LIVE FEEDS IN AQUACULTURE

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ABSTRACT

Over the past two decades intensive larviculture of several fish and shellfish species has expanded into a multimillion dollar industry. Although much progress has been made in identifying the dietary requirements of the larvae of various aquaculture species, the mass culture of their early larval stages still requires the use of live feeds. Selected either through trial and error approaches or because of their convenience in mass production and use, hatcheries are relying today on three groups of live feed, *i.e.* various species of microscopic algae, the rotifer *Brachionus* and the anostracan brine shrimp *Artemia*.

Various species of microalgae are used in feeding mollusc and shrimp larvae and/or in greenwater fish larviculture. As their mass production remains a complex and costly task, and because their dietary value is not always predictable, various types of supplementation and/or substitution products are used in combination with live algae.

Selected strains of the rotifer *Brachionus* are mass-cultured in shrimp and fish hatcheries. The need for microalgae in rotifer culture can be greatly reduced as yeast-based products can be used as more cost-effective diets. The lipid and vitamin composition of *Brachionus* can be adjusted with selected enrichment products in order to better meet the dietary requirements of the fish larvae.

Of all live foods used in fish and crustacean larviculture, the brine shrimp *Artemia* is the most widely used, not least because of the practical convenience of hatching this zooplankton substitute from commercially available dry cysts. With the fast expansion of shrimp and marine fish hatcheries all over the world, the consumption of *Artemia* cysts has recently climbed to over 2,000 mt annually. Selected strains are used as starter feeds, whereas nutritionally less-suitable varieties, such as the Great Salt Lake (Utah, USA) strain can be enriched with emulsified or microparticulate products so as to better meet the dietary requirements of the older larval stages of fish and shellfish. *Artemia* metanauplii can also be used as convenient carriers for oral delivery of chemotherapeutics, vaccines and hormones. Adult *Artemia* biomass harvested from solar saltworks is used as an excellent source of food in shrimp and fish nurseries.

INTRODUCTION

Much progress has been made in the last few decades in the industrial farming of several species of fish and shrimp. The pioneering country has been Japan with the red seabream, *Pagrus major*, and the *kuruma* shrimp, *Penaeus japonicus*. In the last decade Europe made quick progress as well with seabass *Dicentrarchus labrax* and with the gilthead seabream *Sparus aurata*. In terms of quantities of output, the biggest success has been achieved with a few species of Penaeid shrimp in tropical Asia and Latin America.

The most critical factor in this industrialisation of fish and shellfish farming has been the dependable availability of quality fry, produced in so-called hatcheries. Today the larviculture industry of marine fish and shrimp can be valued at several hundred million US dollars annually for the production of more than 50 billion shrimp postlarvae and about 400 million marine fish fry.

Although the commercial hatcheries are much more cost effective and their outputs much more predictable than ever before, we need to admit that the methods applied are still very empirical. This has a lot to do with the very primitive nature of the early larval stages of marine fish and shrimp. Only a few species like the salmon have a big yolk sac that provides endogenous food for the first weeks of the larvae. Most marine fish have very small and primitive larvae: the yolk sac is providing food for only a couple of days and the digestive tract is still very inefficient.
In nature the larvae of most fish and shellfish species eat small phyto- and zooplankton. This diet does not only provide a very diversified composition but because of its autodigestion characteristics will facilitate nutrient uptake in the larvae.

The early pioneers in larviculture of fish and shrimp had to look for a suitable and practical substitute for natural plankton, taking into account nutritional quality as well as production costs in the selection

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process. Over the years a limited number of algal species, the rotifer *Brachionus plicatilis* and the brine shrimp *Artemia* have become the live feeds used on a worldwide scale in the industrial farming of fish and shellfish larvae.

MICROALGAE

Today the most costly and perhaps least understood live food are the unicellular algae; *i.e.* some 15 species of diatoms and green algae, ranging in size from 5-25 microns.

Microalgae are an essential food source in the rearing of all stages of marine bivalve molluscs (clams, oysters, scallops), the larval stages of some marine gastropods (abalone, conch), larvae of marine fish (cod, halibut) and shrimp (*Penaeus*), some fish species (tilapia, milkfish), and zooplankton (rotifers, copepods, cladocerans, brine shrimp). The last are fed in turn to the late-larval stages of various species of fish and crustaceans (prawns, shrimp, crab, lobsters).

