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VIRGINIA RECREATIONAL FISHING DEVELOPMENT FUND
SUMMARY PROJECT APPLICATION*

NAME AND ADDRESS OF APPLICANT:	PROJECT LEADER (name, phone, e-mail):
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PRIORITY AREA OF CONCERN:	**PROJECT LOCATION:**
Research	Virginia Institute of Marine Science

DESCRIPTIVE TITLE OF PROJECT:

Laboratory investigations of the ability of striped bass to function under low ambient oxygen conditions

PROJECT SUMMARY:

Large areas of water with low dissolved oxygen are now regularly observed during summer months in Chesapeake Bay. These "hypoxic zones" are of concern due to their potential to cause large scale fish and shellfish mortalities, as well as their significant sub-lethal effects on Bay life. The striped bass (*Morone saxatilis*) is an important finfish species that is thought to be adversely affected by seasonal hypoxia. It has been hypothesized that hypoxia in deep water areas forces striped bass to occupy warmer and shallower suboptimal habitats during the summer. This "thermal-oxygen squeeze" is thought to lead to fish stress, and may possibly be linked to emaciation and disease now commonly observed in wild striped bass in Chesapeake Bay. We therefore propose to study the physiological effects of hypoxia on striped bass, and to create models that will allow links between hydrological conditions and fish behavior.

EXPECTED BENEFITS:

Our results will allow better understanding and prediction of the effects of hypoxia on striped bass. This will enable managers to determine the seasonal extent of usable habitat for striped bass, as well as the degree to which this is impacted by hypoxic conditions. Additionally, we will examine the possibly synergistic effects between hypoxia and disease (mycobacteriosis) in order to gain a more robust understanding of how hypoxia may be affecting the striped bass population as a whole. Our study will be of future value to planned field research that will directly examine the movement patterns of striped bass with respect to hydrological conditions (e.g., hypoxia). Our research will provide information on the use/avoidance of hypoxic areas by striped bass and allow the development of guidelines to assist fishers in avoiding capture of diseased and/or stressed fish. This maybe particularly important if the laboratory portion of the project shows that fish in hypoxic areas are less tolerant of the stresses associated with capture-and-release.

COSTS:

We are requesting salary for a post-doctoral scientist to conduct the experiments. All the requisite fish husbandry equipment, surgical tools, and physiological instrumentation are available. We are therefore requesting funds only for acquisition and transport of approximately 30 live striped bass to VIMS holding facilities, animal feed, equipment maintenance, disposable surgical and histological supplies, chemicals, and anesthetics.

VMRC Funding:	$ 81,468
Recipient Funding:	$ 34,486
Total Costs:	**$115,954**

Detailed budget must be included with proposal.

Updated 6/1/05

***This form alone does not constitute a complete application, see application instructions or contact Sonya Davis at 757-247-8155 or sonya.davis@mrc.virginia.gov : Due dates are June 15 (Jul. – Nov. Cycle) and December 15 (Jan. – May Cycle)**

Proposal submission to

THE RECREATIONAL FISHING ADVISORY BOARD

VIRGINIA MARINE RESOURCES COMMISSOIN

By

THE VIRGINIA INSTITUTE OF MARINE SCIENCE

COLLEGE OF WILLIAM AND MARY

Laboratory investigations of the ability of striped bass to function under low ambient oxygen conditions

Dr. Richard Brill
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Dr. David Gauthier
Co-Principal Investigator

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Ms. Jane A. Lopez
Director of Sponsored Programs

Dr. Roger Mann
Director of Research and Advisory Services

December 2006

BUDGET

Laboratory investigations of the ability of striped bass to function under low ambient oxygen conditions

Brill and Gauthier

BUDGET

Personnel	RFAB request	NMFS/NOAA contribution	VIMS contribution
Post doctoral research scientist	40,000	0	0
R. Brill 15% time	0	16,500	0
D. Gauthier 10% time	4,750	0	0
Fringe, 30% salaries	13,425	4,950	0
Supplies			
acquisition and transport of 30 live striped bass to VIMS holding facility	2,000	0	0
animal feed, holding & maintenance	2,000	0	0
chemicals, anesthetics, and disposable surgical supplies	2,000	0	0
Histological supplies	1,000	0	0
Subtotal	65,175	21,450	0
Facilities & Administrative Costs*	16,293	0	13,036
Total	81,468	21,450	13,036

Facilities & Administrative Costs: The VIMS institutionally approved rate is 45%, however, F&A costs for VMRC requests are limited to 25%. The remaining costs are contributed as matching funds for this project.

