Mar Biol (2007) 151:2003–2012
DOI 10.1007/s00227-007-0637-9

RESEARCH ARTICLE

Transfer of brevetoxins to a tellinid bivalve by suspension- and deposit-feeding and its implications for clay mitigation of *Karenia brevis* blooms

Anne-Gaëlle Haubois · V. Monica Bricelj · Jerôme Naar

Received: 20 September 2006 / Accepted: 1 February 2007 / Published online: 22 February 2007

Abstract Blooms of the brevetoxin-producing *Karenia brevis* in the Gulf of Mexico cause massive fish kills, food poisoning and adverse respiratory effects in humans. Sedimentation of toxic cells following inert clay application could reduce toxin incorporation by commercially important suspension-feeding bivalves and thus prevent direct public health impacts, but could potentially lead to brevetoxin (PbTx) accumulation by benthic deposit-feeders. The goal of this study was therefore to compare suspension- and deposit-feeding as pathways for brevetoxins. We investigated: (1) the effect of toxic *K. brevis* on both feeding modes using a facultative deposit-suspension feeding tellinid bivalve, the clam *Macoma balthica*, as a model species and (2) the relative effectiveness of brevetoxin transfer via suspension- and deposit-feeding over 24-h exposure. Sedimentation of *K. brevis* was achieved by treatment with 0.25 g phosphatic clay l^{-1} and brevetoxin concentrations were measured by ELISA. *Karenia brevis* reduced both suspension- and deposit-feeding activity. This study demonstrates that brevetoxins can be rapidly accumulated by a surface deposit-feeding bivalve from sedimented *K. brevis* cells and that comparable toxin levels can be attained by both suspension- and deposit-feeding modes [1.2–1.6 μg PbTx (g tissue wet weight)$^{-1}$]. Deposit-feeding clams generally do not pose a direct threat to humans but may provide a pathway for brevetoxin food web transfer.

Communicated by S.A. Poulet.

A.-G. Haubois (✉) · V. M. Bricelj
Institute for Marine Biosciences, National Research Council of Canada, 1411 Oxford Street, B3H 3Z1 Halifax, NS, Canada
e-mail: aghauboi@ifremer.fr

J. Naar
Center for Marine Science, University of North Carolina at Wilmington, Wilmington, NC, USA

Introduction

The Gulf of Mexico, including the Western coast of Florida (FL), is frequently affected by red tide events caused by the proliferation of the athecate dinoflagellate *Karenia brevis*. Occasional outbreaks have also been reported on the US Atlantic coast (Tester and Steidinger 1997) and in New Zealand (MacKenzie et al. 1995). *Karenia brevis* produces lipid-soluble polyether neurotoxins called brevetoxins (*Ptychodiscus brevis* toxin, i.e. PbTx) and its proliferation is associated with significant environmental, human health and economic impacts. Brevetoxins cause massive fish kills at concentrations as low as 250 cells ml^{-1}, can affect human health through ingestion of contaminated shellfish (Kirkpatrick et al. 2004), and induce adverse respiratory symptoms due to toxin released in marine aerosols (Pierce 1986; Pierce et al. 2003). Blooms of *K. brevis* are also associated with mass mortalities of marine mammals such as the endangered Florida manatees and bottlenose dolphins (O'shea et al. 1991; Flewelling et al. 2005).

The threat to marine ecosystems due to harmful algal blooms (HABs) in general has prompted research on control and mitigation methods (Anderson 1997). One of these methods, involving the use of ecologically inert clays to induce sedimentation of harmful algae, has the potential to be environmentally benign and

cost-effective. It has been successfully used at field aquaculture sites in South Korea to control fish-killing blooms of *Cochlodinium polykrikoides* (Na et al. 1996). Sengco et al. (2001) investigated the effectiveness of 25 clays against the dinoflagellate *Karenia brevis* (Florida red tides) and the picoplanktonic pelagophyte *Aureococcus anophagefferens* (brown tides), two major HAB species occurring in Atlantic waters. Phosphatic clays were found to be particularly efficient in removing *K. brevis* from the water column at a concentration as low as 0.04 g dry weight l^{-1} (80% removal efficiency) and 0.25 g dry weight l^{-1} (90% removal efficiency), but clay did not significantly remove *A. anophagefferens* (<60% removal efficiency with clay at 0.04 to 2 g dry weight l^{-1}). Further experimental studies in the laboratory confirmed the effectiveness of phosphatic clay for removing *K. brevis* cells as well as its extracellular toxins from the water column (Pierce et al. 2004). Clay dispersal might provide a potential method to control recurrent blooms of the dinoflagellate *K. brevis* in nearshore Florida coastal waters. However, the biological impacts of this strategy need to be assessed before it can be used at any significant scale on natural bloom populations. Previous studies investigating the effect of clay on shell and tissue growth rates of the hard clam *Mercenaria mercenaria* (Archambault et al. 2004) suggested that a high-energy environment leading to prolonged *in situ* resuspension of clay might affect suspension-feeding communities. Lewis et al. (2003) investigated the detrimental effect of the *K. brevis*/clay flocculates compared to the toxicity of *K. brevis* alone on several benthic species and showed that clay flocculation would neither increase, nor decrease toxicity to benthic organisms relative to that attributable to an untreated bloom.

The mechanism underlying the clay mitigation strategy is the entrainment, flocculation and sedimentation of clay-cell aggregates following clay dispersal at the surface (Sengco et al. 2001). This method transfers the algae from the water column to the bottom sediment and could thus alter the fate of PbTxs in the food web (Fig. 1). During a *K. brevis* bloom, toxins are primarily transferred to suspension-feeding bivalves that filter out large volumes of water and accumulate PbTxs in their tissues (Poli et al. 2000; Fletcher et al. 1998; Plakas et al. 2002). Suspension-feeding bivalves include many commercially important species, and ingestion of contaminated shellfish is responsible for neurotoxic symptoms (i.e. NSP, Neurotoxic Shellfish Poisoning) and acute gastroenteritis in humans (Kirkpatrick et al. 2004). Direct toxin transfer from shellfish to humans is therefore a major public health concern. Another important concern is the possibility of food web toxin transfer via benthic

