

Potential transport of harmful algae via relocation of bivalve molluscs

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ABSTRACT: Aquaculture and restoration activities with bivalve molluscs often involve moving individuals from one body of water to another. Our study tests the hypothesis that harmful algae ingested by source populations of shellfish can be introduced into new environments by means of these shellfish relocations. Cultures of several harmful algal strains, including *Prorocentrum minimum*, *Alexandrium fundyense*, *Heterosigma akashiwo*, *Aureococcus anophagefferens*, *Karenia mikimotoi* and *Alexandrium monilatum*, were fed to various species of bivalve molluscs, *Crassostrea virginica*, *Argopecten irradians irradians*, *Mercenaria mercenaria*, *Mytilus edulis*, *Mya arenaria*, *Venerupis philippinarum* and *Perna viridis*, to assess the ability of the algal cells to pass intact through the digestive tracts of the shellfish and subsequently multiply in number. Ten individuals of each shellfish species were exposed for 2 d to a simulated harmful algal bloom at a natural bloom concentration. The shellfish were removed after exposure, and maintained for 2 further days in ultra-filtered seawater. Biodeposits (feces) were collected after 24 and 48 additional hours, and observed under light microscopy for the presence or absence of intact, potentially viable algal cells or temporary cysts. Subsamples of biodeposits were transferred into both algal culture medium and filtered seawater and monitored for algal growth. Intact cells of most harmful algal species tested were seen in biodeposits. Generally, harmful algae from the biodeposits collected in the first 24 h after transfer re-established growing populations, but algae could less often be recovered from the biodeposits collected after 48 h. These data provide evidence that transplanted bivalve molluscs may be vectors for the transport of harmful algae and that a short holding period in water without algae may mitigate this risk. Further, preliminary results indicate that emersion may also serve to mitigate the risk of transport.

KEY WORDS: Bivalve mollusc · Harmful algal bloom · Toxic algae · Transport · Clam · Scallop · Oyster

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INTRODUCTION

Introductions of non-indigenous species can have major, unpredictable effects on native populations and communities, leading to fundamental changes in ecosystem function that compromise local economies and human health (Ruiz et al. 2000a). Many introductions of non-native species have occurred in the last decades, and shipping and fisheries activities have been identified as the dominant vectors for marine, invasive introductions (Ruiz et al. 2000a, Ruiz et al.

2000b). Furthermore, occurrences of harmful algal blooms are increasing on a global level in frequency, intensity, and geographic distribution (Hallagraeff 1993). Founding populations of harmful dinoflagellates and other microalgal taxa can be active cells, as well as resting stages or cysts. The role of ballast water exchanges in the introduction of new invasive algal species, including dinoflagellates and other harmful taxa, has been established (Hamer et al. 2000); however, there have been few documented cases of successful invasion by this vector. In addition, many shell-

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fish management practices have the potential to inadvertently introduce harmful algal species into new environments.

Movement of shellfish has been reported or suggested as a potential vector for transport into new environments of various non-native microorganisms such as parasites, pathogenic bacteria (Carriker 1992, Ruesink et al. 2005), and harmful algae (Bricelj et al. 1993, Vila et al. 2001, Lilly et al. 2002, Penna et al. 2005). Recognition that parasites and other pathogens can be transported in the soft tissues of bivalve molluscs (Carriker 1992), has led to regulation of transplant of bivalves across jurisdictional boundaries by agencies such as the Interstate Shellfish Sanitation Committee (ISSC) and the National Shellfish Sanitation Program (NSSP) (www.issc.org/NSSP/Default.aspx), often requiring comprehensive health assessments (Van den Bergh et al. 2002). The risk of co-introduction of harmful algal bloom species (HABs) with transplanted bivalves has been identified (Laabir & Gentien 1999, Bauder & Cembella 2000, Harper et al. 2002), but not investigated systematically. Consequently, no regulatory or risk-mitigation measures are in place to protect coastal and estuarine environments from inadvertent introduction of HABs by this vector.

Harmful algal species vary in their effects on a given bivalve species (Shumway 1990, Landsberg 2002), and behavioral responses of different bivalve species to a given harmful alga also vary. Bivalve molluscs can feed selectively (Ward & Shumway 2004) and thus avoid or limit ingestion of harmful algae; i.e. after shell closure, modified feeding is the next line of defense of bivalves against a HAB. In addition, bivalves can eliminate some harmful algal cells in feces and pseudofeces; biodeposits often contain incompletely digested microalgal and protozoan cells. Springer et al. (2002) demonstrated the passage of intact cysts of toxic *Pfiesteria piscicida* Steidinger et Burkholder through the digestive tract of oysters and their ability to recover from the feces within 24 h of gut passage, yielding viable flagellated cells. Toxic strains of the closely related species *Pfiesteria shumwayae* Glasgow et Burkholder showed the same, although more limited, capability in oysters, scallops, mussels and clams (Shumway et al. 2006). Thus, based upon previous studies (Bardouil et al. 1993, Bricelj et al. 1993, Laabir & Gentien 1999, Bauder & Cembella 2000, Li et al. 2001, Springer et al. 2002) and our preliminary observations, it appears likely that transplanted bivalve molluscs could introduce HABs into new areas; however, the relative risk associated with different bivalve and HAB species remains poorly understood.

