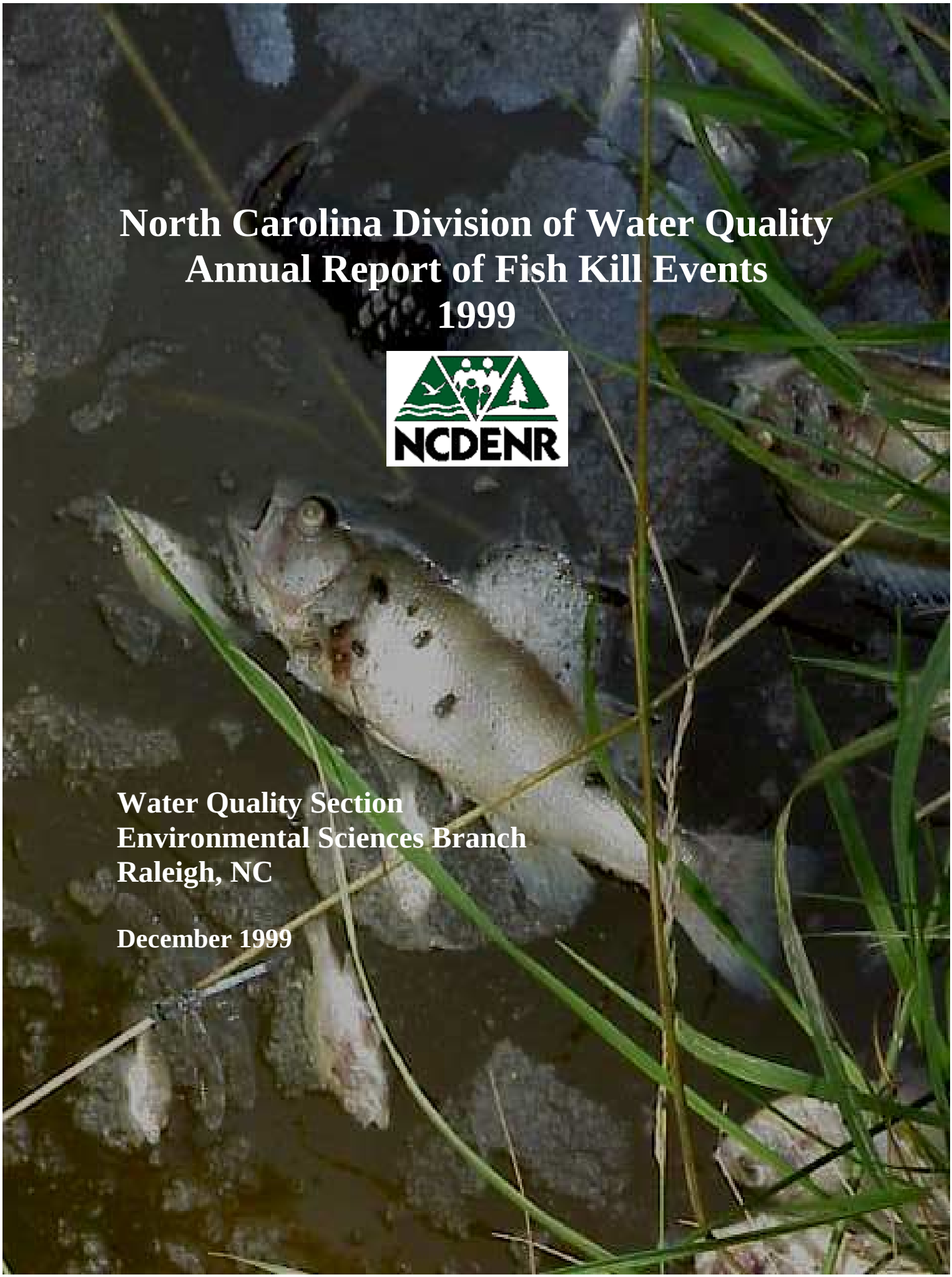


North Carolina Division of Water Quality
Annual Report of Fish Kill Events
1999

NCDENR

Water Quality Section
Environmental Sciences Branch
Raleigh, NC

December 1999

Introduction

This year marks the fourth season where fish kill investigation protocols established by the North Carolina Division of Water Quality (DWQ) have been used to investigate and report kill activity across the state. The protocols have proven successful and have been fully adopted by most of the state's frontline investigators. Fish kill and fish health data are recorded on a standardized form and sent to the Division's Environmental Sciences Branch (ESB) where the data are reviewed. The procedure also requires the notification of appropriate state officials and scientists associated with the investigation of such events. Fish kill investigation forms and supplemental information sent to the ESB are compiled in a central database where the data can be managed and retrieved for use in reporting to concerned parties. Fish kills and disease events often involve a host of factors and underlying causes, therefore, it is the Division's intent to gather as much information as possible surrounding an event from all involved parties.

