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Title of Thesis: "Description of *Fundulus heteroclitus* Ventilatory Data and Water Quality Parameters: A Feasibility Study for Predicting Toxic *Pfiesteria piscacida* and *P. piscicida*-like Events in Estuarine Environments"

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Description of *Fundulus heteroclitus* Ventilatory Data and Water Quality Parameters: A Feasibility Study for Predicting Toxic *Pfiesteria piscicida* and *P. piscicida*-like Events in Estuarine Environments

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ABSTRACT

Title of Thesis: "Description of *Fundulus heteroclitus* Ventilatory Data and Water Quality Parameters: A Feasibility Study for Predicting Toxic *Pfiesteria piscicida* and *P. piscicida*-like Events in Estuarine Environments"

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Master of Science in Public Health, 2000

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Fundulus heteroclitus ventilatory data and water quality data were collected from the Transquaking River of Maryland's Eastern Shore during a non-*Pfiesteria piscicida* event. It is anticipated that a fish ventilatory model reflecting association with water quality parameters can be used as a surveillance strategy in predicting future *P. piscicida* and *P. piscicida*-like events. Four *F. heteroclitus* yielding 1866 observations of ventilation rate, average ventilation depth, cough rate, and physical movement; and water quality parameters consisting of 1866 measurements of temperature, pH, conductivity, and dissolved oxygen were

utilized in this study. When ventilatory data was analyzed according to each individual fish, ventilatory variables showed no significant trend or correlation with any of the water quality parameters that was consistent among all four *F. heteroclitus*. Periods of water flow reduction and stoppage into the mobile Automated Fish Biomonitoring System laboratory likely resulted in increased variability among the data.

DESCRIPTION OF *FUNDULUS HETEROCLITUS* VENTILATORY DATA AND WATER QUALITY PARAMETERS: A FEASIBILITY STUDY FOR PREDICTING TOXIC *PFIESTERIA PISCICIDA* AND *P. PISCICIDA*-LIKE EVENTS IN ESTUARINE ENVIRONMENTS

By

CPT TIMOTHY A. KLUCHINSKY, JR

Thesis submitted to the Faculty of the Department of Preventive Medicine Graduate Program of the Uniformed Services University of the Health Sciences in partial fulfillment of the requirement for the Degree of Master of Science in Public Health, 2000

PREFACE

This study was conducted in collaboration with the United States Army Center for Environmental Health Research (USACEHR), the United States Environmental Protection Agency (USEPA), Johns Hopkins University Applied Physics Laboratory, and Geo-centers Incorporated as part of the Environmental Monitoring for Public Access and Community Tracking (EMPACT) USEPA Project Plan "Real-time Monitoring for Toxicity Caused by Harmful Algal Blooms and other Water Quality Perturbations" directed by William Van der Schalie. This thesis study was designed to determine the feasibility of using *Fundulus heteroclitus* ventilatory patterns to predict toxic *Pfiesteria piscicida* events, utilizing the Automated Fish Biomonitoring System.

DEDICATION

To my wife, Aunika, and my children, Timothy Jacob and Shawn Anthony, for the sacrifices you have made during these last two years of study, I dedicate this thesis to you.

To my mother for the strength, courage, and inspiration you have given me in attaining yet another goal.

To my father and my brother Ricky, thank you for the encouragement and assistance you provided me in meeting the challenges associated with collecting data on Maryland's Eastern Shore.

ACKNOWLEDGMENT

I am grateful to the United States Center for Environmental Health Research (USCEHR), especially United States Army Colonel David L. Danley, Dr. William Van der Schalie, Mr. Tom Shedd, and Mr. Mark Widder for the assistance, guidance, and support you provided me in making this study possible. Your commitment to meeting the environmental challenges associated with military public health is remarkable.

I would like to thank all the members of my committee for their time, patience, and confidence, especially my advisor and committee chairperson LTC Arthur P. Lee who mentored me in being not only a student and a soldier, but a leader as well. Sir I wish you and your family well in your retirement from military service.

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CHAPTER ONE: INTRODUCTION

Statement of Problem

In the Fall of 1996, watermen who had been working along the lower Eastern Shore of Chesapeake Bay began to observe fish with lesions exhibiting unusual swimming patterns such as swimming upside down in circles. Shortly thereafter, some watermen reported symptoms of fatigue, skin irritation, and prospective memory loss.[1] These symptoms subsided with the coming of the winter months when the watermen were less exposed to the water. More fish with lesions and more human health concerns were observed during the following spring. It is believed that the fish kills were associated with the organism *Pfiesteria piscicida* entering a toxic stage of its complicated 24 stage life cycle. Although the organism *P. piscicida* has been identified, its toxic mechanism has not. Scientists are uncertain as to how to predict fish kills believed to be caused by *P. piscicida*. One strategy, as described in this thesis, is to use fish as a sentinel species. If a model were to be developed incorporating fish response variables and water quality parameters, it could be used as a screening tool capable of identifying toxic-*P. piscicida* conditions that could result in fish kill events.

