

# Removal efficiency of the dinoflagellate *Heterocapsa triquetra* by phosphatic clay, and implications for the mitigation of harmful algal blooms

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**ABSTRACT:** An increased incidence of harmful algal blooms (HABs) has been reported in nearshore coastal waters, including economically important aquaculture sites worldwide. This has generated interest in using ecologically inert clays as a means of mitigating HABs at relatively shallow enclosed mariculture sites. This proposed mitigation measure assumes that flocculation and accelerated sedimentation of large algal cells (dinoflagellates) occurs with the application of clays. The objective of the current research was to characterize suspended clay-algal interactions and *in situ* particle size in a laboratory application of the phosphatic clay IMC-P (1.8  $\mu\text{m}$  mean equivalent spherical diameter, ESD) to a simulated *Heterocapsa triquetra* (14.7  $\mu\text{m}$  mean ESD) bloom. Flow speed was adjusted to provide 2 contrasting regimes (mimicking possible field conditions). This research was conducted in a recirculating flume and used a novel *in situ* device, the small volume particle microsampler (SVPM), for particle size characterization. Results indicated that 100 % of the thecate dinoflagellate *H. triquetra* was removed in 48 h by IMC-P in a low-flow ( $<2 \text{ cm s}^{-1}$ ) regime, but remained in the water column in the high-flow ( $>13 \text{ cm s}^{-1}$ ) regime. The mechanism for algal removal is equivocal since SVPM filter photographs indicated that clay aggregated to form particles  $>3 \mu\text{m}$ , but did not aggregate with algal cells under the experimental conditions tested. At this particle size, the clay aggregates become available for retention with 100 % efficiency on the gills of suspension-feeding bivalves, which could be seriously affected by the increased particle flux to the bottom. The mechanism of cell removal by clays has not been identified; however, it may occur via direct effects of clay on cell surface properties and swimming ability. Further investigations on the removal mechanism are required to predict the fate of cells in a field application of clay.

**KEY WORDS:** Harmful algal blooms · Clay · Particle size distribution · Small volume particle microsampler · *Heterocapsa triquetra* · Mitigation · Aggregation

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## INTRODUCTION

Harmful algal blooms (HABs) have increased in their global geographic spread in recent decades (Hallegraeff 1993). In North America, this has prompted research on control and mitigation methods in an effort to reduce the ecological and economic impacts on economically important fisheries and nearshore mariculture operations (Anderson 1997). One of these methods involves the use of ecologically inert clays to control prolific HABs, such as neurotoxic blooms of *Karenia*

*brevis* (formerly *Gymnodinium breve*) in Florida, USA. Clays are relatively inexpensive, can be supplied in large quantities as industrial waste products from mining and drilling operations (Sengco et al. 2001), and are presumed to have little or no direct toxic effects on aquatic organisms (Howell & Shelton 1970, Portman 1970). Clay application 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 t of dry loess clay were dispersed over a

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bloom-affected area (260 km<sup>2</sup>) at an estimated surface aerial loading rate of 400 g m<sup>-2</sup>. Removal rates of *C. polykrikoides* from the upper 2 m of the water column were estimated at 90 to 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. This method was originally used by Avnimelech & Menzel (1984) in low salinity impoundments, and showed that turbid ponds were clarified by the coflocculation of algae and clay.

The reasoning behind the proposed mitigation effort invokes the flocculation and accelerated sedimentation of large algal cells (dinoflagellates) via the application of clays (Avnimelech et al. 1982, Degens & Ittekkot 1984). The process of flocculation consists of 2 discrete steps: transport and attachment (Thomas et al. 1999). The transport step, leading to the collision of 2 particles, is achieved by virtue of local variations in fluid particle velocities. Particle theory identifies several mechanisms by which collisions increase the likelihood that particles will come into contact with each other and effectively aggregate. The 3 main mechanisms are Brownian diffusion, fluid shear (laminar or turbulent) and differential sedimentation (Thomas et al. 1999, Sengco 2001). The dominant collision mechanism changes with particle size: Brownian diffusion is important when particles are <1 µm, while fluid motion begins to dominate for larger particles, depending on the shear rate (McCave 1984a). Jackson & Lochmann (1993) suggested that in a system with flagellated organisms, like many that produce HABs, particle collisions are also generated by swimming. Sengco (2001) calculated that total collision frequency and algal removal were significantly greater in the presence of flagellates than non-motile diatoms and small cocci. Attachment is contingent upon a number of short-range forces largely pertaining to the nature of the surfaces themselves (Thomas et al. 1999).

Transparent exopolymer particles (TEP) have been identified recently and postulated to be essential to the flocculation of diatom blooms and the formation of large settling aggregates (Alldredge et al. 1993, Passow et al. 1994). However, TEP is rarely produced in large quantities by dinoflagellates (common constituents of HABs) and is not considered to be a mechanism for aggregation in such blooms (Alldredge et al. 1993, 1998; Passow & Alldredge 1994). Detailed studies on the possible mechanisms of clay-cell flocculation have been pursued by Avnimelech et al. (1982) and Søballe & Threlkeld (1988). The resulting large aggregated particles have greater settling velocities than smaller unflocculated particles (McCave 1984b). Floccs can also capture more cells as they sink and thus, sweep and entrain particles through the water column.

Clay application is presently being tested experimentally as a potential method for controlling HABs in the United States (ECOHAB research, Woods Hole Oceanographic Institution, Sengco et al. 2001). However, this research on clay-algal flocculation and removal efficiency has been conducted in small scale (10 ml test tubes) static systems, i.e. no flow, showing that clays have specific removal efficiencies depending on the phytoplankton species and cell concentration (Yu et al. 1994a,b, 1995, 1999, Kim 1998, Choi et al. 1999, Sengco et al. 2001). For example, Sengco et al. (2001) determined the removal efficiency of many clay types, including montmorillonites, bentonites and kaolinites, applied to *Karenia brevis* and the brown tide picoplankton *Aureococcus anophagefferens* in test tubes.

The objectives of the current research were: (1) to characterize suspended clay-algal interactions and *in situ* particle size in a laboratory application of clay to a simulated HAB; and (2) to determine the effects of flow on particle (clay and algae) removal. Fluid shear dominates particle contact rates when particles are >1 µm (McCave 1984a) and tears apart aggregates when it is too high at the particle surface (Eisma 1986, Dyer & Manning 1999, Manning & Dyer 1999). Therefore, it was important to include shear to understand if and how aggregates are formed or destroyed, and how this will affect the particle size distribution and sinking rates. Examination of the effectiveness of dinoflagellate cell removal by clay in flowing water has recently been conducted in a large-scale flume (S. E. Beaulieu, M. R. Sengco & D. M. Anderson unpubl. data), but aggregate size characterization was not attempted.

The present research was conducted in a recirculating flume that allowed for flow adjustment and used a novel *in situ* device, the small volume particle micro-sampler (SVPM, Archambault et al. 2001), for particle size characterization. Although aggregation has been observed during laboratory applications of clay to HABs and is theorized as the removal mechanism, past efforts have not quantified the *in situ* particle size spectrum. This information provided by the SVPM was also useful in interpreting results of concurrent research on the effects of this mitigation strategy on the growth of the hard clam *Mercenaria mercenaria*, and allow general predictions about the availability of clay particles to suspension-feeding bivalves exposed to this treatment in the field (Archambault 2002).