This report is a summary of DWQ's efforts to document kill activity in North Carolina during 1999. The following report is mandated under Section 4 of Chapter 633 of the 1995 North Carolina General Assembly Session Laws.

1999 Fish Kill Summary

Field investigators reported 54 fish kill events from February to October, 1999. Kill events were reported statewide from the coastal counties to as far west as Buncombe County in 11 of the states 17 major river basins. Kill events are tracked by the Division when at least 25 fish are affected and the event can be confirmed by investigators.

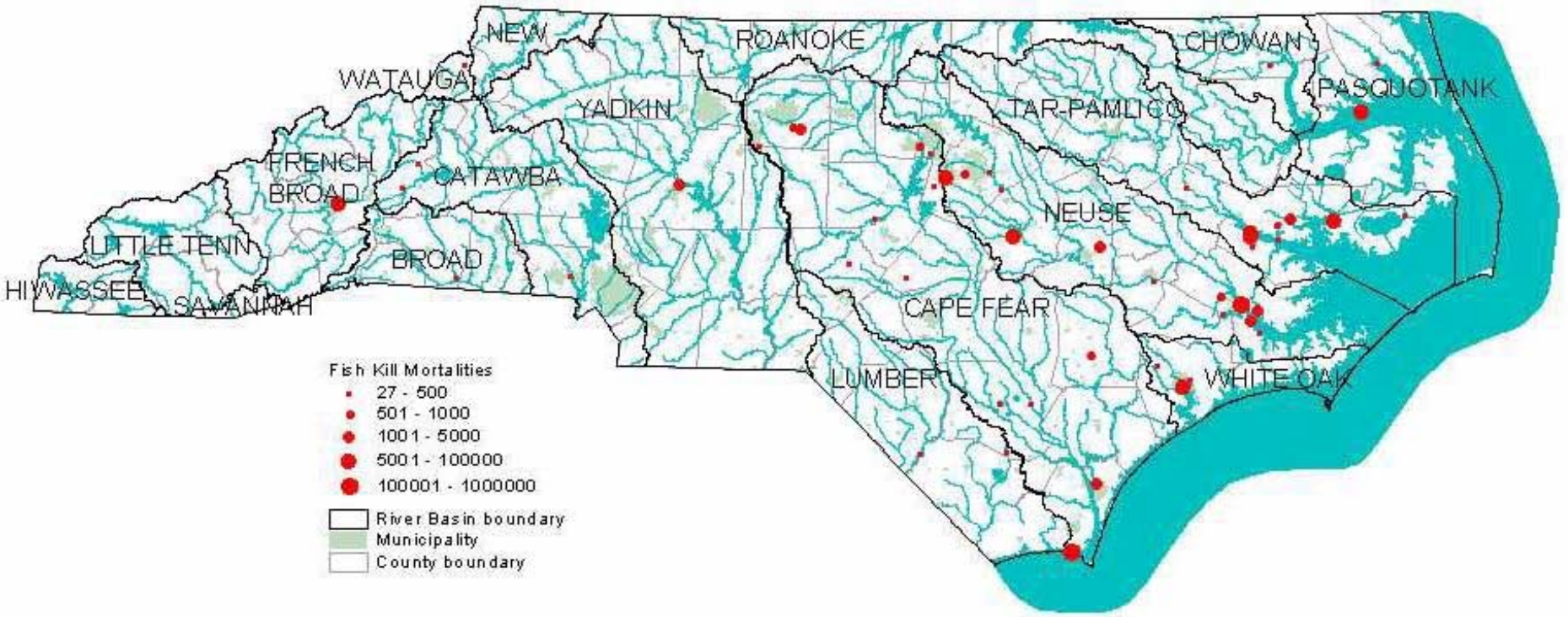
Reported fish mortality figures ranged from 27 to 1 million. Total fish mortality for 1999 (the sum of all 54 events) was about 1.3 million, however, a large portion of the 1999 total was made up of a few large coastal events. The median mortality figure reported in 1999 was 325 and about 18% of the reported events had mortality totals exceeding 5000. A detailed summary of the largest kill events is show in Appendix A.

