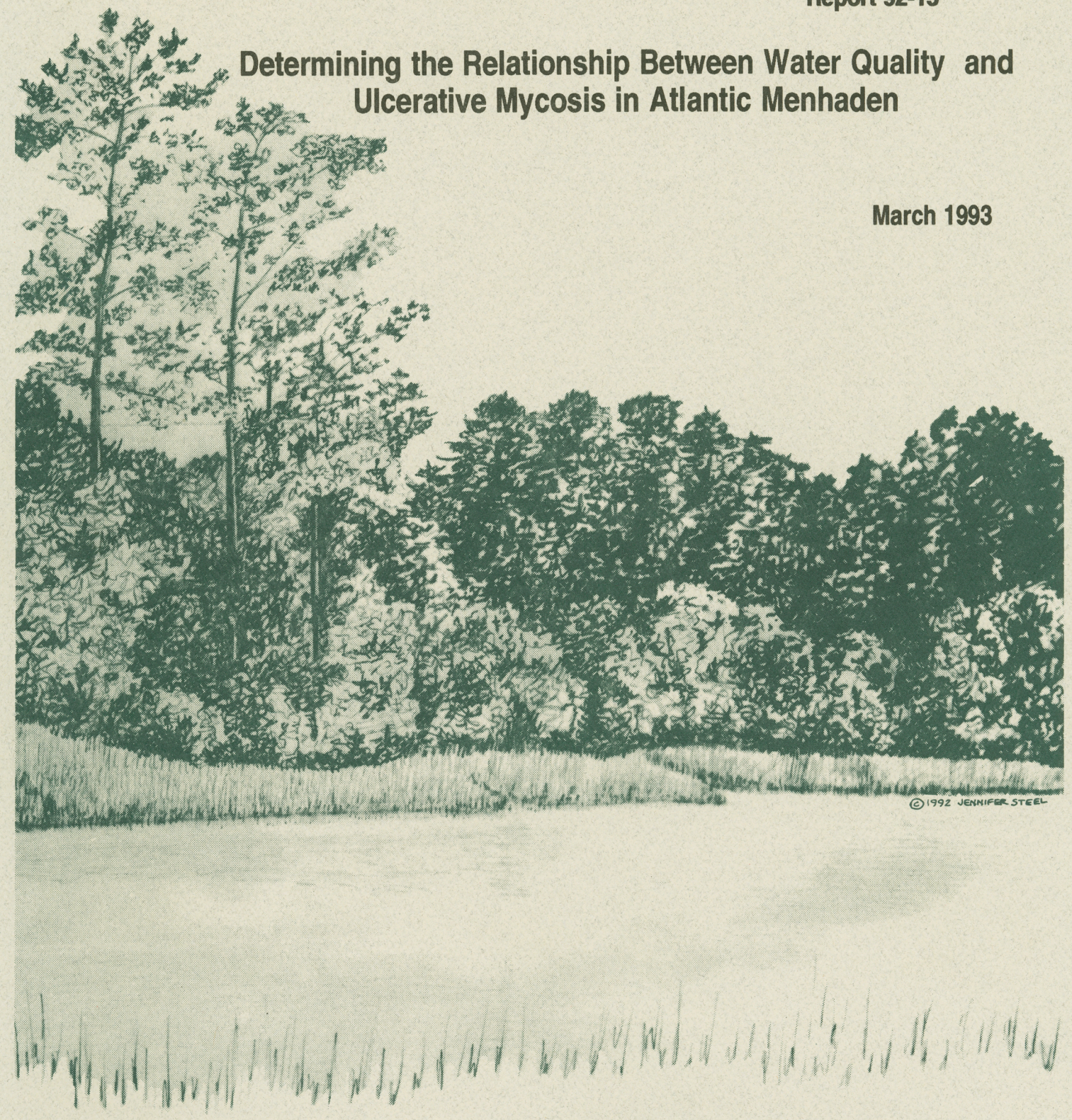
ICMR Tech Report 93-08

Report 92-15

Determining the Relationship Between Water Quality and Ulcerative Mycosis in Atlantic Menhaden

March 1993



ALBEMARLE-PAMLICO ESTUARINE STUDY

NC Department of Environment, Health, and Natural Resources

ALBEMARLE-PAMLICO

Environmental Protection Agency National Estuary Program

DETERMINING THE RELATIONSHIP BETWEEN WATER QUALITY AND ULCERATIVE MYCOSIS IN ATLANTIC MENHADEN

by

Edward J. Noga[1], Stephen E. Johnson[1], David W. Dickey[2], Deborah Daniels[4], JoAnn M. Burkholder[3], and Donald W. Stanley[4]

Department of Companion Animal and Special Species Medicine[1]
College of Veterinary Medicine
North Carolina State University
Raleigh, North Carolina 27606

Departments of Statistics[2] and Botany[3]
College of Agriculture and Life Sciences
North Carolina State University
Raleigh, North Carolina 27606

Institute for Coastal and Marine Resources[4]
East Carolina University, Greenville, NC 27834

"The research on which this report is based was supported in part by the United States Environmental Protection Agency and the North Carolina Department of Environment, Health, and Natural Resources (EHNR), through the Albemarle-Pamlico Estuarine Study."

Project No. 92-15 March 1993

ACKNOWLEDGEMENTS

We wish to thank the personnel of the North Carolina Division of Marine Fisheries for greatly assisting us during this study. Without their generous help, this work could not have been completed. We would especially like to thank Mr. Jess Hawkins and his staff who assisted us in obtaining menhaden for stocking the tanks, as well as assistance in set-up and routine maintainance of the tank systems. We thank Mr. Joe Andrews for assistance with the tank studies at Morehead City and Ms. Nancy Morse who assisted with the early phases of this project. We also thank Ms. Lynn Everett of the North Carolina Division of Environmental Management for assisting in the processing of some water samples and Ms. Martha Jones, Institute for Coastal and Marine Resources, for assistance with water quality analysis. Thanks to the very generous assistance of several private citizens or groups (including Dr. Robert Davis and Texasgulf Phosphate Company), we were able to set up these tank systems on private property and thus have access to secure sites with electrical power. The members of the Pamlico Estuarine Response Team (PERT) informed us as to when and where UM appeared in the river, so that we could correlate this information with changes which we observed in our tanks.

EXECUTIVE SUMMARY

The objectives of this study were to investigate the possible causes of ulcerative mycosis (UM) in the Albemarle-Pamlico Estuary. Ulcerative mycosis is the commonest disease affecting the finfish populations of the Albemarle-Pamlico Estuary. While infectious agents have been isolated from UM lesions, the underlying environmental cause of the disease remains a mystery. We presently know very little of how water quality (including pollution) influences UM prevalence. The difficulty in reproducing the disease by simply challenging fish with the fungal pathogen suggests that environmental stress may play a very important role in disease development.

Previous sampling surveys for UM that simultaneously examined water quality did not always show any consistent relationship to disease prevalence, perhaps because water quality monitored simultaneously with disease sampling may not be representative of the actual conditions that caused the disease outbreak.

To obtain more reliable data on the risk factors influencing the development of UM, we placed clinically normal Atlantic menhaden in tanks at various sites along the Albemarle-Pamlico Estuary and examined them periodically for the development of UM lesions. We also simultaneously measured ambient water quality, including dissolved oxygen, salinity, temperature, pH, ammonia, nitrite, chlorophyll *a*, and prevalence of a new toxic dinoflagellate that we have recently discovered in the Albemarle-Pamlico Estuary.

Our studies have shown that in situ tank culture can be useful for studying water quality factors associated with the development of UM because a) menhaden could be maintained for long periods in these experimental systems, b) classical ulcerative mycosis lesions developed in the tanks, c) our repeated handling of the fish to examine them for lesions, as well as the overall culture system itself, appeared to have negligible effect upon UM development, and d) there was a temporal and spatial difference in UM incidence in the culture systems.

There was a correlation of salinity with the development of UM, with moderate salinity (about 5-6 ppt) being favorable. Decreasing water temperatures in fall were associated with increased UM incidence. As winter approached and temperatures continued to decline, UM incidence also declined. Thus, there was a fall peak as has been observed previously in natural epidemics. There was also some evidence that UM development was associated with fluctuations in dissolved oxygen, as well as with lower pH. There was no correlation of UM incidence with total ammonia nitrogen, nitrite or chlorophyll *a*. The relationship of UM incidence with toxic dinoflagellate density was uncertain, but no

strong evidence for a positive correlation was evident.

Monitoring studies should continue to collect information on the prevalence of ulcerative mycosis and associated water quality factors in various parts of the A/P Estuary in order to substantiate the site-related differences in disease development that have been identified in the present study.

