JOURNAL OF EXPERIMENTAL ZOOLOGY 301A:352–360 (2004)

Seasonal Concentrations of Reproductive Steroids in the Gonads of the Atlantic Hagfish, *Myxine glutinosa*

MICKIE L. POWELL, SCOTT I. KAVANAUGH, AND STACIA A. SOWER*
Department of Biochemistry and Molecular Biology, University of New Hampshire College of Life Sciences and Agriculture, Durham, New Hampshire 03824–2617

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. *J. Exp. Zool. 301A: 352–360, 2004.*

INTRODUCTION

Hagfish are the oldest extant craniates, with a lineage extending over 530 million years (Martini, '98). They are an important link between invertebrates and vertebrates. They and their closest relatives retain characteristics of extinct ancestral species. There are roughly 60 species of hagfish found in benthic marine environments all over the world, except in the Arctic and Antarctic Oceans (Martini, '98; Tsuneki et al., '83). Most hagfish species are found at depths greater than 100 m, making simple observations on their behaviors difficult (Tsuneki et al., '83).

A regular annual reproductive cycle has been identified in only one hagfish species, the Japanese hagfish, *Eptatretus burgeri*, which is known to migrate between shallow and deep water for breeding (Tsuneki et al., '83). The reproductive patterns of most other hagfish are unknown. However, females of most hagfish species are known to produce fewer than 30 eggs. Reproductive behavior has never been observed in any hagfish species, and despite numerous attempts, only a handful of hagfish embryos have been collected from the wild. Only three embryos from Atlantic hagfish have ever been obtained, and all were damaged during retrieval (Gorbman, '97). Martini et al. ('97) suggested, following macroscopic observation, that *Myxine glutinosa* have a limited reproductive potential; a large number of animals lack visible gonad tissue, and only a small number of males and gravid or postovulatory females were found among the fish collected. To date, there have been no intense microscopic and/or histological studies of the gonadal development and sex identification.

Steroid hormones are known to be produced in the mature ovary of the hagfish, and may be correlated with the maturation of the gonad during development (Hirose et al., '75). Weisbart

Grant support: UNH/UME NOAA Sea Grant R/FMS–168. This is Scientific Contribution Number 2165 from the New Hampshire Agricultural Experiment Station.
*Correspondence to: Stacia A. Sower, Department of Biochemisty and Molecular Biology, University of New Hampshire, College of Life Sciences and Agriculture, Rudman Hall, 46 College Road, Durham, New Hampshire 03824–2617. E-mail: sasower@cisunix.unh.edu
Received 21 January 2003; Accepted 8 January 2004
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jez.a.20043

and Kime identified steroids (11–dehydrocortisosterone, 11–deoxycortisol, testosterone) and enzymes responsible for steroid synthesis (5 α reductase, 3 β–hydroxy steroid dehydrogenase) in the Pacific and Atlantic hagfish species (Kime and Hews, '80; Kime et al., '80; Weisbart et al., '80). Estradiol, testosterone, and progesterone have all been measured in the blood sera of the Pacific hagfish by radioimmunoassay (Matty et al., '76), although the plasma steroid levels in hagfish in these early studies were two to three orders of magnitude lower than those measured in other vertebrates (Gorbman, '83). Yu et al. ('81) determined that estrone and estradiol stimulated vitellogenesis in *E. stoutii* and Schützinger et al. ('87) showed that the concentration of estrogens in the plasma of female *M. glutinosa* increases with increasing egg size. Hagfish have also been shown to have a single class of high affinity, low capacity estrogen binding sites in the hepatic nuclei that increase in vitellogenic hagfish (Dickhoff et al., '85).

Estradiol has been identified as an important reproductive steroid in several species of the lamprey, the closest relative of hagfish. In the Japanese river lamprey, *Lampetra japonica*, estradiol levels change in the adult females during the spawning migration (Fukayama and Takahashi, '85). In the river lamprey *Lampetra fluviatilis*, Pickering ('76) hypothesized that estradiol increased the weight of the ovaries by stimulating egg production in the oocytes. Sower et al. ('85) showed that the lowest level of plasma estradiol in the sea lamprey, *Petromyzon marinus,* occurred at the time of ovulation. Plasma concentrations of estradiol in the teleost, *Rhamdia quelen,* increased progressively during oocyte development (Barcellos et al., 2001). We hypothesize that similar correlations may exist between gonadal steroid concentrations and gonadal maturation in the Atlantic hagfish. Therefore, the goal of this research was to identify and characterize the reproductive cycle throughout the year in ocean populations of the Atlantic hagfish, *Myxine glutinosa,* through the quantification of steroid concentrations and histological examination of gonadal tissues.

