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THE USE AND NUTRITIONAL VALUE OF *ARTEMIA* AS A FOOD SOURCE*

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INTRODUCTION

Successful rearing of larval stages of aquatic organisms is a challenge for aquarists, an aim and tool for aquatic ecologists and ecotoxicologists, and the determinant for the commercial success of the aquaculturist.

The primary problem in larval culturing is that of food (May, 1970; Houde, 1973; Barnabé, 1976; Girin & Person-Le Ruyet, 1977; Goodwin & Hanson, 1977). Ideally, one would feed fish and crustacean larvae with their natural diet characterized by a wide diversity of live organisms. Collecting and feeding natural plankton from rivers, lakes and seas may appear evident but already at the beginning of this century this method was designated as hardly dependable beyond aquarium scale (Fabre-Domergue & Bietrix, 1905). On a larger and industrial scale, similarly to intensive cattle and poultry farming where a reliably high culture performance is the objective, a readily available diet has to be selected which is easily accepted and digested and having a reproducibly high nutritional quality. An extensive list of potential organisms may meet the requirements of acceptability, digestibility, and (reproducibly high) nutritional quality. When it comes to availability, however, only a few organisms are left as possible candidates. The provision of adequate numbers of food organisms has been called a "sine qua non" for any rearing attempt (May, 1970) and "the main obstacle" (Barnabé, 1976) or "limiting factor" (Girin & Person-Le Ruyet, 1977) for a successful aquaculture. The provision of adequate numbers of food organisms appropriate to larval rearing has, moreover,

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been quoted as the "only criterion for the success of a larval production system" (Paulsen, 1980).

The property of the small branchiopod crustacean *Artemia** (Fig. 1) of forming dormant eggs, so-called "cysts", may be the reason why it has, to a great extent, been designated a convenient, suitable and excellent larval food source. These cysts are available year-round in large quantities along the shorelines of hypersaline lakes, coastal lagoons, and solar saltworks scattered over the five continents (Persoone & Sorgeloos, 1980; Vanhaecke, 1983; Vanhaecke, Tackaert & Sorgeloos, 1985). After harvesting and processing the cysts are available as storable 'off the shelf' 'on demand' life food. Indeed, upon some 24-hours incubation in sea water the cysts release free-swimming nauplii that can be given directly as a nutritious, live source of food to the larvae of a variety of aquatic organisms.

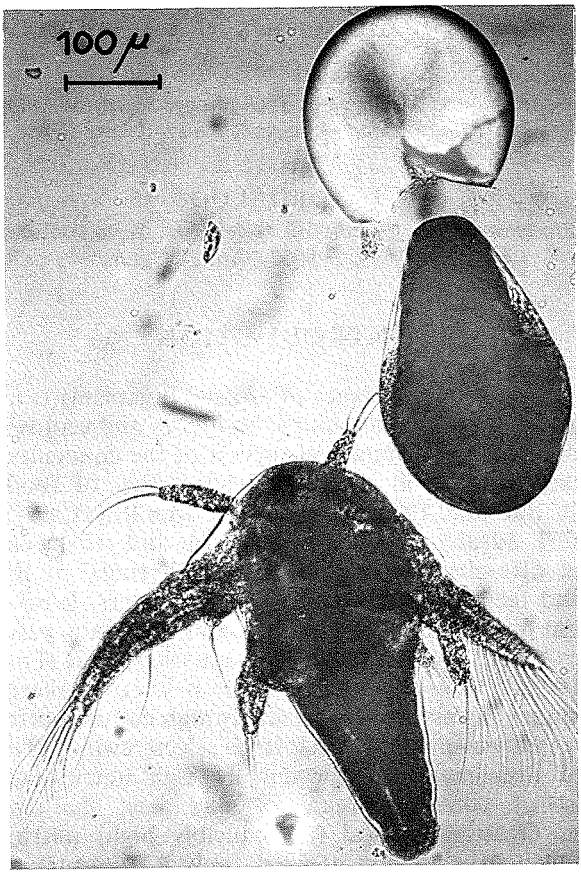

Fig. 1.—*Artemia* prenauplius shortly after breaking of a cyst and a freshly hatched instar I nauplius.

**Artemia* was first described by Schlösser in 1755 and later by Linnaeus in 1758 (Kuenen & Baas-Becking, 1938) under the binomen *Artemia salina*. Because crossing experiments of different *Artemia* populations revealed reproductive isolation of several groups of populations, it is suggested that until speciation in brine shrimp is more clearly understood, only the genus designation *Artemia* should be used (Persoone, Sorgeloos, Roels & Jaspers, 1980).

It is not the intention of the present article to compile all existing records of experiments using *Artemia* as a food source for this and that organism. We will rather go through the different applications of *Artemia*, the different forms of *Artemia* that are being used, the factors determining its nutritional value, its biochemical and chemical composition and, not least, the problems and constraints related to its use as a source of food. A better understanding of the nutritional value and constraints of *Artemia* as a food will, in the first place, lead to an optimized and more dependable culture performance and may ultimately constitute a more comprehensive basis for making it redundant through the formulation of artificial diets of equal merit.

ARTEMIA NAUPLII AND METANAUPLII

ARTEMIA NAUPLII AS A LIVE FOOD SOURCE

Artemia was described in the 18th century and has been extensively studied in the most diverse fundamental disciplines of biological sciences since the 19th century (Sorgeloos, 1980a). Its value as a suitable food organism was discovered only recently. Since Seale (1933), Gross (1937), and Rollefsen (1939) found that freshly hatched *Artemia* nauplii constituted an excellent food source for newborn fish larvae, its application in larval culture has been rampant.

The most diversified groups of organisms of the animal kingdom, *e.g.* foraminifers, coelenterates, flatworms, polychaetes, cnidarians, squids, insects, chaetognaths, fish, and crustaceans have been offered *Artemia* nauplii as a suitable food source (May, 1970; Kinne, 1977; Sorgeloos, 1980c). Kinne (1977) indeed stated that more than 85% of the marine animals cultivated so far have been offered *Artemia* as food source—either together with other foods or, more often, as a sole diet.

The ease with which *Artemia* nauplii are obtained from dry storable cysts has convinced most people involved with larval rearing, *i.e.* aquarists, aquatic ecologists and ecotoxicologists, and aquaculturists. In a digest for aquarists, Rakowicz (1972) stated that all aquarium fishes eat the slow-swimming baby brine shrimp and that those fishes show vigorous growth, excellent survival and best resistance to diseases. When comparing with alternative organisms, including those collected from wild sources or cultured at home, he concluded that brine shrimp nauplii emerge as one of the best of all live foods for most aquarium fishes.

In the cultivation of laboratory animals for scientific and applied purposes nearly all rearing attempts have employed *Artemia* nauplii (May, 1971). This is further confirmed by Kinne (1977), who noted that most investigators engaged in laboratory fish cultivation use *Artemia* nauplii, which in numerous instances proved to be a good food. Most workers culturing decapod larvae have also fed *Artemia* nauplii as a standard laboratory diet (Forster & Wickins, 1967; Provenzano, 1967; Roberts, 1972, 1974; Mootz & Epifanio, 1974; Provenzano & Goy, 1976). These authors cite the following advantages of using *Artemia*: its availability regardless of season, its suitable size for many decapod larvae and the fact that it allows complete development of the juvenile stage or beyond with reasonably consistent survival, intermoult duration and morphogenetic sequence.

Its success as a larval diet for laboratory animals was soon recognized widely among aquaculturists. Carlberg & Van Olst (1976) indeed designate *Artemia* nauplii among the most suitable food items for the controlled culture of larval stages of many commercial fish and shellfish. Girin & Person-Le Ruyet (1977) furthermore remark that 40 years after the first trials with *Artemia* as a food for fish larvae, its freshly hatched nauplii have now become an indispensable link in the larval rearing of most fish and marine crustacean species. More recently, Corbin, Fujimoto & Iwai (1983) agree that in aquaculture production around the world, *Artemia* nauplii are the principal food during the first weeks of larval rearing. Since Hudinaga in 1958 for the first time successfully reared *Penaeus japonicus* using *Artemia* nauplii during mysis and postlarval stages (Liao, Su & Lin, 1983), all commercial cultivation of penaeid shrimp species is at present using this practice (see comprehensive articles by Heinen, 1976; Hanson & Goodwin, 1977; Liao *et al.*, 1983). The culture of the freshwater prawn *Macrobrachium* sp. also heavily depends on the use of *Artemia* nauplii; the nauplii are used as the most successful diet throughout the larval rearing period, after one week mostly in combination with prepared diets (White & Stickney, 1973; Dugan, Hagood & Frakes, 1975; Aquacop, 1977; Hanson & Goodwin, 1977; Murai & Andrews, 1978; Corbin *et al.*, 1983).

Although it is common practice to feed adult *Artemia* to lobster larvae, Castell (1977) noticed better survival, colouration, activity and slightly better growth in *Homarus americanus* larvae raised with *Artemia* nauplii. Other decapod species with aquaculture potential such as spiny lobster (Dexter, 1972; Robertson, in Bardach, Ryther & McLarney, 1972; Roberts, 1974; Tholasilingam & Rangarajan, 1980) and *Palaemonetes* spp. (Broad, 1957; Forster & Wickins, 1967; Reeve, 1969a,b; Campillo, 1975; Sandifer & Williams, 1980; Anonymous, 1984) are also successfully cultured using *Artemia* nauplii.

Intensive larval rearing of commercial non-salmonid fish relies almost completely on the use of living food organisms despite considerable effort to develop artificial diets (Bryant & Matty, 1980; Paulsen, 1980). Nauplii of *Artemia* have most often been used as a convenient food for the larvae of cyprinids (Meske, 1973; Huisman, 1974; Bryant & Matty, 1980; Stroband & Dabrowski, 1981; Dabrowski, 1982), milkfish (Juario & Duray, 1981), flatfishes (Riley, 1966; Shelbourne, 1968; Girin, 1974a,b, 1979; Spectorova & Doroshev, 1976; Bromley, 1977; Gatesoupe, Girin & Luquet, 1977; Kingwell, Duggan & Dye, 1977; Dye, 1980; Fuchs, 1981/1982; Gatesoupe & Luquet 1981/1982; Bromley & Howell, 1983, Olesen & Minck, 1983), bass (Girin, Barahona-Fernandes & Le Roux, 1975; Barnabé, 1976, 1980; Barahona-Fernandes & Girin, 1977; Anonymous, 1978b), bream (Kittaka, 1977; Person-Le Ruyet & Verillaud, 1980), whitefish (Günkel, 1979; Flüchter, 1980, 1982), catfish (Hogendoorn, 1980), rabbitfish (Juario *et al.*, 1985), and sturgeons (Gun'ko, 1962; Gunk'ko & Pleskachevskaya, 1962; Azari Takami, 1976, 1985; Oleinikova & Pleskachevskaya, 1979; Binkowski & Czeskleba, 1980).

THE USE OF PREPARED FORMS OF *ARTEMIA* NAUPLII

In most cases live freshly hatched nauplii are used as a food for immediate use. Several authors, however, report experiments with live cold stored, killed, and other prepared forms of *Artemia* nauplii.

Live cold-stored Artemia *nauplii*

Mock, Fontaine & Revera (1980a) and Mock, Revera & Fontaine (1980b) recommend the use of chilled or frozen nauplii as a back-up to safeguard against a batch of cysts that are inferior in hatching quality. They note that freshly hatched *Artemia* nauplii can be concentrated and stored at 11 °C for several days, although careful monitoring is required to prevent mortality and decomposition. In order to minimize this risk they aerate the suspension of nauplii with an airstone and change the water every day. Léger, Vanhaecke & Sorgeloos (1983) described a technique for high density cold storage of *Artemia* nauplii. They showed that, except for the strains from Chaplin Lake (Canada) and Buenos Aires (Argentina), *Artemia* nauplii viability remains over 90% after 48 hours storage at 4 °C. Subsequent transfer to culture tank conditions (25 °C) did not affect *Artemia survival. Léger et al.* (1983) furthermore demonstrated that cold stored nauplii remained in the instar I stage (Hentschel, 1968) and that energetic losses were minimal (see also p. 587). Decreases in nutritional value of cold stored nauplii used as food for *Mysidopsis bahia* and *Cyprinus carpio* larvae are insignificant after 24-hours cold storage and minimal only for carp after 48 hours. This technique provides opportunities for automation in food distribution (Léger & Sorgeloos, 1982) and offers the possibility of frequent feedings without manual mediation over a two-day period (Fig. 2). Because the labour involved in feeding, especially in large-scale operations, is cumbersome and expensive (Fujimura & Okamoto, 1970; Goodwin & Hanson, 1977), this technique looks worth imitating, be it only to store left-overs of freshly hatched nauplii for later feeding.

Another advantage of using cold-stored nauplii is their initially slower movement from which the predator can benefit. Kahan (1979) indeed noticed that first-feeding mullet (*Mugil capito*) larvae were able to handle the slow-moving refrigerated nauplii, while other authors reported that mullet larvae could not handle *Artemia* nauplii prior to the 7th (Nash, Kuo & McConnel, 1974) or the 16th day (Liao, Lu, Huang & Lin, 1971). Sleet & Brendel (1983) have described a system for flow-through hatching and cold storage of the nauplii. They confirm that during cold storage the nauplii remain in their first larval stage, that viability is not affected even after transfer of the stored nauplii to 25 °C and that naupliar length after 48-hours cold storage only increased by 5·4% compared with 80% in the control (25 °C). It may be noticed that while Sleet & Brendel obtained good results with Canadian (Chaplin Lake) *Artemia*, Léger *et al.* (1983) reported poor storage performance for this strain as compared with others.

Frozen and freeze-dried nauplii

The use of killed forms of *Artemia* nauplii eliminates the drawback that the *Artemia* may compete for food with the predator larvae. Mock *et al.*

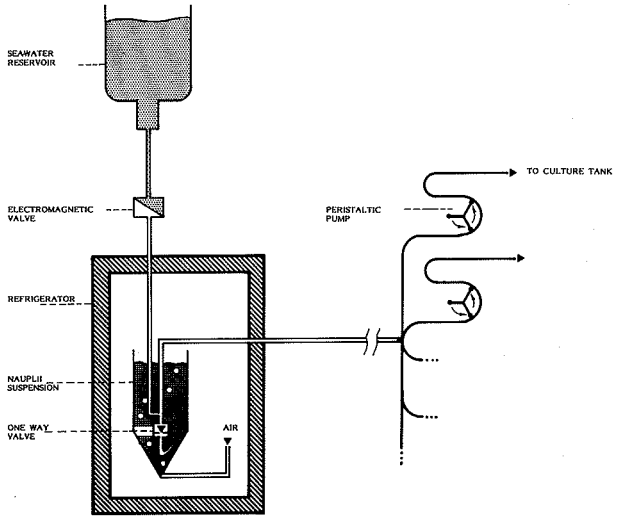

Fig. 2.—Schematic diagram of automatic distribution system for *Artemia* nauplii (modified from Léger & Sorgeloos, 1982).

(1980a,b) observed that *Artemia* nauplii very rapidly consume the algae which are still being fed to the penaeid shrimp larvae when the *Artemia* are first added. This usually results in the on-growing of the *Artemia* to such an extent that, because of their size and swimming speed, they are no longer ingestible by the shrimp larvae which, after all, are not very efficient hunters. To avoid this, Mock *et al.* fed frozen *Artemia* nauplii to zoeal shrimp larvae, *i.e.* a determined amount of *Artemia* was hatched, concentrated and stored after freezing. The frozen block could then either be thawed in sea water before feeding, or the frozen block could be placed directly in the culture tank. According to Mock *et al.* (1980a,b) penaeid shrimp larvae accept frozen nauplii equally well as live *Artemia*. The use of frozen *Artemia* provides, as Mock *et al.* state, a lot of advantages, *e.g.* it ensures a constant food supply, daily food requirements of the predator can be met with higher precision, no more fear that the *Artemia* grow into an unwanted food competitor.

In larval fish rearing frozen *Artemia* nauplii are being used, in the transition of live to artificial diets, aiming to facilitate the acceptance of non-living food. This practice has been described for seabass (*Dicentrarchus labrax*) (Anonymous, 1978b), and sole (*Solea* spp.) larvae (Girin, 1979; Metailler, Menu & Morinière, 1981; Cadena Roa, Huelvan, Le Borgne & Metailler, 1982a; Cadena Roa, Menu, Metailler & Person-Le Ruyet, 1982b; Gatesoupe & Luquet, 1981/1982). Gatesoupe & Luquet also used frozen nauplii as an attractant in re-hydratable extruded pellets.

In his experiments with whitefish (*Coregonus fera*) Günkel (1979) observed that the fry accepted dead nauplii, equally well as live *Artemia*, resulting in similar survival and growth. From these results he assumed that fry could be reared with dry diets. This appeared to be true if they were first fed *Artemia* nauplii and if proper weaning was allowed. Hogendoorn (1980)

reported good results in rearing catfish (*Clarias lazera*) larvae using live or frozen *Artemia* nauplii in combination with a trout starter compared with other diets without *Artemia*. He, nevertheless, noticed significantly better growth and survival in the treatment including live nauplii. Fuchs (1981/1982), aiming to simplify the rearing methods for larval sole of Girin (1978), also compared live *versus* frozen *Artemia* nauplii as a food source. Fuchs also concluded that better survival, growth, and food conversion are obtained with live nauplii (Fig. 3). Similarly, Schauer, Richardson & Simpson (1979) and Seidel, Schauer, Katayama & Simpson (1980a) found largely better results feeding juvenile Atlantic silverside (*Menidia menidia*) with live instead of freeze-dried *Artemia* metanauplii. It was postulated by the last authors that something in the *Artemia* was lost or destroyed during the freeze-drying process.

Kentouri (1980) observed that seabass larvae, offered frozen prey which has been thawed for different times, only ingest the most freshly thawed product. He supposed that possible denaturation of vitamins and proteins, or lipid oxidation eventually aggravated by thawing procedures and especially thawing duration may explain inferior results obtained with a diet of frozen food organisms. Following Flüchter (1980) whitefish larvae metamorphose equally well whether they are fed live or shock-frozen (−196 °C) *Artemia* nauplii, but not when fed slow-frozen nauplii. The fish

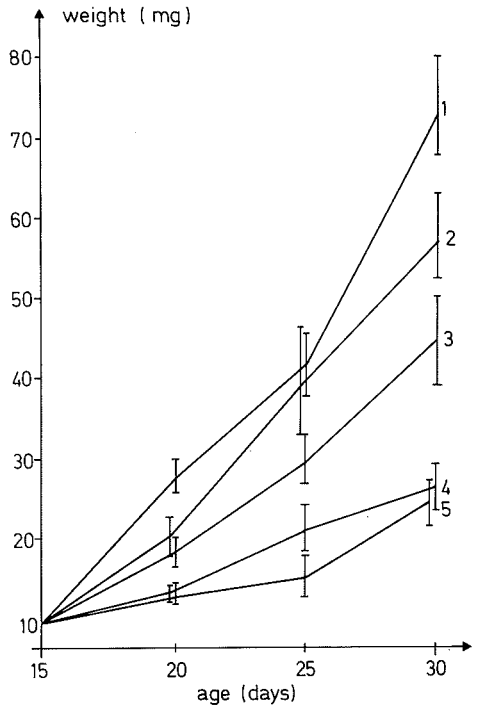

Fig. 3.—Growth of *Solea solea* juveniles from Day 15 to 30 fed different *Artemia* preparations: 1, live nauplii; 2, live plus frozen nauplii; 3, frozen nauplii (4 feeds); 4, frozen nauplii (distributed in 24 h); 5, frozen nauplii (distributed in 15 h); after Fuchs, 1981/1982.

larvae, however, eagerly took the slow-frozen *Artemia* from the bottom of the aquaria and even preferred them to live copepods abundantly present in the aquaria. Flüchter ascribed this feeding response to a strong smell or taste released by dead *Artemia* nauplii and concluded that a substance essential for whitefish larvae is lost during slow-freezing and not during shock-freezing. He assumed this substance to be largely insoluble in water, since during shock-freezing the expansion of the water in the body tissue causes the nauplii to burst. Furthermore, Flüchter postulated that this substance must be connected to the intermediate metabolism and absorbed through enzymatic action which does not stop immediately during slow freezing. Grabner, Wieser & Lakner (1981/1982) indeed proved that activities of proteases as well as enzymes of the intermediate metabolism in food organisms (including *Artemia*) are not diminished by freezing, freeze-drying and by storage at −18 °C even for very long times. He noticed also that during the process of freezing or freeze-drying tissue cells of food organisms experience large scale damage explaining extensive leaching upon thawing, *i.e.* after 10 min at 9 °C about 70–75% of the activities of proteases and of LDH, and an even larger percentage of the free amino acids have disappeared from the food material and can be recovered in soluble form in the water. Following Grabner *et al.* (1981/1982), losses of essential nutrients during thawing are probably the most important reason why frozen food organisms have proved to be unsuitable for rearing the larvae of several fish species.

Other forms of non-living Artemia *nauplii*

In order to prevent food competition with algae, deterioration of water quality as when using frozen *Artemia*, and metabolism of the energy reserves as in live *Artemia*, Wilkenfeld, Lawrence & Kuban (1984) fed *Penaeus setiferus* larvae with UV-killed *Artemia* nauplii as an inactive food. UV-killed nauplii were obtained by exposing freshly hatched *Artemia* nauplii to four 30W germicidal tubes at 10 $mW \cdot cm^{-1} \cdot s^{-1}$ for one hour. Although they noted clumping of UV-killed *Artemia* and algae, they suggest their potential use as a food source during larval stages of penaeid shrimp. Further experimentation, however, is required to confirm their nutritional stability and possible effects on water quality.

When live *Artemia* nauplii were compared with preserved *Artemia* (dried, stored in brine or as a paste) as food for young sturgeons (*Acipenser stellatus*), the superiority of live *Artemia* was striking (Gun'ko & Pleskachevskaya, 1962; Pleskachevskaya, 1963, in Oleinikova & Pleskachevskaya, 1979), *e.g.* final sturgeon weight was 1141% of initial weight after 35 days when fed on live *Artemia* and only 75% when fed on dried *Artemia*; the weight increase was 764·8% and 53·5%, respectively. It was only 28·1% in larvae fed brined- and 22·5% in larvae fed pasted-*Artemia*.

FACTORS AFFECTING THE SUITABILITY AND NUTRITIONAL EFFECTIVENESS OF *ARTEMIA* NAUPLII

Although *Artemia* nauplii have been and are being used as a suitable food in the culture of numerous aquatic species, problems and constraints related to

the use of *Artemia* have been reported by several authors. Besides an undesirable variation in hatching quality (Vanhaecke & Sorgeloos, 1983a) which will not be treated in this article, problems related to unreliable supply and high price, and especially the evidence of a varying nutritional quality have generated intensive research in looking for alternatives for *Artemia*. In this section we shall review and comment on factors affecting the suitability and the nutritional effectiveness of *Artemia* nauplii as a food source; *e.g.* the presence of cyst shells, microbial contamination, nauplius size, effect of feeding starved nauplii, differences in nutritional value of nauplii from different geographical origins.

The presence of cyst shells

Artemia nauplii harvested from the hatching suspension are often contaminated with empty cyst shells (for details on separation problems we refer to Sorgeloos *et al.*, 1983). Although these shells are undigestible (Stults, 1974; Bruggeman, Sorgeloos & Vanhaecke, 1980; MacDonald, 1980), they may be harmful when ingested by larvae. Herald & Rakowicz (1951) indeed observed young seahorses dying through obstruction of their gut by cyst shells. Morris (1956) noticed starvation effects in fish larvae which ingest shells as readily as nauplii and recommended that the nauplii be separated. Shrimp larvae apparently are not affected by the cyst shells as they are often introduced along with the nauplii in some outdoor operations (Heinen, 1976) or as cysts are sometimes incubated for hatching in the culture tank (Mock, pers. comm.). Even when no direct biological effect is seen, this practice is not advised for reasons of water quality. Dissolved hatching products, *e.g.* glycerol (Clegg, 1964) and contaminants carried by the cysts (see below) may indeed affect tank hygiene (MacFarlane, 1969). Several apparatus have been described for separating freshly hatched nauplii from their cyst-shells (Shelbourne, Riley & Thacker, 1963; Riley, 1966; Lenhoff & Brown, 1970; Jones, 1972; Persoone & Sorgeloos, 1972; Nash, 1973; Boyd, 1974; Ward, 1974; Smith *et al.*, 1978). Dissolved wastes and bacteria may be removed by simple washing (Austin & Allen, 1981/1982). The technique of decapsulation of *Artemia* cysts (Sorgeloos *et al.*, 1977, 1983; Bruggeman, Baeza-Mesa, Bossuyt & Sorgeloos, 1979; Bruggeman *et al.*, 1980) makes separation redundant and sterilizes the embryos at the same time.

Microbial contamination

Rakowicz (1972) preferred *Artemia* to natural plankton because the former are free from contagious diseases and parasites. Flüchter (1980) reported a reduced danger for disease introduction by feeding *Artemia* instead of natural zooplankton for coregonid and sturgeon larvae. So far no direct evidence for *Artemia*-borne infections in fish and crustacean larvae has been reported. Nonetheless *Artemia* cyst-shells are known to be contaminated with bacterial and fungal spores (Fig. 4; Wheeler, Yudin & Clark, 1979) and fish or shrimp might be infected *via* introductions with the *Artemia* hatching medium. Heavy bacterial loads have indeed been

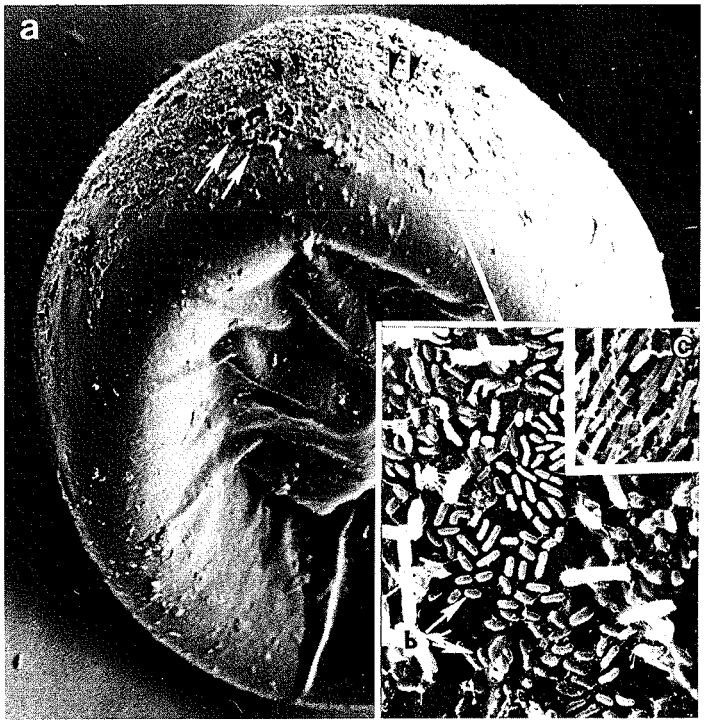

Fig. 4.—Dehydrated *Artemia* cyst covered with microbial material (arrows) a, ×412; b, ×2281; c, ×2500; after Wheeler, Yudin & Clark, 1979.

determined in canned *Artemia* cysts, *i.e.* after 20 to 48 h incubation in sterile sea water from 10^6 to 10^8 colony-forming units·ml^{-1} hatching medium have been counted by Gilmour, McCallum & Allan (1975), Coleman, Nakagawa, Nakamura & Chang (1980), and Austin & Allen (1981/1982). Austin & Allen, however, found no evidence of intimate bacterial colonization of the nauplii themselves and showed that bacteria surrounding *Artemia* nauplii may easily be removed by simple washing procedures. These authors reported the presence of *Bacillus*, *Erevinia*, *Micrococcus*, *Staphylococcus*, and *Vibrio* spp. In this regard several authors prefer to disinfect the *Artemia* cysts prior to their use. Lenhoff & Brown (1970), apprehending bacterial and fungal infections, decontaminate *Artemia* cysts using an 'Antiformin' solution (5·68 g NaOH and 3·2 g Na_2CO_3 in 100 ml of a 5·25% NaClO solution). These authors found the nauplii to be toxic when hatched from cysts disinfected with thiomersal as described by Provasoli & Shiraishi (1959). Sleet & Brendel (1983) sterilize *Artemia* cysts in sequential soakings of 1% sodium hypochlorite, 5% urea, and 13% benzalkonium chloride. After sterilization they resuspend the cysts in sterilized artificial sea water containing 10 μg·ml^{-1} gentamycin sulphate. Disinfection of cysts by hypochlorite treatment is also reported by Corbin *et al.* (1983) and by Artemia Systems (1985). An extreme form of disinfection is obained by decapsulation of the cysts, *i.e.* complete dissolution of the shell in a hypochlorite solution (Sorgeloos *et al.*, 1977,

1983). Coleman *et al.* (1980), in an attempt to increase hatchability, were successful in suppressing bacterial growth during hatching incubation using either 40 $mg \cdot l^{-1}$ veterinary grade chloramphenicol or 50 $mg \cdot l^{-1}$ research grade penicillin-streptomycin. They emphasized, however, the use of antibiotics for experimental testing only, not wishing to propagate their broad application at a production level. Using antibiotics may indeed induce selection and propagation of resistant bacteria and will increase operation costs. For use of *Artemia* on a large scale Coleman *et al.* (1980) suggest other means of suppressing bacterial growth *e.g.* UV-light, chlorination or washing. Oleinikova & Pleskachevskaya (1979) reported the development of moulds *e.g. Penicillium* spp. and *Aspergillus* spp. in unprocessed wet-stored cysts. Because the infested cysts loose their viability and infect the whole lot, the last two authors recommend the removal of mould-infested cysts (application of calcium hypochlorite or burning) and treatment of the rest with a 2% formalin solution before drying.