In order to definitively determine the importance of man-made changes to these effects, more controlled studies need to be performed to ascertain the mechanism(s) responsible for the increased predilection of Atlantic menhaden to develop UM in the riverine areas of the Albemarle-Pamlico Estuary. This should include exposure of clinically healthy menhaden or other fish species to the possible environmental risk factors that have been identified in this project in order to experimentally confirm our findings. Such studies should be conducted in conjunction with examination of how water quality affects the pathogenicity of various microbial agents, as well as challenge with the microbial pathogens that inhabit ulcerative mycosis lesions, especially the fungal and bacterial components.

Once putative water quality factors have been proven to be responsible for UM, management decisions about regulating or otherwise managing (if possible) these risk factors affecting fish of the Albemarle-Pamlico Estuary should be made.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF TABLES (CONTINUED)

INTRODUCTION

A number of deleterious changes have been documented in the Albemarle-Pamlico estuary. Among these are the apparent loss of sea grasses, increased sedimentation/turbidity, increase in nuisance algal blooms, and increased hypoxic/anoxic events (Rader et al 1987). Among the more visible of these problems are the recent epidemics of fish and shellfish diseases, especially those occurring in the Pamlico River estuary. While a number of diseases affect fishes of the A/P Estuary (Esch and Hazen 1980, Noga 1986, Noga et al 1988, Noga 1988), the most prevalent is ulcerative mycosis (UM), a deep skin ulcer having oomycete fungi and several bacterial pathogens (Dykstra et al 1986, Noga et al 1988). It primarily affects Atlantic menhaden Brevoortia tyrannus, although similar lesions have been documented in other economically important estuarine species including southern flounder Paralichthys lethostigma, sea trout Cynoscion regalis, spot Leiostomus xanthurus, croaker Micropogonias undulatus, striped bass Morone saxatilis, and at least 12 other estuarine species in North Carolina (Noga et al 1991). In addition, UM epidemics have also recently occurred in the Chesapeake Bay (Maryland) and St. Johns River (Florida) estuaries (Dykstra et al 1989).

The fungal agents associated with UM are opportunistic pathogens that are not believed to infect normal, immunocompetent individuals (Neish and Hughes 1980). Thus, UM, like many infectious diseases, is probably a secondary manifestation of physiological and/or environmental changes that allow an infectious agent to colonize a host (Ellis 1981). While infectious agents are known to be responsible for the pathological manifestations of ulcerative mycosis, the underlying environmental cause of the disease remains a mystery.

We presently know very little of how water quality (including pollution) influences UM prevalence. Understanding the importance of pollution requires an awareness of how environmental conditions affect the health of the menhaden population.

There is very little information about the relationship between water quality and infectious disease development in natural environments. However, experience with disease problems in aquaculture situations can be helpful in formulating a probable list of risk factors. Two major types of factors should be considered. First are those environmental conditions universally essential to fish health: dissolved oxygen (DO), salinity, temperature, and pH. Second are toxic contaminants (heavy metals, pesticides, etc.) that may be above "normal" limits (i.e., defined as levels that we think may be harmful to the environment). With a myriad number of possible toxic factors

that may affect fish health and the considerable expense required for detection, this list should be based upon any historical trends of anthropogenic inputs into the system.

If a toxin responsible for reducing immunity accumulates in host tissues, determining body burdens in affected individuals may provide clues as to the cause of the problem. However, infectious diseases may also be initiated by environmental factors which may leave no detectable residues. Pollutants resulting in increased nutrient levels, alterations in salinity gradients, or changes in suspended solids may not be directly toxic to fish. Instead, it is their second and third order consequences (e.g., changes in dissolved oxygen due to eutrophication) that may stress fish (Plumb 1984). The rather ephemeral and temporally variable nature of such factors make them especially difficult to study.

There is presently no strong evidence linking any infectious disease in wild fish populations to a specific pollutant. In order to adequately address the pollution-disease relationship, there must be a close linkage between the examination of water quality and disease development.

Over the last several years, we have performed monthly trawl surveys which have documented the prevalence of UM in the Pamlico River (Levine et al 1990a,b; Noga et al 1989). These studies have shown that UM is most prevalent in the upriver areas of the Pamlico River (i.e., away from the river's mouth into Pamlico Sound). While simultaneous examination of temperature, dissolved oxygen, and pH have not shown any relationship to disease prevalence (Noga et al 1989a), we felt that more controlled studies were needed to examine these relationships.

The establishment of an environmental link to ulcerative mycosis in the Pamlico River estuary has been severely hampered by the highly mobile nature of menhaden and other fish populations. Thus, one can never be certain that sick fish collected at one site actually contracted the disease at that site. This problem is compounded by the fact that fish may not develop clinical signs of UM until several days after infection (Noga, Unpublished Data). This also means that water quality monitored simultaneously with disease sampling may not be representative of the actual conditions that caused the disease outbreak.

In order to circumvent these problems, we eliminated fish mobility by placing fish in tanks situated at various on-shore sites on the Pamlico River. This design was chosen because having the tanks on land allowed easy access for frequent water quality monitoring; having the systems in the river would not allow such frequent monitoring because of the effort required to access the fish-holding systems.

During initial studies in Fall 1988 funded by the North Carolina Division of Marine Fisheries, we measured temperature, salinity, dissolved oxygen, and pH. We chose these variables because:

a) They are universally essential to fish health.

b) Certain of these variables have historically been known to be a problem in the Pamlico River estuary (Rader et al 1987). For example, low D.O. has caused many fish kills.

c) In general, the Pamlico River has relatively low levels of toxic contaminants, such as heavy metals, pesticides, etc. While we cannot yet rule out these toxins as being important, we felt that it would be more prudent to examine them (if necessary) after the more basic water quality factors mentioned above.

These preliminary tank studies performed between October and December 1988 provided some evidence that menhaden could be maintained in these systems. Thus, the correlation of fish health with long-term water quality conditions was deemed feasible. We also measured several episodes of sub-optimal water quality, which could be stressful to fish. This initial work formed the basis for the research in this report.

PURPOSE AND OBJECTIVES

The purpose of this project was to determine the relationship between the risk of development of ulcerative mycosis and certain water quality variables, including factors which have a known influence on fish health.

PROCEDURES

A. EXPERIMENTAL DESIGN

In the 1989 experiment, single, 300-gallon, round fiberglass tanks were placed at each of three sites on the Pamlico River. Tanks were situated in low salinity areas of the river, since we previously demonstrated that UM-affected fish were prevalent in this ecological zone. Water was pumped from the river directly into the tanks using a 1 HP centrifugal fiberglass pump. Water was pumped from between 10-15 meters offshore at midwater depth (river depth at the intake was between about 0.6 - 0.9 meters). Raw (unfiltered) water was transmitted in a 3 inch PVC pipe at a

rate of about 250 liters per minute, resulting in a turnover time in the tanks of about 2.5 hr. At this flushing rate, tank water closely approximates ambient conditions in the river (Noga et al 1989b). We also measured DO, temperature, salinity, and pH of river water at the intake pipe in order to validate the similarity of tank water to river conditions.

Previous studies have shown that near-shore water quality measurements are representative of other areas of open water in the river (Hobbie 1974, Stanley 1987). Thus, our data was intended to provide a representative assessment of water quality conditions in the river.

In the 1990 and 1991 experiments, additional funding allowed us to place duplicate tanks at each site and to add another high salinity site (Division of Marine Fisheries dock at Morehead City). This allowed us to have a more broad-ranging spatial comparison.

B. FISH OBSERVATIONS

For each experiment, fish were transported to all tanks as one group. After several days acclimation at the Pamlico Aquaculture Center, we moved fish into experimental tanks. The same group of fish were maintained in the tanks (45-100 fish/tank, depending upon the experiment) throughout the experiment. Fish were not fed because they filtered food directly from the water; incoming water apparently had sufficient food. Tanks were closely examined daily for sick or dead individuals. The presence of acrylic windows in the tanks facilitated such observations. At regular intervals, we drew down the water on all tanks at the same time and removed all fish having UM for confirmation of the disease. Fish affected with UM were immediately fixed in formalin. Representative fish were also examined histologically to confirm that UM was responsible for the lesions (Noga et al 1988).

C. WATER QUALITY ANALYSIS

In situ water quality analysis included daily measurements of temperature, salinity, dissolved oxygen, and pH using electronic probes (Yellow Springs Instruments Corporation). In previous studies where we examined the feasibility of using various fish sentinel systems (Noga et al 1989b), we had demonstrated diurnal fluctuations in water quality factors, such as dissolved oxygen, which are essential to fish health. Thus, initially, all readings were taken in both early morning and late afternoon. However, analysis of the 1989 data indicated that

diurnal fluctuations added little to the analysis, so in the 1990 and 1991 experiments, only morning readings were taken.

