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Endocrine Events Associated with Spawning Behavior in the Sea Lamprey (*Petromyzon marinus*)

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Levels of estradiol, progesterone, and testosterone were determined in plasma of sea lamprey (*Petromyzon marinus*) undergoing certain behaviors associated with spawning in natural and artifical stream environments. Significantly higher levels of estradiol, progesterone, and testosterone were found in males than in females. In the artifical spawning channel, levels of estradiol were significantly higher in females exhibiting resting and swimming behaviors than in fanning, nest building, and spawning behaviors. No significant correlation was found with either progesterone or testosterone levels and the various reproductive behaviors. The data presented are the first experimental evidence that suggest gonadal steroids may be correlated with certain reproductive behaviors in the sea lamprey.

Endocrine control of reproductive behavior has been studied extensively in teleost fish. However, similar studies have not been conducted on Agnathans, the most primitive fish.

Regulation of sexual development mediated through the hypothalamo–hypophysial–gonadal axis has been well defined in vertebrates. The Agnathans, unlike most other vertebrates, lack a hypothalamo–hypophysial portal vascular or innervation system (Gorbman, 1965). Although lampreys lack vascular or neural connections between the hypothalamus and adenohypophysis, the control of the hypothalamus by the pituitary is probable by diffusion of GnRH across the connective tissue to the adenohypophysis (Gorbmam, 1965; Ball, 1981; Nozaki, Tsukahara, and Kobayashi, 1984). The adenohypophysis of lampreys exerts some influence on gonadal tissues at various reproductive phases, based on experiments involving hypophysectomies in adult European river lamprey, *Lampetra fluviatilis* (Evenett and Dodd, 1963; Larsen, 1965, 1980). These studies indicate that a pituitary–gonadal relationship exists in this group. Thus, it may

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be reasonable to assume that certain hormones associated with the reproductive processes in the lamprey may also be associated with reproductive behavior as in other vertebrates.

The sea lamprey reproduces only once in its lifetime, after which it dies. Gametogenesis progresses synchronously as a single event during the lamprey's life cycle. Larval lampreys develop in freshwater streams, migrate to the sea or lake after transformation, and return to streams as adults to spawn. During the time at sea, the lampreys are in their parasitic phase; females are undergoing vitellogenesis and males are undergoing spermatogonia proliferation and development of primary and secondary spermatocytes. Following this period, the lampreys migrate to the streams and undergo their final maturational and spawning phase over a period of 2–3 months. During the final maturation of gonads of both sexes, the steroids, estradiol (Katz, Dashow, and Epple 1982; Sower, Plisetskaya, and Gorbman 1985a,b; Fukayama and Takahashi, 1985), progesterone (Sower, King, Millar, Sherwood, and Marshak, 1987), and testosterone (Larsen, 1974; Weisbart, Dickhoff, Gorbman and Idler, 1980; Sower, Plisetskaya, and Gorbman, 1985b) are considered to be associated with reproductive activity.

Autoradiography studies have established the presence of estrogen target cells in various areas of the lamprey brain providing evidence for the action of gonadal steroids in the brain in the same manner known for other vertebrates (Kim, Stumpf, Reid, Sar, and Selzer, 1980; Reid, Kim, Stumpf, Selzer, and Sar, 1980). These studies indicate a possibility of feedback of steroid hormones affecting brain function of lampreys. The function of this feedback system could be twofold: (1) steroids could act at the hypothalamus or pituitary level to affect the release of gonadotropin-releasing hormone or gonadotropin(s) and (2) steroid hormones could act on the brain to affect reproductive behavior.

There are several studies that have been reported on the reproductive behavior of lampreys (Applegate, 1950; Thomas, 1960; Hanson and Manion, 1978; Manion and Hanson, 1980). These researchers have described normal spawning and nest building behaviors, male and female interactions, fecundity, and timing of maturation. However, there have been no studies concerning the possible endocrine influences on lamprey reproductive behavior. Therefore, the main objectives of this research were to determine steroid levels in plasma of spawning sea lampreys and to correlate these levels with distinct reproductive behaviors associated with nest building and spawning in both natural and artificial stream environments.

