studied during a complete growth season in 1978. Mono Lake, a large (A = 150 km²), moderately deep ($\bar{z}$ = 19 m), alkaline saline lake located on the eastern Sierra slope, contains a physiologically and genetically distinct variant of *Artemia*. The *Artemia* are the most abundant zooplankter and the only crustacean living in the lake. The overwintering cysts were estimated to have hatched in early March, and the cohort reached maturity in late May after a 10 week development. This cohort then reproduced ovoviviparously, giving rise to the second generation which matured in 8 weeks. Only two generations were observed through the summer.

Early in July the females started producing overwintering cysts. Secchi disk readings started to increase from 0.8 m at the end of May by 0.6 m per week, possibly reflecting heavy grazing by the *Artemia*. By early July, readings had reached 3.5 m suggesting substantial depletion of the algal food source. From June to early July the shallow water temperature had remained unchanged ; thus the change in reproductive mode may have been triggered by low food density.

In late August and early September mean densities of 25 000 ind./m² were estimated from ten vertical hauls taken on a 1.2 km transect along a 15 m depth contour. Patchy animal distribution in the near shore areas produced densities ranging between 400 ind./l and less than one ind./l. High densities were associated with sublacustrine springs, foam lines associated with water-mass boundaries and aggregations in thermal currents.

Introduction

Mono Lake is a permanent alkaline saline lake of moderate size (150 km²), located at 1940 m above sea level on the eastern slope of the Sierra Nevada. *Artemia*[1] occupy a prominent position in the ecosystem of this lake. The zooplankton community is limited to *Artemia*, two rotifers, *Brachionus plicatilis* and *Hexarthra jenkinae* and several protozoans (Mason, 1967). In the summer, *Artemia* are the major zooplankton in the lake, attaining high densities and comprising most of the biomass. *Artemia* are also a bridge between the lacustrine and terres-

[1] The Mono Lake *Artemia* have been described as *Artemia monica* (Verrill, 1869). They have been usually referred to as *Artemia salina* (Mason, 1967 ; Dana and Herbst, 1977), since reproductive isolation has not been demonstrated through crosses. Clark and Bowen (1976) and Bowen *et al.* (1978) strongly suggest using a different species name for Mono Lake *Artemia*. Since this issue has not been resolved I will refer to the genus name alone.

trial ecosystems. They, together with the brine fly (*Ephydra hians*) are the major food sources for 50 000 nesting California gulls (*Larus californicus*) and about a million migrating birds including eared grebes (*Podiceps caspicus*) and phalaropes (*Phalaropus tricolor* and *Phalaropus lobatus*) (Winkler *et al.*, 1977).

Although research on *Artemia* has a long and successful history, relatively little is known about this animal's ecology. It has been considered primarily to be a fast reproducing organism inhabiting small, ephemeral salt water ponds, as well as large, permanent saline lakes, with minor seasonal fluctuations in salinity (see review by Persoone and Sorgeloos, 1980). In the latter stable ecosystems, the interaction between *Artemia* and the environment is not unlike that of other zooplankton communities.

The purpose of this research was to investigate several aspects of the ecology of the Mono Lake *Artemia*. I will report their life history which differs from that in other habitats. In attempting to follow population density changes major problems arose due to extreme patchiness, which masked any true density changes. As a result the structure of different patchtypes was investigated. I have also examined vertical distributions, their changes during the year, and their correlation with several physical parameters of the water column.

Study site

Mono Lake is a temperate lake (latitude 38°N, longitude 119°W). During the winter, water temperatures are close to 0 °C. The lake does not freeze, although near shore areas can be covered with a layer of freshwater which freezes. In the spring the lake warms and a pronounced thermocline develops. The thermocline is stable through the summer, yet it deepens as the season progresses. In the fall the lake temperature drops and it becomes nearly isothermal in November (Mason, 1967).

The chemical composition of Mono Lake is unusual as an *Artemia* habitat (Cole and Brown, 1967). The major cation is sodium and major anions are chloride and carbonate (Mason, 1967). The lake also contains unusually high concentrations of sulfate and borate (Mason, 1967). The present pH is about 10 and the specific gravity is 1.08.

Since the 1940s two major streams (about 50% of the water influx) supplying Mono Lake have been diverted into the Los Angeles aqueduct. As a result the lake level has been dropping 30 to 60 cm per year (Mason, 1967 ; Loeffler, 1977). The increasing salinity of the lake can be expected to affect both the phytoplankton and the zooplankton populations. The present ecological study may serve as a base line to measure such effects.

Materials and methods

Two methods were used to sample the *Artemia*. Vertical hauls were made from a selected depth to the surface using a plankton net of 30 cm diameter and 705 μm mesh-size. Discrete samples at a particular depth were made with a trap as described by Schindler (1969). The trap had a 25 cm square cross section, a height of 50 cm, and contained 31.25 l. The attached plankton net had a 150 μm mesh-size ; the cod-end had a strainer consisting of two layers of 705 μm (1978) or one layer of 150 μm (1979) mesh screening. Animals collected were fixed within a few hours in 5% formaldehyde in lake water.

Transparency of the water was measured using a 20 cm Secchi disk as an index of algal densities (other factors influenced Secchi readings, but algal densities were the primary factor). Oxygen and temperature profiles were made with a Yellow Springs Instruments model 54 meter. The oxygen readings were calibrated at lake-level using the Miller method (Walker, 1970).

Standard stations used for sampling are shown on the map in Fig. 1. In 1978, sampling commenced in April and continued through September at approximately one-week intervals. Some data from 1979 and from less regular winter sampling (at non-standard stations) are included.

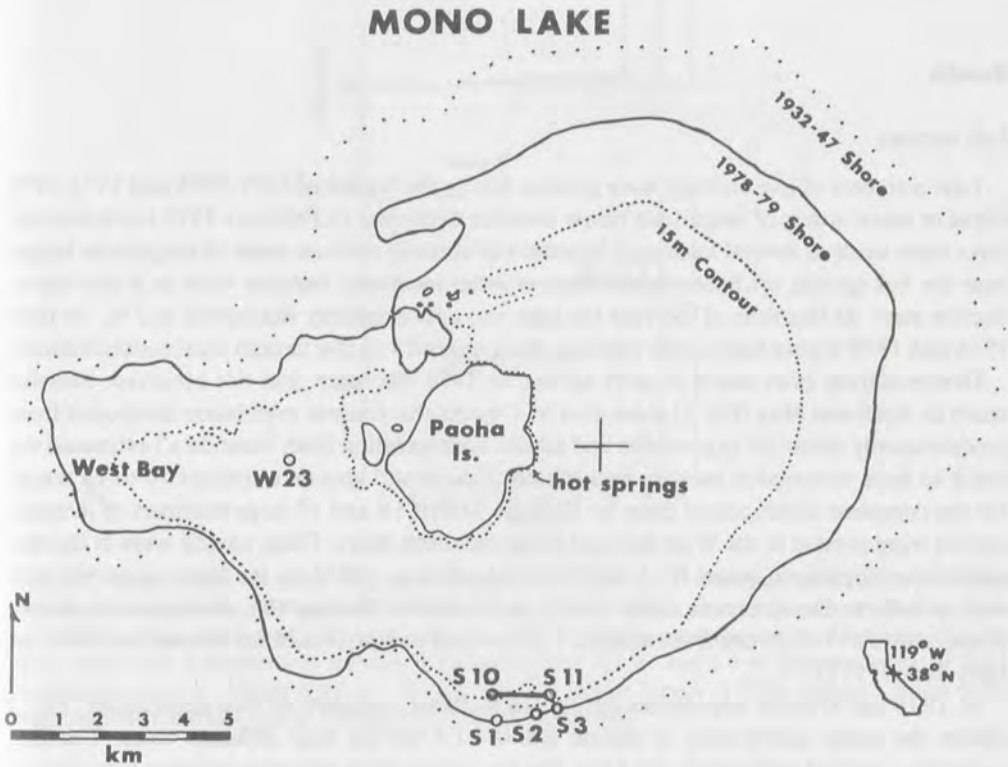

FIG. 1. Map of study site. Solid outline shows estimated 1978-79 shore line based on bathymetric data from Scholl *et al.* (1967) (= 20 foot contour of Plate 2). 15 m contour (inner dotted line) determined similarly (= 70 foot contour). Approximate 1932-47 shore (outer dotted line) derived from topographic maps (US Geological Survey, 15') and a mean lake surface elevation of 1 956 m derived from figures presented by Loeffler (1977) (Table 2-14, in turn derived from Los Angeles Department of Water and Power records). Cross in center corresponds to latitude 38°N, longitude 119°W. Sample stations indicated by circles. S-1, 2, and 3 were near-shore (50 m) locations ; S-1 was in the "South Tufa area" and had a mud bottom ; S-2 was over a spring of *ca* 6 m depth ; S-3 was in a sandy beach area. Station depths (m) : S-1 = 1.8 ; S-3 = 3.5 ; S-10,11 = 15 ; W-23 = 26.

The *Artemia* samples were examined and counted under a disserting microscope. Developmental stages were determined according to Heath's description (1924). However, as Provasoli and Shiraishi (1959) did, I grouped instars 9-11 as "juveniles" and instars 12 and older as "adults". The individual's sex was determined starting at the 7th instar. Adult females were further categorized as possessing or not possessing eggs in some developmental stage. Vertical haul samples were fixed for at least 10 days and then measured using the volume displacement after 5 min settling in a calibrated test-tube or graduated cylinder. Counts of 50 to 500 adults were made and volumes measured to calibrate the method. Each day's samples were calibrated in this way because substantial variation from week to week was found. Density estimates from Schindler trap samples were done by integrating the curve under the sample points connected by a straight line.

Results

Life history

Low numbers of live animals were present during the winter of 1977-1978 and 1978-1979 (three or more orders of magnitude below summer densities). In February 1979 horizontal net tows were made at several locations. Numbers of animals were an order of magnitude higher near the hot springs off Paoha Island than at other locations. Females were in a non-reproductive state. At this time of the year the lake was approximately isothermal at 2 °C. In both 1978 and 1979 winter Secchi disk readings were around 1 m due to high algal concentrations.

Overwintering cysts hatch in early spring. In 1978 the hatch was not observed. Samples taken in April and May (Fig. 2) show that in 4 weeks the *Artemia* population developed from predominantly instar six to juveniles and adults. Extrapolating from these data I estimated the hatch to have occurred in early to mid-March. This would give an estimated 10 to 12 weeks for the complete development time. In 1979 on March 18 and 19 large numbers of *Artemia* nauplii were present in the West Bay and along the south shore. These nauplii were in the two earliest developmental stages (N-1 and N-2 of Anderson, 1967), so the hatch occurred very shortly before. Development time was 8 to 10 weeks. During this development period, temperatures at 1-2 m rose from around 7 °C in April (which is cold for *Artemia*) to 15 °C by early June (1978).

In 1978 the *Artemia* population during the summer consisted of two generations. Fig. 2 shows the instar distribution at station S-1 ($z = 1.8$ m) for four different dates. The first generation reached maturity in late May. The beginning of the second generation was marked by a peak of nauplii in early June. Only in early June did I observe such a peak of nauplii (Fig. 3). Starting at the end of July the population at shallow stations (S-1, S-3) was composed of over 90% adults (Fig. 2 and 3), including early adult instars, suggesting the attainment of adulthood of the second generation by then. Fig. 4 shows the percentage of adult females with developing eggs between May and September at stations S-1 and S-3 ($z = 3.5$ m). In May there was a rapid increase in egg-bearing females and after June 10 greater than 90% of all females were carrying eggs. Since the only major peak of nauplii was in June, this indicates that the first-generation females switched to production of cysts. Most females collected in May produced nauplii in the lab, whereas females collected in mid-summer produced only cysts.

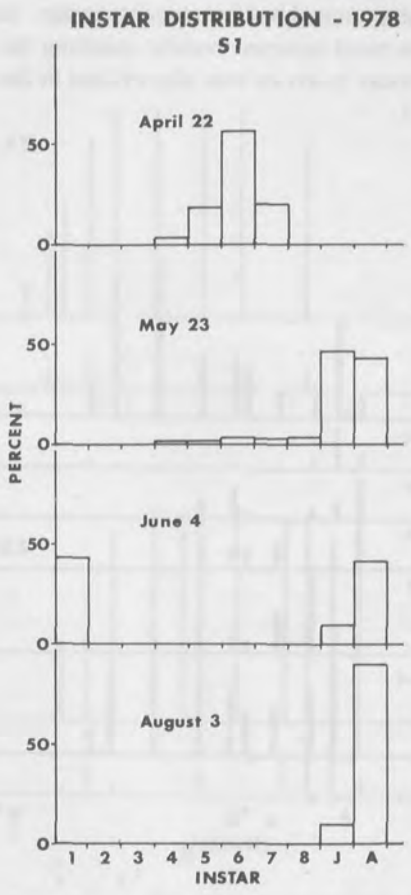

FIG. 2. Distribution of instar classes at station S-1 (see Fig. 1) for four dates in 1978. Instar classification according to Heath (1924) for groups 1-8 ; J = 9-11 ; A = 12 on. April 22 data from subsamples of four horizontal tows taken near the surface with the plankton net (see Methods). Data for other dates from Schindler trap samples at surface and/or 0.5 m, and 1.0 m (distance to top of trap). Total animal counts : 738 for April 22 ; 267 for May 23 ; 562 for June 4 ; 179 for August 3. Some early-stage animals were lost due to coarseness of screen in cod-end.

The variation in animal numbers from one sampling date to the next (Fig. 3 and 4) probably does not reflect true variations in lakewide densities, but is likely due to patchiness (see below).

The lower two graphs of Fig. 3 show temperature, dissolved oxygen, and Secchi changes at S-3 corresponding to the *Artemia* data from May through September, 1978. Mean shallow-water temperature rose to a peak of 22 °C by the beginning of August, and then dropped again. Oxygen dropped from its high initial values to a more-or-less constant level by the beginning of July. Algal densities, as measured by Secchi readings, began to drop rapidly commencing at the end of May. From an initial level of 0.8 m, Secchi readings increased at

over 0,5 m per week at this time. Maximum transparency corresponded in time to the temperature peak ; then the trend reversed rapidly, marking the beginning of the "winter" algal bloom. This shallow-water behavior was also evident in the upper layers of deep-water stations, as shown in Fig. 5.

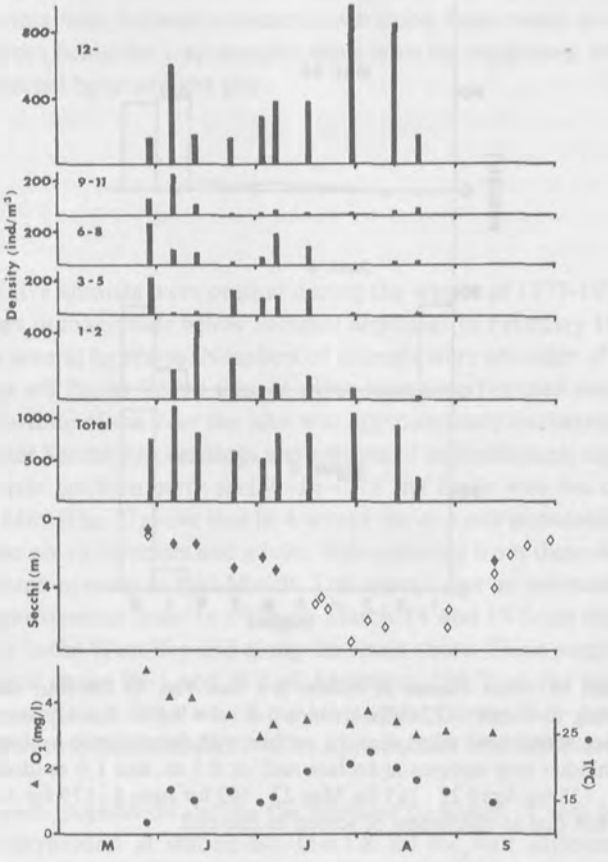

FIG. 3. Instar distributions at station S-3 for late spring and summer, 1978 (upper graphs). Also Secchi disk (◊ ♦), mean temperature (●) and mean oxygen (▲) measurements for the same period. Animal densities calculated by integration of Schindler trap samples (see Methods) from 3 to 5 depths (except July 15, 2 depths). Secchi readings at S-3 (♦) supplemented with data from deeper stations near south shore (◊) since Secchi depth exceeded station depth in mid summer. Temperature and oxygen data are averages of values taken at 1 m depth intervals, since there was little stratification (typically 1.5 °C ; 1.5 mg/l O_2 variation). One sample date with a "foam line" (August 4) was omitted (see text for description of foam-line anomalies). Data were taken between 0700 and 1100 hr Pacific Standard Time (except deep-station Secchi).

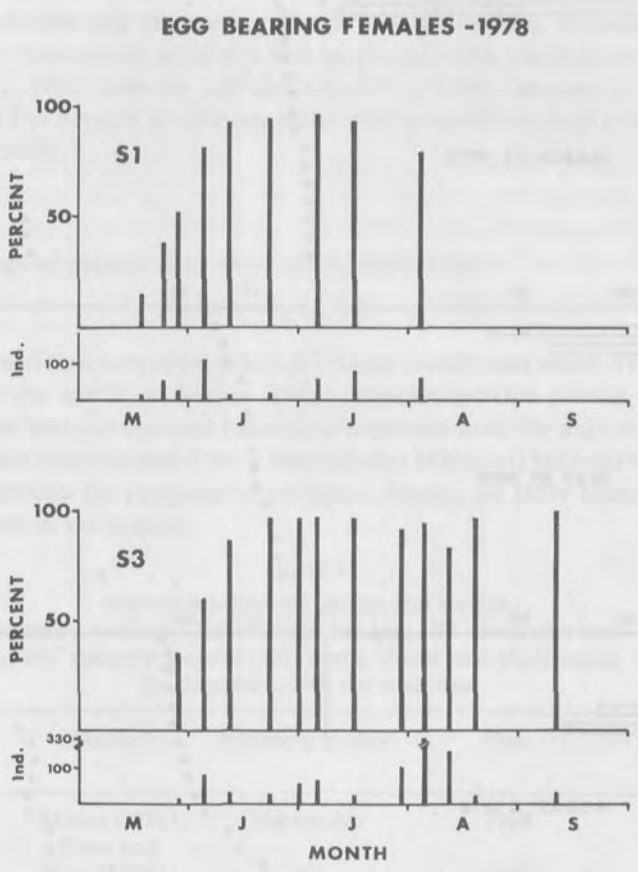

FIG. 4. Numbers of females at two stations (S-1 ; S-3) bearing eggs in some development stage (including oocytes). Percentages given in the upper graph of each pair ; sample numbers in the lower graph. Data based on actual numbers recovered in typically 3 to 5 Schindler trap samples (not integrated).

VERTICAL DISTRIBUTION

Early in the year animals of all stages occur near the surface. As the year progresses increasing numbers are found at greater depths and the upper 0.5 m is usually avoided. Fig. 5 shows the vertical distribution for station S-10 ($z = 15$ m) at three dates in 1978 and W-23 ($z = 26$ m) for March 1979. The deepening of the *Artemia* density peak correlates with the increasing transparency of the lake. In the fall however, no evidence was found for a less deep peak as Secchi visibility decreased.

Artemia density profiles were also compared to temperature and dissolved oxygen profiles. From late spring on, a thermocline develops. The hypolimnion is both anoxic and cold and most animals were found above it. However some animals were consistently recovered from

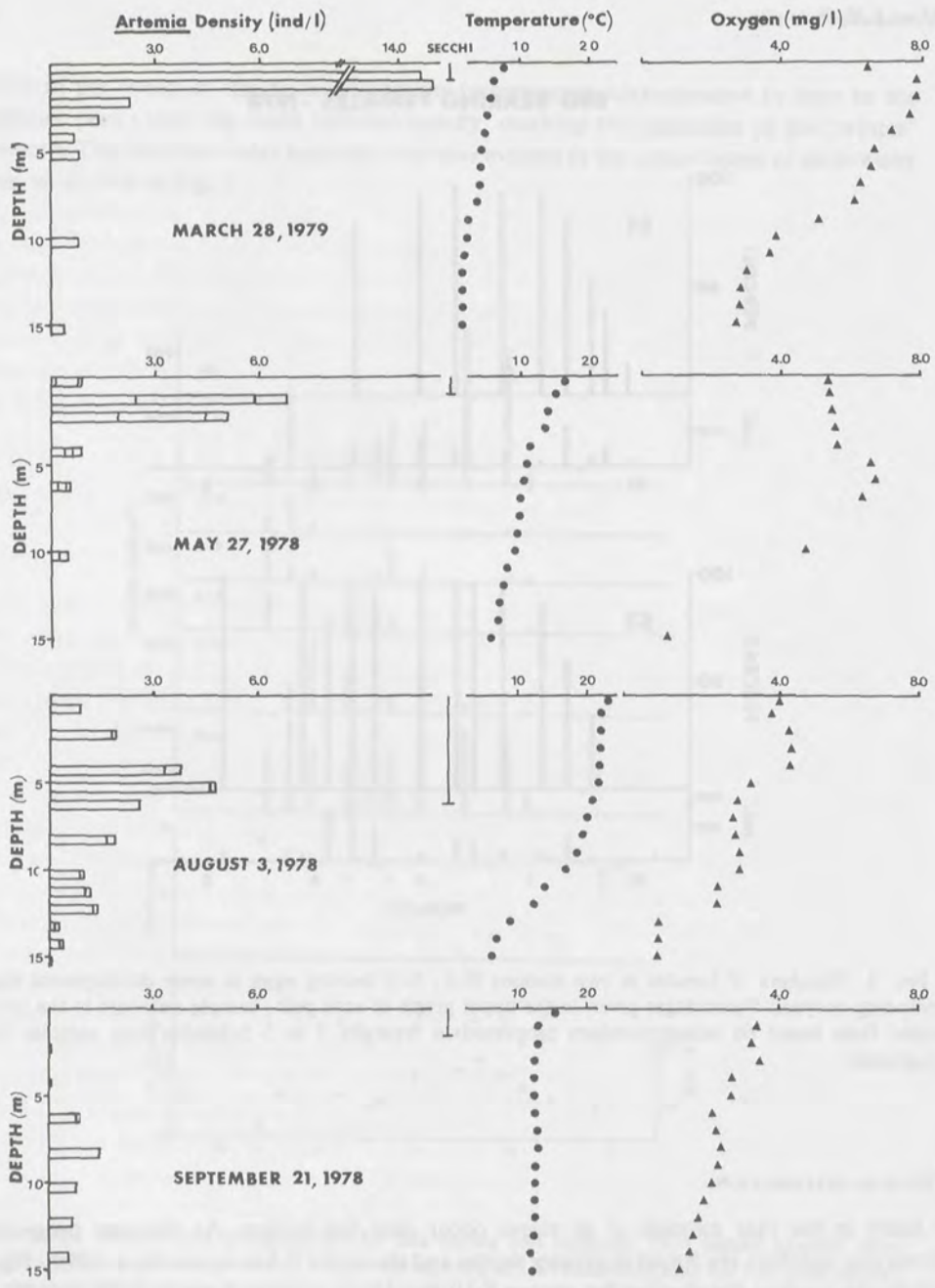

FIG. 5. Vertical distribution of *Artemia* densities (bar graphs), temperature (●), and dissolved oxygen (▲) at four dates. Corresponding Secchi disk readings indicated to right of bar graphs. Instar composition of sample indicated by division of bar into three parts (left to right) : 12 on (= adults) ; 6-11 ; 1-5. The March sample comprises only classes 1-4 ; note also that it is from 1979 since comparable 1978 data are not available. 1978 data from station S-10 ; March, 1979 from W-23. Most *Artemia* densities are based on single Schindler trap samples ; where replicates were taken, results were averaged. Data obtained between 1100 and 1400 PST.

lower layers in Schindler trap samples (*e.g.* Fig. 5, August 3 and Fig. 8). Because temperature and oxygen drops were usually parallel, it was hard to ascertain which factor had more effect on animal density. Note however that on August 3 a sharp decrease in animal densities between 12 and 13 m appears to correlate better with a significant drop in dissolved oxygen than with temperature.

Patchiness

Four major types of patches were observed in Mono Lake :

Plumes

Plumes are dense *Artemia* swarms, which are found usually near shore. Their diameter can vary between 20 cm and 2 m. Mason (1966) hypothesized that plumes resulted from a combination of thermal currents and behavioral responses from the animals. Table I shows density estimates for such a plume. I have also included Mason's (1966) and Dana and Herbst (1977) density estimates for comparative purposes. Plumes are fairly short lived, and only occur on calm days in the summer.

Table I

Artemia densities for plumes and springs.
Sampling methods : VH : vertical net haul ; ST : Schindler trap.
"Non-patch" category presents data from a typical near-shore station (S-3)
for comparisn with the other data

Patch-type	Source	Sampling method	Date	Density (ind./l)
Plume	Mason (1966)	Not known	1964	1 000
	Dana and Herbst (1977)	VH	1976	202
	Present study	ST	July 29, 1978	376
Spring	Dana and Herbst (1977)	VH	1976	38
	Present study S-2	ST 0m	June 11, 1978	10
		ST 2m	June 11, 1978	21
Non-patch	Present study S-3	ST 0m	June 11, 1978	1.3
		ST 2m	June 11, 1978	1.6

Sublacustrine springs

Sublacustrine springs are freshwater springs which create areas of upwelling. The upwelling carries the animals to the surface, where they become concentrated. Schindler trap samples showed that at 2 m, animal densities were even higher (Table I). On the same date *Artemia* densities at S-3 were an order of magnitude lower. The water was consistently more turbid and Secchi disk reading 5-20 cm shallower in the spring compared to S-3. This form of patch is persistent in time and space.

Foam lines

Foam lines are areas marked by concentrated debris including dead *Artemia* floating on the surface. Foam lines often run parallel to the shore for several hundred meters. The debris line is approximately 1 m wide, with one sharp boundary and the other more diffuse. On August 4 a foam line occured near station S-3. Animal densities were extraordinarily high at this station (2 000 ind./m^3). Transects were made across the foam line measuring *Artemia* density, temperature and dissolved oxygen at two different depths at different places (Fig. 6). Note the discontinuity in the surface measurements for all three parameters. Schindler trap samples at 3 m showed no difference in animal densities. Similarly, temperature and dissolved oxygen at 1 m did not change across the foam-line. The surface temperature discontinuity in particular suggests that foam lines are boundaries between watermasses. The density difference for Mono Lake water due to the temperature difference is almost 0.001 g/cm^3 between 24.5 and 22.5 °C (Mason, 1967). Foam lines are moving boundaries, and even during the short sampling period figured some movement of the boundary was detected.

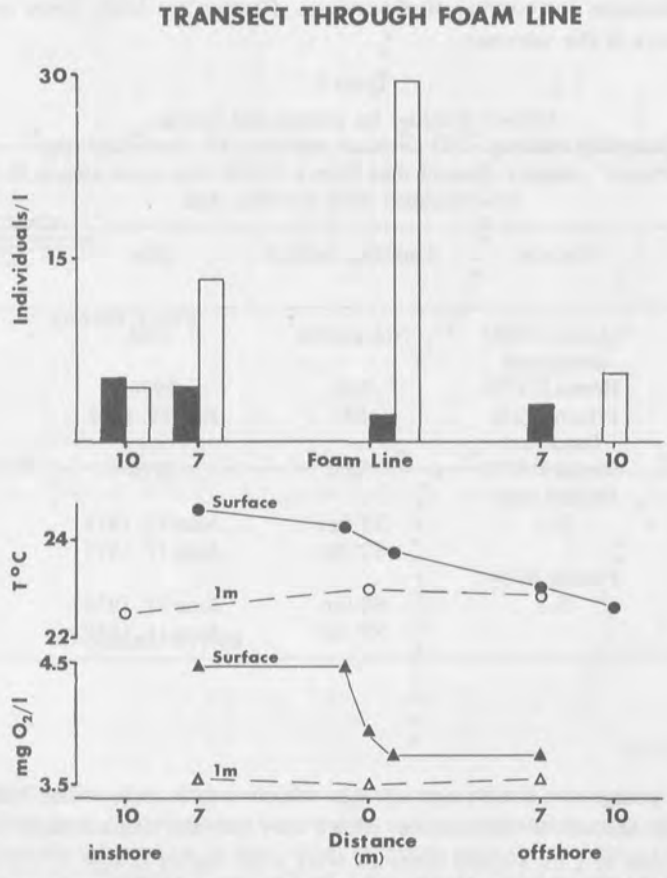

FIG. 6. Transects measuring *Artemia* adult densities (bars), temperature (circles), and oxygen (triangles) across a foam-line. Animal densities were samples at the surface (open bars) and at 3 m depth (filled bars).

Large scale patchiness

The degree of large scale patchiness was first assessed during a 24 hr sampling period at one fixed station. The density observed at this station fluctuated extensively over the 24 hr period (Fig. 7). The corresponding vertical distributions for the *Artemia* are shown in Fig. 8. The patchiness obscured possible vertical migration patterns.

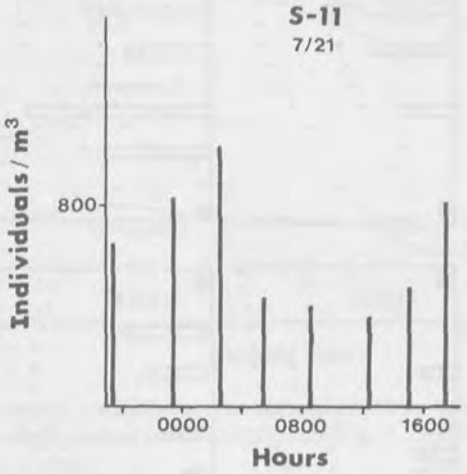

FIG. 7. Measured *Artemia* densities (all stages) over one 24 hr period (July 20-21, 1978). Data from integration of Schindler trap sampes at station S-11. Time given in hours, Pacific Standard Time. Station depth 15 m.

Additional evidence for large scale density variation was obtained by sampling along transects. Fig. 9 shows the *Artemia* densities as sampled with vertical net hauls along a 1.2 km transect between stations S-10 and S-11. *Artemia* densities along this transect showed substantial variation on two dates, whereas the animal densities on September 24 were very uniform. On September 28 the *Artemia* density showed a 60% decline along the transect between two stations. The mean animal densities along this transect were 27 000/m^2 on August 27, 8 500 on September 24, and 11 500 on September 28. On September 24, I also sampled along a transect between the West Bay and the south shore region (Fig. 10) ; these samples showed a major animal density gradient. Estimated densities varied between a peak of over 70 000 ind./m^2 in the West Bay to 8 500 ind./m^2 in the South. The lake was more transparent in the West Bay than in the South, and the boundary between the clear and relatively less clear water was abrupt.

Discussion

TAXONOMY

Determining the taxonomic status of Mono Lake *Artemia* has been impeded by the difficulty of keeping them in media suitable for doing crosses with other strains. San Francisco *Artemia* will hatch in Mono Lake water, but they die soon after hatching (Mason, 1967 ; Clark and Bowen, 1976 ; personal observation). Raising Mono Lake *Artemia* in seawater has

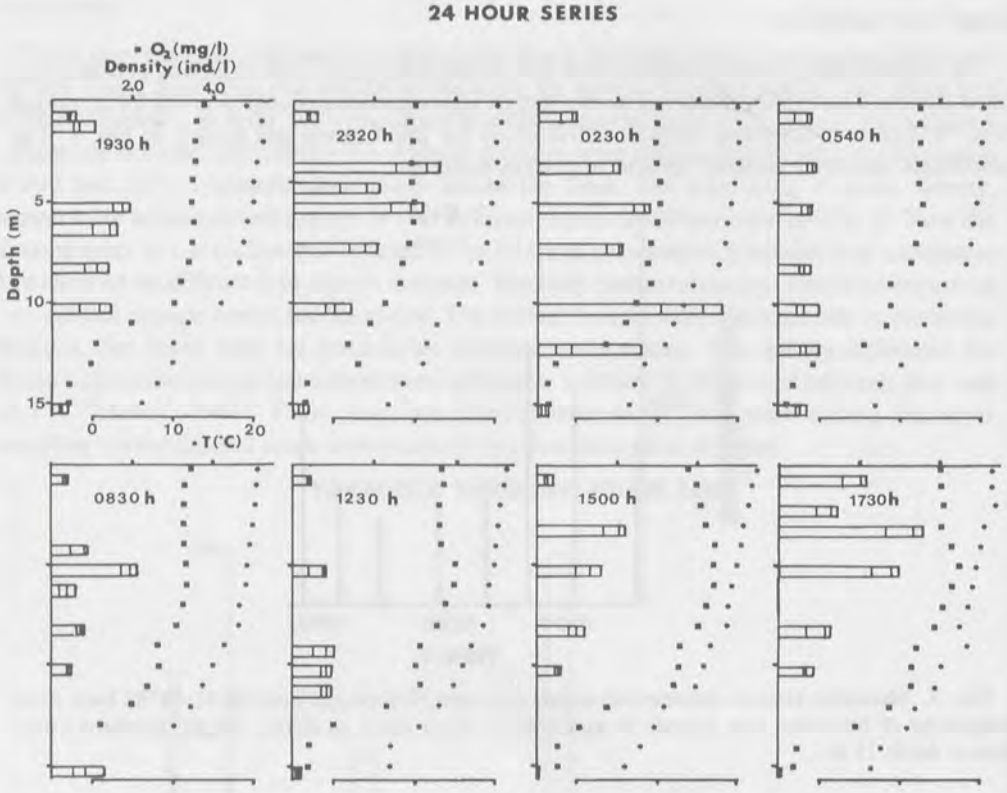

FIG. 8. Vertical profiles for *Artemia* density (bars), temperature (●), and dissolved oxygen (■) over the same 24 hr period as Fig. 7. Note that animal density (ind./l) and dissolved oxygen (mg/l) scales are numerically identical. Bars are divided into instar classes as in Fig. 5. Sunrise was around 0500h PST and sunset around 1930h. High densities at 1730h were associated with a foam-line.

also failed. I have found an *Artemia* variety near Fallon, Nevada, which can live in both Mono Lake and sea water. It is cross-fertile with the Mono Lake *Artemia*, but I have not yet raised the hybrids to maturity. There are marked differences between the cysts of these two varieties. Cysts from Nevada females float (as do those of most *Artemia*) whereas Mono Lake cysts sink in the same medium. Mono Lake cysts still sink when placed in lake water with a double concentration of its salts. Similarly, cysts produced by Mono Lake animals raised at this concentration sink. This is an ecologically very significant characteristic that appears well adapted to a stable lake. Floating cysts may be more appropriate to lakes with substantial seasonal fluctuations in level. One might speculate that the Nevada strain might thus supplant the present strain in a future Mono Lake of reduced and more variable size.

LIFE HISTORY

The life history, especially the reproductive cycle of Mono Lake *Artemia* appears to involve special ecological adaptations and is likely to be particularly sensitive to alterations in environmental conditions :

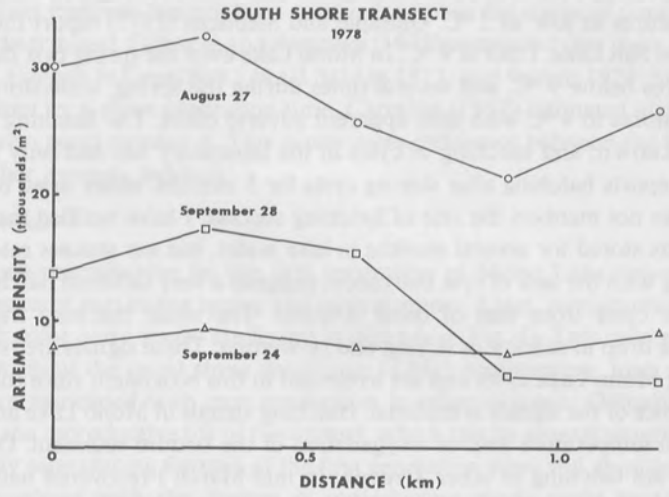

FIG. 9. *Artemia* adult density variation at five stations on three dates along a 1.2 km transect. Stations were at the 15 m depth contour in the south sector (Fig. 1 ; S-10 is the origin). Samples by net hauls from near bottom to surface.

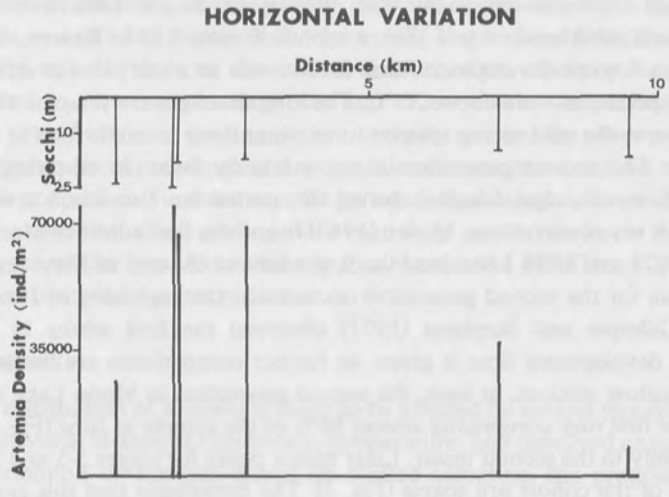

FIG. 10. *Artemia* adult density and Secchi disk variation along a 9 km transect from the West Bay to the region of the 1.2 km transect of Fig. 9 (= last station on right) on September 24, 1978. Note abrupt change in Secchi reading in the west.

Hatching of cysts

Artemia are present in Mono Lake throughout the winter, though in very low numbers, despite temperatures as low as 2 °C. Gillespie and Stephens (1977) report thermal death for *Artemia* in Great Salt Lake, Utah at 9 °C. In Mono Lake even the spring cyst hatch occurred at lake temperatures below 9 °C, and several times during the spring, snowstorms brought the surface temperatures to 4 °C with little apparent adverse effect. The hatching signal in Mono Lake is still unknown, and hatching of cysts in the laboratory has had only limited success. Mason (1967) reports hatching after storing cysts for 5 months, either dried or in lake water, although he does not mention the rate of hatching success. I have verified the hatchability of Mono Lake cysts stored for several months in lake water, but my success rate has been very low. This, along with the lack of cyst bouyancy, suggests a very different hatching mechanism for Mono Lake cysts from that of other *Artemia*. The usual hatching trigger for others involves either a drop in salinity or drying and re-wetting. These signals are relatively ineffective in hatching Mono Lake cysts and are irrelevant in this ecosystem since the cysts sink and natural occurrence of the signals is minimal. Hatching signals in Mono Lake are more likely to involve a rise in temperature and/or oxygenation of the bottom sediment. Oxygenation has been shown to aid hatching in other *Artemia*. In mid-March I recovered nauplii both in the West Bay and along the south shore, suggesting that cysts hatch either synchronously or that the young disperse rapidly throughout the lake. The first hypothesis is supported by the young age of the nauplii.

Development

The development of Mono Lake nauplii is very slow, approximately 70 days to maturity in 1979. Data from 1978 are consistent with this figure. In the laboratory, San Francisco *Artemia* can reach adulthood in less than a month (Heath, 1924 ; Bowen, 1962). Carpelan (1957) reported a 4 week development time for *Artemia* in a salt pond at Alviso, California, when water temperatures were above 20 °C. The long development time for *Artemia* in Mono Lake is likely due to the cold spring temperatures, since there is ample food at this time due to an algal bloom. The second generation is not markedly faster in maturing despite higher temperatures. However, algal densities during this period are also much lower.

In accord with my observations, Mason (1967) found the first adults in Mono Lake in early June. Both in 1978 and 1979 I obtained the first adults at the end of May, and in both years the naupliar peak for the second generation occurred at the beginning of June. In Great Salt Lake (Utah), Gillespie and Stephens (1977) observed the first adults in mid May. No information on development time is given, so further comparisons are limited.

In 1978 at shallow stations, at least, the second generation in Mono Lake seemed to be as numerous as the first one, comprising almost 50% of the sample in June (Fig. 2). However, it was abundant only to the second instar. Later minor peaks for stages 3-5 and 6-8 are evident, but stages 9-11 of the cohort are sparse (Fig. 3). The hypothesis that this generation died is unlikely, since in late June and early August I observed a further apparent peak of adults containing a mixture of younger and older animals, as determined by size. It seems more likely that second generation animals migrated to deeper, offshore water, where there may be more food (J. Melack, personal communication ; see discussion of vertical distribution).

My observations suggest that the summer population in Mono Lake is composed primarily of two generations. The possibility of a third generation in the late summer or fall, when the second generation matures, has not been resolved, nor has the status of young animals found at depth been determined. Gillespie and Stephens (1977) concluded that there were at least five generations of *Artemia* in Great Salt Lake (Utah) in 1973, and four in 1971. Shallow ponds are also characterized by a short generation time. Carpelan (1957) estimated eight generations of *Artemia* in Alviso pond number 6. This is one more difference between the Mono Make ecosystem and other *Artemia* habitats.

Reproductive mode

Ovoviviparous reproduction by the first generation of Mono Lake *Artemia* appear to be confined for the most part to late spring and early summer. Later, reproduction is primarily by cysts, although some early stages are found at depth (*e.g.* Fig. 8). This switch in reproductive mode occurs without the usual stress conditions of high temperature, high salinities, or low oxygen that are associated with cyst production in other *Artemia*. Oviparity is maintained apparently for the reproductive life of the animal, which can be several months, since large (*ca* 1.5 cm), actively reproducing females of the first generation were still abundant in September. One factor associated with the change in reproductive mode could be the drop in algal densities. By early July (1978) Secchi disk readings had reached 3.5 m, suggesting substantial depletion of the algal food source. From June to early July the shallow-water temperature was unchanged ; thus the change in reproductive mode may have been triggered by low food density.

GRAZING

The maturation of the first generation at the end of May corresponds to the sudden drop in algal densities as measured by Secchi disk. Mason (1967) speculates that this algal drop is due to grazing by *Artemia*. If this is so, the relative stability of the algal population prior to May could be ascribed to the smaller animal size and to lower lake temperatures, which could reduce grazing rates. However, grazing may not wholly account for observed algal densities. Correlation between Secchi readings and animal densities as measured on transects such as that of Fig. 10 is not perfect. Furthermore, the "winter" algal bloom still occurs in the presence of high *Artemia* densities, albeit grazing rates may drop as the lake cools in the fall. Mason (1967) ascribes the winter algal bloom largely to a massive die-off of *Artemia*, but in 1978, at least, this bloom preceded any such decline.

VERTICAL DISTRIBUTION

The vertical distribution of *Artemia* is likely to be affected by several physical and biological factors. Three physical factors, transparency, temperature, and dissolved oxygen, were related to vertical distribution of animals. Based on limited sampling, the progressive increase in depth of the animal density peak in spring and summer seems to correlate with the increase in transparency (Secchi reading, Fig. 5). However, when the Secchi trend reverses, there is no evidence that the animal peak follows. Mason (1966) described a negative phototaxis for Mono Lake *Artemia*. This is consistent with the usually low surface densities I observed, and with somewhat higher densities indicated for nocturnal periods (Fig. 8). Exceptions can

usually be correlated with foam lines (Fig. 6, and Fig. 8 at 1730 hr), sublacustrine springs, and plumes. Dana and Herbst (1977) report high *Artemia* densities near the surface through July, whereas Mason (1967) reports otherwise. He consistently found high *Artemia* densities between 10 and 15 m. The vertical profiles I obtained in July over a 24-hour period (Fig. 8) suggest that the distribution at a given date can be quite variable. This variability was also observed by Mason (1967), who attributed it to horizontal water movements. The discrepancies in vertical profiles could possibly also reflect chance variability, due to the small (3 l) sampling bottle used by the other authors.

Dana and Herbst (1977) concluded that the *Artemia* in Mono Lake avoid temperatures below 10 °C, and were therefore not found in deeper waters. My observations do not support this conclusion. I have consistently found animals at 10 °C or less, although in low densities, despite attempts to eliminate these as sampling artifacts. It is not clear whether the decrease in density with depth is a result of the low temperature, low oxygen, or some other factor.

At shallow stations there was no substantial difference in vertical distribution according to instar. The highest density for all instars was consistently found near the bottom. Mason (1967) reported on one occasion a peak of "nauplii" at 16 m on July 2, 1963. My samples from deep stations normally showed no marked differences in instar distribution (Fig. 5) down to 15 m. However, some deep samples had unusually high densities of young (Fig. 8, 0230 and 0830 hr). This latter observation has been corroborated by samples from 1979. The high concentration may correlate with high algal densities in the thermocline (Mason, 1967 ; Lovejoy and Dana, 1977 ; J. Melack, personal communication).

Patchiness

Mono Lake *Artemia* are striking in the horizontal variability of animal density. Of particular interest is large-scale variability, since this can greatly affect estimates of biomass, grazing impact, vertical distribution, and diurnal effects. My density estimates for 1978 are in accord with those of Mason (1967) for 1963, suggesting as yet little impact on this parameter from the dropping lake-level. My mean densities for late August and early September (1978), combining several measurements to minimize patchiness effects, are around 25 000/m^2. If extrapolated to the whole lake, this gives an estimate for total population of almost 4×10^{12} animals and a biomass of 3×10^9 g dry weight. A lake-wide survey in late July, 1979 confirmed the large-scale variability (densities ranging from 8 000 to 30 000/m^2) and gave a total population estimate about 2/3 of the August 1978 value. Mason (1967) reports a sharp drop in population in late August, and my own 1978 data show a similar drop in September for the Southern part (Fig. 9). However, this drop may have been caused by a redistribution of animals, as suggested by the transect of Fig. 10, rather than by a real drop in animal numbers. The structure of this large-scale density variability needs further investigation, but results such as those shown in Fig. 9 indicate that rather abrupt changes can occur over short distances (*e.g.* 0.2 km).

Summary

1. An ecological study was made of the *Artemia* in Mono Lake, California.

2. Overwintering cysts hatched in early March, and the first generation reached maturity in about 10 weeks.

3. The second generation, produced ovoviviparously, reached maturity in 8 to 10 weeks.
4. Only two generations were observed in the spring and summer seasons.
5. Analysis of vertical distributions revealed a peak in animal density which increased in depth from near surface in early spring to over 5 m in late summer.
6. Studies of population parameters were made more difficult by major patchiness in *Artemia* distribution.
7. Concentrations of animals in "foam lines" appear to relate to water-mass boundary phenomena.
8. Large-scale patchiness can involve lake-wide gradients.

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The use of *Artemia* cysts as food by the flamingo (*Phoenicopterus ruber roseus*) and the shelduck (*Tadorna tadorna*)

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Abstract

The aim of this study was to measure the digestibility of untreated dehydrated *Artemia* cysts fed to captive flamingos and shelducks and to determine the effect of the digestive process on the viability of cysts passed unbroken through the gut.

The percent by weight of broken cysts greatly increased as cysts passed through the guts of both flamingo and shelduck (from 6 % in food to 77 % in faeces in one trial). The percent assimilation of organic matter in the cysts (range of 5-36 %) implies that *Artemia* cysts may serve as food for these birds.

The hatching efficiency of intact cysts from faeces was not significantly different from that of non-ingested cysts in both flamingo trials and in one out of two shelduck trials. These results plus the retrieval of cysts in the faeces up to 38 hr after their ingestion indicate the importance of birds as vehicles for dispersion of *Artemia* genotypes between waterbodies.

In addition it was found that the ingestion of sand with cysts significantly reduced the viability of whole cysts passed in the faeces. The results were different for the shelduck and the flamingo : the assimilation efficiencies were generally lower for the former and a greater number of intact cysts were found in the faeces.

Introduction

Because of the commercial importance of *Artemia* harvested in salines around the world, the question has been raised whether or not these cysts have any natural consumers. Preliminary results of feeding ecology on the flamingo (*Phoenicopterus ruber roseus* Pallas) and the shelduck (*Tadorna tadorna* L.) in the Camargue salines, southern France, show that these birds feed on *Artemia* cysts when the latter are concentrated in scums by the wind. Cysts may indeed be an important though limited food source for these shelduck during the winter season (A. Johnson, M. Moser, J. Walmsley, personal communications). Similar feeding behavior has been reported by Savage (1967) for two species of shelduck (*T. tadorna* and *T. ferruginea* Pallas) feeding on lake Rezaiyeh in Iran. The high protein content (64 % in organic content, Nixon 1970) suggests that these cysts would provide a valuable food for birds able to capitalize on these scums. Horne's work (1966), showing that the cyst envelope is impervious and resistant to four avian enzymes : chitinase, trypsin, lipase and pepsin, together with reports of viable *Artemia* cysts from bird faeces (Löffler, 1964 ; Proctor, 1964 ; Proctor and

Malone, 1965) indicate that mechanical breaking of the cyst envelope is an important step in digestion.

Preliminary experiments were carried out on captive birds to evaluate : 1) the assimilation efficiency of organic matter by birds fed whole cysts only, 2) the effect of sand as an abrasive on assimilation efficiency when added to the cysts, 3) the fraction of broken cysts in the faeces and 4) the effect of the digestive process on the viability of cysts passed whole through the gut.

Methods and materials

Experiments were run on two flamingos (adult male, adult female) and two shelduck (one year old female, one year old male). The shelduck had been reared in captivity, while the flamingos were captured as adults, but had been in captivity for over 6 months before the start of the experiments and were accustomed to human proximity. Prior to testing, the birds were isolated individually, the flamingo in a 2 m × 3 m room with a 1 m × 2 m escape room and the shelducks in raised wire mesh cages, 0.75 m × 0.75 m × 1 m, with water and no food for 24 hr. After 24 hr, 70-100 g dry whole cysts were added to the water bowls and clean plastic sheets were placed on the floor and beneath the cages to facilitate faecal collection. When testing the effect of sand in the diet, 30 g coarse sand retained by a 250 μm sieve was added to the food. Food was removed after 8 hr to prevent cysts from hatching in the feeding trays.

Artemia cysts used for assimilation and hatching efficiencies were collected from different Camargue salines, and whole cysts separated and dried following Sorgeloos *et al.* (1978). Commercial cysts from southern France were used in measuring the fraction of broken cysts in the faeces.

Assimilation efficiency

Faeces were collected at regular intervals, taking care not to pick up any cysts spilled from the food tray, and examined to remove any noncyst debris and as much solid white uric acid-urea as possible. Any sand or grit was removed by swilling in freshwater and decanting the faeces, and the extra liquid was boiled down. If too heavily contaminated the dropping was rejected. Four subsamples of food and faeces each were separated into crucibles, dried at 90 °C for 3 days, weighed, then ashed in a muffle furnace ; first at 300 °C to reduce cyst explosion, than at 450-500 °C for 2 hr. The assimilation of organic matter was determined using Conover's formula (1966) :

$$U = \frac{F'\text{-}E'}{(1\text{-}E')F'} \times 100$$

U = percent assimilation (%)
F' = organic fraction in food
E' = organic fraction in faeces

This method was chosen as both species tended to walk in the trays and shake their heads when feeding, spilling food on walls and on the plastic sheets and thus making any quantification of food intake or defecation impossible. The use of glass or plastic beads as markers was avoided for their possible action as grit and preferential retention in the stomach. The introduction of an inert marker such as Cr_2O_3 would have required pulverization of the food

for a homogenous mixture (Billingsley and Arner, 1970), and the use of a naturally occurring marker such as magnesium (Moss, 1973 ; Green, 1978) required the use of an atomic absorbtion spectrophotometer which was not available.

Hatching efficiency

Subsamples of faeces were washed in a 100 μm sieve and oven dried for 24 hr at 30-35 °C, then for 48 hr at 55 °C. Broken cysts were separated from whole cysts by flotation in freshwater. Whole cysts were then floated off in saturated brine letting any mucus and other debris settle out (Sorgeloos *et al.*, 1978). Between 200-500 whole cysts were counted for six to 12 subsamples and incubated in 25 ml of sterilized seawater at 25 °C. Formalin was added after four days and the total number of hatched animals was noted : metanauplii, nauplii, free embryos (antennae not free) and partially excysted embryos. The hatching efficiency of non-ingested cysts taken from the feeding trays prior to removal from the cages was determined by the same method, as a control. Hatching efficiency, X %, was measured as :

$$X = \frac{\text{total number of cysts hatched}}{\text{total number of cysts incubated}} \times 100$$

Fraction of broken cysts in faeces

The degree of cysts broken as a result of digestion was measured as the dry weight of broken cysts in the faeces/dry weight of faeces. It was not possible to determine the actual number of cysts broken as did Löffler (1964) as neither food intake nor the number of broken cysts in the faeces could be quantified.

After 24 hr of starvation birds were fed whole cysts for a period of 8 hr, and the faeces produced were collected, washed in a 100 μm sieve and dried at 60 °C for 3 days. Four to six subsamples were weighed, the broken cysts floated off in freshwater, and the whole cysts in brine. The broken and whole fractions were again dried at 60 °C for 3 days and weighed.

Duration of cyst retention

After the last determination of assimilation and hatching efficiencies, one flamingo and one shelduck were retained in isolation and fed on rice for 24 hr, on cysts for 24 hr, then again on rice. Hourly faecal collections were made up to 38 hr after the birds were returned to a diet of rice, and the presence or absence of cysts in the faeces was noted.

Results

Assimilation efficiency

The results of the assimilation efficiency trials for flamingo and shelduck are presented in Table I. The flamingo assimilated an average of 34.7 % organic matter when fed cysts alone. The average dropped to 19.1 % when sand was added to the food. The percent assimilation by the shelducks was more variable and notably less than that by the flamingo ($\bar{x}$ females, 13.0 % ; $\bar{x}$ males, 20.5 %).

TABLE I

Assimilation efficiency (%) of organic matter

Food	Male flamingo	Shelduck	
		Females	Males
Cysts	34	21	8
Cysts	36	5	33
Cysts			21
Cysts + sand	11		
Cysts + sand	27		

HATCHING EFFICIENCY

No significant difference was found between the hatching efficiency of whole cysts from the faeces of flamingo fed cysts only and that of non-ingested cysts (Table II). However, when the flamingo was fed cysts with sand, the hatching efficiency of faecal cysts was significantly less ($P < 0.001$) than that of the food.

The shelducks again gave varying results. The hatching efficiency of cysts from the faeces of both was not significantly different ($P > 0.25$) in one test, but significantly lower ($P < 0.001$) than that of food in the second trial.

TABLE II

Comparison of hatching efficiencies of whole cysts in faeces and food
(Significance tested by values of t calculated on arc sine transformed data, Sokal and Rohlf, 1972)
NS = not significiant, S = significant, n = sample size

Food type	Flamingo			Shelduck faeces (%)				
	Food (%)	faeces (%)	Significance	Food (%)	Females	Significance	Males	Significance
Cysts	42	41	NS $P > 0.05$ $n = 12$	15	14	NS $P > 0.25$ $n = 24$	15	NS $P > 0.25$ $n = 23$
Cysts	11	8	NS $P > 0.05$ $n = 12$	37	21	S $P < 0.001$ $n = 24$	18	S $P < 0.001$ $n = 24$
Cysts + sand	36	22	S $P < 0.001$ $n = 22$	–	–		–	
Cysts + sand	41	29	S $P < 0.001$ $n = 25$	–	–		–	

FRACTION OF BROKEN CYSTS IN FAECES

Flamingo faeces consisted of an average 74% by dry weight broken cysts and the shelduck 51% (Table III). The original food itself contained only 8% by weight broken cysts. Again the shelduck showed much greater variation in the results than the flamingo.

TABLE III

Percent broken cysts by dry weight in food and faeces.
Confidence intervals (CL) were calculated from values of t obtained from arc sine transformed data

Trial	Food (%)	Flamingo female (%)	Shelduck male (%)
I	6	77	45
II	7	69	37
III	10	78	51
IV	10	70	70
$\bar{x}$	8	74	51
+ 95% CL	4	8	25
− 95% CL	3	9	25

DURATION OF CYST RETENTION

The flamingo produced faeces consisting only of cysts until 3 hr after being returned to a diet of rice and the shelduck until 2 hr after the change. Both birds continued to pass intact cysts in low numbers until at least 38 hr after the return to rice.

Discussion and conclusion

The method used to determine assimilation efficiency is based on the assumption that the inorganic fraction in the food is inert and remains unassimilated. This assumption is not entirely true since the birds are almost certainly assimilating a small proportion of the inorganic matter to satisfy their basic nutritional requirements for minerals and salts (Marshall, 1960 ; Farner and King, 1972). Because the ash content of *Artemia* cysts was found to be only 9-10%, estimated assimilation efficiencies would be sensitive to any small changes in the inorganic content in faeces. It is unlikely that metabolic wastes affected the inorganic content since urine was typically excreted as a solid white layer around the faeces and was easily removed, or frequently excreted separately in the case of the flamingo. Any remaining uric acid and NH_3 would volatilize before 90 °C and not be included in the dry weight, and any urea (comprising only 0-10% of nitrogenous wastes in wildfowl and ducks, Farner and King, 1972) would volatilize at 132 °C and not be included in the ash weight (Hodgman *et al.*, 1960). It is thus felt that any error in the inorganic content in the faeces is likely to be due to absorption during digestion and result in an underestimate of assimilation.

When fed a mixture of cysts and sand the flamingo actually ingested all 30 g of sand the first trial and 20 g the second. The ingestion of heavy quantities of sand has never been reported in wild though Allen (1956) reports finding flamingo stomachs containing mud and gravel. The decreased assimilation efficiency in these two trials may result from a physiological response of the bird to an abnormally high concentration of sand in the gut, perhaps increasing the passage rate to clear the gut and thus reducing the time of digestion. It is also possible that there was a large increase of organic matter in the faeces from a stimulated mucous production or abrasion of the mucous membranes by so much sand.

From these studies it can not be assumed that shelducks are less efficient in digesting *Artemia* cysts than flamingos. Shelducks have been reported to feed much more frequently on scums of cysts in the wild than flamingos (A. Johnson, J. Walmsley, personal communicatijon ; Savage, 1967). The lower values of the shelducks and the greater variation of the results may be due to the possibility that these birds are more sensitive to human handling and isolation than the flamingos.

The large fraction of broken cysts in the faeces indicates that the cyst envelope does not remain a barrier to the digestive enzymes for the majority of cysts ingested. These results are in agreement with Löffler's (1964) report that only 10% of the number of *Artemia* cysts fed to a male mallard via a gelatin capsule were retrieved intact in the faeces. The results in Table III represent percent dry weight, not individual broken cysts, and a figure of 74% may well represent 90% or more of the total number of cysts ingested. The degree of fragmentation of the cysts would depend on the muscularity of the stomach and the retention of grit by the particular species. Both shelducks and flamingos have strong muscular stomachs with ridged keratinous linings. The stomach of one flamingo from the Camargue contained 8.45 g of shell fragments, seeds and small stones. Rooth (1965) reports 300-900 small stones, 7.0-0.5 mm in diameter, in stomachs of the West Indian flamingo (*P. ruber ruber* L.) on Bonaire.

Values of 34.7% assimilation for the flamingo and 20.5% for the shelduck are low for animal matter, and even when compared to similar values (20%-80%, $\bar{x}$: 53%) for plant matter obtained from Billingsley and Arner's (1970) data on digestion of foods by wild turkey. These low values may be due to underestimations because of the method, a high proportion of undigestible cyst shells and a limited embryo surface area exposed to digestive enzymes due to partially intact cyst shells ; they should be considered as minimum possible values for these species. Nonetheless these values are high enough, especially in the light of the high protein and lipid contents of the eggs, for *Artemia* cysts to be considered an acceptable food for both flamingos and shelduck.

The different hatching efficiencies of cysts retrieved from the faeces of the shelducks are difficult to interpret. Both individuals showed the same pattern, but except for the different types (sources) of cysts used there was no difference in method or treatment of cysts or birds. This may simply be a result of individual variation or general nervousness or tension caused by isolation, perhaps heightened by the fact that the two birds had recently formed a pair bond for mating.

The results of hatching efficiency tests on the flamingo fed only cysts confirm the resistance of intact cysts to digestive enzymes as demonstrated in part by Horne (1966). The ingestion of 20-30 g of sand with cysts clearly decreased the viability of intact cysts retrieved from the faeces. This might be due to fine scoring and puncturing of the cyst envelope by the sand, exposing the embryo to gastric juices. However, the cyst shells remained sufficiently intact for these cysts to sink in freshwater after being dehydrated. While it is clear that cysts can pass through the digestive system of a bird still viable, there are certain digestive conditions which effectively decrease the viability of intact cysts.

The importance of birds in the passive dispersal of encysted eggs and spores through faeces has been discussed by Löffler (1964), Proctor (1964) and Proctor and Malone (1965). Their conclusions are supported both by the results of the hatching efficiency tests and the ability of cysts to be retained in the birds system up to at least 38 hr after being ingested. Local movements of both flamingos and shelduck as well as migration of the flamingo between the

African and European Mediterranean coasts may be important in the dispersal of *Artemia* among the saline lagoons of the Mediterranean.

Summary

1. The flamingo (*Phoenicopterus ruber roseus*) can assimilate up to 36% organic matter and the shelduck (*Tadorna tadorna*) up to 33% in diets of whole dehydrated *Artemia* cysts.

2. Faeces of a flamingo fed cysts only, consisted on an average of 74% broken cysts by dry weight. The shelduck faeces averaged 50% broken cysts and showed much greater variation than the flamingo.

3. Hatching efficiency of cysts passed whole through the flamingo neither increased nor decreased, but the ingestion of 20-30 g of sand with the cysts significantly decreased the viability of whole cysts from the faeces.

4. Hatching efficiency of intact cysts from shelduck faeces were significantly lower than that of non-ingested cysts in one out of two trials.

5. The flamingo passed droppings entirely made up by cysts up to 3 hr and the shelduck up to 2 hr after cysts were replaced by rice. Both birds continued to pass cysts in the faeces up to 38 hr after food was changed.

Acknowledgements

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Ecology of *Artemia* in the salt pans of Tuticorin, South India

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Abstract

The present report records the occurrence and distribution of the brine shrimp *Artemia* in the salt pans of Tuticorin, South India. The environmental parameters salinity, temperature, oxygen, pH, particulate carbon, and the nature of the sediment, which influence the distribution of *Artemia*, have been studied. The evolution of the biota occurring in the salt pans, including the bacterial population has been investigated in relation to the brine shrimp population. The ecological significance of brine shrimp in this unique biotope is discussed.

Introduction

The brine shrimp *Artemia* is widely used in aquaculture and the nauplii of this species are employed extensively world over as life food to raise the larvae of crustaceans and fishes. With the stagnating yields of fisheries and the continuously increasing costs of the catches, scientists are setting high hopes on aquaculture practices. In India, with its vast coastline of 5 700 km and a score of potential cultivable finfishes and shellfishes, the perspectives for aquaculture are most promising. As a result there will be a great demand for *Artemia* cysts in this country. Hence, to understand the potential resources of brine shrimp, a detailed survey of saline lagoons and salt pans becomes essential.

In India, *Artemia* has been reported from Bombay (Kulkarni, 1953), Sambhar Lake (Baid, 1968) and the Tuticorin area (Royan *et al.*, 1970 ; Achari, 1971). The above reports excepted, nothing is known about the ecology of this crucially important animal in India. The present paper provides information on the occurrence of *Artemia* in two salt pans of Tuticorin.

Material and methods

Occurrence of *Artemia* around Tuticorin

Royan *et al.* (1970) recorded the occurrence of *Artemia* only at Veppalodai and Achari (1971) found the species in Karsewar Island. During the present survey made around Tuticorin, *Artemia* populations as well as eggs were found in the following five areas (Fig. 1).

Two of the five stations around Tuticorin (latitude 8° 50' N, longitude 78° 8' E) were selected for a detailed study lasting from July 1978 to June 1979.

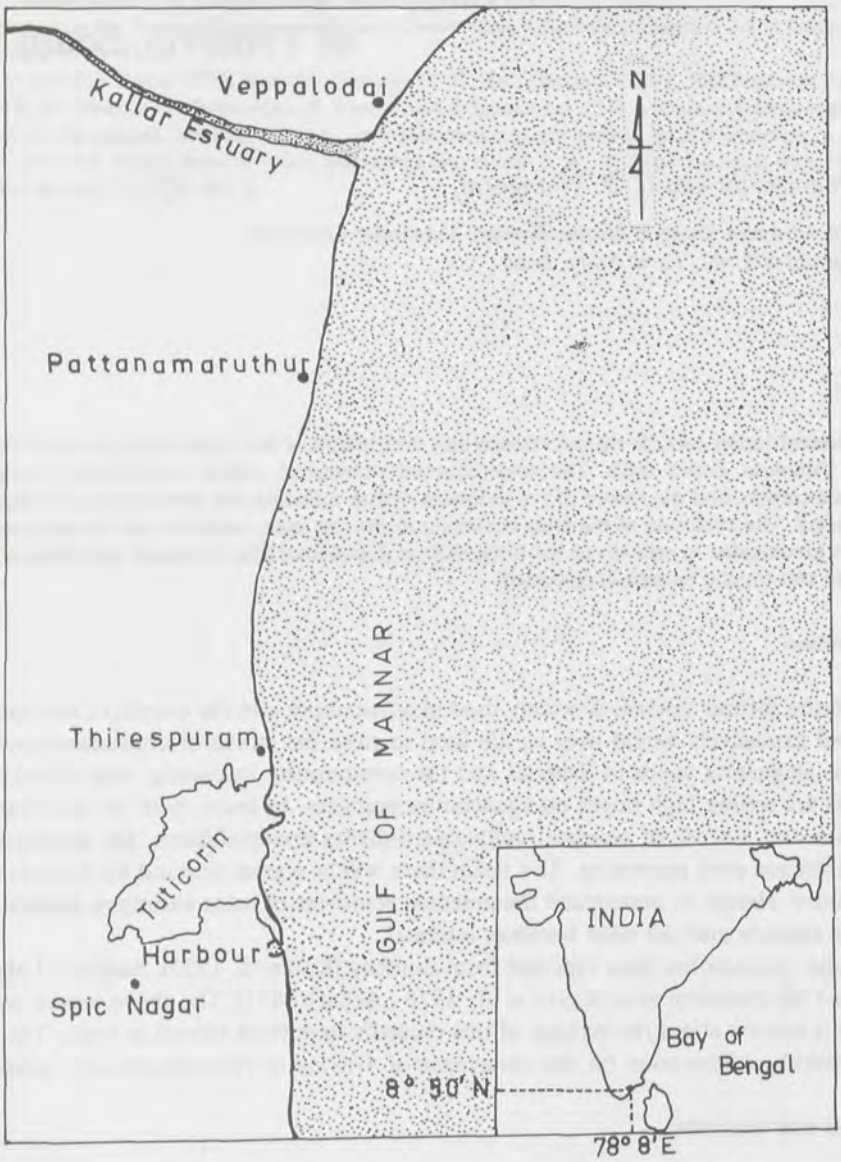

FIG. 1. The locations of study area. a) Salt pans near harbour (Station 1); b) salt pans around Veppalodai (Station 2) – 25 km from Station 1 ; c) salt pans near Pattanamaruthur (Station 3) – 18 km from Station 1 ; d) salt pans near Thirespuram (Station 4) – 5 km from Station 1 ; e) salt pans around Spic Nagar (Station 5) – 3 km from Station 1.

Description of the study area

Station 1, a salt pan near the harbour, covers an area of 0.15 ha with a mean depth of 1.5 m. Station 2 is a salt pan near Vappalodai.

Vappalodai is located about 25 km from Station 1. This station has an area of 0.4 ha with an average depth of 0.5 m. The bottom sediments are composed of a mixture of greyish clay and sand up to 5 cm thick. Below this stratum the clay is hard and black in colour. The alga *Chaetomorpha brachygonia* forms a layer on top of the substratum. When the salinity increases (> 100%) the algal bed is gradually replaced by gypsum.

Monthly collections were made in both stations. The physico-chemical parameters studied were : temperature, pH, dissolved oxygen concentration, and particulate carbon in the water, and organic carbon in the mud. As biological parameters phytoplankton and zooplankton including *Artemia* and bacterial biomass were studied. Temperature measurements were made using a conventional celsius thermometer. Salinity, dissolved oxygen concentration, and particulate carbon were estimated following the standard procedures of Strickland and Parsons (1972). The pH was determined with a Elico Model LI-10 pH meter. Organic carbon in the mud was estimated with the method of El Wakeel and Riley (1956).

For the determination of phytoplankton density a 1 l water sample was fixed with 10% formalin and allowed to settle for 48 hr. The supernatant was decanted at the aid of a siphon and the sedimented material analysed using Utermohl's inverted microscope.

For zooplankton analysis 200 l of water were filtered through a net (No. 10 bolting silk) and the animals counted. For the microzooplankton component, the organisms encountered were enumerated during the phytoplankton analysis. Plankton biomass was estimated and expressed in terms of number of cells or individuals/l.

For the estimation of the heterotrophic bacterial biomass of water and sediments the pour plate technique was employed with ZoBell's 2216^e medium. Random bacterial colonies were selected and identified to the generic level using the scheme proposed by Simidu and Aiso (1962).

Results

Artemia population density

The *Artemia* population showed a clearcut seasonal trend in the two stations (Fig. 2 and 3). The population density varied between 0 (November to June) and 15 ind./l (August) at Station 1 and from 0 (July to December) to 1.9 ind./l (April) at Station 2.

Physicochemical parameters

Table I shows the values for temperature, salinity, oxygen, pH, and particulate carbon in water and organic carbon in mud in the two stations.

The surface temperature ranged from 27.0 °C (November) to 35.5 (June) at Station 1 and 28.0 °C) (November) to 36.5 °C (July) at Station 2.

Salinity ranged between 15.9 ‰ (November) and 151.2 ‰ (August) at Station 1 and from 42.6 ‰ (December) to 221.1 ‰ (August) at Station 2. The decrease of salinity can of course be correlated with the onset of the monsoon season (October-December) (Fig. 2 and 3).

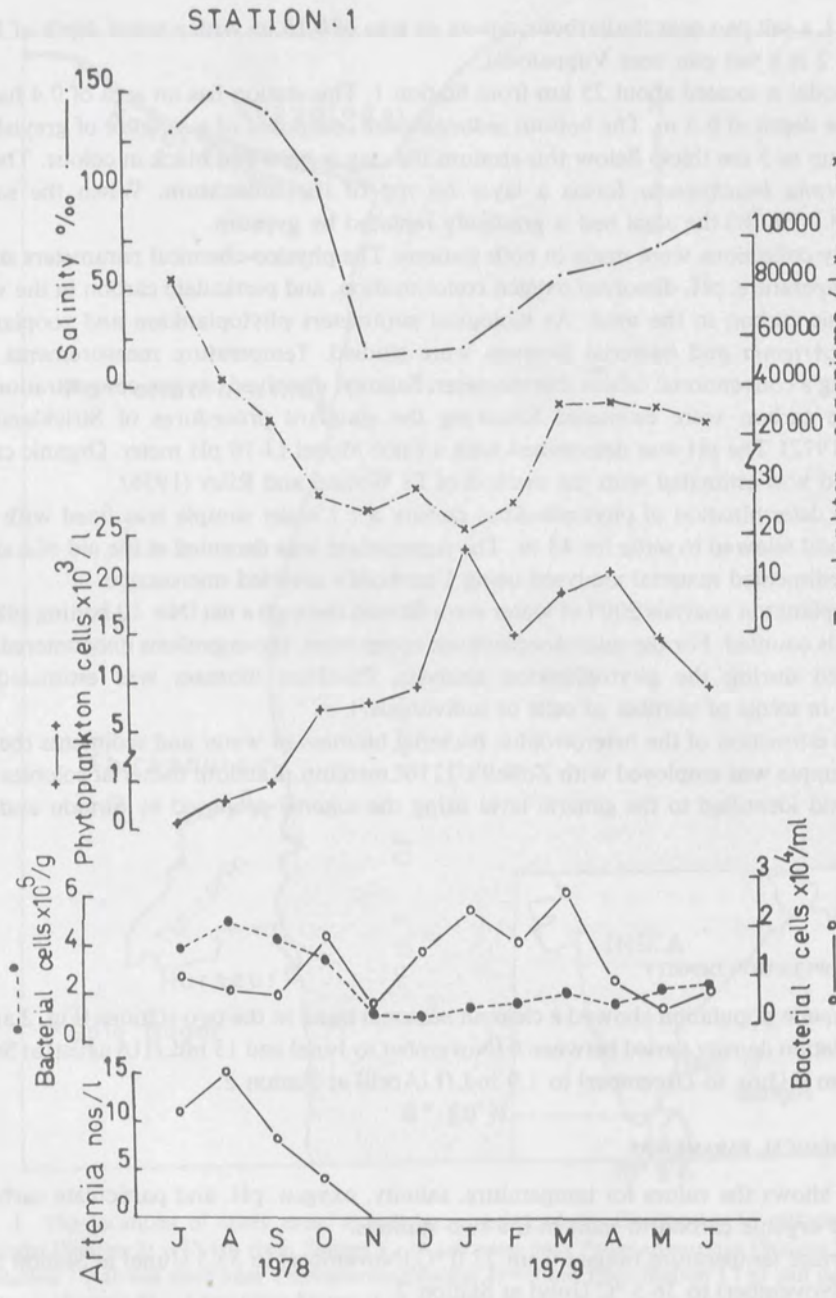

FIG. 2. Seasonal variation of *Artemia*, bacterial population, phytoplankton, zooplankton, and salinity.

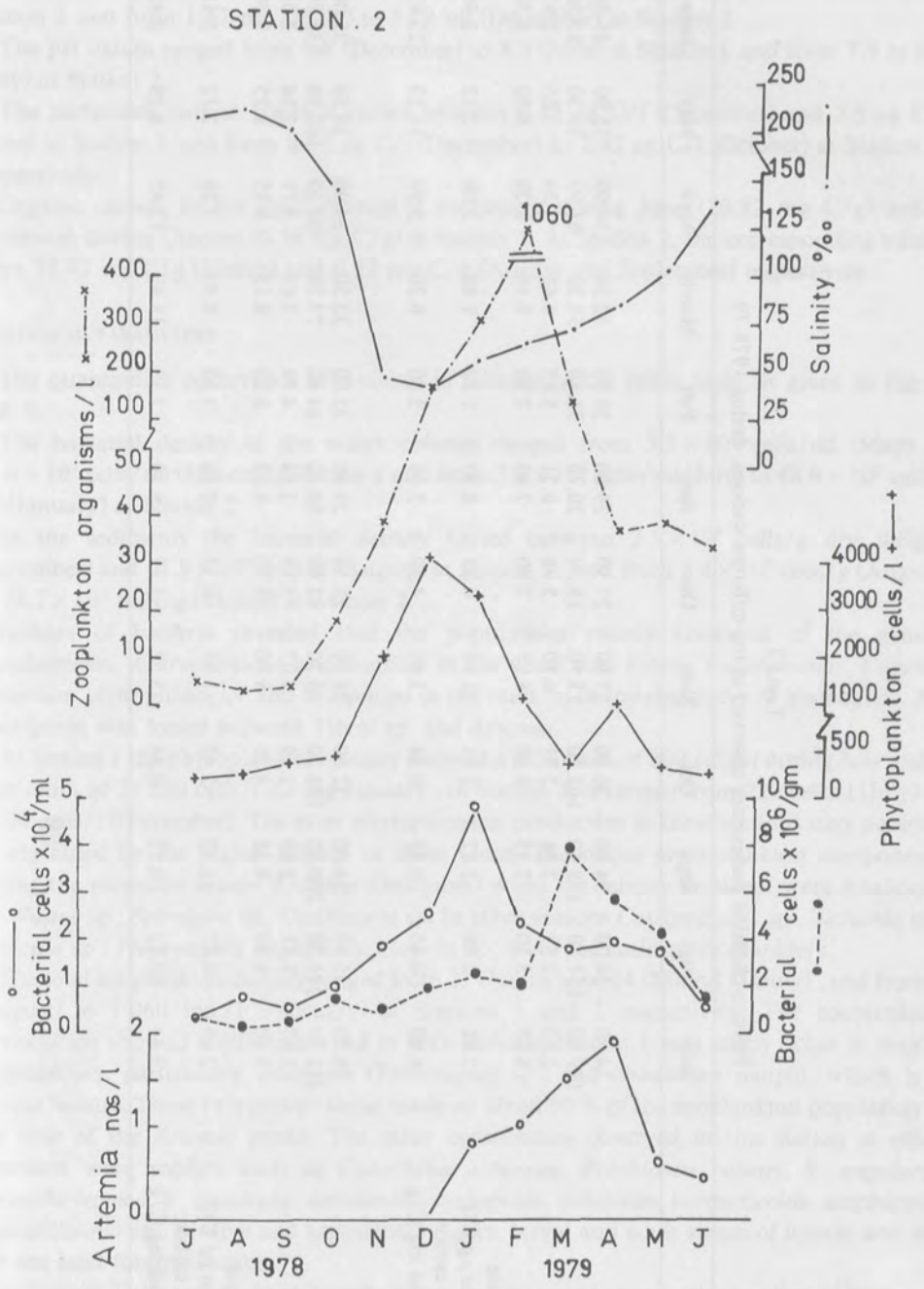

FIG. 3. Seasonal variation of *Artemia*, bacterial population, phytoplankton, zooplankton, and salinity.

TABLE I

Physico-chemical parameters observed in the different stations, during 1978-79

	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June
Station 1												
T (°C)	34.50	34.00	34.00	30.00	27.00	27.50	29.00	30.00	32.50	34.00	35.00	35.50
S (‰)	129.10	151.20	138.40	105.70	15.90	18.30	20.70	36.80	54.20	61.90	70.50	83.30
O_2 ml/l	2.03	2.59	2.26	2.71	6.10	5.98	6.21	5.65	4.68	3.39	3.05	2.93
pH	8.05	8.10	7.85	8.00	8.05	7.80	7.85	7.90	8.10	8.20	8.30	8.30
Particulate carbon μg C/l	2.01	2.08	2.12	2.11	1.18	0.83	0.97	1.12	1.42	2.08	2.12	2.50
Organic carbon in mud mg C/g	6.90	0.45	0.45	0.34	3.60	4.50	5.06	5.50	6.20	5.90	14.23	23.92
Station 2												
T (°C)	36.50	35.50	34.00	32.00	28.00	28.50	29.00	32.00	33.50	34.00	34.50	35.00
S (‰)	211.30	221.10	200.80	166.20	46.70	42.60	56.30	64.40	73.70	84.20	101.90	138.70
O_2 ml/l	1.67	1.81	2.03	2.59	4.07	5.19	3.84	4.74	3.61	3.71	2.14	2.03
pH	8.40	8.35	8.25	8.20	8.00	7.90	8.05	8.20	8.15	8.15	8.25	8.30
Particulate carbon μg C/l	2.09	0.97	1.52	2.42	1.18	0.53	2.16	1.52	0.97	2.38	2.12	1.18
Organic carbon in mud mg C/g	3.44	0.22	0.22	0.34	5.52	5.97	6.30	7.13	18.42	13.45	8.29	0.45

Dissolved oxygen concentration varied between 2.03 ml/l (July) and 6.21 ml/l (January) at Station 1 and from 1.67 ml/l (July) to 5.19 ml (December) at Station 2.

The pH values ranged from 7.8 (December) to 8.3 (June) at Station 1 and from 7.9 to 8.4 (July) at Station 2.

The particulate carbon fraction varied between 0.83 μg C/l (December) and 2.5 μg C/l (June) at Station 1 and from 0.53 μg C/l (December) to 2.42 μg C/l (October) at Station 2, respectively.

Organic carbon in the mud showed a maximum during June (23.92 mg C/g) and a minimum during October (0.34 mg C/g) at Station 1. At Station 2, the corresponding values were 18.42 mg C/g (March) and 0.22 mg C/g (August and September) respectively.

Biological parameters

The quantitative occurrence of *Artemia* in relation to the other biota, is given in Fig. 2 and 3.

The bacterial density in the water column ranged from 3.5×10^3 cells/ml (May) to 27.0×10^3 cells/ml (March) at Station 1 and from 3.0×10^3 cells/ml (July) to 48.0×10^3 cells/ml (January) at Station 2.

In the sediments the bacterial density varied between 2.1×10^5 cells/g dry weight (December) and 41.9×10^5 cells/g (August) at Station 1 ; and from 2.4×10^5 cells/g (August) to 78.7×10^5 cells/g (March) at Station 2.

Isolates of bacteria revealed that the populations mainly consisted of the genera *Pseudomonas*, *Achromobacter* and *Bacillus* in the water and *Vibrio*, *Pseudomonas*, *Corynebacterium*, *Achromobacter* and *Cytophaga* in the mud, in decreasing order of abundance. An association was found between *Vibrio* sp. and *Artemia*.

At Station 1 the phytoplankton density showed a minimum of 100 cells/l during July and a maximum of 23 500 cells/l during January ; in Station 2, it ranged from 220 cells/l (July) to 4 100 cells/l (November). The poor phytoplankton production in these stations may possibly be explained by the higher salinity in these areas. The major phytoplankton components during the monsoon season (October-December) when the salinity declined, were *Anabaena* sp., *Nostoc* sp., *Spirogyra* sp., *Oscillatoria* sp. In other seasons *Coscinodiscus* sp., *Nitzschia* sp., *Navicula* sp., *Pleurosigma* sp. and *Oscillatoria* sp. were the main phytoplankters.

The total zooplankton density ranged from 21 (January) to 84 000 ind./l (July) ; and from 3 (August) to 1 060 ind./l (February) at Stations 1 and 2 respectively. The zooplankton composition showed a different trend in both stations. Station 1 was much richer in microzooplankters, particularly tintinnids (*Tintinnopsis* sp.) and crustacean nauplii, which is a unique feature. These two groups alone made up about 90 % of the zooplankton population at the time of the *Artemia* peaks. The other components observed in this station at other moments were rotifers such as *Conochilus arboreus*, *Brachionus rubens*, *B. angularis*, *Keratella tropica*, *K. quadrata*, nematodes, cyclopoids, calanoids, harpacticoids, amphipods, *Grandidieralla* sp., bivalve and gastropod veligers, larval and adult stages of insects and last but not least foraminiferans.

At Station 2, the zooplankton density was generally poor in comparison to that of Station 1. The chief components observed were crustacean nauplii, harpacticoids, calanoids, nematodes and larval and adult stages of insects.

Further studies in this line are now in progress to understand more about the biology and ecology of this commercially important species in India.

Discussion and conclusions

The present investigation in the Tuticorin salt pans revealed many interesting features.

In both stations a definite relationship can be observed between the evolution of the salinity in the biotopes (which is linked to the climatological conditions, in this case the monsoons) and the occurrence of *Artemia*.

Though brine shrimp were found in both stations in a wide range of salinities (from 56.3 to 151.2 ‰) the population peaks were each time encountered at salinities above 100 ‰.

This corroborates earlier studies made in this area by Royan *et al.* (1978) (see Table II). Baid (1968) reported peaks of *Artemia* at salinities around 80 ‰ in Sambhar Lake.

TABLE II

Peak period of occurrence of *Artemia* in different localities of India

Locality	Period of occurrence	Peak	No./l	Salinity (‰)	Reference
Sambhar Lake, North India	September to April	January	57	28-164	Baid (1968)
Bombay, North India	–	–	–	160	Kulkarni (1953)
Veppalodai, South India	June	–	9	138-188	Royan *et al.* (1978)
Veppalodai, South India	January to June	April	2	56-139	Present study
Tuticorin, South India	July to October	August	15	129-151	Present study

Royan *et al.* (1978) reported the presence of offspring at higher salinities (130 to 160 ‰) ; in this study we found egg-bearing adults and nauplii in salinities from 56 to 101 ‰.

The interpretation of the quantitative evolution of *Artemia* populations in a particular biotope is most complex. The changes in density are indeed function on one hand of the evolution of the physico-chemistry of the salt pan and not at least the salinity changes ; on the other hand they are also basically dependent of the amount of food, and the presence or absence of a specific *Artemia* predating fauna.

As far as the food factor is concerned, *Artemia* which is a unselective particle-feeder, thrives on living (algae-bacteria) as well as on dead (organic detritus) materials of biological origin provided the latter have the size range suitable to be ingested.

Last but not least, depending on the prevailing salinity, the development of brine shrimp can be influenced by other filter feeding zooplankton competing for food.

It is thus clear that the quantities of *Artemia* found in a particular biotope at a particular moment are the resultant of a complex physico-chemical and biological multivariable.

We shall try to pinpoint some of the factors, which, in our opinion, have influenced the evolution of the *Artemia* populations as they were recorded during the course of our study.

STATION 1

An important brine shrimp population is present from July to October 1978 the disappearance of which can probably be explained by the sharp decrease in salinity due to the onset of the moonsoon and the resulting appearance among others of predatory insects in the biotope grazing down the *Artemia* (Kaestner, 1970).

An inverse relation seems to occur between the quantity of zooplankton and *Artemia* on one hand, and the phytoplankton density on the other : the microzooplankton, particularly tintinnids and crustacean nauplii indeed show high densities at the time of the *Artemia* peak but like the brine shrimp these organisms decrease sharply in number when the salinity drops.

After a period of low numbers the zooplankton quantity increases again, apparently in association with the increase in salinity of the water.

The phytoplankton evolves exactly the opposite way : there was but a very low quantity of phytoplankton present at the start of our study. The primary producers increased, however, steadily in number to reach a maximum when all *Artemia*'s had disappeared and when the rest of the zooplankton was at a minimum.

STATION 2

A first striking statement is that the evolution of the *Artemia* population in this station is the reflection of that in Station 1. Whereas in the former the brine shrimp die off completely during the monsoon period and do not reappear again (quantitatively speaking), the *Artemia* population only shows up in February, increasing steadily in importance till April, in parallel with the salinity increase of the biotope.

Noteworthy is that the general trend of the salinity curve in both stations is analogous, but that the water in Station 2 is always much more saline than in Station 1 ; the minimum salinity in December is still above 40 ‰.

As in Station 1 the phytoplankton curve follows a pattern inverse to that of the salinity with a maximum in December. The primary production is, however, as far as we look at the number of cells, at least one order of magnitude lower than in Station 1. In this second station too the *Artemia* food is most probably constituted to a large extent by bacteria and organic particulate matter. Contrary to Station 1 the zooplankton is minimal during the period of highest salinity and reaches a peak after the rainy season. As mentioned in the results the greater richness of Station 1 in zooplankton is due to a large extent to a blooming of tintinnids.

A more detailed interpretation of the relationship between the *Artemia* populations and the other biota on one hand, and the quantitative evolution of the brine shrimp populations in relation to the physico-chemistry is not possible without taking into consideration the dynamics (flow) of the water in the different salt pans which influence to a very large extent the evolution of the biocenoses.

Summary

The occurrence, distribution and seasonal abundance of *Artemia* have been investigated in two salt pans near Tuticorin during the period of July 1978 through June 1979. *Artemia* was found from July to October at Station 1, and from January to June at Station 2.

The maximum population of *Artemia* at Station 1 was 15 ind./l and at Station 2, 1.9 ind./l.

The seasonal fluctuations of the physico-chemical parameters salinity, temperature, dissolved oxygen concentration, pH, particulate carbon in the water and organic carbon in the mud were studied.

The evolution of the *Artemia* population is discussed and interpreted in correlation with the evolution of the physico-chemistry of the biotopes and that of their biota.

The rich bacterial population in the water and the sediments and their high organic content are probably the main source of food for *Artemia*.

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Ecological study of the *Artemia* populations in Boca Chica salt lake, Margarita Island, Venezuela

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Abstract

This paper is the first to record the occurrence of the brine shrimp *Artemia* in Boca Chica salt lake in the Macanao Peninsula of Margarita Island, Venezuela.

This ecological study, made from January to June 1979, relates on the presence, the abundance, the distribution, and the fluctuations of different stages of development of *Artemia* in relation to the hydrological conditions in different parts of the lake.

Wind direction, temperature, salinity, pH, dissolved oxygen, inorganic nutrients, and primary productivity were measured.

Plankton samples were taken at different points and the number of *Artemia* nauplii, juveniles, and adults counted.

The number of eggs per female, the number of setae in the furca, and the size of the adult animals were determined on a fraction of the populations sampled.

Other biota were also investigated, such as larval and adult insects, small fishes and even birds.

The highest density of *Artemia* was found in the SW area of the lake, which can be attributed to lower salinity and temperature, higher oxygen content, and a higher productivity in comparison to the rest of the lake. Population densities varied with depth and distance to the shore, under the influence of wind and drift currents.

Introduction

The brine shrimp *Artemia* is one of the main natural resources used as high quality live food in aquaculture. Especially nauplii and more recently decapsuled eggs of this species are of vital importance for the larval and juvenile stages of crustaceans (shrimps, lobsters, crabs) and fishes which need small living organisms as food.

According to Sorgeloos (1979), the increasing demand of good quality cysts is exceeding the offer, with the expansion of mariculture and might lead to a serious bottle-neck in aquacultural efforts. Scientists are being encouraged to search for natural populations of brine shrimps in their respective countries in order to decrease the high cost of importation of the cysts necessary for the aquacultural industries.

Venezuela has recently initiated programs to promote the culturing of marine shrimps and fishes. Most of these studies were started with juveniles captured from nature (Scelzo *et al.*, in press). Since the next step in shrimp culture will be the development of spawning techniques in controlled conditions, substantial quantities of *Artemia* eggs will be required which will cause serious financial implications.

In Venezuela there are several salt lakes isolated from rivers and from the sea. Most of these lakes are used for commercial production of salt (*e.g.* in the Araya Peninsula) or for local consumption (*e.g.* in Margarita Island and other islands of the Los Roques Archipelago). These environments are suited for the development of natural populations of brine shrimp, especially thanks to the particular environmental characteristics and the absence of predators. The presence of *Artemia* has already been recorded from some of these sites and dry eggs have been collected and sold to aquariologists or have been utilized for experimental purposes in aquaculture. Unfortunately, no ecological study on *Artemia* has been carried out so far in any of the Venezuelian salt lakes or salinas.

To our knowledge, only Sanders (1936), Kristensen (1971), and Kristensen and Hulscher-Emeis (1972) have studied the ecology of *Artemia* populations in Antillean salinas, more specifically in Curaçao, Bonaire and St. Martin, near Venezuela.

This paper is the first ecological study of the brine shrimp *Artemia* in Venezuela, more particularly in Boca Chica salt lake on Margarita Island.

Habitat

Boca Chica salt lake is located in the Macanao Peninsula in the SW area of Margarita Island, (Fig. 1). It has an irregular "V" shape in the east-west direction and covers a surface ranging from 13 to 25 ha (depending on the "dry" and "wet" seasons). The lake is situated about 1 to 2 m below sea level, has a maximum length of about 800 m, a maximum width of 300 m, and a maximum depth of about 4 m near the center of the lake. The bottom sediments vary in composition : black mud and sand in the NW and E, sand in the SW, stones, pieces of dead coral, and mollusc shells in the W and sand plus stones in the S. During dry periods shallow parts of the lake dry out and a firm crust of salts covers the bottom, particularly in the W and NE areas.

The lake is totally isolated from the Caribbean sea located 300-400 m towards the south. In the small landstrip between the lake and the sea, two small "salinas" are found, the largest is used for salt extraction by local people. Seawater filters into the S and SW areas of the lake. Freshwater only comes in by run-off of rain from the surrounding hills in the NW and NE areas.

Materials and methods

This study was carried out during the dry season of 1979 (January-June) ; the lake was sampled at least once a month during the morning hours. Six fixed stations were chosen in different areas with a depth of at least 1 to 4 m. Temperature (°C), salinity (‰), oxygen (ml/l), as well as zooplankton, were measured regularly in the surface waters ; phytoplankton, primary production (mg $C/m^3/day$), pH, hydrogen sulfide, and inorganic nutrients were determined at irregular intervals. Plankton samples were taken with a conical 2 l PVC tube, the bottom of which was covered by a phytoplankton gauze. All samples were taken in duplicate and fixed with formalin. The plankton samples were analyzed under a dissection microscope and the number of juveniles (nauplii and juvenile stages before the sexual differentiation) and adult *Artemia* counted. For 100 adults, the total length (in mm) was measured for both sexes as well as the number of setae in the furca. The number of eggs in the

egg sac of the females was also counted. Observations on the presence of cysts were made in the field but the abundance was not recorded. Plankton samples (marked 1', 2', 5', and 6' in Table I) were taken close to the shore in order to compare the abundance of *Artemia* with the stations in the lake.

