Monitoring Results for *Pfiesteria piscicida* and *Pfiesteria-like* Organisms from Virginia Waters in 1998

Harold G. Marshall, David Seaborn, and **Jennifer Wolny**,
Department of Biological Sciences, Old Dominion University
Norfolk, Virginia 23529-0266

ABSTRACT

Results of an extensive 1998 monitoring program for the presence of *Pfiesteria*-like organisms (PLO) in Virginia estuaries indicate these dinoflagellates are widely distributed in both the water column, and as cysts in the sediment, however *Pfiesteria piscicida* was not detected at this time. The highest concentrations of PLO were in estuaries along the Virginia shore line of the Potomac River, and in western Chesapeake Bay estuaries from the Little Wicomico River to the Rappahannock River. The most common PLO included *Cryptoperidiniopsis* sp. and *Gymnodinium galatheanum*. The lowest PLO concentrations were at ocean side locations. PLO were also present throughout the water column at stations in the lower Chesapeake Bay, being most abundant in waters above the pycnocline.

INTRODUCTION

Pfiesteria piscicida is a predatory dinoflagellate which is capable of toxin production and has been associated with both massive fish kills and human illness (Burkholder et al., 1995; Glasgow et al., 1995). This species has been identified in several estuaries along the U.S. east coast, with its most extensive development to date in North Carolina estuaries (Burkholder et al., 1995).

There are several dinoflagellate species that resemble *Pfiesteria piscicida* in size, morphology, and some even have similar life cycle stages. These species have been placed in a category called the *Pfiesteria*-like organisms (PLO). They consist of a variety of gymnodinioid type cells that may include besides *Pfiesteria*, species within the genera *Gymnodinium, Cryptoperidiniopsis, Gyrodinium, Amphidinium,* and others (Burkholder, 1997; Steidinger et al., 1997). An earlier designation for this group was *Pfiesteria* complex organisms (PCO), with the term toxic *Pfiesteria* complex (TPC) referring to those *Pfiesteria* species known to produce ichthyotoxins. Burkholder et al. (1999) have also identified both toxin producing and non-toxin producing populations of this species. Since the recognition of minute morphological features of a *P. pisicida* cell is necessary for its identification, light microscopy alone is not adequate to distinguish this species from other PLO (and TPC) cells (Steidinger et al., 1996). However, light microscopy is commonly used as the initial step to identify those cells that can be placed in the PLO category. The enumeration of these cells from a water sample will give what is termed the "presumptive cell counts" for *P. piscicida*. These counts do not by themselves indicate the presence of *Pfiesteria*, only that this species may be included in this assemblage. When these counts exceed a predetermined concentration level of concern, subsequent steps are then followed to determine if *P. piscicida* is the dominant constituent of these counts and if a toxic population of this species is present. Burkholder et al. (1995) indicate cell concentrations of the toxic

P. piscicida 250 cells/mL are generally lethal to fish. If presumptive PLO cell counts of this magnitude occur, further examination using scanning electron microscopy (SEM) is then recommended to provide identification of the dominant species. If *P.* piscicida is present, then a fish bioassay will determine if this is a toxin producing population (Burkholder et al., 1999).

The earliest notation of *Pfiesteria piscicida* in Virginia is given by Burkholder et al. (1995) who identified these cells in the lower reach of the York River. Rublee et al.(1999) using a genetic probe in 1998 have identified *P. piscicida* in Mosquito Creek, which is located on the Virginia ocean side of the Delmarva Peninsula. However, no fish bioassays were run in either of these cases to determine if they were toxin producers. In response to the rising concern regarding the presence of *P. piscicida* reported in Maryland estuaries during 1997, the Virginia Task Force on *Pfiesteria* established a monitoring plan for Virginia waters. This included the examination of water and sediment samples for *P. piscicida* during occurrences of fish kills, or when there was a high incidence of fish bearing lesions. This PLO analysis was conducted at the Old Dominion University Phytoplankton Analysis Laboratory. Neither of these events were common in 1997, and there were only several occasions when the examination of water samples identified PLO cell concentrations greater than 250 cells/mL (Marshall et al., 1998; Marshall and Seaborn,1998). These locations were in the Pokomoke River(Virginia region), Rappahannock River, and Great Wicomico River. None of these events, proved to be associated with *P. piscicida* (based on representative SEM analyses by JoAnn Burkholder and Karen Steidinger). The fish bioassays by Dr. Burkholder also gave negative results for *P. piscicida*. Among these sites, the area that received special attention in 1997 was the Pokomoke River in Maryland, which was the site of a fish kill that was associated with toxic *Pfiesteria*. Although this river originates in Maryland, its lower reach forms the border between Virginia and Maryland, with its southern shoreline in Virginia. Subsequent water analysis and fish bioassays of this lower region did not reveal *Pfiesteria* cells in 1997 (Marshall and Seaborn, 1998). One of the major questions that remained at the close of 1997 centered on what extent is *P. piscicida* and other PLO species present in Virginia estuaries. In order to gain information regarding the distribution and abundance of *Pfiesteria*-like organisms in Virginia estuaries, a broad based monitoring program was established in 1998 under the sponsorship of the Virginia Department of Environmental Quality and the Virginia Department of Health.

