

# Chronic Urea Loading: A Correlate of *Pfiesteria* spp. in the Chesapeake and Coastal Bays of Maryland, USA

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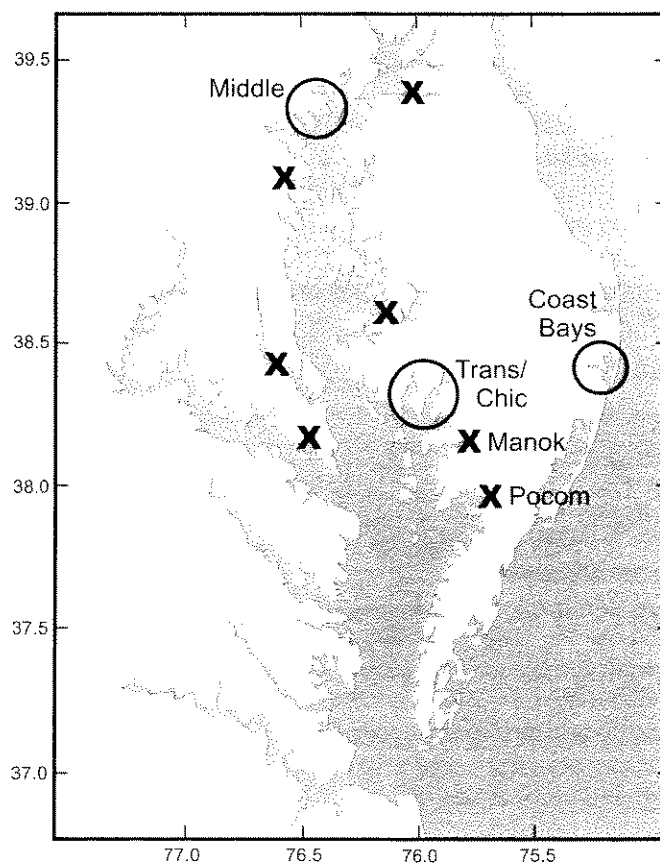
## Abstract

Since the first outbreaks of *Pfiesteria* spp. in the tributaries of the Chesapeake and Coastal Bays of Maryland, USA, a relationship with nutrients from agriculture and intensive animal (poultry) operations has been hypothesized. As an index of these inputs, concentrations of the nutrient urea were examined at >18 stations for several years. Urea is both a commonly used nitrogen fertilizer and is a decomposition product of poultry manure. Distribution of *Pfiesteria* spp. in the sediment and in the water column was also surveyed. The average urea concentration was found to be an excellent predictor of the percent positive detection for *Pfiesteria* spp. in the sediment ( $R^2 \geq 0.94$ ). The correlation with water column *Pfiesteria* spp. presence was also positive but was weaker due to lower overall abundance and the transient nature of the zoospores. The enriched sites had mean concentrations of urea  $>1.0 \mu\text{g at N L}^{-1}$ , and seasonally pulsed concentrations  $>5 \mu\text{g at N L}^{-1}$ . These findings underscore the association of *Pfiesteria* spp. with organic-rich, nutrient-polluted waters. The role of nutrients in *Pfiesteria* growth may either be via direct uptake, or via indirect stimulation of other phytoplankton or bacteria on which *Pfiesteria* may graze.