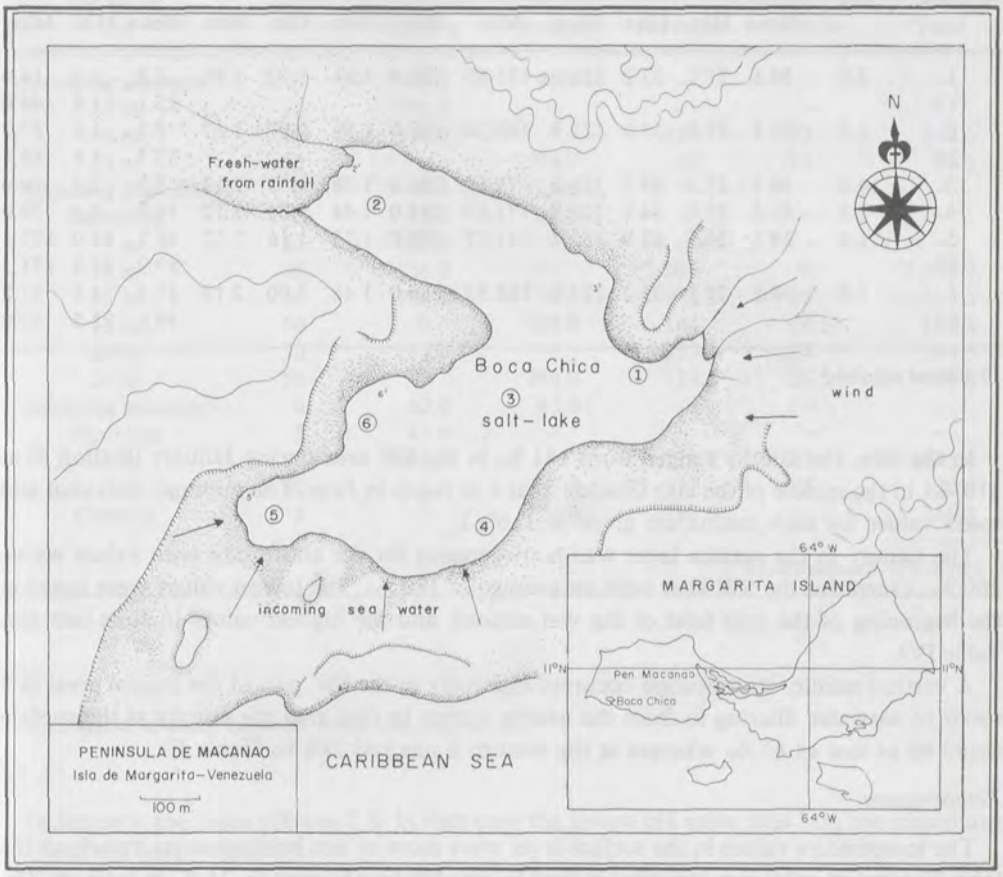

FIG. 1. Location of Boca Chica salt lake in Margarita Island, Venezuela, and position of the sampling stations.

Results

ENVIRONMENTAL CONDITIONS

Salinity

Boca Chica is an hypersaline lake. During the period of this study, the lowest salinity value registered was 42 ‰ (incoming seawater in the SW area); other low values (58 ‰) were found, as a result of local rainfall, in the small river in the NW area.

TABLE I

Temperature, salinity, oxygen, and *Artemia* density at different stations in the lake from January to June 1979 (surface samplings)

Station number	Depth (m)	Temperature (°C)			Salinity (‰)			Oxygen (ml/l)			*Artemia* (ind./l)		
		Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
1	2.0	30.6	27.1	33.2	228.0	171.69	288.0	1.41	1.02	1.99	7.2	2.5	14.0
(1')											23.1	1.8	44.0
2	1.2	30.4	27.0	33.0	227.9	166.14	288.0	1.35	0.97	1.87	9.3	1.8	27.8
(2')											23.3	4.8	88.5
3	4.0	30.7	27.3	33.7	228.6	171.69	284.0	1.38	0.95	2.12	5.5	2.0	10.0
4	1.5	31.3	27.6	34.1	228.8	171.69	286.0	1.44	1.03	2.12	14.9	6.3	29.0
5	1.0	29.3	24.5	32.4	180.7	141.17	220.0	1.73	1.16	2.32	95.7	48.0	173.8
(5')											83.9	32.5	171.5
6	1.0	30.8	27.1	35.2	223.0	168.92	280.0	1.48	1.00	2.10	25.2	4.8	57.8
(6')											30.6	22.5	42.0

(') Littoral samples.

In the lake, the salinity ranged from 141 ‰ in the SW area during January (Station 5) to 316 ‰, in the middle of the lake (Station 3) at 4 m depth in June. The minimal, maximal and mean values for each station are given in Table I.

The salinity in the surface layer was homogeneous for the entire lake with values above 200 ‰, except for the SW area with an average of 180 ‰. The lowest values were noted at the beginning of the year (end of the wet season), and the highest values in June (see also Table IV).

A vertical salinity stratification occurred especially in the SW part of the coastal area, as a result of seawater filtering in from the nearby ocean. In that area the salinity at the surface could be as low as 43 ‰ whereas at the bottom it reached 288 ‰ (Table II).

Temperature

The temperature values in the surface layer were more or less homogeneous troughout the lake. The lowest value was recorded in the SW area (Station 5), namely 24 °C in January. The maximal values were found in June at Station 6. Temperatures fluctuated markedly during the day and from place to place especially in the shallow areas. The mean temperature values recorded for each station are indicated in Table I.

In June extreme conditions of salinity and temperature were found near the shore in the NW area, with values of 320 ‰ and 36 °C which resulted in a high mortality of *Artemia*. The maximal value recorded was 37 °C at Station 3 at 4 m depth and at Station 6 in shallow water nearby the shore.

Oxygen

Dissolved oxygen content ranged between a minimum of 0.95 ml/l (Station 3) and a maximum of 2.32 ml/l in the southwestern area (Station 5). The mean values for each station are given in Table I.

TABLE II

Salinity stratification in the SW area of Boca Chica salt lake, related to depth, distance from the shore, and abundance of *Artemia* in June 1979

Distance from the shore (m)	Depth (cm)	Salinity (‰)		Density of *Artemia* (ind./l)		
		Surface	Bottom	Juveniles	Adults	Total
Incoming seawater	0	46.0	46.0	–	–	–
3 m	20	176.0	–	–	–	–
8 m	40	210.0	–	837	55	842.5
10 m	50	–	274.0	80	16	96.0
Incoming seawater	0	48.0	48.0	–	–	–
Shoreline	2	75.0	75.0	–	–	–
3 m	20	133.0	–	–	–	–
10 m	40	156.0	–	1 084	6	1 090.0
15 m	50	168.0	–	498.5	65	513.5
15 m	50	–	284.0	144	24.5	168.5
20 m	50	184.0	–	555.5	29	584.5
20 m	50	–	280.0	129.5	26.5	156.0
Incoming seawater	0	42.0	42.0	–	–	–
Shoreline	5	43.0	–	–	–	–
5 m	30	208.0	–	274.0	11	285.0
5 m	30	–	288.0	117	5	122.0
Channel	5	–	120.0	710.5	–	710.5
						Mean density 456.9 ind./l

Wind

The wind usually blowed from the NE or SE.

pH

In January, the mean pH was 7.8. In February the lowest pH value was 7.3., the maximum 8.1, and the mean value 7.7.

Hydrogen sulfide

The highest value was detected at Station 3 (3.77 ml/l); Station 2 also had values up to 1.2 ml/l.

Primary productivity

Measurements were only made at Stations 2, 5 and 6. At Station 2 (NW) no net photosynthesis could be observed. At Station 5 (SW) and 6 (W) net photosynthesis was above zero at some ocassions and negative at others.

Inorganic nutrients

Nitrogen (ammonia, nitrite, and nitrate) and phosphate nutrients were only analyzed sporadically. The maximal values for ammonia (45.47 μg-at/l) were detected at Station 3 at

4 m depth, the minimal ones at Station 5 (Table III). In general nitrite and nitrate values were very low ; high phosphate contents on the contrary were found especially at Station 3.

TABLE III

Inorganic nutrients in the salt lake during June 1979

Station number	Depth (m)	Nutrient concentrations (μg-at/l)			
		Ammonia	Nitrite	Nitrate	Phosphate
1	2	19.64	0.04	1.11	2.79
2	1.2	17.66	0.10	0.91	2.54
3	4	45.47	0.25	0.85	10.57
4	1.1	21.29	0.06	1.29	3.15
5	1.5	16.48	0.04	1.29	2.79

BIOTA

Phytoplankton

Sporadic samples of phytoplankton were taken and the following algae were tentatively identified : *Dunaliella* sp. (Clorophyceae) in water of 174 ‰ salinity, *Spirulina* sp. (Cyanophyceae), and *Amphora* sp. (Bacillariophyceae).

Invertebrates other than Artemia

In waters of low salinity, especially in the SW area, insect larvae (*Ephydra* sp. ?) and adult Coleoptera were usually found. In the same area medusae and harpacticoid copepods were also recorded.

Fishes

In the SW and S areas, a small fish was found in the aquatic vegetation close to the shore of the lake, in waters of low salinity (42 ‰). The species was identified as *Cyprinodon dearborni* and is probably penetrating in the lake at moments of low salinity.

Birds

A variety of bird species were common visitors of the lake, such as : the "rayador" or "pico tijera" (*Rhynchops nigra*) and the "viuda" (*Hymantopus hymantopus*).

ABUNCANCE AND DISTRIBUTION OF BRINE SHRIMP IN THE LAKE

Table I gives the abundance of *Artemia* in each station, in relation to salinity, temperature and dissolved oxygen during the period of the study.

The lowest mean value is 5.5 ind./l and was found at Station 3 ; low values were also found at Stations 1 and 2. The highest values were always found at Station 5 (SW). The minimal absolute value found was 1.8 ind./l at Station 2 (NW) and the maximal one was

137.8 ind./l at Station 5. The mean values for the littoral samples were higher than the values recorded for stations with greater water depth, except for Station 5' which was similar to Station 5.

In Table IV, the density and distribution of juvenile and adult *Artemia* is given for each station for the period April-June. The minimal values were found in Stations 1, 2, and 3, and the maximal ones always in Station 5.

TABLE IV

Number (ind./l) and percentage of *Artemia* nauplii (N) + juveniles (J), and adults during April, May, and June 1979, at different stations in the Boca Chica salt lake

Station number	April					May					June				
	N + J		Adults		Total	N + J		Adults		Total	N + J		Adults		Total
	N	%	N	%	N	N	%	N	%	N	N	%	N	%	N
1	3.8	2.36	–	–	3.8	8.5	2.89	0.2	0.07	8.7	6.3	1.15	–	–	6.3
(1')	1.8	1.12	–	–	1.8	23.0	7.81	0.2	0.07	23.2	44.0	8.03	–	–	44.0
2	2.0	1.24	0.3	0.19	2.3	5.4	1.83	0.1	0.03	5.5	27.8	5.07	–	–	27.8
(2')	9.5	5.90	–	–	9.5	33.9	11.51	0.2	0.07	34.1	4.8	0.88	–	–	4.8
3	1.5	0.93	0.5	0.31	2.0	6.8	2.31	0.1	0.03	6.9	4.8	0.88	–	–	4.8
4	8.8	5.47	–	–	8.8	11.9	4.04	0.3	0.10	12.2	29.0	5.29	–	–	29.0
5	44.0	27.33	4.0	2.48	48.0	82.8	28.11	2.8	0.95	85.6	170.0	31.02	3.8	0.69	173.8
(5')	32.0	19.9	0.5	0.31	32.5	71.4	24.24	0.4	0.14	71.8	167.5	30.56	4.0	0.73	171.5
6	30.5	18.94	–	–	30.5	12.5	4.24	–	–	12.5	57.8	10.55	–	–	57.8
(6')	21.0	13.04	0.8	0.50	21.8	33.5	11.37	0.6	0.20	34.1	28.3	5.16	–	–	28.3
Total	154.9	96.23	6.1	3.79	161.0	289.7	98.35	4.9	1.666	294.6	540.3	98.58	7.8	1.42	548.1
Mean abundance (N/l)					16.0					29.5					54.8
Mean temperature (°C)					31.4					30.4					33.5
Mean salinity (‰)					214.52					235.14					274.33
Mean oxygen concentration (ml/l)					1.19					1.12					–

(') Littoral samples.

Juvenile stages of *Artemia* represent more than 96% of the total population (more than 98% in June). In the SW area adults are few in number but they were always present.

The average density of *Artemia* was 16 ind./l in April, 29.5 ind./l in May, and 54.8 ind./l in June. During this period the water showed a steady increase in salinity and temperature and a decrease in dissolved oxygen content. The results from the stratification analyses (Table II) were not taken into consideration for the quantitative analysis.

In June, a detailed sampling exercise was made in the SW area, from the shore to 20 m inside the lake at three different places. Measurements of surface and bottom salinity were made in relation to plankton abundance. The results, summarized in Table II, show a decrease in the *Artemia* density from the surface to the bottom and also a decrease with increasing salinity as related to distance from the shore.

Body size of the adults

The average length of adult *Artemia* measured on 100 individuals was 5.96 mm. No difference in size was detected in relation to sex or salinity.

Number of setae on the furca

The number of setae was determined on the same 100 individuals used for the length measurements. The number of setae fluctuated between 1 + 1 and 7 + 7, but no relation was found with sex or salinity. The number of setae was not constant in the two furcal lobes of the same animal.

Sex ratio

The ratio of males to females was found to be more or less constant.

Number of eggs in the egg sac

The maximal number of eggs found in the egg sac of females sampled in the salinity intervals between 100-150 ‰ was 6 ; in 150-200 ‰ it was 10, and in 200-250 ‰ it was 12 ; the mean number of eggs was 5.5, 6.5, and 6.5 respectively.

Discussion

The values of the parameters salinity, temperature, and oxygen tended to be more or less similar in the surface water layer of the lake, except in the SW area in which seawater filters in from the nearby sea and produces more favorable conditions for the development of *Artemia* than in the rest of the salt lake.

Temperature and salinity tended to increase from January to June, whereas the oxygen content decreased. Extreme environmental conditions were found in shallow waters near the shore where daily fluctuations of temperature were very high.

The maximum depth of the lake is about 4 m ; Station 3 was located in such an area where high values of hydrogen sulfide, phosphate and ammonia were detected, but also high temperatures and salinities. As a result the *Artemia* density was usually very low at Station 3 which can be attributed to the unfavorable environmental conditions in this area.

The NW area (Station 2) is a shallow water area, also with extreme conditions of salinity and temperature. The bottom consists of black mud especially near the shoreline. The wind blowing from the SE tends to accumulate the brine shrimp in the NW part of the lake where high salinities (320 ‰) and temperatures near 36 °C as well as other extreme environmental conditions could be the cause of mass mortality of *Artemia* as was the case in June. In this NW area, but also at Stations 1 and 3 (NE), the *Artemia* density is usually low.

The highest concentrations of *Artemia* were always found in the SW part of the lake. This region always showed lower surface values of salinity and temperature and a higher oxygen content than the rest of the lake. Salinity stratification is common in this area due to the different densities of the water. The infiltering seawater has a salinity of around 42 ‰ but deeper waters some distance from to the shore may reach values above 280 ‰. This area is

characterized by low values of ammoniac, nitrite, and phosphate, and densities of *Artemia* higher than 1 000 ind./l in the surface layer (Table II).

Artemia is usually more abundant in surface than in deep waters, and near the shore than in the middle of the lake. Salinity and wind direction probably play an important role in the distribution of brine shrimp. Variations in density were found to be related to time, with increases with rising salinity and temperature : the highest numbers, however, were always found in the areas of lowest salinity (SW). According to Kristensen and Hulscher-Emeis (1972) "in salines of high salinity brine shrimp are found crowding in the less saline parts". To avoid false interpretations it is necessary to point out that the wind in usually blowing from the NE, and current drift tends to accumulte *Artemia* in the SW area. As is usual in such environments the brine shrimp aggregate in patches of high density in littoral shallow areas : these patches were, however, avoided in our density study. As a result of wind and drift currents, considerable amounts of dry eggs were found on the shoreline in the SW area.

Based on the data for all the stations, the mean brine shrimp density was calculated to be 16 ind./l in April, 29.5 ind./l in May, and 54.8 ind./l in June.

Although no quantitative samples of phytoplankton were taken, the phytoflagellate *Dunaliella* sp. was the most abundant type of microalgae and is probably the most important source of food for the *Artemia*, as found by many other authors (Boone and Baas-Becking, 1930 ; Da Costa, 1972 ; Kristensen and Hulscher-Eimeis, 1972 ; Sorgeloos, 1974).

Coleoptera and the fish *Cyprinodon dearborni* are common predators of *Artemia*. The fish were usually found in the vegetation of the littoral zone at sites where the salinities ranged between 42-46 ‰. Kristensen and Hulscher-Emeis (1972) mentioned that these fishes can live in salinities up to 80 ‰ and are sometimes found in waters of 130 ‰ salinity. *C. dearborni* was also found in the lake in the SW area when the salinity occasionally dropped due to a high tide or local rainfall. In such cases the fish predated heavily on the *Artemia*, but when the salinity increased again they usually died.

The mean size of adult *Artemia* in Boca Chica, measured on 100 individuals, is 5.86 mm. Kristensen and Hulscher-Emeis (1972) stated that in the Antilles, the *Artemia* in the lowest salinity have length of 10-11 mm whereas at higher salt concentrations their maximal length is sometimes only 5.5 mm. In our study no relationship was found between size differences and salinity or sex although it should be said that most of the animals measured were sampled in water of more than 141 ‰ salinity and that the number of animals on which the measurements were made were probably too low for statistical interpretation. The low number of eggs per egg sac found, corroborates the findings of Kristensen and Hulscher-Emeis (1972) who pointed out that the number of eggs is always small in high salinities, seldom surpassing 15.

It has been known for a long time that *Artemia* strains can differ by the length of the abdomen, the shape of the abdominal plates, and the number of the setae. In our study the majority of the animals has a low number of furcal setae, not exceeding 7 in each lobe.

To conclude we hope that this ecological study of *Artemia* with only tentative interpretation of the results because of the limited amount of data, will nevertheless add some useful information to our rather limited knowledge of this species in its natural environment. Unfortunately to date such studies are very scarce. They will, however, become a must in the light of the increased exploitation of *Artemia* cysts necessary for the development of aquaculture.

Summary and conclusions

This study made during the dry season of 1979 in Boca Chica salt lake, records the abundance and distribution of *Artemia* populations in relation to several environmental factors. It represents a first step towards an understanding of the ecology of *Artemia* in Margarita Island, Venezuela.

The major findings are the following :

1. The abundance and distribution of *Artemia* in Boca Chica salt lake varies from place to place in relation to the specific local environmental conditions and productivity.
2. The mean size of adult *Artemia* is 5.86 mm ; the number of setae on the furca is inferior to 7 in most animals. The mean number of eggs in the egg sac of the females is low (average 6, maximum 12) which is typical for the high salinity of this particular biotope. Most of the population collected (96-98 %) were in the naupliar or juvenile stages.
3. High concentrations of hydrogen sulfide and ammonia found in the center of the lake at 4 m depth and near the shore in the NW area are probably at the origin of the low *Artemia* density at these places.
4. The density of *Artemia* is higher in the SW area in which the combination of lower temperature and salinity, a higher content of dissolved oxygen and a higher productivity (due to seawater filtering in from the neighboring ocean) create more favorable conditions for the development of brine shrimp than in the rest of the lake.
5. Wind direction and drift current probably play an important role in the distribution of *Artemia* in Boca Chica salt lake.

This preliminary study should be continued and cover a cycle of at least 1 year. The number of samples and analyses should be increased to obtain a clearer vision of the temporal and spatial interrelationships between the organisms and the environment. Such ecological studies are imperative for the future management and optimal exploitation of saline biotopes from the point of view of production of *Artemia* cysts for aquaculture purposes.

Acknowledgements

We wish to thank M. Sc. J. Pineda for some of the chemical analyses of nutrients and for the primary productivity study. We also wish to thank Dr. F. Cervigón for the identifications of the fishes and Ms. A. Velazquez for the identification of the algae.

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Artemia in the USSR

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Abstract

Numerous water bodies of the arid zone of the USSR have already been surveyed for *Artemia* which has been found in more than 40 lakes and coastal lagoons. These biotopes are located in southern Ukraïne (Odessa region and northern Crimea), in the Caspian area of Kasakhstan and Turkomansa, and in southeastern Siberia which is called the Atlas territory.

The habitats of *Artemia* in the USSR are characterized by a very pronounced continental climate with air temperatures ranging from -30 to -40 °C in the winter, and from 30 to 40 °C in the summer. Atmospheric precipitations are scarce, reaching only 100-150 mm/year in Kasakhstan and Turkomansta, and 250-350 mm in southern Ukraïne.

These water bodies can be subdivided into two groups according to the type of water supply. The first group is composed of a few bodies of water of large surface, fed with the waters of the Azov-, the Black-, and the Caspian Seas respectively. The second group are the land-locked lakes usually of small surface, fed from rivers.

The waters of the first group are shallow, mostly 1.5 to 2 m deep ; their temperature and oxygen regime as well as the water dynamics are most unstable.

The regime of all these parameters is less dynamic in the biotopes of the second group, because of the considerable depths which can reach up to 15-20 m.

Although ecological studies of most of these water bodies are still in progress it already appears now that there are quite a number of *Artemia* strains in the USSR.

The purpose of the investigations made so far was in most cases to determine the possibility to utilize *Artemia* as food for fish larvae at the early stages of their development. Particular attention was therefore given to the natural production of *Artemia* eggs. In most of the water bodies summer and winter concentrations of eggs have been recorded ; the biomasses fluctuate between several mg to several g/m^3. The total allowable catch (TAC) of *Artemia* eggs is presently estimated at several hundred tons.

Exploitation of the resources is, however, restrained by a number of factors. First of all, the major part of the stock of eggs is found in biotopes which are located in the least developed, uninhabited areas of the country. Secondly, the weather conditions in these areas are very severe ; thirdly the harvest of *Artemia* eggs in mid-water turned out to be ineffective, whereas on the other hand there are no reliable technologies available so far to collect the eggs from the shoreline and the available labor force is most limited. Another negative factor is that the procedure for preliminary processing of the *Artemia* cysts is relatively elaborate and labor-intensive.

Nevertheless, the yearly collection and utilization of eggs in the USSR reaches several tons. The quality of the eggs, however, does not meet our expectations, in particular because the hatching rate varies widely. The batches of *Artemia* eggs which are harvested and primarily processed under scientific supervision are known for their high quality, with hatching rates reaching 80 % ; usually this rate is but 20 to 40 %, sometimes up to 60 %.

Much has been said in this Symposium about the high adaptation capacities of *Artemia*. In this connection I would like to mention what seems to us to be an exceptional case.

In the 1940's and 1950's the salt brought from the Sivash lake for salting herring was used at the Vladivostok fishing facilities. In the early sixties this type of work was ceased, but the concrete salting-tanks were kept. They stood in the open air, underwent multiple precipitations and evaporations, heavy frosts, and very high summer temperatures. All of a sudden, in 1976, scientists of the Pacific Research Institute of Fisheries and Oceanography found *Artemia* eggs in these tanks and collected them !

Commercially, *Artemia* is used in the USSR as food for sturgeon larvae which are reared in special tanks. As a rule nauplii are used from late May till early July. Nauplii are reared in special concrete tanks of the type used to grow *Daphnia*.

The biotechnology of rearing *Artemia* nauplii is well developed and satisfies fish culturists. Physiological research has, however, shown that the larvae and fingerlings of sturgeons grown solely on *Artemia* are weaker than those raised on a mixture of *Artemia* + *Daphnia* + Oligochaeta. Some 20-30 million young sturgeons have been grown yearly on *Artemia* since the early 1960's.

In recent years, *Artemia* has also been successfully employed as food for lester (a hybrid of sterlet and huso-huso) and larval carps. Scientists from a number of institutions have also developed methods of partial utilization of *Artemia* for breeding larval sea fish, such as mullets and flounders.

Culturing

The Brine Shrimp Artemia. *1980. Vol. 3. Ecology, Culturing, Use in Aquaculture*
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A note on *Artemia* culture from a local strain in India

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Abstract

The *Artemia* cysts were collected during the summer months from the salt ponds (with 60-80 ‰ salinity) of Bhayander, Bombay.

Hatching experiments were carried out during the months of June, July, August, and September 1977. A 70-80 % hatching was noticed in each experiment, but with a different periodicity : after 36 hr in June and July and after 24 hr in August and September.

During the first week of each experiment the density of nauplii ranged between 2 100-2 500/l after which it decreased to a level of 1 100-1 300 nauplii/l.

The nauplii took about 18-20 days to reach the adult stage in the June and July experiments.

Few nauplii grew out to the adult stage during the August and September trials.

The experimental conditions were : salinity 38-40 ‰, dissolved oxygen 6.0-7.0 ppm, and water temperature 28-30 °C.

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Technological aspects of the batch culturing of *Artemia* in high densities

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Abstract

Contrary to the rearing of brine shrimp at laboratory scale which is current practice and well described in many papers, specific information on the high-density culturing of this species is scanty.

This paper briefly reviews the different culturing methods precognized so far and discusses the advantages of batch culture in air-water-lift raceways ; the latter technology has been optimized during the last 4 years at the Artemia Reference Center and permits a current production of 2 kg biomass/m^3 in 2 weeks culturing at 25 °C.

A detailed description is given of :

1. construction of the raceways ; tank design and characteristics and selection of materials ; construction, positioning and operation of the air-water-lifts ;
2. techniques for water heating and heat insulation ;
3. equipment and methods for (semi-)automatic food distribution ;
4. separation-techniques for continuous removal of faecal pellets from the culturing medium ;
5. culture procedures.

Introduction

During the last decade several techniques have been described for the high-density culturing of brine shrimp in batch systems.

Dohse (1971) is working with a so-called "Artemium" consisting of shallow basins of 100 l contents which are piled up. In each plastic basin, a rotating blade continuously stirs the culture medium and accumulates uneaten food and faecal pellets in a central depression from where they can be siphoned off. The culture medium is not changed during growth of the larvae. The food consists of a commercial mixture of dried algae, yeast, and alfalfa and is distributed manually once a day.

Sorgeloos (1973) proposed a culture system of 30 l transparent plastic columns installed in parallel in metal racks. The working principles, which have also been adopted by Dohse (1973) for the improvement of his "Artemium", are as follows :

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a. intermittent aeration of the medium (10 sec every half hour) which assures a good oxygenation and does not harm the animals ;
b. automatic food distribution once an hour which greatly improves the feeding efficiency of the brine shrimp. As far as the latter factor is concerned it is indeed known that "... the food remains in the gut progressively longer, as there is less pressure on it from the incoming food to move through the gut" (Reeve, 1963) ; Ideally feeding should even be "dropwise" as advised by Provasoli and D'Agostino (1969).

Although excellent growth and survival are obtained with this "column" culturing system its application at a large scale is not economically feasible by some inherent drawbacks such as :
- renewal of the culturing medium every 2nd or 3th day involving extra costs for heating and manual labor ;
- the necessity of using expensive air compressors to aerate "high" water columns (1.5 to 2 m) instead of axial blowers which now are mostly used in aquaculture hatcheries.

The need for repeated water renewals during larval growth was overcome by Person-Le Ruyet (1976) by the gradual decrease of the density of animals from 20 to 1/ml through gradual increase of the volume of the culture. This technology as well as the culturing method in shallow containers proposed by Jahnig (1977), are unfortunately very labor intensive and hard to automatize.

The ideal *Artemia* culturing technique suitable for large scale application implies, in our opinion, the following prerequisites :
a. good oxygenation of the medium to allow culturing at high density (thousands of animals per liter) ;
b. continuous circulation of the medium to maximize food availability to the brine shrimp which are swimming continuously ;
c. shallow water depth (not exceeding 1 m) to allow the use of inexpensive axial blowers ;
d. possibility for automatization, which implies that water-renewals should be restricted as much as possible ;
e. possibility of upscaling which should neither involve major changes in tank design nor in culturing principles.

Of the various techniques which we have tested out for growing *Artemia* larvae in batch culture from nauplius to adult, the air-water-lift (AWL) operated raceway, originally described by Mock *et al.* (1973, 1977) to culture benthonic postlarval *Penaeus*, proved to be the most suitable (Sorgeloos *et al.*, 1977ab). The construction and operation of the culturing tank is indeed simple and very high production results can be obtained.

An air-water-lift operated raceway (AWL-raceway) basically consists of a rectangular tank provided with a central partitioning and air-water-lifts (AWL's). By the specific configuration of the tank and the identical positioning of the AWL's, a screwlike, unidirectional circulation of the culturing medium is achieved, which results in the following effects :
- aeration of the medium is continuous ;
- circulation of the whole medium is almost homogeneous ;
- nearly all particulate matter is kept in suspension ;
- feed added at one place is distributed all over the tank within a few minutes ;

– the system can be scaled up at will, taking in consideration, however, that the height-width ratio of the culturing tank is critical.

It is clear that, prior to consider any adaptation of the original AWL-raceway technique for culturing brine shrimp, it was necessary to find out if the animals (the larvae as well as the adults) are not injured by their intermittent passage through the AWL-pipes. Preliminary tests carried out in small 60 l raceways revealed that high survival and acceptable growth rates could be obtained (Sorgeloos, 1975 ; Bossuyt, 1976).

During the past 4 years we have grown millions of brine shrimp in various types of AWL-raceways, the contents of which ranged from a minimum of 10 l up to a maximum of 5 m^3. In this paper we have tried to summarize the state of the art of our present knowledge on construction and operation of AWL-raceways for batch culturing of brine shrimp.

Construction of the AWL-raceway

Tank design and characteristics

An *Artemia* raceway essentially consists of a rectangular tank with a central partitioning. The corners of the tank may be curved, although this is not absolutely necessary (Fig. 1). In order to assure an optimal water circulation the central partitioning should not be closer to the wall of the small side than 1 to 2/3 of the channel width (W in Fig. 1) and not further than once this width. The partitioning should also be kept 2 to 5 cm off the bottom of the tank either by suspending it from two or more woorden bars resting on the sidewalls of the raceway or by keeping it in its central position on top of two small blocs on the bottom with the aid of rubber bands (*e.g.* cut from an inner tube of a bike) stretched over the top of the partitioning. The most important parameter for the configuration of the tank is the height/width ratio which, in our opinion, should be kept close to 1.

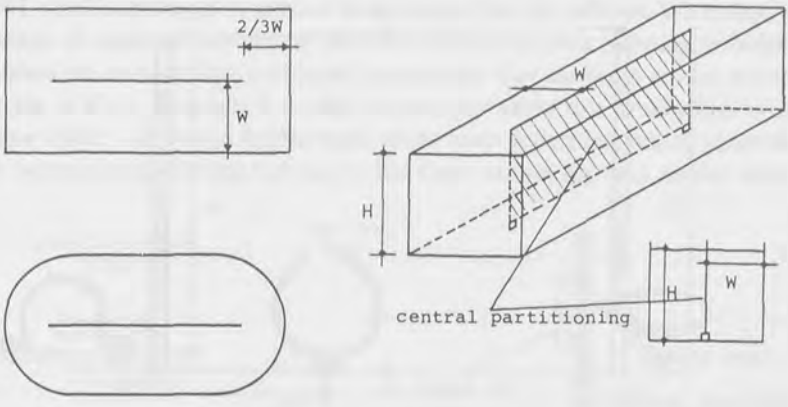

Fig. 1. Top and side views of *Artemia*-raceways illustrating the positioning of the central partitioning (height H and width W of water column).

For optimal water circulation, using axial blowers, the water depth should not exceed 1 m. Deeper tanks indeed require the use of more expensive air-compressors, whereby the resulting widening of the tank (to keep the height/width ratio close to 1) decreases the efficiency of water circulation which can be obtained with one row of AWL's.

Various materials can be used to construct raceway tanks, *e.g.* concrete, marine plywood, fiberglass. We found a very convenient solution for the basic structure by using aluminum plates supported at the outside by angle-irons. The latter are either fixed with bolts into a concrete floor (Fig. 2) or, when constructed on soft bottoms, interconnected at the bottom by an iron frame and by a steelcable at the top (Fig. 3). Several techniques can be considered to make the tanks watertight such as plastic liner or glass fiber coating ; we mostly use PVC-liners, 0.5 to 0.85 mm thick.

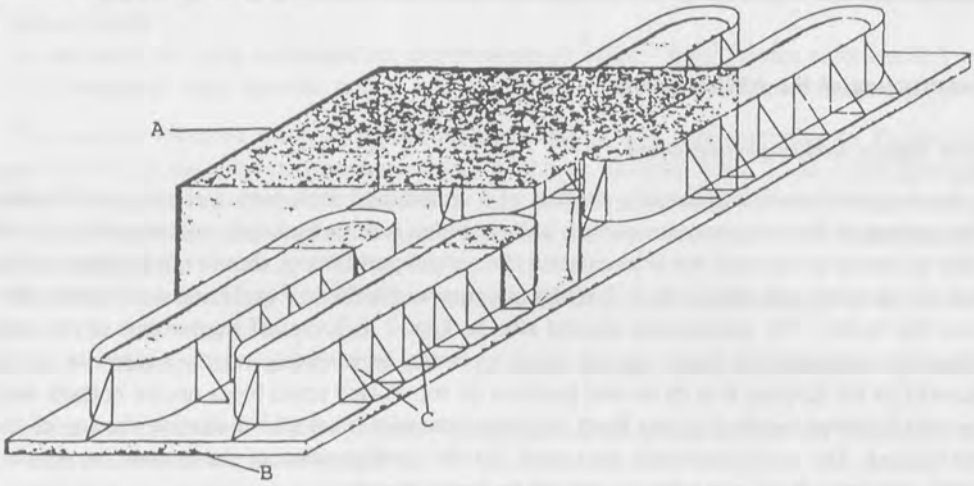

FIG. 2. Scheme of pilot plant of four 5 m^3 raceways for batch culturing of *Artemia* constructed at the Belgian coast. (A) central sheltered workplace, (B) concrete flour, (C) supporting angle irons.

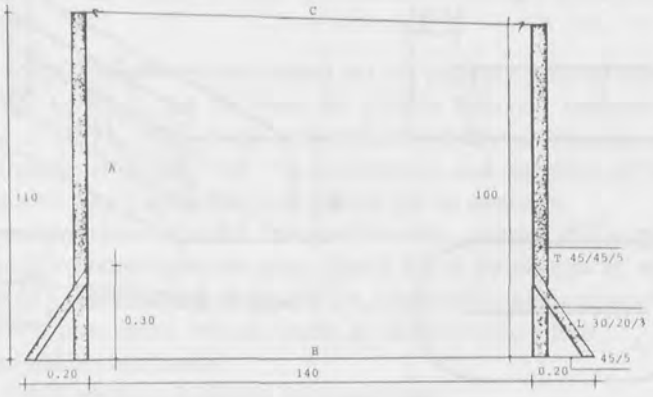

FIG. 3. Alternative frame construction for raceway on soft bottom using angle-irons (A), iron band (B), and steel cable (C), (all lengths in cm).

CONSTRUCTION AND POSITIONING OF THE AWL'S

The easiest way to construct an AWL is to use sanitary PVC-pipes and elbows. The lower part of the tube is cut at an angle of 45° and stands on the bottom of the raceway (Fig. 4). In case elbows are not available, it suffices to cut the upper part of the tube at 45° and seal the two pieces as shown in Fig. 4B.

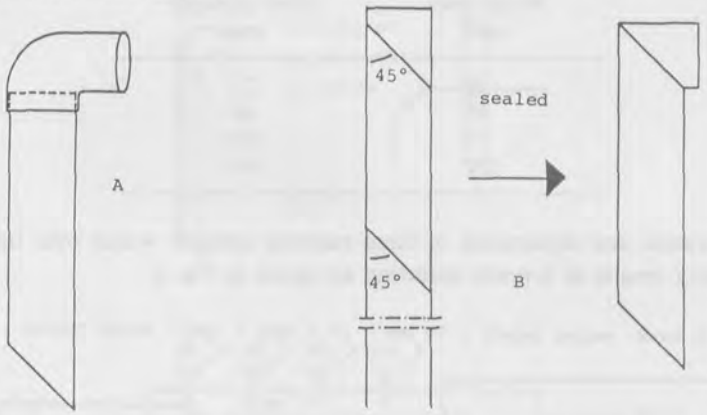

FIG. 4. Air-water-lift (A) assembled with an elbow piece or (B) entirely made of a piece of PVC-tubing.

Depending on the materials available, various systems can be considered to attach the AWL's to the central partitioning to keep them in a well defined position for an optimal watercirculation in the raceway (Fig. 5). For this purpose the elbow outflows should make an angle of 30 to 45° with the central partitioning. Although the oxygenation of the culturing medium is maximal when the AWL's outflow extends a few centimeters above the water surface [according to Spotte (1970) the splash-down of the water back into the tank assures a better aeration than the effect of the air-bubbles within the pipe], we always keep the outflows of the AWL's half submerged to prevent formation of tiny air bubbles. We indeed experienced that ingestion of small air bubbles by the brine shrimp or their trapping between the thoracopods makes the animals float and leads to mortality. For the same reason we never put air stones in the AWL's, although it is well known that these can greatly aid to optimize the oxygenation effect. Air stones furthermore cause foam formation which again can result in mortality because brine shrimp trapped in the foam cannot get back in the water.

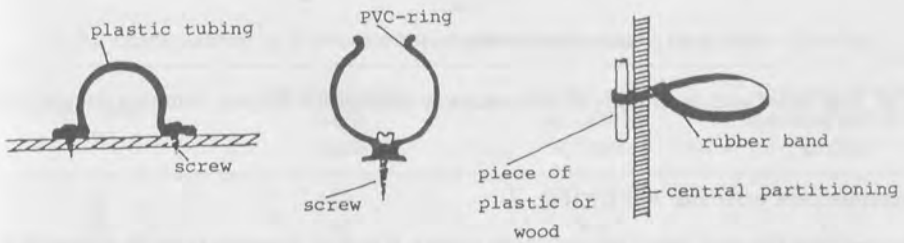

FIG. 5. Three different systems for attaching AWL-pipes to the central partitioning of a raceway.

With regard to the number of AWL's per tank, optimal circulation and aeration is obtained with pipes installed at 25 to 40 cm intervals. In order to assure the maximal water-lift-effect, it is clear that the diameter of the AWL's should be related to the water depth. Based on our experience we can advise the following relationship :

Water level (cm)	Inner diameter (mm)
20	25
40	40
75	50
100	60

The configuration and dimensions of three raceway systems which over the years have given satisfactory results in *Artemia* culturing are given in Fig. 6.

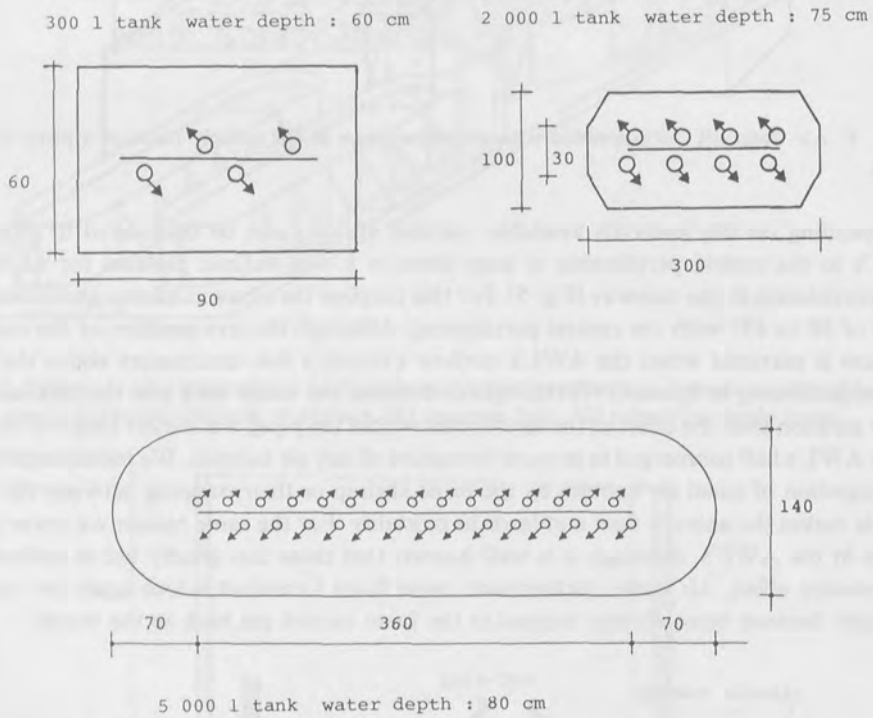

FIG. 6. Top views and dimensions of three raceway-systems for *Artemia* culturing (length in cm).

AIR-DISTRIBUTION INTO THE AWL's (Fig. 7)

The aeration-line (*e.g.* small polyethylene tubing, 6 mm in diameter) can be mounted in the AWL through a hole on top of the PVC elbow. By making this opening slightly smaller than

the outer diameter of the tubing, the aeration-line can be risen or lowered at will and its thight fitting in the elbow prevents any undesired displacement. The aeration lines should extend as deep as possible in the AWL's to assure the best water-lift effect.

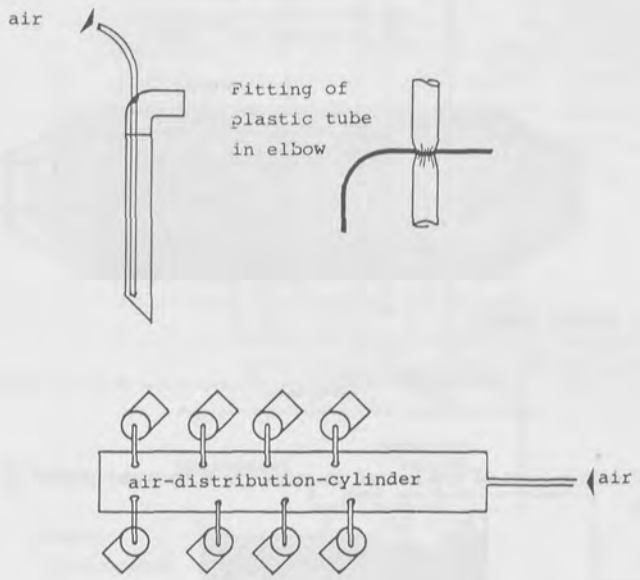

FIG. 7. Details on the installation of airlines in AWL-raceways.

Instead of using expensive valves for each individual aeration line, the different tubes are connected to a central air-distribution cylinder provided with holes wich are smaller than the outer diameter of the plastic air tubes to assure an airtight fitting of the tubes. An identical and constant aeration is obtained by adjusting all air tubes to the same hydrostatic depth in the AWL's. At water depths of 50 cm and more, the adjustment of the air-flowrate in individual AWL's can be greatly facilitated by mounting a small piece of perforated PVC-tubing on the air-outlet (Fig. 8).

The AWL-characteristics of a few raceways operated successfully for *Artemia* culturing are summarized in Table I. Further research is in progress to optimize dimensions and operation conditions.

TABLE I

AWL-characteristics of a few raceways operated successfully for *Artemia* culturing

Water level (cm)	Inner diameter AWL (mm)	Volume of air per AWL (l/min)	Volume of water displaced per AWL (l/min)
20	25	2.7	4.0
40	40	6.6	12.5
75	50	14.0	38.0

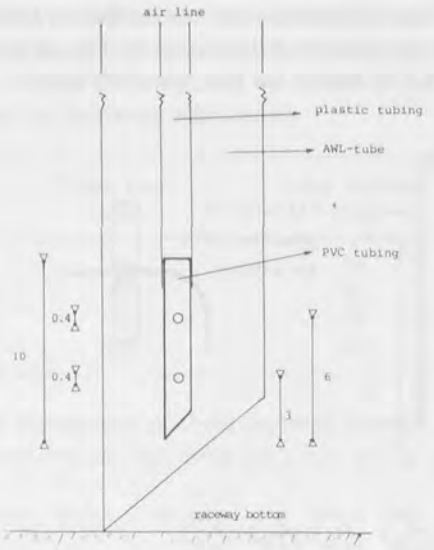

FIG. 8. Detail of bottom part of AWL used in raceways with water depths of 50 cm and more (dimensions in cm).

SELECTION OF MATERIALS

It is clear that all materials which are in direct contact with the culturing medium have to be non-toxic for *Artemia*. Materials withheld so far are : PVC-pipes and tubes ; concrete or wood (marine plywood) coated with epoxy-paint or fiberglass or lined with PVC, or polyethylene-sheet.

HEATING OF THE CULTURE MEDIUM

The optimal culture temperature for brine shrimp is situated in the range 25-30 °C. To heat and keep the culturing medium at such high water temperatures, heaters and thermostats (made of stainless steel, glass or otherwise incorporated in non-corrosive materials) can be directly immersed in the water. Various indirect heat exchangers which are used in mariculture can also be used (Huguenin, 1976).

The first system we worked with, consisted of a copper coil tubing covered by an aluminum plate 1 mm thick and located underneath the PVC-liner on the bottom of the raceway (for more details see Fig. 9 and Sorgeloos *et al.*, 1977b). Freshwater, heated in a separate tank at 50 °C was circulated through this heat exchanger and temperature was controlled by a thermostat immersed in the culture medium.

More recently we experienced that plate radiators, commonly used in domestic central heating are very useful for raceway culturing. Coated with an epoxy-paint they function at the same time as heat-exchanger and central partitioning to which the AWL's can be fixed.

Heat losses can be minimized by proper insulation of the culture tank, *e.g.* with styrofoam or polyurethane. Water evaporation, (which is the major cause of heat losses) can be greatly

reduced by placing an insulated cover (also provided with styrofoam) on top of the raceway ; the non-transparent cover is furthermore beneficial for the food conversion since brine shrimp grow faster in darkness than in light (Sorgeloos, 1972).

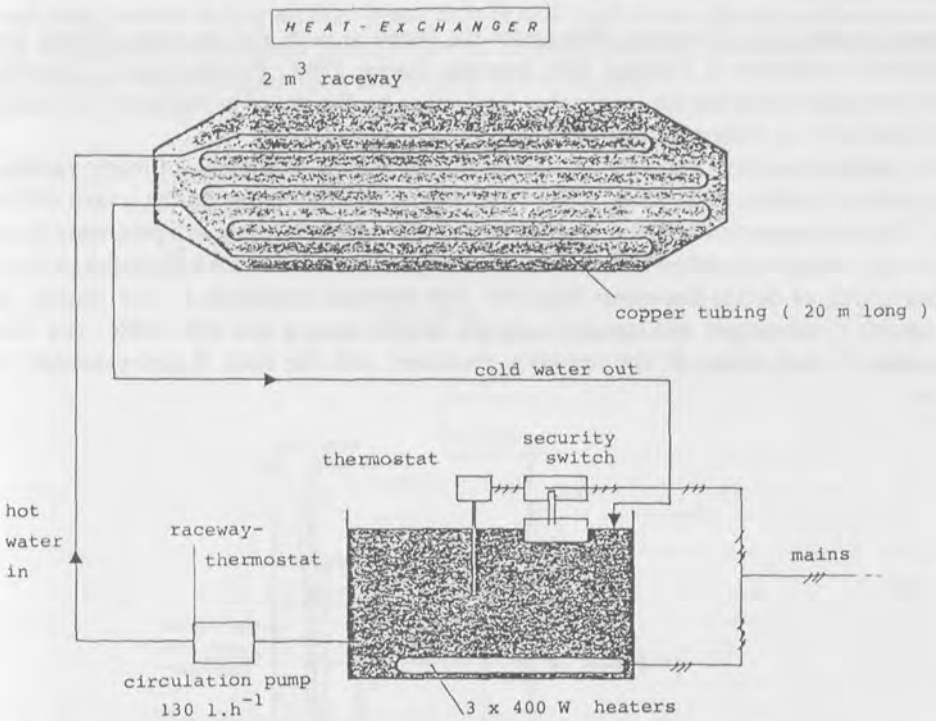

FIG. 9. Schematic diagram of a coppercoil heat exchanger used in a 2 m^3 AWL-raceway.

Feeding conditions

SELECTION OF FOOD

From literature it appears that a wide range of live and inert feeds can be successfully used to culture the brine shrimp (review by Sorgeloos and Persoone, 1975). Since *Artemia* is a non-selective, obligate, particle feeder (Barker-Jörgensen, 1966 ; Provasoli and D'Agostino, 1969) the critical factors in selecting a suitable diet for brine shrimp are its particle size which should be less than 50 μm (Dobbeleir *et al.*, 1980), digestibility, nutritional value (to be tested experimentally) and solubility in the culturing medium (which should be minimal). Soluble products indeed, are not taken up by *Artemia* and, since they will be decomposed by bacteria in the culture medium they contribute to a deterioration of the water quality by the gradual build up of toxic substances such as ammonia (Hanaoka, 1973, 1977).

Different waste products of major agricultural crops or from bio-industries have been found to be a very suitable food source for *Artemia*, *e.g.* rice bran, soybean meal and whey

powder (Dobbeleir *et al.*, 1980). Most experience in raceway culturing was gained so far with rice bran (Sorgeloos *et al.*, 1980).

Food distribution

Since *Artemia* is a continuous filter-feeder the fastest growth and the most efficient food conversion is obtained at constant food densities (Reeve, 1963 ; Provasoli and D'Agostino, 1969). For batch culturing this means that food has to be distributed as frequently as possible either manually or preferably automatically.

The optimal quantity of food to be distributed per feeding is function of many variables, such as larval density, instar-stage, water temperature, *etc.* and makes dosing a very difficult task. We found a practical solution in using the turbidity of the medium as a parameter for the food dose. A rough estimation of turbidity can be made with a self-made turbidistick, which is in fact a type of Secchi-disc-meter (Fig. 10). The practical procedure is very simple : the turbidistick is submerged and lowered until the bottom plate is just still visible ; rice bran-suspension is then added till the turbidity, measured with the stick, is approximately 15-20 cm.

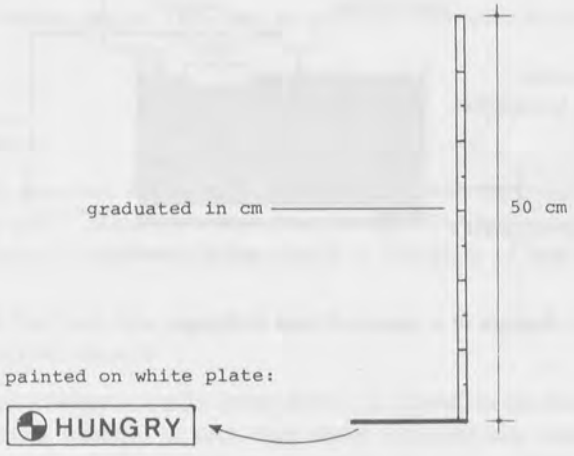

Fig. 10. Turbidistick used in *Artemia* culturing.

In order to maintain the food concentration in the culture medium as constant as possible, we have developed and tested various techniques for automatic food distribution. An example of a cheap and reliable system which we now are using since several years on varous types of raceways is represented schematically in Fig. 11. This system works as follows : in order to prevent particle sedimentation in the food stock, the suspension of rice bran in saturated brine (up to 150 g rice bran/l brine) is gently aerated in a conically shaped container. The latter can eventually be assembled from polyethylene sheet using electrical sealing equipment. Via a

siphon curved at the end (as shown in Fig. 11) or preferably via a water tight connection in the bottom part of the container (using a PVC manifold with screw fitting and rubber rings), the food suspension flows to a T-piece, one end of which is connected to an air inlet, the other one to the food distribution tube extending above the water level in the food reservoir. Every preset time interval during another preset time period, (both to be adjusted in function of the food requirements determined through manual turbidity readings), an electric clock activates the air pump which triggers food distribution to the raceway.

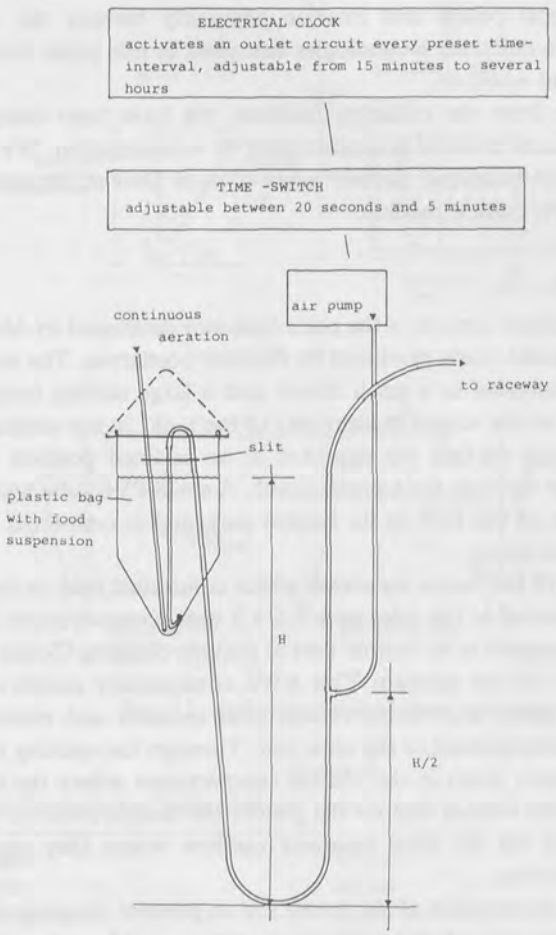

FIG. 11. Schematic diagram of air-lift operated food distribution system.

Full automatic food distribution can be achieved with an electronic turbidimeter. In the self-cleaning system described by Versichele *et al.* (1979), a glass syringue is filled every half an hour with culture medium. When the piston reaches its highest position the optical density of the medium is measured by a photo-electric transistor. The food distribution system is

triggered when the preset critical optical density is surpassed. A few seconds later the piston returns to its original position cleaning at the same time the inside part of the syringue.

Automatic systems for the primary treatment of the culture medium

To culture *Artemia* in high densities (3 000/l) in batch conditions, accumulation of faeces in the culturing tank should be minimized since it seriously interferes with growth and survival of the population. Decomposition of faecal material and uneaten food indeed affects the water quality. Furthermore faecal pellets and exuviae physically hamper the uptake of food particles. Using any of the culturing technologies described in this paper implies removal of the faeces from the second week on.

To separate the waste from the culturing medium, we have been using two different systems into which the faecal material is concentrated by sedimentation. We recently started experimenting with a third technique, namely a new type of filter screen capable to remove particulate matter from the culture medium.

The plate separator (Fig. 12)

This apparatus is a modified version of the plate separator developed by Mock *et al.* (1977) for primary treatment of solid waste produced by *Penaeus* postlarvae. The separator consists of a rectangular tank subdivided in a small inflow and a large settling compartment interconnected by an opening at the sloped bottom part of the tank. In the settling compartment several plates with a rough surface are mounted in an inclined position oriented in the direction of the water flow through the separator tank. A small PVC tube with a longitudinal slit is fixed to the bottom of the tank in its deepest part, and is connected to a removable siphon-tube outside the separator.

The working principle of the faeces separator which is installed next to the raceway is as follows : an AWL surrounded at the inlet by a 0.5-1.0 mm screen-cylinder [and eventually equipped with an aeration collar at its bottom part to prevent clogging (Tobias *et al.*, 1979)] is placed inside the raceway culture medium. This AWL continuously pumps culture medium loaded with particulate matter (*e.g.* faecal pellets, food particles and eventually newborn nauplii) into the inflow compartment of the separator. Through the opening at the bottom of the separator, the suspension flows in the settling compartment where the inert particulate material accumulates on the bottom and on the plates ; the nauplii swim to the surface and flow back in the raceway via the plate separator outflow where they can eventually be harvested in a 200 μm filterbag.

In order to promote sedimentation of the waste and to prevent clogging of the interspace between the plates, the latter are installed at minimum distances of 2 cm from each other at an inclination of 30 to 45°. Sedimentation of the waste is optimized by adjusting the air-water-lift rate since the latter determines the retention time of the suspension in the separator tank. We have experienced that a 20 to 30 min retention time permits a good separation of most of the waste particles while on the other hand small and light food particles do not get a chance to sedimentate and are drained back into the culture medium.

The characteristics of a plate separator currently used to treat a 2 m^3 raceway are summarized in Table II.

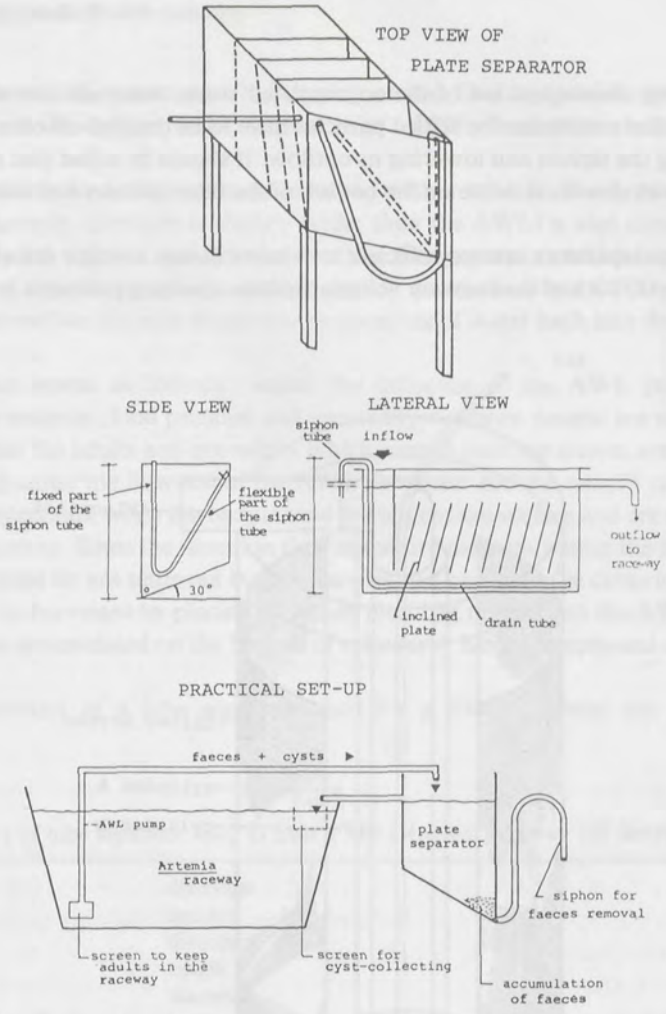

FIG. 12. Different views of plate separator.

TABLE II

Characteristics of plate separator used to treat a 2 m^3 *Artemia* raceway

Dimensions : lenght	60 cm
width	40 cm
depth	50-80 cm
Number of plates	11
Inclination of plates	30-45°
Inclination of bottom part	30-40°
AWL diameter	2.5 cm
AWL pumping rate	3-4 l/min
Filter cylinder : height	50 cm
diameter	10 cm
Faeces drain : diameter	2 cm
Retention time	30-40 min

Since anaerobic decomposition of the accumulated waste materials can affect the water quality of the culture medium, the settled particles have to be drained off once every 2nd day by disconnecting the siphon and lowering its outflow. It should be noted that after a few days of operation faeces also accumulate on the bottom of the filter cylinder surrounding the AWL inlet.

Although plate separators are very efficient to remove faeces, a major drawback, however, is their large size (1/10th of the raceway volume) and the resulting problems in the prevention of heat loss.

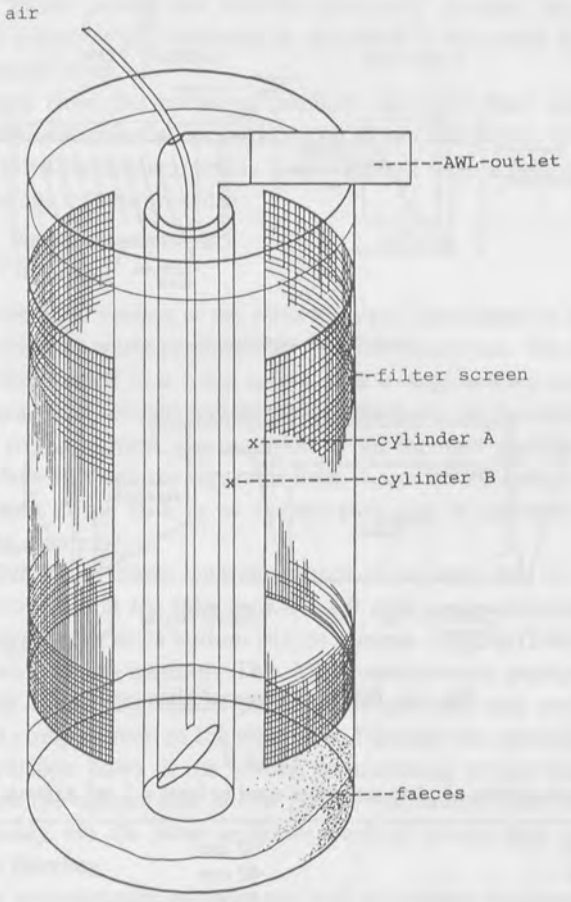

FIG. 13. Schematic diagram of a tube separator.

THE TUBE SEPARATOR (Fig. 13)

Although less efficient than a plate separator we have experienced that a tube separator can often be used as a valuable alternative for waste removal, especially since it can be installed inside the culture tank. This separator consists of a PVC cylinder A, with closed bottom and with very large lateral openings (leaving a type of frame structure) provided with a tightly

fitting filter screen (0.5-1 mm mesh). Clogging of the filter cylinder is prevented by the cleaning effect of an aeration collar (Tobias *et al.*, 1979) fixed at the bottom part of the cylinder.

An AWL tube is mounted inside cylinder A, within tube B. The bottom of the latter cylinder (the diameter of which is slightly wider than the AWL) is also closed.

Dimensioning has to be so that cylinder A of the tube separator, which is placed vertically on the bottom of the culturing tank, extends slightly above the water surface and cylinder B under the water surface (in order to permit the pumping of water back into the culture via the AWL).

The separator works as follows : under the influence of the AWL pumping, culture medium, waste material, food particles and eventually newborn nauplii are sucked into filter cylinder A, while the adults and pre-adults which cannot pass the screen, are retained in the raceway. By adjusting the flowrate of the AWL the waste and the nauplii can be separated, *i.e.* the faecal pellets sink while the nauplii tend to swim to the surface and are returned via the AWL to the raceway. Since the retention time of culture medium within the tube separator is short, food particles do not settle out but are also drained back into the culturing tank. Nauplii can eventually be harvested by placing a 200 μm filter bag underneath the AWL-outlet. Once a day, the waste accumulated on the bottom of cylinder A has to be siphoned off or be poured out.

The characteristics of a tube separator used for a 350 l raceway are summarized in Table III.

TABLE III

Characteristics of tube separator used to treat a 350 l *Artemia* raceway (all dimensions in cm)

Cylinder A	diameter	9.5
	height	45
Cylinder B	diameter	6
	height	40
AWL	diameter	3
Pumping rate (l/min)		2-3

THE CROSS FLOW SIEVE OR WELDED WEDGE WIRE SCREEN

This self-cleaning dewatering sieve is used in waste water treatment for the removal of solids from large volumes of waste water (Poels *et al.*, 1979). As schematically outlined in Fig. 14 it basically consists of a stainless steel welded wedge screen which is placed under a certain inclination and which is provided with slit openings of 150 μm.

Culture medium, screened for larvae and preadults by a filter system similar to that used in a plate separator, is continuously pumped into the head box and evenly distributed over the acceleration deck of the cross-flow-sieve-system. Under optimal conditions of orientation of the screen and speed of the suspension, the thin flowing layer of culture medium is sliced : *i.e.* according to the so-called "Coanda-effect" water and food particles tend to cling to the wall of the preceding bar, which results in their draining through the screen from where they flow back to the raceway culture, whereas oversized particles, *e.g.* faecal pellets, exuviae, *etc.* and

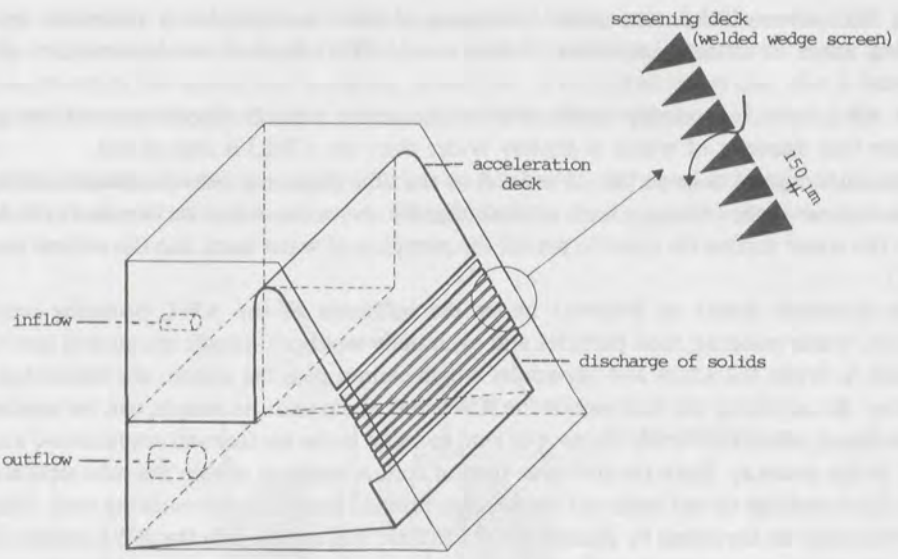

FIG. 14. Cross flow sieve system used for *Artemia* faeces removal.

eventually nauplii are discharged from the lower edge of the screening deck into a small separation tank installed just above the water surface of the raceway. In the latter tank particulate waste settles out (as a result of the long retention time due to the very low volume of water flowing in the separator tank) and the water is drained back into the raceway via the overflow. Nauplii, if present, are attracted by light to the overflow of the separator and also flow back in the culture tank.

Although application of cross flow sieves in *Artemia* culturing has to be studied further we can already point to a major advantage of the cross flow sieve over the other separation systems described : very large volumes of water can be treated on a small screen surface, concentrating the particulate waste into a wet paste. Working with a screen of 15 to 20 cm side, we obtained a satisfactory separation in a 2 m^3 raceway at a pumping rate of 15 to 20 l/min.

Culture procedure

a. natural or synthetic seawater : 30-50 ‰ salinity ; pH : 7.5-8.5
b. density of nauplii : 1 to 3/ml
c. water temperature : 25-30 °C
d. inoculation procedure :
   - incubation of decapsulated cysts for hatching (Bruggeman *et al.*, 1979, 1980 ; Sorgeloos, 1980) ;
   - harvest of instar I population preferably in the late afternoon (the exact timing to start the incubation is function of the water temperature and salinity and the hatching characteristics of the strain used) ;

- eventually counting by subsampling for determination of the exact number of larvae ;
- transfer of a predetermined number of nauplii in the raceway ;
- manual food distribution to a turbidity of 15 cm ;
- no food distribution overnight (the nauplii are first consuming their yolk reserves and there is nearly no food uptake during the first 12 hr) ;
- manual or preferably automatic food distribution from the next morning onwards.

e. food is distributed either manually a few times during the day or by preference automatically at preset time-intervals during the day and at night in order to keep the turbidity of the culture within the range of 15 to 20 cm ;

f. the daily food quantity has to be adjusted to the size of the larvae either by a more frequent food distribution and/or by increasing the quantity of food ;

g. from the second week onwards (when the animals reach a size over 4 mm) waste material should be removed at the aid of a separator, starting with a filter screen of 500 μm and replacing it progressively by screens of 750 and 1 000 μm ;

h. after one or more weeks the aeration lines may have to be checked and eventually cleaned to avoid blockage by salt deposition at the inner side of the airtube ;

i. important water quality parameters should be monitored regularly during the culturing period, *i.e.* dissolved oxygen, pH, and ammonia.

Artemia larvae are very resistant to low oxygen levels and still survive at 2 ppm O_2 ; Provasoli (1969) indicated that brine shrimp do not survive at pH values below 7.0. With regard to the ammonia concentration Hanaoka (1977) reported inhibition of food ingestion already at 2 ppm NH_4^+-N whereas we found that *Artemia* can tolerate levels up to 50 ppm NH_4^+-N. Since ammonia toxicity is greatly influenced by other abiotic parameters, especially pH (Kinne, 1976 ; Bower and Bidwell, 1978), no specific tolerance limit can be given for this important parameter.

A sound management and proper application of the culturing technology as described in this paper has, however, taught us that no specific problems are encountered with water quality for periods up to 6 weeks of batch culturing, without any renewal of the culturing medium.

Production results

The production figures which we obtained in routine batch culturing are summarized in Table IV.

TABLE IV

Production results obtained for various batch culturing tests

Tank volume (l)	Culturing period (days)	Wet weight harvest (g)
350	21	900
1 000	7	1 250
1 500	7	1 800
2 000	21	5 000
2 500	7	3 250
5 000	10	12 000

On the average 2.0 kg wet weight biomass can be produced at 25 °C in a 1 000 l raceway system within about 2 weeks batch culturing starting from approximately 10 g cysts.

Details on the biochemical composition (fatty acids and amino acids) of *Artemia* cultured on rice bran are reported by Dobbeleir *et al.* (1980), respectively Sorgeloos *et al.* (1980).

Conclusion

The present paper only aims at demonstrating the technical feasibility of high-density culturing of *Artemia* on a cheap diet. The results obtained are comparable with the literature data of Person-Le Ruyet (1976) who produced 1 kg of *Artemia* per week per cubic meter using a more complex culturing technology and a more expensive food (*Spirulina* powder) ; they are furthermore by far better than the figures reported by Jahnig (1977) who obtained 0.7 to 1.2 kg per month per cubic meter using polythylene tanks of 280 l content and whole-wheat flour as food. More detailed studies have to be performed to further increase the present production results and to determine the exact cost-benefit of this methodology at a larger scale.

The potential applications of batch culturing in raceways are numerous :

a. The production of bigger larvae, preadults and/or adults as live, frozen, or dried food for aquaculture hatcheries as well as for the petshop market. In comparison to the current technology where only freshly hatched nauplii are used to feed larval stages of crustaceans and fish, rearing of *Artemia* can lead to an important saving of the quantity of cysts needed ; it is known furthermore that in many cases older *Artemias* are more nutritious than freshly-hatched nauplii (Aquacop, 1977 ; Purdom and Preston, 1977) ;
b. The continuous production of offspring by the adult *Artemia*, to be used either as a direct food in the aquaculture hatcheries or for further ongrowth as under (a) by batch culturing for some time ; this application could again result in an important saving of the quantity of cysts needed and should allow *e.g.* to use selected strains with good nutritional characteristics (e.g. parthenogenetic strains) of which only limited quantities of cysts are available ;
c. the conversion of particulate waste products into highly nutritive animal protein which can be valorized either as an ingredient in human food or as a protein-ingredient of feeds for terrestrial and/or aquatic organisms (Sorgeloos, 1980).

In this regard experiments are in progress at or are coordinated by the Artemia Reference Center on the following topics :

- food conversion efficiencies with a micronized rice bran diet in function of larval density, water temperature and density of food particles ;
- cost-benefit analyses of batch and flow-through culturing of *Artemia* on micronized rice bran in AWL-raceways of varying size ;
- culture tests with various other particulate waste-products (technical feasibility studies) ;
- nutritional value analyses of *Artemia* grown on various diets (including diet combinations and/or alterations).

Acknowledgements

Many people have contributed to the continual improvement of the AWL-raceway technique for intensive *Artemia* culturing. In this regard we want to thank the following

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The effect of antibiotics on the hatching of *Artemia* cysts

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Abstract

A problem in the artificial culture of the brine shrimp *Artemia* has been the variability and poor hatchability of the cysts, especially at the high culture densities used under large scale production conditions. Investigators using other invertebrate culture systems have cited fouling bacteria as a causative factor in poor hatchability and survivability of larvae (Oppenheimer, 1955 ; Guillard, 1959 ; Fisher, 1976).

The effects of two antibiotics, chloramphenicol and penicillin-streptomycin, on the hatching of *Artemia* cysts were tested at three cyst densities (2, 4, and 8 g cysts/l). Hatching was significantly improved at the 4 g cysts/l density of the chloramphenicol and penicillin-streptomycin treatment groups and the 8 g cysts/l chloramphenicol treatment group. There appears to be a correlation between bacterial suppression and improved hatching of *Artemia* cysts at certain densities.

Introduction

The yield of brine shrimp *Artemia* nauplii hatched from a given weight of cysts varies between commercial grades, geographical origins, and even individual cans from the same lot of cysts. Hatching percentages from 15-90% have been observed.

Canned *Artemia* cysts carry a large bacterial load (Gilmour *et al.*, 1975) and it has been observed that under high cyst density hatching conditions, bacterial concentrations may reach 10^8 colony forming units (cfu)/ml in less than 20 hr. Gilmour *et al.* (1975) have suggested that these bacteria do not represent a threat to organisms that are fed the *Artemia*, while Glimme (1974) takes the opposite view.

The possibility that bacteria may have detrimental effects on the hatching of *Artemia* cysts has received little investigation to date. Bacteria have been implicated in the poor survivability of oyster larvae (Guillard, 1959), in the poor hatchability of fish eggs (Oppenheimer, 1955) and in the poor hatchability and survivability of Crustacea (Fisher, 1976). An increase in the viability of eggs and larvae of various aquatic species has been demonstrated using a variety of antibiotics, the assumption being that bacterial suppression was responsible for the increase (Walne, 1956, 1966 ; Struhsaker *et al.*, 1973 ; Roman and Perez, 1976).

The purpose of this study was to investigate the effects of cyst density and bacterial suppression on cyst hatching, by treating the cysts with broad spectrum antibiotics at different cyst densities.

Materials and methods

The hatching chambers consisted of sterilized glass intravenous drip bottles (Sorgeloos, 1973), modified for sampling *Artemia* and bacteria (Coleman, 1979). The hatching medium was freshly obtained natural seawater, of 33 ‰ salinity, filtered through glass wool before use. The seawater was tested for bacterial load and was found to contain less that 10 cfu/ml. Dried *Artemia* cysts were obtained from the commercial market, and were used immediately after opening the can.

The hatching chambers were randomly assigned positions on an aeration manifold fed by "Silent Giant" aquarium pumps, model no. 120. All chambers were evenly illuminated by overhead fluorescent lights on an approximate 12 hr photoperiod. Ambient temperature was 28 °C ± 2 °C. Dow Corning Silicone Antifoam A was added to the chambers before the cysts were added to prevent foaming of the medium (Sorgeloos and Persoone, 1975).

The experiment used two antibiotic treatment groups, at three densities of cysts each. One treatment group received 40 ppm veterinary grade chloramphenicol (Pitman Moore) while the other treatment group received 50 ppm research grade penicillin-streptomycin (Gibco). The antibiotics were added at 0 time, along with the dried cysts, then at every 12 hr thereafter for 48 hr total. The cyst densities used were 2, 4, and 8 g cysts/l. Identical controls at each cyst density received no antibiotics. In the penicillin-streptomycin treatment group, the 8 g cysts/l density was not tested.

Forty-eight hours after inoculation with cysts, four 1 ml samples were drawn from each chamber. The nauplii were killed by the addition of 1 ml of 90 % alcohol per sample, and were counted under a dissecting microscope. Only those nauplii that had excysted and shed their hatching membrane, referred to as "swimming nauplii", were counted. Treatment effect at each density was tested by ANOVA (Snedecor and Cochran, 1967).

Bacterial samples were taken at 12 hr intervals, beginning at zero time. Bacteria were cultured on Difco 2216 Marine Agar at ambient temperature and were counted, using standard microbiological technique.

The pH was measured using a Corning pH meter.

Results

In both the chloramphenicol and penicillin-streptomycin treatment groups the mean bacterial counts at 48 hr were at least one log/ml below the control groups. No cyst density effect on bacterial growth was noted (Table I).

At 4 and 8 g cysts/l densities, both treatment groups yielded a significantly higher number of swimming nauplii than the respective controls. At the 2 g cysts/l density, neither treatment group showed a significant difference in yield over their corresponding control group. Means, standard deviations in parentheses and probabilities for each density are presented in Table II.

Average pH value for control groups was 7.9, whereas average chloramphenicol pH was 7.7, with both pH values taken at 48 hr. No pH reading is available for the penicillin-streptomycin group.

TABLE I

Effects of antiobiotic treatment on growth of *Artemia* associated bacteria

Density (g cysts/l)	Number of colony forming units/ml at 48 hr		
	Control	Clor.	Pen-strep.
2	10^6	10^5	10^4
4	10^6	10^5	10^2
8	10^6	10^5	–

TABLE II

Effects of antibiotic treatment on hatching of *Artemia*

Density (g cysts/l)	Treatment effectiveness (P)	Mean swimming nauplii/ml hatched at 48 hr (standard deviation)		
		Control	Chlor.	Pen-strep.
2	N.S.	20 (8.16)	23.75 (2.99)	27.25 (8.63)
4	< 0.01	32.5 (3.81)	52.25 (3.03)	61 (1.41)
8	< 0.01	76.75 (7.76)	112.25 (13.65)	– –

Discussion and conclusions

Analysis of the results indicates a positive correlation between improved nauplii yield and bacterial suppression, at the higher densities tested.

The absence of cyst density effect on the bacteria may be due to the fact that significant changes in bacterial population are noted by logarithmic rather than arithmetic increases. Thus, doubling and quadrupling of bacterial load are considered insignificant increases.

As discussed by Clegg (1974), encysted *Artemia* undergo many biochemical and physiological changes during the first 18-24 hr of rehydration before they hatch. The timing and energetics of these processes are critical and any interference may manifest itself in a decreased ability to hatch and/or survive.

Bacteria have been implicated as the cause of poor hatching of several aquatic species, as mentioned in the introduction. The mechanisms of interference have not been well studied, but Fisher (1976) after examining Dungeness crab eggs suggested that fouling organisms (fungal and bacterial) may cause mortalities by mechanical blocking of pores in the egg membrane, thereby preventing gaseous and aqueous exchange with the environement. It has been the author's experience that the better quality canned brine shrimp cysts contain less

organic detritus and sand which could harbor and nurture fouling organisms. The effects of bacterial exotoxins must also be considered as a possible suppressor of hatching. Suppression of bacterial growth, particularly during the early stages of cyst rehydration, appears to be important to the successful development and subsequent hatch of *Artemia* cysts.

Although antibiotics are designed to work specifically on bacterial systems, there is still the possibility that the improved hatch noted in these experiments and the experiments of others is due to some biochemical effect of the antibiotics on the *Artemia* embryos themselves. These experiments attempted to minimize that possibility by using antibiotics with different mechanisms of action.

Sato (1966ab) discussed the effects of pH and ions on hatching enzymes. Although ions were not measured in this experiment, pH ranges for control and treatment groups were within the range of values that Sato determined to be non-inhibitory. In addition, since both the control and treatment groups experiences similar changes in pH, this could eliminate pH as a factor in these experiments.

The authors would like to emphasize the point that antibiotics were used solely as an experimental technique, and do not wish to suggest broad application on a production level. The danger of inducing antibiotic resistant bacteria, as well as increased production costs, suggest that other means of suppressing bacterial growth such as ultraviolet light, chlorination or washing may more efficiently be employed.

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Successful inoculation of *Artemia* and production of cysts in man-made salterns in the Philippines[1]

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Abstract

The objective of the inoculation described in this paper was to test the feasibility of culturing *Artemia* in man-made earthen salterns and of producing adults and cysts for use in aquaculture projects in the Philippines. San Francisco Bay (California, USA) *Artemia* were inoculated in two concrete tanks and in four earthen ponds which are part of a small local salt factory.

It was found that *Artemia* can be grown (with continuous production of nauplii and cysts) year-round in covered concrete tanks and in open concrete tanks and earthen ponds during the dry season (February to June). Lethal effects of too high water temperatures (> 35 °C) to the cultures were anticipated by the use of green coconut fronds placed on the water surface alongside the walls of the tanks and the earthen dikes.

Rice bran enriched with vitamins and traces of minerals appeared to be a good food for *Artemia* cultured in aerated concrete tanks ; in the earthen salt ponds the brine shrimp grew well on the natural food present.

Over a 3 month production period, 26 kg dry weight cysts and 150 kg live weight adults have been harvested from a total surface of 1.7 ha of salt ponds and brine tanks.

Introduction

It has been repeated at various occasions that the limited provision of *Artemia* cysts may lead to a serious bottle-neck in many aquaculture developments (Sorgeloos, 1979, 1980). The areas in the world where no natural populations of brine shrimp are occurring (Persoone and Sorgeloos, 1980) are most affected, since their aquaculture industry is entirely dependent on cyst import. This is particularly the case in Southeast-Asia where the aquaculture activities are

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increasing exponentially (Anonymous, 1979), *e.g.* "... the inadequate supply of brine shrimp for feeding shrimp larvae remains as the major constraint in the mass propagation of penaeids in Thailand as in the other countries" (ASEAN, 1977).

As explained in detail in a recent review paper on the ecology and biogeography of *Artemia* (Persoone and Sorgeloos, 1980), the pronounced climatic alternations of seasons : dry from November to April and wet during the rest of the year, most probably preclude the perennial establishment of brine shrimp in the Philippines.

The environmental conditions appear, however, to be suitable for *Artemia* during the dry season. Indeed thousands of hectares of saltponds are under operation. As a result, a man-managed inoculation program similar to the one accomplished successfully in the Macau (Rio Grande do Norte, Brazil) salt ponds (Sorgeloos, 1978 ; Sorgeloos *et al.*, 1979) could be considered in the Philippines. Contrary to Brazil where the new brine shrimp population is established permanently, the inoculation in Southeast-Asia will be of a temporary nature, *i.e.* the *Artemia* population will be eliminated by predation at the onset of the rainy reason. Pond production of brine shrimp in Southeast-Asia will thus be a cyclic operation (one cycle per year) and can eventually be integrated with salt production.

The experiments reported in the present paper describe the first *Artemia* inoculation trials in small saltponds in the Philippines.

The 1978 inoculation test and its results

The saltfarm where the inoculation was performed on February 22, 1978 is located in Barotac Nuevo (on Panay Island in the central part of the Province of Iloilo).

Two brine tanks in concrete ($15 \times 20 \times 0.75$ m) and several ponds with walls in concrete and earthen bottom of 3 400 m^2 surface each, were used for the *Artemia* production tests. Twenty million nauplii were hatched out of 100 g San Francisco Bay[6] (California, USA) cysts in the SEAFDEC-hatcheries at Tigbauan. Since it was only discovered at a later stage that freshly hatched nauplii can be transferred from natural seawater into brine of 100‰ and more, without their survival being affected (Sorgeloos, 1978), the nauplii were adjusted gradually to 90 ‰ seawater prior to their transfer to Barotac Nuevo.

The nauplii were transported in two 20 l plastic bags cooled to 4 °C and aerated with battery-operated aquarium pumps. Since the salt ponds were not ready for the inoculation experiment, the nauplii were distributed over two concrete tanks containing seawater of 84 and 130 ‰ salinity respectively.

The water in the tanks was aerated with the aid of 16 airstones connected to a 0.5 HP compressor. The animals were fed twice a week with a suspension of fine rice bran (prepared by squeezing 8 kg pre-soaked rice bran through a flour bag), 50 cc tiki-tiki[7] (a rice bran extract rich in factors of the vitamin B complex) and 10 g poultry vitamin-mineral mix Tra-Phos-D[8]. Rice bran was added till the turbidity of the water reached 20-25 cm (immersion of a graduated stick).

[6] San Francisco Bay Brand Cy, batch 288-2596.

[7] United Laboratories, Inc. of the Philippines.

[8] Interchem Philippines, Corp.

After 10 days the animals had reached the adult stage and half of the population was harvested with nets and inoculated in two salt ponds the salinity of which was about 100 ‰ and the average water depth 30 cm. The animals thrived well in the ponds without aeration and without any supplemental feed. Their natural food was composed of detritus (the salt ponds' intake water from a mangrove-creek) and phytoplankton, mainly diatoms belonging to the genera *Bacteriastrum*, *Chaetoceros*, and *Coscinodiscus*, and the bluegreens *Lyngbya*, *Oscillatoria*, and *Spirulina*.

Water temperature in the ponds ranged from 25 °C at night up to 33 °C during the day. One day the temperature rose to 37 °C, resulting in a mass kill of brine shrimp. About 65 kgs of *Artemia* biomass (wet weight) was collected and frozen for later use.

To avoid a repetition of a mass kill due to intense solar radiation and consequent high water temperatures, coconut fronds (branches) were laid in three parallel rows along the sides of the concrete dikes. They were kept at the water surface by bamboo stakes. No mortality was noticed even when water temperature rose as high as 39 C. It was observed that the animals stayed in the shade of the coconut fronds where the water temperature was 3 to 5 °C lower than in the open water.

Water from an estuary was let into the ponds every 12 to 15 days with the tidal rise to replenish water lost through evaporation and percolation.

When the salinity in the *Artemia* ponds dropped by more than 20 ‰ during water intake, solar salt was added in order to adjust the salinity level. It was furthermore found that the *Artemia* culture did better when the pond bottoms were stirred with bamboo rakes twice daily (7 am and 5 pm). This action probably resulted in a better food distribution.

As the *Artemia* population expanded, more salt ponds were inoculated. At the end of the dry season, a total pond area of 1.6 ha contained brine shrimp.

Over the 3 month production period the water temperature in the ponds ranged from a low 25 °C to a high 39 °C (average 34.5 °C). The salinity fluctuated between 80 and 170 ‰ (average 136 ‰) and the pH between 7.0 to 8.5.

In the concrete tanks 20 % of the culture medium was exchanged once a month by drawing water from the bottom with a pump provided with a fine mesh filter at its suction end (to avoid losses of brine shrimp) and refilling with water from the mangrove creek. The ranges for the abiotic conditions in these concrete tanks approximated 25 to 33 °C respectively (average 31 °C), 84 to 180 ‰ (average 93 ‰) and a pH ranging from 7.5 to 8.0.

From the end of March onwards, cysts were produced in the tanks as well as in the ponds. Whereas the *Artemia* population expanded through ovoviviparity at the lower salinity levels, more cysts were produced when the salinity reached 130 ‰ and higher. Since the wind direction was constant the cysts always accumulated in the same corner of the ponds and tanks.

The daily harvest of the cysts produced in the salt ponds was facilitated and even maximized by the installation of a plastic barrier which prevented the cysts of reaching the irregular dike structure where the harvesting with scoop nets was very difficult.

Processing and drying of the cysts was done following Sorgeloos (1978) and Sorgeloos *et al.* (1978).

At the end of the dry season (June 15th) about 16 kg cysts (dry weight) and some 150 kg adults (live weight) had been harvested from the ponds and the tanks. At the onset of the dry

season, an unquantified biomass of *Artemia* adults was left over in the ponds when these were utilized as nursery ponds for milkfish and prawn fry. The remaining brine shrimp constituted an excellent food for the former species.

During the 1979 dry season the same procedure of *Artemia* production has been repeated in the Barotac Nuevo salt ponds with even better resuls. During the period March-May 1979 cyst prodution averaged 620 gram dry weight/ha/day.

Discussion

The production results obtained at Barotac Nuevo clearly demonstrate the technical feasibility of *Artemia* cyst production in man-made salterns in Southeast-Asia.

It is obvious, however, that the procedures applied so far, can be improved greatly with regard to inoculation procedures, strain selection, water intake regimes, harvesting, etc. It was observed for example in these trials that high water temperatures (exceeding 35 °C) are lethal to the inoculated San Francisco Bay *Artemia*. This corroborates the observations of Baker (1966) for the parental population in the salinas of the San Francisco Bay. For this reason, the water depth in the Barotac Nuevo salt ponds was always maintained at 25 to 30 cm. This might, however, considerably restrict the area where brine shrimp could be produced since most of the salt ponds in Southeast-Asia operate at water depths of maximum 15 cm. To solve this problem, specific geographical strains of brine shrimp could be selected or developed through progressive adaptation to resist water temperatures above 40 °C. An alternative might be a compromise with the salt producers to maintain a higher water depth in order to arrive at an integrated production of salt and *Artemia*.

In view of the recent findings that there are considerable variations from one *Artemia* strain to another with regard to some specific characteristics which limit their use in the aquaculture hatcheries (not at least their nutritional value) the brine shrimp (cysts and adults) produced have to be "evaluated".

From the biometrical analyses of Vanhaecke and Sorgeloos (1980) it appears that the 1978 Barotac Nuevo cysts do not significantly differ from the parental San Francisco Bay stock.

Trial runs on feeding Barotac Nuevo *Artemia* to milkfish fry and juveniles resulted in high survival rates and fast growth. Fed a monodiet of decapsulated cysts, *Chanos chanos* fry grew from 0.024 g to 5 g with 80 to 95% survival. This is a much better figure than the average 65% survival obtained for fry grown in nurseries on a diet of ricebran and bluegreens algae. The daily consumption of decapsulated cysts averaged 30 cysts for larvae weighing 0.024 g. Juveniles with an individual weight of 5 g were fed nauplii and adult *Artemia* and benthic bluegreen algae, and attained an average weight of 250 g per fish after 72 days culturing.

Finally it has been shown that application of the *Artemia* inoculation technique can lead to a substantial increase in the production output of fish and shrimp nursery ponds. About 1 week prior to stock the fry, *Artemia* nauplii are inoculated in the ponds. Through proper screening of the intake waters, the ponds must be kept free from brine shrimp predators (except maybe a few insect species like Corixidae or Notonectidae the predatory activity of which can, however, in a period of one week not interfere too much with the development of the *Artemia* population). By the time the fry are released into the ponds, the *Artemia* have grown into pre-adults which are an excellent diet for the released fry.

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New aspects of the use of inert diets for high density culturing of brine shrimp

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Abstract

Brine shrimp being non-selective filter-feeders, cheap agricultural waste products should be evaluated as potential food sources for their controlled mass culturing.

Preliminary results of screening tests performed with wheat bran, soybean meal, rice bran and whey powder in culturing systems of various sizes are reported. Wheat bran appears to be unsuitable as monodiet for brine shrimp. The other products tested support good growth although specific precautions have to be taken with some foods to avoid problems of water quality during culturing.

Processing techniques for the manual or mechanical preparation of inert feeds for *Artemia* are indicated.

The possibility of influencing the fatty acid pattern of cultured brine shrimp by varying their diet is briefly outlined.

Introduction

The 11 pairs of thoracopods of an adult brine shrimp fulfill several functions : the oar-like endopodites serve for locomotion, the epipodites have a gill-function and the setae on the exo- and endopodites filter small particles from the surrounding medium.

The thoracopods move back and forward without interruption to assure an efficient respiration ; as a result the animals are swimming continuously. The thoracopodal movements induce two water currents along the ventral body surface (Cannon and Leak, 1933 ; Fig. 1) : by moving the limbs towards the head, the interlimb space increases and water is sucked into the midventral groove ; the inner lobes, feathered with dense filtering setae along the margin, collect particles from this incoming water stream. On the backstroke, water is forced out of the interlimb space. The collected particular material is transferred to the midventral food groove and pushed forward by slight spurts of water each time a limb shifts from backstroke to forestroke. In the head region, food particles are packed into a food bolus by a secretion product of the labrum. This bolus is finally pushed into the mouth opening by the action of the first maxillae (Ivleva, 1969).

[4] "Bevoegdverklaard Navorser" at the Belgian National Foundation for Scientific Research (NFWO).

