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# NEW DEVELOPMENTS IN THE HIGH DENSITY FLOW-THROUGH CULTURING OF BRINE SHRIMP *Artemia*

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## ABSTRACT

A closed culture system for the high density culturing of *Artemia* spp. in 300-liter tanks using a new formula of artificial seawater and micronized waste products from agricultural crops as food is described. Culture conditions are greatly improved by applying a new filter technique and an electronic transparency meter which controls food distribution automatically. A total volume of 6,000 liters of culture water is recirculated over a rotating biological contactor, a cross-flow sieve and a plate separator at a flow rate of 3,000 liters per hour.

Production yields obtained with this culture system, when extrapolated to a one $m^3$ tank, indicate that 50 g of brine shrimp cysts can be converted into 20 kg of fresh (wet dry) pre-adult *Artemia* in 14 days. These results are comparable to those reported earlier with open flow-through conditions.

## INTRODUCTION

Although it is known in fish and crustacean farming that adult *Artemia* is a suitable source of food (60% protein content), large-scale commercial use of brine shrimp biomass is still limited to the aquarium pet industry because of the high product cost (Sorgeloos 1980). Since the 1980's, however, the use of juvenile and adult *Artemia* spp. in aquaculture operations is gradually starting: i.e., at a few places in South America and Southeast Asia, brine shrimp biomass is harvested from extensive pond production systems and fed in commercial fish and penaeid shrimp farms (Persoone and Sorgeloos 1982). Nursery yields with both fish and shrimp are significantly increased by using juvenile brine shrimp (De los Santos, Brazil, personal communication; Guimaraes, Brazil, personal communication). Moreover, maturation in various penaeid species is much more effective by using adult *Artemia* than with eyestalk ablation only (Vieira de Castro, Brazil, personal communication).

Extensive pond production of *Artemia*, however, is limited to warm climates with long dry seasons, and may in this way restrict its inte-

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gration into aquaculture systems only to those sites. In this regard, the development of alternative production techniques, whereby the *Artemia* can be cultured on a year-round basis in controlled systems at densities much higher than those in ponds, can overcome this problem and is therefore gaining more interest. Batch production of brine shrimp in air-water-lift operated culture tanks is not commercially attractive because biomass outputs are rather small (5 $kg/m^3$) in proportion to the amount of labor input (Bossuyt and Sorgeloos 1980).

The solution for commercial production of *Artemia* biomass may be found in flow-through culture techniques. These techniques allow an increased use of automation, higher stocking density potential and in general a greater degree of control over environmental parameters. In this way very high production yields may be guaranteed. Initial reports on this culture technique examined open flow-through culturing of *Artemia* using the effluents of simulated OTEC (Ocean Thermal Energy Conversion) algae ponds as a combined culture medium and food source (Roels et al. 1979; Tobias et al. 1979) or using geothermal well water and a micronized rice bran feed (Brisset et al. 1982). Production yields averaged 20 to 25 $kg/m^3$ of culture tank. Commercial applications with these open flow-through culturing systems, however, are again restricted to a limited number of situations, mainly determined by the year-round availability of good quality warm culture water.

We report the adaptation of this production technique for operation in closed culture conditions and introduce some important improvements concerning the filter and automatic feeding system.

## DESIGN AND OPERATION

The new pilot system with a total culture volume of six $m^3$ of seawater and a total flow rate of 3,000 liters/hour is schematically outlined in Figure 1. It consists of four components: six 300-liter culture tanks, a biological treatment system, a particle separation unit and a stock tank with constant head tower. Synthetic seawater is made up with six technical grade salts (Table 1) using automatic brinomat systems and tapwater (Sorgeloos et al. 1983). This new culture medium has a salinity of 35 ppt. Temperatures are kept at 25±1°C using two thermostatically controlled submerged porcelain 2 KW heaters. Heaters are placed at the end of the biological treatment unit (Fig. 1).

