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Observations on the feeding behaviour of *Crassostrea gigas* larvae in the bay of Arcachon (France)

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Abstract

Crassostrea gigas larvae were collected in the bay of Arcachon in July 1985, 1986 and 1987. Larval samples were collected daily at high tide, during at least the first 12 days of pelagic life and every hour during 1 day and night cycle, 6 days after spawning. Epifluorescence microscopy was used for detecting ingestion and digestion of nanoplankton and thin sections were made to try to determine the algal species ingested. The first algal uptake occurred 48 hours after fertilization and different stages or digestion were noted from the 3rd day. No relationship between ingestion or digestion and the age of the veligers was observed. There was no difference in the feeding activity during day and night. Because the nutrition index was high (70%) the feeding behaviour of the larvae was apparently normal. Nevertheless the intensity of algal uptake was weak. The presence of diatoms in the digestive tract suggests that the Bacillariophyceae play a significant role in the nutrition of veligers in nature.

Keywords : *Crassostrea gigas*, larvae, natural surroundings, nutrition, epifluorescence.

Observations relatives à la nutrition des larves de Crassostrea gigas *dans le bassin d'Arcachon (France).*

Résumé

Des larves de *Crassostrea gigas* ont été récoltées dans le bassin d'Arcachon au cours des saisons de reproduction 1985, 1986 et 1987. D'une part, des prélèvements diurnes ont été réalisés quotidiennement, à pleine mer, pendant au moins les 12 premiers jours de la vie pélagique. D'autre part des prélèvements ont été effectués, à intervalle d'une heure, de jour et de nuit, à point fixe, 6 jours après la ponte. L'ingestion et la digestion du nanoplancton par les véligères du milieu naturel ont été étudiées au microscope à épifluorescence et sur coupes semi-fines. La première ingestion est observée 48 heures après fécondation. Les différents stades de digestion sont notés dès le 3^{e} jour. Aucune relation entre l'ingestion ou la digestion et l'âge des véligères n'a pu être mise en évidence. Il n'existe pas de différence de comportement alimentaire des véligères entre le jour et la nuit. L'indice d'alimentation, supérieur à 70%, indique un état nutritionnel satisfaisant. Néanmoins, l'indice d'ingestion et l'analyse des préparations histologiques montrent que la quantité d'algues captées par les véligères du milieu naturel est faible. La présence de diatomées dans leur tractus digestif laissent supposer que les Bacillariophycées jouent un rôle non négligeable dans l'alimentation des véligères *in situ*.

Mots-clés : *Crassostrea gigas*, larves, milieu naturel, nutrition, épifluorescence.

INTRODUCTION

In hatchery conditions, nutrition is one of the predominant factors affecting larval growth of molluscs (Robert *et al.*, 1988). Since the early works of Loosanoff and Davis (1963) and Walne (1970), it has been well established that bivalve larvae are dependent on nanoplankton as their principal source of food (Ukeles, 1980; Webb and Chu, 1981). On the other hand, studies of the abnormalities in the development of oyster larvae in the bay of Arcachon showed that a lack of proper food in nature may affect the pelagic life of veligers (His and Robert, 1985).

The bay of Arcachon is an important oyster spat producing area. It has been previously shown that in certain years spawning of oysters is synchronous within a certain time scale (His and Robert, 1985). Indeed, when heavy spawnings occur and when oysters release most of their eggs during the first spawning, straight hinged stage larvae are detected in large quantities in the plankton 24 hours after fertilization; it is then possible to study the evolution of a single veliger cohort throughout its pelagic life and to determine the age of the larvae at any time.

The application of a new technique for isolating oyster larvae from the plankton enabled us to acquire information on the biology of bivalve larvae in natural surroundings (His and Robert, 1987*a*; His and Maurer, 1988). Because little is known on the subject, our observations were focused on the nutritional requirement of bivalve larvae *in situ*. In the bay of Arcachon the potential role of the nanoplankton in the feeding of *Crassostrea gigas* larvae were shown (Maurer *et al.*, 1984; His and Robert, 1987*a*) and the food value of some algal species ingested by veligers in nature were studied (His *et al.*, 1985; His and Robert, 1987*b*).