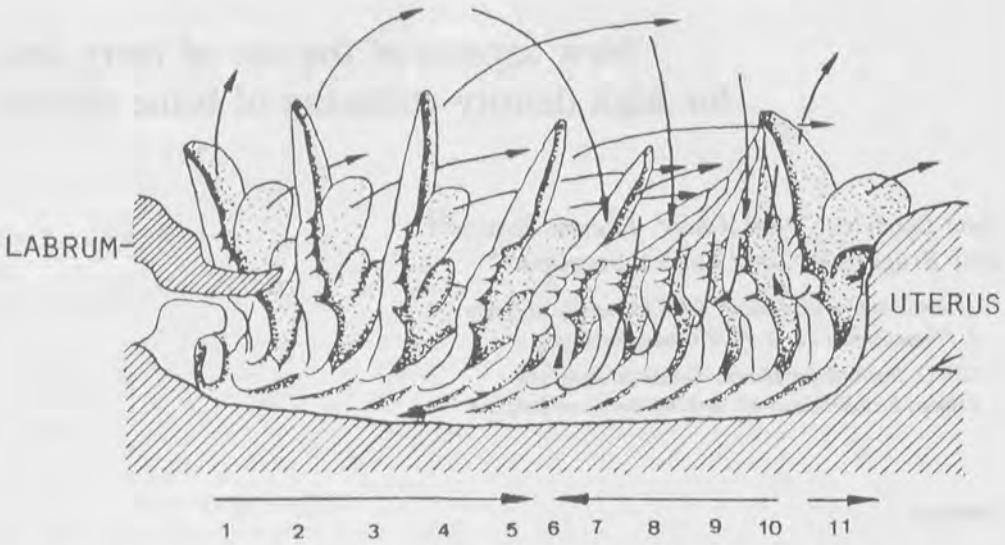

FIG. 1. Schematic diagram of the left row of thoracopods with indication of their separate movements in an adult anostracan shrimp (after Völlmer, 1952). In this phase of movement, thoracopods 1 until 5 and 11 provoke the animal's propulsion whereas the others create an underpressure in the interlimb spaces ; as a result water in sucked in and particles retained on the setae of the exo- and endopodites.

As compared to other Crustacea, *Artemia* has a very primitive feeding mechanism, it is indeed a continuous, non-selective, obligate phagotrophic filter-feeder (Provasoli and Shiraishi, 1959 ; Barker-Jörgensen, 1966). Suspended particles of suitable size, no matter what their nature is (Reeve, 1963), are continuously removed from the culture medium by the beating of the thoracopods. Except for the first larval stages, soluble products cannot be efficiently ingested and as such do not support growth in *Artemia*.

In the early larval stages, the filter-feeding activity of brine shrimp is not very efficient (Tobias *et al.*, 1979) : until instar VI only one pair of appendages, namely the second antennae have a locomotory and filter-feeding function. As molting proceeds, the thoracopods differentiate and become functional one after another, until in stage XII-XIII the maximal filtration capacity is attained when the 11 pairs of thoracopods are fully developed.

From the literature it appears that various food sources both live and inert, have been successfully used to culture *Artemia* from nauplius to adult (Table I). As far as growth rate and production are concerned, best results have always been achieved with a diet of live algae. Most of these tests have, however, been run at a very small scale *e.g.* in test tubes or petri dishes with but a small number of larvae. For high-density mass culturing of *Artemia*, live algae can hardly be taken into consideration since large scale production of the latter is not yet economically feasible. Similarly most inert food sources have either only been used for small-scale testing or cannot be considered for large-scale application because of their limited local availability and/or high price. In this regard the two algal powders, *Scenedesmus* and *Spirulina*, which we thought earlier to be the solution for large scale *Artemia* production

(Sorgeloos 1973, 1974) are unfortunately too expensive «... la recherche d'une nourriture adaptée à bas prix en est d'autant plus nécessaire» (Person-Le Ruyet, 1976).

TABLE I

List of some live and inert food sources known to support good growth in brine shrimp (information from Provasoli *et al.*, 1959 ; Katsutani, 1965 ; Shimaya *et al.*, 1967 ; Takano, 1967 ; Walne, 1967 ; Wolfe, 1971 ; Sorgeloos, 1973, 1974 ; Villacarlos, 1976 ; Jahnig, 1977)

Live algae :
Diatomeae : *Chaetoceros, Cyclotella, Phaeodactylum, Nitzschia*
Chlorophyceae : *Dunaliella, Chlamydomonas, Chlorella, Platymonas, Stichococcus, Stephanoptera, Brachiomonas*
Chrysophyceae : *Isochrysis, Monochrysis, Stichochrysis, Syracosphaera*
Dried algae : *Chlorella, Scenedesmus, Spirulina*
Yeasts : bakers' and brewers yeast
Various inert products : wheat flower, fish meal, egg-yolk, homogenized liver, rice powder

In the framework of a consultancy by one of us (Sorgeloos) in 1977 for the Jepara FAO-project in Indonesia and the SEAFDEC Aquaculture Department in the Philippines, agricultural waste products were screened for their potential use as food for large scale production of brine shrimp. Successfull results were reported with coconut meal, sugarcane molasses and soybean cake (Persoone, 1978 ; Talloen, 1978).

In order to optimise the ingestibility of agricultural products by *Artemia*, we have determined the maximum particle size that can be taken up by nauplii and adults. The paper reports culturing results obtained in feeding brine shrimps with rice bran, soybean, wheat bran and whey powder. Data are given on the fatty acid composition of *Artemia* fed various diets.

Relationship of particle size to ingestion in nauplii and adult *Artemia*

Although we know from the literature that brine shrimp ingest particles from a few micrometers in size [bacteria (Seki 1964)] up to close to 25 μm (Takano, 1967) the maximal size of particles which can be ingested by *Artemia* nauplii, respectively adults has never been defined accurately.

In order to answer this important question, the following experiment was carried out : instar III-IV nauplii (Hentschel, 1968) and adult brine shrimp from San Francisco Bay (California, USA) were offered a mixture of glass microspheres[5] ranging in diameter from a few micrometers up to 120 μm. Plastic test tubes of 5 ml were filled with natural seawater, inoculated with nauplii or adults and given a small amount of glass microspheres. The stoppered tubes were mounted on a rotating device (5 rpm) in order to keep all particles in

[5] Made available by N.V. Glaverbel, Belgium.

continuous suspension. Once the digestive tract was filled with microspheres and before defecation had started (determined to be 3 min for adults and 10 min for nauplii), the animals were fixed with a few drops of lugol's solution. The size determinations of the ingested particles were performed with a microscope equiped with a calibrated eye-piece. For the nauplii the ingested microspheres could be observed easily through the transparent cuticula but for the adults the digestive tract had to be removed by dissection.

In total about 3 500 microspheres have been measured, 2 000 of which were sampled at random from the test tubes, 1 000 ingested by 30 nauplii and 800 ingested by 5 adults.

The size frequency-distribution of the microspheres in suspension in the medium and those ingested by the instar III-IV nauplii, respectively the adults, are represented graphically in Fig. 2 and 3.

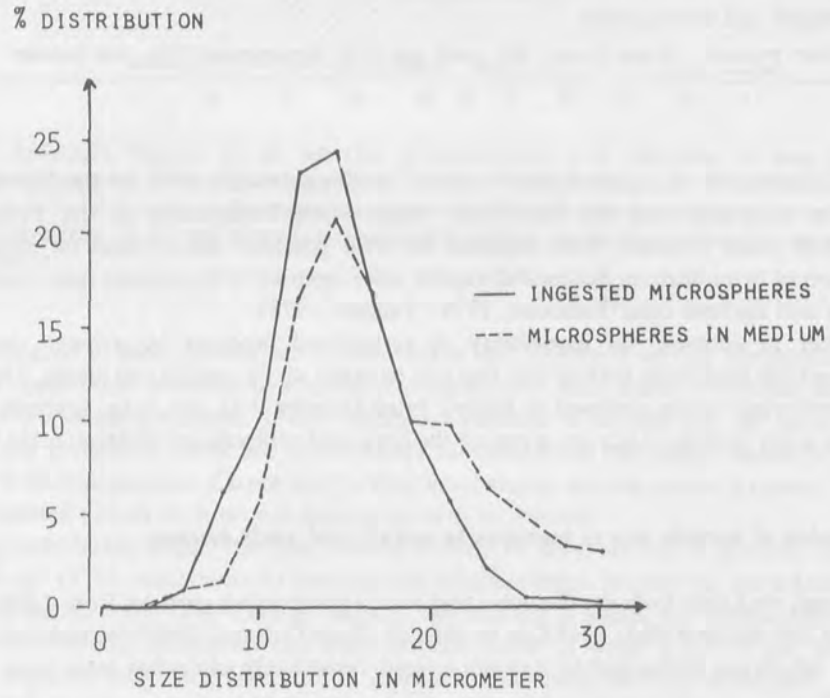

FIG. 2. Size frequency distribution of microspheres present in the medium (broken line) and ingested by instar III-IV nauplii (straight line).

From these results it clearly appears that the maximal size range of particles that can be ingested is 25 to 30 μm for nauplii and 40 to 50 μm for adult brine shrimp. It is interesting to note that there is much overlapping in the size distribution curves which is a further proof of the non-selective nature of the feeding process in *Artemia*.

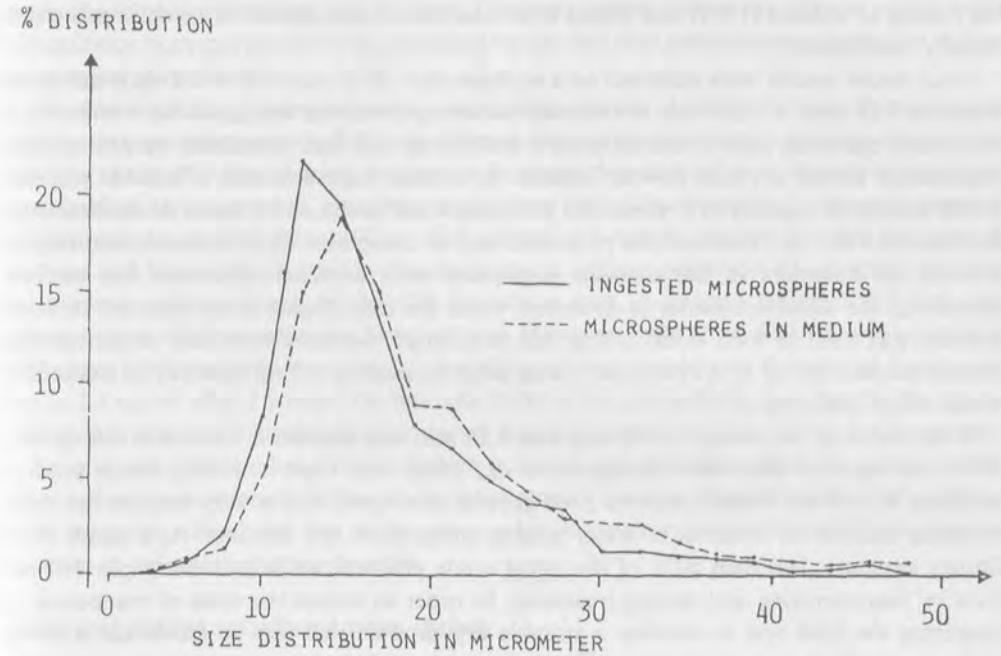

FIG. 3. Size frequency distribution of microspheres present in the medium (broken line) and ingested by adult brine shrimp (straight line).

Evaluation of rice bran, wheat bran, soybean- and whey powder as food for brine shrimp

Initial screening tests were performed in cylindrical-conical glass tubes (10 cm in diameter) : 200 nauplii were incubated in 600 ml natural seawater in each 1 l tube at a constant temperature of 28 °C. In order to assure good oxygenation of the culture medium and to keep the food particles in suspension an intermittant air bubbling from the bottom of the tubes was applied every 3 min for 5 sec. Food suspension was made up as follows : 50 g product was suspended in 1 000 ml seawater, homogenized and passed over a 50 μm screen. Since it could be expected that the quantity of food for optimal growth and survival varies from one type of food to another, the relative value of each product was determined by feeding different amounts of stock suspension in five series of replicate tests. Food was provided twice a day and the culture media renewed every second day. After 10 days culturing the number of survivors was determined. The larval length was measured using a dissection microscope and camera lucida projection. For all cases in which the survival rate exceeded 50 %, raceway-culturing tests were set up following the technique described by Bossuyt and Sorgeloos (1980) to further evaluate the suitability of the respective food sources for mass culturing of brine shrimp.

Poor results were obtained with wheat bran : maximal survival was less than 10 % after 10 days and the larvae reached a length of only 2.08 mm $\pm$ 0.39 mm. Those results corroborate

the finding of Takano (1967) that wheat bran used as a mono-diet for brine shrimp is nutritionally insufficient.

Much better results were achieved on a soybean-diet : 80% survival and a maximal larval length of 3.24 mm ± 0.29 mm. In raceway cultures growth was also good but within 4 to 5 days batch culturing, high ammonia levels were built up and high mortalities were noted. The explanation is that soybean powder appears to contain high amounts of soluble proteins which cannot be ingested by *Artemia* but provokes water pollution by bacterial development. Soybean powder can, however, be processed into an acceptable food for batch culturing of *Artemia* by treatment of the soybean suspension with a cream separator : the outflow containing the dissolved matter is discarded while the centrifugate is resuspended in brine solution and used as food stock. Using this technology, we have been able to convert the nauplii hatched out of 20 g cysts, into 3.8 kg adult biomass in a 2 m^3 raceway in less than 2 weeks batch culturing at 25 °C.

With rice bran, an average larval length of 4.26 mm was attained at a survival rate of over 80%. As reported elsewhere (Sorgeloos *et al.*, 1980) rice bran is a very cheap product available worldwide which supports good growth in *Artemia* and is very suitable for batch culturing because its contents of water soluble components are minimal. As a result of its fibrous structure, less than 50% of the initial crude product can be processed into *Artemia* food by homogenizing and sieving treatment. In order to reduce the costs of manpower in preparing the food and to develop a storable dry product that can be fed to the *Artemia* without any further manual preparations, we have studied the technical and commercial applicability of dry grinding and processing techniques. The best results were obtained with the Ultrafine® mill, the working principle of which is that a current of ambient air entrains crude rice bran powder into a grinding circuit, where three types of physical action (vortexes, changes in pressure, and vibrations) simultanously act on the rice bran particle, provoking a high degree of autogenous grinding of the material. The application of the Ultrafine® mill for rice bran grinding is new. The best results so far were obtained with defatted rice bran : on a weight basis, over 80% of the grounded product has a particle size inferior to 60 μm. The present price of micronised rice bran still amounts to U.S. $ 1.5/kg. Further automatization of the processing should, however, lead to a significant drop in the final price.

The best production results attained in batch culture in air-water-lift raceways were obtained with rice bran : on the average 2 kg *Artemia* biomass are produced per cubic meter of water within a period of 2 weeks, at 28 °C. This culture technique of *Artemia* on rice bran is, by our knowledge, already applied by aquaculture laboratories in Brazil, France, India, Indonesia, Norway, the Philippines, Thailand, the USA, and Venezuela. It is important to add here that although most *Artemia* strains which we tested so far grow well on a rice bran diet, we have been informed that other strains do not thrive on this sole diet : *e.g.* San Pablo Bay (California, USA, Bernardino, personal communication ; Abreu-Grobois, personal communication, and own observations), Lavalduc-France, Eilat-Israel, and Izmir-Turkey (Abreu-Grobois, personal communication). As a result it becomes imperative to study the differences in nutritional requirements between geographical strains of *Artemia*.

Although in earlier feeding tests we had never found a significant difference in food value between crude and defatted rice bran we recently experienced high *Artemia* mortalities after switching to a new commercial lot of crude rice bran. In the meantime we have been informed that depending on the geographical origin, rice bran is sometimes treated with

pesticides for transportation and storage. The good results which we always obtain with defatted rice bran can probably be explained by the fact that pesticides are eliminated during defattening of the product.

We have recently discovered a new interesting source of cheap *Artemia* food, namely Lactoserum or whey powder.[6] This product is the particulate matter resulting from drum drying and conditioning of whey suspension in cheese manufacturing. Although the chemical composition of Lactoserum probably varies from one dairy industry to another, whey is usually rich in carbohydrates (60 to 65% of the dry weight, mainly lactose) but poor in proteins and fatty acids. Its particle size can easily be reduced below the 50 μm upper size limit by hammer mill treatment. In the 600 ml culture tests an average length of 4.6 mm was attained after 10 days culturing. Survival was, however, only 50 to 60%. Repeated tests in 300 l and 2 m^3 raceways have shown that although high production figures can be attained (up to 2.5 kg/m^3 after 3 weeks), the reproducibility is not guaranteed : mass mortalities indeed often occur, especially during the early larval stages. At larval densities below 3 000 animals/l, bacteria and ciliates often develop in high numbers and adversely affect the water quality. Although this aspect needs further study it has already become clear that with whey-powder food dosage is critical and that better chances for success can be guaranteed by starting the cultures at very high nauplii densities.

Fatty acid pattern in cultured brine shrimp

From the literature it is known that fatty acid patterns in marine organisms are strongly influenced by abiotic and biotic parameters, such as temperature, metabolic needs of the animals and not the least dietary composition (review by Conover, 1978). Recent studies by Watanabe *et al.*, (1978, 1979) reveal the dietary need for fish larvae of the following poly unsaturated fatty acids (PUFA), also called essential fatty acids : 18:2ω6, 18:3ω3, 20:5ω3 and 22:6ω3. In view of the importance of these biochemical components in evaluating the nutritional value of *Artemia* cultured on agricultural waste-products, we have compared the fatty acid pattern of *Spirulina*, rice bran and soybean as well as of adult brine shrimp cultured on these food sources. We have also studied the influence of a diet alteration on the changes of the fatty acid pattern in *Artemia*. The *Artemia* were cultured in 300 l raceways at 25 °C. The adult animals were harvested over a 500 μm screen, washed with tap water, freeze dried, and stored under nitrogen. Fat was extracted with a 2:1 mixture of chloroform and methanol (Medwadowski *et al.*, 1971). After esterifying with 5% sulfuric acid in methanol, the methyl-esters were stored at −10 °C. The fatty acids were separated and analysed on two 7 m stainless steel columns packed with 15% diethyl glycocol succinate on chromosorb (acid washed, DMCS, 80-100 mesh) using a Varian Aerograph 2400 gas cromatograph equipped with double flame ionisation detector and temperature programmation from 90 °C to 180 °C at 6 °C/min.

The procentual composition of long chain fatty acids in *Spirulina*, rice bran and soybean meal is summarised in Table II. From these figures it is clear that all food sources contain high amounts of 18:1ω9, 18:2ω6 and, with exception of *Spirulina*, 18:3ω3. The fatty acid data for

[6] Made available by N.V. De Gier, Gierle-Belgium.

the nauplii and cultured brine shrimp are given in Table III. In accordance with Watanabe *et al.* (1978) we observed that the PUFA-concentration increases as the animals grow from nauplii to adults. The very significant drop in 18:3ω3 from 27% in the nauplii to a few percent in the adults is, however, surprising. In rice bran fed *Artemia*, the 18:1ω9 and 18:2ω6 concentration have increased. The low content of 18:3ω3 might be caused by its very low concentration in this specific diet. Although high concentrations of 18:2ω6 are present in soybean meal, this PUFA is metabolised into other fatty acids when taken up by *Artemia*. Comparison of the 18:3ω3 levels in the *Artemia* and their respective diets, reveals that its incorporation in brine shrimp is directly related to its availability in the diet.

TABLE II

Procentual composition of unsaturated fatty acids in *Spirulina*, rice bran and soybean meal (expressed as % of total lipids)

Fatty acid	*Spirulina*	Rice bran	Soybean meal
16:1ω7	12.2	0.1	Trace
18:1ω9	3.0	44.9	15.1
18:2ω6	18.8	36.1	56.6
18:3ω3	trace	1.2	6.4

TABLE III

Procentual composition of unsaturated fatty acids in Great Salt Lake (Utah, USA) *Artemia* nauplii and in adults cultured on rice bran or soybean meal (expressed as % of total lipids)

Fatty acid	GSL-Utah nauplii	Rice bran fed *Artemia*	Soybean fed *Artemia*
16:1ω7	4.6	5.3	10.7
18:1ω9	30.1	47.0	33.5
18:2ω6	5.5	25.1	17.8
18:3ω3	27.4	2.2	4.5
20:5ω3	2.8	Trace	Trace

In the last series of experiments we studied the effect of a diet alteration on the PUFA-changes in *Artemia*. After 14 days culturing on rice bran, the medium was renewed and the animals were fed a *Spirulina*-suspension. Samples of brine shrimp were taken before this change of diet and after 1, 2, and 3 days *Spirulina*-feeding respectively and analysed for their PUFA-contents. The changes in procentual composition are represented graphically in Fig. 4. The drastic change in fatty acid pattern over a period of only a few days is remarkable. The drop in the concentration of 18:1ω9 from 55 to 35% is probably a reflection of its availability in the diet (45% in rice bran, only 3% in *Spirulina*). However, in view of its low content in *Spirulina*, the increase in 18:3ω3 can only be explained by synthesis of this component by the adult brine shrimp.

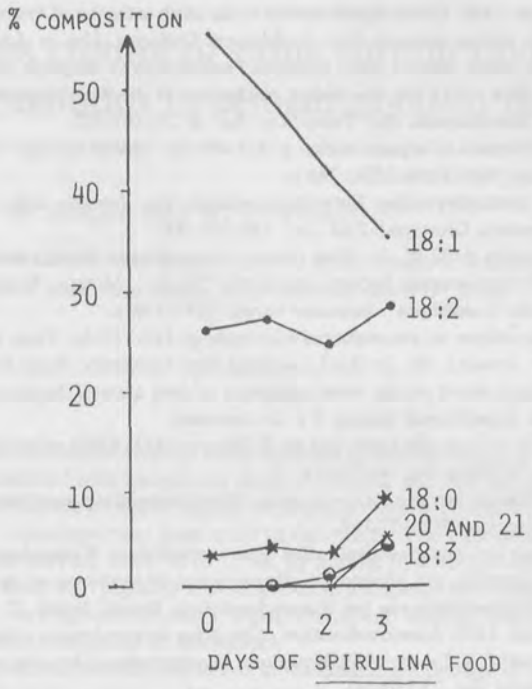

FIG. 4. Changes in fatty acid pattern of *Artemia* switched to a *Spirulina*-diet after 2 weeks rice bran feeding.

Although these data on PUFA composition still need to be completed and interpreted in the light of results obtained in feeding these *Artemia* to fish and crustacean larvae, it is already clear from this experiment that it must be possible to improve the nutritional suitability of *Artemia* through manipulation of the diet. Since brine shrimp can be bulk fed till shortly before their harvest on cheap waste products such as rice bran, a limited feeding period on more expensive formulated diets, should on one hand not have a major impact on the economic feasibility of *Artemia* production, whereas on the other hand it can greatly improve the nutritional value of this invaluable food organism.

Acknowledgement

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Mass culture of brine shrimp under controlled conditions in cement pools at Bombay, India

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Abstract

In 1953 parthenogenetic strains of *Artemia* were recorded in salt pans around Bombay. Cysts collected from these pans are hatched into nauplii for aquaria keeping and rearing of decapod crustaceans.

Since prawn hatcheries require a regular supply of *Artemia* nauplii, continuous mass culture of brine shrimp under laboratory conditions has been tried in this study. The experiments were conducted in brackish water in salinities varying from 30 to 75 ‰, by adding filtered concentrate of crude common salt. The pools were manured with pigdung and single superphosphate and supplemented with groundnut oil cake and yeast in varying combinations. Upon manuring, diatoms (*Navicula* sp., *Nitzschia* sp., *Amphiprora* sp.) and ciliates multiplied in abundance.

The above food combinations proved successful in maintaining a density of 500 adult *Artemia*/l for 30 days. 12-15 days after the inoculation of freshly hatched nauplii a population of adult *Artemia* of about 12 mm in size had developed. Adults of this first generation stock grew further and reached a length of 17 mm 15 days later ; during this period each female released four batches of eggs at an interval of 4-5 days. Gravid females were carrying about 76 to 101 eggs in the brood sac. The temperature during the experiments ranged between 24 °C and 27 °C.

The present series of experiments demonstrate the low input technology in *Artemia* culturing suitable for rural conditions in India and useful for commercial prawn hatcheries.

Introduction

During the last decade, commercial and semi-commercial fish and prawn farming expanded considerably and prawn culture has become important because of the high prices of prawn and fish and good export trade. It has been emphasized that for a quick development of prawn culture, setting up of commercial and semi-commercial hatcheries is necessary so that prawn juveniles are available in large numbers for stocking (Alikunhi 1978 ; Dwivedi, 1978). Management of prawn hatchery involves several problems, amongst which the availability of proper food for the different larval stages (zoea, mysis, and post larvae) has been a limiting factor. The brine shrimp *Artemia* is known to constitute not only the best but also an easily available source of live food (Sorgeloos *et al.*, 1977). There are several advantages in using *Artemia*. The dormant cysts remain viable for several years if they are stored under nitrogen or vacuum. This unique character of the cysts coupled to the nutritive value of the *Artemia* nauplii and their acceptance by the crustaceans has resulted in a flourishing trade in the United States, where the cysts are sold for as much as US $ 60.00/kg. The development of aquaculture and aquaria as a hobby increased the demand of cysts and the actual global requirement exceeds 35 to 40 tons of cysts per year. It has been mentioned that *Artemia* is in

fact the only organism on which about 99 % of aquaculturists have relied for the last decade (Sorgeloos and Persoone, 1975).

In prawn hatcheries prawns are raised through the larval to the juvenile stage during which period the larvae change their food habits from one stage to another. The first stages, which are very small are filter feeders. Therefore, the particle size of the food material and its concentration in the culture have an important effect. At the mysis stage prawn larvae start to feed on *Artemia* nauplii ; the availability of a large number of brine shrimp nauplii in the culture has an important bearing on the survival of the prawn mysis. In this regard continuous culture of *Artemia* at low cost is imperative.

Extensive research on various aspects of brine shrimp is being carried out at various Institutes and mass rearing techniques have been reported from laboratories in Belgium and the United States. In spite of the progresses achieved, Sorgeloos *et al.* (1979) rightly indicate that much fundamental as well as practical research is still necessary to optimize the achievements obtained so far. In the wake of development of coastal zones for brackish water prawn culture, *Artemia* culture has also become more important in India. The present experiments have been conducted to work out an inexpensive system for mass culture of *Artemia*, in view of their use in prawn hatcheries.

Material and methods

Artemia cysts were collected locally from Bahinder near Bombay. They were hatched under laboratory conditions and the nauplii were reared to adults. The effects of different combinations of manures and the influence of salinity on survival, growth, sexual maturity, and fecundity was studied. Cysts were hatched in cylindrical glass funnels. The brackish water was obtained from a well and common salt was added to raise the salinity to 30, 35, 40, 45 and 50 ‰. The water was then filtered through a fine mesh cloth and through glasswool, and moderately aerated for 12 hr, before the immersion of cysts. Rearing experiments were performed in cement pools of 300 l capacity. The first series of experiments were set up with water of 45 ‰ salinity. The pools were enriched with different combinations of manure and waste products 2 days before the innoculation of young nauplii. The manure and foods used were pigdung, groundnut oil cake, single superphosphate and commercial yeast. Six different food mixtures were used in the combinations given in Table I.

TABLE I

Food combinations of manure and waste products used to culture *Artemia*

Combinations	Pigdung (g/100 l)	Single super phosphate (g/100 l)	Groudnut oil cake (g/100 l)	Commercial yeast (g/100 l)
A	30	NIL	NIL	NIL
B	30	6	NIL	NIL
C	30	NIL	10	NIL
D	30	NIL	NIL	3
E	30	6	10	NIL
F	30	6	10	3

Three days after enrichment each pool was inoculated with 550 nauplii/l approximately. The environmental factors, growth and survival were recorded every second day. Before taking samples the pools were covered with a black sheet to obtain a uniform distribution of the nauplii. A sample of 10 l was taken and filtered through a 200 μm filter. The brine shrimp were counted and some specimens were preserved to study growth and fecundity. Since the experiment with food combination F showed the best results, additional tests were conducted with this combination to look at the effect of different salinities (30, 45, 60, and 75 ‰ respectively).

Results

Hatching

A series of preliminary experiments were conducted at salinities ranging from 30 to 50 ‰ with moderate aeration, at a temperature of 26 ± 1 °C. These experiments showed that a salinity of 35 to 40 ‰ gave good results. Hatching started after 12 hr and a maximum hatching efficiency of 70 % was obtained after 18 to 26 hr. Above 40 ‰ salinity the hatching rate decreased to 45 % and hatching took place after 24 hr only.

Rearing

Effect of the different food combinations

Survival

Artemia were reared successfully to the adult stage and maximum survival was obtained with F which induced a high algal production serving as food. Swimming behavior of the brine shrimp was observed to be weak with A ; mortality started to occur after the 3rd day and none of the animals survived after 15 days. With B and C containing single superphosphate and oil cake respectively in addition to pigdung, the animals were healthy and survival rates had increased to 50 %. In combination D with the addition of yeast to pigdung, survival rates of 60 % were recorded. Combination E, a mixture of pigdung, oil cake and superphosphate produced better growth of phytoplankton and the survival rate rose to 80 %. In F finally the addition of yeast to combination E yielded the best results as shown in Table II and Fig. 1.

Growth

The growth achieved with the different food combinations is shown in Fig. 2. Growth was very poor with application of A probably because of poor phytoplankton growth. The growth rates obtained with B and C were almost identical. In D, E, and F the growth rate increased gradually with the further enrichment of the food with different components (Table II). With F, the longest body length attained in two weeks was 12 mm. All the animals continued to grow and attained a size of 17 mm in 30 days. The maximum size recorded so far in Bombay salt pans was only 12 mm (Kulkarni, 1953).

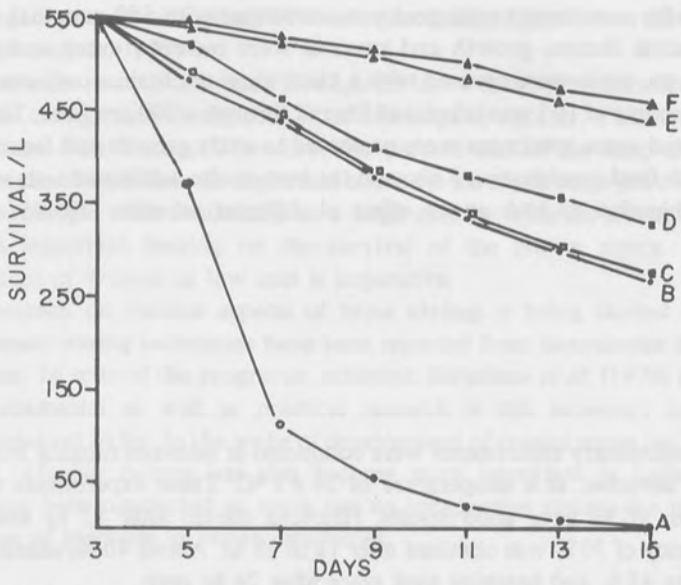

FIG. 1. Survival rates of *Artemia* cultured in different combinations of food (salinity 45 ‰ ; temperature 26 ± 1 °C).

TABLE II

Effect of different food combinations on body length, survival, sexual maturity, and fecundity of *Artemia* cultured in cement pools of 300 l capacity (inoculation : 550 nauplii/l)

Food combination	Age (days)	Length (mm)	Survival (%)	Number of days to maturity	Fecundity (%)	Gravidity (%)
A	3	1.4	100.0			
	9	5.4	10.0	maturity not attained	–	–
	15	6.2	00.4			
B	3	1.8	100.0			
	9	6.1	69.9	18	53	45
	15	8.6	50.0			
C	3	1.8	100.0			
	9	6.2	70.0	18	56	48
	15	8.8	50.0			
D	3	2.0	100.0			
	9	6.6	74.5	16	64	100
	15	9.3	60.0			
E	3	2.1	100.0			
	9	7.6	92.2	14	69	100
	15	10.8	80.0			
F	3	2.3	100.0			
	9	8.0	93.6	14	76	100
	15	11.2	83.6			

Maturity and fecundity of eggs

Maturity and fecundity were also significantly different with the different food combinations. Eggs started to develop when the females reached a size of 8.2 mm. Food combination A yielded poor results and eggs did not develop in any specimen. Fecundity of eggs with B and C showed almost identical results ; 45 % to 48 % of the animals became gravid and the development of eggs started after 18 days. With D, the percentage of gravid females increased to 100 and fecundity of eggs increased to 64 per brood sac. The period required to attain sexual maturity decreased to 16 days. E produced a heavy growth of diatoms which further promoted fecundity and growth and decreased the time span to sexual maturity (Table II). With F all females became gravid within 14 days and the number of eggs in each brood sac increased to an average of 76 (Fig. 3). The experiments were repeated in time and yielded similar results.

TABLE III

Effect of salinity on body length, survival, sexual maturity, and fecundity of *Artemia* cultured on food combination F in 300 l cement pools (inoculation : 550 nauplii/l)

Salinity (‰)	Age (days)	Length (mm)	Survival (%)	Number of days to maturity	Fecundity (%)	Gravidity (%)
30	3	2.1	100.0			
	9	6.8	20.9	18	47	40
	15	8.3	4.4			
45	3	2.3	100.0			
	9	8.0	92.0	14	84	100
	15	11.2	84.4			
60	3	2.4	100.0			
	9	8.4	95.5	12	93	100
	15	11.6	94.0			
75	3	2.4	100.0			
	9	8.6	96.0	12	100	100
	15	12.0	94.5			

Influence of salinity

In order to determine the optimal salinity for growth, survival and sexual maturity, food combination F was tried at salinities of 30, 45, 60, and 75 ‰ respectively. This combination produced good phytoplankton growth in the different salinities tested out, especially diatoms. *Nitzschia* sp. was found in concentrations of 16.10^3/ml, *Navicula* sp. up to 10^3/ml and *Amphiprora* sp. 10^2/ml ; the presence of ciliates (40 ind./ml) was also noted. All these living organisms form a direct food for *Artemia*. Growth and survival were better at 45, 60, and 75 ‰ salinity than at 30 ‰ (Fig. 4 and 5). At 30 ‰ and 45 ‰ salinity sexual maturity occurred in 18 and 14 days respectively (Table III). At 60 and 75 ‰ salinity all the animals matured in 12 days and the first batch of eggs was released in 12-15 days. After 4 to 5 days interval the gravid females released successive batches of nauplii. The fecundity of the eggs

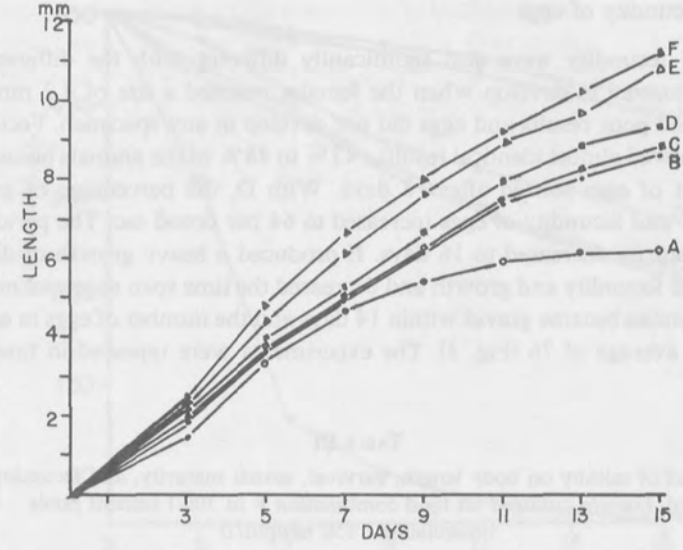

FIG. 2. Growth rate of *Artemia* cultured in different combinations of food (salinity 45 ‰; temperature 26 ± 1 °C).

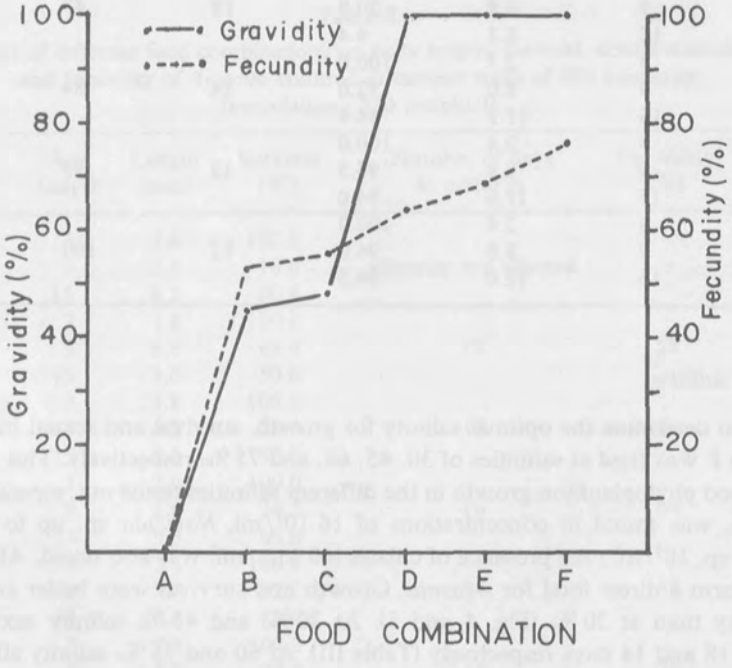

FIG. 3. Effect of different food combinations on maturity and fecundity of *Artemia* (salinity 45 ‰; temperature 26 ± 1 °C).

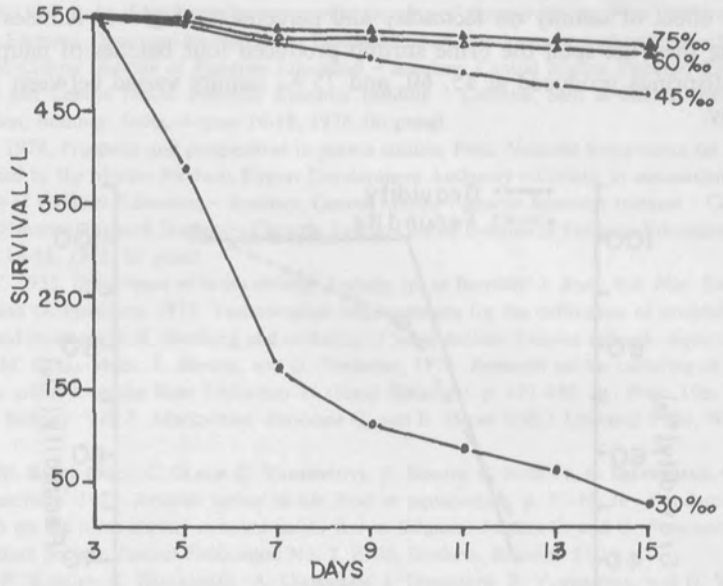

FIG. 4. Effect of salinity on survival rate of *Artemia* (food combination F ; temperature 26 ± 1 °C).

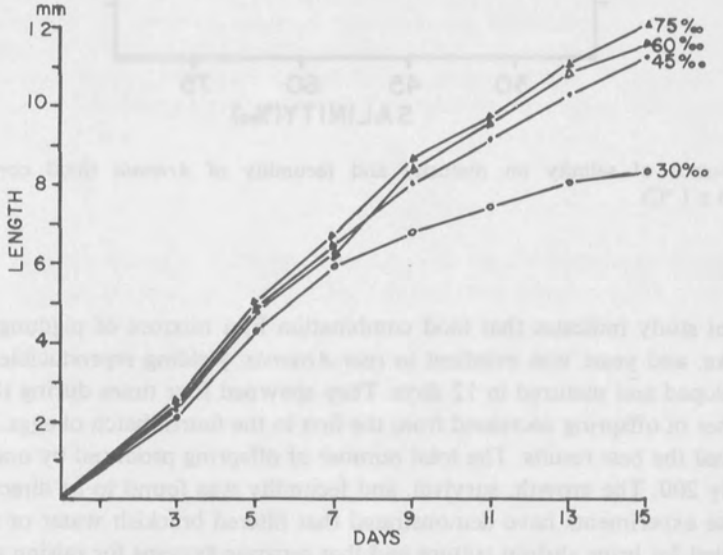

FIG. 5. Growth rates of *Artemia* at different salinities. (food combination F ; temperature 26 ± 1 °C).

increased with increasing salinities. The number of offspring produced in the first batch was 47 in 30 ‰, 84 in 45 ‰, 93 in 60 ‰, and 101 in 75 ‰ and decreased in the subsequent three hatches. The effect of salinity on fecundity and percentage of gravid females is shown in Fig. 6. During their life span the brine shrimp produced four batches of nauplii. The total number of offsprings produced at 45, 60, and 75 ‰ salinity varied between 170 and 200 approximately.

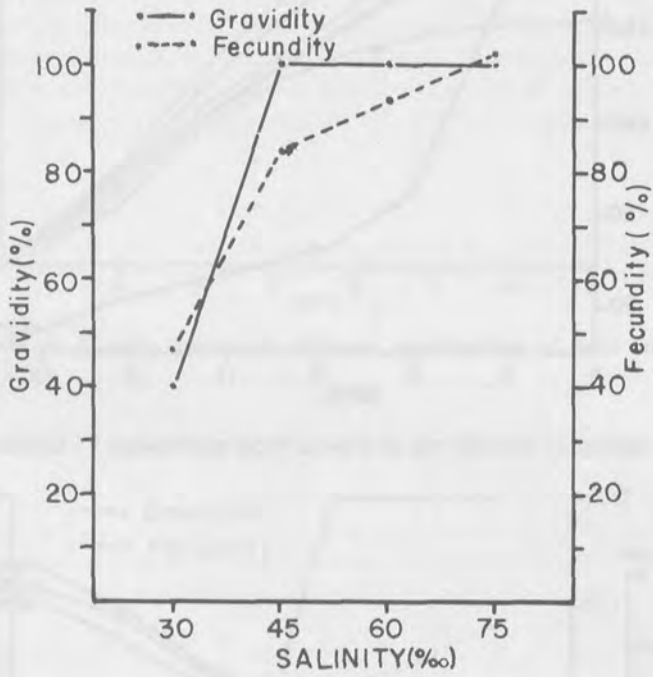

FIG. 6. Influence of salinity on maturity and fecundity of *Artemia* (food combination F; temperature 26 ± 1 °C).

Conclusions

The present study indicates that food combination F, a mixture of pigdung, superphosphate, oil cake, and yeast was excellent to rear *Artemia*, yielding reproducible results. The animals developed and matured in 12 days. They spawned four times during their life span but the number of offspring decreased from the first to the fourth batch of eggs. A salinity of 75 ‰ produced the best results. The total number of offspring produced by one female was approximately 200. The growth, survival, and fecundity was found to be directly related to salinity. These experiments have demonstrated that filtered brackish water or seawater are very well suited for brine shrimp culture and that separate systems for raising algal cultures are not necessary. The technology described is an inexpensive method to culture *Artemia* which can be applied at large scale for supply of *Artemia* nauplii for prawn hatcheries in rural areas in India.

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SALINITY(‰)

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Evaluation of various diets for optimal growth and survival of selected life stages of *Artemia*

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Abstract

Five different types of food, each at six concentrations, were tested to determine their effect on the growth and survival of the brine shrimp *Artemia*. The food types were live *Dunaliella tertiolecta* (microalga), dried *Spirulina* (blue-green alga), freeze-dried *Enteromorpha* (macroalga), freeze-dried *Rhodotorula* (marine yeast) and rice bran. Two different age categories of *Artemia* were examined, animals 1-3 days old and animals 4-7 days old. The first two sets of experiments were run in small flasks with 100 *Artemia* (Brazil lot 11) in 100 ml seawater. Rice bran and *Rhodotorula* yielded significantly better growth than the other diets for animals 1-3 days old. The best concentration of these diets was 0.10 mg dry wt food/*Artemia*/ml seawater/day. For days 1-3 of the 7 day experiment, the *Artemia* were fed the optimum concentration of each diet, which was determined from the 3 day experiments. The concentration of food was then varied from days 4-7. Unlike the 3 day experiment, *Spirulina* was the diet resulting in the best growth and survival of the brine shrimp after 7 days (at a concentration of 0.60 mg dry wt/*Artemia*/ml seawater/day). The diets were then examined in 430 l raceway systems for 7 days. Concentrations of food added were increased daily (0.011 mg dry wt food/*Artemia*/ml on day 1 to 0.066 mg dry wt food/*Artemia*/ml on day 6). *Spirulina* was again the best diet for 7 days. *Spirulina* has the highest protein level of the diets tested (58 %) which may be the reason it is the best diet.

Introduction

The survival and food value of the brine shrimp *Artemia* are important because this species is used extensively in aquaculture as a diet for fish and invertebrates. Until recently almost all brine shrimp were used as food by the instar 3 stage (24 hr after hatching). Benijts *et al.* (1976) showed that from instar 1 to instar 3 the dry weight of *Artemia* decreased by 20 % and the caloric value decreased by 27 % if the brine shrimp were not fed. He suggested using them at instar 1 or else they should have food supplied and be grown to a larger size. Larger *Artemia* have a greater caloric content and provide a food source for larger fish and invertebrates. Sorgeloos *et al.* (1977a) developed methods for mass-culturing brine shrimp to adulthood with good survival and growth.

Artemia are non-selective, obligate filter feeders, and therefore many different oganisms have been used as food for them (Teramoto and Kinoshita, 1961 ; Nimura, 1967 ; Shimaya *et al.*, 1967 ; Jones *et al.*, 1974 ; Sick, 1976). Sorgeloos (1974) has shown that different methods of food preparation : live, suspensions homogenized by Ultrason, frozen and dried algae, do

not affect its nutritional efficiency. However, axenic and non-axenic cultures of food organisms have different nutritive values (Gibor, 1956), the latter yielding better growth of the brine shrimp. Food concentration is also important. Reeve (1963) obtained a maximum growth efficiency with algal concentrations of 25-35 cells/ml ; however, at high concentrations (approximately 5 000 cells/ml), food passes through the gut too fast for efficient digestion and the animals can starve in the presence of excess food.

This study evaluates five diets for *Artemia* to determine both the optimal diet and optimal concentration of each diet, first on a small scale and then in mass-culture systems. Two different age categories of the brine shrimp were used, animals 1-3 days old and animals 4-7 days old.

Materials and methods

The different food types used to feed *Artemia* (Brazil strain Aquarium Products[1]-Lot 11) were : live *Dunaliella tertiolecta*, a green microalga ; dried *Spirulina* sp., a blue-green alga ; freeze-dried *Enteromorpha* sp., a green macroalga ; freeze-dried *Rhodotorula* sp., a marine yeast ; and fat-containing and defatted rice bran. *Dunaliella* was grown in semi-continuous culture in F/2, 35‰ media (Guillard and Ryther, 1962) at 18 °C under continuous light at 5 400 lux. *Rhodotorula* sp. was grown in an 8 ‰ media containing glucose, ammonium nitrate, potassium phosphate, peptone and yeast extract at 25 °C. Both *Dunaliella* and *Rhodotorula* were harvested during log phase. *Enteromorpha* was collected from coastal lagoons in Rhode Island. *Spirulina* and rice bran are commercially available products (*Spirulina* was obtained from Ocean Farming Systems[1], Florida, and rice bran from the Artemia Reference Center at the State Universty of Ghent, in Belgium). *Enteromorpha* and *Rhodotorula* were freeze-dried and then placed in a blender at high speed for 1 min to reduce particle size prior to being used as food.

For the first set of experiments each of the above food types was fed to the brine shrimp at six concentrations (0, 0.0025, 0.025, 0.10, 0.20, and 0.40 mg dry wt food/*Artemia*/ml seawater) for the first 3 days after hatching. One hundred *Artemia*, instar 1 stage, were placed in 100 ml 0.45 μm filtered, autoclaved seawater (35 ‰). The culture flasks were placed randomly on a reciprocal shaker and gently aerated. Air tubes were rearranged daily to minimize any effect of unequal aeration. Experimental treatments were run in triplicate and food was added every day. Percent survival and average lengths (five organisms per flask, each measured from the anterior tip of the ocellus to the base of the caudal furca, significant to 0.05 level) were measured at the end of the third day to determine the optimal food type and concentration. The results were analyzed using 2-way analysis of variance, Duncan's and Bartlett's analysis.

In a second set of experiments each food type was tested again on 4-7 day old brine shrimp. The experimental design was the same as above except that on the first 3 days only the optimal concentrations (determined from the first set of experiments) of the food type being tested were used. For days 4-7, six different concentrations of food were fed the *Artemia* (0,

[1] Mention of trade names does not denote endorsement by the US Environmental Protection Agency.

0.12, 0.36, 0.60, 0.84, and 1.08 mg dry wt food/*Artemia*/ml seawater/day). After day 7, percent survival and average length were measured and analyzed as above.

A final set of experiments were run to test four of the diets on a larger scale (*Enteromorpha*, *Spirulina*, *Rhodotorula*, and rice bran). Raceway systems of 430 l (Sorgeloos *et al.*, 1977b) with an *Artemia* density of 1 per ml were used. All raceways were initially stocked with *Artemia* at the instar 1 stage and experiments were run for 7 days. Each system had increasing amounts of food added each day. The amount of food added was 0.011, 0.022, 0.033, 0.044, 0.055, and 0.066 mg dry wt food/*Artemia*/ml (0.011 mg on day 1 increasing to 0.066 mg on day 6). Percent survival and length were measured daily using three 100 ml samples from each raceway system. The experiments were run in duplicate and analyzed as in the first two sets of experiments.

Two different qualities of the four diets used in the raceways were examined. Forty particles from each diet were measured to obtain an average size and range of sizes for each diet and proximate nutritive analyses were also done. Protein, moisture and ash were analyzed according to Horwitz (1975); protein by the Kjeldhal method (no. 7.016), moisture by drying at 135 °C (method no. 7.008) and ash by method no. 7.010. Lipids were analyzed by the methods described by Schauer *et al.* (1980). Carbohydrates were determined by subtraction.

Results

In the 3 day experiment, *Artemia* fed on rice bran or *Rhodotorula* had the best growth (Fig. 1). At concentrations higher than 0.1 mg food/*Artemia*, increase in length was not significantly different ($P > 0.05$) from *Artemia* fed 0.10 mg food/*Artemia* of *Dunaliella*, *Rhodotorula* or rice bran. *Spirulina* was effective at lower concentrations than the other diets (0.025 mg food/*Artemia*) and *Artemia* fed on *Enteromorpha* never reached a saturation level of food at the concentrations tested.

At concentrations of 0.025 and 0.10 mg food/*Artemia* survival was greater than 93%. At higher concentrations, 0.20 and 0.40 mg, the survival rates decreased slightly (80-90% survival) for all diets except *Spirulina* which decreased rapidly to 30% at 0.40 mg food/*Artemia*. From these data, 0.1 mg food/*Artemia* was chosen as the optimum of the concentrations tested for *Dunaliella*, *Rhodotorula*, *Enteromorpha* and rice bran. For *Spirulina* the optimum was 0.025 mg food/*Artemia*.

In the second set of experiments, *Artemia* were fed the optimal concentrations determined from the first set of experiments for the first 3 days, then concentrations were varied from days 4-7 for each diet. The *Spirulina* diet resulted in the best growth for *Artemia* in this set of experiments (Fig. 2). *Artemia* fed on *Dunaliella* reached a comparable size but at higher concentrations of food, 1.08 mg of *Dunaliella* versus 0.60 mg of *Spirulina*. At concentrations greater than 0.60 mg food/*Artemia* there was either no significant increase in length ($P > 0.05$) or else they decreased in length with the exception of *Artemia* fed *Dunaliella*, which continually increased in length with increasing amounts of food.

Survival rates for the 7 day experiments were very erratic. With the addition of any amount of food, survival fluctuated between 60-90% with no recognizable pattern. Due to a large variation among replicates no statistical analysis could be performed. The optimum concen-

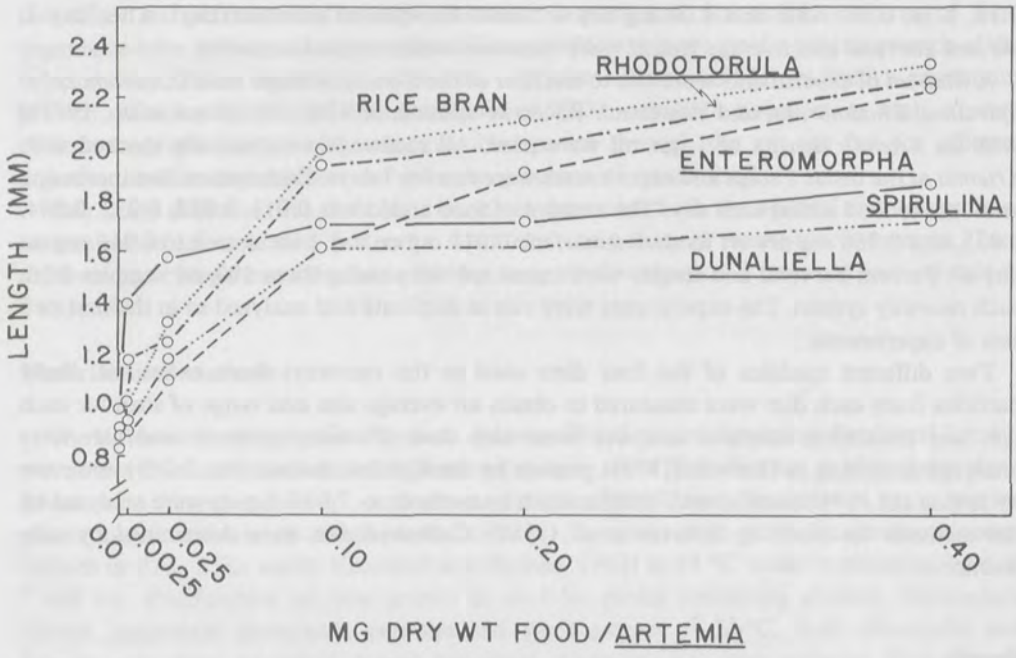

FIG. 1. Length of *Artemia* after feeding for 3 days on five different diets at six concentrations. Culture conditions were 100 *Artemia* in 100 ml seawater.

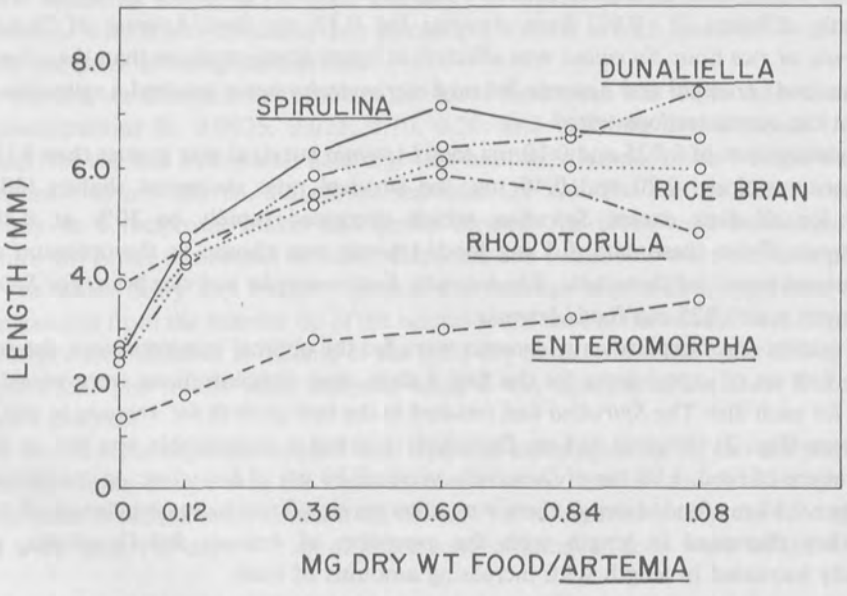

FIG. 2. Length of *Artemia* after feeding for 7 days on five different diets at six concentrations. The *Artemia* were fed 0.10 mg dry wt food/*Artemia*/ml of the diet being tested for days 1-3 except for *Spirulina* which was fed at a concentration of 0.025 mg dry wt/*Artemia*/ml. The diets were then fed at the indicated concentrations for days 4-7. Culture conditions were 100 *Artemia* in 100 ml seawater.

tration for every diet was 0.6 mg food/*Artemia* except for *Dunaliella* which was 0.84 mg food/*Artemia*.

In the previous sets of experiments both fat-containing (17% lipid) and defatted (6% lipid) types of rice bran were used as diets to test for any differences between the two. There was no significant difference ($P > 0.05$) in growth or survival of *Artemia* fed on either type of rice bran.

Extrapolations made from the small flask experiments to the 430 l raceway systems showed that the amount of *Dunaliella* that would be needed as food for the *Artemia* in the raceways was too great to be feasibly cultured ; therefore, this diet was not examined in the large systems. For the other diets the first concentrations utilized were the optimal ones found in the first two sets of experiments. In all cases 100% mortality of the *Artemia* occurred after 3 days and the raceways were anoxic. Apparently there was not enough water circulation in these systems to keep the food in suspension. The amount of food added to the tanks was then sequentially decreased until an amount of food was reached which resulted in good growth and survival after 7 days. The concentrations used each day in the raceway systems are indicated in the materials and methods section. As with the small flask 7 day experiments, *Spirulina* resulted in the best growth and survival of the *Artemia* after 7 days (Fig. 3). *Artemia* fed rice bran, *Enteromorpha* or *Rhodotorula* had significantly ($P < 0.05$) lower growth rates and survival rates than those fed on *Spirulina*. The *Artemia* fed rice bran and *Enteromorpha* had survival rates of 80-85%, but the *Artemia* fed *Rhodotorula* showed only 20% survival after 7 days.

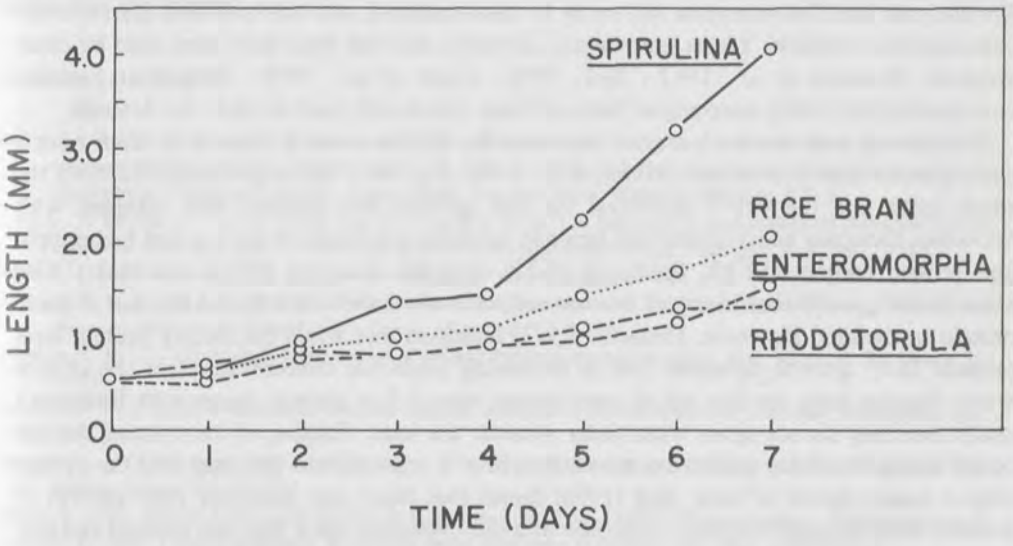

FIG. 3. Length of *Artemia* grown on four different diets in 430 l raceway systems. Concentrations of food fed were 0.011, 0.022, 0.033, 0.044, 0.055, and 0.066 mg dry wt food/*Artemia*/ml on days 1-6 respectively. Density of *Artemia* was 1 per ml.

Examination of the particle sizes of the different diets found that there were large differences among them. An average was made of widths and lengths for the particles of each diet : *Dunaliella* 3-8 μm, *Spirulina* 10-50 μm, rice bran 10-70 μm, *Rhodotorula* 5-100 μm, and *Enteromorpha* 20-120 μm.

Proximate nutritive analyses were run on *Spirulina*, *Enteromorpha*, *Rhodotorula*, and fatted rice bran (Table I). *Spirulina* had a very high protein level of 58 % while the levels for the other diets ranged from 13-31 %. *Rhodotorula* had a very high level of ash (27.8 %). There was difficulty in extracting the lipids from the plants and these data may vary.

TABLE I

Proximate nutritive analyses of *Enteromorpha*, *Rhodotorula*, rice bran, and *Spirulina*. Figures are percent of the dry weight of the diet

Diet	Protein	Lipid	Carbohydrate	Moisture	Ash
Enteromorpha	31.1	2.2	49.2	10.4	7.1
Rhodotorula	27.0	5.0	29.0	11.2	27.8
Rice bran	13.4	16.7	51.8	7.8	10.3
Spirulina	58.1	10.2	12.7	7.2	11.8

Discussion

The five diets represent different classes of plants. *Dunaliella* is a common food organism, *Rhodotorula* and *Enteromorpha* can easily be mass-cultured, and rice bran and *Spirulina* are commercially available. Yeasts, microalgae, *Spirulina* and rice bran have been used by other workers (Shimaya *et al.*, 1967 ; Sick, 1976 ; Claus *et al.*, 1979 ; Sorgeloos, personal communication) while macroalgae have not been previously used as diets for *Artemia*.

Rhodotorula and rice bran are the best diets for smaller *Artemia* (days 1-3). Both have a small particle size and similar carbohydrate levels. For the 7 day experiments (in both the small flasks and the 430 l raceways) the best growth and survival was obtained with *Spirulina*. *Spirulina* had a similar size range to rice bran and *Rhodotorula* but had higher lipid and protein content (10.2 % lipid and 58.1 % protein). Hanaoka (1973) and Sick (1976) showed that growth coefficients of *Artemia* are positively correlated with the amount of crude protein in the diet. However, Hanaoka (1973) indicated that when the dietary protein level exceeds 28 %, growth decreases due to increasing ammonia concentrations in the culture water. Results from the first set of experiments, days 1-3 of growth, agree with Hanaoka's study, but they do not agree when older *Artemia* are used. Fouling of the systems did not occur during the 7 day period examined, however, a high protein diet may foul the system over a longer period of time. Sick (1976) found that there was relatively poor growth of *Artemia* with the alga *Nitzschia closterium* as a diet. *Nitzschia* has a high ash content and low protein and lipid levels. *Rhodotorula* also has a high ash content (28.8 %). Poor growth was obtained when older *Artemia* were fed this diet.

Von Hentig (1971) reported that carbohydrates are the primary energy source for developing embryos of *Artemia*, while during later *Artemia* development lipids and proteins

are the prime source. From this study it appears that concentrations of carbohydrates are also important for the first 3 days of growth. Also, in the early life stages of the brine shrimp, food concentration and particle size of the diet appear to be most critical.

Results observed in the two systems, small experimental systems and mass-culture systems, agreed with each other in that the same diets were the best in both systems. There were differences, however, between the food concentrations needed, the growth of the *Artemia* and percent survival. For these reasons we cannot extrapolate from small scale experimental flasks to mass-culture systems or from short term systems to long term systems.

Summary

1. Rice bran and *Rhodotorula* yielded significantly better growth and survival of *Artemia* for days 1-3 at a concentration of 0.10 mg dry wt food/*Artemia*/ml/day.
2. *Spirulina* is the best diet in 7 day experiments regardless of the culture systems used.
3. *Spirulina* has the highest protein level of the diets tested and a high lipid value which may be a reason it is the best diet for 7 days.

Acknowledgements

I wish to express my appreciation to G. Thursby for continual aid throughout various stages of this study, R. W. Gilbert and P. Schauer for doing the proximate nutritive analyses and P. Marston for helping run the raceway experiments. I am also grateful for the assistance of Drs. M. M. Harlin, G. Klein-MacPhee, R. Steele and Mr. A. Beck for critically reading and editing this manuscript.

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Sequential use of bacteria, algae and brine shrimp to treat industrial wastewater at pilot plant scale

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Abstract

An extended aerated lagoon biotreatment system is used by the Texas Division of the Dow Chemical Company for removing organic contamination from a large-volume hypersaline waste stream. This extended aeration treatment produced large quantities of bacterial suspension that does not settle and requires expensive chemical flocculation for removal.

A sequential two-step algae-brine shrimp and rotifer process has been developed as a tertiary treatment that can clarify up to 89% of the bacteria solids, 89% of the BOD and 88% of the ammonia from the aerated lagoon effluent.

A 0.3 ha pilot plant has been constructed and receives 129 m^3/day effluent from hypersaline waste treatment aeration lagoons. The aeration lagoon effluent flows into a series of ponds where the bacteria serve as nutrients for culturing hypersaline planktonic microalgae (*Chroococcus* sp., *Dunaliella salina*, and *Dunaliella viridis*). The effluent from the algae culture ponds then flows into a temperature-controlled animal culture system where the algae are removed by filter feeding brine shrimp (*Artemia*) and rotifers (*Brachionus plicatilis*). The filter feeders are then mechanically removed and the resulting effluent is suitable for discharge.

Chemical analysis of brine shrimp cultured in the waste treatment system show no significant accumulation of industrial waste chemicals. Also, long-term feeding studies with shrimp (*Penaeus aztecus*) and fish (*Cyprinodon variegatus*) have shown no chronic toxicity from feeding on brine shrimp from the waste treatment system.

A full scale operation of this biological waste treatment system would result in the production of over 1 430 metric tons of brine shrimp and 3 100 metric tons of rotifers per year.

Introduction

Treatment of dissolved organic waste by bacterial oxidation has proven to be a very effective method of waste treatment (Billings and Smallhorst, 1971). However, removal of the resultant suspended bacterial solids from the effluents of these biological treatment systems requires expensive chemical flocculation and mechanical separation followed by landfill of the resulting solids. Such solids removal is usually necessary to prevent environmental degradation by the discharged effluent.

Several studies have been conducted on the use of controlled eutrophication for waste treatment and marine aquaculture production (Dunstan and Tenore, 1972 ; McShan *et al.*, 1972 ; Ryther *et al.*, 1972 ; Goldman *et al.*, 1974). These studies describe processes in which seawater mixed with municipal waste serves as a source of nutrient for culturing marine algae. The algae are then used to feed various aquaculture crops. These studies have shown that controlled eutrophication can be an effective method for removing bacterial solids from a waste treatment system.

The purpose of this paper is to describe a process for bacterial solids removal from hypersaline waste (55-70 g/l NaCl) by controlled eutrophication. The described process, Bioclarification, is a method for settling and decomposing bacteria solids from a hypersaline bacteria oxidation waste treatment system, and then using the nutrients released from the decomposed bacteria to grow unicellular marine algae. Oxygen released by the algae then becomes available for the oxidation of dissolved organic matter released by the decomposing bacteria. Removal of the resulting algae is accomplished by feeding it to high-salt tolerant filter feeding herbivores, specifically brine shrimp (*Artemia*) and rotifers (*Brachionus plicatilis*). The herbivores are then harvested by mechanical separation to leave a clarified effluent acceptable for reuse, recycle, or discharge into the environment. The harvested brine shrimp and rotifers represent a highly desirable protein source which could be marketed to defray the cost of operating the system.

This paper specifically presents data from a one year study, February 1978-January 1979, in which a 0.3 ha waste treatment pilot plant was operated to treat industrial waste from the aeration lagoons of the Dow Chemical Company, Texas Division. The primary objective of this research was to demonstrate at pilot plant scale, a biological tertiary sewage treatment process to reduce bacterial solids in the effluent from a hypersaline aeration lagoon such that an effluent quality equal to or better than the permit requirements for discharge into receiving waters would be achieved. Results from this one year study demonstrated the reliability of the Bioclarification system under various climatic conditions. An average of 89 % of the bacteria solids, (total supended solids, TSS) entering the system were removed. This resulted in a final effluent with an average TSS of 20 mg/l – a level far below permit. In addition to the TSS removal, an average of 89 % of the influent biological oxygen demand (BOD) and 88 % of the influent ammonia was removed.

If the proposed Bioclarification process were used at full scale in the Texas Division of the Dow Chemical Company, it is estimated that over 1 430 metric tons of brine shrimp and 3 100 metric tons of rotifers would be produced each year as a by-product.

Experimental design and methods

Pilot plant design and operation

Influent to the Bioclarification pilot plant came from a 6 ha biological waste treatment aeration lagoon. The salt concentration in the lagoon ranged from 55-70 g/l during the study. This high salt concentration results from chemical processes that use chlorine to make various chemical products and is not atypical of waste effluent from chemical plants. The pilot plant consisted of two separate but sequential systems : an algae culture system where the bacteria solids are converted to planktonic algae and an herbivore culture system where the algae are reduced or eliminated by filter feeding herbivores.

Algae culture system

Aeration lagoon effluent containing a high concentration of bacterial solids entered the front of the Bioclarification algae culture system. A schematic of this part of the pilot plant is shown in Fig. 1. Each pond in the system was approximately 0.10 ha in surface area and 0.9 m deep. Depending on the time of year, one, two, or all three algae ponds were used in series. The number of ponds used depended on the pond detention time needed which in turn depended on the algae growth rate at that particular time of year.

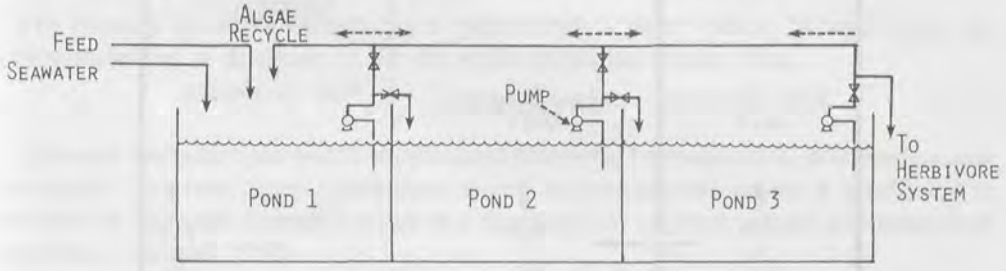

Fig. 1. Schematic of algae culture system. The system was designed so that the number of ponds used could be varied during the year. Algae could be recycled or discharged from any of the ponds.

Raw seawater was added to the first algae pond at a rate that averaged 10% of the pilot plant feed rate. This seawater provided a trace nutrient source for the algae. Typically, about 10% of the effluent from the algae culture system was recycled back to the influent to provide an algae inoculum at that point. If needed, the recycle rate could be increased to provide some mixing in the ponds by accelerating the flow through them. Effluent from the algae culture system was then discharged into the first stage of the herbivore culture system.

Herbivore culture system

The herbivore culture system is shown schematically in Fig. 2. It consisted of two separate fiberglass tanks, one high density and the other low density. The first stage of high density system was 1/10 the volume of the second-stage low-density system. The combined volume of the two systems was 18.9 m^3. The depth of the two stages was 1.8 m.

Effluent from the algae culture portion of the system entered the mixed first stage tank. Contents from this first stage then flowed into the second stage. Final discharge from the pilot plant was then made from the second stage. It was desirable to maintain a high density of herbivores (brine shrimp, rotifers and protozoans) in the first stage. This was done by removing animals from the second-stage low-density system, dewatering them in a liquid-solids separator, and recycling the animals back into the first stage.

A constant supply of dissolved oxygen was maintained by vigorously bubbling air through the tanks. A constant temperature was maintained in the tanks by adding heat in a controlled manner during the colder months with a submerged heat exchanger.

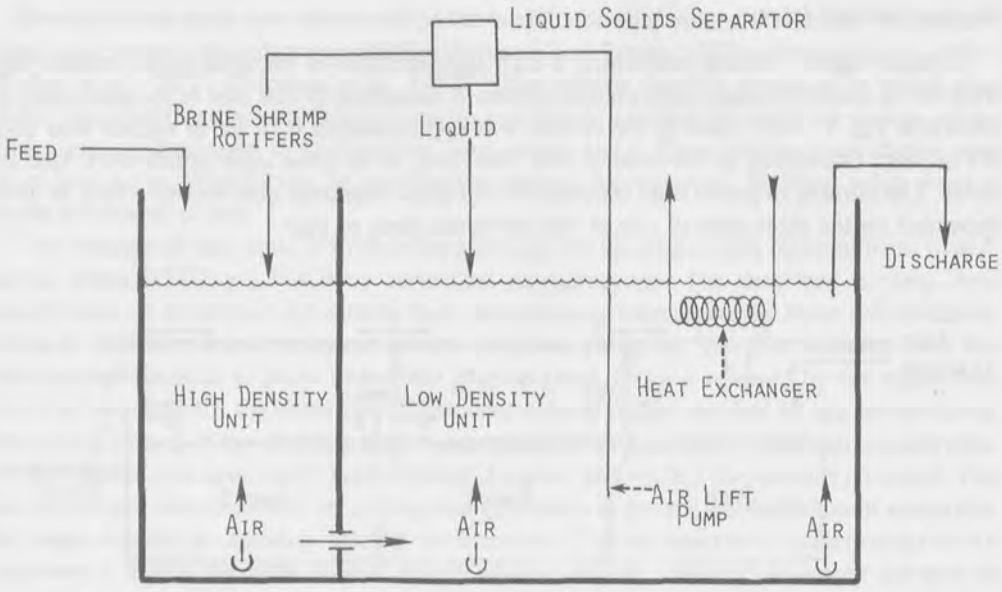

FIG. 2. Schematic of herbivore culture system. The high density unit is 1/10 the volume of the low density unit.

Brine shrimp in the herbivore system were a continuously reproducing population started from an initial inoculum of San Francisco Bay ® brand brine shrimp cysts. The rotifers and protozoans were introduced from the raw seawater being added to the algae culture system.

SAMPLING AND ANALYSIS

A sampling program was initiated to quantitate nutrients, BOD, and herbivores once weekly. Algae concentrations were monitored three times weekly and pH and salinity were monitored weekdays. Algae samples were collected at the effluent points of the culture ponds and counts were made on haemocytometers after fixing 10 ml samples with one drop of Davidson fixative (Klinger and Ludwig, 1957). Brine shrimp biomass measurements were performed by screening 20 l of culture media through a 44 μm Nytex (nylon) screen to separate the animals from the liquid. Any debris was removed from the samples by hand and the samples were washed with fresh water and collected on preweighed filter paper. The samples were then placed in preweighed aluminum dishes and brought to constant dry weight by heating at 100 °C for 1 hr. Brine shrimp biomass was calculated as mg dry weight brine shrimp per liter of culture. The ratio of dry weight brine shrimp to wet weight brine shrimp was approximately 1 to 11. Rotifers were counted by fixing 5 ml samples with five drops of Davidson fixative and counting the rotifers in a petri dish that had fine grids marked on the bottom. Total supended solids (TSS) analysis were performed according to standard methods (Anonymous, 1975), except that the samples were washed with 50 ml aliquots of distilled water after filtering to remove salt residues. Biological oxygen demand (BOD) was also performed according to standard methods (Anonymous, 1975). Total Kjeldahl nitrogen,

ammonia, nitrate, nitrite, and reactive (ortho) phosphorous analysis were performed using a Technicon Autoanalyzer II. Samples were filtered through a 0.45 μm Millipore filter prior to analysis. Salinity was measured with an American Optical temperature compensated Goldberg salinity refractometer and pH was measured on a Leeds and Northrop pH meter. Weather data including wind direction, wind velocity, pond temperature, ambient temperature, humidity and incident and reflected solar radiation were collected and integrated on a daily basis.

Physical relationships

The quantity of waste, Q_I entering a system with a fixed volume, V, determines the approximate time of detention, D, for the waste within the system. Thus :

$$D = V/Q_I \tag{1}$$

The exact detention time would be influenced somewhat by evaporation, precipitation, and percolation. However, except when these conditions are unusually severe it is sufficient to consider the hydraulic detention period as a function only of pond volume and waste flow (equation 1, Oswald, 1960).

Hydraulic loading, H_L, of waste treatment systems is given by the following equation :

$$H_L = \frac{Q_I}{A} \tag{2}$$

Where Q_I is the quantity of waste entering the system in liters per day and A is the surface area of the system in hectares. This gives the quantity of waste in liters per day per hectare that is fed into the system.

The solids load, S_L, applied to culture systems, as kilograms TSS per hectare per day, is given by the following equation :

$$S_L = \frac{Q_I T_I K}{A} \tag{3}$$

The variable T_I is the TSS, in mg/l, entering the system and K is a constant having a value of 1×10^{-6} converting mg/l to kg/l.

The solids removal rate, S_R, is the amount of suspended solids in kilograms removed per day per hectare of area of the treatment system. The equation for S_R is :

$$S_R = \frac{(Q_I T_I K)(Q_E T_E K)}{A} \tag{4}$$

Here Q_E is the quantity of effluent in liters per day leaving the system and T_E is the TSS in mg/l in the effluent of the system.

Results

Algae culture system

Four major species of planktonic algae were observed in the pilot plant. These were *Chroococcus* sp., *Dunaliella salina, D. viridis* and *Navicula* sp. The blue-green algae (*Chroo-*

coccus sp.) and the green algae (*D. salina*) alternated as the dominant species throughout the study.

The pilot plant operating system parameters are given in Table I. Average flow of waste (Q_I)

TABLE I

Pilot plant system parameters
February 1978-January 1979

		Algae culture system	Herbivore culture system
Q_I[1] (l/day)	Average	1.0	1.4
	Range[2]	0.7-1.4 ($\times 10^5$)	0.9-2.0 ($\times 10^3$)
D[3] (days)	Average	16	14
	Range	7-28	9-21
d[4] (m)		0.91	1.8
V[5] (l $\times 10^6$)		.66-2.55[6]	0.0189
A[7] (ha)		.07-.28[6]	8.83×10^{-4}
H_L[8] (l $\times 10^5$)	Average	6.8	16.0
	Range	3.2-12.9	10.1-22.7
T_I[9] (mg/l)	Average	189	80
	Range	112-322	35-136
S_L[10] (kg/ha/day)	Average	120.9	123.9
	Range	54.2-296.0	59.5-287.7
T_E[11] (mg/l)	Average	80	20
	Range	35-136	7-34
Q_E[12] (l/day $\times 10^5$)	Average	1.16	1.4
	Range	0.77-1.54	0.9-2.0
S_R[13] (kg/ha/day)	Average	56.8	98
	Range	8.5-118.2	35.7-244.7
% TSS removed[14]	Average	47	79
	Range	6.9-82.1	60-91
Pond temperature (°C)	Average	23	27
	Range	11-32	8-30
Average incident light (gcal/cm²/day)	Average	417	–
	Range	227-582	–

[1] Quantity of flow entering system.
[2] Range is for monthly averages.
[3] Detention time where $D = V/Q_I$.
[4] Depth of system.
[5] Volume of system (depth × width × length).
[6] The area used for algae culture was changed during the study. From July to October the smaller volume and area was used. The larger area was used during the balance of the year.
[7] Area.
[8] Hydraulic loading, $H_L = Q_I/A$.
[9] TSS in influent to system.
[10] Solids load applied to system, $S_L = Q_I T_I K/A$.
[11] TSS in effluent from system.
[12] Quantity of effluent leaving system, includes seawater addition for algae culture system.
[13] Solids removal rate, $S_R = (Q_I T_I K)-(Q_E T_E K)/A$.
[14] % TSS removed $= S_R/S_L \times 100$.

into the algae ponds was 1.0×10^5 l/day with an average suspended solids level in the effluent (T_I) of 189 mg/l. The solids load (S_L) of the algae culture system portion of the Bioclarification pilot plant averaged 120.9 kg suspended solids/ha/day. The solids removal rate (S_R) by this algae culture system averaged 56.8 kg TSS/ha/day, giving an average TSS removal rate of 47 percent for this portion of the Bioclarification process.

The average detention time (D) for the algae ponds was 16 days with a range of 7 to 28 days. Algae production was continuous during the study, but was limited by solar radiation. It therefore varied with changes in incident light such that average effluent algae density during the study was 10.93×10^6 *Chroococcus* sp. cells/ml and 0.85×10^4 *D. salina* cells/ml (Table II). This algae biomass accounted for the majority (70-97 %) of the algae culture system effluent TSS which averaged 80 mg/l.

TABLE II

Pilot plant algae and herbivore production

		Algae culture system	Herbivore culture system
Chroococcus sp. cell count	Average	10.93	1.37
(no./ml) $\times 10^6$ in effluent	Range	1.97-31.28	0.19-3.64
Dunaliella salina cell count	Average	0.85	0.03
(no./ml) $\times 10^4$ in effluent	Range	0-3.10	0-0.2
Total cell volume	Average	49.8	6.9
($\mu m^3 \times 10^6$/ml)[1]	Range	5.1-131.0	0.4-18.3
Percent algae removed	Average	–	86.1
	Range	–	56.6-99.6
Rotifers/ml	Average	10.7	15.3
	Range	0-50.4	0.6-34.1
Brine shrimp/ml	Average	–	6.4
(mg dry wt/l) in effluent	Range	–	2.3-15.8

[1] Assuming average volume for *Chroococcus* = 4.19 μm^3 and average volume for *D. salina* = 345 μm^3.

Nutrient levels for nitrogen and phosphorous in the algae culture system are given in Table III. At no time during the study did the nutrients in that algae ponds become growth limiting.

The pH of the algae ponds remained relatively stable between 7.8 and 8.2 (average 7.9). Pond temperatures ranged from monthly averages of 11 °C to 32 °C with a yearly average of 23 °C. Rotifers occurred in significant numbers in the algae ponds when pond temperatures remained above 15 °C. The only months in which rotifers were not observed in the algae ponds was during February 1978 and January 1979, when the pond temperatures averaged 12 °C and 11 °C, respectively. Average concentration of rotifers in the algae culture system effluent was 10.7/ml (Table II).

TABLE III

Pilot plant influent and effluent analysis
February 1978-January 1979

		Algae culture system influent		Algae culture system effluent		Herbivore culture system effluent	
		Average	Range	Average	Range	Average	Range
NH_3 (N)	(mg/l)	5.62	3.98- 8.38	6.12	3.83- 9.56	1.14	0.47- 2.56
NO_2 (N)	(mg/l)	0.02	0.01- 0.04	0.05	0.02- 0.17	5.96	0.44- 12.75
NO_3 (N)	(mg/l)	0.31	0.01- 0.69	0.76	0.01- 3.50	30.00	9.30- 76.50
PO_4	(mg/l)	0.62	0.27- 1.43	0.95	0.35- 2.59	1.62	0.84- 2.35
pH		7.90	7.80- 8.20	8.10	7.90- 8.40	8.20	8.10- 8.40
Salinity	(g/l)	64.00	54.00- 70.00	55.00	33.00- 66.00	57.00	42.00- 65.00
TOC[1]	(mg/l)	86.00	47.00-145.00	84.40	53.00-134.00	69.30	43.00-117.00
BOD[2]	(mg/l)	88.60	37.00-225.00	39.40	18.00- 69.00	9.20	4.00- 15.00
TSS[3]	(mg/l)	189.00	112.00-322.00	80.00	35.00-136.00	20.00	5.80- 34.20

[1] Total organic carbon.
[2] Biological oxygen demand.
[3] Total suspended solids.

Herbivore culture system

Due to the relatively small size (18.9 m^3) of the herbivore culture portion of the Bioclarification pilot plant, only a small portion of the effluent from the algae culture system was used as feed.

Average flow into the herbivore culture system (Q_I) was 1.4×10^3 l/day (Table I). With an average influent TSS (T_I) level of 80 mg/l. The suspended solids load (S_L) of the herbivore system was 123.9 kg/ha/day, and the solids removal rate (S_R) averaged 98 kg/ha/day giving an average TSS removal rate of 79 %. The herbivore culture system and algae culture system considered together had an average TSS removal rate of 89 % with an average discharge of 20 mg TSS/l for the 12-month study (Table I). At no time during the study did the effluent discharge exceed permit level for TSS.

The temperature of the herbivore culture system was controlled during the colder months at 26 °C, ± 2 °C (Table II). During January 1979, an interruption in the heat supply allowed the tank temperatures to drop as low as 8 °C. The average water temperature during the 2-week long heat exchanger failure was 11 °C. While there was no noticeable effect on the brine shrimp population, the rotifer population decreased, but recovered rapidly after the system temperature returned to normal.

No effort was made to prevent brine shrimp from being discharged in the effluent of the herbivore culture system. Thus, the brine shrimp production rates were calculated from the amount of brine shrimp screened from a 20 l sample of the herbivore culture system effluent. The average brine shrimp production rate for the 1 year study was 6.4 mg dry weight/l. However, during the first few months of the study, the brine shrimp population was artificially high due to the initial inoculation of *Artemia* cysts into the system. A more realistic

production figure of 3.6 mg dry weight/l can be derived from averaging the monthly production rates for the last six months of the study. The average production of rotifers in the herbivore system was 15.3 animals/ml. Using 0.5 μg as the average dry weight for *B. plicatilis* as reported by Dumont (1977) and multiplying that number times the calculated yearly average number of rotifers/ml produced in the herbivore system, the production rate for rotifers would be 7.7 mg dry weight/l.

Discussion

Algae culture system

Fig. 3 shows schematically an overall view of the biological processes that take place in the Bioclarification waste treatment process described in this paper. Most of the bacterial solids entering the system are removed from suspension by sedimentation and bioflocculation (Lackey and Smith, 1956) and by fecal deposition by herbiverous invertebrates (Oswald, 1960). Once deposited, the bacterial solids are decomposed either aerobically or anaerobically depending on the availability of oxygen at the deposition site. Decomposition may be slow or partial, allowing accumulation in the ponds, or may be complete. In this study, some sediment accumulation was noted near the algae culture system influent point, indicating only partial decomposition of some settled solids. These sediments were noted to increase in the winter and decrease in the summer. The observance of very low numbers of bacteria in the algae culture effluent (3-30% of the influent number) indicated that the majority of the bacterial solids entering the algae culture system were deposited on the pond bottoms or eliminated by autolysing.

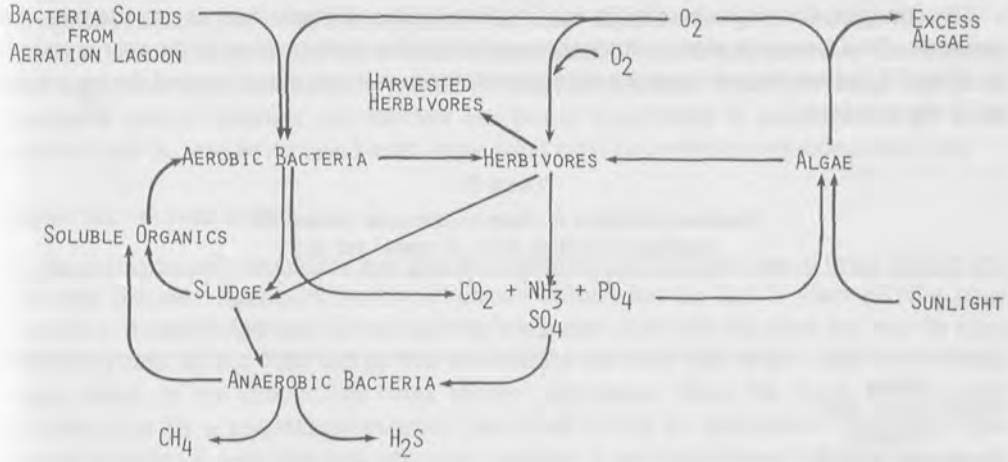

FIG. 3. Schematic of the biological processes that take place in the waste treatment solids removal system.

The carbon dioxide, phosphate, and ammonia resulting from the bacterial decomposition were used as nutrients for algae growing in the ponds. The growing algae released oxygen during the day and this oxygen helped support a herbivore population (rotifers) in the ponds.

Also, some was used for the oxidation of dissolved organic matter by bacteria. Bacterial solids are thus converted into plant cell material (algae) and the water is left relatively free of both bacteria and unstable dissolved organic matter. Nutrient analysis (Table I) of the algae culture system influent and effluent indicated that the algae were not growth limited by nitrogen or phosphorus in the algae culture system. Levels of NH_3, NO_2 and NO_3 (expressed as N content) in the ponds never dropped below 5.0 mg/l and the phosphorus level (as PO_4) did not go below 0.3 mg/l. Studies have shown that nitrogen at a level above 4.2 mg/l and phosphorus at a level above 0.3 mg/l does not limit algae growth (Shelef, 1968 ; Fogg *et al.*, 1973 ; Shiroyama *et al.*, 1975).

Algae production was continuous throughout the year with peak production occurring during the summer months. Correlation of algae production with environmental factors was complicated because of the occurrence of algae feeding rotifers and protozoan flagellate in the algae culture system. However, linear regression analysis of monthly averages of algae number and insolation showed some correlation ($r = 0.64$) suggesting that solar radiation was the major limiting factor for algae growth.

Rotifers were present in the effluent of the algae culture system during all months of the study except January and February. Average monthly pond temperatures during those months was 11.5 °C. During the rest of the year the average monthly pond temperatures ranged from 15 °C during December to 32 °C during July.

The detention time (D) for the algae culture system was varied during the study to maintain an adequate population. Loss of algae in this system causes an oxygen deficit in the ponds causing anaerobiosis and production of disagreeable odors. The average detention time during the study for the algae culture system was 16 days and ranged from 9 days in the summer to 20 days during the winter.

The detention time was changed by varying the number of ponds used during the year. An example of the change in algae culture area needed during various times of the year is shown in Table IV. As can be seen from the table, almost twice as much area is needed during winter as in the summer.

TABLE IV

Seasonal variation in algae culture area needed for treating 10 million liters of waste[1] per day

Season	Area[2] (ha)
Spring March, April, May	23.3
Summer June, July, August	17.5
Fall September, October, November	19.5
Winter December, January, February	28.5

[1] TSS of waste is assumed to average 200 mg/l.

[2] Calculated from average hydraulic load during each season from pilot plant data, February 1978-January 1979.

Since the influent to the waste treatment system was high in salt content (55-70 g/l), the system was selective for algae with high salt tolerance. The predominant algae was the cyanophycean, *Chroococcus* sp., an extremely euryhaline and eurythermal form tolerant to a wide range of nutrient variations (Fogg *et al.*, 1973). This alga was acceptable as food for the brine shrimp but observations and studies indicate that another and larger predominant alga, *D. salina*, was preferred (D'Agostino and Provasoli, 1968). This chlorophycean alga is halophylic preferring optimum salt concentrations between 4 and 8%, but it can tolerate much higher concentrations (Smith, 1950). Also, *D. salina* is a motile alga and tended to concentrate in various areas of the ponds making accurate population estimates difficult. The extremely low concentrations of *D. salina* in the effluent from the herbivore system attested to its desirability as feed by the brine shrimp and rotifers.

The compensation limit (level of penetration of 1% of the surface illumination) in the pilot plant algae ponds was typically about 20 cm. Theoretically, maximum algae production can be achieved if the ponds are no deeper than this photic zone (Rabinowitch and Govindjee, 1969). Pilot plant results demonstrated, however, that when the ponds were operated at depths less than 0.9 m, environmental effects such as heavy rainfall, low temperatures, and high winds had significant detrimental effects on the algae growth. Although the increased pond depth theoretically limits algae production, the greater stability of environmental conditions actually enhanced long-term algae production. These findings are consistent with those for stabilization ponds. Oswald (1960) states that the depth to be employed in (stabilization) pond design should be from one to three times the photic zone.

Another important factor for pond depth determination is maximization of anaerobic digestion of settled solids in the ponds. If the ponds have adequate depth to consistently limit dissolved oxygen at the bottom of the ponds, accumulated sludge there will undergo methane fermentation (Oswald, 1960). However, if the pond depth is not great enough to allow a constant environment on the pond bottom, putrification rather than methane fermentation will take place with concomitant release of unpleasant odors. Thus, providing that pond loading is neither excessive nor deficient and proper pond depth is maintained, high solids removal rate (S_R) can be achieved in the algae pond without producing objectionable odors.

