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# The nutritional value of *Artemia*: a review

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**Content**

## Introduction

Successful rearing of larval stages of aquatic organisms is a challenge for aquarium hobbyists, an aim and tool for aquatic ecologists and ecotoxicologists, and a necessity for the success of the aquaculturist. All these people will agree that the primary problem in any type of larval rearing is that of food. Ideally, one would prefer to feed larvae their natural diet, which is characterized by a wide diversity of nutritious live organisms. Although not a "natural" food, *Artemia* have been successfully used by many as food for larval organisms. It is perhaps surprising that such success could be attained with a food from such an unusual (*i.e.* hypersaline) environment. Some recent experiences suggest that the use of *Artemia* does not absolutely guarantee success (Sorgeloos, 1980; Simpson *et al.*, 1983). Explanations for and remedies to this variable success will be covered in this review through an analysis of the larval organism's requirements for food (Fig. 1). A more complete review on the nutritional value of *Artemia* was presented by Léger *et al.* (1986).

REQUIREMENTS OF A FOOD FOR
LARVAL ORGANISMS

FOR THE CULTURIST

consistent availability

simple production procedures

euryplasticity and versatility :
- salinity/temperature tolerance
- handling
- disinfection
- different sizes and forms
- use as a carrier

FOR THE PREDATOR

physical requirements :
- clean
  - no alien materials
  - no diseases
- acceptable
  - perceptible
  - catchable
  - palatable
  - ingestible

nutritional requirements :
- digestible
- nutrient requirements

FIG. 1. Summary of the requirements a food must meet to be a suitable diet for larval organisms.

## Practical requirements for the culturist

A food organism must first meet the nutritional needs of the predator. In addition, other practical requirements have to be met to satisfy the culturist. The consistent availability of food organisms is of utmost importance for continuous cultures. In this respect, *Artemia* outvies all other food organisms since it is available as an off-the-shelf food in the form of dormant cysts. From those cysts, nauplii are obtained through simple hatching procedures (Sorgeloos *et al.*, 1983). Ideally, a food organism should also be hardy and easily cultured. *Artemia* nauplii fulfill this last requirement quite well, since they are very tolerant to various culture environments, they resist even rough handling, and may be disinfected resulting in a biologically uncontaminated life food (Sorgeloos *et al.*, 1983).

The wide size range of *Artemia* and their different physical forms (Fig. 2) make them very versatile in the use. Since they are easily cultured, *Artemia* nauplii and later stages may be fed according to the growth and development of the predator. Also a smaller food particle may be used in the form of decapsulated cysts, which are some 50 % smaller than freshly-hatched nauplii and have several other advantages : 1) they are disinfected and separated from the cyst shells during the decapsulation process (Sorgeloos *et al.*, 1977) ; 2) the hatchability of the embryos is improved (Bruggeman *et al.*, 1980), so that otherwise unhatchable cysts can be valorized ; 3) the energy content is higher (Vanhaecke *et al.*, 1983), resulting in a higher naupliar biomass production per gram of cysts and a smaller, more energy-rich food particle for the larval organism.

A last example of the versatility of *Artemia* as a food consists in the possibility of using *Artemia* (nauplii or adults) as a carrier for components which are otherwise difficult to administer to fish and crustacean larvae. Indeed, essential nutrients, pigments, prophylactics, and therapeutics may be bioencapsulated in *Artemia* and introduced into the consumer organism (Fig. 3) (Léger *et al.*, 1987).