Successful estuarine fish biomonitoring in a natural setting, as performed in this study, has proven in the past to be extremely challenging due to water quality degradation and data variability. The Automated Fish Biomonitoring System designed by the United States Army Center for Environmental Health Research, coupled with the signal enhancement strategies of Geo-Centers Incorporated, has given scientists an opportunity to overcome these challenges.

Background

On 19 July 1999, the United States Army Center for Environmental Health Research deployed the mobile Automated Fish Biomonitoring System laboratory to a Department of Natural Resources boat launch. The boat launch was located in Bestpitch, a small rural town along the Transquaking River of Maryland's Eastern Shore.

The intent of the deployment was to assess the mobile Automated Fish Biomonitoring System laboratory's capability of simultaneously monitoring ventilation rate, average ventilation depth, cough rate, and physical movement of *Lepomis macrochirus*, while in an estuarine environment. *L. macrochirus*, commonly known as the bluegill, has been studied extensively by researchers of the United States Army Center for Environmental Health Research located at Fort Detrick, Maryland. Also evaluated was the mobile Automated Fish Biomonitoring System laboratory's hydrolab capability of providing average measurements of temperature, pH, conductivity, and dissolved oxygen over fifteen minute intervals.

The deployment, Phase I of the two phase Environmental Monitoring for Public Access and Community Tracking (EMPACT) USEPA Project Plan titled

"Real-time Monitoring for Toxicity Caused by Harmful Algal Blooms and other Water Quality Perturbations" provided for the data collection and analysis used in this thesis.

Conditions brought about by extreme climatic conditions such as drought, Hurricane Floyd, high salinity, low dissolved oxygen, and excessive boat launch activity due to duck hunting seasons and commercial crabbing, caused unforseen challenges. These challenges, all of which could not be immediately overcome, proved to be lethal to the survivability of the *L. macrochirus*. Signals produced by the *L. macrochirus* were monitored; however, *L. macrochirus* survivability lasted only a couple of days instead of several weeks as shown in similar laboratory conditions. *L. macrochirus* mortality was probably a result of poor water flow through the Automated Fish Biomonitoring System, low dissolved oxygen, and changes in salinity. The unexpected mortality prevented adequate acclimatization and baseline data collection prior to the planned two week monitoring period. An alternative species of fish capable of being biomonitored was then selected.

Research Questions

The specific aims of this research were to: (1) collect data describing the association between *Fundulus heteroclitus* ventilatory response values (ventilation rate, average ventilation depth, cough rate, physical activity) and water quality parameters (pH, temperature, conductivity, dissolved oxygen), (2) determine the association between the *F. heteroclitus* ventilatory response variables and water quality parameters, and (3) narrow the focus of how toxic *P. piscicida* events may be predicted.

Definition of Terms

Automated Fish Biomonitoring System (AFBS) - a system developed by the U.S. Army Center for Environmental Health Research to identify developing toxic conditions in water by continuously monitoring the ventilation and movement patterns of selected fish species.[2]

Mobile Automated Fish Biomonitoring System Laboratory - the Automated Fish Biomonitoring System, fish holding tanks, water holding tanks, and hydrolab mounted inside a trailer.

"Dancing cat fever" - a condition developed by domestic cats in Japan during the 1950's. It was determined to have been caused by high mercury concentrations that had bioconcentrated in fish and shellfish of Minamata Bay. Effluent, discharged from a nearby industrial facility that had been using mercuric chloride in the production of vinyl chloride, was found to be the source of the contamination.[3]

Environmental Monitoring for Public Access and Community Tracking (EMPACT) - a four-year program enacted by Presidential initiative. The goal of the program is to make easily understood, time-relevant environmental information available to residents of 86 of the largest metropolitan areas of the

United States, so that people can make informed day-to-day decisions about their lives.[4]

Hydrolab® - a commercial water quality measuring device capable of continuously measuring temperature, pH, dissolved oxygen and conductivity.

Pfiesteria piscicida - a single celled dinoflagellate that is approximately 7 microns wide and found along the east coast of the United States. It appears to use a toxin to stun fish which it then feeds on. The organism is believed to be associated with human health concerns involving exposure to estuarine waters exhibiting fish kills.