Food species of microalgae have been selected on the basis of their mass culture potential, cell size, digestibility, and overall food value, much more by trial and error than any other scientific selection process. Easy to culture "weeds", such as *Chlorella sp.* appear to have poor food value (*i.e.* poor digestibility) for many species with aquaculture potential. The most suitable species still pose many problems for large scale culture, *i.e.* their culture implies the use of complex culture media, made up with germ-free seawater treated by microfiltration and/or UV-irradiation.

Algae are progressively cultured in a multi-stage back-up system (Figure 1). Starting with sterile test-tubes the algae are inoculated in larger glass containers and carboys, then to bigger vessels or plastic bags and finally to large, indoor or outdoor tanks. As a result most farms still apply labour intensive and expensive batch production systems. Even when production targets can be maintained with regard to cell numbers produced, shrimp farmers for example, have experienced temporal variations in algal food values resulting in inconsistent hatchery outputs. Olsen (1989) illustrated that the content of the (n-3) highly unsaturated fatty acids (HUFAs) eicosapentaenoic acid (EPA) 20:5(n-3) and docosahexaenoic acid (DHA) 22:6(n-3) can vary greatly not only among algal species but even from culture to culture within a given species (Figure 2). Using *Penaeus stylirostris* as a test organism, Léger *et al.* (1985) could demonstrate that the content of EPA and DHA in the zoea diet had a major impact on survival and growth in later stages, when animals had already been switched to another diet.

This provided the rationale to look for alternatives or supplements to live microalgae. Today different approaches and formulations are already being applied at the commercial level, and many new developments in producing more cost-effective products are to be expected: *ie* freeze-dried algae, manipulated yeast, microencapsulated feeds, and different kinds of microparticulate diets (for reviews see Léger and Sorgeloos, 1992 and

Figure 1: Schematic outline of steps involved in the mass production of microalgae

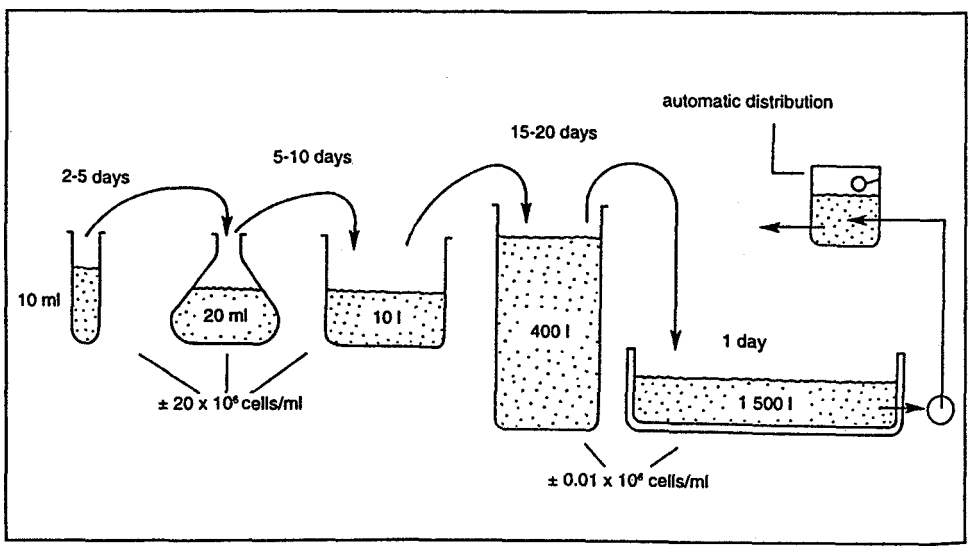
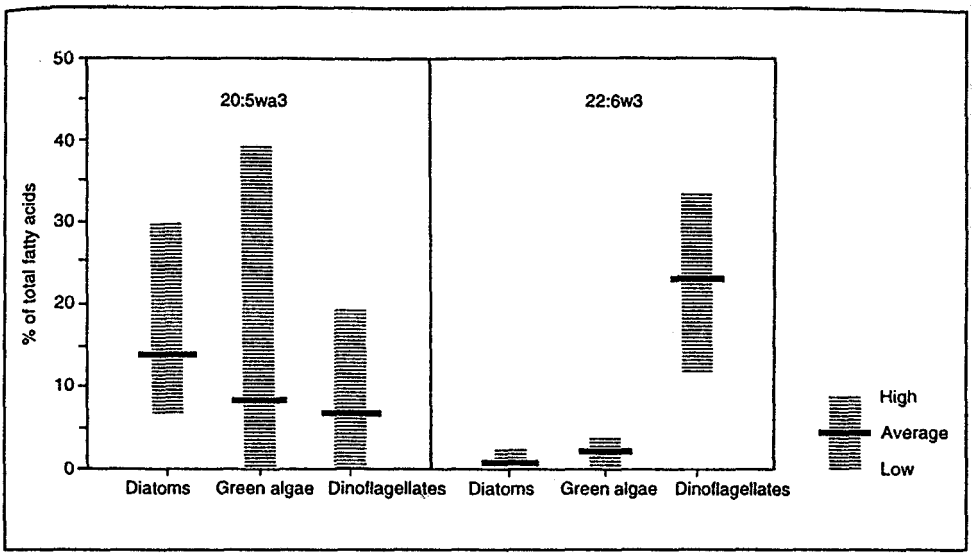


