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Design, operation, and potential of a culture system for the continuous production of *Artemia* nauplii[1]

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Abstract

A flow-through culture system is described for the controlled production of *Artemia* nauplii. High densities of adult brine shrimp are induced to ovoviviparity through diet manipulation, optimal culture water exchange, and high constant oxygen levels.

The nauplii produced are separated from the culture tank effluents in a special recuperation filter system. The culture water is recirculated over a rotating biodisc, a cross-flow sieve, and a plate separator.

The potential of this nauplii production system for application in aquaculture hatcheries is discussed.

Introduction

The application in aquaculture of ongrown *Artemia* as a food source is steadily increasing (Lai and Lavens, 1987). Research efforts of the Artemia Reference Center, Belgium, have resulted in improved techniques for the production of brine shrimp biomass (Brisset *et al.*, 1982 ; Lavens *et al.*, 1985). The same techniques which have been developed for intensive *Artemia* rearing from nauplius to adult under flow-through conditions can be used to maintain cultures of reproductively active animals. By applying cyclic oxygen stresses Lavens and Sorgeloos (1984) managed to induce and to maintain the oviparous reproduction mechanism. This paper reports how an exclusive nauplii-production can be achieved by minimizing stresses or fluctuations in culturing conditions. A recuperation system for the continuous harvesting of liveborn nauplii is also described.

Description and operation of the system (Fig. 1, 2)

The nauplii production system is integrated in the biomass culture unit of an existing flow-through recirculating installation, which has been described by Lavens *et al.* (1985).

[1] Technique protected by patent no. 83201335-3.

Artificial seawater of 25 °C and 50 ‰ salinity flows continuously into the 100 l culture tanks at a flow rate of 100 l/h, *i.e.* a retention time of 1 h as to remove as quickly as possible all soluble and particulate waste (eventually including freshly-born nauplii) via the cylindrical welded-wedge screen filter with a slit opening of 550 µm. Oxygen levels are kept around 4 ppm by the air-bubbling from the aeration collar surrounding the bottom part of the central filter. Following the culture procedures outlined in Lavens *et al.* (1985, 1987a) Great Salt Lake *Artemia franciscana* are fed on a micronized corn byproduct and cultured in the biomass production unit until 2-weeks-old. After harvesting at day 14, the adults are transferred to the 100 l tanks at densities of 5 000 brine shrimp/l.