The majority of kill events occurred in freshwaters during 1999. Reports noted 35 events affecting freshwater species, 18 involving estuarine fauna, and one event in ocean waters. Although freshwater kills were more numerous in 1999, they accounted for only 2.5 % of the years total mortality. Kill events investigated in the state's estuaries and along the coast were fewer in number than freshwater events but were responsible for over 97% of the year's mortality.

Freshwater species most commonly identified during investigations included largemouth bass, sunfishes, and catfishes. Estuarine species most commonly encountered included menhaden and flounder. Menhaden, which have historically been the subject of large fish kills along the coast, were cited again during 1999 in the larger coastal events. Blue crabs were observed in addition to finfish species in 7 of the year's 54 events.

1999 Fish Kill Summary	
Total Kill Events for 1999	54
Total Mortality for 1999	1.3 million
Mortality Range	27 to 1 million
Median Mortality	325
Basins with Activity	11 (of 17)
Freshwater Kills	35
Estuarine Kills	18
Ocean Kills	1

Figure 1: fish kills reported to the DWQ during 1999



Basin Activity

Investigators reported fish kill events in 11 of the state's 17 major river basins during 1999. Most events occurred in the Cape Fear, Neuse and Tar/Pamlico watersheds. The Neuse Basin exhibited the most kill activity, replacing the Cape Fear as the most active watershed during the previous three years. (Table 1). The most intense kill activity in 1999 was generally reported along the coastal plain and in the central part of the state. Activity in the Tar/Pamlico watershed increased from previous years, while events in the Yadkin and Lumber basins were fewer than seen in the past. The total number of statewide events has remained relatively consistent since 1996. Reported annual kill events for North Carolina have numbered between 54 and 60 for the past four years, regardless of yearly weather and environmental conditions and a series of severe hurricanes. Detailed summaries of 1999 fish kills are presented in the Appendix B.

Table 1: Fish kill events by basin

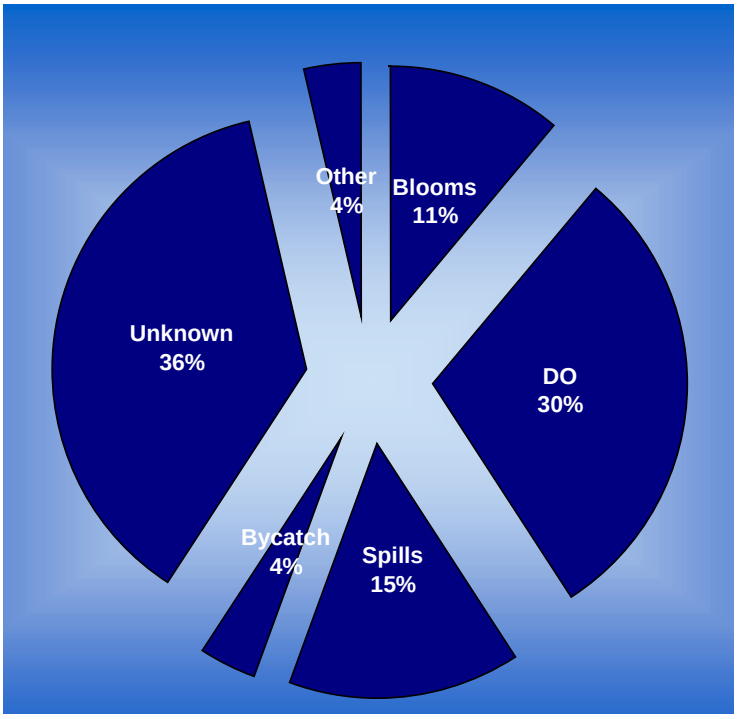
	Year			
River Basin	1996	1997	1998	1999
Broad	None	None	None	1
Cape Fear	21	16	23	14
Catawba	None	3	1	3
Chowan	2	2	1	1
French Broad	None	2	3	1
Neuse	14	12	8	16
Lumber	4	3	5	None
Pasquotank	10	2	8	2
Roanoke	2	None	1	None
Tar/Pamlico	3	6	5	11
Watauga	None	None	None	1
White Oak	3	3	1	3
Yadkin	1	10	2	1
Totals	**60**	**57**	**58**	**54**

Suspected Causes of 1999 Fish Kill Events

Specific causes of fish kill events may or may not be obvious to investigators depending on a host of factors. Many causes may quickly be defined, but others remain unconfirmed or unclear due to investigations occurring after the fact. Kill events often result from many environmental conditions, and sorting out the major reason(s) why fish die is frequently a difficult and often subjective task. The NCDWQ reviews and tracks suspected causes of fish kills conveyed by field investigators. Cause observations aid in evaluating potential water quality trends and problems, and assist scientists and decision-makers with formulating future courses of action. Cause observations should not be viewed as a definitive label for a particular event.

