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THE USE OF *ARTEMIA* IN MARINE FISH LARVICULTURE

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ABSTRACT

Among the live diets used in the larviculture of fish and shellfish, the brine shrimp *Artemia* nauplii constitute the most widely used food item; i.e., over 700 metric tons of dry *Artemia* cysts are annually marketed worldwide for on-site hatching into 0.4 mm nauplii.

Although the use of *Artemia* appears to be simple, considerable progress has been made in the past decade in improving and increasing its value as a larval diet for marine fish larvae. The improvements include: identification of the most appropriate strains and batches; new techniques for cyst disinfection; decapsulation and hatching; and enrichment and cold storage of nauplii. Using particulate or emulsified products rich in highly unsaturated fatty acids or n-3 HUFAs, the nutritional quality of *Artemia* can be further tailored to suit the predators' requirements by bio-encapsulating specific amounts of these products in the *Artemia* metanauplii. Application of the method of bioencapsulation, also called *Artemia* enrichment or boosting, has had a major impact on larviculture output, not only in terms of survival, growth and success of metamorphosis of the fish, but also with regard to their quality, e.g., reduced malformations, improved pigmentation and increased stress-resistance. Nonetheless, in many species survival rates are still marginal.

For several marine fish species, the optimal dietary levels of n-3 HUFAs are still not met by enriched *Artemia* . Furthermore, while n-3 HUFAs might have proven most critical, it is very likely that other nutrients (e.g., other lipid classes, vitamins and free amino acids) might appear equally important and in some species even more critical. The bio-encapsulation technique can also be used for the oral delivery of hormones and therapeutics to the fish larvae.

Artemia juveniles, grown to a size that suits the feeding behaviour of the growing predator and/or adult biomass collected from local saltworks or mass-production units, can be used as an excellent nursery and weaning diet for most species of marine fish, eventually reducing fish mortalities, cannibalism and heterogenous growth.

INTRODUCTION

Larviculture nutrition, particularly in the early stages, appears to be the major bottle-neck for the industrial upscaling of the aquaculture of fish and shellfish. With selected species such as salmon, problems were to be overcome and during the past few years, billions of hatchery produced fry have been available for the commercial production of several 100,000 tons of marketable salmon per year. Salmon do not have feeding problems in the early development stages because the larvae at hatching carry a big yolk sac with enough food reserves for the first three weeks of their development. Once the yolk is consumed and exogenous feeding can start, the larvae already have a large mouth and can thrive on formulated feeds. With most other marine fish, egg production might not pose a major problem, but larval size at hatching and first feeding are the initial obstacles in larval rearing (Table 1). Bass, bream, turbot, grouper, mahi and many other marine fish with aquaculture potential all have very limited yolk reserves which usually last not more than one or two days after hatching. At first feeding, they have small mouths, often with an opening of less than 0.1 mm.

The natural diet of most aquaculture fish species consists of a wide diversity of phytoplankton species (diatoms, flagellates, etc.) and zooplankton organisms (copepods, cladocerans, decapod larvae, etc.), found in great abundance in natural plankton.

This abundance, biochemical composition and maximal diversity of food organisms of different sizes provide maximal opportunities to meet all the nutritional requirements of fish larvae. The first culture trials on a laboratory scale were carried out by feeding freshly-collected wild plankton, aiming to imitate a natural habitat, free of predators. Collecting and feeding natural plankton may appear evident, but it is hardly dependable beyond the laboratory. On an industri-

Table 1. Size of eggs and larval length at hatching in different species of fish (after Jones and Houde 1981).

