Pfiesteria piscicida and Pfiesteria shumwayae in coastal waters of Long Island, New York, USA

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Abstract:
Water and sediment samples were collected during summer and early fall 1999–2004 from coastal waters of New York State, USA, to test for the presence of Pfiesteria piscicida and Pfiesteria shumwayae. Physical and chemical conditions were characterized, and real-time polymerase chain reaction assays were conducted. Both species were relatively common and found at most sites at least once, and the frequency of positive assays was higher in sediments than in the water column. In a subset of the data from Suffolk County, Long Island, the presence of Pfiesteria was related to high chlorophyll a and relatively high nutrient concentrations. Partial SSU rDNA sequences of four PCR amplicons generated using P. shumwayae primers indicated two sequences: three were identical to GenBank P. shumwayae entries, but one showed enough sequence difference (15 positions in a 454 bp amplicon) to suggest a possible new species. Three isolates were tested for toxicity, and one was found to kill fish in bioassays. Despite the widespread presence of both Pfiesteria species and demonstration of potential to harm fish, no blooms of these dinoflagellates have been observed, nor has there been evidence of Pfiesteria-related fish or human health problems in these waters, likely related to colder temperatures than optimal for Pfiesteria species.
Keywords: Dinoflagellates; Long Island; New York; Pfiesteria

Article:

1. Introduction

Prior to 1999, determination of the presence of Pfiesteria species was dependent upon a multi-step process of optical microscopy, fish bioassays, and confirming analysis of suture-swollen or membrane-stripped cells with scanning electron microscopy (Burkholder et al., 2001a). At that time the geographic range extended from a few locations on the Gulf Coast of the USA to the Delaware Inland Bays, with apparent concentration in large estuarine embayments in North Carolina and Chesapeake Bay. With the development of molecular probing methods (Rublee et al., 1999; Bowers et al., 2000; Oldach et al., 2000) it became easier to sample more broadly and knowledge of the geographic range rapidly extended from Texas to Rhode Island in the USA, and then globally (Rublee et al., 1999, 2001, 2004; Jakobsen et al., 2002; Rhodes et al., 2002, 2006).

In addition to demonstrating a global distribution of Pfiesteria species, these studies showed that the environmental tolerance of these organisms was clearly broader than originally observed, although fish and human health risks appeared within a much more restrictive geographic range, the mid-Atlantic Coast of the USA (Burkholder et al., 2001a). The widespread distribution also raised questions about the mechanism(s) of distribution. Recognition of: (1) resistant cyst stages in the life history (Burkholder et al., 2001a); (2) virtual uniformity in ribosomal sequence within species among widely dispersed geographic isolates of Pfiesteria species (Tengs et al., 2003; Rublee et al., 2005); (3) positive assays for Pfiesteria species in the ballast water of coastal and ocean-going vessels (Doblin et al., 2004; Drake et al., 2005) helped to provide some insight into the distribution of these organisms. This study reports the results of five years of molecular assays to detect Pfiesteria species in waters of New York State, an area once considered to be suboptimal because of cold temperatures, or outside the active range of Pfiesteria.

2. Materials and methods

A total of 720 samples (573 water and 147 sediment samples) from more than 100 sites in New York coastal waters were assayed for the presence of the toxigenic dinoflagellates, Pfiesteria piscicida Steidinger et Burkholder (Steidinger et al., 1996) and Pfiesteria shumwayae Glasgow et Burkholder (Glasgow et al., 2001) from 1999 to 2004. Only sites that had tested positive in the previous years were resampled in 2003. Most samples (544) were taken in waters of Suffolk County, Long Island (Fig. 1). Water samples, generally taken during summer and fall of each year, consisted of 50–100 ml of estuarine water drawn through a GFC filter and placed in a CTAB buffer. Surface sediment samples (2–5 cm^3) included sufficient overlying water to prevent desiccation.