MATERIALS AND METHODS

Atlantic hagfish, *M. glutinosa,* were trapped monthly in the Atlantic Ocean from April, 2001, to October, 2002, at Jefferies Ledge (42° 50.6'N, 70° 10.079' W) in the Gulf of Maine using the University of New Hampshire Research Vessel, Gulf Challenger. Samples were not collected for September and October, 2001, due to circumstances beyond our control. As a result of changes in sampling methods, however, data for the posterior gonad is only available from November, 2001 to October, 2002. Hagfish were trapped using modified 200 liter plastic barrels, baited with salted herring and fitted with one-way cones. Traps were set for 45–60 min at a depth of 100–150 m. The hagfish were divided into three size classes: small 20–35 cm; medium 35–45 cm; and large 45–55 cm+. These classes were chosen based on length and weight data from previous studies in the Gulf of Maine (Sower, unpublished observations). Gorbman ('90) proposed that male hagfish occur most frequently in the medium size classes and females dominate the small and large size classes. To increase the probability of including males and females in the sample, twenty hagfish were taken from each of these size classes. Hagfish were maintained in 4°C seawater, transported to the University of New Hampshire, Durham, New Hampshire, and held 24–48 hr. in chilled sea water (4°C) tanks until dissected. Hagfish were anesthetized by immersing in a solution of 0.05g/L tricanine methylsulfonate (MS222), weighed, measured for length, and decapitated. Between April and November, 2001, all gonadal tissue samples were removed from the anterior regions. Preliminary examination of gonad histology, however, showed that the hagfish gonad develops differentially along its length. In order to standardize the sampling, gonadal tissues for histology and steroid assays were sampled from three locations along the gonad in each hagfish, as described by Gorbman ('90) for *Eptatretus stoutii*. Gonadal tissues were sampled in each hagfish from the region of the 5th, 12th, and 44th muscle somite counting from the anterior wall of the cloaca (Fig. 1). Gonadal tissues from the 12th somite were immediately fixed in Bouin's solution. Tissues from the 5th and 44th muscle somite were incubated to determine steroid production, then fixed in Bouin's solution. All tissue samples were prepared for histological examination by embedding in paraffin, followed by hematoxylin-eosin staining. They were evaluated for developmental stage as described by Gorbman ('90) for the Pacific hagfish, *E. stoutii* (Table 1, Fig. 2).

Gonad tissues from the 5th and 44th muscle somite were placed in individual wells of a 24–well culture plate, with 500 μL buffered saline (Hirose et al., '75). The tissues were preincubated for one hour at 4°C on a shaker in an incubator. The

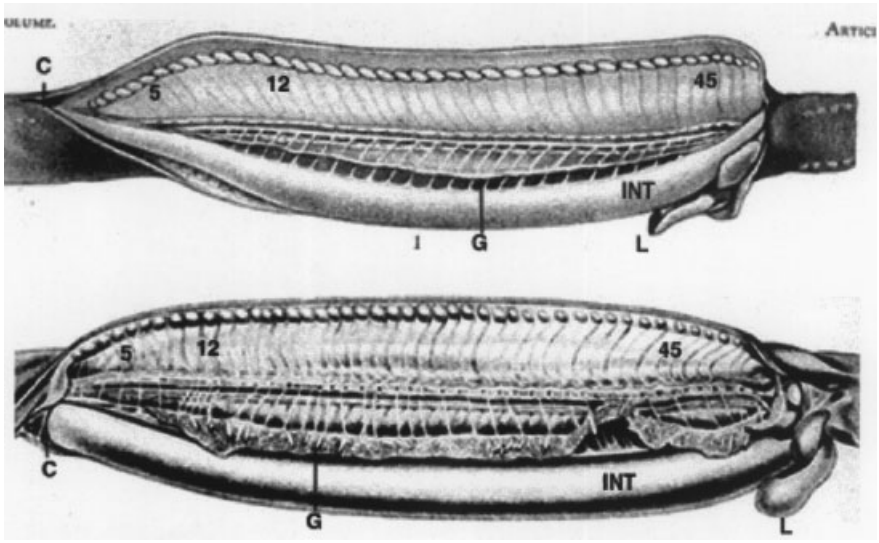

Fig. 1. Drawings of Professor Bashford Dean as referenced in Gorbman ('90) identifying the locations for gonad tissue sampling. (A) represents an immature hagfish with an undeveloped gonad. (B) represents a female with maturing oocytes. L is the liver, INT is intestine, and C is the cloacal vent. Gonad tissue was sampled from the regions of somite 5, 12, and 44. (Used by permission of the American Museum of Natural History).

TABLE 1. Hagfish gonad developmental stages descriptions and photos. Stages 1–10 are viewed at 40×. Oc=oocyst, o=oocyte, Sc=spermatocysts, s=spermatocytes

Stage	Description
1	Completely undifferentiated, small thin gonad.
2	Undifferentiated gonad with slight amount of parenchyma.
3	Small gonad with a few cells cysts or follicles.
4	More numerous gametocysts and a few oocytes
5	Mostly oocytes and a few cysts
6	Ovary containing only oocytes.
7	Ovary with some eggs more than 60μm in diameter
8	Ovotestis.
9	Solid rounded cellular testis, early spermatocysts.
10	Testis, usually with meiotic spermatocytes.

