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Macrobrachium rosenbergii (DE MAN) CULTURE IN POLYNESIA: PROGRESS IN DEVELOPING A MASS INTENSIVE LARVAL REARING TECHNIQUE IN CLEAR WATER

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ABSTRACT

To develop Macrobrachium rosenbergii (de Man) culture in French Polynesia, a high density larval rearing method in clear water for post-larvae mass production has been set up by the "Centre Océanologique du Pacifique".

Based on three years of experiments, a production of 60 post-larvae per liter in 800-liter tank was obtained. The methodology (conical bottom tank, static system with one total daily water exchange, no phytoplankton), the environmental conditions

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(temperature, light, color of the tank), the water quality control (chlorination, antibiotics, aeration), the larval food (quality and quantity) are given and discussed.

The model was used to build a hatchery on a pilot scale (capacity 600,000 post-larvae every 2 months). Due to a high level of control of the system, this method could be used in any environment.

INTRODUCTION

In 1973 the "Centre National pour l'Exploitation des Océans" (CNEXO) and the French Polynesia Territory began a feasibility study on the culture of giant freshwater prawn, Macrobrachium rosenbergii (de Man), in Tahiti. The program included the development of a mass production method for post-larvae and the elaboration of artificial food from local by-products (Aquacop, 1976). An initial stock of 50 adults was obtained from Mr. Fujimura (Hawaii Department of Land and Natural Resources). All the larval rearing experiments were conducted at the Centre Océanologique du Pacifique (COP) in Vairao, Tahiti Island.

Culture of M. rosenbergii could develop widely in tropical areas. Due to omnivorous habit of the species, commercial size animals are easily obtained in semi-intensive conditions with suitable temperature. But the mass production of post-larvae throughout the year at a low cost is often a limiting factor (Goodwin and Hanson, 1975).

The first mass production technique was developped in Hawaii (Fujimura, 1966, 1974; Fujimura and Okamoto, 1970). It is practiced at low density (10 PL/liter) and in large tanks (18 m^3). Algae rich "green water" is used to maintain the quality of the larval rearing water by recycling the waste products of larval metabolism (Cohen et al., 1976) and provide food for Artemia. This technique requires a long experience in the environmental conditions of the hatchery site. The "green water" effect is dependent on its quality which fluctuates according to the meteorological conditions and the variations of fresh and sea water quality.

Our first experiments were set up following Ling (1969) in clear water. Results of 30 PL/liter obtained in 500-liter fiber glass tanks have led us to follow this method which is quite similar in principle to that used for penaeid shrimp in the Galveston Laboratory of the US Marine Fisheries Service (Mock and Murphy, 1970). However, problems of mass mortalities appeared when we attempted to increase the production scale. During the course of about 150 trials, the basic factors of this technique have been determined and production results of 60 PL/liter have been achieved.

This paper presents the main experiments which have led to the definition of the factors affecting larval rearing in high density conditions. The culture technique, which includes a total daily exchange of the water, is described and the sketch of a pilot hatchery is given. This one began operating in October 1976: it may produce 100,000 PL per 2 m^3 tank and enables of production costs.

MATERIAL AND METHODS

Experiments were conducted from October 1973 until April 1976 in a 300 m^2 laboratory oriented N/S with opaque roof and large windows on the west side. No artificial light was used and the animals were under nycthemeral conditions. In October 1976 this laboratory was changed to a pilot hatchery.

The optimum water temperature of larval rearing is around 28 C. In Tahiti this temperature is reached from November until June, which period includes the rainy season. The fresh water[1] source is a small mountain creek, with an annual temperature variation of 19 to 25 C. Sea water[1] is pumped from the lagoon and is of an oceanic type as the swell action above the flat barrier reef allows a rapid renewal of the water mass. Salinity and temperature are quite constant throughout the year.

