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A Simple Semi Flow-Through Culture Technique for the Controlled Super-Intensive Production of *Artemia* Juveniles and Adults

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ABSTRACT

A simple semi flow-through Artemia *culture unit is described for possible integration in marine fish and shellfish hatcheries as source of a cheap nursery diet. The system does not require the use of feeding pumps and involves minimal care. Food preparation and addition to the feeding tank is reduced to one or two manipulations per day during the meta-nauplius stages (day 1–3) and the juvenile stages (day 4–14), respectively.*

Biomass productions are superior to those reported for stagnant culture systems and are comparable to those demonstrated for flow-through culture units. This simple rearing technique offers the possibility of producing brine shrimp populations with a uniform size. Furthermore, by varying the feeding regime with the Artemia *density at the start of the culture, specific* Artemia *prey sizes corresponding to the daily physical requirements in shrimp and fish hatcheries can be obtained.*

INTRODUCTION

Super-intensive *Artemia* culture systems were proposed in the late 1970s with the introduction of batch culture technique using rice bran as food source (Bossuyt & Sorgeloos, 1980, 1981), and a flow-through concept using live micro-algae as food (Tobias *et al.*, 1979, 1980). The latter technique has later been adapted for the use of agricultural by-products as a cheaper food source for brine shrimp (Brisset *et al.*, 1982; Lavens *et al.*, 1985). Depending on production conditions yields up to

5 kg m^3 (wet weight) and 25 kg/m^3 after 14 days culture could be achieved for stagnant and flow-through culture systems, respectively.

Despite these breakthroughs and the documented advantages of using bigger *Artemia* in fish and shrimp larval rearing (Léger *et al.*, 1986; Lavens & Sorgeloos, 1991) the practical impact of super-intensive systems in aquaculture has not become evident so far. Batch production of brine shrimp in air-water-lift operated culture tanks is not very commercially attractive because biomass output is rather small (Bossuyt & Sorgeloos, 1980). On the other hand, the production of *Artemia* biomass under flow-through conditions is characterized by a cost-intensive automation that still requires skilled personnel. Furthermore, in many situations the need for high amounts of warm sea-water implies the use of a water re-use system consisting of mechanical and biological water treatment, which makes the system complex, space-consuming and vulnerable to unexpected mortalities (Lavens *et al.*, 1985).

Despite these problems in integrating such systems into aquaculture facilities, interest in tank produced *Artemia* is still high because of its manifold applications. The indoor culture offers indeed a much better control over diseases and is independent from the season or the climate. Moreover, the easy access to an indoor set-up facilitates frequent harvesting and reinoculation of the system. This means that a strategy, consisting of a controlled production of *Artemia* of specific stages (e.g. metanauplii, juveniles, pre-adults, adults) can be envisaged by programming the length of the rearing period. In this way an appropriate prey of consequently larger size can be offered in accordance to the growth of the predator. The application of feeding ongrown *Artemia* would also contribute to a serious reduction in the expense for larval food (Sorgeloos *et al.*, 1987). The larger prey does not only contribute to a more effective food uptake and earlier satiation of the fish, it is also preferred for its superior nutritional digestibility, i.e. juvenile and adult brine shrimp have a thin exoskeleton, are rich in essential amino acids and constitute an ideal source of animal protein (60% of the dry weight).

Contrary to what is found in wild adults, the fatty acid profile of brine shrimp cultured on feeds of terrestrial origin is low (Dobbeleir *et al.*, 1980). However, the bio-encapsulation technique as described in Léger *et al.* (1986) is very effective in remedying this deficiency, i.e. in less than 1 h essential nutrients as well as pigments, prophylactics, therapeutics or hormones can be incorporated into the *Artemia*.

In order to simplify the *Artemia* culture techniques described in literature and to facilitate its integration into fish and shellfish hatcheries a practical rearing technique has been developed.