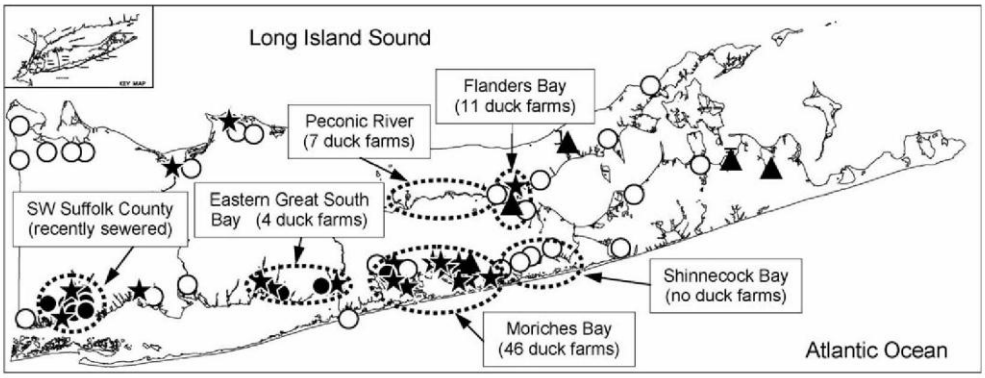

Fig. 1. Map showing sampling locations and *Pfiesteria* spp. detection in coastal waters of Suffolk County, Long Island, New York. Open circles represent locations where neither *Pfiesteria* species was detected; closed circles represent locations were only *Pfiesteria piscicida* was detected; closed triangles represent locations where only *Pfiesteria shumwayae* was present; closed stars represent locations where both *Pfiesteria* spp. were detected.

Samples were assayed for Pfiesteria spp. by polymerase chain reaction (PCR). Prior to 2002, assays were conducted by conventional PCR using primers developed by Oldach et al. (2000). Thereafter, assays were conducted with real-time PCR on a Cepheid SmartCycler using Taqman probes as described by Bowers et al. (2000). Recently, a randomly selected subset of samples from 2004 and 2005 that had previously tested positive for P. shumwayae were amplified with the P. shumwayae specific forward primer (5'-TGCATGTCTCAGTT-TAAGTCCCA-3') and a "universal" 18S reverse primer (508R 5'-TACCGCGGGTGCTGGCAC-3') in order to sequence amplicons generated by P. shumwayae specific primers in 50 μl PCR reactions (300–500 ng genomic DNA; 2.5 mm $MgCl_2$, 200 μM nucleotides, 1 u Taq polymerase [Promega, Madison, WI] 5 μl 10× buffer, 10 μM BSA, and 10 μM of each primer) under the following conditions: 94 °C for 4 m followed by 30 cycles of: 94 °C for 1 m, 57 °C for 1 min, 72 °C for 2 min: and a final extension step of 72 °C for 6 m. Amplicons were obtained from four of the samples, whose reaction products were then cleaned up and directly sequenced on a Megabace automated DNA sequencer (Amersham Biosciences) using the DYE-namic ET terminator kit (Amersham Biosciences) with the appropriate forward or reverse primer. Reaction conditions for dye labeling were: 29 cycles of: 95 °C for 20 s, 50 °C for 15 s, and 60 °C for 2 min. Each sample was sequenced in both a forward and reverse direction.

Table 1
Number of samples from New York coastal waters that tested negative vs. positive for *Pfiesteria* spp.

Year	*n*	Negative	Conventional PCR		Real-time PCR	
			Pfiesteria piscicida	*Pfiesteria shumwayae*	*P. piscicida*	*P. shumwayae*
1999	163	154	5	4	–	–
2000	192	162	17	15	–	–
2001	52	50	1	2	2[a]	6[a]
2002	58	49	7	2	7	2
2003	149	32	96	46	96	46
2004	106	52	43	32	43	32

[a] Number of samples from 25 randomly selected samples taken and analyzed in 2001 that tested positive by real-time PCR in 2001.

Physical and chemical characteristics of water samples were also taken on most dates. In Suffolk County, temperature, salinity and/or conductivity, and dissolved oxygen measurements were made in the field using a YSI Model 85 digital hand held meter (Yellow Springs Instruments, Yellow Springs, OH). Water samples were collected for analysis of ammonium, nitrite + nitrate, total and dissolved Kjeldahl nitrogen, urea, total

phosphorus, total dissolved phosphorus, soluble reactive phosphate, total organic carbon, dissolved organic carbon, silicate, and chlorophyll a. The sampling and analytical methodologies followed Nuzzi and Waters (2004).

3. Results

Approximately 30% of samples tested positive for at least one Pfiesteria species. The percentages ranged from 6% positive during 1999, using conventional PCR, to more than 60% positive in 2003 when the more sensitive real-time PCR and Taqman probes were used (Table 1). Among the samples, 21% of 573 water samples and 62% of 147 sediment samples tested positive for one or both Pfiesteria species. The increase in positive results in 2003 likely was also related to the sampling design, since only sites that had previously tested positive were sampled in 2003. Most samples came from Suffolk County, Long Island, where approximately half of the stations yielded a positive sample for at least one of the two target organisms. Stations on the south shore of Long Island had more positive samples in comparison to north shore stations. A relationship was not discerned between samples testing positive for one or both target species and environmental physical/chemical characteristics, except that Pfiesteria species were more common in sites with higher chlorophyll a content (Figs. 2 and 3), indicating higher phytoplankton abundance.