Preliminary observations using epifluorescence microscopy have been previously reported for samples collected in 1985 (Robert and His, 1987*a*). The main results were the following: the first algal uptake was observed when the larvae were 2 days old. Beyond the 4th day, most of the veligers were found to be stage 2, while the number of larvae belonging to stage 4 was very low.

Epifluorescence observations carried out on umboned larvae (16 and 21 days old) showed that the thickness of the shell does not allow such grazing studies.

The present work attempts to precise the feeding behaviour of *Crassostrea gigas* larvae in natural surroundings. Epifluorescence microscopy was used for detecting ingestion and digestion of unicellular algae and histological sections were made to try identify the species ingested.

MATERIALS AND METHODS

Sampling was performed, at high tide, in the channel of Comprian (*fig.* 1) during the months of July

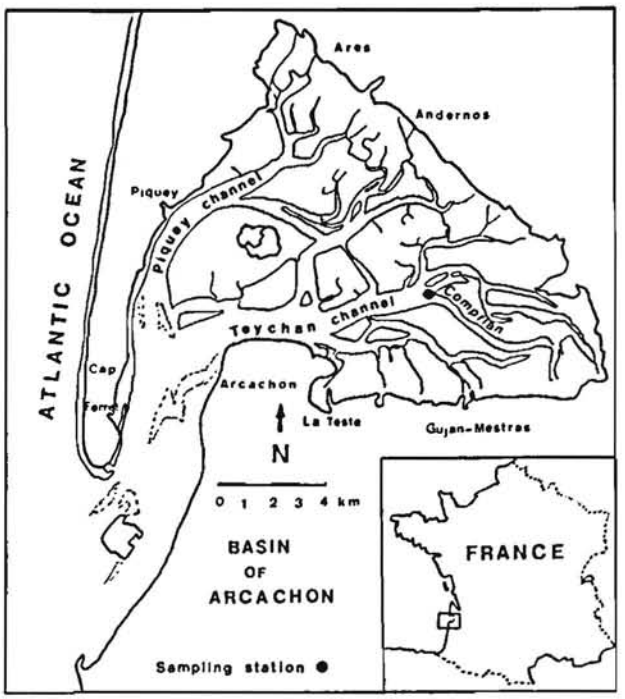

Figure 1. — Map of study area showing sampling station.

1985, 1986 and 1987. In 1985, larvae of 2, to 7, 9, 13 and 21 days old were collected. Because it has been previously shown that such grazing studies on old larvae are unsuccessful (Robert and His, 1987) the samples were only performed during the first 12 days after fertilization in 1986. The samples collected daily included two samples taken at night. The first one consisted of very early umboned stage larvae (3 to 4 day old larvae) and the second one umboned stage larvae (11 to 12 day old larvae). In 1987, observations on nychthemeral feeding behaviour were only carried out on 6 to 7 day old larvae.

All the samples were kept at 0 °C on ice until their return to the laboratory, less than 1 hour later. After isolation, veligers were filtered on black nucleopore polycarbonate 12 µm filters and observed immediately under a 20 E B epifluorescence microscope or fixed for histological preparations.

Epifluorescence microcospe observations were realized on one hundred randomly selected larvae at a magnification of 400.

The Babinchack and Ukeles'scale (1979) was used to define arbitrary ingestion and digestion stages of phytoplanktonic cells by the larvae. The following stages were recognized:

- Stage 1, whole cell stage (ingestion): algal cells are intact and characterized by their well defined outline. They appear as distinct deep red points.

• Stage 2, lysed cell stage (beginning of digestion): intact cells and lysed cells coexist together. The lysed cells appear orange in colour.

• Stage 3, digested cell stage. No more intact cells. Fluorescence becomes very diffused and tends to be fixed in colour.

• Stage 4, empty stage. No cells. No fluorescence signs of algal feeding or digestion. The larvae have never fed or complete digestion of the food has taken place.

The feeding index, number of stage 1 and stage 2 larvae divided by the number of larvae observed per sampling (Salăun, 1987) was also used. To characterize the fullness of the larval stomach (stage 1), the following scale used by Lucas and Rangel (1983) for the young veligers was adopted: when the number of algal cells is less than 10, ingestion is weak. When 10 to 20 algal cells are present, ingestion is medium. When this number is more than 20, ingestion is high.

