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Effects of suspended and sedimented clays on juvenile hard clams, *Mercenaria mercenaria*, within the context of harmful algal bloom mitigation

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Abstract Increased interest in using ecologically inert clays to flocculate, sediment, and thus mitigate harmful algal blooms at nearshore mariculture sites has prompted studies on the effectiveness of this method on prolific blooms, such as those caused by the neurotoxic dinoflagellate *Karenia brevis* in the Gulf of Mexico. Potential repercussions of this control strategy revolve around the increased flux of suspended particles to the benthos. Juvenile suspension-feeding bivalves are potentially vulnerable as they could suffer burial, a decrease in clearance rates, and/or an increase in pseudofeces production in response to suspended clay, leading to reduced growth and delay in attaining size refuge from predators. Here we assess lethal and sublethal effects on juvenile hard clams, *Mercenaria mercenaria*, in a 2-week flume application of phosphatic clay to simulated blooms of the nontoxic dinoflagellates *Heterocapsa triquetra* and *Prorocentrum micans*. Flow regimes simulated two contrasting, “worst-case” field conditions where (1) low flow allowed complete sedimentation and formation of a benthic sediment layer, and (2) high flow allowed complete particle suspension. No clam mortalities occurred in either treatment. The sedimentation treatment showed variable growth inhibition in shell and/or tissue, but effects were not apparent compared to controls (no sediment layer), and clams rapidly resumed siphon contact with the overlying water column. In

contrast, a strong growth effect (~90% reduction in shell and tissue growth) occurred in particle-suspension trials compared to no-clay controls. These results suggest that repeated clay applications in the field are likely more detrimental to clams in a high-energy environment leading to prolonged in situ resuspension of clay than in an environment favoring sedimentation.

Introduction

Harmful algal blooms (HABs) caused by toxin- and non-toxin-producing phytoplankton can harm invertebrates and higher trophic level organisms such as fish and marine mammals via toxin accumulation and/or varying degrees of physiological and ecological damage (Anderson 1997; Landsberg 2002). Concern over an apparent global increase in HABs (Hallegraeff 1993), especially for blooms occurring nearshore and affecting important fisheries and mariculture operations, has prompted research on their control and mitigation in an effort to reduce ecological and economic impacts. Potent toxins produced by some HAB species accumulate in suspension-feeding shellfish and affect human consumers, resulting in outbreaks of paralytic, neurotoxic, amnesic, azaspiracid, and diarrhetic shellfish poisoning. Direct effects on suspension-feeding bivalves include valve closure (Shumway and Cucci 1987), reduced filtration rates (e.g., Bardouil et al. 1996), reduced growth (Nielsen and Strømgren 1991), neurophysiological effects (Twarog and Yamaguchi 1974), burrowing incapacitation (MacQuarrie 2002), and mass mortalities (Matsuyama et al. 1995).

Past efforts to minimize HAB impacts have focused on preventive measures, such as control of eutrophication, selection of aquaculture sites (Anderson 1997), and monitoring to prevent algal toxins from reaching human consumers and causing damage to fish and shellfish (Andersen 1996). Though monitoring and management are effective tools to minimize health and economic

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impacts of HABs, there are no widely accepted strategies to suppress blooms and directly limit the proliferation and distribution of the causative organisms (Sengco et al. 2001). Chemical control methods (Rounsefell and Evans 1958) are deemed too expensive, ineffective, or unacceptable for use in open waters (Anderson 1997).

In recent years there has been increasing interest in using ecologically inert clays as a means of mitigating HABs at relatively shallow fish or suspended shellfish mariculture sites (Anderson 1997). Clays are relatively inexpensive, can be supplied in large quantities (e.g., as industrial waste products from mining and drilling operations; Sengco et al. 2001), and are presumed to have limited or no direct toxic effects on aquatic organisms (Howell and Shelton 1970; see Cranford and Gordon 1992 and Cranford et al. 1999 for effects of bentonite clay, a major constituent of water-based drilling muds). The mechanism underlying this mitigation effort is the flocculation and/or entrainment and accelerated sedimentation of large algal cells via application of clays (Avnimelech et al. 1982; Søballe and Threlkeld 1988). Sengco et al. (2001) determined the removal efficiency of many clay types, including montmorillonites, bentonites, and kaolinites, applied to *Karenia brevis* (formerly *Gymnodinium breve*) and the brown tide species *Aureococcus anophagefferens* in test tubes. Most past research on clay-algal removal efficiency has been conducted in static systems in the absence of flow and showed that clays have specific removal efficiencies and thus varying effectiveness depending on phytoplankton species, and algal cell and clay concentrations (Yu et al. 1994a, 1994b, 1995, 1999; Sengco et al. 2001).

This method is currently being used extensively at fish aquaculture sites in South Korea to control *Cochlodinium polykrikoides* blooms (Na et al. 1996; Bae et al. 1998; Kim 1998). In 1996, approximately 60,000 tons of dry loess clay were dispersed over a bloom (260 km²) at an estimated surface aerial loading rate of 400 g m⁻². Removal rates of *C. polykrikoides* at the surface (upper 2 m) were estimated at 90–99% (Na et al. 1996). No mortality of cultured yellowtail (*Seriola quinqueradiata*) was reported, and the bloom did not return for the remainder of the season. Clay application is presently being tested experimentally as a potential method for controlling HABs in the United States (Sengco et al. 2001). In Florida, *K. brevis* blooms have caused major economic losses due to their impact on tourism and public health, and have caused extensive mortalities of fish, shellfish, as well as manatees (Trainer and Baden 1999).

Potential repercussions of clay dispersal as a bloom-control strategy revolve around the increasing flux of suspended particles to the bottom and their subsequent resuspension. Impacts of this mitigation strategy on benthic organisms have not yet been investigated. Suspension-feeding bivalves, which actively pump large volumes of water, may be particularly vulnerable. Aquaculture operations and efforts to reestablish bivalve populations, such as the bay scallop, *Argopecten*

irradians, in Florida (Arnold et al. 1999), are increasingly affected by HABs. In Florida, hard clams are intensively cultured, with 300 grow-out operations with 1997 estimated sales of US \$10 million (Colson and Sturmer 2000). Aquaculture operations could benefit from clay mitigation; however, bivalves show a wide range of species-specific tolerances and adaptive mechanisms to cope with high water column turbidity (Foster-Smith 1975; Hawkins et al. 1996; Cranford et al. 1999). Bivalves have been categorized into two main groups based on their feeding strategies in the presence of suspended sediments (Bricelj and Malouf 1984): (1) species that primarily reduce clearance or pumping rate, and (2) those that combine high pseudofeces production and ingestion selectivity to optimize ingestion of food particles (e.g., *Mytilus edulis*) and are thus more tolerant of sediment loading (Hawkins et al. 1996). Intermediate strategies may also occur, and the response to suspended sediment concentrations may also vary within a species depending on the prevailing seston characteristics (Navarro and Iglesias 1993).

