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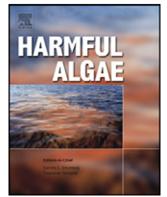

Biofouling ascidians on aquaculture gear as potential vectors of harmful algal introductions

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ABSTRACT

Biofouling ascidians are ubiquitous in coastal ecosystems and are among the main colonizers of aquaculture gear. Our study tested the hypothesis that the transport, removal, and transfer of fouling ascidian species by aquaculturists provide a mechanism for concentration and distribution of harmful-algal cells to new areas. Wild-caught specimens of common, biofouling ascidian species (*Styela clava*, *Ciona intestinalis*, *Molgula manhattensis*, *Botrylloides violaceus*, *Didemnum vexillum*, and *Botryllus schlosseri*) were exposed individually to cultured strains of co-occurring harmful algae (*Prorocentrum minimum*, *Alexandrium fundyense*, *Alexandrium monilatum*, *Karenia brevis*, *Aureococcus anophagefferens*, or *Heterosigma akashiwo*) at simulated bloom cell densities of each HAB species. After feeding, ascidians were transferred to ultrafiltered seawater. Immediately after exposure, and after 24 and 48 h in ultrafiltered seawater, biodeposits were collected and observed microscopically for the presence of intact, potentially viable cells. Subsamples of biodeposits were transferred into culture tubes with ultrafiltered seawater and monitored for algal growth during 8 weeks. Cells of all HAB species were found to pass intact through the ascidian digestive system, remained viable, and in many cases were capable of re-establishing populations at least 48 h post-ingestion. The results of our study will inform industry and managers of the potential threat and ecological impact of spreading biofouling ascidians, and practices to mitigate adverse impacts. Additionally, these management practices have been formally incorporated into a new cost-share program developed to help shellfish producers prevent the further spread of ascidians and associated HAB species.

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1. Introduction

The economic impact of harmful algal blooms (HAB) worldwide has been estimated to be in the range of $50 M annually (Anderson et al., 2000). Both harmful algal blooms (HAB) and biofouling cost the shellfish aquaculture industry and government millions of dollars annually (Shumway et al., 1988; Adams et al., 2011; Matsuyama and Shumway, 2009; Watson et al., 2009). Lost income from closures, as well as expenditures associated with the management and monitoring of HABs account for most of these costs. Harmful algal blooms also pose a human health hazard due to indirect exposure (e.g., asthma) or ingestion (e.g., paralytic shellfish poisoning; see Landsberg, 2002). The geographical extent and duration of HAB have been increasing, attributed in part to human activities (Hallegraeff, 1993; Vitousek et al., 1997; Anderson et al., 2002; Heisler et al., 2008).

The costs associated with the prevention and control of biofouling represent a significant loss to aquaculture operations (Adams et al., 2011). Fouling organisms readily cover man-made structures, and overgrow and out-compete shellfish species of commercial value such as oysters, mussels, and scallops (Braithwaite et al., 2007; Greene and Grizzle, 2007; Daigle and Herbinger, 2009; Locke and Carman, 2009; Adams et al., 2011; Kripa et al., 2012). Removal of fouling organisms is a regular and costly activity on finfish and shellfish aquaculture sites. On average, ~15% of total annual operating costs of shellfish aquaculturists is associated with removal of biofouling material (Adams et al., 2011). To reduce these costs, numerous control measures have been attempted, such as the development of fouling-resistant shellfish aquaculture equipment (Huguenin and Huguenin, 1982; Dafforn et al., 2011); however, the most common treatment is the labor intensive, physical removal of fouling material from aquaculture gear and shellfish (Braithwaite et al.,

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2007). Because it is expensive and laborious to bring the removed fouling material ashore for disposal, it is often transported a short distance away from the aquaculture sites and dumped overboard. Many fouling organisms removed in this way remain alive and can colonize surfaces at the dump site (e.g., Bullard et al., 2007a).

Ascidians are among the most common fouling organisms on temperate shellfish aquaculture gear, with species often comprising >80% of the organisms in the fouling community (Arsenult et al., 2009; Bullard and Carman, 2009). Ascidians are highly invasive because many species possess strong competitive abilities, wide environmental tolerances, and are readily transported by human activities (see Bullard and Carman, 2009); fouling by ascidians is a serious problem along all coasts of the U.S. and in many regions of the world (see Monniot and Monniot, 1994; Lambert and Lambert, 1998). The Atlantic and Gulf Coasts have been especially vulnerable to the ecological and economic impacts of invasive ascidians because of the many quiet embayments that facilitate ascidian colonization. In New England and Long Island Sound, for example, five ascidian species (*Styela clava*, *Ascidiella adspersa*, *Botrylloides violaceus*, *Diplosoma listerianum*, and *Didemnum vexillum*) have successfully invaded in the last 30 years (Steneck and Carlton, 2001; Bullard et al., 2007b; Dijkstra et al., 2007). In some areas, *D. vexillum* has undergone explosive growth (Bullard et al., 2007b). In laboratory and field experiments, introduced ascidians competed with shellfish (especially recruits) for space (Osman et al., 1989) and food (Osman et al., 1989; Zajac et al., 1989; Lesser et al., 1992), and overgrew spat causing mortality (Osman et al., 1989; Osman and Whitlatch, 1995). Additionally, once introduced, sea squirts can rapidly expand their geographic range (Bullard et al., 2007a; Hopkins et al., 2011).