Water samples were also collected thrice weekly, at sunrise, for total ammonia nitrogen, nitrite, and chlorophyll a. All samples were placed on ice and transported to the Washington field office of the Division of Environmental Management, where measured volumes of each sample were filtered through precombusted Whatman 934-AH (glass fiber) filters. A portion of the filtrate was frozen for later analyses of nitrate nitrogen and ammonia nitrogen, and the filter pads were stored frozen for chlorophyll a analysis. Such storage conditions do not significantly affect the accuracy of analysis (Stanley, Unpublished Data).

All chlorophyll a and nitrogen samples were analyzed by the Institute for Coastal and Marine Resources, East Carolina University. Standard colorimetric analyses for chlorophyll a and the two nitrogen fractions were made using an Orion Scientific Instruments Corporation analyzer (EPA 1979).

D. PHYTOPLANKTON ANALYSIS

Although not part of the original study, we felt that it would be useful to archive Lugol's-preserved water samples that could be collected during the course of our ambient water quality sampling. During the course of this project, we subsequently discovered a new, ichthyotoxic dinoflagellate that we have subsequently implicated in over 10 fish and invertebrate kills in the Albemarle-Pamlico Estuary (Smith et al 1988, Burkholder et al 1992).

We subsequently decided to examine the archived water samples for the possible presence of this toxic dinoflagellate, in order to determine if there may be any discernible relationship between its prevalence and incidence of ulcerative mycosis. This organism has a number of life stages and we wished to have a high degree of confidence in the retrospective examination of samples. Thus, we limited our counts to the sexual reproductive (gamete) stage of this organism, even though this stage appears to be non-toxic (Burkholder, Unpublished data). However, this stage has very distinctive features that allowed its identification, including small size (5-7 um for males, 6-9 um for female), having a gentle "figure-8" shape and being poorly pigmented with a dark center. Samples were examined with an Olympus research microscope and the number of individual dinoflagellate cells recorded.

E. TIME COURSE OF EXPERIMENTS

We ran a total of three experiments, one each in 1989, 1990 and 1991. However, none of the fish in the 1991 experiment developed UM lesions, so we were could not assess the relationship between water quality and UM in this experiment. Fish were either stocked into tanks in October-November and then monitored until December, or stocked in April and then monitored until June. These time periods were chosen because of the typically high prevalence of UM during these times (Noga et al 1989a, Levine et al 1990b). New fish were used for each stocking. We wanted to have the fish acclimated to the tanks well before any disease problems appeared in the river, to try to ensure that fish were exposed to the disease-causing stresses. As mentioned, UM may take several days to become grossly visible and we cannot predict exactly when a UM outbreak will occur.

It is also important to know ambient water quality conditions during the absence of a disease outbreak, since this can provide critical "baseline" data which is useful in ascertaining what combination of water quality conditions may lead to UM. However, we experienced some problems with stocking fish during parts of the year, especially when temperatures were very high or very low. Fortunately, these are times when UM is not prevalent (See DISCUSSION, Noga et al 1989a, and Levine et al 1990b). We also encountered problems with acclimating fish to tanks during Spring 1990, which prevented us from performing an experiment during this time period.

F. DATA ANALYSIS

The methods used for the water quality measurements were identical to those used during the past ten years in ongoing studies of water quality and nitrogen cycling in the Pamlico and Neuse River estuaries (Stanley 1987, 1988). Correlations were performed between UM incidence and summary water quality statistics for the periods preceding the fish examinations. Correlations among the environmental variables was also done. Overall correlations and correlations after normalizing for site-to-site variation was also done using standard procedures (Steel and Torrie 1980).

RESULTS

A. 1989 STUDIES

In Fall 1989, we set up tanks at three sites on the Pamlico River: Site A (Crystal Beach), Site B (South Creek at the Pamlico Aquaculture Center) and Site C (Pamlico River at the Texasgulf Phosphate Recreation Area)(Figure 1). One hundred and five fish (from a pool of about 600 fish) were stocked into each tank between 11 October and 13 October 89 (Table 1). Salinity, temperature, dissolved oxygen and pH were measured twice daily (sunrise and sunset). Dead fish were removed from each tank daily.

After about two weeks (on 28 October 89), we gently drew the water down on all 3 tanks and individually examined each fish by hand. All fish with UM were removed and recorded (Table 1). The remaining clinically healthy fish were returned to the tank. This procedure was repeated on 18 November 89. On 4 December 89, the experiment was terminated due to extreme cold weather which was killing fish in the tanks.

Thus, the experiment consisted of three tanks and three time periods with measurements at two times of day, A.M. and P.M.. There were ten potential explanatory variables: temperature, salinity, dissolved oxygen in the tank, dissolved oxygen in the river, pH, chlorophyll a, total ammonia nitrogen, nitrite, and the exponentials of pH and total ammonia nitrogen. For each period in each tank, a measurement of percent infection was the target variable of interest. Note that there are 9 values (3 periods x 3 tanks) of this variable.

During each period of the experiment, many measurements were made on the water characteristics (explanatory variables). We first computed four summary variables for each water characteristic (e.g., temperature), each period, each tank, and each time of day. These summary variables were the mean, standard deviation, maximum, and minimum value (Table 2). Thus, for example, we had nine A.M. and nine P.M. means of water temperature. In tank A for period 1 (11-28 October 89), we averaged all the A.M. readings and all the P.M. readings for water temperature and did the same for the other 8 tank and period combinations.

We then computed the correlations of the column of 9 infection percentages with each column of summary water measures. The correlations are listed in Table 2 with and next to them in parentheses are the p-values. Exponentiating the pH and total ammonia nitrogen values did not appear to make much difference, so this was omitted in the subsequent analyses.

FIGURE 1.

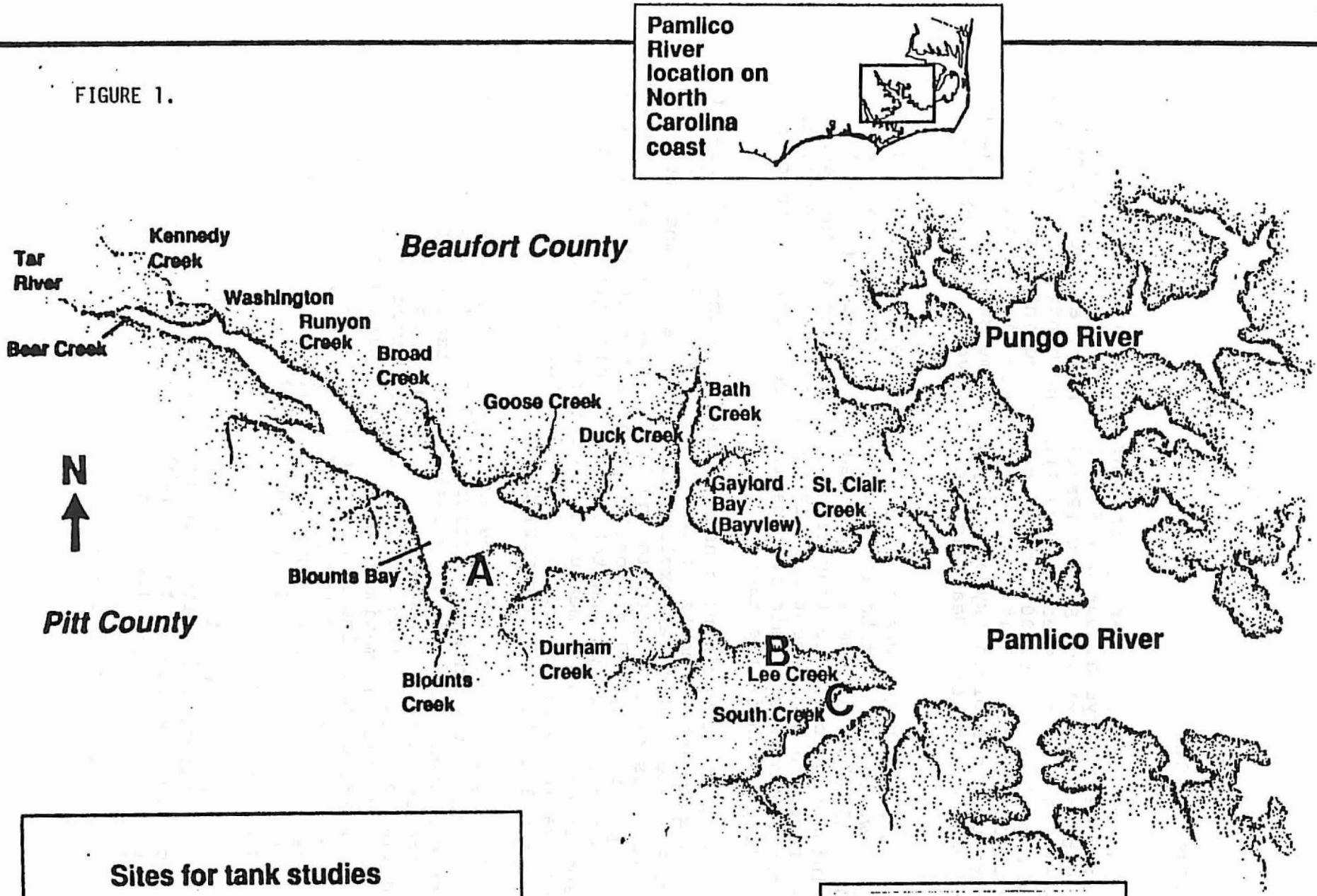

Pamlico River location on North Carolina coast
Beaufort County
Kennedy Creek
Tar River
Washington
Runyon Creek
Bear Creek
Broad Creek
Goose Creek
Duck Creek
Bath Creek
Pungo River
Gaylord Bay (Bayview)
St. Clair Creek
N
Blounts Bay
A
Pitt County
Durham Creek
B
Lee Creek
Blounts Creek
South Creek
C
Pamlico River
Sites for tank studies
0 2 4 6 8 10 12 14 16 18
Kilometers
Site A = Crystal Beach
Site B = South Creek
Site C = Texasgulf

Table 1. 1989 Experiment: Incidence of ulcerative mycosis in experimental tanks placed at various sites on the Albemarle-Pamlico Estuary.

