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Interactions between the toxic estuarine dinoflagellate *Pfiesteria piscicida* and two species of bivalve molluscs

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ABSTRACT: Toxic strains of *Pfiesteria* spp. produce toxin(s) that can cause finfish death, but much less is known about impacts of *Pfiesteria* on shellfish. Here we conducted 4 experiments to examine interactions between shellfish and toxic (actively toxic or TOX-A from finfish-killing cultures and potentially toxic or TOX-B from cultures without finfish) and non-inducible (NON-IND, apparently incapable of killing fish via a toxic effect) strains of *P. piscicida*. First (Expt 1), we documented direct physical attack by *P. piscicida* TOX-A, TOX-B, and NON-IND zoospores on larvae of the bay scallop *Argopecten irradians* (Lamarck, 1819) and the eastern oyster *Crassostrea virginica* (Gmelin, 1791). Within 5 min zoospores swarmed around larvae that had discarded their vela, and attached with their peduncles. Within 15 min they had penetrated into the shellfish visceral cavity and had begun to feed aggressively; after 30 min all shellfish tissues except the adductor muscle had been consumed. Second, we tested the response of scallop larvae to *P. piscicida* (TOX-A or TOX-B) or cryptomonads (as controls) that were held in dialysis tubing (0.22 µm porosity) to prevent direct contact. After 60 min larval survival was 0% in the TOX-A treatment, 100% in the cryptomonad control, and intermediate in TOX-B and TOX-B + cryptomonad treatments. The data indicate a toxic effect of *P. piscicida* zoospores on the larvae, separate from the physical effect shown in Expt 1. Third, we compared grazing by juvenile and adult oysters on TOX-A, TOX-B, and NON-IND *P. piscicida* zoospores from the medium. After 60 min, grazing by juvenile oysters significantly differed as NON-IND ≫ TOX-B ≫ TOX-A. In contrast, adult oysters grazed significantly fewer TOX-A zoospores and maintained comparable grazing on TOX-B and NON-IND zoospores. Thus juvenile oysters, but not adults, were sensitive to residual toxicity of TOX-B zoospores, and both life-history stages were sensitive to TOX-A zoospores. The adverse effects of toxic strains on larval survival and juvenile grazing indicate that *P. piscicida* could potentially affect shellfish recruitment. Fourth, we assessed zoospore survival after passage through the digestive tract of adult oysters. The feces contained many temporary cysts from zoospores, and within 24 h >75% of the cysts produced motile cells. The data indicate that adult oysters would be poor biocontrol agents of *P. piscicida*, given the high survival of ingested zoospores following gut passage and fecal elimination; and that oysters could act as vectors of toxic *P. piscicida* strains if transported from affected estuaries to other waters.

KEY WORDS: *Argopecten irradians* · *Crassostrea virginica* · Oyster · *Pfiesteria* · Scallop · Shellfish · Temporary cyst · Toxic dinoflagellate

INTRODUCTION

Toxic dinoflagellates include species that, when accumulated by shellfish consumed as seafood, can lead to illness and death of humans (Shumway 1990, Bricelj & Shumway 1998, Burkholder 1998, Matsuyama et al. 1999). Although shellfish had been regarded as unaffected vectors of these toxins, it is now known that

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toxic dinoflagellates can significantly affect the survival and physiology of bivalve molluscs, including species-specific changes in feeding, respiration, shell valve closure, mucus production, and cardiac activity (Shumway et al. 1985, 1987, Shumway & Cucci 1987, Gainey & Shumway 1988a,b, Shumway 1990, 1995, Matsuyama et al. 1999).

Among the more recently known toxic dinoflagellate species is *Pfiesteria piscicida* Steidinger & Burkholder, which was first detected in the early 1990s as an ichthyotoxic organism in the Albemarle-Pamlico Estuarine System of the southeastern US (Burkholder et al. 1992, 2001a). Toxic *Pfiesteria* spp. outbreaks have been linked to the death of >1 billion fish in that system, which is the second largest estuary on the US mainland (Burkholder & Glasgow 1997, Burkholder et al. 1999, Glasgow et al. 2001a). Several small outbreaks also have occurred in the largest US mainland estuary, Chesapeake Bay (Magnien et al. 2000, Magnien 2001), and toxic *Pfiesteria* spp. strains have been documented from other geographic regions as well (Jakobsen et al. 2002, Rhodes et al. 2002). Toxicity in *Pfiesteria* spp. is activated by substances in fresh finfish tissues, excreta and secreta (Burkholder et al. 1992, 2001a, Marshall et al. 2000). Like other toxic algae (Gentien & Arzul 1990, Anderson 1991, Skulberg et al. 1993, Bates et al. 1998, Edvardsen & Paasche 1998), *Pfiesteria* spp. include both toxic and 'benign' strains (the latter, non-inducible or apparently incapable of causing fish death with toxin as live cells: Burkholder et al. 2001a). Also like other toxic algae, many toxic strains of *Pfiesteria* spp. have lost toxicity over time in culture, possibly because required organic substrates and/or bacterial cofactors from the natural environment are lacking (Burkholder et al. 2001a). A potent water-soluble toxin has been purified from *Pfiesteria* spp. (Drs. J. Ramsdell & P. D. R. Moeller, National Ocean Service, Charleston, South Carolina, USA, pers. comm.), and fish-killing and pharmacological activity demonstrated (Kimm-Brinson et al. 2001, Melo et al. 2001) but, as for other toxic dinoflagellates, the factors controlling toxin production are poorly understood.

