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Gut passage times in two bivalve molluscs fed toxic microalgae: *Alexandrium minutum*, *A. catenella* and *Pseudo-nitzschia calliantha*

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Abstract – The occurrence of new phytoplankton species in a coastal area may be explained by the import of shellfish containing whole live algal cells in their digestive tracts. Indeed, shellfish containing toxic algal cells can induce both primary contaminations in safe areas (initially free from toxic microalgae), and secondary contaminations of other shellfish growing in the same area via the faeces of the imported animals. To mitigate this problem, shellfish need to be placed in a separate holding tank and their intestinal content purged. For a deeper understanding of the risks associated with transferring contaminated shellfish, oysters (*Crassostrea gigas)* and mussels (*Mytilus edulis)* were purposely fed either *Alexandrium minutum or A. catenella* (Dinophyceae) or *Pseudo-nitzschia calliantha* (Bacillariophyceae) toxic algae for 2 h. They were then transferred into individual tanks where they were continuously fed with a non-toxic alga, *Tetraselmis suecica*. Biodeposit production, faeces composition, and filtration rates were monitored for the shellfish over a 6-h period. The effect of temperature differences and different initial toxic algae concentrations were compared. This study revealed a relationship between temperature and cell lysis in the oyster digestive tract. It also indicated that toxic algae concentration did not seem to influence gut passage time in oysters, while a significant effect was observed in mussels, and confirmed the existence of a difference between oyster and mussel feeding patterns.

Key words: *Crassostrea gigas* / *Mytilus edulis* / Gut passage / Uptake / Toxic phytoplankton / Risk assessment

Résumé – Temps de transit intestinal chez deux mollusques bivalves nourris de microalgues toxiques : *Alexandrium minutum, A. catenella* et *Pseudo-nitzschia calliantha*. L'apparition de nouvelles espèces phytoplanctoniques dans des zones côtières peut être expliquée par le transfert de coquillages contenant des cellules entières vivantes dans leur tractus digestif. En effet, des coquillages contenant des cellules algales toxiques peuvent entraîner une contamination primaire d'une zone exempte de ce phytoplancton, ainsi qu'une contamination secondaire via les fèces des autres coquillages cultivés dans cette zone. Pour pallier à ce problème, il faudrait mettre en bassin les coquillages afin de vider leur contenu intestinal. Pour une évaluation plus précise du risque lié au transfert de coquillages contaminés, des huîtres (*Crassostrea gigas)* et des moules (*Mytilus edulis*) sont placées en contact avec des algues toxiques (*Alexandrium minutum, A. catenella* et *Pseudo-nitzchia calliantha*), durant 2 heures, puis transférées dans des bacs individuels, dans lesquels ces bivalves sont nourris avec un apport en continu de *Tetraselmis suecica*, une algue non toxique.Leur production de biodépôts, la composition de leurs fèces ainsi que leur taux de filtration sont suivis pendant 6 heures, à différentes températures et à différentes concentrations initiales en algues toxiques. Cette étude permet de mettre en évidence une relation entre l'augmentation de la température et la lyse des cellules dans le tube digestif. De plus, la concentration en algues toxiques ne semble pas influencer le temps de transit. Cette étude confirme l'existence d'un comportement alimentaire différent entre huîtres et moules.

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1 Introduction

Any given marine phytoplankton community is generally made up of both indigenous and exotic species. Exotic species (i.e. from other areas) can be accidentally introduced into a community by a number of different means: transport by coastal currents, faeces of migratory birds (Nehring 1998) or water ballast discharges (Burkholder et al. 2007). The toxic dinoflagellate species *Alexandrium catenella* (West Pacific) was probably introduced to the Thau Lagoon in France in this last way (Lilly et al. 2002). New species may also be introduced as a result of shellfish importation (Van der Bergh et al. 2002). The diatom species *Coscinodiscus wailesii* and *Thalassiosira punctigera*, both native to the Indo-Pacific area, were introduced to the North Sea in this way with importation of the Pacific oyster *Crassostrea gigas* (Nehring 1998). Local increases in phytoplankton diversity have thus been seen to result from ballast water discharges or shellfish farming activities. When invading phytoplankton species are also toxic or harmful, they may damage the marine ecosystem, and have consequent adverse effects on aquaculture, fisheries and public health.

French coasts have been affected by seasonal blooms of toxic microalgae for many years. The first recorded incidence of diarrheic toxins was in 1983 (Lassus et al. 1988; Marcaillou et al. 2001), that of paralytic toxins in 1988 (Masselin et al. 2001; Séchet et al. 2003) and that of amnesic toxins (ASP) in 1999 (Amzil et al. 2001). When shellfish are contaminated by these toxic microalgae to a level exceeding the sanitary threshold, they are no longer harvested and marketed. Such market closures can have serious economic consequences. Diarrheic, paralytic and amnesic toxins are produced by the dinoflagellate genera *Dinophysis*, *Alexandrium* and *Pseudo-nitzschia* respectively. In France, *Alexandrium minutum* is found along the north Brittany coasts, while *Alexandrium catenella* occasionally blooms in the Thau lagoon. *Pseudo-nitzchia pseudodelicatissima* (renamed *Pseudo-nitzchia calliantha*) and *Pseudo-nitzchia multiseries* have been responsible for episodes of ASP shellfish contamination in western Brittany (Amzil et al. 2001).