Given the frequency and regularity of shellfish transfers (Powell & Ashton-Alcox 2004), it is important to know which HAB species might be transferred inad-

vertently to new geographic locations by shellfish transplantation so that risk management strategies can be developed. The purpose of this study was to assess the risk of several harmful algal species being transported within several bivalve species by exposing bivalves to HAB taxa in the laboratory, moving the shellfish to ultrafiltered seawater, examining biodeposits for intact, potentially viable algal cells, and finally testing for the re-establishment of HAB populations from the biodeposits of bivalves moved from the simulated HABs to ultrafiltered seawater. The 6 microalgal taxa included in the study are common components of phytoplankton assemblages in waters used for shellfish aquaculture, and have been shown to be toxic or otherwise harmful to shellfish. The bivalve species tested are ecologically and/or commercially important on the USA East Coast. Moreover, the shellfish species from the USA East Coast or the French coast that we studied have been moved from one body of water to another as part of routine aquaculture management.

MATERIALS AND METHODS

Bivalve molluscs. Experiments with bivalve molluscs were conducted in 3 locations: Connecticut in the northeastern USA, the Brest area of France, and North Carolina in the southeastern USA. The HAB–bivalve combinations tested are presented in Tables 2 & 3. For the experiments in Connecticut (5 bivalve species) and France (3 bivalve species), shellfish were obtained from the same general location as the experiments to avoid transport of bivalves across state or country boundaries. Experiments conducted in North Carolina included 2 bivalve species that were imported from other states and 1 species obtained from local waters. Juveniles or young adult stages were used to minimize potential for spawning to interfere with results. An assumption across all experiments was that the individual shellfish had had no prior exposure to blooms of the harmful algal species tested (See ‘Materials and methods; Algal cultures’ below).

Experiments in Connecticut were conducted between May and October, 2005, at the National Oceanic and Atmospheric Administration–National Marine Fisheries Service (NOAA NMFS) in Milford. Three species: blue mussel *Mytilus edulis* Linnaeus (shell length [SL]: 35 to 45 mm), eastern oyster *Crassostrea virginica* Gmelin (SL: 35 to 45 mm), and soft-shell clam *Mya arenaria* Linnaeus (SL: 60 to 70 mm), were obtained from local shellfish harvesters. Northern quahogs (hard clams *Mercenaria mercenaria* Linnaeus) (SL: 45 to 55 mm), were collected from local waters by a recreational diver, and northern bay scal-

lops *Argopecten irradians irradians* Lamarck (SL: 35 to 45 mm) were provided by the Milford Laboratory. All shellfish were maintained in the NOAA Milford Laboratory for several weeks in running, unfiltered seawater prior to the experiments.

Experiments conducted in France were completed during October through December, 2005, at the Institut Universitaire Européen de la Mer (IUEM). Adult *Mytilus edulis* (SL: 35 to 45 mm) were harvested at Pointe du Diable adjacent to the IUEM and acclimated for 1 wk. Manila clams *Venerupis philippinarum* Adams and Reeve (SL: 35 to 45 mm) were from the Island of Bailleron in the Morbihan Golfe in Brittany, France. Bivalves were acclimated for 1 wk in unfiltered seawater.

Experiments conducted in North Carolina were completed during summer of 2005 at the Center for Applied Aquatic Ecology (CAAE), North Carolina State University in Raleigh. Green mussels *Perna viridis* Linnaeus (means \pm SD; juveniles, SL: 26.7 \pm 2.8 mm; adults, SL: 48.9 \pm 8.4 mm) were collected off the Gandy Bridge in Tampa Bay, Florida, USA. *Crasostrea virginica* (juveniles, SL: 29.1 \pm 0.9 mm; adults, SL: 69.8 \pm 2.3 mm) were obtained from the Pemaquid Oyster Company, Waldoboro, Maine. Adults of *Mercenaria mercenaria* (SL: 44.1 \pm 0.8 mm) were supplied by J and B AquaFood, Jacksonville, North Carolina and juveniles (SL: 13.3 \pm 0.4 mm) were obtained from Millpoint Aquaculture, Core Sound, North Carolina. Shellfish were acclimated to laboratory conditions at 22 to 25°C and in 30 to 32 ppt artificial seawater (ASW) in separate 950 l (adults) and 500 l (juveniles) recirculating systems for at least 1 wk prior to experiments. The shellfish were fed Instant Algae® Shellfish Diet 1800® (Reed Mariculture, Campbell, California, USA) or cultured *Pavlova* sp. during the acclimation period.

Algal cultures. Among the 6 harmful algal species used in this study, the dinoflagellates *Alexandrium fundyense* Balech, *Alexandrium monilatum* (Howell) Balech and *Prorocentrum minimum* (Pavillard) Schiller can be toxic to finfish and shellfish, and the raphidophyte *Heterosigma akashiwo* (Hada) Hada ex Sournia can be toxic to finfish (Burkholder 1998, Landsberg 2002, Pate 2006). The dinoflagellate *Karenia* (= *Gymnodinium*) *mikimotoi* (Miyake & Kominami ex Oda) Hansen et Moestrup is a common phytoplankton off the coast of Brittany, France, and blooms in locations where *Venerupis philippinarum* or *Mytilus edulis* are harvested. The pelagophyte *Aureococcus anophagefferens* Hargraves et Sieburth is responsible for the brown tides on the east coast of the United States. The cultures were unialgal and contained bacteria.

