

SEA GRANT PROJECT FINAL 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 with one-year extension

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

Professional Development:

The following were involved or assisted 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 was actively involved and primarily responsible for performing the hagfish experiments and histology.

Scott Kavanaugh, MS graduate student. His project involved the assessment of GnRH associated with reproduction in hagfish and assisted with the other hagfish experiments.

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, Byron Pedler and Jen Gleico who assisted 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. Powell, M. L., T. Heyl, A. Baukus and S. A. Sower. 2001. Gonad Development in the North Atlantic hagfish, *Myxine glutinosa*, Amer Zool. 41: 1560 Abst.
4. Powell, M.L. and S. A. Sower. 2001. Seasonal Estradiol Production in Three Size Classes of Atlantic Hagfish, *Myxine glutinosa*. Amer Zool. 41: 1560 Abst.
5. Kavanaugh, S.I., M. L. Powell, J. E. Davis, and S. A. Sower. 2001. Sexuality and Embryogenesis of the Atlantic Hagfish, *Myxine glutinosa*. Amer. Zool. 41: 1490-1491. Abst.
6. Powell, M.L. and S.A. Sower. 2002. The identification of corpus luteum and determination of reproductive steroids in the gonad of the Atlantic hagfish, *Myxine glutinosa*. Integr. Comp. Biol. 42: 1296-1297.
7. Powell, M.L., S.I. Kavanaugh, and S.A. Sower. 2002. Seasonal gonadal development in the Atlantic hagfish, *M. glutinosa*. Integr. Comp. Biol. 42:1297.Abst.
8. Powell, M.L., S.I. Kavanaugh, and S.A. Sower. 2003. Reproductive hormone concentrations in the Atlantic hagfish *Myxine glutinosa*. Integr. Comp. Biol. 43:814. Abst.

9. Powell, M.L., S.I. Kavanaugh, and S.A. Sower. 2004. Seasonal concentrations of reproductive steroids in the gonads of the Atlantic hagfish, *Myxine glutinosa*. J. Exp Zool. 301A:352-360.
10. Powell, M.L., S.I. Kavanaugh and S.A. Sower. Current knowledge of hagfish reproduction: implications for fisheries management. Integrat. Comp. Biol. In Press.
11. Powell, M.L., S.I. Kavanaugh and S.A. Sower. Preliminary identification of a functional corpus luteum in the Atlantic hagfish, *Myxine glutinosa*. In Revision

Outreach or Industrial Impacts:

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

One oral talk and one poster were presented at the 28th New England Endocrine Conference, Univ. Conn, Oct 27, 2001.

One oral talk and two posters were presented at the Annual Meeting of the Society of Integrative and Comparative Biology, Anaheim, Jan 2002.

One oral talk and one poster were presented at the Annual Meeting of Society of Integrative and Comparative Biology, Toronto, Canada, Jan., 2003.

One oral talk and one poster were presented at the American Fisheries Society Meeting, Baltimore, Md, August 2002.

One oral talk was presented at an NSF-JSPS workshop in Tasmania, Australia, Oct 2002.

One oral talk and one poster were presented at the Annual Meeting of the Society of Integrative and Comparative Biology, New Orleans, LA, 2004.

A representative from Sea Grant, fishermen, my postdoc and myself met with Yang Cho (President and the buyer of hagfish) three times for a discussion on how to develop fisheries guidelines.

My postdoc and I participated in a Hagfish Working Group with the New England Fishery Council in determining information needed for fishing regulations for a hagfishery, 2002-2003. We obtained the fishing regulations on the hagfish fishery from California Fish and Game to use as a starting point. A document was produced identifying the needed areas of research and the steps necessary to take in developing a fisheries management plan.

However, NH Sea Grant Program has declined to fund any more research on hagfish. Thus, the efforts for establishing a fishery management plan have ceased.

Channel 6 News, Portland, Maine, videotaped one of our hagfish trips and broadcasted it in Sept, 2002. The videotaped another one of our trips on May 29, 2004 and broadcasted this in May 2004.