The objectives of the current research were to determine lethal and/or sublethal effects of clay application to a simulated HAB of the nontoxic dinoflagellates *Heterocapsa triquetra* and *Prorocentrum micans* on juvenile northern quahogs or hard clams, *Mercenaria mercenaria*, in a laboratory flume where flow could be adjusted to mimic possible field conditions. Two contrasting flow conditions were chosen to simulate worst-case scenarios in the field. At low flow, a thick depositional sediment layer is formed after clay dispersal. Potential detrimental effects on growth and survival include (1) substrate effects, i.e., hard clams grow poorly in fine-grained sediments compared to coarse sand (Newell and Hidu 1982; Coen and Heck 1991); (2) bioturbation/near-bottom resuspension leading to feeding disturbance (Davis 1993); and (3) suffocation or burial of clams, e.g., if short siphons are unable to reach the sediment-water interface (Peterson 1985). In higher flows or under tidal and wind action, which may induce prolonged resuspension of clay particles, potential detrimental effects include (1) a food dilution effect where high inorganic particle concentrations result in lower clearance rates and higher pseudofeces production, leading to reduced phytoplankton ingestion (Bricelj and Malouf 1984; Cranford and Gordon 1992; Ward and MacDonald 1996; Grizzle et al. 2001); and (2) physical effects, such as clogging and/or damage of the gills (Morse et al. 1982) or valve closure and thus cessation of feeding. Parallel research tested the removal efficiency of a dinoflagellate species by phosphatic clay in the aforementioned flow conditions and characterized particle size distributions in terms of the availability of these particles to suspension-feeding bivalves (Archambault et al. 2003). Results of the present study will be used as a first step to identify and predict potential impacts on benthic communities from clay application to coastal HABs. Specifically, we examine the consequences of this mitigation strategy on a commercially important

infaunal bivalve suspension feeder. This study is also relevant to the assessment of impacts by other anthropogenic activities that cause increased resuspension of fine sediment, such as boating, dredging, and especially oil and gas drilling activities, which use water-based drilling muds dominantly composed of bentonite clay. Applications of clay to nontoxic dinoflagellates were conducted initially to investigate the effects of sediment and organic loading in the absence of confounding effects of algal toxicity. The synergistic effects of clay and sedimented toxins from toxigenic dinoflagellates will be examined in subsequent studies.

Materials and methods

Sequential paired experiments, each consisting of a control and treatment, were conducted in the two selected flow regimes to identify the potential lethal and sublethal effects of clay application to a simulated HAB on juvenile *Mercenaria mercenaria*. The growth experiments, which lasted 2 weeks each, were conducted in a small (1.5 m long) propeller-driven, recirculating seawater flume maintained at the Aquatron facility, Dalhousie University, Halifax, Nova Scotia (see Archambault et al. 2003 for further details). The flume (90 l at 0.2 m water depth) consists of a sand-filled 375 cm² working area (0.9 m downstream of the rectified flow). Constant temperature (20°C) was maintained by means of temperature-controlled seawater recirculated through the insulated double wall of the flume. Preliminary studies were conducted to determine the experimental flow conditions and optimal clay loading required to simulate the two selected treatment conditions.

Test organism

Juvenile hard clams, *M. mercenaria*, notata strain [5.0–11.0 mm in shell height (SH); mean shell length to height = 1.14] were used for all sedimentation and suspension trials. Hatchery-reared clams were obtained at approximately 3.0 mm shell length (SL) from the Fisheries and Aquaculture Shippagan Hatchery, NB (fall 2000), the Aquaculture Research Corporation (ARC), Dennis, MA (summer 2001), and Atlantic Marine Seed, PEI (fall 2001). They were acclimated to 20°C ambient seawater (salinity = 29–30 ppt) and grown to sizes selected for the experiment. They were fed a mixture of cultured algae added continuously via a peristaltic pump to achieve 60 cells μl^{-1} of *Isochrysis galbana* [T-Iso, 4.1 μm mean equivalent spherical diameter (ESD)] equivalent biovolume in a recirculating upweller system at the Institute for Marine Bioscience Aquaculture Research Station (ARS), Nova Scotia. Algal species used during acclimation included *I. galbana* (T-Iso), *Pavlova lutherii* (CCMP 1325), *Chaetoceros muelleri* (CCMP 1316), *Tetraselmis striata* (strain PLAT-P, Milford Lab NMFS), and *Thalassiosira weissflogii* (CCMP 1336, Actin).

Juvenile clams were used for experiments because early stages were expected to be the most vulnerable to the effects of bottom deposition and particle suspension. It was hypothesized that in the sedimentation trials, juveniles, which have shorter siphons and burrow to shallower depths than older clams, would have greater difficulty in maintaining contact with the overlying water column and therefore would be more likely to experience detrimental effects. *M. mercenaria* was chosen as the test species because it is a representative infaunal bivalve, commercially important on the Atlantic coast of North America, and is considered to be of intermediate sensitivity to turbid environments (Bricelj et al. 1984; Bricelj and Malouf 1984; Grizzle and Lutz 1989; Grizzle and Morin 1989; Turner and Miller 1991). In the suspension trials conducted in the present study, it was postulated that growth inhibition would result from the food dilution

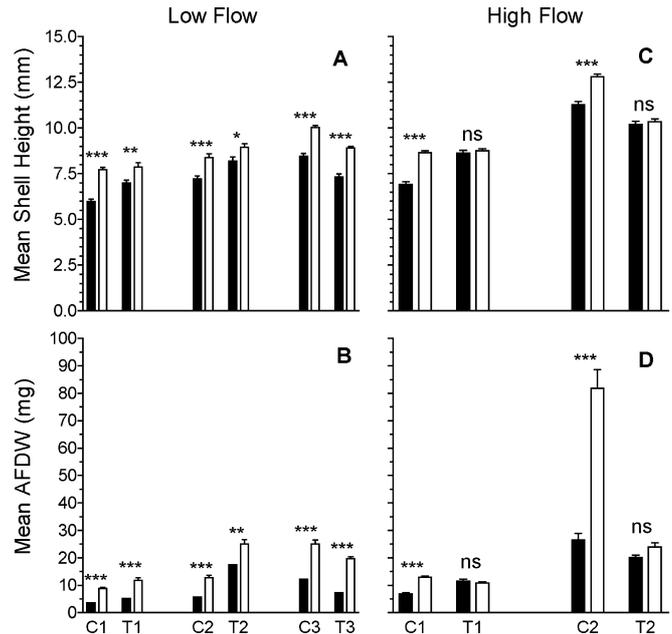


Fig. 1A–D Clam population mean initial and final metric for all trials: *filled bars* initial metric, *unfilled bars* final metric. *C* Controls, *T* clay treatments for each paired trial. **A** Low-flow sedimentation trials: mean shell height (SH). **B** Low-flow sedimentation trials: mean total tissue body weight in ash-free dry weight (AFDW). **C** High-flow suspension trials: mean SH. **D** High-flow suspension trials: mean AFDW. *Error bars* = standard error (SE)

effect, where high sediment concentrations cause a reduction in clearance rates and thus reduce algal ingestion rates (reviewed by Grizzle et al. 2001).