Nauplius size

The nutritional effectiveness of a food organism is in the first place determined by its ingestibility, and as a consequence by its size and configuration. This was clearly demonstrated by Sulkin & Epifanio (1975) who evaluated rotifers (*Brachionus plicatilis*, 45–180 μm), urchin gastrulae (*Lytechinus variegatus*, 110 μm) and *Artemia* nauplii (250 μm) as food sources for blue crab (*Callinectes sapidus*) larvae. Survival rates averaged 50, 5 and 0%, respectively, the last result being similar to that for the unfed control. They concluded that 110 μm was the maximum prey size for early larvae of the blue crab and suggested feeding rotifers during the first two zoea stages prior to a switch to *Artemia* nauplii (see also Sulkin, 1978). This confirms the observation of Roberts (1972) that *Callinectes sapidus* larvae (stages I, II and III) cannot capture nor ingest *Artemia* nauplii. The same author notes that some decapod species are indeed too small to handle *Artemia* nauplii or have mouth parts that are better suited for handling smaller food organisms. Roberts (1972) cites the example of hermit crab (*Pagurus longicarpus*) larvae which are able to capture *Artemia* nauplii but are often only removing and ingesting its appendages, leaving the body of the nauplius behind. The same observation was made for early zoea stages of *Penaeus marginatus* (Gopalakrishnan, 1976). With the further exception of all *Penaeus* spp. larvae which initially are phytoplankton filter-feeders, most decapod larvae can be reared on *Artemia* nauplii for their complete development (Rice & Williamson, 1970; Provenzano & Goy, 1976). On the contrary, most marine fish larvae cannot be fed *Artemia* nauplii at first-feeding. Morris (1956) indeed stated that the size of *Artemia* nauplii is a serious restriction to their use as food for marine fish larvae, and according to Houde (1973) most fish larvae, including those with relatively large mouths, begin feeding on organisms in the 50–100 μm range (size range of *Artemia* nauplii: 428–517 μm, Vanhaecke, 1983).

In his experiments with lemon sole (*Microstomus kitt*), Howell (1971) found that the fish larvae will first select small mussel trochophores and thereafter rotifers prior to the start of feeding on *Artemia* nauplii. In addition, Hirano & Oshima (1963) observed differences between fish species

in the age at which they start to feed on *Artemia*. May (1970) relates this difference to varying morphometry and mouth size. He does not, however, exclude the fact of size differences between strains of *Artemia*. This was effectively demonstrated by Smith (1976) in his feeding tests with bluegill (*Lepomis macrochirus*) larvae. He indeed attributed early larval mortality using freshly hatched Great Salt Lake and older San Francisco Bay *Artemia* nauplii to the size of the *Artemia* nauplii. He observed starvation effects in the larvae fed Great Salt Lake nauplii. These bluegill larvae, however, resumed feeding when they were subsequently fed small freshly-hatched San Francisco Bay nauplii. This and other experiments with both *Artemia* strains allowed Smith to conclude that San Francisco Bay nauplii are smaller than Great Salt Lake nauplii, both varieties are smaller 4 h after hatching than they are when 2 days old, and within any of these groupings there is a substantial range in size.

Size differences between different *Artemia* strains have been reported by D'Agostino (1965), Claus, Benijts & Sorgeloos (1977) and Claus, Benijts, Vandeputte & Gardner (1979) and have been studied extensively by Vanhaecke & Sorgeloos (1980). Beck, Bengtson & Howell (1980) compared the biological effectiveness of freshly hatched nauplii from five geographical strains for the larvae of the Atlantic silverside (*Menidia menidia*). They observed an increasing mortality during the first three days, parelleling the results in the starved control, in the series fed the largest *Artemia* (Margherita di Savoia, Italy). After this critical period further mortalities did not differ from the ones observed in the treatments fed the smaller nauplii. From later culturing tests with the same species, offered eight different *Artemia* strains ranging in size from about 440 to 520 μm, Beck & Bengtson (1982) extrapolated a high correlation between early larval mortality and length of *Artemia* nauplius (Fig. 5). They calculated that the use of *Artemia* nauplii bigger than 480 μm could be expected to result in over 20% mortality in *Menidia menidia* larvae.

When size of freshly hatched *Artemia* nauplii is not normally limiting for ingestion by the predator, it may become so when no adequate feeding regimes are applied (see p. 533). Because prey catching, handling, and ingestion (*e.g.* swallowing compared with biting into species) differ from

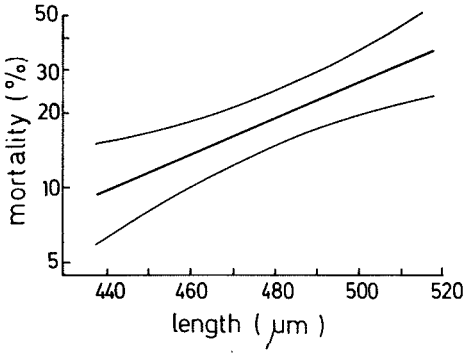

Fig. 5.—Correlation of mortality rate of *Menidia menidia* larvae and naupliar length of *Artemia* fed to the larvae: ln mortality = 15·103 + 0·0168 × length, or mortality = 0·006 × $e^{0·0168}$ × length, r^2 = 0·792; after Beck & Bengtson, 1982.

species to species, size in terms of length may not be the only criterion for morphometrical differences. Body volume of *Artemia* nauplii was considered important by Vanhaecke (1983) who noted very significant differences between strains, *e.g.* the largest difference as found between San Francisco Bay and Italian nauplii was as high as 80%.

Finally an advantage of *Artemia*, when trying to feed optimal sized prey, is that it can be reared to a larger size according to the requirements of the older predator larvae, which for energetical reasons need a larger prey (Sick & Beaty, 1974, 1975; Bryan & Madraisau, 1977). For this the use of on-grown *Artemia* looks most convenient (San Feliu, 1973; Kelly, Haseltine & Ebert, 1977; Girin, 1979; Paulsen, 1980). It was indeed found by Sick & Beaty (1974) that energy intake in *Macrobrachium rosenbergii* stage VIII is directly proportional not only to *Artemia* concentration but also to *Artemia* size. They demonstrated that, in the given experimental conditions, *Macrobrachium rosenbergii* stage VIII attained a maximum energy ingestion of 0·0066 cal·mg animal dry $wt^{-1}\cdot h^{-1}$ when fed 0·7-mm *Artemia* metanauplii, 0·062 when fed 1·5-mm *Artemia* larvae, and 1·014 when fed 5·5-mm *Artemia* juveniles.

Feeding regime

Various aspects related to feeding or 'food addition' *s.l.* appear to play an important rôle in successful shrimp- and fish-farming. The *Artemia* concentrations that are being applied will affect feeding rate, energy uptake and consequently growth, and survival of the predator. Besides, over-feeding may result in fouling stress and under-feeding in cannibalism (Gopalakrishnan, 1976) (Fig. 6). Sick & Beaty (1974) showed that *Macrobrachium rosenbergii* stage VIII larvae did not ingest *Artemia* metanauplii when fed at a concentration of $0\cdot1\cdot ml^{-1}$. Increasing this up to $2\cdot ml^{-1}$ gradually

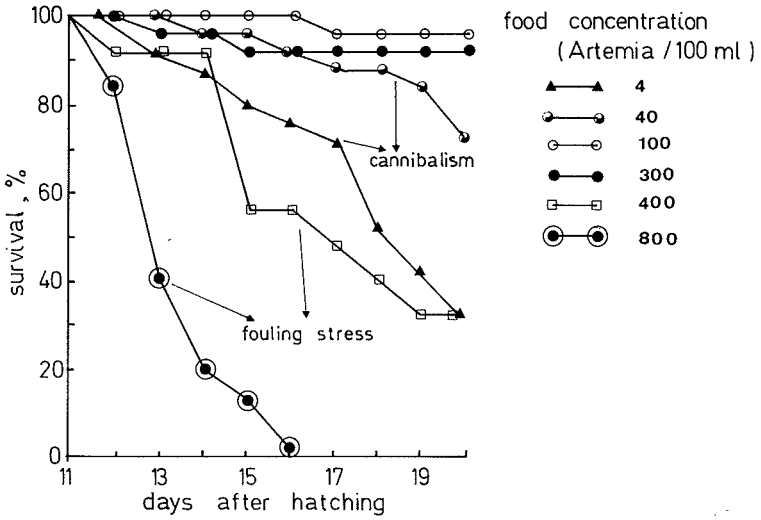

Fig. 6.—Effect of *Artemia* concentration on survival rate of *Penaeus marginatus* (after Gopalakrishnan, 1976).

improved ingestion rate and consequently energy uptake. Other authors (Reeve, 1969a,b; Mootz, 1973; Mootz & Epifanio, 1974; Vijayakumaran & Radhakrishnan, 1980) stress the importance of *Artemia* concentration on developmental rate in decapods. In this regard, Welch & Sulkin (1975) used an *Artemia* concentration of 40 nauplii $\cdot ml^{-1}$ and showed that lower levels increased developmental time; feeding 2 nauplii $\cdot ml^{-1}$ resulted in a significant delay in developmental rate.

Riley (1966) also showed that growth and survival of plaice larvae are markedly affected by the amount of nauplii available. High feeding levels are recommended for first-feeding fish larvae because of their low efficiency in prey catching (Flüchter, 1965; Rosenthal, 1969). Barahona-Fernandes & Girin (1977) agree with the low predatory efficiency in first-feeding fish larvae but advise strict limitation of daily rations of *Artemia* nauplii to match the intake capacity of the fish larvae. They observed that fish larvae eat more when more food is available, but do not grow faster; *i.e.* food conversion ratios appear to be about twice as good at the lowest feeding level as at the highest. Feeding excess food not only results in a lower feeding efficiency, it is a wasteful practice because of the cost of *Artemia* and may even be more dangerous, as a result of the accumulation of metabolites (Houde, 1975), than useful. Riley (1966) also cautioned that although higher feeding rates may increase survival in plaice larvae, excess food is detrimental due to fouling of the culture tanks. Similar observations have been reported in the culture of *Penaeus mondon* larvae (Gopalakrishnan, 1976) and of *Siganus lineatus* larvae (Bryan & Madraisau, 1977). High feeding levels were found to increase consumption in *Penaeus aztecus* mysis but this resulted in poorer survival in postlarval stages (Cook & Murphy, 1969). Roberts (1972) recommended high feeding levels (20 nauplii $\cdot ml^{-1}$) for crab larvae, but added that excessive amounts (80 $\cdot ml^{-1}$) may lead to oxygen depletion in static systems.

Another aspect in feeding practices is the progressive adjustment of the food concentration to the changing requirements of the developing larvae. It is logical to assume that the predator as it grows and develops will require more food. In this regard, Bryant & Matty (1980) have determined optimal *Artemia* rations for developing carp larvae, *i.e.* carp larvae were fed on quantified numbers of *Artemia* nauplii and growth rate was monitored for a 10-day period (Fig. 7). For optimal growth and food conversion, carp larvae were found to require 200–250% of their body weight of nauplii per day during the first 5 days of feeding and only 100–120% per day for the following 5 days. They claim that adjusting food concentrations according to changing requirements with age not only results in a faster growth of the larvae but also in considerable savings of *Artemia* cysts.

Food consumption rates also increase with progressive larval development in decapod larvae (Mootz & Epifanio, 1974), for several species of which daily consumption rates have been determined (*e.g.* Cook & Murphy, 1969; Reeve, 1969a; Omori, 1971; Uno, 1971; Zimmerman, 1973; Rodriguez, 1975; San Feliu, 1973; Shigueno, 1975; Gopalakrishnan, 1976; Heinen, 1976; Emmerson, 1977, 1980, 1984; Vijayakumaran & Rhadakrishnan, 1980; Yufera, Rodriguez & Lúbian, 1984). Differences found by these authors may reflect species specificity, experimental variability, as well as the use of different stages or strains of *Artemia* (*e.g.* varying size,

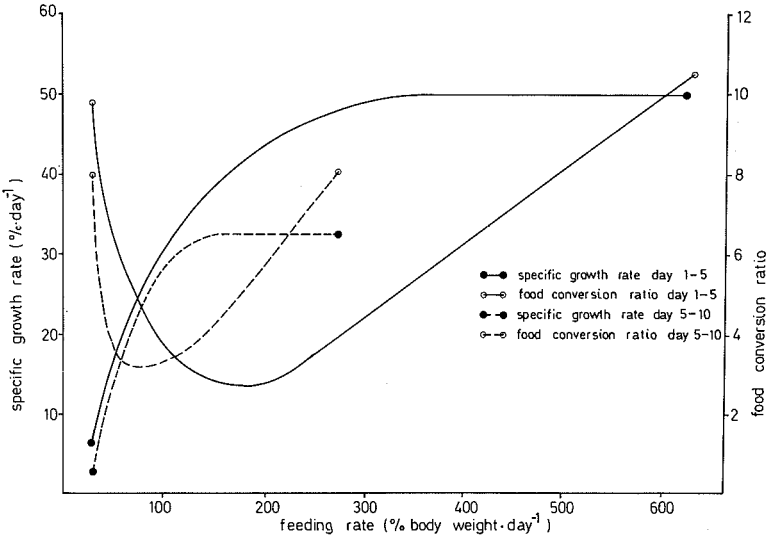

Fig. 7.—Specific growth rate and food conversion ratio of *Cyprinus carpio* larvae fed measured numbers of *Artemia* nauplii during two consecutive periods of five days each (after Bryant & Matty, 1980).

weight, energetic content, and possibly biochemical composition). Optimal feeding levels as established in laboratory studies cannot always be extrapolated to large scale cultures; *e.g.* in most experimental cases *Artemia* nauplii remaining from the previous feeding are removed daily or every other day; a practice which is inconceivable in production situations. Non-ingested *Artemia* nauplii, when not removed before moulting into the second instar stage, will start growing even when no food is available (D'Agostino, 1965; Hentschel, 1968; Sorgeloos, 1975; Smith, 1976; Claus *et al.*, 1979), swim faster (Miller *et al.*, 1979), and may reach a size which is no longer acceptable for the predator (Smith, 1976; Rollefsen in Morris, 1956). Even when acceptable, starved *Artemia* are not as nutritious as freshly hatched ones (see later). Furthermore, when food is available in the culture tank (*e.g.* algae) *Artemia* will not only grow but might also compete with the predator larvae for food and pollute the culture tank with its metabolites. This problem of the on-growing of *Artemia* is classical in penaeid shrimp farming and is aggravated when *Artemia* nauplii are fed during early protozoea stages. These stages eat little and are not very effective in catching and handling prey (Gopalakrishnan, 1976). Feeding protozoea II stage penaeids with *Artemia*, as suggested by Wilkenfeld *et al.* (1984), may indeed give better culture results on a laboratory scale; its application on a commercial scale, however, looks hardly feasible. A convenient solution to that may be the early administration of killed nauplii or decapsulated *Artemia* cysts as suggested by Mock *et al.* (1980a) and Wilkenfeld *et al.* (1984).

Instar-stage

In many cases the retention time in the culture tank of at least a part of the *Artemia* nauplii may exceed 24 h before they are ingested by the predator. This is particularly so when feeding is done *ad libitum* or when inappropriate feeding regimes are applied (see above). As a result part of the *Artemia* are in the second or third larval stage. Several scientists report storage of the freshly hatched nauplii for one or more days prior to feeding them to the predator (*e.g.* Jones, 1972; Tabb, Yang, Hirono & Heinen, 1972; Meske, 1973; Salser & Mock, 1974; L'Herroux, Metailler & Pilvin, 1977; Bengtson, Beck & Poston, 1978; Schauer *et al.*, 1979; Seidel *et al.*, 1980a; Duray & Bagarinao, 1984). Although this practice may be applied unintentionally some authors explicitly state that starvation of the *Artemia* for a few days enhances their nutritional value at least for some predators. Hauenschild (1954, 1956) indeed noticed that polyp stages of the hydrozoan *Hydractina echinata* did not do very well on a diet of freshly hatched *Artemia* but that metanauplii starved for 2 days constituted a better food for the polyps. He attributed this nutritional enhancement of the *Artemia* to a depletion of their fat reserves as a result of starvation. Werner (1968) also allowed *Artemia* nauplii to use up part of their energy-rich reserves prior to feeding them to hydrozoans.

Contrary to these observations with hydrozoans several authors have demonstrated that starved nauplii are nutritionally inferior to freshly hatched nauplii. In his experiments, Morris (1956) observed that when fish larvae were fed only older nauplii they did not grow well, although their guts were properly filled. He attributed this poorer nutritional performance of starved nauplii as food source to their reduced yolk reserves which were exhausted within 2 or 3 days. He noticed that the disappearance of the orange-red yolk was conspicuous in the nauplii even before transition to the second instar stage. Comparing newly hatched and starved nauplii he found the latter empty and chitinous and concluded that one of the primary attributes of the early nauplius, for at least some marine fishes, appears to be its yolk content. Similarly, Wickins (1976) postulated that when *Artemia* nauplii are starved, a depletion of their yolk reserves may result in qualitative or quantitative changes in their normally adequate amino-acid profile which may lead to a chronic nutritional deficiency in *Macrobrachium* larvae.

Dye (1980) and Paulsen (1980) also recommended the use of newly hatched nauplii rich in yolk reserves as food for fish larvae. Devrieze (1984) compared 24-h starved *Artemia* nauplii with newly hatched nauplii of the same strain (Macau, Brazil) as a food source for newborn carp (*Cyprinus carpio*) larvae. At the end of the first week only a slight reduction in growth was noticed in the series fed with starved mentanauplii but the difference became significant in the second week, *i.e.* 37% reduction in individual carp weight after 14 days as compared with the series fed with newly hatched nauplii. In order to satisfy their caloric requirements, the carp larvae apparently have to spend more energy in capturing enough metanauplii which in 24-h old Macau *Artemia* (25 °C) contain 32% less energy when compared with freshly hatched nauplii (Vanhaecke, 1983). This assumption confirms the earlier observations of Radhakrishnan & Vijayakumaran

(1980) that the ingestion rate of *Panulirus homarus* phyllosomae increases when fed with 2-day old instead of 1-day old *Artemia*, *i.e.* 19·3 and 15·1 nauplii $\cdot day^{-1}$, respectively. They further found that phyllosomae fed on 2-day old *Artemia* moulted to the fifth stage in 34 days while it took 31·2 days in the other case. Ablett & Richards (1980) also compared 1-day and 2-day starved *Artemia* nauplii for Dover sole (*Solea solea*) larvae. After 40 days mean length increase in fish was 10·4% higher in the 1-day old *Artemia* treatment and after 85 days this difference had grown to 16·3%. They also attributed this difference to the reduced carbohydrate and lipid levels in starved nauplii, *i.e.* even when fed *ad libitum* a greater feeding effort is required to maintain the same level of nutrition. The major reason for the reduced nutritional value of starved *Artemia* metanauplii is indeed the drastic reduction of their individual dry weight and consequently of their energy content during starvation (Paffenhöfer, 1967; Benijts, Vanvoorden & Sorgeloos, 1976; Oppenheimer & Moreira, 1980; Royan, 1980; Vanhaecke, Lavens & Sorgeloos, 1983). Von Hentig (1971) stated that from the onset of embryonic metabolism, the organic content in *Artemia* decreases until food uptake starts in the second instar stage (Hentschel, 1968; Benesch, 1969). Benijts *et al.* (1976) detected a drop in individual dry weight, organic content, energy content, total lipid and fatty acid content of, respectively, 20, 24, 27, 28 and 26% and an increase in ash content of 88% in San Francisco Bay nauplii which had moulted from the first into the second and third instar stage. Similarly, Oppenheimer & Moreira (1980) found a decrease in individual dry weight of approximately 18% in San Francisco Bay nauplii. Vanhaecke (1983) and Vanhaecke *et al.* (1983) studied decreases in individual dry weight and energy content from instar I to instar II and from II to III metanauplii in 15 different *Artemia* strains and measured differences from 16% (Shark Bay, Australia) to 34% (Buenos Aires, Argentina) in the first case and from 22% (Bahia Salinas, Puerto Rico) to 39% (Buenos Aires, Argentina) in the second. Vanhaecke *et al.* (1983) also noticed that for these various strains the dry weight and energy content of instar II-III metanauplii do not follow the ranking for the same characteristics in instar I nauplii; this allowed them to conclude that the rate of dry weight and energy consumption differs from strain to strain, eventually related to differences in swimming behaviour (Miller *et al.*, 1979). The data for dry weight decrease during nauplius starvation as reported by Paffenhöfer (1967) and Royan (1980) do not correspond well with those from the previous authors. Paffenhöfer noted a weight decrease of only 4% after 24 h and Royan reports a 50% decrease from instar I to instar III. It is to be noted, however, that Paffenhöfer did his experiment at 20 °C while Benijts *et al.* (1976) used 28 °C and Vanhaecke *et al.* (1983) 25 °C. Due to this lower temperature it is not impossible that only instar II metanauplii have been measured while Benijts *et al.* (1976) and Vanhaecke *et al.* (1983) analysed a mixed population of instar II-III. Royan does not report the temperature he used but his value applies to metanauplii which were all at instar III.

Oppenheimer & Moreira (1980) observed a 50% decrease in carbon and approximately 12% in nitrogen as *Artemia* moults from the instar I into instar II stage. They ascribe these changes to a period of "self-absorption" in *Artemia* during development of the rudimentary mandibles and of a feeding mechanism.

Claus *et al.* (1979) also studied starved compared with freshly hatched nauplii and reported an increase in protein and ash content and a decrease in carbohydrate and lipid content. Furthermore, they observed a change in fatty-acid profile; some fatty acids increased while others decreased. This was also noticed by Watanabe *et al.* (1978c), while Benijts *et al.* (1976) found that the relative proportions of the fatty acids were almost unchanged. The changes in the fatty-acid profile cannot be of great significancc in explaining the lower nutritional value of starved metanauplii for marine larvae *i.e.,* the essential fatty acid 20:5ω3 even increases during starvation (Watanabe *et al.*, 1978c; Léger *et al.*, 1983). Claus *et al.* (1979), furthermore, found that the amino-acid profile changed little, but the essential amino acid methionine appeared absent in starved nauplii. Dabrowski & Rusiecki (1983) also analysed amino-acid profiles and contents in starved nauplii, and found some free amino acids to remain constant upon starvation while others decreased 4- to 2-fold; contrary to Claus *et al.* (1979), Dabrowski & Rusiecki measured some increase in methionine content in starved nauplii.

These observations do not minimize the first assumption that, provided their increased size does not interfere with ingestion problems, the reduced nutritional value of starved *Artemia* metanauplii is primarly determined by their reduced energy content. Proper attention has to be paid to the observation of Miller *et al.* (1979) that older nauplii swim faster than freshly hatched *Artemia*. This may indeed constitute an additional increase in energy demand and consumption for prey catching. Similarly important is the suggestion of Dendrinos, Dewan & Thorpe (1984) that loss of orange colour thus reducing the visibility of starved nauplii may to some extent explain their poorer nutritional effectiveness.

The assumption that viability of nauplii may be affected as a result of starvation (Forster & Wickins, 1972) has been rejected by Vanhaecke *et al.* (1983), who found starved *Artemia* nauplii to be very resistant; *i.e.* depending on the strain tested, median lethal time (LT_{50}) values ranged from 73 h to 177 h ($\bar{x}=118$ h) for animals submitted to starvation conditions at 20 °C, and from 42 h to 70 h ($\bar{x}=62$ h) at 30 °C. Even when starved in fresh water, Vanhaecke (1983) recorded LT_{50} values between 16 and 38 h ($x=29$ h).

From all these data it nevertheless looks evident that, perhaps with the exception for some Hydrozoa, freshly hatched instar I nauplii should be fed as a more nutritious food source than starved metanauplii. In order to achieve this prerequisite, application of standard hatching and harvest conditions, as well as proper knowledge of the hatching rate and hatching synchrony of the *Artemia* cysts used is essential. In this context application of the earlier mentioned techniques of cold storage and automated distribution for freshly hatched nauplii is very relevant (Léger & Sorgeloos, 1982; Léger *et al.*, 1983; Sleet & Brendel, 1983; see above). When size is not limiting, the use of fed or enriched metanauplii may be prefered because it solves the problem of nutritional deficiencies (see later).

Strain differences

Table I summarizes the results of culture tests evaluating different strains of *Artemia* for different predators; not all experiments treated in this table are discussed here.

Kuenen (1939) pointed out that the differences which he had observed among different geographical sources of *Artemia* were a potential source of significant variability in experiments in which *Artemia* were used as a food source. His prediction was eventually borne out by Shelbourne (1968) who had to switch from San Francisco Bay *Artemia*, because of their unavailability in early 1966, to Great Salt Lake nauplii for feeding his flatfish (*Pleuronectes platessa* and *Solea solea*) larvae; heavy larval mortality occurred 3 weeks after introducing Great Salt Lake nauplii in the culture tanks. In the same year, Slobodkin (1968) confirmed the poor nutritional value of Great Salt Lake *Artemia* for plaice larvae. He suggested that their "toxicity" could be related to bioaccumulation of residual insecticides from the lake area. Not only flatfish seemed to suffer from a Great Salt Lake *Artemia* diet. *Palaemon serratus* larvae during the first days of their life did equally well on Great Salt Lake as on San Francisco Bay *Artemia*, until metamorphosis, when heavy mortalities occurred in the former (Forster & Wickins, 1967). Forster & Wickens also demonstrated that the food value of Great Salt Lake *Artemia* could be improved in various ways, *e.g.* by mixing with San Francisco Bay nauplii, adding *Isochrysis* in the culture tanks or by feeding the nauplii for 4 days on this alga. They also noticed that no deleterious effects were encountered when Great Salt Lake nauplii were offered during the first 12 days only, followed by a diet of San Francisco Bay nauplii. Reeve (1969a) confirmed these findings with *Palaemon serratus* larvae which became lethargic on a Great Salt Lake diet and died during metamorphosis. Little (1969) and Reed (1969) described similar observations for other decapod larvae (*P. macrodactylus* and *Cancer magister*). In addition, Bookhout & Costlow (1970) reported that four crab species survived better on San Francisco Bay nauplii than on a Great Salt Lake diet; they ascribed the difference to the 3-fold higher concentration of DDT in the Great Salt Lake nauplii.

Wickins (1972) reviewed the available information, on the deleterious effects of Great Salt Lake nauplii as food for marine larvae; in general, negative effects (*e.g.* lethargy, lack of co-ordination, abnormal development, mortality) were manifested around the time of metamorphosis of the predator species. Wickins' (1972) own experiments with *Palaemon serratus* showed that newly hatched or starved Great Salt Lake nauplii were an inadequate food, but the same nauplii could be acceptable when fed on *Isochrysis*. His comparison of the chemical composition of newly hatched nauplii from Great Salt Lake and San Francisco Bay, in terms of pesticides, heavy metals, carotenoids, sterols, and fatty acids, yielded no differences that "could be confidently labelled as the cause of the poor food value of the Utah (Great Salt Lake) *Artemia* nauplii". In any case, the fact that feeding nauplii on *Isochrysis* improved their food value was an indication that the Great Salt Lake *Artemia* problem might be one of nutritional deficiency rather than of contamination.

