Haliotis **27**: 29-34 (1998) Société Française de Malacologie

Nutritional inadequacy of *Nannochloris atomus* and *Stichoccocus bacillaris* for the oyster *Crassostrea gigas* (Thunberg) larvae

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ABSTRACT

The evaluation of *Nannochloris atomus* and *Stichoccocus bacillaris* as nutrient sources for oyster larvae has been attempted in the present work. After a study of their growth and size distributions, these two species were used as food for *Crassostrea gigas* larvae. Despite proper sizes (2.5 to 20 μm^3) the larval development of *Crassostrea gigas* was poor. Evaluation of ingestion and digestion of both algae, by means of epifluorescence microscopy, on 2 day and 6 day old larvae showed that they were normally ingested but weakly digested. Consequently, their nutritional inadequacy for larval molluscs may be also related to their indigestibility.

RÉSUMÉ

Qualité nutritionnelle inadéquate de *Nannochloris atomus* et *Stichoccocus bacillaris* pour les larves de *Crassostrea gigas*.

L'évaluation de *Nannochloris atomus* et de *Stichoccocus bacillaris* comme algue fourrage pour des larves d'huîtres a été réalisée au cours du présent travail. Après une étude en milieu contrôlé de la croissance et de la distribution en taille de ces algues, ces deux espèces de phytoplancton ont servi d'aliment à des larves d'huître *Crassostrea gigas*. Malgré la petite taille du nanoplancton (2,5 à 20 μm^3) le développement larvaire de l'huître est médiocre. L'étude de leur assimilation et dégradation en microscopie à épifluorescence montre que ces algues sont bien ingérées mais peu digérées par des larves de 2 et 6 jours. Leur pauvre valeur alimentaire peut donc être également reliée à leur faible digestibilité.

INTRODUCTION

Despite recent progress in artificial diets (Nell and O'Connor, 1991 ; Robinson, 1992 ; Southgate *et al.*, 1992 ; Laing and Millican, 1992 ; Laing and Lopez Alvarado, 1994 ; Coutteau *et al.*, 1994 ; Robert *et al.*, 1996 ; Nell *et al.*, 1996), phytoplankton is still today the only available food for rearing molluscs. The suitability of a phytoplankton species employed as an algal diet may be characterized by its size, mobility, digestibility and biochemical composition. Moreover, to be commonly used in a hatchery-nursery, the mass culture of microalgae must be easy, fast growing and

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reliable (Robert and Trintignac, 1997) Few nanoplankton organisms fit these criteria (Chrétiennot-Dinet *et al.*, 1986 ; Coutteau and Sorgeloos, 1992) and in order to increase this number. several unicellular marine algae have been isolated from an oyster spatting area, the Bay of Arcachon (Chrétiennot-Dinet *et al.*, 1991). The food value of two of them, *Nannochloris atomus* and *Stichoccocus bacillaris*, as single species, for the larvae of *Crassostrea gigas* is reported in the present work.

MATERIALS AND METHODS

The growth and size distributions of *Nannochloris atomus* and *Stichoccocus bacillaris*, grown in batch culture in 5 liter flasks, were studied, for 6 weeks by means of a Coulter counter ZB ZBi connected to a channelyser C1000, following the techniques previously described (Robert and His, 1987). These two chlorophyceae were isolated from the Bay of Arcachon, and their identification previously made (Chretiennot *et al.*, 1991). Their effects on growth rate and survival of *Crassostrea gigas* larvae were investigated following the techniques reported by Robert *et al.* (1989). Moreover, ingestion and digestion rates of these unicellular algae by *Crassostrea gigas* larvae were compared to those of *Isochrysis galbana* (control: strain originated from Conway, Chretiennot *et al.*, 1991). Epifluorescence microscopy was used to detect uptake and lysis of each phytoplankton species on 2 day and 6 old larvae fed on a single diet according to Babinchack and Ukeles (1979).