Figure 2: Content of the essential fatty acids 20:5 (n-3) and 22:6 (n-3) in marine diatoms, green algae and dinoflagellates (from Olsen, 1989)



Coutteau and Sorgeloos, 1992).

Microalgae might have unique antibacterial and/or immunostimulatory properties. Austin *et al.* (1992) demonstrated that extracts from *Tetraselmis suecica* inhibited bacterial activity within 15 minutes upon addition to the fish tanks and for periods up to 4 hours; supplementation of spray-dried heterotrophically grown *Tetraselmis* to the diet of Atlantic salmon prevented the outbreak of infections with *Vibrio anguillarum, V. salmonicida* and *Serratia liquefaciens.*

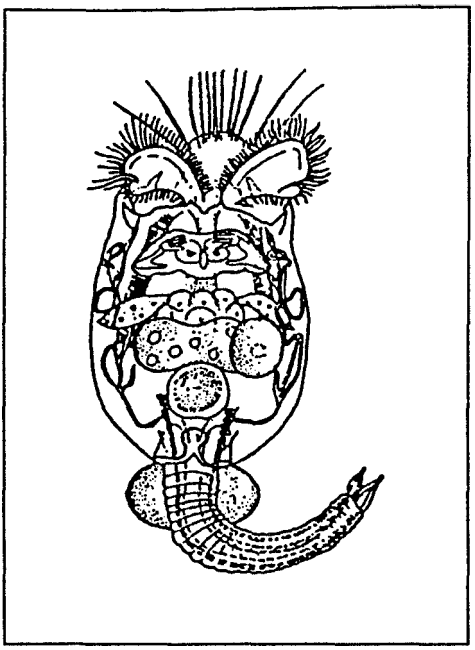
The popular practice in marine fish hatcheries of adding cultures of one or more algal species in the larval rearing tanks, known as 'the green water technique', also deserves further study. Results in marine fish larval production under these conditions are often superior and have been attributed to several factors. Cod and halibut directly ingest the algae as a first food at start of feeding. For other fish larvae, accidental ingestion may provide micronutrients which are not transferred through the zooplankton or supply exogenous enzymes facilitating digestion in the early larval stages. In addition, algae may stimulate enzymatic synthesis and onset of feeding in young larvae. Specific polysaccharides present in the cell wall of the algae might stimulate the non-specific immune system in these early larval stages. Indirectly the algae may serve as food for the remaining rotifers in the fish tank. Microalgae may furthermore act as a water conditioner by stripping off nitrogenous substances or by changing the properties of the incoming light by creating more shaded conditions.

ROTIFERS

The rotifer *Brachionus plicatilis* is an important and essential food source in the early part of the commercial larval rearing of many marine fish and a few shrimp species. This brackishwater zooplankton (Figure 3) was identified by Japanese aquaculturists as a suitable starter diet in marine fish larviculture (Fukusho, 1989). Depending on the mouth size of the cultured fish larvae, a small *Brachionus* strain (50-100 ìm length) or a large one (100-200 ìm) is used. With the exception of harvesting wild populations of *Brachionus plicatilis* in the salt ponds around the Bohai Bay for use as food in local hatcheries of *Penaeus chinensis* (Tackaert and Sorgeloos, 1990), most hatcheries in the world rely on mass production techniques.

Under optimal culture conditions, *Brachionus plicatilis* reproduces by parthenogenesis, *i.e.* each female produces several eggs at a time which upon hatching reach the reproductive stage in a few days only. The widely-applied batch culture procedure appears to be simple, using microalgae and/or baker's yeast or other formulated products as food source. Cylindro-conical tanks are inoculated at a starting density of approximately 200 animals/mt (20-25 ppt seawater). Homogenous mixing and oxygenation of the water is achieved by using airstones. When feeding yeast or

Figure 3: Microscope drawing of *Brachionus plicatilis* (from Walker, 1981)



other formulated feeds, airstones should be placed at a certain distance above the tank's bottom so as to allow sedimentation of waste particles that can be siphoned off once a day. Food should be distributed over several rations a day. Best results are obtained when applying continuous pumping of concentrated food from a refrigerated stock suspension via a centrifugal pump into the culture tanks. Cultures are harvested after about 5 days when the rotifer density has reached a maximum of about 600 animals/ml. Although rotifers can be reared at much higher densities it should be emphasised that at very high densities the egg ratio and protein content of the rotifers decreases.

