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Intensive culture of the Manila clam (*Ruditapes philippinarum*) in marine ponds. The impact of rearing densities and feeding levels on growth rates and yields

J.P. Baud[1], J. Haure[1] & A. Bodoy[2]
[1] *IFREMER, URRA, Polder des Champs, 85230 Bouin*
[2] *IFREMER, URRA, B.P. 133, Mus de Loup, 17390 La Tremblade*

Key words : *Ruditapes philippinarum, Skeletonema costatum*, intensive rearing, marine pond, growth rate, yield.
Mots-clés : *Ruditapes philippinarum, Skeletonema costatum*, élevage intensif, marais maritime, croissance, rendement.

Abstract

Intensive ongrowing (from 28 mm to the commercial size, 35 mm) of the Manila clam *Ruditapes philippinarum* was studied in experimental raceways with a natural bottom of clay. The factors controlled were rearing density (100, 200, and 300 individuals/m^2) and food concentration (34.0 and 68.1 µg.l^{-1} chlorophyll *a*). Food was produced from a batch culture of *Skeletonema costatum* in a large volume (50 m^3), thanks to the fertile ground and saline water. Best results regarding final size were obtained in the vicinity of the inflow with a density of 100 individuals/m^2. However it was a density of 300 individuals/m^2 which appeared from the following results to be the optimal choice for intensive rearing: the survival rate was 84 %, the final biomass was 4 kg/m^2 and the food chain efficiency was 0.16. Production was ten times higher than that which has usually been obtained in these marine ponds, by means of extensive culture.

The design of the hydraulic system and the raceways needs to be improved though, for they induced some changes in growth rates, related to the distance from the water inflow.

Élevage intensif de la palourde japonaise (*Ruditapes philippinarum*) en marais maritime. Impact des densités d'élevage et de la quantité de nourriture sur les vitesses de croissance et les rendements de production

Résumé

Une étude sur l'élevage intensif de la palourde japonaise (*Ruditapes philippinarum*) de 28 mm à la taille marchande (> 35 mm) en bassins à fond de terre a été réalisée à partir d'une production séquentielle en grands volumes (50 m^3) de *Skeletonema costatum* cultivée avec de l'eau salée souterraine. L'influence de la densité (100, 200 et 300 individus/m^2) et de la biomasse phytoplanctonique (34,0 et 68,1 µg.l^{-1} de chlorophylle *a*) sur la croissance et le rendement a été testée. Les meilleurs résultats exprimés en longueur de coquille ont été obtenus pour la densité de 100 individus/m^2. Cependant, la densité de 300 individus/m^2 semble être un meilleur choix pour l'élevage intensif. En effet, le taux de recapture est alors de 84 %, la biomasse finale de 4 kg/m^2 et le rendement d'assimilation atteint 0,16. La production obtenue est ainsi 10 fois supérieure à celle de la technique extensive habituellement utilisée dans les marais atlantiques français. Toutefois, le mode de distribution de l'eau enrichie et la configuration des bassins n'apparaissent pas optimaux, car elles engendrent des croissances hétérogènes en fonction de l'éloignement des individus par rapport à l'injection de nourriture.

Introduction

Along the French Atlantic coasts, Manila clam culture has developed both in marine ponds and in intertidal areas. This culture has been extensive, since no food is added to the environment. The usual rearing densities have been of 30 individuals/m^2 in ponds (Goulletquer *et al.*, 1988) and 200/m^2 in intertidal areas (IFREMER, 1988). However, in previous studies, standard conditions for intensive rearing of the Manila clam (*Ruditapes philippinarum*) were established at the nursery stage (Baud & Bacher, 1990) and at the first ongrowing stage (Baud & Haure, 1989) in marine ponds along the Atlantic coasts of France. A preliminary study of the final growing stage, from a size of 28 mm to a commercial size, at least 35 mm, was conducted later (Baud *et al.*, 1990). These authors showed that, at a rearing density of 100 individuals/m^2, food supplementation produced a final biomass of 1.8 kg/m^2, twice higher than in extensive rearing conditions. The food (*Skeletonema costatum*) was produced in batch cultures (50 m^3) from a saline groundwater. The clams, fed with three different concentrations, did not exhibit any significant differences in growth.