INTRODUCTION: To develop the scientific basis for a sound management plan, the main goal of this two year project was to further our understanding of the growth and reproduction of Atlantic hagfish. The specific objectives of the proposed research were to determine the size of onset of reproductive maturity, rate of reproduction, reproductive fecundity, and age and growth in Atlantic hagfish. In addition, we investigated 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.

During the duration of our project, we determined annual changes of gonadal steroid concentrations, brain GnRH and correlated these changes with gonadal development and maturation of gonad tissues during an eighteen month period in from Atlantic hagfish. We have published one full paper, two papers are In Press and one is in revision. We completed our objectives and goals of this project. In addition, we did more than expected on this project. Funding from Sea Grant did not cover all costs of these experiments. Additional funds were used from indirect cost support and grants of the PI and donated time from the PI.

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

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.

MAJOR RESEARCH FINDINGS:

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 supported studies.

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 re-circulating 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 mg 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. Seasonal concentrations of reproductive steroids in the gonads of the Atlantic hagfish, *Myxine glutinosa*. Powell ML, Kavanaugh SI, Sower SA. J Exp Zool. Part A Comp Exp Biol. 2004;301(4):352-60. **Abstract:** Changes in gonadal morphology, gonadal estradiol, and progesterone were examined in Atlantic hagfish, *Myxine glutinosa*, during a period of 17 months, beginning in April, 2001. Atlantic hagfish were captured from the ocean on a monthly basis. A total of 60 hagfish were divided into three different size classes of twenty hagfish each (small 20-35 cm, medium 35-45 cm, large 45-55+cm) and transported to the University of New Hampshire for sampling. Overall, in the medium and large size hagfish, estradiol and progesterone had significantly elevated peaks in January, 2001. There were significant increases in estradiol concentrations in January, with relatively low fluctuations in levels for the rest of the sampling period. Progesterone concentrations increased significantly in January, 2002, in medium and large hagfish, and remained elevated until June and April, 2002, for the two size classes respectively. The majority of hagfish sampled were females or hermaphrodites; few true males were identified in any of the samples. The number of females with large eggs increased following the estradiol peak in January and hermaphrodites with mature sperm were identified in the July, 2002, sample. These data represent the first evidence for a seasonal reproductive cycle in *M. glutinosa* and only the second seasonal reproductive cycle identified in any hagfish species.

3. Seasonal changes of gonadotropin-releasing hormone in the Atlantic hagfish *Myxine glutinosa*. Scott I, Kavanaugh, Mickie L, Powell, Stacia A, Sower Gen. Comp. Endo. (In Press). **Abstract:** To investigate seasonal reproduction in *Myxine glutinosa*, we measured total brain gonadotropin-releasing hormone (GnRH) and determined gonadal stages of hagfish collected from the Gulf of Maine once a month for 12 months. Thirty hagfish from each of three different size classes of small (20-35 cm),

medium (35–45 cm), and large (50–60+cm) were sampled for brains and gonads. In the medium and large class hagfish there was an increase in GnRH concentrations during April and May that correlated with male and female gonadal maturity. Also in these size classes of female hagfish, there was a similar rise in GnRH in November and then again in January that preceded the highest incidence of large eggs (stage 7). The elevated GnRH may be influencing the onset of ovarian recrudescence which has been shown in other vertebrates. These data suggest an association of the concentration of brain GnRH with gonadal maturity and provide supportive evidence of a possible seasonal reproductive cycle in *M. glutinosa* shown in recent studies of [J. Exp. Zool. 301A (2004) 352], correlating steroid production with gonadal maturation.

4. Current Knowledge of Hagfish Reproduction: Implications for Fisheries Management. Mickie L. Powell, Scott I. Kavanaugh and Stacia A. Sower. (In Press) Integr. Comp. Biol. **Abstract:** This review briefly summarizes the latest findings on reproductive endocrinology of Atlantic hagfish (*Myxine glutinosa*) and implications for fisheries management. In response to a major decline or collapse of the fisheries (groundfish and anadromous species) industry in the Northeast, species that were once considered alternative or underutilized have and are being identified that may be suitable for commercial harvest, one such example is the hagfish. Hagfish in recent years have been sought after as valuable fish not only for their flesh, but also their skin. Currently, there are no regulations governing the harvesting of hagfish along the East Coast. There has been little to no information of the life history of hagfish including growth rate, age determination, reproductive biology, life span, and larval size at hatching. Thus, the level at which a sustainable fisheries for this species can be maintained is unknown. In some parts of the world, hagfish stocks are being depleted due to overfishing. In order for fisheries management to manage its hagfish stocks and develop a sustainable commercial hagfish fishery, critical information is needed to assist in determining the optimal use of this valuable resource.

