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DIVERSE FEEDING RESPONSES OF FIVE SPECIES OF BIVALVE MOLLUSC WHEN EXPOSED TO THREE SPECIES OF HARMFUL ALGAE

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ABSTRACT Shell closure and restriction of filtration are behavioral responses by which bivalve molluscs can limit exposure of soft tissues to noxious or toxic agents, including harmful microalgae. In this study, we assessed the clearance rates of five species of bivalve mollusc—the northern bay scallop *Argopecten irradians irradians*, the eastern oyster *Crassostrea virginica*, the northern quahog *Mercenaria mercenaria*, the softshell clam *Mya arenaria*, and the blue mussel *Mytilus edulis*—exposed for one hour to each of three harmful-algal strains: *Prorocentrum minimum*, *Alexandrium fundyense*, and *Heterosigma akashiwo*. Clearance rates of harmful-algal cells were compared with clearance rates of a benign microalga, *Rhodomonas* sp., and to a Mix of each harmful alga with *Rhodomonas* sp. Qualitative observations of valve closure and production of biodeposits were also assessed during the exposure experiments. Feces and pseudofeces were collected and observed with light and fluorescence microscopy for the presence or absence of intact, potentially-viable algal cells or temporary cysts. Results increase our understanding of the high variation between the different bivalve/harmful alga pairs. Responses of bivalve species to the different harmful algae were species-specific, but in most cases indicated a preferential retention of harmful algal cells, probably based upon different characteristics of the algae. Each shellfish species also reacted differently to the harmful-algal exposures; several remained open; whereas, others, such as oysters exposed to the toxic raphidophyte *Heterosigma akashiwo*, closed shells partially or totally. Similarly, production of feces and pseudofeces varied appreciably between the different bivalve/alga pairs; with the exception of softshell clams *Mya arenaria*, intact cells of most harmful-algal species tested were seen in biodeposits of the other four bivalve species. These results extend our understanding of the high species specificity in the interactions between harmful algae and bivalve molluscs and confirm that generalizations about feeding responses of bivalves to harmful algae cannot easily be made. In most cases, however, there was at least some ingestion of the harmful algae leading to exposure of soft tissues to the algal cells.

KEY WORDS: Filtration, clearance, biodeposits, bivalve mollusc, mussels, oysters, clams, scallops, harmful algal bloom, HAB, toxic algae, *Alexandrium fundyense*, *Heterosigma akashiwo*, *Prorocentrum minimum*, *Argopecten irradians*, *Crassostrea virginica*, *Mercenaria mercenaria*, *Mya arenaria*, *Mytilus edulis*

INTRODUCTION

Suspension-feeding, molluscan shellfish obtain food and a portion of their oxygen by passing water across the gills, within the shell, with water currents generated by gill ciliary activity. Molluscs can respond to the presence of noxious chemical or particulate components in seawater by stopping or modifying the movement of water through the shell—by stopping filtration and closing the shell to protect soft tissues within (Harrison et al. 1984, Doherty et al. 1987, Gainey & Shumway 1988, Katticaran & Salih 1992, Ait Fdil et al. 2006). Alternately, movement of water through the shell can be continued for oxygen acquisition, but reduced retention and ingestion of particles can provide protection when noxious microorganisms are present (Galtsoff 1964, Wildish et al. 1998, Matsuyama et al. 1997). Cells can be rejected prior to ingestion without being incorporated into pseudofeces (Sellner et al. 1995, Bricelj et al. 1998). Additionally, ingestion of useful food particles can be continued, whereas microbes or particles with inimical characteristics can be rejected by sorting functions of gill feeding structures or labial palps surrounding the mouth (Shumway et al. 1985a; Shumway & Cucci 1987, Ward et al. 1997, Ward & Shumway 2004). Finally, postingestive selection can also occur in bivalves (Brillant & MacDonald 2002, Ward & Shumway 2004), with selective sorting of unwanted particles within the stomach followed by rapid elimination.

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Among the naturally-occurring, potentially-noxious particles that occur in many shellfish habitats are toxic microalgae. In evolutionary time, suspension-feeding molluscs have had to accommodate the occasional appearance in the plankton of toxic microalgae that may cause damage to soft tissues or impact metabolic functions. Modified respiratory and feeding behaviors minimizing contact between toxic phytoplankton cells and tissues represent one possible adaptation (Bardouil et al. 1993, Bardouil et al. 1996, Bricelj et al. 1990). For example, Bardouil et al. (1996), showed total inhibition of biodeposit production by Pacific oysters, *Crassostrea gigas,* when exposed to the toxic dinoflagellate *Alexandrium tamarense*. Another adaptation may involve activation of cellular defense functions in digestive and hemocytic cells of bivalves exposed to HABs (Wikfors & Smolowitz 1993 and Wikfors & Smolowitz 1995, Hégaret & Wikfors 2005a, Hégaret & Wikfors 2005b, Hégaret et al. in press [b]). The latter response could be more advantageous, in that nutritional and energetic status can be maintained during harmful-algal events, which often are episodic and ephemeral.

Various behavioral, physiological and cellular responses of bivalves to harmful algae have been described. Several studies assessed the response of different bivalve species to toxic dinoflagellates, measuring changes in valve closure, filtration rate, feeding rate, byssus production, oxygen consumption, and cardiac activity or neurophysiological effects (Shumway et al. 1985a, Shumway & Cucci 1987, Gainey & Shumway 1988, Lesser & Shumway 1993). These publications show a wide range

of responses by which bivalves can protect themselves from deleterious effects of various harmful algal species, but prevalence of species-specific responses makes broad generalizations difficult.

When bivalves limit contact between toxic or noxious algae and soft tissues, then cellular responses are less-likely to be important in these trophic interactions. Previously, we described changes in hemocytes of 3 molluscan species: the eastern oyster *Crassostrea virginica,* the northern bay scallop *Argopecten irradians irradians,* and the northern quahog *Mercenaria mercenaria*, exposed *in vivo* to the dinoflagellate, *Prorocentrum minimum* (Hégart & Wikfors 2005a, 2005b; Hégart et al. in prep). It is unclear, however, whether these kinds of cellular responses are limited to these specific taxa. Thus, the main objective of this study was to screen for changes in feeding responses of different bivalve species to sudden exposures with several HAB species so that the likelihood for hemocyte contact could be assessed. The lists of bivalve and harmful-algal species were based on geographic co-occurrence of managed shellfish species and HABs. An experimental protocol was established and applied consistently to screen shellfish/HAB pairs for filtration and consumption of the algae.

