

# Feeding responses of *Crassostrea gigas* (Thunberg) to inclusion of different proportions of toxic dinoflagellates in their diet

*Crassostrea gigas*  
*Thalassiosira weissflogii*  
*Alexandrium tamarensense*  
Ecophysiology  
Paralytic shellfish poison

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Toxine paralysante des coquillages

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## ABSTRACT

An experimental flow-through system allowing determination of the feeding behaviour of individual molluscs was used to study oysters exposed to mixed diets composed of varying proportions of the diatom *Thalassiosira weissflogii* and two strains (toxic and nontoxic) of the dinoflagellate *Alexandrium tamarensense*. Our results show that, when compared to a *T. weissflogii* unialgal diet, even a diatom/toxic dinoflagellate ratio as low in biomass as 90/10 reduced clearance rates and biodeposit production by oysters. Consumption was slightly but significantly decreased for a 50/50 diatom/toxic dinoflagellate mixture. For the toxic dinoflagellate unialgal diet, ingestion, absorption and consumption were completely inhibited. Thus, the inclusion of low amounts of toxic *A. tamarensense* in a diet composed of *T. weissflogii* significantly altered pseudofaeces production and probably oyster filtering capacity, whereas no significant effect was observed with the non-toxic dinoflagellate.

## RÉSUMÉ

Réponse écophysiologique de *C. gigas* (Thunberg) à l'introduction de proportions variables de dinoflagellés toxiques dans le régime alimentaire.

A l'aide d'un dispositif expérimental en circuit ouvert permettant de mesurer le comportement alimentaire individuel de bivalves, des huîtres (*Crassostrea gigas*) ont été exposées à des régimes alimentaires mixtes comprenant des proportions variables de la diatomée *Thalassiosira weissflogii* et de deux souches, l'une毒ique, l'autre non toxique, du dinoflagellé *Alexandrium tamarensense*.

Les résultats montrent que même pour un rapport aussi faible que 90/10 en biomasse du régime diatomée / dinoflagellé toxique, le taux de filtration et la production de biodépôts sont réduits par rapport au témoin (diatomée seule). La consommation n'est diminuée de façon significative que pour un mélange 50/50 du même mélange tandis que l'ingestion, l'absorption et la consommation sont complètement inhibés pour un régime composé uniquement du dinoflagellé toxique. Il apparaît donc que l'introduction, même faible, d'*Alexandrium* toxique dans un régime constitué de *T. weissflogii* altère la capacité des huîtres à produire des pseudofèces et probablement aussi à filtrer, alors que l'introduction dans le même régime d'un *Alexandrium* non toxique ne produit aucun effet significatif.

## INTRODUCTION

In a previous study (Bardouil *et al.*, 1993), we showed that the feeding habits of the oyster *Crassostrea gigas* (Thunberg) are especially affected by toxic dinoflagellates of the genus *Alexandrium* (*A. tamarensense* and *A. minutum*), particularly through inhibition of clearance rates and reduced production of biodeposits. However, we found that absorption efficiency was markedly better for the highly toxic (*A. tamarensense*: 7200 ng STX eq./ $10^6$  cells) than the moderately toxic species (*A. minutum*: 500 ng STX eq./ $10^6$  cells), *i.e.* respectively 78% more and 61% less than the two nontoxic controls used in these experiments.