This monitoring program emphasized two plans for PLO sample analysis. The first indicated that water and sediment samples would be examined for PLO during significant fish kill events, or when there was a high incidence of fish having lesions. For instance, if more than 20% of a certain fish population had lesions, and there were at least 50 fish in the count, this would warrant sample analysis for PLO. The second approach involved monitoring representative estuaries in Virginia for PLO. In both plans, any high concentrations of PLO would initiate subsequent SEM analysis and toxic fish bioassays.

There were originally two major objectives of this study. The first was to identify the presence and distribution of *Pfiesteria* and other PLO in Virginia estuaries. The other was to determine if there are relationships in the abundance and distribution patterns of PLO to water quality conditions at these sites. Many of these PLO have co-existed with *P. piscicida* during fish kill events (Burkholder et al. 1997; Steidinger,

1997). This infers similar environmental conditions and locations that support PLO development may also apply to the more elusive *Pfiesteria* spp. In support of this second goal, personnel from the Virginia Department of Environmental Quality (VDEQ) analyzed water samples taken during each collection for a broad survey of water quality parameters. This data in relation to the PLO abundance are presented by Weber and Marshall (1999). In general, they found no high correlations between these two sets of parameters. This may be a result of the low cell concentrations and variety of many of these PLO over the 6 month period. Rather than having a single species to relate to these water quality parameters, the PLO were composed of a group of species that may have had different environmental cues and requirements for their development. Also, due to the multiple life stages associated with the PLO, relationships between these different stages and the environmental variables that would influence their development, may not be clearly defined with only one year of data. Other related reports regarding PLO results in the Virginia *Pfiesteria* monitoring program, are those by Marshall et al. (1998a; 1998b; 1999), Seaborn and Marshall (1999), and Seaborn et al. (1999).

METHODS

Personnel from VDEQ collected water and sediment samples during fish kill events, and when a high occurrence of fish lesions were reported. The fish lesion information was provided by the Virginia Institute of Marine Science (VIMS). VDEQ also made monthly collections within Virginia estuaries at 14 stations from June through November 1998, and twice monthly at 20 stations in the Virginia Department of Health COHORT program (Fig. 1), which were sampled from June through October 1998. VIMS personnel also made concurrent fish collections at sites to determine the incidence of fish lesions and reported these figures to VDEQ. VDEQ also provided samples for PLO analysis on a less frequent basis from 29 other sites between April and November 1998. All of these collections included either triplicate, or at times replicate sets of preserved and non-preserved water samples (1l) at each site. Lugol's solution was used as the preservative. There were also 500 mL surface sediment samples taken at the same time at each station with no preservative added.

In addition, personnel from the Virginia Department of Health, Division of Shellfish Sanitation, provided monthly collections of Lugol's preserved water samples (1l) from 105 stations scattered among the Virginia estuaries and Bays (Fig. 2). These collections were made from June through October 1998. One sediment sample (500 mL) was also collected from each station during this period.

Another set of water samples (500 mL) preserved with Lugol's solution were taken at 7 stations located in the Chesapeake Bay between May and October 1998 (Fig. 3). These were monthly collections from the Chesapeake Bay Phytoplankton Monitoring Program and included composite samples taken from waters both above and below the pycnocline.