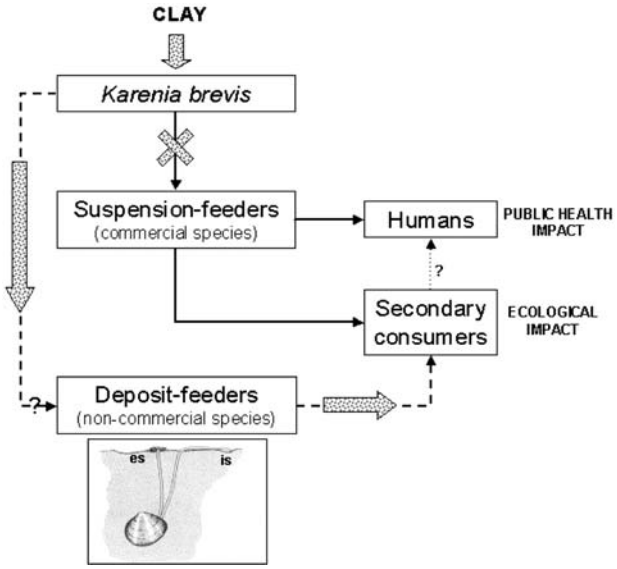

Fig. 1 Schematic flow diagram illustrating potential food web pathways of brevetoxin-producing *Karenia brevis* cells: sedimentation of toxic cells following inert clay application could reduce toxin incorporation by commercially important suspension-feeding bivalves (indicated by the *striped X*) and thus prevent direct public health impacts, but could potentially lead to brevetoxin (PbTx) accumulation by benthic deposit-feeders. *Striped arrows* indicate unknown pathways prior to this study. Drawing shows a tellinid bivalve; *is* and *es* = inhalent and exhalent siphons, respectively

invertebrates such as blue crabs and whelks (Poli et al. 2000), or omnivorous and carnivorous fish (striped burrfish, longnose killfish, pinfish, spot, puffer fish) that feed on bivalves. Flewelling et al. (2005) indeed demonstrated that massive dolphin mortalities in Florida were due to consumption of brevetoxin contaminated fish that can accumulate toxins from their food source. This routing of toxins through secondary consumers might potentially lead to human contamination but no evidence of such poisoning has been reported. Clay treatment of *K. brevis* blooms could reduce direct public health impacts by changing the fate of the PbTxs from suspension- to deposit-feeders as those are generally not commercially exploited for human consumption. However, the ecological impact due to food web transfer from deposit-feeders to higher trophic levels needs to be assessed. This risk assessment will primarily depend on the ability of deposit-feeders to accumulate and retain significant concentrations of PbTxs in tissues, which has not been previously demonstrated.

The main objective of this study was therefore to determine whether surface deposit-feeding could provide a pathway for toxins from deposited clay-*K. brevis* aggregates. Toxin uptake via deposit-feeding was

investigated and compared to that via suspension-feeding using the same model organism, the tellinid bivalve *Macoma balthica*. This species is mainly a deposit-feeder but can facultatively adopt suspension-feeding in response to flow and food availability in the water column (Hummel 1985; Levinton 1991). We determined the toxic effects of *K. brevis* on both feeding modes and compared the effectiveness of brevetoxin-transfer. For both feeding modes, *Karenia brevis* was offered alone or mixed with a non-toxic diatom (*Fragilaria familica*) to test whether the presence of non-toxic algae stimulates feeding activity [as previously shown by Bricelj et al. (1991) and Bardouil et al. (1996) for the toxic dinoflagellate *Alexandrium tamarense*] and to investigate conditions of maximal toxin incorporation.

Materials and methods

Test organism and algal culture

Macoma balthica was chosen as a model species for tellinids, the main family of deposit-feeding bivalves (Ward and Shumway 2004), represented by species such as *Tellina versicolor, Tellina lineata* or *Macoma constricta* in Florida (Camp et al. 1998). Clams were collected from an intertidal mudflat of the Minas Basin, Bay of Fundy, along the northwestern shore of Nova Scotia, Canada. They were acclimated to experimental conditions in a flow-through raceway provided with ambient seawater at 20°C and were maintained in sieved (500-µm mesh) natural sediment from the sampling site. Natural benthic microflora present in the sediment was supplemented once a week by sedimentation of the cultured diatom *Fragilaria familica.* This local species isolated from Mahone Bay, Nova Scotia, was chosen because diatoms constitute the main component of *M. balthica*'s diet (Rossi et al. 2004). We also observed that once added in the water this diatom rapidly settled at low flow and formed a surface biofilm that was readily ingested by *M. balthica* via deposit-feeding.

The toxic dinoflagellate *Karenia brevis* (Wilson strain, CCMP 718) was grown in 2.8 l Fernbach flasks containing autoclaved filtered seawater (0.22 µm millipore membrane filters) enriched with L1 nutrient medium (Guillard and Hargraves 1993). Algae were grown on a 14:10 h light–dark cycle, at 22°C and 30‰ salinity. The non-toxic dinoflagellate *Heterocapsa triquetra* (CCMP 449) was batch cultured in aerated 20 l carboys in L1 medium at 16°C and a 14:10 h light–dark cycle. *Fragilaria familica* was batch cultured in aerated 20 l carboys in F/2 + Si medium, at 16°C, under continuous illumination. All cultures were harvested in late-exponential phase.

Effect of *Karenia brevis* on suspension- and deposit-feeding and comparison of brevetoxin transfer effectiveness

The objective of this experiment was to (1) determine whether clay treatment may provide a trophic pathway for toxins by deposit-feeding of settled clay-cell aggregates and (2) to compare brevetoxin trophic transfer from deposited aggregates (deposit-feeding following clay treatment) to that from suspended cells (suspension-feeding during an untreated bloom). For this, clams (~13 mm shell length, SL) were exposed to suspended (~300–400 cells ml^{-1}) or settled *K. brevis* cells (loading of $\sim 3 \times 10^5$ cells cm^{-2}, equivalent to removal of 300 cells ml^{-1} from a 1 m-deep water column) and sampled for toxin content after 24 h. This interval was chosen to determine whether deposit-feeders were able to accumulate a significant level of PbTxs over a short period of time as we assumed that in the field, winds and currents would rapidly spread the clay layer following deposition.