## MATERIALS AND METHODS

Sequential paired experiments were conducted to determine the efficiency of removal of a HAB species subjected to an application of clay dispersion at 2 flow

regimes. Preliminary trials were conducted to determine the experimental flow conditions, optimal clay and algal concentrations, and various instrument calibrations. The 2 selected flow regimes represent contrasting conditions in the field where low flow characterizes a low-energy environment that encourages complete sedimentation and high flow characterizes a high-energy environment that maintains particle suspension.

**Experimental setup.** Experiments were conducted using a small recirculating seawater flume (1.5 m long  $\times$  0.2 m wide  $\times$  0.25 m high) in the Aquatron facility at Dalhousie University, Halifax, Nova Scotia (Pilditch et al. 1998) (Fig. 1). Ambient seawater at 32 psu was supplied from the Northwest Arm of the harbor by the Aquatron, through a series of pumps and filters, to obtain 0.22  $\mu\text{m}$  filtered seawater (FSW). At a 0.20 m water depth, the total capacity of the flume was 90 l. The working area, located 0.9 m downstream, was 0.25 m long  $\times$  0.15 m wide  $\times$  0.10 m deep and was filled with well sorted clean beach sand. An insulated recirculating water jacket controlled by a heating-cooling system pumped temperature-regulated seawater around the perimeter of the flume. An insulating cover limited heat loss and ambient light to a 12 cm diameter port-hole on the side of the flume and 10 cm square on top of the cover. Water entering the flume through a 10 cm (in diameter) pipe, was rectified by 2 polycarbonate core flow straighteners (0.64 cm in diameter, 1.27 cm in width) (Plascore) located at 5 and 25 cm downstream of the entrance. An airline was inserted in the return flow pipe for gentle water aeration. At 1.35 m downstream, water exits the flume and enters the descending arm of the return pipe, where a propeller drives the flow. Flow speeds can range from 0 to 30  $\text{cm s}^{-1}$ , with sufficient shear stress to resuspend sand size sediments at higher flows (Pilditch et al. 1998). The propeller motor is regulated by a rheostat from which values of 500, 1000 and 2000 rpm were selected; these correspond to current speeds (10 cm above bottom) of  $1.8 \pm 0.02$  (mean  $\pm$  SD),  $5.7 \pm 1.3$  and  $13.6 \pm 2.7$   $\text{cm s}^{-1}$ , respectively, as determined using a Nixon Streamflo<sup>®</sup> current meter calibrated on a rotating arm in a circular tank (Stewart 1999). Because of a short horizontal distance, the flume tends to have a central jet due to flow from the incoming pipe. The height of 10 cm above bottom corresponds to the center of the jet.

**Particles.** The thecate dinoflagellate *Heterocapsa triquetra* (Strain Het,  $14.7 \pm 2.2$   $\mu\text{m}$  equivalent spherical diameter [ESD]) (Fig. 2A) was grown in batch cultures in 20 l carboys in modified L1 medium (Guillard & Hargraves 1993, 50  $\mu\text{M}$   $\text{NH}_4\text{Cl}$  added) at 16°C under 14:10 h light:dark cycle. The removal experiments were performed using cultures in mid- to late-exponential growth phase. *Heterocapsa triquetra* was chosen as a non-toxic proxy for toxic dinoflagellates, such as *Karenia brevis*. This species can be grown easily in large quantities and has previously been shown to be removed effectively by the supplied phosphatic clay IMC-P (see below) (M. R. Sengco pers. comm.). The concentration of *H. triquetra* used in the trials was 4000 cells  $\text{ml}^{-1}$ , as cells at this concentration have been shown to be efficiently removed ( $>80\%$ ) by IMC-P in smaller scale static trials (M. R. Sengco pers. comm.). Typical bloom concentrations reported for *H. triquetra* in the field range from 2000 (Kim et al. 1993) to 5000 cells  $\text{ml}^{-1}$  (Lindholm & Nummelin 1999). Prior to the trials conducted with *H. triquetra*, the batch was moved to a 20°C room with 24 h light for acclimation to prevent temperature shock. Algal stock concentrations were measured using a Coulter Multisizer (Model II, 100  $\mu\text{m}$  aperture). In the clay and algae trials, *H. triquetra* was added to the flume downstream of the working area and allowed to mix for 1 min at 5.7  $\text{cm s}^{-1}$ . Prior to the addition of clay and flow adjustment, an initial sample of the algal suspension was taken 10 cm off the bottom with a 10 ml micropipette and counted by microscope to determine the accuracy of the stock dilution. Cell motility (Fig. 3) was qualitatively as-

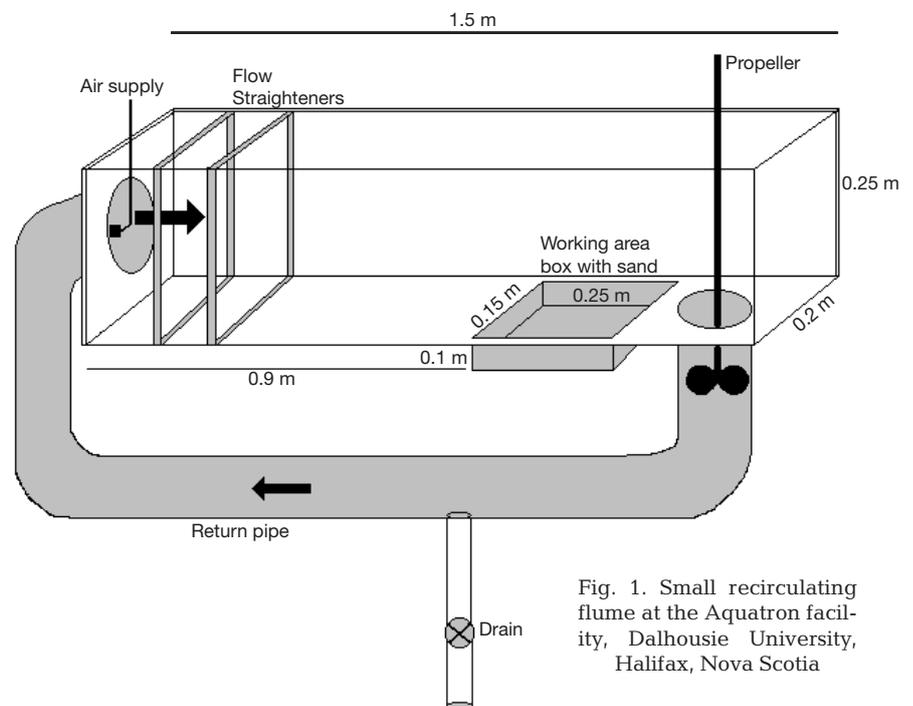


Fig. 1. Small recirculating flume at the Aquatron facility, Dalhousie University, Halifax, Nova Scotia