The majority of the sample collections in the Virginia estuaries were taken in the upper 1 meter of the water column. When collected, Lugol's preserved samples were stored separately from the non-preserved samples which were placed in coolers. Sediment samples were collected by a Ponar Grab lowered to the sediment. When brought to the surface the upper layer of the sediment sample was transferred to
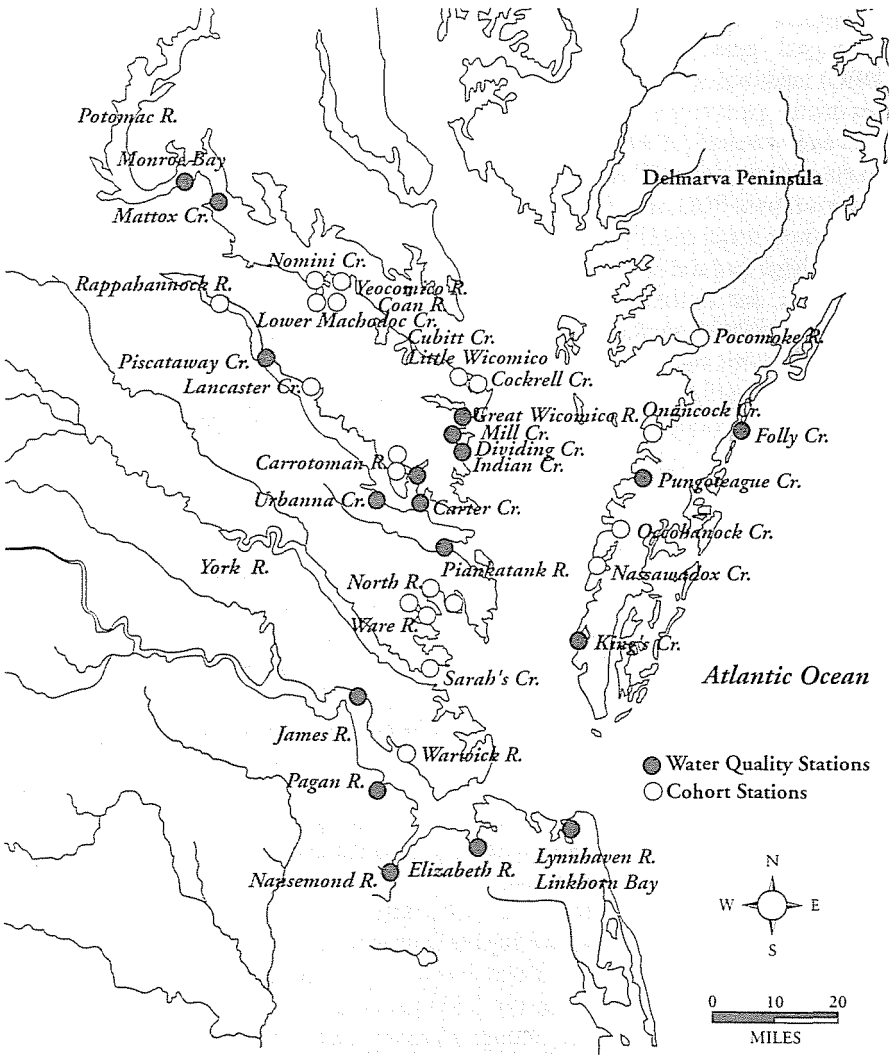
FIGURE 1. Locations for *Pfiesteria* monitoring stations within the DEQ and COHORT programs

collection bottles. All samples were delivered to the Old Dominion University Phyto-plankton Analysis Laboratory for examination.

