

ABSTRACTS FOR ORAL PRESENTATIONS

***PFIESTERIA* – NC, SC, FL SESSION**

NUTRIENT ENRICHMENT AND THE TOXIC *PFIESTERIA* COMPLEX: COMPARATIVE STIMULATION BY SWINE EFFLUENT, POULTRY MANURE LEACHATE, HUMAN SEWAGE, AND OTHER SOURCES

J. Burkholder, C. Zheng, H. Glasgow, N. Deamer-Melia & M. Parrow
Center for Applied Aquatic Ecology, North Carolina State University, Raleigh, NC 27606

Toxic *Pfiesteria* outbreaks have been documented in poorly flushed eutrophic estuaries impacted by anthropogenic nutrient loading from poorly treated animal wastes, human sewage, and other sources. A frequently asked question in efforts to develop management strategies to reduce *Pfiesteria* activity is the relative importance of various nutrient forms and sources in stimulating *Pfiesteria* populations. Here we report a series of short-term (5-day), semi-continuous experiments designed to separately test the response of *Pfiesteria piscicida* and *P. shumwayae* (sp. nov.) zoospores to N versus P enrichment; and to nutrient sources including swine effluent, poultry wastes, and human sewage.

Pfiesteria piscicida and *P. shumwayae* were isolated from the Neuse Estuary, cloned, and confirmed as toxic to fish (uni-dinoflagellate clones confirmed by the Heteroduplex mobility assay of D. Oldach, U.MD; species identifications from suture-swollen cells with SEM, cross-confirmed by molecular probes from our lab. and the FISH probe of P. Rublee, UNC-G; toxicity cross-confirmed by H. Marshall, ODU). To eliminate the confounding influence of nutrient-rich fish excreta, we tested each *Pfiesteria* species in the absence of fish (TOX-B functional type), with/without nutrient-deplete cryptomonads (N,P-limited; cloned from multi-species material of the CCMP listed as *Rhodomonas 757*) as a prey source. Swine effluent was collected from a depth 0.5 m in a lagoon at a swine operation near NCSU; poultry waste leachate was collected from a waste pit at an NCSU research facility; and raw human sewage was supplied from the Raleigh municipal WWTP. These nutrient sources were sterile-filtered and then diluted in series (1:10, 1:50, 1:100, 1:1000) using 15-psu filtered Instant Ocean water. The nutrient content (N,P,C – organic, inorganic forms) of each source was characterized. Controls consisted of cryptomonad prey, *P. piscicida*, *P. shumwayae*. Treatments included [cryptomonads + nutrient source]; [each *Pfiesteria* species + nutrient source]; and [each *Pfiesteria* species + nutrient source + cryptomonads]. To gain additional insights about the stimulatory effects of nutrients, a similar experimental design was used to separately test effects of enrichment with P versus N on zoospore production of each *Pfiesteria* spp.

We documented significantly higher *Pfiesteria* zoospore abundance in all treatments with each nutrient source, relative to abundance in controls without nutrient additions. Cell production increased with increasing nutrient source concentration except at the lowest dilution (10:1) where there was a longer lag effect before zoospore production increased. *P. piscicida* showed higher stimulation by P than by N enrichment, and maximal cell production with swine wastes. In contrast, *P. shumwayae* cell production was higher with N than with P enrichments, and maximal with poultry wastes. Using fluorescent markers for each species (*P. piscicida* – Alexa Fluor 488; *P. shumwayae* – Alexa Fluor 350), we are continuing this effort by examining the comparative response of *P. piscicida* and *P. shumwayae* to nutrient sources in mixed-species trials. These data have provided insights to explain observations about *Pfiesteria* abundance and toxic activity over the past decade in estuaries draining urbanized versus agricultural watersheds.

TOXIC *PFIESTERIA* PROMOTES ACUTE AND CHRONIC LESIONS IN FINFISH, IN CONTROLLED EXPERIMENTAL TRIALS

H. Glasgow,¹ J., R. Smolowitz,² N. Deamer-Melia¹ & J. Burkholder¹

¹Center for Applied Aquatic Ecology, North Carolina State University, Raleigh, NC 27606

²Marine Biology Laboratory of Woods Hole, Woods Hole, MA 02543

Bioactive substances from the toxic *Pfiesteria* complex have been shown to destroy fish epidermis, and to render fish susceptible to opportunistic bacterial and fungal pathogens in lesion formation. Here we report the findings from repeat trial experiments (n=12) in which we (i) characterized acute lesion development and other pathology in tilapia (juveniles, t.l. 5-8 cm) exposed to toxic clonal *Pfiesteria piscicida* (TOX-A functional type); and (ii) tracked chronic lesion development in tilapia following 'recovery' from exposure to toxic clonal *P. piscicida*.