The effect of *Karenia brevis* on *Macoma balthica*'s suspension-feeding behavior was assessed by measuring the clams' individual clearance rate (volume swept clear of particles per unit time). Three experiments were performed in feeding chambers (450 ml capacity, without sediment) placed in a recirculating system (Fig. 2). The algal suspension was delivered from an aerated 60 l header tank. Cell concentration was monitored using a Beckman-Coulter Multisizer (100 µm aperture), and algae were added to maintain the cell concentration within the target range in the system (300–400 *K. brevis* cells ml^{-1}). Cell concentration was monitored every 30 min during the 2 h preceding each CR measurements and every hour the rest of the time. During nighttimes, the system was supplemented with algal culture with a peristaltic pump, and cell concentration was adjusted to the target range in the morning when necessary. The suspension was gravity-fed from the header tank (~120 ml min^{-1}) and outflow from the chambers was collected in a trough and returned to the header tank with a Masterflex peristaltic pump. The flow generated by the pump was gentle enough to maintain the integrity of dinoflagellate cells. Two chambers without animals were used as controls for cell settlement or lysis and five additional chambers contained five juvenile clams each (13.58 mm SL ± 0.85 standard deviation, SD). Algae were held in suspension with motor-driven magnetic stirrer mounted at the top of the chamber, allowing mixing without

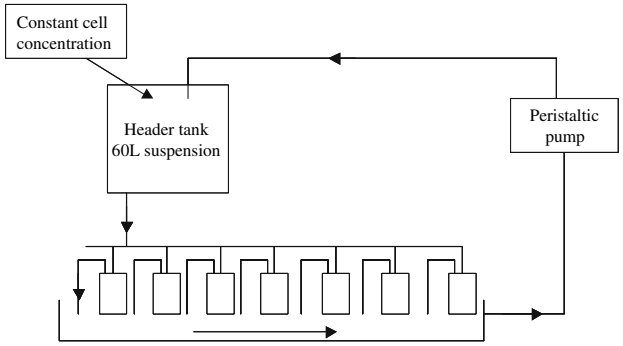

Fig. 2 Experimental design of the suspension-feeding experiments. These were performed in seven feeding chambers placed in a recirculating system. Clams ($n = 5$) were placed in five feeding chambers and two chambers were used as controls for cell settlement

resuspension of biodeposits. Before the experiments, clams were allowed to clear their gut of sediment in filtered seawater for 24 h. Experiments were conducted in a temperature-controlled walk-in environmental chamber held at 20°C.

Clearance rate (CR) was determined from the depletion of cells over a 60 min incubation (period corresponding to ~30% depletion). CR was estimated from the equation: $CR = (LnC_0\text{-}LnC_f) \times V/t$ where C_0 and C_f = initial and final cell concentration (cells ml^{-1}), respectively, V = volume of suspension (ml) and t = time interval (h). CR was standardized to the mean dry body mass of experimental animals, equal to 22.91 mg, using the following equation (Bayne et al. 1993): $CRs = CR \times (22.91/W)^b$, where CR = measured clearance rate and W = dry body mass, and b = slope of the allometric equation relating to CR and body mass. The value of 0.6 was used for b, corresponding to that determined for the tellinid, facultative suspension- deposit-feeder, *Scrobicularia plana* (Hughes 1969).

For each experiment, the clams were exposed successively to a volume equivalent concentration of the conditioning suspended diet of *Fragilaria familica* (18×10^3–21×10^3 cells ml^{-1}, equivalent spherical diameter, ESD = 7.52 ± 2.5 μm SD) and an experimental diet: (1) the non-toxic dinoflagellate *Heterocapsa triquetra* (800–900 cells ml^{-1}; ESD = 17.19 ± 1.55 μm SD) (2) the toxic dinoflagellate *Karenia brevis* (300–400 cells ml^{-1}; ESD = 21.96 ± 2.78 μm SD) or (3) a 50:50 mixture of *K. brevis* and *F. familica*. *Heterocapsa triquetra* was used as a control to test whether the clams were affected by a change in diet from diatoms to non-toxic dinoflagellates. Mixed versus unialgal suspensions were used to determine conditions of maximal toxin incorporation. After 2 h of acclimation, two consecutive CR on the conditioning diet were determined; the animals were then switched to the experimental diet for 24 h with measurement of CR at t_{2h}, t_{4h} and t_{24h}. The *K. brevis* stock was replaced 4 h before the t_{24h} measurement. Finally clams were exposed again to the conditioning diet to determine whether they could recover their initial CR. For diets (2) and (3), five replicates (1×10^6 cells each) of the initial *K. brevis* cell suspension were sampled for toxin analysis and two chambers were sampled after toxic exposure to determine toxin concentration in clam tissues. Soft tissues were dissected, rinsed with filtered seawater and weighed (wet weight). Tissues of the five individuals contained in each chamber were pooled and stored at −80°C until toxin analysis.

For the deposit-feeding experiment, juvenile clams (average size 13 mm SL ± 0.7 SD) were exposed to the following three treatments: (1) 3×10^5 *K. brevis* cells cm^{-2} of substrate (Wilson strain, CCMP 718), equivalent to removal of 300 cells ml^{-1} from a 1-m-deep water column, (2) a mixed diet of ~3×10^5 *K. brevis* cells cm^{-2} and a 20% volume equivalent concentration of *F. familica*, or (3) a control diet of *F. familica* (at a concentration equivalent in cell volume to treatment 1). For each treatment, 3 microcosms (made out of Plexiglas, W6 × L12 × H8 cm^3) were placed in a recirculating system. Microcosms were filled with a 2 cm bottom layer of muddy substrate obtained from the clam sampling site and pre-sieved through a 500 μm sieve. The application of toxic cells and clay was repeated twice to attain the desired bottom loading. Each time, 400 ml of suspension at the appropriate concentration was added to the microcosms and treated with phosphatic clay (IMC-P2, International Mining Corporation, Bartow, FL, USA) at a concentration of 0.25 g dry weight l^{-1}. Five replicates (1×10^6 cells each) of *K. brevis* suspension were previously sampled for toxin analysis. Once *K. brevis* cells had completely settled, the water column in each microcosm was sampled to estimate the removal efficiency. After renewal of water by flushing, five clams were placed in each microcosm and allowed to burrow; t_0 was taken once all the animals had burrowed (~1 h). After 24 h exposure, animals were collected, soft tissues dissected, rinsed with filtered seawater and weighed (wet weight). The five individuals contained in each chamber were pooled and stored at −80°C until toxin analysis.

Toxin extraction and analysis

Toxins from *K. brevis* cells were extracted by elution of water samples through C-18 solid phase extraction tubes (Supelco) and eluted from the tubes with 15 ml methanol under gentle vacuum. The methanol was

dried in a rotary-evaporation unit, and samples were then brought to a final volume of 3 ml methanol.