An aliquot was taken from each water sample (preserved and non-preserved samples) for placement in a Palmer-Maloney cell and examined with light microscopy at 400x magnification. This entire cell was systematically scanned for counting the recognizable PLO cells. Depending upon whether replicate or triplicate samples were taken, each station date provided either 4 or 6 samples for examination. The mean values of these samples were used to appraise the PLO status at each site during fish kill or fish lesion events. If counts exceeded 100 cells/mL in any one of these samples, another reader would re-count the cells for verification. When high cell counts (>100
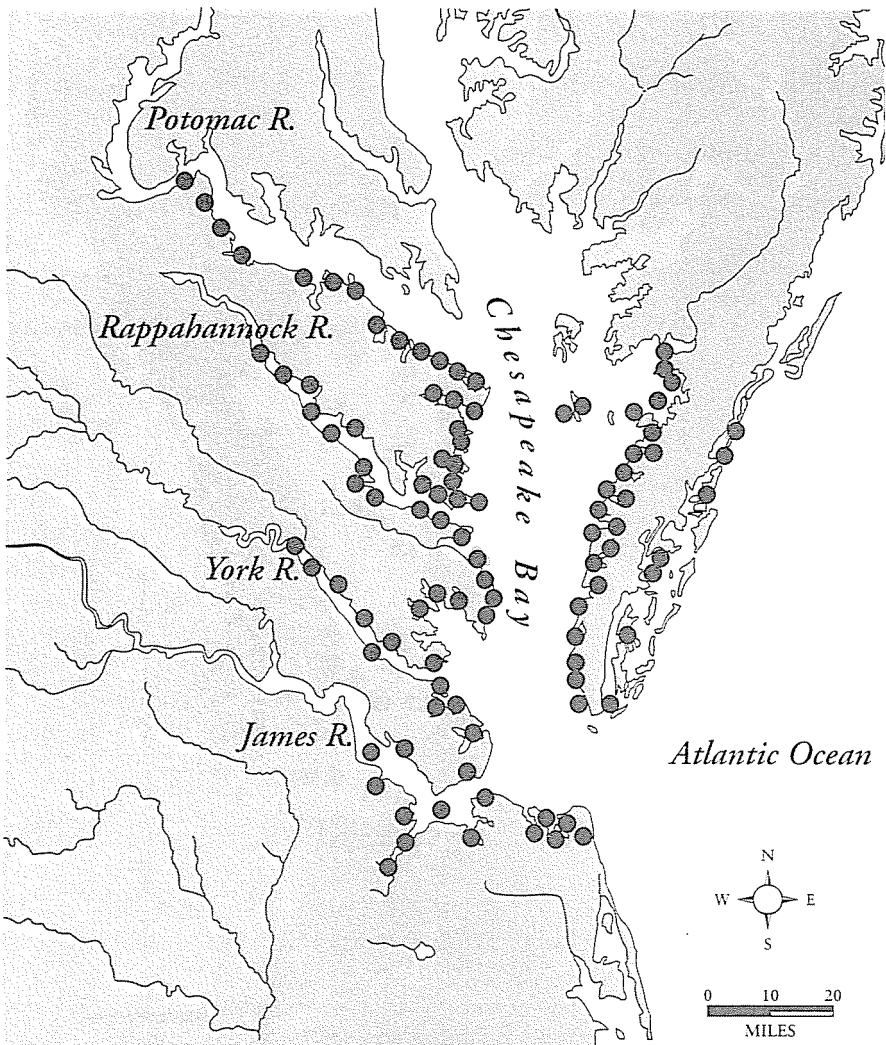
FIGURE 2. Locations for *Pfiesteria* monitoring stations provided by the Virginia Department of Health Shellfish Sanitation Division.

cells/mL) were noted at a site, cell cultures were established by the isolation of the PLO cells from the non-preserved water or sediment samples. Their development in culture flasks was then initiated with the addition of the algal cells of *Cryptomonas* sp. A similar protocol was followed during any incident involving a fish kill or high fish lesion event. These cell cultures were maintained for subsequent SEM analysis and toxic fish bioassays. SEM protocols that may be followed are described by Burkholder and Glasgow (1995). Lewitus et al. (1995), Steidinger et al (1996) and Truby (1997). In contrast, only presumptive cell counts were conducted on the preserved water samples provided by the Division of Shellfish Sanitation and those collected during the Chesapeake Bay cruises.
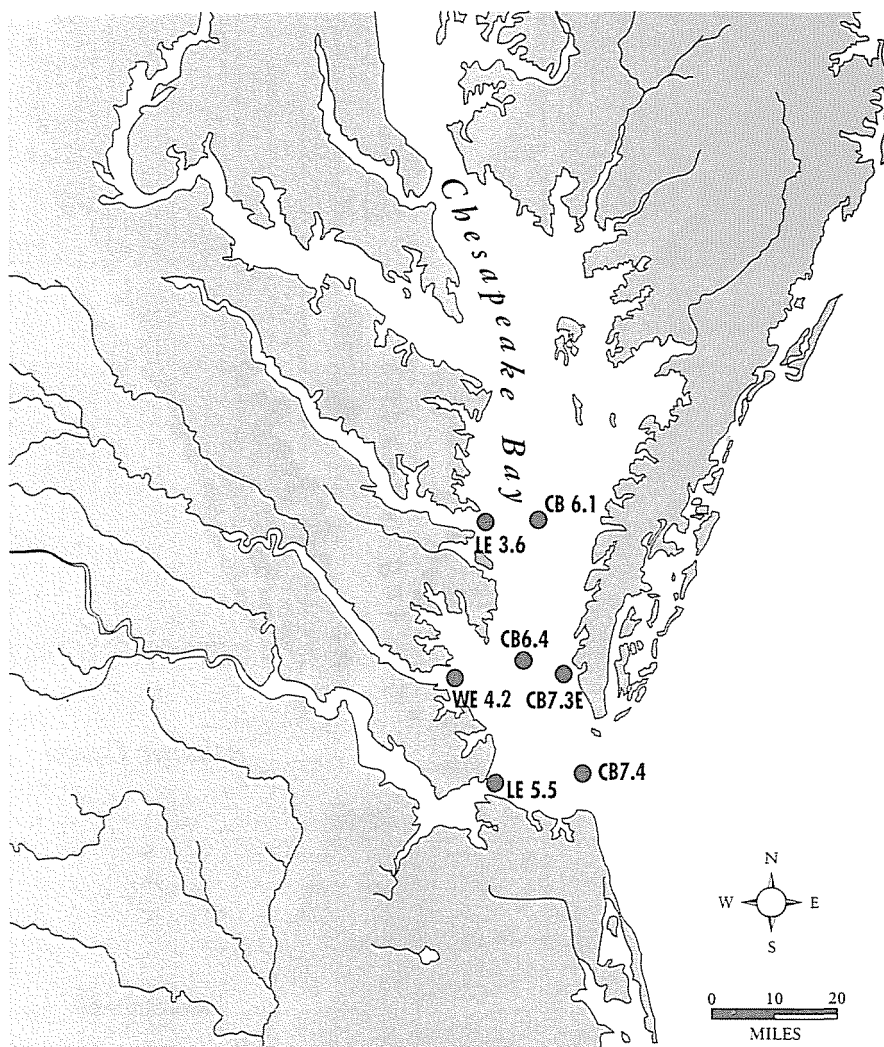
FIGURE 3. Locations for *Pfiesteria* collection sites in the southern Chesapeake Bay.

