

SEA GRANT PROJECT PROGRESS REPORT

PROJECT TITLE: Age, Growth and Reproduction in Atlantic Hagfish

PRINCIPAL INVESTIGATOR: Stacia A. Sower (Dept Biochemistry and Molecular Biology, UNH)

INITIATION DATE: Feb 1, 2001

COMPLETION DATE: Jan 31, 2003

TOTAL AWARD: \$168,000 (\$34,000/year 1: \$134,000/year 2)

Professional Development:

The following are involved or assisting with this project:

Dr. Mickie Powell, a postdoctoral research associate. Half of her salary was supported by our Dean's office for year 1. She is actively involved and primarily responsible for performing the hagfish experiments and histology.

Scott Kavanaugh, MS graduate student. His project involves the cloning of GnRH from hagfish brains.

Ocean Tech Undergraduate Students: Joanne Davis, Samantha Meservey, Amy Agulay, Jen Wishinski, Lyn MacNevin, Taylor Heyl, and Adam Baukus

Undergraduate Students Emily Violette, Jocelyn Sanford and Jen Gleico who are assisting in the Hagfish experiments and histology.

Publications:

1. J. Davis, S. Meservey, A. Agulay, J. Wishinski, L. MacNevin, M. Powell and S.A. Sower. 2001. Sexuality and Embryogenesis of the Atlantic Hagfish, *Myxine glutinosa*. Ocean Tech 797, Final Report, May 2001. 51 pages.
 2. A. Baukus, T. Heyl, N. Maggio, M. Powell and S.A. Sower. 2001. Trap to Rear Eggs from Newly Caught Hagfish, Ocean Tech 797, Final Report, May 2001.
 3. M. L. Powell, T. Heyl, A. Baukus and S. A. Sower. 2001. Gonad Development in the North Atlantic hagfish, *Myxine glutinosa*. Amer Zool. Abstr. (In Press).
 4. M. L. Powell and S. A. Sower 2001. Seasonal Estradiol Production in Three Size Classes of Atlantic Hagfish, *Myxine glutinosa*. Amer Zool. Abstr. (In Press).
 5. S. I. Kavanaugh, M. L. Powell, J. E. Davis, and S. A. Sower. 2001. Sexuality and Embryogenesis of the Atlantic Hagfish, *Myxine glutinosa* Amer Zool. Abstr. (In Press).
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Outreach or Industrial Impacts:

One poster and one oral presentation at the 10th Annual COLSA Undergraduate Research Conference, May 2001.

Two oral presentations and one poster presentation at the New England Endocrine Meetings, Oct., 2001.

Two oral presentations and one poster presentation will be presented at the Annual Meeting of Society of Integrative and Comparative Biology, Jan., 2001.

A meeting is planned with Rollie Baranby and Yang Cho (the buyer of hagfish) in the next couple of months.

We have obtained the fishing regulations on the hagfish fishery from California Fish and Game.

INTRODUCTION: To develop the scientific basis for a sound management plan, the main goal of this two year project is to further our understanding of the growth and reproduction of Atlantic hagfish. The specific objectives of the proposed research are to determine the size of onset of reproductive maturity, rate of reproduction, reproductive fecundity, and age and growth in Atlantic hagfish. In addition, we will investigate the seasonal relationships between changes in hypothalamic gonadotropin-releasing hormone (GnRH) and activity of the gonad. This information is critical to prevent the exploitation of the Atlantic hagfish off the New England Coastline.

We are characterizing and attempting to stimulate the reproductive cycle of the Atlantic hagfish. During the first eight months of our project, we have determined steroid concentrations and have correlated these concentrations with gonad development and maturation of gonad tissues throughout the five months in ocean populations of *M. glutinosa*. Hagfish have also been starved in an attempt to stimulate reproduction. In addition, we did a preliminary study of accelerating reproduction with implants of gonadotropin releasing hormone (GnRH). These results indicated that GnRH could accelerate reproduction in female hagfish. We will continue our studies much more extensively in Year 2 to determine reproductive maturity, age and growth in Atlantic hagfish. In addition, we will continue to do further studies on attempting to stimulate reproduction with GnRH.