Gorbman ('90).

preincubation media was removed and replaced with 500 μL of buffered saline supplemented with pregnenolone (150 ng/mL media), to insure excess substrate for steroid synthesis reactions. The tissue was incubated for 48 hrs at 4°C on a shaker in an incubator. After 48 hr the tissue was removed, blotted, and weighed; the culture media was collected and frozen at −80°C until extracted and assayed for estradiol by radioimmunoassay (RIA) following the procedures described by (Sower and Schreck, '82). The RIA for estradiol–17β used antiestradiol–17β (S–244) obtained from G. Niswender (Colorado State University, Fort Collins, CO). The estradiol antiserum was used at a dilution of 1: 85,000. The lower limit of detection was 0.488 pg/0.1 mL. The intra-assay and inter-assay coefficients of variation for the estradiol RIA were 5.4% and 6% respectively. The RIA for progesterone used antiprogesterone (337) obtained from G. Niswender (Colorado State University, Fort Collins, CO). The progesterone antiserum was used at a dilution of 1:8,500. The lower limit of detection was 7.8 pg/0.1 mL. The intra-assay and inter-assay coefficients of variation for the progesterone RIA were 2% and 5% respectively. Cross-reactivities of progesterone antiserum 337 were shown to be 100% progesterone; 3.7% pregnenolone; 0.8% testosterone, and n.d. estradiol (Koligan and Stormshak, '77). Subsequent unpublished studies using in vitro incubations with pregnenolone has shown non-detectable concentrations of pregnenolone in our progesterone assays (Sower, unpublished observations).

Differences in estradiol and progesterone concentration were analyzed by ANOVA followed by Tukey's test to determine significance ($p<0.05$) using Statview 5.0 ('98).

RESULTS

Reproductive stages- all size classes

Fifty-eight percent of all hagfish examined from all size classes (n=1080) contained only female gonad tissue, 41% were hermaphrodites with both

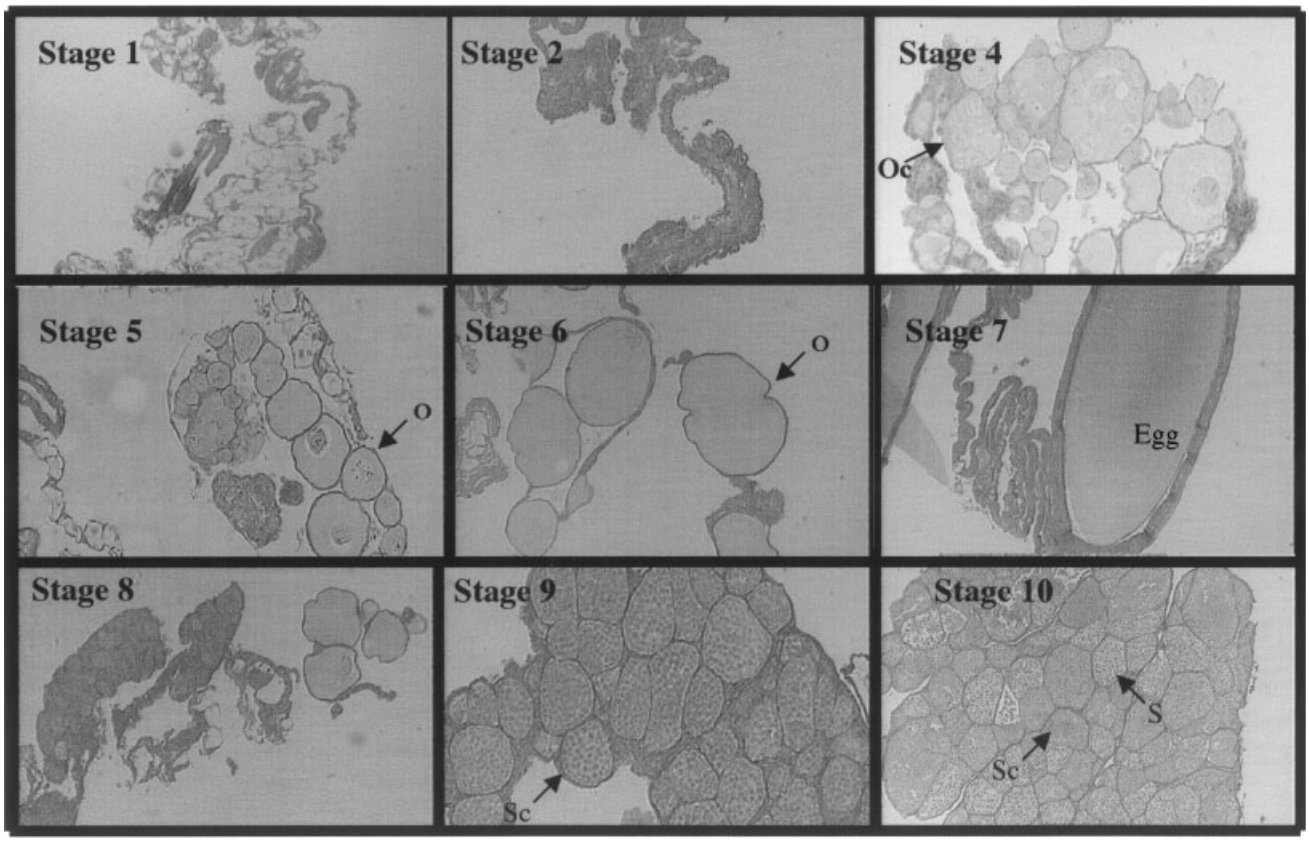

Fig. 2. Hagfish gonad developmental stages descriptions and photos. Stages 1–10 are viewed at 40 ×. Oc=oocyst, o=oocyte, Sc=spermatocysts, s=spermatocytes.