Laboratory investigations of the ability of striped bass to function under low ambient oxygen conditions

Introduction

Aquatic habitats in the Chesapeake Bay are changing in major ways not previously observed. The effects of such changes on fish populations are unknown, but the ability of managers to sustain healthy fisheries in Virginia will depend on understanding the consequences of aquatic habitat alteration on fish populations. During the last 50 years, nutrient inputs and subsequent eutrophication have resulted in significantly reduced dissolved oxygen concentrations in the deeper portions of the Bay (Kemp et al. 2005). In recent years, increases in the geographic extent and volume of hypoxic "dead zones" (< 2 mg O_2 L^{-1}) appear to be adversely impacting the value of estuarine habitats (Eby and Crowder 2002; Adams et al. 2003; Breitburg et al. 2003; Kemp et al. 2005). Periods of hypoxia in the Bay and its tributaries typically occur in mid-summer, with the greatest volume of hypoxic water occurring in July (Figure 1A). During these times, suitable habitat for many of the Bay's fishes decreases.

One of the species likely to be most affected by hypoxic waters is the striped bass (*Morone saxatilis*). This anadromous fish is one of three dominant piscivores in the Chesapeake Bay and fills a critical ecological niche in estuarine food webs (Hartman & Brandt 1995). The striped bass also forms the basis for highly valued recreational and commercial fisheries. Although the abundance of striped bass is currently high, concerns have developed about the condition of individual fish. Capture of emaciated striped bass with ulcerative skin lesions is becoming commonplace in Chesapeake Bay and mycobacteriosis, a chronic bacterial disease, is found with high prevalence (Overton et al. 2003; Hartman and Margraf 2003; Rhodes et al. 2004). Recent tag-based assessments of adult striped bass in the upper Chesapeake Bay show an approximate 20% increase in natural mortality rates since 1999 (Jiang 2005). The coincident increase in natural mortality rates and prevalence of mycobacteriosis has led scientists to hypothesize a possible relationship, and the magnitude of disease-associated mortality in the population is currently under study.

One hypothesis for the observed decrease in condition indices and increase of disease in striped bass is the loss of so-called "thermal refuges." These are areas of deep water in the tributaries and mainstem of Chesapeake Bay which remain relatively cool during summer months. These deep water areas are now often showing low levels of dissolved oxygen during summer months (Figure 1B), and thus are potentially no longer available as fish habitat. It is thought that loss of thermal refuges leads to an annual "temperature-oxygen squeeze", whereby non-migratory striped bass are forced to "choose" between low oxygen in cool deep water or high temperatures in shallower water. Adult striped bass are thought to prefer temperatures below 25°C (Coutant 1985), and the higher temperatures typical of shallow areas of Bay tributaries during the summer may cause significant stress. The combination of elevated temperature and low ambient oxygen may be involved in decreasing the fitness of striped bass in Chesapeake Bay, possibly leading to increased mortality due to disease or other stressors.

(A) (B)