Clam stocking densities were 300 animals for all trials (i.e., 0.8 clams cm²), except for sedimentation trial 1 and suspension trial 2, which used 400 and 200 clams, respectively, to compensate for small differences in initial clam size (SH plotted in Fig. 1). The approximate percentage of sand coverage by the clams was calculated from the mean clam surface area (approximated from mean length \times mean height) multiplied by the total number of animals and then divided by the total surface area of the working box area. Thus, clam areal coverage ranged from 47.1% to 53.2% in sedimentation trials and from 49.4% to 76.4% in suspension trials and differed only by 4–18% between the control and treatment of any given trial.

For the third set of sedimentation trials and all suspension trials, one half of the experimental clams were initially treated with a solution of 5 ppm alizarin red (alizarin sodium monosulfonate) for 24 h in an aquarium containing 1.0 μm filtered and aerated seawater (FSW) maintained at 60 cells T-Iso μl^{-1} . This staining method was previously used by Hidu and Hank (1968) and Newell and Hidu (1982) on both juvenile and adult *M. mercenaria*. Preliminary studies with juvenile *M. mercenaria* in this study indicated that at this concentration and staining time survival, burrowing activity and subsequent growth were not affected. Staining was employed to calculate individual growth rates and to increase the statistical power of analysis of experimental growth data. Clam growth was measured at the end of the 2-week period from subsamples of 50 animals as the instantaneous growth coefficient, k (% day⁻¹), in order to reduce the effects of initial clam size and provide a relative measure of growth:

$$k = \frac{(\ln H_F - \ln H_0)}{\Delta t} \times 100 \quad (1)$$

where H_F is the final metric [SH, total body dry weight (DW), ash-free dry weight (AFDW)], H_0 is the initial metric, and Δt is the duration of the experiment (15 days). For the unstained trials, k was calculated on the basis of mean initial and final clam sizes of

the experimental population. For stained clams, individual growth (SH only) was back-calculated from the unstained band to determine the initial size compared to the measured final height. The calculated individual growth rate was plotted against the derived initial height to determine the effect of size on relative shell growth rate. Initial and final individual condition indices (CI) were also calculated:

$$CI = W/SL^3 \quad (2)$$

where W is the initial or final AFDW in mg and SL is the initial or final length of the clam in micrometers.

An estimate of clam population ingestion rate (IR in cells h⁻¹ clam⁻¹) was calculated for all the suspension trials from the total volume and change in concentration of food stock added to the flume over a timed interval.

Particles

The thecate dinoflagellates *Prorocentrum micans* (strain CCMP21) and *Heterocapsa triquetra* (strain Het) were used as proxy for toxic dinoflagellates, such as *Karenia brevis*. Nontoxic strains were used to eliminate the confounding effects of cell toxicity on clam growth. *P. micans* [26.6 µm ESD ± 5.0 (mean ± SD)] and *H. triquetra* (14.7 µm ± 2.2) were grown in batch cultures in 20 l carboys in modified L1 medium (50 µM NH₄Cl, Guillard and Hargraves 1993) at 16°C and a 14:10 h light/dark cycle. Cultures were harvested in mid- to late-exponential growth phase. The concentration of *P. micans* used in the sedimentation treatment trials was 1,000 cells ml⁻¹. Maximal *P. micans* bloom concentrations in the field can range from 1,100 cells ml⁻¹ (Pybus 1990) to as high as 8,100 cells ml⁻¹ (Sarno et al. 1993). Subsequent test tube trials by Mario Sengco (WHOI) indicated that *P. micans* did not readily flocculate with the phosphatic clay IMC-P, but that *H. triquetra* flocculated and was efficiently removed. Algal removal efficiency by clay has been shown to increase to an asymptotic level with increasing algal concentration (Sengco 2001; Sengco et al. 2001). The concentration of *H. triquetra* used in the suspension trials was 4,000 cells ml⁻¹, since at this concentration cells were efficiently removed (>80%) by IMC-P in static test tube trials (M.R. Sengco, WHOI, personal communication). Typical bloom concentrations reported for *H. triquetra* in the field vary from 500 cells ml⁻¹ (Kononen et al. 1999) to 2,000 cells ml⁻¹ (Kim et al. 1993) or 5,000 cells ml⁻¹ (Lindholm and Nummelin 1999), to maxima ranging from 12,850 to 97,600 cells ml⁻¹ (Bodeanu and Ruta 1998). Prior to use in experiments, *H. triquetra* cultures were maintained overnight at 20°C with 24 h light for acclimation to the flume experimental temperature (20°C). Stock concentrations were measured using a Coulter Multisizer (model II, 100 µm aperture tube), and subsequent food concentrations in the flume were maintained by frequent monitoring with the Multisizer and by adjustments of peristaltic pump speed.

The phosphatic clay IMC-P (1.8 µm ESD ± 0.7, 50% water content) was used for the removal of *P. micans* and *H. triquetra* in the sediment and suspension treatment trials, respectively. IMC-P (IMC Phosphates) is a montmorillonite phosphatic clay produced as a waste product from the phosphate mining industry (Bromwell 1982). The clay [180 g and 45 g wet weight (WW) for the sedimentation and suspension treatment trials, respectively] was

processed in a household blender at high speed for 2 min after suspension in 900 ml of 18–20°C seawater. The suspension was then sieved through a 63-µm stainless steel mesh to produce a fine particle slurry. The final clay suspension added to the flume yielded concentrations of 1.0 g DW l⁻¹ and 0.25 g DW l⁻¹ for the sedimentation and suspension treatment trials, respectively. The clay loading used in the sedimentation trials accommodated the limitations of the optical backscatter (OBS) turbidity sensor (maximum sensitivity = 1.0 g DW l⁻¹) and was selected to produce a relatively thick sediment layer of ~7 mm (150.6 g DW m⁻² ± 5.65). The concentration in the suspension trials was selected because it was the minimum level shown to remove *H. triquetra* efficiently (>80%) in static test tube trials [M.R. Sengco (WHOI), personal communication].

Experimental design

The two contrasting flow conditions established in the flume reflect end-member conditions of a continuum likely to be experienced in the field. The low-flow regime characterizes a low-energy environment that results in complete sedimentation of the clay and algae, and the high-flow regime characterizes a higher-energy environment, which allows continuous suspension of particulates. Specific trials and combinations of conditions and the variables measured in each trial are identified in Table 1. Clams of the same origin (cohort and hatchery) were used for each paired sequential trial that consisted of a control and a treatment.

Low-flow regime: Sedimentation

Two paired experimental trials, each consisting of a no-clay control and a clay treatment, were performed in the fall of 2000 with clams from New Brunswick, and the third paired experiment was completed in the summer of 2001 with clams from the Massachusetts hatchery. The clay treatments consisted of both IMC-P at 1.0 g DW l⁻¹ and an initial batch addition of *P. micans* at 1,000 cells ml⁻¹, as well as the food alga *I. galbana* (T-Iso) at 60 cells µl⁻¹ added once the sediment layer was formed (24 h) and continuously delivered with a peristaltic pump thereafter. Clams in the controls were exposed to the food alga only. *I. galbana* was offered as food, as it has been shown to support the highest growth rates of *M. mercenaria* compared to other unialgal diets (Wikfors et al. 1992). *P. micans* was not added to the controls because it was determined in short-term preliminary trials that in the presence of clay and at low flow the cells are rapidly removed and incorporated in the sediment layer, and thus would not serve as a suspended food source for the clams.

