Chapter 3

Biology, Tank Production and Nutritional Value of *Artemia*

*Jean Dhont and Gilbert Van Stappen*

### 3.1 Introduction

*Artemia* has probably been known and used within its natural distribution areas for centuries. However, its fame elsewhere only began to rise in the 1930s when some investigators adopted it as a convenient replacement for the natural diet of fish larvae, thus realising the first breakthrough in the culture of commercially important fish species (Sorgeloos 1980). In the 1950s, *Artemia* cysts were still predominantly marketed for the aquarium and pet trade at costs as low as US $10 kg\(^{-1}\). There were only two commercial sources: the coastal saltworks in the San Francisco Bay (SFB, California, USA) and the Great Salt Lake (GSL, Utah, USA). With fish and shrimp aquaculture developing from the early 1960s, new marketing opportunities were created for *Artemia* cysts. However, by the mid-1970s, increased demand, declining harvests from the GSL, high import taxes in some developing countries and, possibly, an artificial cyst shortage created by certain companies resulted in a severe price rise of up to US $50 or 100 kg\(^{-1}\) *Artemia* cysts by the end of the 1980s (Bengtson *et al.* 1991).

The dramatic impact of the cyst shortage on the expanding aquaculture industry encouraged research on rationalising the use of *Artemia* and exploration of new cyst resources. During that period, the commercial exploitation of several other natural sources (Argentina, Australia, Canada, Colombia, France, PR China) and managed *Artemia* production sites (Brazil, Thailand) occurred. On the initiative of the *Artemia* Reference Center (Ghent University, Belgium) the International Study on *Artemia* (ISA) was established to co-ordinate a variety of different research initiatives (Sorgeloos 1979). The cyst shortage also simultaneously encouraged the search for alternatives for *Artemia* such as microencapsulated diets (Jones *et al.* 1993; Samocha *et al.* 1999) with the aim of eliminating live feed in fish larval nutrition, a process that continues today with slow but steady progress.

With the development of improved techniques for cyst and nauplii applications (Léger *et al.* 1987a) and the exploitation of new natural resources, cyst prices returned to normal and annual market supply reached over 50 t by the 1980s. During the 1980s, improved techniques for harvesting from the open water and favourable hydrological and climatic conditions enabled a 10-fold increase in the yields from GSL (>200 t processed product; Fig. 3.1), while the hatching quality was also improved. This led to the precarious situation where, by the end of the 1980s, cyst supply was more than 90% dependent on one resource, namely
GSL. Despite its size, the GSL remains a natural ecosystem subject to climatic and other influences, and this has been illustrated by unpredictable and fluctuating cyst harvests. This situation led producers in the 1990s to explore new sites such as Lake Urmia in Iran, Aibi Lake in China, Bolshoye Yaroyevo in Siberia, Kara Bogaz Gol in Turkmenistan, and several lakes in Kazakhstan (Lavens & Sorgeloos 2000). In addition, numerous managed ponds and saltworks worldwide provided small quantities (1–20 t each). Although these sites did not necessarily contribute substantially to the world supply of cysts, they provide interesting opportunities for local commercial development.

New insights into hatching characteristics and nutritional essentials gave rise to the segregation of different cyst qualities. Whereas in the early 1990s, cysts with good hatching quality could be purchased for as low as US $20 kg⁻¹, prices of small sized cysts with high eicosapentaenoic acid (EPA; 20:5n-3) levels could reach over US $100 kg⁻¹ at times of short supply owing to their critical role as starter food for marine fish larvae. With the severe cyst shortage in the mid-1990s and at the end of the twentieth century (Fig. 3.1), cyst prices inflated to levels around US $100 kg⁻¹ for GSL product and nearly US $200 kg⁻¹ for the EPA-rich product. Following the superharvest of over 9000 t of raw cysts from the GSL in 2000–2001, prices started to fall again.

Since the early 1990s, cyst consumption has increased exponentially as a consequence of the booming shrimp and marine fish industries. In 1997, some 6000 hatcheries required over 1500 t of cysts annually. Some 80–85% of the total sales of Artemia went to shrimp hatcheries, primarily in China and south-east Asia, as well as Ecuador and a few other Latin American countries; the remainder went to marine fish larviculture in Europe, China and Japan, as well as to the pet fish producers. However, the rationalisation of the use of Artemia in hatcheries (Sorgeloos et al. 1998, 2001) has enabled a dramatic reduction in the required amount of cysts per unit of fish or shrimp produced. For instance, formerly, a typical Mediterranean sea bass and sea bream hatchery would have been using some 150 kg cysts to produce 1 million fry, whereas nowadays the required amount of cysts is only 90 kg for bass and 70 kg for bream. Likewise, in shrimp hatcheries, the consumption of cysts dropped from 10 kg per million postlarvae to less than 5 kg. Although there is no doubt that Artemia will gradually be replaced by formulated diets, it is obvious that the use of nauplii will continue to be market driven for at least a few more years and that record harvests at GSL and new locations may relieve the pressure or even reverse the current trend.

Comprehensive literature reviews on the use of Artemia as live food in fish and shellfish larviculture have been published by Léger et al. (1986) and Sorgeloos et al. (1998, 2001). Today, Artemia is used in the mass culture of different sea bream species, sea bass species, wolf fish, cod, turbot, halibut, flounder species and other flatfish, milkfish, sturgeon, different carp and catfish species and whitefish species. The same is true for commercially important crustaceans such as several shrimp and prawn species, crawfish, several edible crab species and lobster.

Nauplii in instar I and II stages are, undoubtedly, the most widely used forms of Artemia in aquaculture. They are also the easiest and earliest live food, being obtained directly from the cysts. However, it should be borne in mind that any farmer would switch to formulated feed as soon as this proves to be more cost-effective than Artemia. This switch will be triggered not only by the constantly improving quality of formulated feed, but also by price and quality of Artemia. In general, most fish and shrimp larvae accept formulated feed more easily as they grow bigger. This is not only a matter of size of mouthparts and particle size, but also a matter of the developmental stage and efficiency of the digestive system. As a consequence, Artemia is essential only for those species that require live food in their early life stages.

Brine shrimp are mostly used as freshly hatched nauplii or as ‘enriched’ nauplii (see Section 3.5.5). Hatching procedures can be simplified and improved through prior ‘decapsulation’ of cysts (see Section 3.5.4), a process that also improves the quality of poor or non-hatching cysts. Juvenile and adult Artemia, often referred to as ‘biomass’, can be obtained through culturing (see Section 3.3.1) or can be harvested from salt ponds or lakes (Baert et al. 1996). In China, thousands of tonnes are collected on an annual basis from the Bohai Bay salt ponds and are used in the local culture of Chinese white shrimp, Penaeus chinensis (Tackaert & Sorgeloos 1991). Although live biomass has a higher nutritive value, most of the 3000 t that is harvested annually is marketed in frozen form. Part of it is also flaked, dried or incorporated in compound diets.

### 3.2 Biology of Artemia

#### 3.2.1 Morphology and life cycle

In its natural environment under certain conditions, Artemia produces cysts that float at the water surface and are driven ashore by wind and waves. These cysts are metabolically inactive and do not develop further as long as they are kept dry. Upon immersion in seawater, the biconcave cysts hydrate and become spherical and, within the shell, the embryo resumes its interrupted metabolism. After about 20 h the outer membrane of the cyst bursts (‘breaking’) and the embryo appears, surrounded by the hatching membrane (Fig. 3.2). While the embryo hangs underneath the empty shell (the ‘umbrella’ stage) the development of the nauplius is completed and within a short period the hatching membrane is ruptured (‘hatching’), giving rise to the free-swimming nauplius (Fig. 3.3).
The first larval stage (instar I; 400–500 μm in length) has a brownish-orange colour, a red nauplius eye in the head region and three pairs of appendages: the first antennae (sensory function), the second antennae (locomotive plus filter-feeding function) and the mandibles (food uptake function). The ventral side is covered by a large labrum (food uptake: transfer of particles from the filtering setae to the mouth). The instar I larva does not take up food as its digestive system is not yet functional; it relies completely on its yolk reserves. After about 8 h, the animal moult into the second larval stage (instar II). Small food particles (e.g. algal cells, bacteria, detritus) ranging in size from 1 to 50 μm are filtered out by the second antennae and ingested into the now functional digestive tract. The larva grows and differentiates through a number of moults; although there has been considerable disagreement about the exact number of larval stages, generally one naupliar, four metanaupliar, seven postmetanaupliar and five postlarval stages have been described (Hentschel 1968; Scherhardt 1987).

Paired lobular appendages appear in the trunk region and differentiate into thoracopods (Fig. 3.4). On both sides of the nauplius eye lateral complex eyes begin to develop.

(Figs 3.4, 3.5). From the 10th instar stage onwards, important morphological and functional changes begin to take place, i.e. the antennae lose their locomotive function and undergo sexual differentiation. In males (Figs 3.6, 3.7) they develop into hooked graspers, while the female antennae degenerate into sensorial appendages (Fig. 3.8). The thoracopods are now differentiated into three functional parts (Fig. 3.9): the telopods and endopods (locomotory and filter-feeding), and the membranous exopods (gills).

Adult Artemia are typical primitive arthropods (8–12 mm in length) having an elongated segmented body with two stalked complex eyes, a linear digestive tract, sensorial antennae, a pair of functional thoracopods on each of the 11 thoracic segments (Figs 3.7, 3.8) and a furca on the last abdominal segment. The entire body is covered with a thin, flexible exoskeleton of chitin to which muscles are attached internally. The male (Fig. 3.7) has a paired penis on the first of the eight abdominal segments (Fig. 3.10). Female Artemia can easily be recognised by the brood pouch or uterus situated in the same segment, just behind the 11th pair of thoracopods (Figs 3.8, 3.10). The female reproductive system consists of ovaries and oviducts leading into the single, median uterus, wherein several clusters of shell glands open. The ovaries are paired tubular structures extending into the abdomen
6.6 Head of an adult male. (1) Antenna; (2) antennula; (3) lateral complex eye; (4) mandible.

6.7 Adult male.

6.8 Adult female.

Fig. 3.9 Detail of anterior thoracopods in adult Artemia. (1) Exopodite; (2) telopodite; (3) endopodite.

Fig. 3.10 Artemia couple in riding position. (1) Uterus; (2) penis.

Fig. 3.11 Uterus of ovoviviparous Artemia filled with nauplii (first larvae are being released). (1) Ovary with eggs.
(Fig. 3.11). Adult females ovulate approximately every 140 h, depending on rearing conditions and whether development of embryos occurs oviparously or ovoviviparously. In females, spawning is followed by a moult, after which ovulation takes place. The oviducts emerge from the ovaries near the anterior part of the third abdominal segment (Cassel 1937). Each oviduct empties into the anterolateral border of the uterus. The lateral pouches function as seminal receptacles during the time between copulation and fertilisation (within 1 h) (Benesch 1969; Criel 1980a, b). Once ripe, the eggs developing in the ovaries become spherical and migrate via two oviducts into the unpaired uterus.

Fertilised eggs normally develop into free-swimming nauplii (ovoviviparous reproduction) (Fig. 3.11), which are released by the male. In extreme conditions (e.g. high salinity, low oxygen levels) the embryos only develop up to the gastrula stage. At this point they are surrounded by a thick shell (secreted by the brown shell glands located in the uterus), enter a state of metabolic dormancy (diapause) and are then released by the female (oviparous reproduction) (Fig. 3.12). The shell glands consist of several cell clusters, and can vary from dark brown to white or even colourless, depending on reproductive strategy.

In principle, both oviparity and ovoviviparity are found in all Artemia strains, and females can switch reproductive modes from one ovulation to the next. Although females may differ in their genetic tendency to reproduce either oviparously or ovoviviparously, no Artemia are known to lack completely the ability to produce ovoviviparous nauplii. The cysts usually float in the high-salinity waters and are blown ashore where they accumulate and dry. As a result of this dehydration process the diapause mechanism is generally inactivated; cysts are now in a state of quiescence and can resume their further embryonic development when hydrated in optimal hatching conditions. Under optimal conditions brine shrimp can live for several months, grow from nauplius to adult in only 8 days and reproduce at a rate of up to 300 nauplii or cysts every 4 days.

However, not all encysted embryos produced by ovoviviparous animals enter diapause, and nauplii emerge from some cysts without dehydration or other treatment (Jensen 1918; Mathias 1937; Lochhead & Lochhead 1940; Dutrieu 1960a, b; Morris & Aulfeld 1967; Benesch 1969; Anderson et al. 1970). These cysts are surrounded by a much thinner shell than those that enter diapause (Lochhead & Lochhead 1940). Moreover, changes in cysts post release suggest that diapause is established gradually after release (Jardel 1986).