Many fish hatcheries have experienced significant problems in maintaining large cultures and producing on a predictable basis the massive numbers of rotifers that are needed to feed the hundred thousands to millions of baby fish they have in culture. The food of the rotifers appears to be one of the key elements in their successful mass production; for convenience fresh bakers' yeast is mostly used as the main diet ingredient. However, its freshness, a criterion which is difficult to evaluate by the farmer, can greatly influence the dietary value of the yeast for the rotifers and, as a consequence, determine rotifer culture success.

Many farmers supplement the baker's yeast with microalgae, a procedure which at the same time ensures an increase in the level of (n-3) essential fatty acids in the rotifers. This (n-3) HUFA enrichment is critical in optimising the food value of the rotifers for marine fish larvae. Different microparticulate (Rimmer and Reed, 1990) and emulsified (Watanabe *et al.*, 1983; Léger *et al.*, 1989) formulations are used for this boosting with essential fatty acids and other components. The (n-3) HUFA enrichment of rotifers is performed either in the culture vessel or more often in a separate tank. The enrichment diets are fed over 6-8 hrs at a dose of 100-250 ppm, in two rations, depending on the type of product. Rotifer density is about 200-500 per ml and temperature is kept between 20-25°C. Maximal (n-3) HUFA levels that can be reached presently in 6-hr enriched rotifers are 117 mg Ó-(n-3) HUFA per gram dry weight rotifers or 57 % of total fatty acids when starting at an initial (n-3) HUFA level of 5.8 mg/g or 8.3% of total fatty acids (Léger *et al.*, 1989).

A further improvement of the nutritional quality of *Brachionus* can be accomplished with respect to vitamin C supplementation. Ascorbylpalmitate (AP) can be used as the vitamin C source because of its stable and lipophilic characteristics and its bioavailability. Direct incorporation of AP (0, 0.099, 0.33 and max. 0.55% w/w) into the artificial diet CULTURE SELCO® (CS) can yield ascorbic acid (AA) concentrations of 150, 150, 225 and 280 ppm respectively in the rotifers after three days. Separate supplementation of CS with higher (5, 10, 20 and 30% w/w) percentages of AP can increase the AA levels in *Brachionus* after three days of culturing up to 600, 900, 1200 and 2000 ppm respectively. The AA content of the rotifers does not decrease during a 24 hour storage period. Apparently the vitamin C inclusion does not result in any effect on the production nor on the HUFA-content of the rotifers. Elevated levels of a bio-active vitamin C source can thus be transferred through the live food chain towards the fish or shrimp larvae, in this way providing an important tool to build up stress and disease resistance during larviculture (Merchie *et al.*, in press).

The need for continuous maintenance of live stock cultures of *Brachionus* involves the risk of bacterial contamination. Treatment with antibiotics might lower the bacterial load, but implies the risk of build up of antibiotic resistance. Commercial availability of resting eggs could be the solution by eliminating the need to maintain stock cultures and reduce the chances of contamination with ciliates or pathogenic bacteria. Furthermore, the resting rotifer eggs could also be disinfected prior to hatching out in a new culture inoculum.

A new approach, that is being evaluated as a possible prophylactic treatment, is the use of probiotics, *i.e.* the addition of beneficial bacteria in the *Brachionus*

Figure 4: 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 Léger et al., 1986)

cultures and/or in the fish/shrimp rearing tank to stabilise and improve larval rearing by providing a good microbiologically balanced system and by suppression of detrimental, pathogenic bacteria (Bogaert *et al.*, 1993). Although already routinely applied in some Ecuadorian shrimp hatcheries (Sorgeloos and Van Stappen, 1994), the research and development of probiotics for hatchery applications is still in the developmental stage.

ARTEMIA

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 hours release free-swimming larvae (nauplii) of about 0.4 mm length (Figure 4). Actually more than 2000 mt of dry *Artemia* cysts are marketed annually for worldwide production of freshly hatched *Artemia* nauplii to be used as food in the hatchery phase of fish and crustacean aquaculture.

Considerable progress has been made in the past decade in improving and increasing the value of *Artemia* as a larval diet. A better understanding of the biology of *Artemia* was the key to the development of methods for cyst disinfection and decapsulation (Sorgeloos *et al.*, 1986).