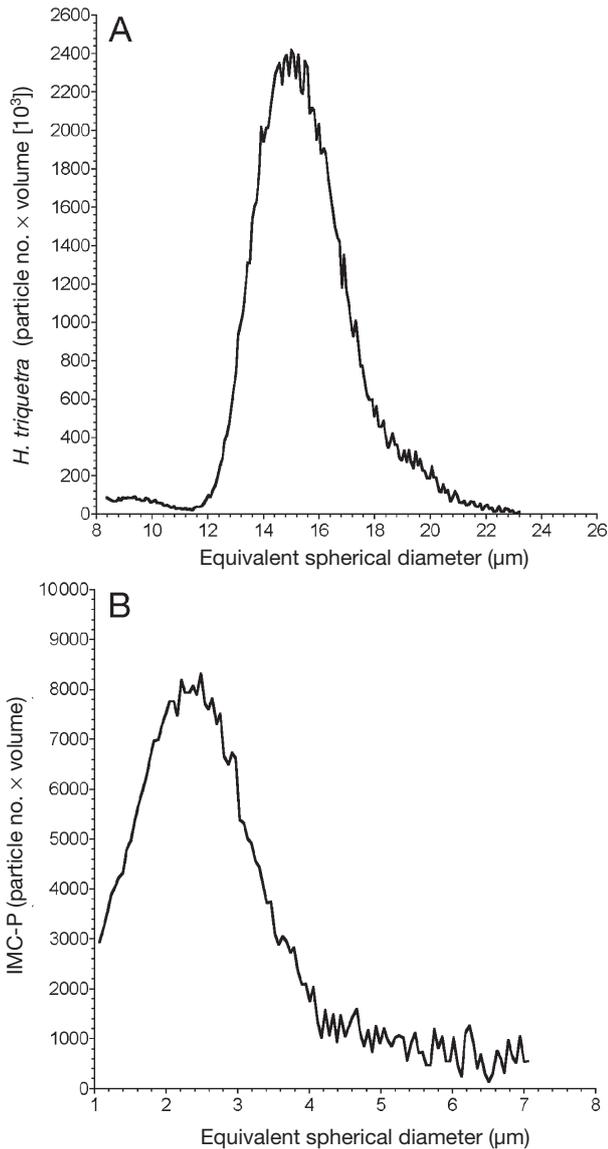


Fig. 2. Coulter Multisizer size distributions as the product of particle number and volume by equivalent spherical diameter (ESD). (A) Thecate dinoflagellate *Heterocapsa triquetra* size distribution ( $14.7 \pm 2.2 \mu\text{m}$  ESD). (B) Phosphatic clay IMC-P particle number times volume distribution ( $2.7 \pm 1.0 \mu\text{m}$  ESD). The mean particle size from the particle number distribution is  $1.8 \pm 0.7 \mu\text{m}$  ESD

essed as relative proportions of total cells moving in a Palmer-Maloney counting chamber (0.1 ml) under a compound microscope, where H, M and L indicate a high, medium and low proportion of actively swimming cells respectively.

The phosphatic clay IMC-P ( $1.8 \pm 0.7 \mu\text{m}$  ESD, 50% water content; Fig. 2B shows the particle number  $\times$  volume distribution) was used for the removal of *Heterocapsa triquetra* in the clay treatment trials. The montmorillonite phosphatic clay IMC-P (IMC Phosphates) is

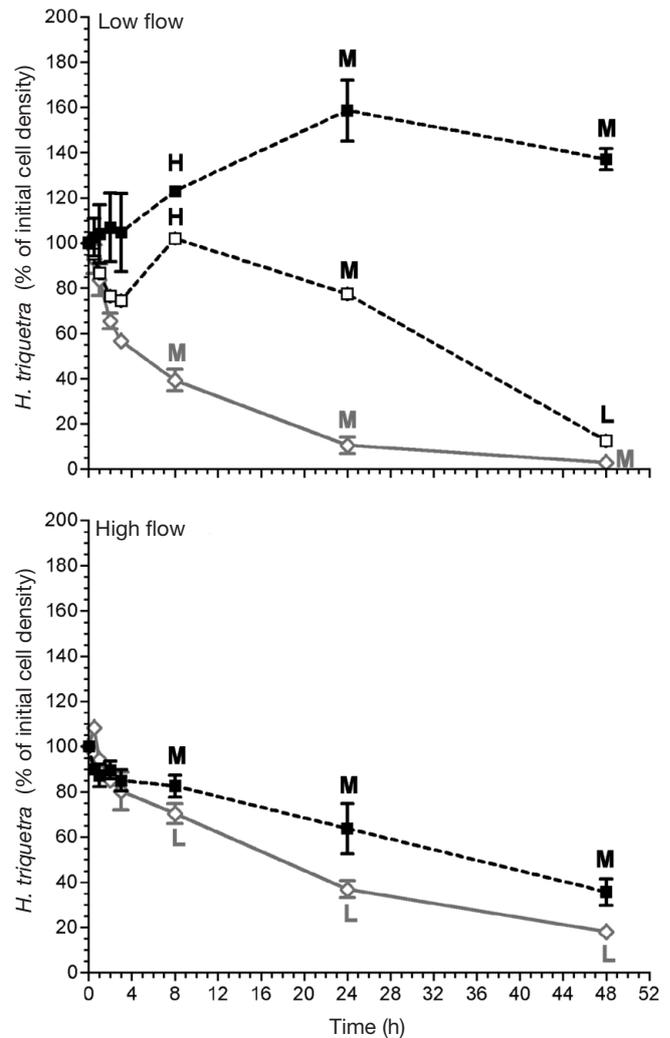


Fig. 3. *Heterocapsa triquetra* removal efficiency by the phosphatic clay IMC-P, as the % of algal cells remaining in the water column relative to the initial cell concentration over 48 h. Algae-only control, no IMC-P present 2 trials averaged ( $\blacksquare$ ); algae-only controls, no IMC-P present ( $\square$ ); clay-algae treatment, IMC-P present at  $0.25 \text{ g DW l}^{-1}$  ( $\diamond$ ). The letters indicate a qualitative measure of algal cell swimming ability, where H, M and L indicate a high, medium and low proportion of cells actively swimming, respectively. Error bars are SE on triplicate trials

produced in large quantities as an industrial waste product from the phosphate mining industry and is composed of 30 to 50% silt/sand and up to two-thirds  $<2 \mu\text{m}$  size fraction (Bromwell 1982). The clay displays strong propensity for exchanging ions in its crystal structure (Sengco 2001). It was prepared for experiments by processing 45 g wet weight (WW) of clay in 900 ml of 18 to  $20^\circ\text{C}$  flume seawater in a blender at high speed for 2 min and sieving through a  $63 \mu\text{m}$  stainless steel mesh to produce a fine particle slurry.

This process is comparable to WHOI procedures for laboratory experiments and for future field trials (M. R. Sengco pers. comm.). The final clay suspension added to the flume (90 l) was 0.25 g dry weight (DW) l<sup>-1</sup>. This IMC-P concentration was used because it was the minimum concentration shown to remove *H. triquetra* efficiently (>80%) in static test tube trials (M. R. Sengco pers. comm.). Preliminary trials indicated that when deposited this clay suspension produced a substantial sediment layer 4 to 5 mm in depth. Following the addition of *H. triquetra*, the slurry was added downstream of the working area and mixed through the water column at a flow of 5.7 cm s<sup>-1</sup> for 1 min prior to any change in experimental flow conditions. This time was defined as time 0.