MATERIALS AND METHODS

Bivalve Molluscs

Experiments were conducted in Milford, Connecticut, USA, between May and October, 2005, at the National Oceanic and Atmospheric Administration, National Marine Fisheries Service Laboratory. Three bivalve species: the blue mussel (*Mytilus edulis* Linnaeus—shell length (SL) 35–45 mm), the eastern oyster (*Crassostrea virginica* Gmelin—SL 35–45 mm), and the softshell clam (*Mya arenaria* Linnaeus, shell height 60–70 mm), were obtained from local shellfish harvesters. Northern quahogs (hard clams, *Mercenaria mercenaria* Linnaeus—SL 45–55 mm), were collected from local waters by a recreational diver, and northern bay scallops (*Argopecten irradians irradians* Lamarck—SL 35–45 mm were provided by the Milford Laboratory aquaculture program. All shellfish were maintained in the Milford Laboratory for several weeks in flowing, unfiltered seawater prior to the experiments. An assumption across all experiments was that the individual shellfish had no prior exposure to blooms of the harmful algal species tested (below).

Algal Cultures

Three harmful-algal species were tested in this study: the dinoflagellates *Alexandrium fundyense* Balech, and *Prorocentrum minimum* (Pavillard) Schiller, which can be toxic to finfish and shellfish (Shumway & Cucci 1987, Shumway 1990, Luckenbach et al. 1993, Wikfors & Smolowitz 1993, Wikfors & Smolowitz 1995), and the raphidophyte *Heterosigma akashiwo* (Hada) Hada ex Sournia, which is known to be toxic to finfish (Landsberg 2002). The algal strains used were obtained from the Milford Microalgal Culture Collection and included: *P. minimum* strain JA-98-1 (isolated from the Choptank River, Maryland, USA.), *A. fundyense*, strain BF2 (isolated from the Gulf of Maine, USA), and *H. askashiwo* strain OL (isolated from New Jersey, USA). In addition, the RHODO strain of *Rhodomonas* sp. was used as a nontoxic, control alga. The RHODO strain is smaller (9 μm) than the three other algal species; *Alexandrium fundyense* diameter is approximately 25–35 μm, *Prorocentrum minimum* diameter is in the range of 15–20 μm, and *Heterosigma akashiwo* diameter ranges between 12 and 15 μm.

The microalgae were cultured in 20-L glass carboy assemblies using aseptic technique (Ukeles 1973). Cultures were harvested semicontinuously to maintain consistency in culture quality over the course of the study and were harvested in late-log or early-stationary phase. Cultures of *Prorocentrum minimum* were grown in EDL7 medium, a modified version of the enriched-seawater E-medium (Ukeles 1973) that contains L-1 trace metals (Guillard & Hargraves 1993), double the EDTA of the standard E formulation, KNO_3 rather than $NaNO_3$, and 10-ml L^{-1} soil extract. The harmful alga *Alexandrium fundyense* was grown in F/2-enriched (Guillard et Ryther 1962, Guillard 1975) Milford seawater; and *Heterosigma akashiwo* and *Rhodomonas* sp. were cultured in E-medium. Cultures were maintained at 20°C with 24-h light. Algal cell densities were determined by hemocytometer counts with the light microscope. The cell densities of harmful-algal strains used for bivalve exposures were equivalent to those of natural blooms of these taxa (Table 1).

Rhodomonas sp. was chosen as a control, benign alga because it is easily mass-cultured, and it contains both chlorophyll and phycoerythrin fluorescence useful for microscopic and flow-cytometric analyses. Further, previous filtration studies used *Rhodomonas* sp. as well (Ward et al. 1998).

Experimental Methods

Exposure experiments were conducted in replicate, 4-L plastic containers holding ultrafiltered (0.2 μm) Milford Harbor seawater to which algal cultures were added. Mixing and oxygenation were accomplished with gentle aeration in each container. Prior to experiments with live bivalve molluscs and

TABLE 1.

Concentrations of harmful algae used for the experiments simulating natural blooms.

Algal Species	Bloom Concentration	Reference(s)
Prorocentrum minimum	10^4 cells mL^{-1}	Hégaret & Wikfors (2005a)
Alexandrium fundyense	5–2 *10^3 cells mL^{-1}	Shumway et al. (1988) Townsend et al. (2005)
Heterosigma akashiwo	10^4 cells mL^{-1}	Rensel & Whyte (2003) Keppler et al. (2005)
Rhodomonas sp.	10^4 cells mL^{-1}	Levinton et al. (2002) Ward et al. (2003)

harmful algae, experiments were run with the same containers and empty shells of the bivalves (or no shells) to determine the removal of microalgal cells from suspension by settling and sticking to container walls, so that clearance-rate measurements with bivalves could be corrected, if necessary. For this purpose, thirty individual containers holding *Rhodomonas* sp at a cell density of 10^4 cells.mL^{-1} were sampled before and after 1 h of incubation with the following additions:

- -empty scallop shell *Argopecten irradians irradians* (5 containers)
- -empty oyster shell *Crassostrea virginica* (5 containers)
- -empty quahog shell *Mercenaria mercenaria* (5 containers)
- -empty softshell clam shell *Mya arenaria* (5 containers)
- -empty mussel shell *Mytilus edulis* (5 containers)
- -no shell present (5 containers)

Water samples, 4 mL from each container, were collected before immersion of the bivalve shells and after one hour.

An additional experiment was done testing loss of harmful-algal cells in the experimental containers with no shells added. Sampling of containers ($n = 7$ for each condition) to determine algal concentration was done before and after 1h of incubation of containers holding:

(1) The equivalent of a natural bloom concentration of each harmful alga (Table 1, 10^4 cells.mL^{-1} for *Prorocentrum minimum* and *Heterosigma akashiwo*, 2–5 $\times$ 10^3 cells.mL^{-1} for *Alexandrium fundyense*—according to the cell availability: mussels and quahogs were exposed to 5 $\times$ 10^3 cells.mL^{-1}, oysters were exposed to 3 $\times$ 10^3 cells.mL^{-1}, and scallops and softshell clams were exposed to 2 $\times$ 10^3 cells.mL^{-1}).
(2) The equivalent of a natural bloom concentration of *Rhodomonas* sp., as a control for the clearance rate (Table 1, 10^4 cells.mL^{-1} for *Rhodomonas* spp.). (3) A Mix of *Rhodomonas* sp. and each harmful alga used for clearance rate determination, containing the sum of the concentrations detailed in the previous sections.