The cryptobiotic cyst shell has two important layers in addition to the hypochlorite-soluble, double-layered outer chorion secreted by the shell glands, and the hypochlorite-resistant embryonic cuticle (Morris & Aulfeld 1967) (see also Section 3.2.5.1). These are the outer cuticular membrane, separating chorion from embryonic cuticle, and the inner cuticular membrane, which delineates the embryo from the fibrous layer of the embryonic cuticle (Fig. 3.13).

### 3.2.2 Ecology and natural distribution

Artemia populations are found in about 500 natural salt lakes and artificial salterns scattered throughout the tropical, subtropical and temperate climatic zones, along coastlines as well as inland. The distribution of these sites over the continents is very uneven, mainly reflecting sampling and exploration activities (Fig. 3.14). As such, it does not give a precise picture of the actual global occurrence of Artemia. The decline of Artemia cyst harvests from the GSL in Utah, USA, since 1977 (Lavens & Sorgeloos 2000) has intensified the search for alternative resources, especially in inland lakes that are sufficiently large and productive to justify commercial exploitation. As a result, several sites, especially in continental Asia, are exploited occasionally or on a regular basis (with some local investment), and these cysts are being used world-wide in aquaculture. The identity or location of these sites has still not reached scientific literature, and attempts are seldom made to perform a systematic
characterisation of the respective strains. A continued survey will undoubtedly lead to the discovery of many more Artemia biotypes in different parts of the world.

Two critical factors determine the population dynamics of Artemia and its biogeographical distribution: first, whether water body conditions allow the animals to survive throughout the year and, secondly, whether or not the seasonality of the environment is predictable (Lenz 1987; Amat et al. 1995).

The common feature of all Artemia biotypes is their high salinity. Salinity is without doubt the predominant abiotic factor determining the presence of Artemia and consequently limiting its geographical distribution. Its physiological adaptations to high salinity provide a very efficient ecological defence against predation, as brine shrimp possess:

- a very efficient osmoregulatory system
- the capacity to synthesise very efficient respiratory pigments to cope with the low oxygen levels at high salinities
- the ability to produce dormant cysts when environmental conditions endanger the survival of the species.

Other variables (temperature, light intensity, primary food production) may have an influence on the quantitative aspects of the Artemia population, or may cause only a temporary absence of brine shrimp.

For physiological reasons the salinity optimum is situated towards the lower end of the salinity range, as higher ambient salinity requires higher energy costs for osmoregulation. Ambient salinity also plays a role in cyst metabolism, as Artemia cysts will only start to develop when the salinity of the medium drops below a certain threshold value.

No Artemia are found in cold tundra or frost climates, as the year-round extremely low temperatures preclude Artemia development. Most strains do not seem to survive prolonged temperatures below 5°C unless in the form of cysts. The maximum temperature tolerated by Artemia populations has repeatedly been reported to be close to 35°C, a temperature often attained in the shallow tropical salt lakes that constitute a large part of the Artemia habitats. This tolerance threshold is, however, strain dependent. Moreover, physiological adaptation of SFB Artemia to high temperatures (40°C) after a number of generations in Vietnamese salt ponds has also been reported (Clegg et al. 2001). As for salinity, temperature optima are difficult to define and are strain dependent; in general, however, the optimum for Artemia is in the range 25–30°C. The ametabolic dehydrated cysts are resistant to a wider temperature range than would ever occur in nature.

Artemia is a non-selective filter feeder of organic detritus, microscopic algae and bacteria. The Artemia biotypes typically show a very simple trophic structure and low species diversity; the absence of predators and food competitors allows brine shrimp to develop into monocultures.

As Artemia is incapable of active dispersion, wind and waterfowl (especially flamingos) are the most important natural dispersion vectors. The floating cysts adhere to feet and feathers of birds and, when ingested, they remain intact for at least 2 days in the digestive tract of birds. Consequently, the absence of migrating birds is probably the reason why certain areas that are suitable for Artemia (e.g. salinas along the north-east coast of Brazil) are not naturally inhabited by brine shrimp.

3.2.3 Taxonomy

The brine shrimp Artemia comprises a group of zygogenetic and parthenogenetic, morphologically similar species very likely to have diverged from an ancestral form living in the Mediterranean area some 5.5 million years ago (Abreu-Grobois & Beardmore 1982; Abreu-Grobois 1987; Badaracco et al. 1987). Speciation in the genus should be regarded as a complex, multidimensional process involving a variety of environmental and genomic factors. The identification of zygogenetic Artemia species has been established by a multi-disciplinary approach, including cross-breeding tests, morphological differentiation, cytogenetics, allozyme studies, and nuclear and mitochondrial DNA sequencing. With the exception of cross-mating, all of these techniques have also contributed to identifying the parthenogenetic types described as A. parthenogenetica by Barigozzi (1974), as well as to gaining insight on population structure, origin and amount of clonal diversity.

In 1755 Schlosser described the brine shrimp based on material collected from the solar saltworks near Lymington, England (no longer in existence) (Kuenen & Baas-Becking 1938). Linnaeus in 1758 classified it as Cancer salinus, and Leach in 1818 renamed the brine shrimp as Artemia salina (Artom 1931). Very often authors have named all brine shrimps A. salina. While for some time the name A. tuiiana was used, Artemia salina is now only recognised as a valid name for the zygogenetic species found in the Mediterranean area (Mura 1990; Triantaphyllidis et al. 1997b).

The differentiation of seven zygogenetic species, defined primarily by the criterion of laboratory reproductive isolation, and many parthenogenetic strains is currently acknowledged. Endemic to the Old World are the parthenogenetic types designated by Barigozzi (1974) as A. parthenogenetica (with different levels of ploidy, found in Europe, Africa, Asia and Australia), the zygogenetic A. salina, Leach 1819 (Mediterranean area) (Triantaphyllidis
et al. 1997b), A. urmiana (Günther, 1890) (Iran), A. sinica (Cal 1989) (continental China), Artemia sp. (Pilla & Beardmore 1994) (Kazakhstan) and A. tibetana (Abatzopoulos et al. 1998) (Tibet). Endemic to the New World are A. persimilis (Piccinelli & Prosdocimi 1968) (Argentina) and A. franciscana (Kellogg 1906) (North, Central and South America), with A. franciscana monica being a special case of a population described for an ecologically unique habitat (Mono Lake, USA).

The genus Artemia is thus a complex of sibling species and superspecies, defined largely, but not completely, by the criterion of reproductive isolation. Very rarely, it has been shown that genetically extremely distinct and allopatric species can produce laboratory hybrids (Pilla & Beardmore 1994).

Coexistence of two species in the same saline habitat is possible: mixtures of parthenogenetic and zygogenetic populations have been reported in Spain, Italy, and central and northern China. Parthenogenetic types tend to predominate in more disturbed, stressful, conditions of salinity, temperature and food availability (Browne & Bowen 1991; Lenz & Browne 1991). Laboratory competition experiments where Artemia adults, belonging to different species, were raised together and reproduced for a maximum of 3 months (25°C, ±0.5°C, fed Dunaliella) resulted in the dominance of A. franciscana over parthenogenetic populations on the one hand, and parthenogenetic populations over A. salina on the other (Browne 1980; Browne & Halanych 1989).

### 3.2.4 Strain-specific characteristics

The world-wide distribution of the brine shrimp Artemia in isolated habitats (about 500 natural salt lakes and artificial salters) with specific ecological conditions has resulted in numerous geographical strains, or genetically different populations within the same sibling species. The parthenogenetic Artemia with their great clonal diversity, as evident from morphology (Hontoria & Amat 1992; Triantaphyllidis et al. 1997a), and cytological and allozyme studies (Abreu-Grobois & Beardmore 1982; Abreu-Grobois 1987; Abatzopoulos et al. 1993) and different ploidy levels (diploid, triploid, tetraploid, pentaploid), display a wide genotypic variation.

While the nutritional value can be manipulated through enrichment, other qualities favourable for aquaculture can be obtained by selection of strains and/or their crosses. In spite of the fluctuations in the harvest, over 90% of all marketed cysts originate from the GSL, but Artemia cysts are commercially available from various production sources in America, Asia, Australia and Europe. Knowledge of the characteristics (both genotypic and phenotypic) of a particular batch of cysts can greatly increase the effectiveness of its use in a fish or shrimp hatchery. Among these strains a high degree of genetic variability as well as a unique diversity in various quantitative characteristics have been observed (Browne et al. 1991). Some of this variability is phenotypic, such as the nutritional composition of the cysts (Léger et al. 1986), and changes from batch to batch. Other characteristics such as cyst diameter and resistance to high temperature are considered strain-specific and remain relatively constant (Vanhaecke & Sorgeloos 1980a), i.e. they have become genotypical as a result of long-term adaptations of the strain to the local conditions.

<table>
<thead>
<tr>
<th>Cyst source</th>
<th>Length (mm)</th>
<th>Dry weight (g/1000)</th>
<th>Energy content (J)</th>
</tr>
</thead>
<tbody>
<tr>
<td>San Francisco Bay, USA</td>
<td>428</td>
<td>1.63</td>
<td>366</td>
</tr>
<tr>
<td>Macau, Brazil</td>
<td>447</td>
<td>1.74</td>
<td>392</td>
</tr>
<tr>
<td>Great Salt Lake, USA</td>
<td>458</td>
<td>2.42</td>
<td>541</td>
</tr>
<tr>
<td>Shark Bay, Australia</td>
<td>458</td>
<td>2.47</td>
<td>576</td>
</tr>
<tr>
<td>Chaplin Lake, Canada</td>
<td>475</td>
<td>2.04</td>
<td>448</td>
</tr>
<tr>
<td>Tanggu, Bohai Bay, PR China</td>
<td>515</td>
<td>3.09</td>
<td>681</td>
</tr>
<tr>
<td>Abi Lake, PR China</td>
<td>515</td>
<td>4.55</td>
<td>—</td>
</tr>
<tr>
<td>Yuncheng, PR China</td>
<td>460</td>
<td>2.03</td>
<td>—</td>
</tr>
<tr>
<td>Lake Urmiah, Iran</td>
<td>497</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

#### 3.2.4.1 Size and energy content

The nutritional effectiveness of a food organism is primarily determined by its ingestibility and, as a consequence, by its size and form. Data on biometrics of nauplii from various Artemia strains are given in Table 3.1. In spite of small variations between batches of the same strain, possibly caused by environmental and/or processing factors, generally the cyst diameter of different production batches of the same strain remains rather constant. Other biometrical characteristics, such as cyst volume, cyst dry weight, instar I naupliar length, individual naupliar weight and naupliar volume, and energy content, show a high correlation with the cyst diameter (Vanhaecke et al. 1983). As a consequence, biometrical parameters, in particular cyst diameter, are good tools to characterise Artemia strains, and to help define the origin of unknown or even mixed cyst samples (Vanhaecke & Sorgeloos 1980a). Some general correlations can also be made between sibling species and size: parthenogenetic Artemia produce large cysts; A. salina, large cysts with a thick chorion; A. franciscana and A. persimilis, small or intermediate cysts with a thin chorion.

#### 3.2.4.2 Hatching quality

Comparative studies of hatching behaviour of cysts of different origin show a considerable variation in hatching percentage, rate and efficiency (Vanhaecke & Sorgeloos 1982, 1983). However, none of these parameters is strain-specific as they are influenced by a wide array of factors such as harvesting, processing, storage and hatching techniques, as well as production conditions affecting the parental generation. For optimal use of Artemia in aquaculture the hatching characteristics of each batch of cysts being used should be known.

#### 3.2.4.3 Diapause characteristics

As diapause can be considered as a life-cycle strategy to overcome temporarily adverse conditions, and to synchronise population developments to the variations of their specific biotype, the process of diapause and its deactivation is likely to be adapted to the population’s habitat (Lavens & Sorgeloos 1987). Adaptations to local conditions may have contributed to strain-specific differences in diapause sensitivity. In response to simple dehydration by storage in a highly saline medium or by air-drying, SFB-type cysts are gradually released.
from diapause (Versichele & Sorgeloos 1980; Vu Do Quynh & Nguyen Ngoc Lam 1987), while cysts from inland salt lakes (GSL, southern Siberia) need a period of cold storage or hibernation of several weeks to break diapause. In addition, differences in tolerance and responsiveness of different strains to hydrogen peroxide (H₂O₂) treatment during diapause deactivation (see Section 3.2.5.3) may be partially genetic and thus strain-specific. Although general recommendations can be formulated with regard to H₂O₂ concentration and exposure time, a limited screening of different combinations is therefore needed when the hatching of new batches is being optimised (Van Stappen et al. 1998).