Pfiesteria piscicida was isolated from the Neuse Estuary (isolate ND-PP990708), cloned, and confirmed as toxic to fish (uni-dinoflagellate clones -- Heteroduplex mobility assay of D. Oldach, U.MD; species identifications from suture-swollen cells with SEM, cross-confirmed by molecular probes from our lab. and the fluorescent *in situ* hybridization probe of P.Rublee, UNC-G; fish bioassay process, following Koch's postulates for modified for toxic rather than infectious agents; toxicity cross-confirmed by H. Marshall, ODU).

Toxic zoospore densities ≥ 100 cells/mL induced epithelial destruction and lesions. In repeat trials, acute lesions formed within ≤ 12 hr (sometimes in ≤ 2 hr, typically in < 8 hr), generally with hemorrhaging (sometimes within minutes) and often culminating in rupture of the peritoneal sack with exposure of the viscera. Dermatological lesion formation involved intra- and extracellular edema and necrosis of epithelium (with pyknotic and eosinophilic cytoplasm), progressing to erosions that extended through the basement membrane (50-80% loss of epidermis, depending on exposure duration). Epidermal and skeletal muscle tissues were characterized with mild to severe multifocal granulocytic and lymphocytic epidermatitis; moderate dermal edema; marked diffuse lymphocytic epidermatitis; and/or mild to marked necrotizing lymphocytic epidermatitis. Other pathology was documented in the gill (cytomegalic bacterial inclusions and mild to severe edema); cornea (mild to severe erosion); pharynx (mild to severe edema); hepatopancreas (mild multifocal lymphoplasmacytic, granulocytic, hepatopancreatitis [sometimes necrotizing]); kidney (mild multifocal tubular mineralization [\pm granuloma formation] and minimal multifocal lymphohematopoietic necrosis); and brain (moderate subacute to chronic multifocal meningitis, mild to acute granulocytic optic neuritis, and encephalitis). Control fish, maintained similarly except without exposure to toxic *Pfiesteria*, remained healthy, and did not show pathologies.

Recovery from sublethal exposure to toxic *Pfiesteria* was tested by removing fish with mild to moderate lesion development from additional exposure to toxic *Pfiesteria* (basis: no detection of *Pfiesteria* from daily analysis of water samples in culture vessels, using light micro-scopy at 600x) and tracking fish health for 6 wk. Control fish were treated similarly except for no prior exposure to toxic *Pfiesteria*, the test fish sustained 'easy infections' from bacteria and fungi. About 80% of the fish developed ulcers with moderate to severe, acute myonecrosis and mixed gram-negative bacterial infections (predominantly *Aeromonas hydrophila*). Ulcers were not observed in the control fish. These findings are consistent with Noga et al. (1996, *Marine Pollution Bulletin* vol. 32), and indicate that exposure to toxic *Pfiesteria* can promote chronic as well as acute lesion development in finfish.

PFIESTERIA, PFIESTERIA-LIKE SPECIES, AND FISH HEALTH IN FLORIDA: AN UPDATE

Jan Landsberg¹, Karen Steidinger¹, Susan Cook¹, Elizabeth Singh¹, Emilio Sosa¹, Ann Forstchen¹, Robin Wood¹, Parke Rublee², Paula Scott¹, Jennifer Wolny¹, and Brian Bendis¹

¹Florida Marine Research Institute, Florida Fish and Wildlife Conservation Commission, St. Petersburg, FL 33701

²Department of Biology, University of North Carolina at Greensboro, Greensboro, NC 27402

