General and Comparative Endocrinology 170 (2011) 276–282

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Seasonal changes of brain GnRH-I, -II, and -III during the final reproductive period in adult male and female sea lamprey

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ARTICLE INFO

Article history:
Received 28 May 2010
Revised 2 August 2010
Accepted 6 August 2010
Available online 13 August 2010

Keywords:
Lamprey gonadotropin-releasing hormone-I
Lamprey gonadotropin-releasing hormone-II
Lamprey gonadotropin-releasing hormone-III
GnRH
Lamprey
Petromyzon marinus
Seasonal changes of GnRH
Estradiol
Ovarian and testicular morphology
Temperature changes on neuroendocrine system

ABSTRACT

Sea lampreys are anadromous and semelparous, i.e., they spawn only once in their lifetime, after which they die. Sexual maturation is thus a synchronized process coordinated with the life stages of the lamprey. Recently, a novel gonadotropin-releasing hormone, lamprey GnRH-II (lGnRH-II), was identified in lampreys and suggested to have a hypothalamic role in reproduction (Kavanaugh et al., 2008). To further understand the role of lGnRH-II, changes in ovarian morphology, brain gonadotropin-releasing hormone (lGnRH-I, -II, and -III), and plasma estradiol were examined during the final two months of the reproductive season of adult male and female sea lamprey. The results showed significant correlations between water temperature, fluctuation of brain GnRHs, plasma estradiol and reproductive stages during this time. In males, lGnRH-I concentration increased early in the season, peaked, then declined with a subsequent increase with the final maturational stages. In comparison, lGnRH-II and -III concentrations were also elevated early in the season in males, dropped and then peaked in mid-season with a subsequent decline of lGnRH-II or increase of lGnRH-III at spermiation. In females, lGnRH-III concentration peaked in mid-season with a drop at ovulation while lGnRH-I remained unchanged during the season. In contrast, lGnRH-II concentrations in females were elevated at the beginning of the season and then dropped and remained low during the rest of the season. In summary, these data provide evidence that there are seasonal and differential changes of the three GnRHs during this final reproductive period suggesting specific roles for each of the GnRHs in male and female reproduction.

1. Introduction

Sea lampreys (*Petromyzon marinus*) are anadromous and semelparous, i.e., they spawn only once in their lifetime, after which they die. The sea lamprey begin their lives in freshwater as blind, filter-feeding larvae called ammocoetes. After approximately five to seven years in freshwater streams, metamorphosis occurs and they become free-swimming, sexually immature lamprey. Lamprey metamorphosis is a highly synchronized and programmed process that involves major physiological and morphological changes, reviewed in Youson and Sower (2001). In the parasitic sea lamprey, sexual maturation is a seasonal, synchronized process. During the approximately 15 month-long parasitic sea phase, gametogenesis progresses. In males, spermatogonia proliferate and develop into primary and secondary spermatocytes, in females, vitellogenesis occurs. After this period, sea lampreys return to freshwater streams and undergo the final maturational processes resulting in mature eggs and sperm, spawning and then death. Lampreys are seasonal and temperature-responsive in the timing of these migrations, maturation, and reproduction (Bolduc and Sower, 1992; Fahien and Sower, 1990; Linville et al., 1987; Sower, 2003; Sower et al., 1983).

The life stages and reproductive processes are coordinated by the neuroendocrine axis through a number of different hormones. As previously described, there are progressive changes in hormonal levels over time that occur in a changing endocrine environment (Sower, 2003; Sower and Kawauchi, 2001). As an indication of the complex simultaneous processes in lampreys undergoing metamorphosis, there is an increase of brain lamprey gonadotropin-releasing hormone -I and -III (lGnRH-I and -III) that coincides with the acceleration of gonadal maturation (Youson and Sower, 1991). In this Youson and Sower paper, lamprey GnRH-III was called the 2nd form. Subsequent studies confirmed that the lamprey 2nd form was lamprey GnRH-III. There is relatively little known of the circulating reproductive hormones coinciding with the reproductive processes during the parasitic phase. However, in adult lampreys, there are seasonal correlations between changes in brain GnRH-I and gametogenic and steroidogenic activity of the gonads in adult male and female sea lampreys (Bolduc and Sower,

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doi:10.1016/j.ygcen.2010.08.005

1992; Fahien and Sower, 1990). In these studies, lamprey GnRH-II or III had not yet been isolated and sequenced. Lamprey GnRH-III was identified in 1993 (Sower et al., 1993). Following the identification of lamprey GnRH-III, it was subsequently shown that lGnRH-III is present in higher concentrations than lGnRH-I during the final stages maturation of adult female lampreys while both lGnRH-I and -III are potent in stimulating ovulation and spermiation (Deragon and Sower, 1994; Gazourian et al., 2000; MacIntyre et al., 1997). These results suggested that lamprey GnRH-III may be a major form regulating reproductive processes in the female sea lamprey during the period of final reproductive maturation.