The available data indicate that toxicity status in *Pfiesteria* spp. zoospores includes 3 'functional types'. Toxic strains may be either actively toxic (TOX-A, in the presence of live fish under conductive culture conditions: Burkholder et al. 2001b) or temporarily nontoxic (TOX-B, without live fish, sometimes retaining residual toxicity and capable of becoming TOX-A when live fish become available [Burkholder et al. 2001c], sometimes referred to as nontoxic [e.g. Burkholder & Glasgow 1997]). Non-inducible (NON-IND) strains, in contrast, are incapable of causing fish death as a toxin effect as mentioned, based on the present understanding of these organisms (Burkholder et al. 2001b). The 3 functional types of *P. piscicida* have shown distinct behaviors in response to nutrients (Burkholder et al. 2001b), algal prey (Parrow et al. 2002), microfaunal grazers (Stoecker et al. 2002), and finfish (Burkholder et al. 2001a, Cancellieri et al. 2001). Toxic *Pfiesteria* spp. strains have caused death in some shellfish species (Burkholder & Glasgow 1997), but the response of shellfish to toxic versus non-inducible *Pfiesteria* strains has not been tested. The objectives of this study were to examine interactions between toxic (TOX-A, TOX-B) and NON-IND strains of *P. piscicida* and 2 commercially and ecologically important shellfish species, the bay scallop *Argopecten irradians* (Lamarck, 1819) and the eastern oyster *Crassostrea virginica* (Gmelin, 1791).

MATERIALS AND METHODS

***Pfiesteria piscicida* cultures.** Using a standardized fish bioassay procedure (Burkholder & Glasgow 1997, Burkholder et al. 2001c), we isolated a toxic clone of *P. piscicida* (defined as in the *Pfiesteria* Interagency Coordination Working Group: *P*ICWG 2002) from the mesohaline Neuse Estuary near Minnesott Beach, North Carolina, USA, a tributary of the Albemarle-Pamlico (Glasgow & Burkholder 2000). The clone (Center for Applied Aquatic Ecology [CAAE] NE121, zoospore diameter 6 to 8 µm) was obtained following flow cytometric procedures described by Glasgow et al. (2001b), using a COULTER® EPICS® ALTRA™ flow cytometer with HyPerSort™System (Coulter), equipped with a water-cooled INNOVA™ Enterprise II™ Ion Laser (Coherent). *P. piscicida* was identified from suture-swollen zoospores using scanning electron microscopy (SEM; procedure of Burkholder & Glasgow 1995, Glasgow et al. 2001b), additionally checked with a species-specific polymerase chain reaction (PCR) probe (Rublee et al. 1999, 2001). The identification was cross-corroborated by Dr. P. Rublee (PCR: University of North Carolina-Greensboro, Greensboro, North Carolina, USA), and by Dr. H. Marshall (SEM: Old Dominion University, Norfolk, Virginia, USA; Marshall et al. 2000).

All cultures were maintained at 21°C and 100 µmol photons m^{-2} s^{-1} at a 14:10 h light:dark ratio. An actively toxic (TOX-A) sub-culture from this clonal culture was maintained with live fish in ultra-filtered (0.2 µm-porosity Versapor filtration capsules, Pall Gelman Corporation) natural seawater that had been collected ca. 40 km off Cape Hatteras, North Carolina, USA; salinity was adjusted to 15 or 25 ppt using sterile-filtered Millipore Q water). The TOX-A sub-culture was maintained in a biohazard III containment system that was specially designed to ensure safe conditions when working with toxic *Pfiesteria* species (required by the

Department of Environmental Health and Safety, North Carolina State University [NCSU], Raleigh, North Carolina, USA; Burkholder et al. 2001c). A TOX-B subculture from the actively toxic clonal culture was grown with clonal cryptomonad algal prey (*Cryptomonas* sp. HP9101; source, Dr. A. Lewitus, University of South Carolina, Charleston, South Carolina, USA) for 6 wk prior to the experiments (TOX-B zoospore diameter 6 to 8 µm). The cryptomonad culture was maintained at 10^4 cells ml^{-1}, and grown in f/2-Si media (Guillard 1975) made with sterile-filtered natural seawater (salinity 15 or 25 ppt). The TOX-B subculture was retested 6 wk before the experiments in fish bioassays to confirm toxicity. A second clonal *P. piscicida* culture (CAAE #98B) was, of necessity, needed for NON-IND zoospores. The clone had been isolated as a toxic strain from the mesohaline Neuse ca. 6 mo earlier, but had become non-inducible over time in culture. The NON-IND strain (zoospore diameter 6 to 7 µm) had also been maintained on cryptomonad prey, and was acclimated to a salinity of 15 or 25 ppt for the experiments.

