Harmful algae can be transported via relocation of bivalve shellfish

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Abstract

Our study tested the hypothesis that harmful algae can be introduced into new environments by shellfish relocations, a common practice for commercially-exploited bivalve molluscs. We identified which managed shellfish species and HABs co-occur geographically and established a protocol to assess the potential of the bivalve species as vectors for transport of harmful algae. Cultured strains of harmful algae, *Alexandrium fundyense*, *Heterosigma akashiwo*, *Prorocentrum minimum*, and *Karenia mikimotoi* were fed to bivalve molluscs for two days at natural bloom concentrations to assess the ability of the algal cells to pass intact through the digestive tract and subsequently grow. After feeding, the bivalves were kept for two days in ultrafiltered seawater. Biodeposits were collected and observed under the microscope after 24 and 48 h to evaluate the presence or absence of intact, possibly-viable cells or temporary cysts. Subsamples of biodeposits were transferred into both algal culture medium and filtered seawater and monitored microscopically for algal growth. Intact algal cells of the various harmful algae were seen in biodeposits (feces) and generally these re-established growing populations.

Introduction

During the last decade, many introductions of non-native species, including toxic and ecosystem-disruption microalgae, have occurred. The dominant vectors are thought to be shipping, and fisheries activities for marine, invasive introductions (Ruiz *et al.* 2000a, b).

Occurrences of harmful algal blooms are increasing worldwide in intensity, frequency, and geographic distribution (Hallegraeff 1993). Ballast water has been suggested as one of the important vectors transporting harmful algae (Hallegraeff 1998).

Displacement of shellfish molluscs has been described or suggested as another possible vector for introduction into new environments of non-native microorganisms, including parasites (Carriker 1992) and harmful algae (Bricelj *et al.* 1993; Scarratt *et al.* 1993 Vila *et al.* 2001; Lilly *et al.* 2002; Penna *et al.* 2005).

This study assessed the potential introduction of new harmful algal species associated with the transport of bivalve molluscs. Bivalves are moved from one body of water to another very frequently, and better risk-management strategies can be developed with knowledge of which HAB species may be transferred inadvertently to new geographic locations by shellfish transplantation, potentially with negative consequences to the transplanted bivalves themselves (Shumway 1990).

Materials and Methods

Six species of bivalve molluscs (softshell clams *Mya arenaria*, northern quahogs *Mercenaria mercenaria*, bay scallops *Argopecten irradians*, eastern oysters, *Crassostrea virginica*, blue mussels *Mytilus edulis* and Manila clams *Venerupis philippinarum*) were fed cultured HAB species obtained from the NOAA, Milford Laboratory (CT USA) collection: *Alexandrium fundyense* strain BF2, *Prorocentrum minimum* (strain JA-98-01), *Heterosigma akashiwo* (strain OL). A fourth species of harmful algae was also tested, *Karenia* (= *Gymnodinium*) *mikimotoi* (Stock GM95TIN), isolated at Tinduff (Rade de Brest, France); this was obtained from the culture collection of IFREMER, Brest, France.

Each of six species of bivalve molluscs was exposed to the four different species of harmful algae (Table 1) experimentally for 48 h in an 80-l basin, at a concentration equivalent to a natural bloom: 10^3 cells ml^{-1} for *Alexandrium fundyense* (Shumway *et al.* 1988; Townsend *et al.* 2005), 10^4 cells ml^{-1} for *Karenia mikimotoi* (Nakamura *et al.* 1995; Matsuyama *et al.* 1999), 10^4 cells ml^{-1} for *Heterosigma akashiwo* (Rensel & Whyte 2003), 10^4 cells ml^{-1} for *Prorocentrum minimum* (Hégaret & Wikfors 2005).