The clams were distributed uniformly on the sediment surface and allowed to burrow in the sand-filled working area of the flume before the start of any experimental trial. Clams that had not burrowed within 4 h were replaced with new clams.

In the clay treatments, the dinoflagellates were added first to the flume downstream of the working area and allowed to mix for 1 min at a moderate current speed of 5.7 cm s⁻¹ ± 1.3. Prior to the addition of clay and flow adjustment, an initial sample of the suspension was taken with a 10-ml pipette (10 cm off-bottom), and dinoflagellate cells were counted by microscope to

Table 1 Summary of experimental clay application trials to determine the effects on growth and survival of juvenile hard clams (*Mercenaria mercenaria*) (see text for explanation)

Condition		Dinoflagellate species and concentration (cells ml ⁻¹)	IMC-P concentration (g DW l ⁻¹)	Food concentration (cells ml ⁻¹)
Sedimentation:	Control	0	0	<i>Isochrysis galbana</i> (T-Iso) 60,000
	Low flow (1.8 cm s ⁻¹)	Treatment <i>Prorocentrum micans</i> 1,000	1.0	T-Iso 60,000
Suspension:	Control	<i>Heterocapsa triquetra</i> 4,000	0	<i>Thalassiosira weissflogii</i> (Actin) 2,360
	High flow (13.7 cm s ⁻¹)	Treatment <i>H. triquetra</i> 4,000	0.25	Actin 2,360

determine the accuracy of the stock dilution. Following the dinoflagellate addition, the IMC-P slurry was added downstream of the working area and mixed through the water column at the 5.7 cm s^{-1} flow for 1 min before decreasing the propeller speed to the experimental flow ($1.8 \text{ cm s}^{-1} \pm 0.02$). The flume water was exchanged 1 week into the experimental trial to reduce potential buildup of ammonia in the water column. The flume was gently drained until 5 cm of water remained above the clams and then gently refilled with 20°C FSW, without disturbing the sediment layer.

Ammonium (NH_4^+) was measured spectrophotometrically at 655 nm using the procedure outlined by Hach (Loveland, Colo.) and calculated from a standard (ammonium sulfate) curve of known NH_4^+ concentrations during the third sedimentation trials. Initial NH_4^+ levels were $2.6 \mu\text{M} \pm 0.8$ (mean \pm SE), and at 1 week they were $13.5 \mu\text{M} \pm 0.12$. Epifanio and Srna (1975) showed that both juvenile and adult hard clams had a high tolerance for ammonium and that their clearance rates were not significantly affected up to concentrations of $400 \mu\text{M}$. Therefore, this water-exchange step was not undertaken during the subsequent suspension trials.

High-flow regime: Suspension

Two paired sequential experimental trials were performed in the fall of 2001 with clams from the Prince Edward Island hatchery. The clay treatments were composed of *H. triquetra* at an initial concentration of $4,000 \text{ cells ml}^{-1}$, IMC-P at 0.25 g DW l^{-1} , and the food alga, *Thalassiosira weissflogii* (Actin, $12 \mu\text{m}$ mean ESD) at $2,360 \text{ cells ml}^{-1}$ (equivalent in volume to $60 \text{ cells } \mu\text{l}^{-1}$ T-Iso), drip-fed with a peristaltic pump. Controls were conducted under the same conditions as the clay treatments but consisted of only the food alga after an initial batch addition of *H. triquetra*. *T. weissflogii* was used instead of T-Iso in these trials because its size-distribution was distinguishable on the Multisizer in the presence of IMC-P.

Preparation for the experiment was identical to that described in the low-flow regime; however, the experimental flow was $13.6 \text{ cm s}^{-1} \pm 2.7$. The suspended particle concentration was maintained at 0.25 g DW l^{-1} by batch addition of the IMC-P slurry, based on a deficit calculated from OBS records.

Measured variables

Clam activity and behavior were recorded 3 and 14 days into the third sedimentation treatment trial (summer 2001) using a SV-DSPZOOM color underwater video camera (Shark Marine Technologies) and a DSR-V10 recorder with DVCAM format (Sony). Temperature was recorded at 5-min intervals using a submersible Onset Optic StowAway temperature probe for the duration of the experiments. Oxygen was also monitored using a dissolved oxygen meter (Orion Mode 840), and levels were maintained with gentle aeration in the return pipe of the flume.

All statistical analyses were conducted using NCSS 2000 software (<http://www.ncss.com>). The data were tested for normality and transformed with appropriate measures before performing Student's *t*-tests. Comparisons in the results are made within trials, such that treatments are compared to their corresponding controls; however, in the discussion all treatments and controls between trials are averaged to illustrate general trends in the data. Significance levels in the figures are as follows: ns not significant; * $P \leq 0.05$; ** $0.001 < P \leq 0.01$; *** $P \leq 0.001$.

Results

Low-flow regime: sedimentation effects

No clam mortality occurred in either controls or clay treatments for all three experimental pairings. In all

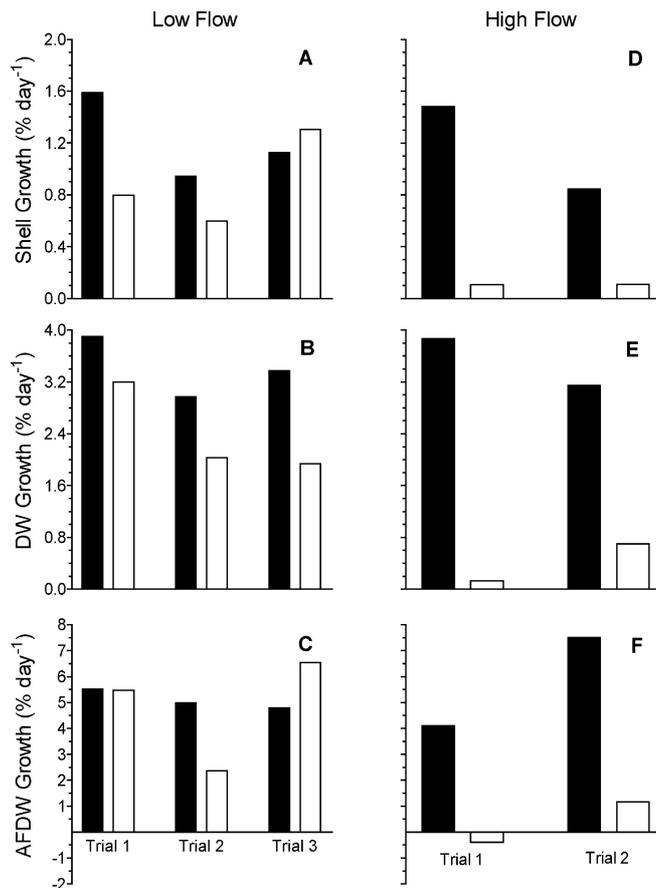


Fig. 2A–F Clam growth rate (instantaneous growth coefficient, k in % increase day⁻¹) for sequential trials: filled bars control, unfilled bars clay treatments. **A–C** Low-flow sedimentation trials, growth in SH, total body dry weight (DW), and AFDW, respectively. **D–F** High-flow suspension trials, growth in SH, DW, and AFDW, respectively