Species	Egg diameter (mm)	Length of larvae (mm)
Salmon (*Salmo salar*)	5.0 - 6.0	15.0 - 25.0
Trout (*Salmo gairdneri*)	4.0	12.0 - 20.0
Carp (*Cyprinus carpio*)	0.9 - 1.6	4.8 - 6.2
Bass (*Dicentrarchus labrax*)	1.2 - 1.4	7.0 - 8.0
Bream (*Sparus aurata*)	0.9 - 1.1	3.5 - 4.0
Turbot (*Scophthalmus maximus*)	0.9 - 1.2	2.7 - 3.0
Sole (*Solea solea*)	1.0 - 1.4	3.2 - 2.7
Milkfish (*Chanos chanos*)	1.1 - 1.25	3.2 - 3.4
Grey mullet (*Mugil cephalus*)	0.9 - 1.0	2.2 - 3.5
Grouper (*Epinephelus tauvina*)	0.77 - 0.90	1.4 - 2.4
Bream (*Acanthopagrus cuvieri*)	0.78 - 0.84	1.8 - 2.0

al scale, similar to intensive cattle and poultry farming, a readily and consistently available, practical and performing diet is optimal.

Selection of a suitable and nutritious diet may be carried out according to a number of criteria selected from the viewpoint of both the culturist and the predator (Fig. 1). From the practical viewpoint of the culturist, a good diet should be readily available, cost-effective and simple as well as versatile in application. The consistent availability of sufficient numbers of food organisms is of utmost importance for continuous cultures. In this respect, the collection and feeding of wild plankton has proven unreliable and not practical. Over the past two to three decades, trial-and-error approaches have resulted in the adoption of selected larviculture diets, taking into account the different criteria listed in Figure 1. Today, three groups of live diets are widely applied in the industrial larviculture of marine fish and crustaceans:

1) Different species microalgae ranging from 2 to 20 μ m
2) The 50 to 200 μ m rotifer *Brachionus plicatilis*
3) The 200 to 500 μ m brine shrimp *Artemia*

In recent years different formulas of supplemental and substitute products have become available.

Of the live diets used in larviculture, brine shrimp *Artemia* nauplii constitute the most widely used species. Technically speaking, the advantage of using *Artemia* is that one can produce live food "on demand" from a dry and storable powder, i.e., dormant *Artemia* cysts (embryos) which, upon immersion in seawater, regain their metabolic activity and within 24 hrs release free-swimming larvae (nauplii) of about 0.4 mm length (Fig. 2). In this paper we will report about the present status in the production and use of *Artemia* as a larval food source in the industrial larviculture of various species of marine fish.

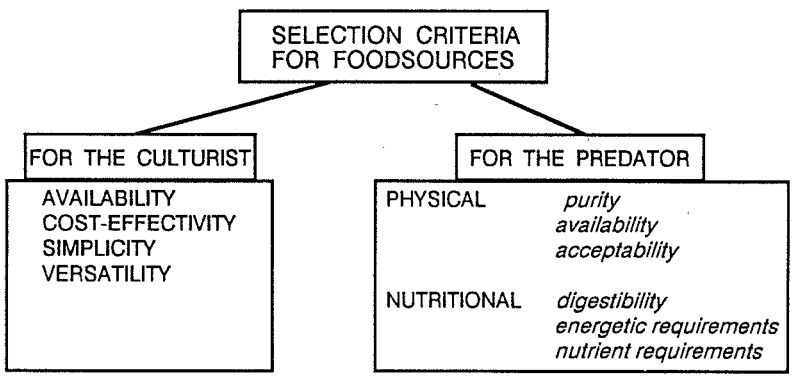

Fig. 1. Selection criteria for larval foodsources from the viewpoint of the culturist and the cultured larva (from Leger et al. 1987a).