Clam tissue samples were extracted in 3–5 ml methanol using a Brinkmann Polytron homogenizer. Slurries were centrifuged (10 min at 3,000 rpm), and the supernatant was collected and dried in a rotary-evaporation unit. The dried residues were re-solubilized in acetone at a concentration of 0.2 g to 1 g wet tissue ml^{-1} by bath sonication and vortex mixing.

Pre-weighed sediment samples were first centrifuged at ca. 2,500 rpm for 2 min to discard any remaining water. Toxins were extracted in ca. 10 ml acetone using an ultrasonic probe. Samples were centrifuged and the supernatant was decanted into a flask. The acetone sonic extraction and centrifugation was repeated until the solvent layer was colorless and successive extracts were pooled in the flask. The extracts were then dried in a rotary-evaporation unit and re-solubilized in 3 ml acetone.

The toxin content of *K. brevis* cells, sediment and clam tissues was analyzed by competitive Enzyme-Linked Immunosorbent Assay (ELISA) as described in Naar et al. (2002). This method allows detecting brevetoxins accurately in a variety of sample types without matrix effects at a level as low as 4 ng PbTx g^{-1}. The anti-brevetoxin goat polyclonal antibodies used are specific for the last H–K rings of the PbTx type-2 brevetoxins (PbTx-2, PbTx-3, PbTx-9) and recognize all of these compound derivatives (Naar et al. 2004; Dickey et al. 2004; Plakas et al. 2004). As ELISA analysis cannot differentiate between toxins present in the sample tested, results are expressed as PbTx-3 equivalent and reflect the overall concentration of brevetoxins and brevetoxin-like compounds present in the sample.

Statistical analysis

Measurement of clearance rates (CR) during suspension-feeding experiments consisted of repeated measurements over time from the same sampling units (i.e. 5 chambers containing 5 individuals each). Such repeated sampling of the same population might introduce risks of temporal non-independence of data and statistical analysis can proceed only if it can be assumed that there is no interaction among times and measurements (Underwood 1997). This interaction might be expected to occur during the toxic treatments (suspension-feeding with *Karenia brevis* and the mixed diet) as CR may be more affected with increasing exposure time. Interactions among time and CR measurements during *K. brevis* exposure were tested by linear regressions. For both treatments, the slope of the regression line was not significantly different from zero ($P > 0.05$) and the coefficient of determination R^2 was low (<0.2) indicating that CR and time were not interdependent. For further statistical analysis we assumed that each measurement was independent and analyses of variance (ANOVA), followed by multiple comparison tests (Tukey), were performed. Normality of data and homogeneity of variances were also tested.

Results

For all treatments and both feeding modes, no clam mortality occurred during the course of the experiments. The first suspension-feeding experiment (Fig. 3a) showed that *Macoma balthica* maintained a constant weight-standardized clearance rate (ANOVA, $P = 0.07$) of ~41 ml h^{-1} when successively exposed to the diatom *F. familica* and the non-toxic dinoflagellate *H. triquetra*. When exposed to the toxic dinoflagellate *K. brevis* (300–400 cells ml^{-1}; 46.8 pg PbTx-3 eq $cell^{-1} \pm 1$ SD), *M. balthica* showed a significant decrease in its mean clearance rate (ANOVA, $P < 0.05$) from ~38 to 12 ± 4 ml h^{-1} (68% reduction) over 24 h (Fig. 3b). It was also observed that the inhalant siphon was less extended in the water column when the clams were exposed to the toxic dinoflagellate. The animals rapidly recovered their initial clearance rate after toxic cells were replaced with the *F. familica* conditioning diet. When exposed to a mixed diet of *F. familica* and *K. brevis* (30.5 ± 3.2 pg PbTx-3 eq $cell^{-1}$), clearance rates were less affected (37% reduction) attaining a mean value of 24 ± 4 ml h^{-1} over 24 h (Fig. 3c), which was twice as high as that obtained with *K. brevis* alone (12 ± 4 ml h^{-1}). The clams cleared *K. brevis* cells and *F. familica* cells from the mixed suspension at similar rates (24 ± 4 ml h^{-1} for *F. familica* and 33 ± 14 ml h^{-1} for *K. brevis*, *t* test, $P > 0.05$) indicating that the animals cleared both toxic and non-toxic algae without showing selective feeding behavior.

The *K. brevis* cell removal efficiency by clay in the deposit-feeding experiment was 98% ± 2 SD for both applications. During the 24 h exposure, clams showed reduced feeding activity when exposed to *K. brevis* (21.2 ± 6.1 pg PbTx-3 eq $cell^{-1}$) compared to that of the non-toxic control (*F. familica*). In the latter, the inhalant siphon exhibited wide movements and explored the sediment over a large surface area, and the algal biofilm deposited on the sediment surface was completely removed after 24 h exposure. In contrast, in the presence of *K. brevis* the inhalant siphon did not emerge much and only a small area of the biofilm was removed in the vicinity of the orifice of the siphonal

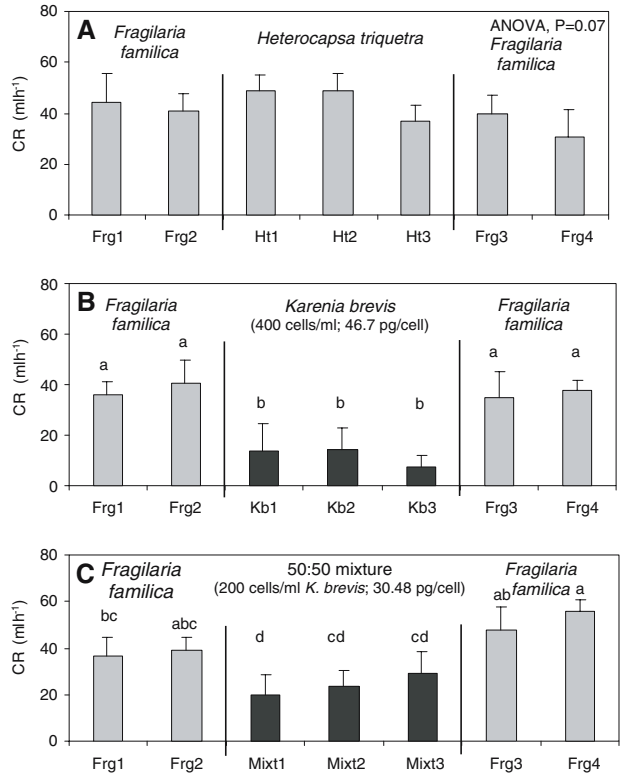

Fig. 3 Mean wet weight-standardized clearance rate (ml h^{-1}; mean ± SD) of *Macoma balthica* successively exposed to a control non-toxic diet of *Fragilaria familica* and an experimental diet (cell volume equivalent concentrations). Experimental diets were **a** the non-toxic dinoflagellate *Heterocapsa triquetra*, and **b** the toxic dinoflagellate *Karenia brevis* and **c** a 50:50 mixture of *K. brevis* and *F. familica*. Toxic diets are indicated by *black bars*. *Letters* (*a*, *b*, *c* and *d*) correspond to the results of Tukey multiple comparison test and indicate statistically significant differences at $P > 0.05$