Table 1. Composition of the Artificial Seawater Used in *Artemia* spp. Culturing

| Components | Concentration in tap water (g/l) |
|---|---|
| Evaporated sea salt (NaCl) | 31.08 |
| $MgSO_4$ | 7.74 |
| $MgCl_2$ | 6.09 |
| $CaCl_2$ | 1.53 |
| KCl | 0.97 |
| $NaHCO_3$ | 2.00 |

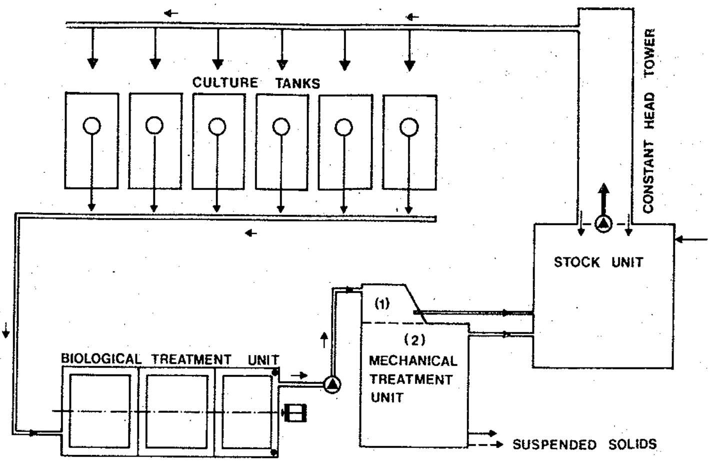


Figure 1. Schematic view of closed flow-through system for *Artemia* culturing. (1) cross-flow-sieve, (2) plate separator. ● = heaters Arrows denote direction of flow.

## CULTURE SYSTEM

Water continuously flows into each rectangular culture tank (100 x 70 x 60 cm) via a supply pipe connected at its end to the constant head tower. Water retention times in the culture tanks can be adjusted for different amounts of animal biomass from 3 hours to half an hour by valves connected to the water intake pipe. Culture effluents, loaded with fecal pellets, food wastes and discarded exoskeletons, are drained by gravity from the culture tanks' filter system into a central drain pipe that feeds the biological treatment unit, and in a second step the mechanical treatment unit.

Instead of cylindrical or rectangular nylon screen filters as reported by Tobias et al. (1979), Brisset et al. (1982), and Lavens and Sorgeloos (1984), a new type of cylindrical filter system (Fig. 2) is used to separate effluents from the *Artemia* population in the culture tank. It consists of a vertically placed stainless steel welded wedge screen cylinder (13.5 cm diameter), the bottom and top part of which extends into PVC rings. A circular aeration collar, fixed to the bottom ring, produces a curtain of rising air bubbles which help to keep the filter screen from clogging. At the same time it assures sufficient culture aeration and mixing. The upper PVC ring extends until just underneath the water surface, preventing the cultured larvae to be foamed off. The filter system, furthermore, contains an inner PVC cylinder which assures a kind of laminar flow pattern. As a result the self-cleaning capacity of the filter is greatly improved.

Depending on the growth stage of the brine shrimp larvae, and in order to have a maximal separation of the suspended solids from the *Artemia* cultures, the central filter cylinders are exchanged every second to fourth day for one with a larger slit opening. The first filter has a slit opening of 150 μm, the last one of 400 μm.

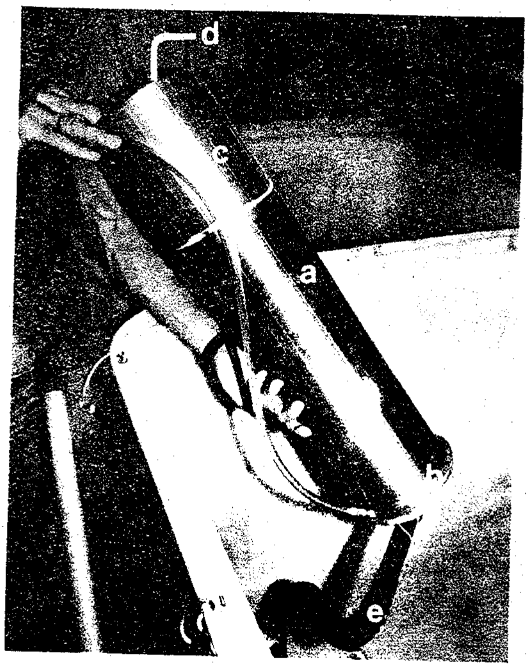


Figure 2. Photograph of the new filter system for selective draining of culture water and waste particles, consisting of (a) stainless steel welded wedge screen cylinder, (b) aeration collar, (c) upper PVC ring, (d) inner PVC cylinder and (e) drain tube.