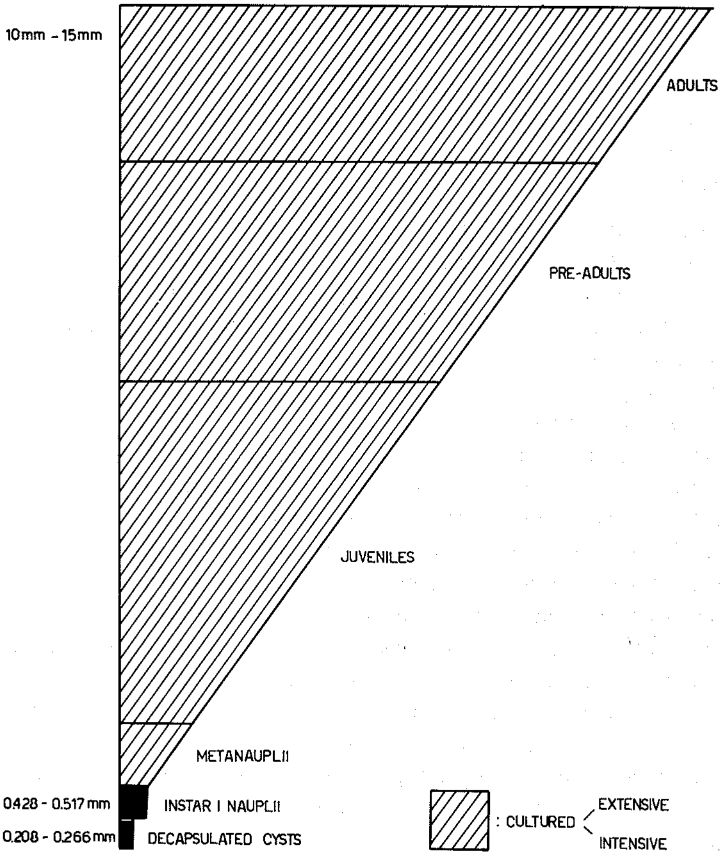

FIG. 2. The size ranges of various *Artemia* life stages.

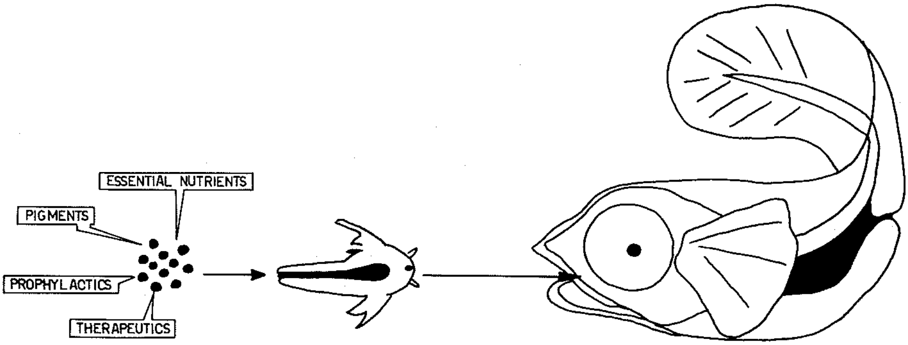

FIG. 3. Schematic outline of the technique of using *Artemia* as a carrier for various nutritional, prophylactic, and therapeutic components.

## Requirements for the predator

The characteristics a food organism must possess to meet the requirements of the predator are of great importance. Physical and nutritional requirements must be considered.

### PHYSICAL REQUIREMENTS

Physically, a food organism has to be clean, free from alien organisms and materials and especially free from contagious diseases. *Artemia* may be disinfected and fed as a clean food. Although cysts are often heavily loaded with microorganisms, Austin and Allen (1981) did not find an intimate microbial contamination of the nauplii and they demonstrated that bacteria surrounding the nauplii may be easily removed by simple washing procedures or even better by disinfecting the cysts prior to hatching incubation by a dipping procedure. No direct evidence exists for *Artemia*-borne infections in larvae.

A second physical requirement is that a food organism has to be accepted by the predator. Acceptability of food is determined by several factors. The bright color of *Artemia* nauplii and their continuous movement make them easily perceptible. Perceptibility may even be enhanced by staining techniques, as demonstrated for sole larvae (Dendrinos *et al.*, 1984). *Artemia* nauplii are easily caught because they lack an effective escape response. Palatability is apparently adequate, since *Artemia* is often used as a gustatory attractant in artificial diets (Barahona-Fernandes *et al.*, 1977 ; Gatesoupe and Luquet, 1981/1982). Ingestibility of a palatable food is governed primarily by its size. The size of *Artemia* nauplii is therefore the first real consideration. Indeed, most first-breeding marine fish species and some decapods, such as penaeids, cannot ingest (or handle) *Artemia* nauplii. Even species which may accept *Artemia* nauplii as a first food sometimes face ingestion and prey-handling problems. Vanhaecke and Sorgeloos (1980) have demonstrated considerable variation in naupliar size (422-517 μm) and volume (7 638-13 604 $\times 10^6$ $\mu m^3$) among different geographical strains. The effect of size in feeding nauplii to fish has been described by Beck and Bengtson (1982). They fed freshly-hatched nauplii from eight different *Artemia* strains to Atlantic silverside (*Menidia menidia*) larvae. The correlation between the size of nauplii and mortality of the fish larvae indicated that 20 % mortality or more could be expected when nauplii larger than 480 μm were fed as a first food.

### NUTRITIONAL REQUIREMENTS

In addition to physical requirements, a food organism also has to meet certain nutritional requisites, including digestibility. Watanabe *et al.* (1978a) found high digestibility rates for *Artemia* fed to carp and rainbow trout and reported high values for net protein utilization and protein efficiency ratio. Enzymes such as amylase and trypsin that are found in *Artemia* (Samain *et al.*, 1980) may also play an important role in enzymatic autolysis during the transit of the nauplii through the larval gut, and thus contribute to digestion.