METHODS AND MATERIALS

Field and laboratory studies were conducted between June 5 and July 28, 1984 to study lamprey spawning behavior. The field study was conducted in the Ocqueoc River, Presque Isle County, Michigan, and the laboratory study was conducted at the Hammond Bay Biological Station, Michigan.

Description of Behaviors

Six types of behavior exhibited by lampreys during the spawning period were studied. These behaviors were based on Applegate's (1950) description of spawning lampreys and included resting, swimming, fanning, nest building, the spawning act, and male aggressive behavior.

Resting. Lampreys remain motionless in the nest and are usually attached to a rock with their oral sucker. This behavior was exhibited by both sexes between spawning acts and by spent individuals.

Swimming. Swimming is an undulating movement of the entire body. This behavior varied from a slow persistent movement to a rapid burst of speed.

Fanning. This behavior occurs during nest building and between spawning acts. The lamprey attaches to a rock with its oral sucker, turns on its side, and with rapid vibratory movements, stirs up the substrate and deposits a layer of silt and sand over the newly spawned eggs. Both males and females participate in fanning the nest.

Nest building. Nest construction consists partly of the removal of sand, rocks, and debris from a circular area in a section of stream with a unidirectional flow of water. Nest building is usually initiated by the male after which one or more females join him and assist in nest building. After spawning begins, both sexes continue to move rocks between spawning acts. The lamprey grasps onto a rock with its oral sucker and, with slow twists and undulations, deposits it at the lower edge of the nest where a crescent-shaped ridge is formed (Fig. 1). The ridge serves to catch drifting, newly spawned eggs and provides protection for developing embryos.

Spawning act. The female grasps onto a large stone with her oral sucker and the male moves up her body with his mouth and fastens

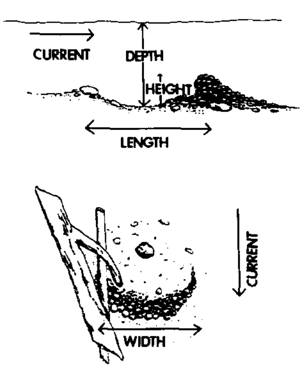

FIG. 1. Morphology of a representative sea lamprey nest: side and overhead views including measurement parameters.

firmly to her head. This stimulates the female to arch her body and begin strong vibratory movements, at which time the male wraps his tail around the posterior half of her body bringing the two gonopores into apposition. Both individuals then vibrate and contract their bodies, releasing eggs and sperm simultaneously. The number of eggs released per spawning act has been estimated at 20 to 40 eggs (Applegate, 1950). The entire spawning act lasts from 2 to 5 sec and occurs numerous times throughout the 2- to 3-day spawning period.

Aggressive behavior. Observed only in males, this behavior consists of attaching to another male (occasionally a female), shaking it vigorously, and forcibly removing it from the nest. At times, they may drift a considerable distance downstream, firmly attached to each other. Eventually, one usually returns to the nest to continue spawning.

Sampling Procedure

A lamprey exhibiting one of the six behaviors was immediately taken from the nest and a blood sample (300–500 μl) was collected by cardiac puncture using a heparinized tuberculin syringe and a 1 × 21-gauge needle. The lamprey was then anesthetized by being placed in water containing 13 mg/liter of tricaine methylsulfonate (MS 222) for approximately 3–5 min. At that time, the blood sample was transferred to a 1.5-ml heparinized centrifuge tube and placed on ice. Samples were centrifuged within 1 to 2 hr and the plasma was separated and frozen at −20°C. Statistical analyses were performed only on samples taken at the same time each day.

Following the blood sampling, the anesthetized animal was sexed, weighed, and measured. Females were checked for degree of maturity by applying gentle pressure to the abdominal wall to determine degree of softness and presence of ovulated eggs. Male maturity was not determined. A Floy tag was injected into the muscle just posterior to the second dorsal fin and the dorsal fins were clipped in a distinctive pattern to allow easy identification of the individual in the event of tag loss. The lampreys were usually handled for no more than 60–70 sec during the tagging procedure; any longer period of time was considered as an increased stress to the animal.