**Experimental design.** Paired sequential experimental trials (n = 3) included an algae-only control (*Heterocapsa triquetra*) and clay-algae treatment (IMC-P and *H. triquetra*). This experimental design was implemented to determine the effectiveness of IMC-P in removing *H. triquetra* from the water column (0.22 µm FSW, 20°C) in 2 flow regimes. A clay-only control was also conducted in both flow regimes to determine the clay particle characteristics in the absence of algae. Two flow conditions, low and high, were chosen to reflect contrasting conditions in the field, where the low-flow regime (1.8 ± 0.2 cm s<sup>-1</sup>) characterizes a low-energy environment that encourages complete sedimentation of the clay and algae, and the high-flow regime (13.6 ± 2.7 cm s<sup>-1</sup>) characterizes a high-energy environment which maintains clay in suspension. Trials and combinations of conditions, with the variables measured per trial, are identified in Table 1.

The removal efficiency of *Heterocapsa triquetra* was quantified as the percent of initial cell numbers remaining in the water column over time, determined with a

0.1 ml Palmer-Maloney counting chamber under a compound microscope. The removal efficiency of IMC-P from the water column was quantified as the percent of the initial suspended particulate matter (SPM) concentration remaining in the water column over time, using an optical backscatter (OBS) probe positioned in the middle of the water column (10 cm off-bottom). The IMC-P concentration was assumed to be ~100% of the total SPM, given that algal cells were rare compared to clay particles and that the OBS could not distinguish the cells. The OBS was calibrated using known dry weight concentrations of IMC-P suspensions determined by filtering known volumes through GF/F filters. The probe face was cleaned occasionally with a soft brush to dislodge any buildup of particles on the sensor. The data shown were adjusted by removing the portions of the logged record affected by particle buildup.

Particles were captured and their size distributions were analyzed using the small volume particle micro-sampler (SVPM) described in Archambault et al. (2001) and image analysis specified below. The SVPM is a sampling device that captures a 1 ml sample and its individual particles *in situ* on filters with minimal disturbance. It is especially effective in capturing particles in the size categories that are ineffectively sampled by most *in situ* cameras (<50 µm) (Archambault et al. 2001). Each trial lasted 48 h, and SVPM and algal samples were obtained at 0, 0.5, 1, 2, 3, 8, 24 and 48 h for the algae-only and algae-clay trials, and 0, 3, 24 and 48 h for the clay-only control trials. Data for the particle size distributions from times 3 and 24 h are reported.

In the low-flow trials, material sedimented to the flume bed was collected after 48 h using three 10 ml beakers acting as sediment traps positioned down-

Table 1. Summary of experimental trials characterizing particle aggregates

Condition	Treatment	<i>Heterocapsa triquetra</i> density (cells ml <sup>-1</sup> )	IMC-P concentration (g DW l <sup>-1</sup> )	Variables measured
Low flow (1.8 cm s <sup>-1</sup> )	Algae-only (Control)	4000	0	Removal efficiency of <i>H. triquetra</i>
	Clay-only (Control)	0	0.25	Removal efficiency of IMC-P Particle size
	Clay-algae	4000	0.25	Clay loading Removal efficiency of <i>H. triquetra</i> Removal efficiency of IMC-P Particle size Clay loading
High flow (13.7 cm s <sup>-1</sup> )	Algae-only (Control)	4000	0	Removal efficiency of <i>H. triquetra</i>
	Clay-only (Control)	0	0.25	Removal efficiency of IMC-P Particle size
	Clay-algae	4000	0.25	Removal efficiency of <i>H. triquetra</i> Removal efficiency of IMC-P Particle size

Table 2. ANOVA on the removal efficiency of *Heterocapsa triquetra* by IMC-P clay over times 0, 3, 24 and 48 h. Treatment conditions are: algae-only controls and clay-algae trials for both low and high flows

	df	SS	MS	F-ratio	p-value
A: Time	7	2.82	0.40	20.58	<0.001*
B: Treatment	3	1.70	0.57	28.96	<0.001*
AB	21	1.62	$7.71 \times 10^{-2}$	3.93	<0.001*
Error	64	1.25	$1.96 \times 10^{-2}$		
Total (adjusted)	95	7.40			
Total	96				

\*Significant at  $\alpha = 0.05$

stream of the working area during the clay-only and clay-algae treatments. Bottom clay loading was measured by filtering the collected sediment on pre-weighed glass fiber filters (2.5 cm GF/F, 0.2  $\mu\text{m}$  pore size) and drying in an 80°C oven for 24 h. Filters were weighed, and sediment mass was calculated and then converted to g DW  $\text{m}^{-2}$ .

**Imaging technique.** Photographs of SVPM filters (20 fields of view [FOV] per filter, 7% of the filter area) were taken under a Leitz Laborlux D fluorescence microscope (10× Fluotar objective, 100× magnification) with a Megaplus Camera Model 1.4i (Kodak) and the XCAP-LITE v.0.5B frame grabber software (Epix). Each field of view was photographed under both light and fluorescence (excitation 270 to 380 nm, emission 410 to 580 nm filter), to capture algal cells and clay particles simultaneously. Microscope resolution at this magnification was a particle diameter of 1.94  $\mu\text{m}$ , based on a convention of 3 screen pixels (Russ 1995). The SVPM filters were placed on CytoClear slides (Osmonics), which eliminated background filter noise and allowed for higher particle resolution. Photographs were analyzed with SigmaScan Pro version 5.0 (SPSS). To minimize any observer bias associated with image analysis, e.g. 'blooming' of the image and differences in illumination, all photographs were enhanced (intensity, contrast) equally.

Particle area was quantified and converted to the equivalent circular diameter (ECD) ( $\mu\text{m}$ ), the diameter of the particle assuming a 2-dimensional circular shape. The ESD is the diameter of a particle assuming a 3-dimensional sphere. Particle distributions are expressed as the product of the number of particles and the particle volume per ECD bin size. To distinguish algal cells from large aggregates in the particle size spectrum, the fluorescence images were used to determine those particles known to be algal cells and to delete them from the distribution. Control photographs using filtered seawater indicated that SVPM filter photographs did not require correction for noise or extraneous particles.

**Statistical analyses.** All statistical analyses were conducted using NCSS 2000 software (www.ncss.com). The data were tested for normality and transformed before performing 2-way analyses of variance (ANOVA) with flow and particle types as the treatments (Table 2), and regressions (linear and non-linear) were used to compare the rate of loss (algae and clay) in each trial. Data that violated normality and could not be transformed were subjected to the Kolmogoroff-Smirnoff non-parametric test for differences in distributions.

## RESULTS

### *Heterocapsa triquetra* removal efficiency

Experiments conducted under the low-flow regime indicated a rapid removal of *Heterocapsa triquetra* from the water column in the presence of IMC-P, but not in the algae-only controls. The mean of the first 2 algae-only trials indicated that in this flow regime there was an increase in cell concentration, indicative of algal growth, with little removal of the cells over time (Fig. 3; ■). There was also a high-to-medium proportion of cells swimming. The third algae-only control, however, showed that after 8 h, a progressive decrease in the proportion of motile cells was associated with a higher net removal of *H. triquetra* cells from the water column than in the 2 previous controls (Fig. 3; □). Removal from the water column may have been accelerated in the anomalous control by poor overall condition of the cell culture, which may have resulted in a higher percentage of non-motile and senescing cells. Had loss in the system occurred via other mechanisms, i.e. capture by flow straighteners or return pipe, it would have been consistent in the first 2 controls. In direct contrast to the algae-only controls, the clay-algae trials indicated that there was an exponential decline described by:

$$C_t = C_0 \exp(-kt) \quad (1)$$

where  $C_t$  is the % algal concentration at time  $t$  (h),  $C_0$  is the % initial algal concentration and  $k$  is the exponential decay rate (%  $\text{h}^{-1}$ ) in *H. triquetra* cell concentration in the water column over time (slope =  $-0.16\% \text{ h}^{-1}$ ,  $R^2 = 0.95$ ). Algal concentrations were reduced by 90% in 24 h and over the 48 h of the experiment, a total of 95% of *H. triquetra* cells were removed from the water column in the presence of IMC-P clay. The algal cells showed moderate swimming ability in the presence of IMC-P, in contrast to high motility observed at 8 h in the algae-only controls, and were not able to maintain net growth or remain suspended in the water column.