The fresh water and the sea water are mixed inside the laboratory 24 hours prior to use in an elevated 10 m^3 tank; the brackish water flows by gravity to the rearing tanks. The water temperature changes according to the season; electric heaters had been previously used in the colder months to heat the fresh water in a first storage tank. In January 1976 the hatchery was completely enclosed to avoid heat loss during the night. Electric heaters are no longer used and temperature of the hatchery is maintained by using the hot exhausted air from the air conditioners of the adjoining laboratory. Within 24 hours the temperature of the water in the storage tank reached the same level as in the rearing tanks, so thermal shocks are avoided when renewing the water. The stored water flows continuously over a sand filter by means of an airlift device. More recently a new treatment has been used: chlorination at 1.5 ppm of total chlorine followed by dechlorination with UV light and strong air bubbling for 24 hours.

Fiber glass tanks of 500 liters and 800 liters capacity with a conical bottom (slope 45°) are used for rearing the larvae (Figure 1). They include a double wall where the lagoon water is circulated if necessary to avoid temperature variations. PVC filters (Figure 2)

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[1]Water chemical analysis are available from the authors.

are used during water exchange. Concentration devices made of PVC tubes (250 and 300 mm diameter) (Figure 3) allow collection of the larvae when cleaning the tank or harvesting of post-larvae. These apparata are fitted with different mesh screens from 335 µ to 1,200 µ according to the size of the larvae. Aeration is supplied by 4 air stones at the center of the conical tank bottom: air flow rate is between 1.5 and 2.6 m^3/hour. Water is exchanged daily at approximately 4:00 P.M. according to the scheme of Figure 4. The rearing sequence and the daily maintenance routine of the last experiments are given in Figures 5 and 6.

Different larval food have been tested: newly hatched Artemia nauplii (5 to 10/ml); frozen skipjack flesh precooked at 60 C for 20 min; frozen squid flesh; frozen adult Artemia reared on a diet of 50% dried Spirulina algae and 50% soluble fish protein concentrate; eggs of dolphin and skipjack; and frozen Macrobrachium flesh. All these foods, except for Artemia nauplii, are washed and screened on sieves to obtain suitable size particles from 300 to 1,000 µ according to the larval stages.

Larvae were counted daily in five one-liter samples taken from each tank after aeration was increased to produce a random distribution of the animals. Newly hatched larvae are counted in 200 ml samples from a 50-liter tank; the same technique is used when the rearing tank is emptied for a complete cleaning. Numbers of post-larvae are estimated by weighting the total quantity harvested in a small nylon bag and comparing it to the weight of a group of 2,000 individuals, weighed in exactly the same conditions after dripping of water (so this technique is reliable at 10%).

The behavior of the larvae is often checked after stopping aeration, to determine if they are in good health (presence of swarms at the surface, no cannibalism, actively feeding, red brownish pigmentation) or in bad condition (accumulation on the bottom, corpses, uneaten food, blue pigmentation). Stages are determined under a binocular stereo-microscope according to the criteria of Uno and Soo (1969). Mean stage of the tank is given by the larval stage index (LSI) from Maddox and Manzi (1976). Microscopic observations of the larvae (Figure 7) allow more accurate determination of larval health. The general condition is shown by the pigmentation, the stomach content and the cleanliness of the gills. Necrosis due to bacterial infections appears first on the tip of antennules and antennae and later on pereiopods, pleopods, telson spines and uropodal setae. Filamentous bacteria (Leucothrix sp.), which are a sign of bad water quality, are easily seen on the eye surface, at the basis of the pereiopods and between the pleopods; they are often mixed with punctiform bacteria and ciliates (Aquacop, 1977a). In the trials of January-March 1976, streptomycin and bipenicillin were used at 1.25 to 2.5 ppm every two or three days during all the rearing for preventive treatment or at 5 ppm when necrosis or filamentous bacteria increase was followed

by mortality. Other antibiotics have been tested (furanace, erythromycin, terramycin) but their use needs further study.