To minimise economic losses during the periods when shellfish sales are prohibited in a particular area, one solution is to transfer shellfish considered to be "safe" to areas free of toxic phytoplankton as soon as a toxic bloom is detected. However, as soon as the faeces of contaminated shellfish are released into a safe zone, all surviving cells trapped in the mucus as pellicular cysts or hypnospores (although such structures have not yet been described for *Pseudo-nitzschia* spp.; Mann 2002) may be subsequently revived from this vegetative state and thus initiate a secondary bloom (Carriker 1992; Scarrat et al. 1993; Laabir and Gentien 1999; Laabir et al. 2007). Moreover, these cysts can be transferred into the biodeposit layers on the sea bottom, and thus give rise to a toxic cell seed stock that may later be re-suspended in the water column and result in further episodes of seeding when environmental conditions become favourable. In mussel *Mytilus edulis* faeces, Scarrat et al. (1993) demonstrated the presence of intact *Alexandrium tamarense* and Bricelj et al. (1993) found intact vegetative *Alexandrium fundyense* cells. Laabir and Gentien (1999) observed that *Alexandrium minutum*, *Alexandrium tamarense* and *Gymnodinium mikimotoi* dinoflagellate cells were revived after they had passed through the digestive tract of *Crassostrea gigas*. Similarly, *Prorocentrum lima*, a benthic dinoflagellate, has been shown to be able to survive in the digestive tube of *Argopecten irradians* seastar and subsequently to divide (Bauder and Cembella 2000).

To reduce risks arising from the transfer of contaminated shellfish, the recommended procedure is to store them in quarantine tanks over the period of time needed to empty their digestive tract of undamaged toxic cells before transferring them to non-contaminated areas. However, to limit the extra cost incurred by this kind of procedure, it is vital to keep the quarantine period as short as possible. We therefore need to be able to predict how long the algae will remain in the bivalve digestive system. The objective of the present study was (i) to determine the presence or absence of intact *A. minutum*, *A. catenella* and *P. calliantha* cells in the faeces of *Mytilus galloprovincialis* and *Crassostrea gigas*, (ii) to predict the influence of environmental parameters (temperature and algal concentration) on the gut passage time for algae, and (iii) to predict the time required by a bivalve to eliminate all toxic cells from its digestive tube.

2 Materials and methods

2.1 Biological material

Alexandrium minutum, *Alexandrium catenella* and *Tetraselmis suecica* were cultivated in 10 L tanks containing filtered autoclaved seawater (121 °C; 15 min) enriched with either Guillard F/2 medium for dinoflagellates, or Provasoli's ES medium for *T. suecica*. The prasinophyceae *T. suecica* is frequently used as a feed in aquaculture and its deep colour makes it easy to detect in faeces, indicating any change in content. Fernbachs tanks containing 1.5 L of filtered sea water enriched with L1 silica medium were inoculated with *Pseudo-nitzschia calliantha* and later grown on in 10 L tanks until cellular concentrations reached 250 000 or 300 000 cells ml^{-1}. The cultures were kept at 16 ± 1 °C under 50 ± 4 μmol photons m^{-2} s^{-1} light, with a 12:12 (light:dark) photoperiod.

Pacific oysters *Crassostrea gigas* with a mean total individual weight of 50 g were used in the experiments, and Mediterranean mussels *Mytilus galloprovincialis* with a mean length of 45 mm. All bivalves were sexually immature.

2.2 Experimentation (Fig. 1)

The experiments were performed at 16 or 20 °C, a summary of the treatments is given in Table 1. Oysters or mussels were put in 35 L recirculating raceways filled with natural sea water that had been decanted but not filtered. At this stage, the bivalves were fed *Alexandrium* spp. (*A. minutum* or *A. catenella*) at 200 or 5000 cells ml^{-1}, or *P. calliantha* at 2000 or 20 000 cells ml^{-1}.

As soon as the first faeces appeared, the bivalves were transferred into individual tanks in a second circulating flume. During this second stage of the experiment, the bivalves were continuously fed *Tetraselmis suecica* and the behaviour of

Table 1. Summary table showing the results for the different experiments, with gut passage time and faeces type (*a*, *b*, *c* or *d*) observed over a 6-h period of contact with *Tetraselmis suecica* (see Fig. 3).

Bivalve	Algal species	Concentration (cell ml^{-1})	Temperature (°C)	Code on Fig. 3	Number of bivalves	Gut passage time (min)	% of bivalves with faeces of each type over a 6-h period			
							a	*b*	*c*	*d*
Oyster	*A. minutum*	200	16 °C	A1	18	172 ± 92	0	78	22	0
	A. minutum	5000	16 °C	B1	16	213 ± 70	5	40	55	0
	A. minutum	200	20 °C	C1	17	164 ± 93	0	0	41	59
	A. minutum	5000	20 °C	D1	20	205 ± 69	0	25	70	5
	P. calliantha	2000	16 °C	E1	17	158 ± 50	0	0	6	94
	P. calliantha	20 000	16 °C	F1	6	136 ± 40	0	0	0	100
	A. catenella	200	16 °C	G1	20	231 ± 84	0	5	68	26
	A. catenella	5000	16 °C	H1	16	263 ± 65	5	0	69	25
Mussel	*A. minutum*	200	16 °C	A2	20	358 ± 45	10	50	0	40
	A. minutum	5000	16 °C	B2	20	284 ± 68	10	20	5	65
	P. calliantha	2000	16 °C	C2	20	130 ± 48	0	0	10	90
	P. calliantha	20 000	16 °C	D2	20	203 ± 52	0	0	0	100