male and female tissue, and 0.05% were males with no female tissue present (Fig. 1). The most frequently observed developmental stages in the anterior gonad of all size classes were 5 and 6 (Fig. 2; Table 1). For all size classes prior to November, 2001, the gonadal tissue was sampled from only the anterior region. Beginning in November, 2001, through October, 2002, gonadal tissue samples were taken from the anterior (44th muscle somite), middle (12th muscle somite), and posterior (5th muscle somite). Development of the middle gonad tissue was usually indeterminate; therefore, only data for the anterior and posterior gonad are reported (Fig. 3).

Small size class

Stages 1, 2, 3, 4, 5, and 6 were identified in the gonads of the small size class (25–35 cm) (Fig. 2; Table 1). Hermaphrodites (stage 8) and developing male tissue (stage 9) were also identified in the small hagfish. Male tissue occurred most frequently in the region of the 5th muscle somite. There were no mature eggs (stage 7) identified in the small size class (Fig 3a).

Medium size class

The medium size class (35–45 cm) gonadal development differed from the small and large size classes in the number of males identified. The majority of the posterior gonad tissue (muscle somite 5) from this size class was identified as male tissue (stage 9) (Fig. 3b). Stages 5 and 6 were the dominant stages in the anterior region of the gonad (muscle somite 45) (Fig. 3b).

Large size class

The large hagfish (45–55+ cm) were almost exclusively female and had the most advanced gonadal development. Hagfish with developing eggs (stage 7) and eggs over 10mm in length occurred only in the large size class (Fig. 3c). Stages 1 and 2 that consisted of undifferentiated gonad tissue were observed in both the small and large size classes. However, in the large size class they were most frequently in the posterior region