Larval samples of 2 to 6 and 7 days old were immediately fixed, decalcified and included in resin (Cragg, 1976). Semi thin sections colored with Sahli blue (Langeron, 1949) were made for detailed observations under the microscope at a magnification of 400 to 1 000. Such examinations were only realized on larvae collected in July 1985.

RESULTS

The nutritional state of *Crassostrea gigas* larvae collected during the 1986 breeding season is illustrated in *figure* 2. No ingestion and digestion of nanoplankton cells were observed on 24 hour larvae (100% stage 4). The first algal uptake took place 48 hours following fertilization. Nevertheless more than half the population exhibited empty stomachs. Digestion took place on the 3rd day where a low percentage of larvae belonging to stage 1 was observed (3%). Such observations were made on 5 day and 9 day old larvae (respectively 5 and 8% of stage 1).

Beyond the 2nd day no relationship between ingestion and the age of the veligers was noticed. From the 3rd day the percentage of stage 2 was high. With the exception of the 4th and 5th days the percentage of stage 2 was greater than 30% and rates greater than 60% were noticed (3rd, 8th, 9th day). Apart from the 5th day, the percentage of stage 3 larvae was always 30% lower. Beyond the 2nd day, the number of empty larvae was very low ($\geq 10\%$). The only exception was noticed on the 11th day (44% of stage 4). Beyond the 2nd day, the feeding index was high. This was generally found to be higher than 70%.

On days 2, 3-4, 4, 6, 8, 11 and 11-12, the percentage of stage 1 larvae was higher than 15% and so we studied the fullness of the larval stomach. The results of larval ingestion are shown in *table* 1. Ingestion is generally weak except on day 3-4 where ingestion was medium (68%). This last result, as well as the high feeding index which characterized the night samples (95 and 98% respectively on the 3rd-4th day and on the 11th-12th day) led us to perform a short day and night cycle in July 1987, 6 to 7 days after fertilization.

The nutritional state of these veligers is illustrated in *figure* 3. There was no difference in the grazing activity during day and night. In all cases feeding index was very high ($\geq 80\%$). The results of larval ingestion are shown in *table* 2. More than 60% of the population ingested a low number of algae. The histological sections of larvae collected in 1985 confirmed the weakness of ingestion. The number of nanoplankton cells found in the stomach never exceeded three. The average size of the ingested material was 2 to 4 µm diameter. In its smallest dimension the minimum cell size was approximately equal to 1.5 µm and the maximum to 6 µm. Nevertheless in its longest dimension particles as long as 8 µm were observed. A diatom of 14 µm long was even noticed in the stomach of a 5 day old larvae. Round and ovoïd shape algae accounted for 80% of the ingested particles. Because we did not notice the flagella (absence or loss) no identification of these forms was made. However, pennated diatoms were observed.

DISCUSSION AND CONCLUSION

In nature, at an average temperature of 22 °C, the first algal uptake by *Crassostrea gigas* takes place 48 hours after fertilization. This result differs from those reported with the same species under controlled conditions. Lucas and Rangel (1983) showed that, at 21 °C, the first larval feeding takes place 30 hours after fertilization. Our observations realized on two batches of larvae obtained from two different breeding stocks and reared at 22 °C under controlled conditions confirmed this last result (*table* 3): the first algal uptake took place either 26 hours (batch 1) or 30 hours (batch 2) after fertilization, while digestion occurred 48 hours after fertilization.

Because the percentage of larvae at stage 2 is generally high while the percentage of larvae at stage 3 is low, ingestion and digestion of nanoplankton by *Crassostrea gigas* veligers seem to be continuous in the bay of Arcachon. Such results have been reported with the same species under controlled conditions (Lucas and Rangel, 1981).

The number of algal cells detected in the stomach of young larvae collected in the bay of Arcachon is generally less than 10 which means that ingestion is weak in nature. In the hatchery this number is normally more than 20. This last result could be explained by the difference in quantity between food available in nature and that used in experimental conditions. Indeed, in the hatchery, larvae are usually fed daily with a mixture of 10^5 cell.cm^{-3}. The weight equivalent of this amount may be estimated as follows: *Isochrysis galbana*'s mean cell volume is equal