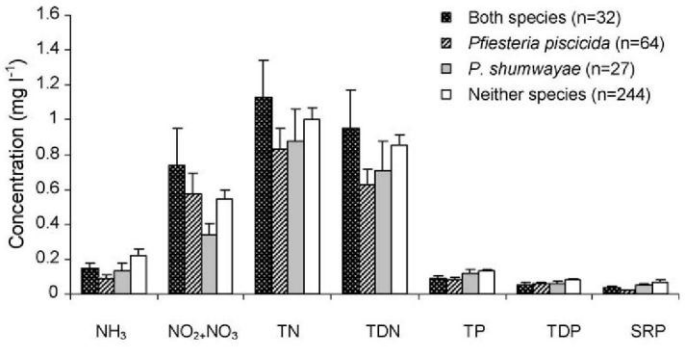

Fig. 2. Nutrient concentrations in coastal waters along Suffolk County that were sampled for *Pfiesteria* spp. (mean ± 1 standard error (S.E.)).

Three samples, collected during 2003 at sites where duck farms had formerly operated, were tested for toxicity using standardized fish bioassays of Burkholder et al. (2001b, 2005). One sample, from Brushy Neck Creek, containing Pfiesteria shumwayae was deter-mined to contain a toxic strain. Four samples collected in 2004 that tested positive for P. shumwayae by real-time PCR were examined further to determine whether a variant sequence of P. shumwayae rDNA was present, given that recent work had indicated the existence of such a variant elsewhere (Rhodes et al., 2006). When PCR amplicons were sequenced, three of the four samples (all sediment samples) yielded sequences (GenBank accession nos. DQ401154–DQ401156) that were identical to the SSU rDNA sequences for P. shumwayae reported in GenBank prior to 2006 (GenBank accession nos. AF080098, AF218805, AY245694). One water sample showed a 452 bp variant sequence (GenBank accession no. DQ401157), which had 100% similarity with P. shumwayae variant sequences found in New Zealand by Rhodes et al. (2006, GenBank accession nos. DQ387443– DQ387449), but only 96% similar to the other three P. shumwayae sequences found in this study and to the P. shumwayae sequences reported in GenBank prior to 2006.

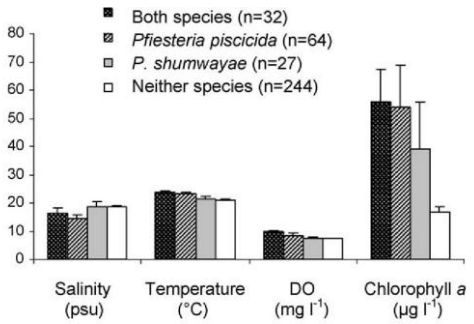

Fig. 3. Salinity, temperature, dissolved oxygen (DO), and chlorophyll a concentrations in coastal waters along Suffolk County that were sampled for *Pfiesteria* spp. (mean ± 1S.E.).

4. Discussion

The initial finding of Pfiesteria species in Long Island waters extended the northern limit of detection of these dinoflagellates (Rublee et al., 1999). Subsequent studies have extended the range globally (Jakobsen et al., 2002; Rhodes et al., 2002, 2006; Rublee et al., 2005), suggesting that their occurrence is both common and widespread. Consistent with this premise has been the detection of Pfiesteria spp. in ballast water of ships transiting the North Atlantic and the US East Coast (Doblin et al., 2004; Drake et al., 2005). Thus, in this study we had expected that Pfiesteria species would be common in New York coastal waters.

The distribution of positive assays in samples from Long Island waters suggests that there are patterns of distribution at regional and local levels. Previous studies have noted a broad range of environmental conditions in which toxic and nontoxic Pfiesteria isolates have been found (Burkholder et al., 2001a,b). There is a narrower range of conditions in which fish kill events have occurred, characterized by low turbulence, low to moderate salinities, warm temperatures, high nutrient concentrations, and abundant fish. The range of physical and chemical environmental variables in this study was narrow, predominantly because nearly all samples were taken from a relatively small region during summer/ early fall (July–October). Overall, two environmental characteristics of the samples are noteworthy. First, nutrient concentrations found in all samples in this study were relatively high, which is known to enhance Pfiesteria growth (Burkholder et al., 2001a). Second, Pfiesteria spp. were more commonly found where algal abundance (indicated as chlorophyll a concentrations) was relatively high (Fig. 2). This is consistent with the trophic mode of Pfiesteria spp.: despite feeding heterotrophically on a wide range of potential prey (bacteria to fish), they are generally consumers of phytoplankton (Burkholder et al., 2001a).