Even when easily digested, a food organism still may not meet the nutritional requirements of the predator. The main problem in evaluating *Artemia* in this respect is the lack of knowledge on the nutrient requirements of most predators to which *Artemia* are fed. A proximate analysis of *Artemia* (Table I) reveals an equilibrated high-protein diet indicating that macronutrient requirements are probably satisfied for most predators. However, several investigators reported considerable variation in larval culture success (Léger *et al.*, 1986).

TABLE I

Average proximate composition (in % ± standard deviation) of *Artemia* nauplii and adults as calculated from data presented in 26 and 15 references, respectively (data compiled from Léger *et al.*, 1986)

| | Nauplii | Adults |
|---|---|---|
| Protein | 52.2 ± 8.8 | 56.4 ± 5.6 |
| Lipid | 18.9 ± 4.5 | 11.8 ± 5.0 |
| Carbohydrate | 14.8 ± 4.8 | 12.1 ± 4.4 |
| Ash | 9.7 ± 4.6 | 17.4 ± 6.3 |

Variation in larval growth rate has been attributed to significant differences in individual energy content (0.0366-0.0725 J) and dry weight (1.61-3.33 µg) of *Artemia* nauplii from different geographical origin (Vanhaecke *et al.*, 1983). Selection of high-energy strains is therefore recommended. Varying growth rates may also be attributed to the use of older unfed instar stages which contain up to 39 % less energy and 34 % less dry weight than freshly-hatched nauplii (Fig. 4) (Vanhaecke *et al.*, 1983). This energy and dry weight loss can be avoided by storing instar I nauplii at lower temperatures, since they survive well for 24 h at 2-4 °C without significant losses in dry weight (Léger *et al.*, 1983). This cold storage technique further allows a complete automation of feeding, permitting a 24 h feeding of energy-rich instar I. Starved nauplii not only

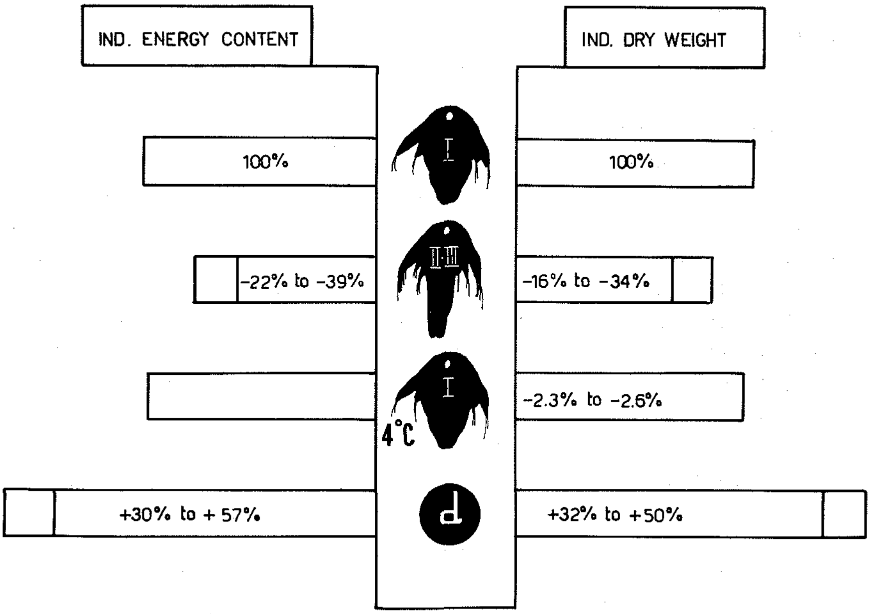

FIG. 4. Change in energy content and dry weight of different forms of *Artemia* (newly-hatched instar I nauplii are considered to have 100 % values for those variables). The percent decrease or increase from 100 % is shown for successively instar II-III metanauplii, cold stored instar I nauplii, and decapsulated cysts.

contain less energy and dry weight, making them less suited to meet the requirements of the predator, but they are also less visible, larger, and faster swimming, and therefore less acceptable. Starved nauplii have a lower free amino acid content (Dabrowski and Rusiecki, 1983), which may reduce their digestibility. All these negative factors may be reflected in poor growth of the larval predator. Decapsulated cysts, on the other hand, constitute the highest energy form of *Artemia* and are preferably used except when a predator feeds only on moving prey.

Besides variable growth rates, other problems have been attributed to the use of *Artemia*. With the availability of different geographical strains of *Artemia*, a relationship has been found between the use of particular strains and the appearance of various symptoms in fish and crustacean larvae, such as lethargy, lack of coordination, abnormal development, problems at metamorphosis, abnormal pigmentation, and even mortality (Wickins, 1972 ; Campillo, 1975 ; Beck *et al.*, 1980 ; Johns *et al.*, 1980 ; Klein-MacPhee *et al.*, 1980, 1982). Several authors have tried to explain these observations and have formulated diverging and sometimes contradictory explanations. For example, Bookhout and Costlow (1970) suspected that high levels of DDT caused the problems, but Wickins' (1972) research suggested that a nutritional deficiency was involved.