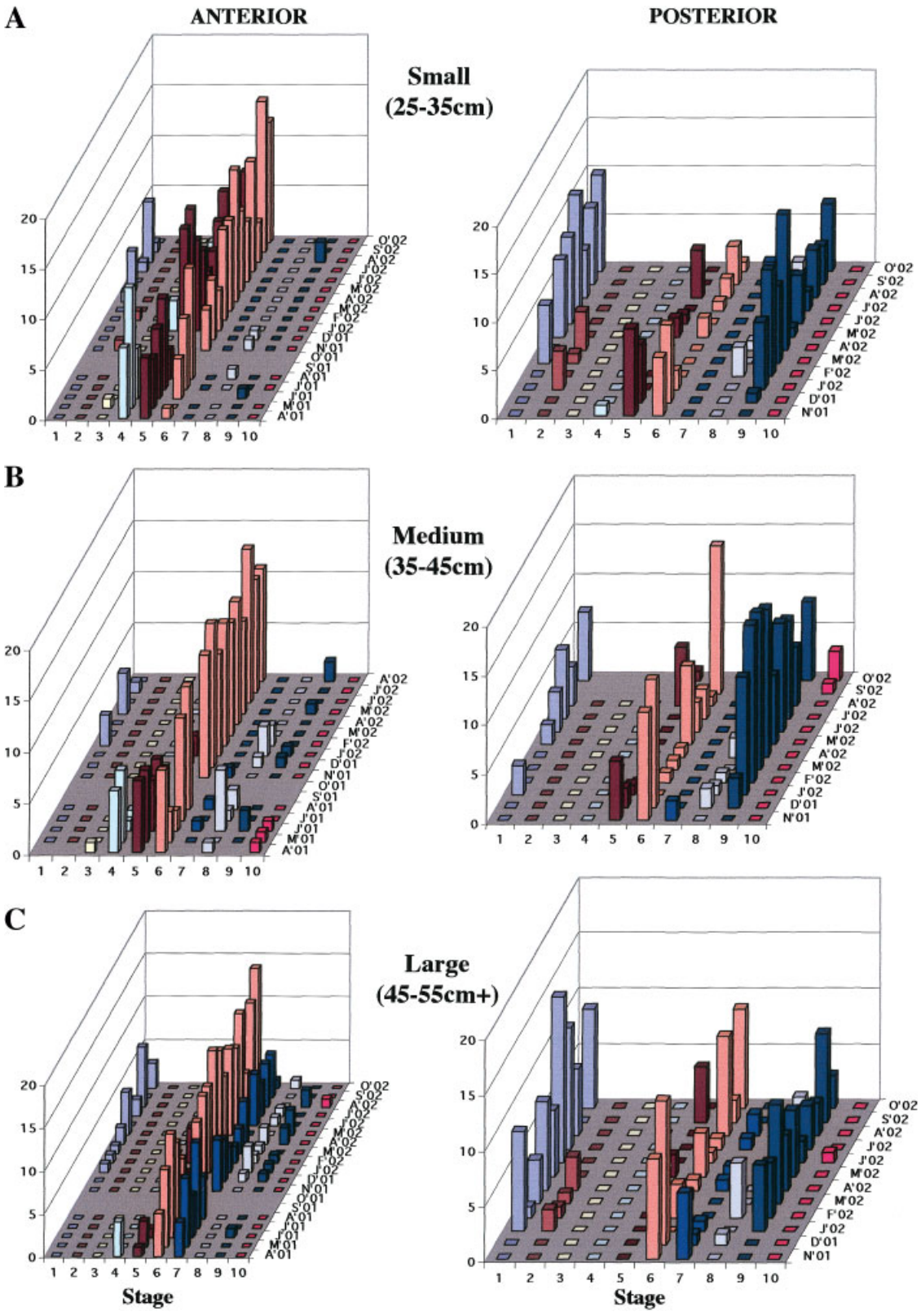

Fig. 3. Occurrence of developmental stages 1–10 in the anterior (muscle somite 44) and posterior (muscle somite 5) gonad of the small (A), medium (B), and large (C) hagfish between April, 2001, and October, 2002, for the anterior gonad and November, 2001, to October, 2002, for the posterior gonad.

(muscle somite 5) of the gonad and differed from stages 1 and 2 in the small hagfish, in that they comprised more vascular and connective tissue.

Reproductive steroids

Estradiol and progesterone were detected in the gonads of all size classes of hagfish examined. For all size classes prior to November, 2001, the gonadal tissue was sampled from only the anterior region. Beginning in November, 2001, through October, 2002, gonadal tissue samples were taken from the anterior (44th muscle somite) and posterior (5th muscle somite). Estradiol and progesterone concentrations in the anterior gonad were lower than those measured in the posterior gonad, but showed similar trends. Only data collected from the incubation of the posterior gonadal tissue (5 muscle somite) are presented for consistency (November, 2001–October, 2002).

Estradiol concentrations

The greatest variability in estradiol concentration was in the small size class (1.8 ± 0.34 to 15.4 ± 3.7 pg estradiol/mg gonad tissue wet wt) (Fig. 4A). Estradiol concentrations in small hagfish from November and December 2001 were significantly higher than all other months except July 2002, which had variability. In January, 2002, estradiol concentrations in the posterior gonad incubation media increased significantly ($p \leq 0.05$) for medium (4.5 ± 2.4 to 11 ± 1.8 pg estradiol/mg gonad tissue wet wt, respectively) size hagfish (Fig. 4B). Estradiol concentrations in the medium size class decreased from 11 ± 1.8 to 3.4 ± 2.4 pg estradiol/mg gonad tissue wet wt between January and February, 2002, and did not return to pre-January levels for the remainder of the sampling period (Fig. 4B). Estradiol concentrations in the posterior gonad incubation media increased significantly ($p < 0.05$) for large hagfish in January, 2002 (0.89 ± 0.62 to 8.2 ± 1.7 pg estradiol/mg gonad tissue wet wt) (Fig. 4C). In large hagfish between January and April, 2002, the estradiol concentration decreased from 8.2 ± 1.7 to 0.73 ± 0.39 pg estradiol/mg gonad tissue wet wt and remained below 4 pg estradiol/mg gonad tissue wet wt through October, 2002 (Fig. 4C). There was no significant difference in estradiol concentrations for the large hagfish between February, 2002, and October, 2002. Based on histological examination of the gonad tissue, all of gonad tissue incubated from the posterior gonad of the medium hagfish contained some male tissue; tissue from the large hagfish was identified as female in stage 5, 6, or 7.

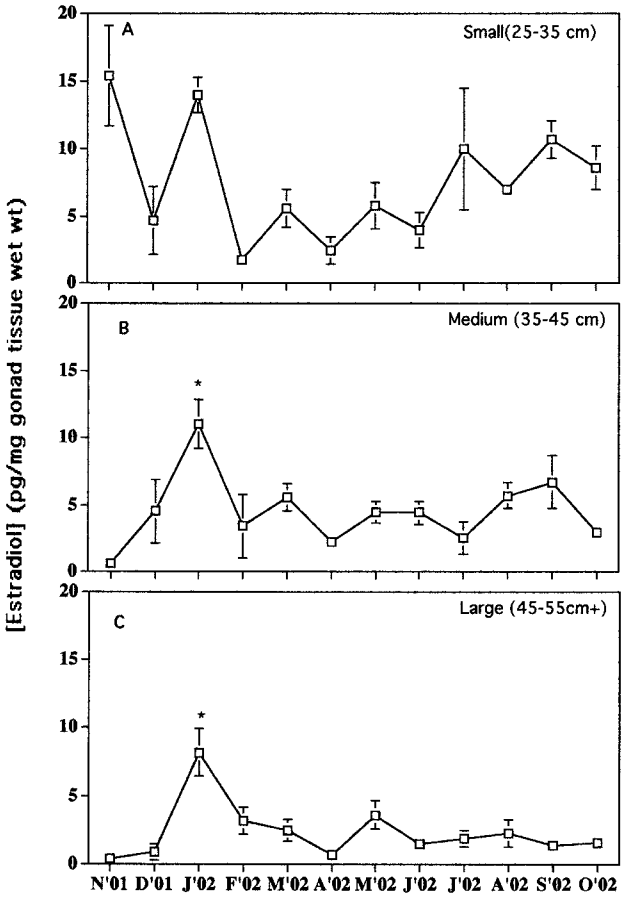

Fig. 4. Concentration of estradiol in the posterior gonad tissue between November, 2001, and October, 2002. Concentration of estradiol was significantly higher (*) in January for medium and large hagfish.