channel (Fig. 4). No obvious behavioral differences were observed between the treatment with *K. brevis* alone and the mixture of *K. brevis* and *F. familica*.

The toxin level in *M. balthica* tissues after 24 h-exposure reached 1.2 ± 0.12 µg PbTx-3 eq (g wet weight tissues)$^{-1}$ by suspension-feeding in the presence of *K. brevis* alone (Fig. 5) and was not significantly different when *K. brevis* was present in a mixture with non-toxic cells [0.9 ± 0.19 µg PbTx-3 eq (g wet weight tissues)$^{-1}$; *t* test, $P > 0.05$].

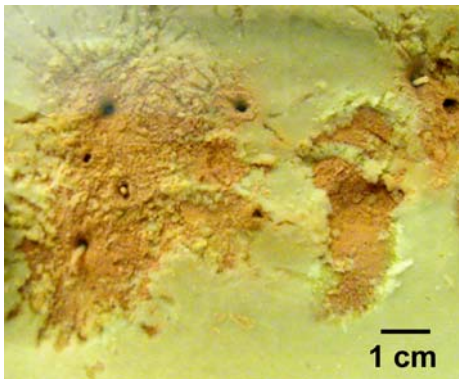

Fig. 4 Partial removal of the clay layer by deposit-feeding after 24 h-exposure (see text)

For the deposit-feeding experiment, toxin accumulation in tissues was not influenced by the addition of the non-toxic diatom *F. familica* (*t* test, $P > 0.05$) and toxin levels reached ~1.6 µg PbTx-3 eq (g wet weight tissues)$^{-1}$ in both treatments. Since the initial toxin loading was the same in both treatments, this suggests that no preferential ingestion of non-toxic cells occurred when clams were exposed to the mixture.

Discussion

Karenia brevis bloom concentrations are highly variable but typically in the range 100–1,000 cell ml^{-1}, although densities up to 10,000 cells ml^{-1} have been reported (Tester and Steidinger 1997; Magnana et al. 2003). The experimental concentration of ~300–400 cells ml^{-1} used in the suspension-feeding experiment was selected to minimize pseudofeces production and maximize ingestion of toxic cells. For the deposit-feeding experiment, the toxic cell loading of bottom sediment aimed to simulate removal of a bloom of a concentration similar to that of the suspension-feeding experiment from a 1 m water column. The brevetoxin concentration obtained in our *K. brevis* cultures using ELISA varied in the range 21–47 pg PbTx-3 eq $cell^{-1}$ for cultures harvested in late log-phase. Roszell et al. (1990) reported brevetoxin concentration of ~11 pg PbTx $cell^{-1}$ for cultures in log-phase and in Florida waters cell toxin content usually varies in the range 10–17 pg PbTx $cells^{-1}$ (Hua et al. 1996; Pierce et al. 2003). Exceptionally high toxin levels have been recorded during a *Karenia* bloom in FL with values in the range 47–126 pg PbTx $cells^{-1}$ (Greene et al. 2000). Our values are consistent with all these data but it is worth noting that toxin content of algae and clam tissues was measured in the present study with the ELISA method, which reacts with PbTx type-2 backbone structure (Naar et al. 2002), including PbTx-2, 3, 9 and derivatives. Values in the study cited above were obtained with high pressure liquid chromatography (HPLC) or liquid chromatography/mass spectrometry (LC/MS) methods, which quantify only the predominant toxins PbTx 1, 2 and 3. Among the nine brevetoxins fully elucidated in terms of structure (PbTx 1 to 9), PbTx-2 is the dominant toxin in *K. brevis* cells (Baden et al. 1988) and represents ~80% of the total brevetoxin content (Roszell et al. 1990; Hua et al. 1996).

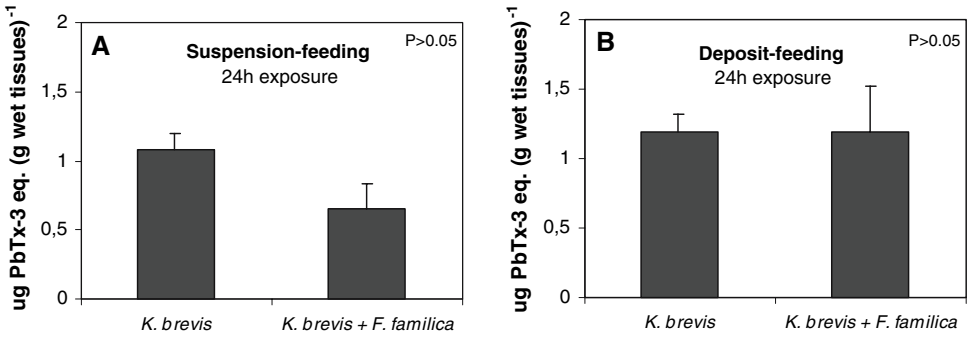

Fig. 5 Brevetoxin concentration in tissues of the clam *Macoma balthica* [μg PbTx-3 eq (g wet tissues)$^{-1}$] after 24-h exposure: **a** to suspended *Karenia brevis* (at 300–400 cells ml^{-1}) and a 50:50 mixed diet including the non-toxic diatom *Fragilaria familica*; **b** to deposited *Karenia brevis* (loading of ~3 × 10^5 *K. brevis* cells cm^{-2}, equivalent to removal of 300 cells ml^{-1} from a 1-m-deep water column) and to a mixture of *K. brevis* (~3 × 10^5 *K. brevis* cells cm^{-2}) and *F. familica* (20% volume equivalent concentration)

We can then assume that suspension- and deposit-feeding experiments simulated two comparable scenarios, representative of field conditions.