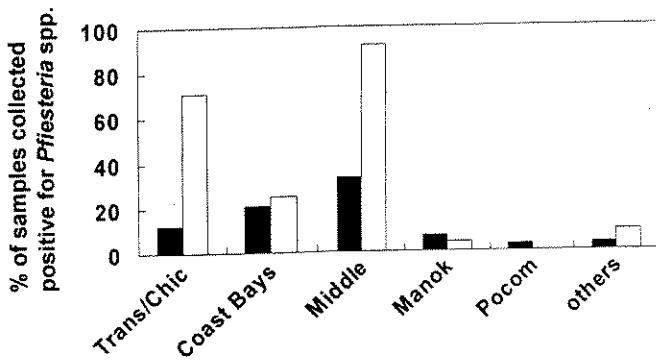
## Introduction

The presence of *Pfiesteria* was first documented in Chesapeake Bay in 1992 (Lewitus *et al.*, 1995), but the first major outbreaks were documented in 1997 (Burkholder and Glasgow, 1997; Magnien, 2001a). Since that time, an intensive monitoring program has been undertaken to record the environmental conditions of the bay and the presence of *Pfiesteria*, using molecular probe techniques. *Pfiesteria* spp. has been found to occur in several tributaries annually, but fish health events have been few in number (Magnien, 2001b).

Since the first documented outbreaks of *Pfiesteria* in both the Chesapeake Bay and the Neuse Estuary, North Carolina, the correlation with high levels of nutrients has been suggested (Burkholder and Glasgow, 1997), and the relationship between *Pfiesteria* and nutrients derived from agricultural and animal operations has been the subject of considerable debate in environmental management. The Chesapeake and Coastal bays of Maryland receive nutrients from agricultural runoff and from point-source discharges. Agricultural inputs of nutrients on Maryland's eastern shore are rich in both phosphorus and nitrogen in inorganic and organic forms (Boynnton *et al.*, 1982; Glibert *et al.*, 2001). In contrast, on the western shore of the Chesapeake Bay, the majority of nutrients are delivered from point source discharges, primarily from the metropolitan regions of Baltimore and Washington, D.C. (Boesch *et al.*, 2001). Many organic nutrients are not typically reported in nutrient monitoring programs. As part of a larger study of the relationship between nutrients and *Pfiesteria* spp., in this report we explore the relationship with one agricultural nutrient, urea. Urea is used as an agricultural nitrogen fertilizer, and it is also a decomposition product of poultry manure, another commonly applied fertilizer. Urea has also been shown to be one of several nitrogen forms taken



**Figure 1** Map of Chesapeake Bay and Coastal Bays of Maryland indicating general regions of sampling. Regions that are circled had more frequent positive identifications of *Pfiesteria* spp. Regions indicated by an X had significantly fewer observations of *Pfiesteria*. Middle River, Transquaking (Trans), Chicamacomico (Chic), Manokin (Manok) and Pocomoke (Poco) rivers, as well as the Coastal Bays, are indicated on the map.



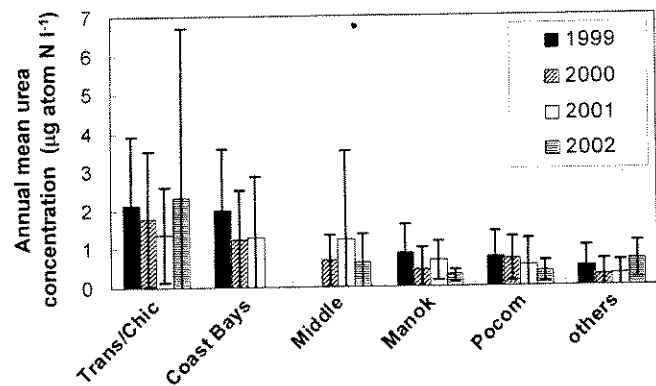
**Figure 2** Percent of samples collected in water (solid bars) and in sediment (open bars) that were positive for *Pfiesteria* spp. in the Transquaking/Chicamacomico rivers (Trans/Chic), Coastal Bays, and Middle, Manokin (Manok) and Pocomoke (Pocom) rivers.

up directly by *Pfiesteria* (Lewitus *et al.*, 1999b).