## International Study on *Artemia*

The elucidation of the nutritional variability of *Artemia* was one of the major concerns of the participants in the International Study on *Artemia* (ISA) (Sorgeloos, 1980). Nutritional bioassays with several larval fish and crustacean species fed various *Artemia* strains confirmed previous reports of variability in food value among different strains of *Artemia* (Table II). However, this variability was not expressed in culture tests with freshwater fish larvae. Major problems seemed to occur only in marine species. Certain *Artemia* strains guaranteed good culture success for all marine species tested (Brazil, San Francisco Bay, and Reference *Artemia*). The 1978 batch San Pablo Bay (SPB) *Artemia* nauplii, on the other hand, were consistently poor food for all species. Also, Utah *Artemia* in some cases gave poor culture results, whereas intermediate success was obtained with the Canadian strain. Generally good results were obtained with the Australian, Chinese, French, and Italian strains, except for Atlantic silverside larvae, which, as mentioned before, were adversely affected by the large naupliar size. Thus, major problems seemed to occur when SPB and Utah nauplii were fed to marine organisms, especially to those species, such as crab and flatfish, whose larvae undergo a pronounced metamorphosis. In those species almost all mortality was suffered at the onset of metamorphosis (Fig. 5 and 6). This information coincides with numerous reports from authors culturing decapod and flatfish larvae, such as crab, turbot, and plaice (Léger *et al.*, 1986).

The ISA has developed various correlations to try to explain strain differences in nutritional value, based on results of the bioassays and the chemical and biochemical analyses of the strains. Abnormalities, effects on growth, and mortality may be an expression either of nutrient deficiency or of toxicity. For this reason a detailed analysis of chlorinated hydrocarbons (CHCs) was carried out on the *Artemia* strains used in the bioassays (Olney *et al.*, 1980 ; Seidel *et al.*, 1982). The most heavily contaminated strains, in terms of total CHCs (the Italian and Chinese strains) gave, however, excellent results in the bioassay. On the other hand, Utah ranked among the cleanest strains. The only similarity between SPB and Utah samples was their higher level of dieldrin. Furthermore, SPB had the highest level of chlordane and high molecular PCBs.

Table II

Summary of nutritional bioassay results for several categories of aquatic organisms fed 10 geographical strains of *Artemia*

| | | | *Artemia* geographical strain | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Australia | Brazil | Canada | China | France | Italy | Utah | San Pablo Bay | San Francisco Bay | RAC |
| Marine | Crustaceans | Metamorphosis | + | + | ± | + | + | + | - | - | + | + |
| | | --------- | + | + | ± | + | + | + | + | - | + | + |
| | Fish | Metamorphosis | + | + | + | + | + | + | - | - | | + |
| | | --------- | ± | + | ± | ± | ± | - | ± | - | + | + |
| Freshwater fish | | --------- | + | + | + | + | + | + | + | + | + | |

Follow-up studies by Johns *et al.* (1981) and McLean *et al.* (1987) have demonstrated that Brazilian nauplii purposely contaminated with the suspected CHCs did not cause mortality in crab larvae or post-metamorphic flounder, although growth was reduced in flounder fed on *Artemia* with moderate levels of CHCs. CHCs therefore were probably not a principal factor controlling the dietary value of the *Artemia* strains tested. Probably heavy metals were also an insignificant factor since no metal common to SPB and Utah was present in dramatic high levels (Olney *et al.*, 1980). One must nevertheless be concerned about the problem of toxic materials in *Artemia* because CHC and heavy metal contamination is anthropogenic and thus subject to variations. For example, copper levels have been shown to vary considerably in Utah *Artemia* (Blust, pers. commun.) and very high pesticide levels have been reported in a batch of Philippine *Artemia* (Simpson *et al.*, 1983). Shelbourne (1968) hypothesized that Utah *Artemia* may have accumulated toxins produced in dinoflagellate blooms, but measurable amounts of paralytic shellfish poison have not been detected in SPB (Olney *et al.*, 1980). Apparently, differences in nutritional value between the *Artemia* sources tested are not related to the presence of toxic contaminants.