Although hatching of *Artemia* cysts appears to be simple, several parameters need to be taken into consideration when hatching out large quantities of cysts as needed on a daily basis in the large fish/shrimp hatcheries. These are: a constant temperature between 25-28°C; salinity of 15-35 ppt; pH 8.0; oxygen levels close to saturation (best hatching results can be achieved with funnel-shaped containers that are aerated from the bottom); cyst densities of not more than 2 g/l; and strong illumination of the cysts (about 2000 lux at the water surface), at least during the first hours following complete hydration, in order to trigger the start of their hatching metabolism (Sorgeloos *et al.*, 1986). All these factors will affect the hatching rate and maximum output, and hence the production cost of the harvested *Artemia* nauplii. Attention should be paid to select *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 one g cyst product), as considerable variation has been demonstrated between cysts of various origins, and even between batches from the same strain.

After hatching, and prior to feeding to the fish/shrimp 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 turned off, cyst shells will float and nauplii will concentrate at the tank bottom; they should be siphoned off within 5-10 minutes. Nauplii should be thoroughly rinsed with freshwater, preferably in submerged filters so as to prevent physical damage to the nauplii. Farmers have long overlooked the fact that an *Artemia* nauplius in its first stage of development cannot take food and thus consumes its own energy reserves. At the relatively 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 therefore important to use first-instar nauplii for feeding rather than starved second-instar metanauplii which are less visible as they are transparent. Instar II *Artemia* are also larger and swim faster than first instars, as a result of which they are less acceptable as a prey. Furthermore they contain lower amounts of free amino acids; so they are less digestible and their lower individual organic dry weight and energy content will reduce the energy uptake by the predator per hunting effort. All this will be reflected in a reduced growth of the larvae, for an increased *Artemia* cyst bill (as about 20-30% more cysts need to be hatched to feed the same weight of starved metanauplii to the predator).

Moulting of the *Artemia* nauplii to the second instar stage may be avoided and their energy metabolism greatly reduced by storage of the freshly hatched nauplii at a temperature below 10°C at densities of up to 8 million nauplii/litre, for periods up to 24 hours.

Table 1: Intra-strain variability of 20:5(n-3) fatty acid content in *Artemia*. Data represents the range (area percent) and coefficient of variation of data as compiled by Léger *et al.* (1986 and 1987a)

Artemia geographical strain	20:5(n-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-Tientsin	1.3-15.4	50.5

Low level aeration is needed in order to prevent the nauplii from accumulating at the bottom of the tank where they would suffocate. Applying 24-hour cold storage (using styrofoam insulated tanks and blue ice packs for cooling), commercial hatcheries are able to economise on their *Artemia* cyst hatching efforts (*e.g.* fewer tanks, bigger volumes, maximum one hatching and harvest per day). Cold storage, furthermore, allows the operator not only to ensure the availability of a better quality product but at the same time to consider more frequent food distribution (feeding). This appears to be very beneficial for fish and shrimp larvae as food retention times in the larviculture tanks can be reduced and hence growth of the *Artemia* in the culture tank can be minimised. For example, when applying one or maximum two feedings per day, shrimp hatchery operators often noticed ongrowing *Artemia* in their larviculture tanks. With poor hunters such as the larvae of turbot *Scophthalmus maximus* and tiger shrimp *Penaeus monodon*, feeding cold-stored *Artemia* results in much more efficient food uptake.

Easy hatching and disinfection procedures, however, are not the only parameters in ensuring the success of using *Artemia* as a larval food source. Several other *Artemia* characteristics can influence the suitability of a particular brine shrimp product for different types of larviculture. One of these is nauplius size which can vary greatly from one geographical source of *Artemia* to another.

Another important dietary characteristic of *Artemia* nauplii when use as food for marine fish/shrimp larvae appears to be their essential fatty acid content, especially EPA content. As can be seen in Table 1, EPA levels in *Artemia* can vary greatly, even from one batch to another within the same strain. Cyst products from inland sources appear to be more constant in composition, even though it may be at suboptimal levels. As a result concentrations of the (n-3) HUFA EPA need to be taken into consideration when selecting the most appropriate batch of *Artemia* cysts. Commercial availability of *Artemia* cysts containing high EPA-levels is limited. Their use, however, should be restricted to the feeding period when size of the prey is most critical. Indeed even the best natural *Artemia* products do not meet all the nutritional requirements of the predator larvae, most particularly with regard to the other essential fatty acid for marine organisms, namely DHA 22:6(n-3), which is never available in significant amounts in *Artemia* cysts (Léger *et al.*, 1986).