These studies were continued in the present work which investigated, in particular, the relative effect of different toxic microalgae when included in a standard diet, *i.e.* a nontoxic microalga [the diatom *Thalassiosira weissflogii* (Grunow) Fryxell and Hasle] currently used in aquaculture and considered to approximate the type of food available in springtime along French and North European coasts (Rich and Morel, 1990; Armbrust and Chisholm, 1992; Goericke and Welschmeyer, 1992). A recent study by Bricelj *et al.* (1991) showed that another filter-feeding shellfish, *Mercenaria mercenaria*, did not ingest a diet composed solely of a highly toxic strain of *A. fundyense*, whereas the addition of a non-toxic diatom, *T. weissflogii*, facilitated absorption of the dinoflagellate. Accordingly, we performed several experiments to study the behaviour of *C. gigas* when exposed to a reference diet (*T. weissflogii* alone) or a mixed diet consisting of 10 or 50% (in equivalent dry weight) of toxic *A. tamarensense* plus 90 or 50% respectively of *T. weissflogii*. The objective was to determine the minimum value at which a change in oyster behaviour toward *T. weissflogii* would be significantly apparent. Similar experiments were also conducted comparing the effects of the lowest ratio of toxic *A. tamarensense* causing behavioural changes with those of an equivalent mixture composed of *T. weissflogii* and non-toxic *A. tamarensense*.

## MATERIALS AND METHODS

Pacific oysters, *Crassostrea gigas*, were obtained from Bourgneuf Bay (French West Atlantic coast) in an area not known to be exposed to toxic blooms of species of the

genus *Alexandrium*. For the first series of experiments, oysters obtained in November from coastal waters had a mean individual dry weight of  $0.83 \pm 0.11$  g. The specimens were acclimated for a week in 35-litre ventilated tanks at the same temperature as coastal waters ( $12^\circ\text{C}$ ), with eight individuals per tank. The oysters obtained in April for the second series of experiments had an individual dry weight (after heating for 72 h at  $60^\circ\text{C}$ ) of  $1.48 \pm 0.9$  g and were acclimated in similar fashion but at  $16^\circ\text{C}$  since the temperature of the bay was higher at that time. During acclimation, the shellfish were fed daily with a culture of *T. weissflogii*, and tank water was changed every other day. The monospecific algal cultures used for experiments were obtained in the conditions summarized in Table 1. *T. weissflogii* and *A. tamarensense* were supplied by the algae collection of the Marine Biology Laboratory (Plymouth, UK), whereas toxic *A. tamarensense* (MOG 835) was provided by Sendaï University (Onagawa, Japan). Cultures were performed with natural sea water filtered on Millipore membrane (porosity 0.22  $\mu\text{m}$ ) and enriched with nutrient solution to obtain the "ES" medium described by Provasoli (1966). Salinity in these conditions was 37, and the lighting provided was 2500 lux ( $50 \pm 4 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ), with a 12/12 photoperiod. The toxicity of the MOG 835 strain was estimated to be 7200 ng STX eq./ $10^6$  cells (Bardouil *et al.*, 1993).

Six experiments were performed at ambient temperature to avoid thermal stress: 3 at  $12^\circ\text{C}$  (*T. weissflogii* alone, *T. weissflogii* + toxic *A. tamarensense* 50%, *A. tamarensense* alone) and 3 at  $16^\circ\text{C}$  (*T. weissflogii* alone, *T. weissflogii* + toxic *A. tamarensense* 10%, *T. weissflogii* + nontoxic *A. tamarensense* 10%). For each experiment, the same series of five oysters was exposed successively to the nontoxic control and a mixture with varying toxicity. Each oyster was placed individually in a 1-litre experimental box continually supplied with decanted but unfiltered natural sea water (36 psu) at the experimental temperature. Sea water containing the microalgae (cell concentrations given in Table 2) was introduced into the boxes at a mean laminar flow of  $3.97 \pm 0.20 \text{ l.h}^{-1}$  for the first three experiments and  $4.78 \pm 0.26 \text{ l.h}^{-1}$  for the last three. Before introduction, algal cultures were mixed with sea water in a homogenization chamber (Anonymous, 1987) to obtain a stable concentration in each box. In addition to the five boxes each containing a living oyster, a sixth box containing empty valves was subjected to the same inflow and served

Table 1

*Characteristics of toxic and nontoxic strains of algae used at the end of exponential growth. Cell size min and max values are indicated between brackets.*

	Optimal temperature	Mean diameter ( $\mu\text{m}$ )	Max algal densities ( $\times 10^6 \text{ cells.l}^{-1}$ )
<i>Thalassiosira weissflogii</i> (Grunow) Fryxell and Hasle	$20^\circ\text{C}$	18.04 (12-28)	200
<i>Alexandrium tamarensense</i> Taylor (Balech) 1. Nontoxic strain (Plymouth, UK)	$16^\circ\text{C}$	36.31 (20 - 48)	17
2. Toxic strain (Onagawa, Japan)	$16^\circ\text{C}$	30.98 (20 - 40)	30

Table 2

Experimental conditions: inputs of microalgae (PIM + POM), and corresponding average cell densities and SD.