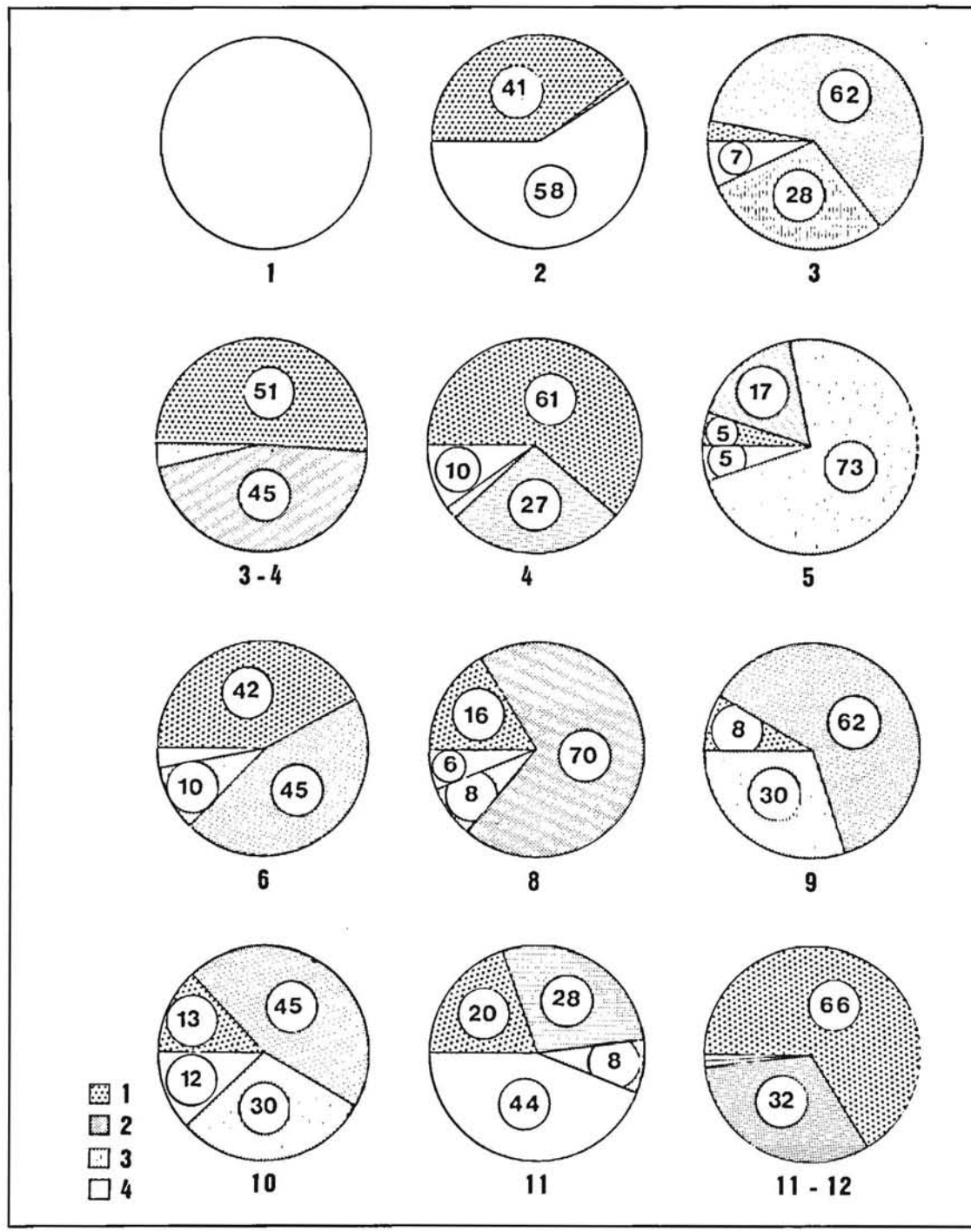

Figure 2. — Percentage of larvae observed at each feeding stage. 1: whole cell stage (ingestion); 2: lysed cell stage (beginning of digestion); 3: digested cell stage (digestion); 4: empty stage (complete digestion or no ingestion). Sampling was performed during the first 12 days after fertilization by day (1, 2, 3, ...) or by night (3-4 and 11-12) in July 1986.

to 40 μm^3 (Robert and His, 1987*b*) and 1 μm^3 corresponds to 10^{-6} µg algal weight (Travers, 1971). So the daily potential amount of food in the hatchery is equal to 4 $\mu g.cm^{-3}$. Nanoplankton biomass, recorded since 1983 in July in the bay of Arcachon, fluctuated from 150 to 700 $mg.m^{-3}$ and average values of 300 $mg.m^{-3}$ were frequently recorded (Guillocheau, 1988). If this last value is retained, the potential amount of food for larvae in the bay of Arcachon is equal to 0.3 $\mu g.cm^{-3}$ which represents 1/10 of the food available under hatchery conditions.