The use of this new filter system greatly improves the culture conditions resulting in increased production yields, especially when non-algal diets are used as feed for *Artemia* culturing. Clogging rates are greatly reduced as compared to the nylon filters and particle removal is much more efficient. This implies also that the daily labor-intensive cleaning of filters is reduced to the smallest screen sizes (150, 200 and 250 µm). The remaining filters (300, 350 and 400 µm screens) used in tanks with bigger brine shrimp only need to be cleaned every other day.

In accordance with Bossuyt and Sorgeloos (1980), Dobbeleir et al. (1980), and Brisset et al. (1982), optimal feeding conditions are created by adding food (e.g., a suspension of micronized rice bran or soybean bran in saturated brine) every 3 minutes using automatic pumps. Time clocks and pumping rates are adjusted daily to maintain a culture medium transparency of about 25 cm. An improved food distribution system is outlined in Figure 3; its main component is a small constant head which assures equal food additions throughout day and night.

Furthermore, a simple electronic transparency meter (Fig. 4) is connected to the effluent of each tank to monitor for eventual overfeeding conditions. When the culture medium transparency drops below a pre-

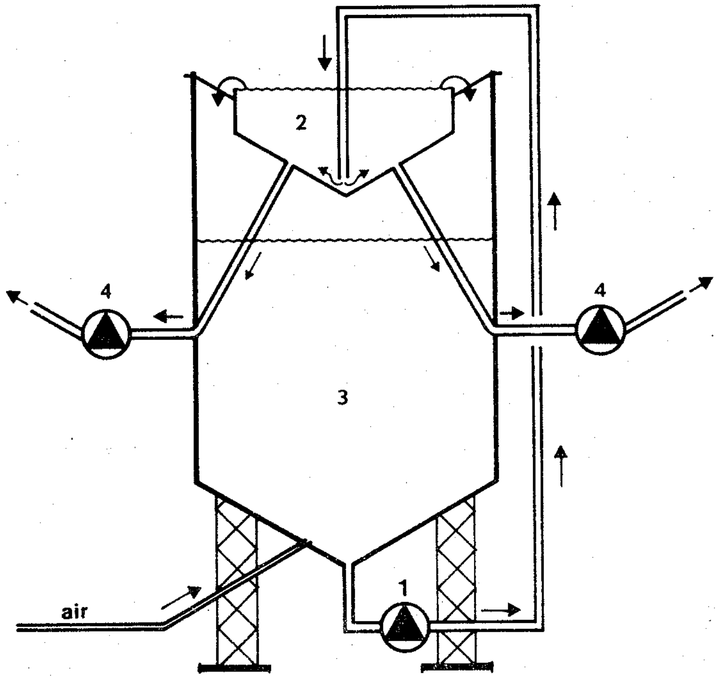


Figure 3. Schematic diagram of the food distribution system: food is continuously pumped (1) into the constant head tank (2), the overflow of which drains into the main reservoir (3); activated by the electronic timers, which can be preset for each culture tank, pumps (4) distribute feed suspension to the respective culture tanks.

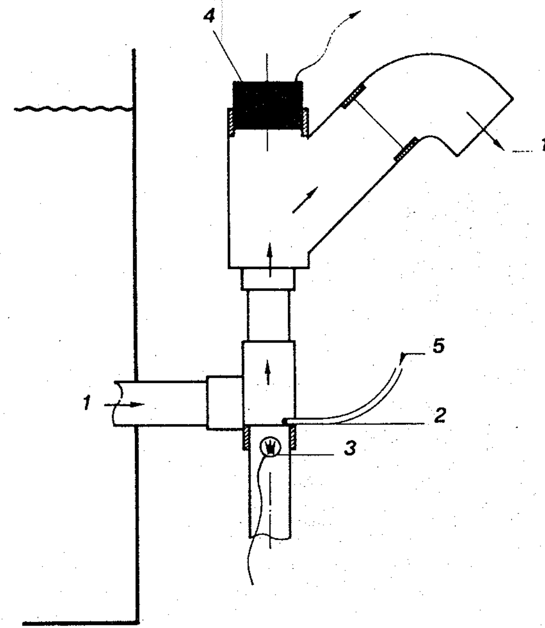


Figure 4. Schematic diagram of the electronic transparency meter: (1) drain of culture water effluent; (2) transparent PVC plate; (3) 6v light source; (4) light receptor (light depending resistance type HS10-Electromatic) connected to electronic detector; (5) air injection.

set critical minimum, the electronic meter will shut off the power for the food distribution pump. The continuous water flow through the apparatus, together with a regular air injection close to the light emitter, limits sedimentation and clogging. Compared to the turbidimeter as described by Versichele et al. (1979) this system has a less complicated construction and may assure more accurate transparency measurements over a 24-hour period.