Estuary - a coastal area at the mouth of a river where fresh water mixes with salty sea water.[5]

Reverse Osmosis - a water purification process which separates water from salt and other contaminants. When the natural osmotic flow through a media is reversed by pressure, minerals are separated from the water.[6]

Animal sentinel - any non-human organism that can react to an environmental contaminant before the contaminant impacts humans.[7]

National Priorities List (NPL) - a list of uncontrolled or abandoned hazardous waste sites, identified for possible long-term remedial actions under Superfund, that present a significant risk to public health or the environment. The NPL list consists of over 1200 sites and has been updated three times a year since its initiation in 1982.[8]

Superfund - a common name for the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). The law was adopted "to provide for liability, compensation, cleanup and emergency response for hazardous substances released into the environment and the cleanup of inactive hazardous waste disposal sites."[8]

Research Goal

The goal of this research is to develop a surveillance mechanism capable of predicting toxic *P. piscicida* events. Modeling fish ventilatory patterns and their association with water quality parameters in the absence of a toxic event as compared to during an event may provide an effective prediction mechanism. The fish ventilation rate, average ventilation depth, cough rate, and physical movement of four *F. heteroclitus* specimens were measured by utilizing the Automated Fish Biomonitoring System. The associated changes in water quality parameters of temperature, pH, conductivity, and dissolved oxygen were also measured.

The Automated Fish Biomonitoring System is currently used to monitor groundwater treatment at a 4.5 acre hazardous waste and ordinance disposal site in the Edgewood Area of Aberdeen Proving Grounds in Maryland. The Automated Fish Biomonitoring System utilizes the ventilatory and movement patterns of the *L. macrochirus* to identify potentially toxic effluent. The system has operated continuously at Aberdeen Proving Grounds since 23 June 1995.[2]

Limitations of Study

Research limitations related to the *L. macrochirus*, as discussed in the background section of the introduction, forced the biomonitoring of fish species other then *L. macrochirus*. *F. heteroclitus*, commonly called the Mummichog, was chosen as the species of interest due to its interesting and unique salinity adaptation capabilities.

Water flow into the mobile Automated Fish Biomonitoring System laboratory appeared to be the major factor responsible for the data variability between each of the *F. heteroclitus* observed. A discontinuance of flow, even for a few minutes, produced fluctuations of ventilatory patterns. Reduced water flow may impact one *F. heteroclitus* or several *F. heteroclitus* depending on the location of the blockage. Variability may be reduced by installing an instrument capable of alerting the mobile Automated Fish Biomonitoring System laboratory operators should reduced flow into the chambers containing the *F. heteroclitus* occur.

The mobile Automated Fish Biomonitoring System laboratory was located between the boat launch and Fishing Bay. The location made the system susceptible to boating activity which resulted in sediment uptake and water flow reduction.

Data from four *F. heteroclitus* were analyzed for this feasibility study. Conclusions drawn from these four specimens may not be generalizable to *F.*

heteroclitus as a species.

Redeployment, due to Hurricane Floyd, inhibited additional study of *F. heteroclitus* at the Transquaking River site. Additional observations may have strengthened the correlation among variables.

Climate and precipitation was variable during the study period. Drought, followed by flooding due to Hurricane Floyd, enabled measurement of water quality data during extremely different climatic periods. It is possible that reduced water flow masked any significant correlation that may have existed between ventilatory data and water quality data.

Toxic *P. piscicida* events resulting in any fish kills or fish with lesions did not occur during this study. Data collected is assumed to be representative of non-toxic *P. piscicida* conditions and should only be generalized to data collected on the Transquaking River under similar conditions.

The toxins associated with *P. piscicida* are assumed to exist and be associated with water quality, but have yet to be fully characterized.

Assuming any *F. heteroclitus* mortality was caused by water flow stoppage, ventilatory data analysis excluded ventilatory and water quality data when a rapid decrease in ventilation rate was accompanied by water flow stoppage.

Stress may have been placed on the *F. heteroclitus* due to maintaining them without food, in small chambers, and in constant light. However, feeding the *F. heteroclitus* and exposing them to light when entering and exiting the mobile

Automated Fish Biomonitoring System laboratory would have elicited a ventilatory response.

The United States Army Center for Environmental Health Research, using the mobile Automated Fish Biomonitoring System laboratory, has studied the sensitivity of the *L. macrochirus* ventilatory response to a variety of chemicals with various levels of toxicity to determine the "time to response" at a range of acutely toxic concentrations. Similar toxicity studies using *F. heteroclitus* have not been performed.