The hypothesis that nutrient deficiency may explain nutritional variability is borne out by the difference in results of feeding *Artemia* strains to freshwater and marine species, which indeed have different dietary requirements. In-depth biochemical profiles of the different strains tested showed first that differences in amino acid profile could not explain differences in culture results (Seidel *et al.*, 1980). All strains were found to meet the essential amino acid requirements for

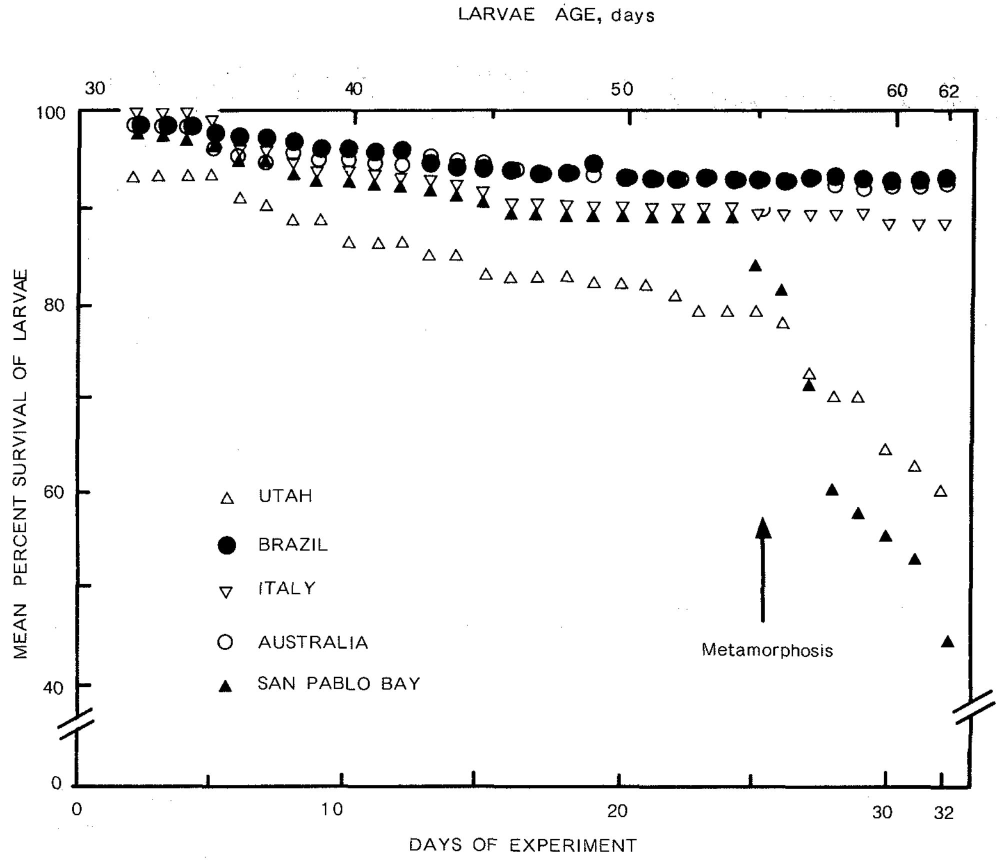

FIG. 5. Percent survival of winter flounder larvae fed on five geographical strains of *Artemia* (from Klein-MacPhee *et al.*, 1980).

chinook salmon, though methionine appeared to be the first limiting amino acid. Similarly, the varying culture success could not be explained by differences in carotenoid (Soejima *et al.*, 1980), mineral (Watanabe *et al.*, 1978a), caloric or lipid content (Schauer *et al.*, 1980). However, pronounced differences were found in fatty acid profiles. As compared to other strains, Utah and especially SPB *Artemia* contained high levels of 18:3ω3 and particularly low levels of 20:5ω3 (Schauer *et al.*, 1980 ; Seidel *et al.*, 1982). The relative lack of 20:5ω3 may explain the poor results in feeding the Utah and SPB strains to marine organisms. The highly unsaturated fatty acid (HUFA) 20:5ω3 is known to be essential for marine fish and crustacean larvae (Teshima, 1978 ; Yone, 1978 ; Kanazawa *et al.*, 1979). Canadian nauplii, in spite of high levels of 20:5ω3, provided intermediate results in the bioassays, which indicates that for this strain other factors, possibly energetic ones, may be involved.

