Manual on the production and use of live food for aquaculture

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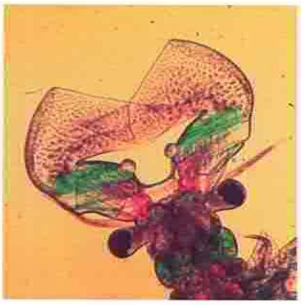

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4. *ARTEMIA*

4.1. Introduction, biology and ecology of *Artemia*

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4.1.1. Introduction

Among the live diets used in the larviculture of fish and shellfish, nauplii of the brine shrimp *Artemia* constitute the most widely used food item. Annually, over 2000 metric tons of dry *Artemia* cysts are marketed worldwide for on-site hatching into 0.4 mm nauplii. Indeed, the unique property of the small branchiopod crustacean *Artemia* to form dormant embryos, so-called 'cysts', may account to a great extent to the designation of a convenient, suitable, or excellent larval food source that it has been credited with. Those cysts are available year-round in large quantities along the shorelines of hypersaline lakes, coastal lagoons and solar saltworks scattered over the five continents. After harvesting and processing, cysts are made available in cans as storable 'on demand' live feed. Upon some 24-h incubation in seawater, these cysts release free-swimming nauplii that can directly be fed as a nutritious live food source to the larvae of a variety of marine as well as freshwater organisms, which makes them the most convenient, least labour-intensive live food available for aquaculture. Although *Artemia* has been known to man for centuries, its use as a food for the culture of larval organisms apparently began only in the 1930s, when several investigators found that it made an excellent food for newly-hatched fish larvae. During the 1940s, most commercially available brine shrimp cysts represented collections from natural saline lakes and coastal saltworks. With the growing interest for tropical hobby fish in the late 1940s, commercial value was attached to brine shrimp, thereby establishing a new industry. Early pioneers exploited in 1951 the cyst production of *Artemia* at the Great Salt Lake in Utah, USA. First harvests of the lake yielded 16 tons of finished product. During the mid-1950s, commercial attention for brine shrimp was turned to controlled sources for production in the San Francisco Bay region. Here it was found that brine shrimp and their cysts could be produced as a by-product of solar saltworks. Since salt production entails management of the evaporation process, yearly cyst and biomass productions could be roughly predicted. In the 1960s, commercial provisions originated from these few sources in North America and seemed to be unlimited. However, with the expansion of aquaculture production in the 1970s, the demand for *Artemia* cysts soon exceeded the offer and prices rose exponentially, turning *Artemia* into a bottleneck for the expansion of the hatchery aquaculture of marine fishes and crustaceans. In particular, many developing countries could hardly afford to import the very expensive cysts.

At the Kyoto FAO Technical Conference on Aquaculture in 1976 it was claimed that the cyst shortage was an artificial and temporary problem. During the following years research efforts were made to prove the possibility of local production of *Artemia* in developing countries.

At present, *Artemia* is being produced and exploited on the five continents. Despite this, a large part of the cyst market is still supplied by harvests from one location, the Great Salt Lake. This situation makes the market still extremely vulnerable to climatological and/or ecological changes in this lake, which has been illustrated by the unusually low cyst harvests in the seasons 1993-1994 and mainly 1994-1995.

Already in the late 1970s it appeared that the nutritional value of *Artemia*, especially for marine organisms, was not constant but varied among strains and within batches of each strain, causing unreliable outputs in marine larviculture. Through multidisciplinary studies in the 1980s both the causes for the nutritional variability in *Artemia* and the methods to improve poor-quality *Artemia* were identified. Genotypic and phenotypic variation (*i.e.* cyst size, cyst hatching characteristics, caloric content and fatty acid composition of the nauplii) determine if a particular cyst product is suitable for hatchery use of specific fish or shrimp species.

By bio-encapsulating specific amounts of particulate or emulsified products rich in highly unsaturated fatty acids in the brine shrimp metanauplii, the nutritional quality of the *Artemia* can be further tailored to suit the predators' requirements. Application of this method of bio-encapsulation, also called *Artemia* enrichment or boosting, has had a major impact on improved larviculture outputs, not only in terms of survival, growth and success of metamorphosis of many species of fish and crustaceans, but also with regard to their quality, *e.g.* reduced incidence of malformations, improved pigmentation and stress resistance. The same bio-encapsulation method is now being developed for oral delivery of vitamins, chemotherapeutics and vaccines.