CHAPTER TWO: LITERATURE REVIEW

Sentinel Species

An animal sentinel is any non-human organism that can react to an environmental contaminant before the contaminant has had some impact on human health.[7] Animal sentinels have long been used as indicators of potential human health hazards. Miners in England during the 1870's took canaries into the mines to warn of potentially lethal levels of carbon monoxide.[9] In Japan, during the 1950's, a neurological disorder in cats was caused by their consumption of methyl mercury contaminated fish and shellfish from Minamata Bay. The methyl mercury affected the central nervous system and caused the cats to act out a dancing-like behavior commonly referred to as "dancing cat fever." The outcome in cats identified a possible human health hazard associated with human exposure to methyl mercury.

There are advantages to using animal sentinels. They represent an early warning system capable of indicating potential human health concerns. Some animals have shorter disease latency periods while others require smaller levels of exposure to develop an outcome. Companion animals, such as dogs, have also been used as animal sentinels for characterizing human exposure patterns.

Sentinel species should not be used as absolute indicators alone. With the current levels of understanding, they can best be used as a screening tool to indicate potential human health hazards warranting further investigation.

Fundulus heteroclitus

The species chosen for this study was *F. heteroclitus* because it is found in the areas that have been commonly contaminated by toxic *P. piscicida* and because of its interesting and unique salinity adaption capabilities. *Brevoortia tyrannus*, commonly known as Atlantic Menhaden, have been extensively studied. Lesions develop on *B. tyrannus* when exposed to the toxins of *P. piscicida*. Little is known of the relationship of the toxin to *F. heteroclitus*.[10]

The *F. heteroclitus* is a small, bony, estuarine fish found along the east coast from Florida to the Gulf of St. Lawrence. It spawns during the high tides by placing its eggs in places such as mussel shells and leaves which provide shelter until the next high spring tide two weeks later. At that time, the high tides provide the water levels that the eggs need for hatching. The *F. heteroclitus* maintains a diverse diet, commonly consuming diatoms, amphipods, mollusks, crustaceans, small fish, fish eggs, and sea grass.[11]

F. heteroclitus are affected by polluted waters in an interesting manner. *F. heteroclitus* exposed to polluted waters from embryonic stage through adulthood have shorter life spans than their controls reared in non-polluted water. *F. heteroclitus* reared in polluted water were found to be faster feeders and better predator-avoiders during their first year of life than the controls; however, these characteristics reversed after the first month of life.[12]

F. heteroclitus have been used as an indicator species in the identification of dioxin-contaminated[13] and creosote-contaminated sites, and has been found to have an increased prevalence of neoplasms suspected of being attributed to creosote's chemical carcinogens.[14] The Atlantic Wood Industries, Inc. wood treatment facility in Portsmouth, Virginia is an Environmental Protection Agency-listed Superfund National Priority List site. Research performed there showed that 93% of the *F. heteroclitus* exposed to high concentrations of polycyclic aromatic hydrocarbons expressed pathological liver disorders. One third of the *F. heteroclitus* taken from the same site demonstrated hepatocellular carcinomas, a prevalence of hepatocellular carcinoma never before seen in any species at a polycyclic aromatic hydrocarbon-contaminated site.[15]

Feeding habits of *F. heteroclitus* vary with their size and level of water salinity. Larger *F. heteroclitus* were found to live longer and consume more food than smaller ones at salinity levels of freshwater; however, smaller *F. heteroclitus* survived longer and consumed more food at high salinity levels than larger ones. *F. heteroclitus* were found to be intolerant of salinity levels greater than 85 parts per thousand.[16]

P. piscicida is predominately found in estuaries which typically have salinity levels of 17 parts per thousand or less; [17] whereas, *F. heteroclitus* have illustrated a unique ability to osmoregulate at varying salinity levels. *F. heteroclitus* are capable of rapidly adapting to saltwater four times the salinity of freshwater [18] and can tolerate water temperatures up to 34 degrees Celsius at a salinity concentration of 14 parts per thousand.[11] They are one of the most stationary species of fish, possessing a home range of only 35 meters.[19] These characteristics support the hypothesis that *F. heteroclitus* have the potential to serve as an indicator species for toxic *P. piscicida* outbreaks in spite of intense temperature, salinity, and home range changes brought about by tidal influences.