Experimental Design: Clearance-rate Measurements

Exposures of each shellfish species to each harmful alga, the *Rhodomonas* control, and the mix of the two, were done synoptically, selecting shellfish haphazardly from the same population acclimated together. Thus, the experiments were designed to answer four separate questions for each shellfish/harmful algal pair:

Q_1: Is clearance of the harmful alga different from that of *Rhodomonas*?
Q_2: Does the presence of *Rhodomonas* change the rate of clearance of the harmful alga?
Q_3: Does the presence of the harmful alga change the rate of clearance of *Rhodomonas*?
Q_4: Is rate of clearance of *Rhodomonas* and the harmful alga equal when they are mixed (preferential retention)?

One-way analysis of variance ($P < 0.05$ or $P < 0.01$) was used to compare experimental treatments addressing these questions. Statgraphics Plus statistical software (Manugistics, Inc., Rockville, MD, USA) was used for statistical analyses of experimental data.

To conduct each experiment, 30 individual bivalve molluscs of one species were held for 48 h in FSW to depurate previously-consumed algae and become acclimated to the experimental conditions (20°C in a temperature-controlled room with 24-h light). Thirty individual shellfish then were transferred, each into a 4-L container holding the algal suspensions defined in the previous section.

1) 10 individual shellfish were transferred into 10 individual containers holding the equivalent of a natural bloom density of harmful alga
2) 10 individual shellfish were transferred into 10 individual containers holding the equivalent of a natural bloom density of *Rhodomonas* sp.
3) 10 individual shellfish were transferred into 10 individual containers holding a Mix of *Rhodomonas* sp. and the harmful alga

Algal Concentration—Clearance Rate Measurements

Measurements of algal cell density in samples collected from experimental containers were made with a flow-cytometer, using methods adapted from Shumway et al. (1985a) and Cucci et al. (1989). A 2-mL water sample was collected from each container before immersion of the bivalves, or of the empty shells, and hourly thereafter for 4 h. Each sample was added to 2 mL of 6% formalin in ultrafiltered seawater containing a known concentration of fluorescent microbeads (Fluoresbrite YG Microspheres, 2.00 μm; Polysciences) to preserve the samples and allow accurate measurements from ratios of algal cells to microbeads. Samples were run the same day on the flow-cytometer; algae and beads were separated according to their different sizes, morphologies and pigments (i.e., by fluorescence). Clearance rates were calculated according to the equation defined by Coughlan (1969), using counts obtained after one hour of exposure to the algae.

$$CR = M/(n * t)\log_e(C_o/C_t)$$

where

CR: Clearance rate (mL/h);
M: volume total of the suspension (mL);
n: number of individuals (here $n = 1$);
t: time of the exposure (here $t = 1$ h);
C_o: concentration at the beginning of the experiment;
C_t: concentration at the end of the experiment, after the time period t.

Observations of the Behavior of Bivalve Shellfish Exposed to Harmful Algae

As bivalves were exposed to the experimental algal suspensions; macroscopic, qualitative observations of the valve opening/closing behavior and biodeposit production were assessed for 4 h. During the 4-h of exposure, production of biodeposits was recorded qualitatively, and subsamples were observed under the microscope to evaluate the presence of intact cells in the biodeposits. Results from these observations are descriptive only.

RESULTS

Controls—Experiments with Empty Bivalve Shells and Rhodomonas *sp.*

No differences were found in the concentrations of *Rhodomonas* sp. before and after 1 h of incubation in either empty

containers or those holding an empty bivalve shell (one-way ANOVA, $P > 0.05$). Therefore, clearance rates based upon concentration of *Rhodomonas* sp. after 1 h incubation with bivalves can be attributed exclusively to filtration of the shellfish, and clearance rate can be assessed without correcting for loss to the container.

Controls—Experiments with no Shells and the Different Algal Species Tested

Algal cell densities were calculated before and after 1 h of incubation of the algae in the containers without shellfish. The containers holding *Rhodomonas* sp, *Prorocentrum minimum*, *Alexandrium fundyense* or the mixes (*P. minimum* and *Rhodomonas* sp. or *A. fundyense* and *Rhodomonas* sp.) did not show any depletion of the algal concentration (individual *t*-tests, $P > 0.05$). Conversely, according to the algal counts, the concentration of *Heterosigma akashiwo* increased in the containers after 1 h of incubation (*t*-tests, $P < 0.05$). Observation of the algae in the containers indicated the presence of aggregated *H. akashiwo* cells, and a very uneven distribution of the algae in the containers resulting from a "swarming" behavior of the algae. Similar results could be observed in the containers holding a Mix of *H. akashiwo* and *Rhodomonas* sp.; no difference was observed for *Rhodomonas* sp., but the concentration of *H. akashiwo* was higher after 1 h of incubation. These results indicated that clearance-rates of *H. akashiwo* could not be quantified reliably with our experimental protocol. Therefore, clearance data were assessed for the five species of bivalves exposed to *Prorocentrum minimum* and *Alexandrium fundyense* only, but observations of biodeposits from the shellfish exposed to *H. akashiwo* were still recorded.

Experiments with Live Bivalve Shellfish: Measurement of Clearance Rate

The different harmful algae, alone and in the Mixes, had very different effects on feeding behaviors of the different bivalve species tested.

Q_1: Is clearance of the harmful alga different from that of *Rhodomonas*? *Are there any differences in clearance rate between HAB – Mix (total cell concentration of HAB and* Rhodomonas*) and* Rhodomonas*?*

The clearance rate of each bivalve tested differed according to the algae to which it was exposed: *Alexandrium fundyense*, *Prorocentrum minimum*, and *Rhodomonas* sp. alone or the total cell densities of the Mix of *A. fundyense* and *Rhodomonas* or *P. minimum* and *Rhodomonas*. Quahogs, *Mercenaria mercenaria,* and northern bay scallops, *Argopecten irradians irradians,* exposed to *A. fundyense* had higher mean clearance rates ($mL.h^{-1}$) than those exposed to a mixture of this harmful alga and the *Rhodomonas* together, and higher than those in the control with *Rhodomonas* sp. alone (ANOVA $P < 0.01$; Table 2). Similarly, oysters, *Crassostrea virginica*, had a higher mean clearance rate when exposed to *P. minimum* alone than in a Mix of this alga with *Rhodomonas* sp., or the control with *Rhodomonas* sp. alone (ANOVA $P < 0.01$; Table 3). Softshell clams, *Mya arenaria*, as well as scallops, *A. irradians irradians*, exposed to *Rhodomonas* sp. had lower clearance rates than those exposed to *A. fundyense*, or mixed microalgae (ANOVA $P < 0.01$; Table 2). Scallops exposed to *Rhodomonas* sp. also presented lower clearance rates than those exposed to *P. minimum,* or mixed microalgae (ANOVA $P < 0.01$; Table 3). No significant differences were observed in the clearance rates of quahogs, mussels, or softshell clams exposed to *P. minimum*, *Rhodomonas* or the Mix of these two algae. Mussels and oysters exposed to *A. fundyense* did not have different clearance rates than when exposed to *Rhodomonas* sp. alone (Tables 2 and 3).