TABLE I

Summary of results of culture tests using different strains of Artemia *for different predators: only those culture tests are considered in which the source of* Artemia *is relevant; +, ± and − refer to relative values, i.e. good, medium and inferior results, respectively, adjudged by interpretation of the given data; these annotations compare values within one experiment, so that no absolute comparison may be made between different experiments even when the same test organism was used; *see Sorgeloos (1980b); n.s., source not specified*

Artemia source	Species tested	Survival	Growth	Development and/or metamorphosis	Remarks	Reference
AUSTRALIA						
Shark Bay (No. 113)	*Menidia menidia*	+	+			Beck *et al.*, 1980
Shark Bay (No. 113)	*Cancer irroratus*	+	+			Johns *et al.*, 1980
Shark Bay (No. 113)	*Rhithropanopeus harrisii*	+	+			Johns *et al.*, 1980
Shark Bay (No. 113)	*Pseudopleuronectes americanus*	+	+			Klein-MacPhee *et al.*, 1980
Shark Bay (No. 113)	*Mysidopsis bahia*	+	+			Johns *et al.*, 1981a
Shark Bay (No. 113)	*Cyprinus carpio*	+	+			Vanhaecke & Sorgeloos, 1983b
BRAZIL						
Macau (No. 871172, 1978)	*Menidia menidia*	+	±			Beck *et al., 1980*
Macau (No. 871172, 1978)	*Cancer irroratus*	+	+			Johns *et al.*, 1980
Macau (No. 871172, 1978)	*Rhithropanopeus harrisii*	+	+			Johns *et al., 1980*
Macau (No. 871172, 1978)	*Pseudopleuronectes americanus*	+	±			Klein-MacPhee *et al.*, 1980
Macau (No. 871172, 1978)	*Mysidopsis bahia*	+	+			Johns *et al.*, 1981a;
Macau (No. 871172, 1978)	*Cyprinodon variegatus*	+				Usher & Bengtson, 1981
Macau (No. 871172, 1978)	*Cyprinus carpio*	+	±			Vanhaecke & Sorgeloos, 1983b
Macau	*Thalamita crenata*	+	+	+		Krishnan, unpubl.
n.s.	*Libinia emarginata*	+				Goy & Costlow, 1980

	Menippe mercenaria	+				Goy & Costlow, 1980
	Palaemonetes pugio	+		+		Goy & Costlow, 1980
	Rhithropanopeus harrisii	+				Goy & Costlow, 1980
n.s.	*Scophthalmus maximus*	+	+		Better growth, survival and acceptance of dry food as compared with San Francisco Bay nauplii.	Howell *et al.*, 1981
n.s.	*Gadus* sp.	+	+		Better nutritional value than San Francisco Bay *Artemia* nauplii, because they contain certain polyunsaturated fatty acids.	Anonymous, 1982
	Scophthalmus sp.	+	+		Better nutritional value than San Francisco Bay *Artemia* nauplii, because they contain certain polyunsaturated fatty acids.	Anonymous, 1982
n.s.	*Morone saxatilis*	+				Westin *et al.*, 1983, 1985
BULGARIA						
Burgas-Pomorije	"freshwater fish"	+	+			Ljudskanova & Joshev, 1972
CANADA						
Chaplin Lake (No. 5002)	*Panulirus interruptus*	+	+		Considerable variation with regard to source of *Artemia*; Chaplin Lake most successful (other sources not defined).	Dexter, 1972
Chaplin Lake	*Palaemonetes pugio*	+		+	At least equal in quality to San Francisco Bay *Artemia* nauplii.	Provenzano & Goy, 1976
Chaplin Lake (1979)	*Menidia menidia*	±	−			Beck & Bengtson, 1982
Chaplin Lake (1979)	*Pseudopleuronectes americanus*	+	±			Klein-MacPhee *et al.*, 1982
Chaplin Lake (1979)	*Rhithropanopeus harrisii*	±	±			Seidel *et al.*, 1982
Chaplin Lake (1979)	*Cyprinus carpio*	+	−			Vanhaecke & Sorgeloos, 1983b
Chaplin Lake (1979)	*Mysidopsis bahia*	±	±			Léger & Sorgeloos, 1984
Lake Saskatchewan	*Pagrus major*	+	±		Good survival but slightly slower growth may be observed.	Fujita *et al.*, 1980 Watanabe *et al.*, 1980

TABLE I—*continued*

Artemia source	Species tested	Survival	Growth	Development and/or metamorphosis	Remarks	Reference
CHINA P.R.						
Tientsin (1979)	*Menidia menidia*	+	+			Beck & Bengtson, 1982
Tientsin (1979)	*Pseudopleuronectes americanus*	+	+			Klein-MacPhee *et al.*, 1982
Tientsin (1979)	*Rhithropanopeus harrisii*	+	+			Seidel *et al.*, 1982
Tientsin (1979)	*Cyprinus carpio*	+	+			Vanhaecke & Sorgeloos, 1983b
Tientsin (1979)	*Mysidopsis bahia*	+	+			Léger & Sorgeloos, 1984
n.s.	*Macrobrachium rosenbergii*	−			Larvae died within a few days, probably due to high levels of BHCs and DDT.	Matsuoka, 1975
n.s.	*Libinia emarginata*	+		+		Goy & Costlow, 1980
	Menippe mercenaria	+		+		Goy & Costlow, 1980
	Rhithropanopeus harrisii	+		+		Goy & Costlow, 1980
n.s.	'Sobaity'	+	+		Better survival and growth than with Great Salt Lake nauplii.	James *et al., 1982*
COLOMBIA						
Galera Zamba	*Mysidopsis bahia*	+	+			Léger, unpubl.
Manaure	*Mysidopsis bahia*	−	−		Good source after HUFA-enrichment.	Léger, unpubl.
Manaure	*Thalamita crenata*	−		−		Krishnan, pers. comm.
CYPRUS						
	Dicentrarchus labrax	+	+		Comparable with San Francisco Bay *Artemia* nauplii.	Person-Le Ruyet & Salaun, 1977
	Solea solea	+	+		Comparable with San Francisco Bay *Artemia* nauplii.	Person-Le Ruyet & Salaun, 1977

FRANCE						
Salins du Midi	*Palaemon serratus*	–	–	–	Abnormal development and lack of coordination.	Campillo, 1975
Salins du Midi	*Solea solea*	+	+		Comparable with San Francisco Bay nauplii and even some better growth noted.	Fuchs, 1976
Salins du Midi	*Dicentrarchus labrax*	+	+		Comparable with San Francisco Bay nauplii and even some better growth noted.	Fuchs & Person-Le Ruyet, 1976
	Scophthalmus maximus	+	+		Comparable with San Francisco Bay nauplii and even some better growth noted.	Fuchs & Person-Le Ruyet, 1976
Salins du Midi	*Dicentrarchus labrax*	+	+		Not different from San Francisco Bay nauplii.	Godeluck, 1981
Salins du Midi, Lavalduc (1979)	*Menidia menidia*	±	+			Beck & Bengtson, 1982
Salins du Midi, Lavalduc (1979)	*Pseudopleuronectes americanus*	±	+			Klein-MacPhee *et al.*, 1982
Salins du Midi, Lavalduc (1979)	*Rhithropanopeus harrisii*	+	+			Seidel *et al.*, 1982
Salins du Midi, Lavalduc (1979)	*Cyprinus carpio*	+	+			Vanhaecke & Sorgeloos, 1983b
Salins du Midi, Lavalduc (1979)	*Mysidopsis bahia*	+	+			Léger & Sorgeloos, 1984
n.s.	*Libinia emarginata*	+				Goy & Costlow, 1980
	Menippe mercenaria	+				Goy & Costlow, 1980
	Rhithropanopeus harrisii	+				Goy & Costlow, 1980
INDIA						
Kutch-Mundra (1979)	*Mysidopsis bahia*	+	+		Cysts originating from SFB2596 inoculation.	Vos *et al., 1984*
IRAN						
Ormia Lake	*Acipenser* sp.	+	+			Azari Takami, 1976, 1985
ITALY						
Margherita di Savoia (1977)	*Menidia menidia*	–	+			Beck *et al.*, 1980
Margherita di Savoia (1977)	*Cancer irroratus*	+	+			Johns *et al.*, 1980
Margherita di Savoia (1977)	*Rhithropanopeus harrisii*	+	+			Johns *et al., 1980*
Margherita di Savoia (1977)	*Pseudopleuronectes americanus*	+	+			Klein-MacPhee *et al.*, 1980

TABLE I—*continued*

Artemia source	Species tested	Survival	Growth	Development and/or metamorphosis	Remarks	Reference
Margherita di Savoia (1977)	*Mysidopsis bahia*	+	+			Johns *et al., 1981a*
Margherita di Savoia (1977)	*Cyprinus carpio*	+	+			Vanhaecke & Sorgeloos, 1983b
Margherita di Savoia (1977)	*Morone saxatilis*	+				Westin *et al.*, 1983, 1985
n.s.	*Libinia emarginata*	–		–	Abnormal megalopae.	Goy & Costlow, 1980
	Menippe mercenaria	–		–	Abnormal megalopae.	Goy & Costlow, 1980
	Palaemonetes pugio	–		–		Goy & Costlow, 1980
	Rhithropanopeus harrisii	–		–	Abnormal megalopae.	Goy & Costlow, 1980
PERU						
Piura	*Mysidopsis bahia*	+	+			Léger, unpubl.
PHILIPPINES						
Barotac Nuevo (1978)	*Mysidopsis bahia*	+	+		Cyst originating from SFB2596 inoculation.	Vos *et al.*, 1984
Jaro	*Mysidopsis bahia*	±	–		Cysts originating from Barotac Nuevo *inoculation (deficient in 20:5ω3).*	Vos *et al.*, 1984
REFERENCE ARTEMIA CYSTS*						
	Menidia menidia	+	+			Beck & Bengtson, 1982
	Pseudopleuronectes americanus	+	+			Klein-MacPhee *et al., 1982*
	Rhithropanopeus harrisii	+	+			Seidel *et al.*, 1982
	Mysidopsis bahia	+	+			Léger & Sorgeloos, 1984
SPAIN						
Cadiz	*Penaeus kerathurus*	+	+	+		Rodriguez, 1975
Cadiz	*Penaeus kerathurus*	+	+	+		Yufera *et al.*, 1984

THAILAND						
Bangpakong (1979)	*Mysidopsis bahia*	+	+		Cysts originating from SFB1728 inoculation.	Vos *et al.*, 1984
TUNISIA						
Mégrine	*Mysidopsis bahia*	+	+			Van Ballaer *et al.*, 1985
Sfax	*Mysidopsis bahia*	+	+			Van Ballaer *et al.*, 1985
TURKEY						
Izmir	*Dicentrarchus labrax*		+			Uçal, 1979
U.S.A.						
Great Salt Lake	*Palaemon serratus*	–	–		Larvae did well until metamorphosis when heavy mortalities occurred; the value of Great Salt Lake *Artemia* could be improved by adding San Francisco Bay nauplii or by pre-feeding them on *Isochrysis*.	Forster & Wickins, 1967
Great Salt Lake	*Pleuronectes platessa*	–	–		Inability to support good growth and survival in flatfish larvae.	Shelbourne, 1968
	Solea solea	–	–		Inability to support good growth and survival in flatfish larvae.	Shelbourne, 1968
Great Salt Lake	*Palaemon macrodactylus*	–	–		Inferior to San Francisco Bay *Artemia*.	Little, 1969
Great Salt Lake	*Cancer magister*	–	–			Reed, 1969
Great Salt Lake	*Palaemon serratus*	–	–		Larvae did well until metmorphosis and then became lethargic and died during metamorphosis.	Reeve, 1969a,b
Great Salt Lake	*Callinectes sapidus*	+		+	Only 1 abnormal crab stage, survival comparable with San Francisco Bay *Artemia* nauplii.	Bookhout & Costlow, 1970
	Hexapanopeus angustifrons	–		–	Abnormal megalopae, none reached 1st crab stage.	Bookhout & Costlow, 1970
	Labinia emarginata	–		–	Only 5% reached first crab.	Bookhout & Costlow, 1970
	Rhithropanopeus harrisii	–		–	Abnormal megalopae, none reached 1st crab stage.	Bookhout & Costlow, 1970

TABLE I—*continued*

Artemia source	Species tested	Survival	Growth	Development and/or metamorphosis	Remarks	Reference
Great Salt Lake	*Palaemon elegans*	–		–	Only a few unhealthy postlarvae passed through metamorphosis compared with those which were fed San Francisco Bay nauplii.	Wickins, 1972
	Palaemon serratus	–		–	*Abnormalities, poor metamorphosis success* and low survival could be significantly improved by adding *Isochrysis* to the culture tank, or by feeding *Artemia* on this alga, or by replacing Utah nauplii by San Francisco Bay nauplii after 12 days; starving the nauplii did not improve their quality.	Wickins, 1972
	Poecilia reticulata	+	+		Good survival but significantly smaller larvae than when fed San Francisco Bay *Artemia*.	Wickins, 1972
Great Salt Lake	*Palaemonetes pugio*	–		±	Appearance of supernumerary stages but not with San Francisco Bay and Chaplin Lake *Artemia*.	Provenzano & Goy, 1976
Great Salt Lake	*Lepomis macrochirus*	–			Early mortality, presumably from starvation due to nauplius size.	Smith, 1976
Great Salt Lake (Lot 185, 1977)	*Menidia menidia*	+	+			Beck *et al.*, 1980
Great Salt Lake	*Libinia emarginata*	+		+		Goy & Costlow, 1980
	Menippe mercenaria	+		+		Goy & Costlow, 1980
	Palaemonetes pugio	+		+		Goy & Costlow, 1980
	Rhithropanopeus harrisii	+		+		Goy & Costlow, 1980
Great Salt Lake	*Cancer irroratus*	–	–		Total mortality.	Johns *et al.*, 1980
Great Salt Lake (Lot, 185, 1977)	*Rhithropanopeus harrisii*	–	–		Total mortality	Johns *et al.*, 1980
Great Salt Lake	*Pseudopleuronectes americanus*	–	±			Klein-MacPhee *et al.*, 1980
	Mysidopsis bahia	+	+			Johns *et al.*, 1981a

Great Salt Lake	'Sobaity'	–	±		Results were inferior compared with Chinese nauplii.	James *et al.*, 1982
Great Salt Lake	*Cyprinus carpio*	+	+			Vanhaecke & Sorgeloos, 1983b
Great Salt Lake (North arm)	*Dicentrarchus labrax*	–	–		Good source after HUFA-enrichment.	Van Ballaer *et al.*, 1985
	Mysidopsis bahia	–	–		Good source after HUFA-enrichment.	Léger, unpubl.
Great Salt Lake (North and South arm)	*Penaeus stylirostris*	–	–	–	Good source after HUFA-enrichment.	Léger, unpubl.
	Penaeus vannamei	–	–	–	Good source after HUFA-enrichment.	Léger, unpubl.
San Francisco Bay	*Palaemon serratus*	+	+	+	Good survival and metamorphosis compared with Great Salt Lake *Artemia*.	Forster & Wickins, 1967
San Francisco Bay	*Pleuronectes platessa*	+	+	+		Shelbourne, 1968
	Solea solea	+	+	+		Shelbourne, 1968
San Francisco Bay	*Palaemon serratus*	+	+	+	Good survival and metamorphosis compared with Great Salt Lake *Artemia*.	Reeve, 1969a,b
San Francisco Bay	*Callinectes sapidus*	+		+		Bookhout & Costlow, 1970
	Hexapanopeus angustifrons	+		+		Bookhout & Costlow, 1970
	Libinia emarginata	+		+		Bookhout & Costlow, 1970
	Rhithropanopeus harrisii	+		+		Bookhout & Costlow, 1970
San Francisco Bay	*Palaemon elegans*	+		+	Good survival and metamorphosis compared with Great Salt Lake *Artemia*.	Wickins, 1972
	Palaemon serratus	+	+	+	Good survival and metamorphosis compared with Great Salt Lake *Artemia*.	Wickins, 1972
	Poecilia reticulata	+	+		Good survival and better growth than when fed Great Salt Lake *Artemia*.	Wickins, 1972
San Francisco Bay	*Palaemon serratus*	+	+	+	Satisfactory source.	Campillo, 1975
San Francisco Bay	*Uca puligator*	+		+		Christiansen & Yang, 1976
San Francisco Bay	*Dicentrarchus labrax*	+	+		Comparable with French nauplii.	Fuchs & Person-Le Ruyet, 1976
	Scophthalmus maximus	+	+		Comparable with French nauplii.	Fuchs & Person-Le Ruyet, 1976
	Solea solea	+	+		Comparable with French nauplii.	Fuchs & Person-Le Ruyet, 1976
San Francisco Bay	*Palaemonetes pugio*	+		+		Provenzano & Goy, 1976
San Francisco Bay	*Lepomis macrochirus*	+	+		Older nauplii too large as first food.	Smith, 1976

TABLE I—*continued*

Artemia source	Species tested	Survival	Growth	Development and/or metamorphosis	Remarks	Reference
San Francisco Bay	*Dicentrarchus labrax*	+	+		Comparable with Cyprus-*Artemia*.	Person-Le Ruyet & Salaun, 1977
	Solea solea	+	+		Comparable with Cyprus-*Artemia*.	Person-Le Ruyet & Salaun, 1977
San Francisco Bay	*Callinectes sapidus*	+		+		Bigford, 1978
	Libinia emarginata	+		+		Bigford, 1978
San Francisco Bay (marine type)	*Pagrus major*	+	+			Watanabe *et al.*, 1978a, 1980, 1982
San Francisco Bay (freshwater type)		–	–		'Freshwater type' nauplii exhibit a high mortality at the 6th day and a shock syndrome during the activity test; their nutritional value is enhanced after feeding on marine *Chlorella*, ω-yeast, or emulsified cuttlefish liver oil; essential fatty acids are the principal factor for the food value of *Artemia* nauplii.	Watanabe *et al.*, 1978a, 1980, 1982
San Francisco Bay	*Gadus morhua*	–			Rearing through metamorphosis is enhanced when nauplii are pre-fed on *Isochrysis* (2 days), plus addition of the same algae and *Pavlova* to the tanks.	Howell, 1979b
San Francisco Bay (No. 198)	*Cyprinus carpio*	+	+			Bryant & Matty, 1980
San Francisco Bay	*Libinia emarginata*	–		+		Goy & Costlow, 1980
	Menippe mercenaria	–		+		Goy & Costlow, 1980
	Palaemonetes pugio	+	+	+		Goy & Costlow, 1980
	Rhithropanopeus harrisii	–		+		Goy & Costlow, 1980
San Francisco Bay (1977)	*Libinia emarginata*	–		–	Abnormal megalopae.	Goy & Costlow, 1980

	Menippe mercenaria	–		–	Higher incidence of supernumerary stages.	Goy & Costlow, 1980
	Palaemonetes pugio	–		–	Higher incidence of supernumerary stages, abnormal megalopae.	Goy & Costlow, 1980
	Rhithropanopeus harrisii	–		–	Abnormal megalopae.	Goy & Costlow, 1980
San Francisco Bay	*Scophthalmus maximus*	±	±		Survival and growth of larvae was inferior to the ones fed with Brazilian *Artemia*; subsequent acceptance of dry food was also inferior; some improvement when nauplii were fed (4h) on *Isochrysis*.	Howell *et al.*, 1981
San Francisco Bay	*Mylio macrocephalus*	–			No larvae survived beyond 18th day; high 18:3ω3 content may be causal.	Lee *et al.*, 1981
San Francisco Bay	*Gadus* sp.	–	–		Inferior to Brazilian *Artemia*; inadequate to rear cod; improved when fed *Isochrysis*.	Anonymous, 1982
	Scophthalmus sp.	–	–		Inferior to Brazilian *Artemia*; inadequate to rear turbot; improved when fed *Isochrysis*.	Anonymous, 1982
San Francisco Bay (No. 236–2016)	*Penaeus stylirostris*	+	+	+		Léger *et al., 1985a*
San Francisco Bay No. 2596)	*Mysidopsis bahia*	+	+			Vos *et al., 1984*
San Francisco Bay (No. 728)	*Mysidopsis bahia*	+	+			Vos *et al.*, 1984
San Francisco Bay (14 different lots)	*Mysidopsis bahia*				Varying results according to 20:5ω3 level in nauplii.	Léger *et al.*, 1985c
San Pablo Bay (No. 1628, 1978)	*Menidia menidia*	–	±			Beck *et al.*, 1980
San Pablo Bay	*Libinia emarginata*	–		–	Abnormal megalopae.	Goy & Costlow, 1980
	Menippe mercenaria	–		–	Higher incidence of supernumerary stages, abnormal megalopae.	Goy & Costlow, 1980
	Palaemonetes pugio	–		–	Higher incidence of supernumerary stages.	Goy & Costlow, 1980
	Rhithropanopeus harrisii	–		–	Abnormal megalopae.	Goy & Costlow, 1980
San Pablo Bay No. 1628, 1978)	*Cancer irroratus*	–	–	–	Total mortality.	Johns *et al.*, 1980
	Rhithropanopeus harrisii	–	–	–	Total mortality	Johns *et al.*, 1980

TABLE I—*continued*

Artemia source	Species tested	Survival	Growth	Development and/or metamorphosis	Remarks	Reference
San Pablo Bay (No. 1628, 1978)	*Pseudopleuronectes americanus*	–	–			Klein-MacPhee *et al.*, 1980, 1982
San Pablo Bay (No. 1628, 1978)	*Mysidopsis bahia*	±	–			Johns *et al.*, 1981a
San Pablo Bay (No. 1628, 1978)	*Cyprinodon variegatus*	+	+			Usher & Bengtson, 1981
San Pablo Bay (No. 1628, 1978)	*Cyprinus carpio*	+	±			Vanhaecke & Sorgeloos, 1983b
San Pablo Bay	*Morone saxatilis*	+			Survival as good as with Brazilian *Artemia*.	Westin *et al.*, 1983, 1985
San Pablo Bay (No. 1628, 1978)	*Penaeus stylirostris*	–	–	+	Survival and growth were significantly improved by HUFA-enrichment, and were also determined by the pre-*Artemia* diet quality.	Léger *et al.*, 1985a
San Pablo Bay (No. 1628, 1978)	*Mysidopsis bahia*	–	–			Léger *et al.*, 1985c
San Pablo Bay (No. 1628, 1978)	*Dicentrarchus labrax*	–	–			Van Ballaer *et al.*, 1985
U.S.S.R.						
n.s.	*Acipenser* sp.	+	+		Live nauplii are better than stored nauplii.	Gun'ko & Pleskachevskaya, 1962

Subsequently, Dexter (1972) noted that growth and survival of *Panulirus interruptus* varied with source of *Artemia* but stated, without mentioning other sources, that the best results were obtained with Chaplin Lake (Canada) *Artemia* nauplii. Provenzano & Goy (1976) found Chaplin Lake *Artemia* nauplii at least equal in quality to San Francisco Bay *Artemia* nauplii. *Palaemon serratus* larvae fed nauplii from France (Salins du Midi) exhibited slower development and less successful metamorphosis to post-larvae than when fed nauplii from California (Campillo, 1975). Metamorphosis was not only retarded, but post-metamorphosis survival was also much lower. Campillo reported several other developmental abnormalities with a diet of French *Artemia*, *e.g.* perturbation of moulting sychronism, abnormal appendices, and rostrum, incomplete pigmentation, lack of co-ordination. None the less, several other authors reported good culture performance with French *Artemia*, *e.g.* Fuchs & Person-Le Ruyet (1976) for seabass (*Dicentrarchus labrax*), sole (*Solea solea*), and turbot (*Scophthalmus maximus*), and Godeluck (1981) also for seabass.

Brazilian *Artemia* have so far not been reported to be nutritionally questionable. Some authors find Brazilian *Artemia* to be even superior to San Francisco Bay *Artemia* (Howell, Bromley & Adkins, 1981; Anonymous, 1982). As to Chinese *Artemia*, Matsuoka (1975) observed that *Macrobrachium rosenbergii* larvae died within a few days when fed *Artemia* from this source, probably due to high levels of BHCs and DDT. James, Bou-Abbas & Dias (1982), on the contrary, observed equal growth and survival in larvae when fed Chinese or Great Salt Lake *Artemia* nauplii.

Investigations of the nutritional adequacy, in terms of essential fatty acids (EFA) in *Artemia* nauplii from San Francisco Bay, South America, and Canada indicated that brine shrimp nauplii can be classified into two categories, *i.e.* high in 18:3ω3, the EFA for freshwater fish, or those high in 20:5ω3, the EFA for marine fish (Watanabe *et al.*, 1978b,c). When the Canada strain (5·2% 20:5ω3) was fed to red seabream, *Pagrus major*, 68% of the fish survived, but when the San Francisco Bay strain (1·6% 20:5ω3) was fed, only 43% survived (Watanabe, Oowa, Kitajima & Fujita, 1980). When the San Francisco Bay nauplii were reared on *Chlorella* or ω-yeast for 24 h, the survival of fish to which they were fed increased to 67% and 86%, respectively. Watanabe, Ohta, Kitajima & Fujita (1982) later confirmed that larval survival in flounder (*Paralichthys olivaceus*) and rock seabream (*Oplegnathus fasciatus*) was also low when fed with low-20:5ω3 San Francisco Bay nauplii but could be improved by feeding the nauplii ω-yeast or cuttlefish liver oil (both rich in 20:5ω3) before presentation to the fish.

A systematic survey of geographical strains by the International Study on *Artemia* (ISA) has provided the bulk of the information on variation in nutritional quality of nauplii. In the ISA survey, a total of eight geographical strains were fed to several fish and crustacean species. The strains tested were from Australia (Shark Bay, lot 114), Brazil (Macau, lot 871172), Canada (Chaplin Lake, 1979 harvest), China (Tientsin, 1979 harvest), France (Lavalduc, 1979 harvest), Italy (Margherita di Savoia, 1977 harvest), and the United States (Great Salt Lake, lot 185, and San Pablo Bay, lot 1628). In addition, an ISA standard reference sample (Reference *Artemia* Cysts RAC, of undisclosed location, Sorgeloos, 1980b) was also tested. All eight strains were fed to three fish species (Atlantic silverside, *Menidia menidia*; winter flounder, *Pseudopleuronectes americanus*; and carp,

Cyprinus carpio) and two crustacean species (mud crab, *Rhithropanopeus harrisii*, and mysid, *Mysidopsis bahia*). Some of the strains were also fed to another fish (sheepshead minnow, *Cyprinodon variegatus*) and another crustacean (rock crab, *Cancer irroratus*). The survival data for the fish and crustacean larvae fed on the various ISA-strains are summarized in Table II. Patterns can be distinguished by reading rows and columns of data. For example, certain species (*Cyprinus carpio*, *Cyprinodon variegatus*) survived well regardless of *Artemia* strain, whereas other species (*Rhithropanopeus harrisii*, *Cancer irroratus*) were profoundly affected by the strains they were fed. Certain strains, *e.g.* Brazil and RAC seemed to be a good food for all the species tested, whereas some strains (*e.g.* Great Salt Lake and San Pablo Bay) were poor for several species; one strain (Italy) was poor for only one species, and one strain (Canada) was mediocre for most species. More information could also be obtained from the time course of mortality for each species. Species that undergo a pronounced metamorphosis (*Pseudopleuronectes americanus*, *Rhithropanopeus harrisii*, and *Cancer irroratus*) suffered almost all the mortality at the time of metamorphosis when fed a poor-quality strain. This phenomenon had been noticed previously for other species (Forster & Wickins, 1967; Shelbourne, 1968; Reeve, 1969a; Bookhout & Costlow, 1970; Wickins, 1972; Campillo, 1975). In most of those cases, survival was excellent up to the time of metamorphosis, when nearly 100% mortality occurred within a very few days. On the other hand, most mortality in culture tests with fish that do not undergo metamorphosis (*e.g. Menidia menidia*) occurred early in the experiment (Beck *et al.*, 1980) indicating that the causes of mortality in the different species may have been diverse.

Johns, Berry & McLean (1981b) designed an experiment to determine whether the nutritional factors in Great Salt Lake and San Pablo Bay *Artemia* causing deleterious effects in *Rhithropanopeus harrisii* larvae were acquired cumulatively or only during certain critical periods of development. They divided the larval development period into three parts: hatching to Day 5, Day 5 to Day 9, Day 9 to Day 11 (metamorphosis). The food source used during each part (Brazil, Great Salt Lake or San Pablo Bay) was varied to produce a total of 11 different feeding combinations, although each combination consisted of a maximum of two sources (*e.g.*, a three-part combination might be Brazil–Brazil–Great Salt Lake or San Pablo–San Pablo–Brazil). They found that total mortality of larvae at metamorphosis occurred only if the larvae received Great Salt Lake or San Pablo Bay for the first 9 days of the development. The type of food being given at the time of metamorphosis was irrelevant to the survival rate compared with what had been given during the first 9 days. This allowed Johns *et al.* (1981b) to conclude that the factor causing mortality was either cumulatively acquired with the diet or was cumulatively deficient in the diet.

In addition to the survival data, the ISA studies also provide results for several fish and crustacean species on growth, rate of development (time to metamorphosis), and reproduction. An examination of growth data for animals raised on the strains that gave poor (Great Salt Lake, San Pablo Bay) or mediocre (Canada) survival results provides a few clear-cut patterns, *i.e.* growth in *Pseudopleuronectes americanus*, *Mysidopsis bahia*, and *Cyprinus carpio* was significantly less when fed San Pablo Bay strain

TABLE II

Per cent survival of seven species of fish and crustacean larvae reared on Artemia *nauplii from eight geographical strains of* Artemia: *(1) Johns* et al., *1980; Seidel* et al., *1982; (2) Johns* et al., *1980; (3) Johns* et al., *1981a; Léger & Sorgeloos, 1984; (4) Beck* et al.,*1980; Beck & Bengtson, 1982; (5) Klein-MacPhee* et al., *1980, 1982; (6) Vanhaecke & Sorgeloos, 1983b; (7) Usher & Bengtson, 1981; (8) Sorgeloos, 1980b*

Artemia source	*Rhithropanopeus harrisii* (1)	*Cancer irroratus* (2)	*Mysidopsis bahia* (3)	*Menidia menidia* (4)	*Pseudopleuronectes americanus* (5)	*Cyprinus carpio* (6)	*Cyprinodon variegatus* (7)
Australia, Shark Bay	78	92	98	60	94	96	—
Brazil, Macau	80	95	95	89	89	96	100
Canada, Chaplin Lake	72	—	74	62	78	95	—
China P.R., Tientsin	84	—	90	71	72	97	—
France, Lavalduc	89	—	94	62	61	95	—
Italy, Margherita di Savoia	92	90	98	44	88	94	—
U.S.A., Great Salt Lake	0	0	98	72	46	93	—
U.S.A., San Pablo Bay	0	0	82	42	39	93	100
Reference *Artemia*(8)	89	—	92	82	86	—	—

(Klein-MacPhee, Howell & Beck, 1980, 1982; Johns, Berry & Walton, 1981a; Vanhaecke & Sorgeloos, 1983b), whereas no signicant differences were obtained in *Menidia menidia* and *Cyprinodon variegatus* (Beck *et al.*, 1980; Usher & Bengtson, 1981). The Great Salt Lake strain (which caused mass mortality in some species) yielded the best growth for *Mysidopsis bahia* (Johns *et al.*, 1981a) and *Menidia menidia* (Beck *et al.*, 1980) and the best reproduction for *Mysidopsis bahia* (Johns *et al.*, 1981a), but resulted in significantly less growth than obtained with the best *Artemia* strains in *Cyprinus carpio* (Vanhaecke & Sorgeloos, 1983b) and *Pseudopleuronectes americanus* (Klein-MacPhee *et al.*, 1980). Growth in Canadian-fed *P. americanus* (Klein-MacPhee, Howell & Beck, 1982), *Rhithropanopeus harrisii* (Seidel, Johns, Schauer & Olney, 1982), and *Cyprinus carpio* (Vanhaecke & Sorgeloos, 1983b) was significantly worse than when the other strains were fed. Although survival of most species was best when they were offered Brazilian *Artemia*, growth of *Menidia menidia* (Beck *et al.*, 1980), *Pseudopleuronectes americanus* (Klein-MacPhee *et al.*, 1980), and *Cyprinus carpio* (Vanhaecke & Sorgeloos, 1983b) on that strain was significantly less than optimal. In summary, concordance of survival and growth data is not necessarily apparent.

Although technically not part of the ISA studies, experiments with the same ISA strains were performed by Westin, Olney & Rogers (1983, 1985) using striped bass larvae, *Morone saxatilis,* and by Goy & Costlow (1980) using three crabs, *Rhithropanopeus harrisii*, *Menippe mercenaria*, and *Libinia emarginata*, and a shrimp, *Palaemonetes pugio*. In general, their results tended to corroborate the ISA results, except that Goy & Costlow observed good survival in organisms fed the Great Salt Lake strain[1] and poor survival in those fed the Italian strain. Westin *et al.*'s (1983) finding that survival of *Morone saxatilis* was equally good with the Brazilian and San Pablo Bay strains agrees with Usher & Bengtson (1981) and Vanhaecke & Sorgeloos (1983b) that the San Pablo Bay strain was an adequate food for organisms that can live in fresh water.

Reasons for the difference between a poor-quality and a good-quality *Artemia* strain are undoubtedly complex, because they must explain different patterns of mass mortality (at metamorphosis compared with during the first few days post-hatch) as well as account for the lack of congruence between growth and survival data. Attempts at explanation are further hampered by the lack of knowledge of the nutritional requirements for the species used in the ISA studies. Nevertheless, an attempt was made to relate the ISA biological data on growth and survival with biochemical data (*e.g.* fatty acids by Schauer, Johns, Olney & Simpson, 1980 and Seidel *et al.*, 1982; amino acids by Seidel, Kryznowek & Simpson, 1980b) and biometrical data (Vanhaecke & Sorgeloos, 1980) in the hope that hypotheses could be developed to explain differences in the food value of the strains.