After 48 h of exposure to the simulated bloom, each of 10 individual bivalves was transferred into a

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1.2-l basin containing only filtered seawater (FSW) to allow the animals to clear their guts and produce feces. After 24 h, each individual bivalve was transferred into a new, 1.2-l basin with FSW for an additional 24 h. Feces were collected and observed under the microscope for presence of intact, possibly-viable cells. Then, 1 ml of concentrated fecal suspension was collected from each shellfish and transferred into a culture tube containing either 5 ml of FSW or 5 ml of the culture medium in which the algal cells had been grown. Growth of harmful algal cells was assessed weekly in each tube under an inverted microscope for up to two months.

Results

Microscopic observations of feces after the bivalves had been in FSW for 24 h showed the presence of intact, possibly-viable cells in almost every interaction, with the exception of softshell clams (Fig. 1, Table 1). Usually the feces produced 24-48 h of depuration in FSW contained fewer intact algal cells (Table 1).

In tubes inoculated with biodeposits, growing populations of harmful algae were usually observed, with first detection after various periods of time. The tubes inoculated with biodeposits from bivalves exposed to *H. akashiwo* and *K. mikimotoi* usually showed a recovery of the cells after 1-2 wk. The tubes inoculated with biodeposits from bivalves exposed to *P. minimum* revealed recovery after 3-4 wk; whereas it took 6-8 wk for the tubes inoculated with biodeposits from bivalves exposed to *A. fundyense* to form detectable, growing populations (Table 1).

Table 1. Bivalve-HAB interactions tested: Grey represents interactions that have not been tested; white shows interactions where algae were not observed in the biodeposits and did not recover; 1. Presence of intact cells in the biodeposits of bivalves after 24 h of incubation in FSW, 2. Presence of intact cells in the biodeposits of bivalves after 48 h of incubation in FSW, 3. Recovery of HAB cells from the biodeposits cultured in the tubes containing FSW after 24 h of depuration in FSW, 4. Recovery of HAB cells from the biodeposits cultured in the tubes containing FSW after 48 h of depuration in FSW, * no biodeposits produced after 24 h of incubation.

Argopecten irradians	1,2,3		1,2,3,4	1,3
Crassostrea virginica	1,2,3		* 4	1,2,3
Mercenaria mercenaria	1,2,3		3	1,3
Mya arenaria				
Mytilus edulis	1,3	3		1,3
Venerupis philippinarum		3		

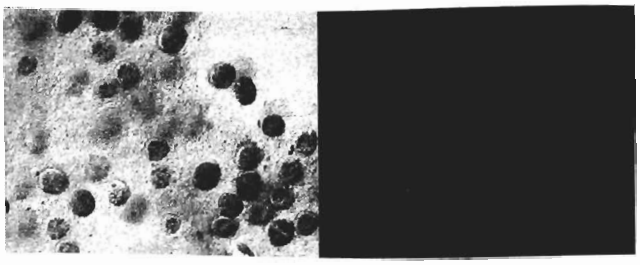

Figure 1. Photomicrographs, paired light (A) and epifluorescence (B) to show chlorophyll a, of fecal pellets produced by hard clams (=quahogs, *Mercenaria mercenaria*) after being exposed to simulated blooms of cultured Alexandrium spp. and then moved to ultrafiltered seawater for 24 h (scale bar = 20 μm): Temporary cysts and cells of *A. fundyense* were seen in fecal material from *Mercenaria mercenaria*.

Biodeposits from the softshell clam, *Mya arenaria*, were thoroughly processed, and not a single intact cell of any harmful alga was observed in the fecal pellets after 24 or 48 h of depuration in FSW. Moreover, no culture tubes inoculated with biodeposits from softshell clams showed any growth of HAB populations (Table 1).

Alexandrium fundyense was observed in the biodeposits in two different morphologies (Fig. 1): as vegetative cells and also as temporary cysts (Persson *et al.* in press), which could have formed during gut passage. The three other harmful algal species did not show any cyst formation; only vegetative cells were observed in the biodeposits or in the tubes where the cultures re-established.