Key elements of the reproductive system have not been elucidated in hagfish. However, there is new evidence from recent reproductive studies that Atlantic hagfish may have a seasonal reproductive cycle. These data include seasonal changes in gonadotropin-releasing hormone (GnRH), gonadal steroids, estradiol and progesterone, corresponding to gonadal reproductive stages along with the putative identity of a functional corpus luteum. This newly acquired data may provide important information to fisheries managers of the East Coast.

5. Preliminary Identification of a Functional Corpus Luteum in the Atlantic Hagfish, *Myxine glutinosa* Mickie L. Powell, Scott I. Kavanaugh and Stacia A. Sower. In Revision.

Abstract: Corpora lutea in nonmammalian vertebrates secrete mainly progesterone thought to be involved in the retention of eggs and down regulation of vitellogenin synthesis. The most ancient vertebrate that is known to have a functional corpus luteum is the dogfish, *squalus acantias*. Brown bodies, hypothesized to be corpora lutea, have been observed by scientists for over 100 years in the gonad of an even more ancient lineage of vertebrate, the hagfish. However, data to date in support of these brown bodies acting as corpora lutea has consisted mainly of observational studies. We examined the steroid production of these corpus luteum-like structures using radioimmunoassays for progesterone and estradiol. Progesterone concentration in incubation media with corpus-lutea like structures was significantly higher (12 ± 1.5 pg/mg gonad tissue wet wt) than incubation media containing only gonad tissues and oocytes (3.6 ± 1.5 pg/mg gonad tissue wet wt) ($p < 0.05$). Estradiol was detected in seven of the 13 samples containing only gonad tissue with oocytes and ranged between 0.6–0.18 pg/mg gonad tissue wet wt and was not detected in any of the media containing only corpora lutea samples. We hypothesize that hagfish have functional corpus-lutea like structures that produce progesterone. We further hypothesize that one possible function of progesterone in hagfish is an increase of vascularization which is an ancestral function and that this function was established at an early stage of evolution in vertebrates.

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Age, Growth and Reproduction in Atlantic Hagfish (S. Sower)**R/FMD -168****Journal Articles:**

Powell, M.L. S.I. Kavanaugh, and S.A. Sower. 2004. Seasonal concentrations of reproductive steroids in the gonads of the Atlantic hagfish, *Myxine glutinosa*. J. Exp Zool. 301A:352-360.

Powell, M.L. and S.A. Sower. Preliminary Identification of a Functional Corpus Luteum in the Atlantic Hagfish, *Myxine glutinosa*. J Fish Biol. Submitted

Powell, M.L., S.I. Kavanaugh and S.A. Sower. Current knowledge of hagfish reproduction: implications for fisheries management. Integrat. Comp. Biol. Submitted.

Kavanaugh, S.I., M.L. Powell and S.A. Sower. Seasonal changes of gonadotropin-releasing hormone (GnRH) in the Atlantic hagfish *Myxine glutinosa*. Gen Comp. Endocrinol. Submitted.

Book Chapters:

Sower, S.A., M.L. Powell and S.I. Kavanaugh. "Myziniformes" IN: Grzimek's Animal Life Encyclopedia, 2nd Edition. Volumes 4-5, Fishes I-II, edited by Michael Hutchins, Dennis A. Thoney, Paul V. Loisel, and Neil Schlager. Farmington Hills, MI: Gale Group, 2003. Vol. 1 77-82.

Technical Reports:

Davis, J., 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.

Baukus, A., 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.

Hathway, L., A. Oberhaus, and S. Thomas. 2002. Self-contained Hagfish Environment for Life-Cycle Learning. Ocean Tech 797, Final Report, May 2002.