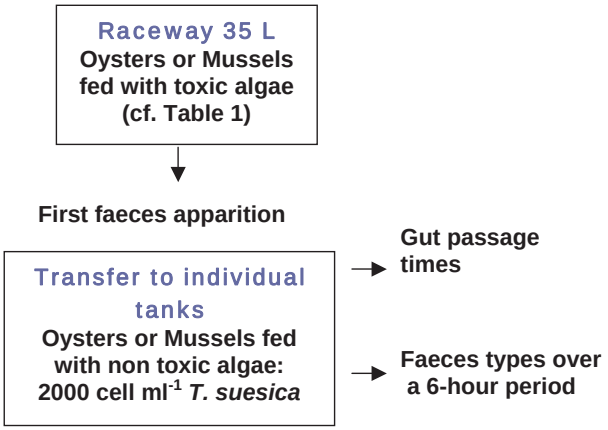

Fig. 1. Experimental design.

each individual was monitored, in particular the retention rate. This parameter, expressed as a percentage of activity, was obtained from continuous fluorescence recording made at the outlets of a control tank ($Fluo_{control}$) and assay ($Fluo_{assay}$) tanks, as defined by the formula (1) below. Control tanks contained only empty oyster or mussel shells. Assay tanks contained a live bivalve.

$$\%\,Activity = \frac{Fluo_{control} - Fluo_{assay}}{Fluo_{control}} \times 100. \qquad (1)$$

According to Soletchnik et al. (1996), gut passage time can be determined by the colour change of the faeces according to the following procedure: oysters are fed *Tetraselmis suecica* for 15 min and this alga acts as a tracer, labelling the faeces with a green colour. The oysters are then fed with natural algae. The time until the appearance of the brown faeces is considered as the gut passage time.

In the present study, the oysters were first fed *Alexandrium* spp. or *P. calliantha*, diets corresponding to brown faeces. They were then given *Tetraselmis suecica*, a green Prasinophyceae, so as to produce green faeces. The gut passage time was determined by macroscopic observations. According to Cognie et al. (2001), faeces can be of different types: intestinal faeces, which appear at first; and glandular faeces, which occur afterwards. The time needed to pass from the first of faeces to the second is approximately 6 h. As the present study only concerned intestinal faeces, composition could be observed and classified after 6 h.

Faeces examinations were made under a microscope and classified into four types according to their state (Fig. 2):

- Type *a* corresponded to faeces made up of only *Alexandrium* spp. cells or *P. calliantha* cells;
- Type *b* represented a mix of *T. suecica* and the toxic algae, where toxic algae represented less than 50% of the faeces content;
- Type *c* corresponded to a significant change in faeces colour, passing from brown to green, with less than 5% toxic algae;
- Type *d*, 99% of the faeces consisted of *T. suecica*.

The time needed to observe faeces corresponding to type *c* was noted, as was the general appearance of the cells. Final observations were made after 6 h feeding with *T. suecica*, and were then subjected to microscopic analysis. The gut passage time corresponded to faeces of the *b* or *c* type.

The gut passage time data were represented by Tukey box plots which allowed different time distributions to be displayed. Following the assumption that the data followed a normal distribution with equal variances, ANOVAs were performed to test for significant differences. Even if normal distributions with equal variances were not systematically observed in our datasets, we chose to use ANOVA as this test is generally recognized to be robust. The ANOVA test was conducted using the *anova* and *multcompare* functions in Matlab 6.5 to obtain *p*-values and graphs with means and 95% confidence intervals.