The most immediately apparent connection that could explain mortality was between the size of the *Artemia* nauplii and mortality of *Menidia menidia* in the first 5 days after hatching (Beck & Bengtson, 1982) (see above). The length of nauplii from eight strains ranged from about 440 to 520 μm and it was calculated that when newly-hatched nauplii >480 μm

[1]It was later found that they were using a different batch of Great Salt Lake cysts.

were fed >20% mortality of *M. menidia* larvae could be expected. Thus, a good part of the mortality when this species was raised on the large, parthenogenetic strains from France, China, and especially Italy was due to the simple fact that many of the fish larvae could not ingest the food. The same phenomenon may account for some of the mortality in *Pseudopleuronectes americanus* reared on the French strain (Klein-MacPhee, Howell & Beck, 1982) and in *Morone saxatilis* reared on the Italian strain (Westin *et al.*, 1985). Because of the hypothesis of Bookhout & Costlow (1970) the ISA group originally suspected that chlorinated hydrocarbons (CHCs) such as DDT might be a cause of mortality. If organisms such as crab larvae (*Rhithropanopeus harrisii*) do accumulate CHCs from their *Artemia* diet, the toxic effect might be expressed as a mass mortality at the time of the major morphological restructuring, *i.e.* at metamorphosis. Olney *et al.* (1980), Johns, Peters & Beck (1980), and Seidel *et al.* (1982) concluded, however, that DDT was unlikely to be the causative agent, because the two strains with the highest DDT concentrations (Italy, 422 $\mu g \cdot g^{-1}$; China, 172 $\mu g \cdot g^{-1}$) yielded excellent survival of *Rhithropanopeus harrisii* larvae, whereas the strains that caused mass mortality of *R. harrisii* at metamorphosis had much lower DDT concentrations (San Pablo Bay, 42 $\mu g \cdot g^{-1}$; Great Salt Lake, 7·3 $\mu g \cdot g^{-1}$). On the other hand, bioaccumulation data for *Menidia menidia* fed on the various strains (Olney *et al.*, 1980) suggested that chlordane or dieldrin, the former found at its highest concentration in the San Pablo Bay strain, might be a causative factor for the observed mortalities. In two follow-up studies (Johns *et al.*, 1981b, McLean, Olney, Klein-MacPhee & Simpson, 1985), *Rhithropanopeus harrisii* larvae and newly-metamorphosed *Pseudopleuronectes americanus* were fed *Artemia* nauplii that had been contaminated on purpose with chlordane and dieldrin. *Rhithropanopeus harrisii* larvae did not die at metamorphosis even when the chlordane and dieldrin levels in the nauplii were one to two orders of magnitude higher than the maximum measured in the eight ISA strains. *Pseudopleuronectes americanus* showed no mortality after having been raised for 30 days on the contaminated *Artemia*, but it should be emphasized that the experiment was started with metamorphosed fish. In summary, it is likely that chlordane and dieldrin, like DDT, were not causative factors for the poor culture performances observed with some ISA strains. Westin *et al.* (1985) fed three strains of *Artemia* (Brazil, Italy, San Pablo Bay) containing different concentrations of four CHCs to *Morone saxatilis* larvae and found that they caused no significant differences in larval survival; what was observed was a parental effect, *i.e.* concentrations of those four CHCs in the eggs from which the fish larvae hatched affected their survival.

Another relationship that merits examination (based on the work of Watanabe *et al.*, 1978c, 1980), is that of the levels of the essential fatty acids, 20:5ω3 and 18:3ω3, with growth and survival of the various species. The strain that had the lowest level of 20:5ω3 (San Pablo Bay, Schauer *et al.*, 1980), an essential fatty acid for marine organisms, normally yielded the lowest survival reates for the marine species tested. Only the species that can live in fresh water (*Cyprinus carpio*, *Cyprinodon variegatus*) exhibited survival rates >90% when fed the San Pablo Bay strain. The strain with the second lowest percentage of 20:5ω3 (Great Salt Lake, Schauer *et al.*, 1980) was similarly very poor at promoting survival in marine species. Low

20:5ω3 levels, however, cannot always be referred to as the sole argument. Indeed, marine species fared somewhat poorly with regard to survival and very poorly with regard to growth when they were fed the Canadian strain, which contains more 20:5ω3 (Seidel *et al.*, 1982) than even the best strains from Brazil and RAC. The culture results with Canadian *Artemia* have to be considered separately since recent experiments (Léger, Sorgeloos, Millamena & Simpson, 1985c) have demonstrated a very good correlation between 20:5ω3 levels in several batches of San Francisco Bay *Artemia* and biomass production in *Mysidopsis bahia* reared on those batches. A similar correlation can be seen in the data of Vos, Léger, Vanhaecke & Sorgeloos (1984), who fed *M. bahia* on *Artemia* nauplii from production ponds in several Asian countries. Thus, the fatty acid 20:5ω3 does seem to be a major factor in the determination of *Artemia* quality, especially as a food for crustaceans, but also as a food for fish.

Further evidence for the importance of 20:5ω3 and 22:6ω3 (another essential fatty acid) to mud crab larvae was obtained from the experiments of Levine & Sulkin (1984). They fed several diets to *Eurypanopeus depressus* larvae and found that best survival to the megalopa stage was attained on a diet of *Artemia* nauplii or a diet of rotifers plus capsules containing *Artemia* lipids. Survival was significantly worse on a diet of rotifers alone or a diet of rotifers plus lipid-free *Artemia*. In a second experiment, they found that the survival achieved on *Artemia* nauplii or rotifers plus capsules containing 22:6ω3 was significantly better than that on rotifers alone. In none of their experiments, however, did they observed the catastrophic mortality at metamorphosis that Johns *et al.* (1980) and Seidel *et al.* (1982) reported.

Schauer *et al.* (1980) remarked that synergistic interaction effects between essential fatty acid and CHC levels may have been operated in the ISA strain studies. Thus, low levels of 20:5ω3 may have combined with high levels of total CHCs in the Great Salt Lake and San Pablo Bay strains to cause mortalities of mud crab larvae. Their argument was supported by the evidence that the Great Salt Lake strain, which had only a slightly lower level of 20:5ω3 than a sample of San Francisco Bay *Artemia* collected in 1975, but also a slightly lower CHC concentration, produced *Rhithropanopeus harrisii* mortalities (Johns *et al.*, 1980) whereas the San Francisco Bay strain did not (Johns, Peters & Beck, 1978). More recent and extensive experiments already mentioned above (Léger *et al.*, 1985c) indicated that the correlation between total CHC concentration and *Mysidopsis bahia* biomass production is very poor and that no interaction effects exist between the 20:5ω3 level and total CHCs with regard to *M. bahia*.

The analysis done on the ISA *Artemia* strains for amino acids (Seidel *et al.*, 1980b), heavy metals (Olney *et al.*, 1980), caloric content (Schauer *et al.*, 1980), and carotenoids (Soejima, Katayama & Simpson, 1980) yielded no data that could be related in any way with the biological data on test species' growth and survival. Thus, the single most important factor so far identified in defining nutritional quality of *Artemia* nauplii for marine fish and crustaceans is the content of essential fatty acids such as 20:5ω3. If one examines all the ISA studies together, a good-quality batch of *Artemia* can be considered to have a fatty-acid profile with a 20:5ω3 content of higher

than 4% of the total fatty acid methyl esters. Batches with a 20:5ω3 content between 3 and 4% may or may not be good depending on other unknown factors. Batches with less than 3% 20:5ω3 consistently yield poor growth and survival of marine organisms. The exception of this rule, however, is the Canadian strain, which was the only sulphate-lake strain tested. As Léger & Sorgeloos (1984) pointed out, more research needs to be done on the sulphate-lake strains to determine what governs their quality as a food for marine organisms. It is important to reiterate here that considerable temporal variation in 20:5ω3 content can exist within a given geographical strain (see later). Watanabe *et al.* (1980, 1982) reported large fluctuations in the quantity of 20:5ω3 during a year or between years for *Artemia* from San Francisco Bay, Brazil, and China. Léger *et al.* (1985c) reported similar variability for batches collected over several years from San Francisco Bay.

THE ENHANCEMENT OF THE DIETARY VALUE OF *ARTEMIA* NAUPLII

The enrichment of Artemia *nauplii as a solution for their nutritional deficiencies*

In the previous section we demonstrated that several factors determine directly or indirectly the food value of *Artemia* nauplii. Indirect factors may be called those that are not immediately related to the nature of the nauplius, *e.g.* presence in the culture tank of unhatched cysts, shells, and other contaminants as a result of insufficient separation and washing of the nauplii. Feeding regime and its attendant use of older instar-stages may also be considered as indirect factors affecting the dietary value of *Artemia*. Reduction of the suitability and dietary value of *Artemia* due to indirect factors may be quite easily corrected as shown above. Direct factors, however, such as size of the instar I nauplii and their nutritional composition may in practice be more problematic. When size of nauplius is critical one should select a strain that produces small nauplii; indeed there is a considerable variation between different strains and cyst size, which is correlated with length of nauplius, and is principally genetically determined.

Small *Artemia* are mainly found on the American continent (Vanhaecke & Sorgeloos, 1980; Vanhaecke, 1983). It is interesting to know that also on other continents one can produce small cysts through inoculation of a properly selected natural strain (Vos *et al., 1984). Thanks to fast developing* progress in the field of genetic selection or manipulation, artificial production of "mini-cysts" and subsequent large scale inoculation and production in suitable environments may offer unique opportunities for the near future.

Nutritional variability between different *Artemia* strains and even between harvests from the same strain may look the most insuperable drawback with regard to the use of *Artemia* in the culture of larvae. Nevertheless, the recent progress in the characterization of *Artemia* and the better understanding of at least some larval nutritional requirements, has resulted in a major breakthrough in the enhancement of the nutritional value of *Artemia*.

Not considering Hauenschild's finding that the nutritional value of *Artemia* nauplii for Hydrozoa was improved by naupliar starvation (Hauenschild, 1954, 1956), the first application of the technique of nutritional enhancement of *Artemia* nauplii was suggested by Morris (1956). As pointed out earlier, he found that marine fish larvae did not prosper in his rearing trials when fed only *Artemia* metanauplii which had consumed their yolk reserves. He noticed however, that the loss in food value in *Artemia* mentanauplii could be restored by allowing them to feed on so-called "secondary foods". These include items which are too small to be directly fed upon by fish larvae, but may be incidentally ingested or delivered by "primary foods", such as *Artemia*. Morris (1956) indeed observed that when an *Artemia* nauplius was ingested by a larva the *Artemia* squirmed violently for some minutes prior to death. These vigorous movements cause the *Artemia* to void much of its gut contents into the alimentary tract of the fish larva. Morris (1956) added algae, *e.g. Stichococcus* and *Dunaliella*, suspensions of Fleishmann yeast or boiled egg yolk to the rearing tank along with the *Artemia* nauplii and observed that these products were readily ingested; as a result the nutritional quality of the *Artemia* was more adequate. One decade later Forster & Wickins (1967) demonstrated that the food value of *Artemia* nauplii of Great Salt Lake origin could be improved for *Palaemon serratus* larvae. Several methods resulted in successful metamorphosis compared with total mortality in the controls fed Great Salt Lake nauplii only, *e.g.* substitution by at least 50% San Francisco Bay *Artemia* nauplii, addition of *Isochrysis* to the culture tank, or 4 days pre-feeding of the *Artemia* with *Isochrysis*. The experiments of Forster & Wickins (1967) further indicated that improved metamorphosis success was achieved by the enrichment of *Artemia* and not through direct ingestion of algae by the shrimp larvae. Wickins (1972) obtained similar improvements in metamorphosis success by 24 h pre-feeding Great Salt Lake nauplii at a density of 10 000·l^{-1} in an algal suspension of 300 cells·μl^{-1}. In order to avoid wastage of expensive algae and to prevent the risk that *Artemia* would grow to an unacceptable size, he determined the time at which newly hatched nauplii started to feed and their feeding rate. He noticed that the number of cells ingested increased continuously in the 48 to 60 hours after cyst incubation at 20 °C. During this period algal consumption increased from less than 500 to over 7000 cells· nauplius^{-1}·h^{-1}; as a result each nauplius could ingest more than 30 000 cells within 24 h. Higher cell densities were not recommended because of the risks of producing too large metanauplii.

The same technique was successfully applied for *Macrobrachium* larvae (Monaco, 1974; Wickins, 1976). On the contrary, Maddox & Manzi (1976) demonstrated that freshly hatched nauplii were a more superior food for *Macrobrachium* than older metanauplii whether they were fed algae or not. The idea of pre-feeding *Artemia* for the purpose of quality enhancement was tested for *Pleuronectes platessa* and *Gadus morhua* by Nordeng & Bratland (1971). Analysing the guts of wild fish larvae, they assumed that phytoplankton could be an essential source of nourishment of which laboratory larvae were deprived when fed *Artemia* nauplii alone. In their culture tests fish larvae were offered additional nutrients by means of *Artemia* which had been pre-fed for 24 h. For this they used marine *Chlamydomonas* sp.,

ω-yeast (*Saccharomyces cerevisiae*), and ground trout food. All three groups of pre-fed metanauplii were given alternately in order to ensure that the larvae received a varied diet. With plaice, metamorphosis, pigmentation, and general condition of the larvae were optimal. Although Nordeng & Bratland (1971) failed with cod, Howell (1979b) obtained a good survival in cod (*Gadus morhua*) larvae when they were given *Artemia* nauplii that were pre-fed for 2 days on *Isochrysis galbana*, while simultaneously adding the same alga plus *Pavlova lutheri* in the tanks. *Artemia* were inadequate when not pre-fed. When Howell *et al.* (1981) pre-fed *Artemia* with *Isochrysis* for only 4 h, *i.e.* a period sufficiently long to fill up their gut, the food value of these *Artemia* for turbot (*Scophthalmus maximus*) larvae improved only appreciably when this alga was also added to the larval rearing tank. Since no evidence was found for direct utilization of the algae by the larval turbot, Howell *et al.* (1981) suggested that the *Artemia*, in order to become an effective diet, had to digest the algae first. This reminds us of the earlier observations of Morris (1956).

Kelly *et al.* (1977) also obtained a better growth in *Pandalus platyceros* by adding *Phaeodactylum tricornutum* to the culture tank along with the *Artemia* nauplii. Bromley (1978) was more successful in weaning *Scophthalmus maximus* when *Pseudoisochrysis paradoxa* was supplemented in the culture tanks as food for the rotifers and *Artemia* nauplii. The beneficial effect of adding algae along with, or 'encapsulated' in *Artemia* was recognized by many authors, but an explanation for the observed nutritional enhancement of the nauplii was not given. In 1979, however, Howell (1979a) pointed out that the choice of algae used was important; *i.e.* much better results were obtained with *Scophthalmus maximus* when using *Isochrysis galbana* instead of *Dunaliella tertiolecta*. This made him suggest that the effect of adding algae was probably more related to nutrition than to their stabilizing action on water quality with which they are often credited (*cf.* green water technique in *Macrobrachium* culturing). The use of algae of inferior 'nutritional-enhancement-quality' may explain some previous reports that no improvement was noticed after pre-feeding the *Artemia* nauplii and/or adding algae. In the same year Scott & Middleton (1979) and Scott & Baynes (1979) confirmed Howell's observation, *i.e.* addition of *Dunaliella tertiolecta* during the live food phase in the culture of *Scophthalmus maximus* larvae resulted in stunted growth and high mortality. It appeared that this effect was not an expression of toxicity but of poor nutrition, probably due to a deficiency of long chain polyunsaturated fatty acids as confirmed by the fatty acid profile of this alga. Several studies in the 1970s have indeed revealed that long chain polyunsaturated fatty acids are essential for a variety of marine animals. More particularly the ω3-highly unsaturated fatty acids (ω3-HUFA) 20:5ω3 and 22:6ω3 seem to be required by marine fish and crustaceans (Owen, Adron, Sargent & Cowey,1972; Owen, Adron, Middleton & Cowey, 1975; Sick & Andrews, 1973; Yone & Fujii, 1975; Castell & Covey, 1976; Cowey, Owen, Adron & Middleton, 1976; Guary, Kayama, Murakami & Ceccaldi, 1976; Sandifer & Joseph, 1976; Gatesoupe *et al.*, 1977; Kanazawa, Teshima & Tokiwa, 1977; Kanazawa, Teshima & Ono, 1979; Yone, 1978; Castell & Boghen, 1979, Léger *et al.*, 1979).

Analyses of the fatty acid profile of different sources of *Artemia* and dif-

ferent lots from the same source by Watanabe and co-workers revealed striking differences in ω3-HUFA content (see Table III). Based on the relationship between the dietary value of *Artemia* and their ω3-HUFA content Watanabe *et al.* (1978c) proposed the following classification: 20:5ω3-rich *Artemia* sources (so-called "marine type" *Artemia*) which are a good food source for red seabream juveniles and 20:5ω3-poor sources (so-called "freshwater type" *Artemia*) which yield poor culture success in red seabream larvae. It was also demonstrated that the ω3-HUFA content in the *Artemia* could be substantially increased by feeding them for 24 to 72 h with ω3-HUFA-rich food sources, such as marine *Chorella minutissima* and ω-yeast (Imada *et al.*, 1979).

As can be seen from Table III, ω3-HUFA-enriched *Artemia* were converted into an excellent food source for red seabream juveniles. On the other hand, the ω3-HUFA content of nauplii fed diets lacking ω3-HUFA, such as baker's yeast, did not differ from starved nauplii, and no improvement in food value was noted for red seabream juveniles. The most pronounced differences between the fish fed marine type or ω3-HUFA-enriched *Artemia* and freshwater type *Artemia* were revealed in the activity test as applied by Watanabe *et al.* (1980), *i.e.* survival is determined in fish larvae 24 h after being scooped out for 5 seconds from the culture vessel and transferred into another tank; (physiologically) weak fish show a shock syndrome and die. Watanabe and colleagues concluded that not protein quality, including amino-acid profile, nor mineral composition, but the presence of essential fatty acids was the principal factor which determined the food value of *Artemia* nauplii for fish larvae. Léger (unpubl.) confirmed those findings for marine crustacean larvae by pre-feeding freshly hatched San Pablo Bay (No. 1628) *Artemia* nauplii for 24 h on micronized and defatted ricebran which was coated (GLC-stationary phase coating technique) with either cod liver oil (CLO) or rice oil (RO). When CLO-rice bran was used for enrichment, the levels of ω3-HUFA in *Artemia*

TABLE III

ω3-HUFA content of "marine type" Artemia (*Canadian and enriched San Francisco Bay* Artemia) *and "freshwater"* Artemia (*San Francisco Bay*) *and their effect on survival and growth of red seabream juveniles* (*data from Watanabe* et al., *1980*): **20:3<ω3* fatty acids

	Artemia treatment			
	Canada	San Francisco Bay		
	Newly hatched	Newly hatched	Fed *Chlorella* for 24 h	Fed ω-yeast for 24 h
ω3-HUFA content				
20:5ω3	5·2	1·6	3·2	3·4
22:6ω3	—	—	—	1·1
Σω3-HUFA*	5·8	2·4	4·1	5·1
Red seabream culture test				
Survival (%)	68·4	43·4	66·8	86·4
Survival after activity test (%)	37·5	24·1	46·1	50·0
Final length (mm)	9·57	10·13	11·13	11·67

markedly increased during pre-feeding and these *Artemia* had a high nutritional value for *Mysidopsis bahia* juveniles; on the other hand, no effect was noticed when rice oil coated ricebran was used for enrichment (see Table IV). Léger, Bieber & Sorgeloos (1985a) confirmed the beneficial effect of using ω3-HUFA-enriched San Pablo Bay (No. 1628) *Artemia* for a commercial crustacean *Penaeus stylirostris* (see Table IV). Furthermore, they observed that the pre-*Artemia* food phase (during protozoea stages) greatly affected post-larval metamorphosis success, *i.e.* the dietary quality differences between ω3-HUFA-rich and -poor *Artemia* nauplii were accentuated or attenuated, respectively, when protozeal food lacked sufficient levels of ω3-HUFA.

It is important to add that both in *Pagrus major* and *Penaeus stylirostris* the best culture results were obtained when enriched *Artemia* contained besides 20:5ω3 also substantial levels of 22:6ω3 (*e.g.* pre-fed with ω-yeast, CLO, AA18 and SEC, see Tables III and IV). In this regard, the better performance with *Acartia clausii* than with marine type *Artemia* nauplii as a food source for red seabream (Watanabe *et al.*, 1980) may thus be related not only to the higher levels of 20:5ω3 but especially to the higher content of 22:6ω3 in this marine copepod. The high amounts of both 20:5ω3 and 22:6ω3 in *Isochrysis galbana* (Watanabe & Ackman, 1974) may indeed explain the nutritional enhancements reported earlier in larval fish culture when this alga was supplemented, either directly or indirectly *via Artemia*. This further explains the improved fish culture success when, besides *Artemia*, *Tigriopus* and *Acartia*, both rich in 20:5ω3 and 22:6ω3 (Watanabe *et*

TABLE IV

ω3-HUFA content of San Francisco Bay and San Pablo Bay Artemia *nauplii, freshly hatched or pre-fed, and their nutritional value for* Mysidopsis bahia *juveniles and* Penaeus stylirostris *larvae (data from Léger* et al., *1985a,b; Léger, unpubl.): RO, rice oil coated rice bran; CLO, cod liver oil coated rice bran; AA18 and SEC, commercial enrichment diets (Artemia Systems S.A.)*

	San Francisco Bay *Artemia* (236–2016)	San Pablo Bay *Artemia* (1628)				
	Newly hatched	Newly hatched	24 h pre-fed RO	24 h pre-fed CLO	24 h pre-fed AA18	24 h pre-fed SEC
ω3-HUFA content (area %)						
20:5ω3	9·3	0·2	0·9	6·3	8·2	9·9
22:6ω3	0·2	—	—	1·5	1·5	5·9
Σω3-HUFA	11·4	0·7	1·9	8·9	10·6	17·8
Culture results with *Mysidopsis bahia*						
Survival (%)	93·3	62·0	60·0	75·0	92·5	95·8
Ind. length (μm)	5532	4587	4285	5029	5375	5254
Ind. dry weight (μg)	354	198	188	259	259	323
Biomass (mg·%)	33·0	12·3	11·3	19·4	24·0	30·9
Culture results with *Peneaus stylirostris*						
Survival (%)	47·5	34·0			45·7	63·9
Ind. wet weight (mg)	1·8	1·7			2·0	2·7
Biomass (mg·%)	85·5	57·8			91·4	172·5

al., 1978b) were also added (Fukusho, 1974). This agrees with Kuhlmann, Quantz & Witt (1981b) who found better results for turbot (*Scophthalmus maximus*) larvae when using *Eurytemora affinis* instead of *Artemia* nauplii. More evidence for the essential requirement of 22:6ω3 has recently been reported for several marine species by Holland & Jones (1981); Léger & Frémont (1981); Léger *et al.* (1985a); Bell, Henderson, Pirie & Sargent (1985); and Jones, Holland & Jaborie (in press). Because *Artemia* nauplii generally contain at most only marginal levels of 22:6ω3, ω3-HUFA-enrichment should be generally recommended for all *Artemia* sources.

The varying and low levels of ω3-HUFAs in *Artemia* are probably related to the exceptional tropical conditions under which the *Artemia* are found in nature, *i.e.* very high and changing salinity levels which favour various species of blue-greens and flagellates; contrary to the diatoms and flagellates usually found in natural sea water the blue-greens are low in ω3-HUFAs (Scott & Middleton, 1979). Indeed several authors have reported that *Artemia* and other zooplankton mainly reflect the fatty acid profile of their food (Kayama, Tsuchiya & Mead, 1963; Jezyck & Penicnak, 1966; Malins & Wekell, 1969; Ackman *et al.*, 1970; Culkin & Morris, 1969; Hinchcliffe & Riley, 1972; Bottino, 1974; Watanabe & Ackman, 1974; Sick, 1976; Claus *et al.*, 1979; Bottino *et al.*, 1980). Using a culture system for the controlled production of *Artemia* offspring (Lavens & Sorgeloos 1984, 1985) it has been demonstrated that the fatty acid profile of *Artemia* cysts and/or ovoviviparous nauplii reflects the profile in the food of the parental population. Moreover the ω3-HUFA content in the cysts and nauplii could be increased by feeding ω3-HUFA-fortified diets to the parental stock (see Table V, Lavens *et al.*, unpubl.).

Vos *et al.* (1984) studied the quality of *Artemia* produced in Southeast Asian saltponds and found that cysts produced in ponds fertilized with inorganic fertilizer had low levels of 20:5ω3 whereas those produced in ponds with water intake from mangrove waters (*i.e.* high food diversity) showed considerable levels of 20:5ω3 and sometimes traces of 22:6ω3; a similar observation was made when organic fertilizers such as poultry manure were applied (Léger, unpubl.). Watanabe *et al.* (1978b) analysed high levels of ω3-HUFA in *Moina* cultured on poultry manure. Similarly *Artemia* might accumulate ω3-HUFA directly from the manure or indirectly from algal blooms induced by this fertilizer; in this regard Jumalon & Ogburn (1985) and Jumalon, Estenor & Ogburn (1985) noticed that *Artemia* production ponds fertilized with poultry manure consistently showed blooms of *Tetraselmis* which is usually rich in ω3-HUFA (Millamena, Bombeo, Jumalon & Simpson, 1985). Fertilizer control of algal composition might be feasible in small production ponds (*e.g.* solar salt operations in Southeast Asia, Central America, *etc.*). This practice is, however, not conceivable in large solar salt operations (*e.g.* Mexico, Brazil, Australia, *etc.*) nor in the hugh lakes found all over the world. In the lakes the available algae may be suitable, unsuitable or subject to a considerable variation in quality. For years the dominant species in the Great Salt Lake (Utah, U.S.A.) has been *Dunaliella* (Stephens & Gillespie, 1976; Post, 1977), which is poor in ω3-HUFA (Scott & Middleton, 1979; Millamena *et al.*, 1985). As opposed to other strains the 20:5ω3 content in Great Salt Lake *Artemia* is remarkably constant, *e.g.* 1·8–3·6% in cysts collected from the Southern arm and 0·2–0·3% in Northern arm *Artemia* cysts (see Table XII, p. 597).

TABLE V

ω3-HUFA content in parental cysts and 1st generation offspring (cysts or nauplii) of Artemia *from two strains cultured on rice bran, either untreated (diet RBO) or HUFA-enriched diet (RBA) (data from Lavens & Léger, unpubl.): a, cysts were analysed after decapsulation; b, sum of ω3-highly unsaturated fatty acids (20:3<ω3 fatty acids); c, per cent fatty acid methyl ester of total fatty acid methyl esters; d, mg fatty acid methyl ester per g dry wt; e, cysts used for the production of the parental population*

Artemia source	Type of material[a]	Diet	ω3-HUFA content 20:5ω3 % [c]	20:5ω3 mg·g^{-1} [d]	22:6ω3 %	22:6ω3 mg·g^{-1}	Σω3-HUFA[b] %	Σω3-HUFA[b] mg·g^{-1}
France, Lavalduc								
	Parental cysts[e]	—	4·8	5·4	—	—	5·7	6·4
	1st generation cysts	RBO	0·2	0·3	—	—	0·5	0·6
	1st generation cysts	RBA	7·5	8·6	0·4	0·4	8·8	10·0
U.S.A., Great Salt Lake								
	Parental cysts	—	2·1	3·9	—	—	3·1	4·6
	1st generation cysts	RBO	0·3	0·3	—	—	0·4	0·4
	1st generation nauplii	RBO	0·5	0·6	—	—	1·3	1·7

The variability in ω3-HUFA content in the other strains may be explained by seasonal changes in algal species composition (*cf.* species diversity in San Francisco Bay and Saskatchewan Lakes, Carpelan, 1957; Haynes & Hammer, 1978) or variability in ω3-HUFA content within the same algal species (*cf.* Scott & Middleton, 1979). It has indeed been demonstrated that the nutritional composition of algae may change according to varying abiotic conditions (D'Agostino & Provasoli, 1968, 1970; Dickson, Galloway & Patterson, 1969; Provasoli, Conklin & D'Agostino, 1970; Moal, Samain & Le Goz, 1978; Scott, 1980; Enright, 1984). As a result man will always be dependent on the caprices of nature, providing ecologists and aquaculturists at one time with a present of excellent quality cysts and at other times with an inferior quality of their preferred live food source. Again, the enrichment of *Artemia* nauplii eliminates the effects of such caprices.

Enrichment techniques

Table VI summarizes the results of enrichment and culture experiments as described in the references cited. Over the past decades several techniques have been elaborated for *Artemia* nauplii enrichment. They may be classified in four groups, *i.e.* the British technique, with algae; the Japanese technique, with ω-yeast or emulsions; the French technique, with compound diets; and the Belgian technique with coated micro-particles or self-emulsifying concentrates.

The British technique. This technique has been pioneered by Forster & Wickins (1967), and Wickins (1972); *Artemia* nauplii are cultured for 24 h (Wickins, 1972) or 4 days (Forster & Wickins, 1967) on an algal suspension, mostly *Isochrysis galbana* at up to 1000 cells·μl^{-1}. The same alga was in many cases also added to the larval culture tank. A density of 10 000 nauplii ·l^{-1} in an algal suspension of 300 cells·μl^{-1} for an enrichment period of 24 h appeared to be a suitable regime to make the nauplii an adequate food for prawn larvae (Wickins, 1972). This technique may well be suited when algae have to be cultured as a food source for first-feeding larvae. Setting up an algal culture only for live food enrichment looks, however, hardly justified, especially as algal quality is variable and alternatives are available (see later).

The Japanese technique. The so-called "indirect method" developed by Watanabe *et al.* (1978c, 1980, 1982, 1983a) at first resembled the British technique. Indeed, marine algae (*Chlorella minutissima*) were used to pre-fed freshly hatched (up to 48 h hatching incubation) *Artemia* nauplii for 24 h (up to 72 h). Algal densities ranged between 14×10^6 to 18×10^6 cells·ml^{-1}. Details on densities of nauplii, however, were not given. A similar procedure was adopted using so-called ω-yeast (0·38 mg·ml^{-1} or 9×10^6 cells·ml^{-1}) as a substitute for the algae. This special yeast preparation is produced by adding cuttle fish liver oil at a 15% level to the culture medium of baker's yeast (*Saccharomyces cerevisiae*) (Imada *et al.*, 1979). Similarly to the application with algae, ω-yeast is pre-fed in newly hatched *Artemia* nauplii for 24 h.