3.2.4.7 Nutritional value

In the late 1970s, when many fish and shrimp hatcheries started to commercialise, switching from one source of Artemia to another provoked unexpected problems. Aquaculturists even noticed highly significant differences when using different batches from the same geographical origin (Léger & Sorgeloos 1984). In particular, the lipid and fatty acid compositions, as well as the metabolism of fatty acids in the Artemia, seemed to differ widely from strain to strain, and even from batch to batch, as a consequence of the fluctuations in biochemical composition of the primary producers (mainly unicellular algae) available to the adult population (Léger et al. 1987a). Cyst products from inland resources are more constant in fatty acid composition, albeit at suboptimal low levels. Appropriate enrichment techniques have thus been developed to improve the lipid profile of deficient Artemia strains, taking advantage of the indiscriminate filter-feeding behaviour of Artemia (Léger et al. 1987b). Applying simple methods, lipophilic compounds can easily be incorporated into Artemia before being offered as live feed (see Section 3.5.5).

A number of other compounds also varies from strain to strain: nutritional components such as total amount of free amino acids, pigments (canthaxanthin), vitamin C, minerals and trace elements, as well as contamination with chemicals such as pesticides and heavy metals. In most cases these variations are not strain-specific, but correspond to different production conditions. Their effects on larviculture success are far less significant than nauplii fatty acid composition.

3.2.5 Cyst biology and diapause

3.2.5.1 Cyst morphology and physiology

A schematic diagram of the ultrastructure of an Artemia cyst is given in Fig. 3.13. The cyst shell consists of three layers:

- **Alveolar layer:** This hard layer consists of lipopolymers impregnated with chitin and haematin. The haematin concentration determines the colour of the shell, i.e. from pale to dark brown. Its main function is to provide protection for the embryo against mechanical disruption and ultraviolet (UV) radiation. This layer can be completely removed (dissolved) by oxidation treatment with hypochlorite (cyst decapsulation, see Section 3.5.4; Bruggeman et al. 1980).

- **Outer cuticular membrane:** This protects the embryo from penetration by molecules larger than the carbon dioxide molecule (multilayer membrane with a very special filter function; acts as a permeability barrier).

- **Embryonic cuticle:** This transparent and highly elastic layer is separated from the embryo by the inner cuticular membrane (develops into the hatching membrane during hatching incubation). The embryonic cuticle is apparently impermeable to non-volatile solutes (De Chaffoy et al. 1978; Clegg & Conle 1980). The embryo is an undifferentiated gutula, which is ametabolic when water content is below 10% and can be stored for long periods without losing its viability. The viability is affected when water levels are higher than 10% (start of metabolic activity) and when cysts are exposed to oxygen; i.e. in the presence of oxygen, cosmic radiation results in the formation of free radicals, which destroy specific enzymatic systems in the ametabolic Artemia cysts.
3.2.5.2 Cyst metabolism and hatching

Given favourable environmental conditions the metabolism and development of encysted embryos are rapidly reinitiated. When incubated in seawater the biconcave cyst swells and becomes spherical within 1–2 h. Respiration, RNA and protein synthesis begin within minutes (Clegg & Conle 1980), supporting the conclusion that encysted embryos contain all the components needed for these activities. After a period of postdiapause development, in the order of 8–24 h depending on temperature and salinity, the cyst shell (including the outer cuticular membrane) bursts (breaking stage) and the embryo surrounded by the hatching membrane becomes visible. The embryo then leaves the shell completely and hangs underneath the empty shell (the hatching membrane may still be attached to the shell). Through the transparent hatching membrane one can follow the differentiation of the pre-nauplius into the first nauplius which starts to move its appendages. Shortly thereafter, the hatching membrane breaks open (hatching) and the free-swimming larva emerges head-first.

Dry cysts (water content 2 to 5%; Fig. 3.15) are very resistant to extreme temperatures; i.e. hatching viability is not affected in the temperature range – 273°C (Skoultchi & Morowitz 1964) to 60°C; above 60°C and up to 90°C only short exposures can be tolerated. Hydrated cysts have far more specific tolerances: mortalities occur below –18°C and above 40°C; a reversible interruption of the metabolism (viability not affected) occurs between –18 and 4°C and between 33 and 40°C, although the upper and lower temperature limits vary slightly from strain to strain. Active cyst metabolism occurs between 4 and 33°C.

Within this range, the hatching percentage remains constant but the nauplii hatch earlier as the temperature increases.

As for other environmental conditions, optimal hatching outputs are reached in the pH range 8–8.5. As a consequence, the addition of NaHCO₃ (up to 2 g L⁻¹) to artificial or diluted seawater to or dense suspensions of cysts, results in improved hatching. This may be related to the optimal pH activity range for the hatching enzyme.

Increased hatching has been reported with increasing oxygen level in the range 0.6–2 ppm, and maximal hatching above this concentration. To avoid oxygen gradients during hatching a good homogenous mixing of the cysts in the incubation medium is required.

As stated above, hatching in a higher salinity medium will consume more of the energy reserves of the embryo. Above a threshold salinity (varying from strain to strain, but >90 g L⁻¹ for most strains), the amount of water that can be taken up is insufficient to support the embryonic metabolism. Optimal salinity for hatching is equally strain specific, but generally situated in the range 15–30 g L⁻¹.

Although the physiological role of light during the hatching process is poorly understood, brine shrimp cysts, when hydrated and in aerobic conditions, need a minimal light triggering for the onset of the hatching process, related to light intensity and/or exposure time. Little is known about the exact light requirements, but generally strong illumination (about 2000 lux at the water surface) is recommended, at least during the first hours after complete hydration.

Finally, hatchability of cysts is largely determined by the conditions and techniques applied for harvesting, cleaning, drying and storing of the cyst material (see Section 4.4). Hatching quality in stored cysts slowly decreases when cysts contain 10–35% water (Fig. 3.15). This process may, however, be retarded when the cysts are stored at freezing temperatures. The exact optimal water level within the cyst is not known, although there are indications that too severe dehydration (down to 1–2%) results in a drop in viability. Water content of about 5% is a reasonable value.

Water levels in the range 30–65% initiate metabolic activity, eventually reducing the energy content to levels insufficient to reach the state of emergence under optimal hatching conditions (Fig. 3.15). Furthermore, a depletion of the energy reserves occurs when the cysts undergo subsequent dehydration/hydration cycles. Long-term storage of such material may result in a substantial decrease in hatching success. Cysts exposed for too long a period to water levels exceeding 65% will have completed their pre-emergence embryonic development. Subsequent dehydration of these cysts will, in the worst case, result in the killing of the now differentiated embryos.

Sufficiently dehydrated cysts only keep their viability when stored under vacuum or in nitrogen. The presence of oxygen results in a substantial reduction in hatching success as a result of the formation of highly detrimental free radicals. Even correctly packaged cysts are preferentially stored at low temperatures. When frozen, the cysts should be acclimatized for 1 week at room temperature before hatching.

3.2.5.3 Diapause

As *Artemia* is an inhabitant of biotopes characterised by unstable environmental conditions, its survival during periods of extreme conditions (e.g. desiccation, extreme temperatures, high salinities) is ensured by the production of dormant embryos. *Artemia* females can indeed easily switch from the production of live nauplii (ovoviviparity) to cyst formation (oviparity) in response to fluctuating circumstances. The basic mechanisms involved in this switch are
3.3 Production Methods: Tank Production of *Artemia* Biomass

3.3.1 Advantages of tank production and tank-produced biomass

Although tank-produced *Artemia* biomass is far more expensive than pond-produced brine shrimp, its advantages may, depending on the local condition, justify its application:

- year-round availability of ongrows *Artemia*, independent of climate or season;
- specific stages (juveniles, preadults, adults) or prey with uniform size can be harvested as a function of size preferences of the predator;
- quality of the *Artemia* can be better controlled (e.g. nutritional content, free from diseases).

High-density intensive culture techniques offer two main advantages compared with pond production techniques. First, there is no restriction with regard to production site or time, since the culture procedure does not require highly saline waters or specific climatological conditions. Secondly, controlled production can be performed with very high densities of brine shrimp, e.g. several thousand animals per litre versus a maximum of a few hundred animals per litre in outdoor culture ponds. As a consequence very high production yields per volume of culture medium can be obtained with tank rearing systems.

Since the early 1990s, several superintensive *Artemia* farms have been established, e.g. in the USA, France, the UK and Australia, to supply local demand. Depending on the selected culture technology and implantation facilities, production costs are estimated at US $2.5–12 kg⁻¹ live weight *Artemia*, with wholesale prices varying from US $25 to 100 kg⁻¹.

In practice, when setting up an *Artemia* culture one should start by listing the prevailing culture conditions and available infrastructure. The abiotic and biotic conditions relevant for *Artemia* culture are:

- physicochemical conditions:
  - ionic composition of the culture media
  - temperature
  - salinity
  - pH
  - oxygen concentration
  - water quality (nitrogen metabolites, particles, etc.)
- *Artemia*:
  - strain selection
  - culture density
- feeding:
  - feeding strategy
  - selection of suitable diets
- infrastructure:
  - tank and aeration design
  - filter design
  - recirculation unit
3.3.2 Physicochemical conditions

Salinity and ionic composition of the culture media

Although, in the wild, *Artemia* only occurs in highly saline waters (mostly above 100 g l\(^{-1}\)), brine shrimp do thrive in natural seawater. The best physiological performance, in terms of growth rate and food conversion efficiency, is at salinities from 32 to 65 g l\(^{-1}\), depending on the cultured strain.

For *Artemia* culture, the use of natural seawater of 35 g l\(^{-1}\) is the most practical. Small adjustments in salinity can be made by adding brine or diluting with tap water free from high levels of chlorine. Beside natural seawater or diluted brine, several artificial media with different ionic compositions are used with success in indoor installations for brine shrimp production. Since ionic composition is so important, concentrated brine (150 g l\(^{-1}\)) from salinas can also be transported to the culture facilities and diluted with freshwater before use.

Temperature, pH and oxygen concentration

Temperature must be maintained between the specific optimal levels of the selected *Artemia* strain. For most strains a common range of preference is 19–25\(^\circ\)C (Table 3.2).

In the literature, it is generally accepted that the pH tolerance of *Artemia* ranges from 6.5 to 8. The pH tends to decrease during culture as a result of denitrification processes. When the pH drops below 7.5 small amounts of NaHCO3 (technical grade) should be added to increase the buffer capacity of the culture water. The pH is commonly measured using a calibrated electrode or with simple analytical test kits. In the latter case, the method must be suitable for seawater.

With regard to oxygen, biomass production will decrease at concentrations below 2 mg l\(^{-1}\). For optimal production, oxygen concentrations higher than 2.5 mg l\(^{-1}\) are suggested. However, continuously maintaining oxygen levels higher than 5 mg l\(^{-1}\) will result in the production of pale animals (low in the respiratory pigment haemoglobin), possibly with a lower individual dry weight, which may therefore be less perceptible to and attractive for the predators. A dark red coloration (high haemoglobin content) is easily obtained by applying regular oxygen stresses (by switching off the aeration for a few minutes several times a day) a few days before harvesting. Oxygen levels should be checked regularly as they may drop significantly, especially after feeding. Oxygen is conveniently measured in the culture tank with a portable oxygen electrode. When oxygen occasionally drops below 30% saturation (i.e. 2.5 mg O\(_2\) l\(^{-1}\) in seawater of 32 g l\(^{-1}\) salinity), aeration intensity should be increased temporarily or air stones added. If oxygen levels remain low, the aeration capacity should be increased. It is important to remember that for a given air flow, the oxygen level is increased more effectively by small air bubbles than by large ones. However, very small air bubbles can become trapped between the thoracopods, causing the animals to float and congregate at the surface.