Karenia brevis reduced both suspension- and deposit-feeding activity. The clams' clearance rate decreased when exposed to *K. brevis* while it remained constant when exposed to *Heterocapsa triquetra*, a non-toxic dinoflagellate of similar size. Clearance rate was also inhibited with the 50:50 mixture of *K. brevis* and *F. familica* and no selective feeding behavior was observed. Similarly, although based only on qualitative observations, deposit-feeding was adversely affected with both diets (i.e. *K. brevis* alone and mixture including *F. familica*) and no preferential ingestion of non-toxic cells appeared to occur based on comparable toxin levels accumulated in tissues. It thus appears that, with both feeding modes, clams respond to the presence of toxic *K. brevis* cells by reducing their feeding rate but have no ability to preferentially sort between toxic and non-toxic cells. According to Colin and Dam (2003), such a feeding response is attributed to a toxic effect, as opposed to a deterrent effect resulting from the grazers' ability to detect a compound in the algae and to select against this specific algal species. Accordingly, Bricelj et al. (1991) showed that the hard clam *Mercenaria mercenaria* did not selectively ingest the non-toxic diatom *Thalassiosira weissflogii* over the PSP-producing dinoflagellate *Alexandrium fundyense* when offered in a mixed suspension. Li et al. (2001) obtained similar results with the clam *Ruditapes philippinarum* and the scallop *Chlamys nobilis* exposed to a mixture of the toxic dinoflagellate *A. tamarense* and the non-toxic diatom *T. pseudonana*. However, feeding responses to toxic dinoflagellates may be highly variable among bivalve species, and clearance rate is either inhibited (Bricelj et al. 1996; Matsuyama et al. 1999; Lassus et al. 1999), enhanced (Lesser and Shumway 1993) or not affected (Shumway et al. 1985; Bricelj et al. 1996). Supplementation with non-toxic cells appears to stimulate suspension-feeding activity compared to that on toxic cells alone in relatively toxin-sensitive species such as hard clam, oysters and soft shell clams (Bricelj et al. 1991; Bardouil et al. 1996). This can lead to accumulation of higher toxin levels in mixed suspensions (Bricelj et al. 1996). Indeed, the clearance rate of *M. balthica* fed with the 50:50 mixture was twice as high as that with *K. brevis* alone and consequently, similar brevetoxin concentrations in tissues were obtained in both treatments (i.e. *K. brevis* alone and mixture including *F. familica*). These results imply that even at relatively low *K. brevis* concentrations in the water column, toxin body burden may reach a significant level in tissues when *K. brevis* is mixed with non-toxic microalgae.

Reduction of feeding activity in the presence of *K. brevis* may ultimately affect bivalve growth and survival. This would particularly affect suspension-feeders as we assume that under most natural conditions the clay layer would be rapidly dispersed by tidal currents or waves after clay application and that consequently deposit-feeders would be only exposed to sedimented brevetoxins for a short period of time. Leverone et al. (2007) showed that clearance rates of juvenile bay scallops, *Argopecten irradians*, were highly affected at concentrations as low as 100 cells ml^{-1}. They suggest that recurring blooms of *K. brevis* could hamper restoration projects for recovery of bay scallop populations in Florida estuaries. By reducing the exposure time to *K. brevis*, clay mitigation could thus prevent the impact on shellfish resources.

Toxin incorporation by *Macoma balthica* occurred by both suspension and deposit-feeding and toxin body burden after 24-hr exposure attained comparable values in both feeding modes [1 to

1.6 μg PbTx (g wet weight)$^{-1}$], exceeding the guidance level of 0.8 μg PbTx-3 eq g^{-1} for edible tissues (based on the established mouse toxicity of PbTx-3). A similar brevetoxin body burden of ~1.8 μg PbTx-3 eq (g wet weight)$^{-1}$ was measured by ELISA in tissues of suspension-feeding bivalves collected in Florida waters (Naar et al., 2003). High levels of contamination achieved by suspension-feeding bivalves result from their ability to concentrate and consume high numbers of cells by filtering large volumes of water (Ward and Shumway 2004). Surface deposit-feeding bivalves use their inhalant siphon to vacuum surface sediment and ingest a mixture of sediment and organic matter including bacteria, detritus and microalgae (Hughes 1969; Hummel 1985). They are able to process large quantities of material to extract the diluted organic food source from the inorganic matrix (Taghon 1981). Clay mitigation of *K. brevis* concentrates PbTxs into a thin layer of clay-cell aggregates at the sediment surface thus making toxins available for deposit-feeders. Toxin levels in shellfish tissues obtained in this study should however be considered with caution as *M. balthica* is a species from North Atlantic and experimental conditions, representatives of Florida water temperatures, are not necessarily optimal for this species. *M. balthica* was chosen for its unique property of allowing test of both suspension- and deposit-feeding with a single species. We then demonstrate that clay mitigation of *K. brevis* blooms may provide a new trophic pathway for toxins by deposit-feeding of settled clay-cell aggregates and that toxin transfer via deposit-feeding could be of comparable magnitude to that attained via suspension-feeding during an untreated bloom (see Fig. 1). Deposit-feeders do not pose a direct threat to humans but may then provide a pathway for brevetoxin transfer to secondary consumers. The present study provides a first demonstration of brevetoxin accumulation by this trophic group and our findings suggest that toxin accumulation by deposit-feeders might also result from natural deposition of particulate matter during a bloom in the absence of clay treatment. This may occur due to settlement of *K. brevis* cells, deposition of copepod fecal pellets (Tester et al. 2000) or accumulation of biodeposits from benthic suspension-feeders. Toxin transfer to deposit-feeders during untreated blooms would then need to be investigated in order to fully assess the ecological impact of *K. brevis* blooms.

Conclusions

This study shows that significant concentrations of brevetoxins may be accumulated in shellfish tissues from deposited clay-cell aggregates after only 24-h exposure. However, the residence time and composition of brevetoxins in the deposit-feeding compartment remain to be determined, and are important in evaluating the potential for toxin transfer to secondary consumers. Furthermore, several studies have shown that shellfish are able to rapidly metabolize brevetoxins (Morohashi et al. 1999; Poli et al. 2000; Plakas et al. 2002; Ishida et al. 2004) and Poli et al. (2000) suggested that PbTx metabolites contribute to shellfish toxicity and may be the true cause of NSP in humans. Finally, this study provides the first evidence of brevetoxin transfer to deposit-feeders. Beyond the context of clay mitigation of HABs, this result highlights that benthic deposit-feeders should be considered as an important compartment in future studies on the fate of algal toxins in the food web.