TABLE VI

Summary of enrichment procedures for Artemia *nauplii, ω-HUFA content in* Artemia, *and results of comparative culture tests with enriched* Artemia: *this table does not include enrichment trials where the enrichment diet (mostly algae in these cases) were only added directly to the tank; experiments without a control treatment (not enriched) were not considered; the quotation +,○,±,−, refer to relative values i.e. very good, good, average, and poor culture results, respectively adjudged by interpretation of the given data; these annotations compare values within one experiment, so that no absolute comparison may be made between different experiments even when the same test organism was used; open spaces refer to same conditions (t, T) as first treatment within an experiment, or to lack of data (t,T,ω3-HUFA content, culture test): SFB, San Francisco Bay, U.S.A.; BRA, Brazil; CAN, Canada; SAM, South America; GSL, Great Salt Lake, U.S.A. (south arm); Na, North arm; SPB, San Pablo Bay, U.S.A.; t, time period in h; T, temperature in °C; %, per cent fatty acid methyl ester of total fatty acid methyl esters (area%); mg·g^{-1}, mg fatty acid methyl ester per g dry wt; Σ ω3-HUFA, sum of ω3 highly unsaturated fatty acids (note: this generally refers to the sum of ω3 fatty acids with 20 or 22 carbons and 3 or more double bounds); na, no pre-enrichment or enrichment diet was applied; tr, trace; nd, not detected; *, dry or wet basis not specified; diets A to J, see Table VII*

Artemia source	Hatching t	Hatching T	Pre-enrichment Diet	Pre-enrichment t	Pre-enrichment T	Enrichment Diet	Enrichment t	Enrichment T	ω3-HUFA-content 20:5ω3 %	ω3-HUFA-content 20:5ω3 mg·g^{-1}	ω3-HUFA-content 22:6ω3 %	ω3-HUFA-content 22:6ω3 mg·g^{-1}	ω3-HUFA-content Σω3-HUFA %	ω3-HUFA-content Σω3-HUFA mg·g^{-1}	Culture test Animal	Culture test Performance	Reference
GSL			na			na									*Palaemon*	−	Forster & Wickins, 1967
			na			*Iso-chrysis*	96									+	
GSL			na			na									*Palaemon*	−	Wickins, 1972
			na			Starved	24									+	
			na			*Isochrysis* 300 cells·μl^{-1} or 500 cells·μl^{-1}										+	
SFB (1976)	48		na			na			2·0		tr				*Pagrus*	−	Watanabe *et al.*, 1978c
			na			Starved	24		3·0		0·1						
							48		5·1		0·4						
							72		7·0		0·7						
			na			Marine *Chlorella* (18×10 cells·ml^{-1})	24		3·5		0·2					+	

TABLE VI—*continued*

Artemia source	Hatching t	Hatching T	Pre-enrichment Diet	Pre-enrichment t	Pre-enrichment T	Enrichment Diet	Enrichment t	Enrichment T	ω3-HUFA-content 20:5ω3 %	20:5ω3 mg·g^{-1}	22:6ω3 %	22:6ω3 mg·g^{-1}	Σω3-HUFA %	Σω3-HUFA mg·g^{-1}	Culture test Animal	Culture test Performance	Reference
							48		7·3		0·1						
							72		10·9		0·3						
			na			Baker's yeast (9×10^6	24		3·0		0·1					−	
						cells·ml^{-1})	72		4·3		0·4						
						ω-yeast (9×10^6	24		4·5		0·4					+	
						cells·ml^{-1})	48		7·3		1·1						
							72		8·8		1·5						
SFB			na			na			9·5		nd		9·9				
(1977)			na			Starved	24		10·7		nd		11·0				
						Marine *Chlorella*			15·0		0·3		15·5				
						Baker's yeast			13·0		0·2		13·3				
						ω-yeast			10·8		2·3		13·8				
SAM			na			na			0·3		nd		1·0				
			na			Starved	24		0·8		nd		1·6				
			na			Marine *Chlorella*			1·7		nd		2·5				
			na			Baker's yeast			0·9		nd		1·6				
			na			ω-yeast			6·6		1·7		8·9				
CAN			na			na			12·1		nd		12·3				
						Starved	24		12·8				12·8				
			na			Marine *Chlorella*			12·0		nd		12·0				
			na			Baker's yeast			14·3		nd		14·3				
			na			ω-yeast			10·5		1·9		13·1				
SFB-1	30-48	23-28	na			na			1·6		nd		2·4		*Pagrus*	−	Watanabe *et al.*, 1980
(78 C)			na			Marine *Chlorella*	24	20	3·2		nd		4·1			±	
						14×10^6 cells·ml^{-1}											
			na			ω-yeast. 0·38 mg·ml^{-1}			3·4		1·1		5·1			+	
			na			Starved			2·4		nd		3·2				
CAN			na			na			5·2		nd		5·8			±	
SFB-2			na			na			7·0		nd		7·5			o	
(78B)			na			*Spirulina* 0·5 mg·ml^{-1}	24	20	5·9		nd		5·9			o	
			na			ω-yeast			7·3		0·9		8·4			+	
			na			Starved			7·6		nd		8·4				

SFB	24	28	na			Starved	4	16-18		*Scophthalmus*	−	Howell *et al.*, 1981
			na			*Isochrysis* (excess)		28			−	
BRA			na			Starved		16-18			+	
GSL (1977)	25-29	25	na			na				*Rhithropanopeus*	−	Johns *et al.*, 1981a
			na			Starved	24				−	
			na			*Isochrysis, cf.* Wickins, 1972					−	
BRA			na			na					+	
SFB			na			A	48			*Dicentrarchus*	−	Robin *et al.*, 1981
			na			B or C					+	
BRA			na			B					o	
			na			A					o	
			F	48		I ($2 \cdot 5$ or $5 \text{ g} \cdot 10^6$ metanauplii)	0·5			*Scophthalmus*	+	Gatesoupe, 1982
SFB	48	24	D	48	24	na				*Dicentrarchus*	−	Robin, 1982
			D			H	0·5				±	
			E			H					+	
			E			na					+	
SFB	48	24-26	na			na			0·5*	*Paralichthys*	−	Watanabe *et al.*, 1982
			na			Cuttle fish liver oil emulsion = 1·5 g cuttle fish liver oil, 0·3 g egg yolk, 20 ml water, Baker's yeast (same weight as nauplii) per 30 l	15-19	24-26	4·0*	(Exp. I)	+	
			na			*Idem*, but corn oil instead of cuttle fish liver oil (= corn oil emulsion)			0·5*		−	
	48	24-26	na			na			1·0*	*Oplegnathus*	−	
			na			Cuttle fish liver oil emulsion *(cf. supra)*	15-19	24-26	3·1*	(Exp. II)	+	
			na			ω-yeast alone			3·0*		+	
			na			Baker's yeast alone			0·8*		−	
			na			Baker's yeast	15-19	24-26	1·2*	*Pagrus*	−	
			na			Corn oil emulsion			0·3*	(Exp. III)	−	
			na			Pollock liver oil emulsion			2·1*		+	
			na			Cuttle fish liver oil emulsion			7·7*		+	
			na			Methyl ω3-HUFA emulsion			7·1*		+	
	48	24-26	na			Baker's yeast	15-19	24-26	0·9*	*Pagrus*	−	
			na			Corn oil emulsion			0·7*	(Exp. IV)	−	
			na			Pollock liver oil emulsion			1·5*		±	
			na			Pollock & cuttle fish liver oil emulsion			3·0*		+	

TABLE VI—*continued*

Artemia	Hatching		Pre-enrichment			Enrichment			ω3-HUFA-content						Culture test		Reference
source	t	T	Diet	t	T	Diet	t	T	20:5ω3		22:6ω3		∑ω3-HUFA		Animal	Performance	
									%	mg·g^{-1}	%	mg·g^{-1}	%	mg·g^{-1}			
			na			Cuttle fish liver oil emulsion								3·3*		+	
			na			Methyl ω3-HUFA emulsion								10·1*		+	
SFB	48	23-24	na			5 ml lipid (pollock liver oil, cuttle fish liver oil or HUFA-methylester mixture), 1 g raw egg yolk or other emulsifier, 100 ml sea water, 12 g baker's yeast per 60 l	24	24-26									Watanabe *et al.*, 1983b
SFB	24	28	na			na			0·5	0·5	nd	nd	2·7	2·8	*Penaeus*	−	Léger *et al.*, 1985a
(No 1628)			na			AA 18 (Artemia Systems S.A.); micronized powder containing 8·5% 20:5ω3 and 9·9% 22:6ω3 0·6 g·3 × 10^5 nauplii (assuming a hatching efficiency of 150 000 n·g^{-1})	24	30	5·9	7·9	3·4	4·6	11·2	15·1		+	
SFB No. 236-2016)			na			na			8·8	11·2	0·6	0·8	9·9	13·1		+	
SFB	48	24	na			na							5·7	9			Robin *et al.*, 1984
(No. 2257)			G	48	24	na							12·1	8			
			100 g (Day 0) + 120 g (Day 1)·m^{-3}; 15 nauplii·ml^{-1}														
			G			J(5 g·10^{-6} metanauplii)	0·5						14·9	16			
SFB			na			na									*Pomatoschistus*	o	Jones *et al.*, in press
			na			Microcapsules (10-30 μm, 300-500 ml^{-1}) containing cod oil	24									±	
			na			*Idem*, containing pollack oil										+	
GSL (Na)	24	25	na			na			0·3	0·5	nd	nd	1·2	1·9	*Mysidopsis*	−	Léger *et al.*, 1985b
	36	28-30	na			Self-emulsifying ω3-HUFA concentrate (SUPAR, Artemia Systems S.A.) added to			4·6	8·0	4·0	6·8	9·7	16·7		+	

				hatching medium from start of incubation											
	24	28-30	na	Self-emulsifying ω3-HUFA concentrate (SELCO Artemia Systems S.A.), 0·6 g·l^{-1} added to hatching medium after 24 h incubation of 1·5 g cysts·l^{-1}	12	28	4·5	6·4	2·4	3·3	8·2	11·2			
					24	28	7·0	12·1	4·4	7·5	13·3	22·1			
					48	28	12·0	22·3	6·4	11·9	21·0.	38·3			
	24	28-30	na	self-emulsifying ω3-HUFA concentrate (SELCO), 0·6 g·l^{-1}, added after separation of nauplii (3×10^5 n·l^{-1})	12	28	5·2	7·9	2·9	4·4	9·8	14·4			
							9·9	21·3	5·9	12·7	17·8	37·4		+	
					24	28	13·5	35·2	7·0	18·1	23·0	58·6			
SPB (No. 1628)	24	25	na	na	48	28	0·5		nd		2·7		*Mysidopsis*	–	Léger unpubl.
			na	Starved	24	24	1·4		0·6		3·5			–	
			na	Cod liver oil coated rice bran (5 cm Secchi-disk reading)			6·3		1·5		8·9			+	
			na	Rice oil coated rice bran (5 cm Secchi)			0·9		nd		1·9			–	
SFB (No. 236-2016)	24	25	na	na			9·3		0·2		11·4			+	

The advantage of using ω-yeast is mainly that one has a better control of the ω3-HUFA content since fish oils are generally rich in both 20:5ω3 and 22:6ω3. The disadvantage of this technique, however, is that as ω-yeast is required to be always in a living condition this technique can only be applied at places close to a production centre (Watanabe, pers. comm.).

Watanabe *et al.* (1982, 1983b) have also developed a "direct method" in which emulsified fish oils in combination with baker's yeast are pre-fed in *Artemia* nauplii. Indeed, *Artemia* nauplii are able to pick up emulsified lipids very easily from their culture medium. After 6 to 12 h enrichment a maximal ω3-HUFA incorporation was demonstrated. The emulsion is made up by blending 1·5 g lipid (*e.g.* cuttle fish liver oil) with 0·3 g raw egg yolk and 20 ml sea water for 3 min for use in a 30-l tank. Baker's yeast is added in an equivalent weight to the nauplii in the tank (Watanabe *et al.*, 1982). In later experiments Watanabe *et al.* (1983b) outlined a similar enrichment technique: 5 ml lipid are emulsified (lipid: egg yolk: water = 5:1:95) with a blender for 1 min and added to a 60-l enrichment tank together with 12 g baker's yeast and *Artemia* nauplii harvested from the hatching tank (48 h incubation); enrichment lasts for 24 h at 24-26 °C. Comparing raw egg yolk, soybean lecithin, and casein-Na as emulsifiers, no significant differences were noted in ω3-HUFA accumulation in the *Artemia* nauplii.

The incorporation of ω3-HUFA in *Artemia* appeared to be much lower than in rotifers: *i.e.* using an emulsified methyl ester mixture containing 85% ω3-HUFA, the incorporation rate in rotifers could yield 60% of total fatty acids within 3 h whereas in *Artemia* nauplii a minimum of 12 h were required to reach the 20% level. When using emulsified cuttle fish liver oil Watanabe *et al.* (1982) report ω3-HUFA levels from 0·31 to 0·77% (dry or wet weight basis not specified), with pollock liver oil 0·15 to 0·21%, and with ω3-HUFA methyl ester mixture 0·75 to 1·01%. They attributed these ranges in incorporation rate to varying culture conditions (*e.g.* water temperature) and density and activity of the nauplii used. It was also observed that the survival rate of the *Artemia* nauplii during enrichment fluctuated, *e.g.* 69·3% with pollock liver oil, 56·2% with cuttle fish liver oil, and 84·0% with ω3-HUFA mixture emulsion.

From their experiments Watanabe and colleagues concluded that *Artemia* containing at least 0·3% ω3HUFA (dry or wet weight basis not stated) may be a satisfactory single feed for marine fish. They added, however, that *Artemia* enrichment should always be applied since lipid contents in *Artemia* gradually decrease after hatching.

The French technique. Robin, Gatesoupe & Ricardez (1981) succeeded in improving the dietary value of San Francisco Bay *Artemia* for seabass (*Dicentrarchus labrax*) larvae by pre-feeding them for 2 days on a compound diet composed of *Spirulina* powder, I.F.P. yeast (a methanol yeast, used to reduce the quantity of the expensive *Spirulina*), DL-menthionine, choline chloride, D-glucosamine HCL, cholesterol, cod liver oil, and a vitamin premix (see Table VII, Diets B and C). No further improvement was achieved when enriching good quality Brazilian *Artemia* with the same diet. In another experiment Robin (1982) and Robin *et al.* (1984) designed a 2-step enrichment technique which consists in pre-feeding newly hatched nauplii (48 h cyst incubation) for 48 h on a compound diet

(Table VII, Diet E or G) after which the nauplii are transferred into another container for a 30-min enrichment with another compound diet, consisting mainly of fish autolysate, cod liver oil, vitamins, and minerals (Table VII, Diet H). The 2 days pre-feeding idea originates from the observation of Anderson (1967) that feeding of *Artemia* is impossible before their second moult which takes place 30 h after hatching at 20 °C. When fish larvae were fed *Artemia* nauplii which were 48-h pre-fed on brewer's yeast (Diet D), survival and growth was inferior to any other case where a compound diet was pre-fed (Diet E) followed or not by a subsequent enrichment batch (30 min, diet H). Application of an enrichment bath (Diet H) after 48 h pre-feeding on brewer's yeast did significantly improve the nutritional value of *Artemia* nauplii but larval growth was superior in those cases where the compound diet was pre-fed. An extra enrichment batch (30 min Diet H) in the latter treatment did not further improve its quality. After 48 h pre-feeding San Francisco Bay nauplii on Diet G, the ω3-HUFA content increased from 5·7% (9mg·g^{-1}) to 12·1% (8mg·g^{-1}); after subsequent enrichment for 30 min with Diet J ω3-HUFA levels reached 14·9% (16mg·g^{-1}; all data expressed on a dry weight basis).

Gatesoupe (1982) demonstrated that for larval turbot (*Scophthalmus maximus*) post-weaning survival and growth are largely improved when live food organisms (*Brachionus* and *Artemia*) are enriched. *Artemia* were first pre-fed for 48 h on a compound *Artemia* diet (Diet F screened through a 48-μm mesh screen) followed by a 30-min enrichment batch (Diet I). The enriched nauplii are offered to the turbot larvae along with the enrichment diet using a drip supply. The feeding of enriched rotifers and *Artemia* is particularly important in stress situations—both occasional stress (*e.g.* an infection) or the inevitable stress of weaning. Incorporation of antibacterial drugs in rotifers as applied by Gatesoupe (1982) using the same enrichment procedures might be equally well applicable to *Artemia*.

The Belgian technique. The Belgian enrichment technique consisted at first in pre-feeding newly hatched *Artemia* nauplii with ω3-HUFA coated micro-particles (5 cm Secchi-transparency or 0·6g·l^{-1} for 3×10^5 nauplii·l^{-1}; Léger, unpubl.). These micro-particles, *e.g.* micronized rice bran, were coated with various fish oils using a similar technique as used in preparing stationary phases for packed column gas-liquid-chromatography. Later, a compound analogue was formulated for larger scale testing in shrimp and fish hatcheries (Léger *et al.*, 1985a; Van Ballaer *et al.*, 1985). Using this compound analogue diet maximal ω-HUFA build-ups in *Artemia* within 24 h after hatching were at least as good as what had been reported in literature (see Table VI). The preparation of coated micro-particles is, however, complex and expensive. Therefore, another even more effective enrichment diet was developed in the form of a self-emulsifying enrichment concentrate (Léger *et al.*, 1985b). This diet is a self-dispersing complex mixture of mainly ω3-HUFA sources, vitamins, carotenoids, phospholipids, steroids, and emulsifiers. After simple dilution in water aerated by an airstone it produces finely dispersed globules which are readily available for ingestion by the nauplii. The advantages of this formulation are its ease in use and its effectiveness, *i.e.* ω3-HUFA accumulation rates in *Artemia* nauplii, especially the levels of 22:6ω3, largely surpass the figures reported in literature (see Table VI).

TABLE VII

Composition (%) of pre-enrichment and enrichment diets used in the French technique for Artemia *enrichment: A, B, C, after Robin* et al., *1981; D, E. H, after Robin, 1982; F, I, after Gatesoupe, 1982; G, J, after Robin* et al., *1984; diets A and D served as control diets*

	Pre-enrichment diets (48 h)							Enrichment bath (0·5 h)		
	A	B	C	D	E	F	G	H	I	J
Spirulina (dry)	100	87·9	40			40				
I.F.P.-yeast			40			40				
Brewer's yeast				100	89·4		89·4			
Fish autolysate								73	73	73
Cod liver oil		4	4		4	4	4	10	10	10
D-glucosamine HCl		0·5	0·5			0·5				
Cholesterol		1	1			1				
Choline chloride		2	2		2	2	2	4	4	4
DL-methionine		1	1		1	1	1	2	2	2
Vitamin premix		3·6	3·6		3	3·2		10	9·6	
Vitamin mineral premix							3·6			11
Corn starch						7·9				
$CaHPO_4$					0·5	0·3		0·8	1	
$FeSO_4 \cdot 7H_2O$					0·1	0·1			0·4	
$FeCl_2 \cdot 7H_2O$								0·2		

Different application procedures have been proposed, *i.e.* enrichment can be done after separation or without separation of the nauplii from the hatching debris. The latter technique indeed simplifies enrichment procedures for large scale applications, for which after all they ought to be developed. A first technique consists in incubating cysts, pretreated with a self-emulsifying concentrate, for 36 h at 28–30 °C. After this, the enriched *Artemia* nauplii are harvested and ready to be fed to the predator. Applying this technique, hatching and enrichment occur in the same tank without extra manipulations. Enrichment levels are high ($\Sigma\omega$3-HUFA = 16·7 mg·g^{-1} dry wt) for a total incubation time which is considerably shorter than the time periods (hatching + enrichment) claimed for the previously described techniques.

A second technique implies the addition of a self-emulsifying concentrate into the hatching tank after 24 h hatching incubation at 28–30 °C. Separation of the enriched nauplii is done after a 36 h total incubation period. After this period enrichment levels ($\Sigma\omega$3-HUFA = 11·2 mg·g^{-1}) will further increase but separation of the nauplii from the hatching debris becomes difficult.

A third technique resembles French and Japanese techniques, *i.e.* after hatching and separation nauplii are incubated in a separate enrichment tank. Nauplius density, however, is higher (up to $3 \times 10^5 \cdot l^{-1}$) and mortality after 24 h enrichment is minimal. Enriched metanauplii are harvested after 12 h enrichment ($\Sigma\omega$3-HUFA = 14·4 mg·g^{-1}), 24 h enrichment ($\Sigma\omega$3-HUFA = 37·4 mg·g^{-1}) or 48 h enrichment ($\Sigma\omega$3-HUFA = 58·6 mg·g^{-1}). For the last case lower naupliar densities are recommended. These high ω3-HUFA accumulation rates, which however may vary according to the ω3-HUFA-source used and to the enrichment conditions (*e.g.* temperature, aeration, naupliar density) are the result not only of optimal diet composition and presentation, but also of proper enrichment procedures. The first difference with other techniques is indeed the shorter hatching incubation period (24 h instead of mostly 48 h). Hatching conditions are optimized and controlled to such an extent that a maximal hatch is achieved within a minimal time. The advantage of this is that the energy decrease in the nauplii will never drop beyond a minimal loss, which inevitably occurs during yolk absorption. Indeed, attention is necessary so that the enrichment diet is available in the hatching medium at the moment of first feeding (instar II stage). Moreover poor hatching synchrony in *Artemia* cysts (*e.g.* time lapse between appearance of first and last hatching nauplius can vary from 5 h to 17 h at 25 °C, Vanhaecke, 1983) implies that first feeding time of nauplii will also be spread. In this regard nauplii should be transferred as soon as possible, before first feeding, into the enrichment medium. Application of these enrichment procedures will result not only in high ω3-HUFA accumulation rates, but also in minimal size increases of enriched nauplii, *e.g. Artemia* enriched according to Japanese and French techniques reach >900 μm, whereas Belgian procedures result in similar and higher enrichment levels in nauplii measuring 660 μm (12 h enrichment) to 790 μm (48 h enrichment).

Conclusions

The application of pre-feeding *Artemia* nauplii on ω3-HUFA enrichment diets has been shown to be effective in enhancing the dietary value of several strains and lots of *Artemia*. Enriched nauplii have an improved nutritional composition since they have a higher energy content and contain all essential fatty acids especially 22:6ω3 which is mostly absent in nauplii from whatever strain. The same enrichment techniques can also be used to transfer other nutrients, prophylactics and therapeutics into the predator larvae *via* the *Artemia*.

The use of enriched *Artemia* in larval culture is reflected in improved performances in terms of both survival and growth. Consequently, culture performance in later stages will also be improved. Fish and shrimp larvae fed enriched *Artemia* are indeed healthier and more resistent to stress conditions, *e.g.* infections, weaning, and transfer from indoor fully controlled hatchery tanks to the wild environment in nursery ponds. The effect of *Artemia* quality on culture performance in later stages has indeed been reported by several authors (New, 1976; Meyers, in Hanson & Goodwin, 1977; Ablett & Richards, 1980; Howell *et al.*, 1981; Gatesoupe, 1982; Bromley & Howell, 1983; Conklin, D'Abramo & Norman-Boudreau, 1983; Wilkenfeld *et al.*, 1984; Geiger & Parker, 1985). The only disadvantage of using enriched *Artemia* is their larger size which may limit their use in the early larval stages. In this cause freshly hatched high quality nauplii should be fed for the first days before gradually switching to enriched metanauplii. Optimized enrichment procedures may, however, reduce the disadvantage of size.

THE SEARCH FOR SUBSTITUTES AND REDUCED DEPENDENCE ON *ARTEMIA* CYSTS

The availability of sufficient quantities of food organisms is a prerequisite for any successful rearing attempt (May, 1970; Barnabé, 1976; Girin & Person-Le Ruyet, 1977; Paulsen, 1980). In this regard, the availability of *Artemia* under the form of storable dry cysts as an off-the-shelf live food has to a great extent accounted for its success in larval rearing. World cyst demand was estimated to be 60 metric tons (MT) in 1981 (Sorgeloos, 1981), 80–90 MT in 1985 and 150–170 (MT) in 1990 (Lai & Lavens, 1985). Current cyst supplies (different quality products) reach over 200 MT (Lai & Lavens, 1985) and thus exceed by far actual demands. In the 1970s the use of *Artemia* in aquaculture was, however, questioned because of an unreliable availability and high price (Bardach *et al.*, 1972; Roberts, 1974; Person-Le Ruyet, 1976; Wickins, 1976; ASEAN, 1977; Gatesoupe *et al.*, 1977; Goodwin & Hanson, 1977; Bigford, 1978; Glude, 1978a,b; Murai & Andrews, 1978; Smith *et al.*, 1978; Girin, 1979; Meyers, 1979; Manzi & Maddox, 1980; Sorgeloos, 1980c). This situation has generated efforts to substitute *Artemia* by other live food organisms and by artificial diets. Furthermore, research has and is being conducted to reduce the dependence on *Artemia* cysts by optimization of feeding levels and techniques, selecting the most bioeconomical strains, using supplemental diets, applying early weaning techniques and using decapsulated cysts and on-grown *Artemia*. A

review of the results of these efforts is beyond the purpose of this review. A brief summary will, however, accentuate once more the versatility in use and nutritional quality of *Artemia* nauplii.

The substitution of Artemia

In summary we may state that for most fish and crustacean species studied complete substitution of *Artemia* nauplii by other food organisms or artificial diets has not been yet achieved.

The collection of wild plankton and other organisms may in some cases indeed provide a welcome supplement to high quality live food, but this method is hardly dependable beyond a laboratory scale (Fabre-Domergue & Bietrix, 1905; Dexter, 1972; Rakowicz, 1972; Houde, 1973; Girin & Person-Le Ruyet, 1977; Nellen *et al.*, 1981). Similarly, the intensive culture of wild food organisms still has to prove its year-round reliability on an industrial scale. None the less, interesting results have been obtained on a small scale with copepods (Kahan, 1980; Watanabe *et al.*, 1980; Kuhlmann *et al.*, 1981a,b, 1982; Kahan, Uhlig, Schwenzer & Horowitz, 1981/1982; Lee, Hu & Hirano, 1981; Kuronuma & Fukusho, 1984; Nellen *et al.*, 1981; Witt, Quantz & Kuhlmann, 1984), amphipods (Good, Bayer, Gallagher & Rittenburg, 1982), mysids (Ogle & Price, 1976; Kuhlmann *et al.*, 1981b), rotifers (Berrigan, Willis & Halscott, 1978; Yamasaki & Hirata, 1982), and nematodes (Kahan, 1979; Wilkenfeld *et al.*, 1984).

Not all trials using other live food as a substitute for *Artemia* nauplii were equally promising or successful for fish and crustacean larvae (Kurata, 1959; Gun'ko & Pleskachevskaya, 1962; May, 1970; Campillo, 1975; Fukusho, 1979; Beck, 1979; Flüchter, 1980; Hogendoorn, 1980; Dejarme, 1981; Anonymous, 1984; Emmerson, 1984). Kanazawa (1984) further stated that the mass culture of other live food organisms not only requires much labour and expensive equipment but its success also fluctuates with climatic conditions. Besides, the nutritional value of planktonic organisms is occasionally variable which restricts their possible utilization on a large scale. Following Kanazawa (1984) the development of artificial diets is one of the most important research areas for intensive larval culture. Along with this author all people involved with larval rearing will agree on the need of developing suitable artificial diets for substituting live food organisms.

Several types of artificial diets have been formulated ranging from natural products, compound diets to micro-encapsulated diets. Artificial diets are indeed appealing because of year-round availability, ease of handling and storage, uniform and constant nutritional quality, optimal size, possible germ-free formulation, no need to wean larvae, *etc.* On the other hand, some inherent problems still have to be solved: *e.g.* optimal nutritional composition (since larval requirements are as yet far from known), buoyancy, nutrient leaching, water quality problems, digestibility, production complexity and cost. Using formulated diets as a substitute for *Artemia*, promising and some successful results have been obtained (Adron, Blair & Cowey, 1974, 1977; L'Herroux *et al.*, 1977; Dabrowski *et al.*, 1978, 1984; Villegas & Kanazawa, 1978; Jones, Kanazawa & Rahman, 1979, unpubl.; Teshima, Kanazawa & Sakamoto, 1982; Levine, Sulkin & Van Heukelem, 1983). More numerous, however, are the less successful trials

and failures (Broad, 1957; Regnault, 1969; San Feliu, 1973; Campillo, 1975; Barnabé, 1976; Gatesoupe *et al.*, 1977; Berrigan *et al.*, 1978; Murai & Andrews, 1978; Hogendoorn, 1980; Beck, 1979; Günkel, 1979; Schauer *et al.*, 1979; Manzi *et al.*, in Manzi & Maddox, 1980; Reddy & Shakuntala, 1980; Sandifer & Williams, 1980; Tacon & Cowey, 1982; D'Abramo, Baum, Bordner & Conklin, 1983; Bengtson *et al.*, 1978; Conklin, Devers & Shleser, 1975; Conklin, Goldblatt & Bordner, 1978; Dabrowski & Kaushik, 1984).

Total replacement of live food, has indeed met with limited success, *i.e.* despite the best efforts of scientists throughout the world, no artificial diet has yet been produced that supports long-term growth and survival comparable with that of live food organisms Bengtson *et al.*, 1978; (Beck, 1979; Cowey & Tacon, 1982; Bromley & Howell, 1983). Even the most advanced artificial diets such as micro-encapsulated diets have achieved only limited success in replacing live food, eventually caused by lack of acceptability due to insufficient gustatory stimulation invoking ingestion (Jones *et al.*, in press). On the other hand, the indirect use of those diets to improve the nutritional value of conventional live food such as *Artemia* and rotifers is proving much more successful. (See also Sakamoto, Holland & Jones, 1982; Jones *et al.*, in press.)

The reduced dependence on Artemia *cysts*

Although substitution of *Artemia* is not realistic yet, a reduced dependence on *Artemia* can be pursued in various ways. Optimizing feeding levels and feeding techniques constitutes the first opportunity for improvements. Indeed, in many cases *Artemia* is fed in excess, often only once a day. The consequences of this wasteful practice have been described earlier. Barahona-Fernandes & Girin (1977), therefore, rightly advise restriction in the daily amounts of *Artemia* nauplii to the intake capacity of the larvae. Bryant & Matty (1980) agree that considerable savings may be achieved by adjusting *Artemia* levels according to changing requirements with larval age.

Besides optimal feeding levels and techniques, Vanhaecke & Sorgeloos (1983b) claim that in the rearing of larval carp 10 to 75% of *Artemia* costs can be saved by selecting the best bioeconomical strain of *Artemia*. Their selection is based on the quantity of cysts needed per gram carp-biomass produced. This quantity is mainly determined by the hatching characteristics of the source of cyst used. For this, besides cyst price, hatching quality may be used as a selection criterion. When price and hatching quality are comparable, they recommend the use of *Artemia* strains producing large nauplii since these guarantee best growth in carp larvae.

As discussed earlier the nutritional quality of *Artemia* does not affect culture results as much in freshwater species as in marine species. For the latter, selection of the most bioeconomical *Artemia* strains should, therefore, also take into account differences in size and nutritional value.

A reduced dependence on *Artemia* cysts, without affecting culture performance, may also be achieved by supplementing a reduced *Artemia* ration with other foods such as artificial diets and other live, freshly killed or conserved food organisms (Meske, 1973; Sick & Beaty, 1974; De Figuei-

redo, 1975; Christiansen & Yang, 1976; Goodwin & Hanson, 1977; Berrigan *et al.*, 1978; Murai & Andrews, 1978; Al Attar & Ikenoue, 1979; Bengtson *et al.*, 1978; Günkel, 1979; Meyers, 1979; Conklin, D'Abramo, Bordner & Baum, 1980; Hogendoorn, 1980; Manzi & Maddox, 1980; Seidel *et al.*, 1980a; Spitchak, 1980; Soebiantoro, 1981; New & Singholka, 1982; Wilkenfeld *et al.*, 1984; Bombeo, 1985).