Published Abstracts:

Powell, M. L., T. Heyl, A. Baukus and S. A. Sower. 2001. Gonad Development in the North Atlantic hagfish, *Myxine glutinosa*, Amer Zool. 41: 1560 Abst.

Powell, M.L. and S. A. Sower. 2001. Seasonal Estradiol Production in Three Size Classes of Atlantic Hagfish, *Myxine glutinosa*. Amer Zool. 41: 1560 Abst.

Kavanaugh, S.I., M. L. Powell, J. E. Davis, and S. A. Sower. 2001. Sexuality and Embryogenesis of the Atlantic Hagfish, *Myxine glutinosa*. Amer. Zool. 41: 1490-1491. Abst.

Powell, M.L. and S.A. Sower. 2002. The identification of corpus luteum and determination of reproductive steroids in the gonad of the Atlantic hagfish, *Myxine glutinosa*. Integr. Comp. Biol. 42: 1296-1297.

Powell, M.L, S.I. Kavanaugh, and S.A. Sower. 2002. Seasonal gonadal development in the Atlantic hagfish, *M. glutinosa*. Integr. Comp. Biol. 42:1297.Abst.

Powell, M.L., S.I. Kavanaugh, and S.A. Sower. 2003. Reproductive hormone concentrations in the Atlantic hagfish *Myxine glutinosa*. Integr. Comp. Biol. 43:814.

Thesis:

Kavanaugh, S.I. 2004. Gonadotropin Releasing Hormone (GnRH) in Chordates: The Cloning of the GnRH Precursor and the Distribution of GnRH in the Tunicate *Ciona intestinalis* and Seasonal Changes of GnRH in the Atlantic hagfish *Myxine glutinosa*. M.S. Thesis. University of New Hampshire

Papers presented at professional meetings (list):

Papers were presented at the Annual Society of Integrative and Comparative Biology, New Orleans, LA Jan 4 to Jan 8, 2004

1. ORAL: M. L. Powell and S. A. Sower. The identification of corpus luteum and determination of reproductive steroids in the gonad of the Atlantic hagfish, *Myxine glutinosa*.
2. POSTER: Powell, M.L., S.I, Kavanaugh, and S.A. Sower. Seasonal gonadal development in the Atlantic hagfish, *M. glutinosa*.

JSPS-NSF Workshop, UNH, May 31, 2004—Only US talks listed here only

3. Welcome and Introduction Stacia A. Sower (Dedication to Aubrey Gorbman and Congratulations to Professor Kawauchi on being the recipient of the Japan Prize of Agricultural Science 2004)
4. Stacia Sower: Current Knowledge of Hagfish Reproduction

Papers were presented at the Annual Society of Integrative and Comparative Biology, Toronto, Canada, Jan 4 to Jan 8, 2003

5. ORAL: M. L. Powell and S. A. Sower. The identification of corpus luteum and determination of reproductive steroids in the gonad of the Atlantic hagfish, *Myxine glutinosa*.
6. POSTER: Powell, M.L., S.I, Kavanaugh, and S.A. Sower. Seasonal gonadal

development in the Atlantic hagfish, *M. glutinosa*.

Papers presented at the American Fisheries Society Meeting, Baltimore, August, 2002.

7. ORAL: Powell, M.L. and S.A. Sower. Seasonal Estradiol Production in Three Size Classes of Atlantic hagfish, *Myxine glutinosa*.
8. POSTER: Powell, M.L. and S.A. Sower. The identification of corpus luteum and determination of reproductive steroids in the gonad of the Atlantic hagfish, *Myxine glutinosa*.

JSPS-NSF Workshop, UNH, May 31, 2003—(hagfish related papers only listed)

9. S.A. Sower, Hagfish Reproduction
10. S. Kavanaugh and S.A. Sower: Seasonal Changes in GnRH in the Atlantic Hagfish

Channel 6 News, Portland Maine, videotaped our hagfish trip in late May and broadcasted it in June, 2004.

Channel 6 News, Portland Maine, videotaped one of our hagfish trips and broadcasted it in Sept, 2002.

Updated: July 14, 2004