RESULTS

From Day 1 to Day 13 the concentration of *Nannochloris atomus* varied from 1.4×10^7 cell. ml^{-1} to 16.4×10^7 cell. ml^{-1} with a daily division rate of 0.298 (Fig. 1). Optimum cell density was noted on Day 28 with 2.3×10^8 cell. ml^{-1}. The culture did not collapse at the end of the experiment, on Day 48 and thus the stationary phase lasted at least 5 weeks. From Day 2 to Day 12 the concentration of *Stichoccocus bacillaris* varied from 1.16×10^7 cell. ml^{-1} to 7.3×10^7 cell. ml^{-1} with a daily division rate of 0.265 (Fig. 1). Optimum cell density was observed on Day 39 with 1.17×10^8 cell. ml^{-1}. The experiment ended on the 46th day with no collapse of the culture.

Similar size frequency distribution of *Nannochloris atomus* was recorded until the 25th day of culture with an average cell volume of 4.5 μm^3 ($\approx$ 2 μm equivalent diameter). Then a slight increase in cell volume was noted. Similar size frequency distribution of *Stichoccocus bacillaris* was recorded during the exponential phase of growth with an average cell volume of 6 μm^3 ($\approx$ 2.30 μm equivalent diameter). Nevertheless, during the stationary phase, its cell volume increased gradually to reach an average cell volume of 14 μm^3 ($\approx$ 3.00 μm equivalent diameter).

No growth occurred when young *Crassostrea gigas* larvae were fed for 8 days with *Nannochloris atomus* or *Stichoccocus bacillaris* (Fig. 2). In contrast, the control fed with *Isochrysis galbana* grew well (Fig. 2) and exhibited a daily width increment of 5.91 μm. High mortalities ($\geq$ 80 %) were recorded at the end of the experiments, on Day 11, when larvae were fed with these chlorophyceae while only 5 % were observed in the controls.

Two hours after feeding, the ingestion of *Nannochloris atomus* and *Stichoccocus bacillaris* by 2 day old *Crassostrea gigas* larvae was equivalent to that of *Isochrysis galbana* (Table 1). However, 24 h later, no digestion of *Nannochloris atomus* occurred and, compared to the control, a delay in that process was equally noted with *Stichoccocus bacillaris* (Table 1). On the other hand, the uptake and beginning of lysis of *Nannochloris atomus* and *Stichoccocus bacillaris* by 6 day old *Crassostrea gigas* larvae were noted 3 h after feeding. In contrast, during the same period, those fed with *Isochrysis galbana* exhibited effective digestion. A greater delay was noted 24h later when complete digestion was only observed with *Isochrysis galbana* (Table 2).

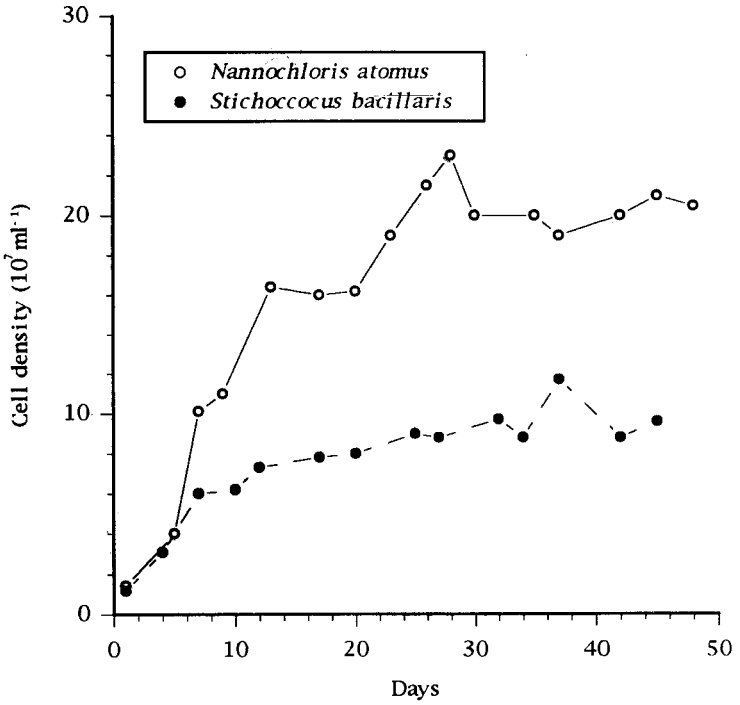

Figure 1. Growth of *Nannochloris atomus* and *Stichoccocus bacillaris* in batch culture (5 l flasks).