Of significant importance in saving on *Artemia* cysts are recent developments in the elaboration of early weaning techniques for fish larvae. These techniques aim to switch from *Artemia* nauplii to inanimate diets (*e.g.* artificial diets, freshly killed or conserved organisms) as early as possible in the development of larvae. Larval development of fish may indeed last from 45 to 90 days compared with a few weeks in shrimp. Several authors report successful trials in this regard (Bromley, 1978; Person-Le Ruyet, Alexandre, Le Roux & Nedelec, 1978; Girin, 1979; Metailler *et al.*, 1981; Cadena Roa *et al.*, 1982a,b; Gatesoupe & Luquet, 1981/1982; Bromley & Howell, 1983; Gatesoupe, 1983; Duray & Bagarinao, 1984). It is noteworthy that weaning success is to a large extent determined by the quantity and quality of *Artemia* fed during earlier development before weaning (Forster & Wickins, 1967; Bromley, 1978; Bromley & Howell, 1983).

Finally, the use of decapsulated cysts and on-grown *Artemia* (see later) may provide extra means of reducing the quantity of *Artemia* cysts needed.

THE USE OF DECAPSULATED *ARTEMIA* CYSTS

Decapsulated cysts are *Artemia* embryos surrounded only by the embryonic cuticle and the protecting outer cuticular membrane (see Fig. 8). Decapsulation is achieved by dissolving the chorion of the cysts in an alkaline hypochlorite solution. When properly carried out, the viability of the embryo is not affected.

The pioneering procedure was described in 1962 by Nakanishi, Iwasaki, Okigaki & Kato for the sterilization of *Artemia* cysts, *i.e.* they used a chilled diluted antiformin solution which was later also used by Lenhoff & Brown (1970, see above). Since then several authors have applied similar techniques; some of them noticed that at higher hypochlorite concentrations the cyst shell dissolved completely (Broch, 1965; Katsutani, 1965; Morris & Afzelius, 1967; Clegg & Golub, 1969; Slobin & Moller, 1976). A routine decapsulation technique for large-scale application was first described by Sorgeloos *et al.* (1977) and improved by Bruggeman *et al.* (1979, 1980) and Sorgeloos *et al.* (1983). This technique involves the following consecutive steps: hydration of the cysts because only fully spherical cysts can be completely decapsulated, treatment with alkaline hypochlorite to remove the chorion, washing and deactivation of the residual active chlorine, followed by direct use or dehydration for storage. The advantages of using decapsulated cysts are numerous.

(1) Decapsulated cysts are sterile thus eliminating the potential risk of introducing germs *via* hatched nauplii into the culture water of the predator. Furthermore, bacterial development during hatching incubation is significantly reduced.

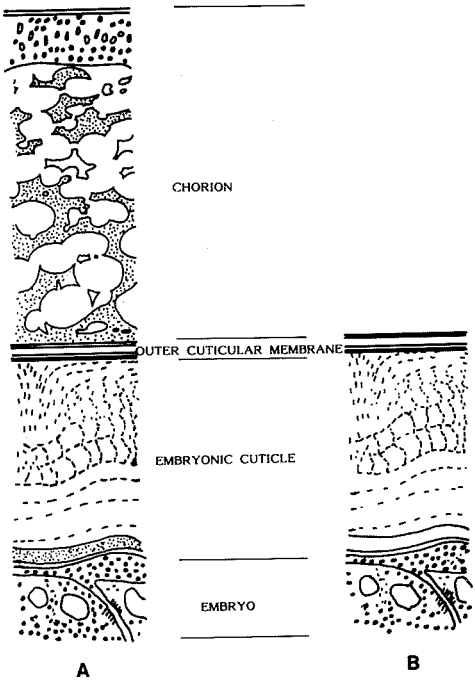

Fig. 8.—Schematic diagram of outer membranes of untreated (A) and decapsulated (B) *Artemia* cyst shell (modified from Morris & Afzelius, 1967).

(2) Because the chorion is removed separation of the nauplii from the hatching debris becomes superfluous. The only membrane discarded by the nauplius at hatching is the thin transparent embryonic cuticle which has proved to be unharmful for crabs and shrimps (Sorgeloos, 1979). As a result, after hatching of decapsulated cysts, the only procedure needed is to rinse the nauplii before feeding them to the predator.

(3) In some strains hatchability of *Artemia* cysts is significantly improved after decapsulation, *e.g.* hatching percentages increase by 1·8 to 230·3%, and because naupliar dry weights are also higher after decapsulation, hatching outputs improve by 2 to 144% (Bruggeman *et al.*, 1980; Vanhaecke & Sorgeloos, 1983a).

(4) Decapsulated cysts may be used as a direct food source for fish and crustacean larvae eliminating the need for hatching of the cysts. Several authors have indeed demonstrated the potential of using decapsulated cysts as a direct food source for decapod and fish larvae, *e.g. Scylla serrata* (Lavina in Sorgeloos, 1979), *Penaeus monodon* (Mock *et al.*, 1980a,b; Lavina & Figueroa, 1978), *P. indicus*, *Metapenaeus ensis*, *M. endeavoori*, *Macrobrachium rosenbergii* (Lavina & Figueroa, 1978), *Metapenaeus monoceros* (Royan, 1980), *Penaeus kerathurus* (Rodriguez, Martin & Rodriguez, 1980, in Sorgeloos *et al.*, 1983), *Penaeus setiferus* (Wilkenfeld *et al.*, 1984), *Chanos chanos* (De los Santos, Sorgeloos, Lavina & Bernardino, 1980; Nanayakkara, Sunderam & Royan, 1985),

Cyprinus carpio (Devrieze, 1984), *Poecilia reticulata* (Sorgeloos *et al.*, 1977), *Oreochromis niloticus, Etraplus suratensis* (Nanayakkara *et al.*, 1985), and many ornamental fish species like black mollies, red sword tails, gouramies, angles, tetras, barbs, and gold fish (Sumitra-Vijayaraghavan *et al.*, 1985). Not all larval species, however, digest decapsulated *Artemia* cysts equally well; larvae of *Solea solea* survive well on a diet of decapsulated cysts but their digestion takes 12 h and as a result growth is retarded (Dobbeleir, 1978, in Sorgeloos, 1979).

The use of decapsulated cysts as a direct food source implies several advantages.

(1) Because their diameter and volume are smaller (30 to 40%) than in freshly hatched nauplii (Vanhaecke & Sorgeloos, 1980; Vanhaecke, Steyaert & Sorgeloos, 1980; Vanhaecke, 1983) they can be fed to earlier larval stages.
(2) The energy content of decapsulated cysts is 30 to 57% higher than in freshly hatched nauplii (Vanhaecke, 1983; Vanhaecke *et al.*, 1983). This means that for an equal hunting effort a high energy intake will be achieved resulting in better growth and considerable savings in *Artemia* cysts (Anonymous, 1980; Devrieze, 1984; Nanayakkara *et al.*, 1985). Devrieze (1984) indeed demonstrated that for the production of the same carp biomass 10 to 23% *Artemia* cysts could be saved during the first week and 32 to 36% during the second week by using decapsulated cysts instead of freshly hatched nauplii.
(3) Cysts that have lost the capacity to hatch may be valuated. About 50% of present cyst stocks have a low commercial value because of their low hatchability (*e.g.* below 50%; Lai & Lavens, 1985) thus their valuation as decapsulated cysts might be more attractive.

The main problem when using decapsulated cysts as a direct food source is their fast sedimentation in sea water which makes them unavailable for planktonic larvae, unless they hatch. Their availability in the water column may be improved, at least in small scale cultures, by using conical tanks equipped with air-water-lifts. The use of dried decapsulated cysts which float and upon hydration sink only slowly may be a better solution, *e.g.* growth in carp larvae was significantly better when using dried instead of freshly decapsulated cysts (Devrieze, 1984). The same author also showed that the addition of dried decapsulated cysts at a ration of 25% of the diet significantly improved weaning success in carp larvae. In conclusion, the application of dried decapsulated cysts provides very interesting opportunities for application in intensive culture systems. A simplification of the decapsulation technique is, however, recommended if application at a larger scale is to be successful.

THE USE OF ON-GROWN AND ADULT *ARTEMIA*

In contrast to the very extensive documentation dealing with the use of *Artemia* nauplii as a food source, similar literature on the application of on-grown and adult *Artemia* (Fig. 9) is very limited. Evident reasons for this

are the worldwide availability of storable *Artemia* cysts and the ease with which nauplii are obtained, whereas commercial availability of adult *Artemia* is very restricted and its cost very high; furthermore, it is only during recent years that reliable techniques have been developed for mass production of pre-adult and adult *Artemia. Nevertheless, several arguments support the use of on-grown and adult Artemia* as a food source.

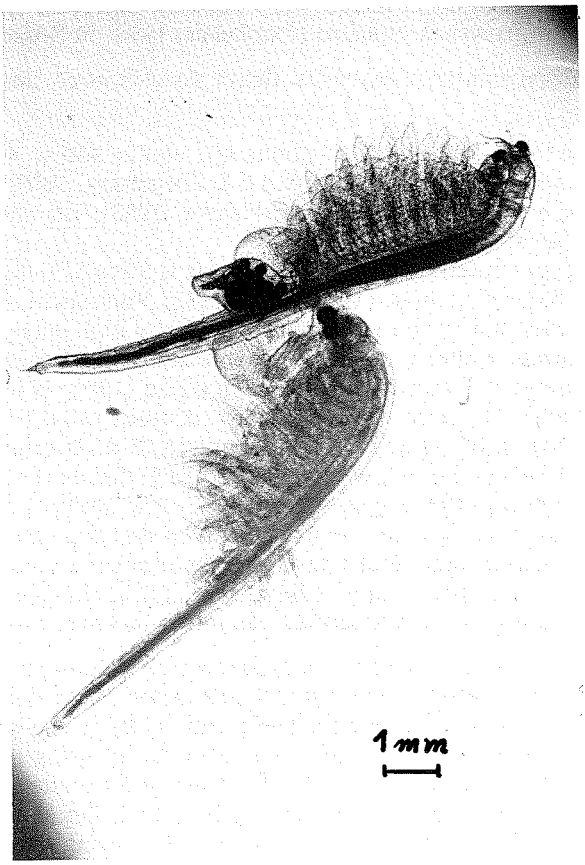

Fig. 9.—*Artemia* pair in precopulation.

NUTRITIONAL QUALITY OF ON-GROWN AND ADULT *ARTEMIA*

As compared with freshly hatched nauplii the nutritional value of on-grown and adult *Artemia* is superior, *i.e.* protein content increases from an average of 47% in nauplii to 60% on a dry weight basis in adults; furthermore, protein quality improves as adults are rich in all essential amino acids (see later). In contrast to other food organisms, the exoskeleton of adult *Artemia* is extremely thin which facilitates digestion of the whole animal by the predator.

Prey size, however, has been the first rationale to switch from nauplius to juvenile and/or adult *Artemia*, e.g. Sick & Beaty (1975) showed that

Macrobrachium rosenbergii stage VIII could not ingest *Artemia* nauplii in sufficient amounts to give a positive energy balance. Better results in terms of relative rates of energy intake and, as a consequence, of prawn growth, developmental rate and survival were obtained with 5·5-mm juvenile *Artemia* as a food source. Purdom & Preston (1977) came to the same conclusion for turbot larvae and several other authors have applied the technique of feeding progressively larger *Artemia* to fish and crustacean larvae, *e.g.* San Feliu (1973), Dugan *et al.* (1975), Smith (1976), Cadena-Roa *et al.* (1982a,b), Ebert, Haseltine, Houk & Kelly (1983). In the case of Person-Le Ruyet *et al.* (1978), *Artemia* metanauplii cultured on dried algae or compound diets (see later) were used to weaning of fish larvae.

All lobster farming relies on adult *Artemia* as food for at least the first four larval stages, e.g. Hughes, Shleser & Tchobanoglous (1975); Van Olst, Ford, Carlberg & Dorband (1975); Carlberg & Van Olst (1976); Stewart & Castell (1976); Rosemark (1978); Conklin *et al.* (1975, 1978); Happe & Hollande (1982); Chang & Conklin (1983); Eagles, Aiken & Waddy (1984). As early as 1907 Williams noticed a better growth in *Homarus americanus* larvae when offered adult *Artemia* instead of a diet of minced clam and naturally available copepods.

Although frozen *Artemia* can be used, best results are obtained with live adults which assure better availability in the water column and do not provoke deterioration of water quality (Schuur *et al.*, 1976). The superiority of live adult *Artemia* to frozen and freeze-dried adults and artificial diets has been demonstrated repeatedly, *e.g.* Botsford, Rauch & Shleser (1974); Serflin, Van Olst & Ford (1974); Hughes *et al.* (1975); Shleser (1976); Schuur *et al.* (1976); Conklin *et al.* (1975, 1978); Happe & Hollande (1982). According to Conklin *et al.* (1978), an essential but water-soluble substance is present in live adult *Artemia* which leaches from frozen or dried brine shrimp.

Live amphipods might be used as a better alternative for adult *Artemia*; *i.e. D'Agostino (1980) reported better growth and pigmentation in lobster* juveniles when using *Calliopius leaviusculus* instead of live *Artemia*, and Good *et al.* (1982) also observed better pigmentation when *Gammarus oceanicus* was fed instead of frozen *Artemia*. Eagles *et al.* (1984), however, caution for quality control of frozen *Artemia*, *i.e.* unpigmented, fragmented and leached frozen adult *Artemia* gave less satisfactory growth and development in lobster larvae. According to Rosemark (1978) culture success in lobster can be enhanced by supplementing the *Artemia* diet with frozen natural products. Nevertheless, Happe & Hollande (1982) claim that a sole diet of *Artemia* guarantees best production results in *Homarus americanus*, *i.e.* market size can be reached in 2 years only as compared with 3 years when *Artemia* is supplemented with red crab flesh. Using only *Artemia* as food, however, makes the production cost of the lobster too high.

Besides lobster, several other species have been offered on-grown and adult *Artemia* with good results, *e.g.* the freshwater prawn *Macrobrachium rosenbergii* (Dugan *et al.*, 1975; Sick & Beaty, 1975; Perrot, 1976; Sick, 1976; Aquacop, 1977; Goodwin & Hanson, 1977; Corbin *et al.*, 1983), marine shrimp such as *Penaeus monodon* (Millamena *et al.*, 1985; Bombeo, 1985; Yashiro, 1985), *P. kerathurus* (San Feliu, 1973; Rodriguez, 1976; San

Feliu *et al.*, 1976), *P. japonicus* (Palmegiano & Trotta, 1981; Camara & De Medeiros Rocha, 1985; Guimares & De Haas, 1985; Trotta, Villani & Palmegiano, 1985), *P. aztecus* (Flores, 1985), *Palaemon serratus* (Wickins, 1972), the crab *Cancer magister* (Ebert *et al.*, 1983), several fish species such as *Pleuronectes platessa* and *Solea solea* (Shelbourne, 1968), *Solea vulgaris* (Cadena Roa *et al.*, 1982a,b), *Scophthalmus maximus* (Aronovick & Spektorova, 1971; Anonymous, 1973, 1978c; Person-Le Ruyet *et al.*, 1978), *Sparus auratus* (Alessio, 1974; San Feliu *et al.*, 1976), *Dicentrarchus labrax* (Allesio, Gandolfi & Schreiber, 1976; Barahona-Fernandes & Girin, 1977; Girin, 1976; Anonymous, 1977, 1978b; Barnabé, 1980; Trotta *et al.*, 1985), *Diplodus sargus* (Divanach, Kentouri & Paris, 1983), *Chanos chanos* (De los Santos *et al.*, 1980; Bombeo, 1985), *Acipenser* sp. (Azari Takami, 1976, 1985; Binkowski & Czeskleba, 1980), *Lepomis* sp. (Smith, 1975, 1976), and ornamental fish (Rakowicz, 1972).

The use of on-grown and adult *Artemia* has mostly been restricted to relatively small scale culture trails. During recent years, however, commercial scale use of *Artemia* biomass harvested from local salt-works (Camara & De Medeiros Rocha, 1985) or produced in manured salt-ponds (De los Santos *et al.*, 1980; Flores, 1985; Jumalon *et al.*, 1985; Tarnchalanukit & Wongrat, 1985) is gaining more and more interest especially in fish weaning and shrimp nursing. The recent finding that a diet of adult *Artemia* may induce maturation in shrimp without application of eyestalk ablation (Camara & De Medeiros Rocha, 1985; Flores, 1985) may also be of major importance in future shrimp farming.

THE USE OF INTENSIVELY PRODUCED *ARTEMIA* BIOMASS

Although the cheapest source of *Artemia* biomass is from natural and man-controlled salt-pond systems, *Artemia* produced in intensive culture systems may become more attractive especially in climates that are unsuitable for outdoor production and when quality control is critical (Sorgeloos *et al.*, 1983; Lavens *et al.*, in press). Recently much progress has been made in the development of new techniques for the high density culturing of *Artemia* using cheap agricultural by-products instead of algae as food (Bossuyt & Sorgeloos, 1980; Brisset, 1981; Brisset *et al.*, 1982; Sorgeloos *et al.*, 1983; De Meulemeester *et al., 1985; Lavens & Sorgeloos, 1985; Platon & Zahradnik*, 1985). Other feeds used are the marine yeast *Candida* (James, Abu-Rezeq & Dias, 1985), organic wastes (Basil & Marian, 1985), clam-meat suspension (Vishnu Bhat & Ganapathy, 1985), and dried algae (Person-Le Ruyet *et al.*, 1978).

Artemia produced in intensive culture systems appeared to be an acceptable food for the larvae of various species of fish and crustaceans (Shelbourne, 1968; Dugan *et al.*, 1975; Smith, 1976; Person-Le Ruyet *et al.*, 1978; Dobbeleir, 1979 in Sorgeloos *et al.*, 1983; Cadena Roa *et al.*, 1982a,b; Chang & Conklin, 1983; Yashiro, 1985; Trotta *et al.*, 1985; Millamena *et al.*, 1985). Contrary to what is found in wild adults, the fatty-acid profile of brine shrimp cultured on feeds of terrestrial origin (*e.g.* agricultural waste products) does not show significant levels of the essential fatty acids 20:5ω3 and 22:6ω3 (see Table XIV, p. 603).

This deficiency can, however, be remedied by application of enrichment

techniques using similar diets as described earlier for the nauplii (Sakamoto *et al.*, 1982; Léger *et al.*, 1985b). In fact this technique of encapsulation provides interesting opportunities to use *Artemia* biomass not only as an attractive food but at the same time as carrier to administer various products, *e.g.* essential nutrients, pigments, prophylactics, therapeutics, hormones, *etc.* to the predator larvae (Léger *et al.*, 1985b). For various reasons *Artemia* produced in intensive cultures may be preferred over wild brine shrimp biomass; *e.g.* being produced at high salinities the latter may not survive equally long when transferred into natural sea water (Sorgeloos, (1979); moreover, wild *Artemia* can be the carriers of infectious organisms such as Cestoda (Heldt, 1926; Young, 1952; Maksimova, 1973), Spirochaeta (Tyson, 1970), Fungi (Kamienski, 1899; Lachance, Miranda, Miller & Phaff, 1976) and intracellular Procaryota (Post & Youssef, 1977). On the contrary, *Artemia* cultured on various agricultural waste products in batch systems have been shown to be relatively clean in terms of microbial contamination (Dobbeni, 1983). Another advantage of using cultured *Artemia* is that any size from 0·5 to >10 mm may be harvested and fed to the predator according to its growth.

OTHER APPLICATIONS OF ON-GROWN AND ADULT *ARTEMIA* AS FOOD SOURCE

Artemia biomass can also be applied as a dietary ingredient or gustatory attractant in artificial diets for fish and crustacean larvae (Sick & Andrews, 1973; Sick, Andrews & Baptist, 1973; Sick & Beaty, 1974, 1975; Sick, 1975, 1976; Barahona-Fernandes, Girin & Metailler, 1977; Girin, Metailler & Nedelec, 1977; Goodwin & Hanson, 1977; Metailler, Mery, Depois & Nedelec, 1977; Cadena Roa *et al.*, 1982a,b; Gatesoupe & Luquet, 1981/1982; Levine *et al.*, 1983). A most interesting application is the complete substitution of freshly hatched nauplii by freeze-dried and micronized *Artemia* biomass in the hatchery production of *Penaeus japonicus* (Guimares & De Haas, 1985), *i.e.* 1 million post-larvae could be produced with 1·8 kg *Artemia* meal.

In the future, *Artemia* biomass may also be considered as a complementary source of animal protein for terrestrial animals and even man (Helfrich, 1973; Stults, 1974; Anonymous, 1978a; Amat, 1980; Webber & Sorgeloos, 1980; Janata & Bell, 1985). A practical example was evaluated by Corazza & Sailor (1982) who tested lyophilized brine shrimp as a promising source of animal protein for broiler diets.

Dobbeni (1983), agreed that adult *Artemia* may have perspectives for human consumption and especially for intravenous feeding since its proteins have an ultra fine texture. Human consumption of brine shrimp may appear futuristic. None the less sun-dried *Artemia* was consumed centuries ago by Indian (Jensen, 1918) and African tribes (Oudney & Clapperton, 1812, in Bovill, 1968; May, 1967; Ghannudi & Tufail, 1978) and still today "pains d'*Artemia*" is on the menu of the Dawada tribe in Libya (Delga, Meunier, Pallaget & Carious, 1960; Monod, 1969; Dumont, 1979).

The idea of using *Artemia* as a food source for man is of particular interest for developing countries where animal protein is scarce and potential *Artemia* production sites abundant. Moreover, because *Artemia*

occupies a lower trophic level than most farmed fish, the use of *Artemia as* a direct food source for man constitutes an economical use of live energy, which in these parts of the world is of critical importance.

THE BIOMETRICS OF *ARTEMIA*

A major advantage when using *Artemia* as food for fish and crustacean larvae is the relatively wide range of sizes from which one can chose. Indeed, in its smallest form, the decapsulated cyst, sizes ranges from around 208 to 266 μm, depending on geographical origin (Vanhaecke & Sorgeloos, 1980), freshly hatched nauplii measure from 428 to 517 μm (Vanhaecke & Sorgeloos, 1980), and when used in its adult form maximum lengths of 10 to 15 mm can be reached.

CYST DIAMETER

Vanhaecke & Sorgeloos (1980) made a detailed comparative study of the cyst biometrics in different batches of cysts from 17 geographical strains of *Artemia*. Data for the same and other strains can be found in D'Agostino (1965), Wickins (1972); Claus *et al.* (1977), Uçal (1979), Amat (1980), Vos *et al.* (1984), Nanayakkara *et al.* (1985), Van Ballaer *et al.* (1985). A compilation of cyst biometrics is provided in Table VIII. Cyst diameters differ widely, *i.e.* from 224·7 to 284·9 μm in hydrated untreated cysts and from 207·3 to 266·3 μm in hydrated decapsulated cysts. Differences between untreated and decapsulated cysts are not consistent revealing a variation in chorion thickness from 3 to 13·35 μm (Vanhaecke, 1983), which is not correlated with cyst diameter. Considering cyst diameter, American *Artemia* are relatively small when compared with the *Artemia* sources from

TABLE VIII

Biometrical data of hydrated untreated and decapsulated cysts and Instar I nauplii of different sources of Artemia *(data from Vanhaecke, 1983; Vanhaecke & Sorgeloos, 1980; Tackaert, unpubl.)*

Artemia source	Cyst diameter (μm)		Instar I nauplii	
	Untreated	Decapsulated	Length (μm)	Volume (10^{-3}μm³)
Argentina, Buenos Aires	238·2	217·4	431	7734
Australia, Adelaide	225·8	209·8		
Rockhampton	231·0			
Shark Bay	260·4	242·2	458	10249
Bahamas, Great Inagua	229·1	210·0		
Brazil, Cabo Frio	233·5	216·1		
Macau	228·7	213·8	447	8314
Bulgaria, Burgas Pomorije	281·0	263·5		
Burma	278·4			
Canada, Chaplin Lake	245·4	234·0	475	8930
China-P.R., Tientsin	274·4	257·8	515	13 097
Tsingtao	270·0	249·2		

Colombia, Galera Zamba	249·9	232·7	480	10 578
Manaure	237·0	220·8	456	8062
Cyprus, Larnaca	261·3	235·6		
Ecuador, Pacoa	226·2			
Salinas	242·3			
France, Aigues Mortes	259·6	240·8		
Lavalduc	276·3	261·5	509	12 724
Salins de Giraud	264·4			
Salins de Hyères	257·8			
Villeroy	261·2			
India, Bhayander, Bombay	258·0			
Kutch, Mundra	254·4	232·4		
Mithapur	267·7	248·0		
Tuticorin	282·9	262·7	509	
Iran, Ormia Lake	258·1	245·7		
Israel, Eilat	274·3	258·4	506	
Italy, Cervia	282·5			
Margherita di Savoia	284·9	266·3	517	13 604
Yugoslavia, Portoroz	291·7			
Kenya, Malindi	228·4			
Mexico, Bahia de Queta	224·9	207·3		
Yavaros Sonora	228·9	213·1		
Netherlands Antilles, Bonaire	236·9	219·0		
New Zealand, Lake Grassmere	231·6	216·7		
Peru, Chilca	246·9	226·7		
Virrila	227·1	208·5		
Philippines, Barotac Nuevo	228·0		429	7991
Jaro	225·2			
Pangasinan	229·7			
Portugal, Alcochete	248·4	233·6		
Puerto Rico, Bahia Salina	253·7	233·4	452	9090
Spain, Barbanera	257·3	230·6		
Delta del Ebro	277·8	258·8		
San Lucar	253·6	237·1		
Santa Pola	248·6			
Sri Lanka, Puttalam	269·8			
Tunisia, Bekalta	251·6		482·3	
Chott Ariana	268·9	245·3		
Mégrine	258·8	234·1	467·7	
Moknine	252·6			
Sfax	235·4	215·1	422·2	
Turkey, Izmir	270·4	252·9		
U.S.A., Great Salt Lake	244·2	234·8	482	9091
Jesse Lake	234·8			
Mono Lake	249·4	243·4		
Playa Tahoka	244·7	225·8		
Quemado	239·7	224·7		
Raymondville	253·9			
San Francisco Bay	224·7	210·0	428	7638
San Pablo Bay	235·6	220·4	433	8144
U.S.S.R., Azov Sea	270·2	258·9		
Bolshoe Jarovoe Lake	273·7	258·⅜		
Kujalnic Lagoon	273·5	255·9		
Mangyshlak peninsula	248·4	229·1		
Odessa	259·7	242·7		
Sivash	251·4	229·6		
Tinaki Lake	280·3	260·9		
Venezuela, Port Araya	249·0	222·6	474	9548
Tucacas	244·3	222·6		
Vietnam, Cam Ranh Bay	242·9			

the Old World. Within the American sources, considerable differences are noticed even between closely located sources, *e.g.* Chilca and Virrila in Peru. On the contrary, several American sources closely reflect the diameter of San Francisco Bay cysts (*e.g.* Great Inagua, Macau, Pacoa, Panama, Bahia de Cueta, Yavaros Sonora, and Virrila) and Great Salt Lake cysts (*e.g.* Galera Zamba, Chilca, Bahia Salinas, and Port Araya), *i.e.* the two oldest commercial strains which may have been used for (non) intentional introductions, *e.g.* San Francisco Bay *Artemia* in Macau, Brazil (Persoone & Sorgeloos, 1980). Cyst size appears to be genetically determined, *e.g.* no appreciable size differences were found between cysts from different harvests from the same source (Vanhaecke & Sorgeloos, 1980) and between cysts produced from the same inoculum in different countries (Vos *et al.*, 1984) or in laboratory-controlled systems (Lavens, unpubl.).

NAUPLIUS DIMENSIONS

Most information on nauplius lengths and volumes results again from the comparative studies of Vanhaecke & Sorgeloos (1980) and Vanhaecke (1983) (see Table XIII, p. 600). Further data can be found in D'Agostino *(1965); Sorgeloos (1975); Smith (1976); Claus et al.* (1979); Amat (1980); and Nanayakkara *et al.* (1985). According to strain origin the size of freshly hatched instar I nauplii ranges from 428 to 517 μm. The largest nauplii are produced in parthenogenetic strains with a high degree of ploidy (Vanhaecke, 1983). Vanhaecke & Sorgeloos (1980) found high degrees of positive correlation between the diameter of decapsulated cysts and nauplius length ($r = 0{\cdot}906$), and between volume of decapsulated cysts and nauplius volume. Cyst size may be an easier criterion for the selection of a proper sized *Artemia* strain either for use as food source (see above) or for *Artemia inoculation (Vos et al.*, 1984).

In view of the high heritability and the large variation in cyst biometrics selective breeding techniques may in the future be successful in the development of strains that produce mini-*Artemia* cysts, which would be a most welcome addition for use in early larval feeding of marine fishes and shrimps.

BIOCHEMICAL AND CHEMICAL COMPOSITION

A review of the literature on the composition of *Artemia reveals considerable* variation in amounts of the various compounds. The causes of the variation are undoubtedly several, *e.g.* different methods of extraction and analysis, different live stages of the *Artemia* studied, and different geographical populations. Although the information presented here could be averaged to portray a generalized *Artemia* composition, the most important message is that the inherent variation makes each commercially obtained *batch of Artemia* different. Scientists or aquaculturists, therefore, have the responsibility to assure that their *Artemia* provide adequate nutrition for the organisms to which they are fed.

INDIVIDUAL DRY WEIGHT AND ENERGY CONTENT

Data on the individual dry weight and energy content of newly hatched *Artemia* nauplii of different geographical origin are summarized in Table IX. The energetic content on an ash-free dry weight basis appears to be very similar for most geographical collections studied. On the contrary, individual energetic content and individual dry weight differ greatly. Not considering variability of a purely analytical origin, differences may be explained by varying hatching conditions. Von Hentig (1971) indeed demonstrated that *Artemia* hatched at a lower salinity and higher temperature contained more energy. When comparing data obtained for different *Artemia* sources hatched under the same conditions, Vanhaecke (1983) and Vanhaecke *et al.* (1983), however, still noticed considerable differences of up to 100% and more. Nevertheless, no significant differences were detected among batches from the same strain nor between cysts originating from the same parental material but produced at different localities, *e.g.* Macau (Brazil), Barotac Nuevo (Philippines) and San Francisco Bay (U.S.A.). This allowed Vanhaecke *et al.* (1983) to conclude that in *Artemia* individual dry weight and energy content are mainly genetically determined and thus strain specific. As a result nauplius dry weight and energy content are important criteria for strain selection; indeed, when size and nutritional composition are acceptable for a predator, *Artemia* with a high energy content will guarantee better predator growth, since less energy will be spent in hunting and food uptake (Vanhaecke & Sorgeloos, 1983b; Nanayakkara *et al.*, 1985).