Field Study

The study site is located approximately 3 miles upstream from the mouth of the Ocqueoc River which flows into Lake Huron, and approximately 0.5 mile downstream from Ocqueoc Lake. The river ranges from 8 to 10 m in width in the study area and consists of a series of small riffles with gravel and sand substrate.

Observations of spawning lampreys were made daily from June 9 to July 20, and every other day from July 21 to July 28. One to three observation times per day were chosen randomly between 0700 and 2100 hr. Lamprey nests were mapped and measured and stream temperature, depth, and velocity were measured during each sampling period.

Each observation period was initiated by examining the nests. Number, sex, position, and behavior of each lamprey were noted. Detailed observations were made on some individuals to determine behavioral patterns and spawning frequencies for different nest situations, temperatures, and times of day. Observations were made using a diving mask to improve visibility so that individual males and females could be distinguished and tagged individuals could be identified.

Laboratory Study

An artificial spawning channel, 4.8 m long, 0.65 m wide, and 0.5 m high, was constructed in a cement raceway at the Hammond Bay Biological Station (Fig. 2). The channel was filled with gravel (ranging from 0.5 to 7.0 cm in diameter) from a nearby Lake Huron beach, to a depth of 10 cm. Two 4-in. diameter logs were placed in the stream to create areas of varying current velocities. The channel was lighted by two fluorescent lights from 0730 to 1700 hr and by some indirect sunlight. Lake Huron water, ranging in temperature from 8.5 to 17.5°C, was delivered to the head of the channel and maintained at a depth of 15–20 cm and a velocity of 0.2 m/sec, which was later increased to 0.5 m/sec by means of inlet gates to help stimulate spawning activity.

Thirty female and thirty male sea lampreys trapped from the Ocqueoc and Cheboygan Rivers were placed in the artificial channel. All dead lampreys were replaced in order to maintain optimal spawning activity. During periods of low spawning activity, spawning lampreys collected from nests in nearby rivers were placed in the channel.

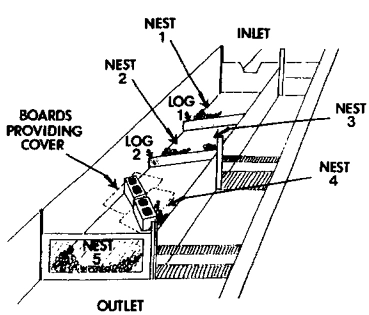

Fig. 2. Artificial spawning channel constructed in a raceway at the Hammond Bay Biological Station, including nest appearance and locations.

Assays

Estradiol, progesterone, and testosterone were measured by radioimmunoassay using specific antibodies. The assays had been previously validated for adult sea lamprey plasma by Sower, Dickhoff, Gorbman, Rivier, and Vale (1983) and Sower, King, Millar, Sherwood, and Marshak (1987). The lower limit of detection for the assays was approximately 60 pg/ml. The interassay and intraassay coefficients of variation were 1.7% (n = 16) and 5.2% (n = 9) for the estradiol assay, 1.8% (n = 6) and 4.3% (n = 7) for progesterone, and 1.4% (n = 6) and 5.5% (n = 9) for testosterone. Estradiol, progesterone, and testosterone were measured in 100 μl of plasma (in duplicate when possible) from both females and males.

Antibodies of estradiol, testosterone, and progesterone were obtained from Dr. G. Niswender, Colorado State University, Ft. Collins, Colorado. All antibodies were diluted in phosphate-buffered saline–gelatin and had binding efficiencies of 55, 35, and 47%, respectively. Estradiol [2,4,6,7-$^3H(N)$], progesterone [1,2,6,7-$^3H(N)$], and testosterone [1,2,6,7-$^3H(N)$], were obtained from New England Nuclear.

Statistical Analyses

One-way analysis of variance was used to determine significant differences in steroid levels associated with behavior and sex of the lampreys in the artificial or natural stream environments. Regression analysis and analysis of variance were used to determine relationships between steroid levels and maturity, temperature, time of day, and season. Tests of significance were conducted at the $P < 0.05$ level.