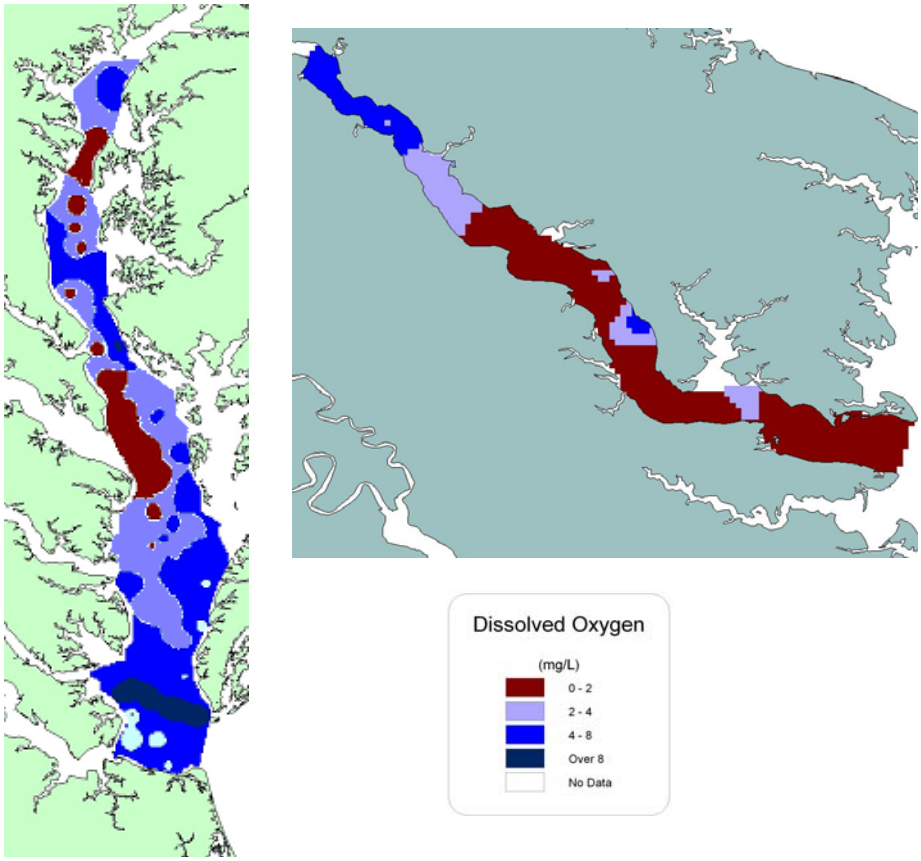

Figure 1. Dissolved oxygen concentration (mg/L) in bottom waters of the Chesapeake Bay (A) and Rappahannock River (B) in July 2005. Data are from the ChesMMAP survey (A) and the VIMS trawl survey (B).

Current Research Needs

Various efforts are currently underway to improve water quality in Chesapeake Bay, and measures of overall Bay health have been devised. Our proposed studies have the potential to provide an additional measure of Bay health because establishing the cutoff levels at which water conditions become inhospitable to striped bass remains a critical question. Establishment of accurate tolerance limits of striped bass for hypoxia would give managers and policy makers information as to the suitability of Chesapeake Bay as habitat for striped bass, as well as how "at risk" various areas are for becoming unsuitable.

The occurrence of seasonal hypoxia (low dissolved oxygen) in large portions of the Chesapeake Bay mainstem and tributaries is well known. This hypoxia has been hypothesized by several researchers to force striped bass to occupy suboptimal habitat, thus increasing stress and decreasing fitness. While a plausible hypothesis, little solid information currently exists regarding this question. For example, the lower tolerable levels of hypoxia are not well known for striped bass, and physiological responses to lowered (but not yet exclusionary) levels of oxygen are poorly understood. Therefore, it is difficult at this point to assess the degree to which striped bass are excluded from hypoxic habitat, as well as the negative physiological

consequences of being unable to escape from hypoxic waters. Understanding the extent to which hypoxia negatively affects striped bass physiologically, and their ability to function under these conditions, is extremely urgent as the extent of hypoxic zones in Chesapeake Bay is likely to increase in the future. As striped bass use the Bay as critical habitat and spawning grounds, the entire population may be adversely affected. Such a link between environmental perturbation (hypoxia) and negative effects on the striped bass stock would have important and direct implications for management efforts.

One of the major goals of our study is to establish baseline data for the physiological response of healthy striped bass to hypoxic conditions. Because of the high prevalence (>50%) of visceral disease (mycobacteriosis) in striped bass of Chesapeake Bay, it is also necessary that we explore the physiological responses of diseased fish. Specifically, we believe study of both healthy and diseased striped bass is important because we strongly suspect that the cardio-respiratory function of the latter may be compromised due to damage to the skin and internal organs.

Objectives

1. Quantify the functional characteristics of the cardio-respiratory system of striped bass under both full oxygen saturation and graded levels of hypoxia. This will provide information as to the levels of hypoxia that wild striped bass in Chesapeake Bay are capable of tolerating.

2. Develop mathematical models describing striped bass cardio-respiratory function under normoxia and hypoxia. These models will allow us to make predictions about the ability of striped bass to function under hypoxic conditions.

3. Compare the physiological responses of striped bass with and without signs of disease (mycobacteriosis). This information will be used to estimate the impact of high disease prevalences on the overall health of the striped bass population.