Shellfish. All shellfish were obtained from Dr. G. Wikfors (National Marine Fisheries Service [NMFS], Milford, Connecticut, USA). Shellfish reared in waters from New York, USA southward (known northernmost extension of *Pfiesteria piscicida* distribution: Allen 2000) were not used to minimize the potential for prior exposure to *Pfiesteria* spp. zoospores or contamination with cysts. Water samples collected adjacent to NMFS-Milford were examined with light microscopy to check for the presence of pfiesteria-like zoospores, and with PCR probes to test for *P. piscicida* and *P. shumwayae* Glasgow & Burkholder (Rublee et al. 1999, 2001, Allen 2000, Oldach et al. 2000), the 2 known toxic *Pfiesteria* spp. (Burkholder et al. 2001a, Glasgow et al. 2001b). *Pfiesteria* spp. were not detected.

The larval bay scallops and eastern oysters had been spawned at the salinities required for experimentation (25 and 15 ppt, respectively), and had been maintained on a mixed algal diet (primarily *Isochrysis* cf. *galbana* or *Rhodomonas* sp. [20°C] prior to shipment. Juvenile and adult oysters (mean shell height 22.3 and 64.2 mm, respectively) were obtained from NMFS-Milford and transferred to separate (15 or 25 ppt) 950 l recirculating seawater systems at 21°C. The shellfish were allowed to acclimate for ≥2 d to clear previously consumed algal prey from their digestive system. Shellfish were gently brushed 24 h before each experiment to remove epibionts, and were held in a tank equipped with ultraviolet sterilization. These steps were taken to minimize transfer of microbial contaminants into experimental treatments.

Sampling design and analyses. Four experiments were carried out as follows:

Expt 1—Pfiesteria piscicida predation on larval shellfish: The 2 shellfish species were maintained separately, and 100 larvae (mean length 61 µm) of each species were exposed to a standardized density of 5×10^3 cells ml^{-1} in each treatment (n = 3). Treatments consisted of larvae exposed to TOX-A, TOX-B, and NON-IND *P. piscicida* zoospores; and to *Cryptomonas* sp. cells (controls) in ultra-filtered natural seawater (0.2 µm-porosity filters; salinity 15 or 25 ppt). Behavioral interactions between the shellfish larvae and *P. piscicida* zoospores were observed over 24 h. Time zero (t_0) was recorded when zoospores were added to an optical-grade glass-bottomed petri dish with larvae. Petri dishes containing the larvae and zoospores were viewed under Nomarski optics (Olympus AX-70 research microscope, Olympus Corporation) using achromatic water immersion (Teflon-coated) lenses (20×, 40×, and 60×). All observations were archived on videotape (S-VHS) using a Sony SVO-9500MD video recorder. Still images were captured and digitized (1280×1024 pixel resolution) using a cooled-chip CCD camera (DEI-750D, Optronics Corporation).

Expt 2—zoospore toxicity to scallop larvae: The ability of toxic strains of *Pfiesteria piscicida* zoospores to induce mortality was determined by exposing 100 bay scallop larvae (in 90 mm petri dishes) to sterile-filtered seawater (salinity 25 ppt) containing *P. piscicida* and/or benign algal prey held within cellulose dialysis tubing (molecular weight cut-off of 12 000 to 14 000 Da, Fisher Scientific) to prevent direct contact. Treatments included TOX-A zoospores, TOX-B zoospores, *Cryptomonas* sp. (controls; benign algal prey as above), and a 50:50 mix of TOX-A zoospores + *Cryptomonas* sp. (n = 3). Observed mortality was considered to have occurred because of the presence of *P. piscicida* toxin (defined as in *P*ICWG 2002, Burkholder & Glasgow 2001, Kimm-Brinson et al. 2001, Melo et al. 2001) that had diffused through the tubing. Mortality was defined as absence of ciliary movement for >1 min, quantified at 15 min intervals for 1 h.

Expt 3—grazing by juvenile and adult oysters on Pfiesteria piscicida: Grazing trials were conducted with juvenile and adult eastern oysters (tested separately), using *P. piscicida* zoospores (TOX-A, TOX-B, or NON-IND) or cryptomonads (controls) at 2.5×10^3 cells ml^{-1} for juveniles (in gently aerated 400 ml glass beakers), and 5.0×10^3 cells ml^{-1} for adults (in aerated 2 l glass beakers). Zoospore sub-cultures were adjusted to the desired density using ultra-filtered seawater (0.2 µm porosity; this experiment was not conducted at salinity 25 ppt because of difficulty in maintaining this strain of TOX-A zoospores over time at that salinity, but see Burkholder et al. 1995). The gentle aeration was sufficient to maintain *P. piscicida* or cryptomonad cells in suspension. Unpreserved and acidic Lugol-preserved

samples (Vollenweider et al. 1974) of fecal material and plankton were taken at t_0 and at 15 min intervals for the first hour. Additional fecal samples were taken at 24 h.

Zoospores were quantified using an Olympus AX-70 light microscope (Nomarski, 600×) and the Utermöhl technique (Lund et al. 1958). Cells were quantified from 20 fields selected at random down a central transect of the Utermöhl chamber (reported as cells ml^{-1}), and were corroborated using a Coulter Multisizer IIe particle analyzer equipped with a 100 µm aperture tube calibrated using 20 µm polystyrene latex beads (L20 size standard; Coulter). Each treatment was analyzed with 20% replication (variation <5% among replicates; US EPA 1998).