	Number of Fish with UM/Total Number of Fish in Tank (Percentage in Parentheses)		
	Tank A	**Tank B**	**Tank C**
11-13 Oct 89	All Tanks Stocked with Fish		
28 Oct 89	13/103(13%)	24/100(24%)	26/103(25%)
18 Nov 89	14/88(16%)	23/76(30%)	35/76(46%)
4 Dec 89	3/74(4%)	7/52(13%)	5/36(14%)

From 100-103 fish were added to each tank on 11-13 October. All fish were removed from the tanks and examined for lesions on 28 October and 18 November 89. The experiment was terminated on 4 December 89.

Tank A = Crystal Beach, Tank B = Pamlico Aquaculture Center, Tank C = Texasgulf Recreation Center

The total number of fish tallied in each tank does not equal 100% since some fish died without any apparent lesions.

Table 2. 1989 Experiment: Correlation of UM infection rate with various water quality summary variables. (p-values are in parentheses)

SUMMARY VARIABLE READINGS
A.M.

	MEAN	STD DEV	MAX	MIN
TEMPERATURE	.56(.12)	-.44(.23)	.38(.31)	.48(.19)
SALINITY	.64(.06)	.37(.32)	.73(.03)	.51(.06)
TANK D.O.	-.26(.51)	-.62(.07)	-.41(.28)	-.20(.60)
RIVER D.O	-.25(.51)	-.16(.69)	-.28(.46)	-.48(.19)
pH	.24(.53)	.06(.88)	.26(.51)	.18(.64)
CHLOROPHYLL	.05(.90)	.08(.84)	.09(.81)	-.24(.53)
AMMONIUM	-.46(.22)	.05(.90)	-.29(.45)	-.67(.049)
NITRITE	.10(.81)	.20(.60)	.20(.61)	-.16(.68)
exp(pH)	.19(.62)	.13(.74)	.22(.57)	.14(.73)
exp (AMMONIUM)	-.50(.17)	-.50(.17)	-.50(.17)	-.52(.14)

SUMMARY VARIABLE READINGS
P.M.

	MEAN	STD DEV	MAX	MIN
TEMPERATURE	.49(.18)	-.25(.51)	.34(.38)	.23(.55)
SALINITY	.64(.06)	.46(.21)	.80(.01)	.60(.09)
TANK D.O.	-.03(.95)	-.14(.71)	-.12(.76)	.07(.85)
RIVER D.O.	-.14(.71)	-.33(.38)	-.43(.25)	-.14(.71)
pH	.30(.43)	.25(.51)	.36(.35)	.36(.34)
CHLOROPHYLL	.	.	.	.
AMMONIUM	.	.	.	.
NITRITE	.	.	.	.

Salinity seemed to have an effect through the mean and more so through the maximum. There was not much of an effect of salinity variability on infection rate within the range of variability observed here, since there was not any significant correlation between infection and the standard deviation of salinity. No other variable, with the possible exception of standard deviation of tank dissolved oxygen in the A.M., correlated well with infection percentage.

Next, we considered relationships among the water quality descriptive variables. There were several hundred of each of these. Table 3 shows correlations in these raw data values. All of these had p-values <0.0034. Removing tank means and correlating the resulting residuals showed how these minor correlations depended upon tank-to-tank variations (Table 4). Interestingly, only minor changes are noted except for salinity. Here we see substantial changes including sign reversals. All elements in Table 4 have p-values less than 0.03 except for the correlation of temperature with salinity ($p = 0.112$). Thus, although salinity and tank D.O. were positively correlated (0.21), when tank differences are ignored, within tanks they were in fact negatively correlated (-0.25).

Finally, we show a table of correlations adjusted for both tanks and time-of-day (Table 5). This adds little to the adjustment for tanks alone. The adjustment for tanks and time-of-day was done by running an analysis of variance (ANOVA) for each water quality characteristic with sources, tank, and time-of-day. The resulting F statistics are reported in Table 6. The previous correlation table (Table 5), then, consists of correlations on the residuals from this ANOVA. Again, it is of interest that salinity, the only variable most strongly correlated with UM incidence, did not depend on the time of day in a significant way.

One last analysis is reported. For each period, tank, and day, we have an A.M. and a P.M. measurement. From these, we created a sum (P.M. + A.M.) and a difference (P.M. - A.M.). Means for each of these created variables for each water quality characteristic were then computed for each tank and period. The previously computed standard deviations for these water quality characteristic variables (Table 2) showed no relationship to infection, but they involved both within-day variation and day-to-day variation. The mean difference between P.M. and A.M., however, reflects only effects due to the two measurements while the sum of represents an overall level (times 2) for each day. Correlations of the means of these created variables with infection rate are given in Table 7. Consistent with our other analyses, we see no correlation between the A.M. to P.M. spread of any water quality characteristic and the UM infection rate. Again, it seemed unnecessary to have both A.M. and P.M. readings at least at the times used. We again did see at least a

Table 3. 1989 Experiment: Correlations of water quality descriptive variables.

	TEMP	SALINITY	TANK D.O	RIVER D.O	pH	CHLOR	AMM	NITR
TEMP	1.00	0.06*	-0.38*	-0.43*	0.16*	0.37*	-0.36*	0.02
SALINITY	0.06*	1.00	0.21*	0.20*	0.36*	-0.30*	0.08	0.18
TANK D.O.	-0.38*	0.21*	1.00	0.88*	0.65*	-0.15	0.09	-0.06
RIVER D.O.	-0.43*	0.20*	0.88*	1.00	0.56*	-0.16	0.18	0.03
pH	0.16*	0.36*	0.65*	0.56*	1.00	-0.03	-0.17	0.02
CHLOR	0.37*	-0.30*	-0.15	-0.16	-0.03	1.00	-0.39	-0.11
AMMONIUM	-0.36*	-0.08	0.09	0.18	-0.17	-0.39	1.00	-0.15
NITRITE	0.02	0.18	-0.06	0.03	0.02	-0.11	-0.15	1.00

All elements with * had p-values < 0.0034.

Table 4. 1989 Experiment: Correlations of water quality descriptive variables normalized for tank effects.

	TEMP	SALINITY	TANK D.O	RIVER D.O	PH	CHLOR	AMM	NITR
TEMP	1.00	0.09	-0.41	-0.47	0.16	0.37*	-0.37*	0.03
SALINITY	0.09	1.00	-0.25	-0.28	0.18	-0.31	0.08	0.28
TANK D.O.	-0.41	-0.25	1.00	0.87	0.59	-0.08	0.17	-0.12
RIVER D.O.	-0.47	-0.28	0.87	1.00	0.48	-0.31*	0.26*	-0.01
pH	0.17	-0.18	0.59	0.48	1.00	0.37*	-0.10	-0.04
CHLOR	0.37*	-0.31*	-0.08	-0.10	0.10	1.00	-0.43*	-0.10
AMMONIUM	-0.37*	0.08	0.17	0.26*	-0.10	-0.43*	1.00	-0.14
NITRITE	0.03	0.28	-0.12	-0.01	-0.04	-0.10	-0.14	1.00

All elements had p-values < 0.03 except for correlation of temperature with salinity ($p = 0.122$).

Table 5. 1989 Experiment: Correlations of water quality characteristics normalized for tank and time-of-day effects.