MATERIALS AND METHODS

The pilot system consists of six oval-shaped raceways (Bossuyt & Sorgeloos, 1980) of 1000 litres content and six resevoir tanks of the same capacity placed above each raceway (Fig. 1). The latter stock tanks hold sea-water and food, and need manually refilling once or twice a day. A siphon with selected diameter drains by gravity the reservoir into the *Artemia* culture tank. The culture effluent loaded with faecal pellets, food wastes and discarded exoskeletons is drained through the cylindrical filter-system which retains the *Artemia* in the culture tank (Lavens *et al.*, 1985). This filter consists of a welded-wedge screen cylinder made of stainless steel, placed vertically in the raceway at the opposite end to the water inlet. The filters are cleaned daily and exchanged for others with larger slit openings when the brine shrimp grow bigger. A set of filters covering the total culture period until adulthood consists of five different units with slit openings ranging from 150 to 400 μm.

The food consists of a rice bran suspension prepared by mixing for 5 min one volume of non-micronized rice bran and four volumes of sea-water. This suspension is subsequently squeezed through a 60-μm plankton net in order to obtain only the particle sizes suitable for the brine shrimp (Dobbeleir *et al.*, 1980; Lavens & Sorgeloos, 1991). Using this methodology, the concentration of the suspended rice bran varies from 20 to 30 g/litre, depending on the quality of the rice bran and the efficiency during squeezing. The exact value can be determined by subtraction of the weight of a volumetric flask filled with the freshly prepared food and the weight of the same flask filled with sea-water only. This food suspension is further diluted to the required concentration in the resevoir and kept in suspension by means of a strong aeration through air lines. Food distribution depends on the requirements of the

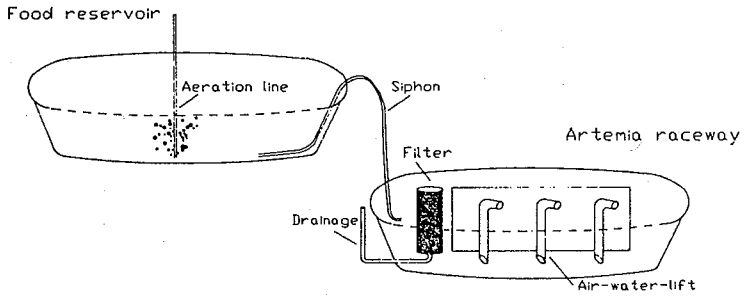

Fig. 1. View of the semi flow-through culture system for *Artemia*.

animals and is basically controlled by transparency measurements in the raceway system (Bossuyt & Sorgeloos, 1980).

Each raceway is inoculated with instar-I *Artemia* of Great Salt Lake (GSL) origin hatched following the procedures described by Sorgeloos *et al.* (1983). Start densities in the different experiments are 2000, 5000 or 8000 animals per litre, depending on the experiments. Water temperature in the tanks is not controlled and follows the ambient temperature of the hatchery. Maximal diurnal temperatures of 30°C are registered after noon time, while the minimum nocturnal temperature attain 25°C. Experiments are only conducted during the dry season when the salinity of the sea-water oscillates between 30 and 32 ppt. Treatments are performed in six replicates and repeated in time. Individual length and survival rates are recorded daily by sub-sampling the culture tanks. Three samples of 60 ml are taken and fixated with lugol solution. After counting the number of *Artemia*, the average length of 30 animals measured from the top of the head to the base of the caudal furca is determined using a dissecting microscope equipped with a drawing mirror. In order to smooth irregular data resulting from density (survival) counts, the data are exposed to the method of proceeding averages. Biomass production yields are calculated by wet weight analysis of the brine shrimp after harvesting and squeezing the *Artemia* in a mesh net.

RESULTS AND DISCUSSION

Standardization of the semi flow-through culture system

The effectiveness of the semi flow-through feeding technique was initially tested by using water/food resevoirs of 300 litres only, which needed to be refilled every 4 h. In this way, feed concentrations could be adjusted frequently in order to maintain the transparency levels in the raceway system which were expected to be optimal (Table 1). Since *Artemia* nauplii only start ingesting food after the first molting, they are initially kept in a stagnant water medium containing rice bran (transparency reading of 15 cm). From the next day onwards, when all larvae have molted into the second and third larval stages and were actively feeding, flow-through conditions were installed by means of a small nalgene siphon (inner diameter 4 mm: flow rate 30–50 litres/h) in order to maintain transparency levels at 15–20 cm. At this larval stage one food resevoir of 300 litres was sufficient to compensate the food uptake of the nauplii. After the third day of culture the animals developed thoracopods, eventually increasing their filtering capacity. As a result water