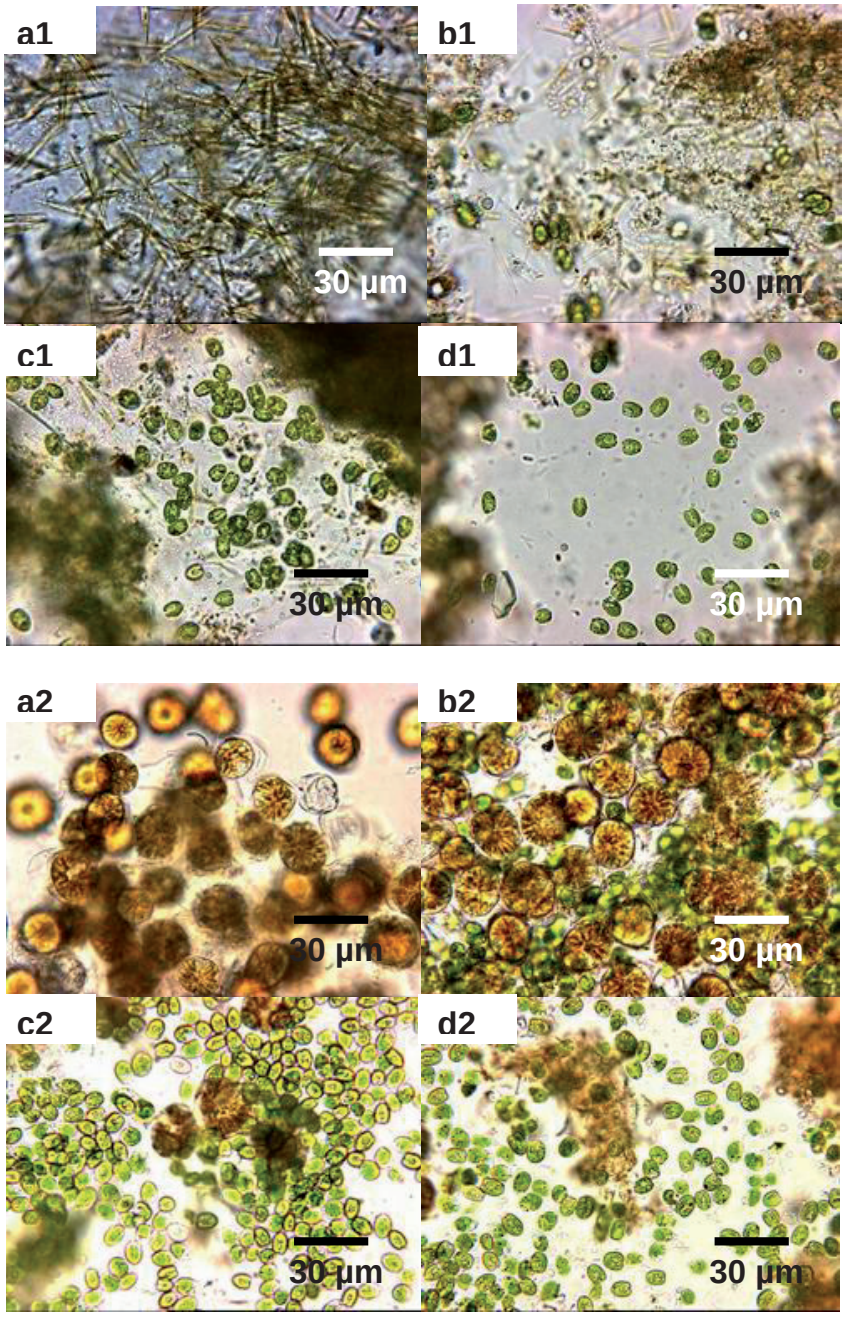

Fig. 2. Light microscope photographs (×250) of faeces produced by *Crassostrea gigas* and by *Mytilus edulis* fed *Pseudo-nitzschia calliantha* (1) and *Alexandrium catenella* (2). Type *a* : faeces composed only of *Alexandrium* spp. or *P. calliantha* cells. Type *b*: a mixture of *Tetraselmis suecica* and the toxic algae species, with the toxic species representing less than 50% of the faecal contents. Type *c*: corresponds to a definite colour change in the faeces, which pass from brown to green, with less than 5% of toxic algae. Type *d*: faeces consist of 99% *T. suecica*.

2.3 Modelling

Digestive tract transit is generally modelled by a linear or exponential function. Some complex multifactoral models are also available. This is the case for fish, where the influence of environmental and intrinsic parameters is well-known and documented. These parameters generally include temperature, species, fish weight, food quality and quantity, or feeding history (Boyce et al. 2000). Penry and Jumars (1987) concluded that the model most frequently encountered in nature (across the whole spectrum of animal species) is one in which the energy gain produced by ingested food is maximized, and combined with this simple tubular intestine featuring a piston-like operation causing axial dispersion of the contents. The model is characterized by a continuous flow of matter through the tube, and gives the following equation:

$$\frac{\partial C(z)}{\partial t} = -U \times \frac{\partial C(z)}{\partial z} + D_a \times \frac{\partial C^2(z)}{\partial z^2} - kC(z) \qquad (2)$$

where C: toxic algal cell concentration, t: time (hours), z: axis, U: speed in the digestive tube (cm h^{-1}), D_a: axial dispersion (cm^2), k: cellular digestion coefficient.

The first term $-U \times \frac{\partial c(z)}{\partial z}$ is the transport term, corresponding to the particle progression speed in the digestive tube.

The second term $D_a \times \frac{\partial C^2(z)}{\partial z^2}$ is the axial diffusion term.

The last term $kC(z)$ corresponds to cell digestion along the digestive tube.

Limiting conditions are:

$$C(z, t = 0) = 0$$
$$\frac{\partial C(z = L, t)}{\partial z} = 0$$
$$C(z = 0, t) = C_{\text{mean}}$$

3 Results

During the contamination process, the first faeces usually appeared 102 ± 27 min following feeding with toxic algae.

3.1 Gut passage times and population response homogeneity

The representation of each bivalve group with Tukey box plots allowed a better understanding of population response homogeneity (Fig. 3). Thus it can be noted that the gut passage times for oysters fed *P. calliantha* (Fig. 3, E1, F1) were more homogeneous than all the other oysters, which had been fed *Alexandrium* spp. (Fig. 3.). The extreme values recorded for gut passage times (when the crosses on the Tukey graphs are above or below the first or third quartile, respectively) for two oysters fed 200 cell ml^{-1} *A. minutum* at 16 °C (Fig. 3, A1) may be explained by a lag period that occurred if the molluscs did not filter the phytoplankton immediately (Fig. 4). Oysters produced *c* type faeces after more than 5 h, but these had a lag period greater than 2 h 30 min (Fig. 4). When this lag period was removed, these oysters had similar gut passage times as the others. This was also true for other oysters behaving in the same way, which had been fed 2000 cell ml^{-1} *P. calliantha* (Fig. 3, E1). With the mussels, an opposite tendency was observed: three mussels (Fig. 3, A2) had gut passage times shorter than the others, an observation which was probably due to greater feeding activity in these three individuals.