Pfiesteria piscicida

P. piscicida is a toxic dinoflagellate that has been shown to be a causative agent of estuarine fish mortality incidents in the Chesapeake Bay of Maryland.[20] During the late summer of 1997, 32 waterman exposed to waterways of fish kill events suspected to have been caused by toxic *P. piscicida*, expressed signs and symptoms indicative of memory difficulties, headache, skin lesions, and skin burning.[21] In 1995, laboratory workers at the University of North Carolina State Laboratory who were exposed to a volatilized form of the toxin while performing laboratory duties, developed symptoms consistent with the apparent effects of exposure to toxic *P. piscicida*.[21] The organism, discovered by Dr. Joanne M. Burkholder and fellow North Carolina State researchers in 1988, has the ability to assume a toxic life cycle stage under appropriate environmental water conditions including optimal salinity, temperature, and nutrient load.[22] The effects of the toxic stage have been documented since 1993. Last year (1999) there were no known fish kills associated with toxic *P. piscicida* outbreaks in the Chesapeake Bay or its tributaries .[23]

Currently, there is no surveillance mechanism for predicting a toxic *P. piscicida* event because the harmful toxin has yet to be isolated. The only evidence that the event has occurred is the presence of large numbers of dead fish, some of which have lesions.[24] Park Rublee of the University of North Carolina, Goldsboro, has developed a test for determining the presence of *P. piscicida*; however, whether or not they are in the toxic stage can only be determined by a method developed by Dr. Burkholder's lab.[25] The method involves placing laboratory-reared fish into waters suspected of containing the toxic *P. piscicida* form. If the toxic form of *P. piscicida* is present in the optimal quantity, the fish will usually die within an hour.

Dr. Burkholder announced at a Pentagon briefing that the toxic form may not be effectively removed by reverse osmosis filtration. Her studies show that the fish still die within two hours after exposing them to previously determined toxic *P. piscicida*-containing water treated by a reverse osmosis filtration.[26]

<u>Water Quality</u>

Water quality, in the context of this thesis, are the water conditions that include temperature, pH, conductivity (salinity), and dissolved oxygen. Toxic *P. piscicida* events have favored conditions where the water quality has demonstrated a salinity of 6 parts per thousand, water temperature of 25 degrees Celsius, high nutrient load of nitrogen and phosphorus, poor water flow, and the presence of large schools of *B. tyrannus*.[27] The presence of large numbers of *B.*

tyrannus found among other species of fish during assumed toxic *P. piscicida*-caused fish kill events suggests an association between *B. tyrannus* and *P. piscicida*.[21]

CHAPTER THREE: METHODS

Automated Fish Biomonitoring System

The Automated Fish Biomonitoring System developed by the United States Army Center for Environmental Research to identify developing toxic conditions in water by continuously monitoring the ventilation and movement patterns of fish, was utilized to collect data for this research. The biomonitoring system was designed to provide an early warning of environmental contamination brought about by a release of a toxic effluent. The biomonitoring system measures fish physiological stress by quantifying fish ventilation and movement patterns associated with changing water quality parameters and developing acutely toxic conditions. The use of continuous fish biomonitoring was recognized by state and federal regulators and accepted as a remediation monitoring strategy.[28]

Site Selection

The Automated Fish Biomonitoring System was installed in a mobile trailer and transported to the Department of Natural Resources Boat launch located in the Fishing Bay Wildlife Management Area on the Eastern Shore of Maryland. The trailer was located 5 meters from the boat launch on the Transquaking River, just downstream of the conjunction of the Transquaking and Chicamacomico Rivers. The site was chosen in collaboration with the Maryland Pfiesteria Study Team due to the historical presence of *P. piscicida*, presence of fish with lesions in 1997 and 1998, and fish kill event that resulted in the closure of the Chicamacomico River in September 1997.[29] The seven-person study team, formed at the request of the Maryland Department of Health and Mental Hygiene, consists of human health experts from the University of Maryland and Johns Hopkins medical schools.[1]

Data Collection

F. heteroclitus specimens were obtained by seining in the immediate vicinity of the trailer location on the Transquaking River and acclimated in the mobile Automated Fish Biomonitoring System laboratory fish holding tanks for four days while under continuous wide spectrum flourescent light. The *F. heteroclitus* were held in constant light and not fed while being monitored by the Automated Fish Biomonitoring System because that would likely initiate a ventilatory response. The ventilatory biomonitoring data of four *F. heteroclitus'* collected from 4-15 September 1999 are presented in this thesis.

The fish parameters measured included ventilation rate, average ventilation depth, cough rate, and physical movement. These parameters were measured by placing two carbon electrodes within each chamber containing a fish. A change of signal was generated when a fish ventilated, coughed, or moved. This signal was amplified and filtered by a customized Dataforth 5B Analog Voltage Imput Modual high-pass low-pass differential amplifier and managed by a DOS-based computer system that used the Automated Fish Biomonitoring System software. The result of this process was a 1000x magnification of the filtered signal.