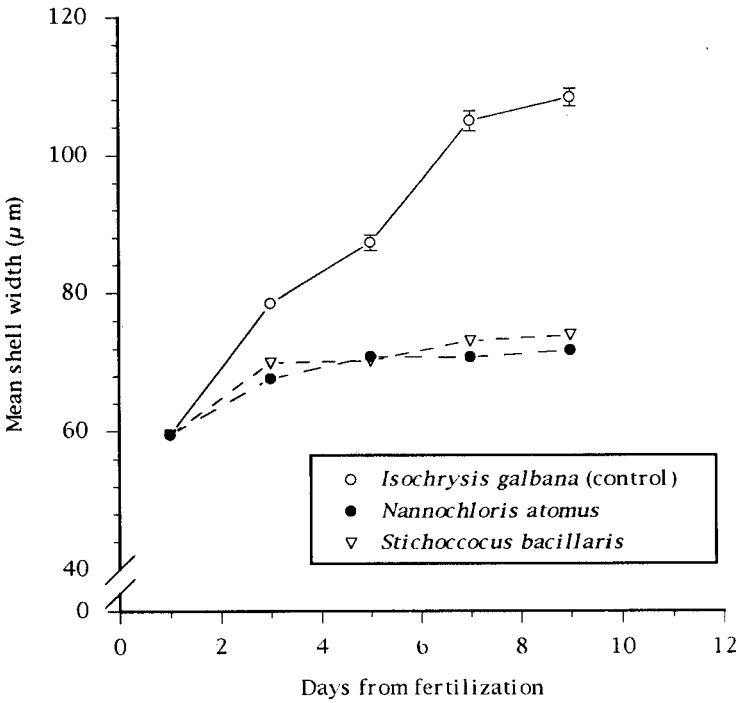

Figure 2. Growth of *Crassostrea gigas* larvae fed with *Isochrysis galbana* (control), *Nannochloris atomus* and *Stichoccocus bacillaris*.

Table 1. Rates of ingestion and digestion of *Nannochloris atomus*, *Stichoccocus bacillaris* and *Isochrysis galbana* (control) by 2 day old *Crassostrea gigas* larvae.

Sampling (hours)	*Nannochloris atomus*				*Stichoccocus bacillaris*				*Isochrysis galbana*			
	% of larvae observed at each feeding stage				% of larvae observed at each feeding stage				% of larvae observed at each feeding stage			
	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4
2	100	0	0	0	100	0	0	0	97	3	0	0
4	100	0	0	0	95	5	0	0	0	40	60	0
24	100	0	0	0	2	38	60	0	0	5	75	20

Stage 1 : Ingestion; Stage 2 : Beginning of digestion ; Stage 3 : Digestion; Stage 4 : Complete digestion.

Table 2. Rates of ingestion and digestion of *Nannochloris atomus*, *Stichoccocus bacillaris* and *Isochrysis galbana* (control) by 6 day old *Crassostrea gigas* larvae.

Sampling (hours)	*Nannochloris atomus*				*Stichoccocus bacillaris*				*Isochrysis galbana*			
	% of larvae observed at each feeding stage				% of larvae observed at each feeding stage				% of larvae observed at each feeding stage			
	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4
3	56	44	0	0	20	78	2	0	10	70	20	0
6	42	46	8	4	0	90	6	4	0	16	74	10
24	23	70	5	2	0	80	10	10	0	0	40	60

