# Handbook for *Artemia* pond culture in Bangladesh

## Introducing circularity through climate-smart aquaculture in Bangladesh

Funded by the European Union

# Handbook for *Artemia* pond culture In Bangladesh

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# Preface

I am very glad to see the both Bangla and English versions of the book entitled "handbook for *Artemia* pond culture in Bangladesh" prepared by the WorldFish led Artemia4Bangladesh project. I sincerely thank the European Commission for funding the project.

*Artemia* is a small branchiopod crustacean widely used in aquaculture all over the world particularly for crustacean and marine fish larval rearing. This is the first book on *Artemia* pond culture published in Bangladesh. *Artemia* research and culture is relatively new in this country compared to that ongoing in other countries including China, Thailand and Vietnam. I am confident that the book will greatly help to improve the knowledge and skill of researchers, extension agents, shrimp/fish hatcheries operators, farmers and policy makers.

I thank the authors for the initiative, patience and hard work in preparing the manuscript.

I hope the readers will benefit from the information presented. Please communicate with the authors with comments and suggestions for further improvement in any subsequent editions.

**(Christopher Price)**
Regional Director
WorldFish Bangladesh and South Asia Office

# Message

Fisheries sector of Bangladesh is flourishing rapidly. Average growth of last 10-12 years about 6% and it is the high time to explore the potentials specially in the coastal and marine aquaculture. Brine Shrimp *Artemia* is an essential live food for crustacean and marine fish aquaculture.

I am pleased to see the initiative of the WorldFish led Artemia4Bangladesh project to introduce. *Artemia* pond culture in the salt farms in Cox's Bazar district. The book entitled "HANDBOOK FOR ARTEMIA POND CULTURE IN BANGLADESH" is an excellent contribution of the project. I am confident that the manuscript will be useful for the capacity building of the aquaculture professionals in research and development, technology dissemination and training on *Artemia* pond culture.

I sincerely thank the authors and WorldFish, for publishing the useful book. I appreciate the European Commission for funding the project.

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# List of acronyms

| | |
|---|---|
| µm | micrometer |
| am | ante meridiem |
| cm | centimeter |
| DO | dissolved oxygen |
| e.g. | exempli gratia |
| FAO | food and agriculture organization of the united nations |
| FCR | feed conversion ratio |
| g | gram |
| g/L | gram per litre |
| h | hour |
| ha | hectare |
| i.e. | in extensu |
| IU | international unit |
| kg | kilogram |
| L | litre |
| mg | milligram |
| mg/L | milligram per litre |
| mm | millimeter |
| MT | metric tons |
| pm | post meridiem |
| USD | united states dollar |
| UV | ultraviolet |
| WW | wet weight |

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# Executive summary

Brine shrimp *Artemia* nauplii constitute the most widely used live-food item for the larviculture of crustaceans and fish. The advantages of *Artemia* nauplii compared to inert diets are size (450 µm), live prey, digestibility, and high nutritional content in terms of protein and highly unsaturated fatty acids. Annually, about 3500 MT of *Artemia* cysts are marketed worldwide. The unique property of this small branchiopod crustacean *Artemia* to form dormant embryos, so-called 'cysts'. The cysts are available year-round in large quantities along the shorelines of hypersaline lakes, coastal lagoons and solar salt works scattered over the four continents. At present, Bangladesh imports 40-50 metric tons dry *Artemia* cysts annually worth an approximate value of USD 4 million (M. Rahman, sourced at shrimp hatcheries). Many countries in the world for example Thailand and Vietnam have successfully adopted technologies for *Artemia* production in solar salt farms. The aim of this manual is to provide technological guidelines to the extension agents, young researchers, salt farmers on *Artemia* production in salt farms in Cox's Bazar. The manual was prepared through review recent of activities in *Artemia* production, the 1996 FAO Manual on the production and use of live food for aquaculture, the 2019 book " Principle of *Artemia* culture in solar salt works", relevant books and published research articles.

The manual covers (i) biology and ecology of *Artemia*, (ii) cyst biology and physiology during hatching process (iii) factors to consider in proper site selection, (iv) different models of *Artemia* culture, (v) steps in proper pond construction, (vi) procedure in shortening the duration of *Artemia* pond preparation through application of concentrated sea water or crude salt, (vii) standard method of *Artemia* cyst incubation and stocking, (viii) *Artemia* pond maintenance and management, (ix) suitable algae production for feeding *Artemia*, (x) preparation of processed feed and supplementary feeding, (xi) diseases and health management and (xii) *Artemia* cyst and biomass harvesting, processing and preservation.

Earlier studies described limited knowledge, improper pond management, and climatic conditions as bottleneck in for dissemination of *Artemia* production in salt farms. Recent improvement in *Artemia* production include deepening ponds more than 50 cm water depth, stocking density of 100 nauplii per litre, stimulate growth of suitable algae species (diatom, green algae), optimum supplementary feeding of green water with fermented agricultural waste products (molasses, mono sodium glutamate by-products), use of formulated shrimp feed, improvement of routine pond management such as raking of pond bottom, health management through application of bioflocs.

# Key messages

The key messages of *Artemia* production in solar salt ponds are following:

## 1.0 Biology and ecology of *Artemia*

*Artemia* is a euryhaline species. The optimum salinity for *Artemia* production in ponds is between 80-150 g/L. It is a non-selective filter feeder and can reproduce two ways either producing cyst (oviparous) or nauplii (ovoviviparous) depending on the environmental condition.

## 2.0 Cyst biology and physiology of the hatching process

The process include hydration, carbohydrate metabolism, cyst breaking, effect of environmental parameters on cyst metabolism.

## 3.0 Proper site selection

The availability of sufficient high saline water is obligatory. Topography, climatic and soil conditions are vital for good site selection for *Artemia* production in salt farms.

## 4.0 Models of *Artemia* culture

*Artemia* culture models can be categorized on the basis of investment, pond management and products. Integration of *Artemia* production with salt and aquaculture are useful culture model for salt farmers in Cox's Bazar.

## 5.0 Pond construction

Calculation of the ratio of reservoir, fertilized pond, *Artemia* pond and salt crystallization yard is useful to maximize the production area (75-80%). Recommended water level is to maintain minimum 30-35 cm in *Artemia* ponds.

## 6.0 Brine preparation

Optimum salinity for starting *Artemia* culture in ponds (80-100 g/L) can be prepared through seawater evaporation, mixing with crude salt or brine stored from last year's salt production.

## 7.0 Incubation and stocking

Application of standard conditions for *Artemia* cyst incubation (temperature, salinity, pH, light, density), acclimatize *Artemia* nauplii with the pond temperature during stocking, distribute the nauplii throughout the pond surface during stocking.

## 8.0 Pond maintenance and management

Life cycle of *Artemia* is 40-60 days depending on water temperature (≤35oC) in pond and availability of sufficient food (algae and supplemental feed). *Artemia* pond management include water level, salinity, water colour, feed, population composition, density, nutritional and health status of female, ratio female carrying cysts and larvae. Weather conditions are particularly concerned for prolonged rainfall, out of season rain, low or too high temperature.

## 9.0 Enhancement of algae production

Combination of organic and inorganic fertilizer with N:P ratio ≥ 3:1, salinity ≤ 50 g/L is suitable for diatom and green algae to develop.

## 10.0 Processed feed and supplementary feeding

Supplementary feeds of *Artemia* in ponds are rice bran, wheat bran, molasses, ami-ami (waste products of mono sodium glutamate factory), fish meal, shrimp feed. Formulated feed (30% protein, 9% fat) can be applied @ 3-6 kg/ha per day.

## 11.0 Diseases and health management

Diseases can cause mass mortality of *Artemia* population in ponds. The health management include optimize rearing condition, changes in salinity, suitable algae production, supplementary feeding, probiotics and biofloc culture.

## 12.0 Harvesting cyst and biomass

Cysts need to be collected daily and cleaned through fine nets using pond water. Then, stored in saturated salt water (250-300 g/L) to remove water and stop cyst metabolism. Cysts can be stored in saturated brine for several months without losing viability. They can be used for nauplii production in hatcheries or be further processed (drying, packaging).

# Handbook for *Artemia* pond culture In Bangladesh

# 1.0 Biology and ecology of *Artemia*

## 1.1 Morphology and life cycle

In its natural environment at certain moments of the year brine shrimp *Artemia* produce cysts that float at the water surface (Figure 1) and that are thrown ashore by wind and waves. These cysts are metabolically inactive and do not further develop as long as they are kept dehydrated (in saturated brine or dry). Upon immersion in seawater, the biconcave-shaped cysts hydrate, become spherical, and within the shell the embryo resumes its interrupted metabolism. After 10 to 20 h (depending on cyst strain and temperature) the outer cuticular membrane of the cyst shell bursts (= "breaking") and the embryo appears, surrounded by the hatching membrane (Figure 2). While the embryo hangs underneath the empty shell (= "umbrella" stage) the development of the nauplius is completed and within a short period of time the hatching membrane is ruptured (= "hatching") and the free-swimming nauplius is born (Figure 3).

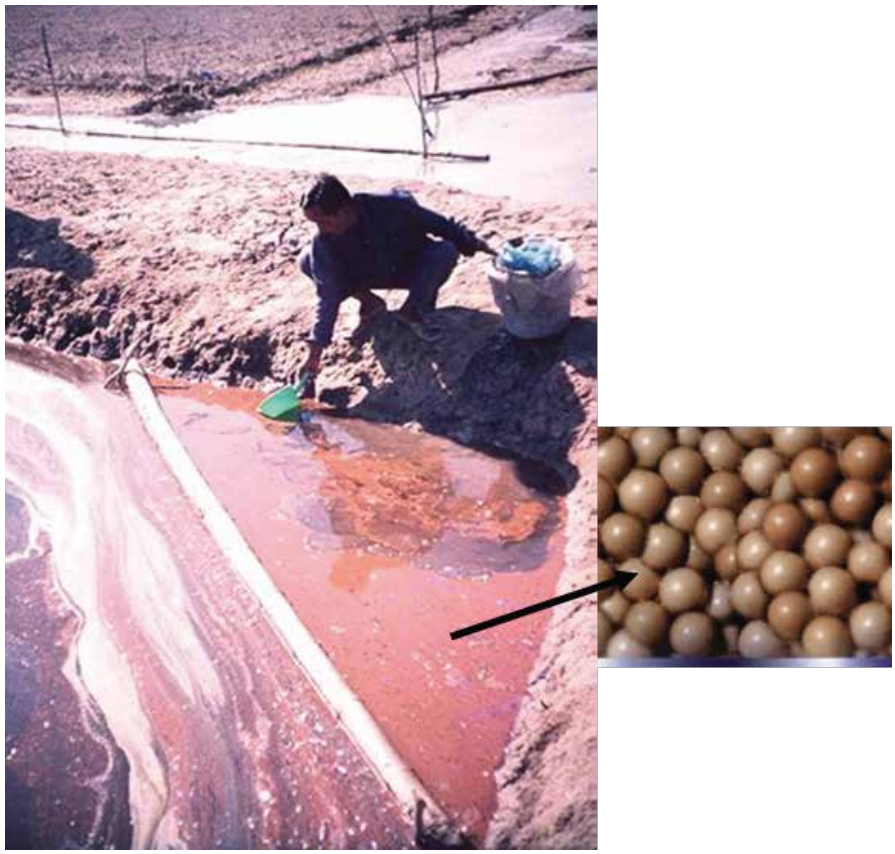

Figure 1: Harvesting of brine shrimp cysts from a salt pond

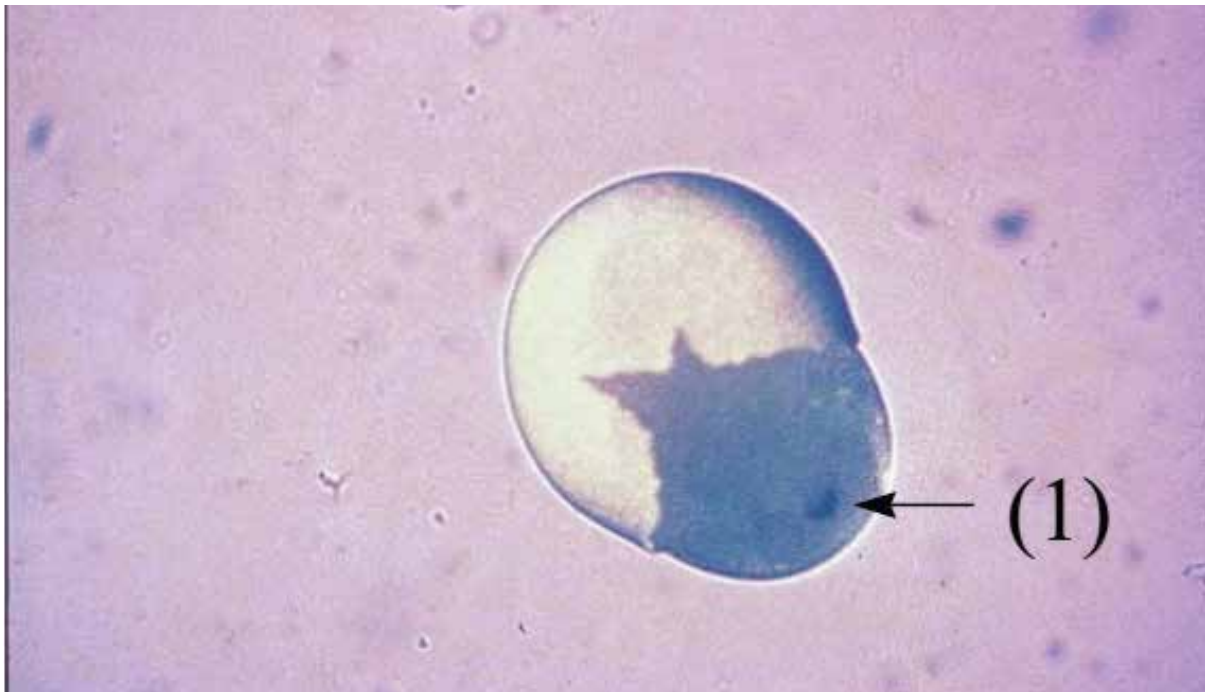


Figure 2: Cyst in breaking stage (1) nauplius eye

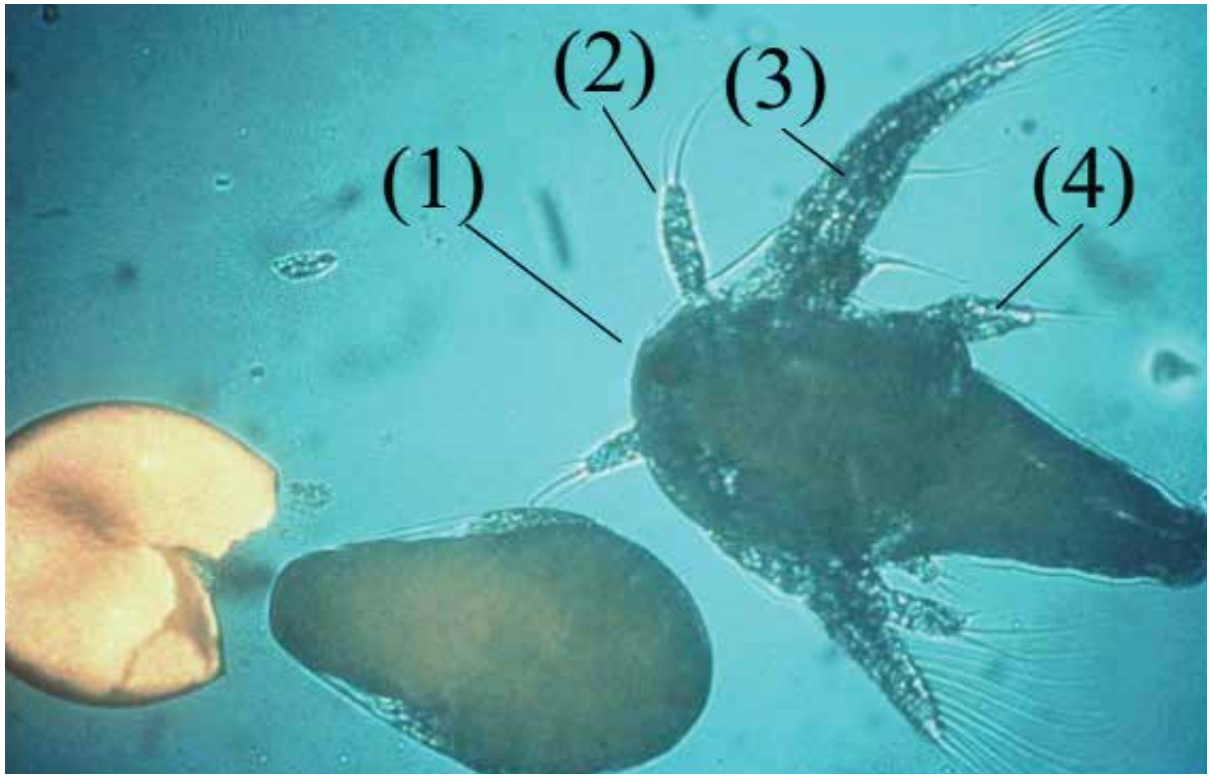


Figure 3: Embryo in "umbrella" stage (left) and instar I nauplius (right), (1) nauplius eye, (2) antennule, (3) antenna (4) gnatobasenseta

The first larval stage (instar I; 400 to 500 µm in length) has a brownish-orange colour, a red nauplius eye in the head region and three pairs of appendages: i.e. the first antennae (sensorial function), the second antennae (locomotory + filter-feeding function) and the gnatobasenseta (food uptake function). The ventral side is covered by a large labrum (food uptake: transfer of particles from the filtering setae into the mouth). The instar I larva does not take up food as its digestive system is not functional yet; it thrives completely on its yolk reserves.

After about 6 to 8 h (strain and temperature dependent) the animal molts into the 2nd 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 2nd antennae and ingested via the mouth into the functional digestive tract.

The larva grows and differentiates through about 15 molts. Paired lobular appendages are appearing in the trunk region and differentiate into thoracopods (Figure 4). On both sides of the nauplius eye lateral complex eyes are developing (Figure 5 and 6). From the 10th instar stage on, important morphological as well as functional changes are taking place: i.e. the antennae have lost their locomotory function and undergo sexual differentiation. In males (Figure 6 and 8) they develop into hooked graspers, while the female antennae degenerate into sensorial appendages (Figure 11). The thoracopods are now differentiated into three functional parts (Figure 13), namely the telopodites and endopodites (locomotory and filter-feeding), and the membranous exopodites (gills).

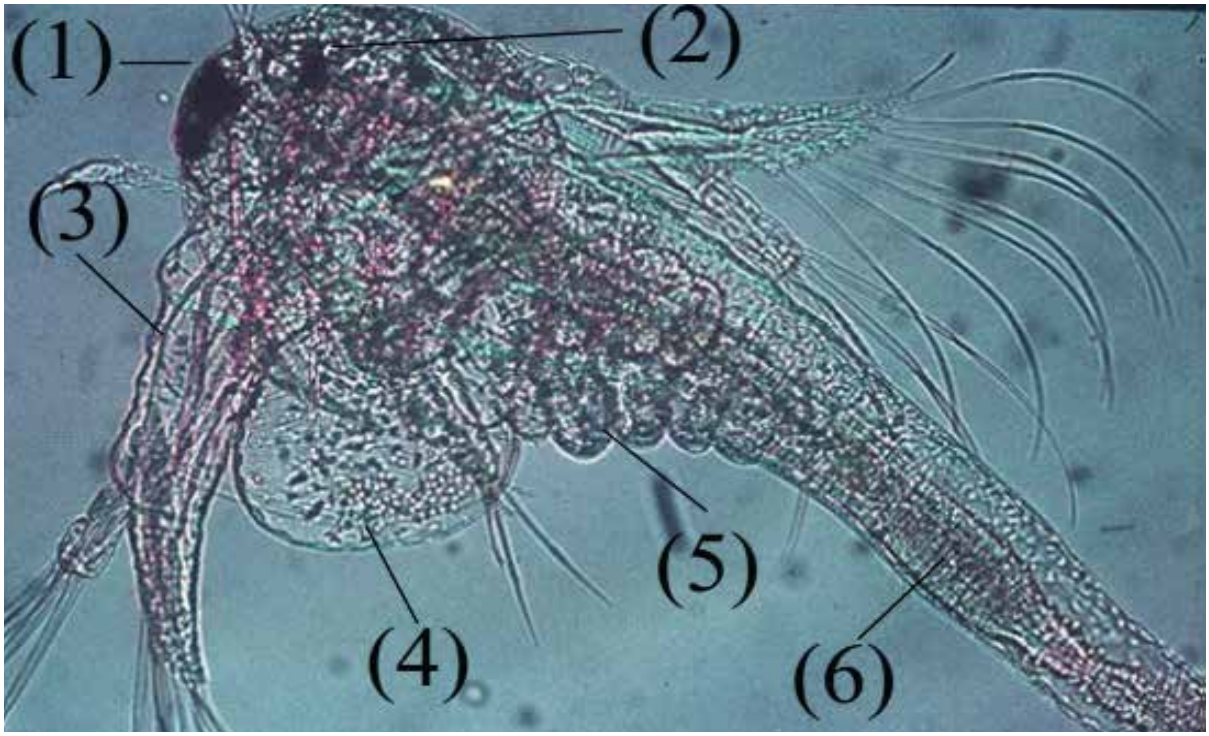


Figure 4: Instar V larva, (1) nauplius eye, (2) lateral complex eye, (3) antenna (4) labrum, (5) budding of thoracopods, (6) digestive tract

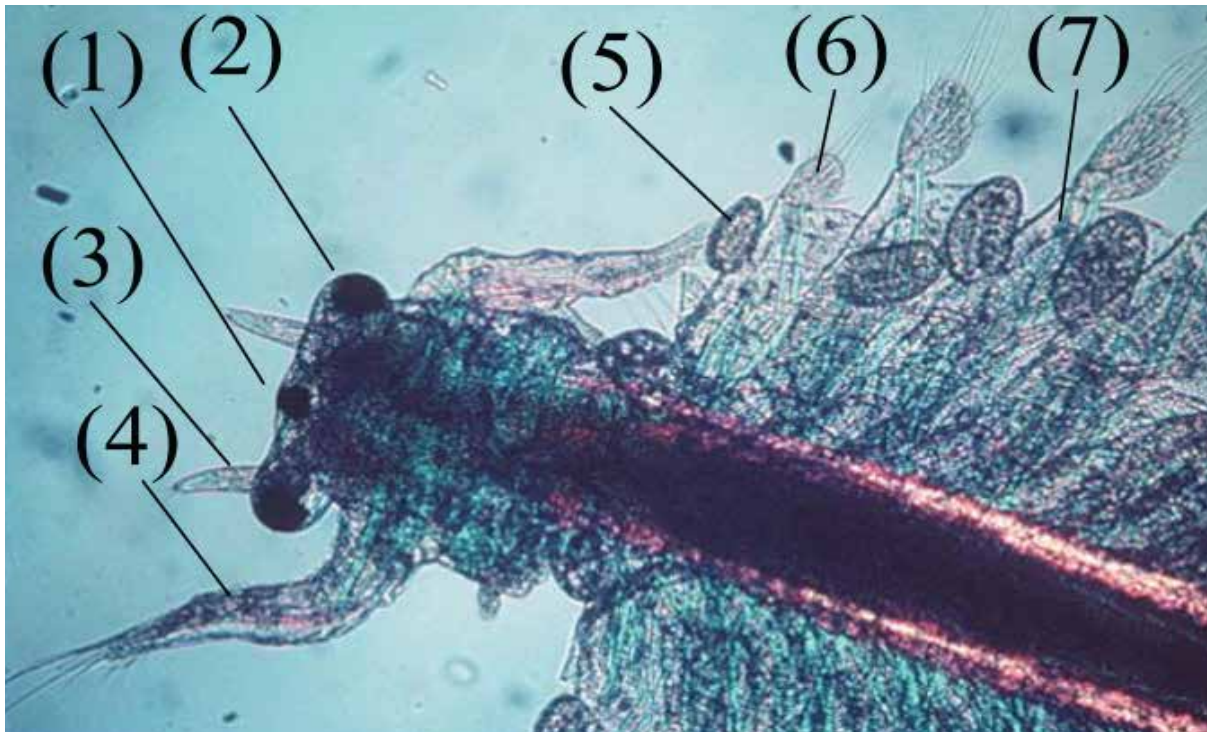


Figure 5: Head and anterior thoracic region of instar XII, (1) nauplius eye, (2) lateral complex eye, (3) antennule, (4) antenna, (5) exopodite, (6) telopodite, (7) endopodite

Adult *Artemia* (± 1 cm in length) have an elongated body with two stalked complex eyes, a linear digestive tract, sensorial antennulae and 11 pairs of functional thoracopods (Figure10 and 11). The male (Figure 11) has a paired penis in the posterior part of the trunk region (Figure 9). Female *Artemia* can easily be recognized by the brood pouch or uterus situated just behind the 11th pair of thoracopods (Figure 9 and 11). Eggs develop in two tubular ovaries in the abdomen (Figure 7). Once ripe they become spherical and migrate via two oviducts into the unpaired uterus.

