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Effects of the estuarine dinoflagellate *Pfiesteria shumwayae* (Dinophyceae) on survival and grazing activity of several shellfish species

Sandra E. Shumway [a,*], JoAnn M. Burkholder [b], Jeffrey Springer [b]

[a] *Department of Marine Science, University of Connecticut, 1080 Shennescossett Road, Groton, CT 06340, USA*
[b] *Center for Applied Aquatic Ecology, North Carolina State University, 620 Hutton Street, Suite 104, Raleigh, NC 27606, USA*

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Abstract

A series of experiments was conducted to examine effects of four strains of the estuarine dinoflagellate, *Pfiesteria shumwayae*, on the behavior and survival of larval and adult shellfish (bay scallop, *Argopecten irradians*; eastern oyster, *Crassostrea virginica*; northern quahogs, *Mercenaria mercenaria*; green mussels, *Perna viridis* [adults only]). In separate trials with larvae of *A. irradians*, *C. virginica*, and *M. mercenaria*, an aggressive predatory response of three strains of algal- and fish-fed *P. shumwayae* was observed (exception, algal-fed strain 1024C). Larval mortality resulted primarily from damage inflicted by physical attack of the flagellated cells, and secondarily from *Pfiesteria* toxin, as demonstrated in larval *C. virginica* exposed to *P. shumwayae* with versus without direct physical contact. Survival of adult shellfish and grazing activity depended upon the species and the cell density, strain, and nutritional history of *P. shumwayae*. No mortality of the four shellfish species was noted after 24 h of exposure to algal- or fish-fed *P. shumwayae* (strains 1024C, 1048C, and CCMP2089) in separate trials at $\leq 5 \times 10^3$ cells ml^{-1}, whereas higher densities of fish-fed, but not algal-fed, populations (>7–8 $\times 10^3$ cells ml^{-1}) induced low (≤15%) but significant mortality. Adults of all four shellfish species sustained >90% mortality when exposed to fish-fed strain 270A1 (8×10^3 cells ml^{-1}). In contrast, adult *M. mercenaria* and *P. viridis* exposed to a similar density of fish-fed strain 2172C sustained <15% mortality, and there was no mortality of *A. irradians* and *C. virginica* exposed to that strain. In mouse bioassays with tissue homogenates (adductor muscle, mantle, and whole animals) of *A. irradians* and *M. mercenaria* that had been exposed to *P. shumwayae* (three strains, separate trials), mice experienced several minutes of disorientation followed by recovery. Mice injected with tissue extracts from control animals fed cryptomonads showed no response. Grazing rates of adult shellfish on *P. shumwayae* (mean cell length ±1 standard error [S.E.], 9 ± 1 μm) generally were significantly lower when fed fish-fed (toxic) populations than when fed populations that previously had been maintained on algal prey, and grazing rates were highest with the nontoxic cryptomonad, *Storeatula major* (cell length 7 ± 1 μm). Abundant cysts of *P. shumwayae* were found in fecal strands of all shellfish species tested, and ≤45% of the feces produced viable flagellated cells when placed into favorable culture conditions. These findings were supported by a field study wherein fecal strands collected from field-collected adult shellfish (*C. virginica*, *M. mercenaria*, and ribbed mussels, *Geukensia demissa*) were confirmed to contain cysts of *P. shumwayae*, and these cysts produced fish-killing flagellated populations in standardized fish bioassays. Thus, predatory feeding by flagellated cells of *P. shumwayae* can adversely affect survival of larval bivalve molluscs, and grazing can be depressed when adult shellfish are fed *P. shumwayae*. The data suggest that *P. shumwayae* could affect recruitment of larval shellfish in estuaries and aquaculture facilities; shellfish can be

* Corresponding author. Tel.: +1 860 405 9282; fax: +1 860 405 9282.
E-mail address: sandra.shumway@uconn.edu (S.E. Shumway).

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adversely affected via reduced filtration rates; and adult shellfish may be vectors of toxic *P. shumwayae* when shellfish are transported from one geographic location to another.