It is fortunate that *Artemia*, because of its primitive characteristics, facilitates a very convenient way to manipulate its biochemical composition. Since *Artemia* is non-selective in taking up particulate matter once it has moulted into the second larval stage, *i.e.* about 8 hours following hatching, simple methods can be developed to incorporate any kind of product into the *Artemia* prior to offering it as a prey to the predator larva. This method of bioencapsulation, also called *Artemia* enrichment or boosting, is widely applied at marine fish and crustacean hatcheries all over the world for enhancing the nutritional value of *Artemia* with essential fatty acids. Various enrichment products and procedures have been developed using selected microalgae, micro-encapsulated products, yeasts and self-emulsifying concentrates (see review of Léger *et al.*, 1986). Highest enrichment levels of 15 mg/g EPA and 72 mg/g DHA after 24 hr enrichment are obtained when using emulsified concentrates.

Freshly hatched nauplii are transferred to the enrichment tank at a density of 100 (for enrichment periods of ≥24 hr) to 300 nauplii/ml (maximum 24 hr enrichment period). The enrichment medium consists of hypochlorite-disinfected and neutralised seawater that is maintained at 25°C. The enrichment emulsion is added at consecutive doses of 300 ppm every 12 hrs. Strong aeration using airstones 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 in order to assure that HUFAs are not metabolised during storage. 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). Furthermore, the size increase occurring after hatching during enrichment will be minimal. *Artemia* enriched by other methods reach > 900 µm, whereas by this method,

higher enrichment levels are obtained in nauplii measuring 660 µm (12-hr enrichment) to 790 µm (48-hr enrichment).

Several European marine fish hatcheries apply a feeding regime, whereby they switch from one *Artemia* diet to another as the fish larvae are able to accept the bigger prey, *i.e.* at the start freshly-hatched nauplii of a selected *Artemia* strain and batch that yields small nauplii with high content of EPA (10 mg/g DW) is fed for a few days, followed by 12-hr and eventually 24-hr (n-3)HUFA emulsion-enriched (Great Salt Lake, Utah) *Artemia* metanauplii.

As with *Brachionus*, very high levels of ascorbic acid (AA) can be incorporated into the brine shrimp nauplii; supplementation of the enrichment emulsion with 20% AP (w/w) increases the AA content to 2000 ppm after 24 hr enrichment. These concentrations do not drop when the 24 hr-enriched nauplii are stored for 24 hours in seawater of 28°C and 4°C. Feeding tests using three enrichment levels in the live food (0%, 10% and 20% AP) for larval *Macrobrachium rosenbergii* (Merchie *et al.*, 1993) show that the vitamin C source, offered through the enriched *Artemia* nauplii, is incorporated as AA in the predator larvae at high concentrations (>500 ppm). If culture conditions are optimal, there is no effect on growth or survival; however, a significantly positive effect of vitamin C-enriched live food on the physiological condition is demonstrated. Moreover, a significant drop in AA-concentration is detected in the postlarvae as compared to the levels found in the larvae. This may reflect a need for vitamin C during metamorphosis, a stress sensitive period. It is expected that under suboptimal conditions (*e.g.*, stress situations), supplementation of high vitamin C levels might also enhance production characteristics.

Artemia can also be used as a vector for oral delivery of antibiotics ingested from an emulsified or particulate preparation. Doses ranging from 20-100 ppm sulphadrugs can be incorporated in seabass and turbot larval tissue within less than 4 hr after feeding *Artemia* metanauplii boosted with the antibiotics. The therapeutic efficacy of this oral delivery system has been documented by Chair *et al.* (1993) and Dhert *et al.* (1993) with turbot larvae challenged with a *Vibrio anguillarum* pathogen.

As was proven in larviculture trials with mahimahi, *Coryphaena hippurus* in Australia (Nell, pers. comm.) and in the USA (Smith, pers. comm.) and in different commercial farms of Asian seabass *Lates calcarifer* in Thailand (Wongrat, pers. comm.) and shrimp *Penaeus chinensis* in PR China (Tackaert and Sorgeloos, 1990), juvenile and adult *Artemia*, on-grown in mass-production units (Lavens and Sorgeloos, 1991; Dhont *et al.*, 1991) and produced and/or collected from local saltworks (Tackaert and Sorgeloos, 1991) are an excellent nursing and weaning diet, eventually reducing larval mortalities and, especially, cannibalism.