Since the late 1970s, numerous fish species from several Florida freshwater and estuarine systems primarily striped mullet (*Mugil cephalus*), silver mullet (*Mugil curema*), and sheepshead (*Archosargus probatocephalus*) - have been affected by lesions, principally ulcerative mycosis (UM). The potential role of *Pfiesteria* as a causative agent in the development of UM has been discussed (e.g. Burkholder et al. 1998). In conjunction with studies on the distribution and etiology of lesions in fish from Florida, we are conducting an intensive statewide survey of the distribution and identification of *Pfiesteria* and *Pfiesteria*-like species (PLS, also known as *Pfiesteria*-like organisms, PLOs). Our most recent surveys have determined that fish affected by UM are found predominantly in low salinity or freshwater habitats where *Pfiesteria* does not usually occur in Florida. Thus far, we have confirmed the presence of *P. piscicida* by molecular probe at only one site in southeast Florida in an area with no historical records of fish kills or lesioned fish. However, repeated surveys (> 35 samples) in this area have failed to reconfirm the presence of *P. piscicida*. *Pfiesteria shumwayae* (proposed nov. sp. Glasgow and Burkholder) has been positively identified by molecular probe at two additional east coast sites in areas that have traditionally had very few or no lesioned fish. Repeated surveys (>40 samples) have also failed to reconfirm the presence of *P. shumwayae*, although samples are still being analyzed. Additional temporal samples in hot spot areas are warranted. The PLS referred to by us as “Lucy” has been confirmed by microalgal assay in the same area that was confirmed positive for *P. piscicida* and in one area confirmed positive for *P. shumwayae*. “Lucy” has been confirmed in the St. Lucie River, but appears to have a limited distribution thus far in Florida. Cryptoperidiniopoids are the most widely distributed PLS around the state and occur in known fish lesion areas such as the St. Johns and St. Lucie rivers. Water quality measurements, sediment profiles, and other environmental data have not yet indicated any significant correlation with PLS distribution. An intensive sampling program has been established in the St. Johns River at seven sites to evaluate environmental variables and occurrence of PLS events. One site includes a floating, automated platform configured with continually recording sensors (NO₃, PO₄, relative fluorescence, salinity, temperature, DO, turbidity, currents, meteorological measurements, and other variables).

Although areas containing fish with UM do not appear to be correlated with areas where *Pfiesteria* has been found, we are investigating possible links between lesioned fish and other potentially toxic PLS. The potential role of bioactive compounds produced by PLS in the initiation of fish lesions, and particularly of UM, cannot yet be ruled out. However, the role of the fungus *Aphanomyces invadans* as a primary pathogen in the etiology of UM is almost conclusive (Kiryu et al. 2000). Although lesioned fish in Florida are often associated with a low incidence of myxosporean (primarily *Myxobolus* or *Kudoa*) or microsporean infestations in deeper-lying musculature, these parasites are not considered a primary cause of skin lesions. Studies are underway to determine if *Aphanomyces*, which are associated with lesions in fish in other areas of the United States and in the Far East, are also associated with UM in Florida's fish.

Burkholder, J. M., H. B. Glasgow, Jr. and A. J. Lewitus. 1998. Physiological ecology of *Pfiesteria piscicida* with general comments on “ambush-predator” dinoflagellates. **In:** *Physiological Ecology of Harmful Algal Blooms*, pp. 175-191. (Anderson, D.M., A. D. Cembella, and G. M. Hallegraeff, Eds.). Springer-Verlag, Heidelberg.

Kiryu, Y., Shields, J.D., Vogelbein, W.K., Zwerner, D.E., Kator, H. and Blazer, V.S. 2000. Infectivity of *Aphanomyces* sp., the putative agent of skin ulcers in menhaden, *Brevoortia tyrannus*, from Chesapeake Bay. Abstr. P. 49, American Fisheries Society, Fish Health Section Annual Meeting, Pensacola Beach, Florida September 6-8, 2000.

PFIESTERIA SPP. AND “PFIESTERIA-LIKE ORGANISMS” IN SOUTH CAROLINA ESTUARIES

Alan J. Lewitus^{1,2}, J.M. Burkholder³, C. Cary⁴, H.B. Glasgow Jr.³, K.C. Hayes¹, A.F. Holland², J.M. Law⁵, and P.A. Rublee⁶

¹Belle W. Baruch Institute, University of South Carolina, Georgetown, SC 29442

²Marine Resources Research Institute, SC DNR, Charleston, SC 29412-2559

³Center for Applied Aquatic Ecology, North Carolina State University, Raleigh, NC 27695

⁴College of Marine Studies, University of Delaware, Lewes, DE 19958-1298

⁵College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606

⁶Biology Department, University of North Carolina at Greensboro, Greensboro, NC 27402-6174.

Pfiesteria piscicida, *P. shumwayae*, and *Cryptoperidiniopsis* spp. are present and predictably widespread in South Carolina estuaries. However, results from routine monitoring and fish kill or lesion event sampling have consistently indicated low abundances compared to estimates from similar programs in North Carolina and Maryland that sample areas with a history of *Pfiesteria* toxic activity. One of the areas targeted in the SC event response efforts is Bushy Park (upper Cooper River, Charleston), a site of annually recurrent menhaden lesions (peak of 25-30% frequency on captured fish in September or October). The finding that “*Pfiesteria*-like organism” (PLO) abundances were always low in samples collected during lesion events in Bushy Park suggested that other causative factors were responsible for lesion development. However, the involvement of *Pfiesteria* spp. in at least lesion initiation could not be discounted if a) the association between toxic *Pfiesteria* blooms and fish populations was short-lived, localized in space, or even intermittent, or b) toxic *Pfiesteria* amoebae were involved. In summer 2000, we expanded our efforts in Bushy Park to include tests of these hypotheses and other hypothetical causes of lesions (e.g. fungi, *Kudoa*). Results will be presented from samples collected prior to and during the lesion event, and analyzed for PLO abundance by automated sampling, and molecular probe identification and quantification of water column and sediment samples. These data will be correlated with findings from gross and histopathological examinations of fish collected at this site.