The Automated Fish Biomonitoring System monitored each fish parameter every 15 seconds. These values were averaged over each 15 minute interval and compared to the water quality parameters which were also measured every fifteen seconds and averaged over 15 minute intervals. This was done to determine trends in fish ventilatory and water quality parameters during the same time intervals.

Water from the Transquaking River was pumped up to the mobile Automated Fish Biomonitoring System laboratory using a five horsepower centrifugal pump at a flow rate of two gallons per minute from an intake located ten meters from the shoreline of the Department of Natural Resources Boat Launch. The pumped water flowed into the mobile Automated Fish Biomonitoring System laboratory, then diverted equally to eight chambers. Four of the chambers contained one *F. heteroclitus* each, while the other four contained fish of another species; thus, allowing simultaneous data collection of *L. macrochirus* (bluegill), *Marone americanus* (white perch), and *B. tyrannus* (menhaden) species. A portion of the Transquaking River water entering the mobile Automated Fish Biomonitoring System laboratory was shunted through a Hydrolab water quality analyzer to measure temperature, pH, conductivity, and dissolved oxygen every fifteen seconds.[30] The Hydrolab water quality analyzer's precision was verified daily with a calibrated handheld water analyzer near the mobile Automated Fish Biomonitoring System laboratory's intake.

Statistical Methods

Univariate statistics were generated for each of the four *F. heteroclitus* in order to compare individual fish response variables to changes in the water quality variables. Since this ecological study involved measurement of individuals of a population group and their individual characteristics, data for the four *F. heteroclitus* were sorted and examined by each individual fish for final analysis.

CHAPTER FOUR: DATA ANALYSIS

Study Variables and Parameters

A total of four *F. heteroclitus* were collectively analyzed in the study. Each of the fish survived different lengths of time; thus, each had a different number of fifteen-minute interval observations of the ventilatory variables: ventilation rate, average ventilation depth, cough rate, and physical movement (Fish 1 = 363 observations; Fish 2 = 912 observations; Fish 3 = 247 observations; Fish 4 = 344 observations). Coupled with each ventilatory observation was a fifteen-minute-interval measurement of the temperature, pH, dissolved oxygen, and conductivity of the water flowing through the Automated Fish Biomonitoring System. Fish ventilatory variables consisting of ventilation rate, average ventilation depth, cough rate, and physical activity are assumed to be biologically related to each other and in violation of the independence assumption needed to utilize a linear regression model. Because of this limitation, data reflecting descriptive statistics of the ventilatory variables were generated and are depicted in Table 1. Results of the water quality parameters are found in Table 2.

Correlation of Variables and Parameters

Fish data were sorted and analyzed individually to evaluate correlation among the ventilatory variables (ventilation rate, average ventilation depth, cough rate, and physical movement) and the water quality variables (temperature, pH, conductivity, and dissolved oxygen). Pearson correlation analysis was used to determine the strength of the relationship between any two variables at a significant level of $p < 0.05$. Correlation among two variables implies a co-relationship; however, correlation does not distinguish them by referring to one as the dependent and the other as the independent variable. The objective was to merely obtain a measure of the strength of the relationship between the two variables. If an attempt were made to build a model or obtain an equation describing the relationship between the two variables, it would be invalid because each intra-fish observation is not independent of the observation measured before or after it.[31]

Pearson correlation values greater than $r = 0.80$ or less than $r = -0.80$ were representative of a strong correlation. Values from $r = 0.50$ to $r = 0.79$ and $r = -0.50$ to $r = -0.79$, were referred to as correlated. Values between $r = 0.50$ and $r = -0.50$ were termed weakly correlated.

Tables 3 to 6 illustrate that fish 1,2,3, and 4 exhibited strong correlation between pH and dissolved oxygen ($r = 0.95$, 0.91, 0.95, and 0.95). Table 3 shows correlation between Fish 1's ventilation rate and physical movement ($r = 0.60$), temperature ($r = 0.58$), pH ($r = -0.63$), and dissolved oxygen ($r = -0.68$), and a correlation between temperature and pH ($r = -0.57$), conductivity ($r = 0.56$), and dissolved oxygen ($r = -0.64$). Fish 2 data, found in Table 4, showed a correlation between ventilation rate and pH ($r = -0.58$) and dissolved oxygen ($r = -0.65$). Additionally, temperature was correlated with pH

(r = -0.51). Fish 3 data can be found in Table 5 and depicted a correlation between physical movement and ventilation rate (r = 0.57) and cough rate (r = 0.54). Fish 4 data, found in Table 6, resulted in a correlation between ventilation rate and average ventilation depth (r = 0.51). Temperature was again correlated with pH (r = -0.52), conductivity (r = 0.51), and dissolved oxygen (r = -0.60).