Progesterone

Progesterone concentrations were most variable in the small size class. Progesterone concentrations, similar to those that were observed for estradiol, peaked in the medium and large size classes in January. However, progesterone concentrations were approximately 100 times greater than estradiol. The peak in progesterone concentrations in January, 2002, was 1300 ± 642 pg progesterone/mg gonad tissue wet wt and 2200 ± 1040 pg progesterone/mg gonad tissue wet wt in the medium and large hagfish respectively. However, unlike estrogen concentrations which returned to near baseline values in February, 2002, progesterone concentrations remained elevated until June, 2002, in the medium size class and April, 2002, in the large size class (Fig. 5B, C).

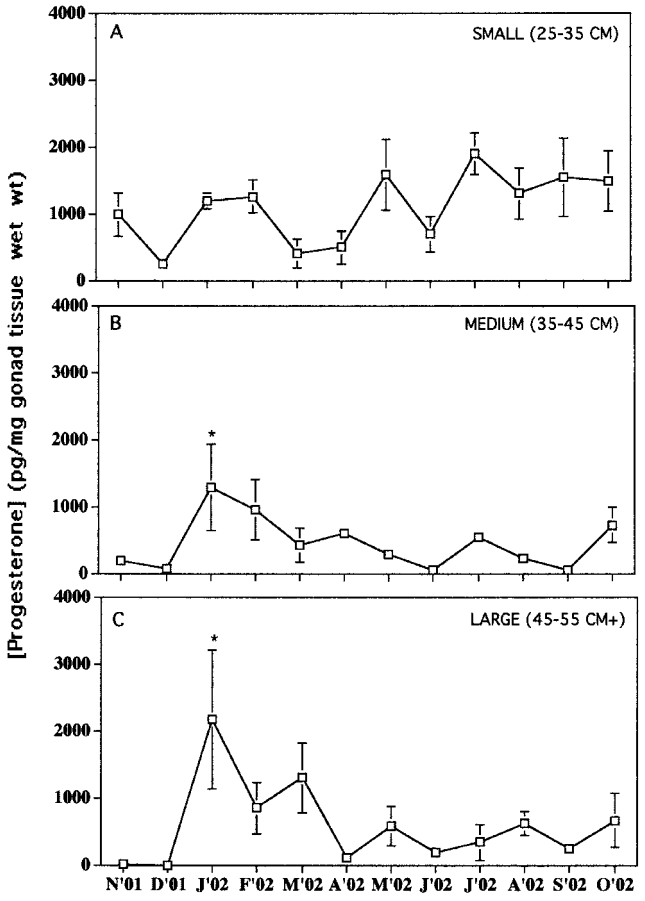

Fig. 5. Concentration of progesterone in the posterior gonad tissue between November, 2001, and October, 2002. Concentration of progesterone was significantly higher (*) in January for medium and large hagfish.

DISCUSSION

The data reported in this study represent the first evidence in support of a seasonal reproductive cycle in *Myxine glutinosa*, and only the second seasonal reproductive cycle identified in a hagfish species. Estradiol was present in the gonad of even the smallest hagfish, suggesting that even early in its development the gonad is capable of steroid synthesis. Schreiner ('55) described germ cells as small as 100 μm in the epithelium of the genital ridge of hagfish. The estradiol concentration in the gonads of all size classes of hagfish had a significant peak in January. The highest estradiol concentrations were measured in the small hagfish, and may be related to gonad growth and development. Estradiol concentrations in the small and medium size hagfish were more variable than in the large hagfish. Histological examination of the gonadal tissue revealed that the posterior region of the gonad incubated for the small size class was female or undifferentiated tissue. The posterior gonadal tissue from the medium size class was frequently male. In male sea lamprey, which spawn only once in their lifetime, plasma estradiol peaked seven distinct times during the final prespawning period, with a significant peak occurring at the time of final spermiation (Sower et al., '85). Estradiol in male hagfish in the present study peaked in January and September, but did not show a correlation with maturation of the sperm after histological analysis. The histology of the gonads from the large hagfish revealed female tissue at varying developmental stages. The greatest increase in the estradiol concentration for the large hagfish occurred in January.