Nevertheless, when heavy spawning occurred in the bay of Arcachon, the density of straight hinged stage larvae is 0.5 to 1 $.cm^{-3}$ (His and Robert, 1985) while

Table 1. — Percentage of various stages of stage 1 larvae (ingestion). Sampling was performed in July 1986 during the first 12 days after fertilization but results were only reported when the percentage of stage 1 was higher than 15% (* night).

Age of larvae from fertilization (days)	Fullness of larval stomach		
	Weak	Medium	High
2	100	0	0
3-4 *	29	68	3
4	87	0	13
6	57	29	14
8	50	12	38
11	100	0	0
11-12 *	100	0	0

Table 2. — Percentage of various stages of stage 1 larvae (ingestion). Sampling was performed at the times shown, in July 1987, 6 to 7 days after fertilization (* night).

Sampling (hours)	Fullness of larval stomach		
	Weak	Medium	High
13.00	81	13	6
14.00	66	18	16
15.00	71	19	10
16.00	92	6	2
2.00 *	57	32	11
3.00 *	71	21	8
4.00 *	83	15	2
5.00 *	65	22	13
6.00 *.	60	22	18

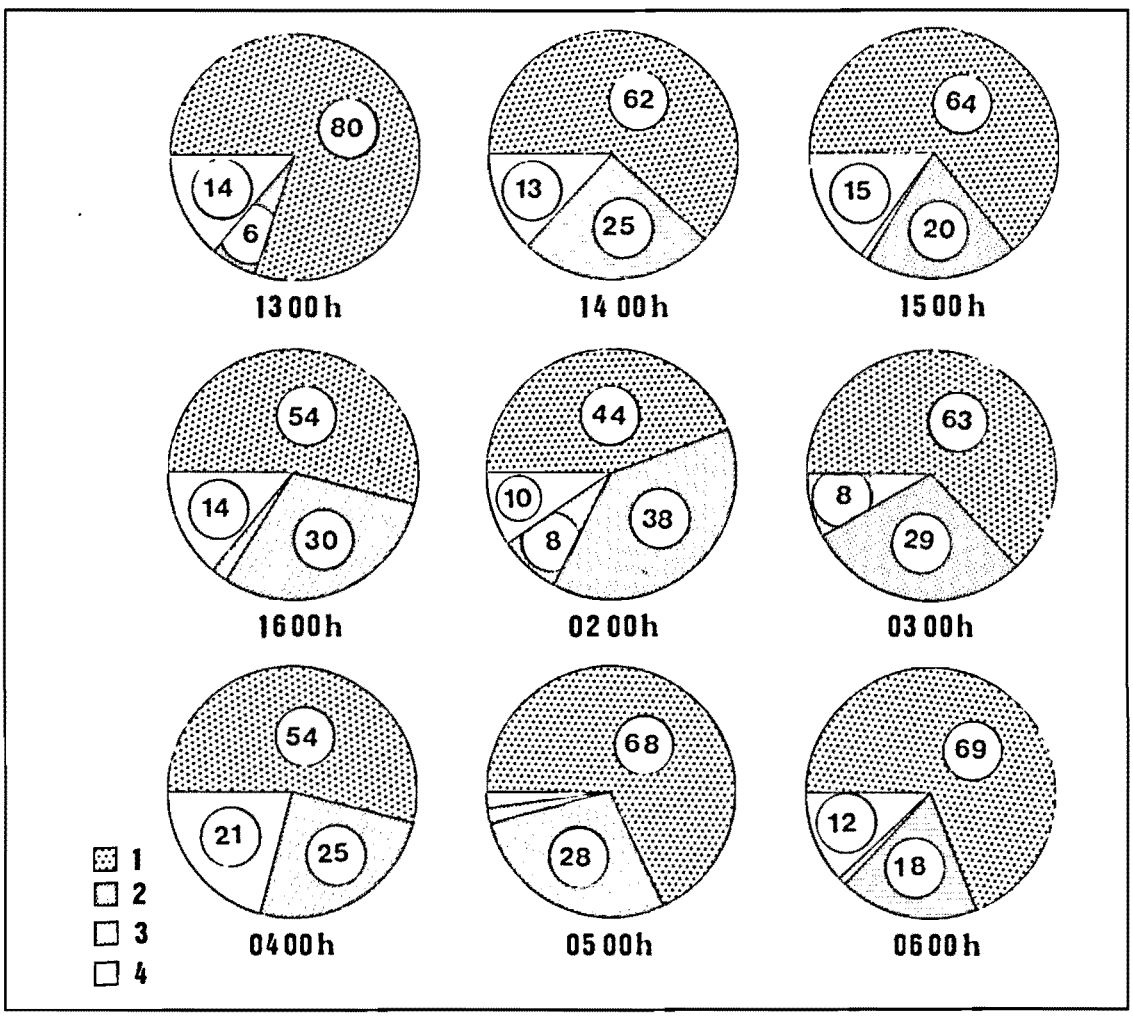

Figure 3. — Percentage of larvae observed at each feeding stage. 1: whole cell stage (ingestion); 2: lysed cell stage (beginning of digestion); 3: digested cell stage (digestion); 4: empty stage (complete digestion or no ingestion). Sampling was performed each hour over a short day-night period in July 1987, 6 to 7 days after fertilization.

the number of larvae usually reared in the hatchery is 5 to 10 cm^{-3} which is 10 times higher. So the potential amount of food per larvae reared in the hatchery or under natural environmental conditions is approximately the same, if competitors are left out of consideration. If the grazing by competitors must be considered, the fact that in the bay of Arcachon nanoplankton bloom and *Crassostrea gigas* spawning occur at the same time has to be kept in mind (Maurer *et al.*, 1984; Guillocheau, 1988). At this moment

Table 3. — Nutritional state of *Crassostrea gigas* larvae under laboratory conditions. The veligers were fed when they were 24 hours old. The observations were made on two batches of larvae (1) and (2) reared at 22°C and obtained from two different breeding stocks.

Age of larvae from fertilization (hours)		Percentage of larvae observed at each feeding stage			
		1	2	3	4
26.00	(1)	17	21	13	49
	(2)	2	0	0	98