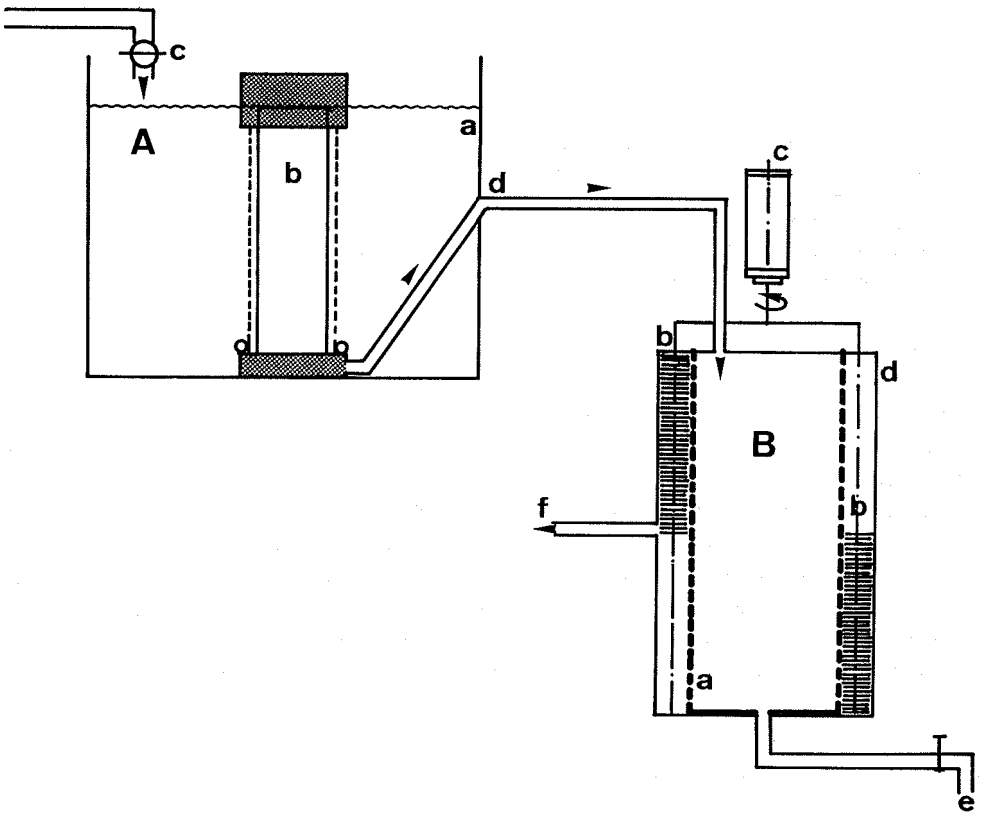
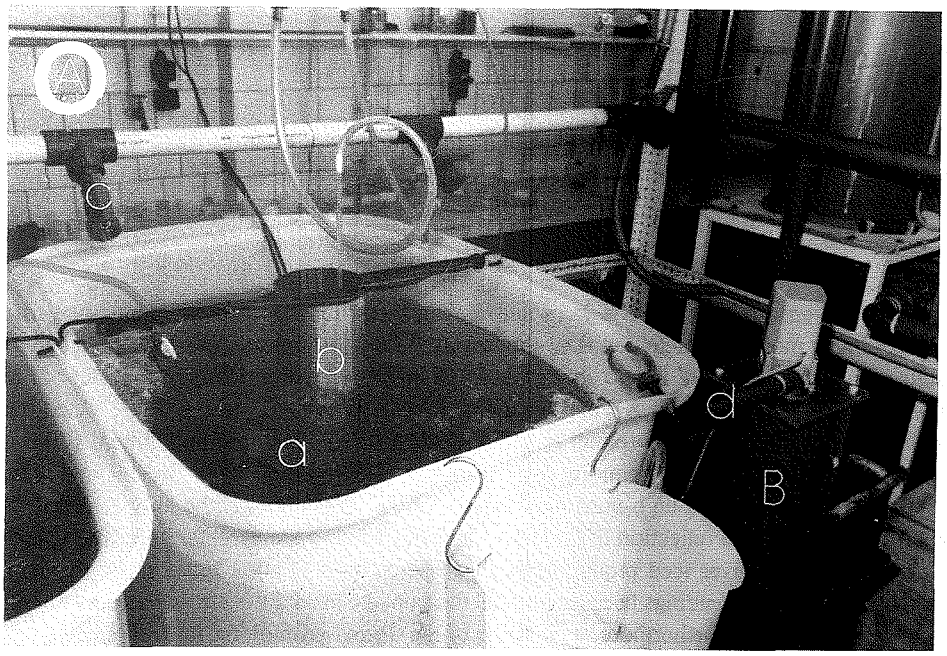


FIG. 1. Schematic drawing of the *Artemia* nauplii production system (not drawn to scale). A. Culture unit with 100 l tank (a), *Artemia* retaining filter (b), and in/outflow (c, d). B. Nauplii recuperation filter with welded-wedge filter (a), cleaning brushes (b) driven by rotor (c), cylindrical holding tank (d), collector drain (e), and effluent drain to recirculation systems (f).



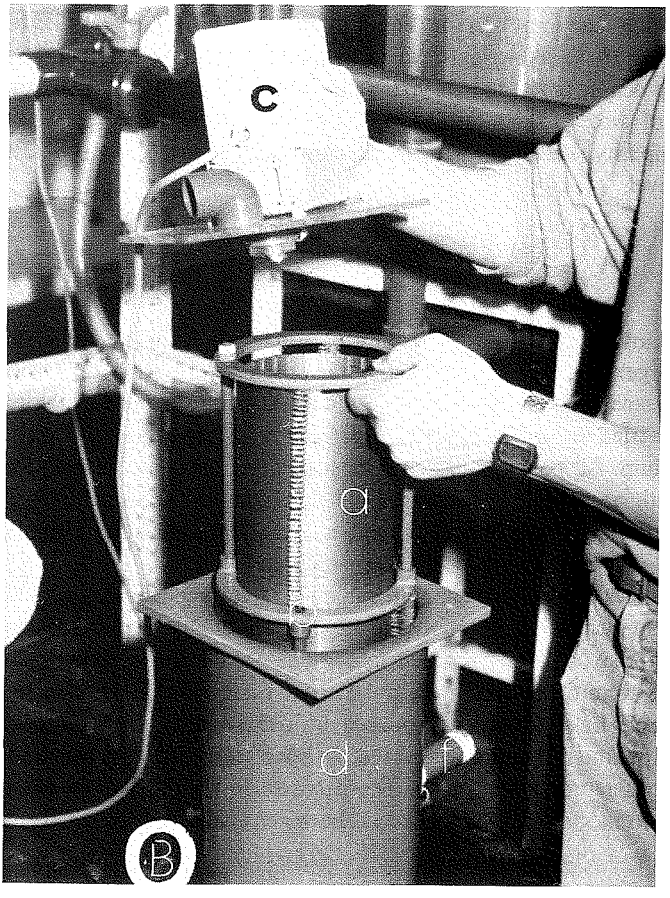


FIG. 2 A,B. Overview of the culture system for continuous production and separation of *Artemia* nauplii (for legend to abbreviations see Fig. 1).