The algae tested in the Connecticut experiments were cultures from the NOAA Milford Laboratory Microalgal Culture Collection and included *Prorocentrum minimum* strain JA-98-01 (isolated from the Choptank River, Maryland, USA); *Alexandrium fundyense*, strain BF2 (isolated from the Gulf of Maine, USA); *Heterosigma akashiwo* strain OL (isolated from New Jersey, USA); and *Aureococcus anophagefferens* strain CCMP1708 (isolated from West Neck Bay in 1995). The microalgae were cultured in 20 l glass carboy assemblies using aseptic technique (Ukeles 1973). Cultures were harvested semi-continuously to maintain consistency in culture quality over the course of the study and used in log 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, double the EDTA of the standard E formulation, KNO₃ rather than NaNO₃, and soil extract. *Alexandrium fundyense* was grown in F/2-enriched Milford seawater; *Heterosigma akashiwo* was cultured in E-medium; and *Aureococcus anophagefferens* was grown in L-1 (enriched with soil extract) culture medium. Cultures were maintained at 20°C on a 12:12 h light:dark cycle and used in log phase. Algal cell densities were determined by hemocytometer counts using the light microscope. For *A. anophagefferens*, cell clumping made it difficult to obtain precise counts; therefore, exposure cell density was quantified based upon the dry weight (dry wt) of the cells in a known volume of culture. The concentration used for bivalve exposures was equivalent to that of a natural bloom of *A. anophagefferens* (Table 1).

For the experiments conducted in France, the toxigenic dinoflagellate *Karenia mikimotoi* (Stock GM95TIN, isolated at Tinduff, Rade de Brest, France) from the IFREMER Brest collection was used and grown in the IUEM laboratory in sterile, 6 l carboys. Medium used for these cultures was F/2-enriched, 1 μ m-filtered Argenton seawater. The cultures were grown at 18°C on a 12:12 h light:dark cycle. Algal cell

Table 1. Bloom concentrations (cells ml⁻¹, except where otherwise indicated) of harmful algae used for the experiments simulating natural blooms

Algal species	Concentration	Source
<i>Prorocentrum minimum</i>	10 ⁴	Hégaret & Wikfors (2005)
<i>Alexandrium fundyense</i>	10 ³	Shumway et al. (1988)
<i>Heterosigma akashiwo</i>	10 ⁴	Rensel & Whyte (2003)
<i>Aureococcus anophagefferens</i>	9 (mg l ⁻¹)	Nuzzi & Waters (1989)
<i>Karenia mikimotoi</i>	10 ³	Matsuyama et al. (1999)
<i>Alexandrium monilatum</i>	5.5 \times 10 ²	Perry et al. (1979) Q. Dortch (unpubl.)

densities were determined by hemocytometer counts using the light microscope.

Experiments conducted in North Carolina used toxic *Alexandrium monilatum* (strain AMO3; cell length 28 to 52 μm) provided by Dr. S. Morton (NOAA, Charleston, SC). *A. monilatum* was mass-cultured in 10 l Nalgene® polycarbonate carboys with L-1 media (Guillard & Hargraves 1993). The strain was sent to the Hollings Marine Laboratory (NOAA, Charleston, SC) for toxin analysis prior to experiments. A GH4C1 rat pituitary cytotoxicity bioassay was conducted on 1 l of culture following Hsia et al. (2005). The *A. monilatum* culture was confirmed to be toxic with the GH4C1 rat pituitary cell line cytotoxicity bioassay. Cultures were grown at 23°C on a 12:12 h light:dark cycle at 170 $\mu\text{E m}^{-2} \text{s}^{-1}$. The growth medium was prepared with salinity 30, sterilized, filtered (0.45 μm pore size), artificial seawater (Coralife® scientific grade marine salt, Pet-Smart Inc.). Cultures of *A. monilatum* were in late log to stationary phase for experiments. Prior to use, cultures were shaken vigorously to break up any long cell chains, a method that has been successful when used for other algal species (G. H. Wikfors unpubl. obs.). Subsamples were preserved with acidic Lugol's solution before each experiment to determine cell concentrations in Sedgwick-Rafter counting slides, using an Olympus BH2 light microscope (Olympus Corporation, Melville, NY).

Presence and recovery of intact harmful algal cells from biodeposits. To assess the presence of intact harmful algal cells and their recovery from biodeposits of bivalves, 10 individual bivalves were held for 48 h in filtered seawater (FSW), to deplete previously consumed algae and to become acclimated to the experimental conditions. Acclimated shellfish then were transferred to an 80 l basin containing a cultured HAB strain at a natural bloom concentration (Table 1); these concentrations were taken from published and unpublished monitoring data. Bivalves were held for 48 h in the algal suspension, with the following exceptions: *Mercenaria mercenaria* and *Crassostrea virginica* were exposed to the toxic dinoflagellate *Alexandrium monilatum* for 24 h, and juvenile and adult *Perna viridis* were exposed to *A. monilatum* for 8 h because the mussels died when exposed to *A. monilatum* for 24 h. After HAB exposure, shellfish were transferred to individual, 3 l containers of FSW for 24 h, followed by an additional transfer to new FSW for 24 h. Feces were collected for microscopic observation. Qualitative microscopic observations of the feces, under white light or blue-light fluorescence excitation (for red chlorophyll *a* fluorescence visualization), allowed determination of whether intact cells were able to pass through the digestive systems of bivalves after 24 or 48 h.

To determine the potential for any intact cells in biodeposits to re-establish viable algal populations, 500 μl sub-samples of fecal slurry produced after 24 and 48 h were collected from each individual basin, mixed by vortexing, and inoculated into test tubes containing (1) for the North Carolina experiments: 5 ml of FSW, or salinity 30 natural seawater (NSW); or (2) for the experiments in Connecticut and France: 5 ml of FSW or 5 ml of the culture medium in which the harmful alga was grown. Tubes were incubated on a 12:12 h light:dark cycle, and were observed weekly for up to 3 mo using an inverted microscope to assess the presence of motile algal cells or viable cysts.