30.00	(1)	19	15	5	61
	(2)	16	0	0	84
48.00	(1)	0	60	20	20
	(2)	3	45	40	12

C. gigas larvae represent the main fraction of the zooplankton lower than 100 µm in size. So the difference in feeding activity of the larvae reared under controlled conditions or in nature can not be explained in this way. There is no relationship between the feeding index and the age of the larvae. This result is not in agreement with those reported by Salaün (1987). Because this feeding index is high (70 to 80%), the feeding behaviour of *C. gigas* larvae was normal during these last two breeding seasons.

No difference in the feeding activity during day and night was noted.

The analysis of larval stomach content on thin sections confirms the weakness of ingestion in natural surroundings. The number of algal cells observed in such conditions never exceeded three. Under laboratory conditions this number is higher: 15 to 20 cells could be easily recognized in the stomach of *Mytilus edulis* and *Pecten maximus* young veligers (Rangel, 1984).

Due to the contact with the crystalline style, the shape of algal cells observed in the stomach of the larvae is generally deformed (Rangel, 1983). Nevertheless the size of ingested algae may be appreciated. In natural surroundings young veligers feed on 1.5 to 6 µm sized particles with a preference for algae of average size of 2 to 4 µm. Diatoms were the only species identified. They accounted for 20% of ingested material. This result confirms those obtained from previous works on oyster larvae extract content (His *et al.*, 1985; Chrétiennot-Dinet and Guillocheau, 1987; His and Robert, 1987*a*).

The excellent food value of *Chaetoceros calcitrans* forma *pumilum* to larvae of *Crassostrea gigas* is well known (Nascimento, 1980; Helm and Laing, 1987). This diatom, as well as *Chaetoceros gracilis* are produced routinely in nurseries because they support good growth of cupped and flat oyster spat (Enright *et al.*, 1986; Laing and Millican, 1986). Because of smaller-sized diatom abundance during summer in the bay of Arcachon (Maurer *et al.*, 1984; Guillocheau, 1988) Baccillariophyceae probably contribute to *C. gigas* larval feeding in natural surroundings.

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