Expt 4—survival following passage through oyster digestive tract: Like many other dinoflagellates, *Pfiesteria piscicida* forms temporary cysts in response to sudden environmental stress (Taylor 1987, Burkholder & Glagow 1997, Burkholder et al. 2001b). In preliminary experiments we noted that TOX-A and TOX-B zoospores produced temporary cysts after passage through the digestive tract of juvenile and adult *Crassostrea virginica*. One hundred cysts per replicate (n = 3) from TOX-A and TOX-B treatments were isolated using an hydraulic micromanipulator (Model MMO-202N, Narishige International USA). Cysts were added to 47 mm glass-bottomed petri dishes containing ultra-filtered natural seawater (0.22 µm-porosity Millipore filters) adjusted to a salinity of 15 ppt. Excystment and subsequent zoospore motility were observed at 1 h intervals for 24 h.

Statistical analyses—Expts 2 to 4. For each experiment, a repeated-measures analysis of variance (ANOVA) model was used to assess the main and interactive effects of treatment and time on the response variable (SAS Institute 1997). The repeated-measures factor was time, and the treatments consisted of *Pfiesteria piscicida* functional type (TOX-A, TOX-B, NON-IND) and/or benign *Cryptomonas* sp. prey. Response variables were larval survival (Expt 2), grazing rate (Expt 3), and excystment (Expt 4).

RESULTS

Zoospore predation: directed attack behavior toward larvae

In Expt 1, *Pfiesteria piscicida* zoospores (all functional types) showed directed attack behavior toward oyster and scallop larvae. These appeared to be chemosensory and involved a rapid, aggressive feeding response—hence, the term 'attack behavior' (also see Riessen et al. 1985, Tjossem 1990, Lewitus et al. 1999). At 25 ppt salinity, zoospores swarmed around scallop larvae within seconds (Fig. 1A). After 2 min, zoospores had extended their peduncles and had begun to attach to the outer edge of the larval shell (Fig. 1B). Larvae began to weaken soon thereafter, generally indicated by a noticeable (10 to 15 µm) valve gape. After 5 to

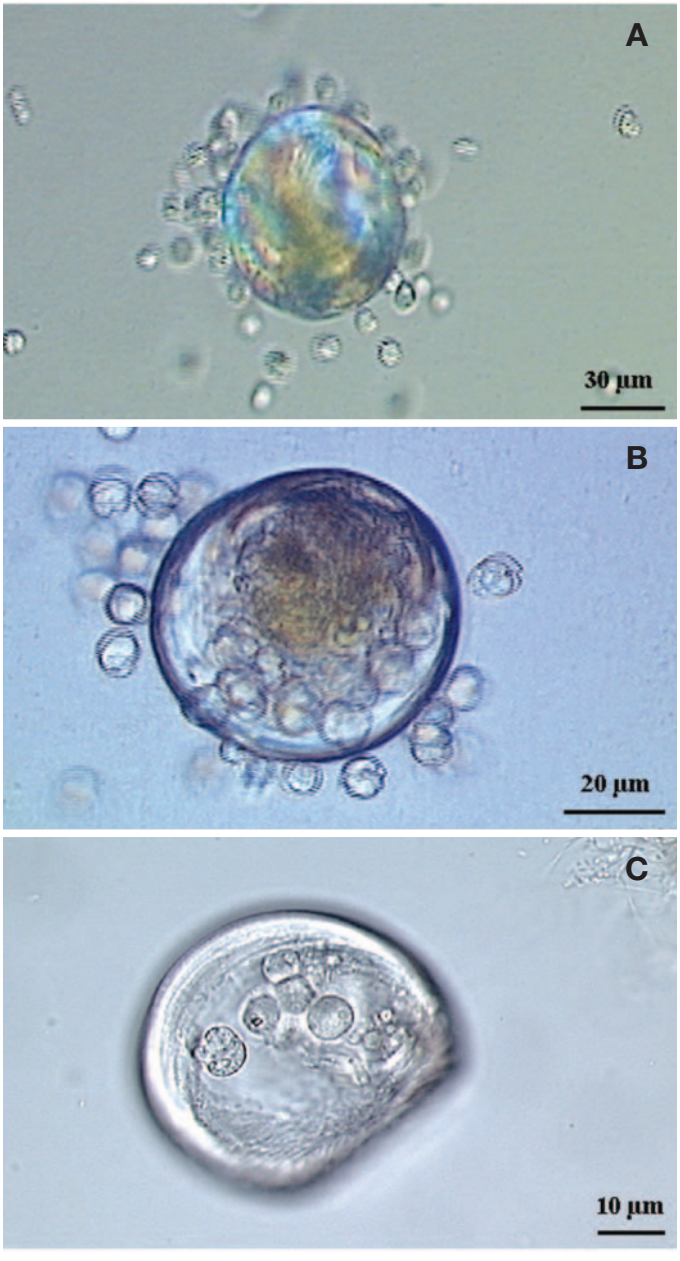

Fig. 1. *Pfiesteria piscicida.* Actively toxic (TOX-A) zoospores preying upon bay scallop (*Argopecten irradians*) larvae (salinity 25 ppt) that had discarded their vela, including (A) zoospores swarming around a larva (150×, t = 60 s); (B) zoospores inside the larva consuming its tissues, with some zoospores still swarming outside (400×, t = 2 min); and (C) zoospores encysted inside a larva after consuming most soft tissues (400×, t = 7 d)

15 min, most zoospores had penetrated into the larval mantle cavity to consume soft tissues, most notably gill and mantle. The observed zoospore behavior appeared to be more aggressive toward oyster larvae (at 15 ppt) and tempered for bay scallop larvae (at 25 ppt), although larvae of both species were consumed. An encystment response was noted in satiated (engorged) zoospores ca. 30 min after introduction. Zoospores that had encysted inside the larvae remained attached to the inside of the shells for up to 1 wk after the conclusion of the experiment (Fig. 1C). It should be noted that only larvae that had discarded their vela were preyed upon by zoospores, whereas larvae with active, extended vela avoided attack. The cilia located on the velar band beat consistently, perhaps acting as an effective deterrent by creating turbulence that prevented zoospore attachment.