TABLE 1
Feeding Conditions and Growth of *Artemia* during the Preliminary Run in a Semi Flow-Through Culture System (Start Density: 2000 *Artemia*/litre)

Day of culture	*Culture medium transparency (cm)*	*Number of refillings of the 300-litre food reservoir*	*Daily food supply (g)*	*Average food concentration per filling (g/litre)*	*Animal length (mm)*
0	15	0	14		0·57 ± 0·09
1	15–20	1	35	0·12	1·02 ± 0·09
2	15–20	2	87	0·15	1·54 ± 0·12
3	15–20	2	89	0·15	1·88 ± 0·10
4	20–25	4	182	0·15	2·73 ± 0·27
5	20–25	4	140	0·12	3·96 ± 0·60
6	20–25	4	140	0·12	4·35 ± 0·43
7	> 30	4	177	0·15	5·39 ± 0·25
8	> 30	4	140	0·12	5·80 ± 0·46

transparency in the culture medium was raised to 20–25 cm, and maintained by increased flow rates which necessitated frequent refillings of the food reservoir. Although the volume of the concentrated rice bran added to the feeding reservoirs was increased daily, a constancy in the daily average food concentration was observed during the complete culture period. The number of refillings was low before the appearance of the thoracopods, but doubled and remained constant after the metamorphosis of the larvae. This may suggest the possibility of rearing nauplii to pre-adult brine shrimp using only one constant daily amount of food which would be distributed in one feeding before the appearance of the thoracopods and which could be divided in two feedings in a double amount of water after the formation of the thoracopods.

Since the results of the first experiment showed that an average food concentration of 0·14 g/litre could support the culture, the size of the food reservoir was scaled up to 1000 litres and 140 g of concentrated rice bran solution was added daily. The larger volume of the food reservoir offered the advantage of reducing the refilling of the reservoirs to once every 24 h. Starting on Day 4 the rice bran supply in the food reservoir was reduced by 50% (70 g) and the siphon replaced by a hose of bigger diameter (inner diameter 8 mm, flow rate at initial full water capacity 150l/h). The increased flow rate reduced the operation time of the siphon to approximately 12 h. Consequently, as of that day a second refilling of the food reservoir was needed, although the daily food supply of 140 g remained constant. The increased water exchange furthermore ensured an optimal water quality. Due to the continuous food supply all animals were exposed to the same feeding conditions, which resulted in a homogenous growth of the population. The linear growth pattern of the *Artemia* ($r > 0{\cdot}99$), combined with the high reliability of producing consistent amounts of brine shrimp, opened the possibility of manipulating the culture conditions (food availability, larval density) in such a way that well-defined *Artemia* prey sizes could be obtained after specific time periods.

Parameters influencing growth and survival

In a second set of experiments the effect of increased/decreased rice bran concentrations was further analysed for three *Artemia* densities (2000, 5000 and 8000 animals/litre, respectively) (Fig. 2). It is clear that animals of identical length can be produced by several combinations of food concentrations and larval densities, however, at high animal densities longer rearing periods are required to reach the same length. Furthermore, the effect of higher food concentration in the beginning of