Q_2: Does the presence of *Rhodomonas* change the clearance of the harmful alga? *Are there any differences in clearance rates of bivalves exposed to the harmful algae alone or the harmful algae in the Mix?*

Clearance rates of the harmful algae were measured for the bivalves exposed to the HAB only or to the Mix of HAB and *Rhodomonas*, by selectively counting the harmful algal cells. No differences were observed between the clearance rates, of the harmful algae alone or in the Mix (Table 2 and 3).

Q_3: Does the presence of the harmful alga change the clearance of *Rhodomonas*? *Are there any differences in clearance rates of bivalves exposed to* Rhodomonas *sp. alone or* Rhodomonas *sp. in the Mix?*

Clearance rates of the *Rhodomonas* sp. were measured for the bivalves exposed to the *Rhodomonas* sp. only or to the Mix

TABLE 2.

Clearance rates ($l.h^{-1}$) of molluscan shellfish exposed to the dinoflagellates *Alexandrium fundyense* alone, to *Rhodomonas* sp. alone or to a Mix of both, data present the clearance rate on the total cells present in the Mix, and on *A. fundyense* only or *Rhodomonas* sp. only. The statistical analyses (ANOVA, $P < 0.05$) answer the fours main questions (Q_1, Q_2, Q_3, Q_4) posed in the Materials and Methods section. NS: non significant, the letters correspond to posthoc least Significant Difference tests from the ANOVA (a > b > c).

	Alexandrium fundyense													
	HAB Alone		*Rhodomonas*		Mix (HAB + Rhodo)		HAB in Mix		Rhodo in Mix		Statistics (one-way ANOVA, n = 10)			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Q1	Q2	Q3	Q4
Argopecten irradians	10.02	1.68	2.02	0.61	7.16	1.66	10.40	2.12	6.80	1.66	aba	NS	ba	NS
Crassostrea virginica	1.18	0.51	0.47	0.19	0.87	0.31	2.42	1.59	0.86	0.30	NS	NS	NS	NS
Mercenaria mercenaria	3.30	1.00	0.63	0.37	0.82	0.21	3.85	0.66	0.71	0.20	abb	NS	NS	ab
Mya arenaria	6.10	0.93	0.48	1.46	4.96	0.64	7.61	0.93	4.93	0.64	aba	NS	ba	ab
Mytilus edulis	5.31	1.42	5.25	0.75	3.43	0.44	4.43	0.70	3.43	0.45	NS	NS	ab	NS

TABLE 3.

Clearance rates ($l \cdot h^{-1}$) of molluscan shellfish exposed to the dinoflagellates *Prorocentrum minimum* alone, to *Rhodomonas* sp. alone or to a Mix of both, data present the clearance rate on the total cells present in the Mix, and on *P. minimum* only or *Rhodomonas* sp. only. The statistical analyses (ANOVA, $P < 0.05$) answer the fours main questions (Q_1, Q_2, Q_3, Q_4) posed in the Materials and Methods section. NS: non significant, the letters correspond to posthoc Least Significant Difference tests from the ANOVA (a > b > c).

	Alexandrium fundyense													
	HAB alone		*Rhodomonas*		Mix (HAB + Rhodo)		HAB in Mix		Rhodo in Mix		Statistics (One-Way ANOVA, $n = 10$)			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Q1	Q2	Q3	Q4
Argopecten irradians	13.83	2.11	2.02	0.61	16.63	1.59	17.42	1.66	16.46	1.60	aba	NS	ba	NS
Crassostrea virginica	1.56	0.21	0.47	0.19	1.01	0.16	1.52	0.21	0.51	0.13	abb	NS	NS	ab
Mercenaria mercenaria	0.41	0.38	0.63	0.37	1.28	0.30	1.39	0.31	1.21	0.30	NS	NS	NS	NS
Mya arenaria	4.79	2.62	0.48	1.46	1.97	0.74	1.22	0.80	2.54	1.04	NS	NS	NS	NS
Mytilus edulis	3.71	0.61	5.25	0.75	4.06	0.34	4.94	0.35	3.37	0.34	NS	NS	NS	ab

of HAB and *Rhodomonas*, by selectively counting the *Rhodomonas* cells. Mussels cleared *Rhodomonas* sp. more rapidly when not mixed with *Alexandrium fundyense*. Conversely, the clearance rate of *Rhodomonas* was higher in the Mix with *A. fundyense* for softshell clams and in the Mix with *A. fundyense* and *P. minimum* for scallops (Table 2 and 3).

Q_4: Is clearance of *Rhodomonas* and the harmful alga equal when they are mixed (preferential retention)? *Are there any differences in clearance rates of bivalves exposed to a mixed suspension of* Rhodomonas *sp. and a harmful algal species?*

Clearance rates of *Rhodomonas* sp. and the harmful algae for bivalves exposed to a Mix were measured separately, by selectively counting the *Rhodomonas* cells or the harmful-algal cells. In bivalves exposed to a mixed suspension of *Rhodomonas* sp. and a harmful algal species, the clearance rates of *Rhodomonas versus* the clearance rates of the harmful algae were compared with assess the preferential retention of one algal species over the other. In the interactions tested, the clearance rates were either not significantly different or were higher for the harmful algae (Table 2 and 3). This is the case for several HAB/bivalve combinations, such as *Mercenaria mercenaria Alexandrium fundyense, Mytilus edulis*/*Prorocentrum minimum, Mya arenaria*/*Alexandrium fundyense,* and *Crassostrea virginica*/*Prorocentrum minimum*.

Observations of the Shell-closure Behavior of Bivalve Shellfish Exposed to Harmful Algae

Results are presented in Table 4. Quahogs, *Mercenaria mercenaria,* exposed to *Alexandrium fundyense* appeared to close shell valves partially, compared with quahogs exposed to *Rhodomonas* sp., *Heterosigma akashiwo* or *Prorocentrum minimum*, for which shells were all fully open. Quahogs exposed to *A. fundyense* still produced biodeposits, which appeared to include both feces and pseudofeces. Microscopic observations of the biodeposits showed presence of many intact, nondigested cells and temporary cysts of *A. fundyense* in the feces and the pseudofeces (Fig. 1). Quahogs exposed to *P. minimum* appeared to be completely open and filtering actively (see Table 3), producing feces and pseudofeces, but microscopic observations indicated that intact cells, even though present in pseudofeces and feces, were much more numerous in the feces (Fig. 1). Finally, quahogs exposed to *H. akashiwo* were open and filtering, and produced only feces devoid of any intact *H. akashiwo* cells containing very low chlorophyll fluorescence.