The technique for controlled production of *Artemia* offspring requires three essential modifications of the biomass production technique :

Specific diet and feeding strategy for adults

In order to ensure high survival rates and maximal reproductive activity adult brine shrimp require a much more complete diet than the one used for larval growth. We have obtained good results with a mixed diet of fresh baker's yeast and an emulsified enrichment product of highly unsaturated fatty acids (HUFA) and vitamins (Selco, Artemia Systems NV), occasionally supplemented with a corn by-product (Lavens *et al.*, 1987a). The lipid enrichment (especially HUFA) not only enhanced fecundity levels, as was also reported by Provasoli and Pintner (1980), but also ensured a high nutritional quality of the offspring ; *i.e.* the fatty acid pattern of the produced larvae is a reflection of the diet composition fed to the parental population (Lavens *et al.*, 1987b). Moreover feed requirements in dense cultures of adult brine shrimp cannot be dosed by transparency readings because optimal food uptake in adults is already achieved at much lower concentrations. Furthermore, as a result of the decreased molting rates in adult *Artemia,* the setae of the thoracopods become easily clogged with food at the higher particle densities. This affects the respiration and feeding functions, and may result in decreased survival rates (Table I). Instead, a daily feeding ratio of 10 % dry weight feed to live weight biomass distributed on a semi-continuous basis (every 5 min over the 24-h period) yielded best production results (Table I). Higher amounts of feed (15 %) resulted in increased mortality rates and stress conditions which induced oviparity. Fecundity and reproductive activity levels were, however, better for the 15 % feeding level.

Induction of ovoviviparity

According to the literature, oviparity or cyst production is basically induced by cyclic stress conditions (Versichele and Sorgeloos, 1980 ; Lavens and Sorgeloos, 1984 ; Berthélémy-Okazaki and Hedgecock, 1987). Since sofar nothing has been reported about controlled ovoviviparity or nauplii production, we have tried to avoid stresses, especially with regard to the oxygen conditions in the culture medium. All potential bacterial substrates (*e.g.* exuviae, uneaten food, faeces) are removed as efficiently as possible by the use of high flow-rates and specific filters. The feed (a Single Cell Protein) has optimal physical properties (maximal ingestibility, minimal probability of bacterial breakdown) and is distributed semi-continuously.

The baker's yeast seems to have a beneficial effect in enhancing ovoviviparity, *i.e.* maybe its deficiency in pigments causes an incapability in the *Artemia* to produce the haem-pigment which is an essential component in the oviparous reproduction mode (under these experimental circumstances only pale adults were observed). From the data in Table I it appears that in all cultures most of the Great Salt Lake females reproduced by ovoviviparity. The differences between day 28 and 35 for experiment B and C reveal that an adaptation period is required to reach a dominant ovoviviparous reproduction mode. The decrease in the number of females carrying nauplii in A was due to mortalities which affected the water quality and initiated stress in the surviving population.

TABLE I

Population characteristics of Great Salt Lake *Artemia* cultures
set up at an animal's age of 14 days and fed baker's yeast under three different feeding regimes

	A In relation to culture medium transparency	B Ratio dry weight food to live weight *Artemia* biomass = 10 %	C Ratio dry weight food to live weight *Artemia* biomass = 15 %
Animal length (in mm) at day 14	4.0	6.1	6.1
day 28	7.1	7.0	7.4
day 35	–	7.3	7.8
Percent survival at day 14	100	100	100
day 28	80	92	84
day 35	40	74	62
Percent of reproductive active females at day 14	0	< 5	< 5
day 21	< 5	8	17
day 28	30	44	66
day 35	10	67	88
Percent ovoviviparous females at day 28	85	–	60
day 35	50	95	90
Fecundity at day 35 (number of nauplii/female)	31	64	76

RECUPERATION TECHNIQUE FOR CONTINUOUS HARVESTING OF THE NAUPLII

The system consists of an inversed type of a welded-wedge screen cylinder (150 μm slit opening) precisely fitted into a cylindrical PVC holding tank (Fig. 1, 2B). The half-submerged filter retains all produced nauplii and particles larger than 150 μm from the culture effluents and drains water and small wastes via the holding tank back into the recirculating unit. Although this 150 μm filter has a high filtering capacity, a mechanical cleaning system is needed if a 24 h autonomy is required : *i.e.* two brushes driven by an electrical rotor (12 rpm) rubb the outer surface of the welded-wedge screen. The nauplii are harvested once or twice a day and separated from the waste materials by taking advantage of the phototactic behavior of the larvae. For this, use is made of a cylindro-conical tank (100 l) with a central overflow tube above which a hole is cut in the cover and a light bulb is installed. The nauplii suspension is poured into the separator-tank and a water flow is adjusted as to let all particles sediment and evacuate the nauplii attracted by the light with the overflowing water.