The sediment samples were used as an additional source of those PLO cells that form cysts. During fish kill and fish lesion events, and at sites when there occurred PLO counts greater than 100 cells/mL, sub-samples of the sediment were placed in culture containers to obtain the motile PLO cells. The dinoflagellate cells in the various cultures were selected and passed through a series of steps to isolate the individual cells which were then placed in culture flasks containing f/2-Si medium (Guillard) at 15 ppt water. This medium was made from water from the Chesapeake Bay, diluted with double de-ionized water and filtered through a 0.2 micron glass filter. Once reaching higher cell concentrations the cells were then processed for SEM analysis and toxic fish bioassays. For corroboration of findings, any PLO suspected to be *P. piscicida*

was forwarded to Dr. JoAnn Burkholder and Dr. Karen Steidinger, with the 1998 fish bioassays conducted by Dr. Burkholder. Subsequently, the toxic fish bioassay facility was completed at ODU in 1998, and these fish bioassays were replicated at ODU thereafter.

RESULTS

Event Responses:

During the 1998 study period there were 5 modest fish kill events in Virginia estuaries. There were another 5 occasions when fish (mostly menhaden) were considered to have a high incidence of lesions. At none of these events were there high PLO cell concentrations. The PLO counts ranged from zero to 40 cells/mL. Low oxygen, or other factors were considered the cause of the fish deaths by the VDEQ. The cause of the fish lesions was undetermined. SEM analysis of cells taken during these events were not identified as *Pfiesteria piscicida*. The most common PLO at these sites were confirmed by Dr. Steidinger to be *Cryptoperidiniopsis* sp. and *Gymnodinium galatheanum*.

VDEQ and VDH Monitoring

The 34 stations that were systematically monitored included eastern and western sites in Chesapeake Bay at locations from the Potomac and Pokomoke Rivers southward to the Chesapeake Bay entrance, plus ocean sites on the Delmarva Peninsula (Fig. 1). From these locations 1437 samples were analyzed, with 34.5% of the samples (496) containing PLO cells. The mean PLO concentrations at the VDEQ and COHORT stations were 10.8 and 11.8 cells/mL respectively. The highest PLO cell counts occurred in the Lower Machodoc River in July 1998 at 270 to 370 cells/mL. Other sites with a single monthly PLO cell counts greater than 100 cells/mL included Little Wicomico River, Great Wicomico River, Nomini Creek, Urbana Creek, Cubit Creek, Dividing Creek, and the York River (Fig. 1). These higher counts also occurred mainly during July, with the sites mentioned above located along the Virginia shore of the Potomac River to sites on the western Chesapeake Bay from the Potomac River to and including the Rappahannock River. Locations that had mean concentrations over the 6 month study period greater than 30 cell/mL were the Little Wicomico River, Lower Machodoc River, Cubit Creek, Urbanna Creek, and the Great Wicomico River. Sites with 6 month values < 2cells/mL were in the Warrick River, North River, Piscattaway Creek, Pagan River, Folly Creek, and mainstream sites in the James River. These locations are further south along the western margin of the Bay, with Folly Creek an ocean side site.

Virginia Shellfish Sites

This group included the analysis of 537 water samples from 105 stations, of which 76.3% of the samples (410) contained PLO. The highest PLO counts of the study occurred at these sites, with 5 stations having monthly concentrations between 200-260 cells/mL. These were located at Cubitt Creek, Urbanna Creek, Onancock Creek, Rappahannock Creek, the Great Wicomico River, and in Linkhorn Bay (See Fig. 1 for locations).

