Widener University: Institutional Animal Care and Use Committee

Project Application Form

1. Name and Department of Applicant (faculty member or course instructor):

Students: Kevin O'Sullivan, Saji Anthony, Jen Wilwert

2. Title of Project:

Concentrations of Myoglobin in two Species of the Family Sciaenidae

3. Is this a new application, revision or renewal?
new application

Date and approval number of last application? n/a

4. If this is a course project, please indicate:

Course Name and Number	Instructors	Estimated Enrollment
Biology 401, fall 2003	Itzick Vatnick and Bruce Grant	16

5. Dates of Project (1 Year):

From: October 2003 To: December 2003

6A. Statement of qualification of applicant. Provide a biographical statement of the experience and training of applicant for the procedures described below.
Students not responsible for care of animals. Dr. David Coughlin assumes full responsibility for caring for animals.

6B . Names and positions of persons (e.g. students) authorized by the applicant to participate in the procedures described below. Also, name the person responsible for instruction in the care and use of laboratory animals of each research participant.

Name	Title	Instruction in Animal Handling by:
Jennifer Wilwert	Research Assistant/Student	Dr. Coughlin
Kevin O'Sullivan	Student	Dr. Coughlin
Sajitha Anthony	Student	Dr. Coughlin

7A. If this is a research project, supply an abstract of the project. Abstract should be written in terms understandable by a non-scientist. Describe the overall purpose of the project and the importance of the research.

Myoglobin is an oxygen-carrying protein found in red muscle. Red muscle is a slow muscle that is often used for non-fast twitch motion. It contains more mitochondria than white muscle, thus it fatigues slower. Therefore, if an organism has more myoglobin in its red muscle tissue, the organism will not fatigue as fast as an organism that does not have as much myoglobin. It has been observed that red muscle is darker in *Leiostomus xanthurus* (Spot) than in the *Micropogonias undulatus* (Atlantic croaker). The darker muscle in the spot may indicate a higher myoglobin content, which affects its swimming performance. Thus, at higher flow speeds, the organism with the greater myoglobin content should fatigue slower. If the spot has a better swimming performance, then its myoglobin content will be greater than the croaker.

In order to perform this experiment, a flume, electrodes and amplifiers will be used to determine the speed at which white muscle is recruited. The white muscle recruitment speed will be taken as the speed that the red muscle reaches its maximum threshold performance. For molecular analysis, the red muscle will then be excised from each fish and placed in phosphate buffer for homogenization. The samples will be centrifuged and bubbled with carbon monoxide. A spectrophotomer will be used to assess myoglobin content in the samples. The importance of this research is to understand how environmental adaptations in muscle properties affect locomotion.

7B. If this is a course project, provide a course description. Also, append a copy of the syllabus of the course.

The course focuses on evolutionary adaptations of physiological aspects of an organism to its environment. The course also will improve students' understanding of the scientific method thoroughly and how to design a research experiment that will reflect critical thinking and an understanding of evolutionary principles.

8.Give the specific reasons why live animals must be used for this study. Are alternative methods available (e.g. computer simulations, cell or tissue culture)? If so, why are they not used?

Live animals need to be used in order to understand locomotive properties in the fish. Computer simulations are not being used because they cannot provide an accurate description of a swimming fish at different flow speeds. Tissue cultures are not reflective of the swimming properties of the organism as a whole.

9A. Animals to be used in this protocol. Numbers used may be estimates. If needed, base estimates on usage in previous years.

Species/Strain	No. / Year	Sex	Age	Weight (grams)
Leiostomus xanthurus	4	n/a	juvenile	100-500
Micropogonias undulatus	4	n/a	juvenile	100-500

9B. How are the animals obtained? Where and how are they housed?

Animals were obtained by Dr. David Coughlin and are housed in a tank in Kirkbride 503 laboratory.

10. Provide complete details on each procedure involving the species listed under section 9A. The description of each procedure should supply the category of animal utilization (see appendix). If drugs or anesthetics are to be used, provide dosage and duration of treatment. As appropriate, identify all aspects of post-procedural care, including euthanasia, and describe procedures for identification and intervention in the care and use of animals if painful or stressful outcomes are anticipated. For course projects, refer to the course syllabus as possible.

The category for animal utilization is A for swimming properties assessment. After swimming properties are assessed, animals will be euthanized in order to extract muscle tissue. The category for animal utilization is A, where the dosage for the euthanasia will be determined by Dr. David Coughlin.

11. I hereby certify that the above information is accurate. The care and use of animals proposed will abide by the National Research Council guidelines published in the *Guide for the Care and Use of Laboratory Animals*.

Name Jennifer Wilwert_____ Title Research Assistant/Student

Name Sajitha Anthony_____ Title Student

Name Kevin O'Sullivan_____ Title Student

Signature_____ Date_____

Signature_____ Date_____

Signature_____ Date_____

The signature of the Associate Dean of Science is required.

Name_____Marc Brodkin_____ Title__Associate Dean of Science__

Signature_____ Date_____

APPENDIX
CATEGORIES OF USE LEVEL FOR APPLICATIONS UTILIZING VERTEBRATE ANIMALS IN RESEARCH TESTING AND INSTRUCTION.

CATEGORY A - Experiments on vertebrate animal species that are expected to produce little or no discomfort.
Mere holding of animals captive for experimental purposes; simple procedures such as injections of relatively harmless substances; blood sampling; physical examinations; food/water deprivation for shore periods (a few hours); standard methods of euthanasia that induce rapid unconsciousness, such as anesthetic overdose or decapitation preceded by sedation or light anesthesia.

CATEGORY B - Experiments that involve some minor stress or pain (short-duration pain) to vertebrate animal species.
Experiments on completely anesthetized animals which do not regain consciousness; with anesthesia and subsequent recovery, exposure of blood vessels or implantation of chronic catheters behavioral experiments on awake animals that involve short-term stressful restraint; immunization employing Freund's Adjuvant; noxious stimuli from which escape is possible; major surgical procedures under anesthesia that result in post-operative discomfort that is treated with analgesics. Category B procedures incur additional concern in proportion to the degree and duration of unavoidable stress or discomfort.

CATEGORY C - Experiments that involve significant but unavoidable stress or pain to vertebrate animal species.

Deliberate induction of behavioral stress in order to test its effect; major surgical procedures under anesthesia that result in significant post-operative discomfort that is not treated with analgesics; induction of an anatomical or physiological deficit that will result in pain or distress; application of noxious stimuli from which escape is impossible for prolonged periods (up to several hours or more) or physical restraint; maternal deprivation with substitution of punitive surrogates; induction of aggressive behavior leading to self-mutilation or intra-species aggression; procedures that produce pain in which anesthetics are not used, such as toxicity testing with death as an end point, production of radiation sickness, certain injections, and stress and shock research that would result in pain approaching the pain tolerance threshold, i.e. the point at which intense emotional reactions occur. Category C experiments present an explicit responsibility on the investigator to explore alternative designs to ensure that animal distress is minimized or eliminated.

CATEGORY D - Procedures that involve inflicting severe pain near, at, or above the pain tolerance threshold of unanesthetized conscious animals.
Use of muscle relaxants or paralytic drugs such as succinyl choline or other curariform drugs used alone or surgical restrain without the use of anesthetics; severe burn or trauma infliction on unanesthetized animals; attempts to induce psychotic-like behavior; killing by use of microwave ovens designed for domestic kitchens or by strychnine; inescapably severe stress or terminal stress.