Acknowledgment Funding for this work was provided by NOAA ECOHAB via a grant awarded to D. Anderson at WHOI, USA. We thank D. Anderson and M. Sengco (WHOI) for providing clay and the *K. brevis* Wilson strain, R. Pierce (Mote Marine Lab, FL, USA) for his helpful advice, and the research team from the shellfish lab at IMB/NRC for assistance in clam sampling.

References

Anderson DM (1997) Turning back the harmful red tide. Nature 388:513–515

Archambault MC, Bricelj VM, Grant J, Anderson DM (2004) Effects of suspended and sedimented clays on juvenile hard clams, *Mercenaria mercenaria*, within the context of harmful algal bloom mitigation. Mar Biol 144:553–565

Baden DG, Mende TJ, Szmant AM, Trainer VL, Edwards RA, Roszell LE (1988) Brevetoxin binding: molecular pharmacology versus immunoassay. Toxicon 26:97–103

Bardouil M, Bohec M, Bougrier S, Lassus P, Truquet P (1996) Feeding responses of *Crassostrea gigas* (Thunberg) to inclusion of different proportions of toxic dinoflagellates in their diet. Oceanol Acta 19:177–182

Bayne BL, Iglesias JIP, Hawkins AJS, Navarro E, Heral M, Deslous-Paoli JM (1993) Feeding behavior of the mussel *Mytilus edulis*. Mar Ecol Prog Ser 55:47–54

Bricelj VM, Lee JH, Cembella AD (1991) Influence of dinoflagellate cell toxicity on uptake and loss of paralytic shellfish toxins in the northern quahog *Mercenaria mercenaria*. Mar Ecol Prog Ser 74:33–46

Bricelj MV, Cembella AD, Laby D, Shumway SE, Cucci TL (1996) Comparative physiological and behavioral responses to PSP toxins in two bivalve mollusks, the soft clam, *Mya arenaria*, and surfclam, *Spisula solidissima*. In: Yasomoto Y, Oshima Y, Fukuyo Y (eds) Harmful and toxic algal blooms. IOC-UNESCO, Paris, pp 405–408

Camp DK, Lyons WG, Perkins TH (1998) Checklist of selected shallow-water marine invertebrates of Florida. Florida Marine Research Institute Technical Report, 239 p

Colin SP, Dam HG (2003) Effects of the toxic dinoflagellate *Alexandrium fundyense* on the copepod *Acartia hudsonica*: a test of the mechanisms that reduce ingestion rates. Mar Ecol Prog Ser 248:55–65

Dickey RW, Plakas SM, Jester ELE, Elsiad KR, Johannessen JN, Flewelling LJ, Scott P, Hammond DG, VanDolah FM, Leighfield TA, Bottein Y, Ramsdell JS, Busman M, Moeller PD, Pierce RH, Henry MS, Poli MA, Walker CS, Kurtz J, Naar J, Baden DG, Musser SM, Truman P, Quilliam MA, Stirling D, Hawryluk TP, Wekell MM, Hungerford JM, Yoshimoto K (2004) Multi-laboratory study of five methods for the determination of brevetoxins in shellfish tissue extracts. In: Steidinger K (ed) Harmful algae 2002, pp 300–303

Fletcher GC, Hay BE, Scott MF (1998) Detoxifying Pacific oysters (*Crassostrea gigas*) of the neurotoxic shellfish poison (NSP) produced by *Gymnodinium breve*. J Shell Res 17:1637–1641

Flewelling LJ, Naar JP, Abbott JP, Baden DG, Barros NB, Bossart GD, Bottein MYD, Hammond DG, Haubold EM, Heil CA, Henry MS, Jacocks HM, Leighfield TA, Pierce RH, Pitchford TD, Rommel SA, Scott PS, Steidinger KA, Truby EW, Van Dolah FM, Landsberg JH (2005) Red tides and marine mammal mortalities. Nature 435:755–756

Greene RM, Walker CC, Murrell MC, Kurtz JC, Stanley RS, Genthner FG (2000) High brevetoxin concentrations in *Gymnodinium breve* blooms along the northwest Florida coast during 1999. J Phycol 36:25–26

Guillard RRL, Hargrave BT (1993) *Stichochyisis immobilis* is a diatom, not a chrysophyte. Phycologia 32:234–236

Hua Y, Lu W, Henry MS, Pierce RH, Cole RB (1996) On-line liquid chromatography-electrospray ionization mass spectrometry for determination of the brevetoxin profile in natural "red tide" algae blooms. J Chromato A 750:115–125

Hughes RN (1969) A study of feeding in *Scrobicularia plana*. J Mar Biol Assoc UK 49:805–823

Hummel H (1985) Food intake and growth in *Macoma balthica* (Mollusca) in the laboratory. Neth J Sea Res 19:77–83

Ishida H, Nozawa A, Nukaya H, Rhodes L, McNabb P, Holland PT, Tsuji K (2004) Confirmation of brevetoxin metabolism in cockle, *Austrovenus stutchburyi*, and greenshell mussels, *Perna canaliculus*, associated with New Zealand neurotoxic shellfish poisoning, by controlled exposure to *Karenia brevis*. Toxicon 43:701–712

Kirkpatrick B, Flemming LE, Squicciarini D, Backer LC, Clark R, Abraham W, Benson J, Cheng YS, Johnson D, Pierce R, Zaias J, Bossart GD, Baden DG (2004) Literature review of Florida red tide: implications for human health effects. Harmful Algae 3:99–115

Lassus P, Bardouil M, Beliaeff B, Masselin P, Naviner M, Truquet P (1999) Effect of a continuous supply of the toxic dinoflagellate *Alexandrium minutum* on the feeding behavior of the Pacific oyster (*Crassostrea gigas*). J Shellfish Res 18:211–216

Lesser MP, Shumway SE (1993) Effects of toxic dinoflagellates on clearance rates and survival in juveniles bivalve mollusks. J Shellfish Res 12:377–381

Leverone JR, Shumway SE, Blake NJ (2007) Comparative effects of the toxic dinoflagellate Karenia brevis on clearance rates in juveniles of four bivalve molluscs from Florida, USA. Toxicon (in press)

