ABSTRACT
During the summer and early fall of 1998, 34 estuarine stations in Virginia were sampled for Pfiesteria like organisms (PLOs) and associated water quality conditions. Stations were sampled either bimonthly (20 stations) or monthly (14 stations) from June to October 1998. At each station, a set of live and Lugol’s preserved samples were collected for presumptive counts of PLOs and water quality conditions were determined. Water quality parameters measured included standard field parameters, nutrients (total, dissolved, and particulate), chlorophyll a, and conventional water quality indicators. Although PLOs were relatively low in 1998 as compared to 1997, presumptive PLO counts revealed higher PLO levels at stations located in the Northern Neck area (Potomac Embayments, Rappahannock River, and other smaller watersheds). The water quality parameters of pH, dissolved oxygen, and temperature were correlated with higher PLO counts, while several nutrients, salinity, and turbidity were negatively correlated with PLOs. In the August 1997, relatively minor fish kills in the Pocomoke River and the Pocomoke Sound on the Virginia and Maryland border were attributed to the toxic dinoflagellate, Pfiesteria piscicida. Pfiesteria piscicida was first identified as a fish-killing dinoflagellate in fish tank at North Carolina State University (Noga et al., 1993). Pfiesteria piscicida has since been implicated in large widespread fish kills in North Carolina’s estuaries (Burkholder et al., 1992, 1995).

INTRODUCTION
In the 1990’s, scientists at NCSU determined that Pfiesteria piscicida was responsible for fish kills numbering in the millions in North Carolina’s Pamlico and Nuese estuaries. These poorly flushed, shallow, nutrient-rich estuaries attracted large schools of menhaden and other coastal fish species. Under the right conditions, Pfiesteria piscicida emerged from benign forms living in the sediments and water into an extremely toxic form. This form, in response to stimuli from the fish schools release a highly potent toxin that stuns its prey, thus allowing the dinoflagellate to feed on epidermal tissue and blood from the fish.

Toxic forms of Pfiesteria piscicida were implicated in fish kills on the Pocomoke River and from several nearby waterways in Maryland. In addition to killing fish, exposure to toxins from Pfiesteria piscicida has been linked to human health concerns. Lab workers, commercial and recreational waterway users, and environmental field staff have reported a variety of symptoms ranging from skin rashes to severe memory loss after exposure to Pfiesteria and its toxins.
EPA Region 3 provided funding to several mid-Atlantic states to monitor for *Pfiesteria* and *Pfiesteria*-like organisms (PLOs) in their coastal estuaries. Concurrently, the Centers for Disease Control (CDC) funded several states to conduct cohort studies to evaluate the effects of exposure to estuarine waters (and potentially *Pfiesteria* and *Pfiesteria*-like organisms). The Virginia Department of Health (VDH) is conducting the CDC sponsored cohort study.
METHODS

In Virginia, 20 stations in 15 watersheds were selected to support the VDH/CDC Cohort study by collecting water samples for PLO counts and determination of a variety of water quality conditions. The VDH is collecting human health data on watermen and others who frequent these coastal waters. The Virginia Institute of Marine Science (VIMS) also monitored these sites for disease incidence in finfish. Cohort sites had either a single monitored station or multiple stations within a watershed site (Figure 1, Table 1) Cohort stations were monitored twice monthly from June to October, 1998. In addition to these stations, another 14 stations in 12 watersheds were sampled for PLOs and water quality conditions on a monthly basis during the same time period as the cohort study (Figure 1, Table 1). These stations, referred to as water quality stations, were not assessed for finfish disease incidence.

All stations were sampled for PLOs by collecting live and Lugols-preserved water samples. Samples were collected in duplicate or triplicate at each station, stored in coolers and delivered to the Old Dominion University Phytoplankton Analysis Laboratory for PLO analysis. Presumptive counts of PLOs were made using light microscopy techniques (Marshall et al., 1999). Sediment samples were also collected from each site and delivered to the ODU Phytoplankton Analysis Laboratory for subsequent culture and identification purposes.

A variety of water quality parameters were determined for each station (Table 2). Field parameters were measured at 1 m beneath the surface using a Hydrolab® multiparameter surveyor. Water samples were collected from 1 m beneath the surface, preserved (if required), packed on ice and shipped to the Virginia Division of Consolidated Laboratory Services (DCLS) for analysis. Samples for dissolved and particulate nutrients were filtered and processed in the field and shipped to DCLS for analysis.