	TEMP	SALINITY	TANK D.O	RIVER D.O	pH	CHLOR	AMM	NITR
TEMP	1.00	0.07	-0.62*	-0.65*	0.01	0.37*	-0.37*	0.03
SALINITY	0.07	1.00	-0.31*	-0.32*	-0.27	-0.31	0.08	0.28
TANK D.O.	-0.62*	-0.31*	1.00	0.84*	0.46*	-0.08	0.17	-0.12
RIVER D.O.	-0.65*	-0.32*	0.85*	1.00	0.37*	-0.31*	0.26*	-0.01
pH	0.01	-0.27	0.46*	0.37*	1.00	0.37*	-0.10	-0.04
CHLOR	0.37	-0.31*	-0.08	-0.10	0.10	1.00	-0.43*	0.10
AMMONIUM	-0.37*	0.08	0.17	0.26*	-0.10	-0.43*	1.00	-0.14
NITRITE	0.03	0.28	-0.12	-0.01	-0.04	-0.10	-0.14	1.00

* indicates significance with p-value < .05.

Table 6. 1989 Experiment: ANOVA of water quality descriptive variables using tank and time-of-day.

VARIABLE	TANK F-TEST (p-VALUE)	TIME-OF-DAY F-TEST (p-VALUE)
TEMPERATURE	1.73 (.1786)	29.51 (.0001)
SALINITY	662.32 (.0000)	1.17 (.2807)
TANK D.O.	29.90 (.0001)	76.32 (.0001)
RIVER D.O.	26.78 (.0001)	46.44 (.0001)
pH	68.63 (.0001)	153.41 (.0001)
CHLOROPHYLL	1.19 (.3100)	.
AMMONIUM	0.46 (.6351)	.
NITRITE	0.38 (.6826)	.

Table 7. 1989 Experiment: Correlations of the means of created mean and difference time-of-day variables.

VARIABLE	A.M. + P.M. (p-VALUE)	P.M. - A.M. (p-VALUE)
TEMPERATURE	.52 (.14)	-.51 (.16)
SALINITY	.64 (.06)	.04 (.91)
TANK D.O.	-.15 (.70)	.37 (.33)
RIVER D.O.	-.19 (.63)	.25 (.51)
pH	.31 (.42)	.29 (.46)

suggestion of correlation (F-value 0.06) between the overall level of salinity and UM incidence.

We also ran regressions of the dinoflagellate's gamete density numbers on a logarithmic scale. The logarithm of density seemed well-explained by a simple linear regression on salinity. The r-square was 27% and the model is:

$$\log(\text{DENSITY}) = 14.44003 - 0.302921*\text{SALINITY}$$

Quadratic terms added little improvement in fit, nor did temperature, pH and dissolved oxygen. A data plot showed that much of the difference in salinity was associated with sites. When we plotted the percentage infected fish for each of the three periods and three sites versus the average density count over the period, there was a clear negative association; that is, the cases with the higher toxic dinoflagellate gamete count had less UM incidence (Table 8).

B. 1990 STUDIES

We repeated the 1989 experiment in spring 1990. In addition to the same tanks and sites used in 1989, we expanded the geographic range of our study to include a very high salinity site, Morehead City. The absence of significant difference between A.M. and P.M. values (Table 2) prompted us to focus on water sampling only in the A.M.

The experiment consisted of two tanks at each of four sites and two time periods (Table 9). The potential explanatory variables were temperature, salinity, tank dissolved oxygen, river dissolved oxygen, pH, total ammonia nitrogen, nitrite, chlorophyll *a*, and toxic dinoflagellate densities. For each tank in each site, a measurement of the percent UM infection was the target variable of interest. Thus, there were 16 values (2 periods x 4 sites x 2 tanks) of this variable.

During each period of the experiment, many measurements were made on the water quality characteristics (explanatory variables). We first computed for each water quality characteristic, each time period, each site, and each tank, four summary variables; namely, the mean, standard deviation, maximum, and minimum value. Thus, for example, we had 16 means of water temperature.

We next computed correlations of the column of 16 UM infection percentages with each column of summary water quality measurements (Table 10). As in the 1989 experiment, salinity seemed to have an effect through the mean and more so through the maximum. There again did not appear to be much of an effect of

Table 8. 1989 Experiment: Mean Algae Density averaged by time periods.

OBS	TANK	TIME PERIOD	FRACTION INFECTED	MEAN ALGAE DENSITY (Gametes/L)
1	A	1	0.12	1117487
2	A	2	0.15	1367463
3	A	3	0.04	1187027
4	B	1	0.24	311708
5	B	2	0.30	543615
6	B	3	0.13	499034
7	C	1	0.25	457711
8	C	2	0.46	447711
9	C	3	0.13	1771219

Table 9. 1990 Experiment: Incidence of ulcerative mycosis in experimental tanks placed at various sites on the Albemarle-Pamlico Estuary.

	Number of Fish with UM/Total Number of Fish in Tank (Percentage in Parentheses)			
	Tank A	**Tank B**	**Tank C**	**Tank D**
7 Nov 90	All Tanks Stocked with Fish			
29 Nov 90				
Replicate 1	1/100(1%)	1/100(1%)	1/100(1%)	0/100(0%)
Replicate 2	3/100(3%)	2/100(2%)	3/100(3%)	0/100(0%)
12 Dec 90				
Replicate 1	0/100(0%)	1/100(1%)	0/100(0%)	0/86(0%)
Replicate 2	1/100(1%)	0/100(0%)	0/99(0%)	0/85(0%)

All tanks stocked with 100 fish on 7 Nov. All fish were removed from the tanks and examined for lesions on 29 November 90. The experiment was terminated on 12 December 90.

Tanks A1, A2 = Crystal Beach, Tanks B1, B2 = Pamlico Aquaculture Center, Tanks C1, C2 = Texasgulf Recreation Center, Tanks D1, D2 = Morehead City

The total number of fish tallied in each tank does not equal 100% since some fish died without any apparent lesions. A total of 29 fish were missing from the Morehead City tanks, possibly due to theft.

Table 10. 1990 Experiment: Correlation of UM infection rate with various water quality summary variables. p-values are in parentheses.

	SUMMARY VARIABLE READINGS			
	MEAN	STD DEV	MAX	MIN
TEMPERATURE	.23(.38)	.08(.76)	.58(.02)	.08(.77)
SALINITY	-.51(.04)	-.18(.50)	-.49(.054)	-.46(.07)
TANK D.O.	.17(.51)	.37(.16)	.30(.26)	-.30(.26)
RIVER D.O	.19(.49)	.42(.11)	.33(.21)	-.04(.87)
pH	-.48(.06)	.43(.09)	-.40(.12)	-.52(.04)
CHLOROPHYLL	-.11(.69)	-.11(.68)	-.06(.83)	-.07(.80)
AMMONIUM	-.09(.74)	-.20(.44)	-.08(.78)	-.14(.599)
NITRITE	-.20(.45)	.21(.44)	.19(.47)	-.39(.14)

salinity variability on UM infection rate, since there was not a significant correlation beteween UM infection and the standard deviation of salinity.

The minimum pH was significantly correlated with UM incidence and the mean pH was close to significance (F-value 0.06). Similar results were obtained when we exponentiated the pH values (to account for the logarithmic nature of pH). The negative sign means that low mean or minimum pH was associated with increased UM infection. Maximum temperature was significant, with a high maximum temperature associated with increased infection. High minimum values of toxic algal density were also associated with increased UM incidence.

We next considered relationships among the water quality descriptive variables. There are several hundred of these. In Table 11 are the correlations in these raw data values. Removing the site means and correlating the resulting residuals shows how these correlations depend upon tank-to-tank variations (Table 12). Several correlations change; for example, the unadjusted correlations between salinity and D.O. variables are significant, but within sites, the significance disappears. However, salinity is correlated with chlorophyll a within sites.

Finally, we show a table of correlations adjusted for both sites and tanks within sites. This adds little to the adjustment for sites alone (Table 13). The adjustment for sites and tanks was done by running an ANOVA for each water quality characteristic, with sources, site, and tank within site. The resulting F-statistics are reported in Table 14. The correlation table (Table 13), then, consists of correlations on the residuals from this ANOVA.

C. 1991 STUDIES

No UM lesions developed in any of the fish used in the Spring 1991 Experiment (Table 15), so we could not analyze for any relationships between UM incidence and water quality.

Table 11. 1990 Experiment: Correlations of water quality descriptive variables.

	TEMP	SALINITY	TANK D.O	RIVER D.O	PH	CHLOR	AMM	NITR
TEMP	1.00	0.24	-0.48*	-0.48*	0.06	-0.11	0.11	-0.50*
SALINITY	0.24*	1.00	-0.49	-0.66	0.73*	0.05	0.25*	-0.11
TANK D.O.	-0.48*	-0.49*	1.00	0.82*	0.05	0.25*	-0.39*	0.24*
RIVER D.O.	-0.48*	-0.66*	0.82*	1.00	-0.18*	0.28*	-0.39*	0.15
pH	0.06	0.73*	0.05	-0.18*	1.00	0.26*	-0.34*	0.06
CHLOR	-0.11	0.05	0.25*	0.28*	0.33*	1.00	-0.39*	-0.10
AMMONIUM	0.11	0.25*	-0.39*	-0.39*	0.03	-0.39*	1.00	0.30
NITRITE	-0.50*	-0.11	0.24*	0.15	-0.06	-0.10	0.30*	1.00

* indicates significance with p-value < 0.05.