RESULTS

Three series of experiments have led to the elaboration of this rearing technique. From October 1973 to July 1974 discontinuous experiments were performed using the larvae hatched from 20 females maintained in separate tanks. Table 1 gives the sequence and the results of one experiment which produced 32 PL/liter thus proving the feasibility of this clear water rearing technique. A total of 180,000 post-larvae were produced. From October 1974 to July 1975 the broodstock increased with the first animals born in Tahiti. Grouped spawnings permitted us to have 5 to 10 larval tanks in production simultaneously. Further comparison of larval development under different conditions pointed out the importance of some factors. But even when these factors were controlled, the consistent results were not obtained and mortalities sometimes destroyed whole batches. Only 120,000 post-larvae were produced during this period. From October 1975 to July 1976 bacterial origin of the losses was demonstrated. Antibiotic treatment combined with a better rearing method allowed better production and a definition of the unit pilot hatchery. Four hundred seventy thousand post-larvae were produced with production efficiency above 50 PL/liter. Chlorination of the rearing water has also improved larval development in comparison with 1 μ filtered water.

Narrow variations in water temperature, light intensity and color of the inner tank walls appear to be primary factors for success in rearing. Best results were always obtained in tanks of dark green interior color, located near a window and maintained at a mean temperature of 28 C without thermal variations during water renewal. In tanks placed in semidarkness the larvae did not develop beyond stage 5. The same phenomenon was observed in suitable light conditions with tanks having white interiors (Table 2).

The hightest mortality encountered during metamorphosis to post-larvae was attributed to the cannibalism. Now microscopic observations of unhealthy larvae correlated mortality with the presence of bacterial necrosis on the appendages. In the trials of January-March 1976, on 8 tanks for a total of 800,000 larvae, preventive or curative treatments using streptomycin and penicillin gave a high survival until stage 10. The previously described conditions were maintained and handling was limited. Larvae reached metamorphosis in a perfect condition. Strong aeration prevented the late stages from sticking to the tank side. Yet the final result was only 60% of survival. Table 3 gives the result of one of the best experiments (75-76/3/A) where the streptomycin-bipenicillin was used once during the first period, the 14th day when filamentous bacteria occured, and every other day during metamorphosis period. Post-larvae were harvested only at the end when very few larvae

remained. Figure 10 presents the evolution of the biomass of the 800,000 larvae in the trials which led to 700,000 stage 10. It gives also the amount of distributed food and the dry weight food necessary to produce 1,000 post-larvae.

DISCUSSION

These results demonstrate that the larval rearing of M. rosenbergii can be achieved in high density using clear water without the help of phytoplankton, as previously shown by Smith et al. (1974). A good production will result of three sets of factors: the first (1) allows the larvae to develop normally (environmental conditions); the second (2) allows the larvae to avoid stress (physical, thermal, physiological); the third (3) allows the larvae to avoid bacterial attacks.

1) Light intensity and color of the tank enhance larval activity, probably due to a better view of the food particles. When the light energy is too low larvae do not develop beyond stage 5 in our rearing conditions, as they do not eat enough to sustain their growth. Direct sunlight also must be avoided because it leads to mortalities by apparent sunburning of the exoskeleton. Larval development is rapid and molts are nearly synchronous if the conditions are near optimum. So the quantity of food must be adapt to the needs of larvae (Figure 10). All the different food used are satisfactory. Some are retained for their easy preparation. Skipjack flesh is better for particles under 500 μ; light cooking and washing on a sieve eliminate the solubles. For particles larger than 500 μ squid flesh is better. Distribution of the right quantity of food can be checked visually by stopping the aeration: each larva should hold a particle.

2) Among the numerous stresses which may occur, the most important to avoid are:

- Mechanical stress during the daily maintenance if the handling is too vigorous; appendages can be crushed or lost.
- Thermal stress during the rearing water exchange, when the temperature of the storage tank is higher.
- Physiological stress if the rearing water is not changed often enough and probably if the aeration is not sufficient; the increase of toxic metabolic products can poison the larvae.

3) The exact role of the antibiotics is difficult to explain. Total bacterial counts in the water over a 24-hour cycle dit not

show a decrease. However, an effect is quite clear on the epiphytic bacterial fauna of the larvae which disappear immediately after treatment (Aquacop, 1977a). To enhance this action the antibiotics are distributed in the water just before the filling of the tank when the water volume is lowest.