Table 1. Repeated-measures ANOVA assessing main and interactive effects of treatment and time on response variables for shellfish exposed to *Pfiesteria piscicida*

Factor	df	SS	MS	*F*	p
Dependent variable: % larval survival (Expt 2; Fig. 2)					
Treatment	3	41402.183	13800.727	2059.81	<0.0001
Error (Treatment)	8	53.600	6.700		
Time	4	25830.900	6457.725	834.15	<0.0001
Time × Treatment	12	14216.566	1184.713	153.03	<0.0001
Error (Time)	32	247.733	7.7416		
Total	59	81750.982			
Dependent variable: cells ml^{-1} (Expt 3; Fig. 3)					
Treatment	2	7504126.711	3752063.356	36.88	0.0004
Error (Treatment)	6	610384.000	101730.667		
Time	4	14775528.58	3693882.14	1122.81	<0.0001
Time × Treatment	8	2505181.96	313147.74	95.19	<0.0001
Error (Time)	24	78956.67	3289.86		
Total	44	25474177.921			
Dependent variable: cells ml^{-1} (Expt 3; Fig. 4)					
Treatment	2	38955093.64	19477546.82	21.68	0.0018
Error (Treatment)	6	5391549.33	898591.56		
Time	4	40277235.24	10069308.81	350.28	<0.0001
Time × Treatment	8	6334651.02	791831.38	27.55	<0.0001
Error (Time)	24	689919.33	28746.64		
Total	44	91648448.56			
Dependent variable: % excystment (Expt 4; Fig. 6)					
Treatment	1	1413.128	1413.128	17.89	0.0134
Error (Treatment)	4	315.897	78.974		
Time	12	54613.461	4551.121	325.35	<0.0001
Time × Treatment	12	1635.871	136.322	9.75	<0.0001
Error (Time)	48	671.435	13.988		
Total	77	58649.792			

Zoospore toxicity to larval shellfish

In Expt 2, there was 100% survival of bay scallop larvae in the controls exposed to *Cryptomonas* sp. held within dialysis tubing. In contrast, significant main effects of *Pfiesteria piscicida* functional type ($p < 0.0001$) and time ($p < 0.0001$) on larval survival were documented when TOX-A and TOX-B zoospores were prevented from direct contact with the scallop larvae (Table 1, Fig. 2). There was also a significant interaction between time and treatment on larval survival ($p < 0.0001$). Highest and second-highest mortalities were observed for larvae exposed to TOX-A and TOX-B zoospores, respectively, indicating that the TOX-B zoospores had retained residual toxicity toward the larvae. Shellfish mortalities observed in the treatment consisting of 50:50 mix of TOX-A *P. piscicida* zoospores: *Cryptomonas* sp. were considered to have resulted from exposure to the toxin from TOX-A zoospores.

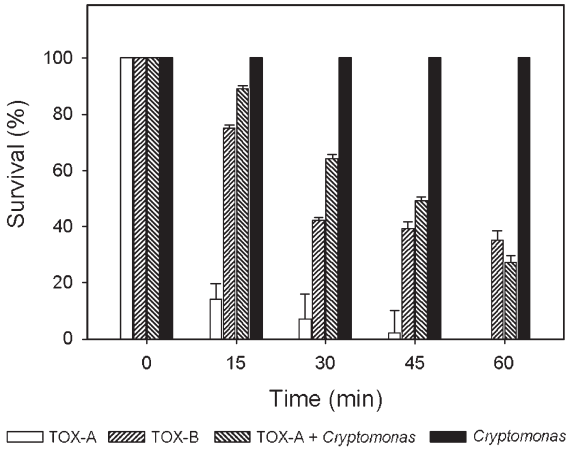

Fig. 2. *Argopecten irradians.* Survival of larval bay scallops assayed with *Pfiesteria piscicida* zoospores that were constrained within dialysis tubing to prevent direct contact, including separate trials with actively toxic (TOX-A) zoospores, temporarily nontoxic (TOX-B) zoospores, and a 50:50 mixture of TOX-A zoospores: *Cryptomonas* sp. (salinity 25 ppt; means ±1 SE, n = 3). Controls were designated as larvae with *Cryptomonas* sp. cells that were constrained within dialysis tubing

Grazing by juvenile and adult oysters on *Pfiesteria piscicida*

In Expt 3, no oyster mortality was observed in cryptomonad controls or *Pfiesteria* treatments throughout the 24 h period. Clearance of benign cryptomonads from the medium by control juvenile and adult oysters

occurred at a rate similar to clearance of NON-IND *P. piscicida*. However, whereas juvenile oysters rapidly cleared NON-IND *P. piscicida* zoospores from the medium, the TOX-A and TOX-B functional types were cleared at progressively slower rates (e.g. 2050 vs 730 cells ml^{-1} h^{-1} for NON-IND vs TOX-A zoospores, respectively, with intermediate clearing of TOX-B cells: Fig. 3). Pseudofeces production was not observed in any of the experimental trials. There was a significant main effect of treatment ($p = 0.0004$) and time ($p < 0.0001$) on grazing rate, and a significant interactive effect between time and treatment ($p < 0.0001$, Table 1).