The aim of this study was to analyse the effect of high rearing densities for clam fed continuously in controlled conditions, in order to reduce the cost of production. The growth and the mortality rate were monitored in a cross experiment based on three rearing densities and two levels of food supplementation. Turnovers were computed from the final yield and from the ratio between mollusc dry weight and phytoplankton dry weight. Their value, compared to available data, provided the basis for an intensive rearing strategy for the Manila clam in these environments.

Materials and methods

The 18 month-old clams, originating from the same batch, were monitored from 30 March to 23 November 1988. They were sowed at densities of 100/m^2, 200/m^2 and 300/m^2 in six differents ponds (Fig. 1). The growth variability within the ponds was assessed by means of random sampling of ten clams in three areas, each of them corresponding to a third of these ponds.

Sampling was performed twice a month during gametogenesis (from June to October) and otherwise every month. At the end of the experiment, 30 clams were collected in the same conditions. Methods for the growth parameters and for physical and chemical parameters of the rearing environment have been described in Baud *et al.* (1990). The data regarding the final capture were expressed as follows : if C is the final catch in percent :

$$C = \frac{NF}{NI} \times 100$$

where NF is the final number of live individuals caught and NF the initial number of individuals sowed in the basin. These numbers were assessed by the formula :

$$N = Wt/wm \times 1/500$$

where Wt is the total weight initially sowed or finally caught in a given basin, and Wm, the average weight of 500 individuals, respectively at the beginning or at the end of the experiment.

Analysis of variance was used to reveal the significance of the different factors (rearing densities, distance from the input, and food levels) and of their interaction, by means of the

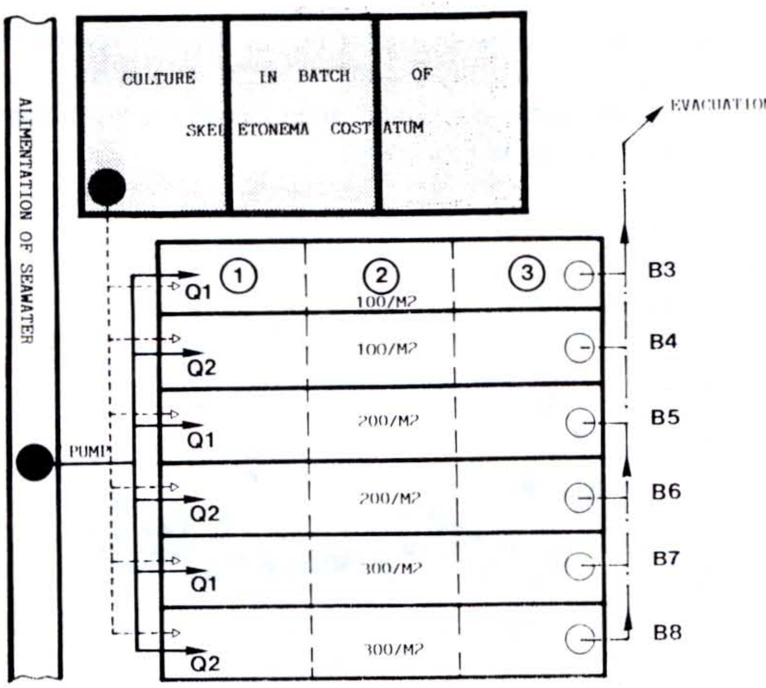

Fig. 1 - Plan of the experimental facilities. Ground bottom basins (30 m^2) had a water depth of 0.80 m. Water input was at a renewal rate of 100 % per day. Flow of phytoplankton in each basin: Q1 = 100 l/h, Q2 = 200 l/h (*Skeletonema costatum*). Distance from the input: **1** = area near the input; **2** = centre; **3** = area near the output. Densities : ponds B3 and B4 = 100 clams/m^2, B5 and B6 = 200/m^2; B7 and B8 = 300/m^2.