In addition to the standard procedure described above, wherein bivalves were held for 24 and 48 h in FSW after exposure to a simulated bloom of *Karenia mikimotoi* (Table 1), 2 other experiments were carried out with *Mytilus edulis* and *Venerupis philippinarum* and this dinoflagellate species. In the first set of experiments, mussels and clams were held for 48 h in a simulated bloom of *K. mikimotoi* and then transferred directly into individual containers (1 bivalve per container) with FSW for 24 h, and then for an additional 24 h. Tubes of FSW inoculated with feces of mussels or clams were checked for motile cells of *K. mikimotoi* after 24 h only. In the second set of experiments, clams and mussels were exposed to *K. mikimotoi* for 48 h followed by dry storage out of water for 24 h, simulating a commonly used transport condition. Shellfish were then placed back in FSW, and tubes were checked for motile cells of *K. mikimotoi* after 24 h. Feces were collected, observed under light microscopy and cultured as described above to check for gut passage and survival of *K. mikimotoi*.

Data presentation and analysis. Results from the microscopic observations of the feces and the reculture of the harmful algae in test tubes inoculated with fecal samples are descriptive only, because the presence of cells is unequivocal, but absence of observed cells in an individual sample was not considered conclusive evidence for their absence—that is, cells still could have been present that were missed in observations.

RESULTS

Presence of intact cells in the feces

Intact cells in the biodeposits were observed after 24 and 48 h in FSW from most of the bivalves (Table 2). The softshell clam *Mya arenaria* was exceptional in that its biodeposits had essentially no intact cells 24 or 48 h post-ingestion. Only once were 2 cells of *Heterosigma akashiwo* observed in a fecal pellet between

Table 2. Presence of intact harmful algae (HAB) cells in the biodeposits of bivalves after 24 and 48 h of depuration in FSW (+: intact cells documented; -: no intact cells detected; nt: HAB/mollusc pair not tested)

Bivalve	<i>Alexandrium fundyense</i>	<i>Alexandrium monilatum</i>	<i>Aureococcus anophagefferens</i>	<i>Heterosigma akashiwo</i>	<i>Gymnodinium mikimotoi</i>	<i>Prorocentrum minimum</i>
24 h						
<i>Argopecten irradians</i>	+	nt	+	+	nt	+
<i>Crassostrea virginica</i>	+	+	+	No feces	nt	+
<i>Mercenaria mercenaria</i>	+	+	-	-	nt	+
<i>Mya arenaria</i>	-	nt	nt	-	nt	-
<i>Mytilus edulis</i>	+	nt	No feces	-	-	+
<i>Perna viridis</i>	nt	+	nt	nt	nt	nt
<i>Venerupis philippinarum</i>	nt	nt	nt	nt	-	nt
48 h						
<i>Argopecten irradians</i>	+	nt	-	+	nt	-
<i>Crassostrea virginica</i>	+	+	+	-	nt	+
<i>Mercenaria mercenaria</i>	+	+	-	-	nt	-
<i>Mya arenaria</i>	-	nt	nt	-	nt	-
<i>Mytilus edulis</i>	-	nt	+	-	-	-
<i>Perna viridis</i>	nt	+	nt	nt	nt	nt
<i>Venerupis philippinarum</i>	nt	nt	nt	nt	-	nt

24 and 48 h in FSW. A single, apparently intact cell of *Alexandrium fundyense* was also seen in a fecal strand after 24 h in FSW. Algal cells in fecal pellets of *M. arenaria* always appeared to have been digested (Fig. 1).

The toxic raphidophyte *Heterosigma akashiwo* was not found in most biodeposits of the bivalve species tested after 24 or 48 h in FSW. Bay scallops were the only bivalves that produced fecal strands with intact *H. akashiwo* cells (Fig. 1A,B). No cysts of *H. akashiwo* were observed in any biodeposits. In contrast, *Prorocentrum minimum* was present in the biodeposits of all bivalve species after 24 h in FSW (Fig. 1C,D), except for *Mya arenaria* (Fig. 1E,F). Intact cells of *P. minimum* were usually not observed after more than 24 h of depuration: intact cells were seen in biodeposits of *Crassostrea virginica* after 24 and also 48 h in FSW, but only after 24 h in feces from *Mercenaria mercenaria* (Fig. 1C,D), *Argopecten irradians* and *Mytilus edulis*.

Intact cells of *Alexandrium fundyense* were present in the biodeposits of *Crassostrea virginica* (Fig. 2E), *Mercenaria mercenaria* (Fig. 2A,B), *Argopecten irradians* (Fig. 2C) after 24 and also 48 h in FSW. Feces from *Mytilus edulis* exposed to *A. fundyense* contained intact cells at 0 to 24 h in FSW, but not after 24 h. Numerous cells of *A. fundyense*, both vegetative cells and temporary cysts (Persson et al. 2006) were observed in the biodeposits of *M. mercenaria* (Fig. 2A,B).

Cells of the brown tide alga *Aureococcus anophagefferens* tended to aggregate and form clumps in the feces. These cells were much smaller (diameter < 2 µm) than cells of the other microalgae tested and were difficult to identify within the fecal debris. Aggregates of *A. anophagefferens* cells were clearly discerned in the biodeposits of *Mytilus edulis* after

48 h only, as *M. edulis* did not produce biodeposits in the first 24 h. Similar aggregates of *A. anophagefferens* were observed in fecal pellets of *Argopecten irradians* after 24 h in FSW but not after 48 h, and in fecal material of *Crassostrea virginica* after 24 and 48 h in FSW. No intact cells of *A. anophagefferens* were found in the biodeposits of *Mercenaria mercenaria* after 24 or 48 h.