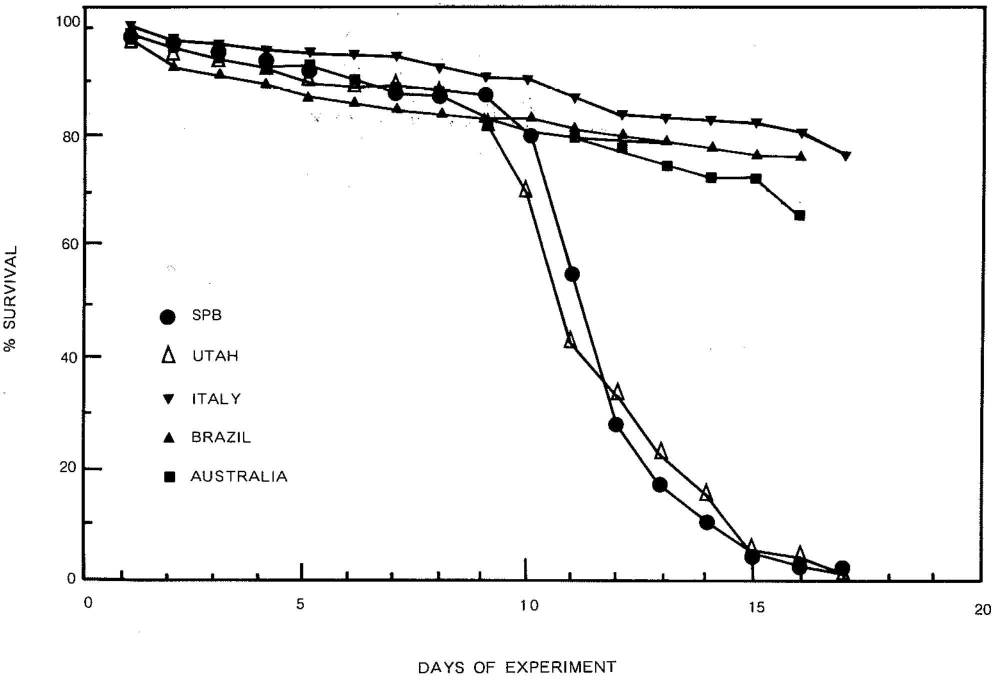

FIG. 6. Percent survival of mud crab larvae fed on five geographical strains of *Artemia* (from Johns *et al.*, 1980).

## Importance of essential fatty acids

Léger *et al.* (1985b) further studied the relationship between 20:5ω3 level and nutritional value of *Artemia* by evaluating different batches of the San Francisco Bay strain together with Reference *Artemia* and SPB as, respectively, positive and negative controls. Levels of 20:5ω3 varied considerably among different batches within the same strain. Batches with high 20:5ω3 levels yielded high biomass production in culture tests with mysids, while batches with low 20:5ω3 levels consistently yielded less biomass (Fig. 7). CHC analyses were also performed on these different batches and again considerable differences were found, but could not be correlated with the biomass figures (Fig. 8). From these studies we may conclude that the content of the essential fatty acid 20:5ω3 seems to be the most important factor determining the nutritional value of *Artemia* nauplii to marine organisms. This is supported by the observation that Utah and San Pablo Bay nauplii provided good survival of freshwater fish which do not require highly unsaturated fatty acids such as 20:5ω3 in their diet.

Watanabe *et al.* (1978b) classified *Artemia* strains into marine type *Artemia*, which contain high levels of 20:5ω3, and freshwater type *Artemia*, which contain low levels of 20:5ω3. They obtained good survival of red seabream larvae fed with the marine type nauplii and poor survival of those fed the freshwater type. However, when the freshwater type nauplii were fed on

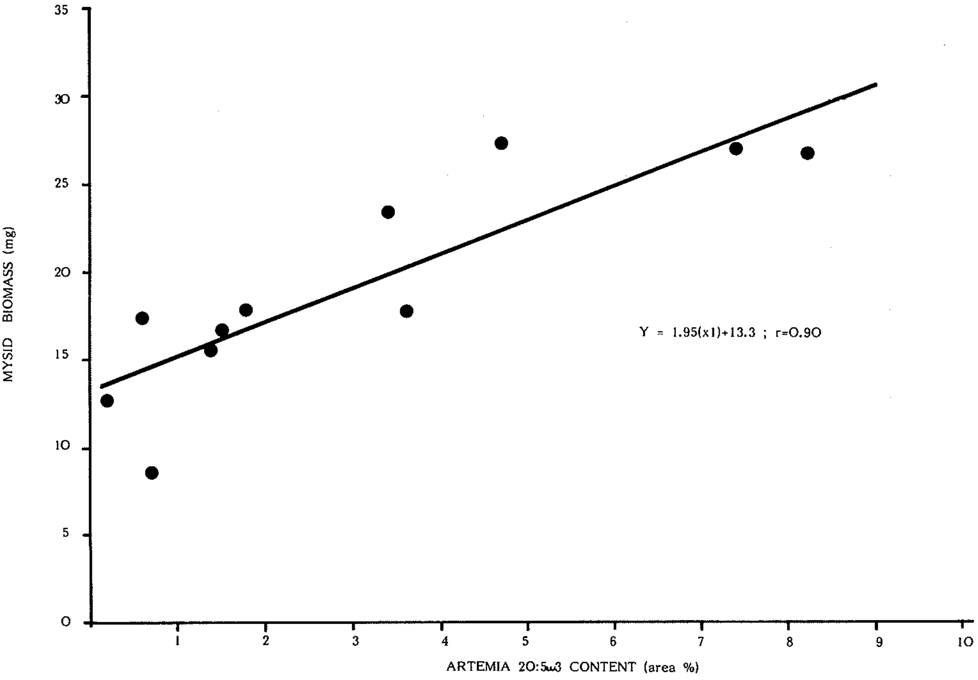

FIG. 7. Linear relationship between the 20:5ω3 content of several *Artemia* collections from San Francisco Bay origin and the biomass of mysids to which the *Artemia* were fed (data from Léger *et al.*, 1987).

