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Use of brine shrimp, artemia spp., in larval crustacean nutrition: A Review

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Abstract

The brine shrimp *Artemia* spp. nauplii have been adopted as a standard diet in the commercial larviculture of several crustacean species, Because of convenience in production and their suitable biochemical composition. The nutritional value of *Artemia* is not constant but varies both geographically and temporally. During the past decade both the causes of *Artemia* nutritional variability and methods to improve poor-quality *Artemia* have been identified. Enriching *Artemia* spp. with emulsified lipophilic products is a technique that has allowed delivery of extra doses of essential nutrients, for example, highly eight unsaturated fatty acids (HUFA) and vitamins, to crustacean larvae. The enrichment technique has limitations, however, because the *Artemia* spp. currently available selectively catabolize some of the nutrients such as docosahexaenoic acid and phospholipids. Decapsulated *Artemia* cysts, juveniles, and adult brine shrimp are also used increasingly as suitable diets for different crustacean species.

Keywords: larviculture, shrimp Penaeus spp., prawn *Macrobrachium rosenbergii*, n-3 HUFA, vitamin C, vitamin E.

Introduction

Although the brine shrimp *Artemia* sp. has been known to man for centuries, its use as a food for the culture of larval organisms apparently began only in the 1930s to the 1940s. Several investigators found that it made an excellent food for newly hatched fish (Seale, 1933) and shrimp larvae (Hudinaga, 1942). The nutritional value of *Artemia*, especially for marine fish

and crustaceans, is not constant, but varies geographically and temporally (Léger et al., 1986).

During the last decade some of the causes of *Artemia* nutritional variability and methods to improve poor-quality *Artemia* have been identified. This article covers historical and present usage of *Artemia* in crustacean larviculture, with particular emphasis on the nutritional aspects of this important food. More in depth reviews of some of the subjects covered here can be found in Sorgeloos (1980), Watanabe *et al.*, (1983), Léger et al. (1986), and Bengtson *et al.,* (1991). Subsequent cytogenic analyses of *Artemia* from 4 Argentinean populations (Lipko *et al.,* 2004) confirmed the presence of *A. franciscana*, and Amat et al., (2004) reported the presence of *A. franciscana* in Mar Chiquita salt lake, Las Tunas lagoon and a shallow lake in Salinas Grandes (Córdoba province). Amat et al., (2004) went on to confirm that *A. franciscana* was distributed north of 36°S and that *A. persimilis* was distributed south of 37°10'S.

Cyst Supply And Demand

The initial sources of commercial cysts were a coastal saltwork in the San Francisco Bay (SFB), California (USA), in the 1950s and later from the inland Great Salt Lake (GSL) in Utah (USA). Cyst prices increased considerably in the mid 1970s as the combined result of increased demands from the emerging hatchery industry, decreased harvests from the Great Salt Lake, and possibly simulated shortages by certain commercial companies. Based on research performed at the State University of Gent in Belgium, the idea was launched at the 1976 FAO Technical Conference on Aquaculture in Kyoto (Japan) that the cyst shortage was an artificial and temporary problem that could be overcome by the exploration for and development of new *Artemia* resources and by the application of improved methods for processing and use of the cysts (Sorgeloos, 1979).

By 1980 the situation was rectified with several new commercial products from natural (e.g., Australia, France, PR China) and man-managed (e.g., Brazil, Thailand) *Artemia* production sites. In the meantime cyst consumption had increased exponentially as a result of the booming shrimp and fish hatchery industry. Presently, some 5000 fish and shrimp hatcheries require over 1000 metric tons of cysts annually (Van Stappen and Sorgeloos, 1993). Strong competition in the market place especially with cyst products that could be harvested very efficiently and cost-effectively from the Great Salt Lake resulted in a new vulnerable situation of dependence on one resource. As cyst prices increase it will become attractive

again to explore and develop new *Artemia* resources or to switch to more cost-effective formulated feeds (Sorgeloos and Léger, 1992).Unsustainable practises, together with climatic phenomena like the El Nino Southern Oscillation (ENSO) and anthropogenic impacts, initiated a decrease in cyst harvesting, which then led to market fluctuations in cyst availability and quality and to increasing prices (Lavens & Sorgeloos 2000).