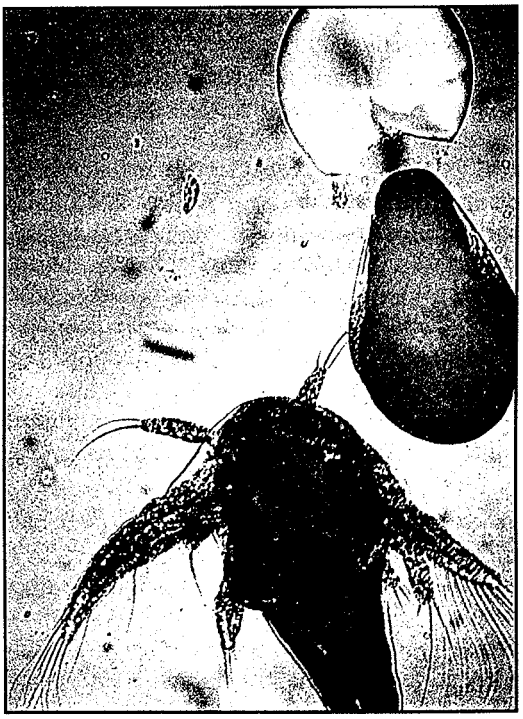

Fig. 2.
Early stage of hatching of *Artemia* cyst (pre-nauplius surrounded by the hatching membrane still attached to the cyst shell or chorion) and free-swimming nauplius (instar I stage) (from Leger et al. 1986).

ARTEMIA SUPPLY

The supply of cysts has varied greatly in the 1970s and 1980s (Bengtson et al. 1991). However, more recently, cyst production, availability and price have been relatively stable due to: efforts in *Artemia* research and development during the first half of the 1980s (Sorgeloos et al. 1986, 1987); application of improved methods for harvesting and processing of cysts; the introduction of *Artemia* cyst quality certificates; and the possibility to enhance the naupliar food value via bio-encapsulation with particulate or emulsified enrichment diets.

The annual cyst consumption in fish and shellfish hatcheries is estimated at over 700 metric tons. Although present commercial cyst supplies generally meet demands, more diverse commercial sources are urgently needed to ensure more, and especially predictable quantities of a commercially available product. Today, over 80% of all marketed cysts originate from the Great Salt Lake (Utah, USA). An exceptionally wet winter in Utah might destroy the *Artemia* population in the Great Salt Lake for an extended period of time. Commercial attention should be focused on the development of alternative/ complementary sources, which, for example, are available for natural harvesting in the Soviet Union and in the People's Republic of China.

Small-scale cyst production in man-made saltworks, although technically very successful in several countries in Southeast Asia and Latin America (Sorgeloos 1987), are not expected to contribute significantly to world cyst supplies. However, they provide interesting opportunities for local commercial developments, especially in third-world countries, like Vietnam (Vu Do Quynh 1989), where import opportunities are restricted and where local availability of *Artemia* cysts is the first requirement in consideration of a viable hatchery industry.

ARTEMIA HATCHING

Although the hatching of *Artemia* cysts appears to be simple, several parameters must be considered when hatching out the kilogram quantities of cysts needed on a daily basis in large fish hatcheries. Essential parameters to trigger the start of their hatching metabolism (Sorgeloos et al. 1986) are: a constant water temperature between 25 and 28°C; salinity of 15 to 35 ppt; pH 8.0 (to increase the buffer capacity of the hatching medium, about 1g $NaHCO_3$ /l should be added) ; oxygen levels close to saturation (best hatching results can be achieved with funnel-shaped containers that are aerated from their bottom); cyst densities of not more than 2 g/l ; and strong illumination of the cysts (about 2,000 lux at the water surface), at least during the first hours following complete hydration. All these factors will affect hatching rate, maximum output and, ultimately, the production cost of the harvested *Artemia* nauplii. *Artemia* cyst-lots with good hatching synchrony and high hatching efficiency (i.e., the number of nauplii that can be produced under standard conditions from 1 g of cyst product) should be selected, as there is considerable variation between cysts of various origin, and among batches from the same strain (Sorgeloos et al. 1986).

After hatching, and prior to feeding them to the fish larvae, *Artemia* nauplii should be separated from the hatching wastes (empty cyst shells, bacteria and hatching metabolites). When the aeration in the hatching tank is stopped, cyst shells will float and nauplii will concentrate at the tank's bottom, from where they should be siphoned off within 5 to 10 minutes. Nauplii should be thoroughly rinsed with fresh water, preferably in submerged filters to prevent physical damage to the nauplii.