From a pratical point of view a production hatchery must be defined to maintain the environmental conditions at optimum level and to minimize stress effects. Figure 9 gives the scheme of a pilot hatchery built to apply this rearing technique. Figure 11 sketches the operations and points out the role of the stability of ambient conditions, the water quality control and the importance of careful daily maintenance in the rearing tank. This maintenance avoids the poisoning of larvae with their own metabolic products and allows control of bacterial activity. The conical bottom of the tank allows a good mixing of the entire water mass and prevents sedimentation of solids and larvae. The strong aeration could also assist in eliminating some volatile toxic products (Figure 8). The small quantity of water used decreases the quantity of antibiotics needed.

Production results using this method could probably be improved. The mortality during metamorphosis (40%) is not explained and should not be so high in comparison with the high survival and good health of the larvae until stage 10. The disappearance of animals is quite surprising as no dead bodies are found. It might be due to cannibalism by post-larvae. On the tank bottom the density is very high, 5 PL/cm^2, at the end of the rearing period. Post-larvae may also be underfed as the aeration keeps the food away from the bottom. To avoid this loss two solutions will be tested: regular separation of post-larvae from late larvae and transfer to a bigger tank before stocking in ponds or use of an other type of tank during metamorphosis.

Compared to the "green water" method, this technique is simpler as it concerns only one trophic level, the larvae. In a 24-hour cycle nitrogen excretion by the larvae, which is a function of the stage and of the total biomass (Aquacop, 1977b), leads to a deterioration of the quality rearing water. Figure 12 gives the theoretical scheme of the evolution of this rearing water: after the exchange the water quality at its best will gradually decrease to the next exchange. A regular development of the larvae will be observed only if the level of nitrogen products stays under a critical limit. This limit, which is represented on the scheme by an horizontal line, probably varies according to larval stage. On maintaining the larvae in stable conditions it should be possible to optimize the method by an accurate knowledge of the density which can be tolerated outside of the critical zone.

CONCLUSION

This rearing technique seems to be well adapted to mass production of Macrobrachium rosenbergii post-larvae; it allows continuous and effective control of the animals. For future work on genetic selection this method permits separate treatment of spawnings and this compact hatchery type only needs low building investment. At the COP, in the new pilot hatchery, efforts will be made to increase the metamorphosis survival, to decrease the time of larval life and to substitute artificial food for fresh food.

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Table 1. - One of the first rearing in 500-liter tank (73-74/03/30/74)

Days	Stages	Larval number	Post-larval number	Density per liter	Survival rate
1	1	35 000		70	
14	6	34 000		68	97
23	8.3	31 000		64	91
26	9	30 000		60	85
36	PL	22 000	1 500		
43			9 400		
49			4 600		
57		3 000	500		
Final result			16 000	32	46

Rearing conditions: mean temperature : 26.3 C; food : *Artemia* nauplii + skipjack; daily water exchange : 300 liters

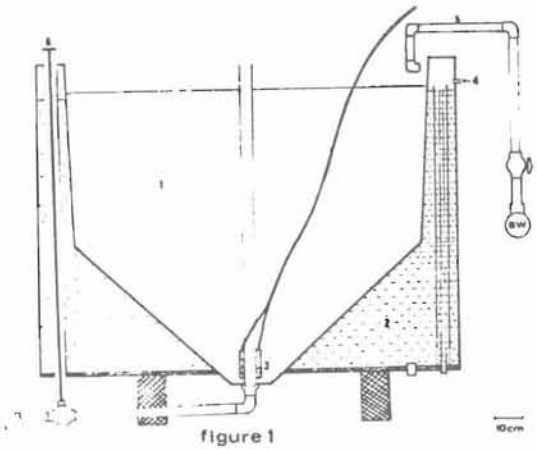
Table 2. - LSI evolution with different conditions[1]

Rearing conditions	Mean Temp	Density per liter	Food	LSI evolution D5	D10	D15	D20
Tank near a window (74-75/1/1)	26.0 C	55	*Artemia* nauplii + skipjack	3	5	6.5	8.3
Tank far from a window (74-75/1/7)				3	4.5	5.3	6.5
Tank near a window (75-76/4/1)	27.5 C	100	*Artemia* nauplii + skipjack + frozen *Artemia*	3	5.2	6.8	8.8
Tank at the darkness (75-76/4/2)				3	4	5	5.1
Dark painted tank (74-75/4/1)	27.5 C	100	*Artemia* nauplii + skipjack + frozen *Artemia*	3	6	7	9.0
White painted tank (74-75/4/5)				3	5	6.1	6.8

[1] The data are based on results from numerous observations of different tanks under each condition.