Although, based on the above conservative interpretation of 1998-1999 results, there is uncertainty regarding *Pfiesteria*'s potential involvement in SC fish events, no evidence supporting toxic activity of the organism in SC estuaries currently exists. Even if *Pfiesteria*-related problems in SC estuaries have occurred, it is clear that the dinoflagellate's impact on fish in SC is historically extremely minor compared to the situation in NC. We hypothesize that the reason why *Pfiesteria* is not abundant in SC estuaries is due to the low phytoplankton biomass that characterize these systems. For example, in a statewide assessment of SC estuaries, nearly 90% of chlorophyll *a* values in samples collected during the spring and summer were $< 20 \text{ } \mu\text{g l}^{-1}$, indicative of oligotrophic-to-mesotrophic conditions, and only 2% exceeded $40 \text{ } \mu\text{g l}^{-1}$. In comparison, the Neuse and Pamlico Rivers, areas most commonly linked to *Pfiesteria* outbreaks, are characterized by annual chlorophyll *a* maxima typically $> 40 \text{ } \mu\text{g l}^{-1}$, and spring mean values $> 20 \text{ } \mu\text{g l}^{-1}$. Correspondingly, NO_3 concentrations also were generally much lower in SC estuaries. Based on the demonstrated positive relationship between PLO abundance, chlorophyll *a*, and inorganic nutrient concentrations (in laboratory experiments and Neuse River field correlations), we hypothesize that relatively low phytoplankton prey abundance in SC estuaries restricts PLO population growth.

Reports of HABs in SC estuaries are rare, contrasting strikingly with the situation in NC, where dinoflagellate red tides, cyanobacterial blooms, and outbreaks of *Pfiesteria*, have been frequently reported over the last decade, and are thought to be linked to high nutrient loading. If the lack of

HAB problems such as *Pfiesteria* in SC is related to the relatively low impact of anthropogenically-related nutrient loading along the SC coast, then it follows that the threat of HABs to SC waters may increase as nutrient inputs from coastal development continue to escalate (the SC coast is among the nation's fastest growing areas). To date, our general assessment is that, whereas *Pfiesteria* spp. are present and potentially widespread in SC estuaries, they typically are in low abundance. Although we cannot discount *Pfiesteria* as a cause of recurrent menhaden lesions in the Cooper River, there is no evidence to suggest their involvement in this or other SC fish kill or lesion events. We therefore consider SC estuaries as reference sites for comparison with more anthropogenically impacted estuaries where higher *Pfiesteria* abundances are typically found, and/or where toxic events have been documented.

PRELIMINARY CHARACTERIZATION OF “CRYPTOPERIDINIOPSOID” CULTURES ISOLATED FROM FLORIDA

Steve L. Morton¹, Tina Mikulski¹, Elizabeth R. Fairey¹, Brad Mitchell¹, Peter D.R. Moeller¹, Bill Richardson², Karen Steidinger², and John Ramsdell¹

¹Marine Biotoxin Program, NOAA/NOS, Center for Coastal Environmental Health and Biomolecular Research, 219 Ft. Johnson Rd. Charleston, SC 29412

²Florida Marine Research Institute, Florida Fish and Wildlife Conservation Commission, 100 Eighth Ave. SE, St. Petersburg, FL 33712

Cultures of different species of “Cryptoperidiniopsoid” dinoflagellates were grown under controlled conditions. Each culture was identified via scanning electron microscopy at the Florida Marine Research Institute before shipment to the Marine Biotoxin Program. Each strain was re-identified after mass culture and toxin analysis. Strains were grown in 100 L batch cultures and harvested at late-log growth phase. Production of biological active substances by each culture was examined from both resulting cell mass and spent culture medium. Both cell mass and spent culture medium were passed through a silica column and eluted with an elutropic solvent series. Totals of 5 samples were collected for both the cell mass extract and spent culture medium. Each of the 10 extracts was tested for the possibility of bioactivity using both live assays and cell based assays. Live bioassays included brine shrimp and sheepshead minnows while cell based assay included the GH4C1 cytotoxicity assay. Solvent fractionation yielded several fractions that were active. A non-polar fraction was active on the shrimp bioassay and the sheepshead minnow bioassay. Subsequent structural analysis of this fraction showed this activity in part was due to DEHP, a man-made phthalate ester. This and other fractions are still under pharmacological characterization. A polar fraction was active on the brine shrimp bioassay and the cytotoxicity assay but was inactive on the sheepshead minnow assay. This data provides initial evidence of bioactive substances from cultures of Cryptoperidiniopsoid. Whether this organism produces a toxic substance is presently unknown and will require future pharmacological and chemical investigations.