In the high-flow regime algae-only controls, there was a gradual decline of *Heterocapsa triquetra* cell concentration with a medium proportion of actively swimming cells in the water column over time (Fig. 3). High shear at this flow regime may have resulted in reduced cell motility compared to the 8 h algae-only control in the low-flow regime. Over the 48 h of the experiment the initial dinoflagellate concentration was reduced by 60%. The clay-algae treatments indicated that in the presence of clay, cells consistently showed low motility and over 48 h, 80% of the cells were removed.

A 2-factorial (Table 2) and Bonferroni post-hoc comparisons indicated that there was a significant effect of the presence of clay on dinoflagellate removal efficiency in the low-flow regime ( $p < 0.001$ ), and a significant effect of flow speed between algae-only controls ( $p < 0.001$ ) and between clay-algae treatments ( $p < 0.001$ ) for both flow types. However, there was no significant effect of the presence of clay between the algae-only controls and clay-algae treatments in the high-flow regime ( $p = 0.236$ ). Thus, clay significantly enhanced dinoflagellate removal relative to controls, but only at the low flow.

### SPM removal efficiency

Paired experiments of clay-only control and clay-algae treatment (IMC-P and *Heterocapsa triquetra* present) were compared in terms of the mean depletion of SPM for all trials recorded by optical backscatter (Fig. 4). Optical backscatter measurements in low flow indicated that approximately 50% of the sediment load was removed within the first 8 h regardless of the presence of *H. triquetra* (Fig. 4). The data were fitted with an exponential decay function:

$$(C_t - C_\infty)/(C_0 - C_\infty) = \exp(-kt) \quad (2)$$

where  $C_t$  is SPM as the % initial concentration at time  $t$  (h),  $C_\infty$  is the asymptotic steady state concentration approached as time goes to infinity,  $C_0$  is the % initial concentration and  $k$  is the exponential decay rate ( $\% \text{ h}^{-1}$ ). The presence of algal cells did not significantly accelerate the exponential decrease in SPM, as tested by the overlap of the 95% confidence limits (CI), where the decay rates at low flow were  $0.07 \% \text{ h}^{-1}$  ( $R^2 = 0.99$ ) and  $0.10 \% \text{ h}^{-1}$  ( $R^2 = 0.99$ ) for the clay-only control and clay-algae treatment, respectively. At the end of the 48 h trial, the total SPM removal for the low-flow trials were approximately 82 and 88% of the original concentration for the clay-only control and mean of the clay-algae trials, respectively.

The deposited particulate matter in the low-flow regimes was not significantly different between the

clay-only control and the combined clay-algae treatments ( $p = 0.437$ ,  $F = 0.66$ ,  $df = 1$ ). The clay-only control indicated an average bottom clay loading of  $13.2 \pm 1.8 \text{ g m}^{-2}$ , compared to a mean clay loading of  $13.9 \pm 1.1 \text{ g m}^{-2}$  for the combined clay-algae trials. All trials produced a sedimented clay layer of approximately 4 mm in depth.

In the high-flow regime, the bulk of the particulate matter remained in suspension regardless of the presence of *Heterocapsa triquetra* (Fig. 4). The greatest loss occurred during the first 8 h, with decay rates (fitted by Eq. 2) of  $0.16 \% \text{ h}^{-1}$  ( $R^2 = 0.89$ ) and  $0.08 \% \text{ h}^{-1}$  ( $R^2 = 0.85$ ) for the clay-only control and clay-algae treatment, respectively. Potential losses in the flume

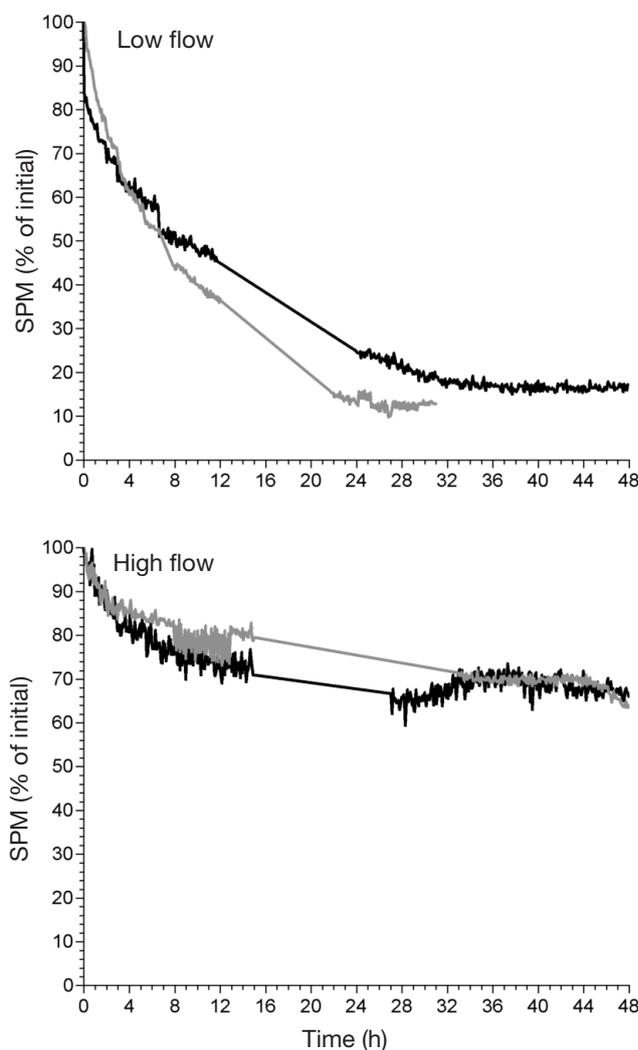


Fig. 4. Removal efficiency of the phosphatic clay IMC-P as the % of total suspended particulate matter (SPM), assumed to be 100% IMC-P at an initial concentration of  $\sim 0.25 \text{ g DW l}^{-1}$ , over time as measured by optical backscatter (OBS). Black lines correspond to the clay-only control trial and gray lines are the clay-algae treatments

system may account for this decline, but likely an equilibrium between sedimentation and resuspension of particles was reached at ~30% removal for these concentrations (4000 cells *H. triquetra* ml<sup>-1</sup>, 0.25 g IMC-P DW l<sup>-1</sup>) and the particular flume system (Fig. 4).

### Particle characteristics

Particle size distributions were determined from the product of particle volume as calculated from the ECD, and particle number counts for the clay-only control and clay-algae treatment trials at 3 and 24 h for both flow regimes (Fig. 5). Regardless of the presence of *Heterocapsa triquetra* or the sampling time, the distributions were similar for both the clay-only control and clay-algae trials within each flow regime. No distinct peak at ~15 μm ECD occurred in the clay-algae treatments, indicative of *H. triquetra* cells; rather the simi-

larity to the clay-only controls suggests that clay-clay flocculation occurs and contributes to the large (>10 μm ECD) particle-size spectrum.