<table>
<thead>
<tr>
<th>Geographical strain</th>
<th>Temperature ((^\circ)C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20.0</td>
</tr>
<tr>
<td>San Francisco Bay, California, USA</td>
<td>97</td>
</tr>
<tr>
<td>Biomass production (g l(^{-1}))</td>
<td>75</td>
</tr>
<tr>
<td>Specific growth rate (g l(^{-1}))</td>
<td>0.43(^a)</td>
</tr>
<tr>
<td>Food conversion (%)</td>
<td>3.89(^a)</td>
</tr>
<tr>
<td>Great Salt Lake, Utah, USA</td>
<td>77</td>
</tr>
<tr>
<td>Biomass production (g l(^{-1}))</td>
<td>69</td>
</tr>
<tr>
<td>Specific growth rate (g l(^{-1}))</td>
<td>0.392(^a)</td>
</tr>
<tr>
<td>Food conversion (%)</td>
<td>3.79(^a)</td>
</tr>
<tr>
<td>Chaplin Lake, Saskatchewan, Canada</td>
<td>72</td>
</tr>
<tr>
<td>Biomass production (g l(^{-1}))</td>
<td>78</td>
</tr>
<tr>
<td>Specific growth rate (g l(^{-1}))</td>
<td>0.42(^a)</td>
</tr>
<tr>
<td>Food conversion (%)</td>
<td>3.42(^a)</td>
</tr>
<tr>
<td>Tonga, PR China</td>
<td>95</td>
</tr>
<tr>
<td>Biomass production (g l(^{-1}))</td>
<td>41</td>
</tr>
<tr>
<td>Specific growth rate (g l(^{-1}))</td>
<td>0.299(^a)</td>
</tr>
<tr>
<td>Food conversion (%)</td>
<td>7.22(^a)</td>
</tr>
</tbody>
</table>

Data compiled from Vanhaecke and Sorgeloos (1989).

*Expressed as % recorded for the Artemia reference strain (San Francisco Bay, batch 288-2956) at 25\(^\circ\)C after 9 days' culturing on a diet of Dunaliella cells. *Specific growth rate k = ln(Wi/W0) T\(^{-1}\), where T = duration of experiment in days (\(\approx 9\)). *Food conversion = F/Wi = W0 = F = \(\mu g\) dry weight Dunaliella offered as food, W\(_0\) = \(\mu g\) dry weight Artemia biomass after 9 days' culturing, and W\(_i\) = \(\mu g\) dry weight Artemia biomass at start of experiment. *Means with the same superscript letter are not significantly different at the p < 0.05 level, na, not analysed.

Water quality

The quality of the culture medium is primarily affected by excess particles as well as by soluble waste products such as nitrogenous compounds. High levels of suspended solids will affect production characteristics, either by their interference with uptake of food particles and propulsion by the *Artemia*, or by enhancing bacterial growth that will compete for oxygen and eventually infest the culture tank. Soluble waste products give rise to toxic nitrogenous compounds. The tolerance levels in *Artemia* for ammonia, nitrite and nitrate in acute and chronic toxicity tests with, for instance, GSI, brine shrimp larvae showed no significant effect on survival [median lethal concentration (LC50)] or growth for concentrations up to 1000 mg l\(^{-1}\) NH\(_3\), 320 mg l\(^{-1}\) NO\(_2\) (Chen et al. 1989). For nitrate, no effects were observed at 1000 mg l\(^{-1}\) and it is therefore considered non-toxic. It is therefore unlikely that N-components will interfere directly with *Artemia* cultures. Nevertheless, the presence of soluble substances should be restricted as much as possible since they are an ideal substrate for bacteria. Excess soluble waste products can only be eliminated by diluting the culture water with clean water, be it new or recycled.
3.3.3 Artemia strain selection and culture density

Strain selection

Based on laboratory results (Table 3.2), guidelines are provided for strain selection as a function of optimal temperature and culture performance. The optimal strain should be selected according to specific culture conditions.

Culture density of Artemia

Unlike other crustaceans, Artemia can be cultured at high to very high densities without affecting survival. Depending on the applied culture technique, inoculation densities up to 5000 larvae per litre for batch culture, 10,000 for closed-flow-through culture and 18,000 for open-flow-through culture can be maintained without influencing survival and growth. Above these densities, culture conditions become suboptimal: (water quality deterioration, lower individual food availability), and growth and survival decrease (Table 3.3). Crowding seems to affect ingestion rate and thus growth. In stagnant systems, a clear decrease in growth rate with increasing animal density has been observed (Dhont et al. 1993). The cost-effectiveness of a culture increases with increasing Artemia density. In an open-flow-through system, maximal densities will be limited by feeding rate, while in recirculating and stagnant system the preservation of water quality will determine a safe feeding level, which in turn determines the animal density at which the individual feed amount still allows a satisfactory growth rate. After some culture trials with increasing animal densities, the maximal density can be identified as the highest possible density where no growth inhibition occurs.

3.3.4 Feeding

Artemia is a continuous, non-selective, particle-filtering organism. Various factors may influence the feeding behaviour of Artemia by affecting the filtration rate, ingestion rate and/or assimilation: the quality and quantity of the food offered, the developmental stage of the larvae and the culture conditions. More detailed information about these processes is given in Couteau and Sorgeloos (1989).

Selection of a suitable diet

Artemia can take up and digest exogenous microflora as part of the diet. Bacteria and protozoans, which develop easily in the Artemia cultures, are able to biosynthesize essential nutrients as they use the supplied brine shrimp food as a substrate. In this way they compensate for possible deficiencies in the diet composition. The interactions with bacteria make it a hard task to identify nutritionally adequate diets per se, and growth tests are difficult to run under axenic conditions. As a consequence, nutritional composition of the diet does not play the most critical role in the selection of diets suitable for high-density culture of brine shrimp. The criteria used generally include:

- availability and cost
- particle size composition (preferentially <50 μm)
- digestibility
- consistency in composition among different batches and storage capacity
- solubility (minimal)
- food conversion efficiency (FCE)
- buoyancy.

Commonly used food sources are listed below.

- Microalgae: These undoubtedly yield best culture results but it is rare that sufficient algae are available at a reasonable cost. Mass culturing of suitable algae for Artemia is most often economically unrealistic, so their use can only be considered in locations where algal production is an additional feature of the main activity. Furthermore, not all species of unicellular algae are considered capable of sustaining Artemia growth (D’Agostino 1980); For example, Chlorella and Stichococcus have a thick cell wall that cannot be digested by Artemia. Coccocloris produces gelatinous substances that interfere with food uptake and some dinoflagellates produce toxic substances.

- Dried algae: In most cases algal meals give satisfactory growth performance, especially when water quality conditions are kept optimal. Drawbacks in the use of these feeds are their high cost (>US $12 kg⁻¹), as well as their high fraction of water-soluble components, which cannot be ingested by the brine shrimp, but will interfere with the water quality of the culture medium.

- Bacteria and yeasts: Single-cell proteins (SCP) have several characteristics that make them an interesting alternative to microalgae:
  - the cell diameter is mostly smaller than 20 μm
  - the nutritional composition is fairly complete
  - the rigid cell walls prevent the leakage of water-soluble nutrients in the culture medium
  - products are commercially available at acceptable cost (e.g. commonly used in cattle feeds).

The highly variable production yields that often occur when feeding a yeast mono-diet are assigned to nutritional deficiencies of the yeast diet and should therefore be met by supplementation with other diets. For some SCP, digestibility by the Artemia can be a problem. Complete removal of the complex and thick yeast cell wall by enzymatic treatment and/or supplementation of the diet with live algae significantly improve the assimilation rate and growth rate of the brine shrimp (Couteau et al. 1992).

- By-products from the food industry: Non-soluble by-products from agricultural crops or from the food-processing industry, such as rice bran, corn bran, soyabean pellets and lactic serum, appear to be a very suitable feed source for high-density culturing of Artemia (Dobbeleir et al. 1980). Their main advantages are their low cost and world-wide availability. Equally important in the evaluation of dry food is the consistency of the food quality and supply, and the possibility for storage without loss of quality. Bulk products must be stored in a dry and, preferentially, cool place.

<table>
<thead>
<tr>
<th>Culture system</th>
<th>Animals/litre</th>
<th>Culture period</th>
<th>Growth</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open flow-through</td>
<td>18,000</td>
<td>To adult</td>
<td>High</td>
<td>Tobias et al. (1979)</td>
</tr>
<tr>
<td>Closed flow-through</td>
<td>&gt;10,000</td>
<td>To adult</td>
<td>Moderate</td>
<td>Lavens et al. (1986)</td>
</tr>
<tr>
<td></td>
<td>5,000-10,000</td>
<td>To adult</td>
<td>High</td>
<td>Dhont et al. (1991)</td>
</tr>
<tr>
<td>Stagnant</td>
<td>5,000</td>
<td>7 days</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20,000</td>
<td>7 days</td>
<td>Low</td>
<td></td>
</tr>
</tbody>
</table>
Feeding strategy

Since *Artemia* is a continuous filter-feeding organism, highest growth and minimal deposition of unconsumed food is achieved when food is distributed as frequently as possible. When feeding SCP, algae or yeast, concentrations should be maintained above the critical minimum uptake concentration, which is specific for the algal (or other) species and the developmental stage of *Artemia* (Abreu-Grobois et al. 1991). Levels of dry feeds, consisting of fragments and irregular particles, cannot be counted in the culture tank. Therefore, a correlation between optimal feed level and turbidity of the culture water has been developed, whereby the feed concentration in a culture tank is determined by measuring the turbidity of the water with a simplified Secchi-disc (Fig. 3.16).

3.3.5 Infrastructure

Tank and aeration design

*Artemia* can be reared in containers of any possible shape as long as the installed aeration ensures proper oxygenation and adequate mixing of feed and animals throughout the total culture volume. However, aeration should not be too strong. Thus, aeration and tank design must be considered together as the circulation pattern is determined by the combination of both. A wide variety of culture tanks has proven to be suitable (Dhont & Lavens 1996).

For cultures up to 1 m³, rectangular tanks are most convenient. They can be aerated either with an air-water lift (AWL) system (Fig. 3.17), by an aeration collar mounted around a central standpipe or by perforated polyvinyl chloride (PVC) tubes fixed to the bottom of the tank.

For large volumes (>1 m³), it is advantageous to switch to cement tanks covered on the inside with impermeable plastic sheets or coated with special paint. These large tanks are traditionally operated as raceway systems. They are oblong, approximately 1.5 m wide and with a height:width ratio kept close to 1:2. The length is then chosen according to the desired volume. The corners of the tank may be curved to prevent dead zones where sedimentation can take place. A partition, to which AWLs are fixed, is installed in the middle of the tank and ensures a combined horizontal and vertical movement of the water, which results in a screw-like flow pattern (Bossuyt & Sorgeloos 1980). If axial blowers are used for aeration, the water depth should not exceed 1.2 m to ensure optimal water circulation.

Filter design

The most important and critical equipment in flow-through culturing is the filter used for efficient evacuation of excess culture water and metabolites without losing the brine shrimp from the culture tank. These filter units should be able to operate without clogging for at least 24 h, to reduce risks of overflowing.

Traditionally, filters are constructed as a PVC frame around which an interchangeable nylon screen is fixed. The aeration is positioned at the bottom of the filter, ensuring a continuous friction of air bubbles against the sides of the filter screen and thus reducing filter-mesh clogging (Fig. 3.18).

A more sophisticated type of cylindrical filter system consists of a welded-wedge screen cylinder, made of stainless steel. This welded-wedge system has several advantages with respect to the filter-screen types:

- Larger particles with an elongated shape can still be evacuated through the slit openings.
- The specially designed V-shape of the slit openings creates specific hydrodynamic suction effects, as a result of which filter particles that are only slightly smaller than the slit opening are sucked through.
3.3.6 Culture techniques

Depending on the objectives and the opportunities, different culture procedures for high-density intensive *Artemia* production may be applied. The final selection of the type of culture installation will be subject to local conditions, production needs and investment possibilities. Decisions need to be made as to: (a) whether or not the water should be renewed (open flow-through); and (b) in the latter case, whether a particular water treatment should be applied (closed flow-through or stagnant or batch system). There are many kinds of transition types, ranging from open flow-through with 0% recirculation to closed flow-through with 100% recirculation. In reality, even at complete recirculation, a small part of the culture water must be regularly renewed. The culture system should be designed in such a way that the water quality can be maintained as close to optimal as possible. This means that the concentration of particles and soluble metabolites should remain minimal to prevent toxicity problems, proliferation of micro-organisms and interferences with the filter-feeding apparatus of the brine shrimp.

Stagnant systems

Stagnant systems are the simplest concept for intensive *Artemia* culture: no wastewater evacuation, filter systems or water treatment are involved. The culture is started in an aerated tank and biomass is harvested after a reduced culture period. The main disadvantage is that high animal densities do not allow for extended culture periods because of the degradation of the water quality. Successful trials with 10 animals £1 on micronised soya pellets yielded *Artemia* juveniles of 3 mm in length and over 75% survival in 7 days (Dhont et al. 1993).