Experiments	Temperature	Seston inputs ( $\text{mg.l}^{-1}$ )	Cells per ml
<i>T. weissflogii</i> alone	12 °C	8.5	$16,284 \pm 410$
<i>T. weissflogii</i> + toxic <i>A. tamarensen</i> (50/50)	12 °C	8.4	$12,276 \pm 521$
Toxic <i>A. tamarensen</i> alone	12 °C	9.4	$2,575 \pm 462$
<i>T. weissflogii</i> alone	16 °C	5.8	$17,332 \pm 734$
<i>T. weissflogii</i> + toxic <i>A. tamarensen</i> (90/10)	16 °C	7.6	$14,060 \pm 250$
<i>T. weissflogii</i> + nontoxic <i>A. tamarensen</i> (90/10)	16 °C	6.5	$15,629 \pm 318$

as a sedimentation control for consumption calculations. At the outflow from the experimental boxes (open circuit), the residual waters containing toxic particles were trapped in a sodium hypochlorite tank.

The effects of the experimental microalgal diets on nutrition were evaluated according to clearance rates, consumption, ingestion and absorption (Bardouil *et al.*, 1993). For measurements of seston inflow or outflow or the production of biodeposits (faeces and pseudofaeces), the quantity of particulate organic matter (POM) and inorganic (PIM) was determined by weighing the cells or filtered biodeposits on Whatman GF/C membranes at 60 °C and then, after combustion, at 450 °C.

Cell concentrations at the inflow and outflow points of the system were measured using a Multisizer particle counter (Coulter) equipped with a 100-µm aperture probe.

The effects of different microalgal diets were quantified according to the following parameters:

1. Clearance rate F, such that  $F (\text{in } \text{l.h}^{-1}) = R \times D$  (Vahl, 1972), where R (in %) = retention  $(C_1 - C_2)/C_1$  and D = flow through the tank in  $\text{l.h}^{-1}$ , with  $C_1$  = cell concentrations ( $\text{cells.l}^{-1}$ ) at control box outflow, and  $C_2$  = cell concentrations at experimental box outflow.
2. Consumption C, such that  $C (\text{in mg.h}^{-1}) = (\text{inflow POM} - \text{outflow POM}) \times D$ .
3. Ingestion I (in  $\text{mg.h}^{-1}$ ) = C - PF and absorption Ab (in  $\text{mg.h}^{-1}$ ) = I - Fe, where Fe and PF = hourly production, respectively of faeces and pseudofaeces in  $\text{mg POM.h}^{-1}$ .

All results are expressed in weight-specific values F/W for 1 g dry weight of oyster meat determined after 48 h of lyophilization. Ecophysiological data thus obtained were statistically tested with a non-parametric method (Wilcoxon test) against a control (*T. weissflogii* alone), due to different size and variance of each data set. Test values greater than 2 are considered significant with confidence limits: 95 %.

Inputs for the different microalgal diets during the six experiments are summarized in Table 2.

## RESULTS

The various experimental diets had different effects on clearance rates. Rates were lower at 12 °C in winter

(Fig. 1a) than spring (16 °C) for *T. weissflogii*, reflecting the role of the temperature on oyster activity (Wilcoxon: 4.09). For the mixture *T. weissflogii*/toxic *A. tamarensen* 50/50 and for the toxic strain used alone mean inhibition of the clearance rate was less than 0.25  $\text{l.h}^{-1}$  for both (Fig. 1b).