Variability in results between authors analysing the same *Artemia* strains is most probably related to differences in hatching incubation time. Indeed, *Artemia* starts utilizing its energy reserves shortly after cyst hydration when the embryonic metabolism restarts (Urbani, 1959; Von Hentig, 1971); food-uptake only takes place after the animal has moulted into the second instar stage (Benesch, 1969). As a result significant drops in individual dry weight and energy contents have been reported in older *Artemia* metanauplii as compared with decapsulated cysts and even instar I nauplii (Paffenhöfer, 1967; Benijts *et al., 1976; Royan, 1980; Vanhaecke et al.*, 1983). According to Vanhaecke *et al.* decapsulated cysts contain 30 to 57% more energy than instar I nauplii which in their turn contain 22 to 37% more energy than instar II-III metanauplii. Metanauplius development and energy loss can be reduced to 2·5% over a period of 24 h when storing the freshly hatched nauplii at 2–4 °C (Léger *et al.*, 1983).

Data on energy content of on-grown and adult *Artemia are scarce, e.g. 7-day old Artemia* reared on *Dunaliella* contain 5854 $cal \cdot g^{-1}$ ($=24\ 499\ J \cdot g^{-1}$) (Paffenhöfer, 1967) whereas only 5100 $cal \cdot g^{-1}$ ($=21\ 344\ J \cdot g^{-1}$) was reported for frozen *Artemia* biomass (Gabaudan, Piggott & Halver, 1980). The latter result is within the same range as reported for newly hatched nauplii (Table IX). Evidently, individual energy content is much higher in adults than in nauplii, for which reason better predator growth is to be expected when on-grown *Artemia* are being fed (Sick & Beaty, 1974, 1975). Individual dry weights of 0·88 and 1·0 mg have been reported by Reeve (1963) and Tobias, Sorgeloos, Roels & Sharfstein (1980), respectively, for sexually mature animals of different origin reared on algae.

TABLE IX

Data on individual dry weight and energy content of newly hatched Artemia *nauplii from different geographical origin: c, calculated; t, transformed to SI-units*

Artemia source	Hatching conditions T(°C)	S(‰)	Individual dry weight (μg)	Energetic content ($J \cdot g^{-1}$ ash-free dry wt)	Individual energy content (J)	Reference
Argentina, Buenos Aires	25	35	1·72	23 506[c]	0·0379	Vanhaecke *et al.*, 1983
Australia, World Ocean (No. 113)	25	30		25 000		Schauer *et al.*, 1980
(No. 114)	25	35	2·47	23 575[c]	0·0576	Vanhaecke *et al.*, 1983
Brazil, Macau (1978)	25	30		23 500		Schauer *et al.*, 1980
(1978)	25	35	1·68	24 116[c]	0·0381	Vanhaecke *et al.*, 1983
(No. 871172)	25	35	1·74	23 927[c]	0·0392	Vanhaecke *et al.*, 1983
(No. 971051)	25	35	1·75			Vanhaecke, 1983
Canada, Chaplin Lake (1978)	25	35	2·04	23 488[c]	0·0446	Vanhaecke *et al.*, 1983
(1978)	25	35	1·97			Vanhaecke, 1983
(1979)	25	35	2·04			Vanhaecke, 1983
China P. R., Tientsin	25	35	3·09	23 616[c]	0·0681	Vanhaecke *et al.*, 1983
Colombia, Manaure	25	35	1·78			Vanhaecke, 1983
Galera Zamba	25	35	2·27			Vanhaecke, 1983
Cyprus	26		2·1			Person-Le Ruyet & Salaun, 1977
France, Salins du Midi	26		2·7			Fuchs & Person-Le Ruyet, 1976
Salins du Midi (Lavalduc)	25	35	3·08	23 156[c]	0·0670	Vanhaecke *et al.*, 1983
India, Tuticorin	30	35	2·80	21 934		Royan, 1980
	25	35	3·17			Vanhaecke, 1983
Israel, Eilat	25	35	3·00			Vanhaecke, 1983
Italy, Margherita di Savoia	25	30		22 400		Schauer *et al.*, 1980
	25	35	3·33	23 191[c]	0·0725	Vanhaecke *et al.*, 1983
Philippines, Barotac Nuevo	25	35	1·68	24 210[c]	0·0382	Vanhaecke *et al.*, 1983
Puerto Rico, Bahia Salinas	25	35	2·10	23 696[c]	0·0470	Vanhaecke *et al.*, 1983
Unknown				28 194[ct]		Slobodkin & Richman, 1961

Sri Lanka, Hambantota	29	35	3·29			Nanayakkara *et al.*, 1985
Tunisia, Bekalta	25	35	2·40			Van Ballaer *et al.*, 1985
Mégrine	25	35	2·61			Van Ballaer *et al.*, 1985
Sfax	25	35	1·97			Van Ballaer *et al.*, 1985
U.S.A., Great Salt Lake	20		1·65	24 913[t]		Paffenhöfer, 1967
	30	32	1·92	24 662[t]		Von Hentig, 1971
(1977)	25	30		22 400		Schauer *et al.*, 1980
(1966)	25	35	2·70	24 549[c]	0·0625	Vanhaecke, 1983
(1977)	25	35	2·42	23 698[c]	0·0541	Vanhaecke *et al.*, 1983
U.S.A., San Francisco Bay	18	30	1·5			Urbani, 1959
	26	33	2·87	27 621[t]		Dutrieu, 1960
	25	20	1·93			Clegg, 1962
	20	33	1·64			May, 1971
	28	35	1·85	23 256		Benijts *et al.*, 1976
	26		1·4			Fuchs & Person-Le Ruyet, 1976
	26		1·45			Person-Le Ruyet & Salaun, 1977
(No. 288–2606)	25	35	1·61	23 852[c]	0·0360	Vanhaecke, 1983
(No. 288–2596)	25	35	1·63	23 999[c]	0·0366	Vanhaecke *et al.*, 1983
U.S.A., San Pablo Bay (No. 1268)	25	30		23 500		Schauer *et al.*, 1980
(No. 1628)	25	35	1·92	23 660[c]	0·0429	Vanhaecke *et al.*, 1983
Venezuela, Port Araya	25	35	2·07			Vanhaecke, 1983

APPROXIMATE COMPOSITION

A summary of available information on the approximate composition of *Artemia* nauplii, pre-adults and adults again reveals considerable variation (see Table X). Protein content in nauplii ranges from 37·4 to 71·4% with an average (excluding extremes) of about 50%. Average protein content in pre-adult and adult *Artemia* is about 56%. Lipid content in nauplii also varies considerably *i.e.* from 11·6 to 30%. Sources of variation are strain differences (Schauer *et al.*, 1980) and nauplius age at analysis (Benijts *et al.*, 1976); the last authors measured a decrease in lipid content from 19·3% in the first instar stage to 13·7% in the instar II-III stage, representing a 26% loss. According to Hines, Middleditch & Lawrence (1980) instar I nauplii contain 33–38% protein, 16–22% lipid, and 8–18% carbohydrate; during 48 h post-hatch development at 18 °C all levels remained relatively constant, but after 24 h at 28 °C levels of lipids and carbohydrates had decreased.

Literature data on carbohydrate and ash content range from 10·54 to 22·7% and 4·2 to 21·4%, respectively in nauplii and from 9·25 to 17·2% and 8·89 to 29·2%, respectively in pre-adult and adult *Artemia*. Variation in ash content is particularly high in nauplii. This may be explained by the large increase in ash content as animals moult from instar I to instar II and III (*e.g.* 88%, Benijts *et al.*, 1976). Ash contents are substantially higher in adults than in nauplii.

MINERALS

The mineral content of adult brine shrimp was reported by Gallagher & Brown (1975), that of cysts was determined by Stults (1974), and that of nauplii was given by Watanabe *et al.* (1978a), Grabner *et al.* (1981/1982) and Bengtson, Beck & Simpson (in press). The studies of Watanabe *et al.* (1978a) indicate that geographic variation in mineral content is apparent, but not particularly large nor significant. Variation in the reported data seems to be due more to the investigator or method differences than to geographic variation. The range of mineral content that has appeared in the literature are: sodium (2·1–51·1 $mg \cdot g^{-1}$), phosphorus (1·1–17·5 $mg \cdot g^{-1}$), potassium (0·73–12·7 $mg \cdot g^{-1}$), magnesium (1·05–6·8 $mg \cdot g^{-1}$), calcium (0·2–4·8 $mg \cdot g^{-1}$), iron (269–2946 $\mu g \cdot g^{-1}$), zinc (75–241 $\mu g \cdot g^{-1}$), manganese (2–139 $\mu g \cdot g^{-1}$), copper (2–32 $\mu g \cdot g^{-1}$), selenium (0·83–1·4 $\mu g \cdot g^{-1}$); values compare well with the mineral content of other natural or cultured zooplankton (Watanabe *et al.*, 1978a; Grabner *et al.*, 1981/1982). At any rate, the nutritional requirements of marine fish and crustacean larvae for minerals are very poorly known and may be partially supplied by the sea water that marine fish drink (Cowey & Sargent, 1979).

AMINO ACIDS

Amino-acid profiles have been reported for *Artemia* by several authors (Gallagher & Brown, 1975; Watanabe *et al.*, 1978b; Claus *et al.*, 1979; Schauer *et al.*, 1979; Seidel *et al.*, 1980a,b; Grabner *et al.*, 1981/1982; Dabrowski & Rusiecki, 1983), but different methods of analysis and

TABLE X

Overview of published data on approximate analysis (% on dry wt basis) of Artemia *nauplii, juveniles and adults: c, recalculated from wet wt basis; n.s. source not specified.*

Artemia source	Protein	Lipid	Carbohydrate	Ash	Reference
Nauplii					
Australia, Shark Bay		18·5			Schauer *et al.*, 1980
Brazil, Macau		20·2			Schauer *et al.*, 1980
Canada	57·6[c]	17·8[c]		12·7[c]	Watanabe *et al.*, 1983a
China P.R.	47·3	12·0		21·4	Duray & Baraginao, 1984
France, Salins du Midi	55·7	12·4		15·4	Fuchs & Person-Le Ruyet, 1976
India, Tuticorin	58·0	23·3	12·8	5·7	Royan, 1980
Italy, Margherita di Savoia		15·6			Schauer *et al.*, 1980
Russia	42·5	23·2			Dutrieu, 1960
South America	71·4[c]	11·6[c]		10·9[c]	Watanabe *et al.*, 1983a
Sri Lanka, Hambantota	66·8	14·1	12·7	6·4	Nanayakkara *et al.*, 1985
U.S.A., Great Salt Lake	41·6	23·1	22·7	6·56	Von Hentig, 1971
	47·24	20·84	10·54	9·52	Claus *et al.*, 1979
		22·4			Schauer *et al.*, 1980
U.S.A., San Francisco Bay	50·3	15·9			Brick, in Helfrich, 1973
	50·0	27·2			Coehn, in Helfrich, 1973
	54·5	17·25		13·78	Fuchs & Person-Le Ruyet, 1976
		19·3		6·03	Benijts *et al.*, 1976
	59·2[c]	19·4[c]		11·7[c]	Watanabe *et al.*, 1983a
	47·26	23·53	11·24	8·17	Claus *et al.*, 1979
		17·4			Schauer *et al.*, 1980
		15·9			Schauer *et al.*, 1980
U.S.A., San Pablo Bay		16·0			Schauer *et al.*, 1980
n.s.	53·6	17·6	18·6	4·2	Coles, 1969
n.s.		30			Sulkin, 1975
n.s.	37·4	17·1		7·4	Grabner *et al.*, 1981/1982
n.s.	47·0	20·8		6·1	Bengtson *et al.*, in press

TABLE X—*continued*

Artemia source	Protein	Lipid	Carbohydrate	Ash	Reference
Adults					
Wild Adults					
U.S.A., Mono Lake	58·5	10·6		20·6	Enzler *et al.*, 1974
U.S.A., San Diego	64·0	12·0		15·4	Millikin *et al.*, 1980
San Francisco Bay	58·0	19·3		20·6	Gallagher & Brown, 1975
	57·9	12·5		12·4	Millikin *et al.*, 1980
	50·2	2·4	17·2	29·2	Good *et al.*, 1982
n.s.	51·00	8·25	9·98	17·40	Capuzzo & Lancaster, 1979
n.s.	69·02	12·84	9·25	8·89	Gabaudan *et al.*, 1980
Cultured juveniles and adults					
Brazil, Macau (at sexual maturity on *Chaetoceros*)	52·77				Tobias *et al.*, 1980
Cyprus, Larnaca Salt Lake (at sexual maturity on *Chaetoceros*)	58·07				Tobias *et al.*, 1980
France, 7 days on *Spirulina*	53·7	9·4		21·6	Fuchs & Person-Le Ruyet, 1976
U.S.A., Great Salt Lake (14 days on defatted rice bran)	56·5	19·5		9·0	Dobbeni, 1983
India, Tuticorin (at sexual maturity on *Chaetoceros*)	51·47				Tobias *et al.*, 1980
Italy, Margherita di Savoia (at sexual maturity on *Chaetoceros*)	52·03				Tobias *et al.*, 1980
U.S.A., San Francisco Bay (7 days on *Spirulina*)	62·5	10·8		19·1	Fuchs & Person-Le Ruyet, 1976
Spain, Santa Pola (at sexual maturity on *Chaetoceros*)	49·73				Tobias *et al.*, 1980

reporting of the data by different authors preclude any comparison of their results. For example, the method used by Claus *et al.* (1979) was not suitable for the detection of proline, cystine, arginine, and tryptophan, which together account for about 25% of the total amino acids reported by other authors. European authors (Claus *et al.*, 1979; Grabner *et al.*, 1981/1982; Dabrowski & Rusiecki, 1983) tend to report the content of each amino acid as a percentage of the total amino acids, whereas Japanese and American authors (Gallagher & Brown, 1975; Watanabe *et al.*, 1978b; Seidel *et al.*, 1980a,b) report it as g of each amino acid per 100 g of protein. The two methods of reporting can be approximately equivalent, but are not necessarily so, depending, for example, on whether all the amino acids can be detected and whether one is working with wet or freeze-dried material. It is appropriate here to plead for standard methods of analysis and reporting of amino-acid data.

The geographical variation in amino-acid content of *Artemia* is not large. Seidel *et al.* (1980b) found that newly-hatched nauplii from five geographical strains were relatively similar in amino-acid composition (Table XI) and that the 10 amino acids considered essential for fish (Anonymous, 1981) were generally present in sufficient quantity in the nauplii. Methionine, however, like other sulphur amino acids (Dabrowski & Rusiecki, 1983), is the first-limiting amino acid. Amino-acid composition is probably genetically controlled, not subject to much environmental variation and not a major problem in the nutritional value of *Artemia*. Dabrowski & Rusiecki (1983) demonstrated, however, that upon starvation the free amino-acid content in *Artemia* nauplii decreases. This may reduce to some extent their digestibility especially for stomachless fish larvae. Digestibility of *Artemia* protein was determined by Watanabe *et al.* (1978a) who found it to be 83% for carp and 89% for rainbow trout. Watanabe *et al.* also found high values for net protein utilization (NPU) and the protein efficiency ratio (PER).

FATTY ACIDS

Newly hatched nauplii and cysts

Although investigators routinely report on levels of 15 or more fatty acids in their profiles of *Artemia*, six of those fatty acids (16:0, 16:1ω7, 18:1ω9, 18:2ω6, 18:3ω3, and 20:5ω3) actually comprise about 80% of the total fatty acids in an *Artemia* sample. Published values (% composition as fatty acid methyl esters or FAMEs) for those six fatty acids are give in Table XII. Most of the analyses have been done on the San Francisco Bay strain, but several other strains have also been studied.

Levels of 16:0 (palmitic acid) range from 5·74 to 26·6% of total FAMEs, although most values for 16:0 approximate the mean value of 13·4%. Thus, levels of this fatty acid in *Artemia* are fairly predictable and constant (overall coefficient of variation of 24·6%, see Table XIII) compared with others that we shall examine. More variable (overall coefficient of variation of 50·4%) are the levels of 16:1ω7 (palmitoleic acid), which range from 3·12 to 30·6% of total FAMEs (overall mean of 11·7%). 44% of the values

TABLE XI

Selected data on amino-acid composition of Artemia *nauplii and adults* (g *amino acid per 100 g protein*): *a, recalculated values; b, Cys + Met; c, destroyed by HCL; d, Phe + Tyr; Ala, alanine; Arg, arginine; Asp, asparagine; Cys, cysteine; Glu, glutamine; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Pe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Tyr, tyrosine; Val, valine; Tryp, tryptophan*

Artemia source	Ala	Arg	Asp	Cys	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Tyr	Val	Tryp	Reference
Nauplii[a]																			
Australia, Shark Bay	4·6	9·2	9·1	b	13·8	4·8	3·2	4·1	6·7	9·0	2·4[b]	6·5	4·6	5·0	4·6	6·2	4·6	c	Seidel *et al.*, 1980b
Brazil, Macau	3·9	9·7	9·3	b	11·1	5·1	4·1	4·7	7·5	9·9	1·9[b]	4·3	4·8	3·8	4·4	8·9	4·5	c	Seidel *et al.*, 1980b
U.S.A., San Pablo Bay	3·6	8·3	11·9	b	8·6	6·3	3·0	4·6	7·1	7·4	2·2[b]	8·8	4·1	6·5	5·1	6·5	4·7	c	Seidel *et al.*, 1980b
U.S.A., Great Salt Lake	4·1	8·2	9·5	b	11·4	5·1	2·3	5·7	8·4	7·8	3·1[b]	7·2	5·0	4·5	4·0	5·6	4·4	c	Seidel *et al.*, 1980b
Italy, Margherita di Savoia	4·1	8·3	9·5	b	12·2	6·1	3·2	5·4	8·5	9·0	3·1[b]	7·2	5·0	4·3	4·6	4·6	2·6	c	Seidel *et al.*, 1980b
Adults																			
U.S.A., San Francisco Bay (wild)	6·9	6·5	9·2	2·2	14·2	5·3	1·8	5·3	8·0	7·6	2·7	4·7	5·2	4·8	4·6	4·5	5·4	1·0	Gallagher & Brown, 1975
U.S.A., Great Salt Lake (14 days cultured on defatted rice bran)[a]	5·8	4·4	9·6		13·1	4·8	2·1	4·6	7·4	7·8	2·1	4·0	4·4	4·4	4·4	2·7	5·0		Dobbeni, 1983
Required levels for Chinook salmon	—	6·0	—	—	—	—	1·8	2·2	3·9	5·0	4·0	5·1	—	—	2·2	d	3·2	0·5	Anonymous 1981

TABLE XII

Data on per cent composition of the six major fatty acids (% fatty acid methyl ester of total fatty acid methyl esters) of Artemia *cysts and newly hatched nauplii: *, analysis performed on* Artemia *cysts; **, may include other monoënes; ***, only polar lipid fraction given; n.s., source not specified*

Artemia source		Fatty acid						Reference
		16:0	16:1ω7**	18:1ω9**	18:2ω6	18:3ω3	20:5ω3	
Australia, Shark Bay	(No. 113, 1979)	13·45	9·97	28·23	5·78	14·77	10·50	Schauer *et al.*, 1980
n.s.	(No. 1980)*	13·9	9·9	33·3	5·2	10·1	8·6	Watanabe *et al.*, 1982
Bahamas, Great Inagua		14·5	17·0	31·7	15·6	2·0	1·3	Léger, unpubl.
Brazil, Macau	(1978)	15·42	10·79	35·86	9·59	4·87	9·98	Schauer *et al.*, 1980
n.s.	(1980A)*	16·0	18·6	21·8	7·2	3·3	3·9	Watanabe *et al.*, 1982
n.s.	(1980B)*	18·2	14·4	23·7	6·4	1·1	3·5	Watanabe *et al.*, 1982
n.s.	(1980C)*	13·7	13·8	28·9	8·5	3·2	5·9	Watanabe *et al.*, 1982
n.s.	(1980D)*	18·0	14·6	16·2	3·1	0·9	4·6	Watanabe *et al.*, 1982
n.s.	(1980E)*	14·7	14·7	26·6	7·7	3·6	5·8	Watanabe *et al.*, 1982
n.s.	(1980E)	12·2	12·8	30·7	9·3	3·3	6·5	Watanabe *et al.*, 1982
n.s.	(1980F)*	13·7	14·1	28·3	11·8	2·7	5·8	Watanabe *et al.*, 1982
Guanabara	(1985)	16·4	13·1	30·5	9·2	2·7	3·3	Léger, unpubl.
Canada, n.s.		8·4	7·3	30·0	6·0	13·5	12·1	Watanabe *et al.*, 1978c
n.s.	(1978A)	9·9	10·1	32·3	5·1	14·1	5·2	Watanabe *et al.*, 1980
n.s.	(1978A)*	13·0	10·0	23·6	6·1	19·8	7·3	Watanabe *et al.*, 1980
n.s.	(1978B)*	13·5	12·8	25·4	6·4	16·0	6·7	Watanabe *et al.*, 1980
Chaplin Lake	(1979)	9·99	9·03	28·24	7·95	19·87	9·52	Seidel *et al.*, 1982
China P. R., n.s.	(1978)	13·9	23·5	23·4	3·7	7·5	7·7	Watanabe *et al., 1980*
Tientsin	(1979)	11·4	19·06	26·81	4·68	7·38	15·35	Seidel *et al.*, 1982
n.s.	(1979A)*	12·1	22·6	26·1	4·1	5·5	9·2	Watanabe *et al.*, 1982
n.s.	(1979A)*	9·7	13·6	33·5	4·4	5·3	13·0	Watanabe *et al.*, 1982
n.s.	(1979B)*	12·7	24·0	20·2	3·8	6·0	10·2	Watanabe *et al.*, 1982
n.s.	(1979)*	9·3	13·4	33·8	4·4	5·1	13·2	Watanabe *et al.*, 1982
n.s.	(1979C)*	12·7	22·4	28·3	4·3	5·1	11·3	Watanabe *et al.*, 1982
n.s.	(1980A)*	23·0	24·7	22·1	1·6	0·4	1·9	Watanabe *et al.*, 1982
n.s.	(1980B)*	21·2	22·8	17·4	2·2	0·6	1·3	Watanabe *et al.*, 1982

TABLE XII–*continued*

Artemia source		Fatty acid						Reference
		16:0	16:1ω7**	18:1ω9**	18:2ω6	18:3ω3	20:5ω3	
n.s.	(1980C)*	12·5	20·1	24·9	4·2	6·4	10·9	Watanabe *et al.*, 1982
n.s.	(1981A)*	13·1	19·1	25·3	5·0	6·6	9·3	Watanabe *et al.*, 1982
Tientsin I	(1984)	13·9	19·3	27·8	4·9	2·9	11·4	Léger, unpubl.
Tientsin II	(1985)	15·2	24·9	30·8	5·6	4·5	13·3	Léger. unpubl.
Colombia, Galera Zamba	(1983)	15·1	7·9	23·2	13·5	13·4	4·7	Léger, unpubl.
Manaure	(1983A)	13·5	9·5	29·2	13·7	1·1	1·2	Léger, unpubl.
	(1983B)	13·3	9·7	29·2	14·3	1·0	1·4	Léger, unpubl.
France, Lavalduc	(1979)	11·90	11·34	24·73	6·14	20·9	8·01	Seidel *et al.*, 1982
Lavalduc	(1981)	14·5	8·6	24·7	6·4	20·0	5·4	Léger, unpubl.
India, Mundra	(1979)	12·7	8·9	27·9	12·0	14·6	5·3	Vos *et al.*, 1984
Mithapur	(1985)	14·4	10·5	26·0	8·4	12·6	8·0	Léger, unpubl.
Tuticorin	(1985)	16·3	16·2	29·3	4·8	3·1	12·3	Léger, unpubl.
Italy, Margherita di Savoia	(1977)	15·23	10·38	29·05	6·79	6·35	13·63	Schauer *et al.*, 1980
Kenya, Malindi	(1985A)	14·1	18·3	32·3	5·5	1·6	6·9	Léger, unpubl.
	(1985B)	12·9	12·5	25·1	6·8	5·0	4·6	Léger, unpubl.
	(1985C)	14·8	17·7	27·8	8·1	1·5	6·8	Léger, unpubl.
Panama, Aguadulce I	(1984A)	14·3	15·4	27·6	8·7	2·5	7·8	Léger, unpubl.
	(1984B)	14·4	16·1	24·6	4·5	1·9	12·0	Léger, unpubl.
Aguadulce II	(1985)	14·5	16·9	27·4	6·0	3·7	9·8	Léger, unpubl.
Peru, Hierba Blanca	(1984)	14·5	10·6	27·2	5·6	11·3	6·4	Léger, unpubl.
Philippines, Barotac Nuevo	(1978)	14·4	15·9	29·6	9·1	4·2	8·6	Vos *et al.*, 1984
Jaro	(1981)	11·4	13·7	27·0	15·0	12·9	1·9	Vos *et al.*, 1984
Puerto Rico, Cabo Rojo		15·1	12·3	31·2	14·0	1·4	1·4	Léger, unpubl.
Reference *Artemia* Cysts	(1980)	12·70	16·78	30·37	9·62	2·55	8·45	Seidel *et al.*, 1982
South America, n.s.		7·9	5·8	26·3	5·2	21·0	0·3	Watanabe *et al.*, 1978c
Thailand, Bangpakong	(1979)	10·1	10·3	31·4	5·5	23·3	5·3	Vos *et al.*, 1984
Chachoengsao	(1983)	14·5	18·5	28·6	4·9	3·2	10·7	Léger, unpubl.
Fam Farm	(1985)	15·5	16·6	29·4	4·9	5·9	10·5	Léger, unpubl.

Tunisia, Sfax	(6, 1984)	15·3	7·5	24·1	8·5	20·0	2·4	Léger, unpubl.
	(502, 1984)	15·6	9·4	26·4	8·6	12·8	4·8	Van Ballaer *et al.*, 1985
Mégrine	(1984)	16·6	15·0	24·0	3·8	7·3	10·2	Van Ballaer *et al.*, 1985
U.S.A., Great Salt Lake		11·75	4·5	23·32	8·81	25·23	?	Wickins, 1972
		11·22	3·52	21·87	3·59	11·22	?	Claus *et al.*, 1979
	(S-arm 1977)	11·78	5·64	28·25	4·60	31·46	3·55	Schauer *et al.*, 1980
		15·06	5·99	30·25	6·69	28·27	1·77	Millamena *et al.*, 1985
	(S-arm 1977–18)	13·1	6·1	25·0	6·6	28·2	2·8	Léger unpubl.
	(S-arm 1977–217)	12·5	5·6	25·8	6·5	28·4	3·1	Léger, unpubl.
	(S-arm 1979–BI–1)	12·5	6·7	26·6	6·4	27·7	2·7	Léger, unpubl.
	(S-arm 1979-WC-4)	13·0	6·0	25·9	6·3	28·2	2·7	Léger, unpubl.
	(S-arm 1979–294)	13·2	5·9	26·0	6·5	28·2	2·7	Léger, unpubl.
	(S-arm 1979–185)	12·3	4·8	27·3	7·6	29·1	2·6	Léger, unpubl.
	(N-arm, 1984A)	10·9	4·1	26·5	8·4	25·5	0·3	Léger, unpubl.
	(N-arm, 1984B)	12·0	5·1	25·8	8·3	24·8	0·2	Léger, unpubl.
	(N-arm, 1984C)	11·8	5·0	23·9	7·7	22·7	0·2	Léger, unpubl.
	(N-arm, 1985A)	11·9	6·3	23·1	7·4	26·3	0·3	Léger, unpubl.
	(N-arm, 1985B)	12·3	6·3	22·9	7·5	26·1	0·3	Léger, unpubl.
U.S.A., San Francisco Bay		13·56	8·20	29·18	6·05	22·27	3·86	Wickins, 1972
		9·5	4·7	25·3	7·8	33·6	1·2	Weaver, 1974
		11·46	9·11	37·5	5·84	15·00	7·22	Schauer & Simpson, 1978
	(1975)	11·2	4·3	25·1	6·1	28·4	3·1	Watanabe *et al.*, 1978c
	(1976)	12·3	3·7	27·4	6·6	27·9	2·0	Watanabe *et al.*, 1978c
	(1977)	9·5	12·0	36·1	3·4	10·3	9·5	Watanabe *et al.*, 1978c
		5·74	3·12	14·00	2·86	7·30	?	Claus *et al.*, 1979
		11·45	16·49	34·34	4·78	4·67	13·31	Schauer *et al.*, 1979
	(No. 313/3006)	10·33	13·27	26·97	9·35	17·33	4·06	Schauer *et al.*, 1980
	(No. 321995)	12·13	19·52	31·20	3·69	5·16	12·44	Schauer *et al.*, 1980
	(1975)*	13·2	4·5	27·8	6·2	27·7	1·8	Watanabe *et al.*, 1980
	(1976)*	12·3	3·7	27·4	6·6	27·9	2·0	Watanabe *et al.*, 1980
	(1977)*	12·0	18·4	31·5	4·0	9·0	7·1	Watanabe *et al.*, 1980
	(1978A)*	19·7	30·6	14·6	5·3	2·6	6·1	Watanabe *et al.*, 1980
	(1978B)*	20·4	20·3	20·1	3·6	7·9	2·0	Watanabe *et al.*, 1980
	(1978C)*	18·9	15·3	29·2	7·8	3·8	5·4	Watanabe *et al.*, 1980
	(1978C)	14·1	13·5	33·3	9·0	3·9	7·0	Watanabe *et al.*, 1980
	(1978D)*	13·3	14·2	18·0	4·4	23·8	1·8	Watanabe *et al.*, 1980
	(1978D)	10·1	7·0	32·7	6·3	24·4	1·6	Watanabe *et al.*, 1980
	(1978E)*	13·3	11·7	27·7	5·4	21·6	1·9	Watanabe *et al.*, 1980
	***	12·6	3·6	33·6	6·9	20·0	1·0	Sakamoto *et al.*, 1982
	(1979)*	13·3	16·4	28·2	8·3	2·3	7·5	Watanabe *et al.*, 1982

TABLE XII-*continued*

Artemia source	Fatty acid						Reference
	16:0	16:1ω7**	18:1ω9**	18:2ω6	18:3ω3	20:5ω3	
(1980A)*	26·6	16·3	25·8	2·6	3·3	3·9	Watanabe *et al.*, 1982
(1980B)*	25·3	15·7	27·6	2·9	4·2	1·7	Watanabe *et al.*, 1982
(1980C)*	25·9	12·9	19·8	2·5	4·8	0·9	Watanabe *et al.*, 1982
(1980D)*	14·9	5·5	28·0	6·3	22·4	2·7	Watanabe *et al.*, 1982
(1980E)*	23·7	7·4	23·7	5·4	14·7	0·6	Watanabe *et al.*, 1982
(1980F)	9·2	14·8	19·1	8·3	5·4	6·8	Watanabe *et al.*, 1982
(1980G)	11·0	3·8	26·7	8·9	27·6	0·3	Watanabe *et al.*, 1982
(1980H)	12·2	10·4	34·9	6·6	17·2	3·5	Watanabe *et al.*, 1982
(1981A)*	15·2	10·5	28·4	7·1	17·2	3·6	Watanabe *et al.*, 1982
(1981B)*	13·6	4·3	27·1	6·1	28·1	2·4	Watanabe *et al.*, 1982
(1981C)*	10·6	5·4	26·3	7·6	27·0	2·1	Watanabe *et al.*, 1982
(1976–No. 236/2016)	12·5	20·85	34·9	3·0	7·0	8·8	Léger *et al.*, 1985c
(1976–2596)	13·0	21·9	34·1	4·7	7·8	7·9	Vos *et al.*, 1984
(1978–1728)	14·4	16·3	28·0	4·5	9·2	13·8	Vos *et al.*, 1984
	11·5	12·6	29·7	7·1	14·8	6·8	Witt *et al.*, 1984
(1976–I)	12·5	20·9	34·9	3·0	5·9	8·8	Léger *et al.*, 1985c
(1976–II)	13·0	20·0	34·7	4·7	7·5	8·2	Léger *et al.*, 1985c
(1978–V)	9·5	4·9	28·5	8·7	27·2	1·5	Léger *et al.*, 1985c
(1978–VI)	10·0	5·0	32·7	9·2	26·3	1·6	Léger *et al.*, 1985c
(1978–VIII)	9·0	4·6	28·3	9·1	27·6	1·2	Léger *et al.*, 1985c
(1978–XII)	11·5	7·7	28·2	8·2	20·9	3·6	Léger *et al.*, 1985c
(1978–XIV)	5·9	7·3	32·8	8·5	25·6	2·8	Léger *et al.*, 1985c
(1979–III)	11·3	3·1	27·6	7·9	23·6	1·8	Léger *et al.*, 1985c
(1979–IX)	10·2	4·3	28·2	9·7	26·3	0·7	Léger *et al.*, 1985c
(1979–X)	10·8	4·2	27·9	8·1	27·7	0·6	Léger *et al.*, 1985c
(1979–XI)	11·6	7·3	28·5	6·9	18·7	4·7	Léger *et al.*, 1985c
(1980–IV)	11·3	6·0	26·8	8·3	23·3	1·7	Léger *et al.*, 1985c
(1980–VII)	11·7	4·4	27·3	10·0	28·0	1·4	Léger *et al.*, 1985c
(1980–XIII)	12·2	7·1	30·8	7·5	22·2	3·4	Léger *et al.*, 1985c
(1983)	12·4	17·4	27·9	5·7	3·6	11·7	Léger, unpubl.
(1984A)	12·9	15·5	28·3	5·7	8·2	9·4	Léger, unpubl.
(1984B)	13·1	9·9	29·1	6·6	16·7	5·8	Léger, unpubl.
(1984C)	14·0	6·2	28·3	8·5	21·2	2·2	Léger, unpubl.