We also note that most of the positive samples were from the south shore of Long Island. This shore has a string of barrier islands that protect the nearshore mainland, resulting in shallow, sheltered embayments that may sustain populations of organisms such as Pfiesteria due to reduced circulation and flushing, which in turn can maintain warm temperatures, high nutrient concentrations, and low turbulence. The lagoonal system receives surface flows from numerous eutrophic tributaries along its northern shoreline, and it has been estimated that 82% of the nitrogen entering Great South Bay is from stream flow (Dennison et al., 1991). The shorelines of a number of these tributaries are sites of former duck farms, and have highly organic sediments (Howes et al., 1998). The site where the ichthyotoxic strain was found is located in a region that was once the site of numerous duck farms that, in at least one case, discharged directly into the tributary (Suffolk County NY Department, Health Services, unpublished data).

Glibert et al. (2004) found positive correlations between urea concentration and the presence of Pfiesteria spp. in both water and sediments of Chesapeake Bay, Maryland, USA. They noted that fertilizers and poultry waste were the predominant urea sources in their study, and that frequent sampling was needed to detect seasonal spikes of urea. In this study, the limited sampling, both temporally and spatially, prevents rigorous assessment of any linkage between urea concentrations and the presence of Pfiesteria spp. Urea concentrations determined for samples from some stations ranged from below analytical detection to 6.5 μg at N^{-1} (grand mean 0.57 μg at N l^{-1}). A partial explanation for the occurrence of Pfiesteria spp. may be found in the relationship to duck farms and to highly polluted groundwater (Fig. 1). For example, Pfiesteria spp. were detected in nearly all stations in Moriches Bay which presently has two operating duck farms, and at one time had 46. In contrast, no samples positive for Pfiesteria spp. were found in Shinnecock Bay (directly to the east), which has never received duck farm effluent. Further, since groundwater is the major source of water for surface streams, nutrient-enriched ground-water in southwestern Suffolk County may be linked to the high number of positive sites for Pfiesteria spp. in western Great South Bay. Other factors likely are involved as well, however, as several of the positive stations did not have discharges directly into the estuaries, and there are stations with duck farms, or that formerly had duck farms, that do not show positive detection of either Pfiesteria spp.

The south bays of Long Island bear some similarity to other areas along the US Atlantic Coast that commonly support Pfiesteria species: sheltered, poorly flushed estuaries of North Carolina, Chesapeake Bay, and Delaware (Burkholder et al., 2001a). In contrast to these other regions, however, there is not an extensive fish nursery

ground in Long Island waters. In addition, the contributing watersheds are much smaller, and steps that continue to be taken to prevent and reduce pollutant loads (Nuzzi and Waters, 2004) likely are yielding significant dividends more rapidly than such efforts in larger systems (e.g. Burkholder et al., 2006).

Although fish and shellfish kills have been reported in waters adjacent to or near Long Island, none have been linked to Pfiesteria species, and no blooms of these dinoflagellates have been reported, likely related to colder temperatures than optimal for Pfiesteria (Burkholder et al., 200 1 a). Nearly all reported fish kills appear to be the result of hypoxia, which is especially common in western Long Island Sound and the Hudson-Raritan estuary (Connecticut Department of Environmental Protection, 2001; Reid et al., 2002; US Environmental Protection Agency (EPA), 2003, 2006). Shellfish kills may result from hypoxia, but declines have also been attributed to the brown tide dinoflagellate, Aureococcus anophagefferens, Hargraves et Sieburth and to pathogens (e.g. Bricelj and Lonsdale, 1997; US EPA, 2003, 2006; Pearce and Balcom, 2005).

The variant of the SSU rDNA sequence found by direct sequencing of amplicons generated using Pfiesteria shumwayae primers is noteworthy. The similarity of this variant sequence to those found in New Zealand (Rhodes et al., 2006) suggests a widely distributed ribosomal gene variant. Further, the similarity of only 96% with GenBank entries listed for Pfiesteria shumwayae prior to 2006 is a value low enough to suggest the possibility of a new species. Further work on isolation and enhanced molecular characterization of this variant is ongoing and essential to establishing whether the variation in sequence is actually of significance.

5. Recommendations

Our findings suggest that, while Pfiesteria species are widespread in the environment, they are likely to become problematic only under specific environmental conditions that are not yet fully understood. Thus, a first recommendation is continued study of the distribution of this organism, especially in relation to blooms and fish kill events. Also, variants of Pfiesteria species sequences may be more common than previously shown. It is unclear at this time whether the variant SSU rDNA sequence represents a new species, but clearly the taxonomic relationships of Pfiesteria and “pfiesteria-like” species require further study. Information on sequences from additional genes in these taxa, combined with morphological analyses, would be especially helpful to advance understanding about Pfiesteria and closely related species across regions.

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