3.1.1 The effect of temperature

The effect of temperature on gut passage time in *Crassostrea gigas* was investigated at two concentrations of *A. minutum* toxic diet: 200 and 5000 cell ml^{-1}. The temperatures tested were 16 and 20 °C. For 200 cell ml^{-1}, the gut passage times (Table 1) were 172 ± 92 min at 16 °C, and 164 ± 93 min at 20 °C. For 5000 cell ml^{-1} the gut passage times were 213 ± 70 min at 16 °C and 205 ± 69 min at 20 °C. Temperature therefore showed no significant effect on gut passage times (ANOVA: $p = 0.81$ for 200 cell ml^{-1}; $p = 0.75$ for 5000 cell ml^{-1}). However, after 6 h contact with *T. suecica*, only oysters in the 20 °C group presented faeces of the

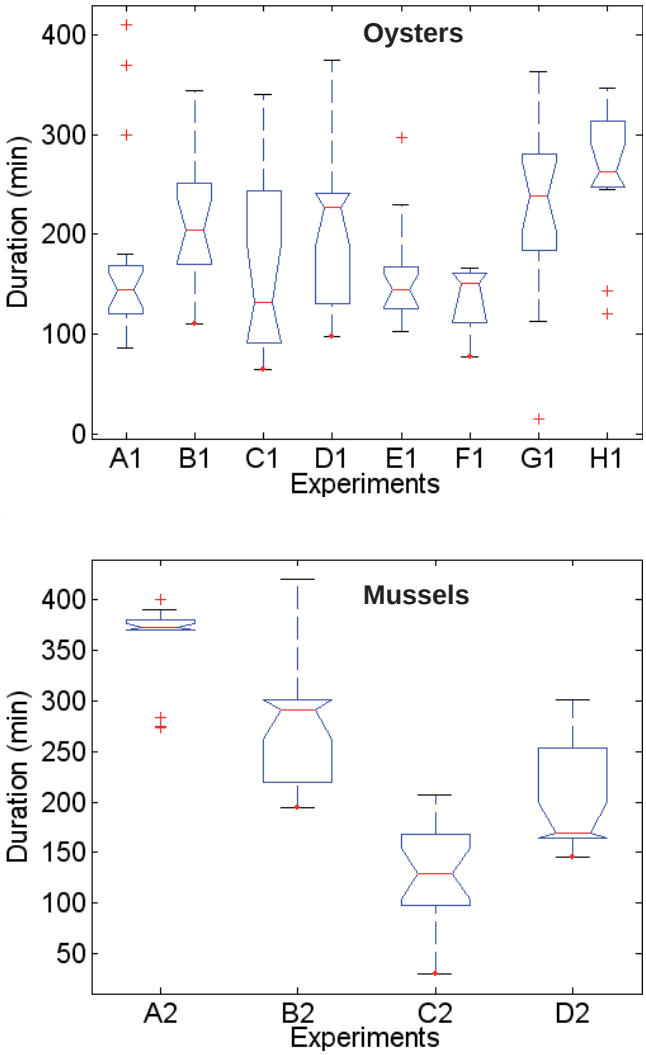

Fig. 3. Gut passage time distribution as a function of the different environmental parameters tested (Table 1) for oysters and mussels.

d−type (consisting of 99% *T. suecica*). In fact, at 200 cell ml^{-1} and 20 °C, 59% of faeces were of *d*-type compared with 0% at 16 °C (Fig. 5c).

3.1.2 The effect of algal concentration

Oysters

Gut passage times, for 200 and 5000 cell ml^{-1} *A. minutum* at 16 °C, were 172 ± 92 min and 213 ± 70 min respectively. Toxic algae concentrations therefore appeared to have no significant effect on gut passage time, which was confirmed by the ANOVA test ($p = 0.1561$). However, the faeces were in a more advanced state for *A. minutum* at the 5000 cell ml^{-1} concentration: 55% of the oysters presented *c* type faeces with 5000 cell ml^{-1}, compared with 22% for 200 cell ml^{-1}. The same result was observed at 20 °C.

The observed gut passage times, for *A. catenella* at 200 and 5000 cell ml^{-1}, were 253 ± 81 min and 268 ± 70 min

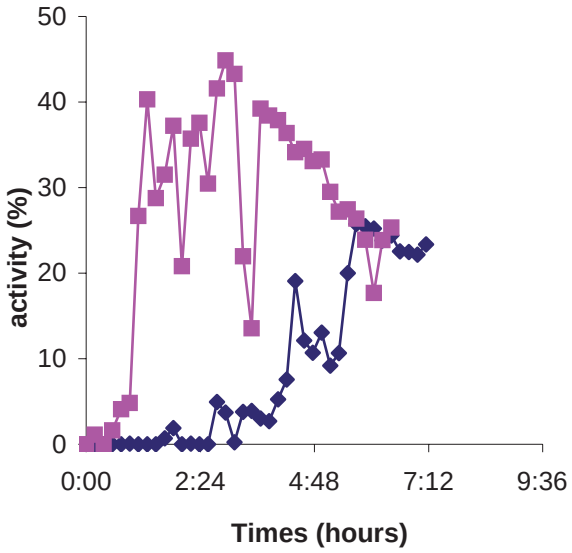

Fig. 4. Activity of two different oysters consuming 200 cell ml^{-1} *Alexandrium minutum* at 16 °C. Oyster-1 (♦) presents a lag period of 1.5 h. Oyster-2 (■) displays a high level of activity from the beginning to the end of the experiment.

respectively. The percentages of oysters, producing *d* type faeces, were also similar between these treatments (26% for 200 cell ml^{-1} and 25% for 5000 cell ml^{1}), as were percentages of *c* type faeces (68% at both concentrations).