The median ECD was calculated by converting the median area from SVPM particle distributions for the low and high-flow regimes over time (Fig. 6). The distributions shown were modified in all cases by deleting areas corresponding to particles < 4 μm attributed to non-aggregated IMC-P particles (see distribution in Fig. 2B) in order to reduce the influence of this large proportion of small particles on the median ECD. The treatment distributions were further modified by deleting the values corresponding to *Heterocapsa triquetra* cells. The particles attributed to algal cells were identified by fluorescence as described in the methods. The resulting size distribution had a median particle ECD ranging between 5 and 8 μm at all times for both the corrected low- and high-flow regimes. In most cases, the median ECD was slightly less for the clay-only control than the

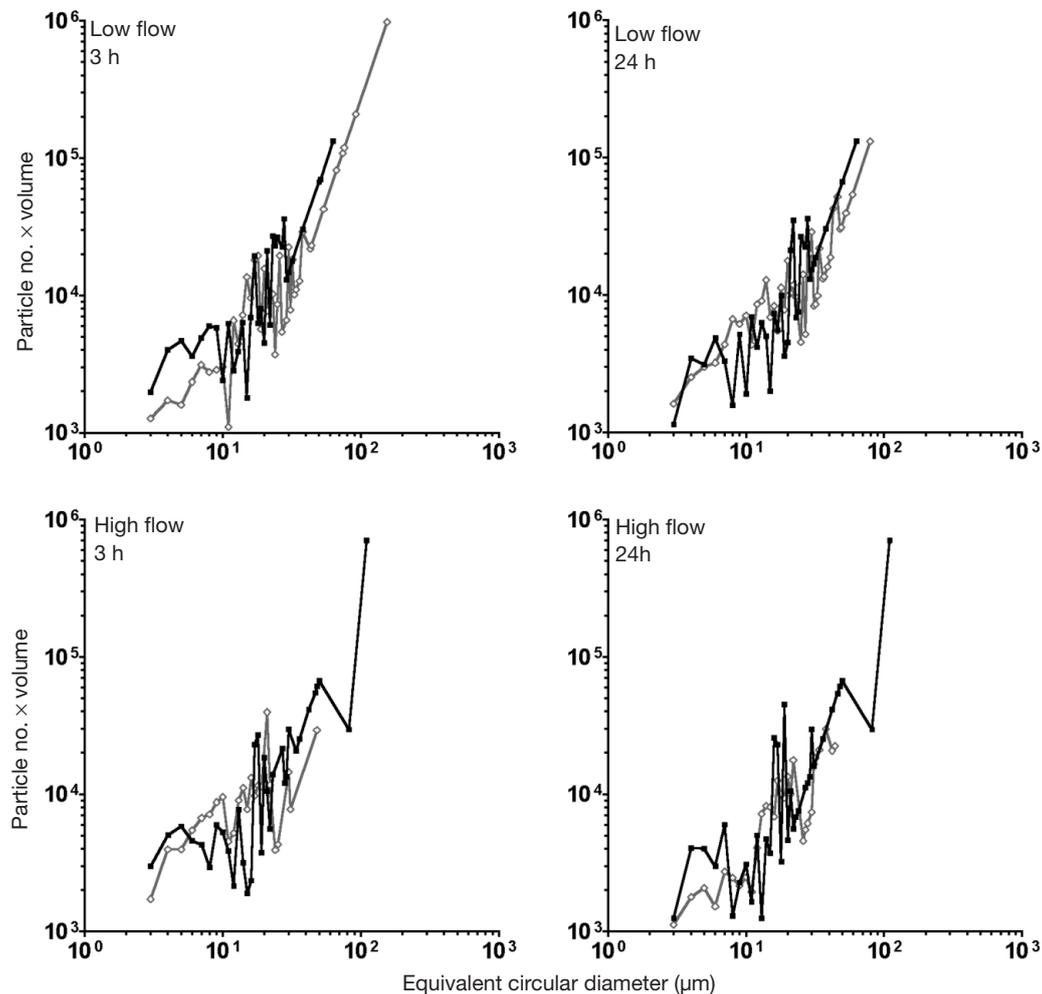


Fig. 5. Particle size distributions as the product of particle number and volume per equivalent circular diameter (ECD) bin size converted from small volume particle microsampler (SVPM) image analysis. Clay-only control (■—■); clay-algae treatment with *Heterocapsa triquetra* cells present in the distribution (◇—◇)

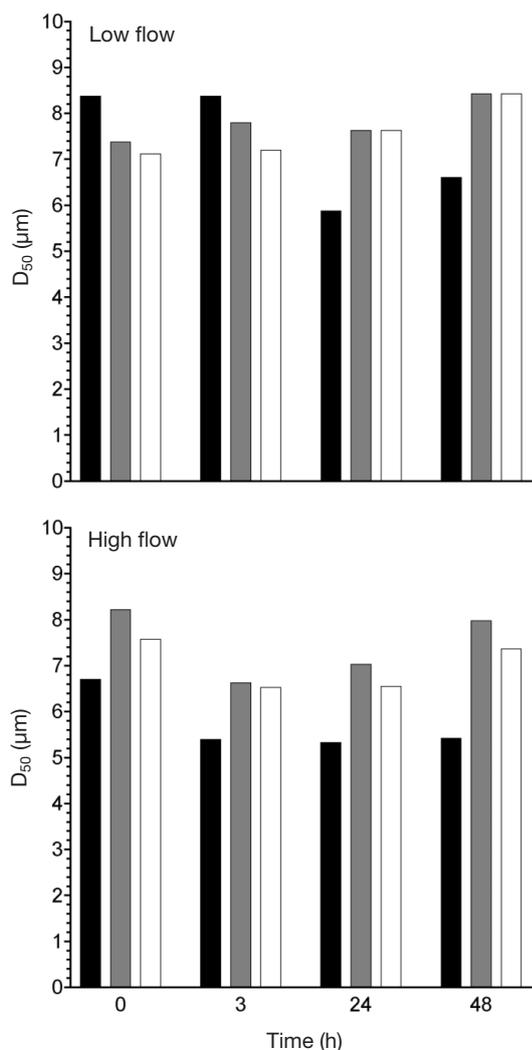


Fig. 6. Median ( $D_{50}$ ) equivalent circular diameter (ECD) determined from the median area of small volume particle micro-sampler (SVPM) particle-size distributions, with clay particles  $<4 \mu\text{m}$  removed. Clay-only control (■); clay-algae treatment with *Heterocapsa triquetra* cells present in the distribution (■); clay-algae treatment without *H. triquetra* cells present in the distribution, where cells were identified through fluorescence (□)

clay-algae treatment in both low flow and high flow (Fig. 6). Inconsistencies in this trend can be attributed to large flocs present early in the trials, which eventually sediment out (Fig. 6). In all cases, modifying the distributions from SVPM analysis by removing the areas attributed to *H. triquetra*, did not significantly change the median ECD. This comparison suggests that the large end of the particle size spectrum did not result from algal-only or clay-algal flocs, but rather large clay-clay aggregates. In both the low- and high-flow regimes, there was no evidence that algal cells were aggregated to each other. Additionally, algal cells did not show the presence of a detectable clay layer on the cell surface.