cases, both shell height (SH) and soft tissue weight (AFDW) in the sedimentation trials showed significant growth of clams over the 2 weeks (Fig. 1A, B). Relative growth in SH measured as the instantaneous growth coefficient (k) showed a decrease (49.8% and 36.6%) in the clay treatment relative to the respective controls in two out of three trials (Fig. 2A), whereas growth in total body dry weight (DW) was consistently reduced in clay treatments relative to controls (by 18.1–42.5%) (Fig. 2B). The one exception to inhibitory clay effects on shell growth occurred in trial 3, where the clay treatment shows a small (15.8%) increase in SH over the control (Fig. 2A). Soft tissue growth, more closely reflected by the change in AFDW, indicated a less consistent response to the application of clay (Fig. 2C), but as with SH, an increase in growth rate occurred with clay in trial 3. Overall, variability in growth responses to clay depending on which metric is considered suggests that differential allocation between shell and soft tissue growth occurred among trials.

A more detailed analysis of trial 3 was possible since individual growth rates from alizarin-stained clams were

Table 2 Results of Student's *t*-test comparing the mean individual clam growth rate for the third low-flow sedimentation experimental pairing and all high-flow suspension trials

Condition		Mean individual shell growth rate ^a ($\mu\text{m day}^{-1} \pm \text{SE}$)	Significance of <i>t</i> -test
Trial 3	Control	109.6 \pm 12.4	n.s.
	Treatment	102.5 \pm 4.3	
Trial 1	Control	106.8 \pm 3.3	***
	Treatment	0.2 \pm 0.1	
Trial 2	Control	94.7 \pm 1.3	***
	Treatment	6.3 \pm 0.7	

* $P \leq 0.05$; ** $0.001 < P \leq 0.01$; *** $P \leq 0.001$

^aBased on alizarin-stained clams ($n = 50$)

measured. Results indicated that individual shell growth rates were not significantly different ($P = 0.54$) between the low-flow control and clay treatment in trial 3 (Table 2). The observed difference in trial 3 may have resulted from a size effect as clams in the third control were slightly larger in SH than the clams in the paired clay treatment, and there was a significant inverse relationship between growth rate and initial size in the control ($P < 0.001$), whereas the effect was only marginally significant in the clay treatment ($P = 0.05$) (Fig. 3A).

The condition index (CI) showed a significant increase over 2 weeks in the control and clay application treatment in trial 1 and in the clay treatment in trial 3 (Fig. 4). However, the control in trial 3 showed no significant change in CI over the 2-week period. In the second paired trial, the CI showed no significant change for either the control or the clay treatment and resulted in consistently lower mean growth rates in SH and body weight measurements compared to the clay treatment (Fig. 4). Thus, the sedimented clay treatment had no detrimental effect on the initial condition index.

Initial video observations of clams in the low-flow clay treatment trials showed that they quickly (≤ 24 h) regained siphon contact with the sediment-water interface and resumed a normal feeding position (Fig. 5A). The siphons were extended sufficiently (> 7 mm) to reach through the clay layer. Over the course of 2 weeks, the clay layer was extensively reworked and large patches of exposed sand could be seen at the surface (Fig. 5B). Some clams were partly exposed with their siphons lying parallel to the clay surface (Fig. 5B, C). This is an anomalous behavior as in nature clam siphons typically extend vertically into the water column. No significant oxygen depletion occurred near-bottom in the sediment treatment trials.

High-flow regime: effects of suspended clay

No clam mortality occurred in either controls or treatments of both experimental pairings. Results obtained between the suspension trials and among parameters

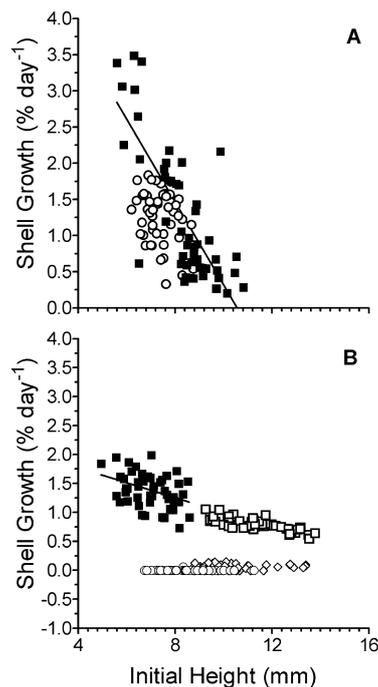


Fig. 3A, B Individual growth rates as the instantaneous growth coefficient (k in % change day^{-1}) against initial height (in mm) calculated from growth bands of alizarin-stained clams. **A** Low-flow sedimentation trial 3: ■ control, $r^2 = 0.61$ ($P < 0.001$, regression line shown); ○ clay treatment, $r^2 = 0.08$ ($P = 0.05$). **B** High-flow suspension trials: ■ control from trial 1, $r^2 = 0.16$ ($P = 0.004$); □ control from trial 2, $r^2 = 0.59$ ($P < 0.001$); ○ and ◇ clay treatments from trials 1 and 2, $P = 0.94$ and 0.73 , respectively

within each trial were more consistent than in the previously described sedimented trials. Clams in the suspension treatments showed no significant increase in SH and AFDW for both trials 1 and 2, in contrast to the highly significant growth shown in the controls (Fig. 1C, D). The clay suspension treatments consistently showed a pronounced decrease in growth rate (k) relative to their respective controls (Fig. 2D–F). The mean reduction in clam growth rate for both clay treatment trials was $90.0\% \pm 2.8\%$ (mean \pm SE), $87.1\% \pm 9.5\%$, and $96.9\% \pm 12.4\%$ in SH, DW, and AFDW, respectively (Fig. 2D–F). In the first paired trial the clams suffered negative soft tissue growth, i.e., a 109% reduction in AFDW relative to its control (Fig. 2F). The second trial showed an 84.5% decrease in growth expressed in terms of AFDW relative to the control. The loss in soft tissue weight in trial 1 is reflected in a significant decrease in CI in the clay treatment over the experimental period not detected in the control (Fig. 6). In contrast, the mean CI of treatment clams in the second clay trial did not change significantly over the 2 weeks, whereas it increased significantly in the control (Fig. 6).