Furthermore, a better knowledge of the biology of *Artemia* was at the origin of the development of other *Artemia* products, such as disinfected and decapsulated cysts, various biomass preparates, which presently have application in hatchery, nursery and broodstock rearing. All these developments resulted in optimized and cost-effective applications of this live food in hatchery production.

4.1.2. Biology and ecology of *Artemia*

4.1.2.1. Morphology and life cycle

In its natural environment at certain moments of the year *Artemia* produces cysts that float at the water surface (Fig. 4.1.1.) 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 dry. Upon immersion in seawater, the biconcave-shaped cysts hydrate, become spherical, and within the shell the embryo resumes its interrupted metabolism. After about 20 h the outer membrane of the cyst bursts (= "breaking") and the embryo appears, surrounded by the hatching membrane (Fig. 4.1.2.). 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 (Fig. 4.1.3.).

Figure 4.1.1.Harvesting of brine shrimp cysts from a saltpond.

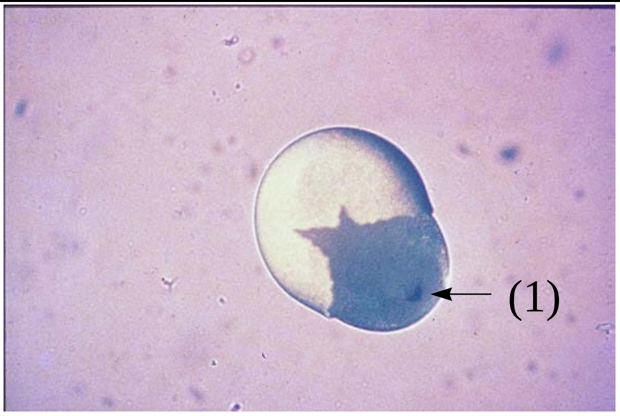

Figure 4.1.2. Cyst in breaking stage. (1) nauplius eye.

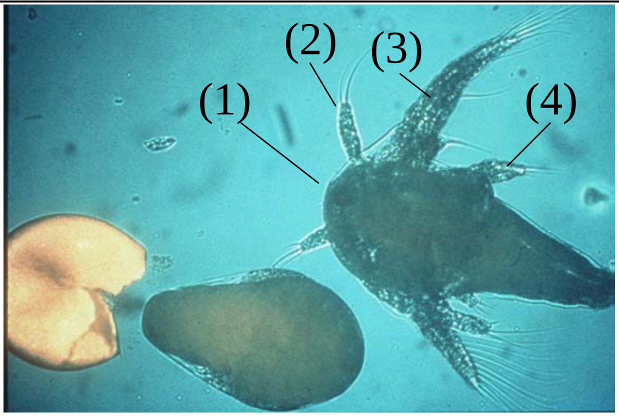

Figure 4.1.3. Embryo in "umbrella" stage (left) and instar I nauplius (right). (1) nauplius eye; (2) antennula; (3) antenna; (4) mandible.

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 mandibles (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 8 h 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 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 (Fig. 4.1.4.). On both sides of the nauplius lateral complex eyes are developing (Fig. 4.1.5.and 4.1.6.). 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 (Fig. 4.1.6. and 4.1.8.) they develop into hooked graspers, while the female antennae degenerate into sensorial appendages (Fig. 4.1.11.). The thoracopods are now differentiated into three functional parts (Fig. 4.1.13.), namely the telopodites and endopodites (locomotory and filter-feeding), and the membranous exopodites (gills).

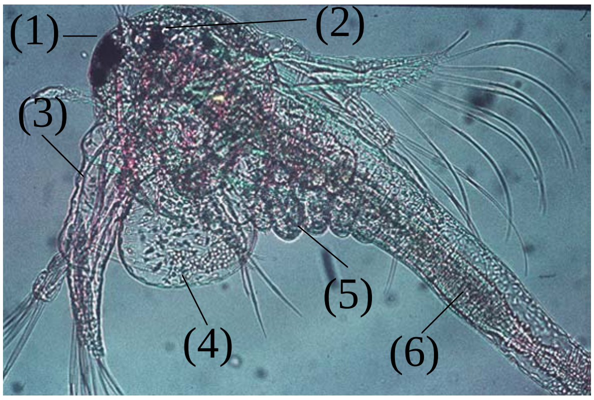

Figure 4.1.4. Instar V larva. (1) nauplius eye; (2) lateral complex eye; (3) antenna; (4) labrum; (5) budding of thoracopods; (6) digestive tract.