There were 5 other sites where there occurred higher concentrations with levels at 330 (Cockrell Creek), 400 (eastern branch of the Carrotoman River), 560 (Lower

Machodoc River), 790 (Little Wicomico River), and 815 (Mill Creek in Ingram Bay) cells/mL. Four of these locations also had the highest monthly mean cell concentrations of all the collections. These were Cubitt Creek, Cockrell Creek, Lower Machodoc Creek, Little Wicomico River, and Mill Creek (in Ingram Bay) 90, 100, 132, 194, and 244 cells/mL respectively. The mean PLO concentration at the shellfish stations was 31.2 cells/mL. The higher mean cell concentrations for these stations in comparison to the DEQ and VDH sites is possibly due to the greater number of sites sampled from shallow areas (more conducive to PLO presence), and the additional stations sampled in regions associated with these higher cell counts.

There were no major events of fish kills or high fish lesion counts at these sites observed in 1998. The higher cell concentrations occurred during June, July, and September. During these peak concentrations, the salinity range was from 6.6 to 22.1 ppt, with oxygen from 4.9 to 6.7 mg/L, and temperature between 21.1 to 39.3°C. The lowest mean PLO cell counts occurred at stations near the Bay entrance, at eastern Bay sites, and along the ocean side of the Delmarva peninsula.

Chesapeake Bay Monitoring Sites

The Chesapeake Bay stations include 3 that are along the mainstem and 3 located off the mouths of the James, York, and Rappahannock Rivers, plus another near the Bay's eastern shore (Fig. 3). A total of 84 water samples were analyzed from waters above and below the pycnocline at these Bay stations. Over the 6 month study 23.8% of the samples above the pycnocline contained PLO. In the waters below the pycnocline, PLO were in 7.1% of the samples. PLO concentrations ranged from zero to a high of 296 cells/mL which was recorded in May above the pycnocline off the mouth of the Rappahannock River.

Sediment Analysis

Sediment samples taken from 43 stations were cultured for PLO. These stations were widely distributed from all three of the monitoring programs, and represented all the COHORT stations and the major estuaries in Virginia. From these samples, there were 36 of the 43 (83%) which produced PLO. The most common PLO were *Cryptoperidiniopsis* sp. and *Gymnodinium galatheanum*. There were several other unidentified (to date) dinoflagellates derived from the sediment. No *Pfiesteria piscicida* cells were noted within this group.

DISCUSSION

The results of this study indicate dinoflagellate species included in the PLO category are common residents in the water column and sediment of Virginia estuaries. They were present at least once during the six month collection period in 90% of the 182 station locations sampled in 1998, and found in 44% of the total water samples examined from June through November. The widespread occurrence of these cells derived from the numerous sediment samples also indicates that the PLO are well established inhabitants in this region and their presence in the estuaries is not dependent upon transport into Chesapeake Bay from neritic waters. In contrast, these sediment concentrations of viable PLO cysts may continue to seed these various sub-estuaries and the Bay.

Within the water column of the lower Chesapeake Bay, PLO cell concentrations were also common and found in greater abundance in waters above the pycnocline than

below the pycnocline. These waters above the pycnocline have a net flow leaving the Bay, thus dispersing these species into neritic waters, and allow their passage to areas outside the Chesapeake Bay system. PLO that enter the Bay from its various sub-estuaries may also be transported via sub-pycnocline waters up the Bay and into other tidal rivers and streams.

Although the PLO were widely distributed in these estuaries there was a greater incidence and higher cell concentrations at locations along the Virginia coastline of the Potomac River and sites on the western shore of Chesapeake Bay from the Little Wicomico River to the mouth of the Rappahannock River. There were also several scattered sites in the Rappahannock River where these cells were abundant. Moving southward to the Chesapeake Bay entrance, the PLO concentrations decreased. On the eastern shore of the Chesapeake Bay from Onancock Creek to the Bay mouth, and along the ocean side of the Delmarva Peninsula, the PLO concentrations were also low.

None of these PLO species produced consistent levels of high cell concentrations in the water column during the study. In contrast, there were several species that produced sporadic (monthly) periods of high abundance at several of the locations (e.g. *Cryptoperidiniopsis* sp.), yet maintained low, but consistent presence at other sites. Whereas, others (as *Gymnodinium galatheanum*) were not typically in high concentrations during the study period, however, their viable cysts were common in the sediment of these estuaries. Overall, the mean PLO concentrations for all stations over the six month period was 19.9 cells/mL. The *Cryptoperidiniopsis* sp. in these Virginia samples has been identified by Karen Steidinger as a different species than *Cryptoperidiniopsis brodyii* found in Florida (personal communication), and this status was further substantiated by the gene sequencing work of David Oldach (U. Md.). However, this species in our cultures had many similarities to *Pfiesteria piscicida* regarding its morphology, in its feeding mechanism using a peduncle, and stages of its life cycle, including amoeboid and cyst stages. In contrast to *P. piscicida*, toxic fish bioassays conducted in our laboratory did not indicate any toxic impact on fish exposed to this *Cryptoperidiniopsis* sp. over a ten week period.