Individual growth rates, calculated for alizarin-stained clams, were significantly different between the clay treatment and control for both trials (Table 2), indicating a pronounced and consistent negative effect of suspended clay on clam growth. Analysis of individual

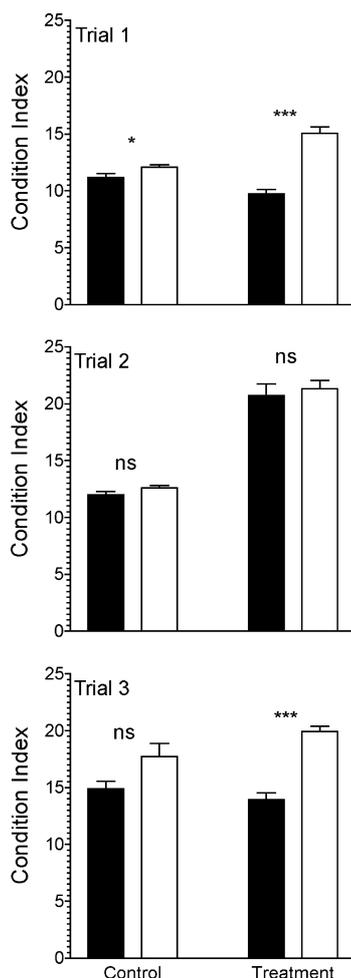


Fig. 4 Condition Index (CI) as AFDW/l³ in mg $\mu\text{m}^{-3} \pm \text{SE}$ for the initial (filled bars) and final (unfilled bars) clams in controls and clay treatments for the three paired low-flow sedimentation trials

growth rates indicated that the initial size significantly affected growth in both controls but did not affect growth during the clay treatments (Fig. 3B). The presence of larger clams in the second control may account for the lower shell growth rate observed in this trial (Fig. 2D), but the relative effect of the clay treatment was still maintained.

The presence of clay greatly affected the ingestion rate of *T. weissflogii* by the clams in the suspension trials. The reduction in *T. weissflogii* concentration in the flume over time in the two control trials was 3.94% and 4.03% h⁻¹, respectively, and was undetectable in the two treatment trials. The measured ingestion rates during the 2-week experiments were $2.15 \times 10^5 \pm 3.09 \times 10^4$ and $2.24 \times 10^5 \pm 2.50 \times 10^4$ cells h⁻¹ clam⁻¹ for the two controls, and < 1.0 cells h⁻¹ clam⁻¹ for the two clay treatments. Feeding and growth interference by suspended IMC-P was likely maximized in the present study by the aggregation of individual clay particles, as the majority of particles were of a size ($\geq 3 \mu\text{m}$) that could be retained by the clam gill with 100% efficiency (Archambault et al. 2003).

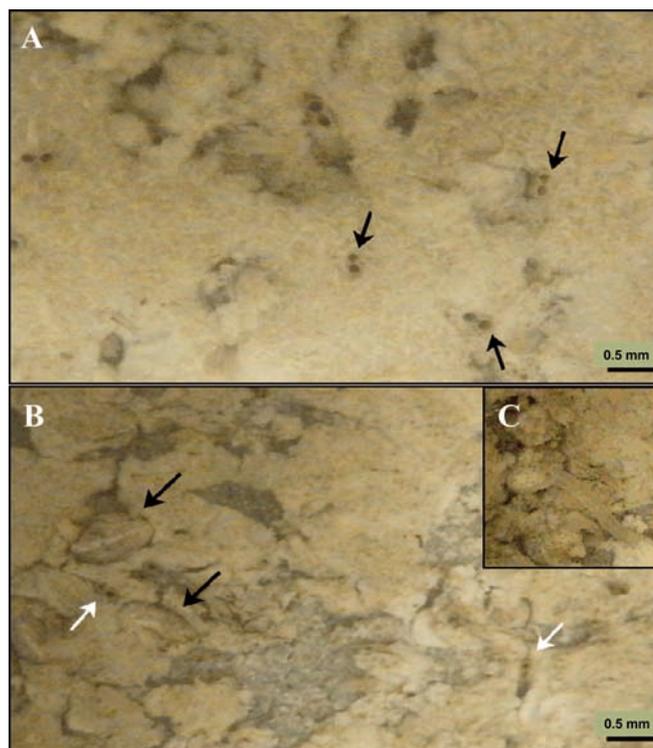


Fig. 5A–C Bottom video images in low-flow sedimentation treatment trials. **A** Three days into the treatment: a relatively undisturbed clay layer ~ 7 mm thick has formed over the sand bed. Arrows indicate the position of representative clam siphons. **B** Evidence of clam activity 14 days into the treatment. Clams have extensively reworked the sediment, exposing large areas of the underlying sand substrate. Black arrows show the position of clams that are partially exposed to the overlying water column, and white arrows indicate siphons from burrowed clams flush with the clay surface. **C** Individual clam 14 days into the treatment. Notice the long siphon extension parallel to the sediment surface rather than vertically into the water column. The scale is identical to frame **B**

Discussion

The application of clays to remove HABs from the water column was shown to be successful under certain conditions for some algal species and concentrations in both laboratory (Sengco 2001; Sengco et al. 2001; Archambault et al. 2003) and field settings (Shirota 1989; Na et al. 1996; Bae et al. 1998). The trade-off in effectively removing HABs with clays revolves around possible negative effects to the marine environment, such as the increased flux of particles to the bottom and the subsequent impact on the benthos. It is possible that some benthic communities, especially those dominated by surface deposit feeders, would benefit from the increased organic-rich particle flux to the bottom (Rhoads and Young 1970) if they were not directly affected by sedimented toxins. However, the mechanisms whereby this mitigation strategy causes cell lysis (release of toxin in the water column) or enhances toxic phytoplankton sedimentation to the bottom and the subsequent trophic transfer of those toxins have not

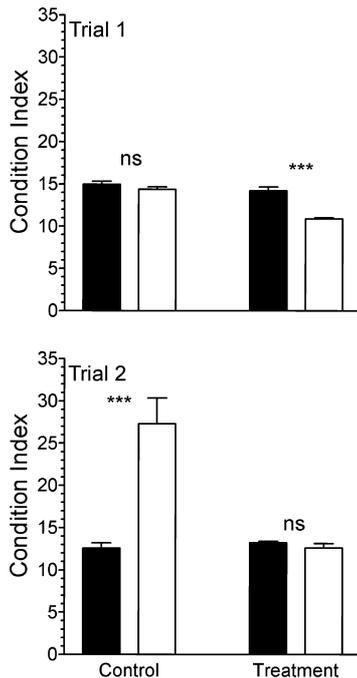


Fig. 6 Condition Index (CI) as AFDW/l³ in mg μm⁻³ ± SE for the initial (filled bars) and final (unfilled bars) clams in controls and clay treatments for the two paired high-flow suspension trials

been addressed. The acute (4–12 days) and chronic (7–28 days) toxicities of IMC-P clay and *Karenia brevis* cells have previously been tested on four estuarine test species (benthic infaunal and epibenthic macroinvertebrates and larval fish) (Lewis et al. 2003). These workers demonstrated that aggregates of clay and *K. brevis* cells were no more toxic than *K. brevis* cells alone. It is not clear, however, whether the results of their small-scale laboratory study are representative of those occurring under natural conditions. Additional studies are needed and are underway in flumes and field mesocosms to investigate the synergistic effects of clay and toxic algae.