*Pfiesteria* spp. are not algae, but rather “heterotrophic” dinoflagellates that primarily depend on grazing to meet their nutritional requirements. It has previously been documented that *Pfiesteria* can graze on bacteria, algae, and bits of fish tissue (Burkholder and Glasgow, 1997; Burkholder *et al.*, 2001). *Pfiesteria* also has the ability to retain chloroplasts from algae that it grazes and can photosynthesize for short periods of time with these intact organelles (Lewitus *et al.*, 1999a,b). *Pfiesteria* spp. have the ability to take up dissolved nutrients directly, albeit at rates significantly slower than would be required to meet the entire growth demand of the cells (Lewitus *et al.*, 1999b). Outbreaks of *Pfiesteria* spp. in Chesapeake Bay tributaries have also been shown to be associated with high organic nitrogen and carbon (Glibert *et al.*, 2001). Here we document exceptionally high concentrations of urea in eastern shore tributaries, and a correlation between high urea loading and the presence of *Pfiesteria* spp. This relationship underscores the notion that *Pfiesteria* spp. is most common in organic-rich waters.

### Materials and Methods

Two types of sampling were undertaken. As part of the established monitoring program for environmental variables in the Chesapeake and Coastal bays and their tributaries, urea concentrations were measured at 18–27 stations on a biweekly or monthly basis from April through October during the years 1999–2002 ( $n = 243$  for 1999; 257 for 2000; 272 for 2001; 122 for 2002; Fig. 1) concomitant with other nutrients and water quality parameters (not reported here). Water samples for urea analysis were collected, filtered, then frozen for later analysis (within about 3 weeks; Parsons *et al.*, 1984). Sediment and water samples for spp. identification ( $n = 1,614$  for water and 156 for sediment; Magnien *et al.*, 2002) were collected in 2000 and 2001, extracted for DNA, then analyzed by Taqman-based PCR assays specific for *P. piscicida* and *P. shumwayae* (Bowers *et al.*, 2000).



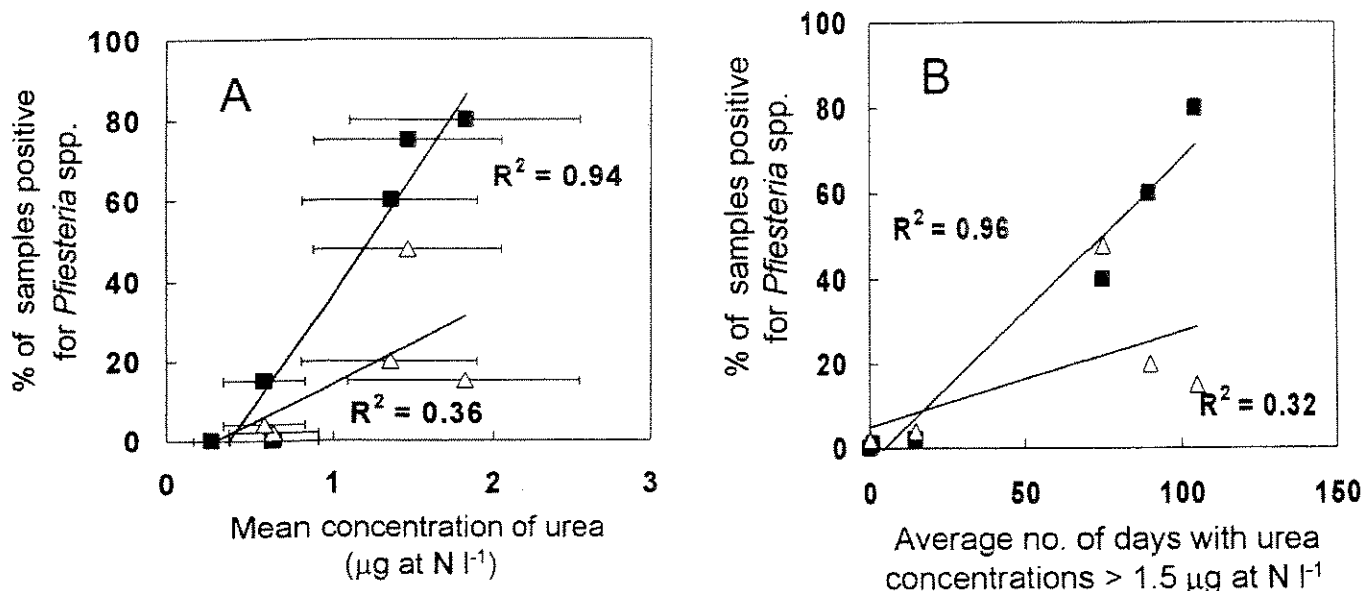
**Figure 3** Annual mean concentration of urea for each of the regions and years shown. Error bars are  $\pm 1$  SD.