In the experiment using 20 000 cell ml^{-1} of *P. calliantha*, oyster filtration rates were probably inhibited, since only 6 out of 40 oysters produced faeces in the contamination raceway. Therefore only these 6 individual oysters were transferred to separate tanks for subsequent study.

Mussels

The gut passage times were 358 ± 45 min for 200 cell ml^{-1} and 284 ± 64 min for 5000 cell ml^{1} *A. minutum*. For *P. calliantha*, gut passage times were 130 ± 48 min (2000 cell ml^{-1}) and 203 ± 52 min (20 000 cell ml^{-1}). The ANOVA test confirmed that the algal concentrations induced significant differences in gut passage time (p = 0.003 for *A. minutum* and p = 0.00005 for *P. calliantha*). The increase in *P. calliantha* concentration resulted in longer gut transit times, whereas the increase in *A. minutum* concentration led to shorter gut transit times. The percentage of mussels producing *d* type faeces at 6 h was greater for the highest cell concentrations (65% versus 40% for *A. minutum* and 100% versus 90% for *P. calliantha*).

3.1.3 The effect of algal species

Gut passage times for the same algal concentrations were significantly greater for oysters fed on *A. catenella* cells than for those fed on *A. minutum* cells (p = 0.04 for 200 cell ml^{-1} and 0.04 for 5000 cell ml^{-1}; Figs. 6 A and B). Moreover, the oysters fed *A. catenella* produced faeces in a more advanced state (Figs. 6 C and D). Only oysters fed with *A. catenella* had *d* type faeces after 6 h.

C. gigas and *M. edulis* presented a shorter gut passage time when fed *P. calliantha* than when fed *Alexandrium* spp., and *P. calliantha* cell degradation was faster in both cases (Table 1).

3.1.4 Results for different bivalve species

For the same concentrations of *Alexandrium minutum*, the mussel gut passage times were longer than those recorded for oysters: 358 ± 45 *versus* 172 ± 92 min for 200 cell ml^{-1} and 284 ± 68 min *versus* 213 ± 70 min for 5000 cell ml^{-1} (Table 1). These times are significantly different, with p-values of 2.65 × 10^{-9} and 0.004 respectively. The gut passage times following contact with 2000 cell ml^{-1} *P. calliantha* were similar for the two (158 ± 50 min for oysters and 130 ± 48 min for mussels; p = 0.090).

3.2 Residence time distribution modelling

When considering the ability of bivalves to maintain toxic phytoplankton cells in a well-preserved state for long periods in their digestive tract, such that the toxic cells can potentially be revived, the risk of contaminating a safe area following transfer is definitely lower for *P. calliantha* than for *Alexandrium* spp. It appears vital to set up a model to determine the time needed to empty the digestive tract of temporary cysts or revived cells. We chose to model the digestive tube as an axial dispersion piston, as recommended by Penry and Jumars (1987). When, for example, the oysters that had consumed *Alexandrium* spp. were tested using this gut passage time model (Fig. 7), the coefficients were as follows: L = 4.6 cm, C_{mean} = 1 unit, U = 1.2 cm h^{-1}, D_a = 0.23 cm^2 and k = 0. The k term corresponds to cell digestion, although the actual dataset did not allow a correct evaluation of this term. The model was based on three observed parameters. The first was mean appearance time of brown faeces after the oysters were fed *Alexandrium* spp. *Alexandrium*-based faeces generally appeared within the first 2 h. The second parameter corresponded to the change in the colour of the faeces due to the change in algae diet (to *Tetraselmis suecica*), previously defined as the mean gut passage time. The observed mean gut passage time was around 3.5 h. The final parameter was cyst presence after 6 h feeding with *Tetraselmis suecica*.

The model shows that faeces containing 100% *Alexandrium* spp. would be produced within less than two hours (Fig. 7a). Within three hours following the *T. suecica* contact, this non toxic alga would then become the dominant species in the faeces (Fig. 7b), which agrees with our observations (data not shown). After 6 h, *Alexandrium* spp. cells would only be present at a low level (lower than 10%). This model represents the observed data, but needs to be refined so it can be integrated into an overall shellfish contamination/decontamination model that can be applied to phycotoxins.

4 Discussion

Whole dinoflagellates and diatoms cells can be found in oyster or mussel faeces. Bardouil et al. (1993) and Laabir and Gentien (1999) observed the presence of intact cells of *Alexandrium minutum* and *Alexandrium tamarense* in oyster faeces.