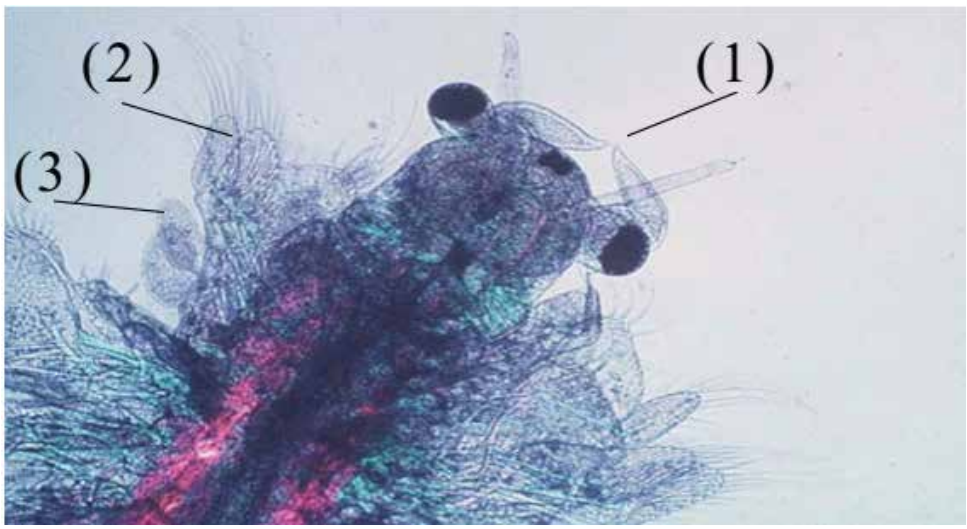


Figure 6: Head and thoracic region of young male, (1) antenna (2) telopodite, (3) exopodite

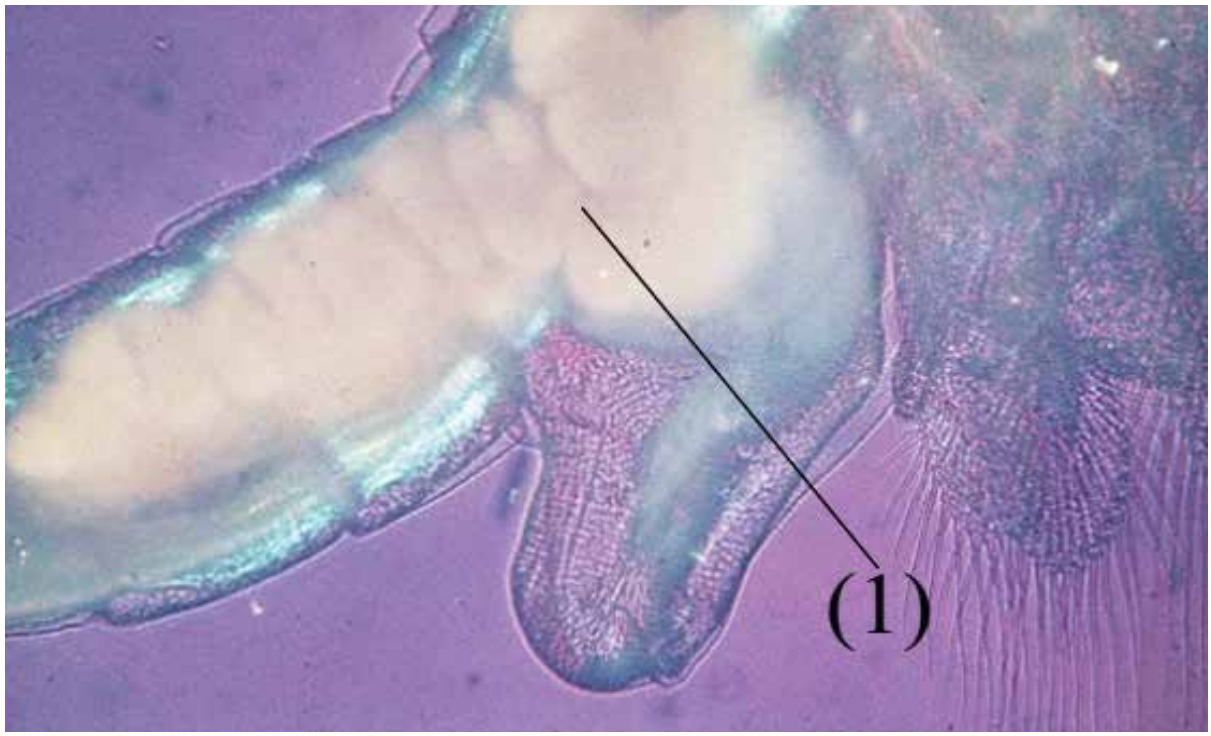


Figure 7: Posterior thoracic region, abdomen and uterus of fertile female, (1) ripe eggs in ovary and oviduct

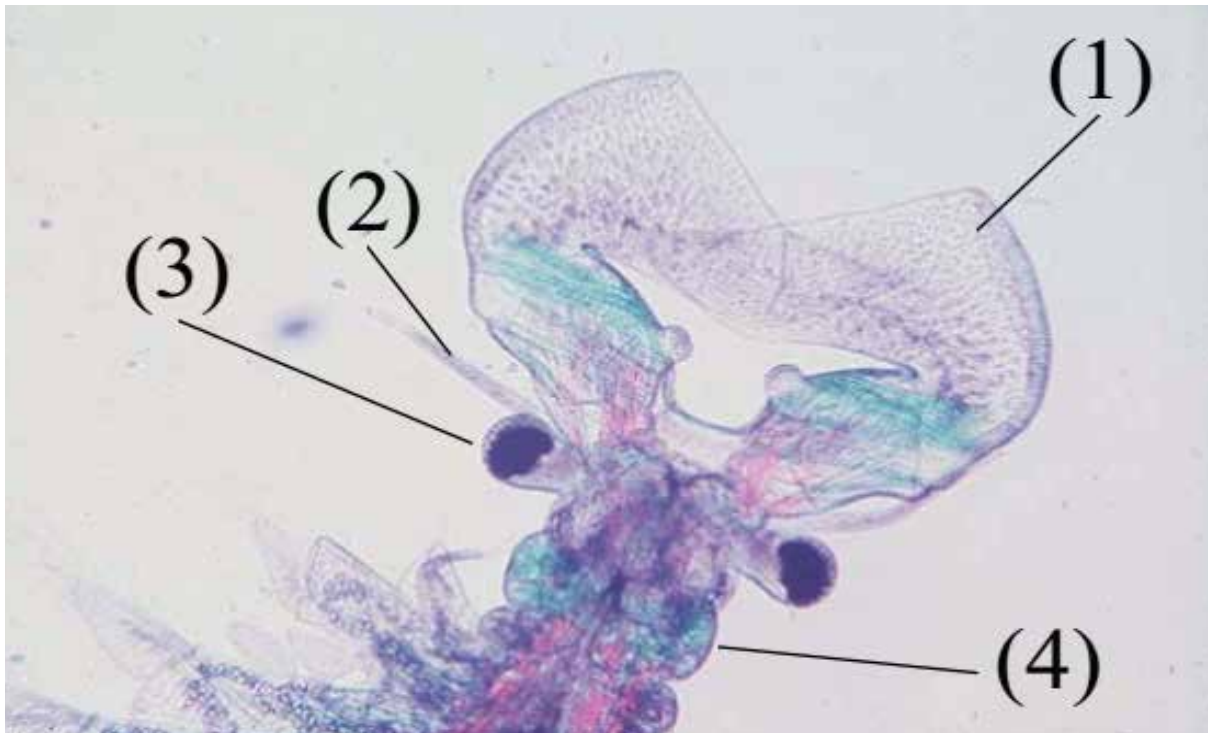


Figure 8: Head of an adult male, (1) antenna, (2) antennule, (3) lateral complex eye, (4) mandible

Fertilized eggs normally develop into free-swimming nauplii (= ovoviviparous reproduction) (Figure 12) which are released by the mother. In extreme conditions (e.g. high salinity, low oxygen levels) the embryos only develop up to the gastrula stage. At this moment they get surrounded by a thick shell (secreted by the brown shell glands located in the uterus), enter a state of metabolic standstill or dormancy (diapause) and are then released by the female (= oviparous reproduction) (Figure 14). In principle both oviparity and ovoviviparity are found in all *Artemia* strains, and females can switch in-between two reproduction cycles from one mode of reproduction to the other. A schematic diagram of *Artemia* life cycle is shown in Figure 15.

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. For more details on the diapause process see chapter 2.

Under optimal conditions brine shrimp can live for several months, grow from nauplius to adult in only 8 days' time and reproduce at a rate of up to 300 nauplii or cysts every 4 days.

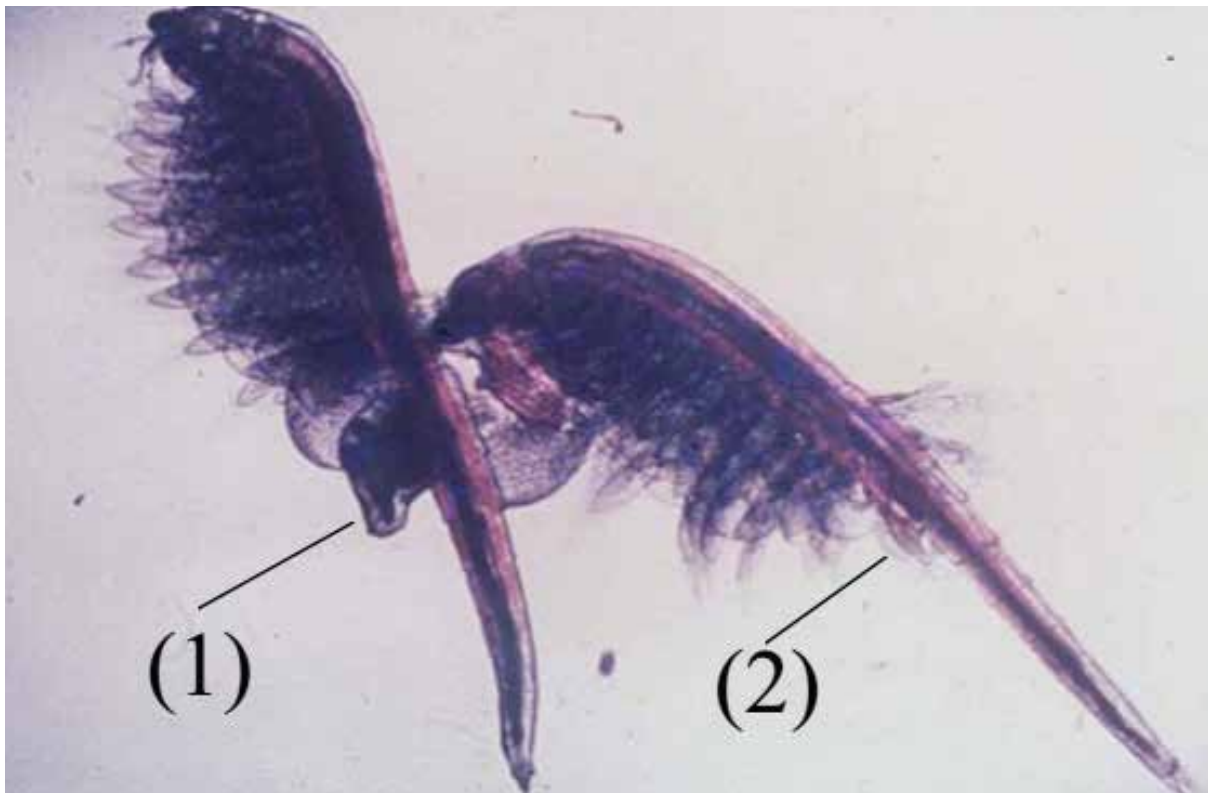


Figure 9: *Artemia* couple in riding position (1) uterus, (2) penis

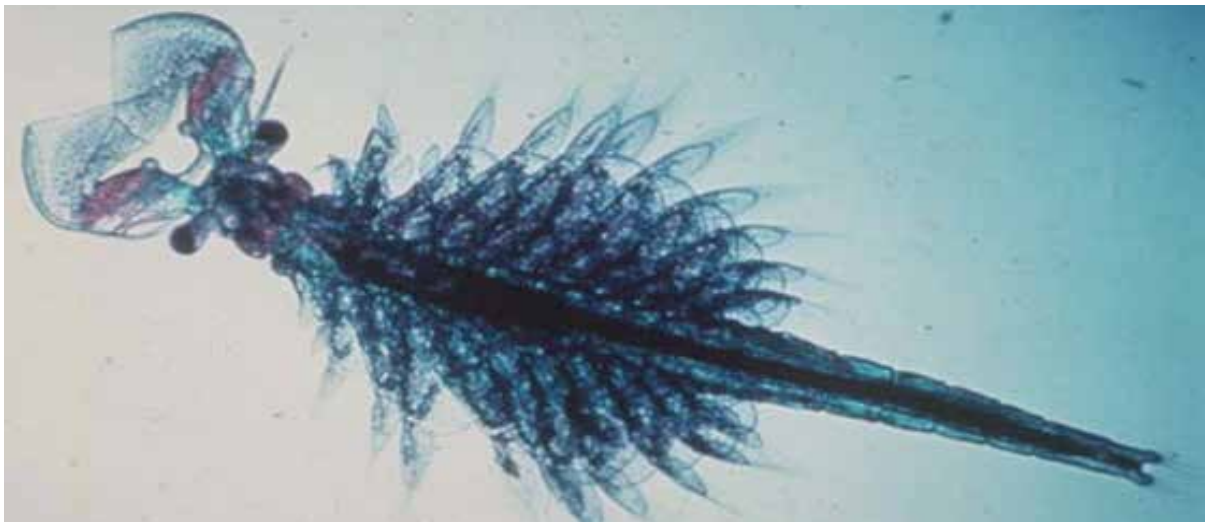

Figure 10: Adult male *Artemia*

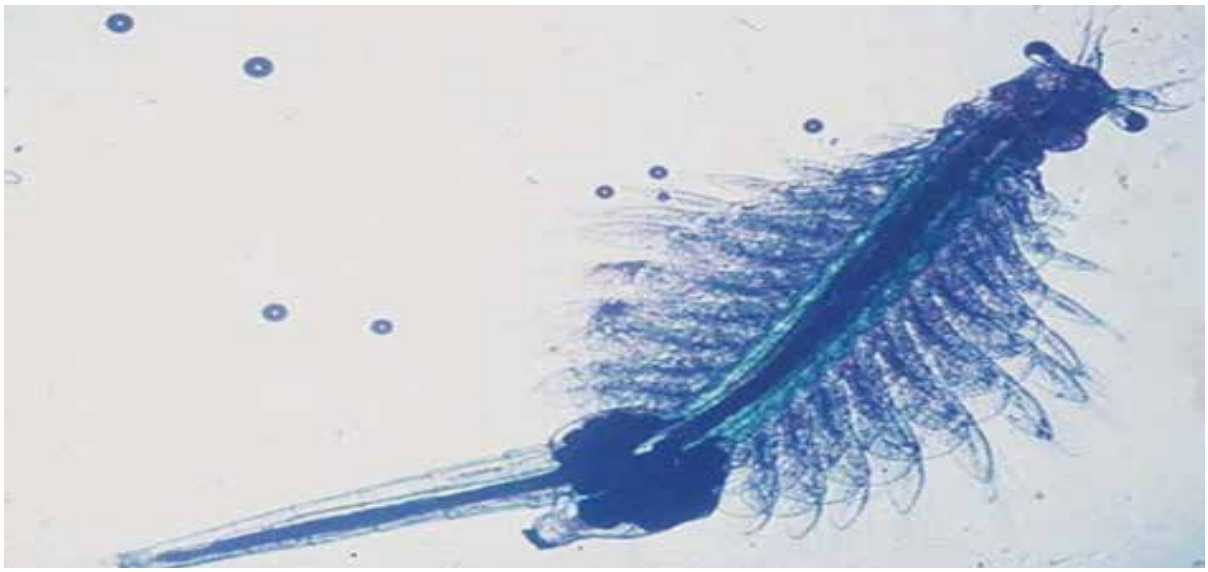

Figure 11: Adult female *Artemia*

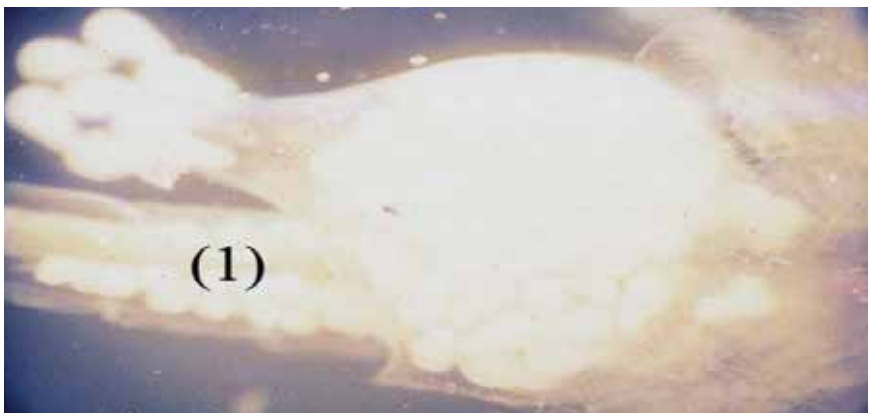


Figure 12: Uterus of ovoviviparous *Artemia* filled with nauplii (first larvae are being released), (1) ovary with eggs

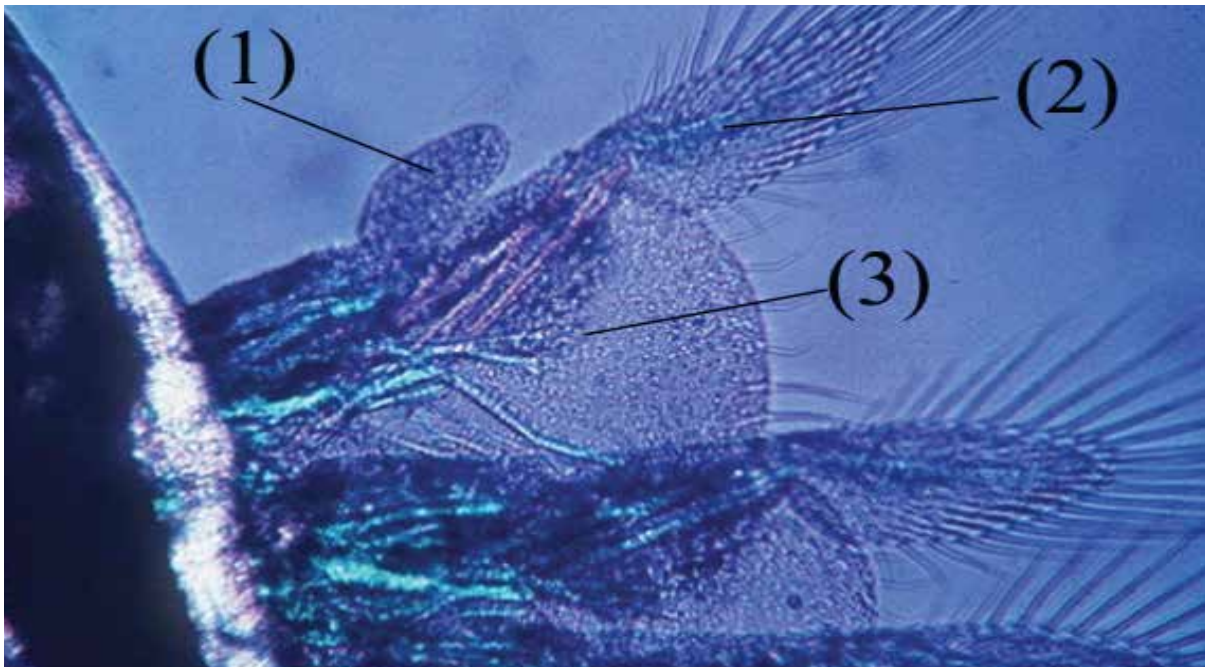


Figure 13: Detail of anterior thoracopods in adult *Artemia*, (1) exopodite, (2) telopodite, (3) endopodite


Figure 14: Uterus of oviparous *Artemia* filled with cysts, 1) brown shell glands (darker colour)

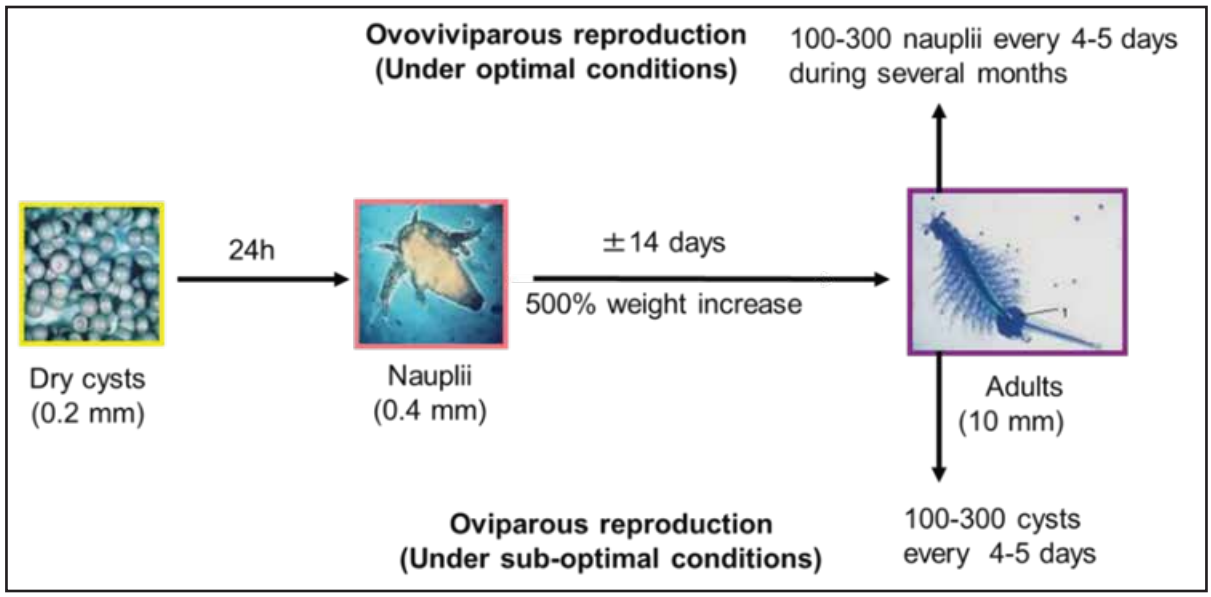


Figure 15: Life cycle of *Artemia*

## 1.2 Ecology and natural distribution

*Artemia* populations are found in about 500 natural salt lakes and man-made salterns scattered throughout the tropical, subtropical and temperate climatic zones, along coastlines as well as inland (for a list of sites of natural *Artemia* populations see FAO Live Food Manual 1996).