### Results and Discussion

Of the sites regularly monitored in Maryland, *P. piscicida* and *P. shumwayae* are most frequently observed in the Transquaking, Chicamacomico, and Middle Rivers, and in the Coastal Bays (Fig. 2). *Pfiesteria* spp. were more frequent in sediment samples than in water samples, reflecting the benthic life stages of this organism as well as the transient nature of zoospore stages.

Annual mean concentrations of urea were significantly higher ( $>1 \mu\text{g at N L}^{-1}$ ) in the same tributaries where *Pfiesteria* sp. were regularly observed: the Transquaking, Chicamacomico and Middle rivers, and in the Coastal Bays (Fig. 3). Maximum observed concentrations of urea for these sites were up to tenfold higher than annual means (not shown). For comparison, concentrations of urea along the main axis of Chesapeake Bay typically do not exceed  $1 \mu\text{g at N L}^{-1}$  in surface waters (Lomas *et al.*, 2002). Focusing on the years 2000 and 2001, the years for which these reported *Pfiesteria* surveys were conducted, both average urea concentration and the average number of days for each tributary during which urea concentration exceeded  $1.5 \mu\text{g at N L}^{-1}$  were found to be excellent predictors of the percent of sediment samples found to be positive for *Pfiesteria* spp. (Fig. 4A,B). For each relationship, the  $R^2$  was  $\geq 0.94$  and was significant at  $P < 0.01$ . The relationships between urea concentration or number of days with elevated concentration and the percent of samples positive for *Pfiesteria* spp. in the water column were also positive ( $R^2 = 0.32$ – $0.36$ ) but were not significant. As *Pfiesteria* spp. is primarily a benthic organism, a weaker relationship with water column presence is not surprising. Presence in the sediment indicates the potential for an outbreak should other conditions be suitable for its proliferation.

This report builds on previous evidence that organic nutrients may be good predictors of *Pfiesteria* spp. Glibert and Terlizzi (1999) observed that dinoflagellate blooms, including *Pfiesteria* spp., in fish aquaculture ponds co-occurred with elevated concentrations of urea. In the Chesapeake Bay, *P. piscicida* was found to occur under conditions of elevated organic carbon, and the ratio of



**Figure 4** **A** Correlations between mean concentration of urea in Maryland eastern shore tributaries during 2000–2001 and the percent of samples positive for *Pfiesteria* spp. in the sediment (squares) and the water column (triangles). Error bars are  $\pm 1$  SD. **B** Correlations between the average number of days (Apr–Oct) with urea concentrations in excess of  $1.5 \mu\text{g at N l}^{-1}$  and the percent of samples positive for *Pfiesteria* spp. in the sediment (squares) and the water column (triangles).

dissolved organic carbon to dissolved organic nitrogen was a better predictor of *P. piscicida* than any inorganic nutrient (Glibert *et al.*, 2001). Locations with higher percentages of samples positive for *Pfiesteria* spp. in the sediment samples reported here were also found to have higher sediment nitrogen, phosphorus and carbon than locations that tested negative for *Pfiesteria* presence (Magnien *et al.*, 2002). Species with a complex nutrition such as *Pfiesteria* spp. may be stimulated by the direct assimilation of dissolved nutrients but more likely by the development of the algal food on which they preferentially graze (Burkholder and Glasgow, 1997; Burkholder and Glasgow, 2001; Parrow *et al.*, 2001). Urea is not the only nutrient which may directly or indirectly stimulate *Pfiesteria*, but elevated concentrations appear to serve as a useful indicator of the presence of *Pfiesteria* spp. in Maryland's agriculturally-influenced tributaries.

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