Production and Use of Freshly Hatched Nauplii

Although using *Artemia* cysts appears to be simple, several factors are critical for hatching the large quantities needed in larval crustacean production. These include cyst disinfection or decapsulation prior to incubation and hatching under the following optimal conditions: constant temperature of 25°C to 28°C, 15 to 35 ppt salinity, minimum pH of 8.0, near saturated oxygen levels, maximum cyst densities of 2 g/l, and strong illumination of 2000 lux (Sorgeloos *et al*., 1986). All these factors will affect the hatching rate and maximum output and hence the production cost of the harvested *Artemia* nauplii.

After hatching, and prior to feeding them to the larvae, *Artemia* nauplii should be separated from the hatching wastes. After switching off the aeration in the hatching tank, cyst shells will float and nauplii will concentrate at the tank's bottom. They should be siphoned off within 5 to 10 min and thoroughly rinsed with seawater or freshwater, using submerged filters (Sorgeloos and Leger, 1992) to prevent physical damage to the nauplii.

Size and Energy Content

In their first stage of development, *Artemia* nauplii do not feed but consume their own energy reserves (Benijts *et al*., 1976). At the high water temperatures that are applied during cyst incubation, freshly hatched *Artemia* naupli develop into the second larval stage within six to eight hour. It is important to feed first-instar naupli rather than starved second-instar metanauplii that are transparent and less visible. Instar II metanauplii are about 50% larger in length and swim faster than first instars. As a result they are less acceptable as prey. Furthermore, they contain lower amounts of free amino acids so they are less digestible and their lower individual dry weights (1.63 µg vs. 2.15 µg in the SFB strain) and energy content (0.0366 Joules vs. 0.0500 Joules in the same strain) reduce the energy uptake by the predator per unit of hunting effort (Léger *et al*., 1986). All this will be reflected in reduced larval growth in the face of increased *Artemia* cyst consumption (20 to 30% more cysts are needed to feed the same weight of starved metanauplii to the predator).

Storing freshly hatched nauplii at temperatures near 4°C, in densities of up to eight million nauplii per liter for up to 24 h (Leger et al., 1983) will greatly reduce their metabolic rate, that is, only a 2.5% drop in individual dry weight vs. 30% at 25°C, and preclude molting to the second instar stage. The 24–h cold storage economizes the *Artemia* cyst hatching effort (e.g., fewer tanks, larger volumes, a maximum of one hatching and harvest per day) and allows not only a constant supply of a high-quality product but also the possibility of more frequent food distributions. This is beneficial for shrimp larvae because food retention time in larviculture tanks can be reduced and hence the growth of *Artemia* in the culture tank minimized.

Nutritional Quality — Fatty Acid Enrichment

In the late 1960s and early 1970s several authors reported problems in larviculture success with shrimp, prawn, lobster, and crab species when using *Artemia* sources other than SFB *Artemia* (for reviews see Sorgeloos, 1980 and Léger *et al*., 1986). High doses of toxic compounds (e.g., chlorinated hydrocarbons and heavy metals), were initially suspected to be the cause of the poor nutritional value of *Artemia* from GSL and the People's Republic of China. A comparative study with eight strains of *Artemia* spp. using crab and mysid shrimp as predator test species confirmed the nutritional variation among *Artemia* sources (Johns *et al*., 1980, 1981; Léger and Sorgeloos, 1984). Léger *et al*., (1985a) documented the nutritional variability in 11 batches of San Francisco Bay *Artemia* nauplii for the mysid shrimp *Mysidopsis bahia*.

Taking advantage of the primitive feeding characteristics of *Artemia* nauplii, it is possible to manipulate the nutritional value of HUFA-deficient *Artemia*, for example, the GSL strain. Because brine shrimp nauplii that have molted into the second instar stage (i.e., about 8 h following hatching) are nonselective particle feeders, simple methods have been developed to incorporate different kinds of products into the *Artemia* prior to feeding to predator larvae. This method of "bioencapsulation", also called *Artemia* enrichment or boosting, is widely applied in marine fish and crustacean hatcheries for enhancing the nutritional value of *Artemia* with essential fatty acids.