The distribution of *Artemia* is discontinuous: not all highly saline biotopes are populated with *Artemia*. Although brine shrimp thrive very well in natural seawater, they cannot migrate from one saline biotope to another via the seas, as they depend on their physiological adaptations to high salinity to avoid predation and competition with other filter feeders. Its physiological adaptations to high salinity provide a very efficient ecological defense against predation, as brine shrimp possess:

- a very efficient osmoregulatory system
- the capacity to synthesize very efficient respiratory pigments to cope with the low $O_2$ levels at high salinities
- the ability to produce dormant cysts when environmental conditions endanger the survival of the species.

*Artemia* therefore, is only found at salinities where its predators cannot survive (> 70 g/L). As a result of extreme physiological stress *Artemia* dies off at salinities close to NaCl saturation, i.e. 250 g/L and higher.

Different geographical strains have adapted to widely fluctuating conditions with regard to temperature (6-35°C), salinity and ionic composition of the biotope. Thalassohaline waters are concentrated seawaters with NaCl as major salt. They make up most, if not all, of the coastal *Artemia* habitats where brines are formed by evaporation of seawater in salt pans. Other thalassohaline habitats are located inland, such as the Great Salt Lake in Utah, USA.

Athalassohaline *Artemia* biotopes are located inland and have an ionic composition that differs greatly from that of natural seawater: there are sulphate waters (e.g. Chaplin Lake, Saskatchewan, Canada), carbonate waters (e.g. Mono Lake, California, USA), and potassium-rich waters (e.g. several lakes in Nebraska, USA).

*Artemia* is a non-selective filter feeder of organic detritus (bioflocs), microscopic algae as well as bacteria. The *Artemia* biotopes typically show a very simple trophical structure and low species diversity; the absence of predators and food competitors allows brine shrimp to develop into monocultures. As high salinity is the common feature determining the presence of *Artemia*, the impact of other parameters (temperature, primary food production, etc.) may at most affect the abundance of the population and eventually cause a temporary absence of the species.

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 a couple of 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 northeast coast of Brazil) were not naturally inhabited by brine shrimp.

Next to the natural dispersion of cysts, deliberate inoculation of *Artemia* in solar salt works by man has been a common practice in the past. Since the seventies man has been responsible for several *Artemia* introductions in South America and Australia, either for salt production improvement or for aquaculture purposes. Additionally, temporal *Artemia* populations were introduced in tropical areas with a distinct wet and dry season (monsoon climate), through inoculation in seasonal salt operations (e.g. the Philippines, Thailand, Vietnam, Myanmar, India, Cambodia in South Asia).

Solar salt operations consist of several interconnected evaporation ponds and crystallizers. In these salt operations, ponds can have sizes of a few to several hundred hectares each with water depths of 0.5 m up to 1.5 m.

Sea water is pumped into the first pond and flows by gravity through the consecutive evaporation ponds. While passing through the pond system salinity levels gradually build up as a result of evaporation. As the salinity increases, salts with low solubility precipitate as carbonates and sulfates (Figure 16). Once the sea water has evaporated to about one tenth of its original volume (about 260 g/L), mother brine is pumped into the crystallizers where sodium chloride precipitates.

Before all sodium chloride has crystallized, the mother liquor, now called bittern, has to be drained off. Otherwise the sodium chloride deposits will be contaminated with $MgCl_2$, $MgSO_4$ and KCl which start precipitating at this elevated salinity (Figure 16). The technique of salt production thus involves fractional crystallization of the salts in different ponds. To assure that the different salts precipitate in the correct pond, salinity in each pond is strictly controlled and during most of the year kept at a constant level.

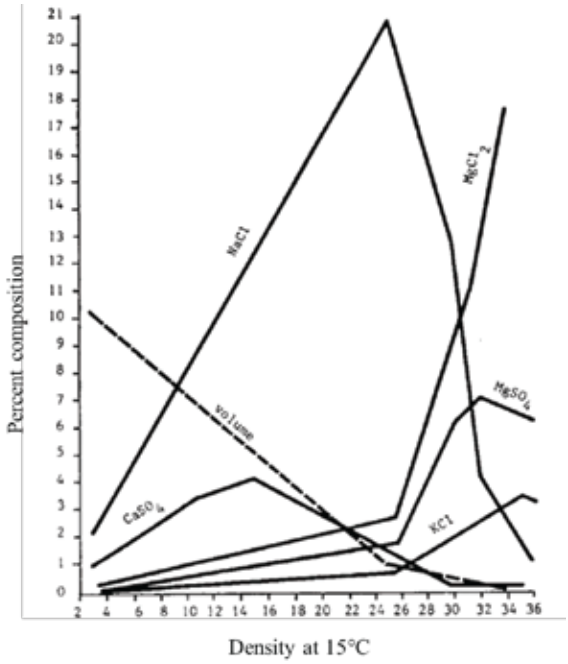


Figure 16: Precipitation of salts with increasing water salinity

Brine shrimp are mainly found in ponds at intermediate salinity levels (Figure 17). As *Artemia* have no defense mechanisms against predators, the lowest salinity at which animals are found is also the upper salinity tolerance level of possible predators (minimum 80 g/L, maximum 140 g/L). From 250 g/L onwards, animal density decreases. Although live animals can be found at higher salinity, the need of increased osmoregulatory activity, requiring higher energy inputs, negatively influences growth and reproduction, eventually leading to starvation and death. Cysts are produced in ponds having intermediate and high salinity (80 g/L to 250 g/L).

The population density depends on food availability, temperature and salinity. The availability of pumping facilities and intake canals allows manipulation of nutrient intake and salinity. Sometimes fertilization can further increase yields. Still, numbers of animals and thus yields per hectare are low.

Moreover the stable conditions prevailing in the ponds of these salt works (constant salinity, limited fluctuations in oxygen as algal concentrations are fairly low, etc.) often results in stable populations in which the ovoviviparous reproduction mode dominates. The selective advantage of ovoviviparous females in these salt works, could also explain the decrease of cyst production which is very typical for stable biotopes (e.g. salt works in NE Brazil).

In salt works *Artemia* should not only be considered as a valuable byproduct. The presence of brine shrimp also influences salt quality as well as quantity. In salt works algal blooms are common, not the least because of the increase of nutrient concentration with evaporation. The presence of algae in low salinity ponds is beneficial, as they color the water and thus assure increased solar heat absorption, eventually resulting in faster evaporation. At elevated salinity, if present in large numbers, algae and more specifically their dissolved organic

excretion and decomposition products will prevent early precipitation of gypsum, because of increased viscosity of the water. In this case gypsum, which precipitates too late in the crystallizers together with the sodium chloride, will contaminate the salt, thus reducing its quality.

Furthermore, accumulations of dying algae which turn black when oxidized, may also contaminate the salt and be the reason for the production of small salt crystals. In extreme situations the water viscosity might even become so high that salt precipitation is completely inhibited and the salt crystals float at the water surface.

The presence of *Artemia* is not only essential for the control of the algal blooms. The *Artemia* metabolites and/or decaying animals are also a suitable substrate for the development of the halophilic bacterium *Halobacterium* in the crystallization ponds. High concentrations of halophilic bacteria - causing the water to turn wine red - enhance heat absorption, thereby accelerating evaporation, but at the same time reduce concentrations of dissolved organic matter. This in turn leads to lower viscosity levels, promoting the formation of larger salt crystals, thus improving salt quality.

Therefore, introducing and managing brine shrimp populations in salt works, where natural populations are not present, will improve profitability, even in situations where *Artemia* biomass and cyst yields are comparatively low. In most of the salt works natural *Artemia* populations are present. However, in some *Artemia* had to be introduced to improve the salt production.

Annually over 3500 MT of dry *Artemia* cysts are marketed worldwide. They are collected from natural sources in North America (Great Salt Lake, Utah-USA), South America (coastal saltworks in Brazil), Central Asia (numerous salt lakes in Siberia-Russia, Kazakhstan, Uzbekistan and China) and Bohai Bay solar saltworks in coastal China.

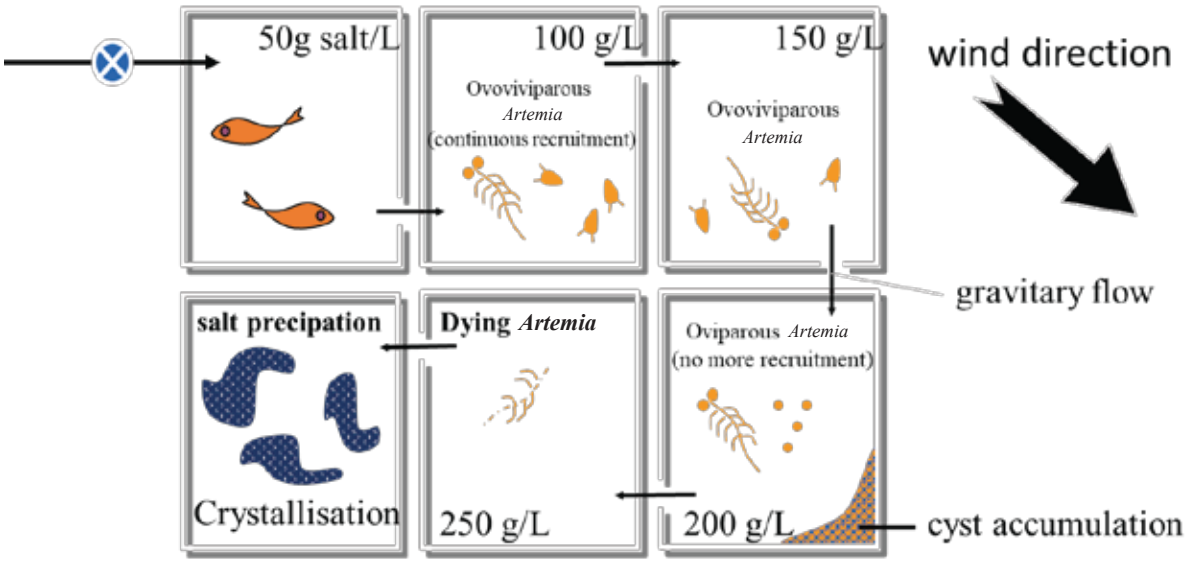


Figure 17: Schematic diagram of solar farm with occurrence of *Artemia*

## 1.3 Taxonomy

Phylum : Arthropoda
Class : Crustacea
Subclass : Branchiopoda
Order : Anostraca
Family : Artemiidae
Genus : *Artemia*
Species : *A. franciscana, A. gracilis, A. monica, A. parthenogenetica, A. persimilis, A. salina, A. sinica, A. tibetiana, A. tunisiana, A. urmiana*

The genus *Artemia* is a complex of sibling species and super species, defined by the criterion of reproductive isolation. Early taxonomists assigned species names to populations with different morphologies, collected at different temperatures and salinities. Later on, the profusion of names was abandoned and all brine shrimp was referred to as *Artemia* salina Linnaeus 1758. Some authors continue this practice today. Generally, different names are assigned to reproductively isolated populations or clusters of populations:

- *A. salina* Linnaeus 1758: Lymington, England (now extinct), Mediterranean area
- *A. tunisiana* Bowen and Sterling 1978 (synonym of A. salina)
- *A. parthenogenetica* Barigozzi 1974, Bowen and Sterling 1978: Europe, Africa, Asia, Australia
- *A. urmiana* Gunther 1990: Iran
- *A. sinica* Yaneng 1989: Central and Eastern China
- *A. persimilis* Piccinelli and Prosdocimi 1968: Argentina and Chile
- *A. franciscana* superspecies: Americas, Carribean and Pacific islands, including populations reproductively isolated in nature such as A.(franciscana) monica Verrill 1869: Mono Lake, California
- *Artemia sp.* Pilla and Beardmore 1994: Kazakhstan.

The coexistence of two species in the same saline habitat is possible: mixtures of parthenogenetic and zygogenetic populations have been reported in Mediterranean salterns. In addition, commercial aquaculture ventures have seeded salterns with imported cysts on many occasions; *A. franciscana* being introduced throughout Asia, Australia, and South America over the last 20 years. Because new populations are constantly being characterised, scientists are urged to use the denomination *Artemia* sp. unless they have sufficient biochemical, cytogenetic or morphological evidence to identify the species name.

The worldwide distribution of brine shrimp in a variety of isolated habitats, each one characterised by its own ecological conditions, has furthermore resulted in the existence of numerous geographical strains, or genetically different populations within the same sibling species; in particular the parthenogenetic *Artemia* with its di-, tri-, tetra- and pentaploid populations display a wide genotypic variation. Among these strains a high degree of genetic variability as well as a unique diversity in various quantitative characteristics have been observed. Some of these characteristics (i.e. the nutritional value of freshly-hatched nauplii) are phenotypical, and change from year to year or season to season. Others, however (i.e. cyst diameter, growth rate, resistance to high temperature) are strain specific and remain relatively constant, (i.e. they have become genotypical as a result of long-term adaptations of the strain to the local conditions; for more details on strain-specific characteristics see FAO Live Food Manual 1996 - chapter 4.1.2.4).

# 2.0 Cyst biology and physiology of the hatching process

A schematic diagram of the ultrastructure of an *Artemia* cyst is given in Figure 18.

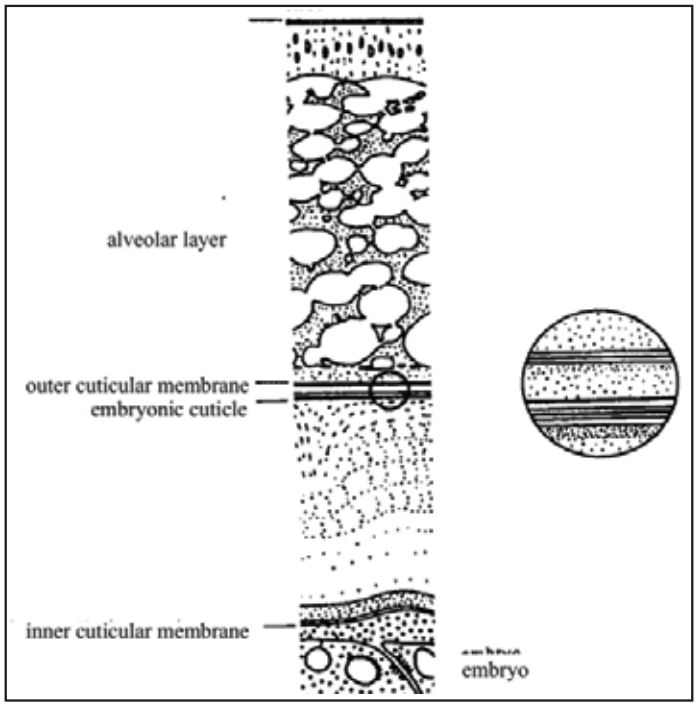


Figure 18: Schematic diagram of the ultrastructure of an *Artemia* cyst.

The cyst shell consists of three layers:

- alveolar layer: a hard layer consisting of lipoproteins 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 UV radiation. This layer can be completely removed (dissolved) by oxidation treatment with hypochlorite (= cyst decapsulation, see Appendix 3).
- outer cuticular membrane: protects the embryo from penetration by molecules larger than the $CO_2$ molecule (= multilayer membrane with very special filter function; acts as a molecular sieve).
- embryonic cuticle: a transparent and highly elastic layer separated from the embryo by the inner cuticular membrane (develops into the hatching membrane during hatching incubation).

The cyst embryo is an indifferentiated gastrula which is ametabolic at water levels below 10% and which can be stored for long periods without losing its viability. The viability is affected when cysts are stored at water levels 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.

The development of an *Artemia* cyst from incubation in the hatching medium till nauplius release is shown in Figure 19.

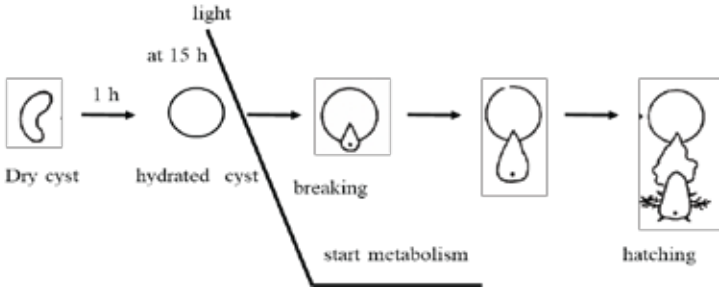


Figure 19: Development of an *Artemia* cyst from incubation in seawater until nauplius release

When incubated in seawater the biconcave cyst swells up and becomes spherical within 1 to 2 hours. After 10 to 20 h hydration, 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 instar I nauplius which starts to move its appendages. Shortly thereafter the hatching membrane breaks open (= hatching) and the free-swimming larva (head first) is released.

Dry cysts are very hygroscopic and take up water at a fast rate (Figure 20). Active metabolism of the embryo is resumed once a water content of 60% is reached and provided all environmental conditions are favourable (oxygen, temperature, see further).

The aerobic metabolism in the cyst embryo assures the conversion of the carbohydrate reserve trehalose into glycogen (as an energy source) and glycerol.

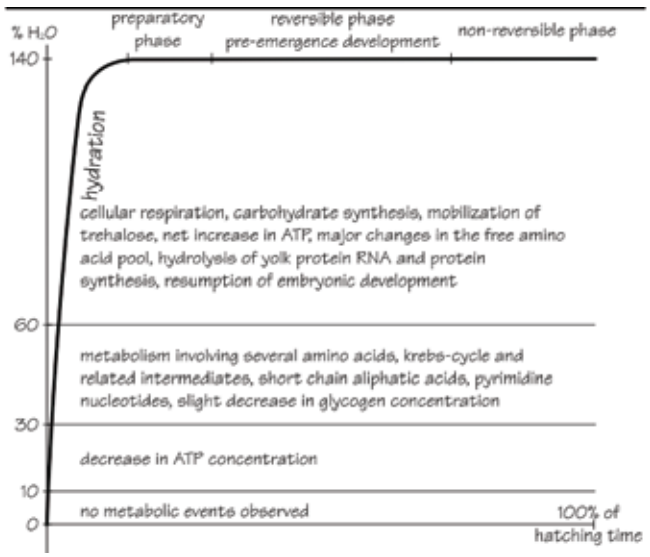


Figure 20: Cellular metabolism in *Artemia* cysts in function of hydration level

Increased levels of the latter hygroscopic compound result in further water uptake by the embryo. Consequently, the osmotic pressure inside the outer cuticular membrane builds up continuously until a critical level is reached, which results in the breaking of the cyst envelope, at which moment all the glycerol produced is released in the hatching medium. In other words the metabolism in *Artemia* cysts prior to the breaking is a trehalose-glycerol hyperosmotic regulatory system. This means that as salinity levels in the incubation medium increase, higher concentrations of glycerol need to be built up in order to reach the critical difference in osmotic pressure which will result in the shell bursting, and less energy reserves will thus be left in the nauplius.

After breaking of the cyst shell, the embryo is in direct contact with the external medium through the hatching membrane. An efficient ionic osmoregulatory system is now in effect, which can cope with a big range of salinities, and the embryo differentiates into a moving nauplius larva. A hatching enzyme, secreted in the head region of the nauplius, weakens the hatching membrane and enables the nauplius to liberate itself into the hatching medium.