RECIRCULATION SYSTEM (Fig. 5)

Culture tank effluents are drained into the rotating biological contactor for biological treatment. Biodisc purification has been selected because of its stable operation under conditions of fluctuating hydraulic and organic loadings (Clark et al. 1977; Autotrol 1978) and because of its successful use in other fish and shrimp culturing closed systems (Lewis and Bynak 1976; Mock et al. 1977; Lavens and Sorgeloos 1984; Rogers and Klemetson 1985). It consists of three units of 40 sand blasted PVC disks (one meter in diameter) that rotate at 6 rpm in interconnected compartments. Total effective contact surface is 190 $m^2$.

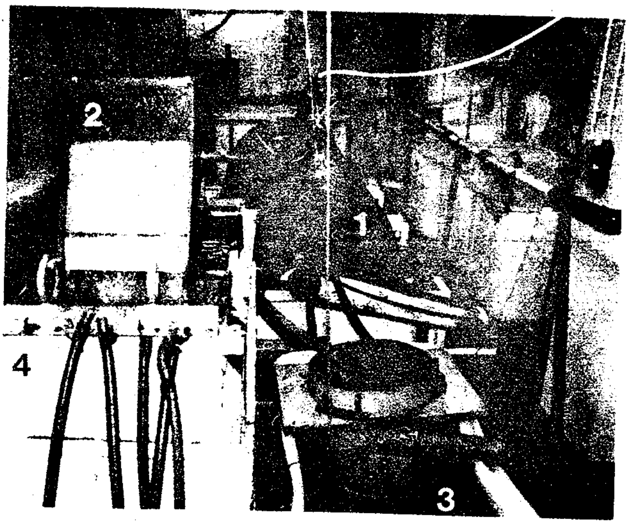


Figure 5. Overview of the recirculation system: (1) biodisc; (2) cross-flow sieve; (3) stock tanks; (4) plate separator.

The biodisc effluent is pumped over a cross-flow sieve with a slit opening of 150 µm (Bossuyt and Sorgeloos 1980). The overflow of the cross-flow sieve is further treated in a plate separator, constructed in accordance to Bossuyt and Sorgeloos (1980). Culture water free from suspended solids is finally drained from the cross-flow sieve and the plate separator into a stock reservoir. From there is is pumped into the constant head tower to which the central supply tube is connected.

The biological and mechanical treatments result in purification rates of less than 5 mg/l BOD-5 at a total flow rate of ±3,000 liters per hour and for a total culture volume of 6 $m^3$ artificial seawater. A small quantity of water is renewed daily resulting in a total change of 100% every month. This also takes into account the water losses due to evaporation. The concentrations of nitrogen compounds always remain below 0.10 ppm for ammonia and 0.15 ppm for nitrite.

## RESULTS AND DISCUSSION

Under standard conditions about three million *Artemia* nauplii, hatched following the procedures described in Sorgeloos et al. (1983), are reared in 300 liters of culture water. As shown in Table 2 for an average culture with Great Salt Lake *Artemia*, survival rates remain above 55% and animal length averages 6 mm at the end of the 2-week culture period.

Table 2. Results of Culture Test Using Great Salt Lake *Artemia franciscana* (25°C; 35 ppt; YM 20 mixed diet manufactured by Artemia Systems)

| Day from start of culture | Survival (%) | Growth (mm) | Biomass production (g/l) |
|---|---|---|---|
| 1 | 100 | 0.5 | -- |
| 5 | 71 | 2.4 | 2.3 |
| 10 | 58 | 4.8 | 10 |
| 14 | 57 | 5.8 | 17 |