CHARACTERIZATION OF A PUTATIVE TOXIN PRODUCED BY *PFIESTERIA PISCICIDA*

J.S. Ramsdell,¹ P.D.R. Moeller,¹ E.R. Fairey,¹ A.C. Melo,¹ K.L. Kimm-Brinson,¹ B. Mitchell,¹ S.A. Morton,¹ N. Deamer-Melia², H.B. Glasgow², and J.M. Burkholder²

¹ Marine Biotoxins Program, NOAA-National Ocean Service, Charleston, NC 29442

² Center for Applied Aquatic Ecology, North Carolina State University, Raleigh, NC 27606

The health hazards attributed to *Pfiesteria piscicida* point to the need to characterize bioactive substances capable of causing adverse effects produced by this organism. Extracts of *Pfiesteria* cell mass as well as filtered culture water have been chromatographically partitioned in reproducible fashion, yielding fractions that demonstrate biological activity. These active fractions contain substance(s) that induce cytotoxicity in GH₄C₁ rat pituitary cells and at non-toxic concentrations induce a c-fos luciferase reporter-gene. The pharmacologic activity of a putative toxin (pPfTx) produced by *P. piscicida* has been examined by characterization of the signaling pathways that induce the c-fos luciferase construct in GH₄C₁ rat pituitary cells. A class of purinergic receptors mediates this c-fos pathway with analog selectivity and functional ionic conductances including elevated cytosolic free calcium and enhanced YOPRO-membrane permeability, consistent with a purinergic receptor of the P2X7 class. The irreversible P2X7 antagonist, adenosine 5'-triphosphate-2',3'-dialdehyde, was used to demonstrate that the pPfTx requires this pathway for activation. P2X7 receptors are found predominantly on myeloid cells including mature macrophages, mast cells and microglial cells. A role of P2X7 receptors in the action of pPfTx is of interest, in consideration of the fact that this toxic dinoflagellate has been reported to cause a range of health impacts in both finfish and humans. The effects linked to *Pfiesteria* toxicity may be related to an inflammatory response, either in macrophages in the periphery or microglia in brain tissue. Implication of P2X7 receptors as a potential target for the bioactive substance produced by toxic *P. piscicida* provides a common basis for the investigation of symptoms that previously have been regarded as unrelated, such as ulcers in menhaden and cognitive dysfunction in humans.

PFIESTERIA FIELD ECOLOGY AND TOXIC ACTIVITY: TRENDS FROM A DECADE OF INTENSIVE STUDY IN NORTH CAROLINA ESTUARIES

R. Reed,¹ H. Glasgow¹, J. Burkholder,¹ N. Deamer-Melia¹ and M. Mallin²

¹ Center for Applied Aquatic Ecology, North Carolina State University, Raleigh, NC 27606

² Center for Marine Sciences Research, UNC-Wilmington, Wilmington, NC 28409

Our research team has amassed a decade of data on toxic *Pfiesteria* outbreaks, extending back to those first documented in 1991. During that time, we have tracked 88 toxic *Pfiesteria* outbreaks in North Carolina waters, most of which affected the Albemarle-Pamlico. This system is the second largest estuary on the U.S. mainland, and is regarded as the most important fish nursery ground on the U.S. Atlantic Coast. Toxic *Pfiesteria* outbreaks have been implicated as the primary cause in the death of well over 1 billion fish, 90% as Atlantic menhaden but also including southern flounder, spot, croaker, striped bass, American eel, and other species.

The ongoing, long-term study has included special focus on the mesohaline Neuse Estuary as the most active system for toxic *Pfiesteria* outbreaks. For the past eight years, the sampling program has included 8 stations weekly and 16 biweekly, with additional sampling during major storm events. Through use of boat-mounted ADCP to obtain improved flow data, and high-frequency sampling to obtain improved water quality data, we have determined that P loading to the mesohaline Neuse has decreased while N loading has significantly increased (especially TN_i, by ca. 40%). We also recently installed a series of seven automated platform stations in the mesohaline Neuse, with maintenance of the stations at ≤ 3 -day intervals. These stations can measure physical, chemical, and biological conditions hourly in depth profiles automated samplers, with real-time data transmitted to a freely accessible website. The stations have been strategically positioned in 'hot spots' for major fish kills (related to *Pfiesteria*, low oxygen stress, and other factors) so that we can strengthen acquisition of 'before' and 'during' data needed to improve diagnosis of the causative factors leading to fish kills.