Oysters, *Crassostrea virginica,* reacted differently to the harmful algal exposures. Indeed, oysters appeared to be mostly closed during the 4 h of exposure. Oysters exposed to *Heterosigma akashiwo* did not produce any biodeposits. Nevertheless, even though they appeared partially closed, oysters exposed to *Alexandrium fundyense* and *Prorocentrum minimum* still produced biodeposits. Minimal fecal production was obtained with *A. fundyense*, and intact cells and temporary cysts were observed in the feces. Oysters exposed to *P. minimum*, on the contrary, produced feces and pseudofeces. Microscopic observations showed that the feces were composed of cohesive material containing numerous intact cells (Fig. 1) as well as many empty cell walls of *P. minimum*. Oyster pseudofeces were less cohesive than the feces and contained an even higher concentration of intact *P. minimum* cells.

Mussels, *Mytilus edulis* were also open with foot extended during the 4 h of incubation with the three different harmful-algal species. When exposed to *Alexandrium fundyense* and *Heterosigma akashiwo*, mussels produced biodeposits; microscopic observations indicated the presence of intact, nondigested cells of both microalgal species in the feces—very few in the case of *H. akashiwo*, but many intact cells and temporary cysts in the case of *A. fundyense* (Fig. 1). Mussels exposed to *Prorocentrum minimum* produced large quantities of feces and pseudofeces, which contained numerous intact, nondigested cells of *P. minimum*.

Scallops, *Argopecten irradians irradians,* were also open and filtering during the 4 h exposures to the different harmful algal species. As with mussels, scallops exposed to *Alexandrium fundyense* produced only feces containing intact cells and temporary cysts, and those exposed to *Heterosigma akashiwo* also produced feces containing some *H. akashiwo* cells (Fig. 1), but very few. Finally, scallops exposed to *Prorocentrum minimum* produced feces and pseudofeces containing many intact cells of *P. minimum*. Two main types of biodeposits were observed: green fecal strands, which contained many intact *P. minimum* cells; and black fecal strands, which did not contain any intact cells.

Softshell clams, *Mya arenaria,* presented consistent responses to harmful-algal exposures. Indeed, they remained open and produced biodeposits in all exposures, with siphons partially

TABLE 4.

Macroscopic and microscopic observations of the status of each of the five different bivalves and of their biodeposits when exposed to the three harmful algal species.

	Alexandrium fundyense	*Heterosigma akashiwo*	*Prorocentrum minimum*
Argopecten irradians	closed at the beginning then opened, but slow	open	open
	few biodeposits produced (feces, no pseudofeces?)	few biodeposits produced (feces, no pseudofeces?)	lots of biodeposits (feces and pseudofeces)
	intact cells and temporary cysts in biodeposits	few intact cells in biodeposits	lots of intact cells in biodeposits
Crassostrea virginica	closed	closed	± closed
	few biodeposits produced (feces, no pseudofeces?)	no biodeposits	feces and pseudofeces
	intact cells and temporary cysts in biodeposits		lots of intact cells in biodeposits
Mercenaria mercenaria	± closed	open	very open
	feces and pseudofeces	feces - no pseudofeces	lots of biodeposits (feces and pseudofeces)
	intact cells and temporary cysts in biodeposits	very few intact cells in biodeposits	lots of intact cells in biodeposits
Mya arenaria		open	very open
		few biodeposits produced	feces, no pseudofeces(?)
	no intact cells in biodeposits	no intact cells in biodeposits	no intact cells in biodeposits
Mytilus edulis	open	open	open
	feces, no pseudofeces(?)	few biodeposits produced (feces, no pseudofeces?)	lots of biodeposits (feces and pseudofeces)
	intact cells and temporary cysts in biodeposits	intact cells in biodeposits	lots of intact cells in biodeposits

extended. Microscopic observations of the biodeposits showed no intact cells.

DISCUSSION

This survey of clearance and behavioral responses of bivalves to sudden exposures with different harmful algae revealed high variability with species-specific reactions. Clearance rates, production of feces and pseudofeces, and presence of intact cells in the feces varied appreciably between the different bivalve/alga pairs, indicating that generalizations about feeding responses of bivalves to harmful algae cannot easily be made.

Q_1: Is clearance of the harmful alga different from that of *Rhodomonas*?

The harmful algal cells tested were often more efficiently filtered by the bivalves; than was the control alga, *Rhodomonas* sp. As size retention efficiencies of the bivalves tested have been reported to be 100% at 7 μm and larger (Møhlenberg & Riisgård 1978, Riisgård 1988; reviewed in Ward & Shumway 2004), microalgal cell size alone cannot explain this higher clearance rate of harmful algae versus the RHODO strain. It is possible that there are some relevant, qualitative properties of the harmful-algal cells tested compared with *Rhodomonas* (Shumway & Cucci 1987; reviewed in Ward & Shumway 2004). Indeed, Shumway et al. (1985a) showed preferential feeding of *Ostrea edulis* on the *Prorocentrum minimum* over the diatom *Phaeodactylum tricornutum*, even though these algal species are roughly the same size. Moreover, Shumway et al. (1997) showed particle selection in three species of scallops fed a mix of several algal species was not based on size only but on other characteristics of the algae. Newell and Shumway (1993) also demonstrated that mussels cleared phytoplankton particles at a higher rate than silt particles of the same size, suggesting a preferential feeding based on qualitative factors. Waite et al. (1995) suggested that interaction between the extracellular matrix of the cells and cilia and mucus of the bivalve ctenidia could increase retention rates. Ward and Shumway (2004) also suggested that cell shape or flexibility can influence particle capture.

Possible reasons that bivalve filtration behavior, in this study, generally was not altered in response to sudden HAB exposure may include: slow induction of behavioral responses, precedence of respiratory needs over protection from harmful-algal effects, or lack of harmful effects. Indeed, several studies reported that the effect of the toxic algae *Alexandrium tamarense* could take up to an hour before having an effect on the clearance rate of bivalves (Gainey & Shumway 1988, Bardouil et al. 1993). Our results only present the clearance rate for one hour of exposure, and thus, delayed induction of response would not be detected. Observations of clearance rates decreasing over time of exposure have been made with *Crassostrea gigas* exposed to *Gymnodinium washingtonensis* (Dupuy & Sparks 1968) and *Mya arenaria* fed *Protogonyaulax tamarensis* (Shumway & Cucci 1987, Cucci et al. 1985). This short-term, screening study was not designed to determine time of induction of any possible chronic effects; however, qualitative observations of biodeposits indicate that rejection of HAB cells before ingestion in pseudofeces, and rapid gut passage of undigested cells, appear to be mechanisms that limit contact between the shellfish tissues and HABs.

