

Southern Connecticut State University  
Lobster-Aging Proposal  
Submitted to the State of Maine

Principal Investigators: Dr. Sean Patrick Grace, Colleen Giannini, Connecticut Department of Environmental Protection

I. Introduction

This research will determine the age of American lobster (*Homarus americanus*) collected from several regions in the Gulf of Maine (GOM) from multiple orbital carapace lengths (mm). The purpose of this research is to document the age and levels of the aging pigment lipofuscin in lobsters from different temperature regimes in the GOM. Data collected will aid in the management of this resource. Recent studies on western rock lobster (*Panulirus cygnus*) indicate that the aging technique proposed in this research would allow independent assessment of population parameter estimates and has shown that most juvenile lobsters are 3-5 years old immediately prior to recruitment (Sheehy 1998). Another study on the European lobster (*Homarus gammarus*) yielded reproducible catch age structures with year-class resolution. In addition, a recent study performed on the European Lobster demonstrated that lipofuscin quantification produced more accurate estimates of age in the species than carapace length (Huglem *et. al.* 2005). This finding has important implications for stock assessments that employ traditional models which include age as an input parameter (Sheehy 1996).

II. Goals

The goals of this research are to:

- a) determine the age of American Lobsters collected from several different regions in the Gulf of Maine population using lipofuscin accumulation in the olfactory lobe cell mass in the brain.
- b) Compare the age structure of wild caught American Lobster from Long Island sound to that of the age structure determined for wild caught American Lobster from Gulf of Maine. The latter, Long Island Sound data has been provided to Dr. Grace from the Connecticut Department of Environmental Protection, Project Director Colleen Giannini.

III. Objectives

1. Establish the effects of temperature on levels of lipofuscin accumulation in the population of American lobster from the Gulf of Maine.
2. Correlate lipofuscin concentrations from wild Gulf of Maine lobsters with age, using data derived from pigment accumulation analyses from known age lobsters.
3. Examine the effects of temperature on lipofuscin accumulation in known age animals, when reared and raised at three distinct temperature regimes.

#### IV. Study Design

The approach employed in this study will be to establish lipofuscin levels from wild lobsters of unknown age from several regions in the Gulf of Maine using levels previously established for laboratory raised, known age animals obtained from various hatcheries. Correlation of pigment levels with age will be determined and compared with published literature. This correlation may then serve as the means by which age can be associated with pigment accumulation in the population of wild lobsters from the Gulf of Maine, and provide insight on the effects of temperature on lipofuscin accumulation, that may aid in the better management of the resource.

#### V. Procedures/Experimental Design

The project will comprise the two tasks listed below:

##### Task 1: Fluorescence examination of Gulf of Maine lobsters

The bidder will isolate tissue samples from the esophageal ganglia of Gulf of Maine lobsters. A maximum of 150 lobsters will be purchased from commercial fishermen from the Gulf of Maine by SCSU, specifically, Dr. Sean Grace. The bidder will purchase 10-20 lobsters from each of the following size class ranges (mm carapace length): 82.5-90.4mm, 90.5-98.4mm, 98.5-106.4mm, 106.5-114.4mm, 114.5-122.5mm, 122.5-130.4mm, and >130.5mm, from each of the following regions within the GOM and detailed as collection regions by the Lobsterman's Association (Figure 1). Rationale for the size ranges chosen is an effort to capture the possibility of 5-8 age classes per molt increment based on research performed by Sheehy (1996) in the United Kingdom. Estimation of approximate prices itemized in the budget were determined using the Atlantic States Marine Fisheries Commission American Lobster Stock Assessment 2000, where the length/weight relationships were published (i.e. 127mm (5") lobster = ~ 4.2 lbs). The bidder will purchase, dissect, store and transport Gulf of Maine lobster brains. Laboratory processing and confocal microscopy will take place at SCSU to determine lipofuscin levels in the neural tissue. Up to five sections (5-7 $\mu$ m) of the cell mass will be analyzed for each lobster. Each of these sections will quantify the percent of the olfactory lobe cell mass occupied by lipofuscin, the density of lipofuscin granules in the olfactory lobe cell mass, and the mean diameter of the lipofuscin granules.

##### Task 2: Examination of temperature on known-age animals

The bidder will rear and raise, or purchase (New England Aquarium) known age animals and 20 animals at one of three temperatures (temp 1, temp 2, temp3). At one year intervals, 10 animals will be examined for lipofuscin accumulation. At the end of three years, the effects of temperature on lipofuscin accumulation will be examined using a two-factor ANOVA with the factors, age (in months) and temperature, and the dependent variable lipofuscin accumulation (as determined by confocal microscopy and image analysis). These results will be used to submit for further funding of the effects of temperature on lipofuscin accumulation over the broad distribution of the American Lobster.