British, Japanese, and Belgian researchers developed enrichment products and procedures using selected microalgae and/or microencapsulated products, yeast and/or emulsified preparations, self-emulsifying concentrates, and/or microparticulate products (Léger *et al.*, 1986). The highest enrichment levels are obtained from emulsified concentrates: freshly hatched nauplii are transferred to the enrichment tank at a density of 100 to 300 nauplii/ml

(for enrichment periods >24 h or <24 h, respectively). The enrichment medium consists of hypochlorite-disinfected and neutralized seawater maintained at 25°C. The enrichment emulsion is added in consecutive doses of 0.3 g/l every 12 hours. Strong aeration using air stones or pure oxygen is required to maintain dissolved oxygen levels above 4 ppm. Enriched nauplii are harvested after 24 or 48 h, thoroughly rinsed and stored at temperatures below 10°C in order to assure that HUFA are not metabolized during storage. Enrichment levels of 50 to 60 mg/g DW n-3 HUFA are obtained after 24 h enrichment with the emulsified concentrates. Nauplii should be transferred or exposed to the enrichment medium as soon as possible before first feeding so they begin feeding immediately after the opening of the alimentary tract (instar II stage). As a result, the increase of nauplius size during enrichment can be minimized, that is, after 24 h enrichment GSL Artemia nauplii will reach about 660 µm, and after 48 h enrichment about 790 µm. Feeding n-3 HUFA-enriched *Artemia* nauplii results in increased larval survival and growth in several *Penaeus* spp. and *Macrobrachium rosenbergii* (Bengtson *et al.*, 1991).

Although the cited studies provided convincing evidence of the importance of the n-3 HUFA in *Artemia* when used as food for shrimp larvae, quantitative dietary requirements as well as the relative importance of selected HUFA (e.g., docosahexaenoic acid, 22:6n-3, DHA) remained to be explored. Rees *et al*. (1994) fed *Penaeus monodon* postlarvae (PL-5 to PL-15) five diets consisting of *Artemia* nauplii enriched with different n-3 HUFA levels.

Phospholipids

Although phospholipid requirements are well documented in juvenile stages for various crustacean species only limited information is available on the role of phospholipids in start-feeding stages (reviewed by Coutteau *et al*., 1997). The few studies on larval requirements of crustacean species used artificial diets (Teshima *et al*., 1982, Kanazawa *et al*., 1985, Camara *et al*., 1997).

Vitamin C

Vitamin C, more specifically ascorbic acid (AA), is generally considered to be an essential dietary component for the various stages of aquaculture organisms. Several biological (e.g., skeletal development, growth, survival) as well as physiological functions (e.g., resistance to toxicants and stress, immunoactivity) are enhanced in larvae from supplemental dietary ascorbate (Dabrowski, 1992). Ascorbic acid 2–sulfate (AAS), a stable derivative of AA, was

discovered in dormant cysts of *Artemia* by Mead and Finamore (1969). Cysts of various batches differed considerably in AAS content: 160 to 517 µg/g DW, expressed as AA (Dabrowski, 1991). The amount of AA, liberated in freshly hatched nauplii reflects the AAS reserve present in the cysts and provides evidence for the conversion of AAS to free AA during completion of embryonic development into nauplii (Golub and Finamore, 1972; Dabrowski, 1991; Nelis *et al*., 1994). The variation in AAS concentration observed in *Artemia* cysts may reflect adult nutrition during egg production as was demonstrated for HUFA content (Lavens *et al*., 1989). However, significantly positive effects on the physiological condition of the post larvae, measured by a salinity stress test, could be demonstrated when vitamin C-boosted live food was administered. Because the AA levels in predator larvae are linked with the enrichment levels in the live prey, it may be assumed that a stress resistance was increased by feeding vitamin C-enriched *Artemia*. Under suboptimal conditions supplementation with high vitamin C levels might also enhance production. These results support the hypothesis that stress creates increased ascorbate requirements for larval fish and crustaceans, and that in this respect body vitamin C concentration may reflect the survival potential more accurately than variation in growth rate (Dabrowski, 1992). Moreover, at day 28, a significant drop in AA concentration was detected in the postlarvae compared with the levels found in the larvae. This may reflect an extra need for vitamin C during metamorphosis, a stressful period as the larvae undergo major morphological and physiological changes. Recent culture tests with vitamin C boosted microbound diets for P. vannamei confirmed the hypothesis that dietary vitamin C improved stress resistance in postlarval crustaceans (Kontara *et al*., 1995b).