Considerable progress has been made in the past decade in improving and increasing the value of *Artemia* as a larval diet. A better knowledge of the biology of *Artemia* was at the origin of the development of methods for cyst disinfection and decapsulation (Sorgeloos et al. 1986; Leger et al. 1991). *Artemia* cyst shells may be loaded with germs of bacteria and fungi which can be removed with bleach. A minimum treatment consists of a 30-minute soak of the cysts in a 200-ppm-hypochlorite solution (fresh or seawater) prior to incubation for hatching. This treatment, however, may not kill germs present in the alveolar and cortical layer of the cyst shell. A more effective treatment consists of the decapsula-

tion of the cysts, i.e., the complete removal of both these layers of the shell by short exposure of the hydrated cysts to a 2%-hypochlorite solution at high pH. Provided that the recommended steps in the decapsulation treatment (cyst hydration, exposure to hypochlorite solution, washing, chlorine deactivation in hydrochloric acid, washing and/or brine dehydration) are followed (Table 2), sterile embryos are produced that have not lost their viability. These procedures are being applied at most large fish and shrimp hatcheries to sterilize the cysts and, at the same time, reduce the problems of separating empty cyst shells at naupliar harvest.

Table 2. Procedure for the decapsulation of *Artemia* cysts (Sorgeloos et al. 1986).

1. Hydrate cysts by placing them in fresh or salt water (100 g/l), bubbled in a buket for 1 hr at 25°C.
2. Collect cysts on a 125 μ m mesh sieve, rinse, and transfer to hypochlorite solution.
3. The hypochlorite solution, prepared in advance, with either liquid bleach NaOCl or bleaching powder $Ca(OCl)_2$, should consist of 0.5g active hypochlorite product (activity is normally labeled on the package) per g of cysts, plus an alkaline product to keep the pH> 10(0.15g technical grade NaOH for liquid bleach; either 0.67g Na_2CO_3 or 0.4g CaO for bleaching powder, per g of cysts. (Dissolve bleaching powder first, before adding the alkaline product).
4. Cool the solution to 15-20°C (some hatcheries place the decapsulation container in a water bath filled with ice water). Add the hydrated cysts and stir for 5-15 minutes. Check the temperature regularly, since the reaction is exothermic; never exceed 40°C, and if needed, add ice to the decapsulation. When microscopic examination shows the outer part of the cyst shells to be completely dissolved, remove the cysts immediately, and rinse them with water on a 120-μ m screen until the chlorine smell disappears.
5. Totally deactivate the hypochlorite by dipping the cysts (<1 min) either in 0.1 N HCl or in 0.1% $Na_2S_2O_3$ solution and rinse again with water.
6. Incubate the cysts for hatching, or refrigerate (at 0 to 4°C) for a few days before hatching incubation. For long term storage, cysts must be dehydrated in saturated brine solution.

COLD STORAGE OF NAUPLII

Farmers have overlooked the fact that an *Artemia* nauplius, in its first stage of development, can not ingest food and thus consumes its own energy reserves { (Benijts et al. 1976;) (Fig. 3)}. At the high water temperatures which are applied for cyst incubation, the freshly-hatched *Artemia* nauplii develop into the second larval stage within a matter of hours. It is important to feed first-instar nauplii rather than starved second-instar metanauplii, which are less visible since they are transparent. Instar II *Artemia* are also larger and swim faster than first instars and, as a result, they are less acceptable as a prey. Furthermore, they contain lower amounts of free amino acids and are less digestible. Their lower individual organic dry weight and energy content will reduce the amount of energy uptake by the predator per hunting effort. This will reflect in reduced growth of the larvae, and an increased *Artemia* cyst cost (as about 20 to 30% more cysts need