- *Schéma des installations expérimentales. Les bassins à fond de terre (30 m^2) ont une profondeur de 0,8 m. Le taux de renouvellement de l'eau est de 100 % par jour. Le débit de phytoplancton dans chaque bassin est de 100 l/h (Q1) et de 200 l/h (Q2) de culture de* Skelotenoma costatum. *Distance depuis l'alimentation : **1** = aire proche de l'alimentation; **2** = centre; **3** = aire proche de l'évacuation. Densités d'élevage : bassins B3 et B4 : 100 palourdes/m^2, B5 et B6 : 200/m^2, B7 et B8 : 300/m^2.*

statistical program STATITCF (1987). The variable used for all the analyses was the shell length, measured along the antero-posterior axis. The following analyses were performed.

Densities × distances × food levels, for all modalities.

Densities × food levels, part nearest to the food input.

Food levels, at density of 200/m^2 and of 300/m^2 nearest to the food input.

From these analyses a hierarchy was established among the factors and the modalities were classified by means of the test of Newman-Keuls. Statistical analyses were performed on log-transformed turnovers and on arcsine transformed mortality rates, in order to stabilize the variances (Lellouch & Lazur, 1974).

Results

Environmental conditions

The average daily temperatures of the water, in the experimental ponds, reached a maximum of 23.5 °C in August and a minimum of 5.7 °C in November. They were however higher than 12 °C, from the end of April to November. Salinity remained within a narrow range (32 ‰ to 35 ‰), except on two occasions, when heavy rainfalls caused salinity to reach 25 ‰, at the beginning of April and the end of May.

The particulate matter added to the water input remained approximately stable during the experiment, for both food levels (Q1 and Q2) (Fig. 2). The average content remained low (21.9 ± 3.3 mg/l). Only on some occasions, higher contents were measured, from 50 to 80 mg/l, mainly during spring and in September.

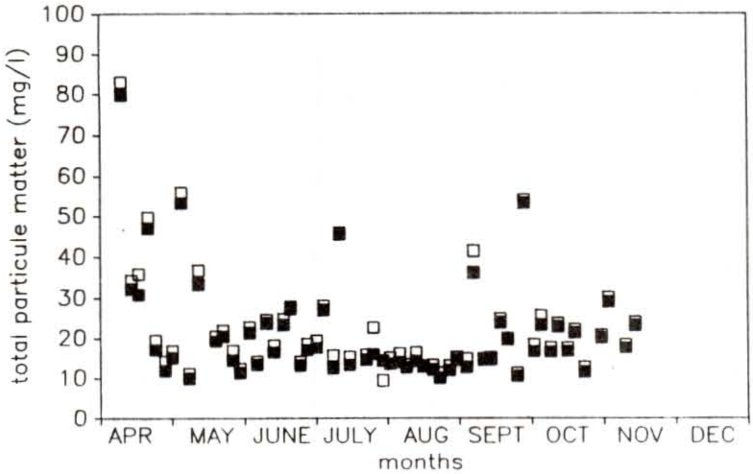

Fig. 2 - Contents in total particulate matter of the water inside the ponds for the two levels of food (full squares: low levels; open squares: high levels).
- *Teneurs en matière particulaire totale de l'eau d'élevage pour les deux quantités de nourriture (carrés pleins : faible niveau; carrés vides : niveau élevé).*

The average content in chlorophyll a pigments differed sharply between the two food levels : 34.0 ± 3.3 µg/l for Q1 and 68.1 ± 6.6 µg/l for Q2 (Fig. 3). But the variability was consecutively higher at the higher level (Q2). Minimum and maximum recorded values were 8.7 and 123.6 µg/l, while for Q1 they were 4.3 and 61.8 µg/l. The batch culture of *Skeletonema costatum* provided enough food for the whole experiment and the nutritional stress during summer reported by Goulletquer *et al.* (1988) for extensive rearing of clams was not observed. The phytoplanktonic biomass may also be assessed through the cell number per volume units (Fig. 4). The counts performed with an hematimetric cell (Malassez) were clearly different for the two food levels. For Q1, they were distinctly $73.5.10^3$ ± $9.0.10^3$ cells/ml and for Q2, $147.1.10^3$ ± 18.10^3 cells/ml.