Herbivore culture system

The herbivore culture system was originally designed for exclusive use of brine shrimp and this was the only significant herbivore species utilized for the first 2 years of pilot plant operation. Rotifers were then introduced into the system from raw seawater fed into the algae culture system. It had been feared that competition between the rotifers and brine shrimp might result in the loss of the brine shrimp population. Since the brine shrimp were considered to be a valuable by-product, this effect would be undesirable. However, after several months of operation and laboratory studies, it became evident that the two species were able to coexist without much loss in brine shrimp population, and that the combination of the two species resulted in a more stable and reliable system.

Prior to the occurrence of the rotifers, any rapid increases in the algae concentration being fed into the herbivore system resulted in higher effluent TSS. This condition would persist until the brine shrimp population increased enough to adsorb the extra algae. Therefore, to prevent producing substandard effluent, extra brine shrimp cysts would have to be added to

the system to speed up the process of population increase. If the algae concentration in the influent rapidly decreased, the brine shrimp population would become stressed and start to die off unless the excess population was harvested.

After the rotifers became established in the herbivore system, it was noted that the effluent quality became much more stable and did not fluctuate greatly or as rapidly as before despite changes in influent algae concentration. Also, the brine shrimp population became stable while the rotifer population varied markedly with influent algae concentration. Linear regression analysis of percent algae removal rate versus herbivore rotifer population level showed a high correlation ($r = 0.8$) while there was no correlation between brine shrimp population and percent algae removal ($r = -0.3$). Rotifers can more than double their population every two days (Thane, 1974). Apparently this faster multiplication rate for the rotifers allows them to more rapidly adjust to the fluctuations in food concentration (Gilchrist, 1960 ; Reeve, 1963a).

The occurrence of rotifers in the herbivore system was not unexpected since *B. plicatilis* is a frequent inhabitant of hypersaline ponds (Beadle, 1943 ; Hutchinson, 1967). In fact, laboratory studies to evaluate the potential of rotifers for use in the waste treatment system had been conducted before they became established in the ponds. The main concern was that the rotifer *B. plicatilis* and brine shrimp had overlapping food size preference ranges and could potentially compete for the same size algae (Pennington, 1942 ; Reeve, 1963b ; Chotiyaputta and Hirayama, 1978). Usually a competition for the same food results in the eventual elimination of one of the competing species (Hutchinson, 1951). The coexistance of the two species suggest that there are some diffences in the actual foods utilized by the two species. Perhaps some relationship similar to that of *B. calyciflorus* and *Daphnia* exist where the rotifers feed on the fecal material of the cladoceran (Dumont, 1977).

Brine shrimp harvested from the waste treatment system were carefully monitored for any accumulation of toxic compounds and were tested in a one year feeding study with the edible brown shrimp, *Penaeus aztecus*. No significant accumulation of toxic compounds were detected in the brine shrimp, brine shrimp cysts, or in the penaeid shrimp used in the feeding study. Brine shrimp harvested from the pilot plant were test marketed through a tropical fish distributor and received a favorable reception.

By-product production

The primary objective of this research was to refine and prove a biological tertiary sewage treatment process that would remove bacterial solids from hypersaline secondary sewage effluent. Thus, the Bioclarification pilot plant was operated to produce an effluent with an acceptable level of TSS for discharge into natural receiving waters. The hervibores were considered as part of the effluent TSS so that if necessary, they would not have to be removed before discharging the effluent into receiving waters. This means that the pilot plant was operated to produce the minimum number of animals necessary to treat the maximum flow possible.

Since brine shrimp and rotifers are valued as food for aquaculture production or as tropical fish food (Bardach *et al.*, 1972 ; Theilacker and McMaster, 1971), it would be possible to harvest and market them to help defray the cost of the waste treatment process. With little additional effort, the production of brine shrimp from the Bioclarification system could be

significantly increased over the production measured in this study. This is supported by the fact that for 3 months after the initial inoculation of the herbivore system with brine shrimp cysts, the brine shrimp population was four times that of a later stable population. This demonstrated that the system was capable of supporting a much larger population of brine shrimp than that maintained in this study. Also, the excess algae ponds available during the summer, spring, and fall, could be used for additional brine shrimp production during those times.

Cysts of brine shrimp also have a high value for use in aquaculture production (Sorgeloos and Persoone, 1975). Although some cysts were frequently observed along the edge of the herbivore tanks and ponds, there was no significant accumulation that allowed an accurate measurement of their production. Most females produce live young rather than cysts when the Bioclarification system is operated properly. To increase cyst production, it might be possible to use a scheme such as that described by Helfrich *et al.* (1975) in which mature brine shrimp are harvested and transferred into ponds with higher salinities. The marked change of salinity triggers the production of cysts rather than live young.

Conclusions

The Bioclarification waste treatment process showed exceptional operational efficiency as measured by the degree of removal of suspended solids to produce a final effluent of less than 30 mg/l TSS. The algae culture portion alone removed an average of 47% of the TSS entering the system with most of the balance being converted into algae biomass. The algae were then fed to a mixed culture of rotifers and brine shrimp resulting in an average overall TSS removal of 89% to produce an effluent with an average of 20 mg/l TSS. In addition to the TSS removal, an average of 89% of the influent BOD and 88% of the influent ammonia was removed.

It should be emphasized that the Bioclarification System is still in an exploratory stage of development, and the economic and engineering feasibility of a full-scale operating system have not been demonstrated by this study. Depending on the geographic location, the heating cost of maintaining a controlled temperature in the herbivore culture system might be prohibitively expensive. Hopefully, additional research will define ways to lower this cost.

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Preliminary trials of combined *Artemia* rearing and salt production in earthen salt ponds in the Philippines[1]

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Abstract

This paper describes trials at combining *Artemia* rearing with salt production during the dry season, in newly-constructed earthen salt ponds (reservoir, evaporation, concentration, and crystallization ponds, total area of 5 000 m^2) at the Leganes, Iloilo Station of the SEAFDEC Aquaculture Department.

Salt production by the solar method amounted to 250 sacks of salt over 30 days or an average of 8 sacks/day (1 sack = 50 kg).

Two successful *Artemia* inoculations were undertaken in May and June 1979 respectively : in both cases the adult stage was reached after 1 week. The May population died off when the salinity was suddenly increased by salt addition. The June population gradually disappeared at the onset of the rainy season.

Introduction

It is a well-known fact that *Artemia* cysts are utilized worldwide in finfish and prawn hatcheries as a primary source of live foods to culture larval and juvenile stages of commercially important species. This results, however, in a total dependence on imported cysts for all countries without their own *Artemia* resources, and purchase of very expensive cysts for developing countries, such as the Philippines, with scarce foreign exchange reserves.

There are only two options which can be considered to solve this dependence on imported cysts : a) the development of substitute foods (live or inert) for aquatic fry or b) the local production of *Artemia*.

Although Southeast Asia is one of the regions in the world where brine shrimp do not occur naturally, the presence of extensive areas of salt ponds makes the second alternative worthy of consideration. In 1971, a total of 8 000 ha was devoted to salt production during the dry season in the Philippines. In the Western Visayas region, a typical solar salt plant consists of 10 to 50 ha of reservoir, evaporation, and crystallization ponds producing an average of 500 to 1 000 kg salt/ha daily.

[1] Contribution No. 43 of the Aquaculture Dept., Southeast Asian Fisheries Development Center, P.O. Box 256, Iloilo City, Philippines.

Pond construction

With the successful *Artemia* inoculation in the salterns of a private fishfarmer (De los Santos *et al.*, 1980), the SEAFDEC Aquaculture Department embarked on a 0.5 ha pilot project that would combine trials of *Artemia* rearing with solar salt production during the dry season and with production of milkfish (*Chanos chanos*) fingerlings and/or prawn (*Penaeus monodon, P. indicus, P. merguiensis*) juveniles during the rainy season.

For this purpose, a series of ponds (reservoir, evaporation, concentration and crystallization) totalling 5 000 m^2, were constructed from November 1978 to February 1979 in Guigui, Leganes, Iloilo (Fig. 1) in an abandoned fish pond area. The proportion of crystallization (salt bed) area to the rest of the pond surface was 1:10, similar to nearby salt ponds (Table I). To permit *Artemia* culture, the water level in the evaporation ponds was, however, increased progressively from 30 to 45 cm. In traditional evaporation ponds water depth only ranges from 2 to 25 cm to obtain a maximum daily salinity increase. Traditionally reservoir ponds are deeper (30-80 cm) because they also serve as milkfish ponds.

A 6-1/2 HP Cooper pump with a capacity of 2 m^3/min was installed near the reservoir pond close to the main supply canal. The inlet of all the pipes was provided with a screen (1 mm mesh size) to keep predators out. For reasons of economy, polyethylene sheets were used to line the crystallization ponds, instead of the usual bricks.

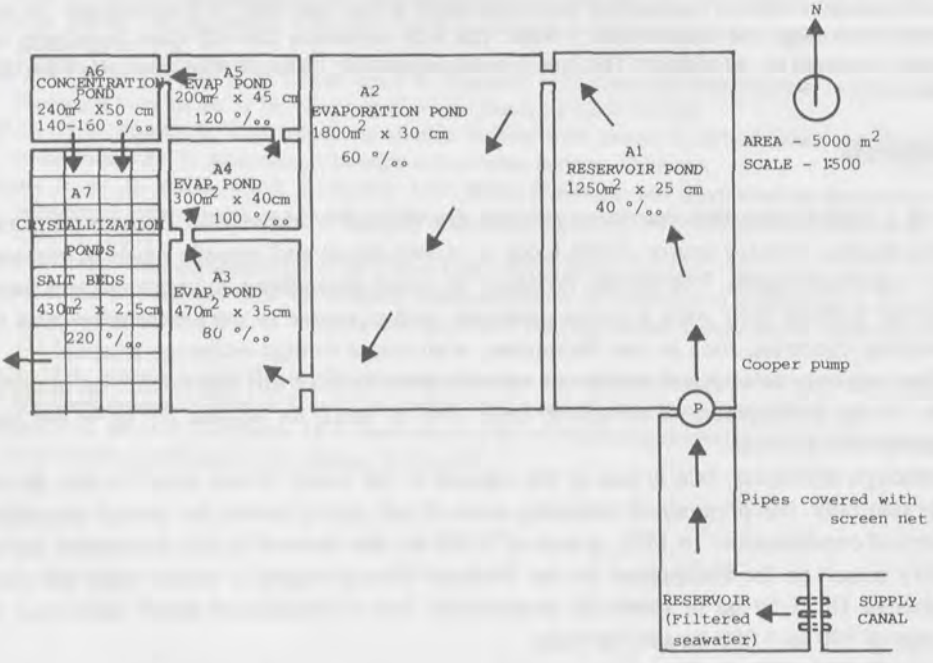

FIG. 1. 0.5 ha earthen pond area for salt production and *Artemia* rearing, Bgy. Gui-gui, Leganes, Iloilo.

Salt production

The classic procedure consisted of daily pumping water from the main supply canal and flow by gravity through the series of evaporation ponds. The original plan was to increase salinity up to 200 ‰ before allowing the water to flow in the crystallization area, for a better and easier harvest of whatever *Artemia* cysts could be produced. However, the water had to be introduced into the salt beds at 140-160 ‰ to produce coarse salt (which requires less salt to fill one sack compared to the fine salt produced at more than 200 ‰). Rough salt is better accepted by the average Filipino because of tradition.

A total of 250 sacks (1 sack = 50 kg) was produced over a 30-day period (March-April 1979) for the 0.5 ha area which means 16 sacks/ha/day. This compares favorably with traditional solar salt production where the average is 4 to 20 sacks/ha/day (Table I).

TABLE I

Data on salt ponds in Iloilo Province, Philippines (June 1979)

	Pond A	Pond B	Pond C	Pond D	SEAFDEC Pond
Pond area (ha)	12	15	10	33	5 000 m²
1. Reservoir	–	6*	(milkfish pond)	(milkfish pond)	1 250 m²
2. Evaporation	11.5	4	9	29	3 010 m²
3. Crystallization	0.5	4-5	1	4-5	430 m²
Ratio reservoir + evaporation area to crystallization area	23:1	2:1	9:1	6:1	10:1
Water depth (cm)					
1. Reservoir	30-60	30-60	30-60	30-60	25
2. Evaporation	2.5-25	2.5-25	2.5-25	2.5-25	30-50
3. Crystallization	5	5	5	5	5
Water supply	Pump and gravity	Pump and gravity	Gravity	Pump and gravity	Pump and gravity
Salt production (sacks/ha/day)**					
1. Minimum	1	7	1	3	8
2. Maximum	25	–	5	15	48
3. Average	21	–	4	10	16
First harvest	Feb. 5-11	Feb. 5-20	Feb. 8-15	Feb. 10-20	–
Last harvest	2nd week June	1st-2nd week June	June 6	1st-3rd week June	–
Total number of days of salt harvest	80-90	90-105	60-120	60-120	–

* With milkfish stock.

** 1 sack = approximately 50 kg.

Assuming an average of 10 sacks/ha/day, 100 operational days in 1 year (Table I), at 10 Philippine pesos[a] per sack (minimum of ₱8/sack in summer and ₱14/sack maximum in the rainy season), there is a gross profit of ₱10,000 or more per ha of salt ponds in 1 year. Clearly, salt-making is a highly profitable enterprise.

Since the salt production rates in the pilot area (despite the higher water depth maintained in the evaporation ponds for *Artemia* culturing) are comparable to those of the classic salt ponds, it appears that the two activities may be successfully combined.

Artemia rearing

Data on the six *Artemia* inoculations in various ponds at the project site are given in Table II.

All inocula were made with "Living World" cysts (San Francisco Bay Brand Co., California, USA) hatched into nauplii or reared to adults at the SEAFDEC Tigbauan Station and transported by motor vehicle to the ponds located in the Leganes Station (duration of transport : 1.5 hr).

Prior to hatching the cysts were decapsulated (Sorgeloos *et al.*, 1977). The adult brine shrimp originated from nauplii reared in 1.5 ton fiberglass tanks and fed with *Tetraselmis chuii* and/or rice bran (Sorgeloos *et al.*, 1980). The inoculations were made in the afternoon to avoid high temperatures (in April and May the water temperature in all the ponds ranged from 27 to 42.5 °C at 11:00 a.m. and from 25 to 38 °C at 4:00 p.m.).

The inoculation on May 16 of both nauplii and adults was relatively successful because after 1 week about half of the females were bearing cysts. The good health of the animals could be attributed to the presence of natural food : lablab as well as phytoplankton (Table III) obtained though fertilization. Lablab is a microbenthic complex of bacteria, diatoms, blue green algae, protozoans, and other microorganisms (Rabanal, 1966). When the first cysts were discovered in the ponds, 30 sacks (1 500 kg) of solar salt were added to the water to increase the salinity and as a result the buoyancy of the cysts. Unfortunately, this action led to a total collapse of the *Artemia* population after a few days, most probably as a result of a high concentration of $CaSO_4$ which may have been toxic to *Artemia* (Vos, 1979). The salinity increase (44 to 53 ‰) also stimulated excessive lablab growth which may have led to unfavorable environmental conditions (low oxygen concentration at high biological oxygen demand, high concentration of ammonium and/or sulfide, *etc.*).

Another noteworthy inoculation was that of June 4. In this case the brine shrimp reached the riding stage and all the animals looked very healthy. A plankton bloom in pond 6 at 94 ‰ on June 16 consisted of a green flagellate tentatively identified as *Dunaliella* sp. (Table II). This species holds promise as a natural food to sustain *Artemia* growth in high salinity ponds. Presently efforts are made to isolate, identify, and mass produce this algal species.

Disappearance of the *Artemia* population, may, in our opinion be attributed to the decline of the phytoplankton in the pond.

The failure of the other *Artemia* inoculations carried out so far are probably due to weak *Artemia* nauplii because of addition of ice to the transport water (March 8), lack of food

[a] 7.14 Philippine pesos (₱) to US $ 1.00.

TABLE II

Summary of data on *Artemia* inoculations in SEAFDEC *Artemia*/salt ponds, Leganes, Iloilo, 1979

Date	Pond number	Salinity at stocking (‰)	Pond preparation	Number and age of *Artemia* stocked	Manner of transport	Remarks
March 8	2	No data	None	5×10^6 nauplii, 1×10^6 adults	In plastic bags containing oxygenated seawater and ice packs.	Nauplii weak after transport. Mass mortality after 4 days ; total mortality by March 19.
March 20	2	No data	None	1×10^6 nauplii, 3×10^6 adults	In plastic pails containing aerated seawater.	Mass mortality after 3-4 days ; total mortality by April 2.
May 16	3	42	Drying ; fertilization with 100 kg dried chicken dung and 15 kg urea ; 45 pieces coconut fronds installed as shelters.	0.55×10^6 nauplii, 3×10^6 adults	In plastic bags containing oxygenated seawater.	Good lablab growth during stocking. Riding adults observed after 1 week. A few cysts found in water samples on May 25 ; 1 000 kg salt added same day. Another 500 kg salt added May 31. Total collapse on June 3 ; excessive floating of benthic lablab.

TABLE II (continued)

Date	Pond number	Salinity at stocking (‰)	Pond preparation	Number and age of *Artemia* stocked	Manner of transport	Remarks
May 29	4	48	Fertilization of water with 10 kg urea ; 30 pieces coconut fronds installed as shelters.	0.5×10^6 nauplii, 0.29×10^6 adults	In plastic bags containing seawater.	Moderate lablab growth. Population gradually died off.
June 7	1	50		10×10^8 nauplii distributed among 6 ponds	In plastic bags containing oxygenated seawater.	Emergency stocking due to excess newly-hatched nauplii at the large tank Prawn Hatchery. Immediate predation by fish, crustacean and insect larvae in ponds 1-5. Nauplii survived for 2 weeks in pond 6 which had abundant phytoplankton.
	2	52				
	3	64	None			
	4	58				
	5	64	20 pieces coconut fronds.			
	6	86	Fertilization with 6 kg urea ; 30 pieces coconut fronds.			
June 14	6	94	None ; pond water green from previous fertilization.	1×10^6 adults	In plastic bags containing oxygenated seawater.	Riding adults observed after 1 week. Pond water very dark green due to bloom of *Dunaliella* sp. on June 16. Strong rains 3rd week June. Population gradually disappeared and no *Artemia* observed by July 1.

(March 8 and 20, June 7), lack of shelter (March 8 and 20, June 7) and predation by finfish and other crustacean larvae (June 4).

TABLE III

Plankton and lablab analysis, SEAFDEC *Artemia*/salt ponds (1979)

	May 25-plankton (cells/ml)	May 29-plankton (cells/ml)	May 29-lablab (cells/m^2)	June 5-plankton (cells/ml)
Chaetoceros sp.	27.7×10^3	65.4×10^3	187.7×10^3	90
Synedra sp.	400	500	2.51×10^6	–
Nitzschia sp.	–	–	10.13×10^6	390
Navicula sp.	–	–	–	90
Diatom 1	–	–	–	–
Oscillatoria sp.	–	–	3.95×10^6	–
Spirulina spp.	–	–	1.77×10^6	–
Filamentous alga 1	600	900	26.1×10^3	495
Filamentous alga 2	400	180	41.4×10^3	–
Filamentous alga 3	200	–	38.0×10^6	–
Filamentous alga 4	–	180	121.5×10^3	–
Filamentous alga 5	–	–	3.37×10^6	–
Dunaliella sp.	3.8×10^3	–	–	28.4×10^3
Flagellate 1	–	414	–	–

Milkfish and prawn nursery

The goal of this phase of the project which was recently started, is to convert the salt ponds into nursery ponds utilizing either or both of two food chains :

a) lablab → milkfish/prawn fry
b) phytoplankton → *Artemia* → milkfish/prawn fry

The first approach is a traditional practice in many salt ponds in the area. The second one, namely the rearing of *Artemia* as food for milkfish and penaeid prawn fry during the rainy season is an innovation based on the success obtained by a private fishpond operator (de Los Santos *et al.*, 1980).

Recommendations

Within the context of integrated solar salt production, *Artemia* rearing and milkfish/prawn nursery, alternating during the dry and wet seasons in a given area, the following guidelines for future salt – *Artemia* production are given, based on our summer 1979 trials :

1) Given the local preference for production of "rough" salt, salinity in the salt ponds (excluding salt beds) should range from 40 to not more than 160 ‰ because higher salinities will produce "fine" salt.

2) Since the fry of some predator species can not be eliminated because of the relatively low salinities, it is advised to inoculate the larger-sized *Artemia* adults instead of the more vulnerable nauplii.
3) Since the incoming water is not rich in food organisms, there is a need to establish natural food through fertilization. Trials should be done using :
   a) organic fertilizers to stimulate lablab production. The advantage of this technique is that the lablab provides shelter for *Artemia* ; the disadvantage is that it decreases the rate of salinity increase for salt production
   b) urea and other inorganic fertilizers for phytoplankton blooming.
4) There is a need to determine experimentally whether all the ponds or only the deeper ponds (30 cm + depth) can support *Artemia* culture.
5) If only the deeper ponds are suitable for rearing *Artemia*, there is a need to determine which kind of water management would best meet the requirements of both salt and *Artemia* production :
   a) a common water source for salt beds and *Artemia* ponds
   b) a common source shared only up to a certain point for salt beds and *Artemia* ponds.

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Design and operation of a recirculating culture system for *Artemia*

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Abstract

Artemia and several species of phytoplankton are reared in a continuously recirculating culture system in conjunction with a coral reef microcosm. The *Artemia* culture system is designed to remove particulate waste and return usable plant and animal nutrition to the reef. It does not require direct access to seawater and may be readily adapted to culture other zooplankters.

Introduction

This report describes a culture system designed to provide a primary food input to a coral reef microcosm, and its role in the success of this unique approach to the study of marine ecology. The model reef community (Fig. 1) has been in operation for over 2 years without

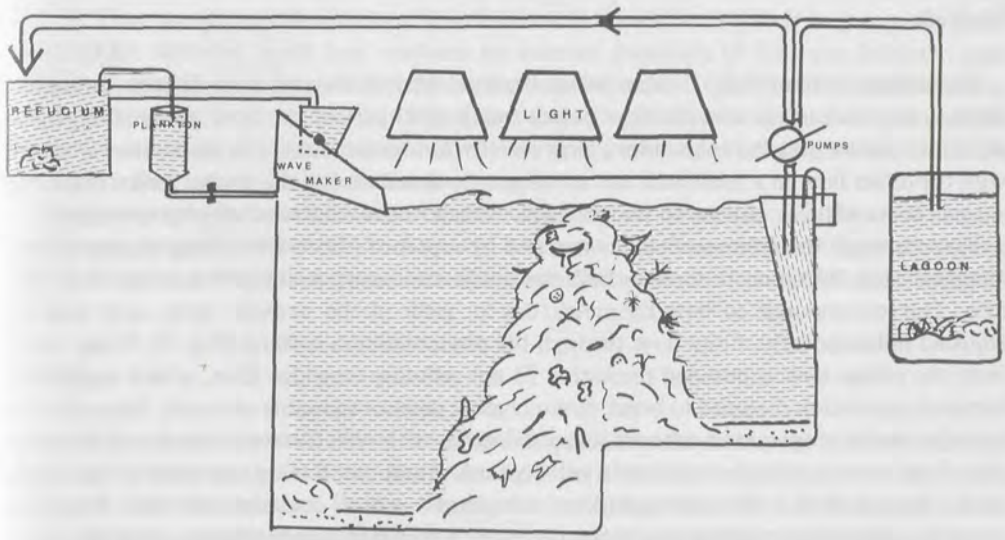

FIG. 1. Schematic diagram of the Smithsonian reef microcosm excluding *Artemia* culture system. With a total volume of 7 200 l, this microcosm supports a complex biological community.

interruption relying on a water exchange of 0.06% daily. By balancing import and export from the reef and matching environmental parameters such as sunlight and wave energy, filters, and other traditional types of waste treatment have been avoided. The diversity of plants and animals in the model approaches that found on natural reefs and includes many of the West Indian corals which have previously resisted domestication. Study of this community in a closed system allows scrutiny of each component of the ecosystem in greater detail than is possible on a wild reef.

Addition of *Artemia* is designed to simulate plankton which wash on to a reef from the open ocean. Studies from Adey (unpublished) show that 2.0 g of *Artemia* per day matches the rate of input to an equivalent size reef. *Artemia* are well suited to this application because they may be easily raised and distributed to the reef over a wide size range, to be preyed on by many of the filter and suspension feeders which predominate the reef environment. *Artemia* have been successfully fed to a variety of corals and anthozoans by several investigators. (Reimer, 1971 ; Porter, 1974 ; Szmantz-Frolich, 1977) and personal observation from the reef model indicate that active predation occurs in corals, gorgonians, zooanthids and anemones.

The objectives of the present study were to develop a culture system which provides a consistent amount of *Artemia* to the reef at levels determined to be washing on to a natural reef. In addition the system design includes continuous removal of particulate matter from the system. This particulate waste may be converted into inorganic nutrients for phytoplankton production. Since the culture system along with the reef microcosm will be put on public display to demonstrate the fundamentals of ecology, geology, and mariculture, it is necessary that the culture system requires a minimum of manual assistance. In operation the system will provide a tool for subsequent quantitive studies involving the role of heterotrophy in coral reef nutrition.

Methods

The culture system (Fig. 2) takes water from an arm of the reef tank. It goes through a detritus trap and ultraviolet sterilizer before being split, part of the flow going to a phytoplankton culture and the remainder going directly to animal tanks. Phytoplankton is mixed with the direct flow in a head tank and subsequently distributed to the animal tanks. From the animal tanks effluent returns to the reef tank with *Artemia* and residual phytoplankton.

Flow through the entire system is regulated by a pair of Masterflex tubing pumps of two channels each. Pump no. 1 controls both the intake and return to the reef at a rate of 35 ml/min. This corresponds to two turnovers/day in each of the *Artemia* tanks and may be adjusted independently of the flow through the phytoplankton carboys (Fig. 2). Water taken from the refuge tank is pumped through a 23 cm cellulose cartridge filter, which supports a bacterial population intended to break down organic detritus to usable nutrients. Separation of bacterial and phytoplankton cultures is maintained by a 15 watt ultraviolet lamp which passes the 35 ml/min in a 5 mm layer into a mixing tank. Pump no. 2 takes a portion of the sterile media from here to a 50 l phytoplankton culture at a rate of one turnover/day. A backup culture is maintained without circulation to insure a constant supply of phytoplankton. After returning to the head tank, media is distributed to the *Artemia* tanks via a manifold tube equipped with hypodermic needles for flow regulation.

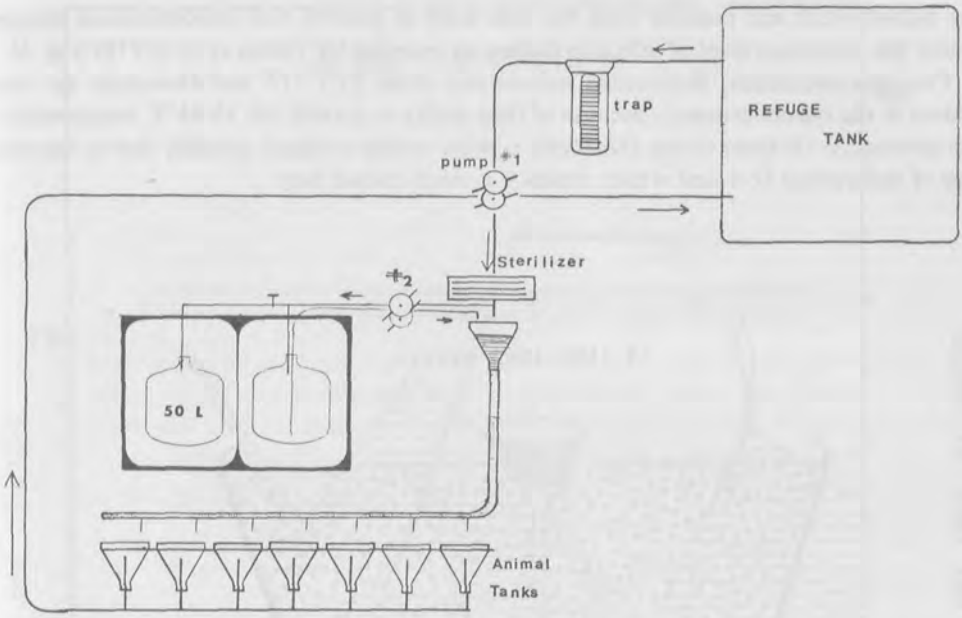

FIG. 2. Schematic diagram of system for culturing phytoplankton and *Artemia*. Refuge tank is directly connected to the reef ecosystem. Pumps are of two channels, each controlling opposing flows.

Seven animal tanks are made from four Erlenmeyer flasks with the bottoms removed. (Fig. 3). They are stoppered and hung upside down in a rack from which they may be easily removed for cleaning. Each tank contains an internal standpipe of 0.65 cm diameter glass cylinder, topped by a Nalgene funnel containing approximately 100 cm^2 of 153 μm screening. Water passing the screen returns to the reef tank through pump no. 1. In case of fouling of the screen the top of the funnel is located below the top of the tank so that animals are fed to the reef before their scheduled appointment.

Artemia are grown in each tank for 7 days, at which point the standpipe is removed and the animals are pumped to the reef over the next 12 hr period. At the same time, the previous days flask, having been washed and reassembled is innoculated with nauplii at a density of 4/ml $\pm$ 0.24.

Results

It was found that around 25% of the animals slipped through the screen in the first 2 days after introduction to the system ; however, densities through the remainder of the week remained relatively constant. On the evening of day 7, approximatey 12 000 animals were harvested or an equivalent of 0.82 g $\pm$ 0.03. Animals were grouped around the seventh instar stage. Tests of growth to day 14 were conducted but yielded only an extra 0.2-0.4 g of harvest. It seems likely that with the present low flow rate food input becomes severely limiting during

the second week and possibly even the first week of growth. Cell concentrations dropped below the minimum level of effective feeding as reported by Tobias *et al.* (1979) (Fig. 4).

Two phytoplankters, *Bellerochea polymorpha* clone STX-114 and *Dunaliella* sp. were grown in the system primarily because of their ability to survive the 33-34 °C temperature in the laboratory. Of these clones *Dunaliella* is more readily stripped, possibly due to the small size of *Bellerochea* (2-4 μm) which makes it a more elusive prey.

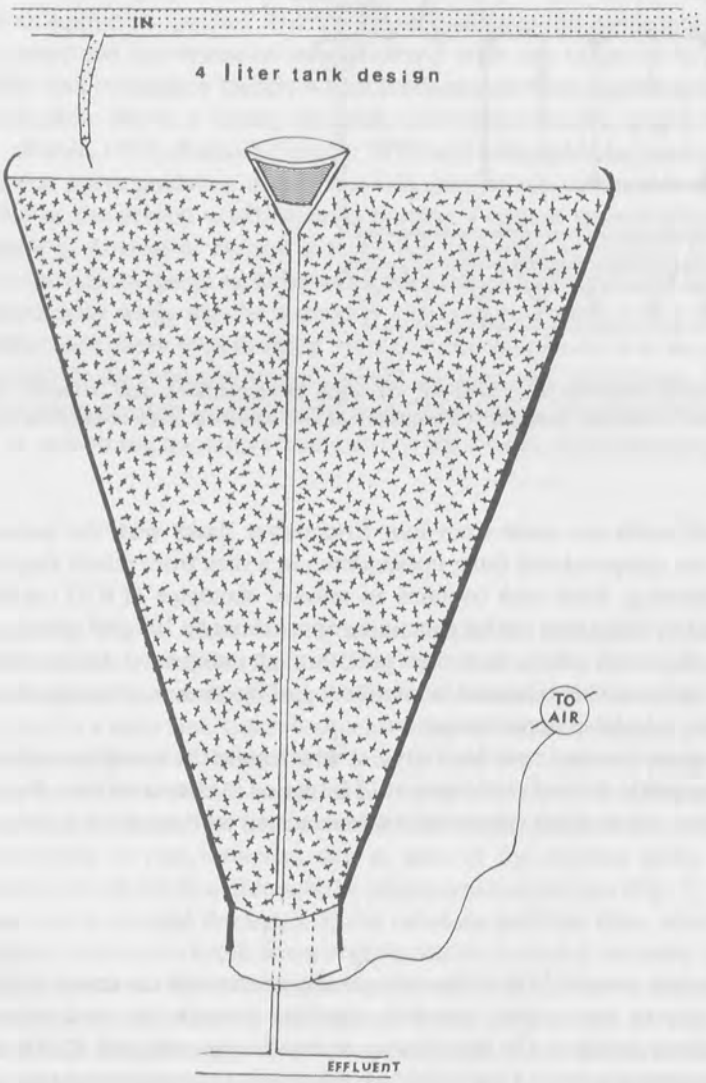

FIG. 3. *Artemia* tanks.

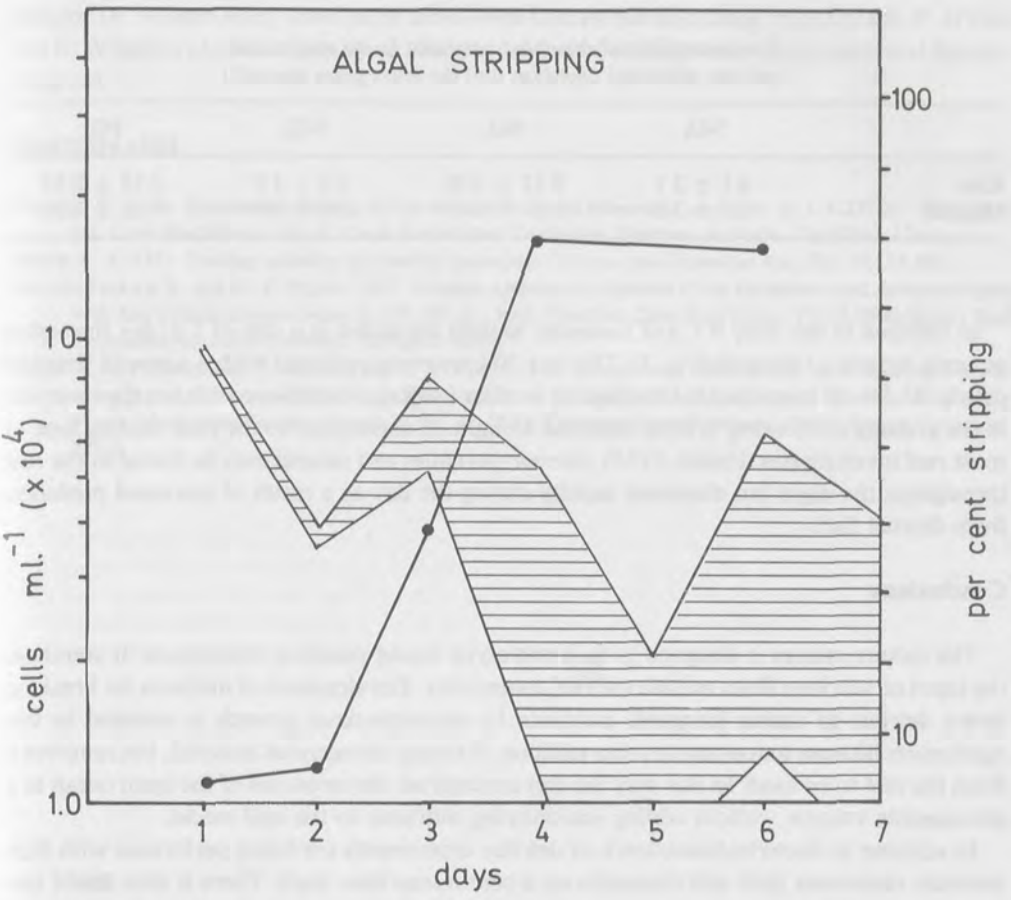

FIG. 4. Difference in cell counts in control tank without *Artemia*, and in animal tanks on days 1-7 (shaded). Per cent difference on days 1-4,6 (line).

Water from the reef model contains approximately 12 mg/l of filterable particulates ; of this a large fraction consists of calcium carbonate; it is, however, felt that this material also holds a sufficient amount of organic detritus that if broken down, would fuel the primary production in this culture system. The present design has yielded a 4-fold increase in nitrite and smaller increases in nitrate and ammonia ; however, the output is inadequate for producing sufficient amounts of phytoplankton for the second trophic level (Table I). Pending development of an effective means of breaking down organics in a continuous flow system, the algal stage is enriched to a level of Guillard's F/8 medium.

TABLE I

Concentrations of dissolved nutrients in the reef model and the microbial digestion unit (in microgram atoms/l)

	NO_3	NO_2	NH_3	PO_4
Reef	4.3 ± 2.1	0.51 ± 0.08	6.2 ± 4.3	0.23 ± 0.05
Digestor	5.2 ± 2.6	1.3 ± 0.6	5.8 ± 3.0	0.25 ± 0.05

In addition to the daily 0.8 g of juveniles, nauplii are added at a rate of 1 g/day through a separate branch of the tank (Fig. 1). This is a 20 l reservoir equipped with a solenoid timed to deliver 87.5% of its contents in the first 12 hr after loading. Nauplii are added to the reservoir in the evening so that they will be delivered at night, to correspond to the peak feeding time of most reef invertebrates (Porter, 1974). *Artemia* juveniles and nauplii may be found in the reef throughout the night but disappear rapidly during the day as a result of increased predation from diurnal fish.

Conclusions

The culture system is designed to be a prototype model plankton microcosm. It simulates the input of nutrition from outside the reef community. Development of methods for breaking down detritus to usable inorganic nutrients for phytoplankton growth is essential to this application because it does not involve addition of excess nitrogenous material, but removes it from the reef to be used. In this way we can concentrate the resources of the open ocean to a manageable volume without adding eutrophying nutrients to the reef model.

In addition to bacterial breakdown of detritus experiments are being performed with high intensity ultraviolet light and ozonation on a continuous flow basis. There is little doubt that by mastering this technology we can eliminate food limitations to the *Artemia* tanks and increase the output of the system.

Since regular addition of *Artemia* began, a greater longevity of many of the corals was noted. Individuals of the genera *Mussa* and *Scolemia* have not survived for long periods and it is possible that the size range of nauplii and juveniles is too limited for these corals, characterized by large polyps. In the future a wider size range will be employed, and possibly expanded to several species of tropical zooplankton which are native to a coral reef. For the moment, the attributes of *Artemia* far exceed the advantages of culturing a variety of less prolific and more fastidious tropical plankton, and it remains the prime choice of reef dwellers and technicians.

Acknowledgements

This work was done at The Smithsonian Institution's National Museum of Natural History. The Marine Systems Laboratory's objective is to develop culture techniques for modeling entire ecological communities under controlled conditions. The inspiration and much of the energy for the development of the coral reef and *Artemia* culture system comes from its

director Dr. Walter Adey. Invaluable advice and interest has also come from Dr. s C. F. D'Elia and F. Wheaton at the University of Maryland, Marine and Estuarine Environmental Studies Program.

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Laboratory and field studies on an Indian strain of the brine shrimp *Artemia*

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Abstract

The Tuticorin strain of the brine shrimp *Artemia* was studied under both laboratory and field conditions. Studies on the survival of the nauplii at different temperatures and salinities revealed that the nauplii prefered a salinity of 35 ‰ and a temperature of 27 °C. Biochemical changes during the larval stages showed a decreasing trend in protein, lipid and caloric contents. The effects of different inert feeds such as *Spirulina*, rice bran and yeast on the growth of *Artemia* are also discussed.

Introduction

In recent years, there has been a great demand for quality brine shrimp cysts in aquaculture, with the demand exceeding the supply. This has necessitated scientists from all over the world to concentrate on the culture of brine shrimp in salt pans and saline lakes. India, having a coastline of 5 700 km and extensive areas of saline ponds, lakes, and salt pans (Fig. 1) offers unique opportunities for the mass culture of *Artemia*. There are many geographical strains of brine shrimp reported from India (Kulkarni, 1953 ; Baid, 1958 ; Royan *et al.*, 1970 ; Achari, 1971 ; Royan, 1979), but none of the strains has yet been completely studied. The present paper summarises the results of some laboratory experiments and a few observations made on natural populations of one of the Indian strains (Tuticorin) of brine shrimp.

Materials and methods

For the laboratory studies, the cysts collected from a natural population of *Artemia* (Tuticorin) were used.

Experiments on the survival of the nauplii in different salinities and at different temperatures were carried out following the methods described by Sorgeloos *et al.* (1976). A *Spirulina* suspension was given as food ; the experiments lasted for 96 hr. Fig. 2 gives the mean percentage of survival of nauplii at different salinities and temperatures.

Biochemical composition and caloric contents of cysts, decapsulated cysts, nauplii-instar I, II, III, and metanauplii were determined by ash-, carbohydrate-, protein-, lipid-, and energy analysis. Ash content was determined by combusting the dry samples in a muffle furnace at

FIG. 1. Salt producing areas along the coastline of India and locations of *Artemia* occurrence.

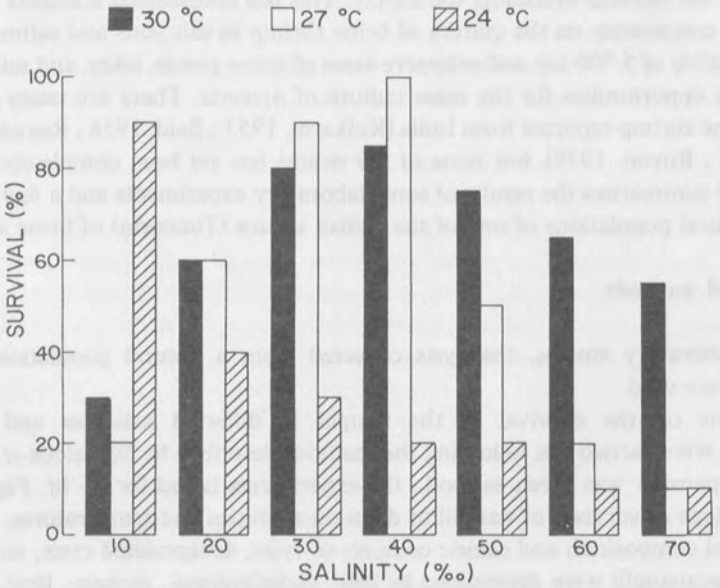

FIG. 2. Percentage survival of nauplii at different salinities and temperatures.

500 °C for 4-5 hr. Biochemical constituents such as carbohydrate, total protein, and lipid were estimated following the procedures of Dubois *et al.* (1956) Raymont *et al.* (1964) and Folch and Stanley (1956) respectively. The caloric content was determined in an adiabatic bomb calorimeter (Parr 1200). Table I shows the variations in biochemical constituents and energy contents at different stages of development.

TABLE I

Changes in chemical composition of eggs, decapsulated eggs, different naupliar instars and metanauplius of *Artemia* (all values are based on dry weights)

	Egg	Decapsulated Egg	Instar			Metanauplius
			I	II	III	
Number/mg	262	296	357	400	714	909
Protein (%)	58.00	61.14	58.00	56.80	51.98	49.91
Lipid (%)	25.64	25.57	23.30	21.00	20.90	18.00
Carbohydrate (%)	7.76	11.05	12.80	10.41	11.21	6.30
Ash (%)	6.23	4.15	5.74	8.30	12.00	20.83
Energy (cal/g ash-free dry wt)	4 924	5 448	5 241	4 915	4 821	3 827

The effect of different types of food on the growth rate of this strain was studied under laboratory conditions. 3 500 nauplii of a length of 0.6 ± 0.05 mm were transferred to plastic troughs containing 7 l of seawater (35 ‰ salinity and 30 °C temperature). Three types of feeds, namely dried *Spirulina*, rice bran, and baker's yeast, were given at a daily ration of 100 mg/100 animals/day during the first week ; during the later part of the experiment, the ration was increased to 400 mg/100 animals/day. Food was given twice daily and the water was changed every second day. Fig. 3 shows the average length (mm) of the animals at different moments for different feeds. Animals were observed daily under a dissection microscope for the formation of an egg pouch and also to find out the exact day of formation of the eggs. The number of eggs per female was counted until the adults attained the maximum body length.

In addition to growth studies in small plastic troughs, large scale rearing experiments were carried out by growing *Artemia* in a large concrete tank (approximately 6 m × 3 m × 0.7 m). The salinity was 35 ‰ at the time of inoculation of the nauplii and 140 ‰ towards the end. The tank was enriched with nitrogen and phosphorus salts in the ratio of 15:1 in order to stimulate growth of a natural population of algae. As the salinity increased due to evaporation, the algae began to die and to sediment. No food was added during the experiment since the decomposing algae provided a good source of nourishment. The *Artemia* population in the tank was about 500 nauplii/l. Fig. 3 shows the average length (mm) observed at different moments. A maximal length of 10.2 mm was obtained at the end of the experimental period.

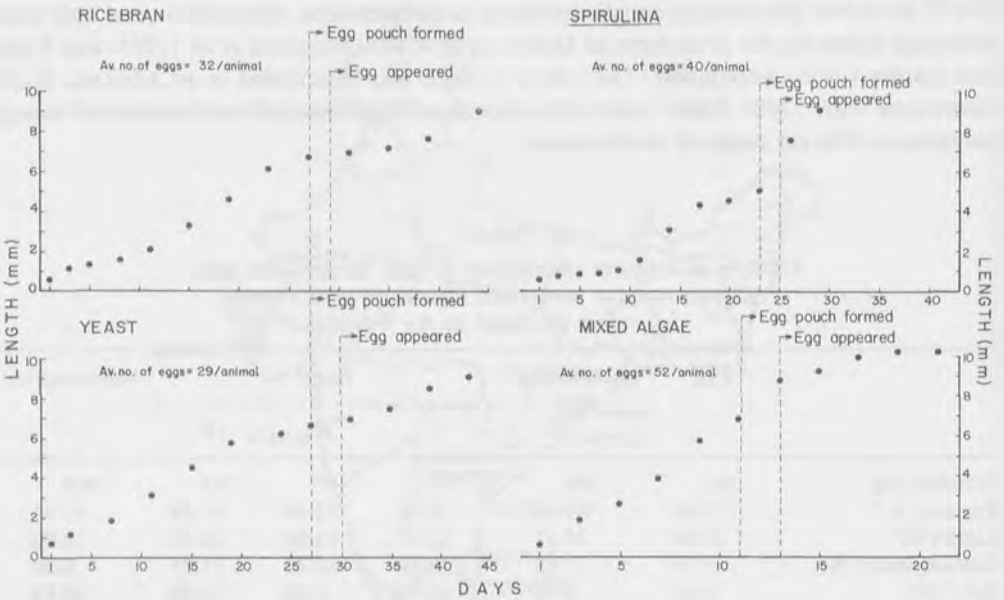

FIG. 3. Average length of the animals at different moments for various feeds.

Results and discussion

It was already reported (Royan, 1976ab) that the Indian strain (Tuticorin) of *Artemia* has a maximum hatching at 35 ‰ salinity and an optimum temperature of 30 °C. Sorgeloos and Persoone (1975) while reviewing the literature showed that different geographical strains of *Artemia* have different salinity and temperature optima for hatching. Similar observations had been reported earlier by Boone and Baas-Becking (1931), Urbani (1959) and Von Hentig (1971).

Experiments on the influence of light on hatching of the cysts showed that variation in light intensities had little influence on the hatching, whereas in the dark the hatching was very low (23 %) (Royan, 1976b). This corraborates the finding of Sorgeloos (1973) who reported that light triggered the hatching mechanism in the dormant cysts irrespective (beyond a minimum threshold) of the light intensity. He also stated that darkness decreased the percentage of hatching to a large extent.

Though *Artemia* has been reported to be euryhaline and eurythermal (Bayly, 1972), Sorgeloos *et al.*, (1976) showed that this was not a general rule ; the latter authors indeed observed that certain strains such as the one of the San Francisco Bay to be euryhaline, while some others (Bulgaria) were stenohaline and stenothermal. The optimal survival of the Indian strain seems to be situated in the salinity range 30 to 40 ‰. However, with a decrease in temperature, the optimal salinity also decreased. The observed preferential temperature was 27 °C, a temperature usually met within peninsular Indian waters.

Benijts *et al.* (1976) reported that in the San Francisco Bay strain the individual caloric content (ash-free) decreased by 27% from the instar I to the instar II stage. Further, as also shown in the present work, they reported a similar increase in ash weight and drop in lipid content with growth. In the present study, the protein content was observed to decrease during growth from instar I to metanauplii. Claus *et al.* (1978) working with Utah and San Francisco Bay strains observed that both in fed and starved conditions, the percentage of protein in the larvae increased during growth in the first 48 hr. They also observed marked differences in the protein synthesis between these two strains. They further reported a decrease in lipid and carbohydrate content during the same time period, pointing to variations of certain biochemical constituents from strain to strain during growth. Variations in temperature and salinity conditions may have a marked influence on the biochemical composition of different stages. Von Hentig (1971) reported a marked difference in protein, lipid, and carbohydrate contents in the Utah strain hatched under different temperature and salinity conditions. Paffenhofer (1967) reported that the Utah strain from Great Salt Lake showed marked changes in its biochemical composition after a starvation period of 96 hr at 31 °C. Thus, the variations in the biochemical composition reported in the present study could be an unique feature of this Indian strain coupled to the influence of temperature (hatching and rearing of the nauplii at 30 °C).

The growth observed with the three different foods showed marked differences. Of the three types of food, *Spirulina* seemed to provide the best growth, as well as the shortest time needed for formation of the egg pouch and eggs. The average number of eggs was also high in comparison to that of the other two sets of animals fed different diets. Animals fed rice bran and yeast showed egg pouch formation on the 27th day, the *Spirulina* fed animals on the 23rd day. Irrespective of the types of food, all the animals showed formation of eggs within 3 days from the date of formation of the egg pouch.

Much work has already been done on the growth of *Artemia* (different geographical strains) fed with different types of food. Ivleva (1969) reported that a Russian strain (food not mentioned) took 35 days to attain the maximum size. Sorgeloos and Persoone (1975) observed that *Artemia* grown on a diet of live algae attained the adult stage within 2 weeks. Teramoto and Kinoshita (1961) even reported that *Artemia* grown on baker's yeast or acetone butanol fermentation waste, grew to the adult size within 6 to 7 days and that spawning took place within 11 days. Reeve (1963) on the other hand reported that the Utah strain fed with live algal cells needed 54 days for the completion of growth under laboratory conditions. Baid (1963) reported that an Indian strain (Rajasthan) fed with yeast took 32 days to become sexually mature.

It thus seems evident that the time taken for the completion of growth and maturity largely depends on the nature of the strain under study, the type of food, the ration and the culture conditions. Our studies showed that *Spirulina* could be preferentially used for growing the Indian strain both under laboratory conditions and for mass culture. Sorgeloos and Persoone (1975) have already recommended the use of dried *Spirulina* as food for brine shrimp.

The results obtained by growing *Artemia* nauplii in a bigger tank were somewhat different from those obtained in plastic troughs. A maximum length of 10.2 mm was attained at the end of the 19th day. Egg pouch and egg formation were observed on the 11th and 13th day respectively. The average number of eggs observed was 52/animal. Thus, a faster growth was observed in the animals reared in the tank.

Different types of feeds have so far been tried out to mass culture brine shrimps. Nimura (1967) reported that the use of any artificial feed in mass culturing resulted in higher mortality in the larval stages as compared to a live algal diet. Sorgeloos and Persoone (1975) have recommended the use of *Scenedesmus* or *Spirulina* for mass culturing purposes.

Recently it has been demonstrated that rice bran as well as other agricultural wastes can be considered as a suitable food for brine shrimp (Sorgeloos, 1978).

In the tank experiments, the food material provided was nothing but settled decomposing mixed natural phytoplankton. The potential food value of mixed phytoplankton had been shown by many workers (Provasoli, 1969).

Table II gives the percentage of assimilation efficiency, gross growth efficiency and net growth efficiency in the shrimp *Metapenaeus monoceros* (Fabricius) fed with different stages of brine shrimp including decapsulated cysts (Royan, 1980). These results prove that the decapsulated cysts could be used profitably in all aquaculture hatcheries. This could be attributed to the high caloric value (Table I) of the decapsulated eggs. Sumitra-Vijayaraghavan *et al.* (1978) have shown that, of the 16 different types of feed tried for the shrimp *M. monoceros*, maximum conversion efficiency was found when the shrimps were fed adult *Artemia*. Table II shows that decapsulated eggs are even superior to adult *Artemia*. The advantages of using decapsulated eggs in aquaculture have recently been summarized by Bruggeman *et al.* (1979).

TABLE II

Percentage values of assimilation efficiency (A/C), gross growth efficiency (P/C or K_1), net growth efficiency (P/A or K_2), relative growth rate (RGR), and relative consumption (RC) in *Metapenaeus monoceros* fed different stages of *Artemia*

Food	A/C	K_1	K_2	RGR (mg/$\bar{w}$/d)	RC (mg/$\bar{w}$/d)
Decapsulated eggs	88.15	45.90	52.07	62.30	135.7
Freshly hatched nauplii	92.28	37.09	40.19	80.30	216.6
Juveniles	89.32	26.67	29.86	62.60	234.7
Adults	79.10	30.00	37.90	39.00	240.0

Finally some observations were made in an earlier study on a natural population of brine shrimp in a salt pan near Tuticorin. The population was traced in different condenser pans and information was obtained on the percentage occurrence of oviparous versus ovoviviparous adults in the population, the density of the animals in different condenser pans, the total cyst production per hectare, *etc.* (Royan *et al.*, 1978).

Summary

From the present study, certain general conclusions can be drawn on the biology of the Indian strain of *Artemia*. Under both laboratory and field conditions reproduction was found to be parthenogenetic with predominant oviparity. For the survival of the nauplii, a salinity of

35 ‰ and a tropical water temperature of 27 °C were close to optimal. Under laboratory conditions, *Spirulina* was found to be the most suitable of the various feeds tried. An experiment in a larger container with a greater depth of the water column, and with decomposing natural phytoplankton as food gave an even faster growth than in the laboratory trials. Decapsulated eggs and first instar nauplii gave a better conversion efficiency than adult *Artemia* in feeding the shrimp *Metapenaeus monoceros*.

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Controlled production of *Artemia* cysts in batch cultures

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Abstract

The possibility to develop a routine batch culturing method for the production of cysts has been examined at laboratory scale.

The influence of abiotic and biotic parameters on the mode of reproduction has been studied and the following facts have been noted :

- cyclic low oxygen tensions induce haemoglobin synthesis with subsequent shift of the mode of reproduction from ovoviviparity to oviparity ;
- addition of ferric-EDTA stimulates haemoglobin synthesis ;
- a correlation exists between the color of the animals and the type of reproduction ;
- neither the food source nor the water salinity *per se* control the mode of reproduction. However, cysts produced at low salinity appear to have a low hatchability.

The effect on the hatchability of various processing techniques applied to the cysts produced has been tested. From these experiments it appears that :

- the cysts must be activated (dehydrated) to allow hatching at a subsequent hydration ;
- the method of dehydration influences to a large extent the viability of the cysts. Of the various methods tested out so far, drying at 40 °C or immersion in a saturated brine solution gave the best results.

A modified version of the classic air-water-lift operated raceway culturing-system is proposed as a standard method for the controlled production of cysts.

Introduction

Although annually thousands of kilograms of *Artemia* cysts are harvested from nature, our knowledge of the phenomena which control cyst production is still very limited.

A factor common to all brine shrimp strains, parthenogenetic as well as bisexual, is that female *Artemia* are capable of producing either live offspring or inactive cysts. A detailed description of the reproductive mechanism and the different processes from oogenesis until deposition of the offspring can be found in Metalli and Ballardin (1972).

The eggs (oocytes) are formed in two string-like ovaria located on both sides of the digestive tract; When ripe, the eggs are transferred through the oviducts into the unpaired broodsac

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(uterus). At this stage bisexual females have to be fertilized to produce viable offspring. As soon as the embryonic process in the uterus has started, the production of new oocytes starts again. When the environmental conditions are "favorable" the mode of reproduction is ovoviviparous ; the fertilized egg hereby undergoes the classical embryonic development and through blastulation and gastrulation the differentiation ends with the release of a free-swimming nauplius. Under influence of specific abiotic and biotic conditions ovoviviparity can, however, shift to oviparity with production of cysts ; the embryonic development is hereby reversibly interrupted at the gastrula stage. Trehalose is stored as carbohydrate reserve (Clegg, 1962) and each gastrula is surrounded by a shell, impregnated with a haematine-like substance (Dutrieu, 1959) secreted by the active brown shell glands (Dutrieu, 1960 ; De Maeyer-Criel, 1978). These encysted gastrulae or so called cysts are finally released in the water.

In the literature much confusion exists about the mechanisms responsible for the shift from ovoviparity to oviparity. Salinity was mentioned as the responsible factor by Abonyi (1915) and by Barigozzi (1939). Dutrieu (1960) correlated cyst production with haemoglobin synthesis. According to Ballardin and Metalli (1963) the mode of reproduction is controlled by environmental factors. D'Agostino and Provasoli (1968) reported that oviparity was induced by the food quality and/or quantity. Others refer to the presence of particular components in the culture medium or the food, *e.g.* iron (Baker, 1966) or chlorophyll (Dutrieu, 1960).

Previous research carried out in our laboratory (Sorgeloos, 1975) revealed a definite influence of low dissolved oxygen levels on the induction of oviparity. Unfortunately the technique applied was too complex to allow long term studies with many replicates excluding as such the possibility of statistical analyses.

For this reason we decided to use open aquaria in which the animals are maintained at densities of 1 000/l. Instead of maintaining low levels of dissolved oxygen, we have submitted the animals to cyclic drops of the oxygen concentration by applying a discontinuous aeration. As the exposures to critically low levels of oxygen were never maintained for long periods of time, the survival rates of the adult brine shrimp were kept high.

The present study deals with the influence of dissolved oxygen, iron, salinity, and type of food on the mode of reproduction of brine shrimp. Our specific goal was to develop a routine method which induces *Artemia* populations to shift from ovoviviparity to formation of cysts. Such a method can indeed become a very practical tool for the further study of the influence of abiotic and biotic parameters on characteristics of cysts. At the same time it may allow production of enough study material for comparative analyses of different *Artemia* strains.

Materials and methods

All experiments were performed with the bisexual San Francisco Bay (California, USA) strain cultured in natural seawater, the salinity of which was increased with natural seasalts.

Three different culture tanks were used :

- 7 l rectangular aquaria equipped with one air-water-lift (AWL) placed in a corner ;
- 40 l rectangular containers converted into AWL-raceways by placing a central partioning and 4 AWL's (see Bossuyt and Sorgeloos, 1980 for more details on AWL-raceways) ;
- one 250 l rectangular AWL-raceway as described by Bossuyt and Sorgeloos (1980).

Reproduction tests were carried out with five different food sources. Stock suspensions were made up as follows :

- *Spirulina* : 50 g spray-dried *Spirulina* (commercially available from Sosa Texcoco, S.A., Mexico) homogenized in 250 ml seawater with a kitchen blender and diluted to 1 l with saturated brine ;
- *Spirulina*/yeast : 25 g *Spirulina* + 25 g bakers' yeast prepared in the same way as the *Spirulina*-suspension ;
- *Scenedesmus* : 15 g drum-dried *Scenedesmus* (made available by the "Kohlenstoff-biologische Forschungsstation", Dortmund, Fed. Rep. Germany) homogenized in 250 ml seawater with a kitchen blender, then grinded in a ball-mill (glass spheres 5 mm in diameter) for 12 hr and diluted to 1 l with saturated brine ;
- *Dunaliella* sp. cultured in natural seawater following De Pauw *et al.* (1978) ;
- rice bran : 100 g Ultrafine® micronized rice bran, defatted product (from the N.V. Brucoma, Ghent, Belgium) homogenized in 250 ml seawater with a kitchen blender and diluted to 1 l with saturated brine.

The following parameters were studied for qualitative and quantitative evaluation of the mode of reproduction of the brine shrimp :

- sex-ratio of 100 animals sampled at random from each culture tank ;
- fertility ratio : approximately 50 females were examined under the dissection microscope for full ovaria, oviducts and/or uterus ; the ratio of the number of mature females to the total number of females observed was used to quantify the fertility of the population. Experiments started if this figure was above 50 % and lasted until it dropped below 50 % ;
- reproductive capacity : 10 females with a full uterus were transferred to individual vials containing 20 ml culture medium. Once a day the vials were checked for deposited cysts or nauplii and the number of offspring was recorded. All females released their offspring within 5 days and the ratio of oviparous to ovoviviparous females as well as the average number of cysts and nauplii produced per female was calculated ;
- color-code of Chow (1968), as a rough estimate of the haemoglobin concentration in *Artemia* :

0 : no color
\+ : light pink
\+ + : light pink, few spots red
\+ + + : red
\+ + + + : dark red.

Experimental results and discussion

Continuous versus discontinuous aeration

A 5-weeks reproduction test was performed with 3 weeks-old adult *Artemia* in two 7 l aquaria at 90 ‰ salinity and 28 °C. The animals were fed with *Spirulina* (20 ml stock suspension per day per aquarium). Every week the medium was renewed.

One aquarium was aerated continuously (average oxygen concentration : 6.0 mg/l). In the other one a 10 min interruption was applied automatically once every hour ; this resulted in a

cyclic drop of the oxygen level to 4.5 mg/l, which is close to the critical level for induction of oviparity (Sorgeloos, 1975).

The results are summarized in Table IA. After two to three reproductive cycles, the animals kept under cyclic oxygen fluctuations shifted from ovoviviparity to oviparity. Of a total of 67 broods, 71% consisted of cysts. The observation that under discontinuous aeration the *Artemia* turn red (as compared to pale in the control) is probably related to the increased haemoglobin production. Gilchrist (1954) and Dutrieu (1960) observed indeed that at low oxygen levels brine shrimp are stimulated to produce haemoglobin. Haematin, a decomposition product of the latter respiratory pigment is then secreted by the shell glands at the moment of cyst production (Fautrez and Fautrez-Firlefijn, 1971).

TABLE I

Influence of different factors on the mode of reproduction of *Artemia*.
A : continuous versus discontinuous aeration ;
B : Fe-EDTA addition in media aerated continuously ;
C : Fe-EDTA addition in media aerated discontinuously

		Number of broods	Mortality rate (%)	% oviparous broods	Color of the animals
A	Continuous aeration	70	20	39	Pale
	Discontinuous aeration	67	45	71	Light pink
B	Fe-EDTA	100	20	67	Dark pink
	Control	100	30	33	Pale
C	Fe-EDTA	100	15	96	Red
	Control	100	30	74	Pink

THE EFFECT OF FE-EDTA ON CYST PRODUCTION

Research carried out in California (USA) revealed a positive correlation between the presence of iron in the medium, increased haemoglobin synthesis, and cyst production (Baker, 1966 ; Chow, 1968). The experimental technique used was, however, difficult to keep under control and often resulted in high mortalities.

To check the influence of iron addition to the medium we carried out experiments in two 7 l aquaria tests (similar culturing conditions as described for the series with continuous aeration). One aquarium was progressively (over a period of 1 week) enriched with Fe-EDTA[2] to a final concentration of 20 mg Fe-EDTA/l medium.

Our results, summarized in Table IB, confirm the theory of Baker (1966) : over a 7 weeks test-period the dominant mode of reproduction was oviparous : 67% *versus* 33% in the

[2] Fe EDTA stock solution (Baker, 1966)

$Fe\ Cl_3\ 6H_2O$	2.4 g
Na_2EDTA	1.86 g
aq. dest.	1 000 ml.

control series. The presence of chelated iron appears to facilitate haemoglobin synthesis ; the animals indeed had a higher haemoglobin content in the iron enriched medium than in the control series. As has been reported earlier by Gilchrist (1954), Dutrieu (1960) and Anonymous (1978), the survival rate of dark-colored animals is higher than that of pale *Artemia*. In this regard it is interesting to note that we often observed "surface respiration" (Baker, 1966 ; Moore and Burn, 1968 ; Horne, 1971) in pale animals but never in dark pink or red adults.

The combined effect of discontinuous aeration and Fe-EDTA addition on cyst production

Two 7 l aquaria, both with discontinuous aeration but one enriched with Fe-EDTA were set up in conditions identical to those previously described. After an adaptation period of 7 days the percentage of oviparous females was determined twice a week over a 5 weeks period. From the data obtained (Table IC) it is clear that cyclic oxygen stresses and the presence of chelated iron, when acting in concert, stimulate cyst production by 20 to 30% respectively compared to their single effect.

Statistical analysis of the effect of cyclic oxygen stresses and Fe-EDTA additions on cyst production

Prior to run this experiment we have determined the minimal oxygen and maximal Fe-EDTA level that should be applied to maximize cyst production.

Three oxygen levels have been evaluated in 7 l aquaria, set up in identical conditions as described earlier, all enriched with 20 mg Fe-EDTA :

- aquarium 1 : continuous aeration (average oxygen concentration of 6.2 mg/l)
- aquarium 2 : every hour, a 10 min interruption of the aeration (minimal oxygen concentration : 4.0 mg/l)
- aquarium 3 : every hour, a 30 min interruption of the aeration (minimal oxygen concentration : 2.5 mg/l)

From the results of a 5 weeks reproduction test (Table II) it appears that a cyclic drop of the dissolved oxygen level to 4 mg/l is sufficient to induce cyst production. A further decrease of the oxygen concentration has no beneficial effect ; on the contrary the animals often concentrated at the water surface even during periods of oxygenation. Survival rate dropped to 40% and the fertility and size of the broods was smallest in the aquarium with the highest oxygen stress.

Table II

Influence of three types of aeration on the mode of reproduction

Aeration mode	% oviparous females
Continuous	33
Discontinuous (minimum 4 mg/l)	78
Discontinuous (minimum 2.5 mg/l)	89

In the next experiment we have examined the effect of 5 concentrations of Fe-EDTA : 10, 15, 20, 25, and 30 mg/l respectively. Five 7 l aquaria were set up in standard culture conditions with continuous aeration. The results in Table III indicate that addition of either 25 or 30 mg Fe-EDTA/l give a maximal cyst production, with no specific differences between the two series.

TABLE III

Influence of five concentrations of Fe-EDTA on the mode of reproduction

Fe-EDTA concentration (mg/l)	% oviparous females
10	22
15	32
20	32
25	68
30	71

Four 40 l raceways were used to test the combined effects of cyclic oxygen stresses (down to 4 mg/l) and Fe-EDTA additions (25 mg/l) :

A : continuous aeration, no iron addition
B : continuous aeration, iron addition
C : discontinuous aeration, no iron addition
D : discontinuous aeration, iron addition.

Each raceway contained about 40 000 *Artemia* in 90 ‰ seawater at 28 °C. Once adult the daily feeding consisted of 100 ml *Spirulina* suspension per raceway. Ten consecutive broods were studied during an experimental period of 2 months. Data on the following parameters are summarized in Table IV : % survival, dissolved oxygen concentration, sex-ratio, fertility ratio, cysts to nauplii or C/N-ratio, and color-code of Chow (1968). The data for the C/N-ratios were submitted to a variance analysis with two factors and replicates within the subclasses. The average were compared using Duncan's Multiple Range Test (Snedecor and Cochran, 1967).

The results of this experiment confirm our previous findings : the highest percentage of oviparous females is found in the presence of extra-iron. The average C/N-ratio for the tests with addition of iron and discontinuous aeration was significantly different from all other combinations at the 0.01 level (Table V, A to D). A definite correlation was furthermore noted between the color of the animals and their mode of reproduction. Our observations confirm the earlier findings of Clegg (personal communication) that females with white shell glands produce nauplii, those with brown shell glands desposite cysts.

DIAPAUZE DESACTIVATION IN *ARTEMIA* CYSTS

Cysts deposited in raceway cultures were harvested and cleaned from large debris by washing through a 350 μm screen and recuperated on a 200 μm filter screen.

TABLE IV

Reproductive characteristics of *Artemia* in 10 consecutive broods as influenced by Fe-EDTA addition and mode of aeration.
A : continuous aeration, iron addition ; B : continuous aeration, no iron addition ;
C : discontinuous aeration, iron addition ; D : discontinuous aeration, no iron addition

	% survival	Dissolved oxygen (mg/l)	Sex-ratio	Number of females from a random sample of 100 adults with full ovaries/full oviducts/full uterus	% fertility	C/N ratio	Color code
	98	6.3	1.43	10/ 4/ 4	40	0.08	+
	91	6.3	1.38	14/10/ 8	55	0.1	+
	97	6.3	1.60	10/12/ 1	54	0.17	+ / + +
	96	6.3	1.52	12/10/ 2	55	0.24	+ / + +
A	96	6.3	1.60	14/16/22	70	0.32	+ / + +
	95	6.2	1.56	16/ 9/10	60	0.60	+ +
	94	6.2	1.50	17/10/ 4	52	0.70	+ +
	93	6.2	1.54	12/ 9/12	55	0.72	+ +
	93	6.2	1.58	16/ 8/ 2	59	1.00	+ +
	99	6.3	1.27	10/ 4/72	29	0.06	0
	98	6.3	1.33	3/ 1/ 3	13	0.07	0
	96	6.3	1.50	10/ 3/11	40	0.04	0
	95	6.3	1.52	12/11/ 4	45	0.08	0
B	95	6.3	1.38	10/ 4/11	43	0.06	0
	94	6.2	1.40	12/ 6/ 4	38	0.05	0
	94	6.2	1.47	12/ 9/10	53	0.08	0
	93	6.0	1.50	14/ 6/11	52	0.10	0
	92	6.0	1.46	11/10/ 4	44	0.06	0
	99	5.6	1.2	12/ 4/ 6	49	1.06	+ / + +
	98	5.4	1.42	14/ 6/ 4	40	1.17	+ +
	96	4.9	1.63	14/10/ 8	52	2.30	+ +
	95	4.6	1.63	12/ 6/ 9	44	3.40	+ +
C	95	4.4	1.58	10/ 4/ 3	44	4.00	+ + / + + +
	93	4.2	1.60	14/ 6/ 9	47	17.0	+ + +
	93	3.8	1.62	16/ 9/ 6	50	14.0	+ + +
	93	3.7	1.62	19/16/ 6	66	24.0	+ + + / + + + +
	91	3.4	1.61	16/ 6/10	51	29.0	+ + + +
	96	5.4	1.3	16/ 4/ 8	49	0.09	0
	94	5.4	1.63	12/10/ 4	42	0.70	0
	93	4.8	1.60	3/ 1/ 3	11	0.06	0 / +
	90	4.6	1.50	10/ 4/ 2	30	0.03	0 / +
D	88	4.3	1.54	6/10/ 4	36	0.07	+
	82	4.0	1.59	14/ 6/ 4	39	0.90	+
	76	3.8	1.62	16/ 4/ 8	45	1.20	+
	74	3.7	1.60	14/ 8/ 4	42	1.00	+
	70	3.4	1.52	18/ 4/ 8	48	1.00	+

TABLE V

Analysis of variance and Duncan's Multiple Range Test for significant differences in C/N-ratios of *Artemia* submitted to combined effects of Fe-EDTA and mode of aeration (see Table IV)

	d.f.	Average SS	F_w
SS total	35	–	–
SS Fe-EDTA	1	243.26	8.85**
SS aeration	1	261.79	9.53**
SS interaction	1	209.86	7.64**
SS replicates	8	32.23	–
SS eror	24	27.48	–

Standard error averages : 1.7474

Duncan's Multiple Range Test

C-B*
C-A* D-B*
C-D* D-A A-B

When incubated in optimum hatching conditions (Sorgeloos, 1980) less than 3% of these cysts gave birth to nauplii. This confirms the theories of Morris (1971) and Clegg (1974) that the hatching metabolism in *Artemia* cysts can only be initiated when the cyst's diapauze has been desactivated. Following Dutrieu (1960) and Morris and Afzelius (1967) this dormancy state can be overcome by dehydration of the cysts.

We have tested this hypotheses in the following experiment : after different time intervals of exposure to saturated brine[3] (4, 6, 12, 24, and 48 hr respectively) the percentage hatching efficiency was determined by incubation of 100 cysts (three parallels) in natural seawater in stoppered plastic vials kept in continuous movement on a rotating axle (Sorgeloos *et al.*, 1978). From the hatching results recorded after 48 hr incubation at 25 °C (Fig. 1), it appears that the hatching efficiency increases as a function of the dehydration time in brine. Although the hatching percentage does not significantly increase after prolongation of the exposure to brine for 2 days, the maximal hatching obtained is still lower than the optimal hatching efficiency data reported in the literature for San Francisco Bay cysts. We do not know if this is related to the incomplete water removal in brine – according to Clegg (personal communication) cysts exposed to saturated NaCl solutions still contain ± 20% water – or to specific effects of the rearing conditions (biotic and/or abiotic parameters) on particular characteristics of the deposited cysts.

Dehydration of *Artemia* cysts can be achieved by various methods. We have analysed the hatching efficiency of cysts harvested from the same raceway cultures but subjected to different drying conditions for varying exposure times :

- oven-drying at 30, 40, 50, and 60 °C respectively ;
- dehydration in excicator over dry $CaCl_2$;
- dehydration in saturated brine solution.

[3] Since upon direct transfer to saturated brine a high percentage of laboratory produced cysts implode, the cysts were initially dehydrated for 1 hr in 115 ‰ brine and then transferred to saturated brine of 280 ‰.

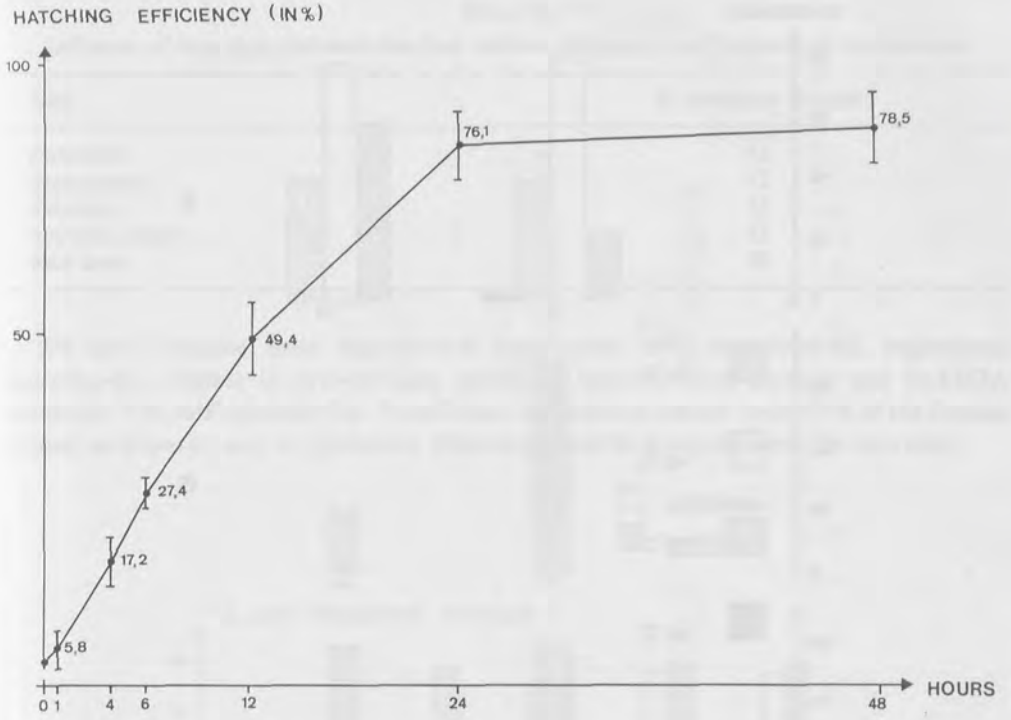

FIG. 1. Influence of dehydration time in saturated brine on the hatching efficiency of laboratory produced cysts.

The results are represented graphically in Fig. 2. Dehydration for 24 hr at 40 °C in an oven or at room temperature over $CaCl_2$ or in a saturated brine solution assures a ± 80% hatchability of the treated cysts. As could be expected exposure to temperatures above 40 °C negatively influence the viability of cysts (Sorgeloos *et al.*, 1976). We are wondering if the low hatching scores recorded for 48 hr drying over $CaCl_2$ might not be related to the extremely low water content of these cysts ; this is at least the explanation which Iwasaki (1958) gave for her finding that cysts dried over $CaCl_2$ are more sensitive than untreated cysts. The best hatching result of 81% obtained with oven-dried cysts is significantly better than the 76% recorded for the brine-activated cysts. Further water removal from 20% to less than 10% thus assures a better desactivation of the diapauze mechanism.

THE EFFECT OF VARIOUS FOOD SOURCES ON CYST PRODUCTION

Five 7 l aquaria were set up with 7 000 adult *Artemia* each in 90 ‰ seawater at 28 °C. No cyst-inducing conditions were created. The following diets known to be good *Artemia* foods (Sorgeloos, 1975 ; Sorgeloos *et al.*, 1980) were administered at specific daily rations :

- 50 ml *Dunaliella* suspension at 1.2 10^6 celles/ml
- 10 ml *Spirulina* suspension

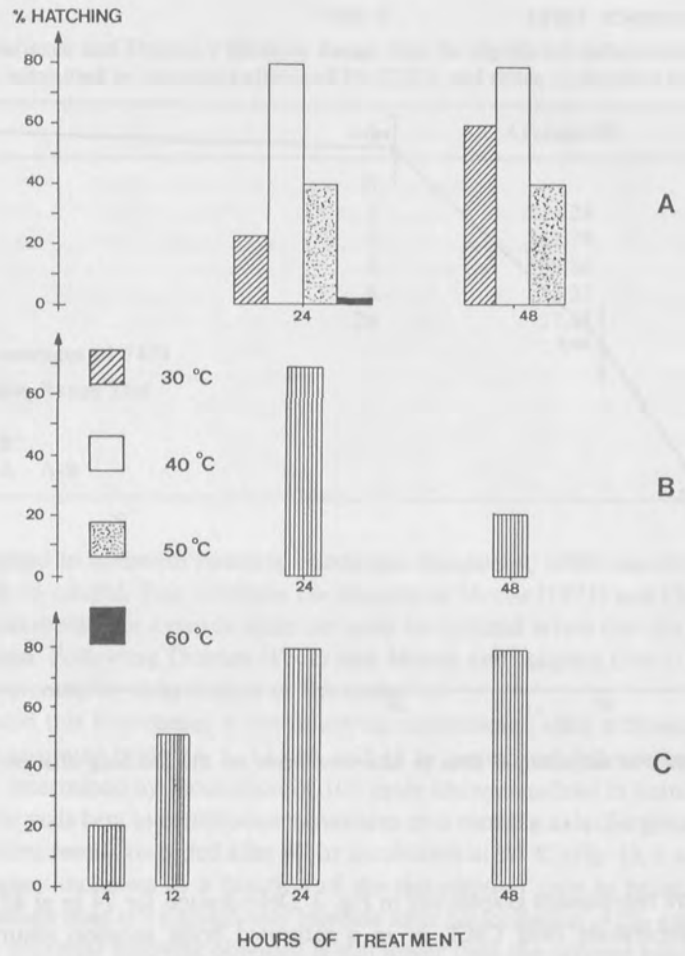

FIG. 2. Influence of different drying methods on the hatching percentage of cysts harvested from raceway cultures. A : oven drying ; B : drying in excicator over $CaCl_2$; C : dehydration in saturated brine.

- 10 ml *Scenedesmus* suspension
- 10 ml *Spirulina*-bakers' yeast suspension
- 10 ml rice bran suspension.

The percentage oviparous females totalized for each diet over a 1 month reproductive period for the various diets is summarized in Table VI. It appears from these data that no typical cyst-inducing food could be found among the diets tested. In the given set of experimental conditions *Dunaliella* and *Scenedesmus* proved to be less efficient in stimulating the oviparous mode of reproduction than *Spirulina* and rice bran. The results obtained with rice bran is in contradiction with Dutrieu (1962)'s theory that chlorophyll in the diets is a *conditio sine qua non* for oviparity.

TABLE VI

Influence of four algal diets and one food without chlorophyll on the mode of reproduction

Diet	% oviparous females
Dunaliella	15
Scenedesmus	12
Spirulina	28
Spirulina/yeast	25
Rice bran	30

We have repeated these reproduction experiments with rice bran-fed, respectively *Spirulina*-fed *Artemia* in cyst-inducing conditions (discontinuous aeration and Fe-EDTA additions). The data obtained (Fig. 3) confirmed our previous results : over 90 % of the females shifted to oviparity and no significant difference could be noted between the two diets.

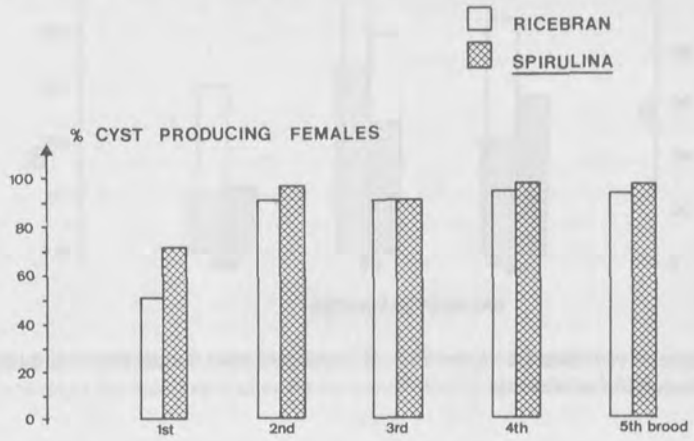

FIG. 3. Influence of two different diets on the mode of reproduction of brine shrimp subjected to cyst-inducing conditions.

THE INFLUENCE OF TWO DIETS, TWO TEMPERATURES AND THREE SALINITIES ON THE MODE OF REPRODUCTION IN *ARTEMIA*

In order to define optimal temperature/salinity combinations for routine production of *Artemia* cysts in small raceways, the following experiment was performed : twelve 40 l raceways containing 40 000 adult *Artemia* each, were maintained under cyst-inducing conditions (discontinuous aeration, Fe-EDTA-addition). The percentage oviparous females was followed over 10 reproductive cycles in function of the following variables :

- temperature : 20 and 28 °C
- salinity : 30, 90, and 180 ‰
- diet : *Spirulina* : 40 ml stocksuspension per day
  rice bran : 50 ml.

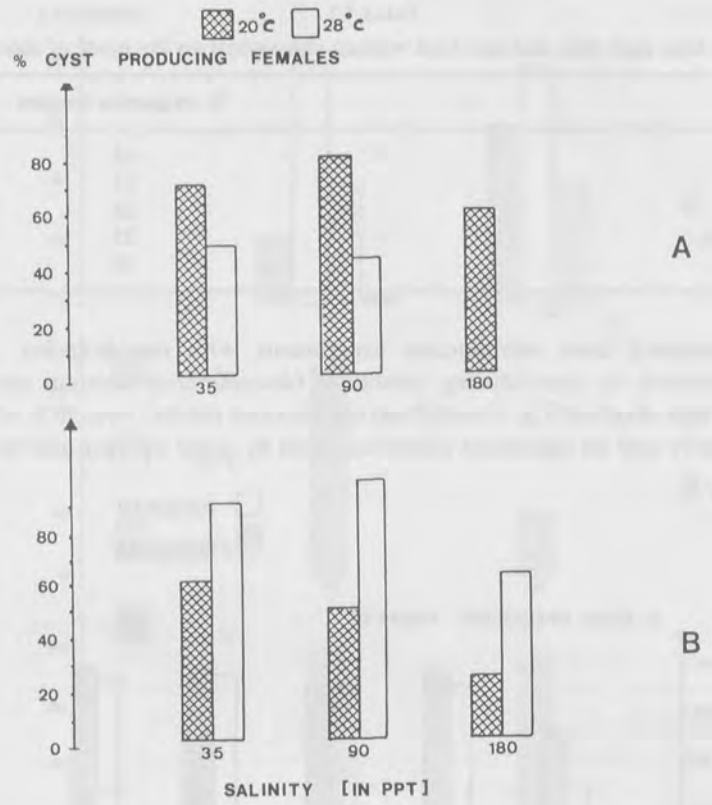

FIG. 4. Influence of two diets (A : *Spirulina* ; B : ricebran), two temperatures and three salinities on the mode of reproduction in *Artemia*.

Cysts harvested from the various experimental combinations were activated in brine solution and incubated at 28 °C in 35 ‰ seawater during 48 hr to determine their hatching efficiency. The results (Fig. 4) indicate that with *Spirulina* maximal cyst production occurred at 20 °C, whereas with rice bran it was at 28 °C. The 180 ‰ cultures were difficult to maintain due to clogging of the aeration lines by cristallization of salt; This phenomenon led to the loss overnight of combination 28 °C/180 ‰/*Spirulina*. Although no statistical difference existed between the 35 ‰ and 90 ‰ parallels, the hatching quality of the cysts produced at 35 ‰ (Fig. 5) turned out to be poor and this for both the 20 °C and 28 °C series. A similar problem in hatching cysts produced in natural seawater was noted by Tobias (personal communication) in his flow-through cultures with live algae. Further research is needed here to find out which factor(s) cause(s) the low viability in these cysts and to define the optimal conditions for activation or hatching in these cysts.

The largest number of cyst-producing females was recorded for the rice bran combination at 28 °C and 90 ‰ ; in this particular set of conditions almost 30 % more cysts were deposited than in the corresponding *Spirulina* series at 20 °C.

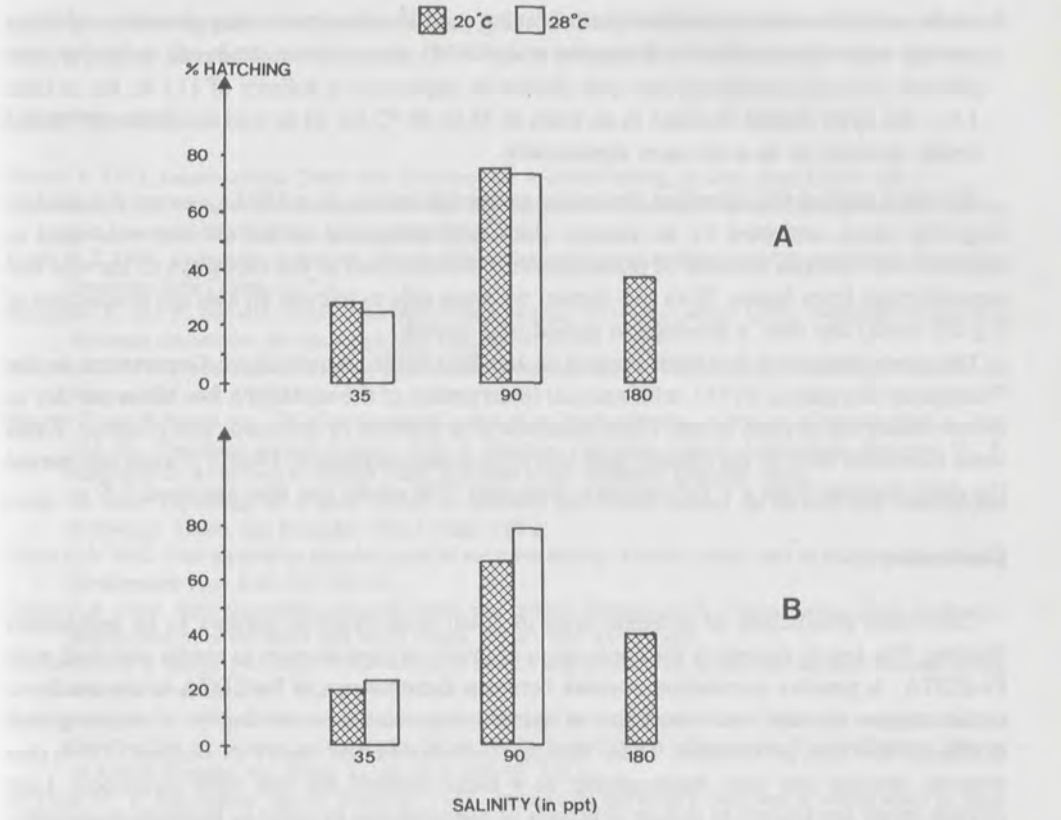

FIG. 5. Hatchability of *Artemia* cysts produced in different combinations of temperature, salinity and diet (A : *Spirulina* ; B : rice bran) after 48 hr incubation in seawater of 35 ‰ salinity at 28 °C.

Standard procedure for controlled cyst production in batch systems

From the information gained in this study the following standard procedure for routine production of *Artemia* cysts in batch systems can be advised :

- brine shrimp are raised to adulthood in densities of 1 ind./l at 28 °C in 90 ‰ seawater using a rice bran suspension as sole source of food. Good growth and survival results are obtained by using "air-water-lift (AWL)-raceways" as described by Bossuyt and Sorgeloos (1980) ;
- after 4 weeks culturing, Fe-EDTA is gradually added in order to reach a concentration of 25 mg/l after 1 week ;
- from the 5th week onwards, the animals are submitted to cyclic oxygen stresses which should not exceed a minimal level of 4 mg/l ;
- within another 1 to 2 weeks, over 90 % of the females will switch to oviparity ;
- the deposited cysts which mostly float in a medium of 90 ‰ salinity should be harvested as frequently as possible ; their cleaning and separation from faecal pellets, respectively empty

shells and other debris is carried out following the two steps processing procedure in brine and tap water as described by Sorgeloos *et al.* (1978). Attention must be paid, however, that prior to the brine treatment the cysts should be exposed to a salinity of 115 ‰ for at least 1 hr ; the cysts should be dried in an oven at 35 to 40 °C for 24 hr and consequently stored under vacuum or in a nitrogen atmosphere.

We have applied this standard procedure in our laboratory in a 250 l raceway. An electromagnetic valve, activated by an electric clock and connected to the air-inlet was used to impose cyclic oxygen stresses. A plate-separator was coupled to the raceways to harvest and separate cysts from faeces. With this system we were able to harvest an average production of 1 g dry cysts/day over a production period of 1 month.

The same technique has been applied in the SEAFDEC Aquaculture Department in the Philippines (Sorgeloos, 1978), with manual interruption of the aeration a few times per day to induce dissolved oxygen drops. Food consisted of a mixture of *Spirulina* and ricebran. Cysts were harvested both in the culture tank and from a plate-separator. Over a 2 week test-period the daily harvest from a 1 m^3 raceway with only 100 adults per liter averaged 5.5 g.

Conclusion

Controlled production of *Artemia* cysts in small scale systems appears to be technically feasible. The key to success is the application of cyclic oxygen stresses in media enriched with Fe-EDTA. A positive correlation appears between the presence of Fe-EDTA in the medium, cyclic oxygen stresses, red coloration of the animals related to the degree of haemoglobin synthesis, and cyst production. These findings corroborate the theory of Dutrieu (1960) that *Artemia* females use their haemoglobin as a basic element for cyst shell formation. Low oxygen levels are known to induce synthesis of haemoglobin in order to facilitate respiration. It appears from our studies that the need for chlorophyll in the diet as an essential factor for the induction of oviparity (Dutrieu, 1962) can be eliminated by addition of chelated iron to the medium.

Since this study was performed with brine shrimp from the San Francisco Bay (California, USA) strain, the results should not be extrapolated to all *Artemia* strains. Considerable variations may indeed exist with regard to specific quantitative factors such as optimal temperature, dosage of Fe-EDTA, sensitivity for oxygen stresses, etc.

It also should be made clear that the procedure worked out cannot be considered, even by scaling up, as an alternative for cyst production in nature. The harvest for aquaculture purposes of tons of cysts produced naturally in salt ponds will always be much cheaper than any "artificial" cyst production. However, the technique of controlled cyst production opens many interesting perspectives in the study of various aspects of the reproductive biology in *Artemia*. It provides the biochemist with a standardized research material of known origin to unravel the mechanisms which induce the shift in the reproductive behavior and allows for the first time the study of the influence of different abiotic and biotic parameters on the characteristics of standard cysts. Application of this technique with various strains, either harvested from natural populations or produced from cross-breeding, will allow to compare their reproductive behavior and to study the heritability of particular characteristics. Finally, in those cases where only a very limited quantity of cysts or animals of a particular strain are

available, the technique described will be a valuable tool in the production of more research material either for direct testing or as inoculation material for further production in nature.