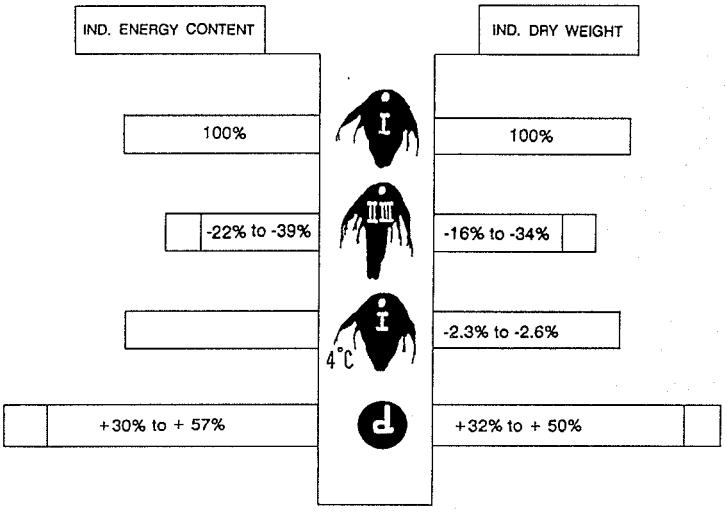

Fig. 3. Change in energy content and dry weight of different forms of *Artemia* (newly-hatched instar I nauplii are considered to have 100 % values for those variables). The percent decrease or increase from 100 % is shown for consequently instar II-III metanauplii, 24-hr cold-stored instar-I nauplii, and decapsulated cysts (from Leger et al. 1987).

to be hatched to feed the same weight of starved metanauplii to the predator). Molting of the *Artemia* nauplii to the second instar stage may be avoided, and their energy metabolism greatly reduced (Fig. 3), by storage of the freshly hatched nauplii at a temperature below 10°C in densities of up to 5 million nauplii /l, for periods up to 24 h (Leger et al. 1983). Slight aeration is necessary to prevent the nauplii from accumulating and suffocating at the bottom of the tank. Commercial hatcheries are able to economize *Artemia* cyst hatching (e.g., fewer tanks, bigger volumes, and a maximum of one hatching and harvest per day) by 24-h cold storage of the nauplii (with styrofoam-insulated tanks and blue-ice packs for cooling). Furthermore, cold storage allows the farmer to ensure the availability of a better quality product and also to consider more frequent food distributions. This is very beneficial for the fish larvae, as food retention time in the larviculture tanks can be reduced and hence growth of the *Artemia* in the culture tank can be minimized. With poor hunters such as the young larvae of turbot (*Scophthalmus maximus*), the feeding of cold-stored *Artemia* results in a more efficient food uptake.

NAUPLIUS FOOD VALUE

Easy hatching and disinfection procedures are not the only parameters to ensure the success of *Artemia* as a larval food source. Several other *Artemia*

characteristics can influence the suitability of a particular brine shrimp product for larviculture. One of these is nauplius size, which can greatly vary from one geographical source of *Artemia* to another. Size is especially critical to several species of marine fish that have a very small mouth and swallow their prey in one bite. Using the marine silverside (*Menidia menidia*) as a test-organism, Beck and Bengtson (1982) were able to illustrate a high correlation between *Artemia* nauplius size (eight *Artemia* strains from 440 to 520 μ m in nauplius length) and larval mortality during early development. With the largest strains of *Artemia*, as many as 50% of the fish could not ingest their prey and starved to death (Fig. 4)

Another important dietary characteristic of *Artemia* nauplii was identified in the late 1970s and early 1980s, when many fish and shrimp farmers began to commercialize. They reported unexpected problems when using different sources of *Artemia* (Sorgeloos 1980). Japanese, American and European researchers studied these problems and confirmed that nutritional value varied in *Artemia* from different geographical sources (see reviews in Watanabe et al. 1983; Leger et al. 1986). The situation became more critical when highly significant differences in production yields were obtained with distinct batches of the same geographical origin of *Artemia*. Multidisciplinary studies in Japan and also by the International Study on *Artemia* (Leger et al. 1987) revealed that the concentration of the essential fatty acid 20: 5n-3 eicosapentaenoic acid (EPA) in the *Artemia* nauplii determines the nutritional suitability of a particular batch of *Artemia* to various species of marine fish larvae. In the meantime, it has been