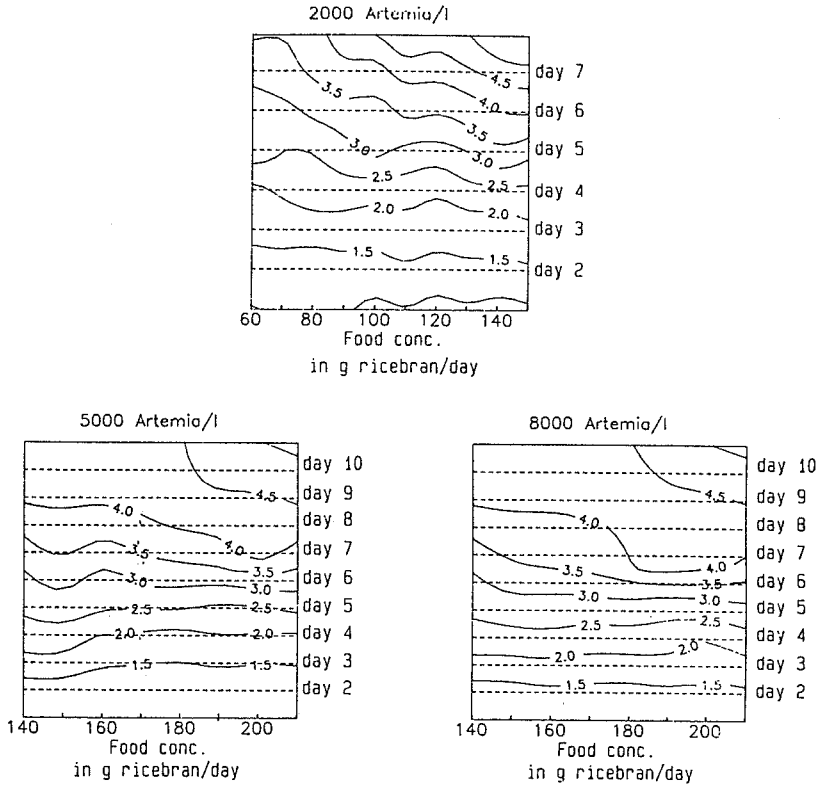

Fig. 2. Growth of *Artemia* reared at different food concentrations and initial stocking densities of 2000, 5000 and 8000 animals/litre (curves connect data points of identical length).

the rearing period does not result in an immediate growth increase of the individual larvae. This probably results from the low filter-feeding efficiency of the larvae (nauplii) at this stage of the development. It is only at the formation of thoracopods (± Day 4) that more efficient food uptake is registered and that the uptake of higher rice bran concentrations results in a significantly better growth of the brine shrimp (curves of increasing negative inclination).

Maximal biomass output was obtained in water of low transparency, but the latter negatively influenced the survival of the animals [Fig. 3(a)]. In this respect it was noticed that for a low larval density (2000 animals per litre) and food concentrations exceeding 140 g/m^3 mortality was caused by overfeeding of the animals.

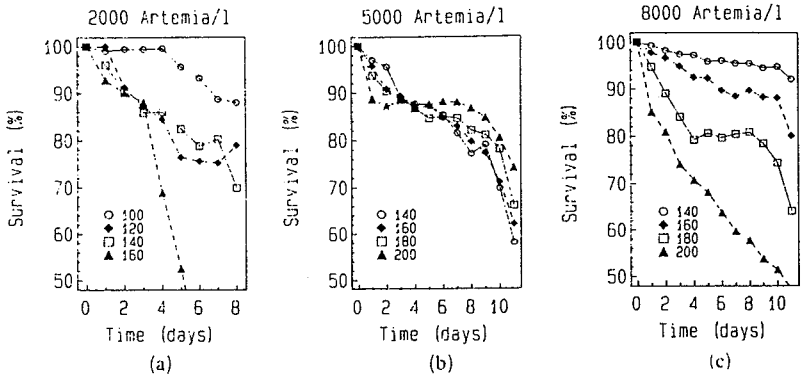

Fig. 3. Survival of *Artemia* reared at different food concentrations (g rice bran/day) and initial stocking densities of 2000, 5000 and 8000 animals/litre.

The size increase of the brine shrimp followed a linear growth pattern which was observed in all combinations of larval density and food concentration. This consistency in *Artemia* growth allowed a growth characterization through a set of linear equations (Table 2). Increased food concentrations resulted in a proportionally larger size increase of the animals reared at low density. Furthermore all animals were uniform in size (standard deviation less than 15% of the average length of the animals in the rearing tank), regardless of the density at which they were grown.

Survival was found to be dependent upon the initial stocking density of the animals (Fig. 3). At low larval density, survival was affected by increasing food concentration, eventually resulting in high mortality due to overfeeding. At larval concentrations of 5000 animals per litre lower survival rates were obtained at increased food concentrations only until Day 4; in the second part of the culture period these culture conditions even proved to be better. Finally, the very dense *Artemia* populations were affected by increased mortality when the food concentrations were raised above 140 g/m^3.