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International Study on *Artemia*[1] V. Nutritional value of five geographical strains of *Artemia* : effects on survival and growth of larval Atlantic silverside *Menidia menidia*

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Abstract

Five geographical strains of brine shrimp nauplii were each fed to larvae of the marine fish, Atlantic silverside (*Menidia menidia*). In each of three experiments, survival was highest and significantly greater on the diet of Macau-Brazil origin and least on the San Pablo Bay, USA strain, respectively, 87% and 58% in Experiment 1, 97% and 83% in experiment 2, 89% and 42% in experiment 3. Survival on the Shark Bay-Australia, Great Salt Lake, Utah-USA, and Margherita di Savoia-Italy strains were intermediate between the above values in all experiments.

Growth was best on the Utah strain in experiment 1. In experiment 2, it was best on Italy. In both, growth was least in fish fed Brazil nauplii.

Two different lots of a San Francisco Bay-USA strain were evaluated in experiment 2 in addition to the other five geographical strains. There was no significant difference in survival between the two lots or between either lot and the other five geographical strains. Growth on the diets of San Francisco Bay-USA was significantly less than on all other strains except Brazil which also supported relatively low growth.

Introduction

Newly hatched nauplii of the brine shrimp *Artemia* are the most widely used food organism for marine larval fishes and invertebrates reared for mariculture or research purposes.

The San Francisco Bay, USA strain has previously provided the bulk of the world's supply of brine shrimp cysts. Demand exceeded supply for a number of years creating serious shortages of the needed cysts. The situation has now been reversed with the exploitation of

[1] International Interdisciplinary Study on *Artemia* Strains coordinated by the Artemia Reference Center, State University of Ghent, Belgium.

several new natural populations. Sorgeloos (1978) now estimates production at about 100 tons/yr.

There is considerable evidence that various geographical strains of brine shrimp differ in their food value due to nutritional composition and/or contaminants (Bookhout and Costlow, 1970 ; Provenzano and Goy, 1976 ; Wickins, 1972 ; Klein-MacPhee *et al.*, 1980 ; Johns *et al.*, 1980). Also, there is a lack of information on the potential suitability of these newly exploited strains as diets for other organisms. This has resulted in the research program of the International Study on *Artemia* (ISA) described by Sorgeloos (1980).

The US Environmental Protection Agency's Environmental Research Laboratory at Narragansett, Rhode Island, USA (ERLN) is participating in the ISA by conducting biological studies to determine relative nutritional value of selected brine shrimp geographical strains. A principal mission of ERLN is to establish the effect of potential pollutants on marine life. Toxicological bioassays are utilized to develop a scientific data base for determining water quality criteria for various hazardous substances. Diet quality can affect the results of both acute (short term) or chronic (whole life cycle) bioassays (Merhle *et al.*, 1977). Anonymous (1978) considers diet as one of the more important experimental variables which can profoundly affect reproducibility and comparability of results. Adequate nutrition of test animals in bioassays is essential to ensure legally defensible toxicological data.

This paper reports on survival and growth of the marine atherinid fish Atlantic silverside *Menidia menidia*, fed on five different strains of *Artemia*.

Materials and methods

Culture methods

Three experiments were conducted, two during the summer of 1978 and the third in summer 1979. Gravid fish for subsequent supply of larvae used in experiments 1 and 3 were collected by beach seine as described in Beck (1980) from Point Judith Pond or Pettaquamscutt River. Both estuaries are located in southern Rhode Island, USA. Larvae for experiment 2 were obtained from gravid stock collected in the same manner from Salem Harbor, Massachusetts, USA.

Experiment 1 and 3 fish were spawned at ERLN and fish collected in Salem Harbor were hand stripped of eggs and sperm at the collection site. In all cases at least three males and five females were stripped and the spawning procedures were as detailed in Barkman and Beck (1976).

Incubation of egg batches was accomplished in modified egg hatching jars (Buss, 1959). Fertilized eggs adhering to 400 μm mesh nylon screens were suspended in the incubation jar which received a 1 l/min continuous flow of filtered seawater and mild aeration. Incubation temperature ranged from 16.4 to 17.4 °C and salinity 29.4 to 31.3 ‰ for experiment 1 fish. For experiment 2, incubation temperature was 19.3 to 21.9 °C with salinity 29.5 to 31.1 ‰ and was 16.4 to 18.4 °C and 29.0 to 30.0 ‰ for experiment 3 fish. Hatching times for groups 1, 2, and 3 were 13, 10, and 13 days respectively. Larvae were reared in 350 l circular tanks according to Beck (1980).

EXPERIMENTAL PROCEDURE

Ten day post-hatch larvae were used for experiments 1 and 2. Fish used in experiment 1 were fed San Pablo Bay-USA strain (Living World lot no. 1628) newly hatched *Artemia* nauplii until the assay started. For experiment 2, the fish were fed on Brazil strain nauplii prior to use. Newly hatched larvae were used for experiment 3 and received the individual geographical strain to be evaluated as the only food. The experiments were designed for a one-way analysis of variance with treatments listed in Table I. All treatments had three replicates except the unfed control with one. Fifty fish were provided in each replicate. Jars were placed in a random order as determined from a random numbers table.

TABLE I

Artemia geographical strains and cyst incubation time to instar I

Treatment number	*Artemia* geographical strain	Time[b] to harvest (hr)
1	San Pablo Bay-USA, Living World, lot no. 1628[a]	24
2	Macau-Brazil, Companhia Industrial do Rio Grande do Norte, CIRNE-Brand, harvest 1978	24
3	Unfed	–
4	Shark Bay-Australia, World Ocean Brand, lot no. 113	29
5	Great Salt Lake (Utah)-USA, harvest 1977	23
6	Margherita di Savoia-Italy, harvest 1977.	29
7	San Francisco Bay-USA, San Francisco Bay Brand Inc., lot no. 313/3006	24
8	San Francisco Bay-USA, San Francisco Bay Brand Inc., lot no. 321995	24

[a] Labeled as San Francisco Bay *Artemia* cysts, Living World, San Francisco Bay Brand Inc. Apparently from San Pablo Bay, California, USA. (Sorgeloos, personal communication).

[b] Cysts were incubated for the required length of time to ensure harvest of instar 1 nauplii.

The experimental assay system was a set of modified acrylic cylindrical fish egg hatching jars, 45 cm in height with a diameter of 15 cm, described by Bengtson *et al.* (1978), and shown in Fig. 1. Experiments 1 and 2 were flow-through with jars receiving a continuous flow of filtered seawater and mild aeration. Experiment 3 utilized the same jars but in a static system with daily replacement of 1/3 the water volume. Illumination was provided by fluorescent lights located above the jars. Light regime was 14 hr light : 10 hr dark. Temperature and salinity were monitored daily. Dissolved oxygen and light levels were taken periodically. Ranges of environmental parameters monitored during the experiment are contained in Table II.

The five geographical strains of *Artemia* nauplii were obtained from cysts provided by the Artemia Reference Center. The criteria used by the ISA group in selecting strains for evaluation as food for cultured marine animals are reported in Sorgeloos (1980). Listing of strains by treatment number are given in Table I. Treatment no. 1 cysts were originally thought to be from San Francisco Bay-USA. Since completion of the study, it has been determined that San Francisco Bay Brand "Living World" lot no. 1628 was actually harvested

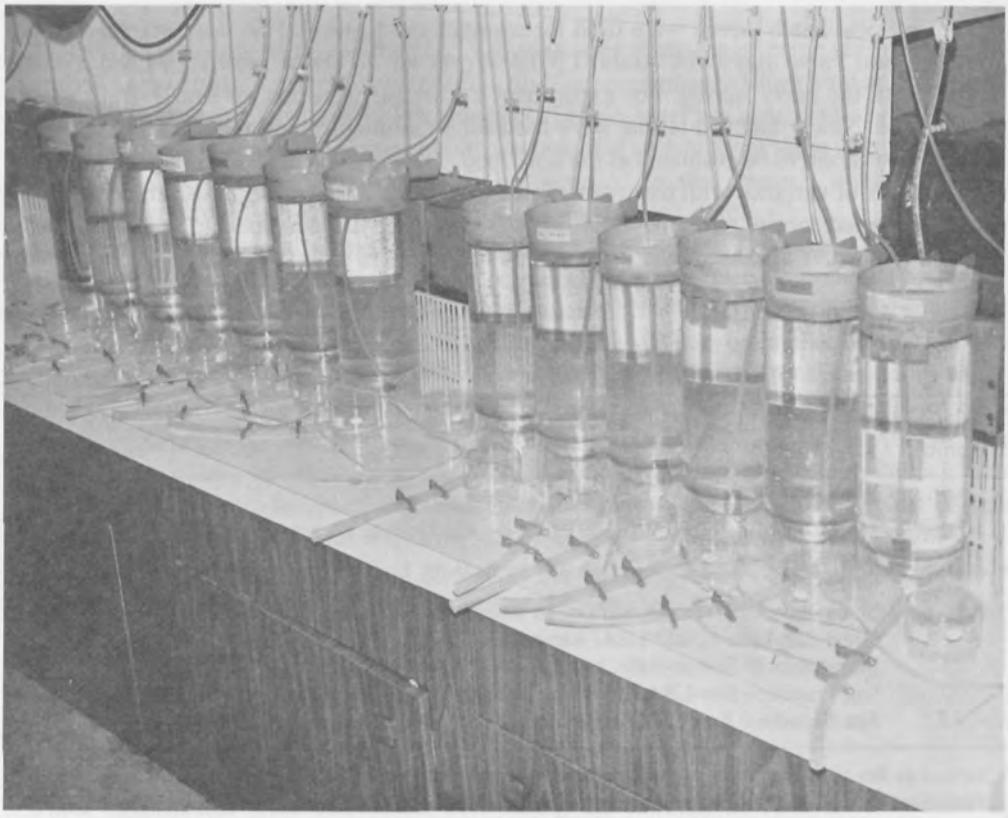

FIG. 1. Physical system used in all three experiments.

TABLE II

Experimental conditions for studies to evaluate nutritional value of *Artemia* strains

Conditions	Experiment 1 July 14-July 31, 1979	Experiment 2 Aug. 4-Sept. 5, 1978	Experiment 3 June 29-July 16, 1979
Age of fish (days)	10	10	Newly Hatched
Length (days) of experiment	18	33	18
Temperature (°C)	18.9-21.8	20.2-22.4	20.0-24.1
Salinity (‰)	30.0-31.7	29.1-31.5	31.8-32.1
D.O. (ppm)	5.2- 6.5	4.7- 7.4	4.4- 6.3
Light (lux)	470-640	470-640	350-470
Flow rate (l/min)	0.12	0.12	Static

from San Pablo Bay-USA (north of San Francisco Bay) (Sorgeloos, personal communication). Treatment no. 2 cysts, harvested in Brazil, are known to be descended from a 1977 transplantation of the San Francisco Bay strain. Cysts were incubated in aerated, filtered natural seawater at 25.0 ± 0.5 °C and 30.0 ± 2.5 ‰ salinity for times listed in Table I. Incubation was in 4 or 6 l glass separatory funnels. Live nauplii were harvested daily and separated from unhatched eggs and debris before being fed to the larval fish.

In experiments 1 and 2, each treatment of fish received an equal ration divided into four daily feedings with 1/4 of the ration given at equally spaced times, between 8 am and 4 pm. Daily ration was 100 % of fish wet weight in a wet weight basis of nauplii, taking into account flushing of food through the continuous flow system. In the static system (experiment 3) the daily food ration of 200 % of wet body weight was added once per day. Fish in all treatments (except unfed) showed vigorous feeding response and ingested nauplii were clearly visible in the gut.

Survival and growth were the responses measured to determine effects of feeding on the varous geographical strains of brine shrimp. Percent survival was determined as the number of survivors at completion of experiment divided by the initial number less any fish lost from the system from causes other than the experimental treatment. Whole body wet weight was determined on an electronic balance to the nearest 0.05 mg after blotting the fish dry.

Survival data was normalized by an arc-sine square root transformation (Sokal and Rohlf, 1969). Separate analyses of variance were conducted on growth and survival data. Where significant differences were found, Student-Newman-Keuls (SNK) *a posteriori* test was used to find individual treatment differences (Zar, 1974).

Results

Survival

Survival data for *M. menidia* in experiment 1 are given in Fig. 2 and Table III. Since two of the three replicates in each of treatments 4 and 5 were lost due to an overflow problem, only the three treatments with complete replication were statistically analyzed. An SNK comparison of treatment means showed that at the $P < 0.01$ level, survival of fish fed Brazil (87 %) and Italy (80 %) strains was not significantly different. Both of these treatments were significantly greater than fish fed San Pablo Bay-USA strain (58 %).

Experiment 2 survival results are provided in Fig. 3 and Table III. All treatments were included in the analysis of variance. Significance was less pronounced and the SNK comparison showed that only Brazil (97 %) and San Pablo Bay-USA (83 %) were significantly different in survival at the $P < 0.10$ level.

In experiment 3 (Fig. 4, Table III), again fish fed the Brazil strain had the greatest survival, significantly higher than any other treatment ($P < 0.05$). The San Pablo Bay-USA and Italy strains supported the least survival, significantly lower than the others.

Growth

Growth results for experiments 1 and 2 are provided in Table IV.

In the first experiment, growth was significantly greater ($P < 0.05$) on the Utah brine shrimp nauplii and least for those fed the Brazil strain. Italy, Australia and San Pablo Bay-

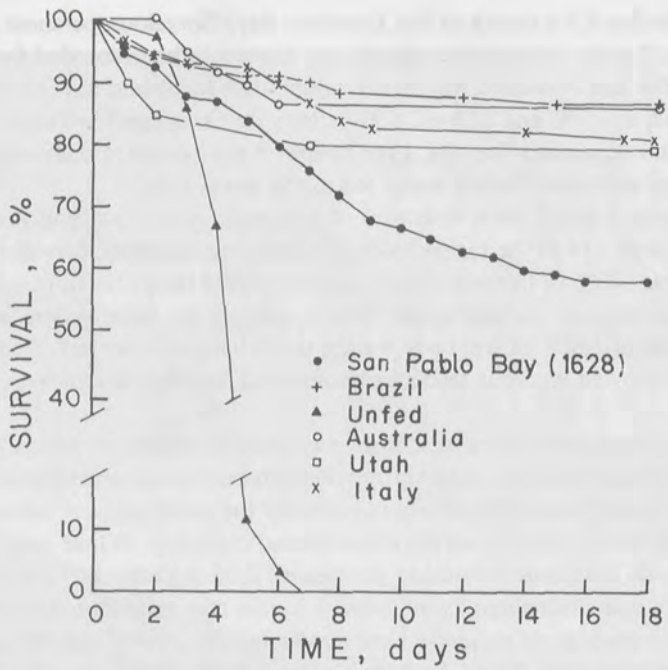

FIG. 2. Experiment 1 (1978). Survival of fish fed the diets of selected geographical strains of brine shrimp *Artemia* for 18 days.

TABLE III

Survival of fish fed diets of the selected geographical strains of brine shrimp nauplii ranked in descending order.
Vertical lines connect treatments with no significant differences
($\alpha = 0.01$ for experiment 1 ; $\alpha = 0.10$ for experiment 2 ; $\alpha = 0.05$ for experiment 3)

Experiment 1		Experiment 2		Experiment 3	
Treatment	Survival %	Treatment	Survival %	Treatment	Survival %
2-Brazil	87	2-Brazil	97	2-Brazil	89
6-Italy	80	8-SF (321995)	95	5-Utah	72
1-SP (1628)	58	4-Australia	94	4-Aust.	60
4-Australia	87[a]	5-Utah	93	6-Italy	44
5-Utah	80[a]	6-Italy	92	1-SP (1628)	42
3-Unfed	0[a]	7-SF (313/3006)	87	3-Unfed	0[a]
		1-SP (1628)	83		
		3-Unfed	0[a]		

[a] Data from one jar in treatment. All other data represent the mean of three replicates.

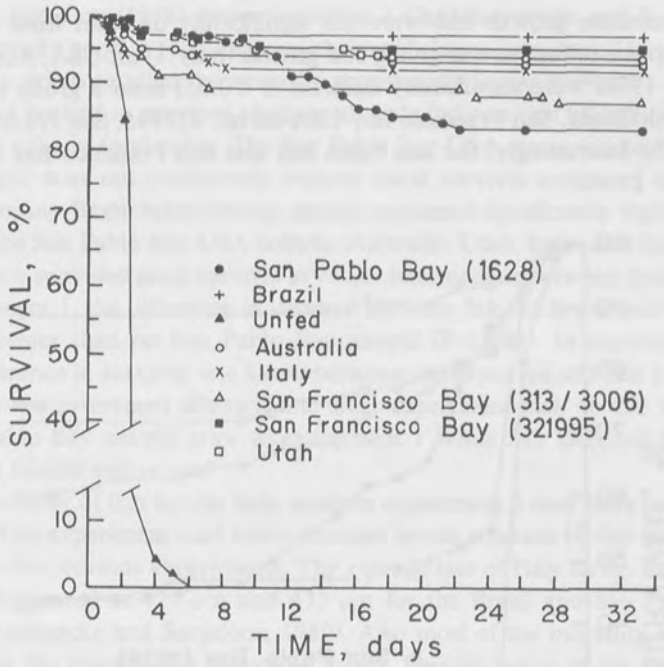

FIG. 3. Experiment 2 (1978). Survival of fish fed the diets of selected geographical strains of brine shrimp *Artemia* for 33 days.

TABLE IV

Growth of fish fed the various geographical strains of brine shrimp nauplii. Vertical lines join treatments that are not significantly different. ($\alpha = 0.05$)

Experiment 1		Experiment 2	
Treatment	Growth increase in wet weight (mg)	Treatment	Growth increase in wet weight (mg)
5-Utah	27.40	6-Itay	81.86
6-Italy	23.90	5-Utah	79.68
4-Australia	23.65	4-Australia	77.58
1-SP (1978)	22.05	1-SP (1978)	76.00
2-Brazil	21.28	7-SF (313/3006)	66.01
		8-SF (321995)	65.78
		2-Brazil	60.04

USA gave intermediate growth and were not significantly different from each other. In experiment 2, a group of four strains giving best growth (Italy, Utah-USA, Australia, and San Pablo Bay-USA 1978) were significantly different ($P < 0.05$) from a group of three strains giving less growth (Brazil, San Francisco Bay-USA-lot no. 321995, San Francisco Bay-USA-lot no. 313/3006). Interestingly, the San Pablo Bay and San Francisco Bay strains fell into different groups.

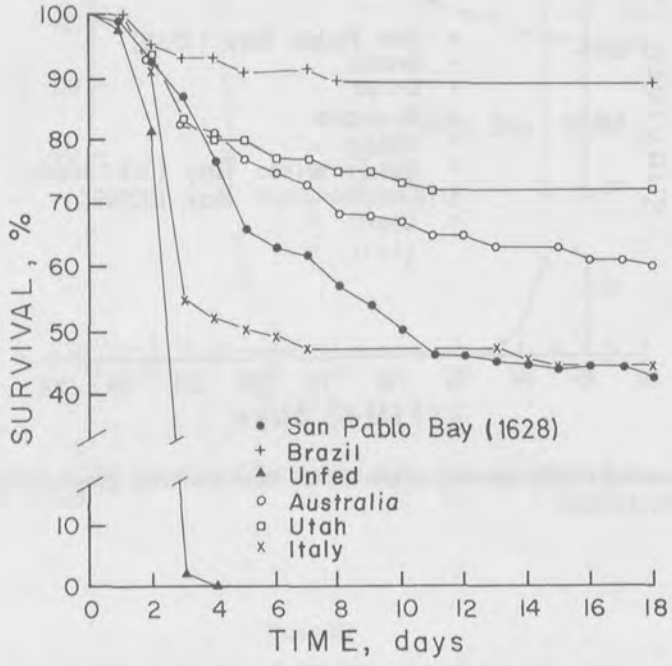

FIG. 4. Experiment 3 (1979). Survival of fish fed the diets of selected geographical strains of brine shrimp *Artemia* for 18 days.

Discussion

These data demonstrate that survival and/or growth can be significantly different among fish fed various geographical strains of live brine shrimp nauplii. This agrees with previous studies using various brine shrimp strains as food for several species of larval marine invertebrates. Bookhout and Costlow (1970) determined that larvae of four species of crustaceans fed on California-USA, *Artemia* nauplii had greater survival than on Utah nauplii. Wickins (1972) found that larvae of the glass prawn (*Palaemon elegans*) had greater survival when fed San Francisco Bay nauplii than those fed the Utah strain. All prawns reared on *Artemia* from San Francisco Bay reached metamorphosis while none of the few remaining survivors fed Utah nauplii had metamorphosed. In a related series of experiments he also observed adverse effects on survival, growth and metamorphosis of *Palaemon serratus* fed the Utah nauplii. Again, the San Francisco Bay strain was clearly a more suitable food.

Provenzano and Goy (1976) determined that a Canadian strain and San Francisco Bay nauplii were equally good as food for grass shrimp (*Palaemonetes pugio*) larvae. Feeding with nauplii of Utah origin resulted, however, in significantly lower survival.

Although not noticed in previous studies, our data indicate that all lots of California-USA nauplii are not alike in food value. The San Pablo Bay-USA strain unlike the San Francisco Bay-USA nauplii does not consistently support good survival compared to other available geographical strains. Brazil brine shrimp nauplii supported significantly higher survival in *M. menidia* than the San Pablo Bay-USA nauplii. Australia, Utah, Italy, and San Francisco Bay-USA strains each provided good survival at values intermediate between Brazil and San Pablo Bay. In experiment 1, the difference in survival between fish fed Brazil and Italy strains was significantly greater than on San Pablo Bay nauplii ($P < 0.01$). In experiment 2, the only significant difference in survival was found between the Brazil fed and San Pablo Bay fed fish ($P < 0.10$). The less prominent effects in the latter experiment may be due to feeding all test fish on San Pablo Bay nauplii prior to experiment 1 while fish intended for experiment 2 received Brazil nauplii before use.

The high mortality of fish fed the Italy strain in experiment 3 may have been related to size of the nauplii. This experiment used newly hatched larvae whereas 10-day old larvae were the test animals in the previous experiments. The average size of Italy strain nauplii at hatching was 517 μm compared to 429 μm and 433 μm for the Brazil and San Pablo Bay strains, respectively (Vanhaecke and Sorgeloos, 1980). Also most of the mortality was in the first 3 days paralleling the mortality in the unfed group. Possibly some of the smaller fish larvae were unable to ingest the relatively large nauplii.

In related ISA studies, Johns *et al.* (1980) and Klein-MacPhee *et al.* (1980) found similar poor ability of San Pablo Bay nauplii to support survival of larval mud crabs and late stage winter flounder larvae, respectively. Winter flounder larvae survived best on Australian nauplii and least on San Pablo Bay with those fed the Utah strain also relatively low in survival. Johns *et al.* (1980) found poor survival with both Utah and San Pablo Bay nauplii. They also indicated that high mortality of crabs on the diet of San Pablo Bay nauplii is not found with those fed San Francisco Bay-USA nauplii.

In each experiment, growth occurred on all diets. Least growth was, however, provided by the diet of Brazil nauplii, contrasting to the relatively high survival achieved.

The reasons for the difference in dietary quality between the various strains are not clearly understood. Bookhout and Costlow (1970) suggested high mortality could be related to the quantity of DDT in the Utah nauplii, about three times the concentration found in San Francisco Bay nauplii. This was not supported, however, by Wickins (1972) who analyzed nauplii from both sources for certain pesticides, heavy metals, carotenoids, sterols, and fatty acids. None of the potential contaminants or nutritional components measured could be adequately related to the comparatively poor food value of the Utah strain. Other possible explanations for poor results obtained with the Utah strain include Slobodkin (1968) who suggested a residual insecticide was responsible. A toxic alcaloid concentrated by the cysts was hypothesized by Shelbourne (in Provasoli, 1969) while Oppenheimer (in Provasoli, 1969) speculated that the Utah strain could be deficient in micronutrients.

In ISA research, preliminary data by Olney *et al.* (1980) on pesticide concentration of the same brine shrimp strains evaluated in our experiments show DDT levels in the Italy nauplii approximatley 10 times higher than San Pablo Bay (1978). In experiments 1 and 2 silversides

fed both strains had significantly higher mortality on the diet of San Pablo Bay nauplii. This suggests DDT is not a causative factor for differences in mortality. Chlordane concentration, however, was lowest in the Brazil nauplii and highest in the San Pablo Bay strain similar to the observed mortality of fish fed the two strains in all three experiments. The pesticide-mortality relationships are speculation at present but will be studied in more detail as ISA data are completed.

A primary objective of the ISA research program is to relate food value of selected brine shrimp strains to individual strain characteristics. Preliminary results of ISA research projects indicate morphological, genetic, nutritional, and as previously mentioned potential contaminant differences between strains. Comparison of the biological data from feeding studies and characterization data should lead to an understanding of specific factor(s) responsible for observed differences in food value to various marine species.

Conclusion

Various geographical strains of brine shrimp nauplii are different in food value as diets for marine organisms. There are significant differences in growth and survival of *M. menidia* when fed certain geographical strains of brine shrimp. Such differences can vary according to species.

San Pablo Bay-USA brine shrimp nauplii appear to be relatively poor in food value as a diet for marine fish larvae.

Fish fed diets of different geographical strains of brine shrimp could perform differently in toxicological bioassay experiments.

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Improvements in the decapsulation technique of *Artemia* cysts

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Abstract

Because bleaching powder is the cheapest source of hypochlorite in many countries of the world, a decapsulation procedure has been worked out in which technical calciumhypochlorite $Ca(OCl)_2$ is utilized.

A standard procedure has been developed for the deactivation of the chlorine residues adsorbed on the decapsulated cysts. Proper dehydration and storage of the decapsulated cysts assure maximum viability of the embryos in diapauze. A protoype system for routine decapsulation and processing of large quantities of cysts is proposed.

Beneficial effects of the decapsulation process on both the hatching percentage and the individual dry weight of the hatched nauplii are discussed in detail.

Introduction

As a result of its beneficial effects on the use of brine shrimp nauplii in aquaculture hatcheries, the decapsulation of *Artemia* cysts (Sorgeloos *et al.*, 1977 ; Bruggeman *et al.*, 1979) is practised more and more. Through the feedback information received from people who apply our methods, we have been able to identify some aspects that needed more study for the further improvement of cyst decapsulation. In this regard, attention was paid to the use of a cheaper source of hypochlorite, a better method to deactivate chlorine residues was developed, and a new system for more simplified routine decapsulation has been worked out. The beneficial effects of the decapsulation treatment on the hatching percentage and the individual dry weight of the hatched nauplii is reported for nine geographical strains of brine shrimp.

The use of calciumhypochlorite

In many countries of the world calciumhypochlorite $Ca(OCl)_2$, also called bleaching powder or chloride of lime, is a cheaper source of active chlorine than liquid bleach NaOCl. It

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can be found in various technical grades and is mostly used for desinfection purposes *e.g.* in swimming pools or culture tanks in aquaculture hatcheries. It is a much more stable product than liquid bleach and can be stored for longer periods. Furthermore its activity is usually correctly mentioned on the label of the commercial products (mostly 70% weight percent activity).

The ratios of cysts to active product (2 g to 1 g) and cysts to volume of decapsulation solution (15 g/200 ml) are identical to the values reported earlier for the treatment with NaOCl (Bruggeman *et al.*, 1979). Neither NaOH nor $Ca(OH)_2$ may be used to stabilize the pH of the decapsulation solution since they interact with $Ca(OCl)_2$ and drastically lower the hatchability of the decapsulated cysts. Optimal results are obtained with 50 g Na_2CO_3 or 30 g CaO/l decapsulation solution ; Tunsutapanich (personal communication) recommends the latter product because of its cheaper price. Since a precipitate is formed in the decapsulation solution, only the supernatant should be used in order to avoid clogging of screens when the treated cysts are washed.

For routine decapsulations we advise the following stepwise procedure : first dissolve the bleaching powder (aerate 10 min) ; add the technical CaO or Na_2CO_3 (aerate another 10 min) ; store overnight for precipitation (and eventually cooling off) ; the next morning siphon the supernatant off and use for decapsulation.

Processing and storage of decapsulated cysts

In a previous paper (Bruggeman *et al.*, 1979) we have described a sodiumthiosulphate treatment for the deactivation of the chlorine residues that remain adsorbed on the decapsulated cysts even after thorough washing with tap water. This method is, however, not entirely statisfactory because upon long-term storage of decapsulated cysts at high densities, the hatchability decreases. A tentative explanation is that the pH at which the cysts are decapsulated, leads to the formation of a saponification layer around the embryos, into which chlorinated compounds are trapped. Apparently the latter are not entirely deactivated by the thiosulphate.

The technique used in the chlorine treatment of plant seeds (Abdul-Baki, 1974) proved to be applicable for brine shrimp cysts : after washing out the hypochlorite, the cysts are treated with a 0.1 N HCl or HAc solution and are then thoroughly washed with tap water. This treatment ensures a higher viability upon storage in brine than the previous manipulation with thiosulphate.

As reported earlier, the storage of decapsulated cysts in saturated brine solution turns out to be a handy technique. Since the preparation of saturated brine is not a very easy task, the use of the Sterling Brinomat® (Spotte, 1970 ; Fig. 1), which can be assembled with very simple materials available in most aquaculture hatcheries, is highly recommended. With this simple apparatus saturated and at the same time filtered brine is made up continuously and automatically by gravity flow of tap water through packed layers of bulk salt.

The storage of decapsulated cysts in brine solutions has nonetheless its limitations. During the first months after decapsulation the cysts keep their maximum hatchability, even when stored at room temperature (± 20 °C). Over longer periods, cyst viability appears, however, to decrease as a function of storage temperature : *e.g.* after 6 months storage at room temperature the hatching efficiency dropped to below 50% whereas after 2 years storage at -4 °C, the

hatching percentage was still about 70 %. According to Clegg (personal communication) the decreased hatchability of decapsulated cysts stored in brine is probably due to their relatively high water content (about 20 %). When the water content ranges from 10 to 35 %, indications of enzyme activity and a slow but significant drop in the ATP concentration have been reported by Clegg and Cavagnaro (1976). We have observed that in function of storage time and temperature, more and more cysts which, after dehydration, have a typical coffee-bean structure, turn opaque and become spherical. Initially these opaque cysts hatch faster than their coffee-bean shaped homologs ; however, after a longer exposure to the brine, they loose their viability.

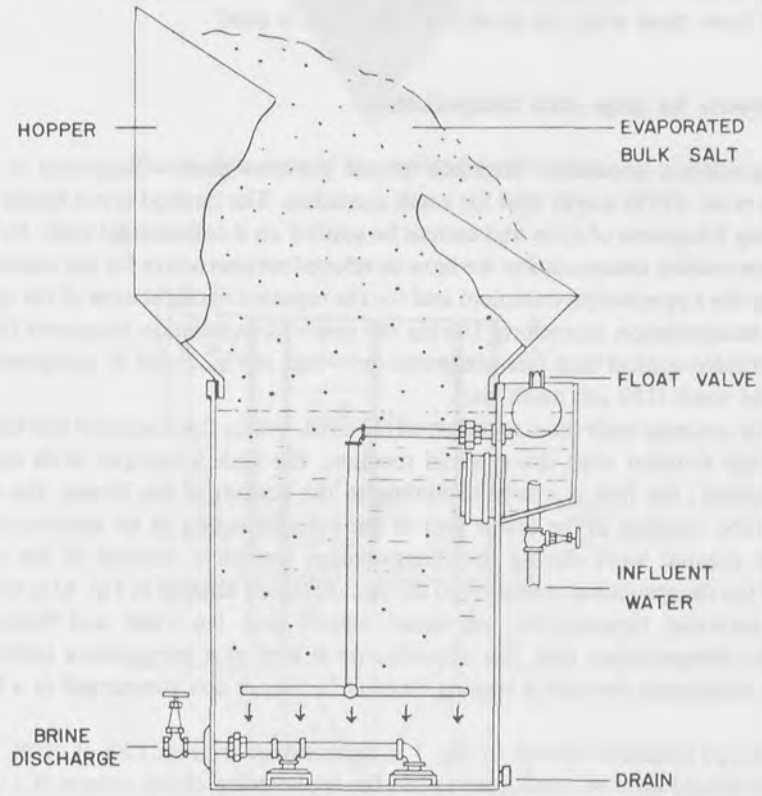

FIG. 1. Schematic diagram of the Sterling Brinomat® (after Spotte, 1970).

The metabolic processes which occur at a 20 % water content are probably delayed to a large extent when the cysts are stored at freezing temperatures : *e.g.* after 1 year storage in brine, the hatching efficiency of untreated cysts had dropped to ± 65 % for two samples stored at respectively 25 °C and 4 °C but only to ± 75 % for a sample stored at -20 °C (Sorgeloos, 1979). Kinne (1977) and Richard (Salins du Midi-France, personal communication) have found that following low temperature storage (-20 to -30 °C) cysts should be

exposed to room temperature for 1 week minimum, prior to incubation, in order to avoid poor hatching yields. We have not yet observed this phenomenon which might be strain specific.

Long-term storage of dried decapsulated cysts (water content below 5 %) is possible when they are kept in a dry and oxygen-free medium (nitrogen flushed or vacuum sealed containers). Air or freeze-drying can be successfully applied – without affecting the hatching efficiency – with decapsulated cysts which, upon dehydration in saturated brine, have been quickly washed free of salt with cold water.

Although we have not studied the phenomenon in detail yet, the major problem which remains to be solved in large scale drying of decapsulated cysts, is the persistent sticking together of these cysts when an air-drying technique is used.

Prototype system for large scale decapsulation

The decapsulation procedure described in our previous papers (Sorgeloos *et al.*, 1977 ; Bruggeman *et al.*, 1979) works well for small quantities. The method is not handy, however, for processing kilograms of cysts and cannot be applied on a commercial scale. In an attempt to automatize routine decapsulation we have developed an alternative for the manual addition of ice during the hypochlorite treatment and for the repeated manipulation of the cysts at each step in the decapsulation procedure. During the entire decapsulation treatment the cysts are kept in a cylindro-conical tank (see schematic drawings in Fig. 2 and 3), completely made of stainless steel mesh (150 μm mesh size).

In order to optimize both the circulation of the cysts within the container and the exchange of the internal solution with the external medium, the tank is equiped with two separate aeration systems : the first is a tube extending to the bottom of the funnel, the second is a perforated tube installed in the lower part of the cylinder acting as an aeration collar.

The only manual work during the decapsulation treatment consists of the consecutive transfers of the decapsulation container to the next bath (see scheme in Fig. 4) in the following sequence : seawater, hypochlorite, tap water, chloric acid, tap water and finally saturated brine. In the decapsulation step, the hypochlorite is kept at a temperature below 35 °C by continuous circulation through a cooling element (a cupper coil submerged in a bath of salt and ice).

The prototype container shown in Fig. 3 is dimensioned to treat 1 kg of cysts. Large scale experiments should now be considered to test the applicability of this system at a commercial scale.

Beneficial effects of the decapsulation treatment

We have reported earlier that the decapsulation technique has at least three major advantages for the use of *Artemia* in aquaculture hatcheries, namely desinfection of the *Artemia*, superfluity of separation of cyst shells from the hatched nauplii, and last but not least the potential use of decapsulated cysts as a direct food source for fish and crustacean larvae (Sorgeloos *et al.*, 1977).

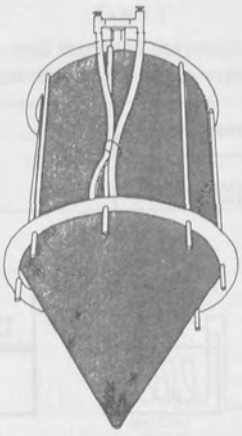

FIG. 2. Side view of the decapsulation container.

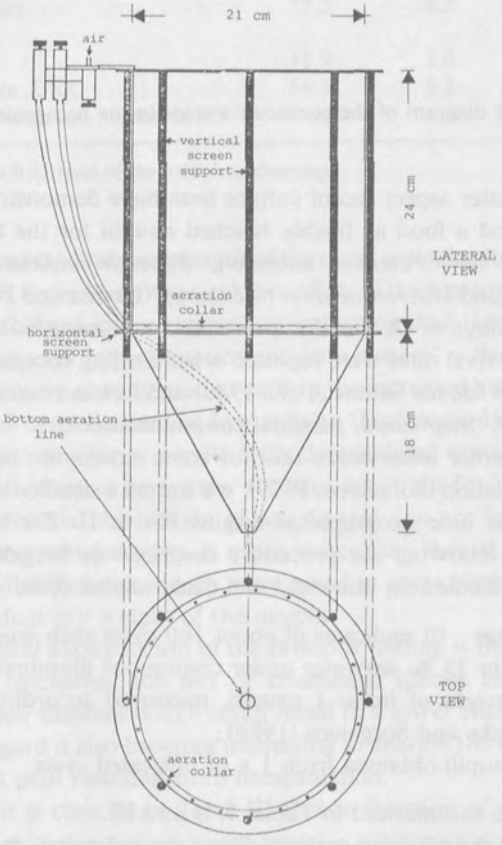

FIG. 3. Lateral and top view of the decapsulation container.

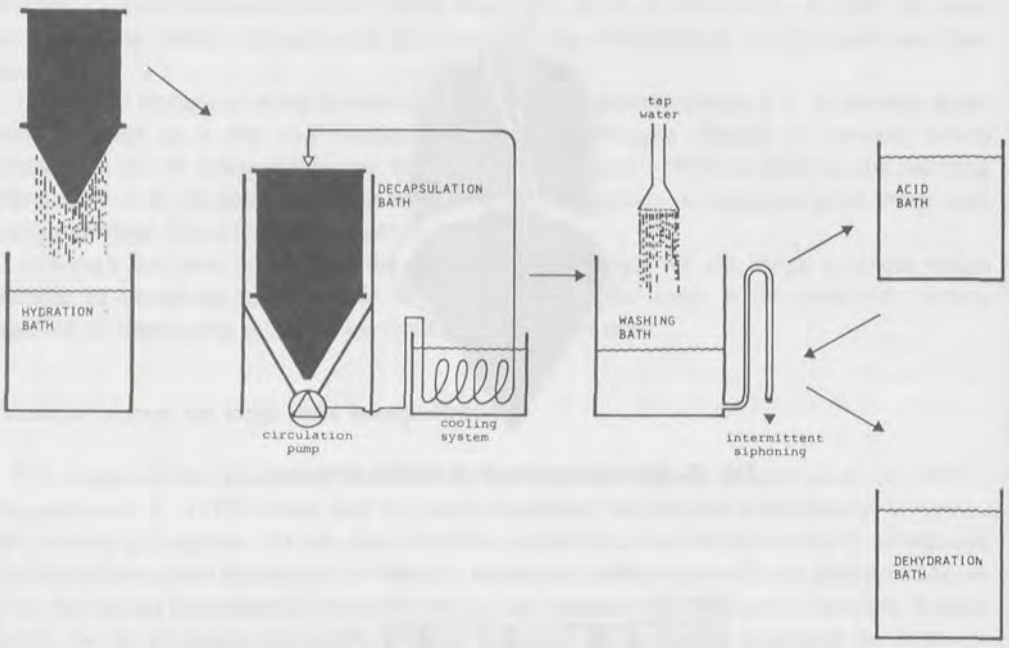

FIG. 4. Schematic diagram of the consecutive steps in the decapsulation procedure.

With regard to the latter aspect recent culture tests have demonstrated that decapsulated cysts are at least as good a food as freshly hatched nauplii for the larvae of *Metapenaeus monoceros* (Royan, 1980); *Penaeus monodon, Penaeus indicus, Metapenaeus ensis, Metapenaeus endevouri* and *Macrobrachium rosenbergii* (Laviña and Figuerosa, 1980), *Scylla serrata* and *Portunus pelagicus* (A. Laviña, personal communication).

Good growth and survival rates were reported when feeding decapsulated *Artemia* cysts to larvae of *Chanos chanos* (de los Santos *et al.*, 1980) and *Lebistes reticulatus, Xiphonophorus helleri* and *X. maculatus* (Stephanou, personal communication).

Departing from an earlier observation that for some strains the hatchability of the cysts increased upon decapsulation (Sorgeloos, 1979), we started a detailed study on this particular subject with cysts from nine geographical origins (Table I). The test material was first processed and cleaned following the procedure described in Sorgeloos *et al.* (1978). The following aspects were studied on untreated and decapsulated cysts :

a) the hatching percentage : 10 replicates of about 100 cysts each were hatched out in glass petri dishes at 25 °C in 35 ‰ seawater under continuous illumination ;
b) the individual dry weight of instar I nauplii, measured according to the methodology described by Vanhaecke and Sorgeloos (1980) ;
c) the total weight of nauplii obtained from 1 g of untreated cysts.

The data obtained are summarized in Tables I, II and III.

For most strains both the hatching percentage and the individual dry weight of the nauplii increase significantly when the cysts have been decapsulated. This can eventually be explained

TABLE I

Hatching percentage (mean $\bar{x}$, and standard deviation s) at 25 °C, in 35 ‰ seawater, of untreated and decapsulated cysts from various geographical origin

Origin of cysts	Untreated cysts		Decapsulated cysts	
	$\bar{x}$	s	$\bar{x}$	s
San Francisco Bay, California, USA (San Francisco Bay Brand,Inc., batch 288-2596)	71.4	4.5	82.1+	7.9
Macau, Brazil (Companhia Industrial do Rio Grande do Norte, batch 871172)	82.0	9.0	91.5+	2.0
Great Salt Lake, Utah, USA (harvest 1977)	43.9	5.2	54.6+	8.0
Shark Bay, Australia (World Ocean Pty, batch 114)	87.5	4.8	90.9	2.4
Buenos Aires, Argentina	62.8	3.9	84.5+	5.0
Galera Zamba, Colombia	80.4	9.3	91.6+	4.1
Margherita di Savoia, Italy (harvest 1977)	77.2	4.7	84.8+	2.9
Chaplin Lake, Canada	11.9	3.0	39.3+	8.6
San Pablo Bay, California, USA (San Francisco Bay Brand, Inc., batch 1628)	84.3	5.2	90.8+	1.2

+ Significant increase at the 0.05 level of the hatching percentage.

by the fact that the resistance and the strength of the outer cuticular membrane are reduced by removal of the chorion, respectively the oxidative effect of the hypochlorite. If this is the case, breaking becomes possible at a lower glycerol concentration than that necessary for untreated cysts. The "trehalose-glycerol hyperosmotic regulatory system" – theory of Clegg (1964) and Conte *et al.* (1977) which we already used to explain the increased hatchability and the higher naupliar dry weight of cysts hatched at low salinity (Sorgeloos, 1980), could thus also be involved here to explain the better results with decapsulated cysts. The differences in the energy reserves of individual cysts within a particular strain (or cyst batch) might be so important, that the individual dry weight of nauplii that can not hatch out from untreated cysts, but only from decapsulated cysts, is much lower than that of the controls. This might explain the rather small differences which were noted in some cases between the two series for the average individual dry weight of the nauplii.

An immediate practical extrapolation of the previous finding is that by using decapsulated cysts in aquaculture hatcheries, the fish or crustacean species cultured have a "bigger" *Artemia* nauplius at their disposal which could result in a lower energy expenditure for their food uptake. In this regard it also becomes interesting to analyze the biochemical composition of the naupliar weight gain resulting from decapsulation.

Last but not least, it is clear from Table III that in function of the *Artemia* strain used, application of the decapsulation technique can lead to a substantial economy of the quantity of cysts needed for aquacultural purposes.

TABLE II

Individual dry weight (in μg, mean $\bar{x}$, and standard deviation s) of instar I nauplii hatched out at 25 °C in 35 ‰ seawater, of untreated and decapsulated cysts from various geographical origin

Origin of cysts	Untreated cysts		Decapsulated cysts		Procentual difference
	$\bar{x}$	s	$\bar{x}$	s	
San Francisco Bay, California, USA (San Francisco Bay Brand, Inc., batch 288-2596)	1.63	0.11	1.74	0.09	+ 6.7
Macau, Brazil (Companhia Industrial do Rio Grande do Norte, batch 871172)	1.74	0.08	1.78	0.05	+ 2.3
Great Salt Lake, Utah, USA (harvest 1977)	2.41	0.11	2.36	0.12	− 2.1
Shark Bay, Australia (World Ocean Pty, batch 114)	2.47	0.13	2.61	0.15	+ 5.7
Buenos Aires, Argentina	1.72	0.07	1.90*	0.10	+10.5
Galera Zamba, Colombia	2.27	0.08	2.26	0.15	− 0.4
Margherita di Savoia, Italy (harvest 1977)	3.33	0.18	3.60*	0.23	+ 8.1
Chaplin Lake, Canada	1.97	0.13	2.05	0.09	+ 4.1
San Pablo Bay, California, USA (San Francisco Bay Brand, Inc., batch 1628)	1.92	0.08	1.99	0.05	+ 3.6

* Significant increase at the 0.05 level of the hatching efficiency.

TABLE III

Naupliar biomass (in mg dry weight) obtained out of 1 g cyst material, untreated, respectively decapsulated, hatched at 25 °C in 35 ‰ seawater

Origin of cysts	Untreated cysts	Decapsulated cysts	Procentual difference
San Francisco Bay, California, USA (San Francisco Bay Brand, Inc., batch 288-2596)	435.5	534.6	22.8
Macau, Brazil (Companhia Industrial do Rio Grande do Norte, batch 871172)	529.0	603.8	14.1
Great Salt Lake, Utah, USA (harvest 1977)	256.5	311.1	21.3
Shark Bay, Australia (World Ocean Pty, batch 114)	537.5	590.0	9.8
Buenos Aires, Argentina	333.0	494.9	48.6
Galera Zamba, Colombia	185.2	210.1	13.4
Margherita di Savoia, Italy (harvest 1977)	458.2	544.1	18.7
Chaplin Lake, Canada	15.8	54.2	243.0
San Pablo Bay, California, USA (San Francisco Bay Brand, Inc., batch 1628)	497.7	555.6	11.6

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The production and efficient use of freshly hatched brine shrimp nauplii (*Artemia*) in the larval rearing of marine fish at the hatcheries of the British White Fish Authority

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Abstract

The rearing of larvae of both sole (*Solea solea* L.) and turbot (*Scophthalmus maximus* L.) is presently dependant upon the use of freshly-hatched brine shrimp (*Artemia*).

Between 1965 and 1976 the British White Fish Authority produced *Artemia* nauplii using established techniques. The relatively low hatching rates achieved (*e.g.* 7.4 g cysts per 10^6 nauplii) were acceptable until 1976 when even lower hatching rates necessitated the development of improved methods.

A modified technique was evolved, incorporating 30 °C incubation under continuous illumination with increased aeration. This increased the naupliar yield to 4.8 g of cysts per 10^6 nauplii. Further improvements in the technique of naupliar extraction greatly simplified the process and reduced the space needed for naupliar production.

Additionally, new methods including the earlier weaning to inert diets, were developed to improve the subsequent utilisation of the freshly hatched nauplii once introduced to the fish rearing tanks. In the case of sole, a 60% reduction was achieved in the weight of *Artemia* cysts used, giving a feed rate per fish larvae equivalent to 0.25 g of cysts.

Introduction

The British White Fish Authority has been involved in the cultivation of marine fish since 1965. Work is currently being carried out at two Scottish Marine Farming Units : Hunterston, where dover sole (*Solea solea* L.) are being reared in heated effluent water from a nuclear power station, and Ardtoe, where turbot (*Scophthalmus maximus* L.) are being grown in floating cages in a sea loch (Kingwell *et al.*, 1977). Both units are equipped with hatcheries and both species are fed extensively on *Artemia* nauplii during their first 30 to 40 days. No on-growing of *Artemia* is carried out. Dover sole consume *Artemia* nauplii as a first feed but larval turbot are too small and are fed during their first seven days on the rotifer *Brachionus plicatilis* for which, in turn, an algal culture unit is needed.

Prior to 1976, the technique for naupliar production closely followed techniques specified by Shelbourne (1964) and Nash (1973). Briefly, this technique consisted of incubating cysts at 1 g/l loading, at 23 °C and in natural seawater ; incubation was in darkness, agitation was assisted by mechanical means (Shelbourne, 1964) and the cropping technique involved submerged lights.

During 1976, the "hatching efficiency" (Sorgeloos *et al.*, 1978) fell from about 7.5 g of cysts per 10^6 nauplii to over 20 g of cysts per 10^6 nauplii and the technique described in this paper resulted from an investigation into this deterioration.

At the same time there was some concern about the nutritional adequacy of the *Artemia* being fed. Benijts *et al.* (1976) had shown a great reduction of caloric content after the first instar and the new technique was calculated to produce the best food value rather than the maximum number of nauplii.

Materials and methods

Investigations into the optimum hatching rates from the "poor" eggs in 1976 gave conditions as follows.

Salinity

Hatching attempted in water of salinity 15 ‰ and 40 ‰ showed no improvement in naupliar production (Smith *et al.*, 1978). In any case, practical considerations excluded any larger-scale salinity modification and it was also thought nauplii would survive longer in the fish tanks if there was no salinity shock on transfer. Therefore untreated seawater continued to be used.

Temperature

The maximum rates of excystment were at 28-30 °C, as noted by Boone and Baas-Becking (1931). This reduced time for maximum excystment from 42 hr at 23 °C to 30 hr at 30 °C.

Aeration, agitation

High aeration rates were not seen to produce very high rates of naupliar damage and aeration rates in the incubators maintained a fairly violent agitation (15 l of air per min, in a 400 l tank). Aeration stones were replaced by open ended glass tubes of 3 mm internal diameter.

Illumination

Following the work of Sorgeloos (1973), incubation after various periods of illumination was tried and it was found that maximum naupliar production followed constant illumination. This is provided by a single fluorescent tube above the incubator giving a light intensity of 400 lux at the surface.

Equipment

The main incubation tank of 400 l capacity was as described by Nash (1973) except for modifications to the air system as described above. The separator bath was reduced to 150 l capacity with a single screen. No underwater lights were used, the extraction process being based on that used by Jones (1972). A 9 l polycarbonate tank was used as a presoack bath (Fig. 1).

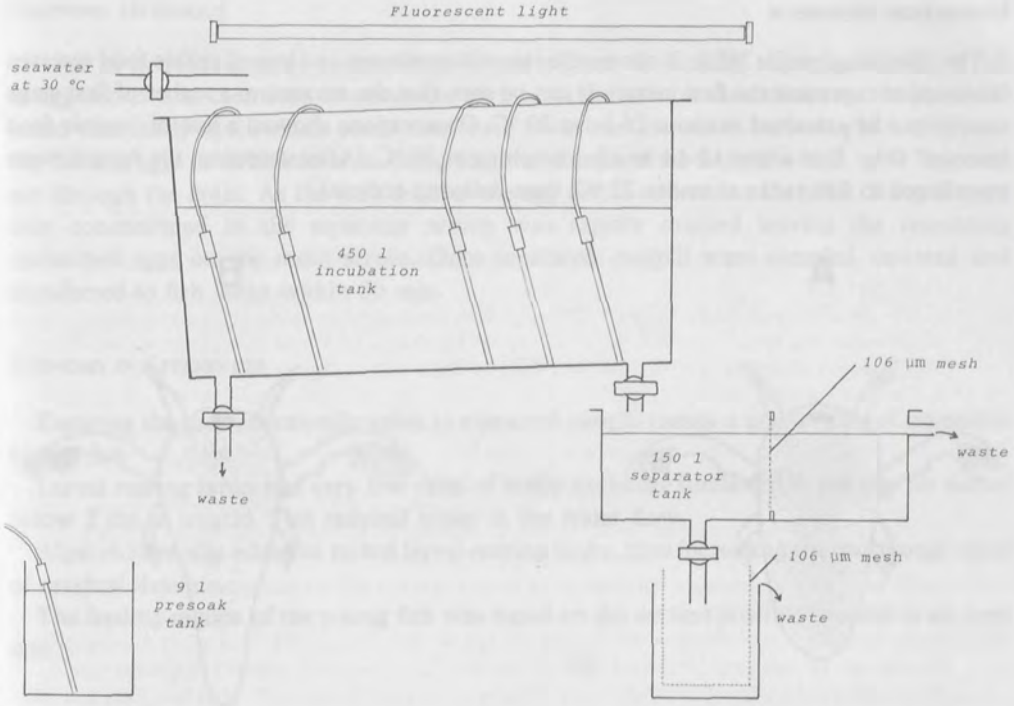

FIG. 1. Components of *Artemia* hatching system.

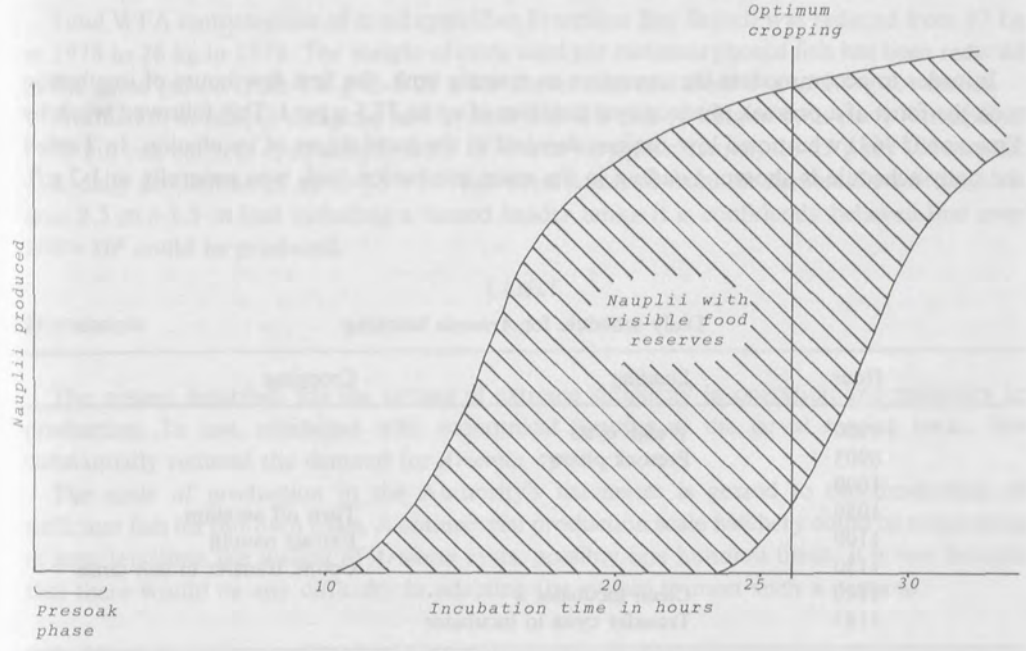

FIG. 2. Hatching profile at 30 °C.

INCUBATION TECHNIQUE

The "hatching profile" (Fig. 2) shows the rate of excystment and loss of visible food reserves (assumed to represent the first instar). It can be seen that the maximum number of first stage nauplii can be extracted at about 26 hr at 30 °C. Observations showed a loss of "visible food reserves" (Fig. 3) in about 12-14 hr after hatching at 30 °C. (After cropping, the nauplii were transferred to fish tanks at under 22 °C, thus delaying ecdysis).

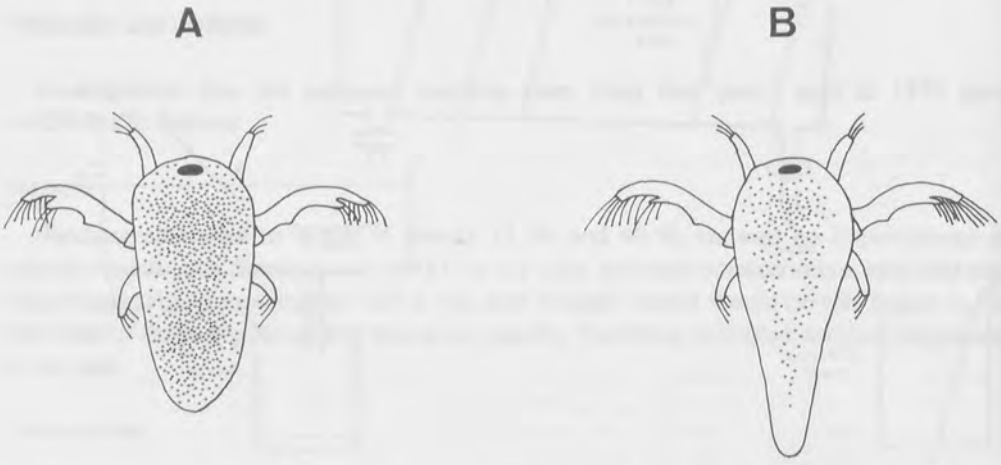

FIG. 3. Visible food reserves. A. Newly hatched nauplius. B. Nauplius 15 hr after hatching.

In order to accommodate the operation in a single tank, the first few hours of incubation took the form of a presoak phase at cyst densities of up to 37.5 g per l. This followed work by Emerson (1963) who noted low oxygen demand in the early stages of incubation. In Table I the daily schedule is shown. Loading in the main incubation tank was generally at 1-2 g/l.

TABLE I

Daily schedule for *Artemia* hatching

Hour	Loading	Cropping
0900	Weigh cysts	
0905	Presoak phase	
1000		
1050		Turn off aeration
1100		Extract nauplii
1130		Count, transfer to fish tanks
1140	Clean incubator	
1145	Transfer cysts to incubator	

Cropping technique

After 26 hr of incubation, aeration was furned off and the tank left static for 10 min. A raft of egg debris was then seen to cover the surface. Above the drain outlet, two drops of oil-based veterinarly multi-vitain compound were placed on the water surface which displaced the floating eggs ; the cleared area, being brighter, attracted the nauplii which were then run out through the drain. As the tank drained the egg debris adhered to the sides. Nauplii were thus concentrated in the separator which was rapidly drained leaving the remaining unhatched eggs on the mesh screen. Once separated, nauplii were sampled, counted and transferred to fish tanks within 30 min.

Economy in *Artemia* use

Ensuring the greatest calorific value in extracted nauplii means a greater rate of utilisation by the fish.

Larval rearing tanks had very low rates of water exchange (under 15% per day for turbot below 2 cm in length). This reduced losses in the water flow.

Algal culture was added to turbot larval rearing tanks, thus increasing the nutritional value of residual *Artemia*.

The feeding regime of the young fish was based on the earliest possible transfer to an inert diet.

Results

Total WFA consumption of dried cysts (San Francisco Bay Brand) was reduced from 93 kg in 1976 to 26 kg in 1978. The weight of cysts used per metamorphosed fish has been reduced in the same period from 0.6 g to 0.25 g for Dover sole and from 3 g to 1.7 g for turbot.

Production of nauplii using the new system was at a rate of 4.8 g of cysts par 10^6 nauplii in 1978 but one batch of cysts used in early 1979 was very poor, namely 20-25 g per 10^6 nauplii.

A daily production of up to 35×10^6 has been achieved from an incubation unit with an area 2.5 m $\times$ 1.5 m (not including a heated header tank). It is confidently believed that over 100×10^6 could be produced.

Discussion

The system described has the virtues of extreme simplicity in operation and reliability in production. Its use, combined with economical running of the larval rearing tanks, has substantially reduced the demand for *Artemia* cysts.

The scale of production in the Authority's hatcheries is geared to the production of sufficient fish for our own trials. A commercial production scale hatchery could be consuming at least ten times the weight of *Artemia* cysts, possibly one hundred times. It is not thought that there would be any difficulty in adapting the system to meet such a demand.

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Nutritional quality of *Artemia* from different localities as a living feed for marine fish from the viewpoint of essential fatty acids

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Abstract

The nauplii of *Artemia* have been widely used as a food in rearing marine fish larvae and crustaceans. However, it has been reported that a single dosage of *Artemia* frequently caused lethargy of larvae, and a consequent high mortality of various marine fishes. The occurrence of this phenomenon varied with the fish species as well as with the place of production of *Artemia*.

We intended to clarify the cause of this phenomenon from the viewpoint of the relationship between the dietary value of *Artemia* nauplii, and the amount of $\omega 3$ highly unsaturated fatty acid ($\omega 3$ HUFA), which are considered to be essential fatty acids (EFA) for marine fish. The fatty acid composition of *Artemia* from different localities was analyzed. It was found to be different from place to place, and sometimes varied from year to year, even when produced in the same place. *Artemia* could be classified into two types on the basis of fatty acid composition : one contains a large amount of 18:3$\omega 3$, which is essential for freshwater fish, and the other contains a large amount of 20:5$\omega 3$, which is essential for marine fish. It was found that the $\omega 3$ HUFA content of freshwater-fish type *Artemia* could be increased by feeding them marine *Chlorella* or ω-yeast, which contain large amounts of $\omega 3$ HUFA.

A number of experiments were conducted, feeding freshwater-fish type *Artemia*, marine-fish type *Artemia*, the improved freshwater-fish type *Artemia*, and a copepod, *Acartia clausi*, which contains a considerable amount of $\omega 3$-HUFA, to the red seabream (*Pagrus major*). It was observed that feeding freshwater-fish type *Artemia* caused a sudden high mortality on the 6th day of the experiment. This type of shock syndrome was also observed during the activity test, with mortalities reaching up to 75.9% at the end of the experiment. Feeding experiments with marine-fish type *Artemia*, improved freshwater-fish type *Artemia*, and *Acartia clausi*, did not result in sudden high mortalities or a shock syndrome.

The above results clearly indicate that the high mortality frequently observed in red seabream culture is induced by the shortage of EFA in *Artemia* nauplii given as a single food. We conclude that the nutritional value of *Artemia* should be determined in terms of the EFA content.

Introduction

Since Rollefsen (1939) discovered that *Artemia* nauplii were a suitable food for postlarval plaice, flounder, and cod, they have been widely used to feed fish larvae (Morris, 1956 ; Kurata, 1959 ; Fujita, 1962 ; Hirano and Oshima, 1963 ; Shelbourne, 1964 ; Riley, 1966). However, it has been observed that a single dosage of *Artemia* nauplii frequently caused

heavy lethargy of larvae and high mortality of various marine fish after they were fed nauplii for a period of 1 or 2 weeks (Fushimi, 1971 ; Fujita, 1973 ; Kitajima, 1978). The occurrence of this phenomenon depended on the fish species as well as the place of production of *Artemia*.

It appears, for instance, that some species of flounders, one species of mullet (*Liza haematocheila*), one salmonoid (*Plecogrossus altivelis*), and some gobiid fish are not easily affected but the larvae of the yellowtail (*Seriola quinqueradiata*) in contrast are very susceptible to this phenomenon (Fujita, 1972 ; 1973). In addition, many investigators have reported a heavy loss of larvae of prawns, crabs, and marine fish fed on *Artemia* nauplii from Utah (Min. Agric. Fish. Food, 1967 ; Slobodkin, 1968 ; Little, 1969 ; Reeve, 1969 ; Bookhout and Costlow, 1970 ; Wickins, 1972).

Bookhout and Costlow (1970) reported that the differences in mortality and the survival rate of the developmental stages of four crab species may be attributed to the difference of the DDT content in the *Artemia* nauplii form California and Utah. There was approximately three times as much DDT in *Artemia* nauplii from Utah as in the nauplii from California.

Wickins (1972) mentioned that the addition of *Isochrysis galbana* to mixed cultures of *Palaemon serratus* larvae and *Artemia* nauplii allowed complete development of the larvae. He noted that feeding *Isochrysis* to the Utah strain of *Artemia*, before feeding the latter to *Palaemon* larvae, also yielded satisfactory results.

The same author reports that *Artemia* eggs and nauplii from San Francisco and Utah were analyzed for the presence of pesticides, heavy metals, carotenoids, sterol, and fatty acids. Although some differences were found, none of them could be confidently labeled as the cause of the poor nutritional value of the Utah *Artemia* nauplii.

Recently, in Japan, a large number of red seabream fry have been produced using rotifers cultured with marine *Chlorella*. It was found that if red seabream larvae are fed rotifers, cultured on baker's yeast instead of *Chlorella*, various syndromes, such as a dark body color, lack of appetite, lethargy shock syndromes and heavy mortalities, were usually observed (Kitajima and Koda, 1976 ; Kitajima, 1978 ; Kitajima *et al.*, 1979 ; Fujita, 1979).

Watanabe *et al.* (1978a ; 1979) indicated that the cause of high mortalities of red seabream fed rotifers, cultured on baker's yeast, is a deficiency in essential fatty acids in the diet.

Since the various symptoms observed in red seabream larvae feeding solely on *Artemia* were very similar to those of red seabream larvae feeding on EFA-deficient rotifers, we assumed that similar causes, *i.e.* the fatty acid composition of the *Artemia* nauplii, were involved.

The present study was carried out from 1975 to 1978. The culture of *Artemia* and feeding experiments with red seabream juveniles were conducted at the Aquaculture Research Laboratory, Nagasaki, Prefectural Institute of Fisheries, by S. Fujita and C. Kitajima. The chemical analyses of *Artemia* and red seabream juveniles were done at the Laboratory of Fish Nutrition, Tokyo University of Fisheries, by T. Watanabe.

Materials and methods

Place and year of production of *Artemia* eggs used in this study

Since the eggs were obtained from commercial sources, it was not always possible to determine the exact place of production. The following data indicate place and year of production to the extent available.

USA	San Francisco Bay	1975, '76, '77, '78A, '78B, '78C, '78D
Canada	Lakes of Saskatchewan	1977, '78
South America		1977
China	From a salt pan near Tientsin	1978

Hatching

Artemia eggs were hatched in a vigorously aerated seawater tank of 30 to 500 l. After 30-48 hr at 23-28 °C, the nauplii were separated from the empty shells and hatching debris and washed with seawater.

Culture of *Artemia* nauplii with various feeds

Newly hatched nauplii from San Francisco, Canada, and South America were cultured in 40 to 100 l tanks. The following diets were used : marine *Chlorella* (*Chlorella minutissima* at 14×10^6 cells/ml), baker's yeast (*Saccharomyces cerevisiae*), and ω-yeast (0.38 mg/ml), which was obtained by adding cuttlefish liver oil at a 15% level to a culture medium of *Saccharomyces* (Imada *et al.*, 1979) and *Spirulina* sp. for 24-72 hr. (Tables IV, V, VI and IX).

Feeding experiments of red seabream with *Artemia* nauplii

Feeding experiment on *Pagrus major* juveniles were conducted twice to compare the dietary value of *Artemia* nauplii cultured with various foods. The juveniles, which were fed rotifers (*Brachionus plicatilis*) cultured with marine *Chlorella*, were randomly divided into several lots of 500 (Experiment I) and 400 fish (Experiment II). Each experimental lot was fed the nauplii cultured with different feeds (Tables VI and IX). The larvae were cultured for 9 days at a water temperature of 19-21 °C in Experiment I, and 11 days at 26-27 °C in Experiment II. The newly hatched nauplii of marine-fish type *Artemia* from Canada (1978), and living *Acartia clausi*, which contains fairly high amounts of ω3 HUFA (Tables VI and IX), were used as reference diets in these experiments.

Results and discussion

Fatty acid composition and classification of *Artemia* from different localities

Artemia eggs from different localities (San Francisco, Canada, South America, and China) were analyzed for fatty acid composition in order to clarify the nutritional value of particular *Artemia* nauplii to marine fish (Watanabe *et al.*, 1978b). The results are summarized in Tables I and II.

Artemia eggs from San Francisco (1975-1978) differed markedly from year to year and from lot to lot (especially 1978). They may be divided into two types with the exception of one lot which is low in both 18:3ω3 and 20:5ω3 amount. Eggs from San Francisco 1975, 76, 78C, and 78D were found to be high in 18:3ω3, while quite low in 20:5ω3. On the other hand, in San Francisco eggs 1977 and '78B, the 20:5ω3 concentration was high and the 18:3ω3 concentration was low. A high concentration of 18:3ω3 and very low 20:5ω3 values were

observed for the eggs from South America produced in 1977. Eggs from China in 1978 were found to be high in the 20:5ω3 concentration and low in 18:3ω3.

From the results outlined above, *Artemia* eggs can be classified into two types according to the fatty acid composition (Watanabe *et al.*, 1978b). One type contains a large amount of 18:3ω3, which is the essential fatty acid (EFA) for freshwater fish, and the other type includes a large amount of 20:5ω3, which is one of the EFA for marine fish (Fujii *et al.*, 1976 ; Fujii and Yone, 1976). As a feed for larval fish, the first type of *Artemia* is suitable for freshwater fish whereas the second type is so for marine fish.

Artemia from San Francisco in 1975, '76, '78C, '78D and South America in 1977, belong to the freshwater-fish type, while eggs from San Francisco in 1977, '78B, Canada in 1977, '78, and China in 1978, are the marine-fish type. San Francisco in 1978A may be ineffective for both freshwater and marine fish because they contain only a small amount of EFA. Although any type of *Artemia* except San Francisco 1978A, may be satisfactory for freshwater fish on the basis of their EFA requirement, it is essential to know the fatty acid composition of *Artemia* used for marine fish larvae.

TABLE I[1]

Certain fatty acids of total lipids from eggs and newly hatched nauplii of *Artemia* from USA (San Francisco, 1975, '76, '77, '78A, '78B, '78C, '78D) (area %)

Fatty acid	1975		1976	1977		1978			
	Egg	Naup.	Naup.	Egg	Naup.	Egg			
						A	B	C	D
14:0	1.1	0.9	1.0	1.4	0.9	3.8	2.7	1.1	2.2
16:0	13.2	11.4	12.3	12.0	9.5	20.4	18.9	13.3	13.3
16:1ω7[2]	4.5	3.2	3.7	18.4	12.0	20.3	15.3	11.7	14.2
16:2 17:0	1.4	2.0	1.5	1.0	0.9	1.3	1.4	0.8	0.8
18:0	4.0	6.0	5.1	3.6	6.8	1.7	2.2	3.0	1.7
18:1ω9[2]	27.8	28.7	27.4	31.5	36.1	20.1	29.2	27.7	18.0
18:2ω6	6.2	6.6	6.6	4.0	3.4	3.6	7.8	5.4	4.4
18:3ω3	27.7	27.9	27.9	29.0	10.3	7.9	3.8	21.6	23.8
18:4ω3 20:0	3.6	3.1	3.8	1.7	1.2	2.0	0.6	4.1	6.0
20:3ω3 10:4ω6	0.6	1.2	1.0	1.6	2.7	1.7	2.3	1.1	1.3
20:4ω3	0.3	0.3		0.9	0.4	0.8	0.2	0.7	1.3
20:5ω3	1.8	2.3	2.0	7.1	9.5	2.0	5.4	1.9	1.8
Lipid %			4.4	6.4	2.0		3.1		3.7

[1] This table is quoted from Watanabe *et al.* (1978b). Culture of the *Artemia* nauplii was carried out at the Aquaculture Res. Lab., Nagasaki Pref. Inst. Fish. by Fujita and Kitajima, and the chemical analyses of the nauplii were done at Tokyo Univ. of Fish. by Watanabe.

[2] Contains small amounts of the other monoenes.

TABLE II[1]

Certain fatty acids of total lipids from eggs and newly hatched nauplii of *Artemia* from Canada (Lakes of Saskatchewan, 1977, '78), South America (1977), China (Tientsin, 1978) (area %)

Fatty acid	Canada				South America		China
	1977		1978		1977		1978
	Egg	Naup.	Egg		Egg	Naup.	Egg
			A	B			
14:0	0.9	0.6	1.3	1.0	0.6	0.5	2.0
16:0	10.2	8.4	13.0	10.5	10.6	7.9	13.9
16:1ω7[2]	9.9	7.3	10.0	12.8	6.4	5.8	23.5
16:2 17:0	1.5	2.2	1.2	1.6	1.7	1.9	2.1
18:0	3.7	7.0	3.0	3.4	5.5	5.9	3.4
18:1ω9[2]	27.8	30.0	23.6	25.4	25.0	26.3	23.4
18:2ω6	7.2	6.0	6.1	6.2	5.6	5.2	3.7
18:3ω3	13.7	13.5	19.8	16.0	18.6	21.0	7.5
18:4ω3 20:0	1.4	0.6	2.1	2.6	4.2	6.5	1.5
20:3ω3 20:4ω6	2.4	3.2	1.9	3.5	0.3	0.6	1.1
20:4ω3	0.3	0.2	0.2	0.4	0.5	0.7	0.7
20:5ω3	10.3	12.1	7.3	6.7	0.2	0.3	7.7
Lipid %	4.8	2.1		6.1	10.5	1.6	5.3

[1] This table is quoted from Watanabe *et al.* (1978b).

[2] Contains small amounts of the other monoenes.

FATTY ACID COMPOSITION OF THE CULTURED *ARTEMIA* NAUPLII

With various feeds

Fatty acid compositions of *Artemia* nauplii from San Francisco (1976) fed on marine *Chlorella*, baker's yeast, and ω-yeast, for a period of 72 hr are presented in Table III (Watanabe *et al.*, 1978b). Fatty acid composition of this *Artemia* was observed as belonging to the freshwater-fish type. Fatty acid composition of the feeds used in culturing these nauplii are shown in Table IV.

Without feed

The amount of total lipids of nauplii starved for a period of 72 hr decreased from 4.4% to 1%. The amount of 18:3ω3 also decreased from 27.9 to 19%. The amount of 20:5ω3, in contrast, increased from 2% to 7%.

TABLE III[1]

Certain fatty acids of total lipids from *Artemia* (San Francisco, 1976) fed respectively marine *Chlorella* (*Chlorella minutissima*), baker's yeast (*Saccharomyces crevisiae*) and yeast supplemented with cuttlefish liver oil (ω-yeast) (area %)

Fatty acid	Newly hatched initial	Feeding period in hours										
		Without feed			*Chlorella*			Yeast		ω-yeast		
		24	48	72	24	48	72	24	72	24	48	72
14:0	1.0	0.8	1.2	1.5	1.0	1.2	4.0	0.8	0.9	0.9	0.8	1.0
16:0	12.3	11.4	10.4	11.6	11.5	11.6	11.1	11.5	8.9	11.4	9.0	9.4
16:1ω7[2]	3.7	3.8	4.1	5.4	4.2	5.3	11.9	3.7	19.1	3.5	6.7	9.3
16:2	1.5	1.8	1.8	1.6	1.8	1.8	0.7	1.9	0.9	1.6	1.1	0.9
17:0												
18:0	5.1	6.4	6.8	5.5	6.0	6.0	1.9	6.8	5.4	5.9	5.4	4.0
18:1ω9[2]	27.4	28.3	32.9	28.6	28.0	28.7	15.1	30.0	35.0	27.9	30.9	32.8
18:2ω6	6.6	6.0	5.1	5.1	6.0	5.0	5.0	5.8	4.9	4.9	3.3	2.4
18:3ω3	27.9	25.7	18.3	19.0	25.9	21.2	22.4	24.1	9.6	22.5	12.0	7.1
18:4ω3	3.8	3.6	3.2	0.6	3.3	2.8	4.7	3.3	1.5	2.4	1.4	1.1
20:0												
20:3ω3	1.0	1.5	1.6	2.0	1.3	2.0	1.7	1.4	1.1	1.1	0.7	0.7
20:4ω6												
20:5ω3	2.0	3.0	5.1	7.0	3.5	7.3	10.9	3.0	4.3	4.5	7.3	8.8
22:1	–	–	tr	tr	–	–	0.3	–	0.5	2.0	6.2	5.9
22:6ω3	tr	0.1	0.4	0.7	0.2	0.1	0.3	0.1	0.4	0.4	1.1	1.5
Lipid %	4.4	2.4	1.4	1.0	2.5	1.7	1.7	2.9	3.1	3.5	4.3	3.8

[1] This table is quoted from Watanabe *et al.* (1978b).

[2] Contains small amounts of the other monoenes.

Group fed on marine Chlorella *for a period of 72 hr*

The total amount of the lipids and 18:3ω3 decreased to a certain extent, while the amount of 16:1ω7 increased from 3.7% to 11.9%, and the amount of 20:5ω3 increased considerably from 2% to 10.9%.

Group fed with baker's yeast for a period of 72 hr

The amount of 16:1ω7 and 18:1ω9 increased remarkably, but the amount of 18:3ω3 decreased from 24.1% to 9.6%. The amount of 20:5ω3 increased from 2% to 4.3%. Baker's yeast have remarkably high 16:1ω7 and 18:1ω9 contents, but are low in 18:3ω3 and contain no 20:5ω3. The nauplii were, as a result, influenced by the amount of fatty acid in baker's yeast.

Group fed with ω-yeast for a period of 72 hr

The amount of 16:1ω7 and 18:1ω9 increased less than in the case of baker's yeast. However, the amount of 20:5ω3 increased considerably and there was a slight increase in 22:6ω3.

TABLE IV

Certain fatty acids of total lipids from marine *Chlorella* (*Chlorella minutissima*), baker's yeast (*Saccharomyces cerevisiae*) and yeast supplemented with cuttlefish liver oil (ω-yeast) and *Spirulina* sp. (area %)

Fatty acid	Yeast		*Chlorella*	ω-yeast		*Spirulina*
	Kaneka 1977	Kyowa 1977	Nagasaki 1977	Kyowa		Mexico 1978
				1976	1977	
14:0	2.2	1.1	4.8	4.2	4.1	0.3
16:0	16.8	11.2	26.1	16.9	13.4	32.8
16:1ω7[1]	32.8	14.2	26.3	6.6	6.6	12.1
18:0	3.4	8.4	1.1	2.6	2.4	1.3
18:1ω9[1]	28.5	38.0	6.2	16.0	16.4	2.3
18:2ω6	7.6	15.1	2.3	1.0	1.1	19.1
18:3ω3	1.8	6.4	0.2	0.9	0.8	25.2
20:1	tr	1.6	0.1	8.4	9.1	–
20:3ω3			3.9	3.1	3.0	0.1
20:4ω6						
20:4ω3			–	0.7	1.1	
20:5ω3			24.8	13.9	17.7	0.2
22:1				5.2	2.1	0.4
22:5ω3				1.0	1.1	
22:6ω3				15.6	12.8	

[1] Contains small amounts of the other monoenes.

As a result, it appears that the composition and amount of fatty acids in *Artemia* nauplii is affected by the fatty acid composition of the foods given. The changes of fatty acid concentrations of nauplii over time showed a similar tendency. Although the amount of total lipids and 18:3ω3 decreased, the 20:5ω3 content increased whereas the amount of 18:2ω6 did not change.

The fatty acid composition of *Artemia* nauplii from San Francisco, South America, and Canada which were fed on marine *Chlorella*, baker's yeast or ω-yeast, for a period of 24hr is shown in Table V (Watanabe *et al.*, 1978b). The *Artemia* from San Francisco and Canada belong to the marine-fish type while South American *Artemia* are the freshwater-fish type.

In the *Artemia* nauplii from South America, which contain very low amounts of 20:5ω3, this level increased rapidly 20-fold from 0.3 to 6.6, when fed on ω-yeast. In the *Artemia* nauplii from San Francisco (1977) and Canada (1977), which contain remarkably high amounts of 20:5ω3, the concentration of this fatty acid did not increase significantly when the animals were fed on ω-yeast. The amount of 20:5ω3 in San Francisco nauplii increased from 9.5% to 10.9%, while in Canadian nauplii it decreased from 12.1% to 10.8%. In the *Artemia* nauplii from San Francisco (1976), the amount of 20:5ω3 increased from 2% to 4.5% (Table III). These results suggest that *Artemia* containing low amount of 20:5ω3 are more easily affected by dietary lipids than *Artemia* containing high amount of this element.

TABLE V[1]

Certain fatty acids of total lipids from *Artemia* fed respectively marine *Chlorella* (*Chlorella minutissima*), baker's yeast (*Saccharomyces cerevisiae*) and yeast supplemented with cuttlefish liver oil (ω-yeast) for 24 hr (area %)

Fatty acid	*Artemia* from San Francisco					*Artemia* from South America					*Artemia* from Canada				
	Initial	Without feed	*Chlorella*	Yeast	ω yeast	Initial	Without feed	*Chlorella*	Yeast	ω yeast	Initial	Without feed	*Chlorella*	Yeast	ω yeast
14:0	0.9	0.7	2.1	1.3	1.0	0.5	1.1	0.4	0.5	1.0	0.6	0.8	0.9	1.1	1.6
16:0	9.5	8.2	8.6	8.6	11.9	7.9	8.6	6.7	6.7	11.0	8.4	8.8	9.8	9.0	13.7
16:1ω7[2]	12.0	9.5	16.7	15.1	6.8	5.8	5.1	5.0	5.1	3.7	7.3	7.3	7.5	8.2	6.1
16:2 17:0	0.9	0.7	0.4	0.7	1.1	1.9	1.0	1.3	1.3	1.2	2.2	1.0	1.2	1.1	1.1
18:0	6.8	8.0	2.0	3.6	6.4	5.9	3.2	7.0	6.0	6.0	7.0	6.2	5.8	5.5	5.6
18:1ω9[2]	36.1	37.3	19.9	28.2	33.3	26.3	22.3	28.9	28.0	30.5	30.0	30.4	27.6	27.0	28.9
18:2ω6	3.4	4.0	4.1	4.1	2.7	5.2	6.1	5.5	5.6	3.3	6.0	6.3	6.6	5.9	3.3
18:3ω3	10.3	8.8	13.5	11.6	5.6	21.0	25.8	20.6	22.0	11.7	13.5	13.3	14.3	14.1	6.6
18:4ω3 20:0	1.2	1.3	2.1	1.8	0.6	6.5	9.8	7.1	8.2	2.3	0.6	0.8	0.9	0.4	0.4
20:3ω3 20:4ω6	2.7	3.8	5.9	4.8	3.2	0.6	2.0	0.6	0.6	1.7	3.2	4.2	3.6	4.3	2.9
20:4ω3	0.4	0.3	0.2	0.1	0.4	0.7	0.8	0.8	0.7	0.4	0.2	tr	tr	tr	0.4
20:5ω3	9.5	10.7	15.0	13.0	10.8	0.3	0.8	1.7	0.9	6.6	12.1	12.8	12.0	14.3	10.5
22:5ω3	–	–	–	–	0.3	–	–	–	–	0.2	–	–	–	–	0.3
22:6ω3	–	–	0.3	0.2	2.3	–	–	–	–	1.7	–	–	–	–	1.9
$\Sigma\omega$3 HUFA	9.9	11.0	15.5	13.3	13.8	1.0	1.6	2.5	1.6	8.9	12.3	12.8	12.0	14.3	13.1
Lipid %	2.0	1.2	1.4	1.5	2.7	1.6	1.6	1.7	1.6	2.0	2.1	1.4	1.5	1.5	2.4

[1] This table is quoted from Watanabe *et al.* (1978b).

[2] Contains small amounts of the other monoenes.

DIETARY VALUE OF *ARTEMIA* NAUPLII, CULTURED ON VARIOUS FEEDS, TO THE RED SEABREAM, *PAGRUS MAJOR*

Feeding of red seabream on Artemia *in Experiment I*

Fatty acid composition of newly hatched *Artemia* and *Artemia* fed on marine *Chlorella* and ω-yeast for a period of 24 hr are shown in Table VI. The marine-fish type nauplii from Canada were found to contain 5.8% ω3 HUFA. The freshwater-fish type nauplii from San Francisco contained a large amount of 18:3ω3 but were low in ω3 HUFA content (2.4%). The ω3 HUFA amount in San Francisco nauplii increased, when fed marine *Chlorella* and ω-yeast, to respectively 4.1% and 5.1%.

TABLE VI

Certain fatty acids of total lipids from *Artemia* and those fed on marine *Chlorella* (*Chlorella minutissima*) and ω-yeast for 24 hr in Experiment I (area %)

Fatty acid	Marine *Chlorella*	ω-yeast	*Artemia* nauplii				
			Canada 78	San Francisco 78C			
			Newly hatched	Newly hatched	Starved for 24 hr	Fed *Chlorella* for 24 hr	Fed ω-yeast for 24 hr
16:0	22.5	12.7	9.9	10.1	10.8	12.9	11.1
16:1ω7[1]	22.5	5.2	10.1	7.0	5.7	6.8	6.3
18:0	1.0	3.9	7.9	3.6	4.8	6.6	5.7
18:1ω9[1]	3.1	19.2	32.3	32.7	32.5	33.8	31.5
18:2ω6	3.4	1.1	5.1	6.3	5.7	4.7	5.0
18:3ω3	0.1	0.9	14.1	24.4	24.3	20.3	22.2
18:4ω3	0.2	1.4	1.4	3.8	3.6	2.6	3.0
20:0							
20:1	0.1	10.9	1.0	0.4	0.6	0.5	2.0
20:3ω3	4.7	1.2	2.1	1.1	1.7	1.3	1.3
20:4ω6							
20:4ω3	0.1	0.9	0.6	0.8	0.8	0.9	0.6
20:5ω3	31.8	9.1	5.2	1.6	2.4	3.2	3.4
22:5ω3	–	1.4	–	–	–	–	tr
22:6ω3	–	15.8	–	–	–	–	1.1
ω3 HUFA[2]	31.9	27.2	5.8	2.4	3.2	4.1	5.1
Lipid %			1.0	1.8	3.1	1.7	2.5
ω3 HUFA %			0.058	0.0432		0.0697	0.128

[1] Contains small amounts of the other monoenes.

[2] $C_{20:3} < \omega 3$ fatty acids.

The results of the comparison of dietary values between newly hatched nauplii and *Artemia* fed on marine *Chlorella* or ω-yeast, are shown in Table VII. Feeding seabream newly hatched nauplii from San Francisco resulted in a rapid dying off of the fish on the 6th day of feeding, and the cumulative mortality of the group reached up to 56.6% at the end. The dietary value of nauplii improved by giving them marine *Chlorella* or ω-yeast and the high seabream mortality was markedly reduced in the fish that were fed these nauplii. The juveniles fed marine-fish type nauplii from Canada also showed a high survival rate. This indicates that marine-fish type *Artemia* are satisfactory as food for red seabream.

The survival rate in activity tests was measured as follows : first, 30-50 larvae were taken from the original stock with a scoop net and kept 5 sec in the air. They were then transferred to a 30 l tank for a period of 24 hr. The survival at this stage was low in the fish fed on the freshwater-fish type newly hatched nauplii from San Francisco. In the fish fed these nauplii a shock syndrome was observed and the mortality during the activity test reached up to 75.9%. As a general result, the dietary value of *Artemia* was found to be effectively improved by raising to ω3 HUFA level.

TABLE VII

Dietary value of *Artemia* nauplii fed on marine *Chlorella* (*Chlorella minutissima*) and ω-yeast, for red seabream juveniles in Experiment I

	Canada	*Artemia* San Francisco 78C		
	Newly hatched	Newly hatched	Fed *Chlorella* for 24 hr	Fed ω-yeast for 24 hr
No. of fish	500	500	500	500
Total length (mm)				
Initial	6.91 ± 0.63	6.91 ± 0.63	6.91 ± 0.63	6.91 + 0.63
Final	9.57 ± 1.35	10.13 ± 1.34	11.13 ± 1.73	11.67 + 2.03
Survival (%)	68.4	43.4	66.8	86.4
Survival (%) at activity test	37.5	24.1	46.1	50.0

Hatching rate was about 10% for Canadian eggs and about 50% for San Francisco eggs.

The fatty acid composition of total lipids in the fish fed on various nauplii is shown in Table VIII. The fatty acid composition of the fish clearly reflected that of the nauplii. The high ω3 HUFA content of 23.3% observed in the fish at the initiation of the experiment decreased to 5.9% after feeding on the newly hatched nauplii from San Francisco for 10 days. At the same time, the usual low level of 18:3ω3 in red sea bream increased from 0.7% to 17.9%.

TABLE VIII

Certain fatty acids of total lipids from red seabream juveniles fed newly hatched nauplii and nauplii cultured with marine *Chlorella* and ω-yeast in Experiment I (area %)

Fatty acid	Initial juveniles	Juveniles fed nauplii			
		Canada 78	San Francisco 78C		
		Newly hatched	Newly hatched	Fed *Chlorella* for 24 hr	Fed ω-yeast for 24 hr
16:0	20.1	17.3	14.9	16.0	17.4
16:1ω7[1]	8.1	6.6	7.8	6.5	8.8
18:0	9.2	7.4	8.0	8.4	4.3
18:1ω9[1]	19.5	21.4	27.2	26.1	18.7
18:2ω6	3.1	4.8	5.8	5.5	4.3
18:3ω3	0.7	8.6	17.9	14.6	18.4
18:4ω3	0.2	0.4	2.3	1.4	2.5
20:0					
20:1	4.4	1.3	1.0	1.2	1.4
20:3ω3	2.5	6.3	2.5	3.5	2.3
20:4ω6					
20:4ω3	1.4	0.9	1.3	1.6	1.7
20:5ω3	8.9	14.4	3.3	6.3	8.8
22:5ω3	4.3	2.3	0.6	1.1	0.9
22:6ω3	8.6	2.1	0.7	1.3	1.4
ω3 HUFA[2]	23.2	19.7	5.9	10.3	12.8
Lipid %	2.1	2.0	2.6	2.3	2.5

[1] Contains small amounts of the other monoenes.

[2] $C_{20:3} < \omega 3$ fatty acids.

Feeding of red seabream with Artemia *in Experiment II*

The dietary value of the marine-fish type nauplii from San Francisco was investigated and compared with that of *Acartia clausi* collected from the sea. As shown in Table IX marine-fish type *Artemia* was found to contain 7.0% 20:5ω3 ; this level was not much affected by feeding on ω-yeast and *Spirulina* for a period of 24 hr. All nauplii groups revealed a similar composition.

Certain fatty acids of the total lipids from the juveniles, fed various *Artemia* nauplii and *Acartia* for 12 days, showed differences originating from the amount of lipids in the diets (Table XI). The concentrations of 18:1ω9 and 18:2ω6 were significantly higher in the fish fed *Artemia* nauplii. The ω3 HUFA concentrations were high in the fish fed on *Acartia* and comparable to those in natural red seabream.

The results obtained in Experiments I and II indicated that feeding on the marine-fish type nauplii and *Acartia*, which both contain a large amount of ω3 HUFA, did not cause a sudden heavy decrease and shock syndrome to the fish. These results are quite different from those obtained in the experiment with the freshwater-fish type nauplii. It can be concluded that the

high mortality observed in red seabream could be induced by the deficiency of EFA in freshwater-fish type *Artemia* nauplii given as a single feed. The food value of *Artemia* is significantly affected by its EFA content.

TABLE IX

Certain fatty acids of total lipids from *Spirulina*, *Acartia*, newly hatched *Artemia* nauplii and nauplii fed on ω-yeast and *Spirulina* in Experment II (area %)

Fatty acid	*Spirulina*	*Acartia clausi*	*Artemia* nauplii 78B			
			Newly hatched	Starved for 24 hr	Fed ω-yeast for 24 hr	Fed *Spirulina* for 24 hr
16:0	32.8	20.5	14.1	12.4	13.2	12.8
16:1ω7[1]	12.1	4.9	13.5	10.8	12.8	9.2
18:0	1.3	6.7	3.2	6.0	3.2	7.8
18:1ω9[1]	2.3	3.2	33.3	35.7	32.4	34.1
18:2ω6	19.1	1.7	9.0	8.4	7.4	10.5
18:3ω3	0.8	2.5	3.9	4.1	5.0	3.1
18:4ω3 20:0	0.3	2.7	0.4	0.2	0.8	0.2
20:1	0.1	0.3	0.4	0.8	1.7	0.5
20:3ω3 20:4ω6	0.1	0.8	2.9	3.5	2.5	2.7
20:4ω3	–	0.5	0.5	0.8	0.2	tr
20:5ω3	0.2	18.7	7.0	7.6	7.3	5.9
22:5ω3	–	0.3	–	–	–	–
22:6ω3	–	20.7	–	–	0.9	–
ω3 HUFA[2]	0.2	39.5	7.5	8.4	8.4	5.9
Lipid %		1.0	2.8	1.3	2.5	1.4
ω3 HUFA %		0.395	0.21		0.21	0.0826

[1] Contains small amounts of the other monoenes.

[2] $C_{20:3} < \omega 3$ fatty acids.