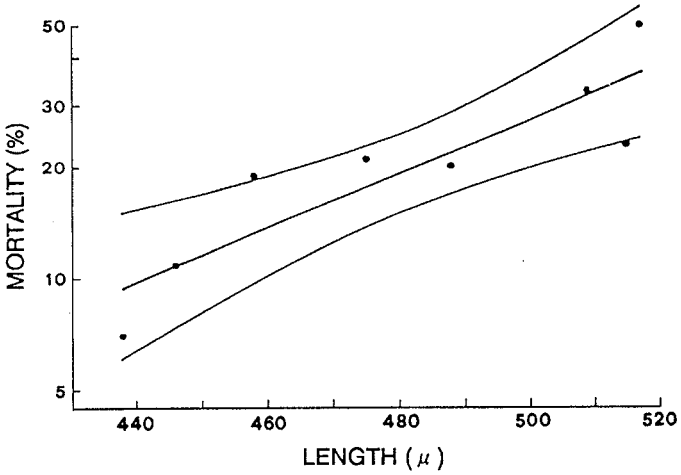

Fig. 4. Correlation of mortality rate of *Menidia menidia* larvae and naupliar length of *Artemia* from eight different geographical sources offered as food to the fish larvae (from Beck and Bengtson 1982).

proven that, for many species of marine fish, high and in some cases total, mortalities occur when feeding *Artemia* deficient in EPA (e.g., the Great Salt Lake *Artemia* strain) for a prolonged period of time. As can be seen in Table 3, EPA levels in *Artemia* can vary greatly from one batch to another within the same strain. Cyst products from inland resources appear to be more consistent in composition, be it however, at suboptimal low levels. As a result, concentrations of the n-3 HUFA EPA must be considered when selecting appropriate *Artemia* cysts for use in marine fish hatcheries.

Table 3. Intra-strain variability of 20: 5n-3 content in *Artemia* . Data represent the range (area percent) and coefficient of variation of data as compiled by Leger et al. (1986 and 1987).

Artemia Geographical Strain	20 : 5n-3 Range (area %)	Coefficient of Variation (%)
USA-California : San Francisco Bay	0.3-13.3	78.6
USA-Utah : Great Salt Lake (S arm)	2.7-3.6	11.8
USA-Utah : Great salt Lake (N arm)	0.3-0.4	21.2
Canada : Chaplin Lake	5.2-9.5	18.3
Brazil : Macau	3.5-10.6	43.2
PR China : Bohai Bay	1.3-15.4	50.5

Commercial provisions of *Artemia* cysts containing high EPA-levels are limited. Their use, however, should be restricted to the feeding period, when the size of the prey is most important. Indeed, even the best natural *Artemia* products do not entirely meet the nutritional requirements of marine fish larvae, particularly with regard to the other fatty acid essential for marine organisms, 22:6n -3 docosahexaenoic acid (DHA) (Bengtson et al. 1991; Watanabe 1991), which is never available in significant amounts in *Artemia* cysts (Leger et al. 1986).

It is fortunate that *Artemia* , because of its primitive characteristics, facilitates a convenient way to manipulate its biochemical composition. Since *Artemia* nonselectively ingest particulate matter after molting into the second larval stage, i.e., about 8 hrs after hatching, a method for bioencapsulation (also called *Artemia* enrichment or boosting) was developed to incorporate products into the *Artemia* prior to offering it to the predator larva. This method is applied at marine fish and crustacean hatcheries throughout the world to enhance the nutritional value of *Artemia* with essential fatty acids. British, Japanese and Belgian researchers developed enrichment products and procedures using selected microalgae and/or microencapsulated products, yeast and/or emulsified preparations, respectively, self-emulsifying concentrates and/or microparticulate products (see review of Leger et al., 1986). The highest enrichment levels are obtained when using emulsified concentrates (Fig. 5): freshly hatched nauplii are transferred to the enrichment tank at a density of 100 (for enrichment periods of > 24 hr) to 300 nauplii/ml for a maximum 24-hr enrichment period. The enrichment me-