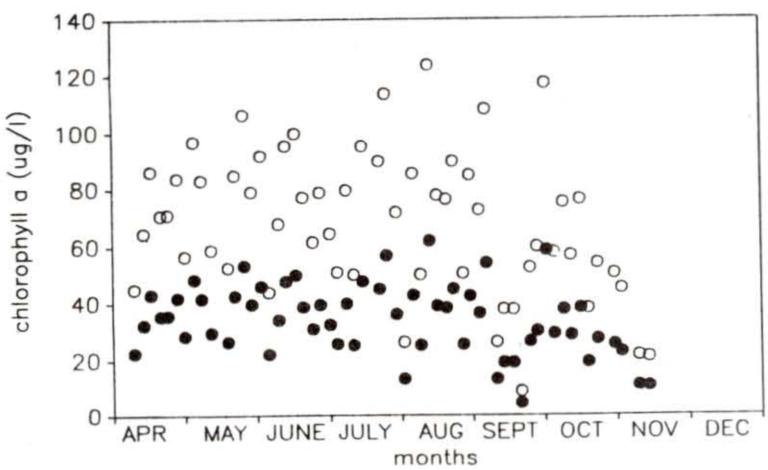

Fig. 3 - Contents in total chlorophyll pigments of the water inside the ponds for the two levels of food (full circles: low levels; open circles: high levels).
- *Teneurs en pigments chlorophylliens totaux de l'eau d'élevage pour les deux quantités de nourriture (cercles pleins : faible niveau; cercles vides : niveau élevé).*

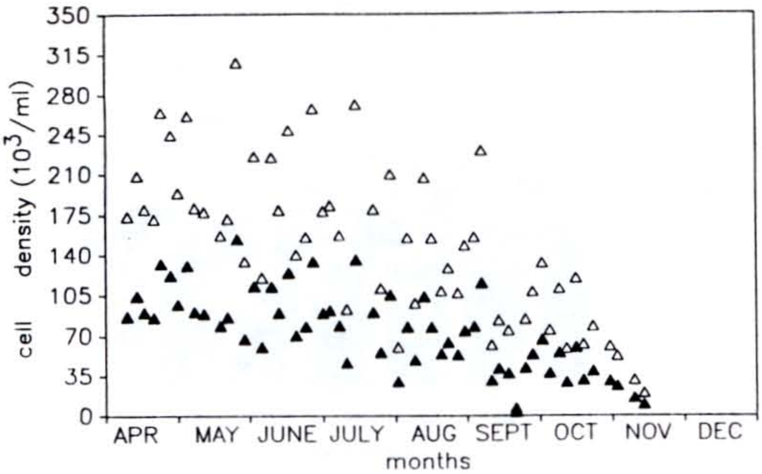

Fig. 4 - Number of phytoplanktonic cells in the water inside the ponds for the two levels of food (full triangles: low levels; open triangles: high levels). - *Nombre de cellules phytoplanctoniques dans l'eau d'élevage pour les deux quantités de nourriture (triangles pleins : faible niveau; triangles vides : niveau élevé).*

Comparison of growth, mortality and turnover at the end of the experiment with regard to the factors studied

A preliminary analysis of variance (ANOVA) was made on the three factors including all conditions on the average final size of the clams. Highly significant actions ($P < 0.001$) were found both for density ($F = 100$) and for distance ($F = 75.8$). A significant action ($P < 0.01$) was also found for the food levels. However, crossed interactions made it impossible to proceed any further with this analysis. These interactions may correspond to cumulative and non-linear effects of the factors on the growth of clams.

Source of variability	Sum of squares	degree of freedom	F ratio	Significant level
Main factors	6 319.38	539	-	
Factor 1 (density)	1 285.95	2	100.00	< 0.001
Factor 2 (concentration)	30.10	1	4.68	< 0.05
Factor 3 (location)	975.25	2	75.84	< 0.001
Interaction 1, 2	251.04	2	19.52	< 0.001
Interaction 1, 3	42.86	4	1.67	NS
Interaction 2, 3	207.33	2	16.12	< 0.001
Interaction 1, 2, 3	170.41	4	6.63	< 0.001
Residual	3 356.44	522	-	-

Table 1

A Newman Keuls'test, performed on the distance factor, showed that significant differences were found for each modality (areas near the water input, at the centre of the basin or near the output). A similar relationship between the growth of clam and the distance between the food input, was found in all basins (Fig. 5).