With the toxic mixture (50/50) or toxic *A. tamarensen* alone, mean filtration activity compared to *T. weissflogii* alone (control) is significantly reduced (Wilcoxon: 6.37 and 5.81). The results obtained later (Fig. 1c) showed that the inclusion of just 10% toxic *A. tamarensen* in the microalgal diet led to about a one-fifth reduction in the mean clearance rate compared to the control (Wilcoxon: 6.73). A mixture with an equivalent ratio (90/10), but composed of *T. weissflogii* and a nontoxic strain of *A. tamarensen*, produces higher filtration activity than the toxic mixture (Wilcoxon: 3.32), though it is lower than with the diatom control alone.

Concerning the formation of biodeposits (faeces and pseudofaeces), the first experiment (Fig. 2a) showed total inhibition of production for toxic *A. tamarensen* alone and a significant reduction in pseudofaeces and faeces for the 50/50 mixture compared to *T. weissflogii* alone (Wilcoxon: 2.83 and 2.87). In the second experiment (Fig. 2b), the

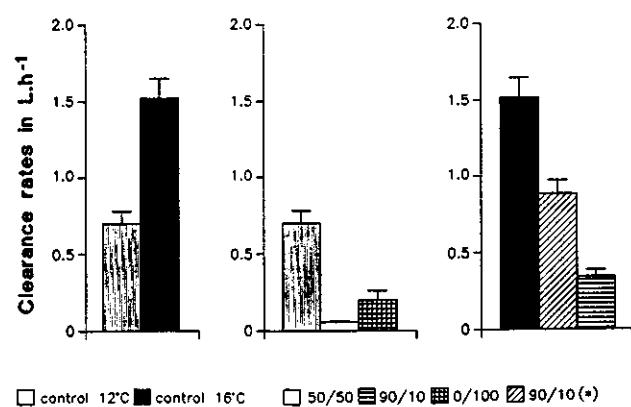


Figure 1

Mean 6-hour clearance rates and standard errors for controls (*Thalassiosira* alone) and toxic (0/100; 50/50; 90/10) and non toxic (\*: 90/10) mixed algal diets (*Thalassiosira* / *Alexandrium*).

Taux de filtration moyens en six heures et erreurs standards pour les témoins (*Thalassiosira* seul) et les régimes mixtes (*Thalassiosira* / *Alexandrium*) toxiques (0/100; 50/50; 90/10) et non toxiques (\*).

results were significant as well (Wilcoxon: 2.13 and 2.09) for pseudofaeces and faeces of the oysters contaminated with the toxic mixed diet. The 90/10 mixture of *T. weissflogii*/nontoxic *A. tamarensense* showed no significant difference when compared to the diatom alone (Wilcoxon < 2).

Mean consumption, ingestion and absorption rates (in  $\text{mg.h}^{-1}.\text{g}^{-1}$  of dry weight) for the five experimental oysters showed higher consumption of *T. weissflogii* in winter than in spring, but roughly the same ingestion and absorption (Fig. 3). These three variables did not display measurable values when the oysters were fed with toxic *A. tamarensense* alone, which is in agreement with the inhibition of biodeposit production previously observed. The 50/50 mixture reduced consumption by one-third compared to the diatom control (Wilcoxon: 2.03) but showed no notable differences for ingestion and absorption. The Wilcoxon test showed no significant differences between the control and each experimental 90/10 diet with the experimental conditions used.

## DISCUSSION

The many observations reported by Shumway *et al.* (1990) indicate that oysters are more sensitive than other shellfish to microalgae producing phycotoxins, particularly paralytic toxins. Although toxicity levels during a contamination event are quite often lower in oysters (Quayle, 1969) than in other molluscs collected in the same area, phycotoxin accumulation in the tissues of several oyster species has been demonstrated on different occasions, particularly during the 1957 event in British Columbia (Canada).

It is well known that some oyster species can sort food particles, and it is also conceivable that they can select non-toxic phytoplankton cells preferentially in a mixed diet. If such selection capability exists, is it related to particular ecophysiological reactions occurring in the presence of toxic species of the genus *Alexandrium*?