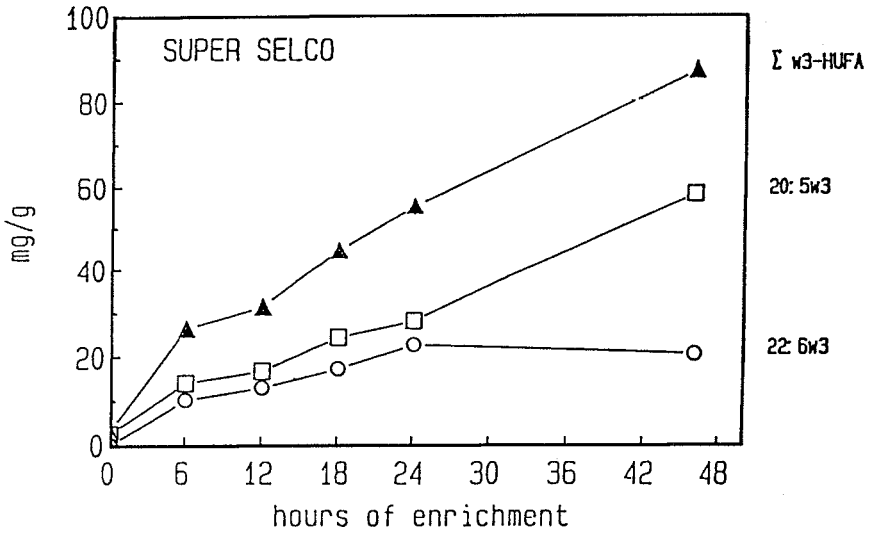

Fig. 5. HUFA levels in Great Salt Lake (UT-USA) *Artemia* (meta) nauplii enriched with the self-emulsifying concentrate SUPER SELCO (*Artemia* Systems NV/SA, Ghent, Belgium) (from Leger et al. 1991).

dium consists of hypochlorite-disinfected and neutralized seawater maintained at 25°C. The enrichment emulsion is added at consecutive doses of 300 ppm every 12 hrs. Strong aeration using air stones is required to maintain dissolved oxygen levels above 4 ppm. Enriched nauplii are harvested after 24 or 48 hrs, thoroughly rinsed and stored at temperatures below 10°C to assure that the HUFAs are not metabolized during storage (Fig. 6). The high enrichment levels obtained with the emulsified concentrates are the result not only of an optimal diet composition and presentation but also of proper enrichment procedures; e.g., nauplii must be transferred or exposed to the enrichment medium as soon as possible before first feeding. In this way, the nauplii begin feeding immediately after the opening of the alimentary tract (instar II stage) and the size increase after hatching and during enrichment will be minimal. *Artemia* enriched according to other procedures reach > 900 μ m, whereas here, higher enrichment levels are obtained in nauplii measuring 660 μ m (12-hr enrichment) to 790 μ m (48-hr enrichment). In view of the high requirement for DHA in particular fish species (e.g., for mahi mahi as demonstrated by Kraul et al. (1991), for turbot by Olsen (pers. comm.) and for halibut by Holmefjord and Olsen (1991) special enrichment emulsions fortified in DHA (yielding *Artemia* metanauplii that contain 44 mg DHA/g naupliar dry weight) are successfully used for the larviculture of several species of marine fish.

Several European marine fish hatcheries apply the following feeding regime, whereby they switch from one *Artemia* diet to the next as the fish larvae are able