PLO and water quality data were evaluated using Statistical software programs.

RESULTS

The mean, minimum and maximum value for temperature, pH, dissolved oxygen (D.O.), and salinity are shown for Cohort and WQ stations in tables 3 and 4, respectively. The stations monitored represent a range of estuarine conditions. Temperatures ranged from a minimum of 15.4°C to 31.0°C during the monitoring season. Salinity ranged from a high mean value of 30.4 ppt at Folly Creek on the seaside of the Eastern Shore to a mean of 6.2 ppt on the James River station. The Pocomoke River on the Eastern Shore had the lowest mean pH (7.3 su), and Monroe Bay off the Potomac River had the highest average pH (8.3 su) value.

Stations were ranked by average presumptive PLO cell counts (live and preserved samples) and the top 15 stations are shown in Figure 2. The maximum cell count and the percentage of samples with greater than zero cells are also shown. A station on the Lower Machodoc had the highest average cell count with 59.4 cells/mL and a maximum cell count of 370 cells/mL. These stations are located in embayments of the Potomac River, the Rappahannock River and tributaries, and coastal embayments of the Northern Neck and Middle Peninsula (Figure 3). The rest of the stations averaged less than nine cells/mL, and even the Pocomoke station, where fish kills occurred in 1997, averaged 5.6 cells/mL with a maximum cell count of 40 cells/mL.

Although PLO cells count were relatively low during the 1998 monitoring season there were several water quality variables that were significantly correlated to PLO cell

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1- Latitude and Longitude are in decimal degrees.

density. None of the correlations are particularly strong. The parameters pH, dissolved oxygen and temperature had a positive relationship with PLO cell density. Salinity, turbidity, and the nutrients, total orthophosphate, total ammonia, and total Kjeldahl nitrogen had a negative relationship with PLO cell density (Figure 4).

SUMMARY
During the summer and early fall of 1998, 34 estuarine stations in Virginia’s coastal waters were monitored for Pfiesteria-like organisms and a variety of water quality parameters. Stations were located on the Eastern Shore (including a seaside station),
TABLE 2. List of water quality parameters measured during the 1998 *Pfiesteria* monitoring season.

**Field Measurements**
- Temperature, D.O., Conductivity, Salinity, pH, Secchi

**Conventional Parameters**
- BOD5, Alkalinity, Turbidity, Sulfate, Solids, TOC

**Nutrients - Total, Dissolved, & Particulate**
- Total - Ammonia, NO2, NO3, TKN, Ortho Phosphate, Phosphorus
- Dissolved - Ammonia, NO2, NO3, NO2&NO3, Ortho Phosphate, Silica, TDN, TDP
- Particulate - Carbon, Nitrogen, Phosphorus

**Chlorophyll a**

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**FIGURE 2.** Top ranked stations based on average PLO cell count during the 1998 monitoring season. Average number of cells, maximum number of cells and percent of samples greater than zero are shown.

Embayments of the western shore of the Chesapeake Bay, and in and along the major rivers (Potomac, Rappahannock, York and James). These stations represented a wide range of physical and chemical conditions that might be expected in estuarine waters.

PLO counts were relatively low during the monitoring season. The maximum cell density recorded was 370 cell/mL and all stations averaged less than 60 cells/mL during the monitoring season. However, stations located in the Potomac embayments, the Rappahannock River, and embayments of the western shore along the Northern Neck and middle peninsula had the highest average PLO counts.
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</tr>
</tbody>
</table>
There were weak relationships between PLO cell counts and some water quality variables. The parameters pH, dissolved oxygen, and temperature were correlated with higher PLO counts, while several nutrients, salinity, and turbidity were negatively correlated with PLOs.

The relatively low PLO counts in 1998 (as compared to levels recorded in the Pocomoke Sound and the Rappahannock River in 1997) hinder the ability to discern the relationship between water quality conditions and *Pfiesteria*-like organisms.
FIGURE 4. Scatterplots of water quality parameters correlated with PLO cell density. Correlations are significant at $p < 0.0500$. 
FIGURE 4 continued. Scatterplots of water quality parameters correlated with PLO cell density. Correlations are significant at \( p < 0.0500 \).
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LITERATURE CITED