BACKGROUND

In response to a major decline or collapse of the fisheries (groundfish and anadromous species) industry in the Northeast, other species that were once considered alternative or underutilized species have and are being identified that may be suitable for commercial harvest. One such example is the hagfish. An East coast Fishery for Atlantic hagfish, *Myxine glutinosa*, started in 1992. The landings for hagfish off the coast of Maine and Massachusetts have ranged from 1 to 3 million lbs. each year during 1996-1999. However, there is virtually little known about reproduction and the reproductive success in hagfish.

Currently, there are no regulations governing the harvesting of hagfish in the East Coast. Since there is little or no information on age determination, age and time of reproduction, seasonality of reproduction and growth of Atlantic hagfish, the level at which a sustainable fisheries for this species can be maintained is unknown. In order for fisheries management to manage its hagfish

stocks and develop a sustainable commercial hagfish fishery, an information base is needed for optimum use of the hagfish resource.

The goal of this proposal is to establish the relative age and reproductive patterns of hagfish to provide an information base for fisheries management. We will test the *hypothesis* that Atlantic hagfish exhibit seasonal reproduction and growth. The specific objectives to test this hypothesis of the two-year proposed research are:

OBJECTIVES:

Objective 1: To determine relative growth rates of hagfish over a one and half year cycle.

Objective 2: To determine gonadal development by histological analysis and gonadosomatic index in relation to weight and length in order to determine size at maturity of Atlantic hagfish.

Objective 3: To investigate seasonal relationships between changes in hypothalamic GnRH and activity of the gonad.

RESEARCH FINDINGS TO DATE:

1. The following two projects were done in collaboration with undergraduate students under my supervision in the Ocean Tech Course in 1999-2000. These projects helped to serve as initial studies for our Sea Grant studies this year and for our planned studies in 2002.

Project One: The objectives of these experiments were to describe gonadal development under controlled laboratory conditions and stimulate gonadal development through injection of lamprey GnRH-III to potentially obtain fertilized eggs. Hagfish *Myxine glutinosa* were obtained from the Gulf of Maine. For the first objective eight hagfish were held for five months at 4 °C in a recirculating saltwater tank at the University of New Hampshire Anadromous Fish and Invertebrate Research Laboratory (AFAIR Lab). Hagfish were sampled once a month for five months from November through March for weight, length and to determine the stage of gonadal development through histological examination. In November, most of the hagfish were classified as undetermined, i.e., they had not undergone sexual differentiation. During the next few months, the majority of hagfish were female. For the second objective, six hagfish were injected in February, 2001, with 48 µg of microencapsulated lamprey GnRH III in an attempt to stimulate gametogenesis. Hagfish were sampled after one month for comparison to a control non-injected group (n=6). Subsequent histological analysis showed that lamprey GnRH-III appeared to stimulate reproductive development in female hagfish compared to controls.

Project Two: Atlantic hagfish, *Myxine glutinosa* were held in modified traps within the Gulf of Maine in order to investigate the reproductive process and embryology of the organisms. The ultimate goal was to obtain a fertilized *Myxine glutinosa* egg under confinement in the ocean. There have been no fertilized eggs discovered since 1891. Eight modified 55-gallon drums were deployed approximately 1 mile west of the Isles of Shoals, in association with open-ocean

aquaculture net pens, each containing 6 hagfish: 2 female, 4 male. The hagfish were maintained for four months. Lengths, weights and gonads were sampled from the hagfish. Overall, the lengths of the female hagfish decreased significantly corresponding with advanced stages of ovary maturation observed in the last month compared to the beginning of the study.