In a mixed diet experiment involving *Ostrea edulis*, Shumway *et al.* (1985 *a, b*) demonstrated selective sorting in favour of a nontoxic dinoflagellate, *Prorocentrum minimum*. In the presence of toxic *A. tamarensense*, despite evident seston uptake, the clearance rate increased for *O. edulis* and decreased for *Crassostrea virginica*. According to Shumway and Cucci (1987) results in the literature are more or less contradictory concerning the effects of PSP-producers on oyster filtration: Dupuy and Sparks (1968) and Ray and Aldrich (1967) found that filtration was inhibited, whereas Connell and Cross (1950) reported that oysters (presumably *C. virginica* in Texas waters) could concentrate toxic *P. catenella* without any obvious ill-effects.

The experimental results reported in the present work concern *Crassostrea gigas* which was shown in a previous study to be sensitive to toxic *Alexandrium species* (Bardouil *et al.*, 1993). The levels of paralytic phycotoxin accumulation in this oyster are low compared to those of other shellfish when it is in the presence of high concentrations (red tide conditions) of *A. tamarensense* (Lassus *et al.*, 1989). Although it was not possible during our experiments to

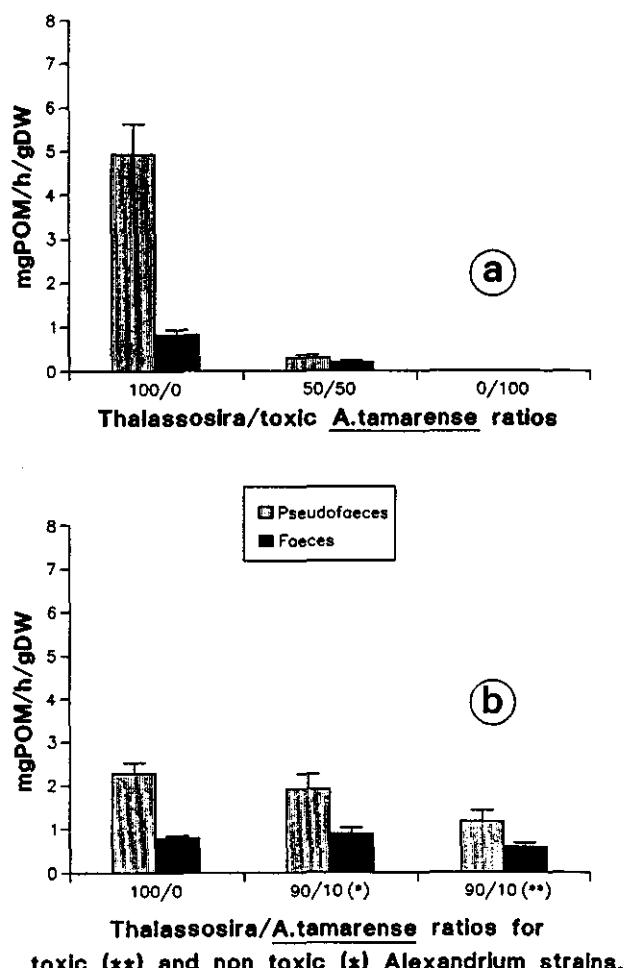


Figure 2

Means (and standard errors) of 6-hour production of biological deposits at 12 °C (a) and 16 °C (b) for *C. gigas* exposed to the different diets tested. A mean was obtained for the two *T. weissflogii* controls.

Moyennes et erreurs standards en six heures des productions de biodépôts chez *C. gigas* exposé à 12 (a) et 16 °C (b) à différents régimes. Les deux témoins *T. weissflogii* ont été moyennés.

work at a single temperature for all trials and to analyse the relative proportions of *T. weissflogii* and *A. tamarensense* in faeces and pseudofaeces, several points seem of interest relative to findings in the literature.