In contrast, adult oysters rapidly cleared TOX-B and NON-IND *Pfiesteria piscicida* and the control *Cryptomonas* sp. from the medium at similar rates (ca. 3000 cells ml^{-1} h^{-1}), whereas clearance of TOX-A zoospores was significantly slower (ca. 1700 cells ml^{-1} h^{-1}; Fig. 4). There was a significant main effect of treatment ($p = 0.0018$) and time ($p < 0.0001$) on grazing rate as well as a significant interactive effect between time and treatment ($p < 0.0001$, Table 1).

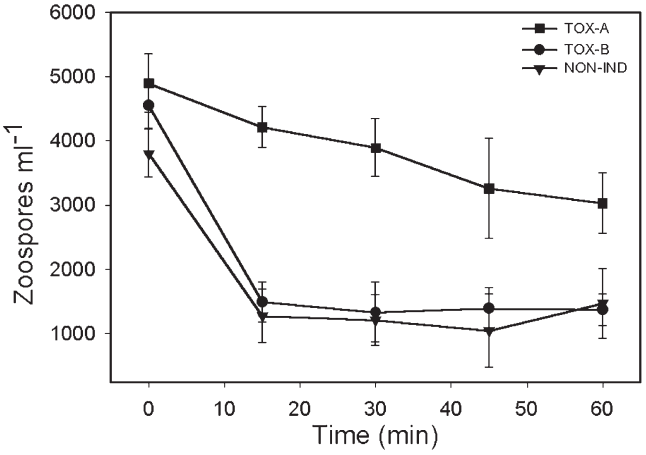

Fig. 4. *Crassostrea virginica.* Grazing of adult eastern oysters on *Pfiesteria piscicida* zoospores, including actively toxic (TOX-A), temporarily nontoxic (TOX-B), and non-inducible (NON-IND) functional types (salinity 15 ppt; means ±1 SE, n = 3). Grazing on cryptomonads (data not shown) was comparable to that on TOX-B and NON-IND zoospores

Excystment of toxic *Pfiesteria picicida* following gut passage

In Expt 4, qualitative analysis of fecal material from adult oysters 1 h after exposure to *Pfiesteria piscicida* indicated that TOX-A and TOX-B zoospores had been packaged into the fecal ribbon (Fig. 5). Many of the ingested zoospores formed temporary cysts as they passed through the oyster digestive tract. These cells rapidly excysted; 50% had regained motility within 6 h, and >75% had regained motility within 24 h (Fig. 6). The remaining temporary cysts did not produce motile zoospores over 5 d of additional observation. There were significant main effects of treatment ($p = 0.0134$) and time ($p < 0.0001$) on excystment, and a significant interactive effect between time and treatment ($p < 0.0001$; Table 1).

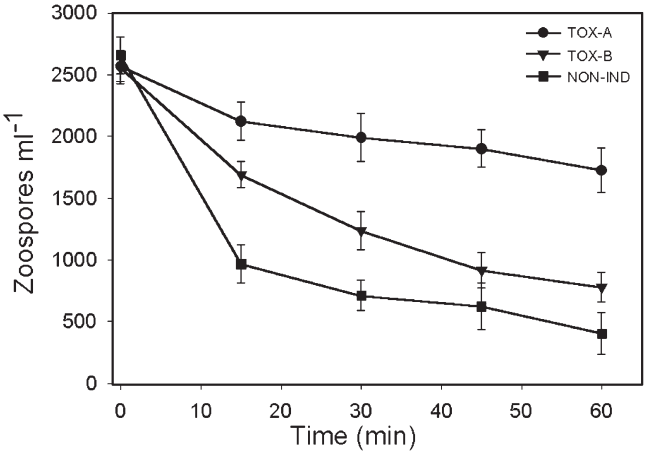

Fig. 3. *Crassostrea virginica.* Grazing of juvenile eastern oysters on *Pfiesteria piscicida* zoospores, including actively toxic (TOX-A), temporarily nontoxic (TOX-B), and non-inducible (NON-IND) functional types (salinity 15 ppt; means ±1 SE, n = 3). Grazing on cryptomonads (data not shown) was comparable to that on NON-IND zoospores

DISCUSSION

This study has documented, for the first time, direct feeding behavior of the toxic dinoflagellate *Pfiesteria piscicida* on shellfish larvae. For these tested strains, both TOX-A and TOX-B zoospores aggressively began to consume bay scallop and eastern oyster larvae within seconds. Such behavior has not been published, to our knowledge, for other toxic dinoflagellate species, and subsequent encystment within the pediveliger shells may provide protection from water-column microfaunal predators known to consume *P. piscicida* (Stoecker et al. 2002).