2. The goal of our studies is to further understanding of growth and reproduction by testing the hypothesis that *M. glutinosa* exhibit seasonal reproductive growth. The specific objective of this study was to determine gonadal development by histological analysis in relation to maturity based on weight and length. *M. glutinosa* were trapped at a depth of 100-150m, 25 miles off the New Hampshire coast. Hagfish were sampled once a month (April thru August) at Jefferies Ledge (42° 50.72'N; 70°10.66'W) in the Gulf of Maine. Traps baited with herring were deployed from the RV Gulf Challenger at a depth of approximately 100m for 45-60 min. After retrieval hagfish were sorted into three-size classes; small (20-35 cm), medium (35-45cm) and large (50-60 cm). Twenty hagfish from each size class were placed into coolers with salt water and frozen salt water for transport to the University of New Hampshire AFAIR laboratory. Hagfish were maintained in the dark in 4°C salt water (32 ppt) for 24-48 hr prior to dissection. Prior to dissection hagfish were anesthetized by placing in MS222 for 1hr. Hagfish were weighed and length and diameter were measured. The brain, pituitary, and liver were removed and frozen on dry ice. Tissue samples of the anterior and posterior gonad were prepared for histological examination by fixing for 24 hr in Bouin's solution. Tissues for histology were embedding in paraffin and sectioned (7 microns). Sections were stained with eosin and hematoxylin to determine reproductive maturity based on the stage of egg or sperm development. Sectioning, staining and examination of the slides for the determination of reproductive stage is ongoing. Hagfish are thought to be protandric, beginning life as females and later differentiating into males. The results are shown in Figures 1, 2 and 3. In our collections males were always the smallest percentage of the sampled animals (25% or less in all samples). The percentage of hagfish with undeveloped gonads decreased from almost 75% in November to zero in January, immature hagfish were also absent in the March sample. Mature females containing ovaries in various stages of development were present in samples. These data showed that females were more abundant and they were usually of equal or greater length than males. There were several hermaphrodites in population -whether they were functional or not needs to be explored further.

3. The specific objectives of this study were to determine gonadal development by histological analysis and estradiol production in the gonads of three size classes (small 20-35 cm, medium 35-45 cm, large 45-55+cm) of *M. glutinosa* trapped 25-30 miles off the New Hampshire coast at a depth of approximately 150 m from April to August 2001. Gonad tissues for steroid incubations were placed in buffered saline (NaCl, 479mM; KCl, 9.4mM; CaCl₂ 9.0mM; MgSO₄ 10.0mM; and NaHCO₃ 4.7mM, pH 7.4) in 24 well cell culture plates at 4°C for 1 hr with constant shaking (Hirose et al. 1975). Saline was removed and frozen at -80°C for determination of endogenous steroid levels. The tissues were then placed in 500 μ l of buffered saline containing 0.15 μ g/mL pregnenolone and incubated for 48 hr at 4°C with constant shaking. Five samples from each size class were placed in 500 μ l of buffered saline containing no pregnenolone as controls. At the end of 48 hrs the media was removed and frozen (-80°C) for determination of steroid production by radioimmunoassay (RIA). The gonad tissues were then blotted, weighed and frozen at -80°C. Estradiol and testosterone released during the 1 hr pre-

incubation and 48 hr incubation were measured by RIA in both pre-incubation media and incubation media. Based on preliminary estradiol measurements and previous reports of low levels of estradiol in other hagfish species (Hirose et al, 1974) estradiol levels were measured using a sensitized RIA assay. Testosterone was initially measured using a non-sensitized assay, however, due to the low levels of testosterone further testosterone assays will be conducted using a sensitized testosterone RIA. The small group contained females and immature hagfish, the medium group contained males and females and the large group contained only females. Mature eggs were found only in the large size class and the greatest number of eggs 1 cm or larger were in the July sample. The greatest change in estradiol production was in the small size class between April and June, increasing from 1.79 to 5.77ng estradiol/g wet wt of gonad tissue (Fig. 4). The highest estradiol production was measured in the small size class in June. This study and earlier studies from this lab indicate that Atlantic hagfish may have annual reproductive cycles.