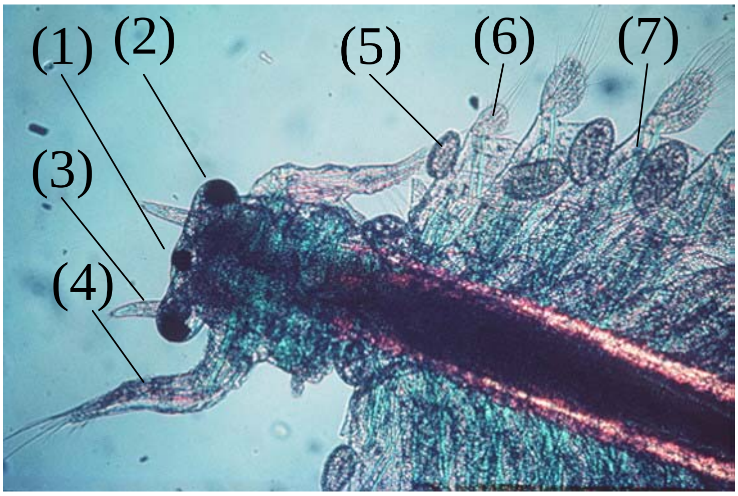

Figure 4.1.5. Head and anterior thoracic region of instar XII. (1) nauplius eye; (2) lateral complex eye; (3) antennula; (4) antenna; (5) exopodite; (6) telopodite; (7) endopodite.

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 (Fig. 4.1.10. and 4.1.11.). The male (Fig. 4.1.10.) has a paired penis in the posterior part of the trunk region (Fig. 4.1.9.). Female *Artemia* can easily be recognized by the brood pouch or uterus situated just behind the 11th pair of thoracopods (Fig. 4.1.9. and 4.1.11.). Eggs develop in two tubular ovaries in the abdomen (Fig. 4.1.7.). Once ripe they become spherical and migrate via two oviducts into the unpaired uterus.

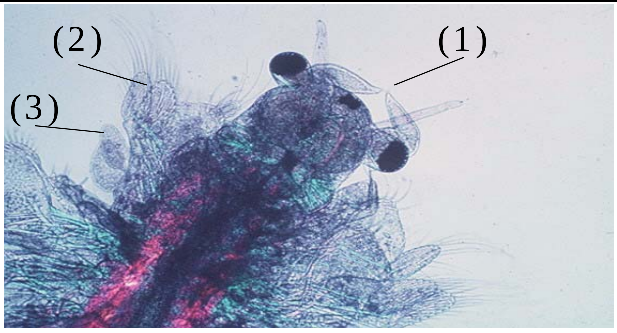

Figure 4.1.6. Head and thoracic region of young male. (1) antenna; (2) telopodite; (3) exopodite.

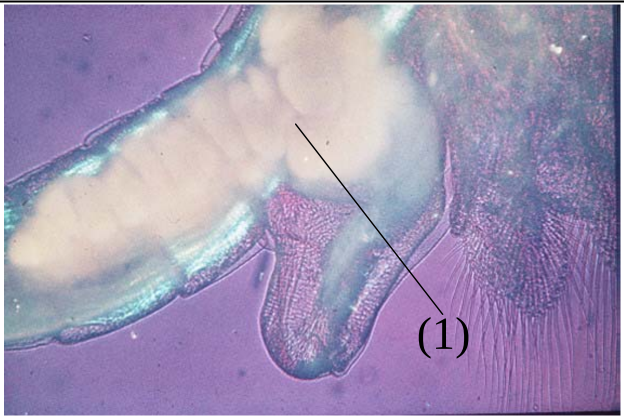

Figure 4.1.7. Posterior thoracic region, abdomen and uterus of fertile female. (1) ripe eggs in ovary and oviduct.