Table 12. 1990 Experiment: Correlations of water quality descriptive variables normalized for tank effects.

	TEMP	SALINITY	TANK D.O	RIVER D.O	PH	CHLOR	AMM	NITR
TEMP	1.00	-0.07	-0.45*	-0.48*	-0.36*	-0.15	0.07	-0.51*
SALINITY	-0.07	1.00	-0.05	-0.03	0.01	0.25*	0.29*	-0.37*
TANK D.O.	-0.45*	-0.05	1.00	0.73*	0.80*	0.21	-0.33*	0.23
RIVER D.O.	-0.48*	-0.03	0.73*	1.00	0.65*	0.17	-0.27*	0.12
pH	-0.36*	0.01	0.80*	0.65*	1.00	0.26*	-0.34*	0.06
CHLOR	-0.15	0.25*	0.21	0.17	0.26*	1.00	-0.46*	-0.20
AMMONIUM	0.07	-0.29*	-0.33*	-0.27*	-0.34*	-0.46*	1.00	0.34*
NITRITE	-0.51*	-0.37*	0.23	0.12	0.06	-0.20	0.34*	1.00

* indicates significance with p-value < 0.05.

Table 13. 1990 Experiment: Correlations of water quality characteristics normalized for tank and time-of-day effects.

	TEMP	SALINITY	TANK D.O	RIVER D.O	pH	CHLOR	AMM	NITR
TEMP	1.00	-0.07	-0.45*	-0.48*	-0.36*	-0.16*	0.07	-0.51*
SALINITY	-0.07	1.00	-0.05	-0.03	0.01	0.25*	-0.30*	-0.37*
TANK D.O.	-0.45*	-0.05	1.00	0.73*	0.80*	0.22	-0.33*	0.22
RIVER D.O.	-0.48*	-0.03	0.73*	1.00	0.65*	0.17	-0.27*	0.12
pH	-0.36*	0.01	0.80*	0.65*	1.00	0.27*	-0.36*	0.06
CHLOR	-0.16	0.25*	0.22	0.17	0.27*	1.00	-0.45*	-0.20
AMMONIUM	0.07	-0.30*	-0.33*	-0.27*	-0.36*	-0.45*	1.00	-0.14
NITRITE	0.51*	-0.37*	0.22	0.12	0.06	-0.20	-0.14	1.00

* indicates significance with p-value < .05.

Table 14. 1990 Experiment: ANOVA of water quality descriptive variables using tank and time-of-day.

VARIABLE	SITE F-TEST (p-VALUE)	TANK (SITE) F-TEST (p-VALUE)
TEMPERATURE	11.61 (.0001)	1.51 (.9939)
SALINITY	1700.05 (.0001)	0.00 (1.000)
TANK D.O.	46.97 (.0001)	0.15 (.9616)
RIVER D.O.	103.92 (.0001)	0.02 (.9991)
pH	315.13 (.0001)	0.47 (.7551)
CHLOROPHYLL	4.14 (.0099)	0.63 (.6465)
AMMONIUM	2.18 (.0989)	0.57 (.6820)
NITRITE	0.48 (.6962)	0.14 (.6820)

Table 15. 1991 Experiment: Incidence of ulcerative mycosis in experimental tanks placed at various sites on the Albemarle-Pamlico Estuary.

	Number of Fish with UM/Total Number of Fish in Tank (Percentage in Parentheses)			
	Tank A	**Tank B**	**Tank C**	**Tank D**
5 April 91		All Tanks Stocked with Fish		
26 April 91				
Replicate 1	0/19(0%)	0/45(0%)	0/44(0%)	0/0(0%)
Replicate 2	0/16(0%)	0/46(0%)	0/43(0%)	0/0(0%)
17 May 91				
Replicate 1	0/19(0%)	0/45(0%)	0/40(0%)	0/0(0%)
Replicate 2	0/16(0%)	0/46(0%)	0/39(0%)	0/0(0%)
7 June 91				
Replicate 1	0/17(0%)	0/45(0%)	0/32(0%)	0/0(0%)
Replicate 2	0/16(0%)	0/46(0%)	0/32(0%)	0/0(0%)

All fish were removed from the tanks and examined for lesions on 26 April and 17 May 91. The experiment was terminated on 7 June 91.

Tanks A1, A2 = Crystal Beach, Tanks B1, B2 = Pamlico Aquaculture Center, Tanks C1, C2 = Texasgulf Recreation Center, Tanks D1, D2 = Morehead City

The total number of fish tallied in each tank does not equal 100% since some fish died without any apparent lesions. All fish transferred to the Morehead City tanks died within 2 days of placement in the tanks.

DISCUSSION

Finding the ultimate cause of ulcerative mycosis requires a multifaceted approach which includes determining the water quality conditions that are associated with disease development. Only after this data has been acquired can a rational plan be developed to prove or disprove the importance of certain water quality factors on disease development. The present studies were intended to provide a knowledge base for the logical formulation of those questions (Perry et al 1987).

It is important to realize that multiple factors are working together to increase susceptibility to UM. Thus, these studies are needed to provide information on not only what factors are important, but also how these parameters may interact to precipitate an outbreak. These studies also can help to rule out conditions that are not important to UM development.

Infectious diseases are not limited to the A/P Estuary, but are of increasing concern to fisheries managers both nationally (Hargis 1985) and internationally (Sindermann 1988). Yet, there is presently no strong evidence linking any infectious disease in wild fish populations to a specific pollutant or water quality factor. In large measure, this is due to the fact that virtually all previous studies have only examined one aspect of this problem (i.e., either only the disease or only water quality). Our tank system allows an integrated approach that may be useful to other fishery disease problems.

These experiments demonstrated that in situ tank culture can be useful for studying water quality factors associated with the development of UM because:

1) We demonstrated that menhaden could be maintained for long periods (at least 60 days) in these experimental systems.

2) Classical ulcerative mycosis lesions developed in the tanks. These lesions were observed during the entire duration of some experiments, indicating that water quality conditions from the estuarine water pumped into the tanks were probably not responsible for their development (i.e., the fish were not simply expressing an infection that had started when they were collected in the river).

3) Our repeated handling of the fish to examine them for lesions appeared to have negligible effect upon our experiments, as indicated, for example, by the low incidence of UM or other disease in Tank A (1989 Experiment) after this manipulation. Indeed, the decrease in disease in Tank A over time suggests that this experimental design is probably not very stressful to the fish. This was also indicated by the active feeding

and normal schooling behavior of the fish during the experiments.

4) There was a marked temporal difference in disease incidence in the culture systems. For example, in the 1989 experiment (Table 1), UM prevalence rose for the first 5 weeks of the experiment and then decreased for the remainder of the experiment.

5) There was a significant spatial difference in UM incidence from one site to another. These spatial differences were correlated with specific water quality factors:

The strongest relationship with the development of UM was salinity. In the 1989 experiment, there was a correlation of UM infection rate with mean salinity and maximum salinity. In Tank A, salinity ranged from 1.6 - 4.0 ppt (mean = 2.3 ppt). At Tanks B and C, it ranged from 4.7 - 7.5 (mean = 6.1 ppt) and 3.8 - 7.5 ppt (mean = 5.3 ppt) respectively. This suggests that fish may not be at the greatest risk of developing UM at very low salinities (i.e., less than about 2 ppt). That is, the highest salinities observed in this experiment (about 5-6 ppt) were most conducive to UM development. Interestingly, 4-8 ppt salinity is the optimum range for growth of Aphanomyces, the fungal pathogen in UM lesions (Dykstra et al 1986). In the 1990 experiment, there was a negative correlation of UM infection rate with mean salinity and maximum salinity. However, this was consistent with the 1989 data because in the 1990 experiment, a much broader and on average higher salinity range was present. In the 1990 experiment, the salinity ranged from 7.0 to 34.0 ppt (depending upon tank location). Again, a salinity of 7 ppt is within the optimum range for Aphanomyces growth (Dykstra et al 1986).

The correlation of greatest UM incidence with low to moderate salinity is consistent with our previous epidemiological evidence of the distribution of ulcerative mycosis in the Tar-Pamlico estuary (Levine et al 1990b) where UM was very prevalent in the upstream areas of the estuary. As mentioned, an oomycete fungal isolate from a UM lesion was also shown to have a maximum growth and reproductive optimum at 4-8 ppt salinity (Dykstra et al 1986).