Approach

We intend use standard procedures (described in Bushnell et al. 1990; Bushnell and Brill 1991, 1992, Steffensen et al. 1982) to quantify differences in cardio-respiratory function of striped bass both under full oxygen saturation and graded levels of hypoxia. In brief, we will instrument fish to monitor simultaneously inhalant and exhalant water, arterial and venous blood, ventilation volume, and cardiac output. These data will allow us to completely characterize the cardio-respiratory function under normoxia and hypoxia, and deficits that result from disease status of the fish. Because of the amount of surgery and instrumentation involved, fish will be restrained, activity levels of fish will be minimal, and metabolic oxygen demands will be fixed. We currently plan to test four levels of ambient oxygen: normoxia, 75% oxygen saturation, 50% oxygen saturation and 30% oxygen saturation. Striped bass for this study will be obtained from pound nets in the Rappahannock River. Obtaining fish in this manner is routine, highly cost effective, and employed by several projects at VIMS.

We have chosen specifically to study both healthy and diseased striped bass. Due to the high prevalence of mycobacteriosis in striped bass in Chesapeake Bay, we believe that any

attempts to understand the impacts of hypoxia on wild striped bass must include study of diseased as well as healthy fish. Mycobacteriosis is most often expressed as a disease of visceral organs, with spleen and kidney being primarily affected. In teleosts (bony fishes), the spleen functions as a reservoir for red blood cells. During periods of stress (e.g., hypoxia) or heightened metabolic oxygen demand (e.g., when escaping predators, chasing prey, or during capture and release), the spleen ejects red blood cells into the circulation thus increasing blood oxygen carrying capacity (Yamamoto and Itazawa 1989). We suspect, however, that splenic function is compromised in diseased fish due to the presence of granulomatous inflammation, resulting in a reduced ability to function during hypoxia or to meet increases in oxygen demand due to activity or feeding.

We also suspect that ulcerated skin associated with mycobacterial infection causes significantly elevated rates of passive salt and water movements across the skin (i.e., an increased osmoregulatory burden). This necessarily results in higher energetic costs for maintaining osmotic balance (Febry and Lutz 1987, Jobling 1995) due to increases in Na^+-K^+ ATPase (responsible for active ion transport) levels in the gills and gut (Colin et al. 1985, Dange 1985, Brill et al. 2001). The net result is likely a decrease in the capability of striped bass to maintain normal activity levels, especially during conditions of decreased dissolved oxygen (Preide 1985, Febry and Lutz 1987). This decreased ability could, in turn lead to impaired ability to capture prey, thus further decreasing fish condition and physiological competence.

For the purposes of our study, disease status of fish will initially be determined on the basis of presence/absence of skin lesions. Such a skin lesion diagnostic has been validated by our group at VIMS, and is currently in use for large-scale tag-and-release studies in the Rappahannock River. At the conclusion of each laboratory trial, fish will be euthanatized and their visceral disease status determined using standard histological techniques (Gauthier et al. 2003). Final determination of disease status will therefore be made by direct histological observations of internal organs. Differences in measured and calculated cardio-respiratory parameters (indicative of the fish's ability to function in normoxia and hypoxia) relative to health status of the fish will be tested using standard two-way analysis of variance procedures. Data from our study will be used to develop mathematical models capable of describing cardio-respiratory function under normoxia and various levels of hypoxia (Bushnell and Brill 1991, Brill 1994). These will, in turn, allow us to make predictions about the ability of striped bass to function (e.g., maintain specific swimming speeds, recover from exhaustive exercise accompanying capture and release) under various combinations of hypoxia and disease status.

Subsequent laboratory studies using instrumented swimming fish would be needed to refine the results we expect from the first year of study (Bushnell and Brill 1991). For example, based on established procedures, activity of fish could be controlled by placing the fish in a swim tunnel respirometer, and applying instrumentation to the fish to measure ventilation volume, heart rate and cardiac output, and/or arterial and venous blood gases (e.g., Jones et al. 1989, Graham et al. 1994). Data from these types of experiments could be used to expand on our work with restrained fish and allow us to further refine our understanding of striped bass cardio-respiratory function in normoxia and hypoxia, as well as the ability of healthy and diseased fish to function during hypoxia and various levels of exercise (i.e., at various levels of oxygen demand). Studies employing instrumented swimming fish are outside the scope of what could be accomplished in one year, and we may approach the Board for funding in subsequent years. We will, however, make every effort to secure funding from alternate sources for research beyond

year one of this project.