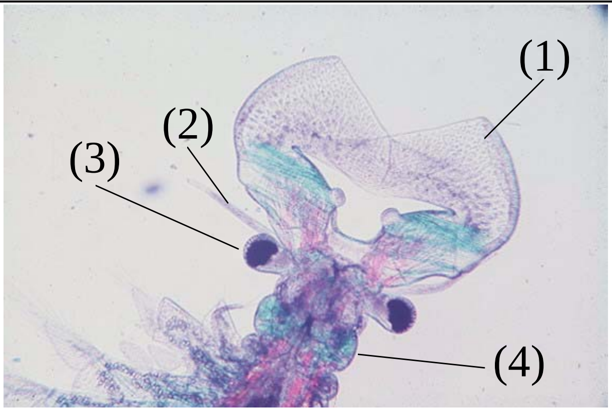

Figure 4.1.8. Head of an adult male. (1) antenna; (2) antennula; (3) lateral complex eye; (4) mandible.

Fertilized eggs normally develop into free-swimming nauplii (= ovoviviparous reproduction) (Fig. 4.1.12.) 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) (Fig. 4.1.14.). 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. The cysts usually float in the high salinity waters and are blown ashore where they accumulate and dry. As a result of this dehydration process the diapause mechanism is generally inactivated; cysts are now in a state of quiescence and can resume their further embryonic development when hydrated in optimal hatching conditions.

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

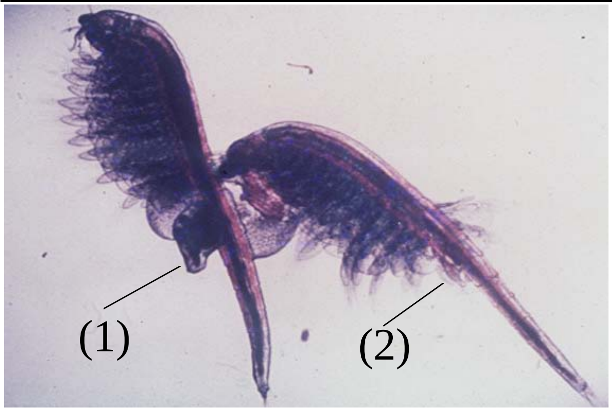

Figure 4.1.9. *Artemia* couple in riding position. (1) uterus; (2) penis.

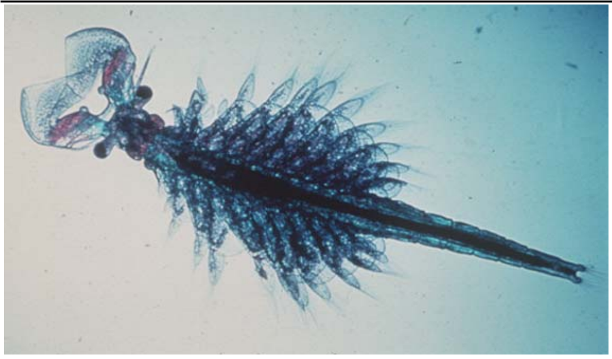

Figure 4.1.10. Adult male.

Figure 4.1.11. Adult female.

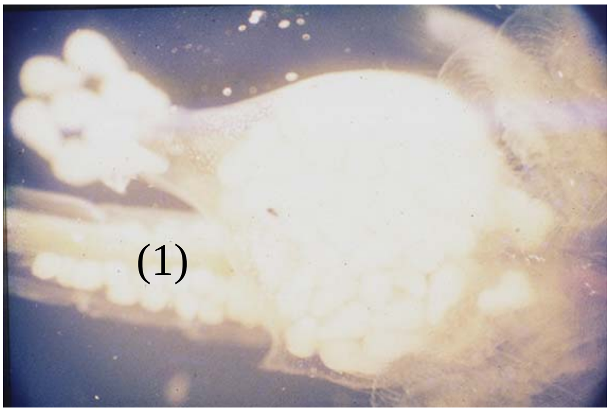

Figure 4.1.12. Uterus of ovoviviparous *Artemia* filled with nauplii (first larvae are being released). (1) ovary with eggs.

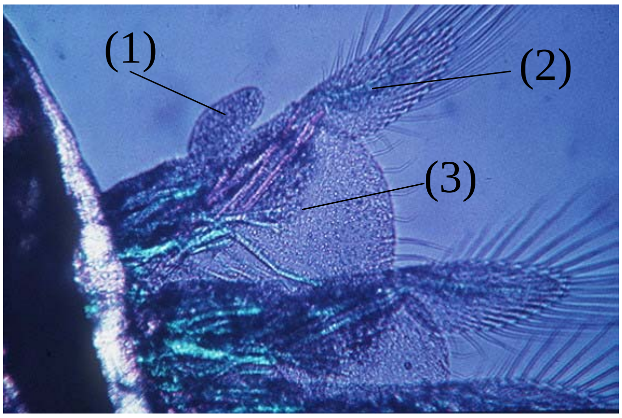

Figure 4.1.13. Detail of anterior thoracopods in adult *Artemia.* (1) exopodite; (2) telopodite; (3) endopodite.