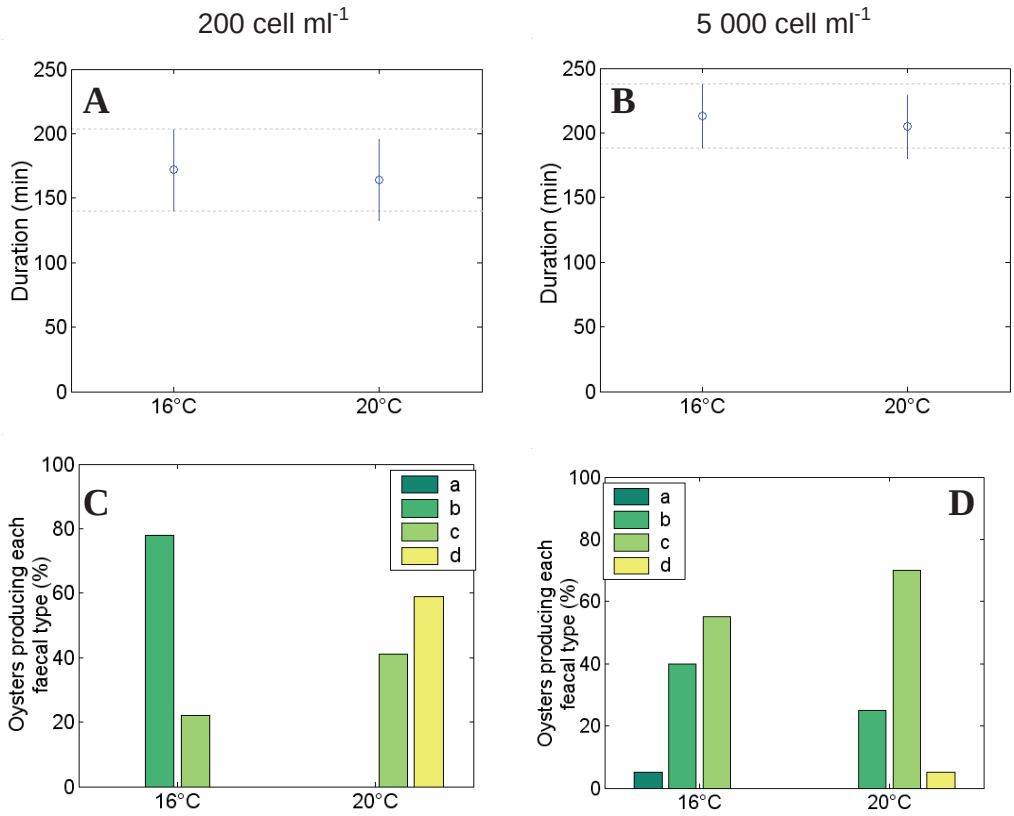

Fig. 5. Oyster gut passage time comparison at two temperatures (16 °C and 20°), with *Alexandrium minutum* fed at concentrations of 200 cell ml^{-1} (A) or 5000 cell ml^{-1} (B). Comparison of faeces (types *a*, *b*, *c*, *d*, see Fig. 2) after 6 h of contact with *Tetraselmis suecica* for oysters previously fed with 200 cell ml^{-1} (C) and 5000 cell ml^{-1} (D) *Alexandrium minutum*.

Bricelj et al. (1993) also found dinoflagellate cells in mussel faeces. Finally, Bauder and Cembella (2000) noted the presence of *Prorocentrum lima* in *Argopecten irradians* faeces. *Alexandrium* spp cysts have also been found in the faeces of other marine invertebrates, such as *Theola fragilis* (Tsujino et al. 2002), polychaetes *Paraprionospio* spp. (Tsujino et al. 2002) and copepods (Reid and Boalch 1987; Montresor et al. 2003).

The presence of intact and viable cells in bivalve faeces does not seem to be related to toxin presence in the phytoplankton cells. This was confirmed by the findings of Bardouil et al. (1993) who found undamaged toxic and non toxic dinoflagellate cells in oyster faeces. Similarly, Barillé and Cognie (2000) observed the presence of intact revived diatoms cells in oyster faeces. Undigested cells may result from the resistance of the cell walls to shellfish gastric juices. Oysters ingesting naked dinoflagellate species (without a theca) usually produce faeces made up of split cells, whereas oysters consuming dinoflagellates with thick thecae produce faeces containing intact or encysted cells in proportions ranging from 50 to 90% (Laabir and Gentien 1999).

Several problems may arise from the presence of toxic phytoplankton cells in the faeces: a possible secondary contamination of other bivalves via re-suspension in the water column (Kikuchi et al. 1996) and, in the event of shellfish transfer, the contamination of another area, previously free of toxic cells. Before any transfer of molluscs to a new area, quarantine storage in recirculating tanks, as suggested by Dukema (1995), therefore seems necessary. To avoid unnecessary expenditure, predictions should first be made on how long the bivalves will take to reject all the whole phytoplankton cells. For this calculation, it is crucial to know the influence of different environmental parameters.

Temperature seems to have an effect on the phytoplankton cells contained in the faeces. Following 6 h feeding with *T. suecica* cells, faeces from oysters at 20 °C presented a lower percentage of whole toxic cells than faeces from oysters at 16 °C. This result does not reveal any convincing evidence that temperature affects phytoplankton cell degradation. However, other studies have highlighted the role that temperature plays in the digestion process. These show that the higher the temperature, the greater the cilia activity along the digestive tube (Boucaud-Camou et al. 1983). In the same way, digestive enzymes are more active at 20 °C than at 16 °C. The toxic algae concentration does not seem to have any effect on gut passage time in oysters, but does in mussels. Moreover, *A. minutum* cells are smaller than *A. catenella* cells, and the gut passage time is longer for *A. catenella* than for *A. minutum* cells. In addition, gut passage time was different according to the type of bivalve used for the experiment. However, for both mussels and oysters, gut passage time is longer with diatoms than with dinoflagellates.