Intact cells of *Karenia mikimotoi* were not seen in the biodeposits of *Mytilus edulis* or *Venerupis philippinarum* after 24 or 48 h in FSW, whether they had been previously held in or out of the water. In contrast, analysis of fecal material collected from *Crassostrea virginica* (Fig. 2F), *Perna viridis* and *Mercenaria mercenaria* (Fig. 2D) after 24 and 48 h in FSW following exposure to *Alexandrium monilatum* indicated that some cells were able to pass intact though the digestive tracts of these shellfish species.

Recovery of HAB cells from shellfish feces

24 and 48 h after bivalves had been exposed to harmful algal species and then held in FSW without algae, feces were collected and cultured. Most tubes inoculated with fecal slurry showed growth of harmful algal species from biodeposits collected during the first 24 h in FSW. Usually, *Heterosigma akashiwo* reformed a 'bloom' after 2 to 3 wk of incubation in FSW, but not in E-medium. Similar lack of growth in culture media was noted for the other harmful algal species tested; cell proliferation was limited to tubes containing FSW. The tubes containing culture media were usually contaminated with diatoms that apparently

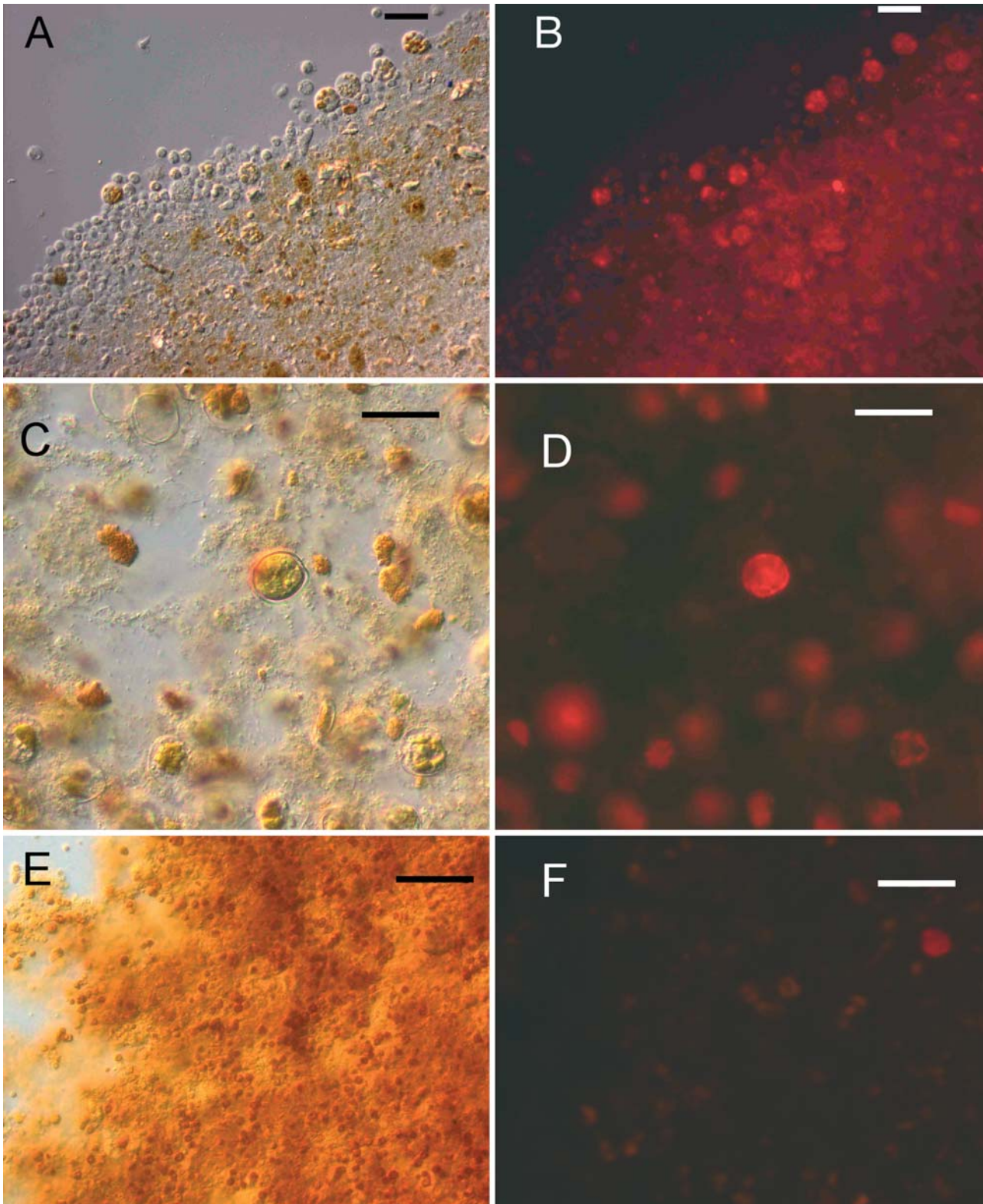


Fig. 1. Photomicrographs with (A,C,E) white light and (B,D,F) blue light/epifluorescence to show chlorophyll *a* of fecal pellets produced by molluscan species after being exposed to simulated blooms of cultured harmful algae bloom (HAB) species and then moved to ultrafiltered seawater for 24 h. (A,B) Intact fluorescent cells of *Heterosigma akashiwo* in fecal material from *Argopecten irradians*. (C,D) Intact fluorescent cells of *Prorocentrum minimum* in fecal material from *Mercenaria mercenaria*. (E,F) Feces from *Mya arenaria* exposed to *P. minimum*, showing no intact cells. Scale bars = 20 μ m