ZOOPLANKTON

Wild zooplankton

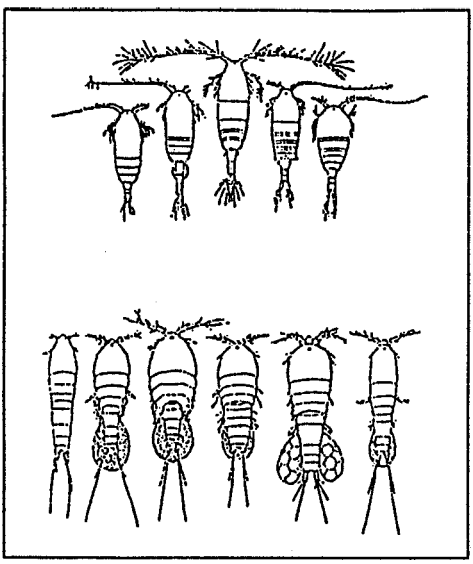
In nature fish and crustacean larvae feed on a broad spectrum of zoo- and phytoplankton, providing the larvae a complete and balanced diet. The heterogenous size distribution of wild zooplankton makes it suitable for all target species. Some hatcheries that are located close to the sea, rivers or large water bodies can make use of this natural food source. The harvested wild zooplankton can be fed to the cultured species as a sole food source, or as a supplement. The poor survival and storage possibilities, however, considerably restrict their use as a fresh diet. A possible solution to this problem is deep freezing the food.

Besides the risk of bringing in parasites to the hatchery system (*e.g.* fish flea, *Argulus foliaceus* for fresh water, *Livoneca* sp. for marine water; and parasitic copepods, *Lernaea* sp. for fresh water, *Lernaeascus* sp. for marine water) the use of natural zooplankton is also restricted by its availability. The quality, as well as the quantity varies between sites and seasons, but the main composition of the plankton stays relatively the same, namely rotifers, copepods and larger rotifers for sea water (*Artemia* for salinas) and rotifers, *Daphnia* and Ostracods for fresh water. The quantity is directly related to the density of plankton at the site. For the Mediterranean and the Atlantic coasts of France, densities of copepods (85 % of the zooplankton) ranges from 500/m³ (winter) to 10,000/m³ (spring and early summer), with an average of 1,000/m³ for the littoral zone. For lagoons and estuaries, this figure can be higher due to the high organic load in the water. Just for the production of a 30-day old seabass approximately 3,000 live prey are needed; for this, 3,000 litres of seawater need to be filtered. For the production of 1 million fry a pumping capacity 1.16 m³/second will be necessary (Barnabé, 1990).

Production of copepods

In nature harpacticoid and calanoid copepods (Figure 5) are the main food source for marine organisms and are used as a starting feed in fish larviculture. They are very rich in free amino acids, except for methionine and histidine. From a nutritional point of view they are far superior to *Artemia* nauplii; their lower proteolytic activity and better fatty acid composition (copepods contain up to three times more DHA than *Artemia* enriched with Super SELCO) makes them an excellent food with a high energy content. It is thus not surprising that their use in aquaculture has often resulted in better growth, survival, development and pigmentation of the fish.

Figure 5: Morphological differences between Calanoida and Harpacticoida



Although copepods have excellent properties their application is still restricted by difficulties in their propagation by mass culture. Harpacticoid copepods are in general easier to culture under intensive conditions than calanoid copepods. They are more tolerant of extreme changes in environmental conditions (salinity tolerance, 15-70 ppt and temperature, 17-30°C), have a higher productivity and feed on a wide variety of food items such as microalgae, bacteria, detritus or even artificial diets. Recently, some laboratories have succeeded in culturing several species of copepods, *e.g Acartia* tonsa (Danish Institute for Marine Fisheries and Research); *Eurytemora* (Kuhlmann *et al.*, 1981); *Euterpina acutifrons* (Kraul, 1989) (Figure 6).