This harvesting technique seems to operate very efficiently. Preliminary production trials with this new set-up yielded 30 g wet weight nauplii/day/100 l culture tank, suggesting a daily reproduction rate of 10 nauplii/female. Moreover, only early larval stages were collected, suggesting an efficient recuperation from the culture tanks.

Potential of an *Artemia* nauplii production system

The technique for the controlled production of ovoviviparous nauplii offers interesting prospects for application in aquaculture hatcheries. It may not only create an independency from the international cyst market with its fluctuating prices, available quantities, and quality but it also provides a far better control of the quality of this live food product, with even the possibility to produce offspring of a far superior quality (Lavens *et al.*, 1987b).

Such a technique also allows a proper integration of *Artemia* in the aquaculture plant : the produced offspring can be used either directly as food source in the hatchery, or for stocking culture tanks which produce juvenile and reproductive active *Artemia* to be fed respectively to nursery and maturation stages of the predator. This vertical integration may, as a result of its beneficial impact on fish or crustacean outputs, greatly improve the cost effectiveness (Lavens *et al.*, 1985), resulting in economically feasible intensive *Artemia* production plants (Lai and Lavens, 1987).

Besides industrial applications, a controlled nauplii-production technique furthermore offers opportunities for biological research with regard to comparative studies (*e.g.* morphological, physiological, and biochemical characteristics) between oviparous and ovoviviparous offspring, produced under identical circumstances ; and the selection of obligate ovoviviparous *Artemia* populations which have a genotypical adaptation towards ovoviviparous reproduction, *e.g.* Laysan Lake *Artemia* (Lenz and Dana, 1987).

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Literature cited

BERTHÉLÉMY-OKAZAKI N. and D. HEDGECOCK. 1987. Effect of environmental factors on cyst formation in the brine shrimp *Artemia*. In : *Artemia* research and its applications. Vol. 3. Sorgeloos P., D. A. Bengtson, W. Decleir, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium.

BRISSET P. J., D. VERSICHELE, E. BOSSUYT, L. DE RUYCK, and P. SORGELOOS. 1982. High density flow-through culturing of brine shrimp *Artemia* on inert feeds. Preliminary results with a modified culture system. Aquacult. Eng. 1(2):115-119.

LAI L. and P. LAVENS. 1987. Workshop report : *Artemia* as a business perspecive. In : *Artemia* research and its applications. Vol. 3. Sorgeloos P., D. A. Bengtson, W. Decleir, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium.

LAVENS P., P. BAERT, A. DE MEULEMEESTER, E. VAN BALLAER, and P. SORGELOOS. 1985. New developments in the high density flow-through culturing of brine shrimp *Artemia*. J. World Maricult. Soc. 16:498-508.

LAVENS P., A. DE MEULEMEESTER, and P. SORGELOOS. 1987a. Evaluation of mono- and mixed diets as food for intensive *Artemia* culture. In : *Artemia* research and its applications. Vol. 3. Sorgeloos P., D. A. Bengtson, W. Decleir, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium.

LAVENS P., P. LÉGER, and P. SORGELOOS. 1987b. Manipulation of the fatty acid profile in *Artemia* offspring produced in intensive culture systems. p. 65-66. In : Book of abstracts. 'Aquaculture Europe 87'. Amsterdam, June 1987, Europe. Aquacult. Soc. 124 p.

LAVENS P. and P. SORGELOOS. 1984. Controlled production of *Artemia* cysts under standard conditions in a recirculating culture system. Aquacult. Eng. 3:221-235.

LENZ P. and G. DANA. 1987. Life-cycle studies in *Artemia* : a comparison between a subtropical and a temperate population. In : *Artemia* research and its applications. Vol. 3. Sorgeloos P., D. A. Bengtson, W. Decleir, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium.

PROVASOLI L. and I. J. PINTNER. 1980. Biphasic particulate media for the parthenogenetic *Artemia* of Sète. p. 231-255. In : The brine shrimp *Artemia*. Vol. 2. Physiology, Biochemistry, Molecular biology. Persoone G., P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 428 p.

VERSICHELE D. and P. SORGELOOS. 1980. Controlled production of *Artemia* cysts in batch cultures. p. 231-246. In : The brine shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in aquaculture. Persoone G., P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 428 p.