The extended period encompassed by this dataset has enabled us to construct a conceptual model of *Pfiesteria* seasonal dynamics in relation to various environmental factors, based on statistically significant interactions from trend analysis. For example, in the Pamlico Estuaries where P loading has decreased by ca. 40%, toxic *Pfiesteria* outbreaks have significantly declined in frequency and duration. On the basis of archived sample analysis with molecular probes that recently have become available, P decline with concomitant N increase has coincided with an apparent shift at *Pfiesteria*-related fish kills from clear dominance by *P. piscicida* to occasional co-dominance by *P. piscicida* and *P. shumwayae* sp. nov. These data support laboratory experiments that have shown comparatively higher P stimulation of *P. piscicida* zoospores, and higher N stimulation of *P. shumwayae*. The conceptual model is guiding collaborative research in progress to construct a quantitative, predictive model of *Pfiesteria* abundance and toxic activity.

This dataset has also enabled detection of a significant effect of high-intensity-storm years on subsequent toxic *Pfiesteria* activity. For 1-2 years following a year with 2-3 hurricanes or severe tropical storms that have passed through North Carolina, toxic *Pfiesteria* outbreaks affect relatively few fish, in comparison to the number of fish affected in the year preceding the high-intensity storms. This trend apparently is related to flooding displacement of resident *Pfiesteria* populations down-estuary to less conducive areas for toxic activity. In addition and with laboratory findings in support, the history of recent toxicity apparently is an important factor influencing subsequent toxicity. Populations that have been engaged in fish-killing activity in the previous season likely are more prone to become actively toxic in the next growing season than populations that have not been in fish-killing mode.

DISTRIBUTION OF *PFIESTERIA* SPECIES: COMPARISON OF RESULTS FROM WATER AND SEDIMENT SAMPLES ACROSS MULTIPLE SCALES, 1998-2000

Parke A. Rublee¹, Eric F. Schaefer¹, Coy Allen¹, Janera Harris¹, Holly Bowers², Torstein Tengs², and D.W. Oldach²

¹Biology Department, Univ. North Carolina at Greensboro, Greensboro, NC, 27412

²Inst. Human Virology, Univ. Maryland, Baltimore, MD 21201

We have used PCR methods for the detection of *Pfiesteria* species from states along the US East and Gulf coasts since 1988. During the first year we tested only for *P. piscicida*. In 1999, we began testing for *P. shumwayae* as well as *P. piscicida*, and we began testing sediments as well as water column samples. During summer and fall of 2000, we tested water and sediment samples collected simultaneously from multiple East and Gulf coast sites. The distribution of *P. piscicida* detected in our studies ranged from New York to Texas. The distribution of *P. shumwayae* appears to be similar. During the summer and fall of 2000 we found a much higher incidence of *P. shumwayae* than *P. piscicida*.

On a fine scale, water and sediment samples are differential indicators of *Pfiesteria* spp. activity. Positive water samples indicate active, though not necessarily toxic, populations of zoospores and/or amoebae at a site. In contrast, sediment samples are likely a better indicator of endemic populations which can serve as the inoculum for planktonic populations, or they may represent residual populations from an earlier event. For example, in one case, a water sample collected from a reported fish kill site, but collected after extensive rainfall, was found to be negative, while a sediment sample collected within a week from the same site tested positive. In many cases we found positive indications of *Pfiesteria* spp. in sediment samples when there was no signal detected in the overlying water. This was further confirmed when we studied fine scale distribution of *Pfiesteria* spp. at sites in the Neuse River, NC where frequent fish kill and lesion events have occurred.

On a regional scale, a major factor affecting the distribution of *Pfiesteria* sp. appears to be tidal flushing of estuarine areas, consistent with previous observations that most fish kill or lesion events occur in poorly mixed waters and that rainfall or storms can dissipate such events rapidly. Overall, results continue to suggest that *Pfiesteria* spp. are a widespread and probably common member of estuarine benthic and planktonic communities.