Recently, a novel GnRH, lamprey GnRH-II (lGnRH-II), was identified in lampreys and suggested to have a hypothalamic role in reproduction (Kavanaugh et al., 2008). Similar to GnRH-II expression in gnathostomes, lamprey GnRH-II was shown to be widely distributed and expressed in a number of tissues. In the brain, lamprey GnRH-II was shown to be located in preoptic area/hypothalamus by *in situ* hybridization and immunocytochemistry. To examine whether the lamprey GnRH-II may have a hypothalamic role, biological studies and functional receptor studies were performed (Kavanaugh et al., 2008). Both lamprey GnRH-I and -III have been shown by extensive functional, physiological, and immunocytochemical studies to control the pituitary–gonadal axis (reviewed in Sower, 2003). As in previous studies, plasma estradiol was measured in the lamprey as potential indicator of pituitary responsiveness to GnRH (Sower et al., 1993). In the Kavanaugh et al., 2008 studies, lamprey GnRH-II was biologically active as determined in stimulating the hypothalamic-pituitary axis using *in vivo* and *in vitro* studies. Lamprey GnRH-II was also shown to activate the inositol phosphate signaling system in COS-7 cells transiently transfected with the lamprey GnRH receptor in these same studies. We proposed from this research that the novel lamprey GnRH-II has a role as a third hypothalamic GnRH. To further understand the potential function of lGnRH-II as a hypothalamic neurohormone, changes in ovarian morphology, brain gonadotropin-releasing hormone (lGnRH-I, -II, and -III), and plasma estradiol were examined during the final two months of the reproductive season of adult male and female sea lamprey.

2. Methods and materials

For these studies, a total of 160 adult sea run lamprey were collected at the Cocheco River fish ladder in Dover, NH from May 22–29, 2008 during their spawning migration from the sea to freshwater. The lamprey were then transported to the Anadramous Fish and Invertebrate Research Laboratory in Durham, NH, where they were maintained in an artificial spawning channel with flow-through reservoir water at ambient temperatures 9–18 °C and natural photoperiod following University of New Hampshire Institutional Animal Care and Use Guidelines. From the artificial channel, the lampreys were sampled once a week during 8 weeks for blood, brain, and gonadal tissue from May 22, 2008 (Week 1) until July 8, 2008 (Week 8).

2.1. Sampling procedures

Twenty lampreys (10 males and 10 females) were sampled each week at the same time in the morning. Each lamprey was removed from the artificial spawning channel and sampled for blood, brain, and gonadal tissue as previously described (Bolduc and Sower, 1992; Fahien and Sower, 1990; Sower et al., 1993). The plasma was separated from the blood and stored at −80 °C until extracted and assayed for estradiol by RIA. Immediately after blood sampling, each lamprey was decapitated, and the brain was removed, flash frozen in liquid nitrogen and placed on dry ice. Individual brains were stored at −80 °C until assayed for each of the GnRHs (lamprey GnRH-I, -II, and -III).

Following decapitation, gonadal tissue was collected from each lamprey, placed in Bouin's solution and processed for histological sections using hemotoxylin and eosin as previous described (Bolduc and Sower, 1992; Fahien and Sower, 1990). The classification of maturation was used as described in Fahien and Sower (1990) and Bolduc and Sower (1992). Four viewing fields were examined and recorded for maturation. The maturation of the testis was divided into seven stages as follows: stage 1 = primary spermatocytes, stage 2 = primary and dividing spermatocytes, stage 3 = primary spermatocytes through spermatids, stage 4 = spermatids and immature sperm, stage 5 = immature sperm, stage 6 = immature sperm and mature sperm, and stage 7 = mature sperm. The maturation of the ovaries were divided into four stages as follows: stage 1 = close association of the follicular envelope and oocyte, stage 2 = initial separation of follicular layer from oocyte, stage 3 = complete separation of the follicular layers from the oocyte, stage 4 = oocyte is no longer associated with the follicular cells.

2.2. Extraction and HPLC

Individual brains and plasma samples were extracted as previously described by Yu et al. (1987). Individual extracts (15 μl) were injected into a 20 μL loop on an HPLC system, which consisted of a Perkin–Elmer Series 100 pump with Pecosphere 3 CR C18 (0.46 × 8.3 cm) reverse-phase column as previously described (Fahien and Sower, 1990). About 110 fractions (1 ml) were collected for each sample. HPLC water was injected onto column after each sample. Duplicate 100 μl aliquots of the peak fractions from each sample were assayed by RIA for lGnRH-I and -III or lGnRH-II.