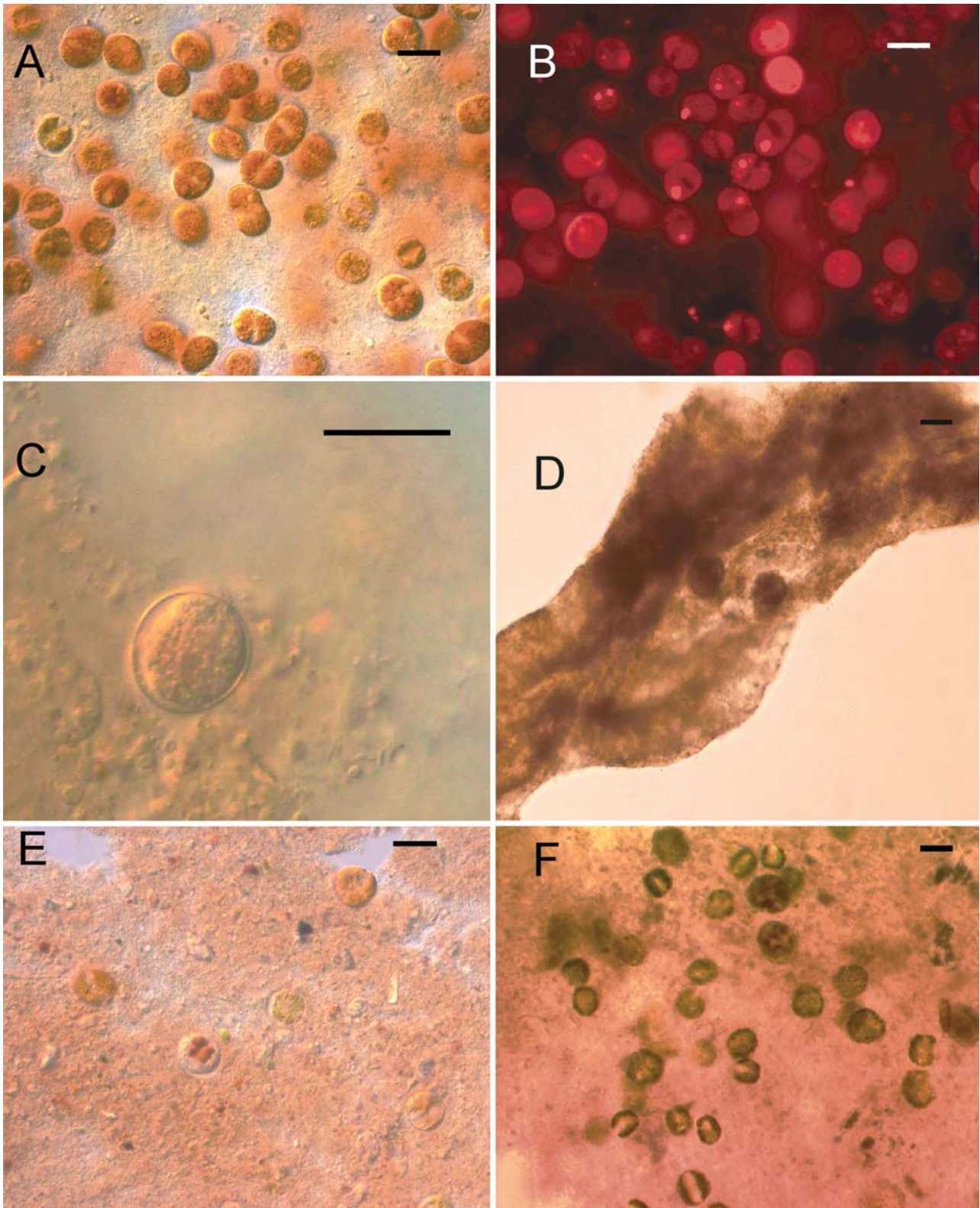


Fig. 2. Photomicrographs of fecal pellets produced by molluscan species after being exposed to simulated blooms of cultured *Alexandrium* spp. and then moved to ultrafiltered seawater for 24 h, except as noted. (A) Temporary cysts of *A. fundyense* in fecal material from *Mercenaria mercenaria*. (B) Epifluorescence of chlorophyll *a*, same field as (A). (C) Temporary cysts of *A. fundyense* in fecal material from *Argopecten irradians* 48 h after removal from the simulated bloom. (D) Intact cells of *A. monilatum* in fecal material from *M. mercenaria* after 24 h. (E) Cells of *A. fundyense* in fecal material from *Crassostrea virginica* after 24 h. (F) Intact *A. monilatum* cells in feces of *C. virginica* produced 48 h after removal from the simulated bloom. Scale bars = 20 μ m

were ingested prior to acclimation and survived gut passage, whereas few harmful algal flagellates were found.

In FSW, *Prorocentrum minimum* produced motile cells from shellfish biodeposits after 24 h, but rarely did so after more than 24 h, except for a few isolated cells in the tubes with feces from one oyster and one clam (Table 3). Populations required 4 to 6 wk to recover. Motile cells of *Alexandrium fundyense* also were found in the FSW after the first 24 h, but not after 48 h (Table 3), and populations usually recovered after 6 to 8 wk. In fact, after 48 h in FSW, observed recovery of most HAB species was almost nonexistent. As exceptions, cysts of *Alexandrium fundyense* from biodeposits of 1 scallop incubated for 48 h produced a few motile cells when cultured in F/2 medium. More notably, motile cells of *Heterosigma akashiwo* were observed from fecal inocula of *Crassostrea virginica* after 48 h in the tubes containing FSW. It should be noted that *C. virginica* did not produce feces during the first 24 h; therefore, the only sampling possible was after 48 h.