Table 3. - One of the rearing (75-76/3/1 A) with antibiotic (SP 2.50: streptomycine-bipenicilline 2.5 g/m^3).

Day	LSI	Larvae number	D/1	Survival rate	Temperature 25 26 27 28 C	Particular maintain microscopic observation	Evening treatment
1	1	90,000	112.5				
2							
3	2						
4							
5	3						
6							
7	4					some necrosis	
8							
9	5	90,000	112.5	100			
10							
11							
12	6						
13							
14	6.5					filamentous bacteria (FB)	SP 5.0
15						no FB, no necrosis	
16	7						
17							
18	8						
19							
20	9					1st total cleaning	
21						some necrosis, broken rostrum	
22							
23	9.5						
24							
25							
26	10						
27							
28		80,000	100	89		2nd total cleaning	
29	(11)					little mortality, dirty gills	SP 2.50
30						no mortality, clean gills	
31	(PL)						SP 2.50
32	10.5						SP 2.50
33							
34							
35						some necrosis on antennae	SP 3.75
36						some necrosis	
37							SP 2.50
38						some necrosis on pleopods	
39							SP 2.50
40							
41							SP 2.50
42							
43							SP 2.50
44							
45							SP 2.50
46							
47							SP 2.50
48							
49							SP 2.50
Final result (PL)		50,000	63.5	55.5			



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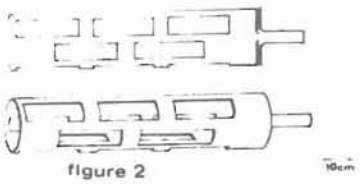

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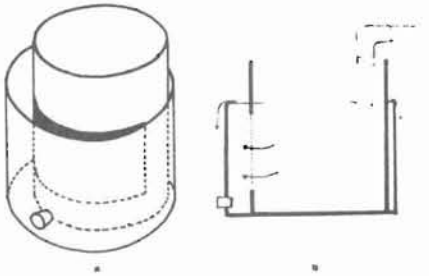

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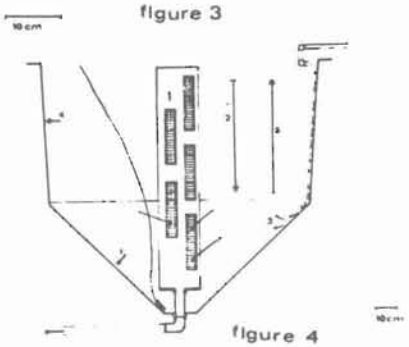


figure 4

Aquacop.

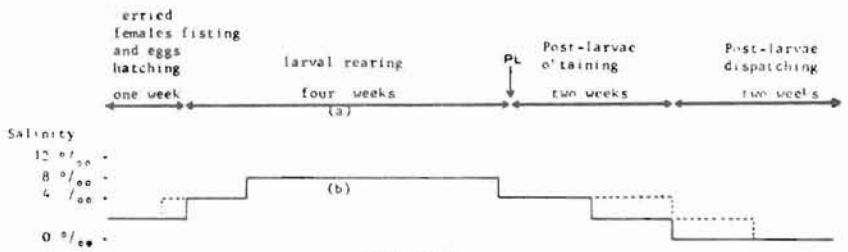
Macrobrachium rosenbergii (de Man) culture in Polynesia
Progress in developing a mass intensive larval rearing technique in clear water.

Figure 1. Eight hundred-liter rearing tank; 1, rearing volume; 2, space where the water of the lagoon circulate to reduce the temperature variations of the rearing water; 3, four airstones to aeration; 4, lagoon water inlet; 5, rearing water inlet; 6, rearing water outlet valve lever.

Figure 2. PVC central filter (Ø 150 mm) used during water renewals, with different mesh screens (0.3 to 1.2 mm).

Figure 3. Larvae collector built with two PVC pipes (Ø 250 and 300 mm) (a); scheme of its use (b), when emptying a tank (⟶ = water outlet).