Other Nutrients

High levels of α-tocopherol can be bio accumulated and maintained in *Artemia* nauplii making this live food delivery system useful for studying dietary requirements and antioxydative effects of vitamin E in larval crustacean nutrition research. The effectiveness of *Artemia* nauplii as a dietary carrier system could be tested for various other nutritional components, such as, liposoluble products administered via an emulsion, water-soluble compounds via liposomes (Hontoria *et al*., 1994), and/or microcapsule delivery (Sakamoto *et al*., 1982).

Other Forms of Artemia Used in Crustacean Nutrition

Aside from the most common regime of feeding freshly hatched and/or 24–h enriched nauplii, the use of dry decapsulated cysts, juveniles, and adult biomass is practiced with various crustacean species (Léger *et al*., 1986; Bengtson *et al*., 1991; Stael *et al*., 1995). Decapsulated cysts (also called de-shelled or shell-free cysts) can be used in start-feeding crab, shrimp, and prawn larvae; however, the rapid settling of the cysts in seawater can make them unavailable for planktonic larvae unless they can be kept in suspension by using conical-shaped culture tanks and strong aeration. Best results are obtained when feeding decapsulated cysts to postlarval shrimp and prawn as a partial or complete substitute for live nauplii (Stael *et al*., 1995). The major advantage here might be, aside from being a directly available off-the-shelf product, that cysts with poor hatching quality can still be used as a food source. Dhert *et al*., (1993b) developed a simple culture system for juvenile and adult *Artemia* as food for postlarval *Penaeus monodon*. The most spectacular example was the use of thousands of tons of fresh brine shrimp biomass harvested from coastal and inland salt works as a supplementary natural food in the pond culture of *Penaeus chinensis* in the Bohai Bay, People's Republic of China (Tackaert and Sorgeloos, 1991). Although the fresh-live form has the highest nutritive value, harvested *Artemia* can also be frozen, freeze-dried, or acid-preserved (Abelin *et al*., 1991; Naessens *et al*., 1995b) for later use, or made into flakes or other forms of formulated feed. *Artemia* biomass is apparently a good food for the maturation of several species of penaeid shrimp. Recent culture tests in Ecuador and the USA have shown that polychaetes, which have been identified as a critical fresh-food component in the maturation diet of *Penaeus vannamei* (Bray and Lawrence, 1992) can be successfully replaced by frozen *Artemia* biomass (Naessens *et al*., 1997).

Conclusion

Empirical applications with the brine shrimp *Artemia* have resulted in quick and successful developments in the commercial hatchery rearing of several crustacean species. The availability of nutritionally different sources of brine shrimp cysts and the use of simple bio encapsulation techniques with the nauplii have in the past, and can in the future, contribute significantly to a better understanding of the nutritional requirements of larval crustaceans. This knowledge will allow further improvement of the formulation and manufacturing of artificial diets, which eventually should completely replace *Artemia* and other live feeds and lead to more reliable and cost- effective hatchery operations.

References

Abelin, P., W. Tackaert, and P. Sorgeloos. Ensiled *Artemia* biomass: a promising and practical feed for *penaeid* shrimp postlarvae. In: Larvi'91 — Fish & Crustacean Larviculture Symposium. pp. 125–127 (Lavens, P., P. Sorgeloos, E. Jaspers, and F.Ollevier, Eds.) Gent: European Aquaculture Society, Special Publication No. 15(1991).

Amat F, Cohen RG, Hontoria F, Navarro JC (2004) Further evidence and characterization of Artemia franciscana Kellogg, 1906 populations in Argentina. J Biogeogr 31: 1–15

Bengtson, D. A., P. Léger, and P. Sorgeloos. Use of *Artemia* as a food source for aquaculture. In: *Artemia* Biology. pp. 255–285 (Browne, R. A., P. Sorgeloos., and C. N. A. Trotman, Eds.) Boca Raton, FL: CRC Press Inc. (1991).