## 2.1 Effect of environmental conditions on cyst metabolism

Dry cysts (water content from 2 to 5% ; see worksheet 1 for determination of water content for practical example) are very resistant to extreme temperatures; hatching viability not being affected in the temperature range -273°C to +60°C and above 60°C and up to 90°C only short exposures being tolerated.

Hydrated cysts have far more specific tolerances with mortalities occurring below -18°C and above +40°C; a reversible interruption of the metabolism (= viability not affected) occurring between -18°C and +4°C and between ±33°C and ± 40°C, with the upper and lower temperature limits varying slightly from strain to strain. The active cyst metabolism is situated between +4°C and ±33°C; the hatching percentage remains constant but the nauplii hatch earlier as the temperature is higher.

As for other environmental conditions, optimal hatching outputs are reached in the pH range 8-8.5. As a consequence, the addition of $NaHCO_3$, up to 2 g/L, to artificial or diluted seawater or to dense suspensions of cysts results in improved hatching. This might be related to the optimal pH activity range for the hatching enzyme.

An increased hatching has been reported with increasing oxygen level in the range 0. 6 and 2 mg/L, and maximal hatching obtained above this concentration. To avoid oxygen gradients during hatching it is obvious that a good homogeneous 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, ± 90 g/L for most strains) insufficient quantities of water can be taken up to support the embryo's metabolism. Optimal salinity for hatching is equally strain-specific, but generally situated in the range 15-36 g/L.

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 meteabolism, related to light intensity and/or exposure time.

As a result of the metabolic characteristics of hydrated cysts, a number of recommendations can be formulated with regard to their use. When cysts (both decapsulated and non-decapsulated) are stored for a long time, some precautions have to be taken in order to maintain maximal energy content and hatchability. Hatchability of cysts is largely determined by the conditions and techniques applied for harvesting, cleaning, drying and storing of the cyst material. The impact of most of these processes can be related to effects of dehydration or combined dehydration/hydration. For diapausing cysts, these factors may also interfere with the diapause induction/termination process, but for quiescent cysts, uncontrolled dehydration and hydration result in a significant drop of the viability of the embryos.

Hatching quality in stored cysts is slowly decreasing when cysts contain water levels from 10 to 35% $H_2O$. This process may however be retarded when the cysts are stored at freezing temperatures. The exact optimal water level within the cyst (around 5%) is not known, although there are indications that a too severe dehydration (down to 1-2 %) results in a drop in viability.

Water levels in the range 30-65% initiate metabolic activities, eventually reducing the energy contents down to levels insufficient to reach the state of emergence under optimal hatching conditions. A depletion of the energy reserves is furthermore attained when the cysts undergo subsequent dehydration/hydration cycles. Long-term storage of such material may result in a substantial decrease of the hatching outcome. 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 differentiated embryos.

Sufficiently dehydrated cysts only keep their viability over long periods (years) when stored under vacuum or in nitrogen; the presence of oxygen results in a substantial depletion of the hatching output through the formation of highly detrimental free radicals. Even properly packed cysts should be preferentially kept at low temperatures. However, when frozen, the cysts should be acclimated for one week at room temperature before hatching.

## 2.2 Diapause status of *Artemia* cysts

As *Artemia* is an inhabitant of biotopes characterized by unstable environmental conditions, its survival during periods of extreme conditions (i.e. desiccation, extreme temperatures, high salinities) is ensured by the production of dormant embryos. *Artemia* females can indeed easily switch from live nauplii production (ovoviviparity) to cyst formation (oviparity) as a fast response to fluctuating circumstances. Although the basic mechanisms involved in this switch are not yet fully understood, sudden fluctuations seem to trigger oviparity (oxygen stress, salinity changes). The triggering mechanism for the induction of the state of diapause is however not yet known. In principle, *Artemia* embryos released as cysts in the medium are in diapause and will not resume their development, even under favourable conditions, until they undergo some diapause deactivating environmental process; at this stage, the metabolic

standstill is regulated by internal mechanisms and it cannot be distinguished from a non-living embryo. Upon the interruption of diapause, cysts enter the stage of quiescence, meaning that metabolic activity can be resumed at the moment they are brought in favourable hatching conditions, eventually resulting in hatching: in this phase the metabolic arrest is uniquely dependent of external factors (Figure 21). As a result, synchronous hatching occurs, resulting in a fast start and consequent development of the population shortly after the re-establishment of favourable environmental conditions. This allows effective colonization in temporal biotopes.

For the user of *Artemia* cysts several techniques have proven successful in terminating diapause. It is important to note here that the sensitivity of *Artemia* cysts to these techniques shows strain- or even batch-specificity, hence the difficulty to predict the effect on hatching outcome. When working with new or relatively unknown strains, the relative success or failure of certain methods has to be found out empirically.

In many cases the removal of cyst water is an efficient way to terminate the state of diapause. This can be achieved by drying the cysts at temperatures not exceeding 35-4°C or by suspending the cysts in a saturated NaCl brine solution (300 g/L). As some form of dehydration is part of most processing and/or storage procedures, diapause termination does not require any particular extra manipulation. Nevertheless, with some strains of *Artemia* cysts the usual cyst processing techniques do not yield a sufficiently high hatching quality, indicating that a more specific diapause deactivation method is necessary.

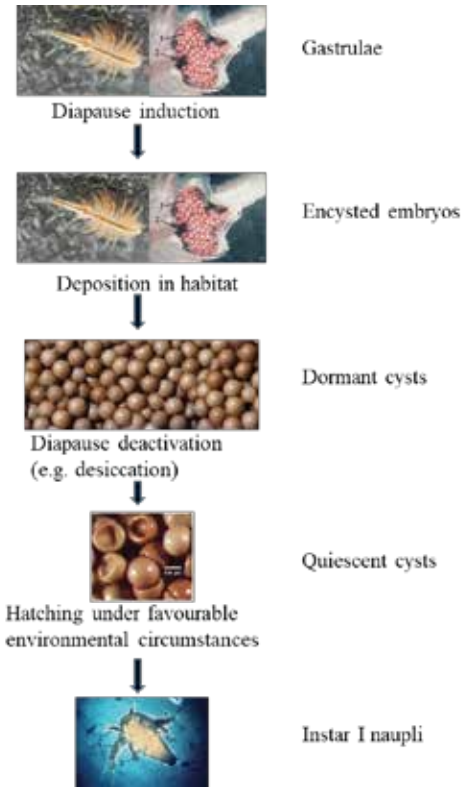


Figure 21: Schematic diagram explaining the specific terminology used in relation with dormancy of *Artemia* embryos

The following procedures have proven to be successful when applied with specific sources of *Artemia* cysts:

- freezing: "imitates" the natural hibernation period of cysts originating from continental biotopes with low winter temperatures (Great Salt Lake, Utah, USA; continental Asia);
- incubation in a hydrogen peroxide ($H_2O_2$) solution. In most cases, the sensitivity of the strain (or batch) to this product is difficult to predict, and preliminary tests are needed to provide information about the optimal dose/period to be applied, and about the maximal effect that can be obtained (Table 1). Overdosing results in reduced hatching or even complete mortality as a result of the toxicity of the chemical. However, in some cases no effect at all is observed.

Table 1: Dose-time effect of $H_2O_2$ pre-incubation treatment on the hatchability of *Artemia* cysts from Vung Tau (Vietnam)

| Time (minutes) | Doses of $H_2O_2$ (%) | | | | | |
|---|---|---|---|---|---|---|
| | 0.5 | 1 | 2 | 3 | 5 | 10 |
| 1 | | | | | 46 | 10 |
| 2 | | | | | 94 | 5 |
| 5 | | | 54 | 69 | 102 | |
| 10 | 47 | | 90 | 81 | 88 | 32 |
| 15 | | 46 | 100 | 76 | | |
| 20 | | | 91 | 94 | 52 | |
| 30 | | 91 | 95 | | | |
| 60 | 56 | 85 | | 6 | 1 | |
| 120 | | 15 | | | | |
| 180 | | | | | | |

Data are expressed as percentage of hatching results obtained at 2 %/15 min. treatment (74 % hatch)

In general other diapause termination techniques (cyclic dehydration/hydration, decapsulation, other chemicals...) give rather erratic results and/or are not user-friendly. One should, however, keep in mind that the increase in hatching percentage after any procedure might (even partially) be the result of a shift in hatching rate (earlier hatching). For more details about diapause deactivation methods see FAO Live Food Manual 1996, Van Stappen et al. 1998).

# 3.0 Site selection

Topography, soil condition, dike system, drain and canal system as well as climate are important parameters for proper site selection of *Artemia* production in solar salt farms (Figure 22). These factors are described below in brief.

Figure 22: Solar salt farms in Cox's Bazar

## 3.1 Topography

Flat lands are suitable to construct ponds of regular shape. A gradual slope facilitates gravity flow through the ponds. It is better to have the level in the *Artemia* pond lower than in all other ponds as to allow higher water flow into the ponds than the outflow. Proper use of gravity or tidal currents will reduce pumping cost. Muddy shores with flat and gentle slope present along the Moheshkhali channel, especially the delta and flood plains of Matamuhuri and Baghkhali rivers where most of the salt pans of Cox's Bazar area are located.

## 3.2 Soil condition

Heavy clay soils (i.e more then 70%) with minimal contents of sand are ideal. The best soil condition is required to prevent leakage or minimal infiltration rates to optimize production of high saline water. Leakage is one of the most common problems in fish/ shrimp farms and salt farms.

Acid sulphate soil or low pH soil must be avoided. pH of acid sulphate soil drops in contact with air. Algae growth is limited at low pH. High organic content in the pond soil might cause oxygen depletion due to decomposition. Soil pH of 8-8.5 with good water holding ability is suitable. Some times solar salt farms were reclaimed from mangrove areas and thst's when low pH problems are encountered.

## 3.3 Dike system

Several dikes are present in salt-*Artemia* integration system. Dikes are to separate from shorelines, different blocks of salt production unit, reservoir and evaporation area, and crystallization area.

Figure 23: Dike system

## 3.4 Drain and canal system

The purpose of the main canal is for intake of seawater for the salt production process and to stock fish/shrimp during the rainy season, it also drains seawater into the reservoir, between the evaporation ponds and finally into the crystallization area.

Figure 24: Drainage system

## 3.5 Climate

Seasonality (dry season during November to April), suitable temperature to accelerate evaporation rates, lower humidity and rainfall, wind, longer duration of sunlight play an important role for *Artemia* production in solar salt farms. Low temperature reduces growth and reproduction whereas too high temperature can be lethal for *Artemia*. The evaporation rate in Cox's Bazar coast is minimum in May-July when cloud cover is high and average wind velocity is near the annual minimum. Evaporation generally reaches its maximum in January to April when temperature is high, skies are clear and the windy season is in full progress (Hossain and Hossain, 2006).

# 4.0 *Artemia* culture model in salt farms

*Artemia* culture system can be categorized on the basis of investment, pond management and products. The profitabilities of *Artemia* farms depend on farming scale, production model and interaction between these factors. Farmer can select the model according to preference. Integration of *Artemia* production with salt and aquaculture is a useful culture model for salt farmers in Cox's Bazar. *Artemia* culture models are described below.

## 4.1 Monoculture

This system uses high saline water of the salt production system for *Artemia* ponds and use waste water of *Artemia* ponds for salt crystallization. *Artemia* filter algae and particulate organic (for example bio flocs).

This model convert all salt field area for *Artemia* culture including ponds, fertilized pond, canal, drainage (Figure 23).

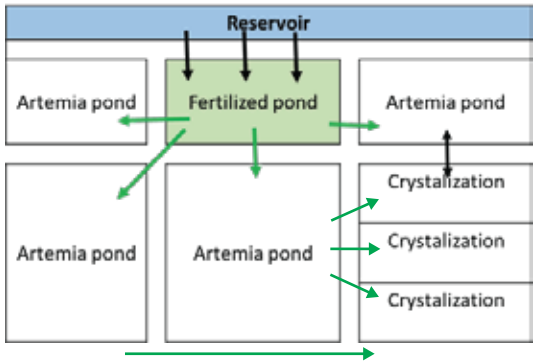


Figure 25: *Artemia* monoculture field

Table 2: Characteristics of *Artemia* monoculture in one ha solar salt farms

| | Number | Area in ha | Area in % | Water depth in meter |
|---|---|---|---|---|
| Reservoir pond/canal | 1 | 0.10 | 10 | 1.0 |
| Fertilized pond | 1 | 0.25 | 25 | 0.5 |
| *Artemia* Production | 4 | 0.80 | 80 | 0.5 |

## 4.2 Integrated culture: this model can be combination of *Artemia* - salt - shrimp/fish

In this system, shrimp/ fish can be produced at low salinity (less than 40 g/L) in the fertilized ponds, canals/reservoirs. The application of fertilizer, feed for shrimp/ fish production will stimulate algae growth. Furthermore, the effluent of shrimp/fish ponds serves as food for *Artemia*. It reduces the demand of organic fertilizer for *Artemia* production. Moreover, fresh *Artemia* biomass can supplement feed for shrimp/fish at different stages. Furthermore, combination of *Artemia*, shrimp/fish open the scope of clean aquaculture environment due to filtration activity of *Artemia* and its beneficial effect on salt quality.

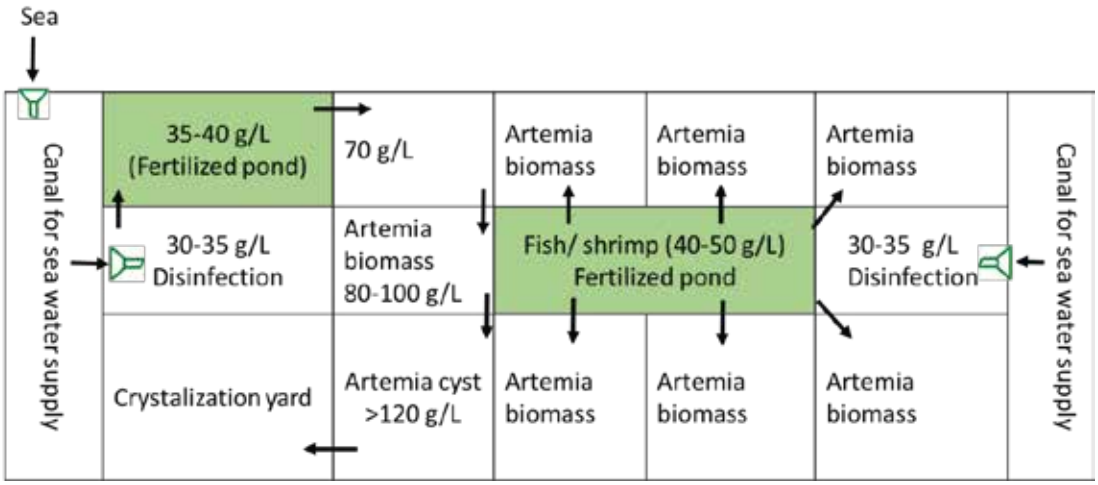


Figure 26: Integrated culture model *Artemia*- salt-shrimp/fish

Table 3: Characteristics of a one ha integrated *Artemia*- salt-shrimp/fish culture in solar salt farm

| | Number | Area in ha | Area in % | Water depth in meter |
|---|---|---|---|---|
| Canal/ reservoir (Fish/ shrimp pond) | 1 | 0.10 | 10 | 2.0 |
| Fertilized pond (Fish/ shrimp pond) | 1 | 0.25 | 25 | 1.0 |
| *Artemia* pond | 3 | 0.55 | 55 | 0.5 |
| Salt crystallization yard | 3 | 0.10 | 10 | 0.1 |

In Vietnam it has been shown that farms that integrate cyst and biomass production are more profitable than only performing cyst production: annual yield of cysts is 60-100 kg WW/ha.

# 5.0 Pond construction

The farm includes reservoir, algae production area (fertilized pond), *Artemia* pond and salt crystallization area. The calculation of ratio of different division is useful to optimize the production area (maximum 75-80% of total area). Usually, salt ponds are shallow with water depth less than 20 cm. This resulting in very high temperature (more than 40°C) and promotes benthic algae production instead of phytoplankton. To integrate *Artemia* production, excavating part of the pond bottom and/or heightening the dikes to increase the water depth should be done. It enlarges the production volume and reduce temperature stress for *Artemia*. Water depths of minimum 40 cm from the pond bottom (preferentially higher) and minimum 50 cm in canals are recommended for *Artemia* ponds (Figure 25). A ratio "pond surface: pond volume" larger then 3:1 is acceptable (pond surface expressed in $m^2$, pond volume expressed in $m^3$).

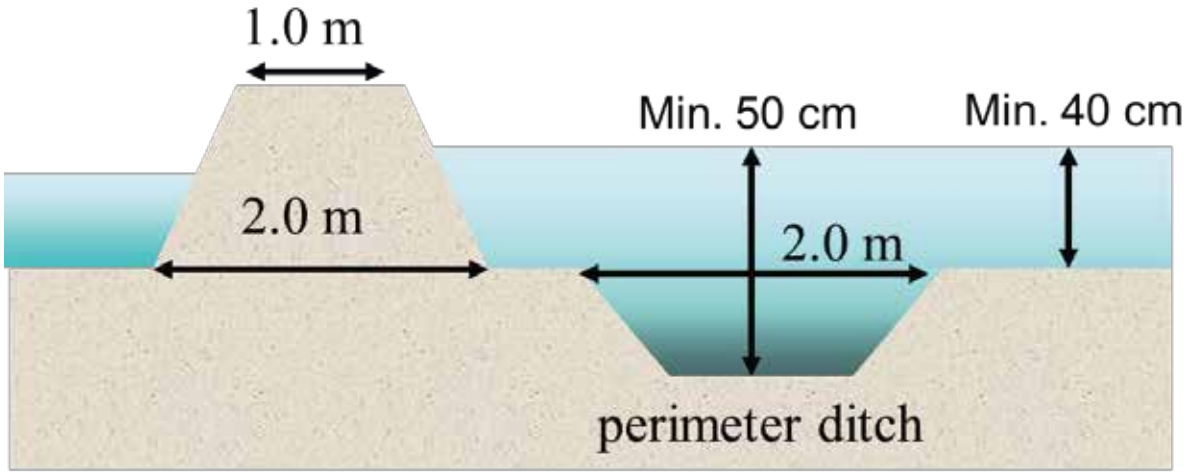


Figure 27: Cross section of *Artemia* pond base

## 5.1 Dike construction

Newly constructed dikes need to be well compacted to prevent leakages (Figure 26 and 27). Leaks mostly occur during heightening old dikes with new soil. Burrowing crabs are often digging holes through dikes. Lime (CaO) and clay are useful to reduce the leaks caused by crabs. Dikes and slopes minimum ratio 1:1 ratio (height: width) is needed to prevent erosion.

Figure 28: Dike construction

Figure 29: Dike compaction

## 5.2 Wave breaker

Windy conditions and high wave action enhance the evaporation. Foam is often formed due to wave action and cysts get trapped in the foam and are lost. Wave breakers using for example bamboo sticks should be installed at downwind side of the pond (Figure 28, 29 and 30).

Figure 30: Wave breaker

Figure 31: Wave breaker

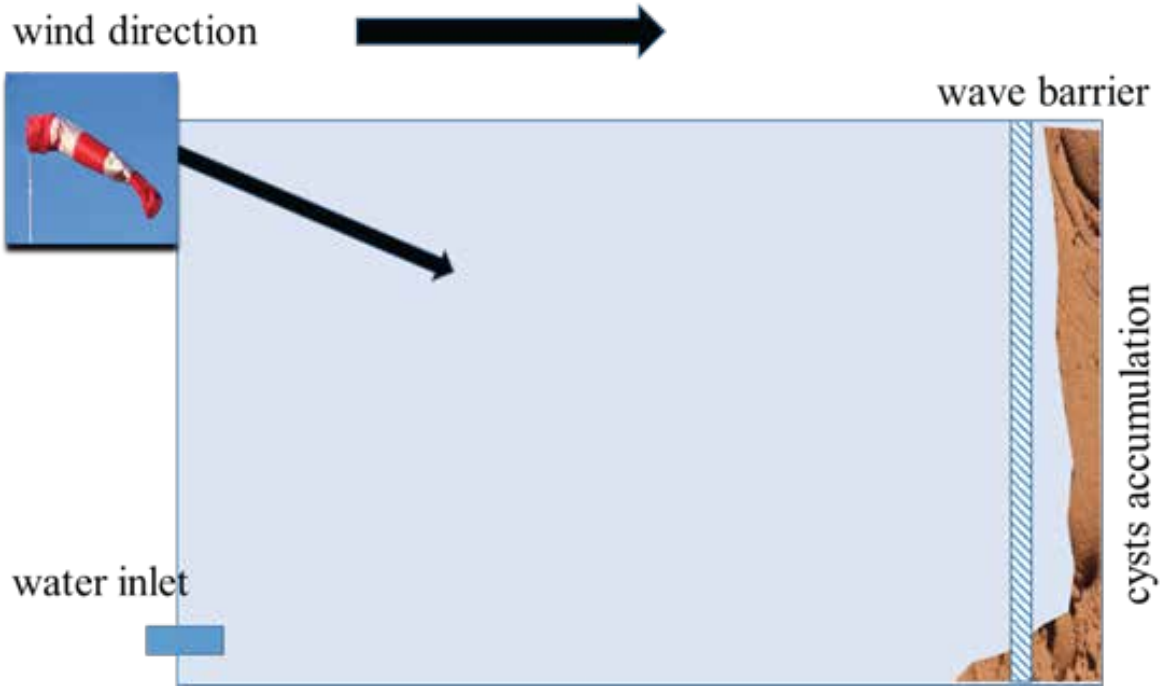


Figure 32 : *Artemia* pond design

## 5.3 Prevention of predators and competitors

Predators of *Artemia* are birds, crab (fiddler crab *Uca sp.*), fishes (tilapia *Oreochromis mossambicus*), aquatic insects (Figure 31). Birds can be prevented by bird traps or scarecrows. Food competitors, aquatic insects and zooplanktons such as copepods (*Acartia sp., Microsetella sp*), rotifers (*Brachionus plicatilis*) and ciliate protozoan (*Fabrea salina*) can be eliminated or prevented to develop in the *Artemia* ponds by screening intake water, use of chemicals and raising salinity level more than 100 g/L before stocking of *Artemia* nauplii or apply derris plant @ 10 kg/100 m3 24 h before stocking of *Artemia* nauplii.