Reported causes 1999 kill events fell into five major categories (see Figure 2). Those events where no specific causes could be determined were assigned to a sixth unknown category.

Figure 2: Major suspected causes for fish kills during 1999



Harmful Algal Blooms: Nutrient enrichment of waterbodies throughout North Carolina has resulted in eutrophication, frequent algal blooms, and an increase in subsequent fish kill events. Algal blooms cause dissolved oxygen (DO) and pH fluctuations as well as DO depletion through diurnal cycles and decomposition. These elements often precede fish kills events. Certain types of algae also release toxins during the course of a bloom that are detrimental to aquatic life. Algal blooms suspected in 6 of the 54 events for 1999. Bloom related kills occurred mainly in freshwater lakes and streams.

Pfiesteria and similar organisms: Since its identification in the early 1990's, the dinoflagellate *Pfiesteria piscicida* ("*Pfiesteria*") and has been reported as the cause for a number of massive fish kill events in North Carolina's estuaries, especially in the White Oak, Neuse, and Tar/Pamlico systems. Scientist believe that the presence of fish, especially schooling species such as menhaden, may induce changes in *Pfiesteria* cells resulting in the emission of a toxin that acts on the schools.

When phytoplankton samples are collected from fish kills, they are viewed under a light microscope by ESB staff members and the cell densities of *Pfiesteria*-like dinoflagellates are calculated. Cell densities reported by DWQ are considered presumptive counts and include *all* cells that resemble *Pfiesteria.* In order to reliably distinguish *Pfiesteria* cells from look-alike cells, samples need to be analyzed under scanning electron microscopy (SEM), but this analysis may take several weeks and is currently performed by only a few laboratories (e.g. NCSU Aquatic Botany Laboratory, Fla. Marine Research Inst.). The DWQ does not currently have this latter capability. Recently, the Environmental Sciences Branch at DWQ has obtained equipment necessary to view phytoplankton samples under epifluorescence microscopy (FM) which causes photosynthetic algae to glow (fluoresce). Most photosynthetic dinoflagellates contain chloroplasts and glow throughout their cell when viewed under FM. These dinoflagellates are considered to be autotrophic because they synthesize their own nutrition by using their chloroplasts to perform photosynthesis. *Pfiesteria*, on the other hand, is considered to be heterotrophic because it does not contain its own chloroplasts and cannot perform photosynthesis. Instead, it must ingest algae and other forms of microplankton in order to survive. In this state, *Pfiesteria* will not glow at all (or glow differently) under fluorescence because it does not contain chloroplasts. When a sample from a fish kill is analyzed by FM, *Pfiesteria*-like dinoflagellates are examined for fluorescence. DWQ staff members observe whether dinoflagellates which visually resemble *Pfiesteria* glow like photosynthetic dinoflagellates, glow as if they are using ingested chloroplasts, or do not glow at all. This method is not as reliable as SEM and both methods are unable to detect whether *Pfiesteria*-like cells are toxic. However, the FM analyses can provide clues that may indicate whether suspected *Pfiesteria* dinoflagellates or *Pfiesteria* look-alikes were present at a fish kill/fish disease site.