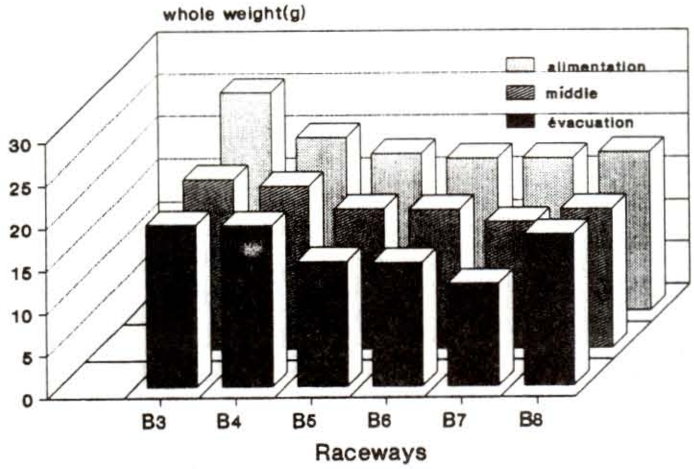

Fig. 5 - Histogram of the final average weight (g) for every basin (B3 to B8) and every distance from the water input.

- Histogramme du poids moyen (g) en fin d'élevage pour chaque bassin (de B3 à B8) et chaque distance à partir de l'alimentation.

Factor	Distance from input	Mean (mm)	Homogeneous groups		
1	input	43.97	A		
2	center	41.89		B	
3	output	40.72			C

Table 2

The best growth observed was always found in areas near the water input. However this was clearer for the lower food level (basin B3, B5 and B7) than for the higher level (basin B4, B6 and B8), where the differences in growth were smaller. Indeed, no significant differences were observed between areas of basin B4 and basin B8.

The next step in the analysis consisted in separate ANOVA in the area near the water input, in order to obtain a higher sensitivity. In these conditions (Table 3) both factors had a significant action on the growth (P < 0.001 for density and P < 0.01 for the food level). A significant interaction was also found in the two factors.

Source of variation	Sum of squares	Degree of freedom	F ratio	Significant level
Main Factors	2 054.59	179	-	-
1. Density	443.04	2	26.14	< 0.001
2. Food	57.69	1	6.81	< 0.01
Interaction 1, 2	79.18	2	4.67	< 0.05
Residual	1 474.68	174	-	-

Table 3

A Newman Keuls'test on the factor density (Table 4) resulted in the determination of two homogenous groups, one at the lower density ($100/m^2$) and the other at the two higher densities (200 and $300/m^2$). In these conditions, best results were obtained for the lower density and the lower food level.

Density	Average (mm)	Homogeneous groups	
100/m^2	46.18	A	
200/m^2	43.01		B
300/m^2	42.71		B

Table 4

In order to obtain more information on the hierarchy between the two higher densities, another ANOVA was performed in the same conditions, after excluding the density of 100/m^2. The results showed (Table 5) that no significant action of the factors was found ($P > 0.05$) and their interaction was not significant. This corresponded to the fact that no differences were observed in the average size of the clam under these conditions (densities of 200 and 300/m^2 and food levels Q1 and Q2) in the area near the water input. However, in the central area a significant difference was observed ($P < 0.05$) between the densities and the food levels. Best results were then obtained at a density of 200/m^2 and for the food level Q2. In the area near the water output, the only significant action was found for the food level factor ($P < 0.001$), while the interaction between the two factors also significantly differed from zero ($P < 0.001$). Thus the food level Q2, as well as the modalities corresponding to the density of 300/m^2 and the food level Q2, gave the best growth in these conditions.

Source of variation	Sun of squares	Degree of freedom	F ratio	Significant level
Main Factors	994.05	119	-	-
1. Density	2.58	1	0.30	NS
2. Food	1.20	1	0.14	NS
Interaction 1, 2	0.97	1	0.11	NS
Residual	989.29	119	-	-

Table 5

The rate of final capture (Table 6) was around 85 % in the different basins. The two extremes were B5, with 72.4 % and B4, with 94.9 % of the initial number of clams caught at the end of the experiment. An ANOVA performed on the capture rate, for the different factors analysed, showed that no significant action of the factor was found, thus indicating that no correlation was observed between the rearing conditions and the mortality of clams.