Consistent with the observation of very few intact cells in biodeposits of *Mya arenaria*, not a single motile cell was observed in any tubes inoculated with biodeposits from this shellfish species (Table 3). *Heterosigma akashiwo* did not recover from fecal pellets of the blue mussels *Mytilus edulis* (Table 3).

Cells of *Karenia mikimotoi* were observed in tubes inoculated with feces from *Mytilus edulis* or *Venerupis philippinarum* after 24 h in FSW, but not after 48 h. In contrast, after dry storage of shellfish followed by return to FSW for 24 h, no cells of *K. mikimotoi* were found in tubes inoculated with fecal samples.

Recovery of *Aureococcus anophagefferens* was not assessed during this study, as it was very difficult to recognize and confirm that *A. anophagefferens* cells were present or absent in cultures with fecal inocula. Further studies are planned to assess the potential recovery of *A. anophagefferens* from bivalve shellfish biodeposits, with immunofluorescent detection techniques to count *A. anophagefferens*.

No *Alexandrium monilatum* cells were observed in tubes inoculated with fecal slurry from juvenile or adult *Mercenaria mercenaria*, or from juvenile *Perna viridis* or *Crassostrea virginica*. During the first week, motile *A. monilatum* cells were found in 2 of 10 tubes with feces from adult *P. viridis* collected after 24 h in NSW. No cells were observed in tubes with feces that were collected after 48 h in FSW, or in tubes with feces in L-1 medium. Tubes inoculated with feces from adult *C. virginica* also contained swimming cells of *A. monilatum* within the first week. Cells were observed in the tubes inoculated with feces collected after 24 h of depuration in L-1 medium (2 of 10 replicates) and in NSW (2 of 10 replicates). Only 1 replicate from the feces inoculated in FSW after 48 h of depuration contained swimming *A. monilatum* cells. After 2 wk and 4 wk, no *A. monilatum* cells were observed in any tubes inoculated with feces. In contrast, *A. monilatum* cell production occurred in the controls with L-1 medium (division rate, $k = 0.19 \text{ d}^{-1}$) or with NSW ($k = 0.24 \text{ d}^{-1}$).

DISCUSSION

This study is the first to compile a screening table of survival and recovery of cells or cysts of harmful algae

Table 3. Recovery of harmful algae bloom (HAB) cells from the biodeposits cultured in the tubes containing FSW after 24 and 48 h of depuration in FSW (+: motile cells documented; -: no motile or intact cells detected; nt: HAB/mollusc pair not tested)

Bivalve	<i>Alexandrium fundyense</i>	<i>Alexandrium monilatum</i>	<i>Aureococcus anophagefferens</i>	<i>Heterosigma akashiwo</i>	<i>Karenia mikimotoi</i>	<i>Prorocentrum minimum</i>
24 h						
<i>Argopecten irradians</i>	+	nt	nt	+	nt	+
<i>Crassostrea virginica</i>	+	+	nt	No feces	nt	+
<i>Mercenaria mercenaria</i>	+	-	nt	+	nt	+
<i>Mya arenaria</i>	-	nt	nt	-	nt	-
<i>Mytilus edulis</i>	+	nt	nt	-	+	+
<i>Perna viridis</i>	nt	+	nt	nt	nt	nt
<i>Venerupis philippinarum</i>	nt	nt	nt	nt	+	nt
48 h						
<i>Argopecten irradians</i>	-	nt	nt	+	nt	-
<i>Crassostrea virginica</i>	-	+	nt	+	nt	-
<i>Mercenaria mercenaria</i>	-	-	nt	-	nt	-
<i>Mya arenaria</i>	-	nt	nt	-	nt	-
<i>Mytilus edulis</i>	-	nt	nt	-	-	-
<i>Perna viridis</i>	nt	-	nt	nt	nt	nt
<i>Venerupis philippinarum</i>	nt	nt	nt	nt	-	nt

after passage through the digestive tracts of several species of bivalve molluscs with a consistent procedure that facilitates comparisons of mollusc–HAB pairs. Of 24 mollusc–HAB combinations tested, 15 showed intact HAB cells in biodeposits. Viability of the algal cells was confirmed in 15 of the 20 mollusc–HAB interactions demonstrated by re-establishment of motile populations from biodeposit samples. The presence of intact cells in fecal pellets was a reasonably good predictor of the risk of HAB population establishment. Evidence of viable gut passage of the majority of the harmful algal species tested, coupled with their broad ecophysiological tolerance and ability to form cysts, suggests that HAB cells released from bivalve biodeposits can have a high probability for survival and growth in nature. Thus, the data support the premise that shellfish transfer from one body of water to another presents a risk of introducing harmful algal blooms into new areas (Table 3). This risk is lowered after a 24 h ‘depuration’ step in FSW (Table 3). In this study, only *Heterosigma akashiwo* was able to recover from passage through *Argopecten irradians* and *Crassostrea virginica* digestive systems after 48 h in FSW. *C. virginica* did not produce feces during the first 24 h in FSW; thus, the only sampling possible was after 48 h.

One notable exception to the general finding that HAB cells can pass intact and viable through the molluscan digestive system is *Mya arenaria*, which seems less likely than other molluscs to be a potential vector for HAB transport. Almost no intact HAB cells could be found in the biodeposits of *M. arenaria*; moreover, no viable populations of any HAB species tested were re-established from *M. arenaria* biodeposits. Thus, risk of transferring HABs during movement of *M. arenaria* appears to be minimal. In this study, softshell clams moved from a simulated HAB species to FSW produced feces containing no intact algae; therefore, digestive processes in *M. arenaria* appear to minimize risk of release of viable algal cells in biodeposits. Similarly, fecal pellets of *Mytilus edulis* did not contain intact cells of *Heterosigma akashiwo*, and *H. akashiwo* populations did not recover from fecal samples, suggesting that there is low risk for *M. edulis* to transport this harmful species.