Maximum temperature during the fall-winter 1990 experiment was also associated with increased UM incidence; temperature during the 1990 experiment ranged from a high of 17.3 C to a low of 5.4 C. In the 1989 experiment, there was no correlation between maximum temperature and incidence of UM, but UM incidence displayed a bimodal response (Table 1), with peak incidence occurring during the middle of the experiment. The lowest UM incidence was seen at the end of the experiment, when temperatures were lowest. Ulcerative mycosis is a distinctively seasonal disease, with typically a spring and a fall peak (Levine

et al 1990a,b). Decreasing presence of disease with the onset of winter has also been seen in natural outbreaks of UM (Noga et al 1989a).

There was also a suggestion of higher tank dissolved oxygen variability being associated with UM incidence (1989 Experiment), although this was not statistically significant. Rapid changes in water quality are a serious threat to fish homeostasis and health (Wedemeyer et al 1976).

The minimum pH was also significant and the mean pH was also close to significance, indicating that both low mean and low minimum pH were associated with increased UM incidence (1990 Experiment). Stress due to low pH is a recognized cause of morbidity and mortality in fish (Wedemeyer et al 1976, Wedemeyer and Goodyear 1984). However, whether this level of pH was stressful for menhaden is speculative, especially since the lowest pH recorded during this experiment was only 6.69. However, lower pH is also conducive to the proliferation of fungi (Neisch and Hughes 1980), which could facilitate the invasiveness of the oomycete pathogens of UM.

There was no discernible correlation of UM incidence with total ammonia nitrogen, nitrite, or chlorophyll a. Ammonia and nitrite are toxins which commonly affect fish health while chlorophyll a gives an indication of phytoplankton biomass. While nitrite's toxicity is inhibited by the presence of chloride in freshwater (Tomasso et al 1979), there is virtually no information on the effect of nitrite on marine and estuarine fishes. Thus, whether similar methods of protection from nitrite intoxication are operative in menhaden is unknown; for this reason, we felt that it was important to investigate nitrite's possible effect on UM development.

The menhaden used in these experiments were certainly not specific-pathogen-free fish but were obtained from the Albemarle-Pamlico estuary, specifically the Pamlico River. No matter where they may have been obtained, since they were feral fish, they certainly could have been exposed to various pathogens prior to their use. To reduce the potential effects of this variable, we collected all fish to be stocked into all tanks from the same geographic site for any one experiment. The fish may have been exposed to the agents responsible for UM, but it is highly doubtful if even cultured fish reared in the laboratory would be free of the potential pathogens, since the infcetious agents associated with UM are ubiquitous agents that are fully capable of a free-living existence (i.e., they can exist in mud, water, etc. and don't need a fish host to survive). Based upon the relatively low prevalence of UM which we observed in our experiments, we feel that it was appropriate to use menhaden, since this species is most susceptible to UM. Also, the similar trends in disease incidence and/or UM risk factors identified

both within experiments (e.g., 1989 Experiment) and between experiments (e.g., 1989 and 1990 Experiments) at various sites suggest that latent infections did not have a large influence on the outcomes.

The lack of disease development in the tanks in 1991 is puzzling. We observed some UM-affected fish in the Pamlico River while this experiment was in progress, so the disease was certainly present in the estuary at the time fish were in the tanks. One possible explanation is that we may have missed placing fish in the tanks during the water quality events that lead to UM development. Epidemics of UM have at times developed very quickly and also subsided very quickly.

Not all fish died of ulcerative mycosis; the cause of these non-UM fish mortalities is unknown. In some cases, mortalities appeared to be due to our inability to acclimate the fish to certain tanks. For example, in the 1991 experiment, all fish placed into the Morehead City tanks (Tanks D1, D2) were dead within two days of placement in the tanks. We also experienced significant early losses at Crystal Beach (Tanks A1, A2), where the salinity was often 1.5 or less. Salinity at the other two sites was at least 5.4 at all times. A large loss of fish in Tanks D1 and D2 in the 1990 experiment appeared to be due to theft. There were other mortalities, which were relatively small, but unexplained. Necropsy examination of these fish failed to reveal any parasites or bacterial agents as the cause of death. We cannot rule out that other, undetected infectious agents may have been responsible (e.g., viral infection). The epidemiological characteristics of the mortalities (a few, episodic losses that varied from time to time) also suggest that some type of sublethal water quality factor may have been stressing the fish, resulting in the occasional loss of a few of the weaker fish in the population. We have recently identified a new toxic dinoflagellate as being responsible for a number of kills of estuarine fish in the Albemarle-Pamlico Estuary (Burkholder et al 1992). Its importance in large-scale acute mortalities most certainly suggests that it can also cause less extensive morbidity and mortality, since we are only assessing the effects of this toxic organism under bloom conditions when we examine its role in fish kills. Thus, the effect on fish of low levels of its toxic stage that may produce sublethal concentrations of toxin should be investigated. The toxic dinoflagellate is certainly very common in the Albemarle-Pamlico Estuary (see below).

The only possible relationship that we discerned between the toxic dinoflagellate's abundance and UM infection was the correlation between the minimum dinoflagellate numbers and UM incidence in 1990. This might be explained by the need for a minimum number of this dinoflagellate to be present to affect the fish. However, this relationship was not supported by the 1989

experiment, where there was a negative correlation between dinoflagellate density and UM incidence. This lack of consistent findings calls into question the significance of the dinoflagellate densities and UM incidence. One possible explanation for this finding may be the fact that we only evaluated the gamete stage of the toxic dinoflagellate, which does not appear to be toxic (Burkholder, Unpublished data). Clearly, more studies are needed before any conclusions can be made about this possible relationship.

However, one striking feature of this data is the extremely high numbers of gametes throughout all areas of the A/P Estuary that we sampled (Table 8). Even in the Morehead City tanks (1990 Experiment), the gametes ranged from 202,737 to 7,260,535 cells/liter. We cannot yet be unequivocally certain that every dinoflagellate cell that we counted was our recently described organism, but the morphological criteria that we used for distinguishing these cells were very conservative and based upon considerable experience in observing this organism in culture.

It is important to realize that water quality refers to all the environmental conditions (including temperature, oxygen, pH, salinity, etc.) that influence the health of a fish population. As such, the environment (i.e., water quality) affects the prevalence of virtually all infectious diseases in fishery populations. Thus, it is not a question of if, but when and which certain water quality factors are proven to affect the development of UM. Anthropogenic influences may or may not be involved and these influences may be direct (i.e., a toxin, such as copper, directly immunosuppressing the fish) or indirect (nutrient enrichment from fertilizers changing algal composition, which then leads to oxygen depletion, increased prevalence of ichthyotoxic algae, etc.).

In order to definitively determine the possible importance of anthropogenic changes, more controlled studies need to be performed to ascertain the mechanism(s) responsible for the increased predilection of Atlantic menhaden to develop UM in the riverine areas of the Albemarle-Pamlico Estuary. This should include exposure of clinically healthy menhaden or other fish species to the possible environmental risk factors that have been identified in this project in order to experimentally confirm our findings that menhaden exposed to riverine water are more likely to develop ulcerative mycosis. Fish should be challenged with the microbial agents present in UM lesions under the environmental conditions that we have identified as "high risk" and "low risk" in the present study. Such studies should be conducted in conjunction with examination of how water quality affects the pathogenicity of various microbial agents, especially the fungal and bacterial components. Since some potentially deleterious anthropogenic agents (heavy metals, herbicides, pesticides) are known to be released into the A/P Estuary,

similar studies should also examine the importance of these agents. Once putative water quality factors have been proven to be responsible for the disease, management decisions about how and whether or not to abrogate these water quality changes affecting the Albemarle-Pamlico Estuary might then be made.

In summary, water quality variables which are associated with UM development should be tested under more controlled conditions. The ultimate goal should be to provide managers with the major water quality factors that are responsible for the development of this disease. With this information and a knowledge of the anthropogenic factors affecting those water quality conditions, administrative decisions can then be made to alleviate the problem.

SUMMARY AND CONCLUSIONS

The major conclusions from this study are as follows:

1) In situ tank culture can be useful for studying water quality factors associated with the development of UM because:

   a) We demonstrated that menhaden could be maintained for at least 60 days in these experimental systems.

   b) Classical ulcerative mycosis lesions developed in the tanks.

   c) Our repeated handling of the fish to examine them for lesions, as well as the overall cuture system itself, appeared to have negligible effect upon UM development.

   d) There was a marked temporal difference in disease incidence in the culture systems.

   e) There was a significant spatial difference in UM incidence in the culture systems.

2) There was a correlation of salinity with the development of UM, with low to moderate salinity (about 5-7 ppt) being optimal.

3) There was a fall peak in UM incidence that occurred with decreasing water temperature. However, as further time passed and temperatures continued to decline, UM incidence also declined.