Bittern

Crab

Tilapia

Ciliate (*Fabrea salina* )

Copepod (*Acartia*)

Figure 33: Predators and competitors of *Artemia*

Filtration screens are required during water intake to prevent predators (for example eggs of fish and crustacean larvae) entering the culture ponds. A filter screen can be installed in the pond monk (Figure 32). Farmers usually install fine filter net at the pumping station (Figure 33). Water is lifted by a pump into an overhead compartment from where the water is drained over the filter screen. Mesh sizes of 120 µm have been tested with good result.

Figure 34: Monk with filter to prevent predators entering in the *Artemia* pond

Figure 35: Screen to prevent predators

# 6.0 Brine (high saline water) preparation

The salinity tolerance of local predators (mainly fish) will determine at what salinity one can introduce *Artemia*. This is mostly above 70 g/L salinity. It may take more than a month to evaporate sea water with initial salinity of 30 g/L to reach the desired salinity of more than 70 g/L. Different approaches can be taken to shorten the period with appropriate salinity for the *Artemia* pond. These approaches are (i) storage of high saline water of previous season, (ii) dissolve crude salt to increase the salinity (Figure 34), and (iii) speed up evaporation by more mechanical turbulence (e.g frequent raking evaporation pond bottom with initial thin layer of seawater about 10 cm to accelerate the evaporation rate).

The commonly used technique is to store brine water at the end of the dry season in a deep pond (2 to 3 m). The storage pond is installed with a wooden sewer (overflowing sewer) to remove the freshwater layer in the water surface after heavy rains (to limits its dissolution with saline water in the storage pond). At the start of the rainy season brine water is pumped from the storage pond to the *Artemia* pond where it is mixed with natural sea water or salt water in evaporation ponds in salt field, so that salt water salinity can be 80-90 g/L and ready for *Artemia* stocking.

In fact, when salt is cheap and unmarketable, it can be used to increase salinity in *Artemia* pond at the beginning of the dry season to shorten the stocking time.

Figure 36: Crude salt can be used to increase salinity of *Artemia* pond

# 7.0 Incubation and stocking

Incubation and stocking process consists of selection of strain, standard procedure for incubation and stocking strategy.

## 7.1 Selection of *Artemia* strain

*A. franciscana* will be used for pond production in solar salt farms in Cox's Bazar. Overall, this species has higher colonizing abilities than other species and it has been successfully used in many similar projects in the Philippines, Thailand, Vietnam, Cambodia, Myanmar, Mozambique, Kenya, India, and Sri Lanka. Cysts of this species have excellent quality characteristics, e.g. small in size, high hatching and nutritional qualities particularly its content of highly unsaturated fatty acids (HUFA). It is recommended to use the Vinh Chau- Vietnam strain as it has a high temperature tolerance after >15 years of selection pressure.

## 7.2 Standard procedure of incubation/ hatching of *Artemia* cysts

For details of the procedure see annex 1.

In brief, the standard conditions for *Artemia* cyst hatching are as follows:

- Dissolved oxygen: Aeration intensity to maintain dissolved oxygen 5 mg/L
- Temperature: 25-30°C
- Salinity: 25-30 g/L
- pH : about 8 (1 g/L $NaHCO_3$ can be used to increase buffer capacity)
- Cyst density: 2 g/L
- Illumination: 2000 lux at water surface

It is critical to inoculate *Artemia* nauplii (hatched out at seawater salinity) in the instar I stage in salt ponds of 70 g/L salinity and higher. Only in this instar I stage *Artemia* nauplii can survive sudden salinity shocks (thanks to their salt gland or neck organ). Approximate incubation time to reach instar I stage should be determined with a small amount of cysts prior to set up the hatching for inoculation and even then close monitoring of the hatching process should allow to harvest nauplii when in instar I stage.

## 7.3 Transportation of newly hatched *Artemia* to stocking into ponds

Upon harvesting and thorough washing, the *Artemia* nauplii are stocked in seawater in plastic bags. When transportation distance is more than 15 minutes between *Artemia* cyst incubation and nauplii stocking location, bags should be filled with oxygen or cooled. With transportation time more than an hour, the newly hatched Instar I *Artemia* nauplii need to be placed in bags with oxygen and water with ice.

## 7.4 Suitable time of stocking

The best stocking time is in the early morning (7-8 am) or in the evening (5-7 pm) considering relatively cool water temperature.

## 7.5 Suitable places to release newly hatched *Artemia*

*Artemia* nauplii suitable to spread evenly all over the pond surface upwind. It is required to check the nauplii density next morning through sampling.

## 7.6 Stocking strategy

Good production result are obtained when inoculating 50-70 nauplii/L into pond water salinity 70 g/L. One can also inoculate at higher densities (up to 500 nauplii/L) in a small pond area under optimal culture conditions (water depth and food availability) and after 1 week of culture transfer the animals to a larger pond.

# 8.0 Pond maintenance and management

*Artemia* pond management requires farmers knowledge, experience in management according to season and circumstances. *Artemia* (cyst and biomass) yield depends on various factors including experience in management, participation in training, weather (salinity, temperature), and cost of feed, fertilizer, labour and renovation.

The pond management starts from the day of stocking and needs to be followed up every day throughout the crop. At the beginning of crop, 7-10 days after stocking the *Artemia* ponds may have "algal bloom" caused by available nutrient in the incoming water and from the pond soil. Therefore, no fertilization is needed during this period. Afterwards, the pond management issues include control of water level, salinity, water colour, feed, population composition, density, nutritional and health status of female, ratio female carrying cysts and larvae. Fertilized ponds should have availability of green water to supply Artemia ponds. Weather conditions are particularly concerned for prolonged rainfall, out of season rain, low or too high temperature.

## 8.1 General management

*Artemia* reach juvenile stage 7-10 days after stocking and start reproducing from day 12-15 onward. Management also depends on the purpose of harvesting cyst or biomass. Biomass harvesting can start from 12-15 days post stocking and cyst can start harvesting after *Artemia* is 15-20 days old. Reproductive activity become stable from 20 days post stocking onwards. The yield mainly depends on availability of food, water temperature, salinity and oxygen levels.

## 8.2 Water level management

Experience in south Asian countries reveal several limitations to integrate *Artemia* production in the salt farm. Most salt farmers slightly renovate salt evaporation ponds for *Artemia* culture without completely change the structure of salt field to save construction cost and to switch *Artemia* and salt production considering market demand and profit model. The deeper water level is suitable for *Artemia* culture requires increased construction cost, longer time for evaporation and need for higher volume of sea water.

In shallow ponds light penetrates to the bottom and stimulate bottom algae development ("lab-lab"). Lab-lab algal mats compete with unicellular algae for nutrients. Lab-lab quickly cover pond bottom surface. After certain period lab-lab dies and, float on surface. This affects cyst and biomass harvesting. Eventually, lab-lab sink to bottom and decompose. It causes oxygen deficiency, release toxic gases (hydrogen sulfide, methane) in anaerobic decomposition.

Considering extreme weather due to climate change, construction of deeper ponds prolong the time (about a month) to prepare salt water 80-100 g/L suitable for *Artemia* stocking. On the other hand, at low water level diurnal temperature fluctuation is high, newly stocked *Artemia* can be trapped in foam (effect of wind) and chances of predation (for example wading water birds) are high.

Therefore, it is necessary to have deep water level and sufficient salinity from the beginning of crop to prolong the culture period and increase productivity.

## 8.3 Salinity management

Optimum salinity management in *Artemia* ponds has significant impact to prolong the crop and increase productivity. Low salinity or too high salinity has negative effect on survival, growth, reproduction and harvesting of cyst. Traditional salt production procedure include natural evaporation, mixing water level of evaporation ponds, and transfer water between evaporation ponds. In the beginning, it might take about a month to increase sea water salinity to the suitable level (70-80 g/L) for *Artemia* stocking. Crude salt or concentrated brine of last crop can be used to shorten the preparatory period. Therefore, at the end of the crop, saline water of culture pond/ evaporation pond will be stored in a brine reservoir. This storage pond needs to be more than 1 meter deep without leakage or contaminants and has drainage or overflowing sewer to remove rain water layer on the surface after heavy rainfall.

Salinity level 80-100 g/L should be maintained and more than 250 g/L is deadly for *Artemia*. Adding low salinity water is useful for salinity management. Amount of water supply required for salinity management can be calculated by the following formula:

$$S = \frac{(V1 \times S1) + (V2 \times S2)}{(V1 + V2)}$$

S = Salinity in the pond after additional water supply
V1 = Water volume in m3 in pond before additional water supply
S1 = Initial salinity of the pond water
V2 = Volume of additional water in m3
S2 = Salinity of additional water

## 8.4 Temperature management

Temperature has great impact on growth and reproduction of *Artemia*. At low or high temperature *Artemia* cannot reproduce and might cause mass mortality. Temperature plays an important role in evaporation, salinity, water quality, algal growth, *Artemia* development and reproduction. Salinity, depth, transparency, wind speed and season all have influence on temperature fluctuation. Long periods of water temperature more than 35°C is not suitable for *Artemia* culture. Different materials such as coconut leaves to cover pond surface partially are useful shelter for *Artemia* at high temperature. Water level management are useful to maintain optimum temperature level.

## 8.5 Dissolved oxygen (DO)

More than 2.5 mg/L DO is suitable for *Artemia* culture in ponds. DO in ponds is related to wind and algae density and population density as well. Supply water in mixing pond water layer is useful to mitigate low DO level at early in the morning.

## 8.6 Feed management

Feed for *Artemia* in ponds include algae from fertilizer ponds, naturally grown in ponds or supplied directly, humus, organic and or inorganic fertilizer, shrimp feed, fish meal. Bio-floc technology can be applied in *Artemia* ponds through adding carbohydrates (to reach carbon nitrogen ratio of 10 and higher) in fertilized ponds or directly to directly to the *Artemia* ponds to stimulate the growth of heterotrophic bacteria and floc materials.

The key to monitor and assess feed suitability is the assessment based on observation on individual or *Artemia* population in pond to stabilize the production process. The observation include -

- Presence of food in *Artemia* gut
- The uniformity in size of *Artemia* population, colour (bright, opaque)
- Swimming behaviour of *Artemia* (active, group versus alone, surface or bottom
- Monitor the development: *Artemia* reach adult stage and participate in reproduction at 15-20 days post stocking in ponds
- Female reproductive efficiency (colour of the ovary bright/dark, fecundity ratio of female carrying empty ovary)
- Dead *Artemia* gathers at the downwind corner

### 8.6.1 Algae bloom

In the Mekong delta in Vietnam the soil of the *Artemia* ponds are rich in nutrients and organic matter, high in calcium and manganese but low in iron. This condition contributes to algal blooms. Ponds without algal bloom inhibit *Artemia* growth and require fertilization.

### 8.6.2 Lab-lab (bottom filamentous algae)

It occurs in ponds with nutrient rich bottom soil or due to excessive fertilization. In the beginning of the *Artemia* crop, when unicellular algae develop and/or turbidity caused by mixing prevent penetration of sunlight to the bottom. Gradually transparency increases due to filtration activity of *Artemia* and no water mixing. This allows sunlight to penetrate to the bottom and contributes to lab-lab growth resulting in low *Artemia* yields. Removing lab-lab and increasing water turbidity take time and are expensive (Figure 35 and 36). Long time presence of lab-lab causes water pollution and *Artemia* population may disappear. Lab-lab can be prevented by managing water transparency to prevent penetration of sunlight to the bottom.

Figure 37: Lab lab at *Artemia* pond bottom

Figure 38: Raking in *Artemia* pond

### 8.6.3 Clay turbidity

A higher amount of sodium in the soil and water than potassium, calcium and magnesium together under high salinity conditions can cause clay particles to disperse and float. Presence of organic substance can reduce clay turbidity. Clay turbidity can be reduced/prevented by following:

- Application of organic fertilizer to increase organic matter content
- Supply green water from fertilized ponds to *Artemia* pond to replace partly of turbid water with brine with the same salinity

## 8.7 Population management

Fluctuation of *Artemia* density in the pond depends on life stages. The pond produces higher yield of cysts and biomass in a favourable environment with sufficient food availability and stability of all life stages in the population. The evaluation of the population also consider the reproductive activity of the female such as female lay cysts/nauplii, number of embryos in the ovary, number of female with empty ovary. Duration of a population depend on salinity, temperature, availability of food. See example in Table 4.

Table 4: *Artemia* population type in culture pond over time

| Type of population | Nauplii | Presence of population compositions | | | |
|---|---|---|---|---|---|
| | | Juvenile | Sub-adult | Adult | Cyst |
| A | ++ | - | - | - | - |
| B | - | - | + | ++ | - |
| C | ++ | - | - | + | - |
| D | + | + | + | + | - |
| E | + | - | - | ++ | - |
| F | - | - | - | ++ | - |
| G | - | - | - | ++ | + |
| H | + | + | + | + | + |

Note: - do not appear; + appear; ++ appear with high density

A: Population at stocking
B: Population developing to sub-adult and adult stage
C: Population with female producing nauplii
D: Population continue to reproduce and pond in good condition
E: Population only has adult, nauplii die off quickly due to lack of food
F: Lack of sufficient food for adult, no recruitment
G: Adult female switch to producing cyst; population no longer increase in density; too stressful condition
H: Population has presence of all life stages, female is producing cysts. Ideal condition.

## 8.8 Harvesting management

*Artemia* can be harvested under 2 forms: cysts and biomass. Harvesting requires to consider suitable time, frequency and ensure best quality of the products. *Artemia* biomass has longer culture period than *Artemia* cyst considering the seasonality and preparation time described earlier. Salinity and feed management require to be in accordance with preference of *Artemia* cyst and or biomass production. Details of *Artemia* cyst and biomass harvesting and processing are described in the chapter 11.

## 8.9 Seasonal management

Cox's Bazar is one of the most vulnerable areas of Bangladesh due to climate induced threats such as temperature fluctuation, out of season rain, heavy rains, thunderstorms, and cyclones. April to October is hot period, November to March is cold period (Table 5). May to September has most rainy days in a year (https://www.weather-atlas.com/en/bangladesh).

Table 5: Climate calendar of Cox's Bazar

| Climate induced threats | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Maximum temperature (26-33°C) | | | | | | | | | | | | |
| Minimum temperature (15-25°C) | | | | | | | | | | | | |
| Rainfall (4.1-924 mm) | | | | | | | | | | | | |
| Rainy days (1-22 days) | | | | | | | | | | | | |
| Humidity (71-89%) | | | | | | | | | | | | |
| Day light (10.8-13.4 average h) | | | | | | | | | | | | |
| Sunshine (average 3-9 h) | | | | | | | | | | | | |
| Radiation (UV index 7-12) | | | | | | | | | | | | |

Note: Deeper colour shows the maximum intensity

The experience in other Asian countries showed 60% reduced yield due to out of season rain, cold wind and extreme temperature in some areas. The following seasonal solutions in *Artemia* culture can be considered to limit the risks due to extreme weather.

### 8.9.1 Season and crop

Climate induced threats might affect *Artemia* culture in ponds in all steps covering pond construction, stocking, management and harvesting. Harvesting of cysts might be feasible from December to March. It might be useful to focus to *Artemia* biomass production for the remaining dry season period. Concurrent and alternative integration with aquaculture of saline tolerant species (shrimp, prawn, crab, sea bass and Tilapia) will be cost effective in the dry and wet season, respectively (Table 6).

Table 6: Seasonal calendar of Integrated *Artemia*-Salt-Fish production in Cox's Bazar

| | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Salt | | | | | | | | | | | | |
| *Artemia* | | | | | | | | | | | | |
| Aquaculture | | | | | | | | | | | | |

# 9.0 Enhancement of algae growth

Microalgae supplied as green water from a fertilized pond is the best food for *Artemia*. Pond water transparency is a good indicator of algae growth. Optimal water transparency should be in the range of 25 to 35 cm. At lower transparency, caused by high algal growth, oxygen depletion might occur particularly at night.

Pond water color is related to the type of phytoplankton present, e.g. green color because of chlorophyta, blue for cyanophyta, tea brown or dark brown by bacillariophyta. Red color in culture ponds at high salinity is caused by the presence of *Dunaliella* or *Halobacterium*. At low salinity (less than 50 g/L) cyanophyta might be dominant over chlorophyta and bacillariophyta. The algae composition will affect growth, reproduction and nutritional value of *Artemia* biomass and cysts. For example the content of the highly unsaturated fatty acids EPA and DHA in *Artemia* cysts is caused by their presence in the diet of the adult *Artemia*. Only few algal species are a suitable food for *Artemia* as the largest size of particles that can be ingested by *Artemia* is about 20 µm for the nauplii and 50 µm for the adults. Suitable algal species for *Artemia* are *Tetraselmis, Dunaliella, Chaetoceros, Navicula, Nitzschia* and *Thalassiosira* (Figure 37).


Figure 39: Algal species food of *Artemia*

The source of algae in the ponds are (i) algae produced in the green-water ponds and transferred from there to the *Artemia* ponds, (ii) algae production in the *Artemia* ponds through fertilization. Advantages and disadvantages of both methods are given in Table 7.

Table 7: Advantages and disadvantages of algae production in green-water ponds and in *Artemia* ponds

| | Advantages | Disadvantages |
|---|---|---|
| Green-water ponds | • Low salinity (<60 g/L) is suitable for algae development<br>• Combination of organic and inorganic fertilizer may limit nutrient accumulation in ponds<br>• Algae water can continuously be supplied to *Artemia* ponds through fertilized pond filled with new water<br>• To sustain and renew the water in *Artemia* ponds an thus to stimulate to growth of *Artemia* population | • Naturally produced algae are mixed with various species. It is difficult to induce suitable species for *Artemia*.<br>• These ponds maintained at low salinity could be source of predators and competitors for *Artemia* ponds.<br>• The ponds are affected by environmental factors such as temperature and salinity that cause variation in algae composition, production of unsuitable algae (example filamentous algae)<br>• Occupies culture area thus reduces *Artemia* production area. |
| *Artemia* ponds | • Expand *Artemia* culture area<br>• Reduces predators and competitors invasion | • Increases accumulation of organic substances in *Artemia* ponds<br>• Fertilization is less effective in *Artemia* ponds as only few algae can grow well at high salinity |

## 9.1 Algae production in fertilized pond

Both inorganic and organic fertilizers can be applied in green-water ponds for algae growth. In Vinh Chau salt field, Vietnam green water is usually pumped into the *Artemia* ponds every 2 days, 1-3 cm each time corresponding to10-15% of *Artemia* pond water volume depending on feed demand and water salinity.

### 9.1.1 Inorganic fertilization

#### 9.1.1.1 Nitrogenous fertilizer

Requirement of nitrogenous fertilizer to enhance algae production vary with geographical location, composition of natural algae population (Table 8).

Table 8: List of nitrogenous fertilizers, recommended dosages and effect in pond water

| Nitrogenous fertilizers | % of nitrogen (N) | Effect on pond water | Application dosages (mg/L) |
|---|---|---|---|
| Ammonium sulphate $(NH_4)_2SO_4$ | 20 | Reduces buffering capacity and stimulates precipitation of phosphate and sulphate | 2.5 |
| Calcium nitrate $Ca(NO_3)_2$ | 15-16 | Increases pH | 3.0 |
| Urea $CH_4N_2O$ | 46 | Reduces temperature, dissolves easily | 1.0 |

#### 9.1.1.2 Phosphate fertilizer

Organic matter and decomposed bacteria are natural sources of phosphate. Phosphate disappears from water quickly as it precipitates in salt water and absorbs in pond bottom. Therefore, it is suggested to apply phosphate fertilizer in low dose and high frequency (usually twice per week). The combination of phosphate and nitrogenous fertilizer determine the growth of algae. Recommended ratio of nitrogenous over phosphate fertilizer (Table 9) is 3-5:1.

Table 9: List of phosphate fertilizers, recommended dosages and effect on pond water

| Phosphate fertilizers | % of phosphate ($P_2O_5$) | Effect on pond water |
|---|---|---|
| Superphosphate $Ca(H_2PO_4)_2.H_2O$ | 16-20 | High solubility |
| Dicalcium phosphate $CaHPO_4.\ 2H_2O$ | 35-48 | Low solubility |
| Triple superphosphate $Ca(H_2PO_4)H_2O$ | 42-48 | Good solubility |
| Sodium polyphosphate $Na_5P_3O_{10}$ | 46 | Liquid |
| Phosphoric acid $H_3PO_4$ | 54 | Liquid |

Excessive application of phosphate fertilizers at temperature more than 28°C with high transparency that allows light to penetrate pond bottom will stimulate growth of bottom algae (cyanophyta). High phosphate content at low salinity stimulate the development of filamentous algae (Lynbya, Oscillatoria). Both algae types are too large for *Artemia*.

### Cautions in application of inorganic fertilizer

- Dissolve in freshwater to ensure solubility then distribute evenly across the pond. It is applicable for liquid fertilizer as well.
- Do not fertilize in a cloudy or less sunshine day. Because algae growth is limited at low light intensity.
- Apply fertilizer in low salinity ponds (less than 60 g/L).
- Stable conditions in terms of salinity, water level, turbidity is required to optimize algae growth.
- Do not apply fertilizer in *Artemia* ponds after stocking. Algae growth is limited at high salinity.
- Daily application is recommended to enhance the algae growth.

### Determine the requirement of inorganic fertilizer

- Calculate the amount of fertilizer required considering type of fertilizer, chemical composition.
- Adjust the application dose and frequency (1-2 per week) considering appearance of algae and maintain water transparency in the range of 30-40 cm.
- Regular water supply to increase $CO_2$ content in water.
- The factors affecting primary production of algae such as salinity, sunlight, water colour and transparency, source of algae need to be considered for fertilization.

## 9.1.2 Organic fertilizer

Poultry waste, quail and duck waste can be applied for *Artemia* culture. Cowdung, goat and sheep waste may not be preferable considering the risk of filamentous algae growth.

The recommended application dosages of organic fertilizer is 0.5 to 1.25 metric tons (MT) per ha in the beginning and then 100-200 kg/ha in every 2-3 days. Organic fertilization stimulates the growth of bacteria and bioflocs which is a good food item for *Artemia*. Quality of organic fertilizer varies with food consumed by cattle, poultry and storage condition. Excessive application of organic fertilization might cause oxygen depletion, stimulate the growth of blue green algae, and lab-lab.

### 9.1.3 Combination of organic and inorganic fertilizer

Combined application of organic and inorganic fertilizer stimulates the development of algae and mineralization of organic fertlizers (lower C:N ratio). Usally inorganic fertilizers are applied in the green-water pond or inlet canal, while organic fertilizers can be applied directly into the *Artemia* pond.