RESULTS

The average velocity of the Ocqueoc River in the study area was 0.71 ± 0.05 m/sec, and the average depth at midstream was 0.43 ± 0.08 m. Midstream depth varied from 0.25 m to over 0.8 m with rainfall. A total of 46 nests were monitored during the study. The nests ranged from 0.40 to 1.30 m (ave., 0.68 m) in length and from 0.32 to 1.57 m (ave., 0.61 m) in width.

Spawning and nest building behaviors in the artificial channel were similar to those observed in the field study. Five nests were built in the channel with the majority of individuals spawning in one large nest at the lower end (Fig. 2).

The interval between spawning acts varied from 20 sec to over 10 min in a total of 40 timed observations. There appeared to be a correlation between the number of females found with a single male in a nest and the interval between spawning acts. The average interval for a monogamous male–female pair was 4.3 ± 0.33 min. One male with two females showed an interval of 2.8 ± 0.27 min, and one male with four females had an interval of 1.8 ± 0.23 min.

Radioimmunoassays

Plasma samples from 224 males and 185 females were measured for estradiol; samples from 41 males and 65 females were measured for progesterone; and samples from 99 males and 32 females were measured for testosterone.

When combining all samples regardless of behavior, estradiol levels were significantly higher in males than in females (means ± SE: 1.75 ± 0.04 ng/ml vs 1.09 ± 0.05 ng/ml). Mean progesterone and testosterone levels in males (1.25 ± 0.08 ng/ml and 0.10 ± 0.01, respectively) were also significantly higher than in females (0.27 ± 0.08 ng/ml and 0.05 ± 0.01). Samples consistently contained very low or undetectable levels of testosterone in both sexes.

As the female approached maturity and ovulation, there was a significant decrease in circulating estradiol levels, with the lowest levels (0.82 ± 0.13 ng/ml) occurring in spent females (Fig. 3). Progesterone levels did not change significantly at the different stages of maturity. Testosterone levels increased slightly as the female lampreys matured.

The steroid levels associated with specific behaviors for males and females in the artificial spawning channel and the Ocqueoc River are

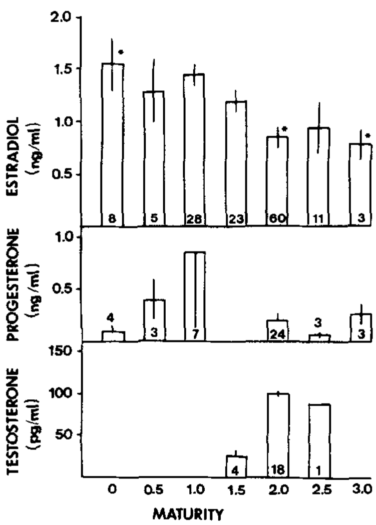

FIG. 3. Relative mean concentrations of estradiol, progesterone, and testosterone associated with determined maturity indices; 0—females that are 1–2 months from ovulation; 0.5—slight softening of abdominal cavity (approximately 1 month from ovulation); 1.0—increased softening; 1.5—mature preovulated female; 2.0—ovulated female; 2.5—one half of egg mass shed during spawning; 3—spent female. These values represent steroid levels of lampreys from both the artificial channel and Ocqueoc River. Error bars represent SE. Starred bars are significantly different from each other. Females at the 0 through 1 maturity levels had nondetectable levels of testosterone and were not included in the graph.

presented in Figs. 4 and 5. Significant differences were found only in estradiol levels with different behaviors in the females in the artificial channel (Fig. 4) and in males in the Ocqueoc River (Fig 5). Estradiol levels in females were lowest (0.59 ± 0.13 ng/ml) when associated with fanning behavior in the channel. These levels, however, were not significantly different from those associated with the spawning act (0.82 ± 0.12 ng/ml) or nest building behavior (1.05 ± 0.12 ng/ml). Resting females had significantly higher estradiol levels than those exhibiting other behaviors. Estradiol levels in swimming females were significantly higher than in females exhibiting spawning and fanning behaviors. In the stream, males exhibiting fanning behavior had significantly lower estradiol levels than all of the other behaviors. Nest building behavior was also associated with significantly lower estradiol levels than resting, swimming, spawning act, or aggressive behavior.