Basins	B3	B4	B5	B6	B7	B8
Recapture (%)	85.2	94.9	72.4	88.5	80.7	86.7

Table 6

The yields obtained in catch basins (final weight of clams caught divided by the surface of the corresponding basin) are shown in Table 7. An ANOVA performed on the results, for the factors already mentioned, revealed that a significant action was found only for the rearing density ($P < 0.05$). A Newman Keuls'test revealed a positive effect of density ($100 < 200 < 300/m^2$) on the yields. With final biomasses of 3.6 and 4.5 kg/m^2, a density of $300/m^2$ resulted in higher yields than a density of $100/m^2$, despite better growth at such a low density. This could indicate that optimal economic conditions may be reached for smaller surfaces and higher densities ; this would lead to reduced costs of maintenance and catch.

Basins	B3	B4	B5	B6	B7	B8
Yield (kg/m^2)	1.8	1.8	2.4	2.9	3.6	4.5

Table 7

The assimilation efficiency was compared between the basins, from the ratio between the net gain in weight (dry weight of the flesh) of the clam and the weight of *S. costatum* in dry weight, injected into the basins during the experiments. The phytoplankton eventually added from the sea water input was not considered in this computation. The weight of *S. costatum* was considered to be fully assimilated by the clams, thus neglecting the phytoplankton sedimentation inside the ponds and the portion of the food which may not be assimilated by the bivalves. The assimilation efficiency showed large differences for a given density (Table 8). It was generally higher for the food level Q1 (between 10.4 and 18.7 %) than for the food level Q2 (between 4.7 and 13.3 %). A positive relation between the assimilation efficiency and the final yield was found only at the density of $300/m^2$.

	B3	B4	B5	B6	B7	B8
Yield (Δ) per raceway (g)	2 585.8	2 013.9	2 224.9	2 505.1	3 996.3	5 691.0
Total weight of phytoplankton given in 217 days Phy (g of organic matter)	21 316.3	42 632.7	21 316.3	42 632.7	21 316.3	42 632.7
Food chain efficiency (%) Δ/Phy	12.13	4.72	10.44	5.87	18.70	13.35

Table 8

Discussion

High densities of molluscs, as well as the food supplementation may cause environmental disturbances of the sediment, since both of these factors could result in an increase in the biodeposition and organic matter on the ground. However, the contents in organic matters and redox potential measurements did not reveal any reduction at the sediment level (Martin, pers. comm.). The pathological state of the clam was not affected : the Brown Ring Disease, corresponding to the pathogenic bacterium Vibrio P1 (Paillard *et al.*, 1990) was not detected on the internal part of the shells, in spite of the high densities, which usually favour it.

The fact that the organic content of the sediment was not modified may be related to the high assimilation efficiency. It was 18.7 % and 13.3 % for basins B7 and B8. This is similar or slightly higher than the value of 13.6 % given by Tenore and Dunstan (1973) and the value of 11.4 % given by Scura *et al.* (1979). These two results were computed for *Crassostrea virginica* fed on *Thalassiosira sp.* The present results confirmed the high nutritional value of the diatom *Skeletonema costatum* reared on subterranean saline waters. They agreed with those of Laing *et al.* (1987) who have shown the adequation of this species as food for Veneridae, when grown in conventional conditions (Walne, 1966). The average yield of 84.8 % for the different basins also confirmed that these intensive rearing conditions were suitable for clam culture.

However, though the average growth rates were satisfactory, a decrease of these growth rates related to the distance from the water and food input was observed in most of the experimental basins. This spatial heterogeneity may be related to the decrease of the vertical flux of food along the basins. These may be assimilated to raceways and, according to Rhodes *et al.* (1981), the hydraulic circuit of such raceways is indeed not optimally designed. On one hand, part of the circulating water is not accessible for bivalves maintained on the bottom. On the other hand, the phytoplankton sedimentation in these laminar systems may partially deprive the bivalves of the food available. It has been shown for *Ruditapes philippinarum* that high food concentrations are regulated by the production of pseudofeces (Goulletquer *et al.*, 1989). This regulation could therefore be found in areas located near the water input, which may be detrimental to the clams located at the centre or at the end.

In the given experimental conditions, density acted in every case on the growth of the clams, with however more or less acute differences related to the food concentrations Q1 or Q2. The average individual weights were higher at a density of 100 individuals/m^2. They were very similar for the two other densities, except that the best results in these conditions were obtained at 300/m^2 and at a food level Q2, i.e. $1.4.10^9$ cells/animal/day. They were even nearer the results obtained at 100/m^2 by Baud *et al.* (1991).