## 9.2 Algae production in *Artemia* pond

Fertlization in *Artemia* pond can stimulate the production of algae. The growth of algae at high salinity is limited by several factors affecting chemical properties of fertilizer, algae development and the composition of algae species present naturally. The factors that affect the chemical properties of the fertilizers are ionic composition of seawater, pH, pond bottom substrate. The development of the algae depends on temperature, salinity, light and transparency.

## 9.3 Constraints with algae in *Artemia* culture

The most common constraints in *Artemia* pond is the presence of bottom algae (lab-lab) and filamentous algae. These algae are unsuitable for *Artemia* culture. Water transparency and water level can be useful to control these algae. Daily mixing by raking is helpful to limit bottom algae production and release nutrients in the water column.

The best way to prevent these problems is to operate the green-water and the *Artemia* ponds at a water depth of 50 cm or more.

# 10.0 Processed feed and supplementary feeding

As *Artemia* is a non-selective filter-feeder, different other products than micro-algae can be used to feed *Artemia*: dried algae, yeast, as well as different agricultural by-products such as ricebran, wheatbran and soybean by-products, provided they can be grinded or sieved until particle size is below 50 µm.

The problem with ricebran and other agricultural by-products is that they contain undigestable matter (for example fibers) that will accumulate on the pond bottom as waste.

In recent years it has been well documented that bacteria and especially bioflocs (accumulations of live and dead organic matter) are an important source of food for *Artemia*.

Biofloc production is enhanced at C: N ratios over 10:1. The following cheap carbon sources are successfully used in *Artemia* culture: molasses, wheat flour, ami-ami (by-product of the monosodium glutamate fermentation).

Application of biofloc technology furthermore enhances the water quality as it converts ammonium and nitrogenous by-products/waste into bacterial biomass containing highly digestible organic matter (35-50% proteins) as well as different bio-active compounds (vitamins, enzymes, immunostimulants, etc.) of benefit for *Artemia* development and health.

Another advantage of the use of bioflocs in *Artemia* pond culture is that they can also be produced at high salinities (contrary to the problem with algae production).

Regular stirring of the green-water ponds and raking of the *Artemia* ponds will prevent settling of biofloc materials and make these better available for filtration by *Artemia*.

# 11.0 Common diseases of *Artemia* and treatments

Occasionally both infectious and non-infectious diseases can cause high mortality of *Artemia* in culture tanks or ponds. The known diseases are caused by *Leucothrix sp*., black spot disease, long faeces trails and white abdomen disease.

It is also important to note that *Artemia*, as a filter-feeder of bacteria, can be vector of *Vibrio* spp occurring in the culture tank or pond and infect the shrimp or fish they are fed to in aquaculture systems (hatchery, nursery, maturation facilities).

## 11.1 Leucothrix sp.

A filamentous flagella bacterium attaches to the appendages of *Artemia*. This usually occurs with very high organic loads in tank cultures at seawater salinities. *Leucothrix* colonies fixe on the exoskeleton, visible from the instar V/VI-stage onwards. The brine shrimp suffer physically, as the movements of their thoracopods become affected and filtration rates consequently are reduced. Eventually, growth and molting are arrested, overfeeding of the tanks occurs, resulting in collapse of the *Artemia* culture. Increasing the salinity to 60 g/L with regular exchanges of water can reduce the presence of *Leucothrix sp*.

## 11.2 Black spot disease

Presence of black spot (necrosis) on the appendages of *Artemia*. This disease consists of the detachment of the epidermis from the cuticula, and is caused by a dietary deficiency which interferes with lipid metabolism (Figure 38). In high density culturing of *Artemia* using agricultural by- products as a food source, the black disease is observed when water quality deteriorates (probably interfering with the composition of the bacterial population and consequently the diet composition) and/or when feeding rates are not optimal. Improving these conditions does not save the affected animals but appears to avoid further losses. Ensure quality feed (algae and supplementary diet) is an useful treatment.

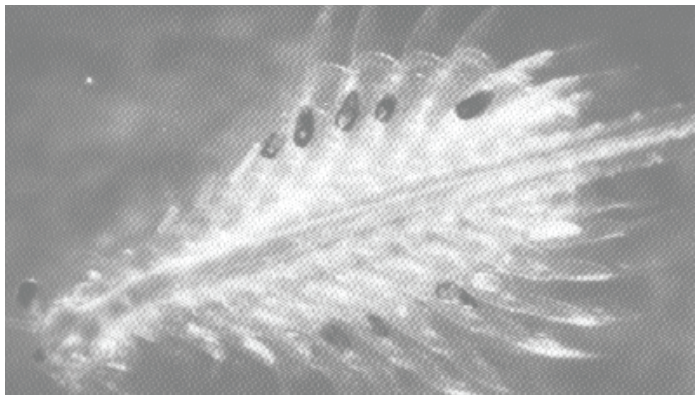

Figure 40: Black spot disease in thoracopods of *Artemia*

## 11.3 Long faeces trails

Presence of long fecal threads in *Artemia* (Figure 39). It is often occurring when the green-pond water contains high concentrations of diatoms. Supply water containing more suitable algal species, or add more supplementary diet are good treatments to overcome this disease.

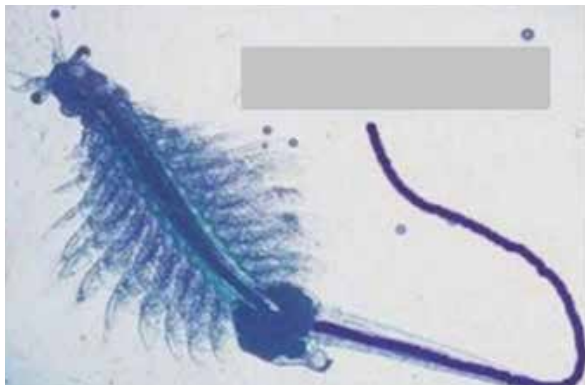

Figure 41: Long faeces trails

## 11.4 White abdomen disease

The digestive tract in the *Artemia* abdomen is opaque as can be seen with the naked eye and under microscope the digestive tract appears empty (Figure 40). The disease might cause due to unsuitable or non-availability of sufficient food item. The *Artemia* population suffer mass mortality within few days of the symptom. Supply green water and partial exchange of water are useful to control the disease.

Figure 42: *Artemia* juvenile affected with white abdomen disease

# 12.0 Harvesting and processing *Artemia* biomass and cysts

## 12.1 Harvesting *Artemia* biomass

2 to 3 weeks post stocking, *Artemia* reach adult stage and females start releasing nauplii, and as a result population densities increase and one can consider partial harvesting of biomass (adult and sub adult *Artemia*). One mm mesh size net is suitable to harvest adult *Artemia*. There is a positive correlation between biomass yield and population density at the time of harvest. Partial harvesting results in a decrease in animal densities and proportionally more food becomes available for the remaining animals to grow and reproduce. In Vietnam salt farmers harvest about 1.5 MT per ha in three months' time with harvesting once every three days (Figure 41).

Harvested biomass needs to be cleaned from particulate impurities by thorough washing over 1 mm mesh nets (Figure 42) ensuring that the biomass is always submerged to minimize physical damage of the animals.

Once cleaned from impurities the biomass can be stored alive in nets (Figure 42) or tanks (Figure 43) at the same or lower salinity as in the production ponds or further treated for different applications:

- depuration from bacteria in recirculation system with protein skimmer (Figure 44) prior to use for live feeding to fish or shrimp in nursery and maturation systems
- live packaging in plastic bags (under air pressure or eventually filled with pure oxygen gas) and stored during transport in coolbox (Figure 45)
- washed in freshwater prior to transfer of biomass paste for freezing in bags exposed in thin layers of maximum 5 mm for quick freezing at -18°C (Figure 46)
- drying in thin layers in oven or in solar oven dryer
- using as fresh ingredient in food recipes (*Artemia* omelet, *Artemia* as partial substitute in fish/shrimp/crab cakes; Figure 47)

Figure 43: Harvesting *Artemia* biomass

Figure 44: Washing of harvested *Artemia* biomass

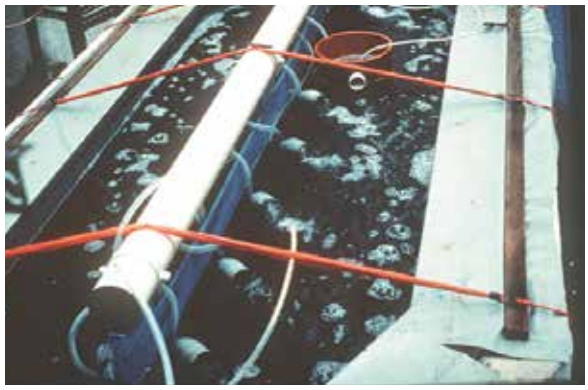

Figure 45: *Artemia* biomass stored in tanks with air-water-lift aeration

Figure 46: *Artemia* biomass depuration in recirculation system with protein skimmer

Figure 47: Live packaging of *Artemia* biomass

Figure 48: Freezing of *Artemia* biomass in bags

Figure 49: *Artemia* omlet as human food

## 12.2 Harvesting of *Artemia* cysts

First cysts appear floating at the water surface 2 to 3 weeks post stocking (Figure 48). Heavy winds (especially in the afternoon hours) create waves resulting in foam formation in which the cysts are trapped and lost when getting airborne. Simple wave breakers should be installed on the downwind side of the ponds, behind which the cysts can accumulate for regular harvesting, up to several times per day (Figure 48).

## 12.3 Primary processing of cysts

Cysts are harvested with a double-screen net: at the top a 1 mm mesh to retain adult biomass and large debris, and underneath a 120 µm net to retain cysts and other fine debris (Figure 49 and 50). At the harvest side further rinse and clean the cysts in the 120 µm net using pond water, squeeze out water and transfer the cyst paste to the cyst storage tank set up in a locked room close to the *Artemia* ponds. The cysts storage tank is filled with saturated brine (300 g/L crude salt) and covered with a lid. When adding harvested cysts (one or more times a day) ensure good mixing of the cysts in the brine tank to facilitate homogenous dehydration of all cysts. Also check the salinity (or presence of salt crystals at the bottom of the tank) as more salt has to be added with the daily addition of (partially hydrated) cysts.

Figure 50: Floating cysts on pond surface (left) and harvesting cysts with scoop net (right)

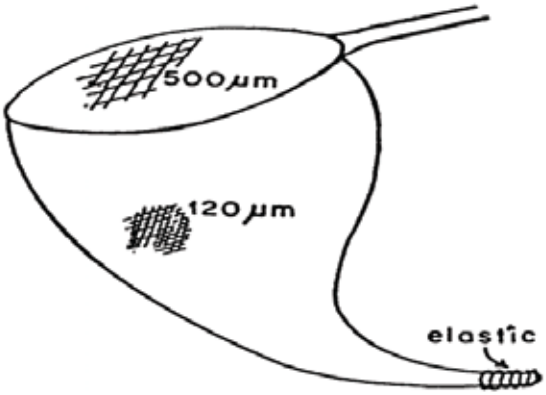


Figure 51: Double screen dip net to harvest *Artemia* cysts

Figure 52: Rinsing cysts

## 12.4 *Artemia* cyst processing and preservation

Once cleaned cysts can be stored under brine-dehydrated conditions for several months and this will be the preferential (and cheapest) method for initial use in shrimp/prawn/fish hatcheries in Bangladesh. Upon arrival at the processing facility brine dehydrated cyst paste will be processed as follows:

- washing and removing of heavy (sedimenting) impurities in brine water
- washing and removing of light (floating) impurities in cold freshwater (using ice packs to minimize cyst hydration)
- removing water in centrifuge
- transfer of cyst paste in ice-cold saturated brine for dehydration; every other day for 3 consecutive times change the saturated brine and make sure to maintain saturated brine conditions
- after that time (or later) harvest cysts over 120 µm filter and mix 1 kg batches of wet-dry cysts with crude salt and pack in closed containers - store in cold room

For long term storage and eventual export cysts can be dried through the following steps (Figure 51):

- washing and removing of heavy (sedimenting) impurities in brine water
- storage in saturated salt water
- washing and removing of light (floating) impurities in cold freshwater
- removing water in centrifuge
- drying of cysts in fluidized bed dryer
- packing of cysts in air-tight bags or containers

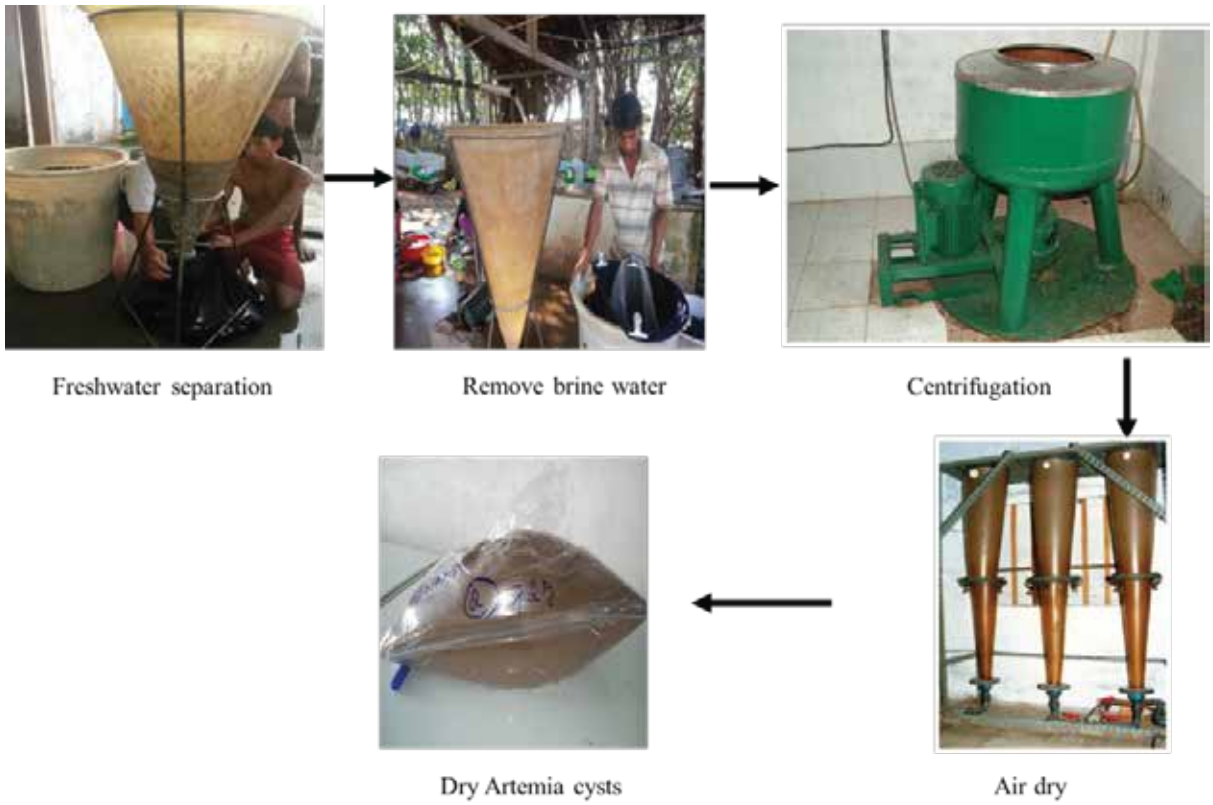


Figure 53: Flow diagram of cyst processing and preservation

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# 14.0 Appendixes

## Appendix 1: Prospects of artisanal solar salt farms in Cox's Bazar, Bangladesh

### Summary

Aquaculture, fisheries and crude salt production are the major sources of income of the population in Cox's Bazar district. The aquaculture and fisheries activities include extensive salt cum fish farms, shrimp hatcheries, Tilapia hatcheries, intensive shrimp/fish farms, small scale artisanal marine fisheries (e.g. Hilsa fisheries), crab farming and dry fish production. At present, the aquaculture and fisheries production of the area meet the demand of the district, supply across the country and contribute in foreign exchange earnings.

The major constraints of the area are low productivity in coastal aquaculture, low profitability in crude salt production and vulnerability of all sectors due to climate induced hazards. The low productivity of coastal aquaculture could be explained by the practice of traditional farming systems, limited availability and low quality of seed, lack of technological improvement in good aquaculture practices, diseases, inefficient coordination within the value chain (e.g. hatchery and grow out), difficulty in organizing small scale aquaculture farmers, limited availability and high price of quality inputs from the international market e.g. *Artemia* cysts, hatchery diets. Many countries have been successful in adopting new technologies for the production of *Artemia* (cysts and biomass), and for the integration of salt and aquaculture production. Similar technological improvements are necessary to upgrade the livelihoods of the thousands of salt farmers in Bangladesh. Climate induced hazards caused by surface warming, sea-level rise, extreme hot and heat waves, floods, cyclone and storm surges, all threaten livelihoods of the local people.

WorldFish has been implementing an EU financed project "Artemia4Bangladesh" to address the constraints mentioned above. The specific objectives of the project are to (i) Introduce an integrated salt and *Artemia* production system and (ii) Increase marine aquaculture productivity and production in the salt farms in Cox's Bazar district.

The expected results of the project (i) *Artemia* and salt integrated production systems will be proven feasible in the Bangladesh context, (ii) *Artemia* and salt integrated production systems will be effectively established and widespread among the salt farmers, (iii) Increased marine aquaculture productivity and production due to low cost of locally produced *Artemia* cyst, (iv) Increased revenue of salt farmers due to adoption of a second profitable activity (e.g. shrimp, fish, crab aquaculture) and (iv) Enhanced saline tolerant species seed production due to adoption of improved technologies.

The results of the project will economically strengthen and stabilize the salt farmers' income throughout the year, reduce climate-induced threats, increase food security and nutrition by improving the ecological sustainability of the marine aquaculture sector in Bangladesh.

## 1. Background and justification

Bangladesh is one of the most densely populated and climate vulnerable countries in the world. The Cox's Bazar District, located on the south-east coastal zone of Bangladesh, is one of the least developed and most vulnerable regions in the country. Its situation is aggravated by the Rohingya refugee crisis which, since August 2017, has resulted in migration of more than 700,000 refugees from Myanmar (Tay et al. 2018) and keeping in mind that already before August 2017 an estimated 500,000 refugees were already present in the region. This crisis has placed enormous extra pressure on the land, local communities and the local and national Government, resulting in increased price inflation, unemployment and higher environmental degradation.

Cox's Bazar area has played a significant role in the Bangladesh economy for aquaculture, fisheries, crude salt production and tourism. But, the area is additionally threatened by the above mentioned pressures. WorldFish is implementing the EU financed project "Artemia4Bangladesh" for technological improvements in integrating *Artemia* production and aquaculture in solar salt farms in Cox's Bazar district.

## 2. Importance of aquaculture and fisheries

Aquaculture and fisheries production in Cox's Bazar district meet the demand of the area, but also supply across the country, contribute in foreign exchange earnings and provide livelihood of millions of people. The major activities of the area are capture marine (e.g. hilsa) and inland fisheries, shrimp hatcheries, Tilapia hatcheries, extensive salt-cum-fish farms, intensive shrimp and fish farms, crab farms and dry fish production (Table 10). In recent years, two shrimp hatcheries in Cox's Bazar have been producing specific pathogen free (SPF) *Penaeus monodon* post larvae (Figure 52).

Table 10: A summary of aquaculture and fisheries resources of Cox's Bazar district

| Aquaculture and Fisheries resources | |
|---|---|
| Extensive shrimp/fish production area | 44,457 ha |
| Number of (#) shrimp hatcheries | 48 |
| # Tilapia hatcheries | 10 |
| # Crab hatcheries | 2 |
| #Tilapia farm | 718 (206 ha) |
| # Intensive shrimp farms | 12 (200 ha) |
| # Crab farms | 146 |
| # Shrimp post larvae production per year | > 10 billions |
| Shrimp production | 16,400 MT |
| Hilsa fisheries production | 15, 256 MT |
| Crab production | 632 MT |
| Dry fish production | 25,178 MT |
| Total fish production | 245, 894 MT |
| # Registered fishermen | 48, 393 |
| # Shrimp/fish farmer | 40,000 |

Figure 54: MKA SPF *P. monodon* hatchery in Cox's Bazar

### 3. Crude salt production

The salt industry is the largest labor oriented cottage industry in the country. The salt farming is a seasonal activity occurring during winter and pre-monsoon months between November to May. The entire practice is dependent on favorable climatic conditions such as limited rainfall, sunny days, high temperature and evaporation rates. The practice is already becoming affected by climatic changes such as pre-monsoon rainfall anomalies, cyclonic surges, tidal inundations, floods, cold weather conditions. These may lead to shorten the salt production season, reduce productivity, income and employment. The climate variability (i.e. high temperatures, cold wave, low/ heavy rainfall) is affecting the aquaculture operations and management decisions. These climatic variabilities need to be assessed for Cox's Bazar to manage for climate induced risks in aquaculture and identify potential climate sensitive management decisions.

95% of the 1.8 million MT crude salt produced per year in Bangladesh is produced in Cox's Bazar area. The crude salt is produced in about 27,000 ha of land operated by 50,000 artisanal salt farmers, providing livelihood to approximately 1.5 million poor people. Land ownerships of salt farms varies into owned, leased and shared land. More than 50% farmers lease the land for salt production, but 90% do not have access to formal credit flow. On an average, 60% of farmer's annual income comes from salt production (Hossain 2018).

The profitability in salt farming varies with different factors, e.g. crude salt price, as well as costs of labor, land lease, loans, polythene, fuel and pump, sluice gate operation and use of insecticides. The low profitability, labour dependency, climatic risk involvement, limited options for livelihood improvement all cause economic vulnerability of the salt farmers.

## 4. Importance of *Artemia* cyst and biomass production

Brine shrimp *Artemia* is a primitive crustacean with a segmented body to which broad leaf-like appendages that greatly increase the apparent size of the animal. The total length is usually about 8-10 mm for the adult male and 10-12 mm for the female. The body is divided into head, thorax, and abdomen. The genus, *Artemia*, consists of bisexual and parthenogenetic strains. Females of most *Artemia* strains reproduce ovoviviparously and oviparously, releasing either nauplius larvae or encysted embryos, respectively. Reproductive mode may switch, in good rearing conditions *Artemia* tend to reproduce ovoviviparously, whereas under adverse situations they reproduce oviparously (Figure 53).