INTERACTIONS BETWEEN *PFIESTERIA* AND REPRESENTATIVE SPECIES OF COMMERCIALY VALUABLE SHELLFISH

S. Shumway,¹ J. Springer,² J. Burkholder² and H. Glasgow²

¹ Natural Sciences Division, Southampton College – LIU, Southampton, NY 11968

² Center for Applied Aquatic Ecology, North Carolina State University, Raleigh, NC 27606

In response to substances in finfish excreta/secretata, species of the toxic *Pfiesteria* complex produce bioactive substances that can cause finfish death and disease. As a unique trait among toxic dinoflagellates, they are also known to exhibit direct attack behavior toward live finfish, but interactions between *Pfiesteria* and shellfish have not been intensively examined. In a series of controlled laboratory trials, we assessed the response of representative adult and pediveliger shellfish to zoospores of *Pfiesteria piscicida*. In addition, we examined attraction of *Pfiesteria* zoospores and amoebae to shellfish tissues, behavioral responses of *Pfiesteria* to larval shellfish, and survival of *Pfiesteria* zoospores consumed by adult eastern oysters. *Pfiesteria piscicida* was isolated from the Neuse Estuary, cloned, and confirmed as toxic to finfish (JB/HG laboratory; uni-dinoflagellate clones, Heteroduplex mobility assay of D. Oldach, U.MD; species identifications from suture-swollen cells with SEM, cross-confirmed by PCR probes from HG/JB and FISH probes of P.Rublee, UNC-G; fish bioassay process, toxicity cross-confirmed by H.Marshall, ODU).

Acute challenges of toxic *P. piscicida* (actively toxic or TOX-A zoospores, 2.5×10^3 /mL) were completed with adult shellfish including bay scallops (*Argopecten irradians*, shell width 5 cm, 3/replicate, n=3), northern quahogs (*Mercenaria mercenaria*, shell width 6-8 cm, n=3), and eastern oysters (*Crassostrea virginica*, shell width 10-12 cm, n=5). Control animals were maintained similarly with benign algae (diatom *Thalassiosira*). Bay scallops showed an extreme escape response (seconds) followed by shell gaping and death (minutes to hours). Quahogs were intermediate in response (shell closure in minutes to hours, death in 1-2 days), whereas adult oysters were narcotized with reduced filtering but were alive at 21 days. *P. piscicida* zoospores and amoebae showed strong attraction to most scallop tissues tested with exception of gonad; strong attraction to quahog siphon (zoospores) and stomach tissues (zoospores, amoebae); and low response to oysters except for gill tissues.

Two other *P. piscicida* clones from the Neuse Estuary were used for other experiments on *C. virginica* and *A. irradians* (1st isolate yielding two functional types as TOX-A and TOX-B [previously tested as capable of toxin production in the presence of live fish, but maintained for 6 wk without fish]; 2nd isolate for NON-IND functional type, benign, i.e., previously tested as incapable of toxicity in the presence of live fish). Both TOX-A and TOX-B zoospores sometimes attacked and consumed oyster and scallop pediveliger larvae that had discarded their vela (only adductor muscle tissue remained; minutes). During 1-hr trials when *P. piscicida* was maintained within dialysis membrane to prevent direct contact with pediveligers, there was high (TOX-A, 90-100%) to moderate (TOX-B, 40-50% larval mortality, but negligible mortality when pediveligers were exposed to benign prey. In another experiment, oyster pediveligers appeared to detect residual toxicity from TOX-B zoospores; their grazing activity was highest on NON-IND zoospores, with intermediate and lowest grazing on TOX-B and TOX-A zoospores, respectively. In contrast, adult oysters grazed significantly less TOX-A *Pfiesteria*, but grazing was comparable on TOX-B and NON-IND zoospores. Examination of adult oyster faeces indicated that zoospores had formed temporary cysts in the digestive tract. Within 20 hr after gut tract passage, 90% of the previously TOX-A zoospores had excysted and regained motility, with lower (ca. 40-70%) survival shown by TOX-B and NON-IND zoospores. These data indicate that toxic *Pfiesteria* zoospores could potentially affect recruitment and survival of commercially important shellfish

species. The demonstrated ability of adult oysters to remove toxic zoospores from the water column indicates a potential, as well, for trophic mitigation/control of toxic *Pfiesteria* outbreaks.