Maximization of biomass production

The effect of food concentration and start density was investigated on biomass yield of 5 mm juvenile *Artemia*. Highest biomass output was obtained by addition of 140 g ricebran/m^3 in the low density system, while 180 g/m^3 offered best results in denser populated raceways (Table 3).

TABLE 2
Equations and Correlation Coefficients (r) Obtained by Linear Regression of the Length (y) versus Age (x) of *Artemia* Fed on Different Rice Bran Concentrations and Reared under Three Different Stocking Densities

Rice bran conc. (g/m^3)	Artemia *stocking density (n/litre)*		
	2000	*5000*	*8000*
60	$y=0{\cdot}382x+0{\cdot}51$ $r=0{\cdot}99$		
100	$y=0{\cdot}518x+0{\cdot}209$ $r=0{\cdot}99$		
110	$y=0{\cdot}578x+0{\cdot}19$ $r=0{\cdot}99$		
120	$y=0{\cdot}587x-0{\cdot}081$ $r=0{\cdot}98$		$y=0{\cdot}266x+0{\cdot}617$ $r=0{\cdot}98$
130	$y=0{\cdot}604x+0{\cdot}058$ $r=0{\cdot}98$		$y=0{\cdot}314x+0{\cdot}553$ $r=0{\cdot}98$
140	$y=0{\cdot}702x-0{\cdot}201$ $r=0{\cdot}97$	$y=0{\cdot}352x+0{\cdot}779$ $r=0{\cdot}98$	$y=0{\cdot}291x+0{\cdot}528$ $r=0{\cdot}97$
150	$y=0{\cdot}707x-0{\cdot}177$ $r=0{\cdot}95$	$y=0{\cdot}370x+0{\cdot}819$ $r=0{\cdot}98$	$y=0{\cdot}322x+0{\cdot}547$ $r=0{\cdot}97$
160		$y=0{\cdot}338x+0{\cdot}931$ $r=0{\cdot}98$	$y=0{\cdot}332x+0{\cdot}451$ $r=0{\cdot}99$
170		$y=0{\cdot}376x+0{\cdot}791$ $r=0{\cdot}98$	$y=0{\cdot}323x+0{\cdot}513$ $r=1{\cdot}00$
180		$y=0{\cdot}388x+0{\cdot}786$ $r=0{\cdot}97$	$y=0{\cdot}321x+0{\cdot}549$ $r=0{\cdot}99$
190		$y=0{\cdot}409x+0{\cdot}749$ $r=0{\cdot}95$	$y=0{\cdot}345x+0{\cdot}534$ $r=0{\cdot}99$
200		$y=0{\cdot}426x+0{\cdot}669$ $r=0{\cdot}95$	$y=0{\cdot}326x+0{\cdot}635$ $r=0{\cdot}99$
210		$y=0{\cdot}432x+0{\cdot}658$ $r=0{\cdot}98$	$y=0{\cdot}399x+0{\cdot}541$ $r=0{\cdot}99$

This optimal feeding regime was maintained in the following experiments in which the appropriate harvesting time for maximal biomass output was determined. For this purpose replicates of identical cultures (same larval density) were harvested on consecutive days and compared for their yield. Figure 4 represents the average length of the individual brine shrimp and the weight of the corresponding biomass obtained during these consequent harvests. The optimal harvesting time was identified as the moment when the last extra input of food was exactly compensated by the extra output in biomass, this is when the food con-

TABLE 3
Artemia Biomass Production (in kg wet weight) in Function of the Food Concentration and Animal Density

Rice bran concentration (g/m^3)	Artemia *density (animals/litre)*		
	2000	*5000*	*8000*
100	2·3	n.m.	n.m.
120	2·4	3·7	4·9
140	3·5	4·3	5·8
160	+	4·1	7·0
180	+	5·2	7·7
200	+	5·1	7·3
220	+	+	+

n.m.: Not measured.
+: Collapse of the culture.

sumption line (dashed lines in Fig. 4b) is tangent to the biomass production line.