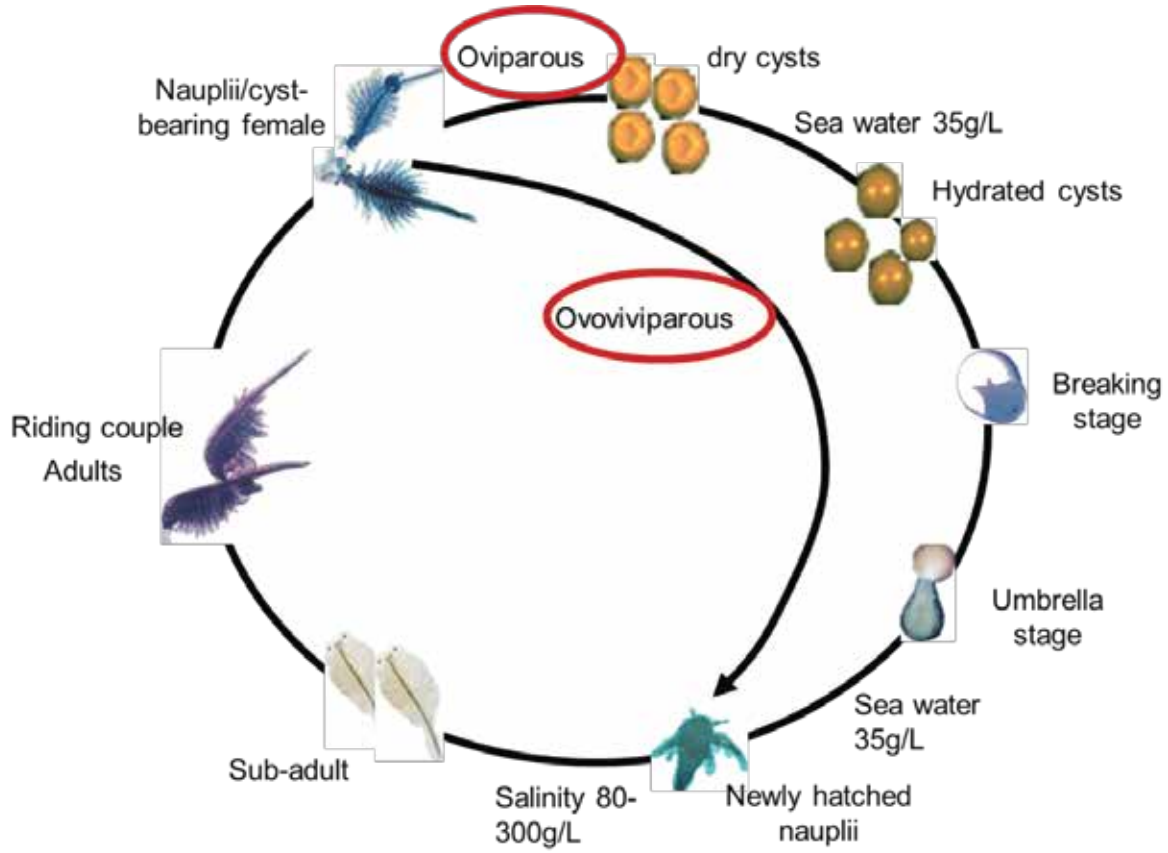


Figure 55: Life cycle of *Artemia*

*Artemia* nauplii constitute the most widely used live-food item for the larviculture of different species of crustaceans and marine fish. Annually, about 3500 MT of *Artemia* cysts are marketed worldwide. Indeed, the unique property of the small branchiopod crustacean *Artemia* to form dormant embryos, so-called 'cysts', accounts to a great extent to the designation of a convenient, suitable, and excellent larval food source that it has been credited with . The cysts are available year-round in large quantities along the shorelines of hypersaline lakes, coastal lagoons and solar salt works scattered over the four continents. After harvesting and processing, cysts are canned for storage and after some 24 h incubation in

seawater the cysts release free-swimming nauplii. These are mostly utilized as a nutritious live-food source for the larvae of crustacean (Figure 54) and marine fish hatcheries (Lavens and Sorgeloos, 1996). At present, Bangladesh imports 40-50 MT dry *Artemia* cysts annually worth an approximate value of USD 4 million (Meezanur Rahman, sourced at hatcheries).

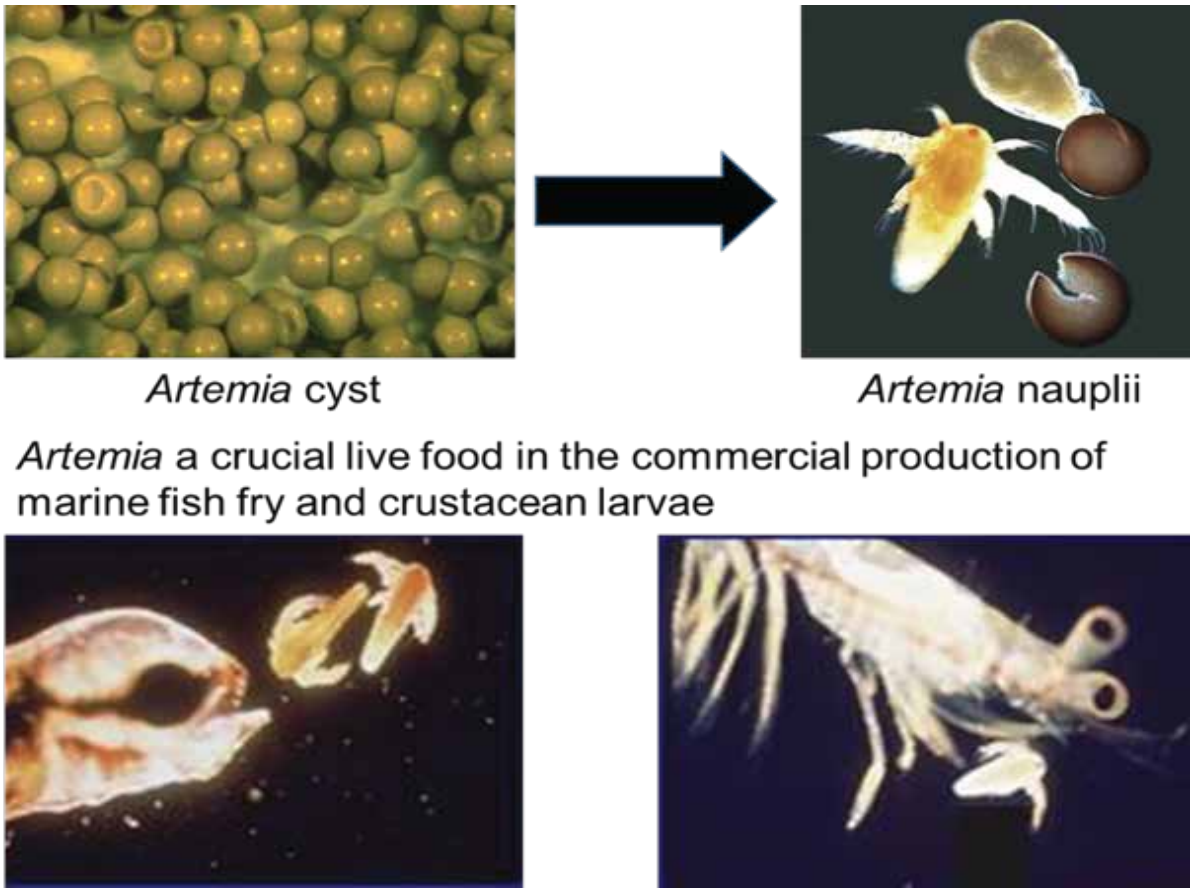


Figure 56: *Artemia* nauplii as live food for shrimp larvae and fish fry

**5. Integrated *Artemia*- Salt-Aquaculture**

Several South Asian countries such as Vietnam have been successful in adopting new technologies for the production of *Artemia* (cysts and biomass), integration of salt and aquaculture production, and alternative use of concentrated sea water to increase income and profitability of the salt farmers. Similarly new projects have been launched in Bangladesh, Cambodia and Myanmar. The technological improvements are necessary to upgrade the livelihoods of the thousands of salt farmers in Bangladesh. Principles of *Artemia* culture in solar salt ponds have been developed following the behaviour of *Artemia* in natural habitats as showed in Figure 17.

At the end of the salt production season, the majority (75%) of salt farmers in Bangladesh transition their land for aquaculture purposes. However, as mentioned the aquaculture production and productivity are very low due to the practice of traditional farming systems.

Recent field visit observations and workshop findings suggest that Bangladeshi salt/aquaculture farmers are unaware of the opportunities with *Artemia* farming, and the consequent technological improvements for saline tolerant crustacean and fish production in the salt farms during the rainy season (Figure 55). Interventions would include improving bio security, compliance of better management practices including stocking of pathogen free seed, nursery rearing, periodic sampling, application of inputs (lime, inorganic fertilizer, feeds), increase water depth, reduce stocking frequency, poly culture; all these are required to improve productivity, profitability and reduce disease incidence (Rahman et al. 2018).

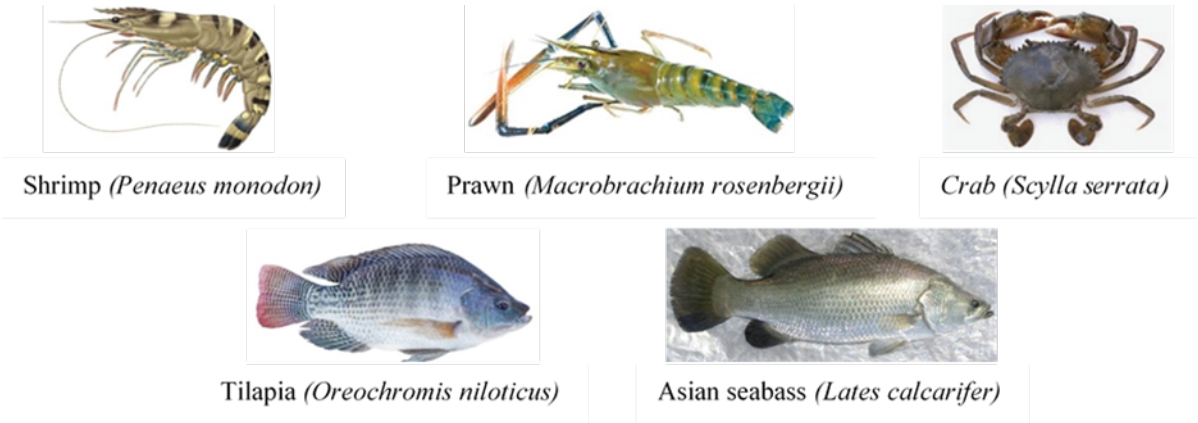


Figure 57: Saline tolerant aquaculture species suitable for culture in salt farms

## 6. Conclusion

The livelihood of the population in Cox's Bazar district has been affected due to climate induced risks, low productivity in coastal aquaculture, low profitability of salt production and rohingya refugee influx in the area. Many countries adopted technologies for *Artemia* cyst and biomass production in the salt farms. Availability of seed, improvement of seed quality, better management practices all can improve productivity and profitability of costal aquaculture in this the area. Artemia4Bangladesh plans to improve the livelihood of salt cum fish farmers through integrated salt, *Artemia* and aquaculture production in the salt farms of the Cox's Bazar district.

## Appendix 2: Standard hatching procedure of Artemia

### 1. Hatching conditions and equipment

Although hatching of small quantities of *Artemia* cysts is basically very simple, several parameters need to be taken into consideration for the successful hatching of large (i.e. kilogram) quantities of cysts, which is a common daily practice within large hatcheries:

- aeration
- temperature
- salinity
- pH
- cyst density
- illumination

For routine operation, it is most efficient to work in standardized conditions (i.e. heaters with thermostat or climatized room to ensure constant temperature) to allow maximal production of a homogeneous instar I population after a fixed incubation time. Instar I nauplii are most nutritious and can withstand sudden salinity shocks when transferred into the salt ponds for inoculation purposes. When nauplii molt into instar II stage their mouth is open and they start ingesting bacteria that are present in the hatching medium and thus can become carriers of opportunistic Vibrio pathogens.

Best hatching results are achieved in cylindroconical containers, aerated from the bottom with aeration tubes. 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.

As a consequence of specific characteristics, the interactions of the hatching parameters might be slightly different from strain to strain, resulting in variable hatching results. The aeration intensity must be sufficient to maintain oxygen levels above 2 mg/L, preferentially 5 mg/L. 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 prior to hatching incubation and/or by the addition of a few drops of a non-toxic antifoaming agent (e.g. silicone antifoam).

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

The quantitative effects of the incubation salinity on cyst hatching are in the first place related with the hydration level that can be reached in the cysts. Above a threshold salinity, insufficient quantities of water can be taken up by the cysts; this threshold value varies from strain to strain, but is approximately 85-90 g/L for most *Artemia* strains. In the second place, 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 point. Optimal hatching can be obtained in the range 5-35 g/L. For reasons of practical convenience natural seawater is mostly used to hatch cysts. However, at 5 g/L salinity, the nauplii hatch faster, as less glycerol has to be built up. For some sources of cysts hatching the cysts at low salinity results in higher hatching efficiencies, and the nauplii have a higher energy content (Table 11). The salinity can easily be measured by means of a refractometer.

Table 11: Effect of incubation at low salinity on hatching percentage, individual nauplius weight, and hatching output for *Artemia* cyst sources from different geographical origin

| | Hatching percentage (%) | | |
|---|---|---|---|
| Cyst source | 35 g/L | 5 g/L | % difference |
| San Francisco Bay, CA-USA | 71 | 68 | -4.8 |
| Macau, Brazil | 82 | 86.4 | +5.3 |
| Great Salt Lake, UT-USA | 43.9 | 45.3 | +3.1 |
| Shark Bay, Australia | 87.5 | 85.8 | -1.9 |
| Chaplin Lake, Canada | 19.5 | 52.2 | +167.6 |
| Bohai Bay, PR China | 73.5 | 75 | +2.0 |
| | Naupliar dry weight (µg) | | |
| San Francisco Bay, CA-USA | 1.63 | 1.73 | +6.1 |
| Macau, Brazil | 1.74 | 1.76 | +1.1 |
| Great Salt Lake, UT-USA | 2.42 | 2.35 | -2.5 |
| Shark Bay, Australia | 2.47 | 2.64 | +6.9 |
| Chaplin Lake, Canada | 2.04 | 2.28 | +11.8 |
| Bohai Bay, PR China | 3.09 | 3.07 | -0.6 |
| | hatching output (mg nauplii/g cysts) | | |
| San Francisco Bay, CA-USA | 435.5 | 440.2 | +1.1 |
| Macau, Brazil | 529.0 | 563.7 | +6.6 |
| Great Salt Lake, UT-USA | 256.5 | 257.0 | +0.2 |
| Shark Bay, Australia | 537.5 | 563.3 | +4.8 |
| Chaplin Lake, Canada | 133.8 | 400.4 | +199.3 |
| Bohai Bay, PR China | 400.5 | 406.0 | +1.4 |

The pH must remain above 8 during the hatching process so as to ensure optimal functioning of the hatching enzyme. If needed, (i.e. when low salinity water is used), the buffer capacity of the water should be increased by adding up to 1 g $NaHCO_3$/L. Increased buffer capacities can also become essential when high densities of cysts are hatched (= high $CO_2$ production).

Cyst density may also interfere with the other abiotic factors that are essential for hatching, such as pH, oxygen, and illumination. The density may be as high as 5 g/L for small volumes (<20 L) but should be decreased to maximum 2 g/L for larger volumes, so as to minimize the mechanical injury of the nauplii and to avoid suboptimal water conditions.

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

## 2. Hatching quality and evaluation

An acceptable cyst product should contain minimal quantities of impurities, such as sand, cracked shells, feathers, and salt crystals, etc. Hatching synchrony must be high; when incubated in 33 g/L seawater at 25°C, the first nauplii should appear after 12 to 16 h incubation (T0; see further) and the last nauplii should have hatched within 8 h thereafter (T100). When hatching synchrony is low (T100-T0 > 10 h), first-hatched nauplii will have consumed much of their energy reserves by the time that the last nauplii will have hatched and harvesting is completed. Moreover, since the total incubation period exceeds 24 h the aquaculturist will not be able to restock the same hatching containers for the next day's harvest, which in turn implies higher infrastructural costs. The hatching efficiency (the number of nauplii hatched per gram of cysts) and hatching percentage (the total percentage of the cysts that actually hatch) often varies considerably between different commercial batches and obviously account for much of the price differences. In this respect, hatching efficiency may be a better criterion than hatching percentage as it also takes into account the content of impurities (i.e.. empty cyst shells). Hatching values may be as low as 100,000 nauplii/g of commercial cyst product, while premium quality cysts can yiels up to 300,000 nauplii per gram of cysts (with an equivalent hatching percentage of >90 %); batches of small (=lighter) cysts (e.g. Vinh Chau, Vietnam *Artemia franciscana* type) may yield even higher numbers of nauplii, (i.e. 320,000 nauplii/g cysts).

To evaluate the hatching quality of a cyst product, the following criteria are being used (see worksheet 6 for practical examples):

- hatching percentage:

= number of nauplii that can be produced under standard hatching conditions from 100 full cysts; this criterion does not take into account cyst impurities, (i.e. cracked shells, sand, salt, etc.), and refers only to the hatching capacity of the full cysts, which in turn depends upon:

  a) degree of diapause termination: cysts that are still in diapause do not hatch, even under favourable hatching conditions
  b) energy content of cysts: may be too low to build up sufficient levels of glycerol to enable breaking and hatching, as a consequence of, for example, improper processing and/or storage, environmental or genotypical conditions affecting parental generation...
  c) amount of dead/non-viable/aborted embryos, due to improper processing and/or storage.

- hatching efficiency:

= number of nauplii that can be produced from 1 g dry cyst product under standard hatching conditions. This criterion reflects:

  a) the hatching percentage (see above)
  b) the presence of other components apart from full cysts in the cyst product (i.e. empty shells, salt, sand, water content of the cysts)
  c) the individual cyst weight (i.e. more cysts/g for smaller strains)

As this criterion can refer to the ready-to -use commercial product, it has very practical implications, since the price of the product can be directly related to its output.

- **hatching rate:**
  this criterion refers to the time period for full hatching from the start of incubation (= hydration of cysts) until nauplius release (hatching), and considers a number of time intervals, including:

  T0 = incubation time until appearance of first free-swimming nauplii
  T10= incubation time until appearance of 10% of total hatchable nauplii, etc. (Figure 56).

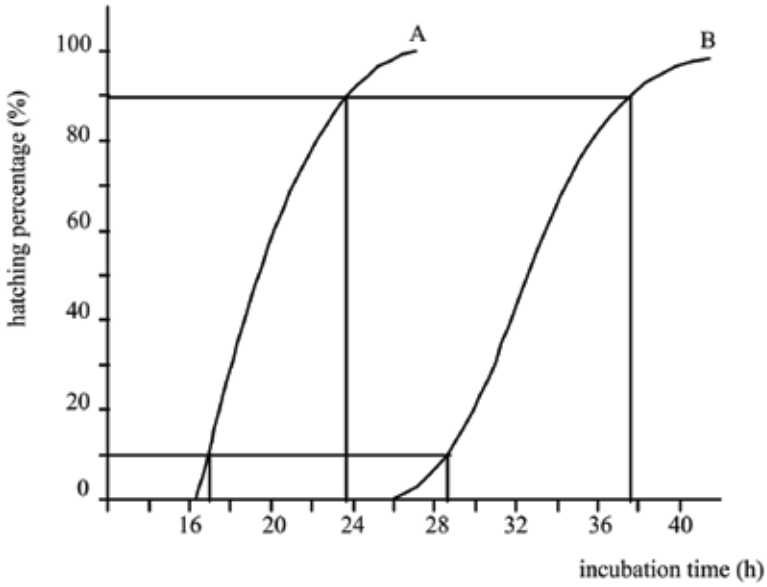


Figure 58: Hatching rate curves from different cyst batches. Curve A: T10= 17 h, T90 = 23.5 h, Ts = 6.5 h; Curve B: T10 = 28.5 h, T90 = 37.5 h, Ts = 9 h.

Data on the hatching rate allow the calculation of the optimal incubation period so as to harvest nauplii containing the highest energy content (Figure 57). It is important that the T90 is reached within 24 h; if not more hatching tanks will be needed so as to ensure a daily supply of a maximal number of instar I nauplii.


Instar I


Instar II


Figure 59: Changes in nutritional content of *Artemia* nauplii molt from Instar I to Instar II

- **hatching synchrony:**

= time lapse during which most nauplii hatch, i.e. Ts = T90-T10

A high hatching synchrony ensures a maximal number of instar I nauplii available within a short time span; in case of poor synchrony the same hatching tank needs to be harvested several times in order to avoid a mixed instar I-II-III population when harvesting at T90.

- **hatching output:**

= dry weight biomass of nauplii that can be produced from 1 g dry cyst product incubated under standard hatching conditions; best products yield about 600 mg nauplii/g cysts. The calculation is made as follows:

= hatching efficiency x individual dry weight of instar I nauplius.

The hatching efficiency only accounts for the number of nauplii that are produced, and not for the size of these nauplii (strain dependent); by contrast the hatching output criterion is related to the total amount of food available for the predator per gram of cyst product (cf. calculation of food conversion)

### 3. Harvesting of nauplii

After hatching and prior to feeding the nauplii to fish/crustacean larvae, they should be separated from the hatching wastes (empty cyst shells, unhatched cysts, debris, microorganisms 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 (Figure 58).