20:5ω3-rich diets, such as marine *Chlorella* or ω-yeast, before they were fed to the fish, superior survival rates of the seabream larvae were achieved. Watanabe *et al.* (1982) have demonstrated that 20:5ω3 could be incorporated into *Artemia* by feeding them for 24 h on 20:5ω3-rich diets. Nauplii prefed ω-yeast can also contain significant levels of 22:6ω3 and red seabream larvae did best on these nauplii. Similarly, Léger *et al.* (1985a) have shown that feeding a HUFA-enrichment diet to San Pablo Bay 1628 *Artemia* increased its levels of 20:5ω3 and 22:6ω3 and markedly enhanced its nutritional value for penaeid shrimp larvae. The nutritional improvement of fatty-acid-enriched Utah *Artemia* has also been demonstrated for mysids, two penaeid species, and seabass larvae (Van Ballaer *et al.*, 1985 ; Amat *et al.*, 1987 ; Léger *et al.*, 1987).

If the abundance of certain essential fatty acids governs the nutritional value of *Artemia* nauplii, what exactly determines their respective levels in *Artemia*? Schauer and Simpson (1985) have demonstrated that *Artemia* have a limited need to produce their own 20:5ω3 and Millamena *et al.* (1985) reported that *Artemia* HUFA levels strongly resembled those of the algal diets on which they were fed. In a recent experiment, Lavens (pers. commun.) has cultured *Artemia* nauplii containing about 5 % 20:5ω3 in a controlled cyst production unit (Lavens and Sorgeloos, 1984). Two different diets were used, one containing 6.7 % and the other one only 0.7 % 20:5ω3. The cysts produced by adults grown on the 20:5ω3-rich diet contained high levels of 20:5ω3 while the others contained very low levels of this fatty acid. This experiment clearly demonstrated that *Artemia* cysts reflect 20:5ω3 levels of the diet available for the parental population.

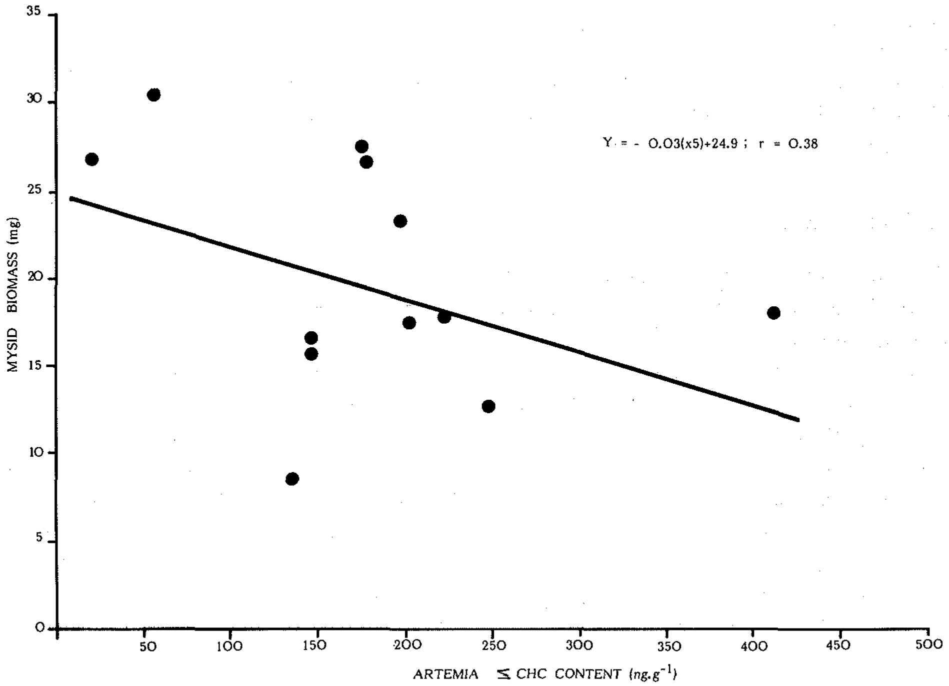

FIG. 8. Linear relationship between the chlorinated hydrocarbon content of several *Artemia* collections from San Francisco Bay origin and the biomass of mysids to which the *Artemia* were fed (data from Léger *et al.*, 1987).