Figure 4. Daily cleaning of the tank during water renewal; 1, brushing the conical bottom; 2, lowering water level till upper limit of the cone; 3, renewal of remaining water; 4, brushing vertical walls; 5, refilling till upper level.



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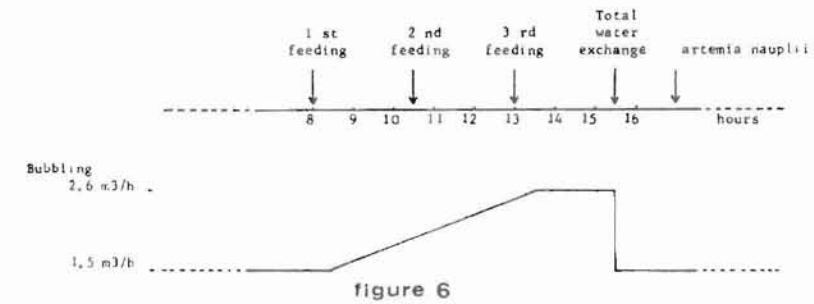
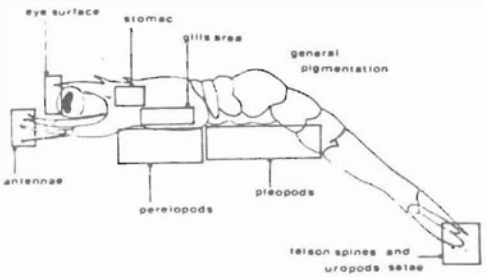


figure 6

Figure 5. The different steps of a post-larvae production (a) and the different salinity values along rearing (b).

Figure 6. Scheme of daily rearing operations with feeding frequency, water renewal, and air flow variations after the second week of rearing.



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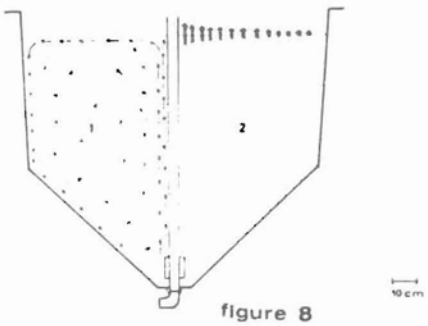

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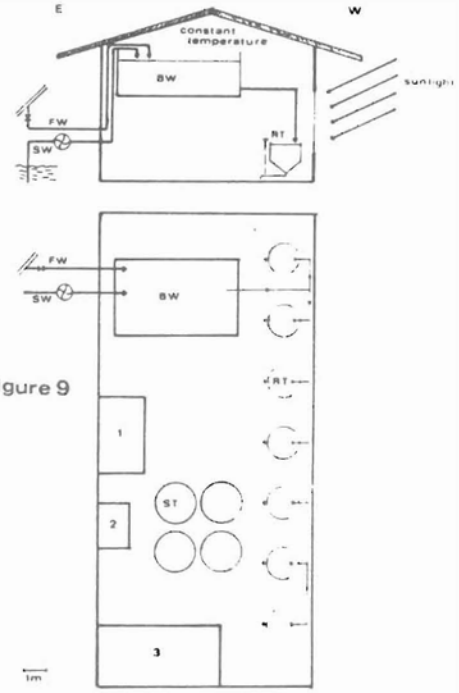

figure 9

Figure 7. Microscopic observations on larvae: main zones checked.

Figure 8. The mechanical part of the aeration in a tank with a conical bottom: a rotary motion of the whole water (1) to increase the gaz exchanges at its surface (2).

Figure 9. Scheme of the COP hatchery; FW, freshwater; SW, sea water; BW, brackish water; RT, rearing tank; ST, spawning and post-larvae stocking tank; 1, preparation of food; 2, incubators of Artemia; 3, laboratory.