In contrast to deposit feeders, suspension feeders, such as commercially important bivalve mollusks, are more likely to be negatively influenced by clay application. Although bivalves have a wide range of tolerances to high concentrations of inorganic particulates (Morse et al. 1982; Bricelj et al. 1984; Bricelj and Malouf 1984), some species are undoubtedly very sensitive to clay loadings, namely the sea scallop, *Placopecten magellanicus* (Cranford and Gordon 1992). This must be taken into consideration when applying clay to an area adjacent to bivalve populations or that is undergoing shellfish restoration, as occurs for the bay scallop, *Argopecten irradians*, on the west coast of Florida (Leverone and Blake 2002). In such situations, management decisions on whether to treat a toxic bloom with clay may require estimates of natural mortality of stocks due to the red tide alone, balanced against the impacts of clay treatment. There is, however, little information on the impacts of *K. brevis* blooms on the

benthos. Such data are clearly needed for an effective evaluation of the relative costs and benefits of HAB mitigation strategies such as clay dispersal. Recent laboratory work shows that *A. irradians* larvae and juveniles are strongly affected by *K. brevis* at concentrations as low as 500 cells ml⁻¹, showing delayed metamorphosis and feeding inhibition, respectively (Leverone and Blake 2002).

It was evident in the present study that the high-flow suspension treatment showed greater and more consistent negative growth effects of IMC-P clay on clams than did the low-flow regime. Although the sedimentation treatments showed on average a decrease in shell growth relative to controls (not shown), the magnitude of the decrease (23.5% ± 20.02% for the three trials) was substantially less than the difference in shell growth between controls and clay treatments of the suspension trials (90% ± 2.85% for both trials). Growth rates in shell height and total body dry weight were overall lower in the clay treatment during the suspension trials relative to the controls, in contrast to the sedimentation trials where differences between averaged controls and treatments were not apparent. Individual measurements of growth rate indicated that the slight increase in SH during the third sedimentation treatment trial in comparison to the control was not statistically significantly compared to the consistent and highly significant negative effect of the presence of clay in the suspension trials (Table 2).

Soft tissue growth (AFDW), showed highly variable results among the three sedimentation trials. However, the overall trend when all trials are averaged (not shown) indicates that relative to the controls, clams in the treatment were not affected by the clay layer, in contrast to the marked differences found in the suspension treatment. The variability among sedimentation trials could have resulted from differential resource allocation to shell versus soft tissues between experiments, which may be a function of initial physiological condition. Studies of resource allocation in freshwater clams, *Anodonta piscinalis*, and mussels, *Mytilus galloprovincialis*, suggested priority rules for energy allocation to shell growth, somatic mass, and reproductive output depending on animal origin, environmental conditions, and developmental stage (Raubenheimer and Cook 1990; Jokela and Mutikainen 1995). The hatchery origin differed for the third sedimentation treatment pairing, and these clams had slightly higher initial condition indices compared to both the control and treatment in trial 1 and the control in trial 2. Clams in the trial 2 treatment had an exceptionally high CI that did not increase over the course of the experiment, which accounts for the consistent decrease in growth rate for all three parameters.

Due to limitations of conducting experiments in a single flume, comparisons could be made only between sequential paired trials, which introduces some variability in the initial conditions and size of experimental clams. Growth in bivalves is size dependent: smaller

clams have higher growth rates per unit size than larger individuals, and the individual growth rates in the sedimentation control trial 3 (Fig. 3A) and for both suspension controls (Fig. 3B) were shown to be dependent on initial clam size. The initial size may thus be directly responsible for the differences in growth among treatments in sedimentation trials and may have overshadowed any possible small negative growth effect of the clay application treatment. Experiments running both control and treatment simultaneously in identical recirculating raceway systems are currently in progress.

It has been hypothesized that a thick sediment layer could inhibit clam growth by a variety of mechanisms. Growth of *Mercenaria mercenaria* is inhibited in fine-grained substrates relative to sand (reviewed by Grizzle et al. 2001). Davis (1993) suggested that extensive bioturbation increases near-bottom resuspension and thus affects feeding of sensitive suspension feeders. Opportunistic surface deposit feeders may benefit from the presence of an organic-rich deposited layer, and their proliferation may lead to increased bioturbation, such that trophic group amensalism may occur (Rhoads and Young 1970). Community dynamics would be an important aspect to consider in a field application trial. Our video observations indicated that over time, clams were partly exposed and caused extensive bioturbation of the clay layer. This is the first documentation that juvenile *M. mercenaria* are capable of this type of bioturbation. The energetic cost of this behavior is not known, but it could result in reduced feeding time and increased metabolic demand over longer-term exposure. It was also hypothesized that juvenile clams with relatively short siphons might be unable to reach the sediment-water interface to feed through a thick deposited clay layer and thus would starve or suffer oxygen depletion. However, in this study the clams readily regained contact with the overlying water column after a clay bottom layer was formed. Effects on newly settled asiphonate clams remain to be determined.

It was also observed that some clams extended their siphons horizontally across the sediment surface, rather than remaining flush at the surface or extending vertically into the water column. This response is unusual, and in the field could potentially subject clams to high rates of siphon-nipping or cropping predation by fish (Irlandi and Mehlich 1996). Coen and Heck (1991) suggested that if siphon-nipping pressures are high, as occurs in southern latitudes, growth of *M. mercenaria* is reduced due to the energetic cost of replacing lost siphonal tissue. This may result in a delay in attaining size refuge from predators, and thus higher mortality rates on smaller size classes (Bricelj 1993; Irlandi and Mehlich 1996).

This research shows that the presence of a sedimented clay layer did not affect clam survival or cause significant growth disturbance. Few studies have focused on the effect of burial from various sediment

types on benthic macrofauna, and as a result less is known about these impacts compared to those by resuspension. Peterson (1985) found that deposition of a 10-cm silt-clay layer substantially increased mortality of the suspension-feeding bivalves *Protothaca staminea* and *Chione undatella*. Previous studies suggest that negative effects from deposited sediments on macrofauna are a function of depth, burial time, and sediments whose particle size-distribution differs from that of the species' native sediment type (Maurer et al. 1980), and also result from changes in sediment geochemistry, such as decreased dissolved oxygen levels and increases in ammonia and sulfide (Maurer et al. 1986). It is important to note that the loading rate and thickness of the sediment layer formed under different conditions in a field application of IMC-P have not been determined. In the 1996 application of clay to a *Cochlodinium polykrikoides* bloom in Korea, surface clay loading was estimated at 400 g m⁻² (Na et al. 1996), but no measurements or calculations were provided of the total suspended particulate matter concentration and thickness of the bottom clay loading, nor was it clear how many repeat applications were made to a given area. In a depositional flow regime, total bottom clay loading is likely to be the determining factor for growth inhibition, possibly due to behavioral responses or indirect effects, e.g., colonization of opportunistic deposit feeders, rather than direct clay effects. Deposited material is subject to tidal- and/or wind-driven resuspension, as well as resuspension due to bioturbation. Concurrent research has demonstrated that resuspension of a 2-mm-thick IMC-P clay layer allowed to consolidate for 3, 9, and 24 h required higher critical shear velocities with increasing time [Beaulieu SE, Sengco MR, Anderson DM (WHOI), in preparation]. This could potentially impact benthic communities as multiple applications of clay can result in a thicker sediment layer that is not removed from the immediate area by relatively low-current speeds.