If these results can be extrapolated to wild populations one can deduce that different food conditions in *Artemia* ponds and lakes probably explain differences in fatty acid profile between *Artemia* strains – and even within the same strain. Compilation of data from literature and our own analyses (Table III) (Léger *et al.*, 1986) show that 20:5ω3 levels may indeed vary considerably among and within strains. Variability is particularly great in strains produced in solar saltworks, *e.g.* San Francisco Bay, Brazilian, and Chinese *Artemia*. Variability is small in

TABLE III

Intra-strain variability of levels of the essential fatty acid 20:5ω3 in *Artemia*. Data are given as percentage of total fatty acid methyl esters and represent analyses of samples taken over several seasons or years (data compiled from Léger *et al.*, 1986)

| *Artemia* geographical strain | 20:5ω3 content (area %) |
|---|---|
| San Francisco Bay | 0.3 - 13.3 |
| Brazil | 3.5 - 10.6 |
| China | 1.3 - 15.4 |
| Canada | 5.2 - 9.5 |
| Utah – southern arm | 2.7 - 3.6 |
| – northern arm | 0.3 - 0.4 |

Canadian and especially Utah *Artemia*, both produced in inland salt lakes which are mostly characterized by a more stable environment. Salinity conditions and food composition in solar-salt ponds are more diverse and often completely different from one pond to another. The nutritional quality of *Artemia* cysts produced in solar-salt ponds is therefore subject to uncontrolled variability and is difficult to predict, being dependent on the caprices of nature.

## *Artemia* enrichment

The technique of feeding *Artemia* nauplii on HUFA enrichment diets markedly increases the nutritional value of inferior strains and batches and hence reduces strain and batch differences. The application of *Artemia* enrichment with algae was pioneered by Forster and Wickins (1967) and Wickins (1972) and further developed by Japanese, French, and Belgian researchers using prepared diets (Léger *et al.*, 1986). Since those methods have been covered elsewhere in these proceedings (Léger *et al.*, 1987 ; Robin *et al.*, 1987 ; Watanabe, 1987), we will not review the techniques here, but summarize the advantages of *Artemia* enrichment. Enriched nauplii have an improved nutritional composition, *i.e.* they have a higher energy content and contain all essential fatty acids including 22:6ω3, which is generally absent in nauplii from all strains. Through enrichment techniques other nutrients, prophylactics, and therapeutics may be passed to the predator via *Artemia* nauplii. The application of enriched *Artemia* is reflected in improved performances in larval culture, in terms of both survival and growth, and consequently improved performances are obtained in later stages (Léger, unpubl. data ; Chamorro, pers. commun.). Larvae fed on enriched *Artemia* are indeed healthier and more resistant to stressful conditions, such as infections, weaning of fish, or transfer of fry/postlarvae from hatchery tanks to nursery ponds. The only disadvantage of using enriched *Artemia* is their larger size, which may be a problem for the early larval stages of the predator. If size is indeed a problem, freshly-hatched high-quality instar I nauplii may be fed for the first few days, followed by a gradual switch to enriched metanauplii as soon as the predator's size permits ingestion of larger particles. Optimized enrichment procedures may also reduce the disadvantage of size by obtaining similar enrichment levels in less time (Léger *et al.*, 1987). Similar enrichment techniques may also be applied for juvenile and adult *Artemia* which may be used as a carrier for essential nutrients and other components to be administered to postlarval shrimp, juvenile fish, and lobster larvae.

## Conclusions and recommendations

To summarize, *Artemia* is an excellent food for a wide variety of cultured marine and freshwater organisms. The major constraint of *Artemia* as a food organism for marine predators is its variable nutritional quality. However, we recommend some measures to remedy this problem :

- for the problem of size and variable energetic content between strains and instar stages, one should :
  a) select suitable strains ;
  b) use freshly-hatched first instar nauplii (*i.e.*, through application of optimized hatching procedures, cold storage of nauplii, and optimized feeding strategies) ;
  c) when possible, use decapsulated cysts.

- for the problem of variable nutritional composition, one should :
  a) apply enrichment techniques ;
  b) select high quality lots for early larval stages and as a reference material in ecological studies.

Especially for those studies where reproducibility of culture results is of utmost importance, an urgent need exists for the fully controlled production of standard high-quality *Artemia* cysts.

Finally, realizing that nutritional quality of *Artemia* may be so poor that mortality of certain predators may result, we wonder how mariculture would have developed if Seale (1933) and Rollefsen (1939) had used a poor quality *Artemia* source when they pioneered the use of *Artemia* for larval fish culture back in the 1930's. Despite variability in size, caloric content, nutritional composition, and contaminants, among geographical strains, *Artemia* has proven to be the most widely used and successful diet for aquaculture purposes.

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