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

Conclusions

The only trend that was consistent among all four fish was the correlation exhibited between the two water quality parameters pH and dissolved oxygen. Because this trend is independent of any ventilatory variable, it tells little of how *F. heteroclitus* ventilatory data can be used to predict toxic algal blooms such as that consistent with *P. piscicida*.

The limitations discussed in the introduction section of this study show why this study is indeed what it's title reads--a "feasibility study." Using the ventilation rates of *F. heteroclitus* as measured with the Mobile Automated Fish Biomonitoring System laboratory to predict future *P. piscicida* and *P. piscicida*-like events may be a feasible surveillance strategy; however, statistical model building encompassing precisely measured variables under controlled conditions and improved ventilatory signal generation should be examined for future study.

Recommendations

A statistical model using repeated measures or time series analysis should be constructed to display the relationship of *F. heteroclitus* ventilatory data and water quality parameters in a non-*P. piscicida* setting. A similar data collection and model building effort should be attained to reflect present time monitoring during a toxic *P. piscicida* event. This would allow a crude comparison of *F. heteroclitus* ventilatory rates and predictor variables in the absence and presence of a toxic *P. piscicida* event.

Much work is needed to determine the associations of ventilatory data and water quality parameters. The mobile Automated Fish Biomonitoring System laboratory must be upgraded to ensure precise measurement capabilities involving challenging environmental conditions. Accurate instruments used to monitor the variables are being employed; however, estuarine dynamics pose many challenges in attempting to mimic estuarine conditions inside the mobile Automated Fish Biomonitoring System laboratory.

Water flow appears to be the cause of much of the variability seen in the data. A constant flow of water into the mobile Automated Fish Biomonitoring System laboratory is crucial to the success of using the system.

Future data collection should involve additional variables such as tidal influence and nutrient loads. Oceanic tides bring about changes in salinity, temperature, pH, dissolved oxygen, and nutrients while mixing with the rivers and streams to form estuaries.

Moving the mobile Automated Fish Biomonitoring System laboratory upstream would minimize several issues causing variability in the data. Moving upstream would result in less boating activity, lower salinity, less tidal influence, and higher dissolved oxygen levels. The conditions would favor *L. macrochirus* as the sentinel species rather than the *F. heteroclitus*. Unlike the *L. macrochirus*, the *F. heteroclitus* generates a variable low amplitude ventilatory signal. *F.*

heteroclitus is also more active than the *L. macrochirus* while in the chamber. This activity, recorded as physical movement, results in less specificity when measuring ventilation rate and can be minimized if *L. macrochirus* is used instead of *F. heteroclitus*.

Table 1. Ventilatory data *of Fundulus heteroclitus* in water from the Transquaking River, Maryland (4-11 September 1999) as measured by the Automated Fish Biomonitoring System.

Fish	Variable	Observations	Mean	Std Dev	Minimum	Maximum	95% LCL	95% UCL
1	VR	363	105.2	14.4	57.1	153.7	103.7	106.7
	AD	363	7.6	2.1	3.9	14.3	6.1	9.1
	CR	363	8.4	4.0	1.5	19.4	6.9	9.9
	PM	363	13.4	20.3	0.0	97.0	11.9	14.9
2	VR	912	94.1	37.3	22.1	187.3	91.7	96.5
	AD	912	3.4	2.4	6.8	16.5	3.2	3.6
	CR	912	2.4	1.6	18.8	9.3	2.3	2.5
	PM	912	5.1	10.3	0.9	91.7	4.4	5.8
3	VR	247	101.3	19.2	52.3	149.2	98.9	103.7
	AD	247	6.7	2.1	1.2	13.9	6.4	7.0
	CR	247	6.2	2.7	0.5	18.1	5.9	6.5
	PM	247	5.3	11.1	0.0	75.0	3.9	6.7
4	VR	344	69.2	17.5	27.9	113.1	67.4	71.0
	AD	344	4.1	3.2	1.3	16.3	3.8	4.4
	CR	344	4.2	2.3	0.1	11.3	4.0	4.4
	PM	344	0.7	1.8	0.0	15.0	0.5	0.9
Total	VR	1866	92.6	31.1	22.1	187.3	91.2	94.0
	AD	1866	4.8	3.0	1.2	13.9	4.7	4.9
	CR	1866	4.4	3.4	0.1	19.4	4.2	4.6
	PM	1866	6.0	12.8	0.0	97.0	5.4	6.6

[1]VR = ventilation rate measured in ventilations per minute
[2]AD = average ventilation depth measured in volts
[3]CR = coughs per minute
[4]PM = percentage of whole body movement averaged over each 15 minute interval

Table 2. Water quality data from the Transquaking River, Maryland (4-11 September 1999) as measured by the Automated Fish Biomonitoring System's Hydrolab.