During the 1998 monitoring study, *Pfiesteria piscicida* was not found in any of the samples. During this period there were no major fish kills that were associated with toxic dinoflagellates in Virginia, nor were high (> 250 cells/L) PLO concentrations common during this period. In Maryland estuaries there were two instances in 1998 when molecular probes and fish bioassays detected the presence of *P. piscicida,* but it was considered to be present at low densities, and not active as a toxin producer (Magnien et al., 1999). Genetic probes were only sparingly used on Virginia water samples in 1998, with this analysis conducted by Parke Rublee (UNCG), and giving negative responses for the presence of *Pfiesteria*.

Environmental factors associated with the presence of the PLO at these sites have been analyzed by Weber and Marshall (1999), and alone did not reveal the significant relationships that may influence their distribution in these waters. These results may be directly influenced by having low concentrations of multiple PLO species, that appear to have different seasonal responses to the existing environmental conditions. In comparing 1997, when toxic *Pfiesteria* was present in Maryland rivers, to 1998 and the absence of this toxicity, Magnien et al. (1999) indicate there were differences in the flow patterns associated with these rivers that were related to periods and amount of rainfall. These changes were associated with 1998 being substantially a wetter year

than 1997. Such differences would impact nutrient entry into the system and could offset seasonal growth patterns among the various phytoplankton. Specific ecological relationships are considered complex and complicated to discern for Pfiesteria due to its varied life stages and options for food intake (Burkholder and Glasgow, 1997).

SUMMARY

The group of dinoflagellates known as the *Pfiesteria*-like organisms are common inhabitants of Virginia estuaries, and the southern Chesapeake Bay. The most frequently encountered species within this category were *Cryptoperidiniopsis* sp., *Gymnodinium galatheanum*, and several yet to be identified gymnodinioid species. *Cryptoperidiniopsis* sp. and *Gmnrodinium galatheanum* possess polymorphic life stages that include cysts, motile bi-flagellate vegetative cells, and amoeboid forms. They have a similar feeding mechanism (peduncle) as *Pfiesteria piscicida*.

The most favorable regions for PLO development in Virginia were the smaller sub-estuaries along the Potomac River and at locations along the western margin of Chesapeake Bay from the Little Wicomico River to the Rappahannock River. There were fewer PLO at the ocean side inlets. Although *Pfiesteria piscicida* was not identified in this year of study, this does not mean *Pfiesteria* is absent from these estuaries. Over a long term monitoring period for phytoplankton in Chesapeake Bay, Marshall (1994) has identified over 700 species. However, this assemblage of species is not found each year in the Bay. The individual phytoplankton species will respond to a variety of environmental conditions favorable for their growth. These conditions vary throughout the year and subsequently initiate different responses among the flora. A similar situation may apply to *Pfiesteria*, where the environmental conditions favorable for its growth did not occur during these sampling occasions. On a broader scale, as suggested by Magnien et al. (1999), the physical configuration of the river basins, or the high amount of rainfall that occurred in 1998, may have produced a sufficient amount of flushing in these estuaries that was not favorable to sustained and high concentrations of the PLO, including *Pfiesteria* spp. Yet, *Pfiesteria* was not found in the sediment samples tested to date. In this study, we cultured sediment from 43 sites from 182 stations (23.6%). Sediment from locations not cultured in this set may contain *Pfiesteria*. In an effort to improve this sampling base, we are currently processing an expanded set of sediment samples, in addition to the use of genetic probes to test waters taken from a broader base of estuarine sites for the presence of *Pfiesteria* spp.

LITERATURE CITED

Burkholder, J. 1997. *Pfiesteria piscicida* and other toxic *Pfiesteria*-like dinoflagellates. Review paper. 8 p.

Burkholder, J. 1999. The lurking perils of *Pfiesteria*. Scientific American (August). pp.42-49.

Burkholder, J. and H. Glasgow. 1995. Interactions of a toxic estuarine dinoflagellate microbial predators and prey. Arch. Protistenkd. 145:177-188.

Burkholder, J. and H. Glasgow. 1997. *Pfiesteria piscicida* and other *Pfiesteria*-like dinoflagellates: Behavior, impacts, and environmental controls. Limnol. Oceanogr. 42:1052-1075.