Previously only low levels of progesterone have been reported in the gonad of the hagfish (Hirose et al., '75). The function of progesterone in hagfish is not clear, however, this is the first study to report a significant concentration of progesterone in the gonad of the hagfish. Progesterone is known to have many functions in vertebrates. In the teleost *Rhamodia quelen* peaks of progesterone correspond to spawning episodes (Barcellos et al., 2001). In reptiles, progesterone produced by the corpus luteum inhibits follicular growth and ovulation (Saidapur, '82). In mammals progesterone acts on the uterus to prepare it for pregnancy and acts on the brain centers to prevent additional ovulations (Juengel et al., '99). Yellow bodies present in the hagfish gonad have been identified as *corpus lutea* based on their histology. We are currently studying the steroid production of these bodies to determine their function in the hagfish gonad. Although these steroid data in hagfish could not be directly linked to a spawning event in males or females, the number of large females with eggs 10 mm in length or larger increased in the months following the estradiol peak. In the October sample, 65% of the large hagfish sampled contained eggs 10mm or larger. Schützinger et al. ('87) measured the concentration of estradiol in the plasma of *M. glutinosa* in an attempt to determine if macroscopic reproductive stages and steroid concentration were correlated. His data showed a correlation between increasing plasma estrogens and increasing egg size in female *M. glutinosa* caught in the spring off the coast of Sweden, and a decrease in plasma estrogens after ovulation. Males showed approximately the same plasma concentration of estrogens as females with small eggs (Schützinger et al., '87). However, the presence of estradiol in the plasma of hagfish

without gonads and in juveniles led Schützinger et al. ('87) to hypothesize that estrogens might be synthesized outside the gonad. Our experiments confirmed the production of estradiol in the gonad of juvenile and adult *M. glutinosa*.

Using other techniques, researchers have been able to detect low levels of estradiol, testosterone, and progesterone in *M. glutinosa* and other hagfish species (Tsunkei, '76; Matty et al., '76; Schützinger et al., '87). Ours is the first study to examine steroid concentrations in hagfish gonads over time in a seasonal context and a natural environment. Factors that may influence estrogen concentrations in hagfish are not known and attempts to study reproduction in hagfish in captivity have not been successful. In early studies, Fernholm ('72) attempted to localize possible steroidogenic sites in *M. glutinosa* and concluded that if there is any steroid hormone formation in the ovary of the hagfish, it is extremely small and not detectable by the techniques used. Schützinger et al. ('87) hypothesized that maintaining hagfish in captivity and handling of hagfish might influence steroid biosynthesis, resulting in the low estrogen concentrations reported by other researchers. The hagfish in this study received minimal handling. They were captured at depths of 150–200 m where light and temperature are nearly constant, held in the dark at 4°C and dissected within 48 hr of capture.

At 150 m, environmental cues that might stimulate reproduction, such as light and temperature, are limited. The only hagfish species known to have a seasonal reproductive cycle, *E. burgeri*, is found in depths of 30m, where environmental factors such as light and temperature could influence behavior. Other factors can provide cues to stimulate reproductive cycles. Evidence for seasonal breeding has been found in 20 species of deep-sea fishes, echinoderms, mollusks, and decapod and mysidaean crustaceans that do not encounter changes in light or temperature (Harrison, '88). Seasonality of the breeding pattern in the deep-sea crustacea (Isopoda: Asellota) is synchronized to the seasonal deposition of organic detritus known to occur during the summer in the NE Atlantic (Harrison, '88). These same seasonal cycles may provide reproductive cues to the hagfish. In deep-sea environments, the breeding patterns may not simply be determined by one or only a few environmental factors, but by a complex coordination of the endogenous events within the individuals of populations and within the environment (Sastry, '83).

The results from the current study support previous studies that have shown estradiol is a functional steroid in hagfish. Hagfish have a single class of high affinity, low capacity estrogen binding sites in the hepatic nuclei. The concentration of these nuclear binding sites is highest in vitellogenic hagfish (Dickhoff et al., '85). The increased estrogen observed in January for *M. glutinosa* may bind with hepatic receptors to stimulate vitellogenesis in the female hagfish, eventually resulting in the increased number of females with large eggs observed in the October sample.

Over the past 150 years, only four embryos of *M. glutinosa* have been recovered, and only one was collected from the western Atlantic (Martini et al., '97). Understanding reproduction in this early vertebrate ancestor has the potential to increase our understanding of reproduction in all vertebrates. The identification of a seasonal breeding cycle is a first step of this process.

ACKNOWLEDGEMENTS

We would like to thank Aubry Gorbman for his assistance with analysis of the histology and review of the manuscript. We would also like to thank Cari Bourn, Mihael Freamat, Nathaniel Nucci, Adam Root, Matt Silver, JoAnne Davis, Jen Glieco, Emily Violette, Jocelyn Sanford, Greg Blaisdell, Kara Lee, and Byron Peddler for assistance in collection of animals and processing of samples. Our appreciation also to the crew of the RV Gulf Challenger, Captain Paul Pelletier, Bryan Soares, Debbie Brewitt for the use of the boat and other assistance. This research was supported by UNH/UME NOAA Sea Grant R/FMS–168.

LITERATURE CITED

Barcellos LJG, Wassermann GF, Scott AP, Woehl VM, Quevedo RM, Ittzes I, Krieger MH, Lulhier F. 2001. Steroid profiles in cultured female jundia, the siluridae *Rhamdia quelen* (Quoy and Gaimard, Pisces Teleostei), during the first reproductive cycle. Gen Comp Endocrinol 121:325–332.