TABLE X

Dietary value of *Acartia*, newly hatched nauplii of the marine-fish type *Artemia* and nauplii fed on *Spirulina* and ω-yeast, for red seabream juveniles in Experiment II

	Acartia clausi	*Artemia* nauplii		
		Newly hatched	Fed *Spirulina* for 24 hr	Fed ω-yeast for 24 hr
No. of fish	400	400	400	400
Total length (mm)				
Initial	8.8 ± 1.2	8.8 ± 1.2	8.8 ± 1.2	8.8 ± 1.2
Final	23.6 ± 4.0	22.1 ± 2.7	22.9 ± 3.0	23.4 ± 2.6
Weight (mg)	207.5	169.7	227.4	222.9
Survival (%)	69.5	67.0	52.4	53.0
Survival (%) at activity test	100	54.1	35.4	61.0

TABLE XI

Certain fatty acids of total lipids from red seabream juveniles fed respectivey *Acartia*, newly hatched nauplii and nauplii cultured with ω-yeast and *Spirulina* in Experiment II (area %)

Fatty acid	Red seabream juveniles fed			
	Acartia clausi	*Artemia* nauplii		
		Newly hatched	Fed ω-yeast for 24 hr	Fed *Spirulina* for 24 hr
16:0	23.8	15.0	19.1	18.7
16:1ω7[1]	6.1	9.7	5.8	4.7
18:0	12.3	7.4	11.1	12.4
18:1ω9[1]	10.4	38.9	34.3	32.3
18:2ω6	0.6	8.9	6.7	8.7
18:3ω3	0.1	2.4	1.4	1.1
18:4ω3	0.2	0.1	0.2	0.1
20:0				
20:1	0.6	0.8	1.3	0.7
20:3ω3	1.9	3.5	4.8	6.0
20:4ω6				
20:4ω3	0.7	0.4	0.3	0.2
20:5ω3	8.8	4.6	6.3	6.6
22:5ω3	2.2	0.7	1.3	1.0
22:6ω3	20.9	0.2	1.5	0.6
ω3 HUFA[2]	39.8	5.9	9.4	8.4
Lipid %	2.0	2.5	2.0	2.0

[1] Contains small amount of the other monoenes.

[2] $C_{20:3} < \omega 3$ fatty acids.

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International Study on *Artemia*[1] VI. Nutritional value of geographical and temporal strains of *Artemia* : effects on survival and growth of two species of Brachyuran larvae

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Abstract

Five geographical strains of *Artemia* were compared for their effects on the survival and growth of *Rhithropanopeus harrisii* and *Cancer irroratus* larvae. High survival during larval development was provided by *Artemia* strains from Macau-Brazil, Margherita di Savoia-Italy and Shark Bay-Australia. The San Pablo Bay, California-USA, and Great Salt Lake, Utah-USA strains did not support survival beyond megalopa despite good survival in earlier development stages. During early development, larvae feeding on San Pablo or Utah strains exhibited either slower development rates (*C. irroratus* larvae) or a supernumerary zoeal stage (*R. harrisii*).

Growth of *R. harrisii* was significantly better in larvae fed Brazilian or Italian *Artemia* than in larvae fed the Australian strain. For *C. irroratus*, growth rate was equivalent in larvae fed Brazilian, Italian or Australian *Artemia*.

In a preliminary study, Utah and Brazilian *Artemia* strains were tested for temporal variability in their nutritional quality. Little short-term temporal variation was found in these two strains. This may result more from an artifact in time of collection than from actual temporal stability.

Introduction

A review of literature dealing with nutritional sources for the laboratory rearing of crustacean larvae reveals that *Artemia* nauplii are by far the most frequently used food source. Commercially available *Artemia* cysts are convenient to use and often support complete larval development. Historically, little attention has been paid to the geographical origin of the *Artemia* cysts used in crustacean culture. As a result, *Artemia* cysts from several regions, considered *a priori* to be equal in terms of supporting larval growth, have been widely used. In the past decade, however, research has indicated that cysts may vary geographically.

[1] International Interdisciplinary Study on *Artemia* Strains coordinated by the Artemia Reference Center, State University of Ghent, Belgium.

Forster and Wickins (1967) and Wickins (1972) report that larvae of *Palaemon serratus* fed *Artemia* nauplii hatched from cysts collected in Utah-USA die during development. Subsequent research has substantiated this claim (Little, 1969, rearing *Palaemon macrodactylus* larvae ; Reed, 1969, rearing *Cancer magister* larvae ; Reeve, 1969, rearing *Palaemon serratus* larvae ; Bookhout and Costlow, 1970, rearing five brachyuran species).

Several theories have been advanced to explain this apparent lack of nutritional quality in Utah *Artemia*. Bookhout and Costlow (1970) have presented evidence of pesticide contamination, while several other investigators (Shelbourne in Provasoli, 1969 ; Oppenheimer in Provasoli, 1969) have suggested biochemical deficiencies.

Other geographical strains of *Artemia* tested have generally proven to be adequate for promoting crustacean larval growth. The most commonly used source is from the San Francisco Bay-USA region, while other sources (several Canadian strains, Kurata, 1967 ; Dexter, 1972 ; Provenzano and Goy, 1976 ; and a San Fernando-Spain strain, Rodriguez, 1975) have also been used with some success.

The purpose of this research is to evaluate the nutritional quality of five geographical strains of *Artemia* for promoting development and growth of the larvae of two brachyuran crabs. In addition, preliminary research was begun to evaluate temporal variation in the nutritional quality of two of the geographical strains.

Materials and methods

Handling of the food source

Geographical strains of *Artemia* to be tested for their nutritional quality were collected from the following areas : 1) Brazilian strain – from Macau, Brazil ; 2) Italian strain – from Margherita di Savoia, Italy ; 3) Australian strain – from Shark Bay, Australia ; 4) San Francisco Bay strain – from San Francisco Bay Brand, California, USA[2] ; 5) Utah strain – from Great Salt Lake Region, Utah, USA. Further details about their origin and lot number can be found in Table I. The criteria used by the ISA-program in selecting the *Artemia* strains used in this study are reported in Sorgeloos (1980). To obtain stage one nauplii, cysts were incubated at 25 °C in 30 ‰ filtered (0.45 μm) seawater for a specified period of time which was dependent on the particular geographical strain (Table I). Hatched nauplii were harvested at the end of this time period, carefully separated from hatching debris, and fed to the crab larvae daily.

In addition to the five geographical strains tested, two other lots of both the Utah strain and the Brazilian strain were used to determine temporal variation in the nutritional quality of *Artemia*. Temporal samples assayed were collected on the following dates : Utah 1-1978 ; Utah 2-1977 ; Utah 3 – mixed years prior to 1977 ; Brazil 1-1978a ; Brazil 2-1978b ; Brazil 3-1979. Handling of these cysts and newly hatched nauplii was identical to that used for the geographical strains.

[2] Upon completion of this study we were informed that this batch is not from San Francisco Bay salt pans but from salinas in San Pablo Bay, located north of San Francisco (P. Sorgeloos, personal communication).

TABLE I

Summary of information on the five geographical strains of *Artemia* used in this study

Artemia strain	Collection site	Incubation conditions	Incubation time (hr)
Brazilian	Macau-Brazil, harvested 1978	25 °C-30 ‰	24
Italian	Margherita di Savoia-Italy, harvested 1977	25 °C-30 ‰	29
Australian	Shark Bay-Australia, World Ocean Brand, lot 113	25 °C-30 ‰	29
San Pablo Bay	San Pablo Bay region, California-USA, Living World, lot 1628[a]	25 °C-30 ‰	24
Utah	Great Salt Lake region, Utah-USA, harvested 1977	25 °C-30 ‰	23

[a] Labeled as San Francisco Bay *Artemia* cysts, Living World, San Francisco Bay Brand, Inc. Apparently collected from San Pablo Bay instead of San Francisco Bay (P. Sorgeloos, personal communication).

HANDLING OF THE CRAB LARVAE

Gravid *R. harrisii* were collected from under rocks in Pettaquamscutt River, Narragansett, Rhode Island-USA during the summers of 1978 and 1979. Gravid *C. irroratus* were collected by otter trawl from the west passage of Narragansett Bay, Rhode Island-USA between March and May, 1979. Females were isolated into either 20 cm finger bowls containing 25 °C, 25 ‰ filtered (0.45 μm) seawater (*R. harrisii*) or into 3 l plastic tubs containing 18-20 °C, 30 ‰ filtered seawater (*C. irroratus*). Newly hatched stage I zoeae (less than 18 hr post-hatching) were subsequently placed into 8 cm finger bowls with 10 zoeae per bowl. There were five bowls per diet for *R. harrisii* and three bowls per diet for *C. irroratus* for each experimental run. Larvae molting into megalopae were individually isolated into 2 cm finger bowls to prevent cannibalism. Experiments using *R. harrisii* were replicated six times (total of 300 larvae per diet) while those experiments using *C. irroratus* were repeated once (total of 60 larvae per diet).

Rearing conditions for the larvae were either 25 °C, 25 ‰ (*R. harrisii*) or 20 °C, 30 ‰ (*C. irroratus*). Daily, larvae were transferred to fresh seawater, examined for mortality, and then were fed an excess of newly hatched nauplii (750-1500 per bowl or 200-300 per megalopa) from the appropriate *Artemia* source. Surviving larvae were reared to the first crab stage (*R. harrisii*) or to the megalopa stage (*C. irroratus*). Then, a portion of the crabs were frozen for biochemical analysis ; and the remaining crabs were rinsed in deionized water, dried to a constant weight at 60 °C and weighed to the nearest 1.0 μg on a Perkin Elmer auto balance[3].

Because early results suggested that high mortality may occur during the stage IV – megalopa molt in larvae fed certain diets, a separate experiment was designed. *R. harrisii* crab larvae were reared on the various *Artemia* strains to late stage IV larvae, and then were sacrified for both biochemical analysis and dry weight determinations.

[3] Reference to trade names does not imply endorsement by the United States Environmental Protection Agency.

Experiments using the temporal collections of *Artemia* as food sources were handled in the same manner as previous experiments. However, only *R. harrisii* larvae were used in these experiments, which were repeated three times (total of 150 larvae per food source).

Data analysis

One-way analysis of variance was computed on an every-other-day basis for larval survival rates. In order to normalize survival data, survival rates were transformed to angles (arcsine $\sqrt{}$ percent) prior to analysis (Snedecor and Cochran, 1967). One-way analysis of variance was also computed for the dry weights of animals at stage IV, megalopa, and first crab stage. If significant differences (at $P \leq 0.05$) were found between crab larvae fed the various food sources, a Student-Neuman-Keuls posterior comparison was used to determine where the differences lay.

Results

Geographical strains – *Rhithropanopeus harrisii*

Food source had a significant effect on the survival of *R. harrisii* larvae (Fig. 1, Table II). Differences between the treatments were not significant until day 10. Thereafter, mortality of crab larvae fed either San Pablo Bay (SPB) or Utah brine shrimp nauplii increased sharply. Larvae fed the other geographical strains continued to survive well after day 10 with little increase in mortality (Fig. 1).

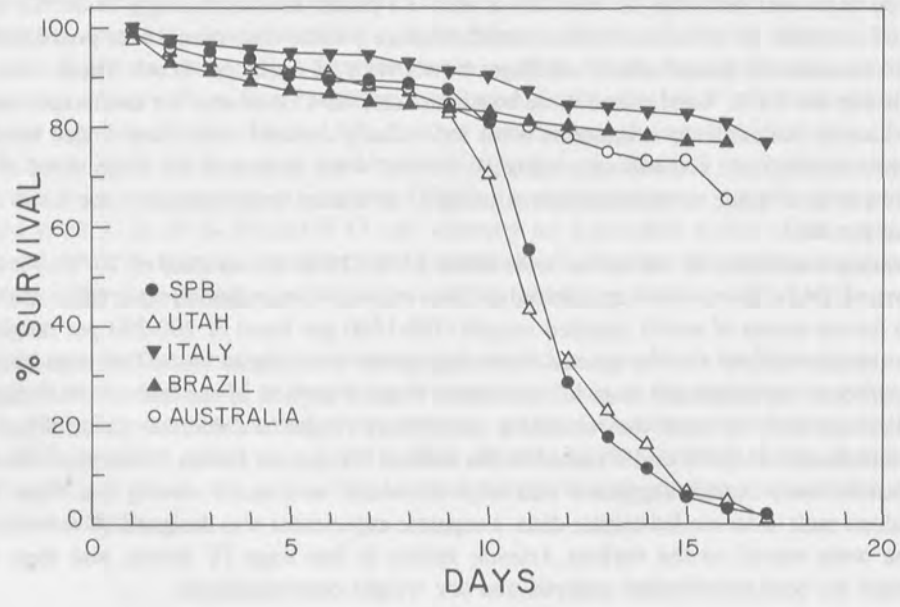

Fig. 1. Survival of *Rhithropanopeus harrisii* larvae fed various geographical strains of *Artemia* during larval development.

TABLE II

Analysis of variance of survival for *Rhithropanopeus harrisii* larvae fed various geographical strains of *Artemia* nauplii. * indicates significant differences in survival of the larvae fed the various food sources ($P \leq 0.05$). SNK = Student-Neuman-Keuls posterior test. *Artemia* strains grouped on the same line are not significantly different from each other while those on different lines are significantly different

Time post-hatch (days)	F-ratio	SNK
2	1.19	
4	1.18	
6	0.81	
8	1.30	
10	4.20*	Utah
		SPB, Brazil, Australia, Italy
12	34.70*	Utah, SPB
		Brazil, Australia, Italy
14	63.35*	Utah, SPB
		Brazil, Australia, Italy
16	116.85*	Utah, SPB
		Brazil, Australia, Italy
18	66.77*	Utah, SPB
		Brazil, Australia, Italy

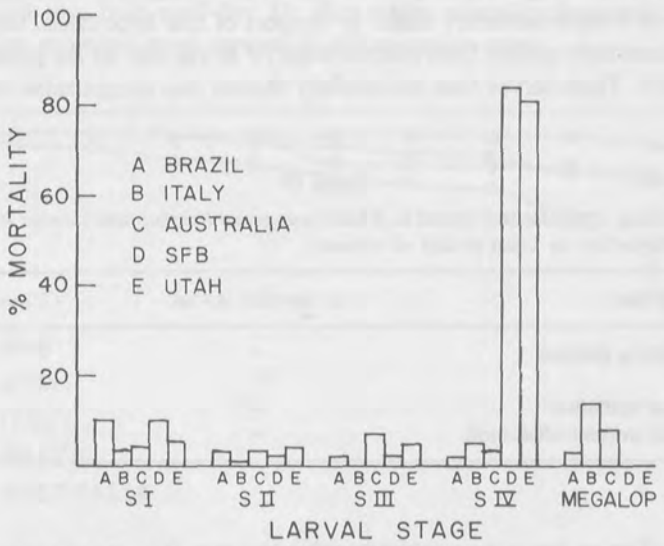

FIG. 2. Stage by stage breakdown of mortality for *Rhithropanopeus harrisii* larvae fed various geographical strains of *Artemia*.

A stage-by-stage breakdown of mortality indicates that patterns of larval mortality differed little in the zoeal stages I, II and III (Fig. 2, Table III). At zoeal stage IV, however, mortality rose sharply for larvae fed SPB and Utah strain *Artemia*. Mortalities during this stage primarily occurred during the stage IV – megalopa molting period rather than during the stage IV intermolt period.

TABLE III

Summary of rearing data for *Rhithropanopeus harrisii* fed various geographical strains of *Artemia*. ND = no data collected ; 100% mortality prior to this stage. Data are presented as a means ± one standard deviation

Parameter	Brazilian	Italian	Australian	San Pablo Bay	Utah
% survival to 1st crab	77 ±13	85 ±10	61 ±26	0	0
% survival to megalopa	80 ±12	92 ± 7	78 ±25	0	0
% survival of megalopa to 1st crab	96 ± 5	85 ± 9	76 ±14	ND	ND
Time from hatch to SIV – megalopa molt (days)	11.1± 1.3	10.3± 0.2	11.1± 1.2	11.0± 1.1	10.8± 0.8
Time from hatch to 1st crab	16.7± 1.2	15.6± 0.2	16.4± 1.1	ND	ND

A number of molting irregularities appeared during the zoeal stage IV – megalopa molting period in those larvae fed either San Pablo Bay or Utah strain nauplii (Table IV). The most common irregularity was the presence of a dorsal spine on larvae after the zoeal stage IV molt. Apparently, the larval stage resulting from this molt was not an abnormal megalopa but rather represents a supernumerary stage. In support of this hypothesis, two larvae fed the Utah strain successfully molted from normal stage IV larvae into larvae possessing the dorsal spine irregularity. These larvae then successfully molted into recognizable megalopae before dying.

TABLE IV

Summary of molting irregularities found in *Rhithropanopeus harrisii* and *Cancer irroratus* larvae fed either the San Pablo Bay or Utah strains of *Artemia*

Molting irregularities	*R. harrisii* larvae	*C. irroratus* larvae
Died during molting process	+	+
Partial molting	+	+
Unusual carapace spination	+	–
Zoeal maxillipeds present after molt	+	–

Diet had no effect on development time to either the stage IV – megalopa molt or to the first crab stage for surviving larvae (Table III). Mean development times to the stage IV – megalopa molt and to the first crab stage were approximately 10.5 days and 16 days, respectively.

The effect of diet on larval growth was determined twice during development, both at late intermolt stage IV and again at the first crab stage (Table V). No significant differences between larvae fed the different geographical strains were found in growth to stage IV. For larvae surviving to the first crab stage, however, those fed the Brazilian and Italian strains of *Artemia* were significantly heavier than crabs fed the Australian strain.

TABLE V

Dry weight of stage IV zoea and the first crab stage of *Rhithropanopeus harrisii* fed various geographical strains of *Artemia*. ND = no data collected ; 100% mortality prior to this stage. Means with the same superscript are not significantly different ($P > 0.05$) while means with different superscripts are ($P \leq 0.05$)

Food source	Stage IV dry weight (μg)	1st crab dry weight (μg)
Brazilian	98 ± 11^a	292 ± 55^a
Italian	94 ± 13^a	285 ± 40^a
Australian	99 ± 12^a	246 ± 45^b
San Pablo Bay	92 ± 8^a	ND
Utah	86 ± 14^a	ND

GEOGRAPHICAL STRAINS – *CANCER IRRORATUS*

As with *R. harrisii*, food source had a significant effect on survival of *Cancer irroratus* larvae (Fig. 3, Table VI). Survival of larvae fed the Utah strain nauplii was similar to that of larvae in the other treatments until day 18, when pronounced mortality during the stage V – megalopa molt and the megalopa stage began to occur. Survival of larvae fed the SPB strain, on the other hand, was high until day 21, after which mortality increased. Larvae fed the other three strains exhibited good survival to the megalopa stage.

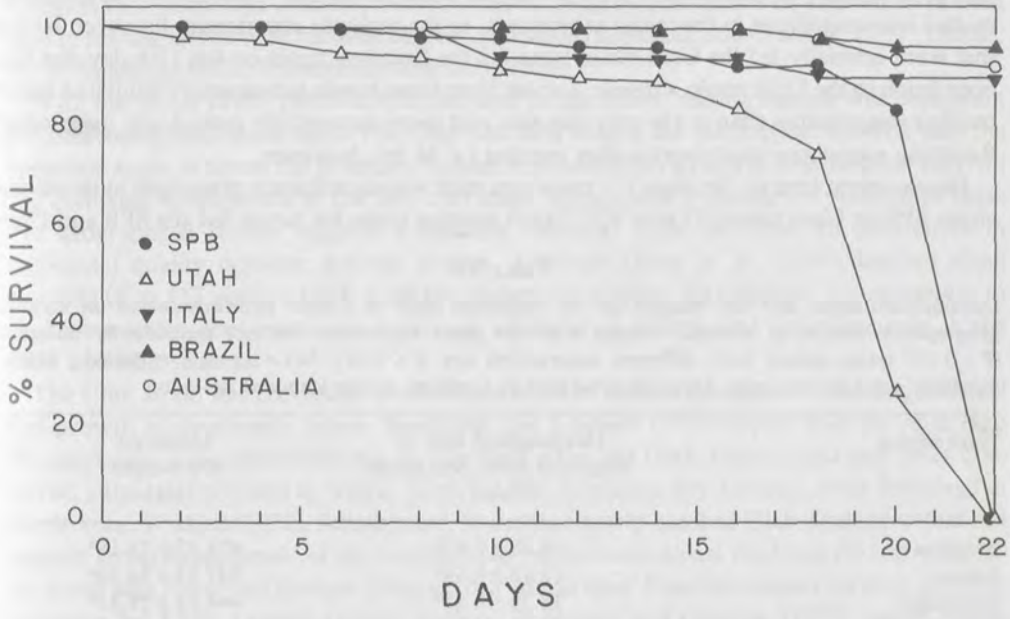

FIG. 3. Survival of *Cancer irroratus* larvae fed various geographical strains of *Artemia* during larval development.

TABLE VI

Analysis of variance of survival for *Cancer irroratus* larvae fed various geographical strains of *Artemia* nauplii. * indicates significant differences in survival of the larvae fed the various food sources ($P \leq 0.05$). SNK = Student-Neuman-Keuls posterior test. *Artemia* strains grouped on the same line are not significantly different from each other, while those on different lines are significantly different

Time post-hatch (days)	F-ratio	SNK
2	1.25	–
4	0.94	–
6	0.98	–
8	1.14	–
10	1.32	–
12	1.53	–
14	1.94	–
16	3.63*	Utah, SPB Brazil, Australia, Italy
18	2.91*	Utah, SPB Brazil, Australia, Italy
20	16.5 *	Utah, SPB Brazil, Australia, Italy
22	83.4 *	Utah, SPB Brazil, Australia, Italy

Larvae fed SPB and Utah strains exhibited certain molting irregularities similar to those seen in *R. harrisii* larvae (Table IV). No larvae fed either the SPB or Utah *Artemia* successfully molted into megalopae in the initial experiment. In the replicate experiment, however, larvae that were normally fed the Utah strain were fed the Brazilian strain on the 17th day due to a poor hatch in the Utah strain *Artemia*. Larvae from these bowls subsequently exhibited fewer molting irregularities than in the previous run, and some successfully molted into megalopae. Resulting megalopae died shortly after molting (< 36 hr), however.

Development time to the stage V – megalopa molt was significantly prolonged in larvae fed either SPB or Utah nauplii (Table VII). Mean molting times for larvae fed the SPB and Utah

TABLE VII

Development times and dry weights for the megalopa stage of *Cancer irroratus* reared on various geographical strains of *Artemia*. Means with the same superscript are not significantly different ($P > 0.05$) while means with different superscripts are ($P \leq 0.05$). ND = no data collected; 100% mortality prior to this stage. Data are presented as a means ± one standard deviation

Food source	Development time to megalopa from post-hatch (days)	Megalopa dry weight (μg)
Brazilian	18.28 ± 0.63^a	472.57 ± 74.18^a
Italian	18.18 ± 0.68^a	447.83 ± 64.20^a
Australian	18.20 ± 0.85^a	444.13 ± 59.83^a
San Pablo Bay	21.84 ± 1.13^c	ND
Utah	19.60 ± 1.01^b	346.14 ± 51.02^b

strains were 21.8 and 19.6 days, respectively ; contrasting with approximately 18 days for all other strains.

Growth to the megalopa stage was not significantly different for larvae fed the Brazilian, Italian or Australian strains (Table VII).

Temporal studies – *Rhithropanopeus harrisii*

Generally, little temporal variation in nutritional quality was found in the two geographical strains of *Artemia* tested. Utah collections supported equally poor growth and survival (Fig. 4), while the Brazilian collections promoted high survival rates (Fig. 5).

Some difference between the Utah temporal strains did appear at the stage IV – megalopa molt. More larvae fed either Utah-mixed or Utah 1977 successfully molted into the supernumerary stage than did larvae fed Utah 1978. One-hundred percent mortality occurred in this stage, however, just as in larvae fed Utah 1978. Furthermore, some minor reduction in survival was observed in larvae fed the Brazilian 1978b strain relative to the other two Macau-Brazil samples.

Discussion

Geographical strains – larval survival

Artemia is generally accepted as the most promising food source in rearing brachyuran larvae. Except for the early zoeal stages of some brachyurans which are too small to feed on brine shrimp, survival of *Artemia*-nourished larvae from hatching through metamorphosis is common (Bookhout and Costlow, 1974). Different geographical strains of *Artemia* have been used in rearing larvae with variable degrees of success (Bookhout and Costlow, 1970 ; Dexter, 1972 ; Wickins, 1972 ; Provenzano and Goy, 1976).

With the larvae of the two brachyurans used in this study, rearing success was dependent on the geographical strain used. The Utah and SPB strains did not support survival past the megalopa stage, whereas the Brazilian, Italian, and Australian strains promoted good survival and complete development to the first crab stage. Chlorinated hydrocarbon analysis of these five geographical strains suggests a possible chemical basis for observed differences in nutritional quality between *Artemia* strains. Although Olney *et al.* (1980) detected small amounts (4 to 433 ppb) of DDT in all five geographical types with highest contamination in the Italian strain, they measured significant amounts of dieldrin and chlordane only in the Utah and SPB strains.

The Utah strain has previously been shown to be of suspect quality in supporting survival and growth of crustacean larvae. Bookhout and Costlow (1970) report that the mud crab *Hexapanopeus angustifrons* did not survive well when fed Utah *Artemia* and that other crab larvae, although surviving as well as those fed San Francisco Bay *Artemia*, were abnormal in appearance. Wickins (1972), furthermore, found that newly hatched Utah *Artemia* would not support larval development of the prawn *Palaemon serratus* unless the brine shrimp were fed the green alga *Isochrysis galbana* prior to their use as food. Possible reasons for poor survival of larvae fed Utah *Artemia* remain unclear. Bookhout and Costlow (1970) report higher pesticide levels in the Utah strain than in the San Francisco Bay strain. In contrast, Wickins (1972), reports much lower pesticide levels with the differences between the Utah and San

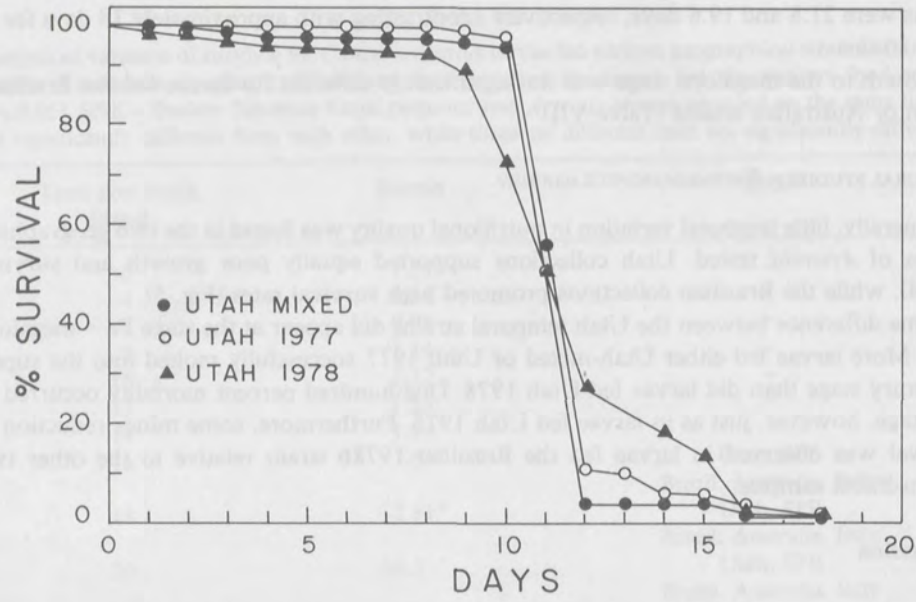

FIG. 4. Survival of *Rhithropanopeus harrisii* larvae fed temporal collections of *Artemia* collected from Great Salt lake, Utah-USA. Utah mixed = mixed years prior to 1977.

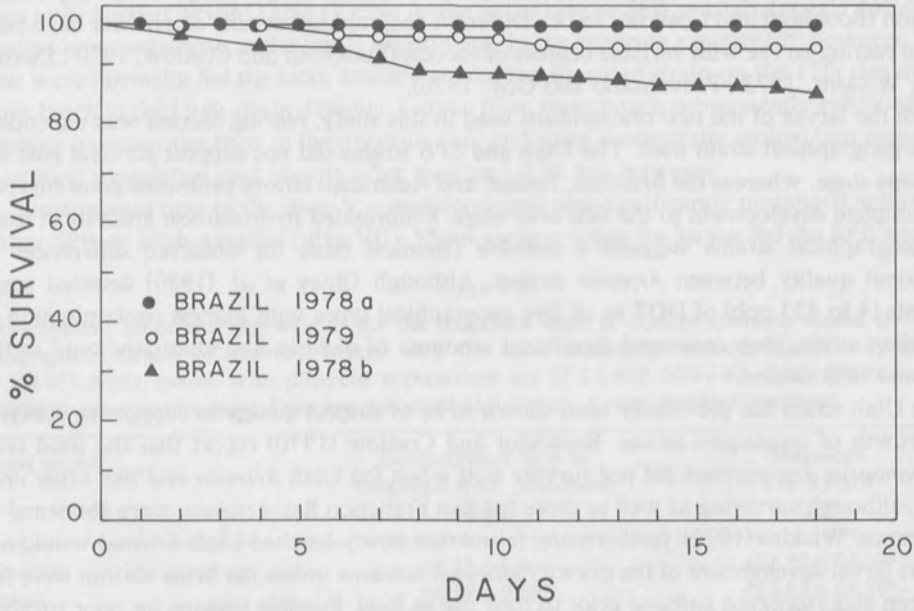

FIG. 5. Survival of *Rhithropanopeus harrisii* larvae fed temporal collections of *Artemia* collected from Macau-Brazil.

Francisco Bay strains very slight. Copper levels, on the other hand, were twice as high in the Utah strain as in San Francisco Bay *Artemia*. Other explanations, primarily dealing with differences in biochemical composition, have been suggested but have not been experimentally assessed (Provasoli, 1969).

Our relatively poor results with SPB nauplii are noteworthy since prior to this study, California-collected *Artemia* have often been used as the standard diet to which all other diets were compared (Sulkin and Epifanio, 1975 ; Sulkin and Norman, 1976). In addition to our data, poor survival of fish larvae fed SPB *Artemia* from the same lot used in this study has also been reported (Beck *et al.*, 1980 ; Klein-MacPhee *et al.*, 1980). A possible explanation for these results is provided by Olney *et al.* (1980) who found substantial levels of dieldrin (1.4 ppb) and chlordane (14 ppb), in nauplii hatched from the same lot of SPB cysts as that used in this study. Low concentrations of dieldrin (5 ppb) are known to be acutely toxic to larvae of the crabs *Leptodius floridanus* and *Panopeus herbstii* (Epifanio, 1971). High levels of pesticide contamination in SPB cysts would not be surprising, since San Pablo Bay is the receiving water for the extensively cultivated Napa Valley Region.

Although the observed correlation between survival results and pesticide levels is suggestive, it may not be causal. Other chemical characteristics of *Artemia* nauplii, such as heavy metal or fatty acid content, may be involved. Long chain polyunsaturated fatty acids are essential in the growth of marine fish (Watanabe *et al.*, 1978). Although the necessity of these fatty acids has yet to be shown for brachyuran larvae, they are present in the Brazilian, Italian and Australian strains and absent in the SPB and Utah strains (Schauer *et al.*, 1980).

Successful rearing of crustacean larvae on the other three strains of *Artemia* (Brazilian, Italian, and Australian) has not been previously reported. Survival values for larvae fed these strains are comparable to literature values for *R. harrisii* larvae (Costlow *et al.*, 1966 ; Bookhout and Costlow, 1970) and for *C. irroratus* larvae (Sastry, 1970 ; S. Vargo, personal communication) reared on San Francisco Bay *Artemia*.

Geographical strains — growth and development rate

Brachyuran larval development follows a set pattern in which there are a predictable number of zoeal stages followed by a megalopal stage before metamorphosis into a juvenile crab. Variation in the number of zoeal stages has been induced in laboratory cultures by varying rearing conditions (Ong and Costlow, 1970) or by adding pollutants to the rearing medium (Bookhout *et al.*, 1972). Under normal rearing conditions, *R. harrisii* larvae pass through four zoeal stages while development of *C. irroratus* involves five zoeal stages.

When fed either the Utah or SPB brine shrimp, some of the *R. harrisii* larvae in this study molted into a supernumerary zoeal stage rather than directly into the megalopal stage. Only twice did the supernumerary stage survive to molt into the megalopal stage, and these larvae died prior to completing larval development. Costlow (1968) postulated that there are at least two neuro-secretory mechanisms involved in controlling larval development. One mechanism controls molting rate while the other, usually synchronized with molting, controls the rate of morphological development within each larval stage. Pesticides, which commonly act as neuro-toxins, (O'Brien, 1967) may affect either one of these control mechanisms independently or both mechanisms together. For example, Epifanio (1971) found that dieldrin in concentrations of 1 ppb increased the duration of crab larval development by 11.4 % without

altering the morphological changes apparent at each molt. Similarly, low concentrations of mirex (0.01 ppb) prolonged the larval development of *R. harrisii* larvae (Bookhout *et al.*, 1972). On the other hand, Bookhout *et al.* (1972). found that mirex did not alter larval development rates in *Menippe mercenaria* but rather caused an increase in the incidence of a supernumerary zoeal stage. It is not predictable, then, what effects a particular pesticide may have, except that alterations in neuro-secretory functions are often involved.

In contrast with most other investigations, pesticides in our study were introduced incidentally as part of the food source rather than directly into the rearing water. Hence, strict comparisons with literature values of pesticide concentrations cannot be made. However, prolonged larval development (*C. irroratus* larvae fed Utah or SPB nauplii) and the appearance of supernumerary stages (*R. harrisii* larvae fed Utah or SPB nauplii) did occur when food sources containing detectable amounts of dieldrin and chlordane were fed to the larvae. Again, a causal relationship is uncertain.

Interestingly, all five strains assayed contained DDT family compounds (up to 422 ppb), but no discernable changes in normal crab larval morphology or in the normal sequence and duration of development were manifest in larvae fed the Brazilian, Italian, and Australian strains. Furthermore, larval growth in terms of dry weight was similar for each decapod on all diets except for *R. harrissi* larvae fed the Australian strain. This contrasts with the findings of Bookhout and Costlow (1970) who reported deleterious effects in crab larvae fed Utah *Artemia* containing 7.05 ppb DDT. However, since DDT was the only pesticide assayed in their study, it may be that other contaminating pesticides were responsible for the observed differences.

Temporal studies – survival

The nutritional quality of an *Artemia* source might be expected to vary temporally as well as geographically. Short-term changes in the diets of adult *Artemia* or changes in the pollutant level of salinas where cysts are harvested could have an impact on the quality of resultant nauplii as a food source. Our preliminary results, however, indicate that there is relatively little short-term variation in the nutritional quality of brine shrimp nauplii from a specific region. Temporal samples of both the Utah strain (collected over a 2 to 3 year period) and the Brazilian strain (collected over a 2 year period) generally promoted similar growth and survival of crab larvae. An exception is the Brazilian 1978b strain which supported somewhat lower larval survival than the other Brazilian temporal strains. An explanation for this reduction in survival may be forthcoming when biochemical analysis is completed on these *Artemia* collections. Johns *et al.* (1978) apparently found significant temporal variation in the nutritional quality of San Francisco Bay *Artemia* when they compared survival and growth of mud crab larvae reared either on brine shrimp harvested prior to 1976 or on *Artemia* cysts harvested in 1978. Since the completion of that study, however, it has been determined that the 1978 collection was actually made from San Pablo Bay rather than from San Francisco Bay. Hence, the possibility of some degree of geographical variability cannot be excluded.

Sources of error

Potential sources of error associated with studies of this type include variability between hatches of crab larvae and seasonal factors involving the collection of the *Artemia* cysts.

Both inter-female and seasonal hatch variability are known to occur with decapod larvae (Sastry and Vargo, 1977). On several occasions we observed poor hatch survival from an egg mass that did not appear to be damaged or parasitized. Sastry and Vargo (1977), furthermore, found distinct seasonal variation in the temperature and salinity tolerance of *Cancer irroratus* larvae. Larvae from crabs collected in the spring tolerated a wider range of temperature and salinity than did larvae from crabs collected in the fall or summer (*loc. cit.*). Such physiological variation may also be reflected in larval survival and feeding response.

Details of collection and processing of commercially obtained *Artemia* cysts are unknown variables. Cyst production in the salinas normally occurs several times per year at intervals associated with episodes of maximum salinity and large diurnal fluctuations in the dissolved oxygen concentration (Sorgeloos *et al.*, 1977). Seasonal variation in the long chain poly-unsaturated fatty acid composition of *Artemia* cysts from a single location has been demonstrated (Watanabe *et al.*, 1978). Seasonal variability, then, may be as important as differences between years in determining the nutritional quality of an *Artemia* source.

Although we examined the nutritional value of cysts collected over a two year period, this time frame may be too short to reflect any major changes in the salinas caused by shifts in local agricultural or industrial activity. Hence, we have not systematically examined either short-term seasonal changes or long-term anthropogenic changes in our temporal analysis.

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International Study on *Artemia*[1] VII. Nutritional value of five geographical strains of *Artemia* to winter flounder *Pseudopleuronectes americanus* larvae

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Abstract

Artemia (brine shrimp) nauplii from five geographical locations (San Pablo Bay, USA ; Great Salt Lake, Utah, USA ; Shark Bay, Australia ; Macau, Brazil; and Margherita di Savoia, Italy) were tested as a food source for late stage winter flounder larvae.

Results showed fish fed Australia, Italy, and Brazil strains had significantly better survival ($P < 0.05$) than those fed Utah and San Pablo Bay (93.9%, 89.1%, 88.4%, 46.1%, and 38.8%) respectively. There was no significant difference ($P > 0.05$) between survival percentages of Italy, Australia, and Brazil and none between Utah and San Pablo Bay.

Total fish length was significantly greater ($P < 0.05$) in fish fed Australia and Italy strains than in those fed Utah, Brazil, and San Pablo Bay (10.22, 9.87, 8.91, 8.58 mm TL) respectively. There was no significant difference between fish fed Australia and Italy or among those fed the other three strains ($P > 0.05$).

Dry weights of fish fed Italy and Australia strains were significantly greater ($P < 0.05$) than those fed Utah, Brazil, or San Pablo Bay (1320.8, 1176.5, 914.4, 800.7, 702.7 μg) respectively. There was no significant difference between fish fed Italy and Australia, none between Brazil and San Pablo Bay or Brazil and Utah ($P > 0.05$) ; but those fed Utah were significantly heavier than those fed San Pablo Bay ($P < 0.05$).

Introduction

Winter flounder larvae and juveniles are used as test animals in toxicological bioassays at the Environmental Protection Agency, Environmental Research Laboratory in Narragansett, Rhode Island. Because of increasing emphasis on long term chronic studies, methods were developed for rearing the fish from eggs obtained in the laboratory. The brine shrimp *Artemia* has been used as a convenient source of food by fish culturists for many years (Sorgeloos and Persoone, 1975) for fish which require a live diet. Because of periodic shortages in *Artemia*

[1] International Interdisciplinary Study on *Artemia* Strains coordinated by the Artemia Reference Center, State University of Ghent, Belgium.

cyst supplies from San Francisco Bay (the most widely used cyst source); other strains, available commercially and from the Artemia Reference Center in Belgium, have been used by the Environmental Research Laboratory as larval food sources. Studies with the Atlantic silverside, *Menidia menidia* (Beck *et al.*, 1980) and the summer flounder, *Paralichthys dentatus* (Klein-MacPhee and Howell, unpublished report) have shown a significant difference in survival between fish fed different strains of *Artemia*. Since nutritional history of the test animal can effect the results of toxic substance testing (Mehrle *et al.*, 1977), this experiment was conducted to test the nutritional value of five geographical strains of *Artemia* to winter flounder in terms of survival and growth.

Materials and methods

Gravid adult winter flounder were collected in West Passage Narragansett Bay, Rhode Island in January 1979, by means of otter trawl. The fish were transported to the laboratory and maintained in a 2.4 m diameter fiberglass tank provided with running seawater at ambient temperature and salinity (1-4 °C, 31 ‰ S). They were allowed to ripen naturally and stripped manually just prior to spawning. The eggs were stripped into plastic dish pans, fertilized and coated with diatomateous earth to prevent clumping after the method of Smigielski and Arnold (1972). Eggs were incubated in acrylic hatching jars (modified from Buss, 1959) in filtered running seawater at ambient temperatures and salinity (4-6 °C, 31-33 ‰ S). The larvae hatched 13 days later and were collected in plastic containers with a screened bottom. Larvae were transferred by dipper to large black plastic containers (60 × 47 × 13 cm) holding 24 l of filtered (0.25 μm Gelman[4] filter) ultra violet treated seawater to which an antibiotic mixture of penicillin and streptomycin in a 1:1 ratio was added at a concentration of 25 ppm. The stocking density was 30 larvae/l. Water temperature varied between 6 and 8 °C and salinity between 30 and 33 ‰. Larvae were fed daily starting 10 days post hatch on wild plankton and laboratory cultured rotifers *Brachionus plicatilis* (1:1 ratio by weight). This amounted to a concentration of 3 000 organisms/l (1 000 wild plankton to 2 000 rotifers). The plankton consisted primarily of copepod nauplii, wild rotifers and polychaete worm larvae screened to collect organisms about 64-75 μm in size. The light regime was 12 hr light/12 hr dark. One third the water volume was changed once a week and replaced with clean, filtered UV treated seawater at the same temperature. A mixture of phytoplankton *Isochrysis galbana* and *Dunaliella tertiolecta* (1:1 by volume) was added periodically as a water conditioner. The larvae were large enough to eat *Artemia* nauplii 42 days after hatch and were then transferred to the experimental containers (black plastic pans 34.0 × 29.5 × 1.5 cm) holding 6 l of filtered UV treated seawater with the same antibiotic mixture as described for newly hatched larvae. The pans were kept in a temperature control box set for 7 °C on a 12 hr light/12 hr dark photoperiod. The pans were covered with 400 μm mesh nylon screens to retard evaporation, and rotated daily to offset temperature, air movement and light gradients in the box. Sixty fish were placed in each container providing a stocking density of 10 larvae/l. Five strains of brine shrimp were tested with three replicates of each treatment. The strains tested were San Pablo Bay, California-USA (Living World, San

[4] Use of trade names does not denote endorsement by the United States Environmental Protection Agency or the University of Rhode Island.

Francisco Bay Brand, Inc., lot 1628[5]); Great Salt Lake, Utah-USA (harvest 1977); Macau-Brazil (Companhia do Rio Grande do Norte, Cirne Brand, harvest 1978); Margherita di Savoia-Italy (harvest 1977). The criteria used by the ISA group in selecting these strains for evaluation as food for cultured marine animals are reported in Sorgeloos (1980). Cysts were incubated in 6 l separatory funnels containing filtered seawater and provided with strong aeration. These were located in a temperature controlled room in constant dim light. Newly hatched nauplii were harvested by pouring them into a plastic box painted black except for a clear "window". Fluorescent light was placed beside the window and the photopositive nauplii siphoned off when they collected near the light. The nauplii were resuspended in clean seawater and aerated until fed to fish.

The larvae were fed 0.1 g wet weight *Artemia* nauplii every other day. This was approximately 5 000 nauplii per container. Preliminary experiments with summer flounder (MacPhee and Howell, unpublished report) showed they consumed about 35 nauplii/day (varying from 5 to 125). Live nauplii were always present indicating an excess of food. Nauplii left at the beginning of the second day were siphoned off before new food was added so that they would not outgrow the size range that flounder would eat. A liter of clean filtered UV treated seawater was added each feeding day to replace water removed with the nauplii. Temperature, salinity and number of mortalities were recorded daily. Fifty ml phytoplankton (1:1 mixture *I. galbana* and *D. tertiolecta*) was added each feeding day as a water conditioner. At the end of 29 days all remaining fish were sacrificed, measured with a filar micrometer attached to a dissecting scope, placed in a drying oven at 46 °C for 24 hr, and weighed on a Perkin-Elmer[4] electrobalance (Model No. AD2Z accurate to 1×10^{-4} mg) to the nearest μg. The dried larvae were then scraped into vials and frozen for pesticide analysis. Length and dry weight measurements as well as mortality data for different treatments were analyzed by one way analysis of variance. If significant differences were found, the means were compared using Duncan's Multiple Range Test (Snedecor and Cochran, 1973).

Results

There was a significantly greater survival in fish fed Australia, Brazil, and Italy strains than in those fed Utah and San Pablo Bay. There was no difference between fish fed Australia, Brazil, and Italy and none between those fed Utah and San Pablo Bay. Mean survival percentages are as follows : Australia 93.9 ; Brazil 89.1 ; Italy 88.4 ; Utah 46.1 ; San Pablo Bay 38.8. Results of the Duncan's Multiple Range test are given in Table I. Mortality in the Utah and San Pablo Bay treatment began to increase 22 and 24 days respectively after the start of the experiment at which time mortality in the other three treatments remained at a constant low rate (Fig. 1).

Total lengths of fish fed Australia and Italy strains were significantly greater ($P < 0.05$) than those fed Utah, Brazil, and San Pablo Bay. There was no significant difference in length between fish fed Australia and Italy or between fish fed Utah, Brazil, and San Pablo Bay. Table II gives results of Duncan's Multiple Range test and Fig. 2A shows mean differences in length.

[5] Labeled as San Francisco Bay *Artemia* cyts. Apparently collected from San Pablo Bay, California-USA. (Sorgeloos, personal communications).

Dry weights of fish fed Italy and Australia strains were significantly greater ($P < 0.05$) than those fed Utah, Brazil, or San Pablo Bay. There was no significant difference between fish fed Italy and Australia, and none between Brazil and San Pablo Bay or Utah and Brazil ($P > 0.05$), but those fed Utah were significantly heavier than those fed San Pablo Bay ($P < 0.05$). Table III gives results of Duncan's Multiple Range test and Fig. 2B shows mean differences in weight between treatments.

TABLE I

Duncan's multiple range test for significant difference in mean survival of fish fed different strains of *Artemia*. Means with same grouping letter are not significantly different ($\alpha = 0.5$)

Treatment	Grouping	Mean (%)
Australia	A	93.86
Brazil	A	89.12
Italy	A	88.40
Utah	B	46.11
San Pablo Bay	B	38.84

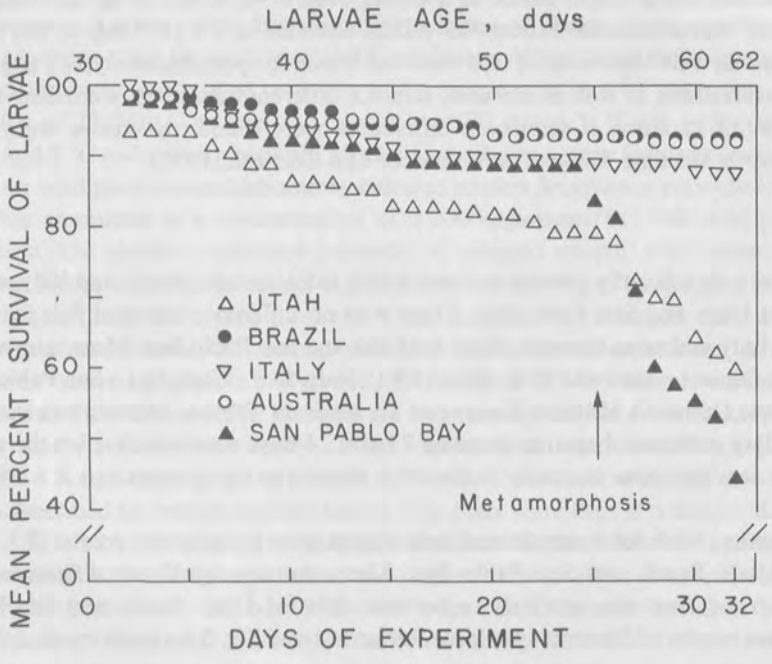

FIG. 1. Cumulative survival curves of winter flounder fed five strains of brine shrimp. Top horizontal axis shows the age of the larvae expressed in days since the first feeding. Bottom shows days after the start of the *Artemia* experiment.

TABLE II

Duncan's multiple range test for significant difference in mean length of fish fed different strains of *Artemia*. Means with same grouping letter are not significantly different ($\alpha = 0.5$)

Treatment	Grouping	Mean
Australia	A	10.22
Italy	A	9.87
Utah	B	8.91
Brazil	B	8.91
San Pablo Bay	B	8.58

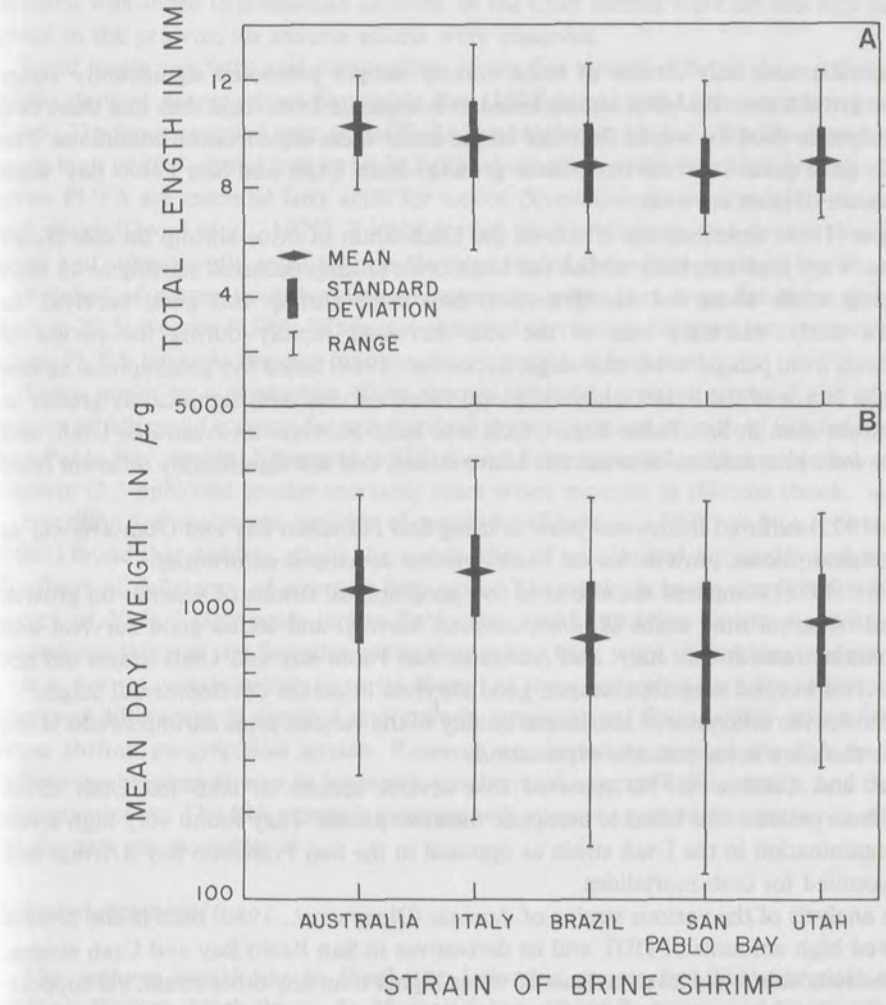

FIG. 2. A. Mean total length of winter flounder fed five strains of *Artemia*. B. Mean dry weight of winter flounder fed five strains of *Artemia*.

TABLE III

Duncan's multiple range test for significant difference in mean dry weight of fish fed different strains of *Artemia*. Means with same grouping letter are not significantly different ($\alpha = 0.05$)

Treatment	Grouping	Mean
Italy	A	1302.79
Australia	A	1176.49
Utah	B	914.39
Brazil	B,C	800.65
San Pablo Bay	C	702.67

Discussion

Both Australia and Italy strains of brine shrimp nauplii promoted significantly better survival and growth than the other strains tested. It is apparent from these data that these two strains are superior food for winter flounder larvae under these experimental conditions. The Brazil strain gave good survival but poorer growth. Both Utah and San Pablo Bay were inferior because of poor survival.

Shelbourne (1968) described the effects of the Utah strain of brine shrimp on sole (*Solea solea*) larvae. Very high mortality of fish fed Utah brine shrimp occurred starting at 45 days after hatching while those fed San Francisco Bay brine shrimp had good survival. In Shelbourne's study, mortality rate of the sole increased rapidly during the period of metamorphosis from pelagic to benthic stage. Beck *et al.* (1980) tested five geographical strains of *Artemia* on larvae of the Atlantic silverside and found survival was significantly greater in the Brazil strain than in San Pablo Bay, which was least. Survival on Australia, Utah, and Italy strains were intermediate between the above strains and not significantly different from one another.

Wickins (1972) cultured freshwater prawns using San Francisco Bay and Utah *Artemia* as food. At metamorphosis, prawns fed on Utah *Artemia* developed deformities.

Johns *et al.* (1978) compared the effects of five geographical strains of *Artemia* on growth and survival of larval mud crabs (*Rhithropanopeus harrisii*) and found good survival and growth in strains from Brazil, Italy, and Australia. San Pablo Bay and Utah strains did not support survival beyond megalopa despite good survival in earlier developmental stages.

The reason for the difference in nutritional quality of the various brine shrimp strains is not known, but there are some possible explanations.

Bookhout and Costlow (1970) reported that several species of crab fed Utah strain developed abnormalities and failed to complete metamorphosis. They found very high levels of DDT contamination in the Utah strain as opposed to the San Francisco Bay *Artemia* and felt this accounted for crab mortalities.

Pesticide analysis of the various strains of *Artemia* (Olney *et al.*, 1980) used in the present study showed high amounts of DDT and its derivatives in San Pablo Bay and Utah strains, however, the Italy strain had levels at least 10 times higher than any other strain, yet supported good survival and growth of winter flounder larvae. On the basis of these findings, DDT does not seem to be the major cause of mortality; however, high concentrations of dieldrin, a chlorinated hydrocarbon insecticide toxic to marine fish (Eisler 1970), were found in San

Pablo Bay and Utah strains in comparison to Brazil, Australia, and Italy *Artemia* (Olney *et al.*, 1980).

Argyle *et al.* (1975) found that dieldrin in the diet of channel catfish *Ictalurus punctatus* at concentrations of 4.0 μg/g of food over a period of 210 days caused reduced growth. No significant mortality was observed although dieldrin was accumulated in the tissues.

The adverse effect of Utah and San Pablo Bay strains of *Artemia* on flounder larvae may be due to nutritional deficiencies. Wickins (1972) in seeking an explanation for his observed results analyzed for pesticides, heavy metals, carotenoids, sterols, and fatty acids. No significant differences in any of these were found between Utah and San Francisco Bay strains. Wickins (1972) therefore felt the difference in nutritional value between the two *Artemia* strains was responsible for the deformity of the prawns because when the alga *Isochrysis galbana* was added to crustacean cultures, or the Utah nauplii were fed this alga before being given to the prawns, no adverse effects were observed.

Lipid levels and fatty acid composition of the five strains of brine shrimp (Schauer *et al.*, 1980) showed *Artemia* from San Pablo Bay (1978 batch) and Utah were low in long chain (20:5ω3) polyunsaturated fatty acids (PUFA) and higher in 18:3ω3. The Brazil and Italy strains were high in 20:5ω3 and low in 18:3ω3. The Australian strain was high in both. The 20:5ω3 series PUFA are essential fatty acids for turbot, *Scophthalmus maximus* (Owen *et al.*, 1975) and plaice (Owen *et al.*, 1972). Turbot do not readily elongate and desaturate dietary fatty acids and subsequently must rely on the presence of these fatty acids in the diet.

Survival of winter flounder larvae was greater when they were fed brine shrimp strains high in 20:5ω3 series PUFA. While this observed correlation between larval survival and long chain PUFA levels in the diet is suggestive, it may not be causal.

There might be a synergistic effect among pesticide levels, nutritional and physiological factors which could account for low survival percentages and growth of fish fed the Utah and San Pablo Bay strains. Silbergeld (1973) found fish pretreated with a sublethal exposure to dieldrin (2.3 ppb) had greater mortality rates when exposed to thermal shock.

Nutrition influences the toxicity of pesticides (Shakman, 1974) and *vice versa*. Durham (1967) found that dieldrin affects the metabolism of unsaturated fatty acids and increases the ill effects of deficiency of essential fatty acids. The pesticide levels combined with the deficiency of 20:5ω3 fatty acids in San Pablo Bay and Utah brine shrimp nauplii might have caused mortality in the flounder at the time when they were undergoing metamorphosis.

It is not yet certain which factor(s), if any, of these suggested here are responsible for the observed differences in survival and growth among larval flounder for diets of the selected brine shrimp geographical strains. Research conducted as part of the ISA had indicated differences between strains in biometry, biochemical composition, genetics, and the presence of contaminants. The ISA research program will attempt to relate diet quality to effects when all the data are available.

Acknowledgements

The authors would like to thank the following people for their technical assistance: William Walton, Mark Peters, D. Michael Johns, David Bengtson and Dierdre Brouton.

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Requirements for *Artemia* nauplii in *Macrobrachium rosenbergii* (de Man) larviculture[1,2]

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Abstract

Minimum requirements of *Artemia* nauplii as primary rations in *Macrobrachium rosenbergii* larviculture were analyzed in a series of experiments from December 1976 to April 1978. Larvae were reared (static culture) in 60 l, black fiberglass, rounded bottom tanks at initial stocking densities of 75 larvae/l. Cultures were maintained at 13 ‰ synthetic seawater and 26.5 ± 1 °C with ambient light and moderate aeration. Results strongly indicate that several fish roes (particularly those of *Cynoscion* spp. and *Mugil* sp.) are not only valuable supplemental rations but can also completely replace *Artemia* nauplii in later larval stages. Results from all experiments show more rapid larval development (approaching differences of 0.9 to 1.5 LSI), higher survival (13-19% better than controls), and greater postlarval production in roe supplemented cultures. In a test production run, using *Cynoscion* sp. roe as supplement, over 36% more postlarvae were obtained than in controls which received *Artemia* nauplii as the singular ration. Together with studies on intermittant or reduced *Artemia* nauplii feeding protocols, alternative ration studies indicate that the amount of *Artemia* nauplii used in the entire larval cycle can be reduced by one-half to two-thirds. A number of conclusions concerning the minimum requirements of *Artemia* nauplii and suggested feeding protocols are given.

Introduction

Adult *Artemia* and their nauplii are widely used and acclaimed as principal rations for the larvae and juveniles of many species of fish and invertebrates. Among these are species showing the greatest potential for commercial mariculture success, i.e., shrimp (*Penaeus spp.*), freshwater prawns (*Macrobrachium spp.*), american lobsters (*Homarus americanus*), and several finfish species including sole (*Solea solea*) and striped bass (*Monrone saxatilis*). Thus, the production of *Artemia* cysts and fresh, frozen, and freeze dried adults can be considered an indispensible mariculture-support industry.

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[2] This work is a result of research supported by NOAA Office of Sea Grant, Department of Commerce. The US Government is authorized to produce and distribute reprints for governmental purposes notwithstanding any copyright that may appear hereon. Reference to trade names in this paper does not imply endorsement by NOAA Office of Sea Grant or the State of South Carolina.

Major concern over the availability of *Artemia* became apparent in Europe in the first years of this decade and became a world wide problem in 1975. This concern was reiterated over the next year particularly at meetings of the mariculture community (World Mariculture Society Workshop, January 1976, San Diego, California ; FAO Technical Conference on Aquaculture, June 1976, Kyoto, Japan). The large demand placed upon existing sources of *Artemia* by the burgeoning mariculture industry resulted in a dramatic increase in price (from ~$ 15.00/kg in 1974 to over $ 70.00/kg in 1977) and the marketing of less viable products. This in turn led to an increase in prospecting for new *Artemia* resources and the eventual formation of several new companies supplying *Artemia* products from a variety of sources world wide. Despite the recent general increase in the supply of *Artemia* cysts the price has not significantly decreased. Thus, aside from labor and certain fixed costs (amortization of capital, taxes, etc.) the principal expenditure in larval culture continues to be food.

Widespread investigations on prawn (*M. rosenbergii*) cultivation have not only significantly increased our knowledge of the biology of this commercially important crustacean but have also resulted in substantially increased efficiency and standardization of larval culture operations (Ling, 1969ab ; Fujimura and Okamoto, 1970 ; Fujimura, 1974 ; Sandifer and Smith, 1974 ; Sandifer, 1975 ; Sick, 1975 ; Manzi *et al.*, 1977 ; Murai and Andrews, 1978). Recent reports have indicated that relatively high survival and postlarval production can be accomplished routinely (Manzi and Maddox, 1976 ; Manzi *et al.*, 1977 ; Sandifer and Smith, 1979). Even at these relatively high production rates, the cost of rearing sufficient quantities of postlarvae for pond stockings is seriously inflated by the cost of *Artemia* cysts. At present, *Artemia* nauplii are the primary ration in *M. rosenbergii* larviculture. Normally it is fed throughout the larval period, but it is generally supplemented with a variety of feeds (fish roe, hen's egg, fish flesh, commercial dry rations, soya meal, mollusk flesh, etc.) during late larval stages.

The literature is strangely mute on the origins of the standard concentrations of *Artemia* nauplii fed to *M. rosenbergii* larvae. Ling (1962) suggested the use of *Artemia* nauplii but did not state optimum feeding concentrations. By 1974, *Artemia* nauplii was established as the "food of choice" for at least the early larval stages of *M. rosenbergii* (Goodwin and Hanson, 1974), but the suggested feeding concentration (one-fourth teaspoon per day per 5 000 larvae at day 3, gradually increasing to a full teaspoon by day 30) was vague. Sandifer *et al.* (1977) in a summary of responses to a survey of *Macrobrachium* hatchery operators, reported that *Artemia* was the major hatchery ration. It was fed at rates of 5-15 nauplii/ml and in several cases the feeding rates were initially high and decreased in the latter half of the larval cycle. The purpose of the present study was to determine the minimum requirements of *Artemia* nauplii as primary rations in *M. rosenbergii* larviculture. If appropriate, the results of this study could be used to alter existing feeding protocols and improve the economics of hatchery production.

Material and methods

Larval culture

Larvae were obtained from brood females maintained in the laboratory or in outdoor brood stock ponds and cultured following standard techniques (Fujimura and Okamoto, 1970 ;

Fujimura, 1974) as modified by Sandifer and Smith (1974, 1979) and Manzi *et al.* (1977). Larvae were reared in 60 l, black fiberglass, rounded bottom tanks at a volume of 40 l and a stocking density of 75 larvae/l. The tanks were fitted on wooden platforms, arranged five to a platform, and placed in a controlled environment room maintained at low intensity light and high humidity. The cultures were supplied with constant mild aeration and maintained at 26.5 ± 1 °C. A synthetic seawater medium (Instant Ocean, Aquarium Systems, Inc.), formulated at 13 ‰, was used exclusively in all larval cultures. All experiments were performed in static culture with water changes at 2 or 3 day intervals. Used culture water was added to a 1 500 l reservoir which continuously exchanged its contents with two external biological filters. The filters were constructed in 170 l rectangular plastic containers and consisted of approximately 0.028 m^3 of dolomite gravel (3.0-5.0 mm diameter) spread over a plastic grid forming an 18 cm layer 13 cm above the container bottom (Manzi *et al.*, 1977). This reconditioned water was used in all culture changes throughout all five experimental periods (a total of 28 months). All tanks were emptied, cleaned, and scalded with hot freshwater once a week.

Nauplii of *Artemia* (World Ocean Brand, Melbourne, Australia) were obtained from cysts hydrated under constant aeration at 28 ± 1 °C and 25 ‰ using standard rearing techniques (Persoone and Sorgeloos, 1975 ; Sorgeloos and Persoone, 1975 ; Sorgeloos *et al.*, 1976). Nauplii were harvested 24 to 36 hr after cyst immersion and appropriately added to cultures to maintain experimental and control densities. Supplemental rations were incorporated into the culture protocol according to specific experimental designs (consult subsequent section) and included roes from *M. rosenbergii*, *Cynoscion* spp., *Rhomboplites aurorubens*, and *Paralichthys dentatus*, as well as poached hen's egg both alone and in combination with squid (*Loligo* sp.). The fish roe were obtained, and the hen's egg prepared using methods outlined by Sandifer and Williams (1980).

Evaluations of diet efficacy were determined by estimates of larval growth and periodic estimates of larval survival and postlarval production. Growth was measured by the use of the larval stage index (LSI) described by Maddox and Manzi (1976). Survival was determined by direct count at the initiation of culture and at weekly intervals.

Total ammonia, nitrite, nitrate, and pH were measured at the initiation of culture using La Motte Chemical Products Company (Chestertown, Maryland) marine aquarium test kits. Thereafter, total ammonia and nitrite were measured two or three times weekly immediately before water exchanges. In addition, total ammonia and nitrite were monitored in the system reservoir before water exchanges.

Experimental design

A total of four different experimental designs were incorporated in five culture studies between December 1976 and April 1978 to determine the minimum requirements for *Artemia* nauplii in *M. rosenbergii* larviculture.

Experiment I (December 8, 1976-January 13, 1977)

The experimental design for this experiment, outlined in Fig. 1, incorporated four treatments and a control, each represented by three replicates. All cultures were maintained on *Artemia* only, at a concentration of 10 nauplii/ml for the first 12 days. Thereafter, the control

retained the common regime while the experimental treatments were subjected to diet modifications involving the introduction of alternative or supplemental rations and a reduction in *Artemia* nauplii concentrations. Treatment one (T_1) received one-half the nauplii ration with an average of 0.75 cc *M. rosenbergii* roe per day, for the remainder of culture. Treatment two (T_2) received only the roe ($\bar{y}$ = 1.25 cc/day); treatment three (T_3) only the roe until day 20 (stage IX), then the combination of nauplii (5/ml) and roe (0.75 cc); treatment four (T_4) only the roe until day 27 (stage XI), then the nauplii-roe combination.

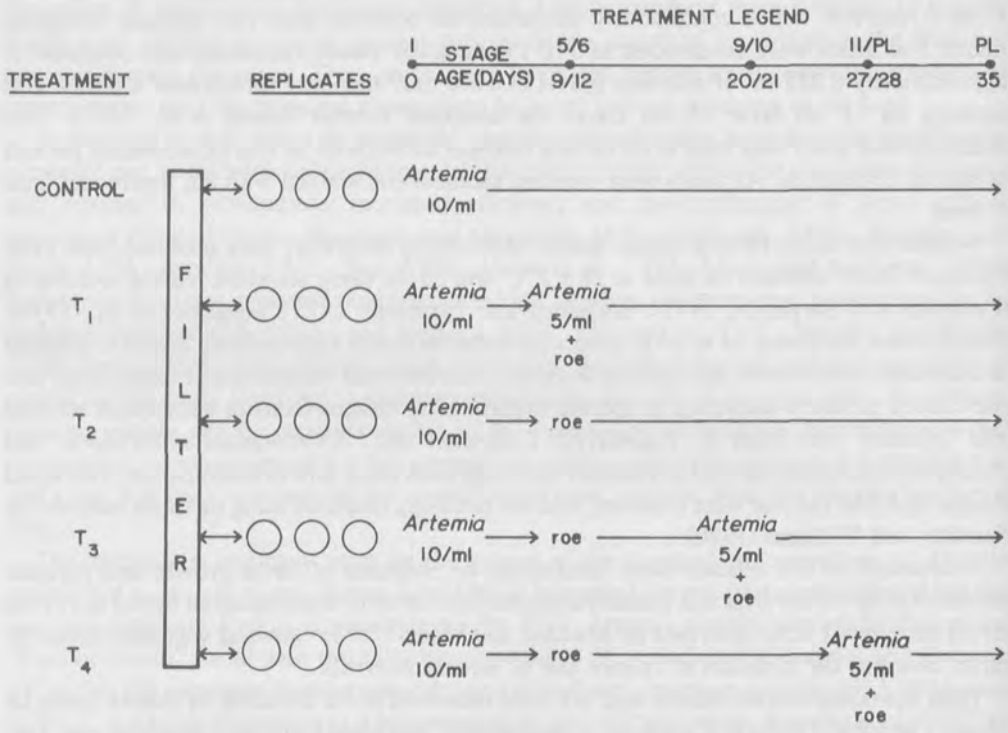

FIG. 1. Experimental design for experiments I and II testing the intermittant use of fish and *M. rosenbergii* roes as replacements for *Artemia* nauplii (see text).

Experiment II (April 23-May 16, 1977)

This experiment was performed under the same design as Experiment I (Fig. 1) with the substitution of *Cynoscion* sp. roe for *M. rosenbergii* roe as the only modification.

Experiment III (August 23-October 5, 1977)

Fig. 2 illustrates the experimental design of this culture series incorporating the intermittant use of *Artemia* nauplii as a supplement to a primary ration of fish roe (*Cynoscion* sp.). Each of the four treatments and the control were represented by three replicate cultures. The control cultures received nauplii (10/ml) as the only ration. Treatment cultures were maintained on

the same common ration until day 9 (stage V). Thereafter they received *Cynoscion* sp. roe ($\bar{y} = 3.50$ ml/day) as the primary ration with *Artemia* nauplii (5/ml) fed daily (T_1), every second day (T_2), every third day (T_3), and every fourth day (T_4) for the remainder of culture.

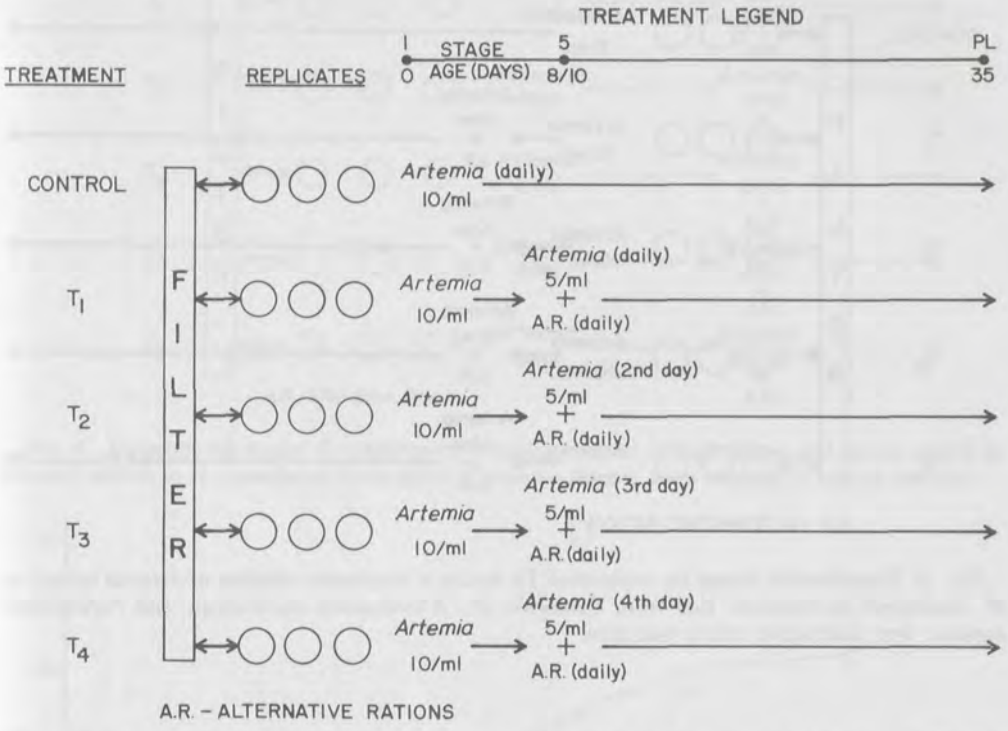

FIG. 2. Experimental design for experiment III testing the intermittant use of *Artemia* nauplii with alternative rations (*Cynoscion* sp. roe) in latter stage *M. rosenbergii* larviculture (see text).

Experiment IV (January 10-February 20, 1978)

This experimental design (Fig. 3) incorporated a progressive deletion of *Artemia* nauplii in four treatments, and a control, each represented by three replicate cultures. Control cultures received the standard ration throughout (10 nauplii/ml/day) while all treatment cultures received the same common ration until day 13 (stage V). Thereafter, treatment one (T_1) received 5 nauplii/ml/day and fish roe ($\bar{y} = 3.5$ cc/day) ; treatment two (T_2) the same (nauplii and roe) until stage 7 (LSI) then just fish roe ($\bar{y} = 5.0$ cc/day) ; treatment three (T_3) received the same regimen with the exception that the fish roe ration was delayed until stage 8.5 (LSI) ; and treatment four (T_4) received the fish roe ration at stage 10 (LSI). Two fish roes (*Cynoscion* sp. and *Rhomboplites aurorubens*) were used as the alternative rations in this study. One replicate of each treatment received *Cynoscion* roe, another *Rhomboplites* roe, and the third a combination of the two. In addition, a fifth treatment (labled T_{1p} in subsequent figures) was run concurrently following the design of T_1 but using *Paralichthys dentatus* roe in all three replicates.

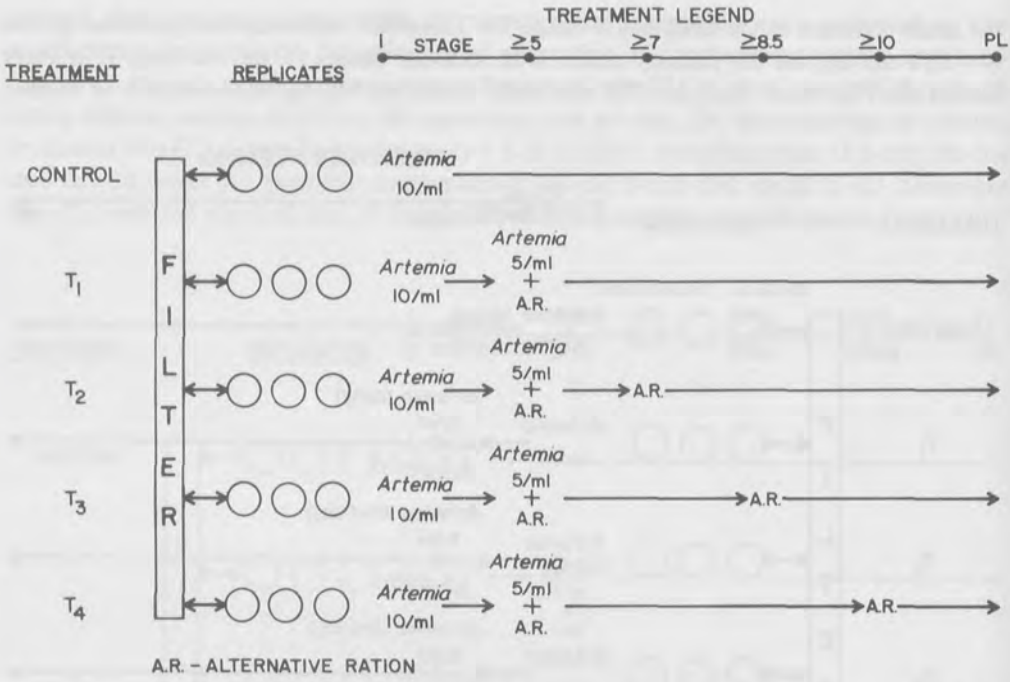

FIG. 3. Experimental design for experiment IV testing a progressive deletion of *Artemia* nauplii in *M. rosenbergii* larviculture. Roe from *Cynoscion* sp., *Rhomboplites aurorubens*, and *Paralichthys dentatus* were alternative rations (see text).

Experiment V (March 2-April 15, 1978)

Fig. 4 illustrates the experimental design of this culture series testing various concentrations of *Artemia* nauplii as primary rations. All five treatments were each represented by three replicate cultures and the alternative ration was *Cynoscion* sp. roe fed at an average concentration of 3.5 cc/day. Treatments one (T_1), two (T_2), three (T_3), four (T_4), and five (T_5-control) received only nauplii at concentrations of 2, 4, 6, 8, and 10/ml, respectively, until day 12 (stage V) at which time roe ($\bar{y} = 3.5$ cc/day) was supplemented to all treatments.

During the entire experimental period (December 8, 1976-April 15, 1978) several additional experimental culture series were performed. Although reported elsewhere (Manzi *et al.*, unpublished) their results are pertinent to the data reported here and will be discussed appropriately in subsequent sections.

Results and discussion

The results of the first two experiments are presented in Fig. 5, 6, and 7. Larval development proceeded much more rapidly in T_1 and control cultures with both groups reaching stage IX almost a week before the other treatments (Fig. 5). Both total survival curves and

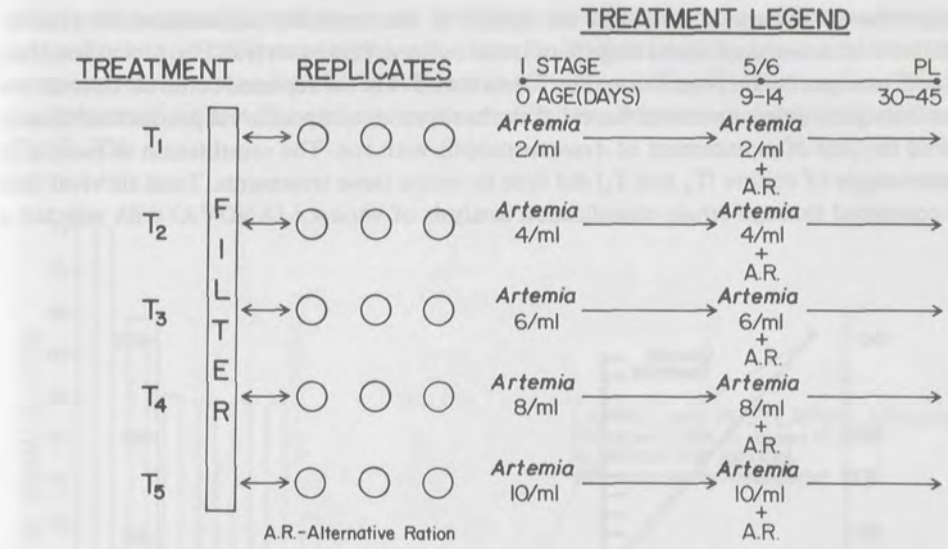

FIG. 4. Experimental design for experiment V testing various concentrations of *Artemia* nauplii as primary rations in *M. rosenbergii* larviculture. *Cynoscion* sp. roe were alternative rations (see text).

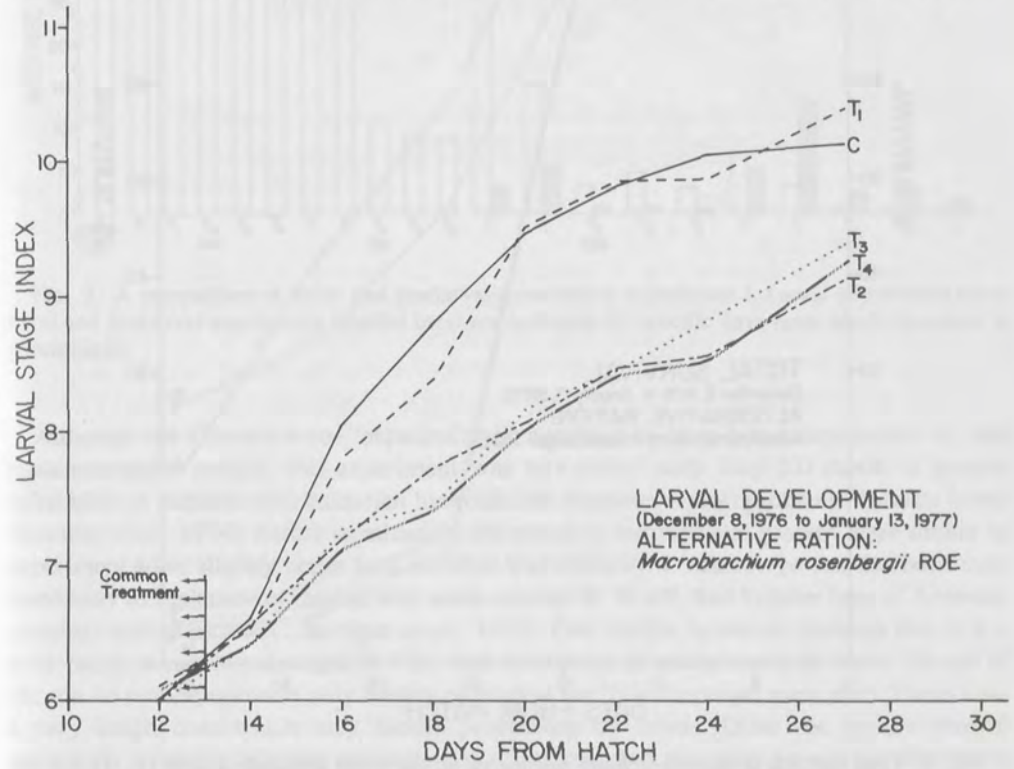

FIG. 5. *Macrobrachium rosenbergii* larval development in experiment I (see text for explanation of LSI).

larval/postlarval dynamics reiterated the failure of the complete replacement of *Artemia* nauplii with *M. rosenbergii* roe at stage V of larval culture. Total survival (Fig. 6) was less than 10 %, and total postlarval production (Fig. 7) less than 5/1 in roe replaced cultures. Overall, the results from parameters measured (larval growth, survival, and postlarval production) closely followed the rate of replacement of *Artemia* nauplii with roe. The reinstitution of nauplii in the latter stages of culture (T_3 and T_4) did little to revive these treatments. Total survival data were compared through single classification analysis of variance (ANOVA) with selected *a*

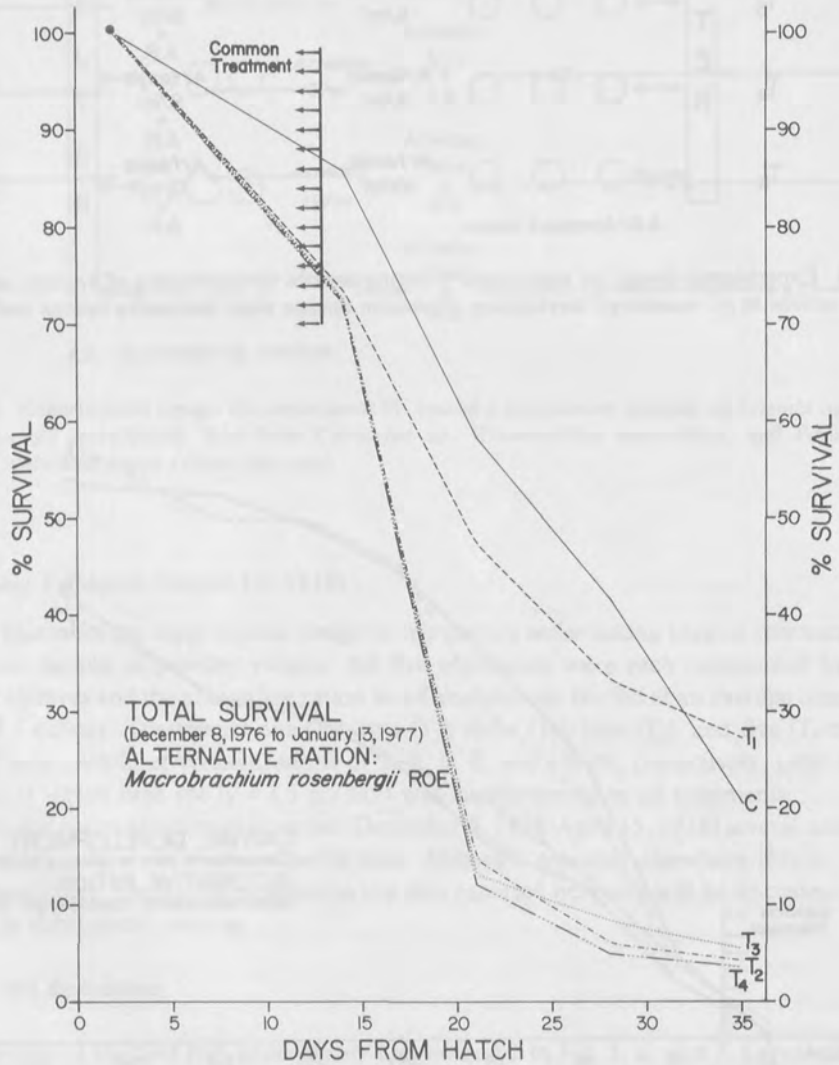

FIG. 6. Total survival of *Macrobrachium rosenbergii* larvae in roe replaced cultures (T_2, T_3, T_4), roe supplemented cultures (T_1), and controls (experiment I).

priori comparisons. Overall treatment variance was significant at the 95% level, supported primarily through differences between T_1-controls and the rest of the treatments ($\alpha = 0.01$). As subgroups there was no significant difference between nauplii maintained cultures (C) and roe supplemented cultures (T_1) and no significant difference between the nauplii deleted cultures (T_2, T_3, T_4).

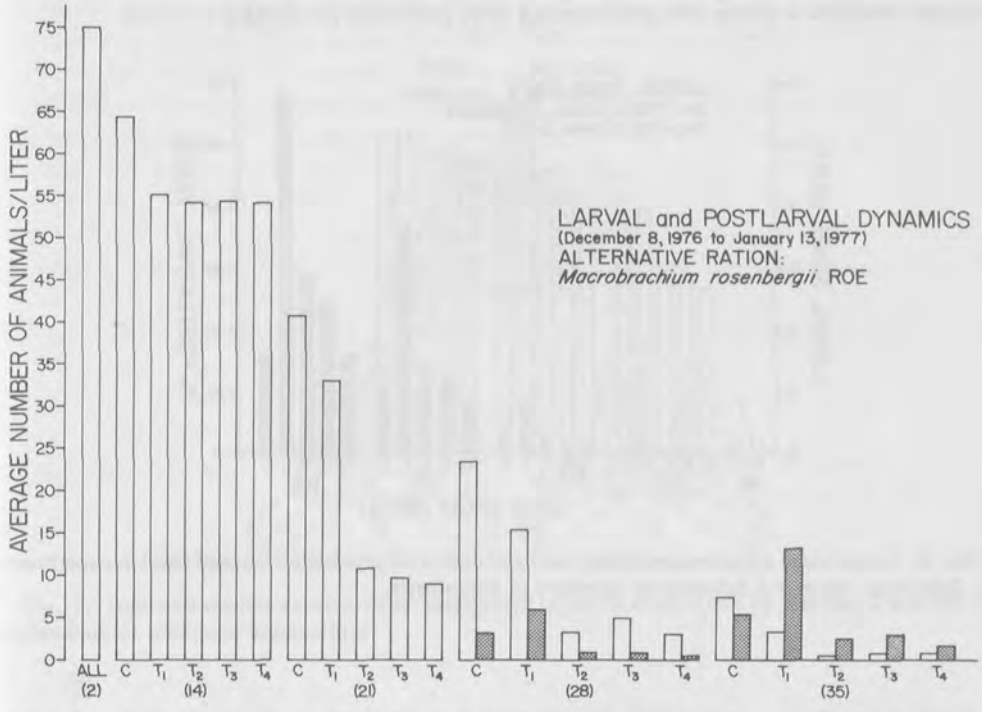

FIG. 7. A comparison of larval and postlarval dynamics in experiment I. Larval populations (clear bars) and postlarval populations (shaded bars) are indicated for specific days from hatch (numbers in parenthesis).

Although the *Cynoscion* roe (experiment II) appeared to be a better supplement to, and replacement for nauplii, this experiment was terminated early (day 23) due to a general infestation of cultures with cnidarian hydroids and medusae – most probably *Moerisia lyonsi* (Sandifer *et al.*, 1974). Before termination, the trends in survival and growth were similar to experiment I but slightly better total survival was evident. *M. rosenbergii* roe has been used previously as replacement rations with some success (R. Wulff, Red Lobster Inns of America, personal communication ; Berrigan *et al.*, 1978). Our results, however, indicate that it is a poor ration at mid-larval stages (V-VII). This divergence of results could be due to the age of the roe. In our experiments only freshly oviposited roe (bright orange) were used. These have a very tough coat which may hinder penetration by larvae. Older roe (yellow-brown) apparently exhibit a thinning and softening of the coat and may thus be a better alternative ration.

Experiment III, which tested the use of nauplii at daily, 2 day, 3 day, and 4 day intervals in conjunction with a daily ration of *Cynoscion* sp. roe, indicated that intermittent feeding of nauplii was acceptable with appropriate supplemental rations. Larval development (Fig. 8) was significantly slower in T_2, T_3, and T_4 by day 21 and postlarval production (Fig. 8) did not peak in these cultures until day 43. Larval stage advancement was more rapid in T_1 and controls and postlarval production peaked by day 35 in both culture sets, although control cultures exhibited a rather low comparative total postlarval production ($\sim 13/l$).

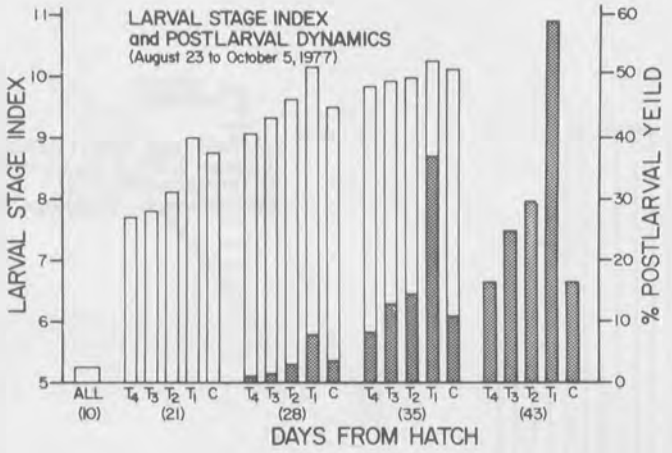

FIG. 8. Larval stage advancement (clear bars) and postlarval production (shaded bars) in experiment III. Days from hatch are indicated by numbers in parenthesis.

Total and relative survival (Fig. 9) indicated that the feeding of nauplii at 3 and 4 day intervals was detrimental for the first 11-17 days of culture. Thereafter the relative survival rates compared favorably with cultures fed nauplii daily and every other day. One possible reason for these results is that the older larvae utilized the alternative ration (roe) more effectively, thus reducing the requirement for nauplii. Thus one alteration to standard feeding protocols would be to use nauplii only on an every two-three day interval after the larvae have reached an average LSI of 7.

Both survival and postlarval production data were analyzed by a single classification ANOVA with selected *a priori* comparisons between means. Overall treatment variance was significant ($\alpha = 0.05$) for both survival and postlarval production. There was no significant difference in survival between T_2, T_3, and controls although all were significantly ($\alpha = 0.05$) higher than T_4. There was also no significant difference between T_2 and T_3 in postlarval production although both were significantly higher ($\alpha = 0.05$) than either T_4 or controls.