Cells of *Alexandrium monilatum* appeared to be intact within the feces of all shellfish species tested. This species showed poor recovery, however, from the feces of juvenile and adult *Mercenaria mercenaria* or of juvenile *Perna viridis* and *Crassostrea virginica*. Cultures with inoculated feces from adult *P. viridis* and *C. virginica* contained swimming cells of *A. monilatum*, but no cell growth was observed. Control cultures of *A. monilatum* grew under the same conditions, indicating that egested *A. monilatum* cells were incapable

of cell division. Owen & Norris (1982) suggested that *A. monilatum* cysts may have been introduced into new areas through the movement of raft-cultured oysters transferred from areas where blooms of *A. monilatum* have occurred. Whether shellfish species can function as vectors for the introduction of *A. monilatum* may depend on the strain of *A. monilatum* or other factors such as the shellfish species and environmental conditions.

The results reported in this paper are qualitative; when a tube inoculated with feces grew the harmful algae, the bivalve–harmful algae combination was considered to be a definite risk. When no viable or motile cells or cysts were observed in either the biodeposits or the tubes, the risk might still exist, although not observed under our experimental conditions. Our tubes were assessed every week for up to 3 or 4 mo, and some algal species consistently took longer to recover than others. We note that diatoms, mainly pennate, often dominated tubes inoculated with bivalve feces. Recovery of vegetative algal populations from fecal slurries occurred only in un-enriched filtered seawater. Enriched media, in contrast, are often inoculated with very high initial populations of microalgae. Typically there is a ‘lag’ phase between inoculation and initiation of cell division. This delay in active growth is attributed to some physiological and chemical accommodation of the cells to the medium, possibly involving the release of ectoenzymes necessary for nutrient or trace-element acquisition (Fogg 1983). Under-inoculated cultures often fail, and a similar lack of initial cell biomass in the cultures with inoculated feces in this study may have been a factor in the observed lack of population recovery in enriched seawater media. In un-enriched seawater, nutrients would have been present at natural concentrations, and macronutrient regeneration by bacterial decomposition of fecal matter apparently was sufficient to support noticeable population growth of most microalgae tested.

Many studies and discussions with shellfish growers suggest a potential risk of transfer of harmful algae via transport of bivalves; however, data to support this hypothesis have been scarce. There is some evidence in the literature that co-introductions of HABs with shellfish transfer have occurred, and that current practices carry a high risk of more introductions of HABs. It has been hypothesized, for example, that *Alexandrium catenella* was introduced in the Thau Lagoon in France several years before it bloomed and produced enough toxicity to be noticed by the public (Lilly et al. 2002). Analysis of radionucleotide data from *Gymnodinium catenatum* cysts in Tasmania indicated that this species was introduced and present for 8 yr before it was detected in the water (McMinn et al. 1997). Previously

published observations of intact harmful algal cells in shellfish biodeposits are widely scattered in articles with various, related objectives and obtained by a wide range of methods (Bricelj et al. 1993, Laabir & Gentien 1999, Bauder & Cembella 2000, Harper et al. 2002, Laabir et al. 2007). In addition, results can depend on specific mollusc–HAB interactions, the length of exposure, and the depuration period. Thus, our systematic approach provides the best assessment to date of risk of transfer.

As transport of molluscan bivalves is common worldwide, our findings underscore the need for caution and vigilance of harmful algal presence in waters from which shellfish are taken. Shellfish growers and aquaculturists regularly move shellfish from site to site for grow-out purposes (Powell & Ashton-Alcox 2004). Further, live shellfish are sold in seafood markets worldwide, increasing the potential for accidental introduction of harmful algal species (Olson 2001), and resilient organisms transported with them, both within and on the shells.

Movement of shellfish for depuration of toxic algae is not commonly practiced; however, it is known in some areas (Silvert & Cembella 1995, Hamer et al. 2000) and has been proposed in others. Our study shows that, in general, harmful algae from the biodeposits collected in the first 24 h after transfer re-established growing populations, but algae were less often able to recover from the biodeposits collected after 48 h. Thus, a holding period in FSW for 24 h was often sufficient for gut clearance, reducing the risk of recovery of harmful algae from biodeposits. Our findings also indicate that a 24 h period out of water could be an effective method to minimize transfer of some harmful algae with shellfish transport. Thus, we propose to mitigate the risk by modification of some shellfish-transfer practices. Specifically, a holding step is supported by our finding of fewer viable cells after 48 h, compared to 24 h, and by the apparent effectiveness of dry storage in one instance. More experiments are necessary to assess the possibility of a 24 h holding period for bivalves out of water. Further studies are underway to test other holding strategies, such as wider application of the 24 h out-of-water step, for bivalve species that can tolerate this treatment; this would be simple and convenient because water and containers for the holding period would not have to be decontaminated before discharge.

CONCLUSIONS

We have demonstrated that transplanted bivalve molluscs can potentially serve as vectors for introduction of harmful algae. This risk is widespread among

the bivalve–HAB species combinations tested. Species-specific shellfish–HAB interactions should be considered in management, restoration, and aquaculture activities involving shellfish transplant activities. Mitigation of the risks of HAB transport by a holding period in seawater or out of water for 24 h appears promising, but will require further development and trials before a final protocol can be recommended.

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