The biomass production line of each of the three different culture systems clearly delimits boundaries where maximal harvest can be expected, i.e. (respectively Day 9, 10 and 12 for densities of 2000, 5000 and 8000 animals per litre) (Fig. 4b). In short rearing periods only high yields can be reached with low density systems. After 1 week, respectively 10 days of culture, systems with initial stocking densities of 5000 or 8000 nauplii per litre become more productive. The higher absolute productivity of the dense system should, however, be extrapolated to an optimal economical profit since the input as cyst consumption and length of the rearing period may have important economical restrictions. When those two important cost parameters are eliminated by expressing the production (yield) per unit of input, the low density system proves to be more profitable (Table 4).

In conclusion, the use of the developed semi flow-through system can be recommended for high density culturing of *Artemia* as a cheap alternative for the more sophisticated super-intensive systems. Indeed, investment costs are low compared to traditional systems (Bossuyt & Sorgeloos, 1980; Brisset *et al.*, 1982; Lavens *et al.*, 1985). It has a high reliability in producing consistent amounts of biomass and, moreover, provides the advantage of producing juvenile or pre-adult *Artemia* of a well defined prey size. The duration of the rearing period for obtaining the desired *Artemia* prey can be calculated by means of the equations corresponding to culture conditions. Finally the choice of the density in

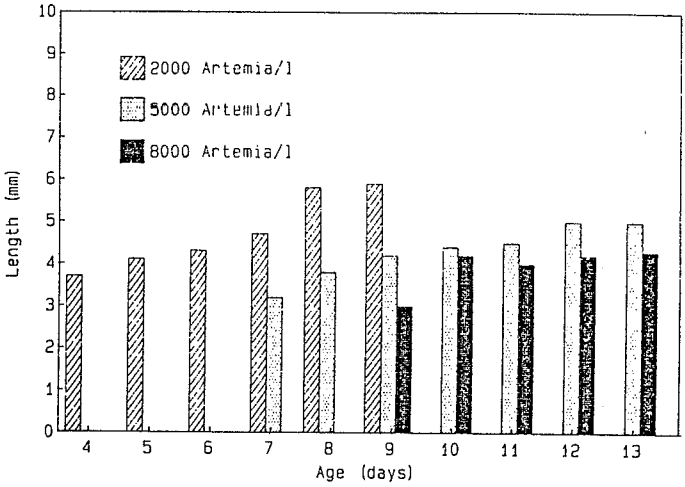

Fig. 4a. Average length of *Artemia* in function of the age and larval density of the population.

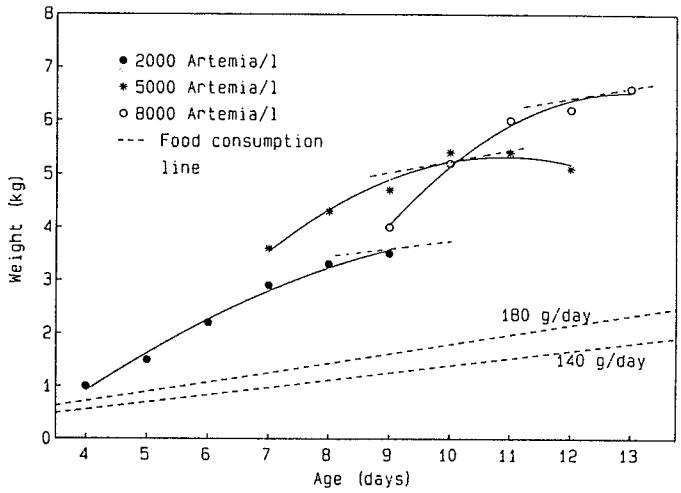

Fig. 4b. *Artemia* biomass production in function of the age and larval density of the population.

the raceway can be chosen in function of the final purpose of the produced biomass. Shrimp growers, for instance, prefer to feed small prey which can easily be caught by the predator and result in less detritus when torn by the predator. Fish growers, on the other hand, prefer to

TABLE 4
Rentability of the Semi Flow-Through Culture System Used at Three Different Initial *Artemia* Stocking Densities