Burkholder, J., H. Glasgow, and C. Hobbs. 1995. Fish kills linked to a toxic ambush-predator dinoflagellate: distribution and environmental conditions. Marine Ecology Progress Series 124:43-61.

Burkholder, J., H. Glasgow, and K. Steidinger. 1995. Stage transformations in the complex life cycle of an ichthyotoxic "ambush-predator" dinoflagellate. *In* P. Lasus et al. (eds.) Harmful Marine Algal Blooms. Technique et Documentation, Lavoisier, Intercept Ltd., pp.567-572.

Burkholder, J., M. Mallin, and H. Glasgow. 1999. Fish kills, bottom-water hypoxia, and the toxic *Pfiesteria* complex in the Neuse River and estuary. Marine Ecol. Prog. Ser. 179:301-310.

Glasgow, H., J. Burkholder, D. Schmechel, P. Tester, and P. Rublee. 1995. Insidious effects of a toxic estuarine dinoflagellate on fish survival and human health. J. Toxicology and Environmental Health, 46:501-522.

Lewitus A, R. Jesien, T. Kana, J. Burkholder, H. Glasgow, and E. May. 1995. Discovery of the "Phantom" dinoflagellate in Chesapeake Bay. Estuaries 18:373-378.

Magnien, R., D. Goshorn, B. Michael, P. Tango, r. Karrh, and R. Nelson. 1999. Findings for water quality, habitat and *Pfiesteria*-related organisms-1998. Summary of Maryland's *Pfiesteria*-related comprehensive assessment and rapid response efforts in 1998. Sp. Rpt., Prepared by the Maryland *Pfiesteria* Study Team, February 1999. pp. 13-15.

Marshall, H. 1994. Chesapeake Bay phytoplankton: I. Composition. Proc. Biol. Soc. Washington. 107:573-585.

Marshall, H. and D. Seaborn. 1998. The presence of *Pfiesteria piscicida* and other potential toxin producing phytoplankters in Virginia rivers and regions of Chesapeake Bay. Proc. 16th Intern. Conf. of the Coastal Society. Williamsburg, Va., Addendum Volume, pp. 24-28.

Marshall, H., D. Seaborn, and J. Wolny. 1998a. Monitoring Virginia estuaries for *Pfiesteria piscicida* and other similar dinoflagellates. Poster presentation.

Marshall, H., D. Seaborn, and J. Wolny. 1998b. Species within the *Pfiesteria piscicida* complex in Virginia waters during 1997. Virginia J. Science, Abs. 49:71

Marshall, H.G., D. Seaborn, and J. Wolny. 1999. Monitoring results from Virginia waters for species within the *Pfiesteria* complex. Virginia J. Science, Abs. 50:138.

Rublee, P.A., J. Kempton, E. Schaefer, J.M. Burkholder, H.B. Glasgow, and David Oldach. 1999. PCR and FISH detection extends the range of *Pfiesteria piscicida* in estuarine waters. Virginia J. Science. 50(4):325-336.

Seaborn,D.W. and H.G. Marshall. 1998. The culturing and life history of *Cryptoperid-iniopsis* sp.: A member of the *Pfiesteria* complex. Virginia J. Science, Abs. 49:71.

Seaborn, D.W., A.M. Seaborn, W. Dunstan, and H. Marshall. 1999. Studies of growth and feeding by the dinoflagellate *Cryptoperidiniopsis* on algal prey. Virginia J. Science, Abs. 50:139.

Steidinger, K., J. Landsberg, and E. Truby. 1997. *Pfiesteria piscicida*, other *Pfiesteria* species, and *Pfiesteria*-like species: A Question of Recognition and Toxicity. Information Handout. 10 pp.

Steidinger, K., J. Landsberg, E. Truby, and B. Blakesley. 1996. The use of scanning electron microscopy in identifying small "gymnodinioid" dinoflagellates, NOVA HEDWIGIA 112:415-422.

Steidinger, K., J. Burkholder, H. Glasgow, C. Hobbs, J. Garrett, E. Truby, E. Noga, and S. Smith. 1996. *Pfiesteria piscicida* gen. et sp. nov. (Pfiesteriaceae Fam. nov.), a new toxic dinoflagellate with a complex life cycle and behavior. J. Phycology, 32:157-164.

Truby, E. 1997. Preparation of single-celled marine dinoflagellates for electron microscopy. Microscopy Research and Technique, 36:337-340.

Weber, E.P. and H.G. Marshall. 1999. Water quality relationships to concentrations of *Pfiesteria*-like organisms in Virginia estuaries for 1998. Virginia J. Science, 50(4):365-380.