Open flow-through

A discontinuous or continuous renewal of culture water by clean seawater, with consequent dilution of particulate and dissolved metabolites, will result in the best possible culture conditions and highest production capacities. Application of an open flow-through culture technique, however, is limited to those situations where large volumes of sufficiently warm seawater (or brine) are available at relatively low cost, or where large quantities of algal feed are available, such as from effluents from artificial upwelling projects, tertia treatment systems or intensive grow-out ponds of shrimp. The water retention time is chosen so as to reach an optimal compromise between efficient evacuation of wastewater and minimal food losses.

A very simple semi flow-through system has been developed by Dhert et al. (1992). The system does not require the use of feeding pumps and involves minimal care. The pilot system consists of six oval raceway tanks of 1 m² and six reservoir tanks of the same capacity placed above each culture tank. These reservoir tanks hold seawater and food (squeezed rice bran suspension), and need manual refilling only once or twice a day. They are slowly drained to the culture tanks, and flow rate is easily adjusted by means of a siphon of a selected diameter. Retention time is at least 12 h. The culture effluent is drained using welded-wedge filters as described above. This technique involves minimal sophistication and appears to be very predictable in production yields, which are between those obtained in batch and flow-through systems (see Section 3.3.9 for production figures).
3.3.8 Harvest and processing of cultured Artemia

Harvesting of high-density cultures of *Artemia* can be facilitated by taking advantage of the surface respiration behaviour of the animals. When the aeration in the culture tank is not sufficient for the animals to maintain their respiration, they will stop feeding and the oxygen levels in the water will drop. When the oxygen levels drop below 2 mg/l, the Artemia will start to swim at the surface of the water, where they can be harvested by skimming or siphoning. The harvested Artemia can then be washed and dried to remove excess water.

3.3.9 Production figures of intensive Artemia cultures

Figure 3.19 provides a summary of average production data expressed as *Artemia* survival and length, obtained in the different culture systems described in this chapter. After...
2 weeks of culturing, pre-adult or adult Artemia with an average length of 5 mm or more can be harvested. In a flow-through culture there is a slight but continuous mortality during the whole of the culture period. No significant differences in survival are observed between open flow-through and recirculating cultures (Fig. 3.19). In stagnant cultures, there is a significantly higher mortality during the end of the first week of culture, which can be explained by the deterioration of the water quality, probably because the early naupliar stages are more sensitive to the juvenile or pre-adult stages.

Average production yields harvested after 2 weeks (live wet weight Artemia biomass relative to tank volume) amount to 5, 15 and 25 kg m⁻³ for batch production, and flow-through systems using micronised feeds and live algae, respectively. These differences in production figures are mainly the result of differences in maximum stocking density at the start and survival at the end of the culture trial.

### 3.4 Biochemical Composition

#### 3.4.1 Proximate composition

Dealing with the biochemical composition of any living organisms usually involves a great deal of generalisation. Presented figures inevitably consist of average values that may conceal subtle changes or intriguing differences. In the case of Artemia, distinctions between species, sources and life-stage must be made, owing to its specific nature and the range of its applications. Unlike algae or rotifers, different life stages of Artemia are used as larval food: from the embryonic form (as decapsulated cysts), through non-feeding nauplii and enriched nauplii, to adult biomass. These life stages may show important biochemical differences. Different Artemia strains or even Artemia from the same strain but from another batch or a different seasonal harvest may show variation in their composition. However, the most important differences in composition can be observed with the exogenous feeding stages (instar II and later). Since Artemia is a non-selective filter-feeder with a relatively high ratio of gut content to body volume, its composition is highly dependent on its diet. This section aims to give a comprehensive picture of the composition of various Artemia forms (see Table 3.5).

#### 3.4.1.1 Cysts and decapsulated cysts

Since the chorion of Artemia cysts is completely indigestible by all known cultured species, the biochemical composition of intact cysts could be considered irrelevant, since they cannot be used as a food source. However, once this chorion has been removed by chemical decapsulation (see Section 3.5.4) the remaining embryo is digestible, and has been used successfully in the larviculture of carp, catfish, milkfish and some marine shrimp.

#### 3.4.1.2 Nauplii

There is a crucial distinction between the first larval stage or instar I, which is non-feeding, and subsequent stages (instar II and further), which have a functional digestive system. Instar I nauplii survive by depleting their yolk reserves, and gradually decrease in nutritional value and in energetic content. Their initial composition reflects the parental characteristics, both genetic and phenotypic, while from instar II nauplii onwards, the composition will also be influenced by the diet.

Several publications offer data on naupliar composition, but few authors make a clear distinction between instar I and instar II stages. This is understandable given the fact that asynchronous hatching produces batches of nauplii consisting of different stages.

#### 3.4.1.3 Juveniles and adults

Ongrown Artemia are used much less frequently in aquaculture than nauplii, for the reason that, while nauplii can easily be obtained through simple hatching of widely available and storable cysts, culturing Artemia requires a considerable amount of labour and infrastructure. Even though various culture techniques have been developed to suit all kinds of local conditions (Section 3.3; Dhont & Laven 1996), they often remain a significant extra investment. However, the nutritional value of ongrowing Artemia compared with freshly hatched nauplii is superior, at least with respect to its protein quality and individual energetic content.

#### Table 3.5 Proximate composition of different developmental stages of Artemia (% on a dry weight basis)

<table>
<thead>
<tr>
<th>Artemia stage</th>
<th>Source (refs)</th>
<th>Protein (g/100g)</th>
<th>Lipid (g/100g)</th>
<th>Carbohydrate (g/100g)</th>
<th>Ash (g/100g)</th>
<th>Fibre (g/100g)</th>
<th>DW (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cysts</td>
<td>GSL, USA (a)</td>
<td>55.8</td>
<td>11.2</td>
<td>6.9</td>
<td>5.9</td>
<td>—</td>
<td>4.83</td>
</tr>
<tr>
<td></td>
<td>SFB, USA (c)</td>
<td>53.2</td>
<td>3.9</td>
<td>36.3</td>
<td>5.2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Mexico (e)</td>
<td>41.1–50.2</td>
<td>0.3–4.0</td>
<td>36.1</td>
<td>5.6–12.6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Decapsulated</td>
<td>GSL, USA (a)</td>
<td>50.6</td>
<td>14.7</td>
<td>6.6</td>
<td>10.6</td>
<td>—</td>
<td>3.42</td>
</tr>
<tr>
<td></td>
<td>SFB, USA (d)</td>
<td>67.4</td>
<td>15.7</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Nauplii</td>
<td>GSL, USA (a)</td>
<td>56.2</td>
<td>17.0</td>
<td>3.6</td>
<td>7.6</td>
<td>—</td>
<td>2.31</td>
</tr>
<tr>
<td></td>
<td>GSL, USA (b)</td>
<td>41.6–47.2</td>
<td>20.9–23.1</td>
<td>10.5</td>
<td>9.5</td>
<td>—</td>
<td>1.65–2.70</td>
</tr>
<tr>
<td></td>
<td>GSL, USA (c)</td>
<td>61.9</td>
<td>14.4</td>
<td>10.6</td>
<td>7.1</td>
<td>3.9</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>PR China (b)</td>
<td>47.3</td>
<td>12.0</td>
<td>21.4</td>
<td>—</td>
<td>—</td>
<td>3.09</td>
</tr>
<tr>
<td></td>
<td>France (b)</td>
<td>55.7</td>
<td>12.4</td>
<td>15.4</td>
<td>—</td>
<td>2.7–3.1</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>SFB, USA (d)</td>
<td>41.9–59.2</td>
<td>15.9–27.2</td>
<td>11.2</td>
<td>8.7</td>
<td>—</td>
<td>1.45–2.87</td>
</tr>
<tr>
<td>Adults: wild population</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>San Diego, USA (b)</td>
<td>64.0</td>
<td>12.0</td>
<td>—</td>
<td>20.6</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>SFB, USA (b)</td>
<td>50.2–58.0</td>
<td>2.4–19.3</td>
<td>17.2</td>
<td>29.2</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Italy (g)</td>
<td>41.9</td>
<td>3.5</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Adults: cultured</td>
<td>GSL, USA (b,e,f)</td>
<td>50.8–67.4</td>
<td>10.8–30.6</td>
<td>4.0–12.3</td>
<td>5.2–13.6</td>
<td>4.2</td>
<td>—</td>
</tr>
<tr>
<td>France (c)</td>
<td>53.7</td>
<td>9.4</td>
<td>—</td>
<td>21.6</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>SFB, USA (d)</td>
<td>39.4–64.0</td>
<td>4.5–12.1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Italy (g)</td>
<td>55.4</td>
<td>4.0</td>
<td>20.0</td>
<td>20.6</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

References: a, García-Ortega et al. (1998); b, Léger et al. (1986); c, Lim et al. (2001); d, Dendrinous and Thorpe (1987); e, Correa Sandoval et al. (1993); f, Correa Sandoval et al. (1994); g, Trota et al. (1987).

DW, dry weight; GSL, Great Salt Lake; SFB, San Francisco Bay; —, not mentioned.
3.4.2 Lipids

The lipid fraction has undoubtedly received most attention in marine larviculture, yielding a wealth of published lipid analysis on all Kind of Artemia strains and life stages. Published values seem to exhibit important differences (Table 3.6). Besides natural fluctuations, these differences may also arise from different analytical methodologies or inaccurate definitions of what exactly was analysed (e.g., exact strain or larval stage).

3.4.2.1 Cysts and nauplii

The lipid content and profile of cysts and instar I nauplii are not affected by diet or environmental conditions. The differences in lipid profile that are observed between strains (Triantaphyllidis et al. 1995; Han et al. 2000b) can reflect either genetic characteristics or the lipid profile of the food of the parental population. However, the lipid profile is considered to be environmentally rather than genetically determined, as several authors have demonstrated that the fatty acid profile of Artemia adults and their offspring clearly reflects the composition of the parental diet, regardless of the strain (Vos et al. 1984; Millamena et al. 1988; Lavens et al. 1989; Navarro & Amat 1992). Many publications include values for 15 or more different fatty acids in Artemia nauplii, but according to Léger et al. (1986) only six fatty acids (16:0, 16:1n-7, 18:1n-9, 18:2n-6, 18:3n-3 and 20:5n-3) actually make up about 80% of the total fatty acid pool in an Artemia sample. In later publications, some authors also detected significant levels of 18:1n-7, which was not reported earlier, probably reflecting increased chromatographic resolution (Estevez et al. 1998; Han et al. 2000b). Léger et al. (1986) compiled data on almost 150 fatty acid analyses of nauplii from about 20 different Artemia sources and came to the following conclusions. Oleic acid (18:1n-9) is often the most abundant fatty acid. It also has the most stable occurrence (lowest coefficient of variation). Together with palmitic (16:0) and palmitoleic acids (16:1n-7), it accounts for 40-60% of the total fatty acids in Artemia. The level of 16:0 is fairly constant over different strains, but levels of 16:1n-7 are more variable. Léger et al. (1986) concluded that Artemia nauplii contain between 0.4 and 33.6% linoleic acid (18:2n-6), but that the distribution of these levels is actually bimodal: 36% of the samples contain more than 20% of their total fatty acids as linoleic acid, while 43% of the samples contain less than 10% linoleic acid. The levels of EPA (20:5n-3) seem to be inversely related to linoleic acid levels. Most of these patterns can be recognized in the more recent compilation presented in Table 3.6. Only data expressed in mg g⁻¹ dry weight have been listed here, as these not only reflect the relative proportions of the various fatty acids, but also indicate their quantities with reference to Artemia body weight.

Table 3.6  Fatty acid composition of different developmental stages of Artemia (mg g⁻¹ dry weight).