Benijts, F., E. Vanvoorden, and P. Sorgeloos. Changes in the biochemical composition of the early larval stages of the brine shrimp, *Artemia salina* L. In: Proceedings of the 10th European Symposium on Marine Biology, Vol. I. Research in Mariculture at Laboratory and Pilot Scale. pp. 1–9 (Persoone, G. and E. Jaspers, Eds.) Wetteren: Universa Press (1976).

Dabrowski, K. Some aspects of ascorbate metabolism in developing embryos of the brine shrimp. Can. J. Fish. Aquat. Sci. 48: 1–3 (1991).

Dabrowski, K. Ascorbate concentration in fish ontogeny. J. Fish Biol. 40: 273–279 (1992).

Dhert, P., R. B. Bombeo, and P. Sorgeloos. Use of ongrown *Artemia* in nursery culturing of the tiger shrimp. Aquaculture International 1: 170–177 (1993b).

Golub, A. L. and F. J. Finamore. Ascorbic acid sulfate metabolism in the brine shrimp. Fed. Proc. 31: 706 (1972).

Hontoria, F., J. H. Crowe, L. M. Crowe, and F. Amat. Potential use of liposomes in larviculture as a delivery system through *Artemia* nauplii. Aquaculture 127: 55–264 (1994).

Hudinaga, M. Reproduction, development, and rearing of *Penaeus japonicus* Bate. Jpn. J. of Zool. 10: 305–393 (1942).

Johns, D. M., W. J. Berry, W. Walton. International Study on *Artemia*. XVI. Survival, growth and reproductive potential of the mysid, *Mysidopsis bahia*. J. Exp. Mar. Biol. Ecol. 53: 209–219 (1981).

Johns, D. M., M. E. Peters, and A. D. Beck. International Study on *Artemia*. VI. An evaluation of five geographical strains of shrimp *Artemia*: effects on survival and growth of two species of Brachyuran larvae. In: The brine shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in Aquaculture. pp. 291–304 (Persoone, G., P. Sorgeloos, O. Roels, and E. Jaspers, Eds.). Wetteren: Universa Press (1980).

Kontara, E. K., G. Merchie, P. Lavens, H. Nelis, A. De Leenheer, and P. Sorgeloos. Improved larviculture outputs of postlarval shrimp *Penaeus vannamei* through supplementation of l-ascorbyl-2–polyphosphate in the diet. In: Larvi'95. pp. 230–234 (Lavens, P., E. Jaspers, and I. Roelants, Eds.) Gent: European Aquaculture Society, Special Publication No. 24 (1995b).

Lavens, P., P. Léger, and P. Sorgeloos, P. Manipulation of the fatty acid profile in *Artemia* offspring produced in intensive culture systems. In: Aquaculture: a Biotechnology in Progress. pp. 731–739 (De Pauw, N., E. Jaspers, H. Ackefors, and N. Wilkins, Eds.) Bredene, European Aquaculture Society (1989).

Lavens P, Sorgeloos P (2000) The history, present status and prospects of the availability of Artemia cysts for aquacul- ture. Aquaculture 181:397–403

Léger, P., D. A. Bengtson, K. L. Simpson, and P. Sorgeloos. The use and nutritional value of *Artemia* as a food source. Oceanogr. Mar. Biol. Ann. Rev. 24: 521–623 (1986).

Léger, P., and P. Sorgeloos. International Study on *Artemia*. XXIX. Nutritional evaluation of *Artemia* nauplii from different geographical origin for the marine crustacean *Mysidopsis bahia*. Mar. Ecol. Prog. Ser. 15: 307–309 (1984).

Léger, P., P. Vanhaecke and P. Sorgeloos. International Study on *Artemia*. XXIV. Cold storage of live *Artemia* nauplii from various geographical sources: Potentials and limits in aquaculture. Aquacultural Eng. 2: 69–78 (1983).

Léger, P., P. Sorgeloos, O. M. Millamena, and K. L. Simpson. International Study on *Artemia*. XXV. Factors determining the nutritional effectiveness of *Artemia*: the relative impact of chlorinated hydrocarbons and essential fatty acids in San Francisco Bay and San Pablo Bay *Artemia*. J. Exp. Mar. Biol. Ecol. 93: 71–82 (1985a).