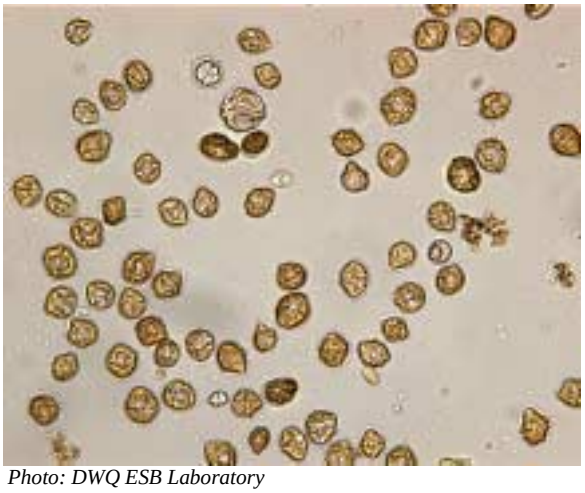


Photo: DWQ ESB Laboratory

Pfiesteria-like cells were identified by ESB staff members in samples from 9 fish kills during 1999 (see Appendix B). *Pfiesteria*-like cell counts ranged from 210 to 32000 cells per milliliter. Although counts of *Pfiesteria*-like organisms were often elevated in the samples, further investigations of the cells using FM revealed that none of the look-alikes fluoresced as true *Pfiesteria piscicida* (ie: they all glowed as autotrophic cells). Additional fish bioassay results were also provided on samples from two fish kills by the NCSU Aquatic Botany Laboratory. These results were also negative for toxic forms of *Pfiesteria*.

Fish kill investigations coupled with analyses of water samples from kill sites failed to suggest that toxic forms of *Pfiesteria* were a factor in any kill events during 1999.

Dissolved Oxygen Depletion: Fish kills associated with low dissolved oxygen (DO) levels were reported statewide during 1999. DO depletion prior to kill events often occurred as a result of heavy rain or following periods of drought and low flow. Anoxic conditions also occurred in estuaries as nutrient and organic loading coupled with water column stratification depleted DO levels during the summer months. DO depletion was cited as a factor in 16 (29%) of the kill events during 1999. This figure is substantially lower than the 1998 total where nearly one half of reports cited DO as a major factor. Fewer DO related events in 1999 may be the result of more favorable environmental and meteorological conditions throughout the season (see below). DO related events during 1999 were most frequent in the Neuse and Tar/Pamlico basins.

Hurricane Dennis. Photo courtesy Nasa.

Since 1995, several large storm events in North Carolina have resulted in widespread flooding, depletion of DO, and subsequent fish kills throughout many river basins, especially on the coastal plain. Hurricanes Bertha and Fran (1996) and Hurricane Bonnie (1998) were a direct factor in 75% and 38% of the fish kill events for each year, respectively. Several large storms also occurred in 1999 producing effects equal to or greater than those seen in previous years. Hurricanes Dennis, Floyd and Irene all resulted in record rainfall and flooding, however, fish kills attributed to the 1999 storms were suprisingly few. Only one event was tied to the effects of Dennis, and one possibly to the prolonged flooding from Floyd. No reported events were attributed to Irene.

The absence of post- hurricane fish kills during 1999 may be the result of several factors. Massive flooding such as what was seen after Floyd may have quickly dispersed dead and dying fish, thus preventing investigators from making accurate counts of identifying discreet events. However, since there were few reports and little anecdotal information outside the investigating agencies regarding dead fish sightings after the storms, a more likely reason is a consistent period of favorable weather and environmental conditions following the hurricanes. Cooler, windier conditions, as well as dilution effects from record flooding may have ameliorated factors that lead to DO depletion following storms in 1996 and 1998. Furthermore, the timing of each hurricane during 1999 may have been sufficient to keep many waterbodies “stirred up” and thus prevent anoxic conditions due to organic loading from occurring. Whatever the reasons, hurricanes appear to have played a relatively insignificant role in causing fish kill events during 1999.

Spills: Waste spills either deplete DO levels in receiving streams or induce kills outright through physical or chemical toxicity. Spills were reported as a cause in 8 (15%) of the kill events during 1999. Spilled substances included pesticides and chlorine, as well as

agricultural and industrial wastes. Most kill events related to spills were located in the Neuse and Cape Fear Basins.

Bycatch: Discarded fish from nearby fishing operations were reported as a cause or possible factor in two kill events during 1999. A large kill of menhaden in the Atlantic Ocean off Yaupon Beach (Brunswick Co.) was definitely tied to purse seine activity in the area. Investigation of a protracted kill in the New River near Morgan Bay (Onslow Co.) revealed net marks on as many as 10% of the dead fish. A bycatch cause was suspected but never confirmed.

Unknown and Other Causes: Causes for kill events are listed as unknown when investigators fail to report specific reasons for an event. Investigations may not provide definitive causes when they are conducted too long after an event and no clear factors are determined, or when causes are suspected but not confirmed. Investigators reported 20 (36%) of 1999 kills without clear causes. Most were located in the Neuse, Tar/Pamlico, and Cape Fear basins.