Q_2: Does the presence of *Rhodomonas* change the clearance of the harmful alga?

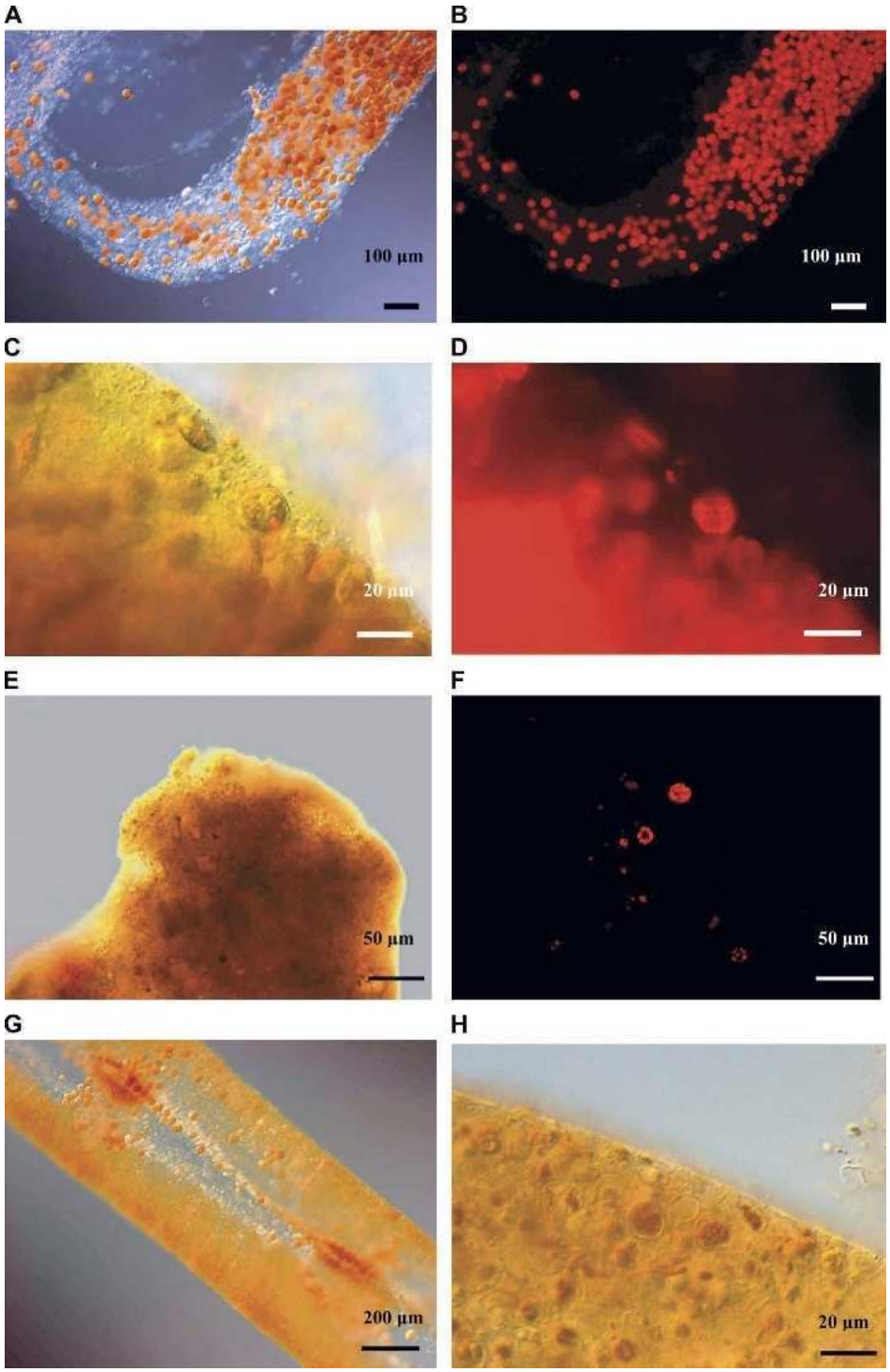

Figure 1. Photomicrographs with white light, and blue light/epifluorescence to show chlorophyll *a*, of fecal pellets produced by molluscan species during exposure to simulated blooms of cultured HAB species. (A, B) Intact, fluorescent cells of *Alexandrium fundyense* in fecal material from *Mercenaria mercenaria* (scale bar = 100 µm). (C, D) Intact, fluorescent cells of *Prorocentrum minimum* in fecal material from *Crassostrea virginica* (scale bar = 20 µm). (E, F) Intact, fluorescent cells of *Heterosigma akashiwo* in fecal material from *Argopecten irradians* (scale bar = 50 µm). (G) Intact cells of *Alexandrium fundyense* in fecal material from *Mytilus edulis* (scale bar = 200 µm). (H) Intact cells of *Prorocentrum minimum* in fecal material from *Mercenaria mercenaria* (scale bar = 20 µm).

Concerning the question of whether co-occurring microalgae influence the filtering behavior of bivalves exposed to a HAB, the present study indicates that the clearance rate of the harmful alga alone and in the Mix was almost always the same. Moreover, in this study, the presence of *Rhodomonas* sp. in the water did not influence the clearance rate of the harmful algal cells by the different bivalve species. Conversely, Bricelj et al. (1991) demonstrated that hard clams, *Mercenaria mercenaria*,

exposed to a highly-toxic dinoflagellate, *Alexandrium* sp., did not filter unless the diet was complemented with a nontoxic alga, such as *Thalassiosira weissflogii*. An explanation for this difference between studies may be attributable to variable toxicity of the strains. Indeed, Bricelj et al. (1991) used a very toxic strain of *Alexandrium* sp., whereas, our strain was less toxic and therefore may not have affected the quahogs as much.

This study also demonstrated that hard clams exposed to a mildly-toxic *Alexandrium* sp. ingested and absorbed the algal cells in a monopsecific diet. The *Alexandrium* strain used for this experiment was probably not highly toxic, which might explain the fact that the bivalve molluscs cleared it in the presence or absence of nontoxic algae. Although we did not quantify toxin content in any of the harmful-algal cultures used in this study, the cultures were tested using a bioassay exposing juvenile scallops. Scallops exposed to *A. fundyense* were killed in less than 24 h, and mortality occurred in less than 4 d for scallops exposed to *Prorocentrum minimum* and *Heterosigma akashiwo* at 10^5 cells mL^{-1} (Hégaret & Wilkfors 2005b), indicating a fairly low level of toxicity. The question of whether filtration in the mixed algal treatments is selective or repressive is relevant to field exposures of bivalves to harmful-algal blooms, because these blooms are essentially never unialgal. Co-occurring, benign phytoplankton cells would, thus, not be expected to affect the filtration of harmful-algal cells, based on these results.