With respect to suspended clay effects, bivalves exposed to high concentrations of particulate inorganic matter (PIM) may suffer direct physical effects such as clogging of gills (Rhoads 1974; Ward and MacDonald 1996) and permanent damage by disruption of ciliary junctions between gill filaments in the case of filibranch bivalves such as scallops at >100 mg l⁻¹ of attapulgite clay (Morse et al. 1982), leading to reduced feeding and possibly to mortalities. Results of the present study indicated that clams in the suspended clay treatments were not feeding, in comparison to the normal consumption rates in the controls. Removal of *Thalassiosira weissflogii* in high-flow controls was attributed to clam feeding, as additions of algae were not required during the suspended clay trials, and algal losses within the flume system are unlikely at the relatively high flows used. Weight-specific clearance rates of clams in the controls (10.1 l h⁻¹ g tissue AFDW⁻¹) were also comparable to literature values for *M. mercenaria* at the same temperature (7.3 l h⁻¹ g tissue AFDW⁻¹) (Bricelj

and Malouf 1984). Although the flume experiments were not specifically designed to measure feeding rates, they provide a measure of relative feeding activity between controls and treatments.

Some bivalves, such as mussels, *Mytilus edulis*, show enhanced growth rates in the presence of relatively low (5–25 mg l⁻¹) silt concentrations (Kjørboe et al. 1981). This effect was not observed for juvenile *Mercenaria mercenaria* (Bricelj and Malouf 1984). Bricelj et al. (1984) found that the final mean tissue weight of juvenile hard clams (9 mm) subjected to sediment (silt) concentrations from 0 to 44 mg DW l⁻¹ for 3 weeks was not significantly affected up to 25 mg DW l⁻¹, but was significantly lower than controls at the highest concentration (16.4% reduction). A threshold of 23 mg DW l⁻¹ seston concentration has been suggested for reduced shell growth of juvenile hard clams (Murphy 1985). During a simulated storm event lasting 1.5 days, during which total particulate matter was 190 mg DW l⁻¹, shell growth rate of hard clams (20 mm) was reduced by 31% compared to a non-storm acclimation period (Turner and Miller 1991). When exposed to bentonite clay for ~1 month, adults sea scallops (*P. magellanicus*) showed 7% and 60% mortality rates at 2 mg l⁻¹ and 10 mg l⁻¹, respectively, and exhibited no significant shell growth (Cranford and Gordon 1992). Animals exposed to 10 mg l⁻¹ suffered significant soft tissue weight loss, but those exposed to 2 mg l⁻¹ showed no significant difference compared to a non-clay control. A flow regime that encourages prolonged suspension of particles in the benthic boundary layer would likely result in decreased clearance rates and increased pseudofeces production in bivalves. Ultimately, if the seston concentration is high, as might occur in repeated field applications of clay or due to in situ resuspension of a single application, bivalves may experience reduced feeding, thus resulting in decreased growth rates, as shown in the present study in which no recovery in feeding or growth occurred over 2 weeks of sustained clay loading. The significance of this effect will depend on the duration of exposure to high suspended clay concentrations and on the spatial extent of clay application. In their natural habitat, juvenile *M. mercenaria* (< 20 mm SL) are subject to high size-specific predation pressure (Bricelj 1993; Kraeuter 2001). Therefore, although clams suffered no mortalities in suspended clay treatments, reduced growth rates will cause a delay in attaining refuge from predators and could thus result in higher mortalities in the field.

In the present study exposure to clay occurred over a prolonged, 2-week period, considered an extreme or worst-case condition for the field. Hard clams inhabit shallow estuaries affected by tidal- and/or wind-driven sediment resuspension and experience pronounced temporal and spatial changes in PIM concentrations (Grizzle and Morin 1989). Ambient PIM levels exceeding 100 mg l⁻¹ have been documented in Great South Bay, N.Y., waters (Bricelj et al. 1984), and in the Chesapeake Bay these levels have ranged from 20 to 200 mg l⁻¹ over a tidal cycle (Schubel 1971). Mean

current speeds 2–8 cm off-bottom at eight sites in a New Jersey coastal lagoon ranged from 2.4 to 12.5 cm s⁻¹ (Grizzle and Lutz 1989), and even lower-flow velocities are expected at the sediment-water interface within the clam-feeding zone, which extends only a few cm above the seabed (Grizzle et al. 2001). Thus, the current velocities used in this flume study are realistic of those experienced by *M. mercenaria* in its natural habitat. Although hard clams grow faster in sandy substrate, they occur in a wide spectrum of sediment types, including sediments characterized by a relatively high silt-clay content [e.g., up to 20–47% in a North Carolina estuarine system (Peterson and Beal 1989) and up to 98% silt-clay and 57% clay in Great Sound, N.J. (Grizzle and Lutz 1989)]. Therefore the sedimentation scenario created in the present study is representative of ambient conditions that may be experienced by hard clams in relatively protected bays.

Clay concentration and duration of exposure are the key factors that will determine species-specific physiological and ecological effects on benthic organisms. A change in particle size distribution also has consequences for bivalve feeding, as clams, for example, retain particles $\geq 3 \mu\text{m}$ on the gills with 100% efficiency and formation of large aggregates could lead to increased pseudofeces production. The majority of particles in both flow regimes described in this study ranged from 3 to 15 μm equivalent circular diameter, indicating that small individual clay particles (1.8 μm ESD) aggregated and could become available for retention by bivalve gills (Archambault et al. 2003). In this context, White (1997) showed that adult sea scallops, *P. magellanicus*, consistently removed water-based drilling fluid waste particles that were flocculated at greater rates than unflocculated particles.

Additional laboratory and field research on benthic organisms belonging to different functional groups (passive and active suspension feeders, surface deposit feeders, etc.) and more sensitive bivalves than *M. mercenaria*, e.g., bay scallops, is necessary to understand how benthic communities will cope and recover from clay application. The present study did not use toxic dinoflagellates in order to minimize the confounding effects of cell toxicity on growth. In field applications, toxic bloom species such as *Karenia brevis* are likely to be the first targets of clay mitigation due to their extreme impacts on economies and the environment. However, harmful species that do not produce known toxins, such as *Cochlodinium polykrikoides* and *Prorocentrum minimum*, also cause detrimental effects and may potentially be treated with clays. Feeding inhibition of bivalves by harmful algae is typically dependent on cell density (Bricelj et al. 2001), and therefore even their partial removal from suspension by clay would have beneficial effects. However, the fate and availability of water- and lipid-soluble sedimented toxins in terms of their release, bioturbation, and trophic transfer remain an important consideration in field applications of clay to toxic dinoflagellate

blooms that is not addressed by the present study and needs future research. Intracellular toxins (e.g., brevetoxins from *K. brevis*) may be released following sedimentation and adsorb onto organic and inorganic particles, thus affecting the persistence of toxins in local sediments. Repeated clay applications during a bloom may also lead to organic enrichment of sediments. Lastly, economic impact assessments should be undertaken to weigh the costs/benefits of this mitigation strategy in the presence of valuable mariculture operations, wild fisheries, and marine mammals or endangered species.

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