Dickhoff WW, Sower SA, Gorbman A. 1985. Hepatic estrogen binding during vitellogenisis in Pacific hagfish *Eptatretus stoutii*. In: Lofts B, Holmes WN, editors. Current Trends in Comparative Endocrinology. Hong Kong: Hong Kong University Press. p 347–349.

Fernholm B. 1972. Is there any steroid hormone formation in the ovary of the hagfish, *Myxine glutinosa*? Acta Zool (Stockh) 53:235–242.

Fukayama S, Takahashi H. 1985. Changes in serum levels of estradiol–17beta and testosterone in the Japanese river

lamprey, *Lampetra japonica*, in the course of sexual maturation. Bull Fac Fish Hokkaido Univ 36:163–169.
Gorbman A. 1983. Reproduction in cyclostome fishes and its regulation. In: Hoar WS, Randall DJ, Donaldson EM, editors. Fish Physiology, vol. IXA. New York: Academic. p 1–29.
Gorbman A. 1990. Sex differentiation in the hagfish *Eptatretus stoutii*. Gen Comp Endocrinol 77:309–323.
Gorbman A. 1997. Hagfish development. Zoolog Sci 14:375–379.
Harrison K. 1988. Seasonal reproduction in deep-sea crustacea (Isopoda:Asellota). J Nat Hist 22:175–197.
Hirose K, Tamaoki B, Fernholm B, Kobayashi H. 1975. In vitro bioconversions of steroids in the mature ovary of the hagfish, *Eptatretus burgeri*. Comp Biochem Physiol 51:403–408.
Juengel JL, McIntush EW, Niswender GD. 1999. Corpus Luteum. In Encyclopeia of Reproduction, vol. 1 A-En. Knobil E, Neill JD, editors. p 703–709. San Diego: Academic Press.
Kime DE, Hews EA. 1980. Steroid biosynthesis by the ovary of the hagfish *Myxine glutinosa*. Gen Comp Endocrinol 42:71–75.
Kime DE, Hews EA, Rafter J. 1980. Steroid biosynthesis by testes of the hagfish *Myxine glutinosa*. Gen Comp Endocrinol 41:8–13.
Koligian KB, Stormshak F. 1977. Nuclear and cytoplasmic estrogen receptors in ovine endocmetrium during the estrous cycle. Endocrinology 101:524–533.
Martini F. 1998. The ecology of hagfishes. In: Jorgensen JM, Lomholt JP, Weber RE, Malte H, editors. The Biology of Hagfishes London: Chapman and Hall. p 57–77.
Martini F, Heiser J, Lesser M. 1997. A population profile for Atlantic hagfish, *Myxine glutinosa* (L.), in the Gulf of Maine. Part 1: Morphometric and reproductive state. Fish Bull 95:311–320.
Matty AJ, Tsuneki K, Dickhoff WW, Gorbman A. 1976. Thyroid and gonadal function in hypophysectomized hagfish, *Eptatretus stoutii*. Gen Comp Endocrinol 30:500–516.
Pickering AD. 1976. Stimulation of intestinal degeneration by oestradiol and testosterone implantation in the migrating river lamprey, *Lampeetra flaviatilis* L. Gen Comp Endocrinol 30:340–346.
Saidapur SK. 1982. Structure and function of postovualtory follicleds (corpora lutea) in the ovaries of nonmammalian vertbrates. Int Rev Cytol 75:243–285.
Sastry AN. 1983. Ecological aspects of reproduction. In: Vernberg FJ, Vernberg WB, editors. Environmental Adaptations, vol. 8. London: Academic Press. p 179–270.
Schreiner K. 1955. Studies on the gonad of *Myxine glutinosa* L. Univ i Bergen Arbok Nat rek 8:1–40.
Schützinger S, Choi HS, Patzner RA, Adam H. 1987. Estrogens in plasma of the hagfish, *Myxine glutinosa* (Cyclostomata). Acta Zool (Stockh) 68:263–266.
Sower SA, Plisetskaya E, Gorbman A. 1985. Changes in plasma steroid and thyroid hormones and insulin during final maturation and spawning of the sea lamprey, *Petromyzon marinus*. Gen Comp Endocrinol 58:259–269.
Sower SA, Schreck CB. 1982. Steroid and thyroid hormones during sexual matureation of coho salmon (*Oncorhynchus kisutch*) in seawater or freshwater. Gen Comp Endocrinol 47:42–53.
Tsuneki K, Ouji M, Saito H. 1983. Seasonal Migration and Gonadal changes in the hagfish, *Eptratretus burgeri*. p 429–440.
Tsunkei K. 1976. Effects of estradiol and testosterone in the hagfish *Eptatretus burgeri*. Acta Zool (Stockh) 57:137–146.
Weisbart M, Dickhoff WW, Gorbman A, Idler DR. 1980. The presence of steroids in the sera of the Pacific hagfish, *Eptatretus stoutii,* and the sea lamprey, *Petromyzon marinus*. Gen Comp Endocrinol 41:506–519.
Yu J, L Y, Dickhoff WW, Swanson P, Gorbman A. 1981. Vitellogenesis and its hormonal regulation in the Pacific hagfish, *Eptatretus stoutii* L. Gen Comp Endocrinol 43:492–502.