Although the present flow-through recirculation system has not yet been optimized for various culture parameters, e.g. *Artemia* strains, animal densities, etc., production yields are comparable to results reported earlier in open flow-through cultures in the Virgin Islands (Tobias et al. 1979, 1980) and in Belgium (Brisset et al. 1982). Depending on the *Artemia* strain (Great Salt Lake *Artemia franciscana*) and the quality of the food used (YM 20 diet), one 300-liter tank yields 6 kg live weight pre-adult brine shrimp after 2 weeks. A total 4.6 kg micronized *Artemia* food (special mixed diet YM 20, manufactured by Artemia Systems N.V.) will be consumed during this culture period. Extrapolated to a total culture volume of one $m^3$ this means a production capacity of 20 kg *Artemia* for a food consumption ratio of 0.75. Further detailed results obtained with different feeds and animal densities are discussed in De Meulemeester et al. (1985).

Although this flow-through recirculation system has been functioning properly for a year and a half, heavy losses of pre-adult *Artemia* have occurred from time to time due to infections with filamentous *Leucothrix* bacteria. A partial solution was first found by continuously oxidating the culture water with hydrogen peroxide at 10 ppm. These infections are now controlled by increasing the culture water salinity to 50 ppt by adding sea salt.

The nutritional composition (e.g. fatty acids, vitamins, carotenoids) of the produced animals can be manipulated by application of the simple bioencapsulation technique of Léger et al. (1985). Using this enrichment procedure the nutritional composition of the adult *Artemia* can be modified in less than one hour's time. Thus, foods of varying nutritional value for specific predators can be developed.

## CONCLUSIONS

This flow-through recirculation system has proven to be a reliable technique for the high-density culturing of *Artemia* spp. Present yields of 15 to 20 kg live weight *Artemia* per $m^3$ culture tank per 2 weeks are comparable to those obtained in open flow-through cultures.

Culture success is mainly ensured by the use of the new filter concept. As compared to conventional mesh-screen filters, cross-flow sieve cylinders are much more efficient in particle removal and in the reduction of clogging rates. We are convinced that this filter system has much potential for use in large-scale culturing of zooplankton and shrimp/fish larvae. As compared to the flowing-bed reactor of Brune (1982), designed to solve the clogging problems of classical filters, our device may be more simple for upscaling. In fact, the commercial applicability of the present technique for closed flow-through culturing of *Artemia* is now being tested in Belgium by the company Artemia Systems N.V. for a production capacity of 100 kg brine shrimp per week.

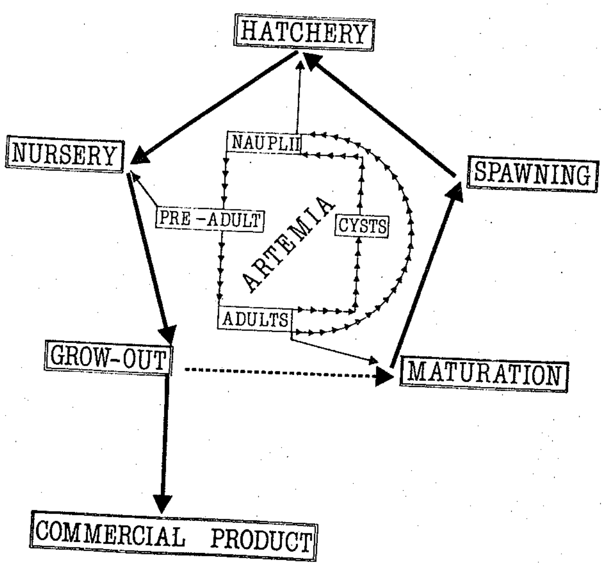


Figure 6. Outline of the integrated use of various *Artemia* products in fish/shrimp production.

Significant increases in production capacity can be expected through specific research and development with regard to *Artemia* strain selection, physical and nutritional characteristics of the feeds used, and the animal density.

Considering the beneficial rôle of the integrated use of various *Artemia* products in fish and shrimp farming, as mentioned above and as is schematically outlined in Figure 6, the present culture system may offer potential for worldwide application in the field of mariculture. When applied as an integral part of the hatchery, nursery and maturation operations, its cost effectiveness might greatly improve as a result of the significant increase in fish and crustacean outputs. In this regard upscaling of the promising laboratory technique for continuous nauplii production (Lavens and Sorgeloos 1985) might increase the possibility for a total integration of the brine shrimp culture system and in this way might create a complete independence for this valuable live food, *Artemia*.


## ACKNOWLEDGMENTS

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