##### Task 3: Data Analyses/Written Reports/Work Schedules

The bidder will complete analyses of the pigment data gathered in Task 1 and Task 2 to determine if size and pigment accumulation are related as previously reported in similar studies from published literature and to examine the effects of temperature on pigment accumulation, a

study that has yet to be completed using the American Lobster . If so, these data will be used to correlate pigment accumulation in wild Gulf of Maine lobsters with known age lobsters whose lipofuscin concentrations have already been established.

Work Schedules are delineated and provided in the Milestone Chart, Attachment B. Final report of all completed work will be provided on/before May 31, 2007.

#### VI. CT DEP Responsibilities

Colleen Giannini, Project Director, American Lobster Project, CT DEP Marine Fisheries Division will provide data on lipofuscin concentration of known age American lobster purchased from hatcheries. These samples were fixed and stored in 10% buffered formalin and maintained in labeled air-tight vials until delivered to the SCSU's histology laboratory for processing. All samples were analyzed using the protocol described above and these controls will be used to correlate the results from lobsters collected from the Gulf of Maine. Letter of collaboration from Colleen Giannini is attached. In addition, a letter from support for the proposed research from Penelope Howell, CT DEP Marine Fisheries Biologist, American Lobster project and chair of the Atlantic States Marine Fisheries Commission Lobster Technical Committee is attached.

#### VII. Literature Cited

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Stock Assessment Report No. 06-03 (Supplement) of the Atlantic States Marine Fisheries Commission (*American Lobster Stock Assessment for peer review*) January 2006.

Wahle RA, Tully O and O'Donovan V (1996) 'Lipofuscin as an indicator of age in crustaceans: analysis of the pigment in the American lobster *Homarus americanus*' *Mar Ecol Prog Ser* 138:117-123

#### VIII. References Reviewed

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**Grant Proposal Specifics: As outlined in the instructions for proposals.**

1. The proposal must include detail on the experience and knowledge required as previously stated, including a description of experience.

Description of Southern Connecticut State University's Experience:

Previous awards have been granted to Southern Connecticut State University to support the Master's thesis research of Connecticut Department of Environmental Protection (CT DEP) biologist, Colleen Giannini. These awards have resulted in the purchase and use of a BIORAD Confocal Microscope, Laserssharp 2000 imaging software and related laboratory supplies necessary for histological preparation and paraffin wax sectioning. In addition, the brains of 50 laboratory raised, known age lobsters, ranging in age from 6 months to 5+years, have been analyzed. Lipofuscin concentrations in these animals were quantified using a National Institute of Health (NIH) analysis package and provide the benchmark by which lipofuscin concentrations from wild animals can be correlated with age. SCSU has a sophisticated histological facility dedicated to the ongoing research of fluorescence microscopy and cell imaging. The University recently obtained state of the art digital imaging equipment to compliment their research projects. The investigators have demonstrated specific skills required to isolate the olfactory lobe cell mass from the brain and process the material for lipofuscin detection. Dr. Sean Grace is also familiar with the analytical procedures required to quantify and correlate the laboratory results. They have demonstrated considerable accessibility to the CT DEP's project director, Colleen Giannini, a graduate student under their thesis advisory at SCSU. This association allowed time sensitive samples to be obtained and processed opportunistically, an important factor in meeting the collection requirements to satisfy the contract with the CT DEP. This past history has provided the basis for all future research aimed at lobster aging at SCSU. The equipment is present, the protocol has been established, the experimental design is well tested and the methods are consistent and repeatable.

Bidder's Specific Experience:

Example 1:

Dr. Grace has been a committee member for the Master's thesis of Colleen Giannini, which involves aging American lobster collected from Long Island Sound. The results of this research have been presented to the Atlantic States Marine Fisheries Commission's American Lobster Stock Assessment, Modeling and Technical Committees in Old Lyme, CT, December 2004, at the Retirement Symposium of Dr. Stann Cobb at the Graduate School of Oceanography at the University of Rhode Island in July 2005, and presented at the Benthic Ecology Meeting to held in Quebec, Canada on March 9, 2006. Dr. Grace has the experience necessary to carry out the histological procedures, perform confocal microscopy and to analyze the digital images of the cell mass. Dr. Grace has also extended the study begun by Colleen Giannini (scheduled for completion in August 2006), by examining the effects of temperature on lipofuscin concentrations in known age animals. The future focus of this research will be the collection of wild lobsters from specific size classes, along a temperature gradient (from Maine to New Jersey) to determine the effects of temperature on natural concentrations of lipofuscin.

Example 2:

Dr. Grace was also just awarded monies from the State of Maine, Department of Natural Resources, in the amount of \$6,000.00 for a study entitled:

Age Determination of the American Lobster in the Gulf of Maine (GOM) using lipofuscin concentrations in the olfactory lobe cell mass of the brain.