The growth of clam appeared to be non-linear with density. Similar observations were made by Saint-Felix *et al.* (1984) in extensive rearing conditions. They showed that the growth of clam was higher for rearing densities of 50 and 100/m^2 than for densities of 200, 300 and 400/m^2. This non-linear effect can be explained by different hypotheses. First, dissolved organic matter (dissolved organic carbon and nitrogen, amino acids) are usually excreted both by phytoplanktonic species and by molluscs. These could be directly absorbed or reabsorbed by the clams, thus resulting in a better growth, noticeably during summer, when concentrations are higher, as demonstrated by several authors (Feuillet *et al.*, 1979 ; Frankboner & De Burgh, 1978 ; Bamford & Gingles, 1974 ; Pequignat, 1973 ; Jorgensen, 1983 ; Sielves & Winkler, 1984 ; Melaouah, 1985).

Secondly, an indirect ecological effect may be evoked. As demonstrated by Vincendeau (1987), the excretory products of the clam may increase the fertility of the water, corresponding to an autostimulation of primary productivity. The corresponding fertilization could result, either in an increase of the bacterial populations (Prieur, 1981 ; Martin & Mengus, 1977), or in an increase of the phytoplanktonic biomass. Robert *et al.* (1982) demonstrated that this may result from the dissolved organic nitrogen excreted by molluscs. In recirculating waters, and at high densities, a positive feedback could then be established between mollusc excretion, and primary production, in accordance with the simple pattern given in Figure 6, the final result being an increase in the yields.

The high growth rates, in length, in live weight and in flesh weight (Baud *et al.*, 1991), which were found at the end of basin B8, may thus result from the higher organic excretion

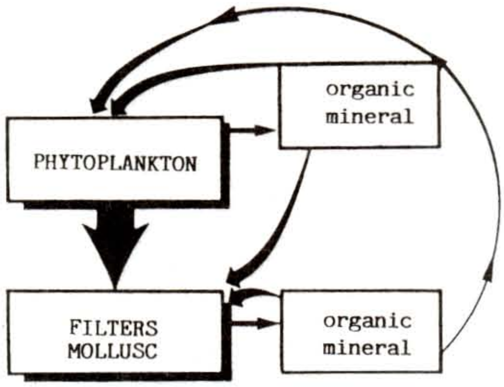

Fig. 6 - Simplified and schematic structure of the ecosystem relationships in a semi-closed environment. The large arrows correspond to consumption and the narrow ones to excretion. Microphytobenthos, bacteria and exchanges with the sediment were not considered.

- Schéma simplifié de fonctionnement de l'écosystème dans un environnement semi-fermé. Les flèches épaisses correspondent à la consommation et les flèches fines à l'excrétion. Le microphytobenthos, les bactéries et les échanges avec le sédiment n'ont pas été pris en compte.

produced by a high density of clam (4 to 4.5 kg/m^2), especially during summer. This hypothesis was not demonstrated in the experiment owing to the lack of adequate data on dissolved organic matter. However, it may suggest an explanation for the fact that growth was depleted in the central part, and slightly stimulated at the end, near the water output of the basin B8, for high densities and high food levels.

Conclusion

The experimental conditions tested aimed at the optimization of intensive rearing of the Manila clam. Despite better growth obtained for the lower density of 100/m^2, a rearing density of 300/m^2 may constitute the best compromise. With 3.6 and 4.5 kg/m^2 caught at the end of the experiment, the corresponding yield is ten times higher than the one recommended by Goulletquer (1988) i.e. 0.4 kg/m^2 in extensive rearing ponds. This yield is also twice higher than the 2 kg/m^2 obtained in intertidal areas of the bay of Bourgneuf (Baud *et al.*, 1988). Further studies should be undertaken to understand the spatial patterns and the distribution of the bulk diatom, *S. costatum*, within the ponds. Finally, the food levels should be optimized, in order to improve the food distribution, and to reduce the inputs of sea water and phytoplanktonic culture. A homogenous growth and lower production costs should then be reached for the intensive rearing of the Manila clam.

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