## DISCUSSION

The concept behind mitigating HABs by treatment with clays is based on the mutual aggregation between the algae and the mineral particles leading to the formation of large flocs that settle to the bottom (Avnimelech et al. 1982, Degens & Ittekkot 1984). A number of studies have demonstrated that fine dispersions of clay minerals can effectively remove algal cells from seawater (Avnimelech et al. 1982, Søballe & Threlkeld 1988, Sengco et al. 2001). Several of these studies have shown that some species are removed more efficiently than others and that the removal rate depends on clay type, clay concentration, algal type and algal concentration (Avnimelech et al. 1982, Sengco et al. 2001). The mechanism underlying this differential removal is poorly understood. Although these studies suggest that flocculation between algal cells and clay particles occurs, no effort has been made to quantify the *in situ* particle-size distribution or composition in turbulent flow. These studies were often conducted in static systems, e.g. test tubes, where flow and shear were not considered as agents of particle aggregation and disaggregation, and wall effects are known to occur (Sengco et al. 2001). In our laboratory study, differential removal of the dinoflagellate *Heterocapsa triquetra* by the phosphatic clay IMC-P in 2 flow regimes is demonstrated, pointing to the importance of flow conditions in evaluating the site-specific effectiveness of clay mitigation. It is difficult to make direct comparisons with previous work given the differences in scale and experimental design. Further studies are needed to assess the relevance of scale and of varying flow conditions in predicting HAB removal by clay in the field.

### *Heterocapsa triquetra* removal efficiency

The phosphatic clay IMC-P effectively removes the thecate dinoflagellate *Heterocapsa triquetra* in a low-flow environment ( $<2 \text{ cm s}^{-1}$ ). In this flow regime, the population of 'healthy' algal cells grew over time in the absence of clay. The data suggest that cell physiological condition plays an important part in determining cell removal from the water column. The third algae-control showed that if cells are in poor condition, for example with old or 'shocked' cultures, removal by the clay may be amplified. In the current research, swimming ability was used as an indicator of cell physiological condition. This is an important factor in determining the removal efficiency of algal cells and needs to be considered as a potential source of variability in future studies. Many phytoplankters, including dinoflagellates, are classified as 'strong swimmers' and may avoid or break free from sticky clay particles, and

ultimately remain in the water column. It was shown that freshwater *Euglena gracilis* had only a slight tendency for flocculation, probably because of its vigorous motility (Avnimelech et al. 1982). However, *Chlamydomonas*, a less motile alga, formed clusters and sedimented readily with clay. *H. triquetra* is a fairly strong swimmer with an average swimming speed of 0.21 mm s<sup>-1</sup> (Sengco 2001); however, results of the present study suggest that it quickly shows a reduction in swimming ability in the presence of clay and increased flow. Similar experiments to those described in the present study were conducted in the flume using the thecate dinoflagellate *Prorocentrum micans* as the test organisms (unpubl. data), showing that the swimming ability of cells was not affected and that they were not removed effectively by IMC-P in static conditions (also M. R. Sengco pers. comm.).

Sengco (2001) examined collision frequencies and algal removal rates as a function of swimming speed for flagellates, non-motile diatoms and small cocci in the presence of clay in static systems. Clay concentration was an important factor in cell removal efficiency. At relatively low clay concentration (<0.03 g l<sup>-1</sup>), *Karenia brevis* cells were able to free themselves from the clay, swim away and resume vegetative growth; however, in contrast, mortality resulted at high clay loadings (>0.50 g l<sup>-1</sup>) (Sengco 2001). It was concluded that mortality was caused by direct physical contact between the cells and clays, but the exact mechanism by which surface interactions lead to cell death is unknown. It is suggested that clay potentially disrupts motility by adhering to the flagella (M. R. Sengco pers. comm); however, this has yet to be examined conclusively. Avnimelech et al. (1982) speculated that the mutual aggregation of algae and clay particles was a widespread phenomenon and that the variable flocculating potential of different algae (Cuker 1987, Søballe & Threlkeld 1988, Sengco et al. 2001) depends on the composition and properties of the cell wall, on the extent and type of excretions, physiological conditions, age, and other factors.

The present study shows that at higher flow (~13 cm s<sup>-1</sup>), IMC-P is not as effective in removing algal cells, as indicated by the similar removal of *Heterocapsa triquetra* in the water column in the presence or absence of clay. Irrespective of the presence of clay, the algal cells suffered stress indicated by the decrease in swimming ability. Therefore, in this flow regime, the removal mechanism appears to be dictated by the physiological stress on *H. triquetra* resulting from the effects of high current speed and/or high fluid shear and not the presence of IMC-P. Smayda (2002) indicated that in the field there are zones of vertical velocity rates (increased turbulence) at which, based on their motility, dinoflagellates would survive and bloom.

A review of 71 clones and species of dinoflagellates, phytoflagellates and protozoans, showed that the majority (59%) swim at rates which exceed an upwelling velocity of 0.125 mm s<sup>-1</sup>. The results suggest that *H. triquetra* cells swam easily at low flow, but were unable to overcome shear rates at high flow. Several studies have shown that increased turbulence results in a decrease in cell growth rates and an increase in mortality (Thomas & Gibson 1990, Berdalet 1992, Juhl et al. 2000, 2001). Berdalet (1992) suggested that the physiological processes linked to nutrient uptake, biosynthetic metabolism and cellular growth are responsible for observed decreased growth rates.

Turbulence, which breaks apart as well as aggregates particles, has been suggested as the mechanism that determines the maximum size of flocs (Tambo & Hozumi 1979, Eisma 1986, Manning & Dyer 1999, Manning et al. 2001). Although an initial application of clay to HABs may aggregate particles in the presence of turbulence, a balance between aggregate formation and aggregate breakup may result in cells escaping floc formation and therefore, resulting in low removal efficiencies. Alternatively, breakup of flocs and cell escape could occur when encountering near-bottom shear after the successful aggregation and removal of cells in a relatively low flow regime. Much of the past research on cell removal efficiencies with clay has been conducted in static systems. These experiments demonstrate that removal efficiencies are highly dependent on flow. This may ultimately be the determining factor in successful removal of HABs, but requires further investigation over a range of current velocities.

### SPM removal efficiency and particle characteristics

Regardless of the presence of algae, the largest fraction of suspended particulate matter was almost completely removed from the water column in the low-flow regime. In contrast, the SPM in the high-flow regime reached a steady state concentration (70% of the initial concentration) over the 48 h irrespective of the presence of *Heterocapsa triquetra*. The initial loss could also be explained by trapped clay in areas of the flume, such as unknown features in the return pipe, and in the boxed sand (Pilditch et al. 1998).