<table>
<thead>
<tr>
<th>Artemia stage</th>
<th>Palmitic acid</th>
<th>Palmitoleic acid</th>
<th>Oleic acid</th>
<th>Linoleic acid</th>
<th>Linolenic acid</th>
<th>EPA</th>
<th>DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source (ref)</td>
<td>(16:0)</td>
<td>(16:1n-7)</td>
<td>(18:1n-9)</td>
<td>(18:2n-6)</td>
<td>(18:3n-3)</td>
<td>(20:5n-3)</td>
<td>(22:6n-3)</td>
</tr>
<tr>
<td>Cysts</td>
<td>16.0</td>
<td>16.1-25.7</td>
<td>16.5</td>
<td>12.2-19.4</td>
<td>3.6</td>
<td>12.6</td>
<td>15.7</td>
</tr>
<tr>
<td>GSL, USA (a)</td>
<td>12.7</td>
<td>11.0</td>
<td>12.0</td>
<td>6.7</td>
<td>5.7</td>
<td>3.6</td>
<td>4.5</td>
</tr>
<tr>
<td>GSL, USA (b)</td>
<td>12.0</td>
<td>12.0</td>
<td>12.0</td>
<td>6.0</td>
<td>6.0</td>
<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td>France (b)</td>
<td>12.0</td>
<td>12.0</td>
<td>12.0</td>
<td>6.0</td>
<td>6.0</td>
<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td>Nauplii</td>
<td>12.0</td>
<td>12.0</td>
<td>12.0</td>
<td>6.0</td>
<td>6.0</td>
<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td>SFB</td>
<td>12.0</td>
<td>12.0</td>
<td>12.0</td>
<td>6.0</td>
<td>6.0</td>
<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td>Perlk, Iran (g)</td>
<td>12.0</td>
<td>12.0</td>
<td>12.0</td>
<td>6.0</td>
<td>6.0</td>
<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td>A. parthenogenetica</td>
<td>12.0</td>
<td>12.0</td>
<td>12.0</td>
<td>6.0</td>
<td>6.0</td>
<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td>Madagascari (i)</td>
<td>12.0</td>
<td>12.0</td>
<td>12.0</td>
<td>6.0</td>
<td>6.0</td>
<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td>A. perminutus, Aeg (g)</td>
<td>12.0</td>
<td>12.0</td>
<td>12.0</td>
<td>6.0</td>
<td>6.0</td>
<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td>A. australis, China (j)</td>
<td>12.0</td>
<td>12.0</td>
<td>12.0</td>
<td>6.0</td>
<td>6.0</td>
<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td>Adult</td>
<td>12.0</td>
<td>12.0</td>
<td>12.0</td>
<td>6.0</td>
<td>6.0</td>
<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td>GSL, USA (k)</td>
<td>12.0</td>
<td>12.0</td>
<td>12.0</td>
<td>6.0</td>
<td>6.0</td>
<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td>GSL, USA (l)</td>
<td>12.0</td>
<td>12.0</td>
<td>12.0</td>
<td>6.0</td>
<td>6.0</td>
<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td>SFB, USA (c)</td>
<td>12.0</td>
<td>12.0</td>
<td>12.0</td>
<td>6.0</td>
<td>6.0</td>
<td>4.5</td>
<td>4.5</td>
</tr>
</tbody>
</table>

References: a, Garcia-Ortega et al. (1998); b, Lavens et al. (1989); c, Estevez et al. (1998); d, Han et al. (2000a); e, Drexler and Thorpe (1987); f, Dhert et al. (1993); g, Triantaphyllidis et al. (1995); h, Han et al. (2006); i, Triantaphyllidis et al. (1996); j, Han et al. (1999); k, Lim et al. (2001); l, Lavens (1989); m, Han et al. (2000b); n, Triantaphyllidis et al. (1996); o, Han et al. (1999); p, Lim et al. (2001); q, Han et al. (2000a); r, Dhert et al. (1993); s, Drexler and Thorpe (1987); t, Estevez et al. (1998); u, Han et al. (2000a); v, Han et al. (2000b); w, Triantaphyllidis et al. (1995); x, Han et al. (1999); y, Lim et al. (2001); z, Han et al. (2000a); {, Dhert et al. (1993); |, Drexler and Thorpe (1987); }.

3.4.2.2 Oogrown Artemia

As soon as brine shrimp start feeding (at instar II), their lipid profile will quickly reflect the profile of their diet. This is the basis of the enrichment technique that is discussed in detail in Section 3.5.5.

Just as for nauplii, the lipid composition of natural Artemia biomass will reflect the composition of its diet. The metabolic pathways and abilities of Artemia to convert one fatty acid to another are not yet entirely elucidated. Indications that Artemia converts docosahexaenoic acid (DHA) into EPA (McEvoy et al. 1995) were proven to be correct by Navarro et al. (1999). The conversion rate seems to vary according to the strain (Evjemo et al. 1997). Thus, it should be borne in mind that data on lipid composition of Artemia biomass reflects a variety of influences such as diet, season, geography, strain, life stage and physiological stage, and as such the figures presented only offer an indication of possible levels.

3.4.3 Proteins

Some confusion appears when reviewing amino acid profiles of Artemia because of different methods of analysis or reporting the data. Nevertheless, it seems correct to state that protein levels and amino acid profiles show much less fluctuation between strains or life stages than, for example, the lipid fraction (Table 3.7).

Adult Artemia have a slightly higher protein content than nauplii and contain slightly more essential amino acids than nauplii. The higher protein content of cysts compared with decapsulated cysts or nauplii is due to the presence of the chorion, which is composed of lipoprotein impregnated with chitin and haematin (García-Ortega et al. 1998). Nauplii contain markedly lower levels of free amino acids compared with wild cypodids (Tonheim et al. 2000) but, generally, Artemia naupli as well as adults contain sufficient levels of the 10 amino acids that are considered essential for fish larvae. Even so, methionine seems to be the first
 limiting amino acid when feeding nauplii to fish larvae (Fyn et al. 1993; Conceição et al. 1997; Helland et al. 1999). Most protein in Artemia nauplii consists of small size proteins with a molecular weight between 7.4 and 49.2 kDa (Garcia-Ortega 1999). The presence of these low molecular weight peptides and free amino acids in nauplii, together with their autolytic capacity and high solubility, accounts for the easy digestion of the proteins by fish larvae.

3.4.4 Vitamins

Most data on vitamins relate to Artemia franciscana (Table 3.8). An account of differences between strains could only be found for ascorbic acid (AscA) forms in cysts in Merchier et al. (1995). They observed considerable differences in ascorbic acid 2-sulfate (AscAS) concentrations (296–517 µg g⁻¹ dry weight expressed as AscA) when comparing 10 different strains. Furthermore, they provided further evidence for the complete conversion of the ascorbic acid 2-sulfate form to free ascorbic acid. Whether AscAS serves as a storage form of AscA to satisfy the brine shrimp’s larval requirements after hatching (Mead & Finmore 1969), or acts as a sulfating agent during embryonic development (Mead & Finmore 1969; Bond et al. 1972) is unclear.

With the exception of AscA and thiamine, Artemia nauplii contain higher vitamin levels than natural marine zooplankton (Mæland et al. 2000) and according to the NRC standards (NRC 1993; Kaushik et al. 1998) Artemia seems to cover the minimal dietary requirements of fish larvae. However, exact dietary vitamin requirements have not been established for many marine fish larvae.

3.5 Applications of Artemia

3.5.1 The future use of Artemia in aquaculture

Although there is no doubt that Artemia will gradually be replaced by formulated diets, the use of nauplii will continue to be market driven for at least a few more years (see also Section 3.1). Increased harvests at GSL and new locations may relieve the pressure or even reverse the current trends, but it seems far from redundant to improve and promote the existing applications of Artemia in live food production.

3.5.2 Hatching

Although hatching Artemia cysts appears to be simple, several factors are critical for the successful hatching of the large quantities needed in larval fish production. Optimal hatching conditions are (Lavens & Sorgeloos 1996):

- constant temperature of 25–28°C
- 15–35 g l⁻¹ salinity
- pH around 8.0
- minimum oxygen levels of 2 mg l⁻¹, preferably 5 mg l⁻¹ (see below)
- maximum cyst densities of 2 g l⁻¹
- strong illumination of 2000 lux.

All of these factors will affect the hatching rate and maximum output, and hence the production cost of the harvested Artemia nauplii. Best hatching results are achieved in containers
with a conical bottom, aerated from the bottom with air-lines (Fig. 3.20). Cylindrical or square-bottomed tanks will have ‘dead spots’ in which Artemia cysts and nauplii accumulate and suffer from oxygen depletion. Transparent or translucent containers will facilitate inspection of the hatching suspension, especially when harvesting.

The underlying physiological processes of hatching are described in Section 3.2.5.2, but practical implications are reiterated here.

The aeration intensity must be sufficient to maintain oxygen levels above 2 mg l⁻¹, preferably 5 mg l⁻¹. The optimal aeration rate is a function of the tank size and the density of cysts incubated. Excessive foaming can be reduced by disinfection of the cysts before incubation and/or by the addition of a few drops of a non-toxic antifoam agent (e.g. silicone antifoam).

The temperature of the seawater should be kept in the range of 25–28°C; below 25°C cysts hatch more slowly and above 33°C cyst metabolism is irreversibly stopped. The effect of more extreme temperatures on the hatching output is largely strain specific.

Quantitative effects of the incubation salinity on cyst hatching are related primarily to the hydration level that can be reached in the cysts. Above threshold salinity, the cysts do not absorb sufficient quantities of water. This threshold value varies from strain to strain, but is approximately 85–90 g l⁻¹ for most Artemia strains. Secondly, the incubation salinity will interfere with the amount of glycerol that needs to be built up to reach the critical osmotic pressure within the outer cuticular membrane of the cysts. The fastest hatching rates will thus be noted at the lowest salinity levels, since it will take less time to reach breaking. Optimal hatching can be obtained in the range 15–70 g l⁻¹. For practical convenience, natural seawater is most often used to hatch cysts. For some sources of cysts, hatching at low salinity results in higher hatching efficiencies, and the nauplii have a higher energy content (Table 3.9).

The pH must remain above 8 during the hatching process for optimal functioning of the hatching enzyme. If necessary, for instance when low-salinity water is used, the buffer capacity of the water should be increased by adding up to 1 g NaHCO₃ l⁻¹. Increased buffer capacity is also essential when high densities of cysts are hatched (because of high carbon dioxide production).

Cyst density interferes with other abiotic factors that are essential for hatching, such as pH, oxygen and illumination. The density may be as high as 5 g l⁻¹ for small volumes (<20 litres) but should be decreased to maximum 2 g l⁻¹ for larger volumes, to minimise mechanical injury to the nauplii and to avoid suboptimal water conditions.

Strong illumination (about 2000 lux at the water surface) is essential, at least during the first few hours after complete hydration, to trigger the start of embryonic development. Although this level of illumination can generally be attained in daytime by using transparent tanks set up outdoors in the shade, it is advisable to keep the hatching tanks indoors and to provide artificial illumination, so as to ensure good standardisation of the hatching process.

When hatching large quantities or high densities of cysts, an impressive bacterial load rapidly develops (Dehasque et al. 1993). This is a potential source of pathogens, a competitor for oxygen and a general threat to hatchery hygiene. Reducing bacterial development during hatching will improve the hygienic status of nauplii and may result in better hatching yields. It can be achieved through simple disinfection of the cysts using liquid bleach solution, through decapsulation (see Section 3.5.4) or through the use of recently
developed cyst and enrichment products that achieve disinfection during the course of the hatching process (Sorgeloos et al. 2001).

Attention should be paid to the selection of Artemia cyst batches with good hatching synchrony (less than 7 h between hatching of first and last nauplii) and high hatching efficiency (more than 200,000 nauplii per gram product), as considerable variation has been demonstrated for cysts from different sources, and even among batches from the same strain (Vanhaecke & Sorgeloos 1982).

### 3.5.3 Harvesting hatched nauplii

After hatching and before feeding to fish/crustacean larvae, the nauplii should be separated from the hatching wastes (empty cyst shells, unhatched cysts, debris, micro-organisms and hatching metabolites). Five to ten minutes after switching off the aeration, cyst shells will float and can be removed from the surface, while nauplii and unhatched cysts will concentrate at the bottom (Fig. 3.21). Since nauplii are positively phototactic, their concentration can be improved by shading the upper part of the hatching tank (use of cover) and by focusing light on the bottom part of the conical tank. Nauplii should not be allowed to settle for too long in the bottom of the conical container, as they will quickly suffer from oxygen depletion.

First, unhatched cysts and other debris that have accumulated underneath the nauplii are siphoned or drained when necessary (i.e. when using cysts of a lower hatching quality). Then the nauplii are collected on a filter with a fine mesh screen (<150 μm), which should be submerged at all times to prevent physical damage to the nauplii. They are rinsed thoroughly with water to remove possible contaminants and hatching metabolites such as glycerol. In commercial operations the use of a concentrator/rinser (Fig. 3.22) allows fast harvesting of large volumes of Artemia nauplii and complete removal of debris from the hatching medium.