DISCUSSION

Endocrine control of reproductive behavior in higher vertebrates is believed to be mediated by the hypothalamus which has the capacity to integrate signals from internal stimuli such as steroid feedback and stimuli from the external environment. Recent studies have indicated hypothalamic influence in the control of certain reproductive processes in lamprey (Sower, Dickhoff, Gorbman, Rivier, and Vale, 1983; Sower, Plisetskaya,

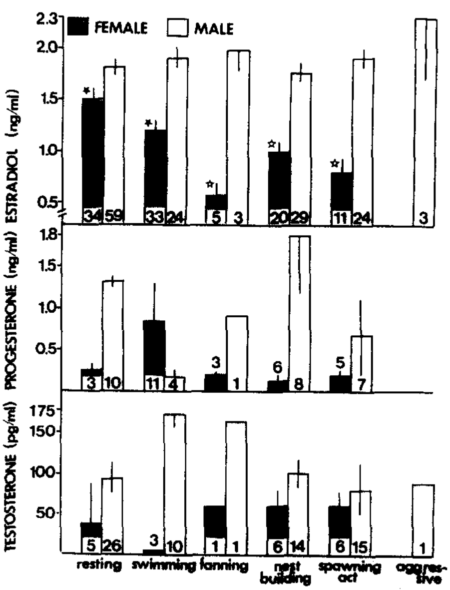

FIG. 4. Concentrations of estradiol, progesterone, or testosterone associated with behavior of lamprey in the artificial spawning channel. Bars with dissimilar symbols are significantly different.

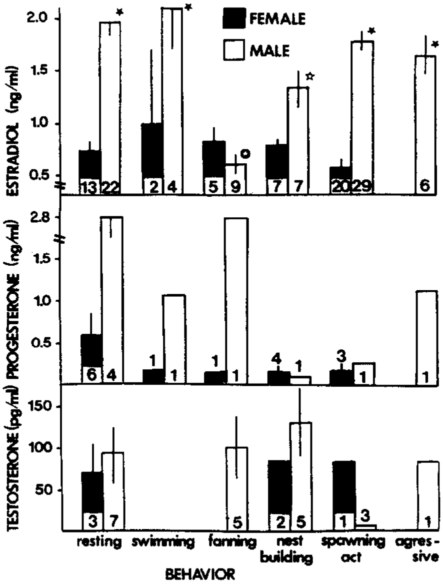

FIG. 5. Concentrations of estradiol, progesterone, or testosterone associated with behavior of lamprey in the Ocqueoc River. Bars with dissimilar symbols are significantly different.

and Gorbman, 1985a), which suggests that there may be hypothalamic involvement in certain reproductive behaviors of these animals. The data presented here comprise the first experimental evidence that gonadal steroids, particularly estradiol, may be correlated with certain reproductive behaviors in the sea lamprey.

The highest estradiol levels were associated with resting behavior and the lowest levels were associated with fanning, nest building, and spawning in females in the artificial stream, and were lowest in the males exhibiting fanning and nest building in the natural stream. These data indicate estradiol may be involved in certain reproductive behaviors in both male and female sea lampreys. The lack of significant correlations of estradiol levels with behavior of females in the natural stream and of males in the artificial channel is interesting and merits further study.

There were higher levels of estradiol in males regardless of the behavior exhibited. This agrees with Sower *et al.* (1985b) and Fukayama and Takahashi (1985) who found that estradiol levels in the final maturation stages of the sea lamprey and the Japanese river lamprey, *Lampetra japonica,* respectively, were very similar in males and females until the time of spermiation and ovulation at which time estradiol levels dropped dramatically in the females and rose in the males. These data suggest a possible role of estradiol in male sexual behavior in the sea lamprey.

Testosterone levels did not vary significantly in lamprey exhibiting different types of behaviors, however, testosterone was found to be significantly higher in males than in females. Testosterone is known to be involved in male aggressive behavior in many vertebrates. It is not known from this study whether testosterone may be involved in agonistic behaviors because testosterone was only measured in one male exhibiting aggressive behavior.