In addition to the larval mortality from zoospore feeding, we also demonstrated a toxic effect of *Pfiesteria piscicida* zoospores on bay scallop larvae. In previous research conducted with *P. piscicida* and adult bay scallops, 100% mortality of adult bay scallops was observed <20 min after the shellfish had been added to actively toxic *P. piscicida* cultures with live finfish (Burkholder et al. 1992, Burkholder & Glasgow 1997). *Pfiesteria* spp. behavior has been shown to differ substantially among strains, including strains within the same functional type (Burkholder et al. 2001a). Thus,

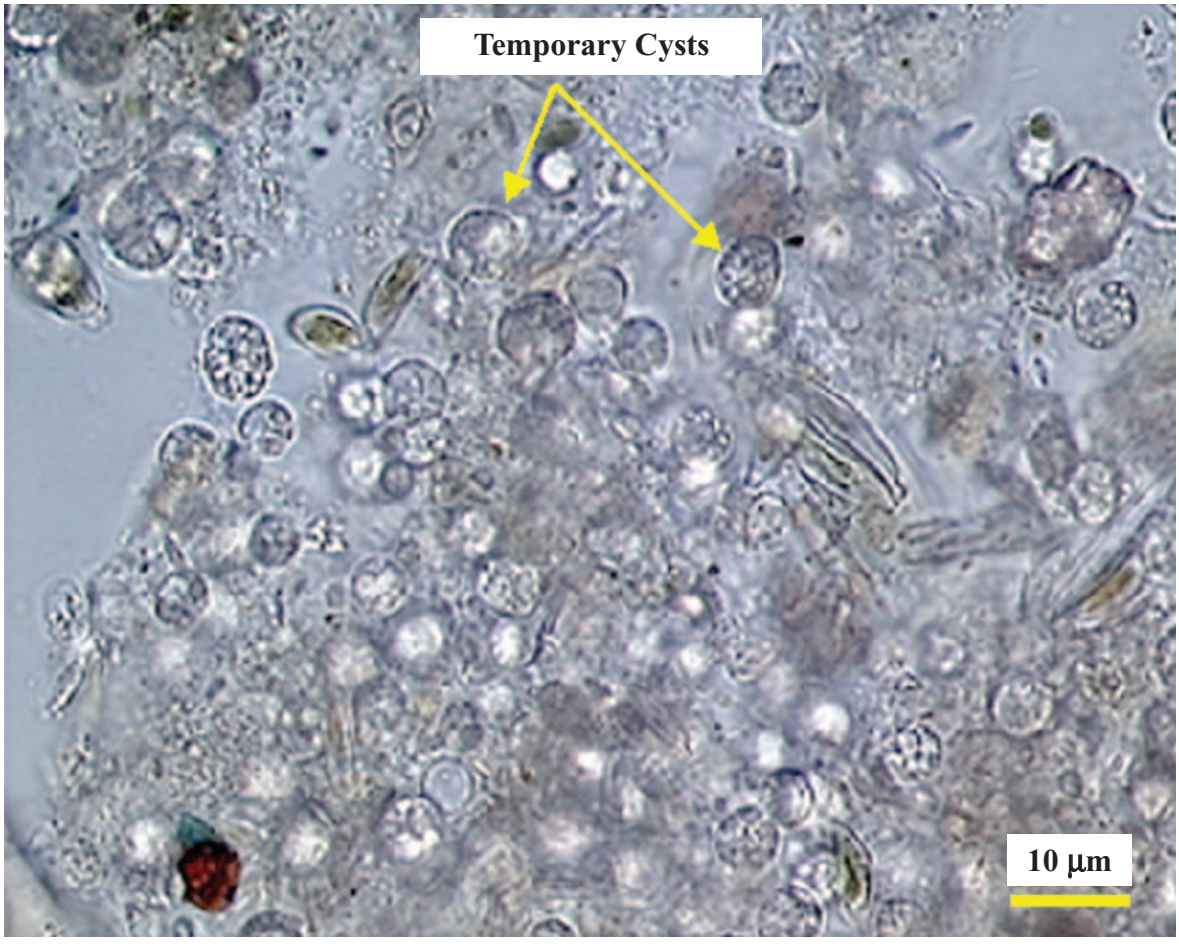

Fig. 5. *Pfiesteria piscicida.* Fecal strand containing TOX-A zoospores that had formed temporary cysts after passage through the digestive tract of an adult eastern oyster (*Crassostrea virginica*) (400×, salinity 15 ppt, t = 24 h)

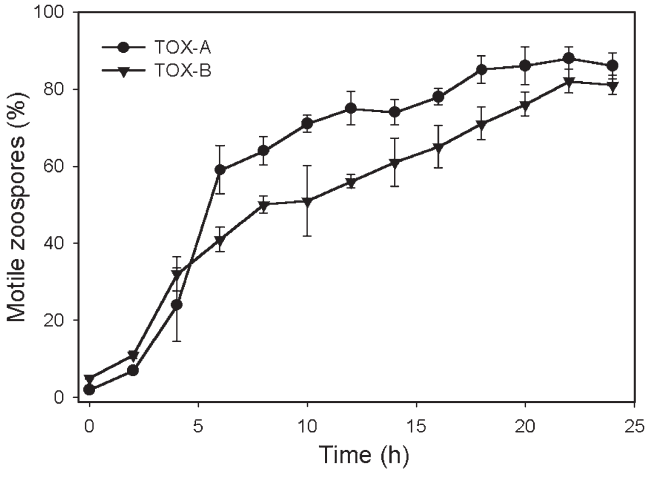

Fig. 6. *Pfiesteria piscicida.* Excystment of zoospores after passage through the digestive tract of adult eastern oysters (salinity 15 ppt; means ± 1 SE, n = 3)

some *Pfiesteria* toxic strains have been lethal to finfish when held within dialysis membranes to prevent direct contact, as in this study with shellfish, whereas others have required close proximity or direct contact for lethal effects (Burkholder et al. 2001b). We expect that such variability in impacts among *Pfiesteria* strains, which may be related to production of different toxin(s) as in other dinoflagellates (Hallegraeff et al. 1995), also extends to shellfish.