DISCUSSION

It is widely accepted that phytoplankton constitutes the principal food of bivalve larvae but not all phytoplankton species are equally suitable. Firstly, the good food value of some algae is related to their cell size. Concerning the upper size limit, algal cells have to be less than the larval gut diameter and most of the algal species used in mollusc hatchery are less than 15 µm accordingly (Robert and Trintignac, 1997). Concerning the lower size limit the ability to ingest cells less than 2 µm was shown to be weak until recently (for reviews see Bayne, 1983, Strathmann, 1987). Nevertheless on natural seston *Crassostrea virginica* larvae are able to consume lower size food particles (0.2-0.8 µm: Baldwin and Newell, 1991). Moreover, when fed on cultured algal mixtures (size range:1 to 11 µm) small larvae (< 150 µm) preferred 1 µm algae while large larvae preferred 11 µm algae (Baldwin, 1995). *Nannochloris atomus* and *Stichoccocus bacillaris* are in the average range of 1 to 3 µm and have therefore the appropriate size to be well ingested by young *Crassostrea gigas* larvae. Epifluorescence microscopic observations, used in the present study, confirmed this assumption, both chlorophyceae being ingested at an equivalent rate than *Isochrysis galbana*, known to be highly consumed by mollusc larvae.

Secondly, to be commonly used in a hatchery-nursery, the mass culture of microalgae must be easy, fast growing and reliable (Robert and Trintignac, 1997). Despite high cell densities (from 60 to 100. million. ml^{-1}) at the end of the first week, the growth of *Nannochloris atomus* and *Stichoccocus bacillaris* was moderate. Indeed, their division rates, 0.265 and 0.298 were relatively low compared to other phytoplankton species grown in similar conditions (Robert and His, 1987). In contrast, both species did not collapse after 1.5 months of culture. This hardiness is noteworthy and may explain the excellent adaptability of *Nannochloris* sp. to open air conditions (Witt *et al.*, 1981).

Thirdly, to allow the transfert of nutrients to the larvae the algae must be well digested. In young larvae, no digestion of *Nannochloris atomus* occurred 24 h after feeding and a delay was

noted with *Stichoccocus bacillaris*. Similar trends were also observed in older larvae. When fed 24 h before with *Isochrysis galbana*, the whole larval population had digested this algae while few larvae were digesting the chlorophyceae in the meantime. Similar results have been reported with *Chlorella autotrophica* on *Crassostrea virginica* (Babinchack and Ukeles, 1979) and with *Stichoccocus* sp on *Artemia* (Gibor, 1956). Because both chlorophyceae are weakly digested by young *Crasssostrea gigas* veligers, no larval growth occurred. Such absence of growth was alsc observed on *Tapes philippinarum* and *Pecten maximus* larvae fed, respectively as single species, with fresh *Nannochloris atomus* (Laing *et al.*, 1990) and fresh *Chlorella minutissima* (Robert *et al.*, 1996).

Moreover, when fed *Nannochloris atomus*, growth was also poor in *Mercenaria mercenaria* spat while reasonably good development of *Ostrea edulis* juveniles was obtained at high ratio (500 cells.μl^{-1}: Walne, 1970). However, the latter author reported that *O. edulis* spat growth was only equal to the half of the *Isochrysis* control.

In contrast, Laing *et al.* (1990) have shown that the equivalent of *Nannochloris atomus* as dried algae allowed larval development. This result suggests that drying process may induced a fragility of the cell wall making the diet more easily digested.

Lastly, several authors have reported a lack or low levels of polyunsaturated fatty acids (PUFA) in these chlorophyceae (Dunstan *et al.*, 1992 ; Jeffrey *et al.*, 1994). PUFA are well-known to be essential compounds in the growth of marine molluscs (Brown *et al.*, 1989) and the biochemical composition of *Nannochloris atomus* and *Stichoccocus bacillaris* may also explain their poor quality for *Crassostrea gigas* larval development.

CONCLUSION

Despite proper sizes and hardiness of the phytoplankton cultures, neither *Nannochloris atomus* nor *Stichoccocus bacillaris* are good food organisms for *Crassostrea gigas* larvae. This inadequacy may be related to their weak digestibility, shown especially on young veligers, linked to their inadequate biochemical composition. Similar results have been reported on *Chlorella* and therefore, it may be confirmed that chlorophyceae are unsuitable, as single diets, for mollusc larval rearing.

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