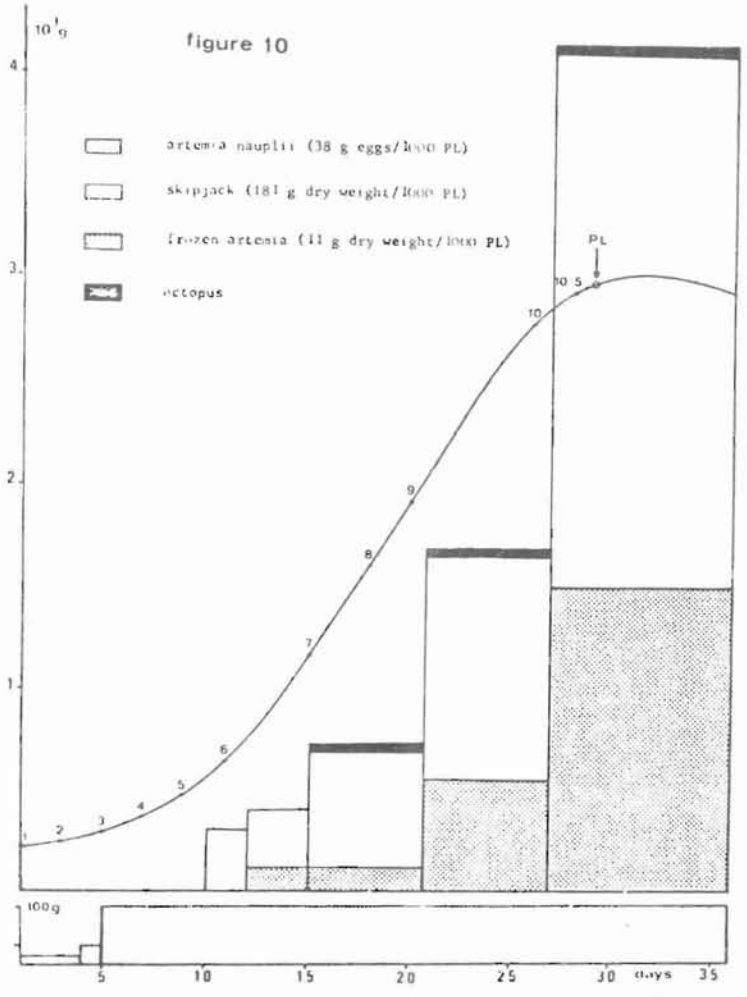

Figure 10. Larval biomass evolution (800,000 stage 1; 700,000 stage 10) and food amounts evolution (wet weight after preparation) during the experiment of January-March, 1976.

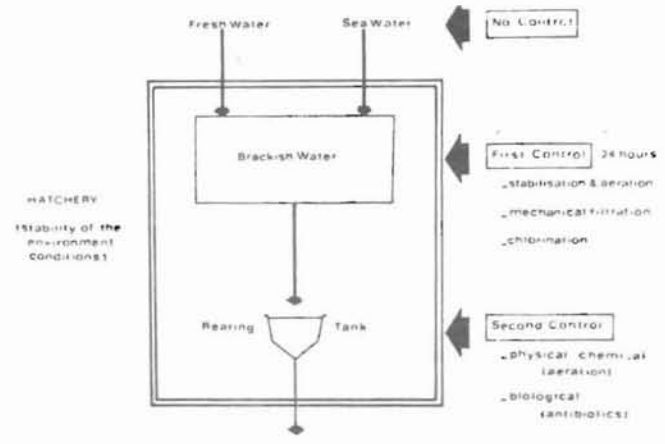

figure 11

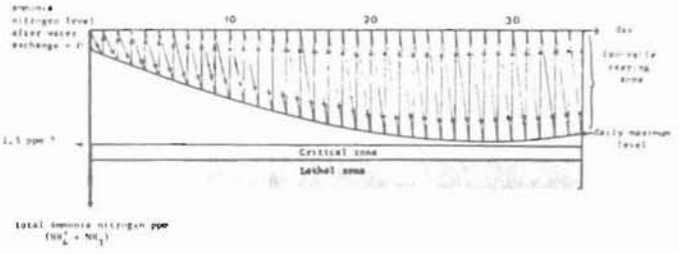

figure 12

Figure 11. Design of the COP hatchery.

Figure 12. Scheme of the evolution of the ammonia concentration ($NH_4^+ + NH_3$) in a rearing tank during the whole larval period, in a static system with one daily total water exchange.