Compared to results for a nontoxic control with a theoretically higher food value (*T. weissflogii*), the inclusion of 50% or even 10% of toxic *A. tamarensense* in the algal diet led to inhibition of the clearance rate, producing levels similar to those with toxic *A. tamarensense* alone. It would thus appear that the genus *Crassostrea* is more sensitive to toxic microalgae than the genus *Ostrea*, a condition already reported by Shumway *et al.* (1990) for other species. Our results are similar concerning the production of biodeposits: total inhibition with toxic *A. tamarensense* alone and a considerable reduction (particularly in pseudofaeces) with the 50/50 and 90/10 mixtures. However, these findings are not in agreement with those of Dupuy and Sparks (1968) concerning increased production of pseudofaeces in *C. gigas* exposed to *Gonyaulax washingtonensis*.

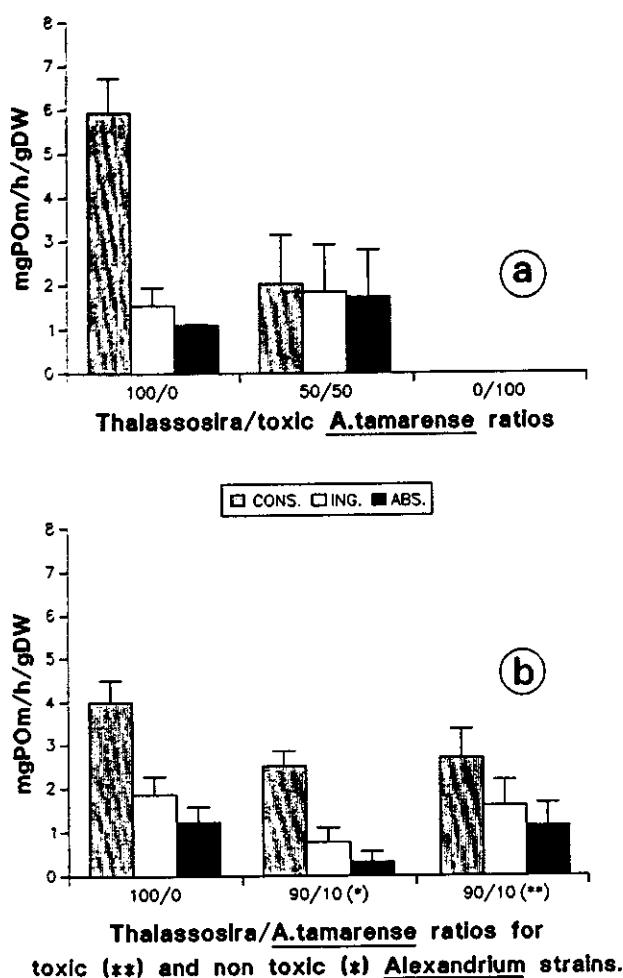


Figure 3

Means (and standard errors) of 6-hour consumption, ingestion and absorption for *C. gigas* exposed to the different diets tested. A mean was obtained for the two *T. weissflogii* controls.

Moyennes et erreurs standards en six heures des consommation, ingestion et absorption de *C. gigas* exposé à différents régimes. Les deux témoins *T. weissflogii* ont été moyennés.