Transplanted shellfish can introduce fouling organisms to new areas and cultured bivalves can release undigested, viable HAB cells to new sites (see Naylor et al., 2001; Hégaret et al., 2008 and references therein). The movement of bivalve molluscs for management or aquaculture purposes presents a very real risk that harmful algae may be introduced into receiving waters. Hégaret et al. (2008) demonstrated that cells of numerous HAB species can survive passage through the guts of bivalves and emerge intact and viable. Thus, moving shellfish between locations could lead to the introduction of new harmful algal species to new sites, and potentially to new HAB outbreaks. Fortunately, due to the rapid gut clearance of bivalves, keeping shellfish out of the water for a period of 6 h (scallops) to 24 h (mussels, oysters, quahogs) was found to mitigate this risk.

The present study was undertaken to determine if ascidians could serve as vectors of harmful algal species. If so, this would suggest that ascidians fouling shellfish could transport HABs to new areas when the shellfish are moved between sites for management or culture purposes.

2. Materials and methods

To determine whether ascidians can act as vectors for the transport of viable HAB cells, we systematically exposed six species of harmful algae to six common (both native and non-native) species of fouling ascidians (see Table 2 for pairings). Experiments were conducted between July and August, 2010, and between June and October, 2011, at the National Oceanic and Atmospheric Administration-National Marine Fisheries Service (NOAA NMFS) Laboratory in Milford, CT using seawater from Milford Harbor.

2.1. Experimental animals

Ascidians (the solitary species *Styela clava*, *Molgula manhattensis*, and *Ciona intestinalis*, and the colonial species *Botrylloides violaceus*, *Didemnum vexillum*, and *Botryllus schlosseri*) were collected from sites near Avery Point, Groton, CT. Species were harvested from PVC panels suspended from the Avery Point docks or collected by SCUBA divers from the docks and nearby submerged structures. All ascidians were maintained in 0.22-μm filtered seawater (FSW) for 24 h prior to experiments to allow purging of gut contents.

Four of the algal species used were obtained from the NOAA Milford Laboratory Microalgal Culture Collection (Milford, CT). These algae were cultivated as follows: *Prorocentrum minimum*, JA-9801 strain, was grown in EDL7 medium with soil extract (Ukeles, 1973); *Alexandrium fundyense*, BF2 strain, was grown in F2 enriched SW medium (Guillard, 1975). These species were cultivated at 20 °C with a 14:10, light:dark cycle; *Heterosigma akashiwo*, OL strain, was grown in E-medium (Ukeles, 1973); *Aureococcus anophagefferens*, CCMP1708 strain, was grown in L-1 medium (Guillard and Hargraves, 1993), and both *H. akashiwo* and *A. anophagefferens* species were cultivated at 18 °C with a 12:12, light:dark cycle. The remaining two algal species used were obtained from NOAA, Marine Biotoxins Program, Charleston, SC. Both *Karenia brevis*, Wilson strain, and *Alexandrium monilatum*, strain AM-02, were cultured in 20 L carboys using L-1 medium (Guillard and Hargraves, 1993) at 23 °C, at a salinity of 35 under a 14:10, light:dark cycle. All cultures were harvested semi-continuously to maintain consistency in quality over time, and were used in log phase of growth. Algal cell densities were determined using a FACScan flow cytometer prior to use in the experiments.

2.2. Exposure assays

Exposure assays were performed according to methods outlined by Hégaret et al. (2008) with minor modifications. During each assay, individual ascidians were placed in 1 L beakers and exposed for 24 h to a single harmful algal species at a concentration simulating a natural bloom (Table 1). All animals but those exposed to *Karenia brevis* were placed in filtered seawater from local waters. All experiments were conducted at 21 °C. Water was gently aerated to keep algal cells suspended and to maintain oxygen levels. A 500 μL sub-sample of the water was taken at T_0 and T_{60} of the exposure assay for algal cell counts and flow cytometry was used to calculate algal cell concentrations. After exposing the ascidians to algae for 24 h, ascidian feces were collected. Fecal samples were placed in 10-mL test tubes containing autoclaved FSW with no added nutrients and incubated to assess cell viability (see below). Due to the inability of the Milford laboratory to culture *K. brevis* using Milford Bay water, coastal South Carolina water was used in the culture tubes to

Table 1
Cell concentrations of harmful algae used in the experiments to simulate bloom concentrations.

Algal species	Cell concentration (cells L^{-1})	Source
Prorocentrum minimum	10^4	Hégaret and Wikfors (2005)
Alexandrium fundyense	10^3	Shumway et al. (1988)
Heterosigma akashiwo	10^4	Rensel and Whyte (2003)
Aureococcus anophagefferens	10^6	Gobler et al. (2005)
Karenia brevis	10^5	Tester and Steidinger (1997)
Alexandrium monilatum	5.5×10^2	Perry et al. (1979)

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