The fourth experimental series (Fig. 3) tested the use of alternative rations (fish roes) as complete replacements for nauplii at progressively later stages of the larval cycle. Larval stage advancement was strikingly similar in all treatments until day 25, thereafter treatments T_2 and T_3 advanced at a slightly slower rate. Total survival, however, deviated greatly between treat-

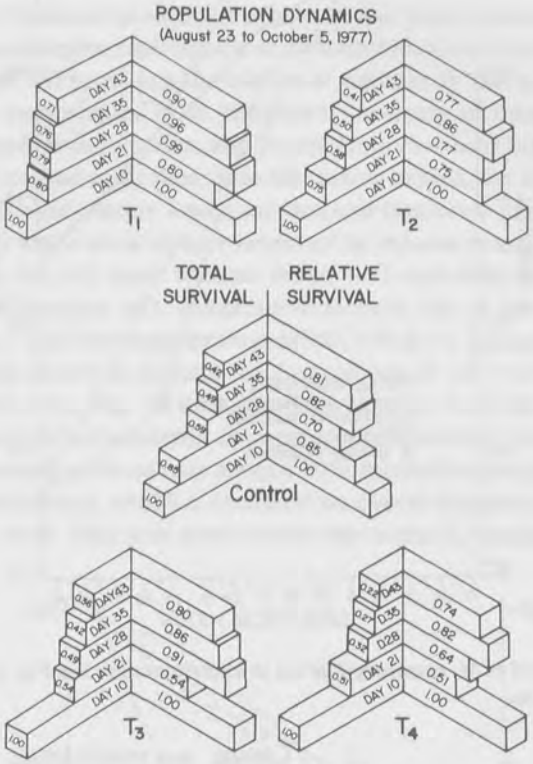

FIG. 9. Total and relative survival of *M. rosenbergii* larvae in experiment III. See Fig. 2 and text for explanation of treatment designations.

ments (Fig. 10) with those subjected to the earliest deletion of nauplii (T_2, T_3) exhibiting the lowest survival. The controls and treatment T_4 (nauplii removed at stage X) had higher and similar survival rates (76.4 and 74.8%, respectively) and treatments T_1 and T_{1p} (nauplii and roe throughout) exhibited exceptional survival (84.2 and 85.8%, respectively) throughout the tenure of the study. Analysis by single classification ANOVA with *a priori* comparisons of means indicated an overall significant difference ($\alpha = 0.01$) of survival data between treatments. The well defined survival curves illustrated in Fig. 10 were justified by the analysis which indicated significant differences ($\alpha = 0.01$) between (T_1 and T_{1p}), (C and T_4), and (T_2 and T_3) but no significant differences within each subgroup.

Postlarval production is illustrated in Fig. 11. Production reached its highest rate in the T_1, T_3, and T_4 cultures by day 34 and by day 40 in the remainder. Total postlarval production was exceptional in the T_1, T_{1p}, and T_4 cultures (79.2, 76.0, and 70.1%, respectively), somewhat lower (65.3%) in the controls, and lowest in the T_2 and T_3 cultures (43.4 and 52.7%, respectively). A single classification ANOVA with *a priori* comparisons among means indicated no significant differences in postlarval production among the T_1, T_{1p}, T_4, and control cultures but indicated that these treatments had significantly higher production ($\alpha = 0.01$) than

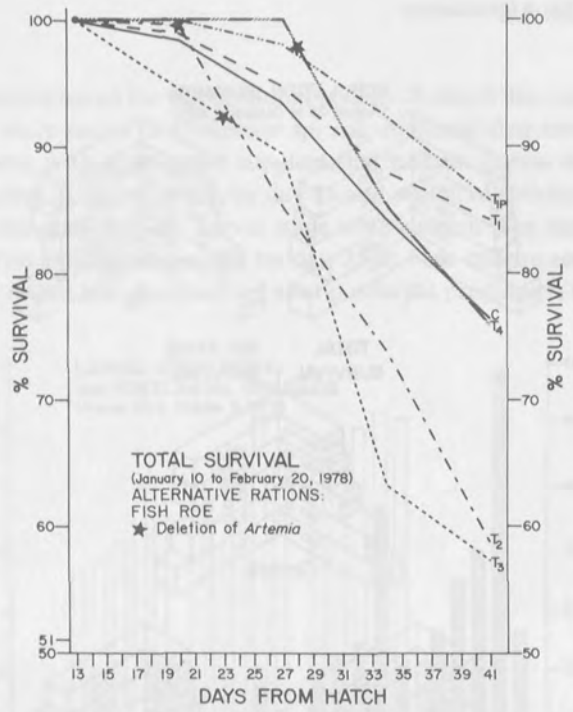

FIG. 10. Total survival of *M. rosenbergii* larvae in experiment IV. See Fig. 3 and text for explanation of treatment designations.

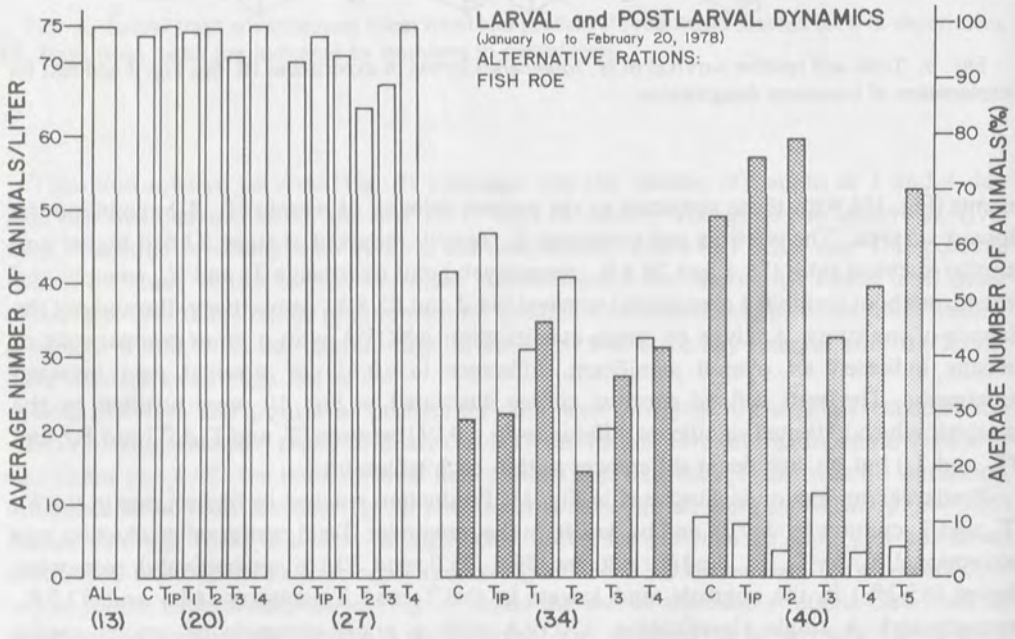

FIG. 11. Larval (clear bars) and postlarval (shaded bars) dynamics in experiment IV. Days from hatch are indicated by numbers in parenthesis.

the T_2 and T_3 cultures. Postlarval production in general was high in this experiment (> 43 % or 32/l) indicating that complete replacement of nauplii with a suitable alternative ration is feasible as early as stage VII providing the culture is vigorous at that point.

Experiment IV also incorporated in its design a comparison of three different fish roes (*Cynoscion* sp., *Rhomboplites aurorubens*, and *Paralichthys dentatus*) both separately and in combination. While all proved to be acceptable alternative rations, the snapper roe (*R. aurorubens*) fed cultures exhibited slightly lower survival and postlarval production.

Experiment V (Fig. 4) was a larval culture series testing reduced nauplii rations (2, 4, 6, and 8/ml) against controls (10/ml) both without (stage I-V) and with (stage VI-postlarvae), supplemental rations (*Cynoscion* sp.). Initial survival from day 0 to day 12 showed no significant difference ($\alpha = 0.05$) between treatments (all $\geqslant 91.1\,\%$). Larval stage advancement, however, was somewhat slower in cultures fed 2 nauplii/ml ($\bar{y}$ LSI = 5.73) than in the other treatments ($\bar{y}$ LSI = 5.82-5.91). Fig. 12 illustrates total survival from day 12 to termination. Only treatment T_1 (2 nauplii/ml) exhibited any appreciable mortality (71.6 % survival by day 33) while all other treatments suffered only about a 10 % mortality throughout the larval cycle (day 40). A single classification ANOVA indicated an overall treatment variance that was significant at the 99 % level. This was undoubtedly due to the T_1 treatment variation.

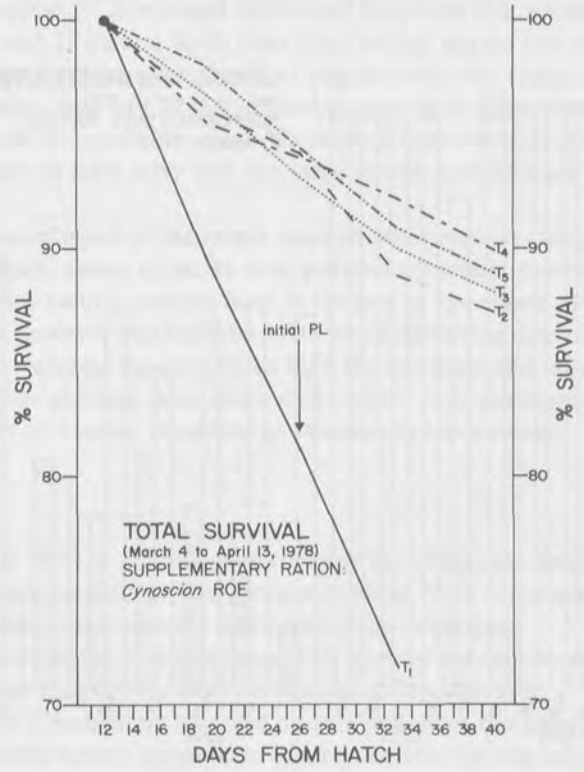

FIG. 12. Total survival of *M. rosenbergii* larvae in experiment V. See Fig. 4 and text for explanation of the treatment designations.

Larval and postlarval dynamics are presented in Fig. 13. Postlarval production was again exceptional in this culture series with highest production (T_4) exceeding 81% (61 postlarvae/l). A single classification ANOVA showed no significant difference ($\alpha = 0.05$) between the postlarval production of treatments T_2, T_3, T_4, and T_5 while indicating that the production of T_1 was significantly lower ($\alpha = 0.01$) than the other treatments. Deleting the T_1 cultures it is possible to show the exceptional production of this culture series by reporting that over 26 000 postlarvae were produced in a volume of approximately 480 l of culture space (twelve 40 l culture containers).

The results of this experimental series indicate that as few as 2 nauplii/ml can sufficiently support early stage (I-V) development of *M. rosenbergii* larvae stocked at initial densities of 75 larvae/l. With appropriate supplemental rations a protocol using 4 nauplii/ml can be used to rear larvae from stage 5 to metamorphosis. This translates to only 20% of the traditional nauplii ration for early larval stages and only 40% for later larval stages.

In addition to the above, several coincidental studies were performed during the time frame of this investigation. While they are reported elsewhere (Berrigan *et al.*, 1978 ; Murai and Andrews, 1978 ; Sandifer and Williams, 1980 ; Manzi *et al.*, unpublished) some data are pertinent to this investigation and should be mentioned for comparative reasons.

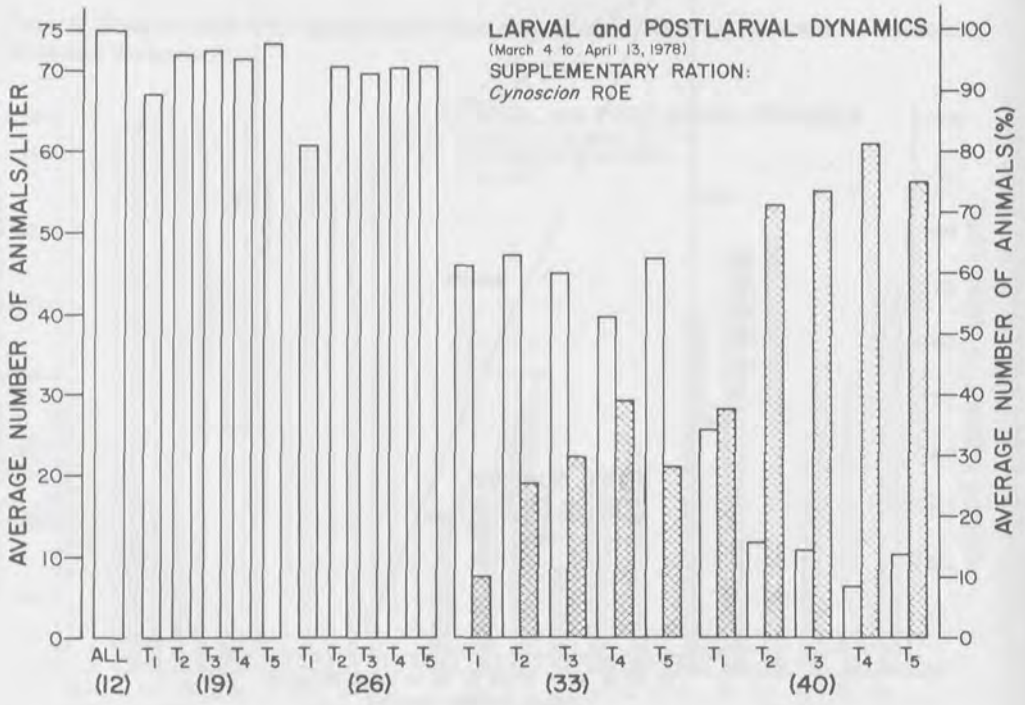

FIG. 13. Larval (clear bars) and postlarval (shaded bars) dynamics in experiment V. Days from hatch are indicated by numbers in parenthesis.

The general shortages and high price of *Artemia* cysts generated a significant amount of interest in the development of non-living rations. Sandifer and Williams (1980) examined a total of 18 non-living test diets segregated in four type classes : 1) frozen natural products (fish roes), 2) freeze-died natural products (beef organs, earthworms, oyster flesh, euphausiids, prawn, and shrimp), 3) "dry" prepared rations (Purina MR-25, Tetramin, Colvin's, and Meyer's), and 4) "wet" prepared diets (poached egg with and without squid). Although they had limited success rearing *Palaemonetes* spp. larvae with two diets (freeze-dried squid and euphausiids) none of their non-living diets approached the growth and survival of the *Artemia*-fed controls. Manzi *et al.* (unpublished) repeated many of these same diets with *M. rosenbergii* larvae with similar results. Alone, none of the non-living diets were comparable replacements for *Artemia* nauplii in early larval culture. In later culture (> stage V), however, several diets (fish roes, poached hen's egg both alone and with squid) were acceptable either alone or in combination with reduced nauplii rations. The results of Murai and Andrews (1978) had similar connotations, indicating that freeze-dried oyster and catfish flesh were acceptable supplements to, but not suitable replacements for *Artemia* nauplii in *M. rosenbergii* larviculture.

Berrigan *et al.* (1978) tested a large number of alternative rations for early stage (to stage V and VI) culture of *M. rosenbergii* larvae. These diets included : 1) a live food organism (the rotifer, *Brachionus plicatilis*), 2) formulated diets (dried flakes and gels, encapsulates and two egg based granulates), and 3) natural foods (roes from mullet, prawn and lobster, hen's egg and clam flesh). The live food organism (rotifers) was the only diet comparable to *Artemia* nauplii in this study. Other diets had, at best, limited success, although several appeared to be beneficial as supplements (primarily the roes). Manzi *et al.* (unpublished) tested many of the same formulated rations on both early and late stage larvae, and obtained much the same results.

The general consensus of much of the recent research performed on alternative rations for *M. rosenbergii* larviculture, seems to be, at best, guarded optimism. Results to date, show considerable success with natural product diets in the mid to late stages of the larval cycle. Little to limited success, however, has been the norm for all non-living diets tested on the early larval stages. The study reported here reiterated both the successes and failures of the more recent diet studies while defining and delineating, albeit in a preliminary manner, the minimum requirements of *Artemia* nauplii in *M. rosenbergii* larviculture.

Conclusions

1. Early larval stages (I-V) of *M. rosenbergii* appear to subsist and develop on as low a concentration as 2 *Artemia* nauplii/ml/day (larvae stocked at 75/l). Although a concentration of 4 nauplii/ml/day was a more suitable non-limiting ration protocol.

2. These same concentrations in conjunction with appropriate supplemental rations also appeared to be a suitable protocol for later larval stages (VI-postlarvae).

3. A concentration of 5 nauplii/ml allocated on an every other day schedule was suitable for later larval stages when a daily supplement of an acceptable fish roe was incorporated in the feeding regime.

4. The same concentration (with the supplement) allocated on an every 3 or even 4 day schedule was suitable for very late stage larvae (⩾ VIII).

5. Complete replacement of *Artemia* nauplii is feasible as early as stage VII with suitable alternative rations (fish roes).

6. Exceptionally high postlarval production (81.1 %, 61 postlarvae/l) can be obtained with intensive feeding protocols of 8-10 nauplii/ml/day supplemented with an average of 3.5-5.0 cc fish roe/tank (40 l)/day.

7. Larval cultures completely denied of nauplii (maintained only on fish roes) between stages V and VII, could not be significantly revived by the readdition of nauplii after stage VII.

Acknowledgements

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Improvements in rearing larval penaeid shrimp by the Galveston Laboratory method[1]

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Abstract

Improvements in rearing larval penaeid shrimp by the Galveston Laboratory method are presented in this paper. The use of froze algae and frozen *Artemia* nauplii as food for larval penaeid shrimp in hatchery systems is discussed. Also presented are culture techniques for the rotifer *Brachionus plicatilis*, and descriptions of its use as a shrimp larvae food intermediate to algae and *Artemia* nauplii. A lengthy discussion concerning problems and their solutions encountered in the Galveston Laboratory shrimp hatchery is presented.

Introduction

Whether it be for research or commercial intent, one of the more crucial links in attempting to produce cultured penaeid shrimp is the hatchery facility. Without the ability to produce large numbers of healthy postlarvae, growout and maturation are useless. In the past 15 years a vast array of equipment and techniques have been tried and developed for rearing larval penaeid shrimp. Chief among these are the systems developed by the National Marine Fisheries Service, Galveston Laboratory, Galveston, Texas, that are now known worldwide as the "Galveston" method.

The penaeid shrimp rearing techniques developed at Galveston have been described by Mock and Murphy (1970), Salser and Mock (1972), and Mock and Neal (1974). These techniques produced survival rates ranging from 65 to 85% while maintaining population densities as high as 260 postlarvae/l. Problems inherent in the use of live *Artemia* still remained, however, including larval mortalities incurred during the transition from frozen algae to live feed.

Since the first paper by Mock and Murphy (1970), experimentation at Galveston has shown that similar densities and survival rates can be achieved using frozen rotifers, *Brachionus plicatilis*, and frozen brine shrimp, *Artemia* as foodstuffs. These feeding modifications have

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simplified the rearing process considerably by eliminating the need for simultaneous live cultures and the crises of aborted shrimp hatches due to the failure of live foodstuff.

This paper reiterates methods used at Galveston and discusses the preparation and use of frozen rotifers and frozen brine shrimp for use as foodstuff in the hatchery. Also presented are several other items of interest related to the Galveston Laboratory culture system. Recently, bakers' yeast has been successfully substituted for frozen algae as foodstuff in the Galveston hatchery ; its use is described in another paper (Mock *et al.*, 1980).

Materials and methods

Hatchery

The conical fiberglass hatchery tanks used at Galveston have a capacity of 2000 l and are fitted with airlift pumps, center screens, and external in-line filters. The airlift pumps keep both the larval shrimp and foodstuff evenly distributed throughout the water column while the water in the tank can either be discharged or recirculated with the center screen in place by operating the drainage or filter housing valves. Detailed descriptions of this equipment have been described by Salser and Mock (1972).

Foodstuff organisms

Algae culture

The basic techniques for culturing algae at the Galveston Laboratory have been described by Kenslow (1970) and Griffith *et al.* (1973). A 10 ml innoculum of either *Skeletonema costatum* or *Tetraselmis chuii* may be expanded to a 320 l culture in 16 days. The algal cultures are then concentrated by harvesting through a cream separator and the resulting concentrate is frozen in plastic cups as described by Mock (1971). *S. costatum* is frozen in quantities providing densities of 25 000, 50 000, or 100 000 cells/ml while *T. chuii* is packaged in quantities providing densities of 5 000, 10 000, or 20 000 cells/ml when either is added to the 2000 l hatchery tank.

Rotifer culture

Rotifers, primarily *B. plicatilis*, are cultured in rectangular fiberglass tanks (1.0 m × 0.6 m × 0.6 m ; working volume of 400 l) under a fiberglass shelter using natural light and ambient temperature as described by Fontaine and Revera (1980). Best growth occurs on sunny days at salinities of 28-32 ‰, temperature of 27-29 °C, and pH of 7.7-8.7. The rotifers are fed a water slurry of torulose yeast at a rate of 0.2 g/l. The culture tanks are stirred each morning after feeding but are not aerated, which allows the tanks to become anaerobic. It appears that anaerobic conditions are essential for rapid proliferation of rotifers. Population densities of 500 to 700 rotifers/ml are reached within 15-20 days. The rotifers will aggregate at the surface 1 to 2 hr after stirring the tanks and those cultures that have attained a density of 500/ml are harvested. Rotifer cultures apparently can be harvested daily for a period of 45 days, once production density has been achieved, without causing a permanent reduction in the standing crop.

Harvesting is accomplished by skimming the surface of the tank. The rotifer harvest is concentrated by mixing thoroughly with an equal volume of deionized water in a 2 l separatory funnel. The deionized water shocks the rotifers that then settle to the bottom where they may be removed and frozen. Currently, however, drained rotifers are resuspended in clean seawater (28-34 ‰) and aerated gently for 24 hr. The rotifers are poured through a 69 μm filter, diluted to 1 l with fresh seawater and fed frozen *T. chuii* at a level of 10^6 cells/ml after microscopic examination has ascertained that the rotifers' digestive tracts have been emptied. The rotifer-alga solution is gently stirred and after 2 hr the rotifers are again harvested by filtering through a 69 μm net. An aliquot count is made and the rotifers are frozen in seawater in plastic cups in quantities yielding levels of 5, 10, and 20/ml when added to the 2 000 l hatchery tanks.

Artemia *hatching*

An earlier hatchery system for *Artemia* has been described by Salser and Mock (1972) but decapsulated *Artemia* cysts, as documented by Bruggeman *et al.* (1979) are also used. Once the *Artemia* concentration is established with the method of Salser and Mock (1972), a predetermined amount is measured into a container for freezing. The container should be deep enough to allow for 2 to 3 cm of liquid. Convenient densities for use with the 2 000 l tank are 0.5/ml, 1.0/ml, 2.0/ml, and 3.0/ml.

Preparation for feeding

S. costatum and *T. chuii* may be thawed in either distilled or deionized water. The algae are poured through a fine mesh screen (45 μm) as they begin to thaw. Large particles that are retained are mashed and then washed through the screen. Rotifers must be thawed in seawater to prevent lysing before being passed through the 45 μm screen for clump elimination.

Examination of frozen *Artemia* containers has shown that freezing induces a physical separation of unhatched cysts and nauplii. The unhatched cysts are stratified in the surface layer. If enough water is initially put in the container before freezing, the surface layer containing the unhatched cysts may be flushed away with tap water after freezing. The frozen block of nauplii can then be either thawed in seawater and poured in the hatchery tank or the frozen block itself may be placed in the hatchery tank where it will thaw in about 5 min.

Water quality and treatment

All seawater used for shrimp culture at Galveston is filtered through a 5 μm filter and treated with ethylene-dinitrilo tetra-acetic acid disodium salt (EDTA) at 0.01 g/l and erythromycin[2] at 5 mg/l. Salinities are adjusted to a minimum of 28 ‰ with Instant Ocean (a synthetic sea salt) and temperatures are maintained at 28 °C with a thermostatically controlled immersion heater.

[2] Reference to trade names in this paper does not imply endorsement by the National Marine Fisheries Service, NOAA.

Hatchery procedures

The Galveston hatchery systems have been used successfully to culture a wide variety of crustaceans and finfish (Table I). The most serious fault with the system in regards to crustaceans has been the dependency upon wild gravid females collected by shrimp boats from offshore for spawning stock. In August 1979, the Galveston Laboratory successfully matured, mated, and spawned the blue shrimp, *P. stylirostris* (Brown *et al.*, 1980).

Table I

Crustaceans and Finfish reared to the postlarval stage utilizing the Galveston Laboratory technique

Scientific name	Common name
Crustaceans	
Penaeus aztecus aztecus	brown shrimp
P. brasiliensis	Brazilian shrimp
P. brevirostris	pink shrimp
P. californiensis	brown shrimp
P. duorarum duorarum	pink shrimp
P. japonicus	banded shrimp
P. merguiensis	banana prawn
P. monodon	sugpo
P. occidentalis	blue shrimp
P. schmitti	white shrimp
P. setiferus	white shrimp
P. stylirostris	blue shrimp
P. vannamei	blue shrimp
Xiphopenaeus kroyeri	sea bob
Sicyonia brevirostris	rock shrimp
Macrobrachium rosenbergii	river shrimp
Finfish (with aeration modifications)	
Scienops ocellatus	redfish
Pogonas cromis	black drum
Morona saxatilis	striped bass

Spawning

The female shrimp that appear to be gravid are separated and placed in individual containers. The segregation of females allows for discarding of incomplete spawns, separation of spawns occurring at different times to reduce cannibalism, and provides for a more homogeneous population in the hatchery tanks. After spawning, the female shrimp is removed, the spawning water is replaced with fresh seawater, and the eggs are counted. The eggs remain in the spawning vessel until hatching is complete. A larval population count is then made and the nauplii transferred to the hatchery tanks. The transfer is accomplished by one of two methods : 1) the entire contents of the spawning container is emptied into the hatchery tank, or 2) the nauplii are attracted to the surface by light-attraction and removed by siphoning.

LARVAE

Nauplius

The water in the hatchery tank is filtered through a central screen (69 μm) and is recirculated through an external in-line filter (10 μm) during early larval stages. Water filtration continues until the addition of food, at which time it must be discontinued to prevent food loss.

The larval shrimp are absorbing their yolk sac throughout naupliar stages I-V and do not require feeding. Their metamorphosis to protozoea should be anticipated, however, and food (algae) added to the tank during the later naupliar stages. Under the temperature regimes described, nauplius IV of *P. aztecus* and *P. setiferus* usually occur in the afternoon; nauplius V in the evening; and protozoea I the following morning. *S. costatum* is added at a level of 50 000 cells/ml at late N-IV or early N-V (usually in the afternoon) in preparation for the first larvae to metamorphose. Only *S. costatum* is fed until 75% of the population has metamorphosed to the late stage of protozoea II. *T. chuii* is introduced at a level of 5 000 cells/ml once this stage is reached. The *T. chuii* level is increased to 20 000 cells/ml while allowing the remaining *S. costatum* to be grazed.

Rotifers, *B. plicatilis*, are added to the tank at a level of 20/ml once the entire population has metamophosed to protozoea III. Care must be taken, thereafter, to prevent the rotifer density from dropping below 10/ml.

Mysis

The density of rotifers should be maintained at a level of 20/ml during mysis I and early mysis II. Frozen *Artemia* may be introduced at a level of 1.0/ml at late mysis II. The *Artemia* level should be increased to 3.0-4.0/ml when it is evident that the level of rotifers has dropped and that the *Artemia* are being consumed. At mysis III, live *Artemia* are substituted for frozen *Artemia* at the same level.

Postlarvae

An *Artemia* level of 3.0-4.0/ml is maintained until the postlarval shrimp are 3 days old (PL-3). If the standard 2 000 l conical tanks are being used, the population must be harvested at this time to prevent widespread cannibalism.

Feeding levels

It is of paramount importance to maintain the required density of food in the tanks at all times. The shrimp must never be allowed to graze the food below these specific levels. Therefore, food density must be increased as food requirements become greater. A suggested feeding regime for all stages of larval development is illustrated in Appendix (actual experimental data).

Discussion

In the decade following the publication of Cook and Murphy (1969) research efforts at the Galveston Laboratory were guided primarily by the need to solve known problems. During

this period of experimentation, each new study evolved its own set of new problems to be overcome. These problems, difficulties and their solutions are discussed here.

Hatchery

Although the conical hatchery tank has proven satisfactory for the culture of larval shrimp at Galveston, it is necessary to harvest the postlarvae after they are 2-3 days old to reduce cannibalism. It would be economically advantageous, however, to rear the postlarval shrimp to a larger size in the hatchery vessel before stocking in ponds or raceways. Semi-closed penaeid shrimp culture raceways, as described by Mock *et al.* (1973), used as hatchery tanks may be the answer. In Natal (Brazil), Dr. Tupan de Souza, General Coordinator of the Shrimp Culture Project, has built six concrete raceways each with a working volume of 3 m^3. They routinely culture 800 000, 20-25 day old postlarvae (*Penaeus brasiliensis*) per raceway beginning with a stocking density of 300/l or a total of 10^6 larvae. It should be pointed out that the raceway as a hatchery tank has not been tried in Galveston, although we plan to do so in the near future.

Regardless of whether a conical tank or a raceway is used as the primary hatchery vessel it is necessary to use airlift pumps. The airlifts continuously return food from the bottom to the surface resulting in an even food distribution throughout the hatchery tank. An even distribution of foodstuff in the water column is absolutely essential for early larval stages of penaeid shrimp that are primarily pelagic.

Larval shrimp do not have the ability to search for food, but must come in contact with it. Therefore, it is the density (particles/ml) throughout the water column that determines if the larvae are going to graze or starve.

Algae

Initially, seawater was used to thaw frozen algae and the algae were added to the hatchery tank without regard to possible effects on water temperature. Under these conditions the algae tended to mass in clumps near the surface. Elimination of this clumping tendency as well as of the worry of undesirable temperature variations was achieved by thawing the algae in distilled water before feeding and adjusting the temperature to within ± 2.0 °C of the hatchery water.

Rotifers

In the mid-1970's, the demand for *Artemia* cysts exceeded the supply, resulting in a sharp increase in price. In fact, between 1969 and 1976 the price of a 1 280 g can of *Artemia* cysts increased from US $19.80 to $70.00. When a search was begun in 1975 for an *Artemia* substitute, economics was an important consideration; however, it was not the sole factor. A large difference exists between the sizes of food particles of algae and *Artemia* nauplii as indicated in Table II. There existed a need for a food organism intermediate in size to algae and naupliar *Artemia*. Such a feed would not only increase survival rates but would also reduce the required amounts of *Artemia*.

Michel (personal communication Michel Alain, Centre Océanologique du Pacifique, B.P. 7004 Taravoo, Tahiti, Polynésie Française) noted that there is usually a portion of the penaeid shrimp population that does not survive the transition from algae to *Artemia*, perhaps as a

result of the change in particle size. Ito (1960) suggested that rotifers could be used as food for a variety of marine organisms, and Hudinaga and Kittaka (1966) reported that rotifers were a good intermediate food to bridge the gap between algae and *Artemia* in penaeid and finfish cultures. They introduced live rotifers, usually along with the culture medium, directly into hatchery tanks.

TABLE II
Size comparison of various feed items

Species	Size
Skeletonema costatum	2-4 μm diameter
Tetraselmis chuii	13-15 μm diameter
Brachionus plicatilis	50-300 μm (total length)
Artemia (San Francisco Bay Brand)	
Cyst Decapsulated	210 μm diameter
Non-decapsulated	225 μm diameter
Instar 1	0.43 mm (total length)
Instar 2	0.6 mm (total length)
Instar 3	0.7 mm (total length)

In our studies at Galveston, once rotifers are concentrated in deionized water they must be put into a salt solution to be frozen. Comparison studies of thawed rotifers that have been frozen in distilled water and in seawater showed that those frozen in distilled water tended to disintegrate while those frozen in seawater did not.

Artemia

There are a number of problems associated with the feeding of live *Artemia* to larval shrimp. A great deal of time, effort, and equipment must be invested daily to insure that adequate *Artemia* are available. It is necessary to maintain a costly surplus stock to safeguard against a batch of cysts that are inferior in quality or quantity.

While freshly hatched *Artemia* can be concentrated at 10 000 to 13 000/ml and stored in refrigerators at 11 °C for several days, careful monitoring is still required to prevent spoiling. Some development still takes place although the lower temperature slows down metabolism. The containers must also be provided with an airstone providing bubbles continuously and water changes are required every other day. *Artemia* have been held in volumes of 20 l successfully for up to 6 days at the Galveston Laboratory.

Live naupliar *Artemia* compete biologically with the larval shrimp production. They not only consume algae and dissolved oxygen but they also add their waste products to the system. A portion of the live *Artemia* fed into hatchery tanks are not consumed and survive to adulthood. This greatly complicates hatchery tank conditions as it results in a tri-partite culture including three distinct populations: larval shrimp, *Artemia* destined to be consumed by the shrimp, and mature *Artemia* immune to the predation of larval shrimp.

Tetraselmis levels in the hatchery tank required very close monitoring before rotifers were used as an intermediate food between algae and *Artemia*. *Artemia* accept and will very readily graze *Tetraselmis*; later, if *Artemia* are introduced into a system with high levels of *Tetraselmis* (20 000 cells/ml) the algae can be very rapidly consumed. In early experiments this phenomenon provided a perplexing problem. The larval shrimp still requiring *Tetraselmis* had to compete with the *Artemia* and any increase in the level of *Tetraselmis* resulted in dramatic increases in the *Artemia* population and consequently, *Tetraselmis* consumption. The larger larval shrimp were grazing *Artemia* at the same time necessitating their presence. Removing the *Artemia* population from the hatchery tanks is presently impracticable.

Artemia were hatched, concentrated, frozen, and stored before hatchery start-up. The frozen *Artemia* were then fed at the same rate as the live *Artemia* and were found to be equally acceptable to the larval shrimp. Originally, *Artemia* cysts were hatched and then fed directly to the larval shrimp. If the cysts have been decapsulated, however, the larval shrimp will consume not only the hatched *Artemia* but the decapsulated cysts as well. Furtherfore, decapsulated cysts will hatch more readily than the non-decapsulated, even within a hatchery tank.

In recent experiments in the Philippines, at the Southeast Asian Fisheries Development Center, *Artemia* that had been decapsulated and frozen for 24 hr were thawed and fed to larval shrimp of *P. monodon*. A number of the cysts were consumed and after a 24 hr period the remaining cysts began to hatch [Mock and Sorgeloos, (personal communication Patrick Sorgeloos, Artemia Reference Center, State University of Ghent, Ghent, Belgium)]. Although the decapsulated cysts sink, the action of the airlift pumps keep them suspended in the hatchery tank. Mock and Sorgeloos furthermore observed that postlarvae *P. monodon* feeding upon adult *Artemia* ate only the head and the thoracopods, ignoring the digestive tract. If the adult *Artemia* were placed in clean water and allowed to discharge the contents of their digestive system, however, the shrimp consumed them entirely (Sorgeloos and Mock, personal communication).

Biological control

The free-swimming dinoflagellate, *Oxyrrhis marina*, has been observed in the hathery water on occasion. In large quantities it may compete with the larval shrimp for *Tetraselmis* for food although its presence is not directly harmful. Reproduction is by fission and is very rapid when associated with *Tetraselmis*. Attempts were made to reduce the density of *O. marina* in the initial bloom by changing water. The dinoflagellate remained in the tank, however, and continued its consumption of the algae. Microscopic examination of individual dinoflagellates showed recent ingestion of 10-15 *Tetraselmis* cells.

On another occasion when we observed this condition in the hatchery tank the water was not changed but, instead, the feed was switched from *Tetraselmis* to *Skeletonema*. The larval shrimp began to graze the *Skeletonema* once the remaining *Tetraselmis* was consumed but the dinoflagellate rejected the *Skeletonema* and died out. When the *Tetraselmis* was continued, the larval shrimp reverted to the larger feed while the dinoflagellate did not reappear. Population counts of the larval shrimp indicated that they had survived. This technique has since been used successfully several times.

The dinoflagellate, *O. marina*, may also be eliminated from hatchery tanks by recirculating the water through the interscreen to an external in-line UV-light. The external filter is

bypassed during this operation. A germicidal intensity (microwatts/cm², μWs/cm²) of 2 537 Å at pumping rates of 68 and 454 l/hr have been successful in eliminating *O. marina* as described by Mock *et al.* (1980). The UV-light is not detrimental to the algae since the algae are fed dead.

Air stripping

A yellowish-brown foam will begin to accumulate on the surface of the hatchery water after the addition of food and after the larval shrimp begin feeding and feces are observed. This foam is indicative of a buildup of dissolved organics and should not be confused with the foam sometimes present after the addition of algae.

Early attempts to deal with this problem involved replacing 1/3 to 1/2 of the water but more recently another solution has been devised. The water level of the tank is lowered about 15-20 cm below the tops of the airlift pumps or until only bubbles and no water are being discharged. The airlift acts as a protein skimmer and the foam may be removed by simply lifting it from the tank. The foam should be examined to make certain that the larval shrimp are not being trapped during this procedure. The water level is raised to its original height in the tank after about 30 min or until the bubbles are colorless.

Daily hatchery tank inspection

Although the configuration of the hatchery tank and the action of the airlift pumps keep most of the particles suspended in the water column, some deposition does occur. The interface between a particle and the bottom becomes anaerobic and formation of hydrogen sulfide and methane begins once a particle has settled to the bottom of the tank. Since many of the compounds thus formed are toxic, this condition must be eliminated. The bottom of the hatchery tank must be inspected daily and brushed lightly when a buildup is noted.

Conclusions

The techniques for preparing and using frozen foodstuffs promises better efficiency to some operations previously requiring live feeds. Hatchery projects can now be undertaken with an assurance of an abundant, high quality food supply that could never be absolutely guaranteed with simultaneous live culture procedures. *Artemia*, when used in frozen form, gain a new dimension of versatility. They can now be used without fear of creating an unwanted extra tier in a hatchery tank population. Frozen foodstuffs also provide a new level of efficiency in meeting feeding requirements. Daily food requirements can be met with precision, and loss of a shrimp hatch does not mean the sacrifice of a costly live food culture. Conversely, the loss of a live food culture does not result in a lost hatch. The most economical use of space and equipment, in those circumstances where they are at a premium, can now be realized by committing scheduled periods once or twice a year for intensive food culturing.

Development of rotifer culture techniques has filled an important gap in the hatchery feeding cycle of penaeid shrimp larvae. Although the economic desirability of an *Artemia* substitute has become less critical due to the worldwide proliferation of producers and the concomitant advancements in *Artemia* culture and usage techniques, the use of rotifers as a transitional food solves the problem of abrupt changes in food particle sizes.

Recent developments have served to reiterate the benefits of the airlift pump in crustacean culture. In those instances where the feeding needs of pelagic organisms require the maintenance of food density levels, it is the method of choice. They make possible full utilization of frozen feeds and decapsulated *Artemia* cysts. In addition, they have some applications to water quality maintenance problems in the hatchery. For instance, the air-stripping of proteins resultant from the buildup of dissolved organics.

The development by the Galveston Laboratory of techniques to mate, mature and spawn the blue shrimp, *P. stylirostris* (Brown *et al.*, 1980) has been a major breakthrough in respect to hatchery technology. With an abundant year-round supply of nauplii we will now be able to more effectively place our hatchery in a research mode. Although the systems presently being used have worked well for us in the past, we believe there is room for improvement.

Given a dependable supply of shrimp larvae we will now be able to conduct statistically valid, replicated hatchery studies on such items as conical tanks compared with raceways, UV-light treatments, and a variety of other equipment modifications and developments. Additionally, testing of baker's yeast against frozen algae as larval foodstuff may proceed, and further testing of rotifers, nematodes, tardigrades, and lugworms as food for shrimp larvae may be done. Research, particularly that involving hatchery and maturation technology, can now proceed at an accelerated rate so that penaeid shrimp aquaculture can become more cost effective.

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Appendix

August 1976. Daily log of hatchery tank I.
Penaeus setiferus, nauplii (N), protozoa (P), mysis (M), postlarva (PL) ;
Skeletonema costatum (S), *Tetraselmis chuii* (T)

Day	Hour	Larval Stage	Larval Count	Algal Cells/ml		Remarks
				Residual	Feeding	
4	0800					Set-up 2 000 l tank, 28 ‰, 28 °C, add EDTA and erythromycin
5	2000	N-I	400 000			
6	0915	N-I-II	380 000			
	2140	N-III-IV	380 000			
7	0745	N-IV-V	380 000			
	0930				50 000S	
	1200	N-V - P-I		44 000S	94 000S	
	1500			70 000S	170 000S	
8	0800	P-I	380 000	84 000S		
	0830				184 000S	
	1345			123 000S	173 000S	
	1500	P-I		135 000S	185 000S	
9	0800	P-I-II	376 000	80 000S	180 000S	
	1700	P-II		120 000S	170 000S	
10	0800	P-II		80 000S	180 000S	
	1200			150 000S		
	1700	P-II		110 000S	5 000T	
11	0800	P-II-III	350 000	70 000S		
				2 500T	12 500T	
	1200			65 000S		
				8 000T	18 000T	
	1700	P-II		10 500T	20 500T	
				60 000S		

APPENDIX (continued)

Day	Hour	Larval Stage	Larval Count	Algal Cells/ml		Rotifers/ml		*Artemia*/ml				Remarks
								Frozen		Live		
				Residual	Feeding	Residual	Feeding	Residual	Feeding	Residual	Feeding	
12	0800	P-II		7 500T 50 000S	17 500T							Brush tank bottom
	1700			12 000T 45 000S	22 000T		10					Change 400 l H_2O
13	0800	P-III - M-I		13 000T 37 000S		5	10					
	1700	M-I		7 500T 34 000S		6	26					
14	0800	M-I		2 500T 32 000S		12	22					Brush tank bottom Change 400 l H_2O
	1700	M-I-II		2 500T 28 000S		14	24					
15	0800	M-II		2 000T 22 000S		12	22	0.2	0.5 2.2			
	1700			18 000S		18	28	1.0	3.0			Change 400 l H_2O
16	0800	M-II-III		18 000S		13	23	1.5	3.5		1.0	
	1700					10	20	1.5	3.5	0.5	3.5	
17	0800	M-III				16		1.5		2.5	3.5	
	1700	M-III, PL				10		1.0		1.5	3.5	
18	0800	PL	320 000					0.5		1.0	3.0	Harvest 80% survial

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International Study on *Artemia*[1] VIII. Comparison of the chlorinated hydrocarbons and heavy metals in five different strains of newly hatched *Artemia* and a laboratory-reared marine fish

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Abstract

Newly hatched nauplii of *Artemia* from Brazil, Australia, Italy, and the United States (Utah and San Pablo Bay, California) were analyzed for chlorinated hydrocarbons. The Brazil and Australia nauplii contained very low levels of PCB and chlorinated insecticides. Italian nauplii contained the highest levels of HCB, BHCs and DDTs, while San Pablo nauplii were highest in chlordanes, dieldrin and PCBs. With the exception of 188 ppb pp-DDT in Italy nauplii, none of the residues exceeded 100 ppb on a wet weight basis.

Atlantic silversides (*Menidia menidia*) and mud crabs (*Rhithropanopeus harrisii*) from two *Artemia* feeding studies were analyzed. Residue levels in the organisms generally reflected the concentrations in the nauplii.

Attempts to correlate nauplii residue levels with the survival of laboratory-reared marine fish (Atlantic silversides and winter flounder *Pseudopleuronectes americanus*) and crabs (mud crab and rock crab *Cancer irroratus*) disclose no obvious component(s) which could totally account for the poorer performance of the Utah and San Pablo strains.

The satisfactory performance of the Italian strain seems to eliminate HCB, BHCs and DDTs as causative agents *per se*. If chlorinated hydrocarbons are involved with the poorer survival of marine fish and crabs fed San Pablo nauplii, chlordane, dieldrin or high molecular weight PCBs would appear to be the most likely suspects.

Twelve metals, including copper, lead, and cadmium, were measured by atomic absorption and neutron activation analysis. Differences between nauplii were small and no particularly high concentrations were observed.

Introduction

In 1970, Bookhout and Costlow reported that *Artemia* nauplii from salt pools near San Francisco, California supported better survival of four crab larval species than nauplii from

[1] International Interdisciplinary Study on *Artemia* Strains coordinated by the Artemia Reference Center, State University of Ghent, Belgium.

the Great Salt Lake in Utah, and suggested that differences in performance might be due to different DDT levels in the nauplii (7.5 ppm *versus* 2.3 ppm, wet weight basis). Two years later, Wickins (1972) fed newly hatched *Artemia* from Utah and San Francisco to prawn larvae and again found that Utah nauplii did not support growth to the post larval stage. DDT levels in *Artemia* eggs and nauplii were considerably lower than those reported by Bookhout and Costlow (1970) (< 0.8 ppm for cysts and < 0.2 ppm for nauplii, dry weight basis). He also reported measurable quantities of PCBs (0.04-0.24 ppm). Analysis of eggs and nauplii for several metals revealed detectable amounts of cadmium (0.87-1.15 ppm, dry weight), zinc (51-66 ppm), and copper (4.4-27.5 ppm) in eggs and nauplii. PCB, cadmium and copper levels were somewhat higher in the Utah samples, but "none were confidently labeled as the cause of the poor food value of the Utah nauplii".

As part of a cooperative effort (International Study on *Artemia*) to characterize and compare *Artemia* cysts from different geographical locations, we have analyzed freshly hatched nauplii and two marine organisms fed the nauplii for residues of chlorinated hydrocarbon insecticides and PCBs. We have also analyzed cysts and nauplii from the five locations for heavy metals and the results are reported here.

Methods and materials

Materials

Dehydrated cysts from five geographical locations were provided by the Artemia Reference Center (Ghent, Belgium). The origins of the cysts were Macau, Brazil (Companhia Industrial do Rio Grande do Norte, CIRNE-Brand, harvested 1978); Shark Bay, Australia (World Ocean, lot no. 113); Margherita di Savoia, Italy (harvested 1977); Great Salt Lake, Utah, USA (harvested 1977); and San Pablo Bay, California, USA (Living World, San Francisco Bay Brand, Inc., lot no. 1628). In addition, two 1975 lots of San Francisco Bay *Artemia* (San Francisco Bay Brand, Inc., lot no. 313/3006 and lot no. 321995) were analyzed and were used in biological tests at the Narragansett EPA laboratory.

Artemia nauplii were hatched as described by Beck *et al.* (1980).

Samples of *Menidia menidia* were obtained from experiment I of Beck *et al.* (1980). Newly hatched larvae were fed San Pablo Bay nauplii for 10 days. The fish (*circa* 2.4 mg) were then fed newly hatched nauplii from the five geographical locations for 18 days. At termination, the fish weighed approximately 30 mg.

Newly hatched (stage I) zoeal *Rhithropanopeus harrisii* larvae were fed *Artemia* nauplii from four of the geographical locations for 9 days and sacrificed before the stage IV megalopa molt (Johns *et al.*, 1980).

All biological samples were stored at -20 °C until analysis.

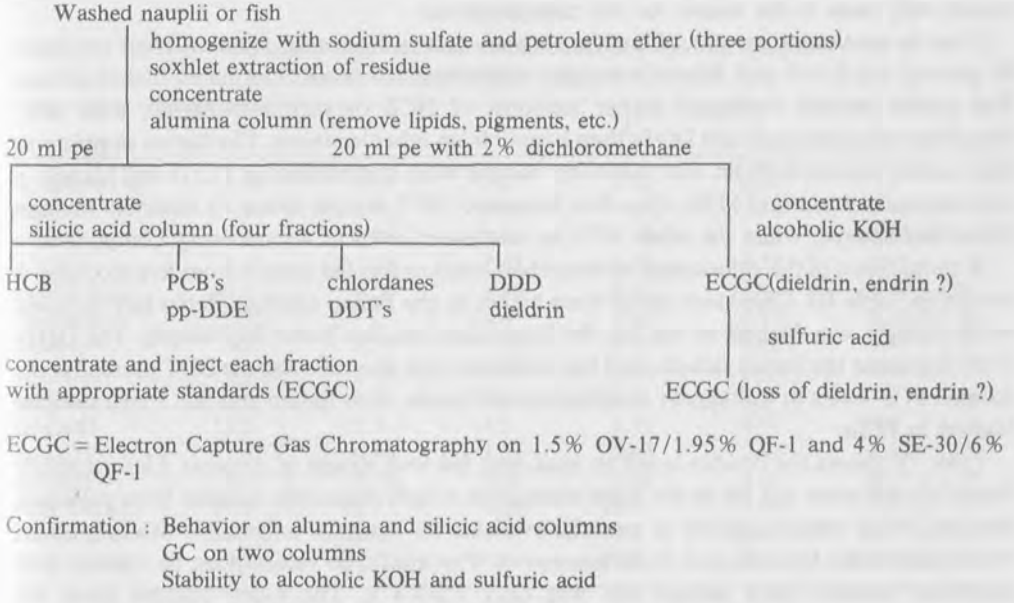
Chlorinated hydrocarbon analysis

The analytical scheme for chlorinated hydrocarbons in fish and *Artemia* nauplii is summarized in Fig. 1. Frozen samples were thawed and representative subsamples weighed. The samples were blended with anhydrous sodium sulfate and three successive portions of petroleum ether followed by extraction in a Soxhlet apparatus and concentration by Kuderna-Danish evaporative concentrator. The extracts were chromatographed on Woelm alumina,

activity grade III, to remove lipids, pigments and other co-extractives. Two fractions were collected : the first, containing PCBs and the majority of insecticides, was further fractionated on silicic acid (Bidleman *et al.*, 1978), primarily to separate PCBs from other components. The second fraction from alumina was checked for dieldrin and endrin before and after treatment with alcoholic potassium hydroxide, in which dieldrin and endrin are stable, and concentrated sulfuric acid, in which they are not. Sulfuric acid and, less frequently, alcoholic potassium hydroxide were also employed for pesticide confirmation and clean-up of the third and fourth silicic acid fractions.

The crab samples were extracted and cleaned-up by a slightly different procedure. Small samples (0.2-0.4 g wet weight) were ground with sodium sulfate and dichloromethane in a Potter Elvehjem tissue homogenizer and chromatographed on florisil instead of alumina to obtain better separation of dieldrin and endrin from other chlorinated hydrocarbons.

Individual column fractions were analyzed by dual column electron capture gas chromatography (Tracor MT-220 gas chromatograph, Ni-63 detectors, 180 × 0.4 cm glass columns packed with 1.5% OV-17/1.95% QF-1 or 4% SE-30/6% QF-1 on 100/120 mesh Supelcon AW-DCMS). Peaks were identified by retention time and quantitated using peak heights as compared with standards. The residues reported have been corrected for reagent blanks which were run with each set of five samples. Nauplii values represent duplicate analyses of 2-6 g samples, *Menidia* values are from a single analysis of 1-2 g samples, while the mud crab data are from a single analysis of 0.2-0.4 g samples.



ECGC = Electron Capture Gas Chromatography on 1.5% OV-17/1.95% QF-1 and 4% SE-30/6% QF-1

Confirmation : Behavior on alumina and silicic acid columns
GC on two columns
Stability to alcoholic KOH and sulfuric acid

FIG. 1. Flow chart of chlorinated hydrocarbon analysis

Metal analysis

Screening for metals in washed cysts and freshly hatched nauplii was accomplished by atomic absorption spectrometry and neutron activation analysis. Freeze-dried samples were digested with nitric and perchloric acids, filtered and diluted to 25 or 100 ml for analysis by flame atomic absorption (Fe, Zn, Pb, Cu, Cr, Cd, and Ni). For neutron activation, freeze-dried samples were sealed in polyethylene vials and irradiated, along with appropriate standards, for 7 hr with thermal neutrons (flux approximately 4×10^{12} neutrons/cm^2/sec) at the Rhode Island Nuclear Science Center reactor. After a 21-day cooling period, gamma spectra were measured with a Ge(Li) detector and 4096 channel pulse height analyzer (Fe, Zn, Cr, Co, Sc, Sb, Se, and Rb).

Results

The chlorinated hydrocarbon contents of nauplii of *Artemia* from the five locations are presented in Table I, while the results of five analyses of nauplii from one lot of cysts are shown in Table II to illustrate the variation encountered. In Table II, the coefficients of variation for individual compounds vary from 16 to 85%, with the majority less than 40%. The five samples were analyzed over a 6-month period and therefore reflect biological as well as analytical variation. Because of the manner in which they are formed, collected, pooled and processed, it appears to us that cysts within a given lot might well contain variable amounts of environmental contaminants. For comparing nauplii from different locations (Table I) the San Pablo Bay values are based on the first two replicates listed in Table II, which turn out to have means very close to the means for five determinations.

It can be seen that there are differences between nauplii from cysts from different locations. In general, the Brazil and Australia samples were very low in all chlorinated hydrocarbons. The Italian nauplii contained higher amounts of HCB (hexachlorobenzene), total BHC (hexachlorocyclohexane) and DDTs than nauplii from other locations. The Italian sample was also second highest in PCBs. San Pablo Bay nauplii were intermediate in DDTs and highest in chlordanes, dieldrin and PCBs. One San Francisco 1975 sample seems to resemble the San Pablo Bay sample, while the other 1975 lot has lower levels of almost every component.

A comparison of the chlorinated hydrocarbon levels in fish fed nauplii from five locations is shown in Table III. Chlordane levels were higher in the Italian and San Pablo Bay samples, while dieldrin was highest in the fish fed Australian and San Pablo Bay nauplii. The DDTs were highest in the Italian sample, and San Pablo-fed fish also reflected the DDT present in the nauplii. PCB levels in fish appear to reflect nauplii levels, with Italian and San Pablo samples highest in PCBs.

Table IV shows the residue levels in mud crab fed four strains of *Artemia*. Unfortunately Italian nauplii were not fed in the experiment from which these crab samples were collected. Because of the limited amount of material available for analysis, only single determinations were possible for *Menidia* and *Rhithropanopeus*. The mud crab values must be viewed with additional caution since sample size was only 0.2-0.4 g. The 0-day column gives the chlorinated hydrocarbon content of a sample of crab larvae taken at the start of the feeding period. With a few exceptions, the 9-day crabs contained more residues than any of the 0-day crabs. However, the bioaccumulation of the dietary pesticides by the crabs was less than that

TABLE I

Chlorinated hydrocarbons in *Artemia* nauplii (ng/g wet weight)[1]

	Brazil	Australia	Italy	Utah	San Pablo Bay	SFB-75a (313)	SFB-75b (321)
HCB	0.1	0.2	4.4	0.4	0.5	0.5	0.7
α-BHC	1.1	0.6	5.2	5.9	3.1	2.8	1.4
γ-BHC	0.8	–	5.0	1.9	3.2	1.0	2.8
c-chlordane	0.1	–	2.2	0.8	14.	1.3	6.6
t-nonachlor	0.03	–	–	1.2	5.5	1.4	8.3
dieldrin	0.2	0.1	–	0.7	1.4	0.2	1.2
op-DDE	0.4	–	3.9	0.7	5.3	0.9	6.7
pp-DDE	1.2	1.3	100.	2.2	18.	2.0	10.
pp-DDD	0.4	–	74.	3.0	13.	3.8	22.
op-DDT	0.4	0.2	60.	0.2	1.0	–	2.0
pp-DDT	1.9	4.3	188.	1.2	4.6	1.9	2.0
Σ DDT's	4.3	5.8	422.	7.3	42.	8.7	43.
PCB (1016)	5.3	2.0	5.3	3.2	14.	6.4	18.
PCB (1254)	1.6	0.9	19.	6.5	29.	3.5	23.
PCB (1260)	–	–	7.5	4.9	22.	3.5	2.8
Σ PCB's	6.9	2.9	32.	15.	66.	13.	43.

[1] Two determinations.

TABLE II

Chlorinated hydrocarbons in San Pablo Bay *Artemia* nauplii.
Results of five analyses (ng/g wet weight)

	1	2	3	4	5	$\bar{x}$	s^1
Sample wt (g)	3.13	2.04	6.07	2.00	2.00		
HCB	0.67	0.41	0.58	0.34	0.39	0.48	± 0.14
α-BHC	3.73	2.45	3.15	1.15	2.19	2.53	± 0.98
γ-BHC	4.19	2.24	2.48	1.18	4.74	2.97	± 1.47
c-chlordane	20.4	7.50	5.86	6.42	11.9	10.42	± 6.06
t-nonachlor	9.27	1.72	14.4	2.88	3.32	6.32	± 5.38
dieldrin	NA	2.15	1.88	0.65	1.01	1.42	± 0.70
op-DDE	8.35	2.18	6.45	3.32	6.75	5.41	± 2.57
pp-DDE	22.6	12.8	19.1	14.1	16.5	17.0	± 3.94
pp-DDD	13.6	12.0	14.9	9.75	14.7	13.0	± 2.15
op-DDT	1.53	0.46	1.51	3.03	3.95	2.10	± 1.38
pp-DDT	8.51	0.79	6.35	2.88	5.00	4.71	± 3.00
PCB (1016)	17.2	10.1	8.80	12.4	7.90	11.3	± 3.72
PCB (1254)	38.0	20.9	17.7	30.5	38.4	29.1	± 9.55
PCB (1260)	27.8	17.1	36.6	20.8	11.2	22.7	± 9.83

[1] Standard deviation $s = [\frac{\Sigma(x-\bar{x})^2}{n-1}]^{1/2}$

TABLE III

Chlorinated hydrocarbons in Atlantic silversides *Menidia menidia* fed freshly hatched nauplii of *Artemia* from five locations (ng/g wet weight)[1]

	Brazil	Australia	Italy	Utah	San Pablo
HCB	0.2	–	1.2	0.2	0.7
α-BHC	1.5	2.7	1.2	1.1	3.0
γ-BHC	0.4	–	0.6	0.4	0.8
c-chlordane	1.7	–	8.0	2.1	7.1
t-nonachlor	1.8	–	–	0.9	34.
oxychlordane	–	–	–	–	1.9
dieldrin	1.6	3.9	1.9	1.5	7.5
op-DDE	–	–	3.0	–	13.
pp-DDE	3.8	5.7	178.	5.8	55.
pp-DDD	2.2	2.5	46.	19.	27.
op-DDT	–	0.2	20.	1.5	1.3
pp-DDT	1.5	3.0	360.	2.8	13.
PCB (1016)	11.	17.	21.	16.	62.
PCB (1254)	19.	22.	91.	30.	66.
PCB (1260)	4.7	4.5	17.	7.0	122.
% survival[2]	87	87	80	80	58

[1] Single analysis. Samples from experiment I (Beck *et al.*, 1980).

[2] Beck *et al.* (1980).

TABLE IV

Chlorinated hydrocarbons in mud crab (*Rhithropanopeus harrisii*) fed freshly hatched nauplii of *Artemia* from four locations (ng/g wet weight)[1]

	0-day	Brazil	Australia	Utah	San Pablo
HCB	0.1	0.7	–	1.2	0.6
α-BHC	0.8	1.2	5.2	2.3	1.3
γ-BHC	–	0.2	1.8	0.6	0.3
c-chlordane	0.3	0.1	1.3	0.9	3.5
t-nonachlor	–	–	–	–	–
oxychlordane	0.9	0.3	0.7	0.6	3.4
dieldrin	0.8	–	–	0.8	1.8
op-DDE	–	–	–	–	6.3
pp-DDE	2.2	2.4	6.1	4.0	19.
pp-DDD	–	–	–	2.1	6.5
op-DDT	–	–	1.5	–	–
pp-DDT	0.3	1.0	2.4	1.1	1.8
PCB (1016)	4.0	21.	29.	30.	16.
PCB (1254)	10.	14.	20.	20.	33.
PCB (1260)	2.5	3.2	2.0	6.1	25.
Survival	75	77	61	0[2]	0[2]

[1] Single analysis. No crab sample available for Italy.

[2] Larvae did not survive beyond megalopa stage (Johns *et al.*, 1980).

found in silversides. Major differences between the crabs fed various geographical strains were the higher levels of BHC in the Australian treatment and chlordane, DDT and PCB 1260 in the San Pablo Bay treatment.

The results of the metal analyses are shown in Table V. Because of variations in blanks and sample size, we have not attempted to insert values for minimum detectable levels, but only report positive values after correcting for blanks. All cysts were high in iron, much of it associated with the chorion. Zinc was the second most abundant element. The Utah cysts contained three times more copper than any other cyst, but the nauplii copper concentration was lower and similar to that in the nauplii from Brazil, Italy, and San Pablo Bay. This is true for most of the metals ; nauplii levels are lower than cyst levels and generally show less variation between strains.

TABLE V

Metals in cysts and nauplii of *Artemia* from five locations (μg/g dry weight)

	Brazil		Australia	Italy		Utah		San Pablo Bay	
	c[1]	n[1]	c[2]	c	n	c	n	c	n
Fe	804.	62.	820.	1 860.	70.	800.	47.	1 380.	46.
Zn	59.	89.	144.	125.	104.	81.	102.	78.	98.
Pb	8.9		2.1	17.	3.0	5.1	6.2	6.6	3.8
Cu	4.7	6.3	4.6	14.	9.2	42.	8.2	10.2	10.8
Cd	0.02	0.15	0.15	0.09	0.12	0.28	0.14	0.26	0.10
Cr		1.4	1.8	3.7	0.66	2.0	1.1	4.4	0.48
Ni	0.23	0.29	0.50	4.0	0.09	2.9	0.70	6.6	0.12
Co	0.35	0.38	0.20	1.6	0.14	0.34		1.9	
Sc	0.02		0.01	0.41		0.03		0.31	
Sb	0.53		0.53						
Sc	1.4		1.2	1.2	1.0	1.5	2.1	2.3	0.83
Rb	6.5	12.			7.5	23.	23.	9.1	6.5

[1] c = cyst ; n = nauplii.

[2] Australian nauplii not analyzed (insufficient sample).

Nauplii concentrations, which should give a better indication of potential problems in using decapsulated cysts or nauplii as food for other organisms, range from none detected (ND) to 6.2 ppm for lead, 6.3-10.8 ppm for copper, 0.10-0.15 ppm for cadmium, 0.48-1.4 ppm for chromium, 0.09-0.70 ppm for nickel, ND-0.51 ppm for cobalt, ND-2.1 ppm for selenium, and ND-23 ppm for rubidium.

Discussion

These analyses were performed in conjunction with feeding studies in which differences in growth and survival of several marine organisms were observed. Although growth, which would include the effects of naupliar size on acceptance and energy expended in feeding, may

be an important indicator of overall food value, we feel that survival is a more important factor in rating *Artemia* sources as potential live food for animals during the early stages of their development and that survival would also be a more definitive indicator of pollutant-induced stress.

In feeding experiments with two species of crab (Johns *et al.*, 1980) those fed nauplii from Utah and San Pablo Bay did not survive beyond the megalopa stage. With winter flounder, which also undergoes a metamorphosis, survival was also low on Utah and San Pablo nauplii (Klein-MacPhee *et al.*, 1980), while San Pablo Bay nauplii provided the poorest survival in feeding experiments with Atlantic silversides, which does not undergo metamorphosis (Beck *et al.*, 1980).

Since the amino acids (Seidel *et al.*, 1980) and carotenoid contents (Soejima *et al.*, 1980) of all *Artemia* compared in this study were similar, we have scrutinized the lipid, chlorinated hydrocarbon and metal data for possible patterns which might explain the differences in survival of the test organisms fed the various *Artemia* nauplii. Schauer *et al.* (1980) found some differences in total lipid, total fatty acid and 16-carbon fatty acids, but the most striking feature of their data is the high 18:3ω3 and low 20:5ω3 contents of the Utah and San Pablo *Artemia*. Complicating any simple explanation based on fatty acid distribution was the fact that both lots of 1975 San Francisco Bay *Artemia*, one with a low 20:5ω3 content, did promote good growth and survival through the larval development of the mud crab (Johns *et al.*, 1978).

Our analyses clearly show that there are differences in chlorinated hydrocarbons between *Artemia* samples from different locations. The samples from Brazil and Australia were lowest in all types of chlorinated hydrocarbons, while those from Italy and San Pablo Bay were highest in several compounds.

In looking for residues that might explain the poorer performance of San Pablo Bay nauplii, chlordane, dieldrin, DDTs and PCBs, especially the more highly chlorinated 1260 type, would be suspect. The Italian nauplii, however, which did support good growth and survival, contained much higher levels of DDT and thus DDT does not appear to be the causative factor. Moreover, the Utah nauplii, which did not support survival of flounder and crabs through metamorphosis, contained less chlordane, DDT, and PCB than the Italian strain. Of the chlorinated hydrocarbons, therefore, only dieldrin at low levels is common to both Utah and San Pablo nauplii. Since dieldrin LC_{50}'s for aquatic organisms are generally similar to, or higher than those for DDT, it seems unlikely that dieldrin alone could have the effects noted, unless a dieldrin sensitive mechanism is operating at metamorphosis.

The bioaccumulation data for silversides (Table III) show that chlordanes, dieldrin, DDTs and PCBs were accumulated by the fish and would point to chlordane, dieldrin or high molecular weight PCBs as potential factors in the lower survival under the San Pablo regime. However, the mud crab accumulations (Table IV) are lower and offer no explanation for the poor survival with the Utah diet.

The concentrations of various metals found in these *Artemia* nauplii fall in the range of concentrations for marine organisms summarized by Byran (1976). If dietary toxicities follow the pattern of lethal concentrations in water, one might expect copper, lead, and cadmium to be the most harmful of the metals determined. However, none of the metal concentrations in any nauplii appear to be exceedingly high and there does not appear to be any metal common to Utah and San Pablo which is dramatically higher than that found in other *Artemia*. The

Utah nauplii do contain slightly more lead, nickel, selenium, and rubidium than other nauplii and the Utah and San Pablo nauplii were slightly lower in iron and slightly higher in copper than the Brazilian or Italian nauplii.

Although LC_{50}'s for chlorinated hydrocarbons and metals have been reported for various freshwater and marine organisms (*e.g.* Eisler, 1970 ; Holden, 1973 ; Mayer *et al.*, 1977) we can find no published values for the toxicities of these compounds introduced by way of the diet to silversides, larval flounder, or mud crab larvae. Eisler (1970) reported LC_{50}'s of 0.4 ppb pp-DDT, 5 ppb dieldrin and 9 ppb γ-BHC for Atlantic silversides, while Epifanio (1971) observed mortality of two crab larvae with 1 ppb dieldrin in seawater. Smith and Cole (1973) subjected adult winter flounder to 2 ppb DDT and 2 ppb dieldrin in water prior to spawning. Mortality of the eggs was related to residue levels in the ova and was higher in the DDT treatment. All flounder larvae, including controls, died at onset of feeding, so residual pesticide effects, if any, could not be assessed.

Variable results have been obtained in bioaccumulation studies where dietary *versus* environmental (water) inputs were compared. Macek *et al.* (1979) conclude that except for DDT, uptake from the diet will be very small compared to bioconcentration from water. Reinert *et al.* (1974), however, found that lake trout over several months accumulated similar levels of pesticide from water containing 0.01 ppb DDT or dieldrin *versus* food containing 2 ppm DDT or dieldrin. When the fish were placed in pesticide-free water, dieldrin was gradually eliminated, but DDT was not. In most comparisons, water concentrations have been much higher than those normally encountered in natural waters. Furthermore, levels of pollutants added or found in dietary studies have been much higher than the levels we found in the *Artemia* nauplii from five geographical locations. Thus it is difficult to predict the possible effects of the relatively small amounts of chlorinated hydrocarbons and metals fed as live *Artemia* in our studies.

Finally, Soejima (personal communication, 1979) isolated chlorophyll from the San Pablo cysts in the course of a study on the carotenoids in *Artemia* (Soejima *et al.*, 1980). This would presumably indicate a higher level of algae in the diet of the San Pablo Bay *Artemia*. Analysis of Brazilian and San Pablo cysts for toxin from dinoflagellates, especially *Gonyaulax sp.*, disclosed no measurable amounts of paralytic shellfish poison (Y. Shimizu, personal communication, 1980).

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Comparison of *Artemia* nauplii and non-living diets as food for larval grass shrimp *Palaemonetes* spp. : screening experiments[1]

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Abstract

A total of 18 non-living test diets were presented once daily to larval *Palaemonetes pugio* or *P. vulgaris* in a series of replicated screening experiments. Four kinds of preparations were represented among these diets : 1) frozen fish eggs (mullet roe, trout roe and a mixture of the two) ; 2) freeze-dried natural products (beef heart, beef liver, earthworm, oyster, "plankton" [euphausiids], prawn and squid) ; 3) "dry" prepared diets (Colvin's, Meyer's, Purina MR-25 and Tetramin) ; and 4) "wet" prepared diets (poached hen's egg, poached egg with freeze-dried plankton, poached egg with freeze-dried squid and poached egg with frozen squid). Starved and *Artemia*-fed groups of *Palaemonetes* larvae were maintained as controls.

In all cases, survival of *Artemia*-fed larvae was much (at least 1.8 times) higher and development significantly more rapid than for larvae fed any non-living diet. Nevertheless, 26.7% of larvae fed freeze-dried squid and 13.3% of those fed euphausiid particles survived through metamorphosis. Other diets which produced small numbers of postlarvae or showed special promise in experiments not continued through metamorphosis included freeze-dried beef heart, mullet and trout roes, and all the poached hen's egg preparations. Poorest results were obtained with the freeze-dried oyster and prawn flesh and the dry Purina and Tetramin rations. Slightly better survival and development were observed among larvae fed the freeze-dried earthworm and beef liver diets and Colvin's and Meyer's dry rations.

No relation between diet lipid content or gross fatty acid composition and performance was apparent.

Introduction

For a number of years, the basic food items used in both laboratory and commercial rearing of decapod crustacean larvae (other than the early stages of penaeid shrimp) have been the nauplius larvae of the brine shrimp *Artemia*. These animals have become so widely accepted as food for decapod larvae in culture that diet, as a factor influencing survival and development of larvae, often has been ignored. Despite its undeniable suitability as food for many forms, *Artemia* obviously is a substitute for natural foods, and it does not provide optimal nutrition for all decapod larvae (*e.g.* Sandifer, 1972 ; Sulkin and Epifanio, 1975).

[1] Contribution No. 104 from the South Carolina Marine Resources Center. References to trade names in this paper do not imply endrosement.

Also, there is increasing evidence that the nutritional value of *Artemia* nauplii may differ with the source (Reeve, 1969 ; Bookhout and Costlow, 1970 ; Wickins, 1972 ; Beck *et al.*, 1980 ; Fujita *et al.*, 1980 ; Johns *et al.*, 1980 ; Klein-MacPhee *et al.*, 1980).

Partly because of tenuous supplies and rapidly rising costs of *Artemia* cysts in recent years, considerable interest has developed in identifying substitutes for *Artemia* nauplii as diets for larval decapods. One approach, most frequently taken with crab larvae, has focused on living foods (Brick, 1974 ; Roberts, 1974 ; Sulkin, 1975, 1978 ; Sulkin and Epifanio, 1975 ; Christiansen and Yang, 1976 ; Sulkin and Norman, 1976 ; Bigford, 1978). A second approach, more frequently taken with shrimp larvae, has focused on non-living foods, alone or as supplements to *Artemia* (Regnault, 1969 ; Jones *et al.*, 1974, 1979 ; Campillo, 1975 ; De Figueiredo, 1975 ; Hirata *et al.*, 1975 ; Sick, 1975 ; Sick and Beaty, 1975 ; Murai and Andrews, 1978 ; Villegas and Kanazawa, 1978 ; Manzi and Maddox, 1980 ; Mock *et al.*, 1980). As yet, however, little success has been reported in rearing caridean shrimp larvae on diets lacking *Artemia*, and little is known about what other foods are accepted by larval shrimp. The purpose of the present study was to screen a variety of non-living food items as possible diets for larval palaemonid shrimp, with a view of ultimately incorporating promising materials in compounded particulate or microencapsulated diets.

Materials and methods

The larvae of *Palaemonetes* shrimp were chosen for these experiments because of the ease with which they may be reared in the laboratory and their general similarity to larvae of commercially important caridean species such as *Macrobrachium rosenbergii*.

A total of 18 non-living test diets representing four general kinds of preparations were presented to larval *P. pugio* and *P. vulgaris* in a series of screening experiments (Table I). Larvae were reared in 1 l plastic funnels equipped with central air lines. Very gentle aeration was applied to help maintain the non-living food particles in suspension. Nevertheless, there was a strong tendency for many of the food particles to sediment on the sides of the cones. Therefore, several times daily the funnels were stirred gently to resuspend the food particles.

Two or three replicates of 20 or 30 larvae each were reared for each treatment. All larvae were reared in Instant Ocean (Aquarium Systems, Inc., Eastlake, Ohio) artificial seawater at 15 or 20 ‰ salinity. Temperature varied considerably among experiments due to a faulty room thermostat (see Tables II-V). The culture water was changed and the larvae were fed and counted daily. Periodically, the larvae were examined microscopically and staged following the descriptions of 10 zoeal stages given by Broad (1957a). These data were used to calculate a Larval Stage Index similar to that used by Manzi *et al.* (1977) except that the post-larval stage was included as stage 11. The index was calculated by multiplying the number of larvae at each stage on a given day by the stage number and then dividing the sum of the products by the total number of larvae sampled.

Since the diets tested in this study differed so much in unit dry weight, moisture content and other characteristics, it was difficult to standardize amounts fed. Therefore, the diets were simply fed at levels equal to or exceeding (usually) the number of particles provided to the *Artemia*-fed control larvae (5-10 nauplii/ml) but not in such quantities as to cause massive fouling of the water. For the dry diets this generally amounted to $\sim$ 0.1 mg/ml of rearing water.

TABLE I

Test diets presented to larval *Palaemonetes pugio* and *P. vulgaris*

Type of diet	Particle size
1. Frozen fish eggs	
Mullet (*Mugil* sp.)	0.66-0.71 mm[1]
Trout (*Cynoscion* sp.)	0.41-0.51 mm[1]
Mixture of the two	
2. Freeze-dried natural products	
Beef heart	< 425 > 250 μm
Beef liver	
Earthworm	
Oyster (*Crassostrea virginica*)	
"Plankton" (euphausiids[2])	
Prawn muscle (*Macrobrachium rosenbergii*)	
Squid (*Loligo pealii*)	
3. Dry prepared diets	
Colvin's flake[3]	< 425 > 250 μm
Meyer's flake[4]	
Purina Marine Ration 25 pellet[5]	
Tetramin flake[6]	
4. Wet prepared diets	
Poached egg	< 425 > 250 μm
Poached egg + frozen squid	
Poached egg + freeze-dried squid	
Poached egg + "plankton"	

[1] Egg diameters.

[2] Breedmore Aquarium Products, Ltd., Shohola, PA 18458.

[3] Diet II-C, Table I, in Brand and Colvin, 1977 ; courtesy of Dr. L. B. Colvin, University of Arizona.

[4] Diet 1023-77-1 HP courtesy of Dr. S. P. Meyers, Louisiana State University.

[5] Ralston Purina Co., 900 Checkerboard Square, St. Louis, MO 63188 ; courtesy of Dr. W. MacGrath.

[6] TetraMin Staple Food (Tetra Werke).

Diets were prepared by a variety of means. Fish ovaries obtained from local suppliers were thawed, split down the middle, folded open to expose the eggs and placed in stacked US Standard Sieves (no. 20 and no. 60). Then a steady stream of water was passed back and forth over the eggs to disconnect them from the ovarian membrane and other tissue. The size 60 sieve (250 μm) caught the eggs while the no. 20 sieve (850 μm) retained the other tissues. The eggs were then washed into a beaker and stored in a refrigerator until used. Fresh ovaries were thawed and prepared about every 3 days. All freeze-dried products except the "plankton" were obtained either fresh or frozen, lyophilized in the laboratory, and then ground through a no. 40 sieve (425 μm). Particles retained by a no. 60 sieve were used as food. The "plankton" (euphausiid) particles were obtained as a commercially freeze-dried product and sieved as above. All freeze-dried diets were stored in capped vials at room temperature for the life of the project. Prepared dry diets were sieved as above and also stored in capped vials. The hen's egg diet was prepared by homogenizing a raw egg by vigorous stirring, poaching it in a

standard poaching cup, and then sieving it as above. Egg particles were washed into a beaker and maintained in a refrigerator until used. Supplemented hen's egg diets were prepared by stirring 1 g of the desired supplement (chopped squid, sieved freeze-dried squid, or sieved freeze-dried euphausiids) into the raw egg before poaching. Fresh egg diets were prepared every 2 or 3 days. *Artemia* cysts (San Francisco Bay Brand) were hatched daily for the control diet.

Lipid content and composition were determined for several of the freeze-dried diets. Lipids were extracted from the diets by the Bligh and Dyer (1959) method. The lipid extracts were evaporated to dryness and made up to 10.0 ml with chloroform. Two 1.0 ml aliquots were taken of each extract for gravimetric determination of total lipid. Additional aliquots were transmethylated with boron trifluoride in methanol (14% w/v) with additional methanol and benzene, as recommended by Morrison and Smith (1964).

Fatty acid methyl esters (FAME) were analyzed by gas-liquid chromatography in a Hewlett-Packard 7610 instrument equipped with a flame ionization detector. The FAME were separated on a wall-coated, open tubular stainless steel capillary column (45.7 m long, 0.25 mm I.D.) coated with Silar 5-CP. The column was operated isothermally at 185 °C. Injector and detector temperatures were maintained at 240 °C. Helium at 50 psig was used as the carrier gas.

Provisional identifications of the FAME were based upon comparisons of relative retention times ('18:0) of the chromatographic peaks with those of known FAME and with those of a secondary standard, cod liver oil (Ackman and Burgher, 1964). The chromatograms were quantitated by electronic integration.

Results

In all cases, survival of *Artemia*-fed larvae was much (at least 1.8 times) higher and development more rapid than for larvae fed any non-living diet (Tables II-V). Nevertheless, 26.7% of the larvae fed freeze-dried squid and 13.3% of those fed "plankton" (euphausiid) particles survived through metamorphosis. However, larvae fed these diets required substantially more time to reach the postlarval stage than did those fed *Artemia* (Tables II, IV). Other diets which produced small numbers of postlarvae or showed special promise in experiments not continued through metamorphosis included freeze-dried beef heart, mullet and trout eggs, and all the poached hen's egg preparations (Table II, III, IV). Supplementation of the poached egg diet with squid or euphausiid particles had no discernible effect on larval survival or development rates (Table V).

Poorest results were obtained with the freeze-dried oyster and the dry Purina and Tetramin rations. Survival and development of larvae fed these rations were but little better than for starved animals (Table III). Slightly better survival and development were observed among larvae fed freeze-dried beef liver, freeze-dried prawns, Meyer's and Colvin's diets (listed in approximate order of decreasing performance).

Lipid analyses were conducted on several of the freeze-dried diets (Table VI). However, there was no apparent correlation between total lipid level or gross lipid composition and diet performance.

TABLE II

Survival and development of grass shrimp (*Palaemonetes pugio*) larvae fed different diets in the laboratory (rearing conditions : salinity, 20 ‰ Instant Ocean artificial seawater ; temperature, 31.1 ± 1.8 °C ; duration, 30 days)

	Diet treatments							
	Artemia	Mullet roe	F.D.[1] squid	F.D. earthworm	F.D. beef heart	Meyer	Colvin	Starved
Mean % survival :								
Day 1	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
5	93.3	90.0	93.3	76.7	96.7	93.3	85.0	3.3
10	88.3	73.3	85.0	13.3	86.7	23.3	25.0	0[4]
15	48.3	60.0	75.0	6.7	48.3	5.0	5.0	–
20	48.3	41.7	58.3	3.3	26.7	1.7	1.7	–
25	48.3	41.7	40.0	1.7	6.7	1.7	1.7	–
30	48.3	35.0	30.0	0	3.3	1.7	0	–
Mean % PL[2] :	48.3	1.7	26.7	0	1.7	0	0	0
Days to PL :								
Mean ± S.D.	15.4 ± 2.2	24	22.3 ± 3.3	–	19	–	–	–
Range	13-21	–	18-29	–	–	–	–	–
Larval Stage Index[3] :								
Day 1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
7	8.3	3.9	3.9	2.4	4.1	2.8	2.8	–
14	9.4	5.4	7.0	4.3	7.2	5.0	6.0	–
20	10.9	6.4	9.8	6.5	8.8	6	9	–
27	11	7.8	10.7	7	8.7	6	–	–
30	11	8.3	10.9	–	10.5	6	–	–

[1] F.D. = freeze-dried.

[2] PL = postlarvae.

[3] Larval Stage Index $= \frac{\text{no. animals} \times \text{stage 1} + \text{no. animals} \times \text{stage 2} + \ldots + \text{no. animals} \times \text{stage 11}}{\text{total no. animals sampled}}$

[4] All dead by day 6.

TABLE III

Survival and development of grass shrimp (*Palaemonetes vulgaris*) larvae fed different diets in the laboratory (rearing conditions : salinity, 20 ‰ Instant Ocean artificial seawater ; temperature, 25.0 ± 0.9 °C ; duration 28 days)

	Diet treatments											
	Artemia	F.D.[1] prawn	F.D. oyster	F.D. beef liver	F.D. beef heart	F.D. squid	Mullet roe	Trout roe	Trout + mullet roe	Purina 25	Tetra-min	Star-ved
Mean % survival :												
Day 1	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
4	100.0	82.5	97.5	95.0	97.5	100.0	92.5	97.5	90.0	97.5	100.0	87.5
8	95.0	47.5	0	87.5	90.0	87.5	80.0	87.5	75.0	57.5	17.5	0
12	87.5	17.5	–	65.0	75.0	72.5	77.5	82.5	65.0	2.5	0[3]	–
16	62.5	5.0	–	27.5	65.0	55.0	42.5	55.0	47.5	0[4]	–	–
20	47.5	2.5	–	20.0	57.5	45.0	30.0	32.5	22.5	–	–	–
24	42.5	2.5	–	17.5	50.0	40.0	22.5	30.0	12.5	–	–	–
28	42.5	0	–	5.0	22.5	27.5	17.5	10.0	12.5	–	–	–
Mean % PL[2]	42.5	0	0	0	0	0	0	0	0	0	0	0
Days to PL :												
Mean ± S.D.	24.2 ± 3.4	–	–	–	–	–	–	–	–	–	–	–
Range	21-28	–	–	–	–	–	–	–	–	–	–	–
Latest stage reached	PL	4	1	5	6	5	5	5	6	2	2	1

[1] F.D. = freeze-dried.

[2] PL = postlarvae.

[3] All dead by day 10.

[4] All dead by day 14.

TABLE IV

Survival and development of grass shrimp (*Palaemonetes vulgaris*) larvae fed *Artemia* nauplii and freeze-dried "plankton" (euphausiid) particles in the laboratory (rearing conditions : salinity, 20 ‰ Instant Ocean artificial seawater ; temperature, 26.3 ± 1.2 °C ; duration, 37 days)

		Diets	
		Artemia	F.D.[1] plankton
Mean % survival :			
	Day 1	100.0	100.0
	5	4.7	82.7
	10	92.0	68.0
	15	92.0	61.3
	20	72.0	56.0
	25	57.3	46.7
	30	54.7	37.3
	35	54.7	28.0
	37	54.7	17.3
Mean % PL[2] :		54.7	13.3
Days to PL :			
Mean ± S.D.		23.3 ± 2.7	27.8 ± 4.9
Range		18-29	20-37
Larval Stage Index[3] :			
	Day 1	1.0	1.0
	5	2.8	3.0
	11	7.4	5.2
	17	9.0	6.4

[1] F.D. = freeze-dried.

[2] PL = postlarvae.

[3] Larval Stage Index $= \frac{\text{no. animals} \times \text{stage 1} + \text{no. animals} \times \text{stage 2} + \ldots \text{etc.}}{\text{total no. animals sampled}}$

Discussion

Results of the present study show that palaemonid larvae can be reared successfully without *Artemia*. Non-living animal materials which appear to have significant potential as diets or diet components for larval palaemonid shrimp include freeze-dried squid, euphausiids and beef heart, fish eggs (mullet and trout), and poached hen's egg, alone or with supplements. Of these, the freeze-dried squid (especially) and euphausiid particles showed by far the most promise. Similarly, Fenucci and Zein-Eldin (1979) and Lim *et al.* (1978), among others, have recommended squid as a component of prepared diets for postlarval panaeid shrimp. Our results suggest that squid and euphausiids also deserve consideration as dietary components for larval shrimp.

TABLE V

Survival and development of grass shrimp (*Palaemonetes vulgaris*) larvae fed *Artemia* nauplii and four poached hen's egg preparations in the laboratory (rearing conditions : salinity, 15 ‰ Instant Ocean artificial seawater ; temperature, 27.2 ± 1.5 °C ; duration, 19 days)

	Diets				
	Artemia	Egg	Egg + fresh squid	Egg + F.D.[1] squid	Egg + F.D. plankton
Mean % survival :					
Day 1	100.0	100.0	100.0	100.0	100.0
5	94.4	88.9	91.1	91.1	88.9
10	90.0	42.2	70.0	52.2	46.7
15	83.3	16.7	27.8	15.6	20.0
19	73.3	15.6	23.3	11.1	16.7
Mean % PL[2] :	25.6	0	0	0	0
Days to PL :					
Mean ± S.D.	18.3 ± 0.8	–	–	–	–
Range	17-19	–	–	–	–
Larval Stage Index[3] :					
Day 1	1.0	1.0	1.0	1.0	1.0
8	4.7	2.8	2.7	2.6	2.8
11	7.3	3.2	3.4	3.5	3.9
14	8.4	4.8	4.2	4.4	4.5
19	9.7	5.8	5.3	5.7	5.8

[1] F.D. = freeze-dried.

[2] PL = postlarvae.

[3] Larval Stage Index $= \dfrac{\text{no. animals} \times \text{stage 1} + \text{no. animals} \times \text{stage 2} + \ldots \text{etc.}}{\text{total no. animals sampled}}$

TABLE VI

Lipid content (percent dry weight) and gross fatty acid composition (expressed as percent of total fatty acids by weight) of six freeze-dried diets presented to larval grass shrimp (*Palaemonetes* spp.) (diets arranged from left to right in order of increasing performance)

	Freeze-dried diets					
	Oyster	Prawn	Earthworm	Beef liver	Beef heart	Squid
Total % lipid	6.6	4.6	2.6	11.2	11.5	3.7
% saturated FA[1]	49.8	31.4	17.7	45.5	38.2	38.2
% mono-unsaturated FA	18.6	23.8	17.2	20.4	23.8	13.0
% poly-unsaturated FA	20.1	37.1	44.7	29.4	25.3	45.4
Total % ω3 FA	10.0	10.5	16.6	6.0	3.7	39.9
Total % ω6 FA	3.3	26.6	27.8	23.4	21.6	5.5
ω3:ω6 ratio	3.0	0.4	0.6	0.3	0.2	7.3

[1] FA = fatty acids.

Relatively few previous studies have dealt with the rearing of shrimp larvae through metamorphosis without *Artemia*. Broad (1957b) found that, compared to *Artemia* nauplii, feeding non-living animal matter (freshly killed zooplankton) to *Palaemonetes* larvae resulted in very low survival (< 1 %) through metamorphosis. Supplementation with living algal cells increased survival only to 2.6 %. Later, Regnault (1969) fed *Hippolyte inermis* larvae a variety of animal materials (fertilized *Arbacia* eggs, infusyl, dried liver powder, *Pleuronectes* blood cells and finely chopped mussel mantle) and several species of algae, with *Artemia* nauplii as the reference diet. Of the animal materials, only the mussel gave any promising results compared to *Artemia*, and it had to be abandoned after the larvae passed through one molt because it fouled the water so badly. In contrast, Campillo (1975) found that freeze-dried mussel was a very poor diet for larvae of *Palaemon serratus*, as were sea urchin (*Sphaerechinus granularis*) eggs. Campillo also found that shrimp (*P. serratus* and *Crangon crangon*) and fish (*Gadus morhua*) eggs were unsuitable as food for early larval stages, probably because of their relatively large size. However, when the *P. serratus* larvae were reared to stage III on *Artemia* nauplii and then switched to the egg diets, quite good results were obtained, especially with the shrimp eggs. Similarly, De Figueiredo (1975) found that a diet of *Artemia* nauplii supplemented with eggs of *C. crangon* gave better results than did *Artemia* nauplii alone for rearing larval *P. serratus*.

In work on prepared foods for *Macrobrachium rosenbergii* larvae, Sick and Beaty (1975) reported that, of their compounded preparations, only a formula diet containing *Artemia* meat and presented in freeze-dried form supported development through metamorphosis (4 % survival). In the same study, they obtained 11 % survival through metamorphosis among larvae fed freeze-dried catfish flesh. Murai and Andrews (1978) found that a diet of *Artemia* nauplii supplemented with freeze-dried oyster flesh gave excellent results with *M. rosenbergii* larvae, and supplements of freeze-dried catfish and commercial trout fry feed were also acceptable. However, when the freeze-dried oyster meat and trout feed were substituted completely for *Artemia* after the 10th day of larval culture, very slow growth and low survival through metamorphosis (< 2 %) resulted. In the present study, freeze-dried oyster meat alone was found to be a very poor food for *Palaemonetes* larvae.

Several studies have also dealt with the culture of penaeid shrimp larvae on non-living diets without *Artemia*. Hirata *et al.* (1975) reared *Penaeus japonicus* through its zoeal stages on soy cake particles with good results. Best results were obtained with a mixture of soy cake and diatoms. Villegas and Kanazawa (1978) reared *P. japonicus* larvae to mysis stages on an artificial diet (diet-B, Kanazawa *et al.*, 1971), *Tapes* and mysid meals, and *Artemia* nauplii + diatoms. Fastest growth was obtained on the *Artemia*-diatom diet, but highest survival to the mysis III stage was observed among the larvae fed diet-B. Larvae reared on the mysid meal attained only the mysis I stage. Those fed *Tapes* meal developed almost to mysis III, but development was slower than for larvae fed *Artemia* + diatoms or diet-B. More recently, Jones *et al.*, (1979) fed both microencapsulated and particulate non-living foods to *P. japonicus* larvae. Of the encapsulated diets tested, only a *Tapes*-hen's egg mixture supported complete larval development though metamorphosis (50 % survival). Hen's egg alone in encapsulated form did not support good growth and survival, indicating that *Tapes* was the important component. In contrast, no marked differences in survival and development of *Palaemonetes* larvae fed poached hen's egg alone or supplemented with squid or euphausiid particles were noted in the present study. Jones *et al.* (1979) found that diet-B (Kanazawa *et*

al., 1971) and "commercial diet" fed as particulates gave somewhat better results than the encapsulated preparations but required more care in feeding because of their tendency to foul the rearing water.

In studies on living diets for larvae of the blue crab, *Callinectes sapidus*, Sulkin (1975) found that *Artemia* nauplii and polychaete (*Hydroides dianthus*) larvae would support complete larval development while rotifers (*Brachionus plicatilis*) did not. He speculated that the success of the polychaete and *Artemia* diets might be related to the fact that they contained two-three times more lipid per unit dry weight than rotifers. However, Villegas and Kanazawa (1978) found no obvious relationship between diet chemical composition (including lipid levels) and growth and survival of larval *P. japonicus*. Similarly, results of the present study indicated no relation between diet lipid content or gross fatty acid composition and performance as food for larval *Palaemonetes*.

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International Study on *Artemia*[1] IX. Lipid level, energy content and fatty acid composition of the cysts and newly hatched nauplii from five geographical strains of *Artemia*

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Abstract

Artemia cysts and newly hatched nauplii from Australia, Brazil, Italy and the United States (California and Utah) were analyzed for their total lipid level, total fatty acid level and composition, and their energy content in an effort to evaluate their lipid nutritional value as diets of marine organisms. Results are compared to biological data from a nutritional evaluation of these five *Artemia* strains on various marine organisms.

The total lipid, fatty acid methyl ester and energy levels of all strains appeared to be adequate to promote good growth and survival of the marine organisms. The fatty acid spectrum of the cysts and nauplii were nearly identical, indicating that the cyst shell contains little fatty acid-type lipids. However, significant differences were found in the fatty acid composition between the various strains.

Artemia were classified into two groups based on their $\omega 3$ polyunsaturated fatty acid composition. The major difference between the two groups was that one group contained predominantly 18:3ω3 and 18:4ω3, while the other group contained chiefly 20:5ω3. Considering the importance of 20:5ω3 to marine organism nutrition, the Australia, Italy, Brazil, and San Francisco Bay 321 strains which contained the higher level of this fatty acid would provide the best nutrition of the five strains. The San Pablo Bay 1628, San Francisco Bay 313 and Utah strains would probably be of less nutritional value due to the low level of 20:5ω3 and/or the excess amount of 18:3ω3. It is possible, however, that there is an interaction between an essential fatty acid (20:5ω3) deficiency and a dietary contaminant. This possibility is discussed with reference to biological results obtained when these five *Artemia* strains were fed to three different marine organisms.

Introduction

Artemia (brine shrimp) is a widely used food source in laboratory and commercial rearing of many marine organisms. Several new geographical *Artemia* strains have recently become

[1] International Interdisciplinary Study on *Artemia* Strains coordinated by the Artemia Reference Center, State University of Ghent, Belgium.

available and a subsequent need has developed for their analysis as potential supplements or replacements for the present commercial sources.