This study will examine the lipofuscin concentrations in wild-caught Maine lobsters and use this information to compare with already collected data on known-age and wild-caught Connecticut lobsters. All the techniques used for this collaboration will be extended if the present grant is successfully funded.

Confirmation of award subject to the Freedom of Access Laws of the State of Maine (1 MRSA 401):

We understand that our bid will be available for public viewing following the bid award.

PROJECT PROPOSAL SUMMARY

**Project Title: Temperature effects on lipofuscin concentrations in the olfactory lobe cell mass of the brain of the American Lobster in the Gulf of Maine (GOM).**

*Summary of Scope of Work (see above for complete details)*

Task 1 – Determine lipofuscin concentrations for wild GOM lobsters

The bidder will quantify lipofuscin accumulations from a minimum of 100 wild caught American lobsters collected from GOM waters by commercial fishermen. A minimum of 100 up to a maximum of 150 lobsters will be purchased from fishermen by Southern Connecticut State University, specifically, Dr. Sean Grace's lab. The bidder will dissect and fix the tissues immediately in 10% neutral buffered formalin, dehydrate, clear and embed the tissues, serially section the brains at 5-7 $\mu$ m and slide mount as described in the literature (Simpson *et al* 2005). The bidder will then use confocal microscopy to detect the naturally fluorescent pigment and quantify its presence using digital image analysis (ImageJ™).

Task 2 – Compare lipofuscin concentrations between known age and wild caught.

The bidder will use the results obtained from Task 1 and compare them to the published literature of similar studies done on known age American lobster and also on other crustaceans. This comparison will determine the appropriateness of correlating the results with accumulation levels from wild animals to determine age.

Deliverables

The bidder will complete analyses of the data gathered in Tasks 1 and 2 to determine if the correlation between age and lipofuscin quantities in known age animals can be used to correlate lipofuscin levels in wild GOM lobsters with age.

2. The following individuals are designated to serve as Project Directors:

Southern CT State University  
Dr. Sean Patrick Grace  
Biology Department  
Southern CT State University  
501 Crescent Street  
New Haven, CT 06515-1355  
203-392-6216

3. The following individuals are designated to serve as contacts for business matters:

For the University:  
Delinda Conte  
Assistant Director of Pre-Award Services  
Sponsored Programs and Research (SPAR)  
Southern Connecticut State University  
501 Crescent Street  
New Haven, CT 06515-1355  
1-203-392-5066

4. Total project costs for the period of the Agreement shall not exceed \$125,000.00.

SOUTHERN CT STATE UNIVERSITY

by \_\_\_\_\_  
Authorized signature

\_\_\_\_\_  
Typed Name and Title

\_\_\_\_\_  
Date

**ATTACHMENT A**  
Cost Proposal

1. Provide a fixed cost proposal to deliver the research and final product previously detailed.

Proposed Costs: Bidders would use monies awarded for the following items, please note that no salary is being requested. Drs. Grace and Crawford have access to excellent graduate students, whom will assist in the dissection, laboratory processing and digital imaging and analysis of lobster tissues.

Item 1: Laboratory costs:

Histological processing equipment. ....	\$67,000.00
Laboratory chemicals and sectioning supplies. . . . .	\$1,500.00

Item 2: Travel Expenses . . . . . \$1600.00  
(to Maine to collect, dissect and store neural tissue; to provide support for travel, lodging and registration to the Benthic Ecology Meeting and PEI Lobster Meeting for myself and student)

Item 3: GOM Lobster purchases (estimating wholesale cost at \$6.00/lb): (Collected from Regions 1-8)

10-20 lobsters 82.5-90.4mm CL (approximately 2.7lbs) x \$6.00/lb x 8 . . . . .	\$2,608.00
10-20 lobsters 90.5-98.4mm CL (approximately 2.9lbs) x \$6.00/lb x 8. . . . .	\$2,864.00
10-20 lobsters 98.5-106.5mm CL (approximately 3.2lbs) x \$6.00/lb x 8 . . . . .	\$3,120.00
10-20 lobsters 106.5-114.4mm CL (approximately 3.8lbs) x \$6.00/lb x 8. . . . .	\$3,376.00
10-20 lobsters 114.5-122.4mm CL (approximately 4.0lbs) x \$6.00/lb x 8. . . . .	\$3,624.00
10-20 lobsters 122.5-130.4mm CL (approximately 4.2lbs) x \$6.00/lb x 8. . . . .	\$3,880.00
10-20 lobsters >130.5mm CL (approximately 4.3lbs) x \$6.00/lb x 8. . . . .	\$4,120.00

Total \$93,692.00