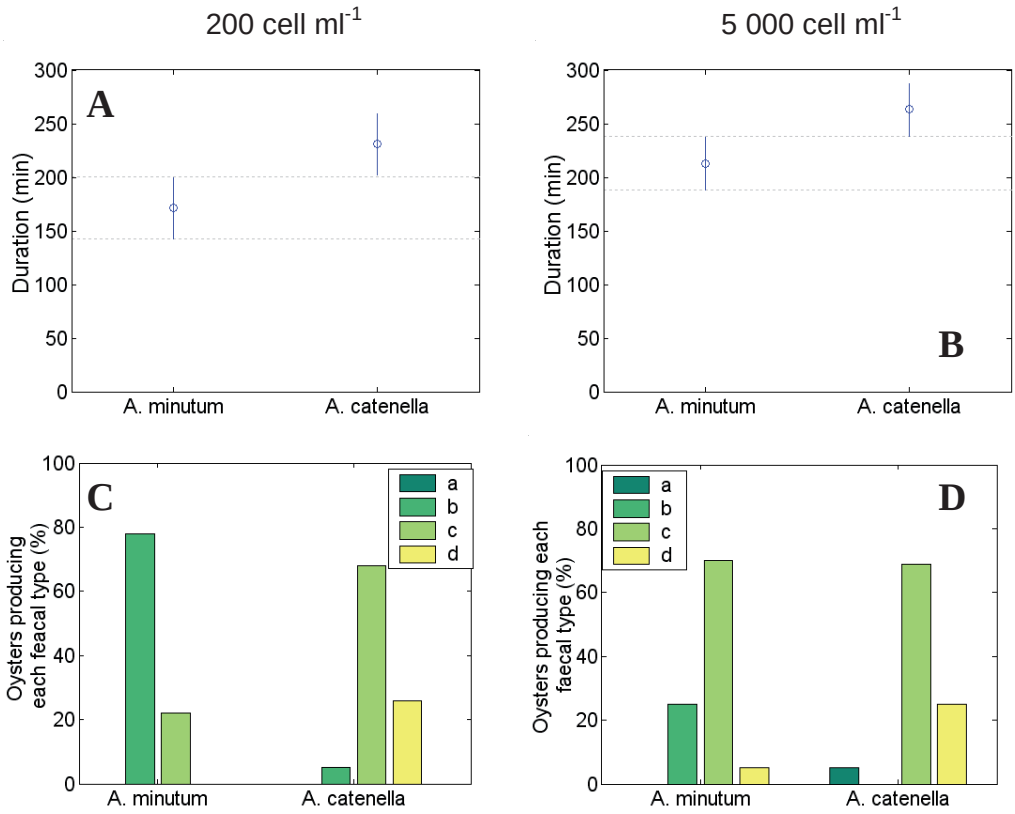

Fig. 6. Oyster gut passage time comparison for two toxic algae (*A. minutum* and *A. catenella*) fed at concentrations of 200 cell ml^{-1} (A) or 5000 cell ml^{-1} (B). Comparison of faeces (types *a*, *b*, *c*, *d*, see Fig. 2) after 6 h of contact with *Tetraselmis suecica* for oysters previously fed with 200 cell ml^{-1} (C) and 5000 cell ml^{-1} (D) *Alexandrium minutum* or *A. catenella*.

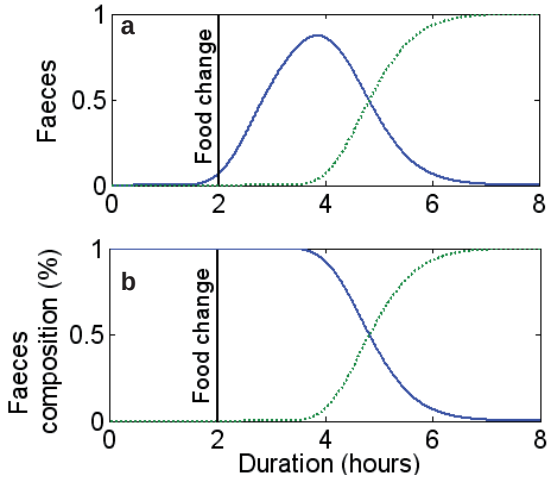

Fig. 7. Modelling the apparition of faeces in oysters (a) containing *Alexandrium* spp. (- - - -) and *Tetraselmis suecica* (—) and the faecal composition (b) over time.

The model agrees with data on first faeces appearance time, gut passage time (colour change) and cyst presence after 6 h feeding with *T. suecica*. Moreover, it gives a representation of the potential risk of transferring oysters to the natural environment: thus, a 6 h period was not enough time to empty the oyster gut.

To sum up, temperature, cell concentration, cell size and bivalve species seem to be the key-parameters that should be taken into account when trying to set up a model to predict the time required to free the digestive tube of revived and whole toxic algal cells. To improve the model, it would be interesting to integrate different environmental factors (temperature, cell concentration, cell size and bivalve type) into the initial model, based upon the "intestinal transit" parameters (U: velocity, D_a: axial dispersion and k: cell digestion).

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