Q_3: Does the presence of the harmful alga change the clearance of *Rhodomonas*?

The clearance rate of *Rhodomonas* sp. was, in most cases, no different or higher when mixed with harmful-algal cells. Indeed, scallops retained *Rhodomonas* sp. better when mixed with *Alexandrium fundyense* or *Prorocentrum minimum;* similar results were observed with softshell clams and *A. fundyense*. These results suggest that feeding may be more efficient with these harmful algal cells, or that the shellfish exposed to harmful algae were processing more particles, rejecting the harmful cells and retaining the nutritionally-useful cells. Conversely, the clearance rate of *Rhodomonas* sp. alone by mussels was higher than when mixed with *A. fundyense*, indicating that *A. fundyense* may have a negative effect on the clearance rate of mussels. Bardouil et al. (1993, 1996) found similar results with *Crassostrea gigas* exposed to a Mix of *A. tamarense* and *Thalassiosira weissflogii*. Exposure to *Alexandrium* spp. has been shown to paralyze adductor and siphon muscles in several species of bivalve (Bricelj et al. 2005, Hégaret et al. in press [b]), but not necessarily pumping activity.

Observations of the Shell-closure Behavior of Bivalve Shellfish Exposed to Harmful Algae

The measurement of clearance rates and behavioral responses of each shellfish species to the harmful-algal exposures shows that complete valve closure to isolate soft tissues from the harmful algae is very rare. Only eastern oysters exposed to *Heterosigma akashiwo* responded by closing the shell and ceasing filtration. The results confirm the observations from Hégaret et al. (in press [a]), which showed that oysters exposed to *H. akashiwo* did not produce any biodeposits for the first 24 h of exposure to this harmful alga. Similarly, Keppler et al. (2005) observed a very low rate of consumption of *H. akashiwo* cells by oysters *C. virginica* exposed to a similar concentration for 48 h. The low rate of consumption can also be attributed to the behavior of the *H. akashiwo* cells, indeed, the swarming effect may have made them less available to the bivalves.

In the presence of *Alexandrium fundyense*, shell valves of quahogs, *Mercenaria mercenaria*, were not as open as when these cells were absent; siphons were partially retracted, and the valves partially closed. Similar observations have been made in the past with *Gymnodinium tamarensis* (and *Protogonyaulax tamarensis* = *A. fundyense*) (Shumway et al. 1985b, Shumway & Cucci 1987). The dinoflagellate *A. fundyense* also affected the valve closure of oysters, *Crassostrea virginica*, which remained closed during the entire experiment. In contrast, scallops and mussels did not seem to be affected, in terms of shell-valve closure. These observations are also similar to previous experiments exposing mussels and oysters to *G. tamarensis* (Shumway et al. 1985b, Shumway & Cucci 1987). In these studies, oysters presented an initial valve closure response before reopening slowly; whereas, mussels, according to their provenance (originated from site where blooms of *G. tamarensis* occur regularly), remained open in the presence of *G. tamarensis*. Our results also confirm the findings of Bricelj et al. (1990), who observed that *M. mercenaria* exposed to *A. fundyense* alone closed shells and did not resume pumping until a low density of another alga was present in the water. These results were confirmed by Bricelj et al. (1991), who also showed that, when toxic and benign algae were present, the quahogs did not show any ingestion selectivity. Furthermore, Bricelj et al. (1991) also showed that mussels exposed to toxic *A. fundyense* did not stop feeding, had a much higher ingestion rate than clams with no *A. fundyense*, and accumulated PSP toxins.

Cells of *Prorocentrum minimum* were present intact and in very high concentration in biodeposits (feces and pseudofeces) in all the bivalve species tested (except *Mya arenaria,* discussed further). Shumway et al. (1985a) also observed the presence of intact cells of *P. minimum* in the biodeposits (feces and pseudofeces) of *Crassostrea virginica* and *Mytilus edulis*. Leibovitz et al. (1984) recorded the first observation of intact *Prorocentrum* cells in the open vascular system of bay scallops feeding in a natural bloom. These authors hypothesized that scallop mortalities were caused by physical damage from the apical tooth present on some *Prorocentrum* species. Wikfors and Smolowitz (1993) observed that survival and growth rates of clams fed a mixed diet of *P. minimum* and T-ISO were no different from those of unfed clams. Further, undigested *P. minimum* cells were seen in clam biodeposits, similar to our results. These authors hypothesized that the clams probably rejected the *P. minimum* cells in pseudofeces. Luckenbach et al. (1993) recorded mortalities and poor growth of oysters *C. virginica* exposed to *P. minimum* blooms in the Chesapeake Bay. A recent study by Grzebyk et al. (1997), using the mouse-bioassay, demonstrated neurotoxic activity in extracts from some isolates of *P. minimum*, when they were in the "decline" phase of the growth cycle. Thus, it appears that *P. minimum* is capable of producing some uncharacterized toxins under some conditions (Wikfors 2005). The biodeposits of the several bivalve molluscs tested (except softshell clams) contained a high concentration of intact *P. minimum* cells in feces and pseudofeces. Indeed, *P. minimum* cells seemed to be rejected as intact cells in the pseudofeces, through modified preingestive feeding, but they also seemed to undergo post-ingestive selection (Brillant & MacDonald 2002), because many *P. minimum* cells were still present intact in the feces.

One could hypothesize that the strategy of the bivalve to limit their exposure to toxic *P. minimum* is through pre and post-ingestive selection.