Discussion

Results of these experiments clearly demonstrate that harmful algal cells can be found intact in the biodeposits of bivalves which have been feeding upon these, and at least some of these cells have the ability to recover from the feces and re-establish new populations. These findings demonstrate a clear risk of introducing new species of harmful algae through movement of shellfish.

These results also demonstrate that a period of 24 h or more in seawater allows partial clearing of gut contents. Indeed, usually when the bivalves were held in seawater for 24 h or more, the harmful algae did not

recover from the feces. Thus, keeping the animals for 24 h in seawater before reintroducing them into a new environment may be one way to mitigate the risk of introducing harmful algae into new environments.

These findings present but a small number of bivalve-harmful algal bloom interactions; many others are known to occur, thus a systematic approach is needed to test for specific bivalve-algal interactions. Indeed, the responses observed in our experiments are species-specific: softshell clams for example do not seem to present a risk of introducing HABs; whereas, oysters and scallops seem to present more risk of transfer.

Information generated in the present study may be used in shellfish hatcheries, but it will also be applicable to public education as recreational fishing or harvesting may precipitate introductions.

These results demonstrate a clear potential for transplanted bivalves to be vectors transporting HAB species into new areas, but they also show that a 24-h depuration period in seawater may mitigate this risk. Current studies are investigating alternative, less expensive or more convenient methods, to mitigate the risk of harmful algal introductions through movement of bivalve shellfish.

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References

Bricelj, V.M., Greene, M. & Cembella, A.D. (1993). In: Toxic Phytoplankton Blooms in the Sea, Smayda, T.J. & Shimizu, Y. (eds), pp. 371-376.

Carriker, M.R. (1992). J.Shellfish Res. 11: 507-510.

Hallegraeff, G.M. (1993). Phycologia 32: 79-99.

Hallegraeff, G.M. (1998). Mar. Ecol. Progr. Ser. 168: 297-309.

Hégaret, H. & Wikfors, G.H. (2005). Harmful Algae 4: 201-209.

Lilly, E.L., Kulis, D.M., Gentien, P. & Anderson, D.M. (2002). J. Plankton Res. 24: 443-452.

Matsuyama, Y., Uchida, T. & Honjo, T. (1999). Fish. Sci. 65: 248-253.

Nakamura, Y., Suzuki, S.Y. & Hiromi, J. (1995). Mar. Ecol. Progr. Ser. 125: 269-277.

Penna, A., Garcés, E., Vila, M., Giacobbe, M.G., Fraga, S., Luglie, A., Bravo, I., Bertozzini E. & Vernesi, C. (2005). Mar. Biol. 148:13-23.

Persson, A., Smith, B.C., Wikfors, G.H., Quilliam, M. (in press). Harmful Algae.

Rensel, J.E. & Whyte, J.N.C. (2003) In: Manual on Harmful Marine Microalgae, Hallegraeff, G.M., Anderson, D.M. & Cembella, A.D. (eds), UNESCO, Paris, pp. 693-722.

Ruiz, G.M., Fofonoff, P.W., Carlton, J.T., Wonham, M.J. & Hines, A.H. (2000a). Ann. Rev. Ecol. Syst. 31: 481-531.

Ruiz, G.M., Rawlings, T.K., Dobbs, F.C., Drake, L.A., Mullady, T., Huq, A. & Colwell, R.R. (2000b). Nature 408: 49-50.

Scarratt, A.M., Scarratt, D.J. & Scarratt, M.G. (1993). J. Shellfish Res. 12: 383-388.

Shumway, S.E. (1990). J. World Aquacult. Soc. 21: 65-104.

Shumway, S.E., Sherman-Caswell, S. & Hurst, J.W. (1988). J. Shellfish Res. 7: 643-652.

Townsend, D.W., Pettigrew, N.R. & Thomas, A.C. (2005). Deep-Sea Research Part I -Topical Studies in Oceanography 52: 2603-2630.

Vila, M., Garcés, E., Maso, M. & Camp, J. (2001). Mar. Ecol. Progr. Ser. 222: 73-83.