Particle size is an important variable to quantify in these experiments and relevant to characterize exposure conditions in subsequent benthic impact studies on lethal and/or sublethal effects of clay mitigation on juvenile bivalves. The data from the size-frequency distributions were used to identify the size range of particles that benthic organisms would be exposed to during the mitigation event in the 2 flow regimes. This parameter becomes important in predicting the feed-

ing behavior of suspension-feeding fauna exposed to increased particle flux to the bottom. For example, bivalve particle retention efficiency and subsequent selection and/or rejection of particles depend partly on particle size. White (1997) showed that adult sea scallops *Placopecten magellanicus* consistently removed water-based drilling fluid waste particles that were flocculated at greater rates than unflocculated particles. Most bivalves including hard clams retain 100% of particles  $>3 \mu\text{m}$  on the gill, but the minimum size at which scallops attain 100% retention efficiency is  $\sim 5 \mu\text{m}$  (reviewed by Grizzle et al. 2001). The present study indicated that in both the low- and high-flow regimes, clay particles, via formation of clay-clay flocs, are available for retention on gills of suspension-feeding bivalves and thus, potentially could affect feeding behavior and particle availability (Archambault 2002).

Particle size distribution did not vary between the clay-control and treatments (IMC-P with *Heterocapsa triquetra*) within flow regimes and sampling times. Several explanations for the lack of clay-algal aggregation are suggested, as evidenced from SVPM filter photographs. Shear disturbance in the flume could account for the lack of large aggregates. Thomas et al. (1999) suggested that in a flocculator stirred with an impeller, high values of strain occur close to the tip of the impeller. It is probable that breakage occurs in these regions of high shear intensity rather than in the more quiescent areas away from the impeller. The airline inserted in the return flow pipe could potentially also account for some breakage of particles via production of air bubbles.

The flume used in the experiment did not allow for the formation of a well defined benthic boundary layer. At higher rheostat speeds ( $>500 \text{ rpm}$ ), a central jet was developed as the water entered the main body of the flume from the return pipe. The flow straighteners did not fully rectify the flow, resulting in potentially static flow areas where material may have deposited, as evidenced by the slight removal of clay in the high-flow regime. Despite these caveats (turbulent flow), clay was effective in removing *Heterocapsa triquetra* cells from the water column in the absence of algal-clay flocs; thus, supporting the hypothesis that other mechanisms (e.g. surface interactions) may be contributing to HAB removal. Few laboratory flocculators, including flumes, operate with laminar flow (Thomas et al. 1999), and both laminar and turbulent flow are of interest in the context of clay control of HABs. Turbulent particle interactions are still poorly understood, but the isotropic model has been successfully adopted in flocculation modeling. The model describes turbulence as a cascade of eddies of diminishing size (Thomas et al. 1999). Based on this model and experimental data, Casson & Lawler (1990) concluded that smaller particles were far

more likely to collide with one another than with larger particles; therefore, the smaller clay particles ( $\sim 2 \mu\text{m}$  ESD) were more likely to aggregate together (as shown in the present study) than with *H. triquetra* cells. Sengco (2001) speculated that in the application of clay to the brown tide alga *Aureococcus anophagefferens*, the small clay particles aggregated quickly and were removed without colliding and flocculating with the cells, resulting in less effective removal of *A. anophagefferens* from the water column. Increasing the agitation (mixing) kept the initial clay size small, i.e. no initial clay aggregation and removal, and increased collision rates with cells, thus increasing the removal efficiency of *A. anophagefferens*.

Previous research conducted by Sengco et al. (2001) on the removal efficiencies of clay on *Karenia brevis* and *Aureococcus anophagefferens* suggested that the use of coagulants or flocculants in the clay treatment increases cell removal efficiency. In theory, coagulants and flocculants promote flocculation by affecting the surface chemistry (stickiness) of the particles, thus resulting in increased particle aggregation. Ideally the use of these substances would decrease the total clay loading required to remove HABs and might reduce benthic impacts from sedimenting or resuspended particles. Sengco et al. (2001) suggested that polyaluminum chloride (PAC) at 5 ppm was the most effective in removing *K. brevis*. The addition of PAC to clay treatments could potentially increase the removal efficiency of *Heterocapsa triquetra* by IMC-P clay, especially in the high-flow regime, by increasing the occurrence of large clay-algal aggregates.

Here, we observed that the SVPM did not effectively capture dinoflagellate cells which led to the underestimation of the absolute concentration of dinoflagellate cells. Therefore, the particle distributions may not accurately describe the relative contribution of *Heterocapsa triquetra* cells to the large end of the particle size spectrum. Archambault et al. (2001) implied that the SVPM could be used to calculate particle concentration, but suggested that, depending on particle type, concentration thresholds of the suspension determined the effectiveness of the SVPM in calculating particle concentration. At  $4000 \text{ cells ml}^{-1}$ , the concentration of *H. triquetra* cells could have been too low for the SVPM to sample accurately. Motile algal cells may also actively avoid the solenoid mechanism and this could result in reduced capture of cells. Further investigations on the effectiveness of the SVPM in determining absolute particle concentrations of large motile cells and its dependence on particle concentration thresholds are required. Despite these caveats, the findings using the SVPM method that dinoflagellate cells were never incorporated in larger aggregates even at low flow remains valid.

In summary, the application of clay to HABs is a promising method for reducing environmental and economic impacts from HABs, but is more likely to be effective in relatively low-energy environments. Despite the inherent limitations in flume design outlined above (most notably in terms of propeller-driven flow and associated shear stress), several important findings emerge from this study which will be useful in guiding future research and interpreting results of clay applications in the field. At low flow, *Heterocapsa triquetra* cells sedimented from the water column in response to clay addition despite the demonstrated lack of formation of clay-algal aggregates. This suggests the existence of alternate mechanisms for clay-induced algal removal, e.g. entrainment by clay-clay aggregates or direct contact of clay particles on the cell surface leading to physico-chemical interactions that could impair cell motility. Evidence of the latter was detected in our study although motility was only determined qualitatively. *H. triquetra* sedimentation was also found to occur in 1 out of 3 low-flow controls, and the anomaly was associated with reduced cell motility. This finding suggests that differences in the physiological condition of cells may lead to variable results both in the laboratory and in the field, and need to be considered in future work. In contrast to results obtained in the low-flow regime, no significant removal of *H. triquetra* by clay occurred at high flow. This may be due to the high current speed and/or high shear generated in the experimental flume in this treatment. Since turbulent mixing and shear may occur in the field, it will be important in future studies to determine the effects of both of these variables, horizontal current speed and shear, potentially using less disruptive experimental flumes, in controlling the effectiveness of HAB removal by clays.

**Acknowledgements.** We thank R. Jordan, M. Anderson, S. McKenna, D. van der Meer and S. MacQuarrie from the Institute for Marine Biosciences (IMB), National Research Council of Canada (NRC), and B. Schofield from Dalhousie University for their technical support and assistance in various aspects of this work. Thanks to B. Hargrave at the Bedford Institute of Oceanography for the use of his fluorescent microscope for the past 2 yr. P. Hill, Department of Oceanography, Dalhousie University, provided valuable advice which greatly benefited this work. We extend our thanks to Don Anderson, Woods Hole Oceanographic Institution (WHOI), for his comments and input into the research, and WHOI postdoctoral fellows Stace Beaulieu, Aishao Li and especially Mario Sengco for providing the clay as well as valuable unpublished information used in experimental design. This project was funded by ECOHAB-EPA (US EPA grant No. R-82551-01-01) via a sub-contract from WHOI (D. Anderson) to V.M.B. at IMB, NRC. Supplementary student support was provided by a Dalhousie University Graduate Fellowship and a Natural Sciences and Engineering Research Council of Canada (NSERC) Graduate Research Award.

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Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

Submitted: July 9, 2002; Accepted: January 13, 2003  
Proofs received from author(s): April 23, 2003