Expected Benefits

Striped bass in Chesapeake Bay are currently at or near historical levels, and recreational fishers are enjoying the benefits of this recovered fishery. Despite these positive signs, there are some troubling indications that all is not well with the striped bass population. The presence of disease and emaciated fish is well known to those familiar with the fishery, and there is currently much concern among those who use this resource. Also of considerable concern to those who live in the Chesapeake Bay watershed is the seasonal occurrence of large areas of water with severely diminished dissolved oxygen levels.

By quantifying the ability of striped bass to function under various levels of dissolved oxygen, we will provide better understanding of the effects of hypoxia on natural mortality, movements, etc., and possibly post-release survival of striped bass. This knowledge is important for several reasons. If, as we suspect, large areas of the Bay are becoming unavailable during summer months due to hypoxia, fish are required to seek out suboptimal habitat. This would suggest hypoxia as a major risk factor for the striped bass population and would increase the urgency with which we must deal with water quality issues in the Bay. Previously, the relationship between water quality, especially hypoxia, and fish health has been somewhat vague. Our study would provide direct linkage between water quality parameters and fish condition, and would generate the means by which decreases in usable habitat for striped bass could be meaningfully measured.

In the future, we also intend to integrate this laboratory project with field studies that will directly examine the movement patterns of striped bass with respect to hypoxic areas. We strongly feel that the combined results from these two projects will effectively define suboptimal and unusable habitat and allow us to determine exactly when fish will be forced out of cool summer refuges by oxygen depletion in bottom water, or are blocked from exiting river mouths by hypoxic barriers. Conclusions as to the effects of hypoxia on the biology and behavior of striped bass, and any resultant recommendations, will obviously be far more robust with this field and laboratory approach than if either were pursued alone. The end result of these complementary field and laboratory projects will be a better understanding of negative effects of seasonal hypoxia on the survival, growth, short-term movement patterns, and seasonal migrations of striped bass in Chesapeake Bay, as well as how these negative effects may be exacerbated by disease.

Perhaps more important, our efforts will also provide information that would be of direct benefit to fishers. For example, our results could be used to develop guidelines by which conservation-minded fishers could help ensure low levels of post-release mortality in healthy and diseased striped bass. Additionally, our study will take steps toward answering the common question of why striped bass become emaciated and/or diseased in Chesapeake Bay.

Dissemination of results

Results of this study will be made available to fisheries managers (e.g., VMRC) through quarterly and annual reports, and to the scientific community through publication in peer-reviewed journals. Results will also be presented to regional fisheries management boards as requested (e.g., Atlantic States Marine Fisheries Commission and Mid-Atlantic Fisheries Management Council), and at professional meetings (e.g., American Fisheries Society). We also intend to share these data with fishery professionals developing ecosystem-based models. Results from this project will likewise be made available to the general public through preparation of articles for recreational fishing magazines and presentation at recreational fishing clubs.

ESTIMATED COST AND BUDGET JUSTIFICATION

Personnel

We are requesting salary for a post-doctoral scientist to conduct these physiological experiments. The surgical and instrumentation procedures require high skill levels and it is unlikely that a graduate student would be able to conduct the research effectively in the time allotted for the project. The PIs' time will be covered either by the federal government (Brill) or funds from this project (Gauthier).

Supplies

We are requesting funds for:

1. the acquisition and transport of 30 live striped bass to VIMS holding facility, plus animal feed;

2. maintenance of pumps, tanks, filters, and temperature controllers;

3. purchase of chemicals, anesthetics, and disposable surgical supplies, and histological supplies

Equipment

All the requisite fish husbandry equipment, surgical tools, and physiological instrumentation are available at VIMS through the Cooperative Marine Education and Research Program (NMFS, NOAA, U.S. Department of Commerce). Accordingly, we are not requesting funds to obtain this expensive equipment. The replacement cost of this equipment is estimated at $40,000.

Facilities & Administrative Costs

VIMS Facilities & Administrative Costs will be capped at the reduced rate of 25%. VIMS will provide the difference of this reduced rate versus the institutional rate (45%) as matching funds.

Location

All experiments will be performed at the Virginia Institute of Marine Science.

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