	Stocking density (animals/litre)		
	2000	*5000*	*8000*
Yield (kg)	3·3	5·4	6·2
Duration of production cycle (days)	9	10	12
Cyst consumption (g)	10	25	40
Rentability (biomass prod./day per g)	37	20	12

offer prey sizes which are only slightly smaller than the mouth size of the fish in order to assure a better energy balance in food uptake and assimilation. A solution to the first problem can be given by rearing *Artemia* at high densities (8000 per litre) and moderate food supply; the rather small animals will be an excellent food which can be scooped and fed to the shrimp for consecutive days. The drop in density by repetitive sampling will certainly affect the growth of the brine shrimp but can be corrected by lowering the feeding levels. In the second application low initial stocking densities (5000 per litre) combined with maximum food supply will produce fast-growing *Artemia* which will meet the requirements of the growing fish. In order to obtain a prey which has the best size characteristics to feed the fish, an appropriate choice of the culture parameters (prey density and food concentration) has to be made to ensure optimal growth of the predator. In the event of slow-growing prey, partial removal of the *Artemia* population can be considered in order to benefit from the growth increase caused by the abrupt decrease in the prey density. In all other situations where adult *Artemia* are required the producer should be guided by the higher rentability of a low density (2000 per litre) system.

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REFERENCES

Bossuyt, E. & Sorgeloos, P. (1980). Technological aspects of the batch culturing of *Artemia* in high densities. In *The Brine Shrimp* Artemia, Vol. 3, ed. G. Persoone, P. Sorgeloos, O. Roels & E. Jaspers. Universa Press, Wetteren, Belgium, pp. 133–52.

Bossuyt, E. & Sorgeloos, P. (1981). Batch production of adult *Artemia* in 2 m^3 and 5 m^3 air-water-lift operated raceways. In *Book of Abstracts. World Conference on Aquaculture*, 21–25 September, 1981, Venice, Italy.

Brisset, P. J., Versichele, D., Bossuyt, E., De Ruyck, L. & Sorgeloos, P. (1982). High density flow-through culturing of brine shrimp *Artemia* on inert feeds — preliminary results with a modified culture system. *Aquaculture Eng.*, **1**, 115–19.

Dobbeleir, J., Adam, N., Bossuyt, E., Bruggeman, E. & Sorgeloos, P. (1980). New aspects of the use of inert diets for high density culturing of brine shrimp. In *The Brine Shrimp* Artemia, Vol. 3, ed. G. Persoone, P. Sorgeloos, O. Roels & E. Jaspers, Universa Press, Wetteren, Belgium, pp. 165–74.

Lavens, P. & Sorgeloos, P. (1991). Production of *Artemia* in culture tanks. In *Artemia Biology*, ed. R. A. Browne, P. Sorgeloos & C. N. A. Trotman. CRC Press, Boca Raton, FL, USA.

Lavens, P., Baert, P., De Meulemeester, A., Van Ballaer, E. & Sorgeloos, P. (1985). New developments in the high density flow-through culturing of brine shrimp *Artemia*. *Journal of World Mariculture Society*, **16**, 498–508.

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

Sorgeloos, P., Bossuyt, E., Lavens, P., Léger, P., Vanhaecke, P. & Versichele, D. (1983). The use of brine shrimp *Artemia* in crustacean hatcheries and nurseries. In McVey, J. P. (Ed.), *CRC Handbook of Mariculture*, Vol. I. *Crustacean Aquaculture*, ed. J. P. McVey. CRC Press, Boca Raton, FL, USA, pp. 71–96.

Sorgeloos, P., Léger, P., Lavens, P. & Tackaert, W. (1987). Increased yields of marine fish and shrimp production through application of innovative techniques with *Artemia*. *Aquaculture et Développement, Cahiers Ethologie Appliquée*, 7, 43–50.

Tobias, W. J., Sorgeloos, P., Bossuyt, E. & Roels, O. A. (1979). The technical feasibility of mass-culturing *Artemia salina* in the St. Croix 'artificial upwelling' mariculture system. *Proc. World Maricult. Soc.*, **10**, 203–14.

Tobias, W. J., Sorgeloos, P., Roels, O. A. & Sharfstein, B. A. (1980). International study on *Artemia*. XIII. A comparison of production data of 17 geographical strains of *Artemia* in the St. Croix artificial upwelling-mariculture system. In *The Brine Shrimp* Artemia, Vol. 3, ed. G. Persoone, P. Sorgeloos, O. Roels & E. Jaspers. Universa Press, Wetteren, Belgium, pp. 383–92.