An important determinant of the overall nutritional value of any food stuff is its lipid content. Triglyceride-type lipids are a major source of a diet's metabolizable energy and are directly linked to the growth of the consumer organism (Pandian, 1975). The dietary fatty acid composition ultimately determines the fatty acid composition of the structural phospholipids (Castell *et al.*, 1972ab ; Norred and Wade, 1972). Phospholipids are functionally active in maintaining proper membrane fluidity and cellular transport mechanisms. Recent research has demonstrated that the $\omega 3$ polyunsaturated fatty acids (PUFA) are required for lobsters (Castell and Covey, 1976), prawns (Guary *et al.*, 1976 ; Kanazawa *et al.*, 1979) and for several marine fish, including plaice (Owen *et al.*, 1972), red sea bream (Yone and Fujii, 1975a) and turbot (Cowey *et al.*, 1976).

The purpose of this research was to determine the total lipid content, fatty acid composition and energy content of cysts and newly-hatched nauplii of *Artemia* from five geographical regions.

Materials and methods

Sources and culturing of *Artemia*

Dehydrated cysts from five geographical locations were provided by the Artemia Reference Center (Ghent, Belgium). The origin of these cysts were Shark Bay, Australia (World Ocean, lot no. 113) ; Macau, Brazil (Companhia Industrial do Rio Grande do Norte, CIRNE-Brand, harvested 1978) ; Margherita di Savoia, Italy (harvested 1977) ; Great Salt Lake, Utah, USA (harvested 1977) ; San Pablo Bay, California, USA (Living World, San Francisco Bay Brand, Inc., lot no. 1628) and two samples from San Francisco Bay, California, USA (San Francisco Bay Brand, Inc., lot no. 313/3006 and lot no. 321995). The latter three Californian samples will be referred to by their respective full or abbreviated lot numbers : SP 1628, SF 313 and SF 321.

Stage one *Artemia* nauplii were hatched from cysts incubated at 25 °C in 30 ‰ filtered (0.45 μm) seawater for a specific period of time that was dependent on the particular strain (Johns *et al.*, 1980).

Samples were either held at -20 °C until lipid analysis or dried at 60 °C to a constant weight for determination of the energy content.

Lipid extraction and analysis

Total lipids

Cysts were ground in a mortar and pestle and extracted twice with chloroform/methanol/water (20 ml/40 ml/16 ml) (Bligh and Dyer, 1959). The remaining solids were repeatedly extracted in acetone until the supernatant lacked pigmentation. The lipid in the acetone solution was transferred to petroleum ether (PE) and along with the chloroform fraction from the Bligh and Dyer extraction was evaporated to dryness at 30 °C. Total lipid weight was determined gravimetrically after which the lipids were dissolved in benzene and stored at -20 °C until analysis. Total lipid weight of the nauplii was determined in a similar manner. Lipid weights are presented as mg lipid/g dry weight sample.

Fatty acids

The fatty acid composition of each sample was determined by gas-liquid chromatography on two independent columns as described by Schauer and Simpson (1978). Results are presented here as fatty acid methyl esters (FAME) weight percent of total lipid. Quantification of total FAME weights were made by co-injecting the FAME 20:2ω6 as an internal standard and are presented as mg FAME/g lipid of the dry weight samples.

Determination of energy content

Energy content of newly hatched nauplii was determined using wet oxidation in the presence of an acid-dichromate mixture (Maciolik, 1962). These values are reported as Joule/gram ash-free dry weight.

Data analysis

One-way analysis of variance was computed for total lipids, FAME and energy content. If significant differences ($P < 0.05$) were found, a Student-Neuman-Kuels posterior comparison was used to determine where the difference lay (Snedecor and Cochran, 1967).

Results

The Australian cysts' lipid level was statistically greater than the other four strains while the means of the Utah, SP 1628 and Brazilian strains were greater than that of the Italian cyst strain (Table I). The nauplii lipid levels, however, were all statistically different from one another except for the SF 321 and SP 1628 strains. The highest level of lipid in the nauplii was found in the Utah strain, and in descending order, the other strains were ranked as follows: Brazil, Australia, SF 313, SP 1628, SF 321 and Italy.

Table I

The amount of total lipid and fatty acid methyl esters in the cysts and newly hatched nauplii of five strains of *Artemia*[1]

	Artemia sources						
	Australia	Brazil	SF 313	SF 321	SP 1628	Italy	Utah
mg total lipids[2]							
Cysts	$157^{a} \pm 8$	$134^{b} \pm 8$	N.A.[3]	N.A.	$134^{b} \pm 22$	$91.0^{c} \pm 6$	$136^{b} \pm 1$
Nauplii	$185^{c} \pm 9$	$202^{b} \pm 8$	$174^{d} \pm 4$	$159^{e} \pm 13$	$160^{e} \pm 3$	$156^{f} \pm 2$	$224^{a} \pm 14$
mg fatty acid methyl esters[4]							
Cysts	$510^{b} \pm 18$	$502^{b} \pm 12$	N.A.	N.A.	$573^{b} \pm 39$	$704^{a} \pm 34$	$490^{b} \pm 19$
Nauplii	$751^{a,b} \pm 41$	$854^{a} \pm 26$	$716^{a,b} \pm 30$	$602^{b} \pm 15$	$711^{a} \pm 58$	$739^{a} \pm 12$	$742^{a,b} \pm 10$

[1] Values within each row which bear the same letter are not significantly different at $P < 0.05$.

[2] Per gram dry weight of sample.

[3] N.A. = not analyzed.

[4] Per gram lipid.

Although the total lipid level of the Italian strain was the lowest in both cysts and nauplii, it contained significantly greater levels of FAME (mg/g lipid) than the other statistically similar cyst strains. The FAME levels for the nauplii of the Brazil, Australia, Utah, Italy, SP 1628 and SF 313 strains were all statistically similar as were the Australian, Utah, SF 313 and SF 321 nauplii, but the Brazilian, Italian and SP 1628 strains were significantly greater than the SF 321 strain.

The fatty acid composition of cysts and nauplii is presented in Table II and Table III, respectively. The relative proportion of fatty acids in cysts and nauplii remain the same, indicating that the fatty acid content of the chorion is small.

TABLE II

Fatty acid composition of whole cysts of five strains of *Artemia*

FAME	Aust.	Brazil	SP 1628	Italy	Utah
14:0	1.80	2.04	0.65	1.79	1.20
14:1	2.11	1.03	2.88	3.55	1.94
15:0[1]	1.02	0.95	0.22	0.14	- - - -[1]
15:1	0.44	0.38	0.55	0.56	0.50
16:0	15.11	16.35	9.80	14.15	12.39
16:1	10.66	12.88	6.49	13.05	6.00
16:2ω7	0.58	0.68	1.67	2.04	1.68
16:3ω4/17:1ω8	3.98	3.93	2.69	3.47	1.47
18:0	2.66	2.34	2.38	3.31	3.55
18:1ω9	26.71	33.50	27.43	26.05	28.03
18:2ω6	6.22	9.17	5.30	7.08	5.58
18:3ω3	13.19	4.39	31.85	6.24	28.16
18:4ω3	4.41	0.97	5.15	1.55	3.52
20:1ω9	0.27	0.49	0.46	0.31	0.21
20:2ω6/ω9	0.08	0.29	0.15	0.62	0.16
20:3ω6	0.77	2.30	0.04	1.35	0.27
20:5ω3	9.32	8.35	1.66	12.61	3.23
22:6ω3	0.26	0.11	trace	- - - -[1]	trace

[1] No value (- - -) means the fatty acid was not found.

The major fatty acids in all *Artemia* strains tested were 16:0, 16:1, and 18:1ω9. In addition, levels of 18:3ω3 and/or 20:5ω3 were substantial in various strains. Docosahexaenoic acid (22:6ω3) was found in only small amounts in the Australian and Brazilian *Artemia* nauplii. The *Artemia* strains were divided into two groups based on their most predominant long chain ω3 PUFA. Strains that contained mostly 18:3ω3 and 18:4ω3 included the SF 313, SP 1628, and Utah strains, while the group that contained predominantly 20:5ω3 included SF 321, Italian and Brazilian strains. The Australian strain contained substantial amounts of both 18:3ω3 and 20:5ω3 and therefore could be included in either of the two groups.

TABLE III

Fatty acid composition of newly hatched *Artemia*

FAME	Australia	Brazil	SF 313	SF 321	SP 1628	Italy	Utah
14:0	1.34	1.57	0.99	1.57	0.43	1.53	0.93
14:1	2.23	0.81	1.27	0.74	2.26	3.30	1.45
15:0	0.34	0.67	0.16	0.58	0.25	0.11	0.11
15:1	0.15	0.24	0.20	0.13	0.46	0.54	0.37
16:0	13.45	15.42	10.33	12.13	7.79	15.23	11.78
16:1	9.97	10.79	13.27	19.52	5.24	10.38	5.64
16:2ω7	- - - -[1]	- - - -[1]	- - - -[1]	- - - -[1]	1.51	2.94	- - - -[1]
16:3ω4/17:1ω8	3.87	3.88	2.09	2.32	2.44	3.28	2.90
18:0	3.07	2.79	6.83	2.90	3.08	3.17	4.07
18:1ω9	28.23	35.86	26.97	31.20	29.15	29.05	28.58
18:2ω6	5.78	9.59	9.35	3.69	4.60	6.79	4.60
18:3ω3	14.77	4.87	17.33	5.16	33.59	6.35	31.46
18:4ω3	4.37	0.96	3.26	1.28	4.88	1.01	3.10
20:1ω9	0.37	0.52	0.41	0.35	0.35	0.42	0.37
20:2ω6/ω9	0.12	0.06	0.06	- - - -[1]	0.24	0.20	0.09
20:3ω6	0.79	2.76	1.01	2.23	0.05	1.47	0.48
20:3ω3/20:4ω6	- - - -[1]	- - - -[1]	1.48	2.69	1.48	- - - -[1]	- - - -[1]
20:5ω3	10.50	8.98	4.06	12.44	1.68	13.63	3.55
22:6ω3	0.26	0.06	- - - -[1]	- - - -[1]	- - - -[1]	- - - -[1]	- - - -[1]

[1] No value (- - - -) means the fatty acid was not found.

Energy content for the geographical strains is presented in Table IV. Using the same statistical tests as in the lipid and FAME evaluations, differences in energy content between the various strains are significant ($P \leq 0.05$). The Australian strain contained the most energy with 2.50×10^4 J/g ash-free dry weight (5.961 Kcal/g); the Italian strain contained the least with 2.24×10^4 J/g ash-free dry weight (5.370 Kcal/g).

TABLE IV

The energy content[1,2] ($\times 10^4$) of five strains of *Artemia* nauplii

Australia	Brazil	SP 1628	Italy	Utah
$2.50^a \pm 0.16$	$2.35^{a,b} \pm 0.04$	$2.35^{a,b} \pm 0.11$	$2.24^b \pm 0.06$	$2.34^{a,b} \pm 0.08$

[1] J/g ash-free dry weight (ash content 5.4% dry weight).

[2] Values which bear the same letter subscript are not significantly different at $P \leq 0.05$.

Discussion

Major differences were found in the lipid level, FAME levels, fatty acid composition and the energy content of the cysts and nauplii of the five geographically different strains. These differences could have been caused by variations in the genetic make-up or the previous

dietary history of the parental stock which produced the cysts. Clark and Bowen (1976) have isolated six separate species from 27 different geographical strains of *Artemia* and Bowen *et al.* (1978) have shown a genetic variation in the hemolymph proteins of various strains. More recently, Abreu-Grobois and Beardmore (1980) found evidence for speciation between populations of *Artemia* and Seidel *et al.* (1980) showed a variation in the total protein electrophoretic patterns of the same five strains analyzed here.

The total FAME analysis provided an indication of the type of lipids associated with the various strains. A disproportionately high level of FAME for the Italian and a low value for the Utah strains indicated the two extremes. The Italian strain must have contained a higher level of triglyceride lipids per gram sample whereas the Utahan strain probably contains a greater proportion of phospholipids and/or sterol-type lipids.

The loss of the chorion, upon cyst hatching, resulted in a greater amount of lipid material per unit weight nauplii, except in the Italian strain. This would indicate that the chorion of the Italian strain contained more lipids than the shells of the other strains. The relative proportion of fatty acids of the cysts and nauplii remained the same before and after hatching and, when related to the increased lipid and FAME levels in the nauplii, indicates that the shell contains practically no fatty acids, with a possible exception of the Italian strain.

The nutritional quality of the five geographical strains used in this study has been recently determined for several species of marine larvae (Johns *et al.*, 1978, 1980 ; Beck *et al.*, 1980 ; Klein-MacPhee *et al.*, 1980). Johns *et al.* (1980), working with the larval stages of the mud crab *Rhithropanopeus harrisii* and the rock crab *Cancer irroratus* found marked differences in the ability of the geographical strains of brine shrimp nauplii to promote high survival and growth. Crab larvae did not complete larval development when fed SP 1628 or the Utah strain but did so when fed the Brazilian, Australian or Italian strains. Klein-MacPhee *et al.* (1980) found similar results when winter flounder *Pseudopleuronectes americanus* larvae were reared using the five geographical strains as food sources. Survival through metamorphosis was high in fish larvae fed Australian, Brazilian or Italian strains while it was markedly lower for larvae fed SP 1628 or Utah brine shrimp.

Beck *et al.* (1980), also working with the larvae of a marine fish (*Menidia menidia*), found varying results. Survival and growth of the fish larvae were dependent on several factors including the previous dietary history of the fish larvae. Differences in survival between the fish fed the various geographical strains were less prominent when all larvae were fed the Brazilian strain rather than the SP 1628 strain prior to the start of the experiment. In an earlier study, Johns *et al.* (1978) had found that both SF 313 and SF 321 promoted good survival throughout larval development of the mud crab. Beck *et al.* (1980) also showed that these two strains promoted higher (but not significant) survival in *Menidia menidia* than the SP 1628 strain.

The relatively good survival and growth of the marine organisms fed the Italian strain indicates that the lipid levels and energy content are probably adequate. The total energy values of the five strains were in close agreement with those of Paffenhöfer (1967) who found a level of 5 953 calories (2.49×10^4 J) per gram organic substance for an unidentified brine shrimp strain. Because the lipid levels and total energy values for the SP 1628 and Utah strains were greater, all strains were considered sufficient in energy and are probably not a significant contributor to the poorer growth and survival of the SP 1628 and Utah fed organisms.

The fatty acid composition of the nauplii appeared to be much more closely related to the biological effects of the five *Artemia* strains. The fatty acid composition of the strains evaluated here may have been different enough to affect their potential nutritional value. Strains that are higher in 20:5ω3 (Italy, SF 321, Brazil, and Australia) would presumably be better diets for marine organisms (Owen *et al.*, 1972 ; Yone and Fujii, 1975b ; Guary *et al.*, 1976 ; Watanabe *et al.*, 1978). Furthermore, the high level of 18:3ω3 (> 30% of the total FAME) and the low level of 20:5ω3 in the Utah and SP 1628 nauplii may have induced a nutritional stress in marine consumer organisms.

Watanabe *et al.* (1978) stated "that the class of EFA (essential fatty acids) contained in *Artemia* (principally 20:5ω3 and 22:6ω3) is the principle factor in the food value of *Artemia* to fish". This was determined by modifying the fatty acid composition of a San Francisco Bay Brand *Artemia* (1976), deficient in 20:5ω3 and 22:6ω3, by feeding them either a marine *Chlorella* or a yeast-supplemented squid liver oil, both of which are rich in these long chain ω3 PUFA. The enriched *Artemia* induced better growth and survival of their test organism, the red sea bream *Chrysophys major*.

Artemia have generally been shown to contain either a fatty acid predominance of 18:3ω3 or 20:5ω3 (Enzler *et al.*, 1974 ; Benijts *et al.*, 1976 ; Gallagher and Brown, 1975 ; Claus *et al.*, 1977, 1979 ; Watanabe *et al.*, 1978 ; Fujita *et al.*, 1980). Similar results were obtained in this study. Also, a variation was found in the fatty acid composition of cysts collected within the same year at a similar location (Table III, SF 313 *versus* SF 321) (personal communication, A. Schmidt, 1978).

One other important factor which relates to the lipid character of a food organism is the interaction of lipids with contaminants. This is important because a number of cyst strains are collected from salinas in locations near commercial and agricultural regions (*e.g.* San Francisco Bay, San Pablo Bay, and Great Salt Lake). An analysis of the five geographical strains for chlorinated hydrocarbons revealed that all are contaminated to some extent ; the Italian strain contained more of the DDT family of pesticides, the Utah and SP 1628 contained higher levels of dieldrin and the SP 1628 contained substantially higher levels of dieldrin and much higher chlordane levels than the other strains (Olney *et al.*, 1980).

The lipid content of a diet can affect the accumulation of pesticides which can thereby alter lipid metabolism. For example, Phillips and Buhler (1979) found that rainbow trout fed dieldrin-contaminated tubificid worms (15% lipid) experienced a decrease in lipid accumulation while fish fed an artificial diet (10% lipid) containing dieldrin exhibited normal rates of lipid accumulation. These authors suggested that the increased dietary lipid level created a greater reservoir for pesticide accumulation which, in turn, may have altered lipid metabolism. Furthermore, Durham (1967) has shown that dieldrin affects the metabolism of unsaturated fatty acids and accentuates the symptoms of a deficiency of essential fatty acids.

Considering the biological results of the mud crabs (Johns *et al.*, 1980) and winter flounder (Klein-MacPhee *et al.*, 1980) that were fed SP 1628 and Utahan strains of *Artemia*, it is possible that the excess 18:3ω3 and/or minimal quantities of 20:5ω3 could result in a nutritional stress which, in the presence of a dietary contaminant, could be manifested in a synergistic fashion. Both a nutritional stress and/or a pesticide contaminant could potentially exert a physiological response which may be elevated to a critical point at the time of metamorphosis. This may explain why the results of the five strains as diet on survival of Atlantic silversides (Beck *et al.*, 1980), which has a much less distinct metamorphosis, was not as

dramatic as in the mud crab and winter flounder. However, specific investigations will be required to evaluate these conditions.

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International Study on *Artemia*[1] XI. Amino acid composition and electrophoretic protein patterns of *Artemia* from five geographical locations

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Abstract

The cysts of five geographical strains of *Artemia* (Australia, Brazil, Italy, Utah-USA, and San Pablo Bay, California-USA) were hatched and the newly hatched nauplii were acid hydrolyzed and analyzed for their amino acid content (Technicon Amino Acid Analyzer). Strains from Australia, Brazil, Italy, and Utah were similar in amino acid content, whereas the San Pablo Bay, California strain exhibited several values different from the other four strains. Essential amino acid levels for all five strains of *Artemia* were considered adequate in terms of the dietary requirement levels for Chinook salmon and levels that appear to be adequate for Atlantic silversides (Seidel *et al.*, 1980).

Electrophoretic protein patterns for the five strains of *Artemia* were compared in an effort to differentiate the strains. No two strains, when paired, showed identical banding patterns. Strains from San Pablo Bay and Utah had the most bands in common (81%) of any pair, and strains from Australia and Brazil had the fewest bands in common (30%). Whether this variation in electrophoretic protein patterns may occur among batches of one strain of *Artemia*, as well as among many strains, remains a question. Based on our preliminary study, there seems to be a need for future work in this area to test for the effect of temporal and environmental factors on the protein patterns of *Artemia*.

Introduction

Considerable dependence has been placed on brine shrimp (*Artemia*) as a food source in culturing fish and crustacean larvae. As a result, *Artemia* cysts have become in great demand, creating commercially high prices. Despite their relatively limited occurrence in nature, a number of geographical strains of brine shrimp are known and available.

In the past, success in fish culture operations has varied according to the specific strain of brine shrimp used. Whether this is due to toxic contamination (Bookhout and Costlow, 1970),

[1] International Interdisciplinary Study on *Artemia* Strains coordinated by the Artemia Reference Center, State University of Ghent, Belgium.

biochemical composition (Seidel *et al.*, 1980) or nutritional quality (Wickins, 1972), however, remains a question.

With a variety of brine shrimp strains now available, there is a need to provide aquaculturalists with reliable information in order that they may choose the most effective diet for a particular species. The diet's nutritional quality is an area of considerable importance. Dietary protein, for example, accounts for the maintenance of fish body tissues, the repletion of depleted tissues and the formation of new, additional protein (Cowey and Sargent, 1972). In view of its importance, this paper presents a biochemical analysis of amino acids and electrophoretic patterns of protein for five geographical strains of brine shrimp. Other biochemical characteristics of *Artemia* strains, such as lipids and caloric content (Schauer *et al.*, 1980), pigments (Soejima *et al.*, 1980) and pesticides and heavy metals (Olney *et al.*, 1980) will be covered separately.

Materials and methods

Sources and culturing of brine shrimp

Dehydrated cysts from five geographical locations were provided by the Artemia Reference Center (Ghent, Belgium). The origin of these cysts were Shark Bay, Australia (World Ocean, lot no. 113); Macau, Brazil (Companhia Industrial do Rio Grande do Norte, CIRNE-Brand, harvested 1978); Margherita di Savoia, Italy (harvested 1977); Great Salt Lake, Utah, USA (harvested 1977); San Pablo Bay, California, USA (Living World, San Francisco Bay Brand, Inc., lot no. 1628).

The cysts were rehydrated and hatched into nauplii according to procedures of Beck *et al.* (1980) at the Environmental Protection Agency (EPA), Environmental Research Laboratory (ERLN), Narragansett, Rhode Island, USA. Each strain was harvested as newly hatched nauplii, labeled and frozen at -20 °C until the time of analysis.

Protein hydrolysis and amino acid analysis

Frozen nauplii of each strain of brine shrimp were thawed to room temperature and were then acid hydrolyzed in duplicate. The procedure involved the *in vacuo* hydrolysis of protein material at 110 °C for 22 hr with 3.0 ml of 6.0 N HCl. Heavy walled, vacuum hydrolysis tubes were prepared for use by washing in a chromic acid cleaning solution, then in a mixture of H_2SO_4 and HNO_3 (3:1), rinsed overnight in deionized water and oven dried. The hydrolysate was filtered through a Buchner funnel (size 15-M) and rotary evaporated *in vacuo* at 37 °C. To insure removal of HCl, the residue was wet three times with 1 ml of deionized water and dried in the rotary evaporator. The free amino acid residue was then dissolved in 3-5 ml of pH 2.2 sodium citrate buffer, filtered through a 0.45 μ Millipore filter and stored under nitrogen at -20 °C until final dilution was made just prior to time of analysis.

Amino acid analyses were performed on a NC-2P Technicon Auto Analyzer equiped with a 25×0.5 cm cationic exchange column packed with Chromobead Resin, Type C-3 (ion exchange capacity of 5 meq/mg ; 8% cross-linked). The column was operated at 55 °C with a flow rate of 0.5 ml/min. A Columbia Scientific Instruments Supergrator-2 Integrator/Calculator was used to compute the absolute amount of each amino acid using color response

factors as determined from an authentic standard mixture of amino acids obtained from Pierce Chemical Co., Rockford, Illinois, USA.

Electrophoresis

Artemia nauplii from Australia, Brazil, Utah, Italy, and San Pablo Bay-California were run according to two electrophoretic techniques. The first was thin layer polyacrylamide gel isoelectric focusing (TLIEF), a technique developed by Kryznowek and Wiggin (1979). *Artemia* nauplii were focused in this manner at the National Marine Fisheries Service Gloucester Laboratory, Gloucester, Massachusetts, USA. Samples were applied to the gel in two forms : as a 'drip' and as the 'whole nauplii'. Preparation of the 'drip' was as follows : Each sample was thawed to room temperature, then approximately 2-4 g (wet wt) was centrifuged at 3 000 rpm for 30 min at -2 °C. The supernatant (liquid from thawing process) was spotted onto filter paper tabs on the gel in 10 and 20 μl amounts. The solid residue in the centrifuge tube (freshly prepared as above) was the 'whole nauplii' sample. It was spotted into gel wells twice for a total of 14 μl in the first 3-4 hr of the run in order to concentrate the protein. All runs were at 1 Watt constant power for 20 hr to a maximum of 500 volts. Gels were stained according to Righetti and Drysdale (1974).

The second electrophoretic technique tried was that of Saravis and Zamcheck (1979) and Saravis *et al.* (1979), and it involved rapid isoelectric focusing of proteins in a thin layer agarose gel. The thawed *Artemia* nauplii (5 μl) were applied directly to the gel in this method and were focued 10 min at 10 mA constant power, then 15 additional min at 25 W constant power. The five *Artemia* strains were focused in this manner at Boston City Hospital, Boston, Massachusetts, USA.

Results

Amino acids

The amino acid profiles for five strains of *Artemia* nauplii are presented in Table I. Values for essential and nonessential amino acids vary only slightly between strains from Australia, Brazil, Utah, and Italy. There are, however, several variations between the above four strains and the San Pablo Bay, California strain, including values for serine, glutamic acid, proline, phenylalanine, and lysine.

The essential amino acid requirements for Chinook salmon are presented in Table II. All five strains of brine shrimp appear to meet these requirement levels with the exception of phenylalanine in the Brazilian strain and valine in the Italian strain. These two exceptions, however, are only very slightly lower than the requirement levels.

Electrophoresis

Brine shrimp nauplii were run in a polyacrylamide gel (Kryznowek and Wiggin, 1979). Preliminary focusing was tried in the pH 5-7 range with San Pablo Bay *Artemia*, however bands were seemingly too numerous to be meaningful (Fig. 1). Fewer, and therefore more potentially unique bands were seen in basic pH ranges than in acidic pH ranges. The most useful banding patterns were seen in the range of pH 7-9 (Fig. 2).

No two strains of brine shrimp showed identical banding patterns in the pH 7-9 range when 'whole nauplii' samples were focused. The San Pablo Bay and Utah strains had 81% of their bands in common, followed by Italy-Brazil (74%), San Pablo Bay-Brazil (71%), Italy-San Pablo Bay (70%), Italy-Utah (64%) and Brazil-Utah (63%) (Fig. 3). No other combinations of the five strains showed over 50% common bands. Brine shrimp from Australia and Brazil had only 30% of their bands in common, which was the least of any two strains in the pH 7-9 range.

TABLE I

Amino acid profiles for *Artemia* nauplii from five geographical locations expressed as g amino acid/100 g protein (tryptophan destroyed by HCl)

Amino acid	Australia	Brazil	San Pablo Bay	Utah	Italy
Aspartic acid	10.8	11.0	14.1	11.3	11.2
Threonine	5.5	5.2	6.0	4.8	5.5
Serine	5.9	4.5	7.7	5.4	5.1
Glutamic acid	16.3	13.1	10.2	13.5	14.5
Proline	5.4	5.7	4.9	5.9	5.9
Glycine	5.7	6.0	7.4	6.0	7.2
Alanine	5.4	4.6	4.2	4.9	4.9
Valine	5.4	5.3	5.5	5.2	3.1
Methionine	2.8	2.2	2.6	3.7	3.7
Isoleucine	4.9	5.6	5.4	6.8	6.4
Leucine	7.9	8.9	8.4	10.0	10.1
Tyrosine	7.3	10.5	7.7	6.6	5.4
Phenylalanine	7.7	5.1	10.4	8.5	8.5
Histidine	3.8	4.9	3.5	2.7	3.8
Lysine	10.6	11.7	8.7	9.3	10.7
Arginine	10.9	11.5	9.8	9.7	9.8

TABLE II

Essential amino acids for *Artemia* nauplii from five geographical locations and Chinook salmon dietary requirements for these amino acids (expressed as g amino acid/100 g protein)

Amino acid	Chinook salmon	Australia	Brazil	San Pablo Bay	Utah	Italy
Threonine	2.5	5.5	5.2	6.0	4.8	5.5
Valine	3.25	5.4	5.3	5.5	5.2	3.1
Methionine	1.5	2.8	2.2	2.6	3.7	3.7
Isoleucine	2.5	4.9	5.6	5.4	6.8	6.4
Leucine	4.0	7.9	8.9	8.4	10.0	10.1
Phenylalanine	5.25	7.7	5.1	10.4	8.5	8.5
Histidine	1.75	3.8	4.9	3.5	2.7	3.8
Lysine	5.0	10.6	11.7	8.7	9.3	10.7
Arginine	5.75	10.9	11.5	9.8	9.7	9.8

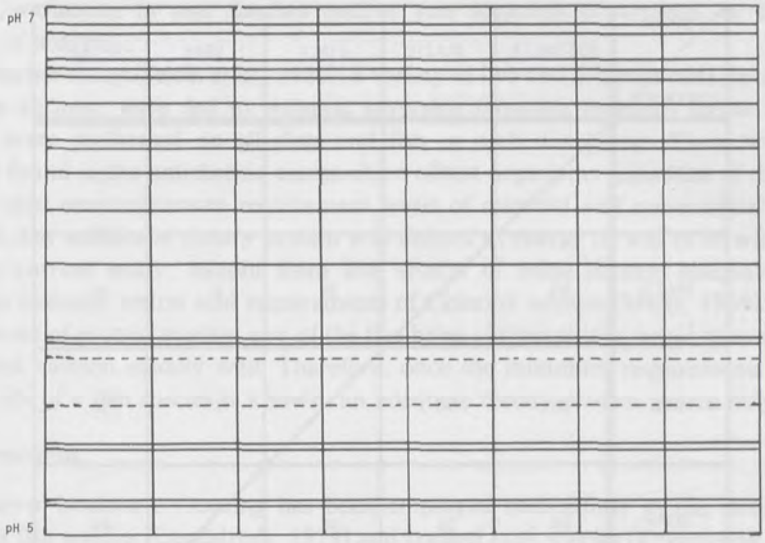

FIG. 1. Electrophoretic banding patterns of San Pablo Bay *Artemia* nauplii in polyacrylamide gel at pH 5-7 (8 replicates). Dotted lines indicate lightly stained protein bands. (Sketched from photograph of gel).

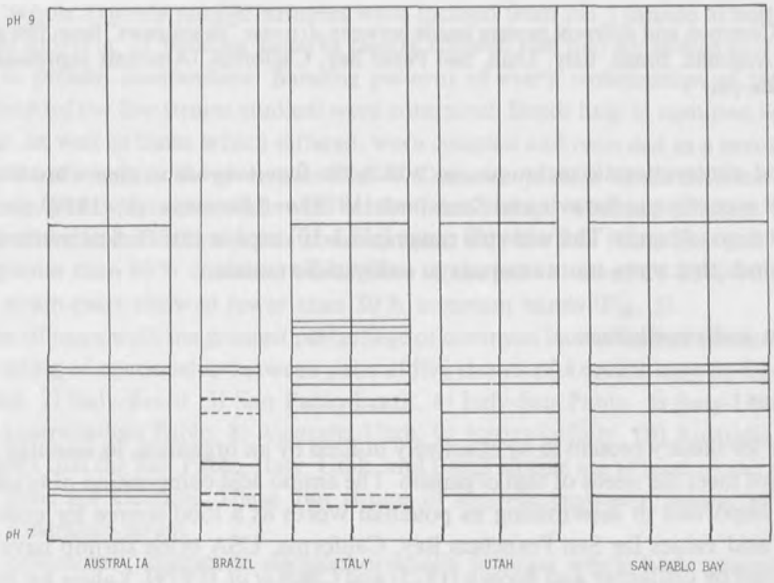

FIG. 2. Electrophoretic banding patterns of *Artemia* nauplii from five geographic locations in polyacrylamide gel at pH 7-9 : Australia, 2 replicates ; Brazil, 1 replicate ; Italy, 2 replicates ; Utah, 2 replicates ; San Pablo Bay, 3 replicates. Dottes lines indicate lightly stained protein bands. (Sketched from photograph of gel).

COMMON / DIFFERENT	AUSTRALIA	BRAZIL	ITALY	UTAH	S.P. BAY, CALIF.
AUSTRALIA		15	16	20	23
BRAZIL	69		35	31	36
ITALY	68	30		32	35
UTAH	60	31	36		44
S.P. BAY, CALIF.	54	29	30	13	

FIG. 3. Common and different protein bands between *Artemia* "strain-pairs" from five geographical locations: Australia, Brazil, Italy, Utah, San Pablo Bay, California. (Amounts expressed as % total bands/"strain-pair").

A second electrophoretic technique, in which the five *Artemia* strains were focused in an agarose gel according to Saravis and Zamcheck (1979) and Saravis *et al.* (1979), provided very rapid resolution of bands. The wide pH range (pH 3-10) used in this clinical method, however, showed bands that were too numerous to easily differentiate.

Discussion and conclusions

AMINO ACIDS

In order for dietary protein to be effectively utilized by an organism, its essential amino acid pattern must meet the needs of that organism. The amino acid composition of brine shrimp is, therefore, important in determining its potential worth as a food source for culturing fish.

Amino acid values for San Francisco Bay, California, USA brine shrimp have previously been reported by Gallagher and Brown (1975) and Claus *et al.* (1979). Values for brine shrimp from Great Salt Lake, Utah have also been reported by Claus *et al.* (1979). In comparing the above amino acid profiles with those of the current study for San Pablo Bay, California and Utah strains, differences may be found in values for glycine, alanine, valine, methionine, tyrosine and phenylalanine. These differences may be due to variations in the age of *Artemia*

analyzed, variations in year batches and/or year locations of *Artemia*, or differences in methods of analysis.

In an earlier study (Seidel *et al.*, 1980), a variety of live and artificial diets, including 3-day-old brine shrimp, were fed to Atlantic silverside (*Menidia menidia*) larvae. Biochemical analyses were performed on all diets and fish in each diet group. There was very little variation found in the amino acid composition of test organisms regardless of diet treatment, implying that once minimum requirement levels of essential and nonessential amino acids were met, any additional dietary protein was utilized as energy or was otherwise eliminated.

In the current study, nauplii from five strains of brine shrimp adequately meet the minimum essential amino acid requirements of Chinook salmon (Mertz, 1969). This implies that in terms of protein quality, any of the five brine shrimp strains could support a fish such as Chinook salmon equally well. Therefore, once the minimum requirements for essential amino acids of a fish species is known, an adequate dietary protein source may be selected.

Electrophoresis

Thin layer isoelectric focusing has been employed successfully in the differentiation of uncooked fish species (Lundstrom, 1979) and cooked crab species (Kryznowek and Wiggin, 1979). In our study, *Artemia* were similarly focused in an attempt to differentiate strains on the basis of protein patterns.

Protein banding patterns in the pH 5-7 range were observed in preliminary focusing of San Pablo Bay *Artemia* (Fig. 1). The great number of bands in this range led to focusing in the pH 7-9 range since it seemed very unlikely to be able to see unique bands in the acidic range.

When 'whole *Artemia* nauplii' samples were focused from pH 7 (anode at bottom) to pH 9 (cathode at top) (Fig. 2), banding patterns clearly showed that no two strains of *Artemia* were identical in protein composition. Banding patterns of every combination of two strains of brine shrimp (of the five strains studied) were compared. Bands held in common between each strain-pair, as well as those which differed, were counted and recorded as a percentage of the total number of bands in the given pair (Fig. 3). Common protein bands between members of a pair were positively correlated with strain relationship, whereas different bands were negatively correlated. Of the ten pair combinations, only the San Pablo Bay and Utah strains showed greater than 80% common bands ; five strain-pairs showed 60-80% common bands and four strain-pairs showed fewer than 50% common bands (Fig. 3).

In terms of pairs with the greatest percentage of common bands, and in descending order, a tentative listing of relationship between pairs of five strains of *Artemia* may be formed : 1) San Pablo-Utah, 2) Italy-Brazil, 3) San Pablo-Brazil, 4) Italy-San Pablo, 5) Italy-Utah, 6) Brazil-Utah, 7) Australia-San Pablo, 8) Australia-Utah, 9) Australia-Italy, 10) Australia-Brazil. This data suggests that the San Pablo, Italy, Utah, and Brazil strains are at least moderately related, although none are identical. These four strains of *Artemia*, however, appear to be different from the Australian strain.

In this preliminary attempt to electrophoretically separate whole body proteins from five strains of *Artemia*, several objectives were achieved. The basic pH range (7-9) showed more potentially unique bands than acidic pH ranges, a 'whole nauplii' sample applied directly into the gel gave better protein concentrations and resolution of bands than a 'drip' sample, and a urea-based gel was more effective than an agarose gel. Future work with *Artemia* electrophoresis may further refine the above objectives as well as advancing the technique in other

ways. The present findings, however, seem to provide useful 'fingerprinting' data which could be used in the quick identification of unknown *Artemia* strains. It is possible, however, that electrophoretic data would show as much variation among several batches of one *Artemia* strain as it does here among several strains. If this is the case, its reliability as a quick identification method may be limited.

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International Study on *Artemia*[1] XIII. A comparison of production data of 17 geographical strains of *Artemia* in the St. Croix Artificial Upwelling-Mariculture System[2]

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Abstract

Seventeen geographical strains of *Artemia*, 13 bisexual and 4 parthenogenetic, were cultured through sexual maturity to select a strain which gives the best production in the St. Croix Artificial Upwelling-Mariculture System.

Individual strains were compared for survival, growth, and time to sexual maturity. Dry and ash weight were determined on 100 couples (bisexual) or 200 females (parthenogenetic) from each strain. Total biomass (dry weight) production per strain has also been determined. Five strains were compared for the percent protein content of sexually mature individuals.

On the basis of the results obtained, the best strains for use in the St. Croix System are the bisexual *Artemia* from Macau (Brazil), San Pablo Bay (California, USA), San Francisco Bay (California, USA) and Adelaide (Australia).

Introduction

The St. Croix Artificial Upwelling Project utilizes a constant flow of nutrient-rich water (Antartic Intermediate Water) pumped from a depth of 870 m below the surface into two ponds (100 m^2, 1 m deep). This system produces 100 000 l/day of unialgal cultures of *Chaetoceros curvisetus* (STX-167) at 4.5×10^4 cells/ml, grown in Antarctic Intermediate Water, salinity 34.8 ‰ at a temperature of 23-29 °C (Roels *et al.*, 1976).

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This upwelling project has been basically designed for shellfish production whereby the phytoplankton suspension is pumped continuously to filter-feeding shellfish (Roels *et al.*, 1979).

Since 1978 research has been focused on the production potential of brine shrimp as secondary producer. The technical feasibility of *Artemia* culturing in the St. Croix Artificial Project has already been demonstrated (Tobias *et al.*, 1979). From the experience gained so far, this new culturing technique offers unique possibilities for the mass production of brine shrimp in flow-through systems (Sorgeloos, 1980).

Although *Artemia* populations have been reported from over 100 geographical locations in the world (Persoone and Sorgeloos, 1980), only a few strains have been analyzed to date for their biometrical, genetical, nutritional, and chemical characteristics (see papers of the "International Study on *Artemia*", this symposium) and apparently no information is presently available on the production performances of different strains in controlled culture systems.

The purpose of this study was to screen various geographical strains of *Artemia* to select one or more, either bisexual or parthenogenetic, for their production performance in the St. Croix Artificial Upwelling-Mariculture System.

Strain selection was based on survival, growth, time to sexual maturity, dry and ash weights, and protein content.

Materials and methods

The tests were conducted in an experimental rack designed to accommodate two rows of 12, 2 l plastic aquaria. Each aquarium was equipped with an internal standpipe-filter, 3.8 cm in diameter and 11.4 cm in height (Fig. 1).

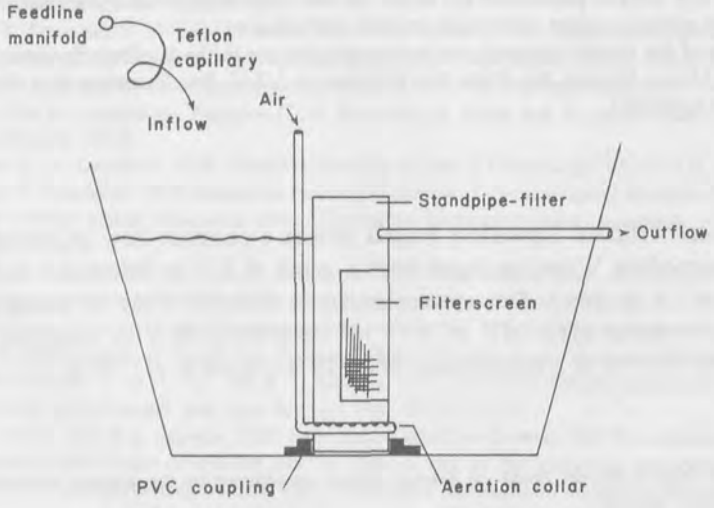

FIG. 1. Cross section through 2 l flow through culturing unit.

Two-thirds of the standpipe area was cut away in the shape of a rectangle and the opening covered with a monofilament bolting cloth (TET-Kressilk Products, New York) as a filter. Two mesh sizes, 190 and 350 μm, were used for the filters. A circular piece of flat PVC plate, 3.8 cm in diameter, was glued to the base of the standpipe-filter to provide a watertight seal. The effluent water passing through the filter leaves the tank via a 1.27 cm diameter overflow tube fitted snugly through the standpipe-filter and the tank wall.

Each 2 l tank was equipped with an aeration collar, consisting of a pre-formed piece of perforated teflon tubing, to prevent the fine mesh internal standpipe-filter from clogging with cells and to provide additional aeration and adequate culture mixing. As the *Artemia* became larger, the internal standpipe filters were changed from 190 to 350 μm to prevent clogging by the increasingly larger *Artemia* molts.

The experimental *Artemia* tanks received continuous flow of unialgal cultures of *Chaetoceros curvisetus* (STX-167) via gravity feed from overhead reactors, [2 000 l concrete culture tanks, (Malone *et al.*, 1975)]. The algal cultures flowed into a PVC mixing chamber before entering the *Artemia* feedline manifold on the experimental rack. The flow rates to the individual *Artemia* tanks were regulated at 6 l/hr by pre-cut lengths of black teflon capillary tubing from the feedline manifold.

Seventeen geographical strains of *Artemia* were tested using up to eight strains at a time. Three runs were made, based on expected hatching times ; the San Pablo Bay, California, USA strain was repeated in each of the three runs as a control (Table I).

TABLE I

The 17 geographical strains of *Artemia* grouped into three test runs. The letter (B) denotes a bisexual strain and (P) a parthenogenetic strain

Test 1	Test 2	Test 3
San Pablo Bay, California, USA (B)	San Pablo Bay, California, USA (B)	San Pablo Bay, California, USA (B)
(San Francisco Bay Brand Inc., batch 1628)	(San Francisco Bay Brand Inc., batch 1628)	(San Francisco Bay Brand Inc., batch 1628)
Great Salt Lake, Utah, USA (harvest 1977) (B)	Macau, Brazil (B)	San Francisco Bay, California, USA (B)
Shark Bay, Australia (P)	Port Araya, Venezuela (B)	(San Francisco Bay Brand Inc., batch 236-2016)
Adelaide, Australia (B)	Bonaire, Netherlands Antilles (B)	Great Salt Lake, Utah, USA (B) (harvest 1966)
Buenos Aires, Argentina (B)	Larnaca Salt Lake, Cyprus (B) (sample 1)	Bahia Salinas, Puerto Rico (B)
Lavalduc, France (P)	Tuticorin, India (P)	Margherita di Savoia, Italy (P)
Galera Zamba, Colombia (B)	Unknown origin, China (B)	Larnaca Salt Lake, Cyprus (B) (sample 2)
	Santa Pola, Spain (B)	

Each strain was tested in triplicate at a stocking density of 2 animals/ml or 4 000 *Artemia* per aquarium. Two different batches of *Artemia* from Great Salt Lake, Utah, USA were tested : harvest 1966 and harvest 1977.

Cysts were hatched out in 1 000 ml glass cones in aerated Antartic Intermediate Water, salinity 34.8 ‰, at 25 to 30 °C under continuous illumination. When enough *Artemia* were available, the animals were transferred to the experimental 2 l tanks, which were filled with STX-167 suspension. It was necessary to batch feed the animals until day 2 (tank cultures changed daily) due to the potential loss of some *Artemia* through the 190 μm mesh filter screen. After that time the 2 l tanks were placed on continuous flow.

Each strain was kept in the experiment until 50% of the animals in each of the three replicate tanks were sexually mature. Sexual maturity was determined as 50% of the population in the riding stage for the bisexual strains, and 50% of the females with oocytes present in the oviducts for the parthenogenetic strains.

Upon reaching sexual maturity, 100 couples of the bisexual strains or 200 females of the parthenogenetic strains were removed from the aquaria, rinsed with deionized water and drained. The animals were placed in tared aluminum weighing tins and dried at 60 °C to constant weight. After cooling for 1 hr in a dessicator, dry weights were determined. Ash weight determination was done according to Claus *et al.* (1979) by placing the samples in a muffle furnace, ashing at 550 °C for 4 hr, cooling in a dessicator for another hour, and weighing.

For the length measurements, 30 *Artemia* were removed from each strain on days 5 (coinciding with the change of the standpipe-filter screens), and 15, and preserved in lugol's solution. Line drawings of animal length were made with the aid of a dissecting microscope and converted to linear measurements with a curvimeter.

Survival was determined on days 5 and 15 by draining the entire tank content down to 1 l and transferring the suspension to a 1 l aerated glass hatching cone ; 10, 2 ml subsamples were than taken to determine the percent survival.

At the end of the experiment 3 to 4 mg dry weight *Artemia* were removed from the dried population for protein analysis, following the method of Dorsey *et al.* (1977). Due to lack of time only five strains could be analyzed, *i.e.* Larnaca Salt Lake, Macau, Margherita di Savoia, Santa Pola, and Tuticorin.

To quantify the algal food supplied to the *Artemia* cultures, algal protein was determined daily on samples taken from the algal culture mixing chamber at 3 p.m. Two 75-ml replicate samples of the algal culture were filtered on glass-fiber filters and algal protein determined on the whole filter by the method of Dorsey *et al.* (1977).

Individual tank temperatures were recorded at 8 am and 2 pm daily and tank flow rates at 2 pm daily.

Results and discussion

Temperature of the algal reactor cultures on the beach varied from 22.9 to 29.3 °C ; salinity remained constant at 34.8 ‰. Inflow algal cell densities ranged from 4.9×10^4 to 1.2×10^5 cells/ml and flow rates were maintained at 94% of the intended flow. The inflow algal protein ranged from 0.007 to 0.017 g protein/hr.

Table II shows the percent survival of the 17 *Artemia* strains on days 5 and 15 and the number of days required for each strain to reach sexual maturity.

If a strain reached sexual maturity before day 15, the percent survival was determined on that particular day and the strain removed from the experiment.

The following strains reached sexual maturity on or before day 15 with a survival larger than 90% : San Pablo Bay, Great Salt Lake (1966), Macau, San Francisco Bay, Great Salt Lake (1977), Bahia Salinas, Adelaide, and Bonaire. Of these eight strains, San Pablo Bay *Artemia* reached sexual maturity in 12 days. The Margherita di Savoia strain was the only parthenogenetic one showing more than 90% survival at sexual maturity.

The Galera Zamba and Buenos Aires strains had significantly lower survivals ; on the other hand they required only 11 days to reach sexual maturity. This is still far from the absolute record figure of Teramoto and Kinoshita (1961) who obtained adult brine shrimp within 7 days after hatching. The Port Araya strain required the longest time to sexual maturity, namely 21 days.

TABLE II

Percent survival of the 17 geographical strains of *Artemia* on day 5 and day 15 and number of days required to reach sexual maturity

Strain	Survival (%)		Time to sexual maturity (days)
	Day 5	Day 15[a]	
(1)[b] San Pablo Bay, California, USA	100	100	12
(1) Great Salt Lake, Utah, USA (harvest 1966)	100	100	13
(2) Macau, Brazil	100	100	14
(3) San Francisco Bay, California, USA	93	100[c]	14
(1) Great Salt Lake, Utah, USA (harvest 1977)	100	100	15
(1) Bahia Salinas, Puerto Rico	89	100[c]	14
(1) Adelaide, Australia	90	100[c]	15
(1) Margherita di Savoia, Italy	100	92	15
(1) Bonaire, Netherlands Antilles	100	100	19
(1) Galera Zamba, Colombia	65	79[c]	11
(2) China	100	72	19
(1) Port Araya, Venezuela	100	61	21
(1) Larnaca Salt Lake, Cyprus (sample 2)	100	54	17
(1) Lavalduc, France	42	42	15
(1) Shark Bay, Australia	60	39	15
(2) Tuticorin, India	43	37	14
(2) Santa Pola, Spain	100	38	21
(1) Buenos Aires, Argentina	33	29	11
(4) Larnaca Salt Lake, Cyprus (sample 1)	100	25	14

[a] If a strain reached sexual maturity before day 15, a growth sample of 30 *Artemia* was taken on that day and the strain removed from the experiment.

[b] Number of times an acceptable test could be made for that strain. The criteria for an acceptable strain test run was maximum survival rate and uncontaminated STX-167 cultures with a mean cell density of 4.9×10^4 cells/ml.

[c] Greater survival on day 15 than on day 5 results from an error in the survival determination.

The biomass production figures, expressed as the dry and ash weight of 100 couples or 200 parthenogenetic females are shown in Table III.

TABLE III

Dry and ash weight production figures for 100 bisexual couples or 200 parthenogenetic females for the 17 geographical strains of *Artemia*

Strain	Production weights (mg)			% Ash of dry
	Dry	Ash	Ash free dry	
Tuticorin, India	176	21	155	11.9
Macau, Brazil	153	18	135	11.8
San Pablo Bay, California, USA	149	19	130	12.7
Shark Bay, Australia	129	13	116	10.1
Lavalduc, France	129	17	112	13.2
San Francisco Bay, California, USA	129	17	112	13.2
Margherita di Savoia, Italy	109	13	96	11.9
Buenos Aires, Argentina	111	18	93	16.2
Great Salt Lake, Utah, USA (harvest 1966)	108	16	92	14.8
Galera Zamba, Colombia	85	12	73	14.1
Great Salt Lake, Utah, USA (harvest 1977)	83	11	72	13.2
Adelaide, Australia	76	10	66	13.4
Santa Pola, Spain	71	9	62	12.7
Bonaire, Netherlands Antilles	67	12	55	17.9
China	63	9	54	14.3
Bahia Salinas, Puerto Rico	57	6	51	10.5
Larnaca Salt Lake, Cyprus (sample 2)	59	8	51	13.6
Port Araya, Venezuela	54	8	46	14.8
Larnaca Salt Lake, Cyprus (sample 1)	27	4	23	14.8

The most striking observation here is the very large variation in dry weight values for adult brine shrimp from various geographical origins.

The highest individual dry weight of 0.88 mg per adult approximates the figures given by Reeve (1963). The data for the ash content are in the same range of values as reported by Ivleva (1969).

The six best producers are the strains from Tuticorin, Macau, San Pablo Bay, Shark Bay, Lavalduc, and San Francisco Bay. The high incidence of parthenogenetic strains (3) among the top biomass producers, compared to the number of bisexual strains tested, can be explained by the morphologically larger females which probably results from their genetic polyploid condition. A similar observation which corroborates the findings described above was made for a number of biometrical characteristics of the cysts and nauplii from the same parthenogenetic strains by Vanhaecke and Sorgeloos (1980).

The two best biomass production strains, namely Tuticorin and Macau had individual dry weights of 0.88 and 0.77 mg respectively. The percent ash weight of these strains was 8.8 and 11.8% respectively.

Total strain biomass production was calculated from the remaining strain population minus 60 individuals removed for growth measurements and 100 couples or 200 parthenogenetic females removed for dry and ash weight analysis. The results are given in Table IV.

TABLE IV

Total biomass production per strain after removal of 60 individuals for growth measurements and 100 couples or 200 parthenogenetic females for dry and ash weight production analysis

Strain	Dry weight (g)
Macau, Brazil	5.741
Adelaide, Australia	4.737
San Francisco Bay, California, USA	4.416
San Pablo Bay, California, USA	4.240
Great Salt Lake, Utah, USA (harvest 1977)	4.109
Great Salt Lake, Utah, USA (harvest 1966)	3.725
Galera Zamba, Colombia	3.271
Lavalduc, France	3.069
Shark Bay, Australia	2.050
Margherita di Savoia, Italy	1.843
Buenos Aires, Argentina	1.483
China	1.073
Tuticorin, India	1.053
Bonaire, Netherlands Antilles	1.018
Bahia Salinas, Puerto Rico	0.524
Larnaca Salt Lake, Cyprus (sample 1)	0.342
Port Araya, Venezuela	0.262
Larnaca Salt Lake, Cyprus (sample 2)	0.242
Santa Pola, Spain	0.150

The greatest biomass was produced by the Macau strain (5.741 g dry weight) followed by five other bisexual strains. As shown in Table II, 100% survival was recorded on day 15 for all but one of these strains. It is interesting to note that for the production performance all parthenogenetic strains follow each other closely. The smallest biomass production was observed for a group of six bisexual strains.

Length measurements of the 17 geographical strains of *Artemia* are shown in Table V.

The numbers between brackets next to day 15 length measurements indicate the actual day the samples were taken if different from day 15. If 50% of a strains' population reached sexual maturity before day 15, a growth sample was taken earlier and the strain removed from the experiment. Day 5 *Artemia* ranged from 1.05 mm for the Santa Pola strain to 3.2 mm for the Great Salt Lake strain. The size range for adult *Artemia* is very large, *i.e.* from 3.6 mm for Port Araya brine shrimp to 8.5 mm for Margherita di Savoia *Artemia*. This maximum figure is however still smaller than the 8.9 mm reported by Walne (1967) for 9 day old (adult) brine shrimp. Since most parthenogenetic strains exhibit a polyploid chromosomal condition, it is not surprising that the largest individuals obtained on day 15 were from the parthenogenetic strains Margherita di Savoia, Lavalduc, and Shark Bay.

TABLE V

Length measurements for the 17 geographical strains, on day 5 and 15, arranged in descending rank order based on day 15 measurements. Each measurement represents the mean length (± SD) of 30 *Artemia*

Strain	Day 5 $\bar{x} \pm SD$ (mm)	Day 15 $\bar{x} \pm SD$ (mm)
Margherita di Savoia, Italy	1.19 ± 0.18	8.52 ± 1.34
Lavalduc, France[a]	NA	8.47 ± 2.53
Shark Bay, Australia	1.33 ± 0.21	6.82 ± 1.67
Great Salt Lake, Utah, USA (harvest 1966)	[b]3.22 ± 0.61	6.63 ± 1.10[c] (13)
China	[b]1.10 ± 0.17	6.46 ± 1.39
Buenos Aires, Argentina	1.66 ± 0.31	6.42 ± 1.76[c] (11)
San Pablo Bay, California, USA	[b]1.99 ± 0.30	6.28 ± 1.33[c] (14)
Great Salt Lake, Utah, USA (harvest 1977)	2.37 ± 0.40	6.25 ± 1.43
Larnaca Salt Lake, Cyprus (sample 1)	1.17 ± 0.26	6.06 ± 0.88[c] (14)
Galera Zamba, Colombia[a]	2.48 ± 0.44	6.05 ± 1.52[c] (11)
Adelaide, Australia	2.23 ± 0.36	5.96 ± 0.63
Macau, Brazil	1.81 ± 0.26	5.91 ± 1.64[c] (14)
Santa Pola, Spain	1.05 ± 0.20	5.85 ± 1.57[c] (16)
San Francisco Bay, California, USA	[b]2.70 ± 0.40	5.58 ± 1.17[c] (13)
Larnaca Salt Lake, Cyprus (sample 1)	[b]2.07 ± 0.42	5.38 ± 0.92[c] (17)
Bahia Salinas, Puerto Rico	[b]1.78 ± 0.34	4.93 ± 0.74[c] (14)
Tuticorin, India	1.10 ± 0.18	4.60 ± 1.06[c] (14)
Bonaire, Netherlands Antilles	1.26 ± 0.30	3.95 ± 0.67[c] (14)
Port Araya, Venezuela	[b]1.56 ± 0.36	3.63 ± 0.51[c] (14)

[a] Complete data not available.

[b] Length measurements taken on day 6.

[c] Length measurements taken on the day in parentheses.

The protein data for sexually mature *Artemia* from 5 geographical strains are shown in Table VI.

The percent protein figures for sexually mature animals range from 49.7 to 58.1 % for the Santa Pola and Larnaca Salt Lake strain respectively. These levels correspond with the ranges reported in the literature, *e.g.* Gun'ko (1962) mentions 41 and 56 % protein in adult brine shrimp from the Soviet Union ; Deshimaru and Shigueno (1972) and Gallagher and Brown (1975) analyzed San Francisco Bay adults and detected 64.0 and 42.5 % protein respectively.

In conclusion, there seems to exist a wide variation between *Artemia* strains with regard to the performance criteria considered in this study. The results obtained, provide a first guideline for the selection of one or more strains for optimal use in the St. Croix Artificial Upwelling-Mariculture System. If one considers the criteria of survival and total production performance as the most important, the strains from Macau (Brazil), San Pablo Bay (California, USA), San Francisco Bay (California, USA) and Adelaide (Australia) should be

selected. The other bisexual as well as all the parthenogenetic strains tested gave less good results for one or more of the criteria selected.

TABLE VI

Percent protein in sexually mature *Artemia* for five strains

Strain	Protein content (as % dry weight) ($\bar{x} \pm SD$)
Larnaca Salt Lake, Cyprus	58.07 ± 5.62 (14)[a]
Macau, Brazil	52.77 ± 4.01 (14)
Margherita di Savoia, Italy	52.03 ± 0.64 (15)
Tuticorin, India	51.47 ± 2.18 (14)
Santa Pola, Spain	49.73 ± 3.35 (16)

[a] The day sexual maturity was reached for each strain.

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International Study on *Artemia*[1] IV. The biometrics of *Artemia* strains from different geographical origin

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Abstract

The cosmopolitan distribution of brine shrimp in coastal lagoons and island salt lakes has resulted in numerous geographical strains.

In the framework of the characterization study of different *Artemia* strains which is the objective of the laboratories participating into the "International Study on *Artemia*" the following biometrical parameters have been studied by the Artemia Reference Center :

- volume and diameter of hydrated, untreated and decapsulated cysts ;
- chorion thickness ;
- length, dry weight and ash free dry weight of freshly hatched nauplii ;
- volume index of freshly hatched nauplii.

For all parameters, important differences were observed between the *Artemia* strains studied. From these data it appears that many strains can be differentiated on the basis of their biometrical characteristics. The small variations observed in a few cases between batches of the same strain might be caused by fluctuating environmental conditions and/or cyst processing techniques.

Highly significant correlations have been found between a number of the biometrical parameters which were taken into consideration ; this will, no doubt, facilitate the further screening of *Artemia* strains.

The impact of the biometrical characterization of *Artemia* strains on the practical use of brine shrimp in aquaculture is discussed.

Introduction

The brine shrimp is a cosmopolitan organism inhabiting coastal lagoons as well as inland salt lakes. Its distribution is not continuous ; the populations are indeed localized in isolated biotopes in both temperate and tropical climates (Stella, 1933). Furthermore it is known that the ecological and physico-chemical characteristics of *Artemia* habitats can differ widely (Cole and Brown, 1967 ; Persoone and Sorgeloos, 1980).

[1] International Interdisciplinary Study on *Artemia* Strains coordinated by the Artemia Reference Center, State University of Ghent, Belgium.

[2] "Bevoegdverklaard Navorser" at the Belgian National Foundation for Scientific Research (NFWO).

The geographical isolation of brine shrimp populations has resulted in numerous geographical strains. The number of different *Artemia* populations described today already exceeds 150 (Persoone and Sorgeloos, 1980). Recently, genetic studies of 27 strains revealed the existence of at least six non-interbreeding species (Bowen *et al.*, 1978).

The geographical isolation and the specific habitat conditions have led to various phenotypes with different biological, chemical, and physiological characteristics. Evidence of this has already been presented in a few studies that have been made on these aspects (Gilchrist, 1960 ; Baid, 1963, 1964 ; D'Agostino, 1965 ; Nimura, 1968 ; Lüdskanova and Joshev, 1972 ; Sorgeloos, 1975 ; Claus *et al.*, 1977). Since a more detailed analysis of the various existing *Artemia* populations might lead to a better characterization of their value for specific applications in aquaculture, the "International Study on *Artemia*" was started in 1978 in which the task of the biometrical analysis of brine shrimp strains of different origin was assigned to the Artemia Reference Center.

Materials and methods

Within the framework of the general characterization study of different *Artemia* populations, 17 strains originating from 14 countries have been studied so far. Details on their exact origin are given in Table I. For the strains from San Francisco Bay, Macau, Great Salt Lake, Port Araya and Tuticorin different batches have been analyzed.

It should be noted that the *Artemia* strains from Macau and Barotac Nuevo originate from San Francisco Bay *Artemia* which were inoculated in the two former areas in 1977 and 1978 respectively (Sorgeloos *et al.*, 1979).

The following biometrical parameters have been analyzed : diameter, volume and chorion thickness of the cysts and length, dry weight, ash free dry weight and volume index of the freshly hatched nauplii. The size analysis of the cysts has been performed on both hydrated untreated and decapsulated cysts.

A routine procedure using Coulter Counter® equipment has been worked out in order to measure the processed *Artemia* cysts and to statistically analyse the data obtained (Vanhaecke *et al.*, 1980).

For the analysis of the biometrical characteristics of the nauplii, cysts were incubated in natural seawater (35 ‰) at a temperature of 25 ± 0.5 °C at 1 000 lux. The larvae were usually harvested when 90 % of the total number of hatchable nauplii had been produced.

For the slow hatching strains nauplii were sampled 8 to 10 hr after the appearance of the first nauplii. In this way a homogeneous population of instar I nauplii was obtained for all the strains. The nauplii were separated from the hatching debris using a separator box (Persoone and Sorgeloos, 1972). The analyses were made immediately after the separation.

Dry weight analyses were carried out on six replicate series of approximately 50 000 nauplii each ; the number of larvae was checked by taking 10 subsamples of 250 µl. The nauplii were rinced thoroughly with distilled water and dried for 24 hr at 60 °C. After cooling for 30 min in a dessicator the larvae were weighed on a microbalance. The dried samples were then incinerated for 4 hr at 550 °C and the ash weights determined.

For the analysis of the naupliar size, 120 instar I larvae, were fixed in lugol's solution (5 volume %) and the length determined using a microscope equiped with a projection system.

TABLE I

Artemia strains and batches studied

Source of cysts[1]	Batch number or year of harvest	Abbreviation used
Argentina : Buenos Aires (Aquarium Products)	1977	ARG
Australia : – Adelaïde	–	AD
– Shark Bay	–	SB1
(World Ocean)	114	SB2
Brazil : Macau	March 1978	MAC1
(Cirne Brand)	May 1978	MAC2
	October 1978	MAC3
	870191	MAC4
	87500	MAC5
	871172	MAC6
Canada : Chaplin Lake	1978	CHA
China : locality unknown	–	CHI
Colombia : Galera Zamba	1977	GZ
France : Aigues Mortes	–	AM
India : Tuticorin	–	TUT1
	1978	TUT2
Italy : Margherita di Savoia	1977	MS
Philippines : Barotac Nuevo	1978	PHIL
Puerto Rico : Bahia Salinas	–	PR
Spain : San Lucar	1978	SL
USA : – Great Salt Lake	1966	GSL1
	1977	GSL2
– San Francisco Bay	288-2596	SFB1
(Metaframe-San Francisco Bay Brand, Inc.)	288-2606	SFB2
	2847	SFB3
	236-2013	SFB4
	933235	SFB5
– San Pablo Bay	1628	SPB
(Living World, San Francisco Bay Brand, Inc.)		
Venezuela : Port Araya	August 1977	PA1
	January 1978	PA2
	May 1978	PA3

[1] For those cysts which were purchased from a commercial dealer the company name is given in parenthesis.

The average volume of approximately 20 000 nauplii was measured using Coulter Counter® equipment. The operation conditions on the Coulter Counter® equipment differ from those reported in Vanhaecke *et al.*, (1980) with regard to :

- tube orifice : 1 000 μm
- 1/amplification : 4
- count range : 100

In order to slow down the movements of the larvae, which interfere with the proper functioning of the Coulter Counter®, 100 ml glycerol was added to 1 l of the conventional 10 ‰ electrolyte solution. The data obtained do not represent the real volume of the nauplii,

but provide us with a volume index, which is the mean of the volumes of the nauplii which pass through the tube's orifice under different orientations.

Results

The data on the cyst diameter and chorion thickness are summarized in Table II. The volumes of the cysts are represented graphically in Fig. 1. The statistical comparison of the results obtained is given in Table III.

TABLE II

Diameter and chorion thickness of cysts from different *Artemia* strains and batches

Strain[1]	Diameter of hydrated untreated cysts (μm)	s[3]	Diameter of hydrated decapsulated cysts (μm)	s[3]	Chorion thickness (μm)
SFB 1	224.7	12.4	210.0	12.7	7.35
SFB 2	224.6	11.9	210.5	12.3	7.05
SFB 3	223.9	11.7	209.7	12.8	7.10
SFB 4	224.3	11.8	207.7	11.1	8.30
SFB 5	228.7	12.3	212.1	11.3	8.30
SPB	235.6	13.0	220.4	14.3	7.60
PHIL	228.0	13.0	213.8	12.2	7.25
MAC 1	232.5	11.1	216.6	11.4	7.95
MAC 2	227.8	11.7	211.2	12.4	8.30
MAC 3	227.4	11.9	213.2	11.3	7.10
MAC 4	227.7	12.5	212.9	11.3	7.40
MAC 5	231.8	12.3	217.6	12.8	7.10
MAC 6	228.7	11.0	213.8	12.0	7.45
MAC lab[2]	226.9	12.4	211.0	12.5	7.95
GSL 1	252.5	13.0	241.6	13.2	5.45
GSL 2	244.2	16.1	234.8	16.0	4.70
SB 1	259.7	9.7	242.9	10.1	8.40
SB 2	260.4	10.4	242.2	11.3	9.10
PA 1	246.7	12.7	226.5	12.7	10.10
PA 2	246.8	13.4	226.4	14.4	10.20
PA 3	249.0	12.6	226.6	12.8	11.20
TUT 1	283.8	10.2	262.0	11.0	10.90
TUT 2	282.9	14.4	262.7	11.5	10.10
AD	225.8	10.9	209.8	9.5	8.00
ARG	238.2	13.2	217.4	13.9	10.40
PR	253.7	13.3	233.4	13.7	10.15
CHA	240.0	16.1	229.3	15.1	5.35
GZ	249.9	12.3	232.7	11.2	8.60
CHI	267.0	19.8	246.6	18.9	10.20
MS	284.9	14.6	266.3	14.8	9.30
SL	253.6	11.7	237.1	12.2	8.25
AM	259.6	14.1	240.8	14.3	9.40

[1] Legend to the abbreviations in Table I.

[2] MAC lab : cysts produced in a laboratory culture of MAC 2 *Artemia*.

[3] Standard deviation.

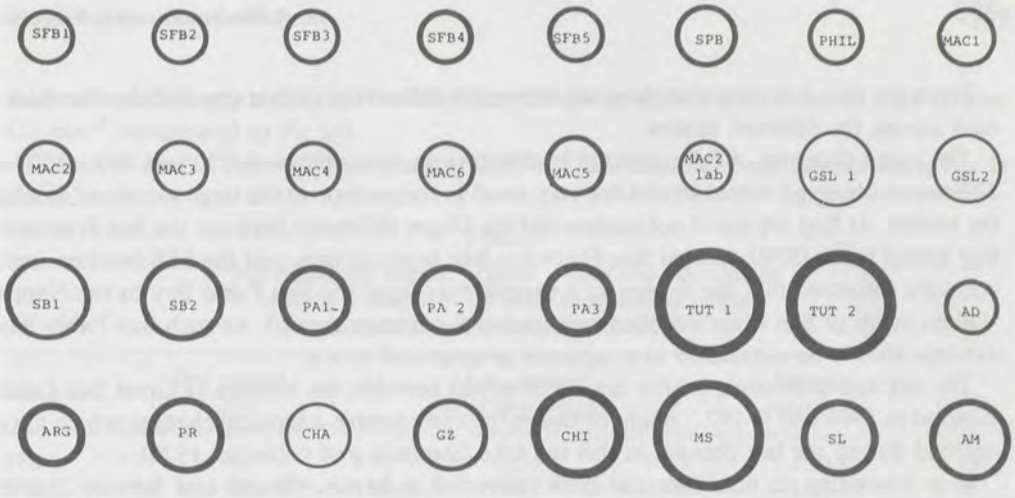

FIG. 1. Schematic diagram of cyst volume (outer circle) and chorion volume (black surface) for different *Artemia* strains and batches. (legend to abbreviations in Table I).

TABLE III

Statistical comparison (0.01 level[1]) of the sizes of *Artemia* cysts from different geographical origin

A. diameter of untreated hydrated cysts
B. diameter of hydrated decapsulated cysts
C. chorion thickness

(Legend to abbreviations in Table I)

A	SFB 3	SFB 4	SFB 2	SFB 1	AD	MAC 2 lab	MAC 3	MAC 4	MAC 2
	PHIL	SFB 5	MAC 6	MAC 5	MAC 1	SPB	ARG	CHA	GSL 2
	PA 1	PA 2	PA 3	GZ	GSL 1	SL	PR	AM	
	SB 1	SB 2	CHI	TUT 2	TUT 1	MS			
B	SFB 4	SFB 3	AD	SFB 1	SFB 2	MAC 2 lab	MAC 2	SFB 5	
	MAC 4	MAC 3	MAC 6	PHIL	MAC 1	ARG	MAC 5	SPB	
	PA 2	PA 1	PA 3	CHA	GZ	PR	GSL 2	SL	
	AM	GSL 1	SB 2	SB 1	CHI	TUT 1	TUT 2	MS	
C	GSL 2	CHA	GSL 1	SFB 2	SFB 3	MAC 3	MAC 5	PHIL	
	SFB 1	MAC 4	MAC 6	SPB	MAC 1	MAC 2 lab	AD	SL	
	SFB 4	SFB 5	MAC 2	SB1	GZ	SB 2	MS	AM	
	PA 1	PR	PA 2	CHI	ARG	TUT 2	TUT 1	PA 3	

[1] Horizontal lines join cyst sizes that are not significantly different.

From the data it is clear that there are important differences in cyst size and chorion thickness among the different strains.

The mean diameter of the untreated hydrated cysts varies between 220 and 285 μm. The differences observed within strains are very small in comparison to the large variations among the strains. At first we could not understand the 10 μm difference between the San Francisco Bay Brand batch (SPB), labeled San Francisco Bay brine shrimp, and the SFB-batches, until we were informed that the former cyst sample was from the San Pablo Bay in the Nappa Valley, north of San Francisco (Schmidt, personal communication). As such San Pablo Bay *Artemia* should be considered as a separate geographical strain.

The marked difference, which can be observed between the batches of Great Salt Lake, sampled in 1966 and in 1977, might be due to the considerable ecological changes which have occured during the last decades in this salt lake (Stephens and Gillespie, 1976).

It is interesting to note that the cysts harvested in Macau (Brazil) and Barotac Nuevo (Philippines) show only minor differences as compared to the parental SFB-stock. Similarly, the cysts produced by MAC 2 adults in laboratory cultures on a rice bran diet (Versichele and Sorgeloos, 1980) are not significantly different from the original MAC 2 batch.

For the decapsulated cysts, a similar range of differences as in the untreated cysts is observed between the different strains. The cyst diameter varies between 203 and 266 μm. The strain sequence from small to big has, however, changed, indicating differences of chorion thickness. Indeed this parameter varies from 4.7 to 11.2 μm in the batches analyzed.

The results of the size analysis of SFB 1 cyts produced in the laboratory under different salinity conditions (Table IV) reveal only minor differences. Nonetheless the statistical analysis indicates that the cysts produced at 180 ‰ are significantly smaller than the cysts produced at lower salinities.

TABLE IV

The influence of salinity on the biometrics of SFB cysts produced in laboratory cultures

Salinity (‰)	Diameter of hydrated untreated cysts (μm)	s[1]	Diameter of hydrated decapsulated cysts (μm)	s[1]	Chorion thickness (μm)
35	223.5	14.6	206.4	13.7	8.55
90	223.7	16.7	206.6	14.6	8.55
180	222.0	15.3	205.2	13.0	8.40

[1] Standard deviation.

Microscopic measurements of 100 cysts produced at respectively 22 °C and 28 °C from SFB 1 parents revealed no significant size differences between these cysts, the mean size being 223.0 respectively 223.2 μm.

The fact that the laboratory produced cysts are slightly smaller than the original SFB 1 stock is probably due to the presence of empty cyst shells in these samples. Indeed, since these cysts were not dried, the separation (in tapwater) of the empty and broken shells from the full cysts

is less efficient. Empty shells shift the frequency distribution of cysts (as analyzed with Coulter Counter® equipment) to the left.

The data on the dry weight, organic weight and ash content of the nauplii are given in Table V.

TABLE V

Individual dry weight, organic weight and ash content of instar I nauplii of different geographical origin (Legend to abbreviations in Table I)

Strain	Dry weight (μg)	s[1]	Ash content (% of dry weight)	s[1]	Organic weight (μg)	s[1]
SFB 1	1.63	0.11	6.33	0.15	1.52	0.10
SFB 2	1.61	0.09	6.17	0.10	1.51	0.09
SPB	1.92	0.08	5.62	0.15	1.81	0.07
MAC 2	1.68	0.11	5.83	1.11	1.58	0.10
MAC 6	1.74	0.08	5.88	0.03	1.64	0.07
PHIL	1.68	0.03	6.07	0.10	1.58	0.03
GSL 1	2.70	0.13	5.74	0.05	2.55	0.12
GSL 2	2.42	0.11	5.69	0.25	2.28	0.11
SB	2.47	0.13	5.28	0.10	2.34	0.13
PR	2.10	0.11	5.51	0.06	1.99	0.11
GZ	2.27	0.08	6.32	0.07	2.12	0.08
PA 3	2.07	0.09	6.39	0.25	1.94	0.08
ARG	1.72	0.07	6.32	0.28	1.61	0.06
CHI	2.76	0.14	6.25	0.26	2.62	0.14
MS	3.33	0.18	6.17	0.15	3.13	0.17

[1] Standard deviation.

From the dry weight data it appears that there are very large differences between the geographical strains analyzed. The percentual differences among the strains studied, vary from 5 to over 100%. Student's t-tests reveal that the variations within a same strain are not significant, exception made, however, for the already mentioned "San Francisco Bay Brand-1628" batch, which in fact is the San Pablo Bay strain, and the Great Salt Lake 1966 and 1977 batches.

Again no significant differences can be observed between the nauplii from the San Francisco Bay strain and those originating from San Francisco Bay inoculations in either Brazil or the Philippines.

From the data it also appears that the variation in ash content is small, *i.e.* from 5.28 to 6.39% of the naupliar dry weight. As a result we can conclude that the differences in organic weight are proportional to the dry weight differences.

From Fig. 2 it is clear that almost the same range of differences as already given for the dry weights, is valid for the volume index of the nauplii. This parameter varies from 7.6 up to 8.3 for the San Francisco Bay, Macau and Barotac Nuevo nauplii, as compared to 11.4 up to 13.6 for the parthenogenetic strains from China and Italy.

The data for naupliar length are represented graphically in Fig. 3. The maximal difference in naupliar size is about 100 μm.

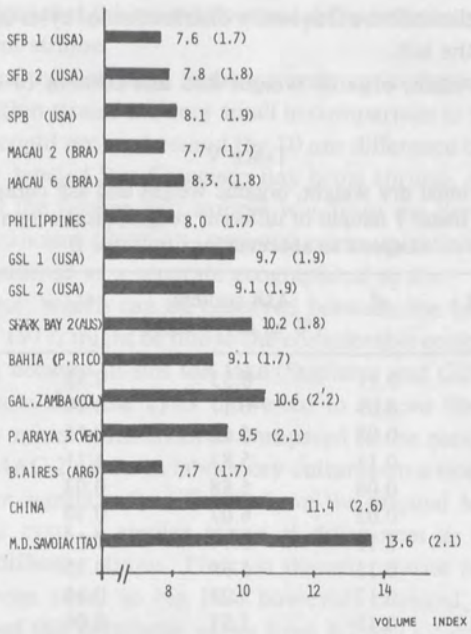

FIG. 2. Volume index of *Artemia* nauplii from different geographical strains (s-values in parentheses ; legend to abbreviations in Table I).

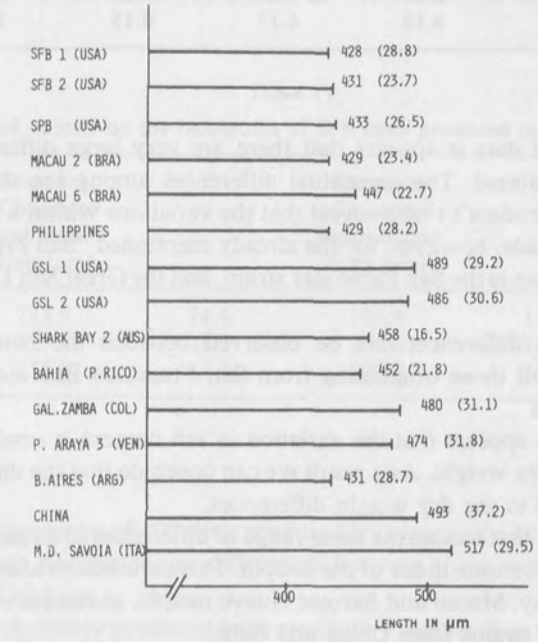

FIG. 3. Length of *Artemia* nauplii from different geographical strains (s-values in parentheses ; legend to abbreviations in Table I).

Discussion

The data obtained so far demonstrate that significant differences exist for several biometrical parameters between *Artemia* strains. Our results confirm the findings of D'Agostino (1965) and Claus *et al.* (1977) with regard to cyst sizes.

The former author reported that within a same strain the mean egg size remains constant between batches which were collected at different periods of the year. Our results, however, indicate that in a few cases the mean cyst size varies significantly among batches from the same strain.

These small variations may be due to changing environmental conditions in the salt ponds. Indeed, it appeared from laboratory tests that a salinity drop from 180 to 90 ‰ causes a ± 1.5 μm increase in the size of both hydrated untreated and decapsulated cysts. Collins (1978) analyzed various *Artemia* strains and did not find a correlation between cyst size and brine density in the natural habitat. Whereas it appears from our studies that temperature, within the experimental range, probably does not influence the cyst size, other factors such as variations in food conditions for the adults or widely separated harvest sites – especially in very large salt ponds – may cause small differences in cyst size among batches.

The packaging technique can eventually also lead to some small differences ; *e.g.* segregation can occur during filling of consecutive cans in function of different cyst densities. Although for most commercial batches analyzed we did not find significant variations, differences ranging from 1.1 up to 1.7 μm were found between cans in specific batches from Macau and San Pablo Bay.

The constancy in cyst size of San Francisco Bay cysts harvested from natural populations and those produced from the same strain but in laboratory conditions confirm similar observations of D'Agostino (1965) with Great Salt Lake *Artemia*.

In conclusion it appears that the biometrical parameters which we studied are mainly strain specific. As a consequence biometrical parameters in general, and more specifically cyst characteristics can be considered as good tools for the characterization of *Artemia* strains. These criteria can from now on be utilized to differentiate strains and to help to define the origin of unspecified cyst samples.

The differences which we have observed for the cyst's characteristics are not correlated with the genetic distances calculated by Abreu-Grobois and Beardmore (1980) for the same geographical strains. More biometrical parameters as well as other criteria (*e.g.* morphological and/or physiological) will probably be needed to evaluate genetic differences between strains.

From our data we distinguish three groups of strains :

1. Those with the smallest cysts produced by the Adelaide-strain and the brine shrimp from the San Francisco Bay area, including the SFB inoculated strains from Macau and Barotac Nuevo ;
2. The parthenogenetic strains from China, France, Italy and India characterized by a large cyst size. This quantitative property might be correlated with the degree of ploidy ;
3. Strains with cysts of intermediate size but with the thinnest chorion characteristic for the *Artemia franciscana* strains described by Bowen and Sterling (1978) from Chaplin Lake and Great Salt Lake.

TABLE VI

Correlations between biometrical characteristics of nauplii and cysts from various *Artemia* strains

Correlation	r-value
Volume of decapsulated cysts – naupliar dry weight	0.986
Volume of untreated cysts – naupliar volume	0.960
Volume of decapsulated cysts – naupliar volume	0.955
Volume of untreated cysts – naupliar dry weight	0.945
Naupliar volume – naupliar dry weight	0.945
(Naupliar length)^3 – naupliar dry weight	0.945
(Naupliar length)^3 – naupliar volume	0.912
Diameter of decapsulated cysts – naupliar length	0.906
Diameter of untreated cysts – naupliar length	0.864

Since one can expect a high correlation between the biometrical characteristics of the cysts and their respective nauplii, a detailed correlation analysis has been carried out for the various parameters studied (Table VI).

Since highly significant correlations were indeed found, the screening of *Artemia* strains can be much simplified with regard to the number of biometrical characteristics to be taken into consideration. For example the correlation between the volume of decapsulated cysts (X) and the naupliar dry weight (Y) is given by the equation $Y = -0.0978 + 3.554.10^{-4}X$. The regression line and its 95% confidence limits are represented graphically in Fig. 4.

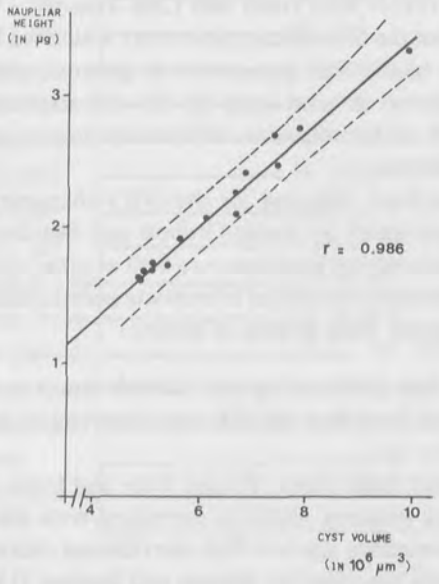

FIG. 4. Regression line and 95% confidence limits for the correlation between the volume of decapsulated cysts and the naupliar dry weight in several *Artemia* strains.

An estimation of the dry weight (with a 95% accuracy, *i.e.* ± 0.22 μg) is now possible by extrapolation from the cyst volume, which can be measured much faster and more precizely using Coulter Counter® equipment.

The highest correlation between the naupliar characteristics is found between the volume index and the dry weight of the nauplii. In fact, for the evaluation of the ingestibility of *Artemia* as a prey, the most realistic criterion to express the amount of food which a nauplius represents is the volume index.

The results obtained so far thus become already of practical value with regard to the selection of the most appropriate strain(s) as an adequate food source for larval fishes or crustaceans in aquaculture hatcheries.

If the size of the *Artemia* prey does not cause ingestion problems for the predator, one might expect that the use of large nauplii with a higher individual organic weight will be beneficial. The predator will indeed spend less energy taking up a smaller number of larger nauplii to fulfill its food demand. This is especially the case for fish larvae which are not very efficient in prey hunting (Rosenthal, 1969). The beneficial effect of feeding bigger *Artemia* is apparent from the experimental results of Beck *et al.* (1980) : *Menidia* larvae indeed grew significantly faster on a diet of large nauplii from Margherita di Savoia, Great Salt Lake and Shark Bay as compared to those silverside larvae fed with the smaller nauplii from San Francisco Bay and Macau.

In a similar comparative study with *Pseudopleuronectes americanus* larvae Klein-MacPhee *et al.* (1980) also noted better growth results for larval winter flounder raised on Shark Bay and Margherita di Savoia nauplii as compared to Macau, San Pablo Bay and Great Salt Lake nauplii. As reported by the same authors the poor growth results obtained with the SPB and especially with the relatively large GSL larvae are not related to prey size but appear to be caused by nutritional and/or toxic factors.

For those cultured organisms where the naupliar size is critical for the ingestion mechanism of the predator, better growh might be expected when using small nauplii. The use of a particular *Artemia* strain may even result in a total failure in culturing a specific predator on brine shrimp because of the inability of the predator to ingest this specific *Artemia* strain (Smith, 1976). As a consequence the larval age of the predator at which a diet of freshly hatched *Artemia* nauplii can be successfully used is function of the strain of brine shrimp used ; *e.g.* Beck *et al.* (1980) compared the biological effectiveness of freshly hatched nauplii from MAC, SPB, SB, GSL and MS in feeding trials with newly hatched *Menidia* larvae ; in the group of fish which were offered large MS nauplii (volume index 13.6) a high mortality, similar to the one noted for the starved fish, was recorded during the first 3 days of the experiment ; after this time the mortality of the MS fed fish larvae did not exceed the mortality of those fed smaller nauplii from the other strains. A selection of specific *Artemia* strains based on biometrical characteristics might thus be very useful to raise the chance of success in rearing specific organisms.

From the foregoing it becomes clear that the comparative study on *Artemia* strains should be continued by screening more strains, taking advantage however, of the earlier mentioned correlations, which facilitate the characterization study. We intend to also study other correlations such as : cyst size versus adult size ; chorion thickness and light intensity threshold at the onset of the hatching metabolism ; naupliar size versus larval growth rate and finally biometrical characteristics in function of the various genotypes.

At a later stage cross-breeding of specific strains with particular characteristics will be considered and the heritability of these parameters in the new strains produced will be studied.

Acknowledgements

We are very indebted to the many people who provided us with cyst samples from various geographical strains of brine shrimp.

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REPORTS ON WORKSHOPS

The Brine Shrimp Artemia. *1980. Vol. 3. Ecology, Culturing, Use in Aquaculture*
G. Persoone, P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 456 p.

Workshop I. Characterization of *Artemia* strains for application in aquaculture

Chairmen

Kenneth L. Simpson, University of Rhode Island
(Rhode Island, USA)

Allan D. Beck, US Environmental Protection Agency
(Rhode Island, USA)

Rapporteur

Patrick Sorgeloos, Artemia Reference Center,
State University of Ghent (Belgium)

At this Symposium brine shrimp were reported to have been isolated from over 150 different locations. Because of the demand for brine shrimp in aquaculture, the exploitation of brine shrimp for commercial production is now practised in several places throughout the world. It was pointed out in this Symposium and in reports previously published that brine shrimp populations can widely vary in size, mode of reproduction, nutritional value, pesticide content, *etc*. Differences in the bio-effectiveness of these populations have usually been suggested to result from their pesticide content.

Clearly there is a need for the analysis of available strains for selected chemical, biochemical, morphological, genetic characteristics and the cultivation response to a predator. This need has been suggested on various occasions (FAO Technical Conference on Aquaculture, Kyoto 1976 ; ACMRR/IABO Working Party on Aquaculture, FAO Rome 1972 ; 5th Conference on Marine Biology, Princeton, NJ, USA 1967). The results of such studies will only be meaningful if all samples from authentic sources are analyzed under the same conditions with the same test organisms. It is also clear that this comprehensive analysis is beyond the grasp of a single laboratory.

In the introduction to this workshop the Chairmen reported on the formation of a group called the "International Study on *Artemia*" (ISA). The ISA group was formed several years ago during informal discussions at the occasion of the Aquaculture Conference "Cultivation of fish fry and its live food" (Szymbark, Poland, September 23-28, 1977). Shortly afterwards, research groups from Belgium, the United Kingdom and the USA undertook the analysis of a small number of *Artemia* populations. These studies included biometrics, hatching characteristics, mass culture techniques, genetic characterization, ability to support predators, and the biochemical and chemical characteristics of various strains.

This initial research was primarily focused on single lots of five geographical *Artemia* strains from California (USA), Utah (USA), Brazil, Australia, and Italy, and the considerable variability between strains has been demonstrated in most parameters although similarities were also observed. Parameters that are essentially similar include amino acid profiles and pigment content. Differences were noted in biological effects (as diet for other species), biometrics, genetics, chlorinated hydrocarbon content, heavy metals, fatty acids, and physiological characteristics.

These differences were demonstrated and discussed in the ten ISA papers given during this symposium, corroborating the results of other papers showing the same variability among populations as well as within populations. These results proved to be more than academic in as much as some lots would not support life in test organisms. Thus the conclusion was reached in the Symposium and restated in the workshop that to say, "*Artemia* was fed" is not the equivalent of saying "casein was fed" *i.e.* that *Artemia* is not a well defined diet.

In view of the unique characteristics of certain strains, much discussion in the workshop centered on the need to identify batches of brine shrimp cysts from the various suppliers. A good dialogue arose between suppliers' representatives and scientists. It was generally accepted that certain minimum information is essential :

1. specific location with, if possible, exact harvest site within the salt work or salina complex ;
2. date of harvest ;
3. identification – lot number ;
4. date of processing.

Other information considered desirable included air and water temperatures, salinity, dissolved oxygen, and pH at the collection site. A one point measurement of these conditions in time and space would not be very determining but might provide some insight as to the hatching conditions and procedures to be used for the cysts and nauplii. A range of environmental parameters occuring during the cyst deposition and the subsequent collection process could prove useful information.

A proposal was made to designate a reference lot of *Artemia* cysts. It was determined that this would not be a mandatory standard that all would be compelled to use in scientific research activities. The reference lot would rather serve as an internal control. The selection of such a strain was discussed but no decision was reached. The reference cysts could be an existing commercial strain, mixture of several strains, or cysts produced specifically for the reference purpose under the auspices of an institutional or semi-governmental body such as FAO.

Another major area of interest discussed was to standardize reporting of procedures in scientific papers concerning the brine shrimp. Little time was left to discuss this point but several necessary aspects of the reporting problem were noted :

- source of *Artemia* cysts, nauplii or adults ;
- hatching conditions and procedures (temperature, salinity, pH, media, *etc.*) ;
- age ;
- diet (if fed) ;
- culture and handling procedures.

Dr. Simpson closed the workshop by recognizing the significant progress in characterization of the species, especially through the ISA effort. All scientists were invited to continued communication.

Note by the Editors

Since the Artemia *Symposium of Corpus Christi, a substantial quantity of so-called "Reference* Artemia *Cysts" has been acquired and is available from the Artemia Reference Center at the State University of Ghent, Belgium, as calibration material.*

For more details we refer to the short note "Availability of Reference Artemia *Cysts" by P. Sorgeloos to be published in the Mar. Ecol. Prog. Ser. and in Aquaculture.*

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Workshop II. Commercial aspects of *Artemia* exploitation

Chairman

Harold H. Webber, Groton BioIndustries,
(Massachusetts, USA)

Rapporteur

Patrick Sorgeloos, Artemia Reference Center,
State University of Ghent (Belgium)

Discussions focused on various aspects dealing with the potential future uses of *Artemia* biomass. From several papers presented at this Symposium, it appeared indeed that large scale production of brine shrimp is now technically feasible in several ways : *e.g.* harvest from natural sources eventually after artificial inoculation (de los Santos *et al.*) ; mass production in high density flow through culture systems either as a tertiary step in industrial waste treatment (Milligan *et al.*) or by feeding the animals with agricultural waste-products (Sorgeloos).

Although presently there is no specific market for an annual extra output of hundreds to thousands of tons of brine shrimp biomass, it was concluded that a considerable commercial demand could soon 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 in cattle breeding and aquaculture) ; *Artemia* protein concentrate ; *Artemia* as raw material to extract interesting biochemical product(s) ; brine shrimp preparations for human nutrition, *etc.*

Many questions on the potentials of mass production of brine shrimp, mostly dealing with commercial feasibility aspects, remained as yet unanswered, pointing to the need of further investigation.

Two major conclusions were formulated at the end of the workshop :

1) *Artemia* has a unique potential and a promising future for large scale biomass production and harvesting, as a direct or indirect food source for man ;
2) there is an urgent need for financial support from international agencies to sponsor pilot-scale studies for mass production, harvesting and processing of brine shrimp biomass in order to make up the cost-benefit of industrial *Artemia* biomass production farms.

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SUBJECT INDEX

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