ABSTRACTS FOR POSTER PRESENTATIONS

***PFIESTERIA* – NC, SC, FL SESSION**

THE ECONOMIC EFFECTS OF *PFIESTERIA* IN THE MID-ATLANTIC REGION

Timothy Haab¹, John Whitehead², Douglas Lipton³, James Kirkley⁴, George Parsons⁵

¹Department of Agricultural, Environmental and Development Economics, Ohio State University, Columbus, OH 43210

²Department of Economics, East Carolina University, Greenville, NC 27858

³Department of Agricultural and Resource Economics, University of Maryland, College Park, MD 20742

⁴Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Point, Virginia 23062-1346

⁵Departments of Economics and Marine Studies, University of Delaware, Newark, DE 19716

While significant amounts of research are currently being conducted to assess the biological, ecological and environmental effects of *Pfiesteria piscicida* and other harmful algal blooms (HABs), very little work has been conducted to look at the economic impacts or lost benefits due to *Pfiesteria* outbreaks or HABs. We report on the results from a 2-year Mid-Atlantic (North Carolina, Virginia, Maryland and Delaware) study of the economic impacts of *Pfiesteria* and HABs, on seafood consumption. The study consisted of a phone-mail survey of 2,000 Mid-Atlantic residents focusing on current seafood consumption patterns and reactions to harmful algal blooms, *Pfiesteria* outbreaks, and various education materials. Questions addressed include: What are the current seafood consumption patterns in the Mid-Atlantic region? What is the effect of a HAB/*Pfiesteria* outbreak on seafood consumption in the Mid-Atlantic region? What are the potential economic impacts of an outbreak (as opposed to welfare losses)? What are the regional similarities or differences in response to outbreaks (Albemarle/Pamlico Sound estuary system versus Chesapeake system)?

Preliminary results suggest that localized *Pfiesteria* associated fish kills significantly decrease the demand for seafood over large geographic regions creating large short-term economic losses. Reducing consumer uncertainty in relation to the risks from a *Pfiesteria* outbreak significantly mitigates the negative consumption effects of an outbreak. Demand models of seafood consumption demonstrate that perceived reductions in risk associated with an outbreak can significantly lessen the economic losses during and after an outbreak. Public distribution of scientific findings regarding the risks associated with *Pfiesteria* can reduce demand uncertainty and consequently reduce the economic losses.

POLYMORPHISMS IN THE ITS REGION OF DINOFLAGELLATES: IMPLICATIONS FOR PHYLOGENY AND PROBE DEVELOPMENT

Wayne Litaker^{1,4}, Kimberly S. Reece², Nancy A. Stokes², Karen Steidinger³, and Pat Tester¹

¹National Ocean Service, NOAA, Beaufort, NC 28516, ²Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, VA 23062, ³Florida Fish & Wildlife Conservation Commission, Florida Marine Research Institute, St. Petersburg, FL 33701-5020, ⁴Program in Molecular Biology and Biotechnology, University of North Carolina, Chapel Hill, NC 27599

Numerous research efforts are underway to develop RNA or DNA based assays for identifying Harmful Algal Bloom Species (HABS). One of the fundamental questions that must be answered in developing reliable molecular assays is whether the target DNA or RNA sequences are species-specific. To answer this question requires sequence information from the target organisms and a suite of related species, as well as a panel of appropriate cultures for cross-reactivity testing. In this study we investigated whether the internal transcribed spacers regions, ITS1 & ITS2, or the 5.8S gene of dinoflagellates is an appropriate target for the species-specific PCR assays. The ITS1 and ITS2 regions of the ribosomal gene complex are more variable than the structural 5.8S, or large and small subunit genes, and have proved useful in phylogenetic studies of closely related species and as targets for primer development in other organisms. Specifically, we amplified, cloned, and sequenced the ITS/5.8S region from *Pfiesteria piscicida* and the following *Pfiesteria*-like organisms: *Pfiesteria* species "B", 3 Cryptoperidiniopsoid species, a Florida "Lucy" species, and "Cell J" - an unidentified PLO that co-occurs in the same environment as *P. piscicida*. Other more distantly related species sequenced for comparison included *Amyloodinium ocellatum*, *Prorocentrum minimum*, and *Heterocapsa triquetra*. Five independent isolates of *Pfiesteria piscicida* and 2 isolates of the *Pfiesteria* sp. B were sequenced to estimate the amount of ITS/5.8S variation between isolates of the same species. Within genome variation was assessed by sequencing 2 - 12 independent ITS/5.8S clones from each isolate. The sequencing results showed the ITS regions of closely related species were sufficiently divergent to provide unique targets for species-specific PCR assay development. In contrast, the 5.8S gene was less divergent and did not yield as many unique primer sites. Sequencing of clones from the same species revealed the existence of species-specific polymorphisms. Hence, each ITS region must be fully characterized to ensure the probes will bind a conserved site present in every genotype. The extensive ITS sequence variability between species means that sequences from closely related species could be aligned reliably, but that more distantly related species could not. Hence, ITS sequences will likely prove useful in determining the phylogenetic relationships among members of the *Pfiesteria* complex, or other closely related groups, but may not prove useful in determining overall phylogenetic relationships among dinoflagellates.