CLADOCERA

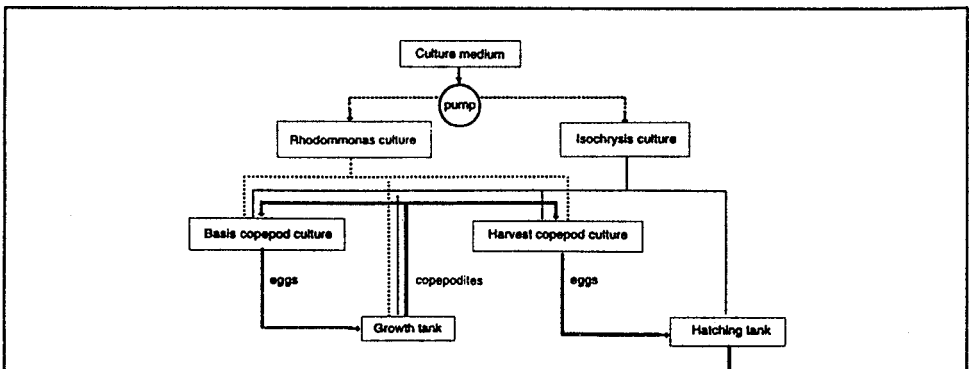
Daphnia

Daphnia is an important food source in the freshwater aquaculture and ornamental fish industry. They can also be used in mariculture as replacement live feed for *Artemia*. The animals are often used in waste water treatment studies and in ecotoxicology.

The nutritional value of the *Daphnia* is highly dependant on the chemical composition of their food source. For example, *Daphnia* cultures tend to collapse after 10-30 generations, if they are fed solely with *Chlorella*, *Chlamydomonas*, mixtures of yeast and *Scenedesmus* or *Scenedesmus* and *Chlamydomonas* (Peters, 1987). This can be overcome by adding vitamin mixtures and trace elements to the culture medium.

In a study on the food quality of freshwater phytoplankton for the production of cladoceran zooplankton, it was found that from the spectrum of blue-green, flagellate and green algae, *Daphnia* performed best on a diet of the cryptomonads, *Rhodomonas minuta* and *Cryptomonas* sp. Only these algae contained long-chain polyunsaturated fatty acids with carbon 20 and 22. More than 50 % of the fatty acids in these two algae consisted of EPA and DHA, while the green algae were characterised by more

Figure 6: Diagram of the algal and *Acartia tonsa* production system



18:3w3 fatty acids. This means that the long-chained polyunsaturated fatty acids are also very important for normal growth and reproduction of *Daphnia* (Ahlgren *et al.*, 1990).

Daphnia contains a broad spectrum of digestive enzymes, such as proteinases, peptidases, amylase, lipase and even cellulase, that can serve as exoenzymes in the gut of the fish (Lampert, 1987).

Moina

Moina also thrives in ponds and reservoirs but are primarily inhabitants of temporary ponds or ditches. *Moina* are smaller in size than *Daphnia* and contain 70 % more protein. *Moina* is often used as an *Artemia* replacement feed in the culture of rainbow trout, salmon, striped bass, *Lates calcarifer*, *Macrobrachium* (Alam *et al.*, 1993) and for tropical ornamental fish production.

The (n-3) HUFA composition of *Moina* varies with the culture medium, but it can be upgraded nutritionally by employing emulsified lipids (Watanabe *et al.*, 1983; Watanabe and Kiron, 1994). *Moina* shows clear sexual dimorphic characteristics, so males (0.6-0.9 mm) are smaller than females (1.0-1.5 mm). Also, the males have long graspers for holding the female during copulation.

NEMATODES

Many free-living nematodes can be cultivated very cheaply under laboratory conditions, especially the soil inhabiting species *Panagrellus revivus* and *Caenorhabditis elegans*. Both these species can be cultured on a medium of breakfast cereal, soy peptone, yeast extract and liver extract mixed with sterile water. The nematodes have a short generation time (life-cycle), ranging from 5-7 days and a high level of reproduction. The experimental use of the free living nematode *Panagrellus revivus* as larval food has been successful for *Crangon crangon*, juvenile king prawn (*Penaeus plebejus*), carp (*Cyprinus carpio*) and silver carp (*Hypophthalmichthys molitrix*).

Nematodes, such as *P. revivus* can be used for bioencapsulation. Experiments done with the antibacterial *Romet-30* have produced good results. Enrichment is simply done by adding the product to the culture medium (direct enrichment) or by placing the nematodes in an emulsion of the product (secondary enrichment). In the case of *Romet-30* enrichment, the nematodes are put in one litre beakers with 500 ml fresh artificial seawater and 5 g of *Romet-30* premix (3,000 ppm of drug). *Romet* premix contains 30 % active ingredient (25% sulfadimethoxine and 5% ormetoprim) and 70% rice bran carrier. After a 4 hr boost period, after which the nematodes have accumulated 0.25 ìg of the drug/ind. (0.1 ìg/individual for *Artemia* nauplii), the nematodes are separated from the antibiotic carrier (rice bran) by resuspending them in seawater and centrifuging at 1500 rpm for 10 min. After a 10-20 min rest the animals would have migrated to the top of the tube, where they can be collected by pipetting onto a 100 ìm mesh screen. After rinsing with seawater, the nematodes can then be fed to the larval penaeid shrimp.

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