Similarly, intact cells and temporary cysts of *Alexandrium fundyense* were observed in the biodeposits of most bivalves, except *Mya arenaria* (discussed further). Our observations indicate that bivalves exposed to *A. fundyense* tend to produce mostly feces and not as much (or none, the data do not always allow us to have accurate results) pseudofeces, which confirms the results of Bricelj et al. (1998), who showed using video-endoscopy that most *Alexandrium* spp. cells are ingested by *M. arenaria* and by the oyster *Ostrea edulis,* but very few are rejected as pseudofeces. Only *Mercenaria mercenaria* seemed to produce feces and pseudofeces and intact cells and temporary cysts of *A. fundyense* were observed in both types of biodeposits. Bardouil et al. (1993) also observed the presence of intact cells of *A. minutum* and *A. tamarense* in the biodeposits of Pacific oysters (principally in the feces). Our observations also showed that oysters and clams were closed during the one hour of the experiment; whereas, scallops were closed at the beginning but reopened after a period of time. Conversely, mussels stayed open during the entire time of the experiment, cleared the *A. fundyense*, and produced biodeposits containing intact cells of *A. fundyense*. Marsden and Shumway (1992) observed similar results with mussels *Perna canaliculus*, which remained open in the presence of toxic *A. tamarense*. These observations of different feeding behaviors most probably explain the differences in the rapidity of accumulation in paralytic shellfish toxins (PSP toxins) between individual bivalve species. Lassus et al. (1989) showed that mussels, *Mytilus edulis* and scallops, *Pecten maximus,* very rapidly accumulated PSP toxin; whereas, clams, *Ruditapes philippinarum*, and oysters, *Crassostrea gigas*, did not accumulate as much toxin and not as quickly. Mussels have been shown to accumulate very large amounts of PSP toxins in very short time (Shumway & Cucci 1987); whereas, oysters tend to be less toxic, because they remain closed for extended periods when exposed to toxic species (Shumway et al. 1990). The presence of intact cells and temporary cysts in the feces can also indicate some postingestive selection (Brillant & MacDonald 2002), which can be an additional strategy to limit the accumulation of toxins in the tissues.

Very few cells of *Heterosigma akashiwo* were observed in the biodeposits of bivalve species tested. Two hypotheses can be suggested. First, the absence of intact *H. akashiwo* in the biodeposits may be a consequence of the fragility of raphidophytes in general, including *H. akashiwo* (Okamoto et al. 2000). Our observations indicate that oysters did not produce any biodeposits; moreover, they remained closed for the hour of the experiment. Because clearance-rate data could not be assessed with *H. akashiwo*, it is not certain that the algae were not consumed, but Hégaret et al. (in press[a]) observed a similar type of behavior in oysters that did not produce feces for 24 h in filtered seawater after they had been exposed to *H. akashiwo* for 48 h, indicating that oysters may not clear much *H. akashiwo*. Similarly, Keppler et al. (2005) observed that oysters, *Crassostrea virginica*, exposed to a similar cell density of *H. akashiwo* for 48 h had a very low rate of consumption. A second alternate hypothesis could be that oysters limit exposure of soft tissues to *H. akashiwo* by partial shell closure and reduced pumping. We were unable to confirm this hypothesis because *H. akashiwo* cells did not remain evenly distributed in containers containing shellfish, but observations of higher cell densities 1 h after introducing oysters suggests that cell removal by the shellfish was minimal. Reduced filtration would also explain the very limited presence of intact cells into the biodeposits. The raphidophyte *H. akashiwo* produces reactive oxygen species (Oda et al. 1997); moreover, brevetoxin-like compounds (HaTX) have also been observed in *H. akashiwo* cells (Khan et al. 1997). Mechanisms associated with *H. akashiwo* toxicity have not been characterized; therefore, it is difficult to interpret the behavior of bivalves when exposed to *H. akashiwo*. Further research seems necessary on interactions between this alga and bivalves, at the cellular level, to be able to characterize the mechanisms of toxicity of *H. akashiwo*.

No intact cells of any kind, whether harmful or not, were observed in the feces of softshell clams, *Mya arenaria*, which is consistent with the findings of Cucci et al. (1985) and of Shumway and Cucci (1987), who demonstrated the absence of intact *Gonyaulax tamarensis* (= *Alexandrium tamarense* = *A. fundyense*) in the feces of *M. arenaria*. These observations have also been made by Hégaret et al. (in press [a]); no intact cells of harmful algae were found in the biodeposits of softshell clams after 48 h of exposure to *A. fundyense*. This phenomenon results from more effective digestive processes in softshell clams, *M. arenaria*. Cucci et al. (1985) also indicated a reduction in pre and postingestive selectivity, reduction in feeding rates, and a slight increase in the feeding rates of mussels, *Mytilus edulis,* after addition of *G. tamarensis* (= *A. fundyense*) to the diet.

The diversity of responses of these bivalve species to three different harmful-algal species indicates that bivalves have evolved different strategies to minimize inimical effects of harmful algae, while maintaining metabolic functions as much as possible. It makes sense, from an evolutionary perspective, that selective pressure to minimize tissue exposure to harmful algae will be dependent on the severity of tissue damage and/or the capacity of digestive processes and cellular defense mechanisms available to protect the individual. Accordingly, results of the present study suggest that differences between bivalve species in their behavioral and filtration responses to harmful algae could offer clues about the importance of digestive and defense cells in mitigating any harmful effects upon tissues of exposed bivalves. Although this study found some quantitative and qualitative evidence for protective changes in filtration and feeding behaviors in bivalves exposed to HABs, in general these responses do not prohibit tissue exposures to the HAB cells. Thus, cellular effects and responses can be expected in bivalves, which experience HABs.

The first line of defense for bivalve molluscs after valve closure is the immune system. Bivalve molluscs, as invertebrates, do not have acquired immunity and are limited to innate immunity, including humoral and a cellular responses (Janeway 1994). The cellular immune response is accomplished by hemocytes, which can be observed in the hemolymph and open vascular system, but also in all the tissues. As ingested microalgal cells pass intact through the digestive system into the feces, they can be in contact with the hemocytes. Indeed, Leibovitz et al. (1984) observed intact *Prorocentrum* cells in the open vascular system of bay scallops feeding in a natural bloom. Moreover, histological observations have shown aggregates of

hemocytes in gill and mantle tissues of bivalve molluscs exposed to *P. minimum* indicating a response of the hemocytes to a harmful algal exposure (Wikfors & Smolowitz 1995). Hemocytes play a role in defense mechanisms involving phagocytosis, production of reactive oxygen species, aggregation, and tissue repair. Therefore, it is important to understand the role of the hemocytes during an exposure of bivalve mollusc to harmful algal species. The present study confirms that encounters between harmful-algal cells and hemocytes will regularly occur in molluscan shellfish exposed to harmful-algal blooms.

This study demonstrates clearance of three harmful algal species by several species of bivalves. Studies have, indeed, shown that harmful algal cells can pass intact and viable through the digestive systems of bivalve molluscs (Laabir & Gentien, 1999, Springer et al. 2002, Hégaret et al. (in press [a]). The presence of these cells in the biodeposits, especially the feces, emphasizes the importance of considering the risk for bivalve molluscs to be vector of transport of harmful algae into new environments because bivalve molluscs are moved from one body of water to another.

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