Progesterone levels were not correlated with distinct reproductive behaviors in either sex. However, as with estradiol and testosterone, progesterone levels were significantly higher in males which may indicate a potential role of progesterone in the final reproductive processes (other than behavior) in the male sea lamprey.

Steroidal control of spawning behavior in the sea lamprey may be possible. Short-term changes in steroid levels associated with changing behaviors have been verified in vertebrates (Moore and Muller, 1977; Harding, 1981; Wingfield, 1984; Ramenofsky, 1984). However, these studies were conducted on species whose endocrine profiles had been previously determined. In the lamprey, estradiol, progesterone, and testosterone may not be the only steroids involved in reproductive behavior. In plasma of river lamprey and in sea lamprey, the 15 hydroxylated steroids were identified (Kime and Rafter, 1981; Kime and Gallard, 1982). The role of these steroids is unknown and merits further study. Stacey (1976) suggested that the prostaglandins may be directly involved in goldfish spawning behavior. It would be of interest to conduct similar studies on sea lamprey. Other studies have showed the importance of neurohypophyseal hormones in the reproductive behavior of newts, *Tricha granulosa* (Moore and Miller, 1983; Moore, Miller, Spielvogel, Kubiak, and Folkers 1982). The existence of a GnRH molecule in the brain of lampreys (Sherwood and Sower, 1985; Sherwood, Sower, Marshak, Fraser, and Brownstein, 1986) and the evidence for lamprey responsiveness to exogenous administration of lamprey GnRH and salmon GTH (Sower, Dickhoff, Gorbman, Rivier, and Vale, 1983; Sower, King, Millar, Sherwood, and Marshak, 1987) indicate GnRH or GTH may influence behavior as shown in studies by Cheng (1977) and Moore *et al.* (1982).

In the Ocqueoc River study, there was a reduced time interval between spawning acts in nests with large numbers of females per male, indicating that it may be the female that controls the timing and frequency of spawning acts. The female will either accept or reject the grasping of the head by the male which signifies the start of the spawning act. The females were normally only responsive to the male's attempt to grasp her head following completion of her nest building behavior. The female was unreceptive to any attempts by the male at any other time. This pattern was also noted in the artificial channel even though the interval between spawning acts was almost twice as long as in the Ocqueoc River. The difference in the interval may have been caused by the limited space and lower water temperature in the artificial channel.

Photoperiod is believed to influence gonadal maturation and migration of lampreys (Bertmar, 1985), however, it seems to have a minor role in specific reproductive behaviors. During their upstream migration, lampreys are the most active at night (Applegate, 1950; Bertmar, 1985), while during the spawning period, they are more active during the day (Bertmar, 1985).

Temperature appears to be an important environmental factor for initiating spawning activity as shown in studies on the European river lamprey (Bertmar, 1985) and in the present study. Decreased spawning activity associated with sudden drops in temperature have been noted by Applegate (1950) and Manion and Hanson (1980). In our study, lampreys were transferred from the Ocqueoc River with a water temperature of 22°C to the artificial channel that had a temperature of 12°C on June 17 over a 2-hr acclimation period. Accompanied with this drastic change of temperature was a sudden and prolonged decrease in level of activity. Lampreys in the artificial spawning channel were exposed to unusually large fluctuations in temperature ranging from 8 to 17°C which seemed to strongly suppress the levels of activity of reproductive behaviors, compared to lampreys having high levels of activity in the Ocqueoc River with water temperatures remaining around 20–22°C. Further studies are warranted to examine the influence of temperature on reproductive behavior.

Stream velocity appears to be another factor that may influence spawning behavior. While water temperature was the critical factor in the maturation of lampreys, the onset of spawning behavior did not occur until, along with higher temperature, the water velocity in the artificial channel was increased to 0.5 m/sec. Manion and Hanson (1980) found that a velocity of 0.5 m/sec was the minimum velocity at which lampreys will spawn; our data support that view.

In conclusion, steroids appear to be correlated with certain lamprey reproductive behaviors. Clearly, further studies including gonadectomy, hormonal replacement therapy, and measurement of other hormones are necessary to further elucidate the role of the endocrine system in the control of reproductive behavior in the sea lamprey.

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