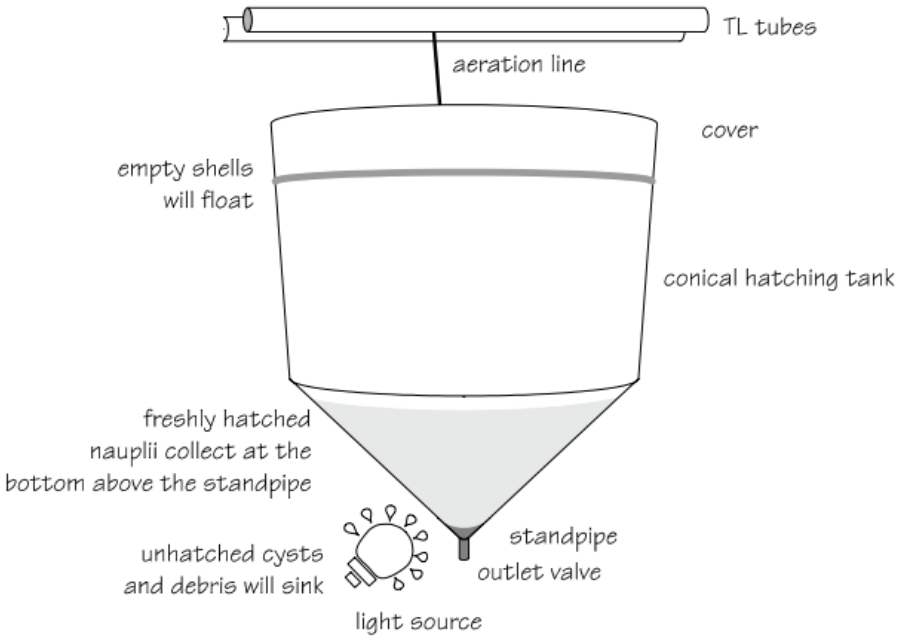


Figure 60: Hatching container at harvest

Since nauplii are positively phototactic, their concentration can be improved by shading the upper part of the hatching tank (use of cover) and focusing a light source on the transparent conical part of the bottom. Nauplii should not be allowed to settle for too long (i.e., maximum 5 to 10 minutes) in the point of the conical container, to prevent dying off due to oxygen depletion. Firstly, unhatched cysts and other debris that have accumulated underneath the nauplii are siphoned or drained off when necessary (i.e. when using cysts of a lower hatching quality). Then the nauplii are collected on a filter using a fine mesh screen (<150 µm), which should be submerged all the time so as to prevent physical damage of the nauplii. They are then rinsed thoroughly with water in order to remove possible contaminants and hatching metabolites like glycerol. Installation of automated systems simplify production techniques in commercial operations, (i.e. by the use of a concentrator/rinser equipped with a stainless steel cross-flow sieve; Figure 59) that enables fast harvesting of large volumes of *Artemia* nauplii and allows complete removal of debris from the hatching medium. This technique results in a significant reduction of labour and production costs.

Figure 61: Concentrator/rinser in use

As live food is suspected to be a source of bacterial infections eventually causing disease problems in larval rearing, microbial contamination should be kept to a minimum.

At the moment of cyst breaking glycerol is released in the hatching water. This carbohydrate is a suitable substrate for bacterial development, and as a result bacterial numbers, especially *Vibrio* spp. increase by $10^3$ to $10^5$ compared to the initial population before the breaking of the cysts.

When harvesting instar I nauplii this bacterial contamination can be removed by thorough washing, however, when the nauplii molt into instar II stage, they start ingesting bacteria and become carriers of the *Vibrio*.

Bacterial development during the hatching incubation can be suppressed by the addition of commercial disinfection products such as ACE (INVE Aquaculture, Thailand). Still one should aim to harvest *Artemia* in its most nutritious and biosecure stage as instar I nauplii. When stored at room temperature second-instar meta-nauplii have already consumed 25 to 30% of their energy reserves within 24 h after hatching (Figure 56). Moreover, instar II *Artemia* are less visible as they are transparent, are larger and swim faster than first instar larvae, and as a result consequently are less accessible as a 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 an increased *Artemia* cyst bill as about 20 to 30% more cysts will be needed to be hatched to feed the same weight of starved meta-nauplii to the predator.

When harvested instar I nauplii cannot be offered immediately as live food they should be stored at low temperature as to slow down their metabolic activity and consequest loss in nutritional value.

Molting of the *Artemia* nauplii to the second instar stage may be avoided and their energy metabolism greatly reduced (Figure 60) by storage of the freshly-hatched nauplii at a temperature below 10°C in densities of up to 8 million per liter. Only a slight aeration is needed in order to prevent the nauplii from accumulating at the bottom of the tank where they would suffocate. In this way nauplii can be stored for periods up to more than 24 h without significant mortalities and a reduction of energy of less than 5%. Applying 24 h cold storage using styrofoam insulated tanks and blue ice packs or ice packed in closed plastic bags for cooling, commercial hatcheries are able to economize their *Artemia* cyst hatching efforts (i.e., reduction of the number of hatchings and harvests daily, fewer tanks, bigger volumes). Furthermore, cold storage allows the farmer to consider more frequent and even automated food distributions of an optimal live food. This appeared 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 minimized. For example, applying one or maximum two feedings per day, shrimp farmers often experienced juvenile *Artemia* in their larviculture tanks competing with the shrimp postlarvae for the algae. With poor hunters such as the larvae of turbot *Scophthalmus maximus* and tiger shrimp *Penaeus monodon*, feeding cold-stored, less active *Artemia* furthermore results in much more efficient food uptake.

An important factor affecting the nutritional value of *Artemia* as a food source for marine larval organisms is the content of essential fatty acids, eicosapentaenoic acid (EPA: 20:5n-3) and even more importantly docosahexaenoic acid (DHA: 22:6n-3). In contrast to freshwater species, most marine organisms do not have the capacity to biosynthesize these EFA from lower chain unsaturated fatty acids, such as linolenic acid (18:3n-3). In view of the fatty acid deficiency of *Artemia*, research has been conducted to improve its lipid composition by prefeeding with (n-3) highly unsaturated fatty acid (HUFA) rich diets. It is fortunate in this respect that *Artemia*, because of its primitive feeding characteristics, allows a very convenient way to manipulate its biochemical composition. Thus, since *Artemia* on molting to the second larval stage (i.e. about 8 h following hatching), is non-selective in taking up particulate matter, simple methods have been developed to incorporate lipid products into the brine shrimp nauplii prior to offering them as a prey to the predator larvae. This method of bioencapsulation, also called *Artemia* enrichment or boosting (Figure 61), is widely applied at marine fish and crustacean hatcheries all over the world for enhancing the nutritional value of *Artemia* with essential fatty acids. Different commercial self-emulsifying products are available for application with specific species.

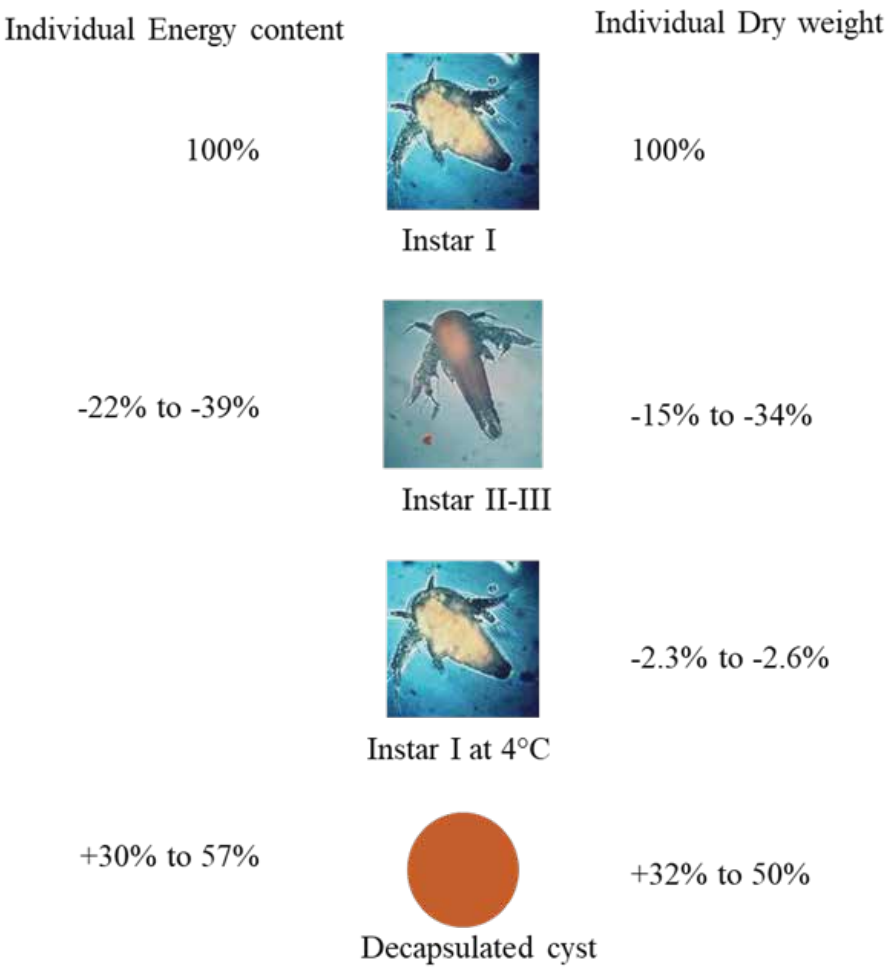


Figure 62: Change in energy and dry weight of different forms of *Artemia* (newly hatched instar I nauplii are considered to have 100 % values for those variables). The % decrease or increase is shown for Instar I, Instar II-III meta-nauplii, Instar I nauplii store

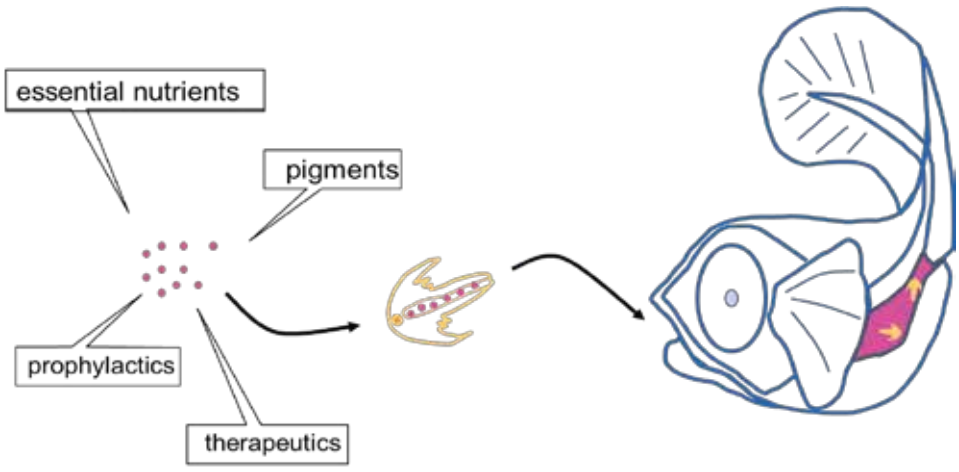


Figure 63: Schematic diagram of the use of *Artemia* as vector for transfer of specific components into the cultured larvae.

## Appendix 3: Cyst decapsulation

The hard shell that encysts the dormant *Artemia* embryo can be completely removed by short-term exposure to a hypochlorite solution. This procedure is called decapsulation. Decapsulated cysts offer a number of advantages compared to the non-decapsulated ones:

- 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 can cause deleterious effects in the larval tanks when they are ingested by the predator: they cannot be digested and may obstruct the gut.
- 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, because they do not spend energy necessary to break out of the shell (Figure 60).
- Decapsulated cysts can be used as a direct energy-rich food source for fish and shrimp

The decapsulation procedure 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 washing and deactivation of the remaining hypochlorite (see worksheets 3 and 4). These decapsulated cysts can be directly hatched into nauplii, or dehydrated in saturated brine and stored for later hatching or for direct feeding. They can be stored for a few days in the refrigerator at 0-4°C without a decrease in hatching. 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 usually release 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. Moreover, since they lose their hatchability when exposed to UV light it is advised to store them protected from direct sunlight.

The direct use of *Artemia* cysts, in its decapsulated form, is much more limited in larviculture of fish and shrimp, compared to the use of *Artemia* nauplii. Nevertheless, dried decapsulated *Artemia* cysts have proven to be an appropriate feed for larval rearing of various species like the freshwater catfish (*Clarias gariepinus*) and carp (*Cyprinus carpio*), marine shrimp and milkfish larvae. The use of decapsulated cysts in larval rearing presents some distinct advantages, both from a practical and nutritional point of view.

The daily production of nauplii is labour intensive and requires additional facilities. Furthermore, *Artemia* cysts of a high hatching quality are often expensive, and decapsulation of non-hatching cysts means valorization of an otherwise inferior product. The cysts have the appearance and the practical advantages of a dry feed and, in contrast to *Artemia* nauplii (470-550 µm), their small particle size (200-250 µm) is more suitable for small predator stages. If they have been dried before application, they have a high floating capacity, and sink only slowly to the bottom of the culture tank. Leaching of nutritional components (for example, with artificial diets) does not occur, since the outer cuticular membrane acts as a barrier for larger molecules).

On the other hand, a possible major drawback of decapsulated cysts is their immobility, and thus low visual attractivity for 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. Extra aeration or drying is therefore needed to keep these particles better in suspension. However, on the contrary, older penaeid larvae are mainly bottom feeders and so do not encounter this problem.

From the nutritional point of view, the gross chemical composition of decapsulated cysts is comparable to freshly-hatched nauplii (Table 13). In addition, their individual dry weight and energy content is on the average 30 to 40 % higher than for instar I nauplii (Figure 60). For example, for the culture of carp larvae during the first two weeks, the use of decapsulated cysts constitutes a saving of over one third in the amount of *Artemia* cysts used, compared to the use of live nauplii.

Table 12: The proximate composition (in % of dry matter) of decapsulated *Artemia* cysts and instar I nauplii

| | GSL | | SFB | |
|---|---|---|---|---|
| | cysts | nauplii | cysts | nauplii |
| protein | ±50 | 41-47 | ±57 | 47-59 |
| lipid | ±14 | 21-23 | ±13 | 16-27 |
| carbohydrate | - | 11 | - | 11 |
| ash | ±9 | 10 | ±5 | 6-14 |

# 15.0 Worksheets

## Worksheet 1: Procedure for estimating water content of *Artemia* cysts.

- Take three small aluminium foil-cups = $T_1$, $T_2$, $T_3$.
- Fill each cup with a cyst sample of approximately 500 mg.
- Determine gross weight (at 0.1 mg accuracy) = $G_1$, $G_2$, $G_3$.
- Place aluminium cups containing cysts for 24°C in a drying oven at 60°C
- Determine gross water free weight (at 0.1 mg accuracy) = $G_{1'}$, $G_{2'}$, $G_{3'}$.
- Calculate water content Wi (in % $H_2O$) $W_i = (G_i - G_{i'}) \times (G_i - T_i)\text{-}1 \times 100$
- Calculate mean value for the three replicate samples.

## Worksheet 2: Disinfection of *Artemia* cysts with liquid bleach.

- Prepare 200 ppm hypochlorite solution: ± 20 ml liquid bleach (NaOCl))/10 L (see cyst decapsulation)
- Soak cysts for 30 min. at a density of ± 50 g cysts/L
- Wash cysts thoroughly with tapwater on a 125 µm screen
- Cysts are ready for hatching incubation

## Worksheet 3: Procedures for the decapsulation of *Artemia* cysts

### Hydration step

- Hydrate cysts by placing them for 1 h in water (< 100 g/L), with aeration, at 25°C.

### Decapsulation step

- Collect cysts on a 125 µm mesh sieve, rinse, and transfer to the hypochlorite solution.
- The hypochlorite solution can be made up (in advance) of either liquid bleach sodium hypochlorite (NaOCl) (fresh product; activity normally =11-13 % w/w) or bleaching powder calcium hypochlorite {$Ca(OCl)_2$ } (activity normally ± 70 %) in the following proportions:
    - 0.5 g active hypochlorite product (activity normally labeled on the package, otherwise to be determined by titration) per g of cysts; for procedure see further);

**an alkaline product to keep the pH>10; per g of cysts use:**

- 0.15 g technical grade sodium hydroxide (NaOH) when using liquid bleach;
- either 0.67g sodium carbonate ($NaCO_3$) or 0.4 g calcium oxide (CaO) for bleaching powder; dissolve the bleaching powder before adding the alkaline product; use only the supernatants of this solution;
- seawater to make up the final solution to 14 ml per g of cysts.
- Cool the solution to 15-20°C (i.e. by placing the decapsulation container in a bath filled with ice water). Add the hydrated cysts and keep them in suspension (i.e. with an aeration tube) for 5-15 minutes. Check the temperature regularly, since the reaction is exothermic; never exceed 40°C (if needed add ice to decapsulation solution). Check evolution of decapsulation process regularly under binocular.

### Washing step

- When cysts turn grey (with powder bleach) or orange (with liquid bleach), or when microscopic examination shows almost complete dissolution of the cyst shell (= after 3-15 minutes), cysts should be removed from the decapsulation suspension and rinsed with water on a 125 µm screen until no chlorine smell is detected anymore. It is crucial not to leave the embryos in the decapsulation solution longer than strictly necessary, since this will affect their viability.

### Deactivation step

- Deactivate all traces of hypochlorite by dipping the cysts (< 1 min.) either in 0.1 N hydrochloric acid (HCl) or in 0.1 % sodium thio sulphate ($Na_2S_2O_3$) solution, then rinse again with water. Hypochlorite residues can be detected by putting some decapsulated cysts in a small amount of starch-iodine indicator (= starch, KI, $H_2SO_4$ and water). When the reagent turns blue, washing and deactivation has to be continued.

### Use

- Incubate the cysts for hatching, or store in the refrigerator (0-4°C) for a few days before hatching incubation. For long term storage cysts need to be dehydrated in saturated brine solution (1 g of dry cysts per 10 ml of brine of 300 g NaCl/L). The brine has to be renewed after 24 h.

## Worksheet 4: Titrimetric method for the determination of active chlorine in hypochlorite solutions

- Principle: active chlorine will liberate free iodine from KI solution at pH 8 or less. The liberated iodine is titrated with a standard solution using $Na_2S_2O_3$, with starch as the indicator.

- Reagents:

acetic acid (glacial, concentrated) KI crystals

$Na_2S_2O_3$, 0.1 N standard solution

starch indicator solution: mix 5 g starch with a little cold water and grind in a mortar. Pour into 1 L of boiling distilled water, stir, and let settle overnight. Use the clear supernatants. Preserve with 1.25 g salicylic acid.

- Procedure:

dissolve 0.5 to 1 g KI in 50 ml distilled water, add 5 ml acetic acid, or enough to reduce the pH to between 3.0 and 4.0;

add 1 ml sample;

titrate protected from direct sunlight. Add 0.1 N $Na_2S_2O_3$ from a buret until the yellow colour of the liberated iodine is almost disappearing. Add 1 ml starch solution and titrate until the blue colour disappears.

- Calculation:

1 ml 0.1 N $Na_2S_2O_3$ equals 3.54 mg active chlorine.

## Worksheet 5: Determination of hatching percentage, hatching efficiency and hatching rate

- Incubate exactly 1.6 g of cysts in exactly 800 ml 33 g/L seawater under continuous illumination (2000 lux) at 28°C in a cylindroconical tube (preferentially) or in a graduated cylinder; provide aeration from bottom as to keep all cysts in suspension (aeration not too strong as to prevent foaming); run test in triplicate.
- After 24 h incubation take 6 subsamples of 250 µl out of each cone.
- Pipet each subsample into a small vial and fixate nauplii by adding a few drops of lugol solution.
- Per cone (i = 6 subsamples), count nauplii (ni) under a dissection microscope and calculate the mean value (N), count umbrellas (ui) and calculate mean value (U).
- Decapsulate unhatched cysts and dissolve empty cyst shells by adding one drop of NaOH solution (40 g/100 ml distilled water) and 5 drops of domestic bleach solution (5.25% NaOCl) to each vial.
- Per cone (i = 6), count unhatched (orange colored) embryos (ei) and calculate mean value (E).
- Hatching percentage $H\% = (N \times 100).(N + U + E)^{-1}$
- calculate H% value per cone and calculate mean value and standard deviation of 3 cones = final value
- Hatching efficiency $HE = (N \times 4 \times 800).(1.6)^{-1}$ or $HE = N \times 2000$* (*conversion factor to calculate for number of nauplii per g of incubated cysts)

calculate HE value per cone and calculate mean value and standard deviation of 3 cones = final value

- Eventually leave hatching tubes for another 24 h, take subsamples again and calculate H% and HE for 48 h incubation.
- Hatching rate (HR): start taking subsamples and calculating HE from 12 h incubation in seawater onwards (follow procedure above). Continue sampling/counting procedures until mean value for HE remains constant for 3 consecutive h. The mean values per hour are then expressed as percentage of this maximal HE. A hatching curve can be plotted and $T_{10}$, $T_{90}$ etc. extrapolated from the graph. A simplified procedure consists in sample taking e.g. every 3 or more h.

Table 13: Practical example of hatching percentage and hatching efficiency

| nauplii (n) | umbrellas (u) | embryos (e) | H% = n*100 (n+u+e)$^{-1}$ |
|---|---|---|---|
| replicate 1 | | | |
| 110 | 3 | 17 | 84.62 |
| 129 | 4 | 14 | 87.76 |
| 122 | 3 | 13 | 88.41 |
| 108 | 2 | 15 | 86.40 |
| 117 | 2 | 16 | 86.67 |
| 101 | 3 | 10 | 88.60 |
| average nauplii = 115 | | | average H% =87.08 |
| replicate 2 | | | |
| 124 | 1 | 14 | 89.21 |
| 122 | 1 | 21 | 84.72 |
| 138 | 0 | 18 | 88.46 |
| 103 | 3 | 7 | 91.45 |
| 142 | 0 | 12 | 92.21 |
| 130 | 4 | 13 | 88.44 |
| average nauplii = 127 | | | average H% =89.03 |
| replicate 3 | | | |
| 127 | 3 | 14 | 88.19 |
| 107 | 4 | 10 | 88.43 |
| 133 | 2 | 18 | 86.93 |
| 135 | 5 | 13 | 88.24 |
| 125 | 1 | 15 | 88.65 |
| 128 | 1 | 15 | 88.89 |
| average nauplii = 126 | | | average H% =88.23 |
| average H% = (87.08+89.03+88.23).3$^{-1}$ x 100 = 88.11 (standard deviation = 0.98) | | | |
| average HE = (115+127+126).3$^{-1}$ x 2000 = 245 300 (standard deviation = 13000) | | | |

Table 14: Practical example to calculate hatching rate

| incubation time (in h) | HE (N/g) | % of maximal HE |
|---|---|---|
| 12 | 0 | 0 |
| 13 | 800 | 0.4 |
| 14 | 9 000 | 5 |
| 15 | 29 400 | 15 |
| 16 | 79 800 | 42 |
| 17 | 144 400 | 76 |
| 18 | 158 200 | 83 |
| 19 | 184 600 | 97 |
| 20 | 185 000 | 97 |
| 21 | 191 000 | 100 |
| Characteristic time-intervals | T10 = 14.5 h | |
| | T50 = 16.2 h | |
| | T90 = 18.5 h | |
| | $T_s = T_{90} - T_{10} = 4.0$ h | |

## About WorldFish

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