Levinton JS (1991) Variable feeding behavior in three species of *Macoma* (Bivalvia: Tellinacea) as a response to water flow and sediment transport. Mar Biol 110:375–383

Lewis MA, Dantin DD, Walker CC, Kurtz JC, Greene RM (2003) Toxicity of clay flocculation of the toxic dinoflagellate, *Karenia brevis*, to estuarine invertebrates and fish. Harmful Algae 2:235–246

Li SC, Wang WX, Hsieh DPH (2001) Feeding and absorption of the toxic dinoflagellate *Alexandrium tamarense* by two marine bivalves from the South China Sea. Mar Biol 139:617–624

Mackenzie L, Rhodes L, Till D, Chang FK, Kaspar H, Haywood A, Kapa J, Walker B (1995) A *Gymnodinium* sp. Bloom and contamination of shellfish with lipid soluble toxins in New Zealand, Jan–April 1993. In: Lassus P et al (eds) Harmful marine algal blooms. Intercept Ltd, France, pp 795–800

Magana HA, Contreras C, Villareal TA (2003) A historical assessment of *Karenia brevis* in the western Gulf of Mexico. Harmful Algae 2:163–171

Matsuyama Y, Uchida T, Honjo T (1999) Effect of harmful dinoflagellates, *Gymnodinium mikimotoi* and *Heterocapsa circularisquama*, red-tide on filtering rate of bivalves mollusks. Fish Sci 65:248–253

Morohashi A, Satake M, Naoki H, Kaspar HF, Oshima Y, Yasumato T (1999) Brevetoxin B4 isolated from greenshell mussels *Perna canaliculus*, the major toxin involved in neurotoxic shellfish poisoning in New Zealand. Nat Toxins 7:45–48

Na G, Choi W, Chun Y (1996) A study on red tide control with loess suspension. J Aquac 9:239–245

Naar J. Bourdelais A, Tomas C, Kubanek J, Whitney PL, Flewelling L, Steidinger K, Lancaster J, Baden DG (2002) A competitive ELISA to detect brevetoxins from *Karenia brevis* (formely *Gymnodinium breve*) in seawater, shellfish, and mammalian body fluid. Environ Health Perspect 110:179–185

Naar J, Flewelling L, Lenzi A, Landsberg J, Jacocks H, Musser S, Bourdelais A, Steidinger K, Baden D (2003) Experimental bioaccumulation of ichthyotoxic brevetoxins in healthy fish. Poster at the Second Symposium on Harmful Marine Algae in the US, Woods Hole, Massachusetts***

Naar J, Weidner A, Kubanek J, Flewelling L, Bourdelais A, Jacocks H, Steidinger KA, Pierce R, and Baden DG (2004) Bioaccumulation, biodepuration, and biotransformation of brevetoxins by shellfish: an issue for shellfish monitoring. In: Steidinger, K (ed) Harmful algae 2002, pp 488–490

O'Shea TJ, Rathbun GB, Bonde RK, Buergelt CD, Odell DK (1991) An epizootic of Florida manatees associated with a dinoflagellate bloom. Mar Mamm Sci 7:165–179

Pierce RH (1986) Red tide (*Ptychodiscus brevis*) toxin aerosols: a review. Toxicon 24:955–965

Pierce RH, Henry MS, Blum PC, Lyons JI, Cheng YS, Yazzie D, Zhou Y (2003) Brevetoxin concentrations in marine aerosol: human exposure levels during a *Karenia brevis* harmful algal bloom. Bull Environ Contam Toxicol 70:161–165

Pierce RH, Henry MS, Higham CJ, Blum PC, Sengco MR, Anderson DM (2004) Removal of harmful algal cells (*Karenia brevis*) and toxins from seawater culture by clay flocculation. Harmful Algae 3:141–148

Plakas SM, El Said KR, Jester ELE, Granade HR, Musser SM, Dickey RW (2002) Confirmation of brevetoxin metabolism in the eastern oyster (*Crassostrea virginica*) by controlled exposures to pure toxins and to *Karenia brevis* cultures. Toxicon 40:721–729

Plakas SM, Wang Z., ElSaid KR, Jester ELE, Granade HR, Flewelling LJ, Scott P, Dickey RW (2004) Brevetoxin metabolism and elimination in the Eastern oyster (*Crassostrea virginica*) after controlled exposures to *Karenia brevis*. Toxicon 44:677–685

Poli MA, Musser SM, Dickey RW, Eilers PP, Hall S (2000) Neurotoxic shellfish poisoning and brevetoxin metabolites: a case study from Florida. Toxicon 38:981–993

Rossi F, Herman PMJ, Middelburg JJ (2004) Interspecific and intraspecific variation in $\delta^{13}C$ and $\delta^{15}N$ in deposit- and suspension-feeding bivalves (*Macoma balthica* and *Cerastoderma edule*): evidence of ontogenic changes in feeding mode of *Macoma balthica*. Limnol Oceanogr 49:408–414

Roszell LE, Schulman LS, Baden DG (1990) Toxin profiles are dependent on growth stages in cultured *Ptychodiscus brevis*. In: Graneli E et al (eds) Toxic marine phytoplankton. Elsevier, Amsterdam, pp 403–406

Sengco MR, Li A, Tugend K, Kulis D, Anderson DM (2001) Removal of red- and brown-tide cells using clay flocculation. 1. Laboratory culture experiments with *Gymnodinium breve* and *Aureococcus anophagefferens*. Mar Ecol Prog Ser 210:41–53

Shumway SE, Cucci TL, Newell RC, Yentsch CM (1985) Particle selection, ingestion, and absorption in filter-feeding bivalves. J Exp Mar Biol Ecol 91:77–92

Taghon GL (1981) Beyond selection: optimal ingestion rate as a function of food value. Am Nat 118:202–214

Tester PA, Steidinger KA (1997) *Gymnodinium breve* red tide blooms: initiation, transport, and consequences of surface circulation. Limnol Oceanogr 42:1039–1051

Tester PA, Turner JT, Shea D (2000) Vectorial transport of toxins from dinoflagellate *Gymnodinium breve* through copepods to fish. J Plankton Res 22:47–61

Underwood AJ (1997) Experiments in ecology: their logical design and interpretation using analysis of variance. Cambridge University Press, Cambridge, p 504

Ward JE, Shumway SE (2004) Separating the grain from the chaff: particle selection in suspension- and deposit-feeding bivalves. J Exp Mar Biol Ecol 300:83–130