Since instar I nauplii rely solely on their endogenous yolk reserves they should be harvested and fed to the fish or crustacean larvae in their most energy-rich form, i.e. as soon as possible after hatching. Farmers often overlook the fact that an Artemia nauplius in its first stage of development cannot take up food and thus consumes its own energy reserves. At the high temperatures that occur during cyst incubation, the freshly hatched Artemia nauplii develop into the second larval stage within a matter of hours. It is important to feed first instar nauplii to the predator rather than starved second instar meta-nauplii which will already have consumed 25–30% of their energy reserves within 24 h after hatching (Fig. 3.23). Moreover, instar II Artemia are less visible as they are transparent. They are also larger and swim more rapidly than first instar larvae. As a result they are less accessible as prey. Furthermore, they contain lower amounts of free amino acids, and their lower individual organic dry weight and energy content will reduce the energy uptake by the predator per hunting effort. All this may be reflected in a reduced growth of the larvae, and increased Artemia cyst usage and cost, as about 20–30% more cysts need to be hatched to feed the same weight of starved meta-nauplii to the predator (Léger et al. 1986).

![Fig. 3.21](image1)

Hatching tank after switching off aeration.

![Fig. 3.22](image2)

Concentrator/rinser used for an efficient harvest of large amounts of hatched Artemia.

![Fig. 3.23](image3)

Energy content and dry weight of instar I, instar II, cold stored nauplii and decapculated cysts. (Modified from Léger et al. 1987a.)
3.5.4 Decapsulation

Decapsulation is the process whereby the chorion that encysts the *Artemia* embryo is completely removed by a short exposure to a hypochlorite solution (Bruggeman et al. 1980). The use of decapsulated cysts as a food source is much more limited than the use of *Artemia* nauplii. Nevertheless, dried decapsulated *Artemia* cysts have proven to be an appropriate feed for larval rearing of various species such as the freshwater catfish (*Clarias gariepinus*), the common carp (*Cyprinus carpio*), and marine shrimp and milkfish larvae (Verreth et al. 1987; Vanhaecke et al. 1990; Stael et al. 1995; Ribeiro & Jones 1998; Sui 2000).

Using decapsulated cysts in larval production offers a number of advantages over nauplii and non-decapsulated cysts:

- The daily production of nauplii, a labour-intensive job that requires additional facilities, is avoided.
- Cyst shells are not introduced into the culture tanks. When hatching normal cysts, the complete separation of *Artemia* nauplii from their shells is not always possible. Unhatched cysts and empty shells cannot be digested by fish or shrimp larvae and may obstruct the gut when ingested.
- Nauplii that are hatched out of decapsulated cysts have a higher energy content and individual weight (30–55% depending on strain) than ‘regular’ instar I nauplii from non-decapsulated cysts, because they do not expend energy breaking out of the shell. In some cases, where cysts have a relatively low energy content, the hatchability may be improved by decapsulation, because of the lower energy requirement to break out of a decapsulated cyst (Table 3.10).
- Decapsulation results in complete disinfection of the cyst material.
- Cysts with poor hatching quality or even non-hatching cysts can still be used as a food source.

Decapsulated cysts, however, have the disadvantage that they are non-motile and thus less visually attractive to the predator. Moreover, decapsulated cysts dehydrated in brine sink rapidly to the bottom, thus reducing their availability for fish larvae feeding in the water column unless adequate mixing of the culture water is applied. Older penaeid larvae, however, are mainly bottom feeders and do not find this a problem.

From the nutritional point of view, the gross biochemical composition of decapsulated cysts is comparable to that of freshly hatched nauplii (García-Ortega et al. 1998) (Tables 3.5–3.7).

<table>
<thead>
<tr>
<th>Cyst source</th>
<th>Hatchability</th>
<th>Naupliar dry weight</th>
<th>Hatching output</th>
</tr>
</thead>
<tbody>
<tr>
<td>San Francisco Bay, USA</td>
<td>+15</td>
<td>+7</td>
<td>+23</td>
</tr>
<tr>
<td>Macau, Brazil</td>
<td>+12</td>
<td>+2</td>
<td>+14</td>
</tr>
<tr>
<td>Great Salt Lake, USA</td>
<td>+24</td>
<td>-2</td>
<td>+21</td>
</tr>
<tr>
<td>Shark Bay, Australia</td>
<td>+4</td>
<td>+6</td>
<td>+10</td>
</tr>
<tr>
<td>Chaplin Lake, Canada</td>
<td>+132</td>
<td>+5</td>
<td>+144</td>
</tr>
<tr>
<td>Bohai Bay, PR China</td>
<td>+4</td>
<td>+6</td>
<td>+10</td>
</tr>
</tbody>
</table>

Modified from Bruggeman et al. (1980).

In addition, their individual dry weight and energy content is on average 30–40% higher than for instar I nauplii (Fig. 3.23).

The decapsulation procedure (Appendix II) involves the hydration of the cysts (as complete removal of the envelope can only be performed when the cysts are spherical), removal of the brown shell in a hypochlorite solution and deactivation of the remaining hypochlorite by washing. These decapsulated cysts can be directly hatched into nauplii, or dehydrated in saturated brine and stored for later hatching or directly feeding. They can be stored for a few days in the refrigerator at 0–4°C without a reduction in hatchability. If storage for prolonged periods is needed (weeks or few months), the decapsulated cysts can be transferred into a saturated brine solution. During overnight dehydration (with aeration to maintain a homogeneous suspension) cysts have released over 80% of their cellular water, and upon interruption of the aeration, the now coffee-bean-shaped decapsulated cysts settle out. After harvesting of these cysts on a mesh screen they should be stored cooled in fresh brine. Since they lose their hatchability when exposed to UV light it is advisable to store them protected from direct sunlight.

3.5.5 Enrichment

The nutritional value of *Artemia* nauplii is easy to manipulate thanks to their primitive feeding characteristics. After about 8h posthatch, brine shrimp molt to the second naupliar stage, instar II, and start filtering particles smaller than 25 μm irrespective of their nature (Makridis & Vjedstein 1999; Gelabert Hernandez 2001). Taking advantage of this non-selective filter feeding, simple methods were developed to incorporate various kinds of products into nauplii before feeding them to predatory larvae (Fig. 3.24). This ‘bioencapsulation’ or ‘enrichment’ is a now very common practice in fish and crustacean hatcheries for enhancing the nutritional value of this live feed or for delivering specific ingredients to cultured larvae.

3.5.5.1 Lipid enrichment

In the early 1970s, several authors reported problems with the larviculture of shrimp, crab, prawn, lobster and marine fish larvae when using *Artemia* sources other than SFB *Artemia*. After considerable efforts by diverse research groups, these striking differences in culture results could be related to the origin of the *Artemia* strain used (Bengston et al. 1991).
Comparative studies with different strains of *Artemia* (Kanazawa et al. 1979) and with other zooplankton (Nellem et al. 1981; Watanabe et al. 1983a) revealed differences in levels of specific polyunsaturated fatty acids. Initially, most attention was focused on the presence of EPA (20:5n-3) in *Artemia* and its importance in the successful production of marine fish and crustacean larvae (Watanabe et al. 1983b; Léger et al. 1985). In the late 1980s and early 1990s, attention shifted to DHA when several authors documented the importance of DHA, more particularly the requirement for high DHA: EPA ratios (Lavenes et al. 1995; Kraul 1993; Reitan et al. 1994; Mourente et al. 1993). A myriad of studies has been carried out on the essentiality of long-chain, highly unsaturated fatty acids (HUFAs) in several fish and shrimp species to gain a better understanding of their true requirements, with the ultimate goal of optimising larval feed and/or enrichment products. For the latest developments and insights into larval fish lipid nutrition, the reader is referred to the reviews by Sargent et al. (1999a,b). To provide fish larvae with adequately enriched *Artemia*, the following points should be considered.

- **Most** marine fish larvae cannot synthesise DHA, EPA or arachidonic acid (ARA, 20:4n-6) from shorter chain precursors and they must be provided preformed in the larval diet (see review by Sargent et al. 1997).
- **ARA (20:4n-6)** is the major precursor for eicosanoids in fish, as in mammals (Castell et al. 1994; Bell et al. 1995).
- **EPA** is present in large amounts in the cellular membranes of marine fish larvae and is also a precursor of eicosanoids (Sargent et al. 1993). Eicosanoids formed from EPA are less biologically active than ARA-derived eicosanoids, and EPA is important in modulating eicosanoid production by competing for the same enzyme systems that convert ARA to eicosanoids (Sargent 1995).
- **DHA** is a major constituent of neural and visual cell membranes and, thus, is essential for a range of physiological processes that are crucial to fast-growing marine fish larvae (Sargent et al. 1999b).
- The requirements for these essential fatty acids cannot be considered separately. Altering the dietary dose of one of them will influence the ARA:EPA:DHA balance owing to competitive interactions and metabolic conversions (Sargent et al. 1999b).
- Besides absolute HUFAs requirements, the importance of polar lipids and the distribution of HUFA between dietary phospholipids and triacylglycerols (TAG) should not be overlooked (see Section 3.5.5.2).
- Dietary HUFA requirements seem to be, at least to some extent, species specific.

These facts have various consequences on the usefulness of enriched *Artemia* as larval food and the modalities of enrichment procedures.

- It has been proven that optimised DHA levels and high DHA:EPA ratios improve growth, stress resistance and proper pigmentation, especially in marine flatfish (Watanabe et al. 1983b; Kraul 1993; Mourente et al. 1993; Reitan et al. 1994; Copeman et al. 1999). While the DHA:EPA ratio in enriched *Artemia* rarely exceeds 2:1, marine zooplankton generally have ratios substantially higher than 1:1, often 4:1 and higher (Shields et al. 1999; see also Chapter 5). Consequently, zooplankton-fed halibut larvae have a much higher DHA level and DHA:EPA ratio than enriched *Artemia*-fed larvae (McEvoy et al. 1998b).

- Although ARA is an essential precursor of eicosanoids, the dietary requirements for ARA are relatively low. Moreover, dietary levels must be carefully chosen, as excessive ARA:EPA ratios tend to exert negative effects on pigmentation (McEvoy et al. 1998a; Estévez et al. 1999; Copeman et al. 1999). Although the exact requirements and effects of ARA in relation to EPA and DHA are not fully understood, they are likely to be species specific.

- It is difficult to maintain high DHA levels in enriched *A. franciscana* because of the rapid retroconversion of DHA to EPA, resulting in decreased DHA:EPA ratios as soon as enrichment is interrupted (Navarro et al. 1999). An interesting solution to this problem may stem from the capacity of some *Artemia* strains to reach high DHA levels during enrichment (Dhert et al. 1993; Velazquez 1996) and to maintain them during subsequent starvation (Ejermo et al. 1997; Han et al. 2000a). Although retroconversion of DHA to EPA may also occur in rotifers, it seems to occur at a lower rate than in *Artemia* and it is easier to maintain high DHA levels in rotifers.

Although *Artemia* is often an inferior food source for fish larvae compared with wild zooplankton, the ability to produce any amount of biomass within 24 h, in contrast to zooplankton, and the constant improvement of enrichment products ensure its continued use in marine fish larviculture.

In parallel to this relentless unravelling of the biochemical pathways, physiological functions and dietary requirements, numerous enrichment products and procedures were developed, using selected microalgae and/or microencapsulated products, yeast and/or emulsified preparations, self-emulsifying concentrates and/or microparticulate products (reviewed by McEvoy & Sargent 1998). Although initially the composition of enrichment products was based on empirical trials of variable components, they are increasingly supported by sound insight into the true dietary requirements of marine fish larvae. Nevertheless, we are still far from understanding every species' requirements and it therefore seems a logical approach to tune the composition of the larval food and associated enrichment products to reflect the natural diets of the larvae; namely, yolk and zooplankton (Sargent et al. 1999b).

Currently, the highest enrichment levels are obtained using emulsified concentrates (Table 3.11). This procedure, developed by Léger et al. (1987b), involves the incubation of freshly hatched nauplii in an enrichment emulsion for a period up to 24 h (for detailed procedures, see Appendix II).