Lipko P, Papeschi AG, Cohen RG, Amat F (2004) Cytogenetic analysis of four Argentinean populations of Artemia (Crus- tacea Branchiopoda: Anostraca). Caryologia 57:283–289

Naessens, E., A. Pedrazzoli, V. Vargas, S. Townsend, M. L. Cobo, and J. Dhont. Evaluation of preservation methods for *Artemia* biomass and application in postlarval rearing of *Penaeus vannamei*. In: Larvi'95. pp. 338–341 (Lavens, P., E. Jaspers, and I. Roelants, Eds.) Gent: European Aquaculture Society, Special Publication No. 24 (1995b).

Naessens, E., P. Lavens, L. Gomez, C. L. Browdy, K. McGovern-Hopkins, A. W. Spencer, D. Kawahigashi, and P. Sorgeloos. Maturation performance of *Penaeus vannamei* co-fed *Artemia* biomass preparations. Aquaculture 155: 87–101 (1997).

Nelis, H. J., G. Merchie, P. Lavens, P. Sorgeloos, and A. De Leenheer. Solid phase extraction of ascorbic acid-2–sulfate from cysts of the brine shrimp *Artemia franciscana*. Anal. Chem. 66: 1330–1333 (1994).

Sakamoto, M., D. L. Holland, and D. A. Jones. Modification of the nutritional composition of *Artemia* by incorporation of polyunsaturated fatty acids using micro-encapsulated diets. Aquaculture 28: 311–320 (1982).

Seale, A. The brine shrimp *Artemia* as satisfactory live food for fishes. Trans. Am. Fish. Soc. 63: 129–130 (1933).

Sorgeloos, P. The brine shrimp, *Artemia salina*: a bottleneck in mariculture? In: FAO Technical Conference on Aquaculture, Kyoto 1976. pp. 321–324 (Pillay, T. V. R. and Wm. A. Dill, Eds.) Farnham: Fishing News Books Ltd. (1979).

Sorgeloos, P. 1980. The use of the brine shrimp Artemia in aquaculture. In: The brine shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in Aquaculture. pp. 25–46 (Persoone, G., P. Sorgeloos, O. Roels, and E. Jaspers, Eds.) Wetteren: Universa Press (1980).

Sorgeloos, P., P. Lavens, P. Léger, W. Tackaert, and D. Versichele. Manual for the Culture and Use of Brine Shrimp *Artemia* in Aquaculture. 319 pp. Gent: Artemia Reference Center (1986).

Sorgeloos, P. and P. Léger. Improved larviculture outputs of marine fish, shrimp and prawn. J. World Aquac. Soc. 23 (4): 251–264 (1992).

Stael, M., T. Sanggontanagit, E. Van Ballaer, N. Puwapanich, A. Tunsutapanich, and P. Lavens. Decapsulated cysts and *Artemia* flakes as alternative food sources for the culture of

Penaeus monodon postlarvae. In: Larvi'95. pp. 342–346 (Lavens, P., E. Jaspers, and I. Roelants, Eds.) Gent: European Aquaculture Society, Special Publication No. 24 (1995).

Tackaert, W., and P. Sorgeloos. Salt, *Artemia* and Shrimp. Integrated production in the People's Republic of China: The Tang Gu Saltworks. World Aquaculture 22 (3): 11–17 (1991).

Teshima, S., A. Kanazawa, H. Sasada, and M. Kawasaki. Requirements of the larval prawn, Penaeus japonicus, for cholesterol and soybean phospholipids. Mem. Fac. Fish. Kagoshima Univ. 31: 193–199 (1982).

Van Stappen, G., and P. Sorgeloos. The cosmopolitan brine shrimp *Artemia*. INFOFISH International 4: 45–50 (1993).

Watanabe, T., T. Tamiya, A. Oka, M. Hirata, C. Kitajima, and S. Fujita. Improvements of dietary value of live foods for fish larvae by feeding them on (n-3) highly unsaturated fatty acids and fat-soluble vitamins. Bull. Jpn. Soc. Sci. Fish. 49: 471 (1983).