Parameter	Observations	Mean	Std Dev	Minimum	Maximum	95% LCL	95% UCL
TEMP	1866	24.8	1.3	22.13	27.59	24.7	24.9
PH	1866	7.1	0.2	6.8	7.6	7.1	7.1
COND	1866	24.2	1.5	18.8	27	24.1	24.3
DO	1866	4.3	1.6	0.9	7	4.2	4.4

[1]TEMP = temperature measured in degrees Celsius
[2]PH = concentration of hydronium ions
[3]COND = conductivity measured in millisiemen per centimeter
[4]DO = dissolved oxygen measured in milligrams per liter

Table 3. Pearson's correlation coefficient for Fish 1.

variable	VR	AD	CR	PM	TEMP	PH	COND	DO
VR	1.00	0.15	-0.35	0.60	0.58	-0.63	0.36	-0.68
AD	0.15	1.00	NS[1]	0.26	-0.16	NS	-0.26	0.12
CR	-0.35	NS	1.00	0.14	-0.11	0.27	-0.16	0.27
PM	0.60	0.26	0.14	1.00	0.43	-0.37	0.26	-0.42
TEMP	0.58	-0.16	-0.11	0.43	1.00	-0.57	0.56	-0.64
PH	-0.63	NS	0.27	-0.37	-0.57	1.00	-0.23	0.95
COND	0.36	-0.26	-0.16	0.26	0.56	-0.23	1.00	-0.38
DO	-0.68	0.12	0.27	-0.42	-0.64	0.95	-0.38	1.00

[1]NS = not significant at $p < 0.05$

Table 4. Pearson's correlation coefficient for Fish 2.

variable	VR	AD	CR	PM	TEMP	PH	COND	DO
VR	1.00	0.48	-0.09	0.42	0.37	-0.58	-0.14	-0.65
AD	0.48	1.00	-0.24	0.36	NS[1]	-0.20	NS	-0.24
CR	-0.09	-0.24	1.00	0.16	0.27	NS	0.11	0.19
PM	0.42	0.36	0.16	1.00	0.19	-0.16	NS	-0.12
TEMP	0.37	NS	0.27	0.19	1.00	-0.51	0.31	-0.37
PH	-0.58	-0.20	NS	-0.16	-0.51	1.00	0.14	0.91
COND	-0.14	NS	0.11	NS	0.31	0.14	1.00	0.22
DO	-0.65	-0.24	0.19	-0.12	-0.37	0.91	0.22	1.00

[1]NS = not significant at $p < 0.05$

Table 5. Pearson's correlation coefficient for Fish 3.

variable	VR	AD	CR	PM	TEMP	PH	COND	DO
VR	1.00	NS[1]	NS	0.57	NS	-0.19	NS	-0.27
AD	NS	1.00	-0.17	NS	0.18	-0.41	NS	-0.33
CR	NS	-0.17	1.00	0.54	-0.18	-0.13	-0.16	NS
PM	0.57	NS	0.54	1.00	-0.23	-0.13	NS	-0.14
TEMP	NS	0.18	-0.18	-0.23	1.00	-0.25	0.26	-0.31
PH	-0.19	-0.41	-0.13	-0.13	-0.25	1.00	0.31	0.95
COND	NS	NS	-0.16	NS	0.26	0.31	1.00	0.23
DO	-0.27	-0.33	NS	-0.14	-0.31	0.95	0.23	1.00

[1]NS = not significant at $p < 0.05$

Table 6. Pearson's correlation coefficient for Fish 4.

variable	VR	AD	CR	PM	TEMP	PH	COND	DO
VR	1.00	0.51	0.46	0.34	0.23	-0.17	NS[1]	-0.26
AD	0.51	1.00	-0.17	0.15	0.32	-0.27	0.28	-0.37
CR	0.46	-0.17	1.00	0.39	NS	0.22	-0.14	0.27
PM	0.34	0.15	0.39	1.00	0.16	NS	NS	NS
TEMP	0.23	0.32	NS	0.16	1.00	-0.52	0.51	-0.60
PH	-0.17	-0.27	0.22	NS	-0.52	1.00	-0.14	0.95
COND	NS	0.28	-0.14	NS	0.51	-0.14	1.00	-0.28
DO	-0.26	-0.37	0.27	NS	-0.60	0.95	-0.28	1.00

[1]NS = not significant at $p < 0.05$

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