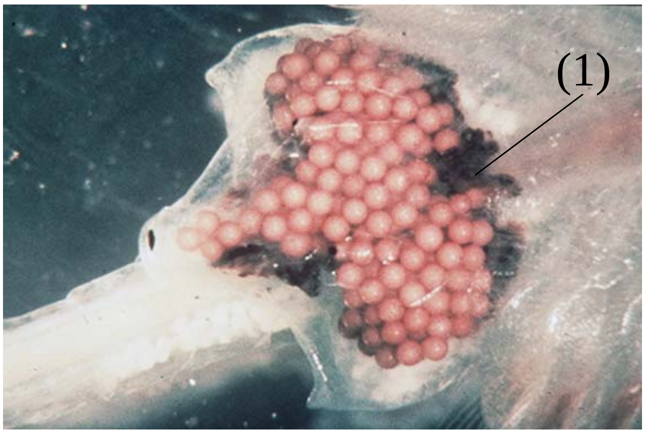

Figure 4.1.14. Uterus of oviparous *Artemia* filled with cysts. (1) brown shell glands (darker colour).

4.1.2.2. 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 (Fig. 4.1.15.). This list still remains provisional as more extensive survey work should lead to the discovery of many more *Artemia* biotopes in different parts of the world (Table 4.1.1.). 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 ($\geq$ 70 g.l^{-1}). As a result of extreme physiological stress and water toxicity *Artemia* dies off at salinities close to NaCl saturation, *i.e*. 250 g.l^{-1} 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, 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) are 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 responsable for several *Artemia* introductions in South America and Australia, either for salt production improvement or for aquaculture purposes. Additionally, temporal *Artemia* populations are found in tropical areas with a distinct wet and dry season (monsoon climate), through inoculation in seasonal salt operations (*e.g.* Central America, Southeast Asia).

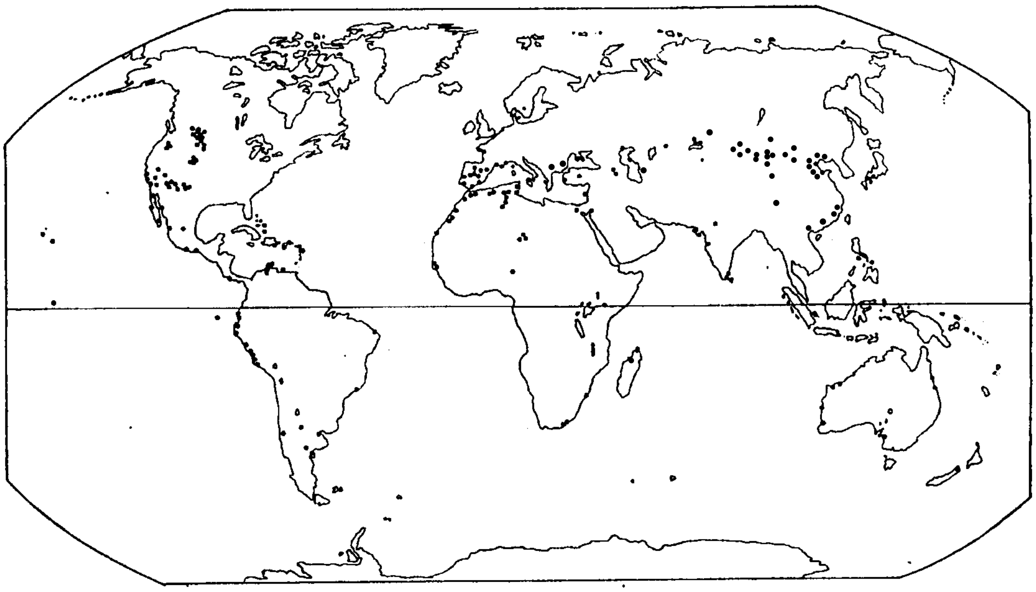

Figure 4.1.15. The world distribution of *Artemia*.