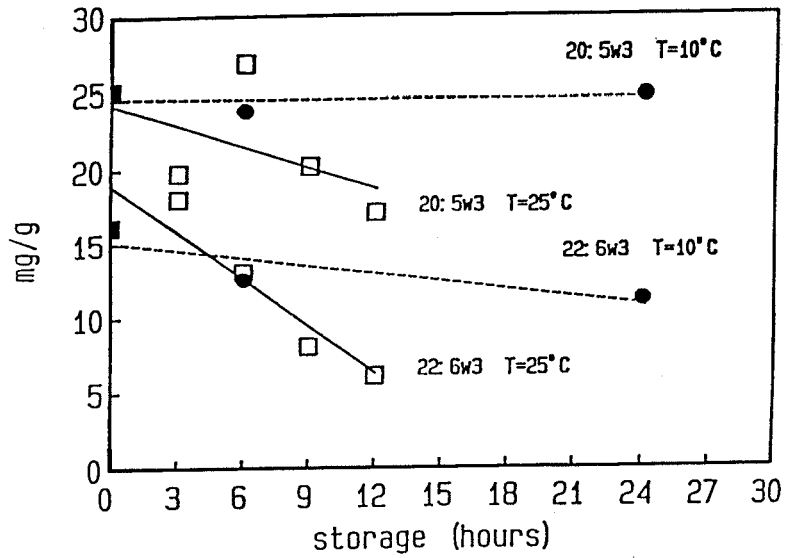

Fig. 6. HUFA levels in SUPER SELCO-enriched *Artemia* metanauplii during storage at 10 and 25°C (from Leger et al. 1991).

to accept bigger prey. During the first few days, freshly-hatched nauplii of a selected *Artemia* strain and batch that yields small nauplii with high content of EPA (≧10 mg/g DW) are fed, followed by 12-hr and eventually 24-hr n-3 HUFA emulsion-enriched (Great Salt Lake, Utah) *Artemia* metanauplii.

The use of n-3 HUFA enriched *Artemia* as a more adequate food source has, without any doubt, been a major breakthrough in the larviculture of many marine fish species (Watanabe et al. 1983; Sorgeloos et al. 1987, 1988) . For example, the adoption of this bioencapsulation methodology has allowed the transition from pilot to commercial larviculture of European bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) (Frenzos and Sweetman 1989). As reported at the recently held Fish & Crustacean Larviculture Symposium, LARVI '91 (Lavens et al. 1991), the feeding of n-3 HUFA enriched *Artemia* not only results in improved survival rates, but also ensures faster growth, better pigmentation, reduced malformations and higher stressresistance in the larvae of several species of both cold- and warmwater marine fish. Nonetheless, with some species such as mahi, turbot, halibut, and grouper, research is ongoing to better define qualitative and quantitative n-3 HUFA requirements; it appears that optimal dietary levels have not been reached yet in *Artemia* . Furthermore, while n-3 HUFAs have proven critical in some species, nutrients such as other lipid classes, particular peptides, free amino acids, pigments, sterols and vitamins are equally or even more important in other species. However, in view of the better results obtained when using natural plankton (consisting of marine copepods) in culturing turbot and mahi (Stot-

ttrup and Kraul, respectively, pers. comm.), the challenge remains to identify the vital components in the copepods so they can be incorporated into a dietary system as convenient as *Brachionus* and *Artemia*.

OTHER APPLICATIONS OF ENRICHMENT

Artemia can also be used as a carrier for the oral delivery of specific components such as hormones and antibiotics into fish larvae. Emulsions enriched with lipid-soluble hormones (e.g., methyltestosterone) can be used for induction of sex-reversal in tilapia. Recently, Verpraet et al. (1991) and Chair et al. (1991), demonstrated the loading of *Artemia* nauplii with doses up to 300 ppm (*Artemia* DW) of the therapeutic mixture Trimetoprim : Sulfamethoxazole. It was reported that levels of antibiotics up to 20 ppm were found in fish larvae tissue only 3 hrs after feeding one dose of antibiotics-enriched *Artemia* metanauplii.

USE OF ONGROWN *ARTEMIA*

As proven in larviculture trials with mahi (*Coryphaena hippurus*) and Asian seabass (*Lates calcarifer*) (Nell, Smith and Wongrat, respectively, pers. comm.), juvenile and adult *Artemia*, on-grown in mass-production units (Lavens and Sorgeloos 1991; Dhont et al. 1991), produced and/or collected from local saltworks (Tackaert and Sorgeloos 1991) are an excellent nursery and weaning diet. The sources of *Artemia* were from mass production and/or collected from local saltworks (Tackaert and Sorgeloos 1991). In addition, it was observed that when used as a weaning diet, larval mortality and especially cannibalism were reduced.

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