Other miscellaneous causes include two thermal-related kills of striped bass in Albemarle Sound and the Pasquotank River during a period of severe heat and drought. High water temperatures, outside the tolerance range for the fish, were suspected as causing stress and death in both events.

NCSU College of Veterinary Medicine Pathology Results

During 1999, Dr. Mac Law along with NCSU College of Veterinary Medicine staff and veterinary students, assisted DWQ investigators by performing gross and microscopic evaluations of stressed/diseased fish. Dr. Mac Law is an American College of Veterinary Pathology (ACVP) board certified veterinary pathologist at the North Carolina State University College of Veterinary Medicine. The pathology team examined more than 60 fish from various sites of fish disease events and fish kills in the Pamlico and Neuse estuaries. Fish species examined included Atlantic menhaden, croaker, spot, flounder, mullet, striped bass, red drum, and speckled trout.

Flounder Lesion. Photo courtesy J.M. Law.

Dr. Law's Findings: Many fish had one to several ulcerative lesions of the skin and underlying muscle tissue which were often circular and centered near the anus. These lesions ranged from 4 mm to >3 cm in diameter, were of variable depth, and occasionally penetrated through the body wall of the fish. Lesions were most common in the Atlantic menhaden, although in May, September, and October small groups of flounder, croaker, and spot were received which had severe ulcerative dermatitis/myositis. Some fish samples were characterized by extensive necrosis of skin and underlying muscle tissue. Within the injured areas, special stains revealed abundant bacteria and fungal infections. All of the ulcerative lesions associated with fish kill and/or

stressed/diseased fish samples contained mature forms of inflammation. This type of inflammatory process takes at least a week to develop to the point observed by the NCSU veterinary staff.

In general, most of the ulcerative lesions in these fish were compatible with the "ulcerative mycosis" lesions of menhaden reported by Noga *et al* in 1986 (Journal of Fish Diseases 9:47-53) and later attributed to the "dermonecrotictoxin" produced by the *Pfiesteria* organism (Burkholder *etal*, Nature 358:407-410, 1992; Noga *et al*, Marine Pollution Bulletin 32:219-224, 1996; Baden, unpublished data, 1997). The small hemorrhagic lesions in the striped bass, however, were probably associated with temperature and/or low oxygen stresses.

The significance of the fungi in the initiation and development of these lesions is uncertain at this time. It is likely that some primary stressor such as a toxin, low dissolved oxygen, trauma, parasitism, etc. causes an initial breach in the fish's skin, allowing secondary invasion of fungi and other organisms.

During the warmest parts of the summer, several striped bass from the Albemarle Sound were received which had shown stress and high mortalities. Their musculature was generally in good condition, but each had sunken eyes, relatively empty GI tract, and large gall bladder (indicating very little recent feeding activity). One fish had hemorrhages of the scale, but no overt ulcers. A second fish had patchy areas of reddening of the gill cover and mouth area, but again no actual ulcers. The reddening and scale pocket hemorrhages were probably caused by bacterial infection, such as Aeromonas, or others. Stress due to high temperature and/or marginal DO levels most likely played a role.

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Summary

Reports of fish kills across North Carolina during 1999 closely matched the number of events seen annually since 1996. The consistency in annual kill event totals is unexplained and suprising considering the differences in environmental conditions from year to year.

Events observed in 1999 were the result of similar causes of fish mortality seen in previous years. Problems with DO depletion continued to be a major cause of events statewide. Coastal rivers and estuaries continued to experience eutrophication, stratification, and associated hypoxia, especially along the shallow, poorly flushed reaches of the Neuse and Pamlico rivers. A number of waste and pesticide spills, as well as harmful algal blooms also caused events. Several events involved the discharge of bycatch from nearby fishing operations, one was confirmed in the Atlantic Ocean, another in the New River (Onslow Co.) could not be verified. Two unusual kills of striped bass occurred in the Albemarle estuary due to elevated water temperatures that eliminated cool water refugia and lead to stress and mortality in the species. The presence of *Pfiesteria*-like organisms was observed in conjunction with a number of events, however, further investigation using epifluorescence microscopy and fish bioassays failed to reveal *Pfiesteria piscicida*.

North Carolina was once again the target of hurricane events that left behind record flooding, especially in coastal counties. The large number of kill events seen after storms in years past did not occur in the wake of the 1999 hurricanes. More favorable conditions, as well as the timing of the storms, may have been responsible for their insignificant role as a cause for kill events during the year.