Keywords: *Argopecten irradians*; *Crassostrea virginica*; Cyst; Dinoflagellate; Grazing; *Geukensia demissa*; *Mercenaria mercenaria*; *Perna viridis*; *Pfiesteria shumwayae*; Shellfish; Toxigenic

1. Introduction

Molluscan shellfish increasingly have been recognized as potential vectors of harmful algae and their toxins (Shumway et al., 1985, 1987; Shumway and Cucci, 1987; Gainey and Shumway, 1988a,b; Shumway, 1990, 1995; Matsuyama et al., 1999). Most previous studies of impacts of harmful algae have focused on photosynthetic species, especially species that attain high cell densities and frequently discolor the water (Shumway, 1995; Landsberg, 2002). With the exception of well-documented impacts on shellfish from certain parasitic dinoflagellates (e.g. crustacean epizootics and mortalities caused by *Hematodinium* and hematodinium-like species; Newman and Johnson, 1975; Taylor and Kahn, 1995; Stentiford et al., 2002), effects of harmful heterotrophic algae on shellfish behavior and survival are poorly understood (Burkholder, 1998).

Reports of toxic shellfish linked to free-living heterotrophic dinoflagellates mostly have been limited to *Dinophysis* sp. (e.g. Suzuki et al., 1996; Sidari et al., 1998; Miles et al., 2004a,b), *Protoperidinium* sp. (James et al., 2003, 2004), and *Pfiesteria piscicida* Steidinger et Burkholder (based on experimental data—Springer et al., 2002). Miles et al. (2004a) described a link between *Dinophysis* spp. and accumulation of pectenotoxin in shellfish, and *Protoperidinium* sp. also has been reported to produce a toxin that accumulates in shellfish (James et al., 2003). *P. piscicida* and a closely related species, *Pfiesteria shumwayae* Glasgow et Burkholder (Marshall et al., 2006), have toxic strains (Moeller et al., 2001; Gordon et al., 2002; Burkholder et al., 2004, 2005; Gordon and Dyer, 2005) that have been linked to fish mortalities and epizootics in estuaries of the mid-Atlantic and southeastern U.S. (Magnien et al., 2000; Burkholder et al., 2001a; Glasgow et al., 2001; Magnien, 2001). Adverse affects on fish have been associated with predation (Burkholder and Glasgow, 1997; Burkholder et al., 2001a,b; Lovko et al., 2003) as well as toxin production (Burkholder et al., 2001a,b, 2005; Melo et al., 2001; Moeller et al., 2001). Recent research also documented adverse impacts from *P. piscicida* on larval bay scallops (*Argopecten irradians* Lamarck, 1819) and eastern oysters (*Crassostrea virginica* Lamarck, 1819) (Springer et al., 2002). In that study, flagellated cells of *P. piscicida* attacked, fed upon, and killed pediveliger larvae of both shellfish species; actively toxic cells held in dialysis membrane to prevent direct contact with *A. irradians* larvae caused high larval mortality as an apparent toxin effect; and actively toxic cells depressed grazing of adult *C. virginica*. Flagellated cells of *P. piscicida* also showed chemosensory attraction toward freshly dissected tissues of *A. irradians*, *C. virginica*, and northern quahogs (*Mercenaria mercenaria* Linneaus, 1758) (Springer, 2000). Moreover, *P. piscicida* survived passage through the digestive tract of adult *C. virginica* by forming temporary cysts, and $\geq$75% of the cysts produced viable flagellated cells when separated from the shellfish feces and placed into fresh culture media.

Impacts on shellfish from the second known toxigenic species of *Pfiesteria*, *P. shumwayae*, have not previously been assessed. The objectives of this study were to: (1) examine interactions between *P. shumwayae* and several shellfish species, based on a series of laboratory experiments and supporting field observations; (2) assess predation by *P. shumwayae* on shellfish pediveliger larvae and the potential for toxic effects on larvae, using different strains of *P. shumwayae*; (3) assess the grazing response of adult shellfish to different strains; and (4) determine the viability of *P. shumwayae* cells recovered from fecal strands.

2. Materials and methods

2.1. Field study

Eastern oysters (*C. virginica*) and ribbed mussels (*Geukensia demissa* Dillwyn, 1817) were sampled on 8 May, 18 August, and 30 August 2002 in the Bay River Estuary, a tributary of western Pamlico Sound (latitude 35°08′39″N, longitude 76°46′15″W, salinity ~15 ppt) where *P. piscicida* and *P. shumwayae* previously had been documented (Burkholder et al., 2004). The field sites included diverse sediment types ranging from fine-particulate sediments in the upper reaches of tidal creeks (sites 2, 3, 7–10, 13, and 14) to consolidated sands (sites 1, 4–6, 11, and 12). On the first sampling date, physical and chemical data were collected from eight sites (sites 1, 2, 5–7, 10, 11, and 14; Fig. 1). Water-