## REFERENCES

- Anonymous (1987). Bilan énergétique chez les Mollusques. Groupe de travail, La Tremblade (1987). *Vie Mar. H. S.* 7, 1-68.
- Armbrust E.V., S.W. Chisholm (1992). Patterns of cell size change in a marine centric diatom : variability evolving from clonal isolates. *J. Phycol.* 28, 2, 146-156.
- Bardouil M., M. Bohec, M. Cormerais, S. Bougrier, P. Lassus (1993). Experimental study of the effects of a toxic microalgal diet on feeding of the oyster *Crassostrea gigas* Thunberg. *J. Shellfish Res.* 12, 2, 417-422.
- Bricelj V.M., J.H. Lee, A.D. Cembella (1991). Influence of dinoflagellate cell toxicity on uptake and loss of paralytic shellfish toxins in the Northern quahog *Mercenaria mercenaria*. *Mar. Ecol., Prog. Ser.* 74, 34-46.
- Connell C.H., J.B. Cross (1950). Mass mortality of fish associated with the protozoan *Gonyaulax* in the Gulf of Mexico. *Science* 112, 359-362.
- Dupuy J.L., A.K. Sparks (1968). *Gonyaulax washingtonensis*, its relationship to *Mytilus californianus* and *Crassostrea gigas* as a source of paralytic shellfish toxin in Sequim Bay, Washington. *Proc. Nat. Shellfish Assn.* 58, 2.
- Goericke R., N.A. Welschmeyer (1992). Pigment turnover in the marine diatom *Thalassiosira weissflogii*. I. The  $^{14}\text{CO}_2$  labeling kinetics of chlorophyll *a*. *J. Phycol.* 28, 4, 498-507.
- Lassus P., J.M. Fremy, M. Ledoux, M. Bardouil, M. Bohec (1989). Patterns of experimental contamination by *Protogonyaulax tamarensis* in some French commercial shellfish. *Toxicon* 27, 12, 1313-1321.
- Provasoli L. (1966). Media and prospects for the cultivation of marine algae. *Proc. U. S. - Japan Conference held at Hakone*, Sept. 12-15, 1966. The Jap. Soc. of Plant Physiologists.
- Quayle D.B. (1969). Paralytic shellfish poisoning in British Columbia. *Bull. Fish. Res. Board of Can.* 168, 1-68.
- Ray S.M., D.V. Aldrich (1967). Ecological interactions of toxic dinoflagellates and molluscs in the Gulf of Mexico. In : Animal Toxins, Russell F.E. and Saunders, P.R. Eds, Pergamon Press. New York: 75-83.

There is little documentation in the literature on ingestion and absorption rates relative to net seston uptake in the context of toxic dinoflagellates and their effects on oysters. Our results indicate inhibition of these three variables with *A. tamarensis* alone and a significant reduction in consumption for the 50/50 but not the 90/10 mixture.

Finally, an attempt was made in all our experiments to compare the results of the toxic 90/10 mixture with an equivalent nontoxic mixture, inasmuch as the effects observed could have been due in part to the nutritive value of the dinoflagellate rather than the intrinsic toxicity of the *Alexandrium* strain tested. In fact, the values for clearance rates and pseudofaeces production were always lower than those for the diatom control, though not as low as for the toxic mixture.

With respect to filtration inhibition and the production of biodeposits, the present study confirms our earlier findings for *C. gigas* obtained in the same experimental conditions (Bardouil *et al.*, 1993). However, the high absorption values reported then (percentages of consumption rated as 100%) were not observed here.

It is likely that observations over longer experimental periods will be required to assess ingestion and absorption more exactly. These variables should ultimately prove to be good indicators of the real capacity of *C. gigas* to store *A. tamarensis* toxins.

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- Rich H.W., F.M.M. Morel (1990). Availability of well defined iron colloids to the marine diatom *Thalassiosira weissflogii*. *Limnol. Oceanogr.* **35**, 3, 652-662.
- Shumway S.E., T.L. Cucci, L. Gainey C.M. Yentsch (1985 a). A preliminary study of the behavioral and physiological effects of *Gonyaulax tamarensis* on bivalve molluscs. In: *Toxic Dinoflagellates*. Anderson *et al.*, Eds. 389-394. Amsterdam, Elsevier Sci. Publi. Co.
- Shumway S.E., T.L. Cucci, R.D. Newell, C.M. Yentsch (1985 b). Particle selection, ingestion, and absorption in filter-feeding bivalves. *J. Exp. Mar. Biol. Ecol.* **91**, 77-92.
- Shumway S.E., J. Barter, Sherman-Caswell (1990). Auditing the impact of toxic algal blooms on oysters. *Environmental Auditor*. **2**, 1, 41-56.
- Vahl O. (1972). Efficiency of particule retention in *Mytilus edulis* L.. *Ophelia* **10**, 17-25.