The behavior and feeding trials of this study indicate that *Pfiesteria piscicida* zoospores could potentially affect shellfish recruitment and survival. Larval and juvenile oysters were sensitive even to residual toxicity from TOX-B zoospores that had been without live fish for an extended period (weeks). In contrast, adult oysters responded similarly to TOX-B and NON-IND zoospores, and appeared to be insensitive to residual toxicity of TOX-B zoospores. Adult oysters actively

grazed bloom concentrations of *P. piscicida* zoospores (5×10^3 cells ml^{-1}; cell densities during toxic outbreaks are $>3 \times 10^2$ to 10^4 cells ml^{-1}; Burkholder & Glasgow 1997, Burkholder et al. 2001a). Burkholder et al. (1995) did not observe adult oyster mortality after 3 wk from feeding toxic *Pfiesteria* spp. continuously at similar densities (2×10^3 TOX-A cells ml^{-1}).

To assess the relevance of these laboratory results, the data should be considered within the context of the potential for overlap of actively and potentially toxic *Pfiesteria piscicida* with the habitat and recruitment periods for bay scallops and eastern oysters. The major period of activity by TOX-A and TOX-B zoospores ranges from March through October (occasionally through December; Burkholder et al. 1995). TOX-B zoospores (algivorous/omnivorous) appear to track the spring phytoplankton bloom in North Carolina estuaries (research in the Neuse Estuary; Burkholder et al. 2001a, Glasgow et al. 2001a), reaching maximal densities in April. TOX-A zoospores (piscivorous) are maximal in late summer to early fall, as Atlantic menhaden (*Brevoortia tyrannus* Latrobe) begin to move downestuary for fall migration out to sea (Manooch 1988, Glasgow et al. 2001a). *Argopecten irradians* has a relatively narrow salinity range and prefers waters ranging from 20 to 30 ppt (Tettelbach & Rhodes 1981), while *Crassostrea virginica* occurs across a fairly broad salinity range from ca. 3 to 31 ppt (Carriker 1951). *P. piscicida* is capable of lethal activity toward fish across a salinity range from ca. 2 to 35 ppt (Burkholder et al. 1995, 2001a), with an optimum of ca. 15 ppt in North Carolina waters. *A. irradians* generally spawns during the late summer months, but can spawn from May through October depending on the latitude and the sub-population (Rhodes 1990). Populations of *C. virginica* on the Gulf Coast and north along the Atlantic Coast to Virginia tend to produce a major spawn in the spring, with minor spawning throughout the summer followed by another major spawn in the fall (Hayes & Menzel 1981, Ortega & Sutherland 1992, Thompson et al. 1996). Therefore, a portion of the spawning cycle of both species overlaps the period of zoospore activity in toxic *P. piscicida* strains. The available evidence suggests that *P. piscicida* could potentially impact both shellfish species in their natural habitat, with a higher likelihood of affecting *C. virginica*.

These data indicate the potential for adult *Crassostrea virginica* to concentrate toxic *Pfiesteria piscicida* cells and, thus, *P. piscicida* toxin through filter-feeding activity. The extent to which this may occur cannot be determined until purified toxin standard becomes available for development of a reliable assay to detect the toxin in shellfish. Such an assay would also facilitate study of chronic, long-term impacts from exposure to toxic *Pfiesteria* strains on major life-history stages of these and other shellfish species in habitats of co-occurrence. This study also showed high survival of toxic *P. piscicida* following passage through the digestive tract of adult *C. virginica* as temporary cysts. Thus, there is a high likelihood that viable *Pfiesteria* toxic strains could be transported from one geographic region to another via movement of shellfish broodstock and relaying activities (see Shumway 1990, Hallegraeff 1993).

In summary, we have demonstrated the potential for planktonic (larval) bay scallops and eastern oysters to be affected adversely by an aggressive feeding response of *Pfiesteria piscicida* zoospores toward soft tissues, and also by toxin from *P. piscicida*. Grazing rates of *Crassostrea virginica* on *P. piscicida* zoospores were dependent on the *P. piscicida* functional type. Of particular importance to natural resource managers is the finding that TOX-A and TOX-B zoospores survived passage through the digestive tract of adult *C. virginica*. Thus, activities of aquaculture operations may inadvertently contribute to the transfer of viable *P. piscicida* cysts between geographic locations. Although *P. piscicida* blooms have been linked to certain major finfish kills in mid-Atlantic US coastal estuaries (Glasgow et al. 2001a), historically little attention has been directed toward the health of shellfish populations in the affected areas. We recommend that field-based monitoring and research activities should include resident shellfish populations to gain further insights about shellfish health in estuarine waters inhabited by toxic *Pfiesteria* species.

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