### 3.5.5.2 Phospholipid enrichment

Several marine fish and shrimp larvae seem to have a requirement for phospholipids (see review by Coutteau et al. 1996). Geurden et al. (1997) demonstrated that the observed positive effect of phospholipid supplementation was not connected to the role of phospholipids as an additional HUFA source, which suggested that dietary phospholipids were necessary to compensate for a limited ability for de novo biosynthesis by the fish larvae. However, enriching *Artemia* with traditional products seems to increase the fraction of TAG at the expense of the phospholipid fraction (McEvoy et al. 1996). Although fish larvae appear to have a certain ability to convert fatty acids between phospholipids and TAG, the relatively high proportion of linolenic acid (LNA, 18:3n-3) in the phospholipids of enriched *Artemia* will hinder fish larvae in assimilating sufficient EPA and DHA (mainly...
Table 3.11 Overview of lipid levels obtained through enrichment of *Artemia franciscana* by various authors (mg g⁻¹ dry weight).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Reference</th>
<th>LNA (18:3n-3)</th>
<th>ARA (20:4n-6)</th>
<th>EPA (20:5n-3)</th>
<th>DHA (22:6n-3)</th>
<th>DHA EPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nauplii</td>
<td>Dhert et al. (1993)</td>
<td>39.8</td>
<td>1.0</td>
<td>10.7-17.8</td>
<td>27.5-45.7</td>
<td>1.2-4.9</td>
</tr>
<tr>
<td></td>
<td>Dhert et al. (1993)</td>
<td>32.7-40.3</td>
<td>2.8-4.2</td>
<td>4.4-19.5</td>
<td>8.9-9.5</td>
<td>1.4-2.5</td>
</tr>
<tr>
<td></td>
<td>Pratula et al. (1995)</td>
<td>—</td>
<td>—</td>
<td>17.7</td>
<td>25.1</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>Everaert et al. (1997)</td>
<td>—</td>
<td>—</td>
<td>17.4</td>
<td>36.6</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>Harel et al. (1999)</td>
<td>—</td>
<td>2.4-2.7</td>
<td>9.5-11.9</td>
<td>21.1-39.1</td>
<td>1.7-3.9</td>
</tr>
<tr>
<td></td>
<td>Estevez et al. (1998)</td>
<td>20.6-21.7</td>
<td>2.1-14.3</td>
<td>10.0-25.1</td>
<td>5.2-16.6</td>
<td>0.3-1.3</td>
</tr>
<tr>
<td></td>
<td>Estevez et al. (1999)</td>
<td>—</td>
<td>1.6-7.9</td>
<td>5.5-35.2</td>
<td>3.0-10.0</td>
<td>0.1-1.3</td>
</tr>
<tr>
<td></td>
<td>Narciso et al. (1999)</td>
<td>—</td>
<td>—</td>
<td>4.1-20.2</td>
<td>14.1-11.1</td>
<td>0.0-0.7</td>
</tr>
<tr>
<td></td>
<td>Hare et al. (2000b)</td>
<td>25.6-45.0</td>
<td>2.4-4.9</td>
<td>29.6-53.2</td>
<td>11.2-28.9</td>
<td>0.3-0.5</td>
</tr>
<tr>
<td>Adults</td>
<td>Han (2001)</td>
<td>14.9-26.0</td>
<td>13.4</td>
<td>23.1</td>
<td>4.6</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Lim et al. (2001)</td>
<td>1.8</td>
<td>—</td>
<td>5.6</td>
<td>1.9</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Dhont (unpubl.)</td>
<td>1.7</td>
<td>3.8</td>
<td>9.5</td>
<td>4.0</td>
<td>0.4</td>
</tr>
</tbody>
</table>

LNA, linolenic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; —, not determined.

Table 3.12 Vitamin levels obtained by enrichment (µg g⁻¹ dry weight).

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>(Ref)</th>
<th>Unenriched nauplii</th>
<th>24h enriched nauplii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astaxanthin</td>
<td>(a)</td>
<td>692</td>
<td>3100</td>
</tr>
<tr>
<td>(c)</td>
<td>1000-12000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiamin</td>
<td>(b)</td>
<td>7.5</td>
<td>8.8</td>
</tr>
<tr>
<td>(c)</td>
<td>20-40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Riboflavin</td>
<td>(b)</td>
<td>47.3</td>
<td>38.0</td>
</tr>
<tr>
<td>Niacin</td>
<td>(b)</td>
<td>187</td>
<td>202</td>
</tr>
<tr>
<td>(b)</td>
<td>86</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>Vitamin B₉</td>
<td>(b)</td>
<td>9.0</td>
<td>6.7</td>
</tr>
<tr>
<td>Biotin</td>
<td>(b)</td>
<td>3.5</td>
<td>4.0</td>
</tr>
<tr>
<td>Folate</td>
<td>(b)</td>
<td>18.4</td>
<td>12.2</td>
</tr>
<tr>
<td>Vitamin B₉</td>
<td>(b)</td>
<td>3.5</td>
<td>3.9</td>
</tr>
</tbody>
</table>

References: a, Mercie et al. (1995); b, Mølgaard et al. (2000); c, Olsen et al. (1999).

Dendrinos and Thorpe (1987) cultured *Artemia* on different types of protein and observed an increase in total protein content when feeding *Candida utilis* and *Saccharomyces cerevisiae*. The pattern for levels of essential amino acids was not very clear: some levels clearly increased (leucine, isoleucine, tyrosine, lysine) but others remained unchanged or decreased slightly (phenylalanine, histidine, arginine). Tonheim et al. (2000) demonstrated that methionine levels in nauplii could be boosted 20-30-fold in 16 h by simply adding dissolved methionine in the water. This increase could be doubled (to 60-fold of the unenriched control) when incorporating methionine in liposome droplets.

3.5.5.4 Vitamin enrichment

Enrichment of *Artemia* for 48 h with DHA-Secuo (Inve, Belgium) led to increased levels of thiamin, niacin and pantothenic acid, but no changes in the content of AsA, riboflavin, or vitamin B₉ or B₉ (Mølgaard et al. 2000). Better results for selected vitamins are obtained using specific enrichment preparations. Tests have been conducted to incorporate extra AsA into *Artemia* nauplii in a stable and bioavailable form (see Table 3.12). In a 24 h enrichment with self-emulsifying concentrates containing 10-20% ascorbyl palmitate (AscP), levels up to 2.5 mg free AsA g⁻¹ dry weight were achieved in brine shrimp nauplii (Mercie et al. 1995). When these vitamin C-enriched *Artemia* were fed to turbot larvae, no differences in growth or overall survival could be detected compared with those fish fed the non-enriched live food containing 500 µg AsA g⁻¹ dry weight. However, the larvae given the high AsA treatment showed a better pigmentation rate compared with the control group. Evaluation of the physiological condition through a salinity stress test also revealed an improvement. Cumulative mortalities after challenge with *Vibrio anguillarum* amounted to 50% for the control versus 40% for the AsA-supplemented fish, with a slower onset of mortality for the AsA-fed fish (Mercie et al. 1995).

High levels of α-tocopherol can be bioaccumulated and maintained in *Artemia* nauplii, making this live food delivery system useful for studying dietary requirements as well as antioxidative effects of vitamin E (Huo et al. 1996).
Vitamin A levels in Artemia nauplii could be raised from 1.3 to 1283 IU g⁻¹ dry weight over an 18 h period through the addition of vitamin A palmitate to an egg-yolk-based emulsion (Dedi et al. 1995). However, Rennestad et al. (1998) found striking differences in vitamin A and carotenoid composition between haliotid larvae fed SuperSelco-enriched Artemia and a species of copepod (Tenora). Although the pigmentation status was not systematically recorded, the authors observed a higher incidence of malpigmentation with Artemia-fed larvae.

3.5.5.5 Enrichment with prophylactics

Although the use of antibiotics in larviculture is rightfully questioned, disapproved of or even banned, it may be of interest to mention that techniques have been developed for oral bio-medication, rather than administration via the culture water (‘bath treatments’). Doses ranging from 20 to 100 ppm sulfadiazines can be incorporated in sea bass and turbot larvae tissue, respectively, within less then 4 h by feeding them with specifically enriched Artemia (Chair et al. 1996; Gapas et al. 1996).

3.5.5.6 Enrichment with other products

The effectiveness of Artemia nauplii as a dietary carrier system could be tested for various other nutritional components, e.g. liposoluble products administered via an emulsion, water-soluble compounds via liposomes (Hontoria et al. 1994) and microcapsule delivery (Sakamoto et al. 1982). However, for each nutrient, the usefulness of the Artemia bioencapsulation method remains to be verified by chemical analysis.

3.5.6 Cold storage

3.5.6.1 Survival at low temperatures

Moulting of the Artemia nauplii to the second instar stage can be delayed and their energy metabolism greatly reduced by storage of the freshly hatched nauplii at a temperature below 10°C at densities of up to 8 million/l (Léger et al. 1983). Nauplii can be stored for more than 24 h without significant mortalities and a reduction in energy of less than 5%. Only slight aeration is needed to prevent the nauplii from accumulating at the bottom of the tank where they might suffocate. Nauplii stored at 20 million/l showed good survival (>70%) even after 72 h when kept at 12°C with a slight injection of pure oxygen (Anbay Almulh 2000). Estevée et al. (2001) kept nauplii after enrichment at moderate densities (<100,0001⁻¹) and recorded survival above 70% for temperatures between 8 and 19°C. At 5°C and above 19°C survival decreased rapidly after 48 h.

3.5.6.2 Maintenance of nutritional value

Initially, it was generally accepted that nauplii stored at low temperatures maintained their biochemical composition (Fig. 3.23), especially the lipid content (Léger et al. 1983). More detailed analysis revealed that protein is fairly well conserved even after 96 h (Estevée et al. 2001), but fatty acid levels, mainly DHA, decrease significantly. Estevée et al. (1998) demonstrated that up to 70% of the DHA obtained through enrichment was catabolised, while losses of EPA were more moderate and depended on the type of enrichment received. The fact that Artemia retroconvert DHA to EPA (Navarro et al. 1999) may also account for the reduced losses of EPA compared to DHA. An interesting observation was made by Estevée et al. (1997): they confirmed that, after enrichment, A. franciscana catabolised DHA at a rate that increased with temperature but demonstrated that, under similar circumstances, DHA levels in A. sinica remained at almost constant high levels after enrichment and at temperatures ranging from 6 to 22°C.

3.5.6.3 Other advantages

Cold storage enables the farmer to reduce hatching efforts (less frequent hatching and harvesting, fewer tanks, larger volumes). Using cold storage also allows for more frequent and automated distribution of nauplii to larvae. This appears to be beneficial for fish and shrimp larvae as food retention times in the larviculture tanks can be reduced and hence growth of the Artemia in the culture tank can be minimised. For example, applying one or two feedings per day, farmers often experienced juvenile Artemia in their larviculture tanks. With poor hunters such as the larvae of turbot, using cold-stored, less active Artemia as live prey resulted in a much more efficient food uptake (Léger et al. 1986).

3.5.7 Use of juvenile and adult Artemia

Besides nutritional and energetic advantages, the use of Artemia biomass for feeding post-larval shrimp also results in improved economics, as expenses for cysts and weaning diets can be reduced. Dhen et al. (1993) developed a simple culture system for juvenile and adult Artemia as food for postlarval (PLa) Penaeus monodon. The growth performance of shrimp reared from PLa-4 to PLa-25 on juvenile Artemia live prey is identical to the growth obtained when feeding newly hatched Artemia, but the PLa-25 reared with juvenile brine shrimp display significantly better resistance in salinity stress tests, i.e. the stress sensitivity index dropped from 138 with freshly hatched nauplii to 36 when feeding juvenile Artemia. Similarly, Olsen et al. (1999) proved that haliotid larvae fed with gradually increasing sizes of nauplii showed the same satisfactory growth and survival as larvae fed short-term enriched nauplii, but the quality of the haliotid larvae (proper pigmentation, eye migration and lack of deformities) was significantly higher.

Lim et al. (2001) developed a pilot-scale culture unit for ongrowing Artemia for use in ornamental fish in Singapore and proved its to be cost-effective, with a payback period of less than 18 months. It offers local fish breeders a cheaper, less labour-intensive and nutritionally suitable alternative to the traditional Moina culture.

Although the fresh, live form has the highest nutritive value, harvested Artemia can also be frozen, freeze-dried or acid-preserved for later use (Abelin et al. 1991; Naessens et al. 1995), or made into flake or other forms of formulated feed (Sui 2000). Artemia biomass is apparently a good food for the maturation of several species of penaeid shrimp. Recent culture tests in Ecuador and the USA have shown that polychaetes, which have been identified as a critical fresh-food component in the maturation diet of Litopenaeus vannamei (Bray & Lawrence 1991), can be successfully replaced by frozen Artemia biomass (Naessens et al. 1997).
3.6 References


Han, K., Geurden, I. & Sorgeloos, P. (2000b) Enrichment strategies for Artemia using emulsions providing different levels of n-3 highly unsaturated fatty acids. Aquaculture, 183, 335–347.