	(1984D)	13·2	16·6	30·7	5·0	4·5	10·0	Léger, unpubl
	(1984E)	12·6	8·7	28·0	6·8	18·7	5·0	Léger, unpubl.
	(1984F)	14·0	7·2	28·3	8·4	21·5	2·0	Léger, unpubl.
	(1985G)	13·6	6·7	28·1	9·1	22·6	1·9	Léger, unpubl.
	(1985H)	14·5	14·6	28·1	6·7	13·2	5·6	Léger, unpubl.
	(1985I)	14·9	8·9	27·5	7·9	19·1	3·8	Léger, unpubl.
	(1985J)	13·0	10·4	27·6	6·4	17·7	5·3	Léger, unpubl.
	(1985K)	13·4	7·3	29·0	8·5	22·2	2·5	Léger, unpubl.
	(1985L)	13·9	6·9	27·1	9·0	20·3	2·2	Léger, unpubl.
	(1985M)	13·9	6·3	28·6	9·3	21·9	1·8	Léger, unpubl.
U.S.A., San Pablo Bay	(1978–1628)	7·79	5·24	29·15	4·6	33·6	1·68	Schauer *et al.*, 1980
	(1978–1628)	9·3	4·8	27·0	9·9	31·0	0·2	Léger *et al.*, 1985c
Vietnam, Cam Ranh Bay	(CR, 1984)	15·4	14·2	34·8	3·6	1·6	11·0	Léger, unpubl.
	(CR, 1985)	14·8	14·4	30·7	4·9	3·9	10·5	Léger, unpubl.
	(CRHT, 1985)	15·6	14·7	31·4	5·1	3·1	10·1	Léger, unpubl.
	(CRVT, 1985)	15·8	15·5	31·1	3·8	1·8	13·7	Léger, unpubl.

TABLE XIII

Coefficient of variation of contents of particular fatty acids in Artemia *nauplii from commercial sources listed in Table XII: Sa, South arm; Na, North arm; *, may include other monoënes; **, value of all* Artemia *strains and samples reported in Table XII*

	16:0	16:1ω7*	18:1ω9*	18:2ω6	18:3ω3	20:5ω3
Coefficient of Variation (%)						
U.S.A., San Francisco Bay	30·2	57·1	16·1	30·9	53·0	78·6
U.S.A., Great Salt Lake (Sa)	8·5	17·9	9·1	22·7	21·1	11·8
U.S.A., Great Salt Lake (Na)	4·5	17·5	6·6	5·9	5·8	21·2
Canada	19·9	20·2	12·5	16·5	18·3	18·3
Brazil	13·1	14·8	21·3	30·2	43·2	43·2
China	28·8	18·4	18·4	26·8	50·5	50·5
Overall value**	24·6	50·4	14·8	57·3	71·7	71·7

for 16:1ω7 fall between 3·0 and 9·9%, whereas another 44% of the values fall between 10·0 and 19·9%. Very often, the most abundant fatty acid in *Artemia* is 18:1ω9 (oleic acid), for which values range from 14·0–37·5% of total FAMEs (overall mean of 27·8%). Of the values listed for 18:1ω9, 96·5% are higher than 20.0%. Over all variance for this fatty acid is the lowest when compared with the other main fatty acids.

To summarize, we find that the major saturated and monoene FAMEs (16:0, 16:1ω7, and 18:1ω9) generally comprise about 40 to 60% of total FAMEs in a sample of *Artemia*. In addition, the major diene, 18:2ω6 (linoleic acid), usually contributes something <10% (range: 1·6–11·8%; overall mean of 7%) to the FAME total.

The major fatty acids of the linolenic series, 18:3ω3 (linolenic acid) and 20:5ω3 (eicosapentaenoic acid), must be considered together because of their importance as essential fatty acids (EFA) and because their levels are mostly interrelated. 18:3ω3 is considered the EFA for freshwater fish and 20:5ω3 an EFA for marine fish (see p. 560). Kanazawa *et al.* (1979) and Schauer & Simpson (1985) demonstrated that 18:3ω3 is readily converted to 20:5ω3 in freshwater fish, but the conversion by marine fish is very slight. It is, therefore, necessary to have adequate amounts of 20:5ω3 in the diet of larval marine fish and crustaceans. Although the range of values for 18:3ω3 is 0·4 to 33·6% of total FAMEs, the distribution of the values is actually bimodal. 36% of the values of 18:3ω3 are 20.0% or greater (of total FAMEs) and 43% of the values are 10.0% or less (of total FAMEs). Thus, 18:3ω3 is usually either very abundant or very scarce. This is reflected in a high overall variance (coefficient of variation of 71·7%) which is mainly due to a high variability in San Francisco Bay, Brazilian, and Chinese *Artemia*. The level of 20:5ω3 is inversely related to the level of 18:3ω3. If one examines the data for all the samples in Table XII in which the level of 18:3ω3 exceeded 20% of total FAMEs, one finds that the values of 20:5ω3 in those samples were consistently low (mean and SD of 20:5ω3 in those samples is 2·1±1·5%). By contrast, in those samples in which the level of *18:3*ω3 was <10%, the values for 20:5ω3 were substantially higher (7·9±3·9). Standard deviations are relatively high because of a few

exceptions to this rule, *e.g. Artemia* from Great Inagua (Bahamas), Cabo Roya (Puerto Rico), Manaure (Colombia), and some samples from San Francisco Bay, Brazil and China have low levels of both 18:3ω3 and 20:5ω3; some *Artemia*, on the other hand, contain relatively high levels of both 18:3ω3 and 20:5ω3, *e.g.* Bangpakong (Thailand), Australia, Lavalduc (France), and Canada. In general, however, Watanabe *et al.* (1978c) were right in dividing *Artemia* samples into two categories: *i.e.* those good for freshwater organisms (high 18:3ω3, low 20:5ω3) and those good for marine organisms (low 18:3ω3, high 20:5ω3).

An examination of the 18:3ω3 and 20:5ω3 data in Tables XII and XIII from the point of view of variability between and within geographical strains is disconcerting. While there is clearly variability among strains (Schauer *et al.*, 1980; Seidel *et al.*, 1982; Léger, unpubl.), there is at least as much variability within the strain, both between years and during one year (Watanabe *et al.*, 1978c, 1980, 1982; Léger *et al.*, 1985c; Léger, unpubl.). Strains from San Francisco Bay, China, and Brazil are particularly variable in levels of 20:5ω3 (see Table XII). On the other hand, 20:5ω3 levels in Utah (Southern Arm and Northern Arm) are remarkably constant.

On-grown and adult Artemia

It is not clear whether adult *Artemia* simply reflect their diet or convert fatty acids irrespective of diet. Both indirect and direct evidence exists to show that *Artemia* can elongate 18:3ω3 to 20:5ω3. Kayama *et al.* (1963) fed phytoplankton (*Chaetoceros simplex*) lacking 20:5ω3 to *Artemia*, but the subsequent fatty-acid profile of *Artemia* included high levels of 20:5ω3. Jezyk & Penicnak (1966) obtained similar results when they reared *Artemia* on an unknown species of green algae that lacked 20:5ω3. Hinchcliffe & Riley (1972) fed *Artemia* on four separate algal species, only one of which (*Chlamydomonas* sp.) lacked 20:5ω3; nevertheless, the *Artemia* fed on *Chlamydomonas* contained 20:5ω3, although at a lower level than when fed the other algal species. The fact that, in most cases, *Artemia* did not resemble very well their diet led Hinchcliffe & Riley to conclude that the metabolic needs and conversion abilities of *Artemia* determine their fatty-acid profile. Schauer & Simpson (in press) have obtained clear evidence *via* radioactive labelling of rice-bran diets that Australian *Artemia* can elongate 18:3ω3 to 20:5ω3; however, recent evidence (Millamena & Simpson, 1985) indicates that the Utah strain may be different. Fatty-acid analyses of Utah *Artemia* grown in ponds in the Philippines show that the *Artemia* very closely resembled their live algal diets, *Chaetoceros* sp. (high 20:5ω3, low 18:3ω3) and *Dunaliella* sp. (low 20:5ω3, high 18:3ω3). These various findings are not necessarily contradictory. *Artemia* is certainly able to convert 18:3ω3 to 20:5ω3 to meet its metabolic needs, but the percentage of 20:5ω3 required to meet those needs may be much less than the levels found in some algae. From culture experiments with *Artemia* fed different diets (*e.g.* Sakamoto *et al.*, 1982; Yashiro, 1982, 1985; Millamena *et al.*, 1985; Léger, unpubl.) it is clear that 20:5ω3 levels in *Artemia* are greatly determined by the food ingested. Indeed, high 20:5ω3 levels in the diet (e.g. *Chaetoceros* sp. and fish oil based diets) are reflected in elevated levels in *Artemia*, while low dietary levels (*e.g. Dunaliella*) result in reduced

concentrations in *Artemia*. Nevertheless, when 20:5ω3 lacking diets are fed (*e.g.* rice bran and other agricultural products) still a minimal 20:5ω3 level will appear in *Artemia*. This is another indication that *Artemia* is able to biosynthesize a minimal amount of 20:5ω3 to meet its metabolic requirements. Biosynthesis in *Artemia* is also noticed for 16:1 and 18:1 while 16:0, 18:2ω6, and 18:3ω3 more closely reflect dietary levels. An interesting experiment in this regard was performed by Léger (unpubl. data, see Table XIV) who cultured three *Artemia* strains (Great Salt Lake—Southern Arm, San Francisco Bay, and San Pablo Bay) that have a very different fatty-acid profile (see Table XII) on rice bran which is deficient in 18:3ω3 and 20:5ω3; after 1 week culturing the three groups of pre-adult brine shrimp ended up with a very similar fatty-acid profile. The same experiment also showed that a 20:5ω3-rich *Artemia* (SFB 236–2016) will consume its 20:5ω3 reserves up to a minimal level when fed a 20:5ω3 -lacking diet (rice bran). Similarly, 18:3ω3-rich strains (San Pablo Bay and Great Salt Lake) consume most of their 18:3ω3 reserve when fed a 18:3ω3-poor diet, even in the presence of high dietary 20:5ω3 levels (cod liver oil).

DIGESTIVE ENZYMES

Among the many explanations suggested for the superior value of live food (compared with artificial diets) for fish and crustacean larvae, one of the most intriguing is that exogenous enzymes may contribute to the digestive process. If the larval digestive tract is incompletely developed, living food eaten by the larvae may contain not only the required nutrients, but also some of the enzymes needed to digest them. The question of exogenous enzymes has been studied for both freshwater fishes (Dabrowski & Glogowski, 1977a,b) and marine shrimps (Maugle, Deshimaru, Katayama & Simpson, 1982).

Artemia nauplii possess some carbohydrase activity (Telford, 1970) with particularly strong activities on the substrates amylopectin, glycogen, maltose, and trehalose. Dabrowski & Glogowski (1977a) found relatively high proteolytic activity in *Artemia* nauplii homogenates at both acid and alkaline pH levels. The activities of amylase and trypsin in various life stages of *Artemia* have been extensively studied by Samain, Boucher & Buestel (1975) and Samain *et al.* (1980, 1985). Osuna *et al.* (1977) showed that the activity of four proteolytic enzymes in *Artemia* nauplii increased sharply after hatching and Olalla *et al.* (1978), Sillero *et al.* (1980), and Burillo, Sillero & Sillero (1982) subsequently characterized the four as alkaline proteases. An acid protease has also been discovered (Nagainis & Warner, 1979) and characterized (Warner & Shridhar, 1980) in dormant *Artemia* cysts. Burillo *et al.* (1982) pointed out that the four alkaline proteases could lyse *Artemia* yolk platelets and calculated that their activity was sufficient to account for the rate of yolk platelet degradation observed in live nauplii. Several recent publications deal with various aspects of digestive enzymes in *Artemia* (Ezquieta & Vallejo, 1985; Munuswamy, 1985; Perona & Vallejo, 1985; Samain *et al.*, 1985). Whether these enzymes operate in the digestive tracts of predators that are fed *Artemia* nauplii is unknown and is a potentially fruitful area for research.

TABLE XIV

Data on per cent composition of the six major fatty acids (as fatty acid methyl esters) of ongrown and adult Artemia: *data are expressed as percentage of total fatty acid methyl esters for each sample: *, may include other monoënes; **, only polar lipid fraction given; SFB, San Francisco Bay, U.S.A.; SPB, San Pablo Bay, U.S.A.; GSL, Great Salt Lake, U.S.A.; Sa, South arm; ML, Mono Lake, U.S.A.; n.s., source not specified*

Artemia	Source	Food	Fatty acid 16:0	16:1ω7*	18:1ω9*	18:2ω6	18:3ω3	20:5ω3	Reference
Wild	ML		17·0	14·5	38·8	3·9	7·4	5·9	Enzler *et al.*, 1974
	SFB		13·5	13·8	35·6	6·2	—	12·0	Gallagher & Brown, 1975
Cultured	SFB	*Chaetoceros***	15·5	19·4	30·6	2·8	3·9	12·7	Sakamoto *et al.*, 1982
		Microencapsulated diets, lipid free**	13·6	6·8	43·2	8·2	7·0	1·6	Sakamoto *et al.*, 1982
		cod liver oil**	9·4	7·2	43·7	7·8	6·9	9·2	Sakamoto *et al.*, 1982
		Tapes oil**	9·4	5·6	40·1	5·5	6·3	8·0	Sakamoto *et al.*, 1982
		soybean oil**	12·4	2·9	35·1	20·7	7·5	3·4	Sakamoto *et al.*, 1982
	SFB	Wheat flour extract	9·61	6·92	28·90	22·80	7·94	2·34	Yashiro, 1982
		Rice bran extract	12·44	4·93	34·36	26·14	4·48	2·18	Yashiro, 1982
		Milled rice extract	12·84	4·03	23·40	10·09	11·16	7·67	Yashiro, 1982
	SFB	Rice bran	15·2	10·9	33·6	21·6	1·7	0·8	Léger, unpubl.
	(No. 236–2016)	Rice bran + cod liver oil	12·2	14·4	36·4	9·1	1·2	9·2	Léger, unpubl.
	SPB	Rice bran	14·4	9·0	30·2	16·5	4·8	1·6	Léger, unpubl.
	(No. 1628)	Rice bran + cod liver oil	11·0	10·7	32·8	6·2	4·1	8·8	Léger, unpubl.
	GSL	Corn	10·62	5·87	39·54	32·03	1·63	2·18	Millamena *et al.*, 1985
		Copra	14·10	11·38	32·93	8·02	0·93	1·34	Millamena *et al.*, 1985
		Rice bran	11·91	6·76	39·17	29·08	1·90	1·19	Millamena *et al.*, 1985
		Soybean	8·97	4·29	37·30	33·12	3·47	0·98	Millamena *et al.*, 1985
		Chaetoceros	11·70	22·51	17·25	5·04	0·94	18·64	Millamena *et al.*, 1985
		Dunaliella	14·76	2·46	27·32	13·43	20·16	4·72	Millamena *et al.*, 1985

TABLE XIV—*continued*

Artemia	Source	Food	Fatty acid 16:0	16:1ω7*	18:1ω9*	18:2ω6	18:3ω3	20:5ω3	Reference
	GSL-Sa	Corn byproduct A	12·0	6·1	33·1	35·8	1·5	0·5	Léger, unpubl.
		Corn byproduct B	12·0	9·6	31·2	27·4	2·1	1·1	Léger, unpubl.
		Defatted rice bran	13·3	9·1	36·1	23·5	1·8	0·9	Léger, unpubl.
	n.s.	*Chaetoceros*	11·6	44·9	18·4	0·7	0·5	12·0	Kayama *et al.*, 1963
	n.s.	*Chlamydomonas*	12·0	4·4	14·0	7·7	11·9	4·6	Hinchcliffe & Riley, 1972
	n.s.	*Monochrysis*	12·9	13·4	17·8	6·5	4·4	17·3	Hinchcliffe & Riley, 1972
	n.s.	*Phaeodactylum*	9·8	9·2	21·6	10·0	9·0	11·0	Hinchcliffe & Riley, 1972
	n.s.	*Platymonas*	12·0	5·0	14·7	6·5	13·9	9·2	Hinchcliffe & Riley, 1972

CAROTENOIDS

The carotenoid composition of *Artemia* has been the subject of some controversy. Gilchrist & Green (1960) concluded that astaxanthin was the only carotenoid pigment in *Artemia*, although Gilchrist (1968) admitted that this was probably a misdiagnosis. Krinsky (1965) reported that canthaxanthin and echinenone were the major pigments present and postulated that *Artemia* converts dietary β-carotene to echinenone and thence to canthaxanthin. Subsequently, Davies, Hsu & Chichester (1965), Czygan (1966), Gilchrist (1968), Hata & Hata (1969), and Wickins (1972) all showed that the main carotenoids in *Artemia* were echinenone and canthaxanthin. Hsu, Chichester & Davies (1970) and Davies, Hsu & Chichester (1970) finally demonstrated conclusively that canthaxanthin and echinenone were the conversion products when *Artemia* were fed β-carotene and that the scheme proposed by Krinsky was most probably correct.

In all the studies mentioned in the preceding paragraph, the investigators used California *Artemia*. The controversy arose when Czygan (1968) suggested that a Canadian *Artemia* strain is able to form astacene and Czeczuga (1971) reported that cysts he had obtained from scientists in France contained mostly β-carotene (53·3%), much astaxanthin (26·8%) and almost no canthaxanthin (1·2%). Czeczuga (1971, 1980) postulated that the qualitatively different results obtained by different authors is due to differences in the food eaten by the *Artemia* and that carotenoid content of *Artemia* "eggs" depends on the carotenoid content of the adult food. Although his contention seems to be invalidated by the experiments of Hsu *et al.* (1970) and Davies *et al.* (1970), the possibility exists that the Canadian strain studied by Czygan (1968) and the (presumably) French strain studied by Czeczuga (1971) are different from the other strains. Unfortunately, Soejima *et al.* (1980) did not examine the French and Canadian strains along with the eight geographical strains that contained only echinenone and canthaxanthin. They did show, however, that astaxanthin in the diet could be absorbed and accumulated by *Artemia*. Subsequently, they also found that *Artemia* could bioaccumulate astacene from the diet (Soejima, Simpson & Katayama 1983). Recently, Nelis *et al.* (1985) analysed 19 different strains of *Artemia* and confirmed that for all strains tested canthaxanthin was the most abundant carotenoid. Some differences between strains were found in amount of total canthaxanthin, which is probably determined by environmental factors. Another difference they noticed was the relative amount of cis- and trans-canthaxanthin. Cis-canthaxanthin, which has not been isolated yet from other animals, was recently discovered by Nelis *et al.* (1984) in *Artemia* cysts and in the reproductive system of female brine shrimps.

STEROLS

Artemia are unable to synthesize sterols from acetate, but can convert several sterols to cholesterol, the only sterol found in the brine shrimp (Teshima & Kanazawa, 1971a). The dietary sterols that have been shown to be bioconverted to cholesterol by *Artemia* are ergosterol (Teshima & Kanazawa, 1971b), brassicasterol (Teshima & Kanazawa, 1972), β-sitosterol and 24-methylcholesterol (Teshima, 1971).

VITAMINS

Stults (1974) analysed *Artemia* cysts (San Francisco Bay) and found high levels of thiamin (7·13 $\mu g \cdot g^{-1}$), niacin (108·68 $\mu g \cdot g^{-1}$), riboflavin (23·15 $\mu g \cdot g^{-1}$), pantothenic acid (72·56 $\mu g \cdot g^{-1}$) and retinol (10·48 $\mu g \cdot g^{-1}$ or 35 IU). These levels are higher for riboflavin and panthotenic acid and almost as high for niacin as those reported by Sparre (1962 in Stults, 1974) for whole fish meal. Stults also mentioned that vitamin losses occurring during storage of fishmeal should be zero in *Artemia* cysts as long as they remain whole and viable.

A stable form of vitamin C (L-ascorbic acid 2- sulphate) was discovered in dormant *Artemia* cysts (Mead & Finamore, 1969); Golub & Finamore (1972), however, found that during embryonic development and hatching the stable form disappears and is replaced by L-ascorbic acid.

A vitamin analysis has also been reported for adult brine shrimp (Gallagher & Brown, 1975; published in corrected form by Simpson, Klein-MacPhee & Beck, 1983). The composition compares very favourably with the minimum dietary requirement for salmonids (Ketola, 1976), but is slightly less than the recommended dietary levels for cold-water fishes (Anonymous, 1981) in niacin, pyridoxine, and riboflavin.

POLLUTANTS

Because *Artemia* grow in many areas of the world close to human populations, anthropogenic inputs to their environment such as chlorinated hydrocarbons (CHCs) and heavy metals are often found in cysts and nauplii. Bookhout & Costlow (1970) measured DDT concentrations of 2·30 $\mu g \cdot g^{-1}$ and 7·05 $\mu g \cdot g^{-1}$ in *Artemia* nauplii from California and Utah, respectively, whereas Wickins (1972) reported DDT levels of 0·0004–0·02 $\mu g \cdot g^{-1}$ and PCB levels of 0·04–0·08 $\mu g \cdot g^{-1}$ for nauplii from those regions. CHC concentrations in nauplii from eight geographical sources and two Reference strains (Olney *et al.*, 1980; Seidel *et al.*, 1982; Bengtson *et al.*, 1985) ranged over about two orders of magnitude (2–422 $ng \cdot g^{-1}$) for total DDTs and more than one order of magnitude (1–66 $ng \cdot g^{-}$) for total PCBs. Nauplii from Italy and China generally had the highest CHC levels and those from Brazil, Australia, and the Reference strains the lowest.

Olney *et al.* (1980) provided the only published data on heavy metal content (12 metals) in *Artemia* cysts and nauplii. They concluded that differences among geographical strains were small and that the levels observed were not particularly high. According to Blust (pers. comm.) and our own unpublished data levels of copper in Great Salt Lake *Artemia* cysts are low in the Northern Arm cysts (around 10 $\mu g \cdot g^{-1}$ on a dry weight basis) and high in commercial batches of Southern Arm cysts (80 $\mu g \cdot g^{-1}$ and more). Cyst samples collected at different sites, 40 to 60 km north of the commercial harvesting area (a major dumping site of copper ore wastes, Sanders Brine Shrimp Cy, pers. comm.) have significantly lower Cu-contents (16 to 20 $\mu g \cdot g^{-1}$); contrary to commercial batches of Great Salt Lake South Arm cysts, the latter samples appear to be an acceptable source of live food for different crab species (Goy, pers. comm.; see also p. 554).

CONCLUSIONS AND PERSPECTIVES

Although *Artemia* nauplii have already been used for a few decades as live food for culturing larvae of various fish and shrimp species, it is only during recent years that the nutritional properties of freshly hatched *Artemia* nauplii have been better understood. It had been known for some time that *Artemia* could not be considered as a 'standard' food. It was, however, only in the late 1970s when several new geographical sources of *Artemia* became available that detailed characterization work in Japan and through the International Study on *Artemia* could compare the suitability of particular sources or batches of *Artemia* cysts as a larval food source with specific *Artemia* characteristics, *e.g.* nauplius dimension, fatty-acid content, contamination level. Probably the most critical factor determining the dietary value of *Artemia*, as a food-source for marine predators, is the presence and concentration of essential fatty acids; *i.e.* the natural prey of marine fish and crustacean larvae mostly contain substantial levels of the highly unsaturated fatty acids 20:5ω3 and 22:6ω3, whereas in *Artemia* their concentration is inconsistent and minimal if present at all. This is due to the extreme as well as highly fluctuating natural environment in which *Artemia* and especially its particular diet are developed. In this regard it is very fortunate that the early pioneers in fish culturing were using a nutritionally adequate *Artemia* product from the San Francisco Bay strain; *Artemia* might never have become a widely recognized 'suitable' diet for marine organisms if Great Salt Lake *Artemia*, deficient in essential fatty acids had been the only source of *Artemia* available at that time.

It is obvious now, more than ever before, that the special value of *Artemia* as a food source is due not so much to its nutritional composition but is related to a large extent to its convenient production, its optimal physical availability as a moving prey of suitable size, and to the opportunities it provides for bioencapsulation of vital components, *i.e.* to convert it from a deficient food into a supra-natural diet. It is clear that as dietary requirements of marine fish and shrimp larvae become better known, the *Artemia* enrichment technique involving bioencapsulation of vital components will be most useful in enhancing larval nutrition. A very recent example being an improved pigmentation in flatfish larvae (Pricket, pers. comm.; Danish Aquaculture Institute, pers. comm.) through HUFA-enrichment of the live foods.

The causal relationship between high contamination levels and low nutritional quality of *Artemia* nauplii was over-estimated in the earliest publications. It is not yet clear, however, to what extent the presence of pesticides, heavy metals or other contamination products may affect the biological effectiveness of *Artemia* as a food source, especially when considering potentially delayed effects expressed in post-*Artemia* feeding stages; *e.g.* toxicity effects in larval fish during weaning when lipids in which pesticides have been accumulating are metabolized. As more and more *Artemia* production is initiated in areas where intake waters may be contaminated with industrial wastes or with the run-off waters from agricultural fields, the risks of contamination of *Artemia* cysts with persistent herbicides, pesticides, *etc.* are increasing. Because of their high

tolerance for various contamination products the *Artemia* population may not be affected but bio-accumulation in the cysts will be the consequence.

The great variability in *Artemia* strains as well as batch characteristics are the origin of much confusion when trying to compare data obtained by different authors using different strains and/or batches of the same strain of *Artemia* for their culture tests. This is particularly critical in ecotoxicological testing where the bioassay results may vary as a function of the type of *Artemia* used as food for the test-animals (Bengtson *et al.*, 1984). In this regard the recommendation of the International Study on *Artemia* to use Reference *Artemia* Cysts (Sorgeloos, 1980b) as inter-calibration material should gain more interest. Reference *Artemia* Cysts are only a temporary solution as their limited stocks (from the wild) are never identical when replaced. It is hoped that the laboratory technique for controlled cyst production of Lavens & Sorgeloos (1984) can soon be scaled up to produce so-called "Standard *Artemia* Cysts" of reproducibly high nutritional quality as the inter-calibration material for future research and applications with brine shrimp.

In view of the large variation in nutritional quality of *Artemia*, not only among strains but even between batches of cysts from the same geographical origin, cyst distributors would do a great favour to their customers by providing more detailed product specifications, *i.e.* not only hatching quality characteristics but also strain origin, biometrical data, fatty-acid profiles and eventually contamination levels. In this regard it is obvious that in the future price differences for cysts will also be determined by the variation in nutritional quality.

Although cysts and nauplii still draw most attention in research on applications of *Artemia*, the potential with brine shrimp biomass is at present *under-estimated, e.g.* in nursery and maturation feeding, eventually after application of bioencapsulation enrichment, and as an animal protein source. Again in this field of research and developments, inter-calibration through product characterization (such as biochemical composition) and product processing (such as freezing technique) will be very important.

Finally, much theoretical information exists on how fish and shrimp *production can be improved, e.g.* strain selection, use of decapsulated cysts, cold stored nauplii, on-grown juveniles, *etc.* A better interaction between the academic world and the aquaculture industry is, however, essential to translate better the research findings into commercial profits. It is our conviction that this will improve as competition in this new bio-industry increases.

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