4) There was some evidence that UM development was associated with fluctuations in dissolved oxygen, as well as with lower pH.

5) There was no correlation of UM incidence with total ammonia nitrogen, nitrite, chlorophyll *a*, or the density of non-toxic gametes of a toxic dinoflagellate that is prevalent in the Albemarle-Pamlico Estuary.

RECOMMENDATIONS

These studies have shown that Atlantic menhaden have an increased risk of developing ulcerative mycosis in areas of low salinity and during the fall season. There was also evidence for a possible relationship of UM development with fluctuations in dissolved oxygen, as well as low pH. Monitoring studies which extend the technology developed in this project, should continue to collect information on the prevalence of ulcerative mycosis and associated water quality factors in various parts of the A/P Estuary in order to substantiate the site-related differences in disease development that have been identified in the present study.

It is important to realize that water quality refers to all the environmental conditions (including temperature, oxygen, pH, salinity, etc.) that influence the health of a fish population. As such, the environment (i.e., water quality) affects the prevalence of virtually all infectious diseases in fishery populations. Thus, it is not a question of if, but when and which certain water quality factors are proven to affect the development of UM. Anthropogenic influences may or may not be involved and these influences may be direct (i.e., a toxin, such as copper, directly immunosuppressing the fish) or indirect (nutrient enrichment from fertilizers changing algal composition, which then leads to oxygen depletion, increased prevalence of ichthyotoxic algae, etc.).

In order to definitively determine the possible importance of anthropogenic changes, more controlled studies need to be performed to ascertain the mechanism(s) responsible for the increased predilection of Atlantic menhaden to develop UM in the riverine areas of the Albemarle-Pamlico Estuary. Studies should include exposure of clinically healthy menhaden or other fish species to the possible environmental risk factors that have been identified in this project in order to confirm experimentally our findings that menhaden exposed to riverine water are more likely to develop ulcerative mycosis. Specific pathogen-free fish should be challenged in closed system aquaria with the microbial agents present in UM lesions under the environmental conditions that we have identified in the present study as "high risk" (e.g., 5-6 ppt salinity, water temperatures of - C, low pH, and fluctuations in dissolved oxygen). Such studies should be conducted in conjunction with examination of how water quality affects the pathogenicity of various microbial agents, especially the fungal and bacterial components. Since some potentially deleterious anthropogenic agents (heavy metals, herbicides, pesticides) are known to be released into the A/P Estuary, similar studies should also examine the importance of these agents. Once putative water quality factors have been proven to be responsible for the disease, management decisions about how

and whether or not to abrogate these water quality changes affecting the Albemarle-Pamlico Estuary might then be made.

REFERENCES

Burkholder, J.M., E.J. Noga, C. Hobbs and H. Glasgow, Jr. 1992. New 'phantom' dinoflagellate is the causative agent of major estuarine fish kills. Nature 358: 407-410.

Dykstra, M.J., E.J. Noga, J.F. Levine, D.W. Moye, and J.H. Hawkins. 1986. Characterization of the Aphanomyces species involved with ulcerative mycosis (UM) in menhaden. Mycologia 78:664-672.

Dykstra, M.J., J.F. Levine, E.J. Noga, J.H. Hawkins, P. Gerdes, W.J. Hargis, Jr., H.J. Grier and D. TeStrake. 1989. Ulcerative mycosis: A serious menhaden disease of the southeast coastal fisheries of the United States. J. Fish Dis. 12: 175-178.

Ellis, A.E. 1981. Stress and the modulation of defense mechanisms in fish. In: Stress and Fish, A.D. Pickering (Editor). Academic Press, New York, pp. 147-170.

Environmental Protection Agency. 1979. Methods for chemical analysis of water and wastes. EPA-600/4-79-020.

Esch, G.W. and T.C. Hazen. 1980. The ecology of Aeromonas hydrophila in Albemarle Sound, North Carolina. University of North Carolina Water Resources Research Institute Final Report # 80-153, 116 p.

Hargis, W.J. 1985. Quantitative effects of marine diseases on fish and shellfish populations. Trans N. Am. Wild. and Nat. Res. Conf. 50: 608-640.

Hobbie, J.E. 1974. Nutrients and eutrophication in the Pamlico River estuary, North Carolina. University of North Carolina Water Resources Research Institute, Report No. 100, Raleigh. 239p.

Levine, J.F., J.H. Hawkins, M.J. Dykstra, E.J. Noga, D.W. Moye and R.S. Cone. (1990a). Species distribution of ulcerative lesions on finfish in the Tar-Pamlico River Estuary, North Carolina. Dis. Aquat. Org. 8: 1-5.

Levine, J.F., J.H. Hawkins, M.J. Dykstra, E.J. Noga, D.W. Moye and R.S. Cone. (1990b). Epidemiology of ulcerative mycosis in Atlantic menhaden in the Tar-Pamlico River Estuary, North Carolina. J. Aquatic Anim. Health 2: 162-171.

Neish, G.A. and G.C. Hughes. 1980. Fungal diseases of Fishes. TFH Publications, Neptune, NJ.

Noga, E.J. 1986. The importance of Lernaea cruciata in the initiation of skin lesions in largemouth bass in the Chown River, North Carolina. J. Fish Diseases 9:295-302.

Noga, E.J. 1988. Determining the relationship between water quality and infectious disease in fishery populations. Water Resources Bulletin 24: 967-973.

Noga, E.J. and M.J. Dykstra. 1986. Oomycete fungi associated with ulcerative mycosis in Atlantic menhaden. J. Fish Diseases 9:47-53.

Noga, E.J., J.F. Levine, M.J. Dykstra, and J.H. Hawkins. 1988. Pathology of ulcerative mycosis in Atlantic menhaden Brevoortia tyrannus. Dis. Aquat. Org. 4: 189-197.

Noga, E.J., J.F. Levine and M.J. Dykstra. 1989a. Fish diseases of the Albemarle-Pamlico estuary. University of North Carolina Water Resources Research Institute Final Report # UNC-WRRI-89-238.

Noga, E.J., H.A. Berkhoff and N.J. Markwardt. 1989b. Water quality and the health of estuarine fishes. Report of Contract #88-0670A, North Carolina Division of Marine Fisheries.

Noga, E.J., J.F. Wright, J.F. Levine, M.J. Dykstra and J.H. Hawkins. 1991. Dermatological diseases affecting fishes of the Tar-Pamlico Estuary, North Carolina. Dis. Aquatic Org. 10: 87-92.

Perry, J.A., D.D. Schaeffer, and E.E. Herricks. 1987. Innovative designs for water quality monitoring: Are we asking the questions before the data are collected? In: New Approaches to Managing Aquatic Ecosystems, T.P. Boyle (Editor), American Society for Testing and Materials, Philadelphia, Pennsylvania, pp. 28-39.

Plumb, J.A. 1984. Relationship of water quality and infectious diseases in cultured channel catfish. Symposia Biologica Hungarica 23:189-199.

Rader, D.N., L.F. Loftin, B.A. McGee, J.P. Dorney and J.T. Clements. 1987. Surface water quality concerns in the Tar-Pamlico River estuary. Water Quality Technical Report No. 87-04, N.C. Division of Environmental Management. 113 p.

Sindermann, C.J. 1988. Epizootic ulcerative syndromes in coastal/estuarine fish. NOAA Technical Memorandum NMFS-F/NEC-54, 37 p.

Smith, S.A., E.J. Noga and R.A. Bullis. 1988. Mortality in

Tilapia aurea due to a toxic dinoflagellate bloom. Proc. Third Int. Coll. Pathol. Marine Aquacult., pp. 167-168.

Stanley, D.W. 1987. Water quality in the Pamlico River estuary, 1986. Institute for Coastal and Marine Resources Technical Report No. 87-01. East Carolina University, Greenville, NC. 77p.

Stanley, D.W. 1988. Water quality in the Pamlico River estuary: 1967-1986. Institute for Coastal and Marine Resources Technical Report No. 88-01. East Carolina University, Greenville, NC. 199p.

Steel, R.G.D. and J.H. Torrie. 1980. Principles and procedures of statistics. 2nd Ed., McGraw-Hill, New York, 633 p.

Tomasso, J.R., B.A. Simco and K.B. Davis. 1979. Chloride inhibition of nitrite-induced methemoglobinemia in channel catfish (Ictalurus punctatus). J. Fish. Res. Bd. Can. 36: 1141-1144.

Wedemeyer, G.A. and C.P. Goodyear. 1984. Diseases caused by environmental stressors. In: Diseases of Marine Animals, Vol. IV, Part I (Pisces), O. Kinne (Editor), Biologische Anstalt Helgoland, Hamburg, FRG, pp. 424-434.

Wedemeyer, G.A., F.P. Meyer, and L. Smith. 1976. Environmental Stress and Fish Diseases. TFH Public., Neptune City, NJ, 192 p.