2.3. Radioimmunoassays

Plamsa estradiol-17β was measured in duplicate 100 μl aliquots of plasma by radioimmunoassay (RIA), as described by Sower and Schreck (1982) and validated for lampreys as described by Sower et al. (1983, 1987). Radioimmunoassays for lamprey GnRH-I, or -III were performed on the HPLC fractions (fractions 1–15) as previously described in Macintyre et al. (1997) and Stopa et al. (1988). Lamprey GnRH-III antiserum (3952) was used at a final dilution of 1:16,000. RIA sensitivity was 19.5 pg/ml. The range of antiserum binding was 46.5–68.9%. Standards used were lamprey GnRH-III. Synthetic lamprey GnRH-I was iodinated using a modification of the chloramine-T method and purified as described in Stopa et al. (1988).

2.4. Lamprey GnRH-II RIA

Lamprey GnRH-II was purchased from American Peptide Co., Inc. (Sunnyvale, CA). Polyclonal antiserum production to lamprey GnRH-II was done by Colcalico Biologicals (Reamstown, PA) following conjugation to BSA via glutaraldehyde and characterized as described in Kavanaugh et al. (2008). The RIA was characterized for lamprey GnRH-II. Lamprey GnRH-II antiserum (135-66) was used at an initial dilution of 1:240,000. RIA sensitivity was 9.8 pg/ml. The range of binding was 43.8–55.8%. Standards used were lamprey GnRH-II. Synthetic chicken GnRH-II (American Peptide Co. Inc., Sunnyvale, CA) was iodinated using a modification of the chloramine-T method and purified as described in Stopa et al. (1988). Lamprey GnRH-II does not have a Tyr in its primary amino acid sequence; therefore chicken GnRH-II (Type 2) was iodinated and characterized for the lamprey GnRH-II assays. It was determined by testing spiked samples with known quantities of GnRH that the use of this radioligand in the RIA detected greater than

Stages of Final Maturation

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Male	III-IV	IV-VI	V-VI	V-VII	V-VII	VI-VII	VI-VII	VI-VII
Female	I	I-II	II-III	III-IV	III-IV	III-IV	III-IV	IV

Female
Stage I: Close association of follicular envelope and oocyte
Stage II: Initial separation of the follicular layers from the oocyte
Stage III: Complete separation of the follicular layers from the oocyte
Stage IV: Ovulation

Male
Stage I: Primary spermatocyte
Stage II: Primary and dividing primary spermatocyte
Stage III: Primary spermatocyte to spermatid
Stage IV: Spermatid and immature sperm
Stage V: Immature sperm
Stage VI: Immature and mature sperm
Stage VII: Mature sperm

Fig. 1. This figure depicts the average range of stages of final maturational stages based on histological examination for the ten male and ten female lampreys sampled each week.

85% of lamprey GnRH-II. Radioimmunoassays were performed on the HPLC fractions 35–55. To determine the fraction numbers to be collected, lamprey GnRH-II standard was eluted on HPLC, fractions collected and assayed for lamprey GnRH-II. The column (Pecosphere 3CR C18, 0.46 × 8.3 cm) used for this standard was a new column and not used for the lamprey brain fractions.

2.5. Statistics

Data for hormone concentrations were analyzed using analysis of variance and significance differences were determined using Fisher's PLSD. In all tests, the level of significance for different groups was $P < 0.05$.

3. Results

3.1. Reproductive cycle

During the 8-week final reproductive period, several stages of spermatogenesis were observed (Fig. 1). In mid May, testes of lampreys generally were in stages I–IV. Final stages of spermatogenesis, mature sperm, were observed by mid June until the end of the season. The females were in the early stage of I–III during the first three weeks, in which the germinal vesicle was located peripherally and the vitelline membrane was doubled as described by Lewis and McMillan (1965) and Bolduc and Sower (1992). This doubling appears to occur at the beginning of the freshwater phase with the inner and outer portions being of about equal thickness (Bolduc and Sower, 1992). Following this period, the theca had begun to separate from the vitelline membrane with ovulation occurring at week 8.

3.2. HPLC elution

The elution profiles for lamprey GnRH-I, II, and -III are shown in Fig. 2. The profiles of lamprey GnRH-I and -III had been done previously (Calvin et al., 1993; MacIntyre et al., 1997). As shown in Fig. 2, lamprey GnRH-III eluted at fractions 2–4; lamprey GnRH-I eluted at fractions 8–9; and lamprey GnRH-II eluted at fractions 38–42.

3.3. Water temperature, lamprey GnRH-I, -II, and -III and estradiol

The results reveal correlations between water temperature, fluctuation of brain GnRHs and plasma estradiol in male and female lamprey (Figs. 2–4). The water temperature started at 11 °C and increased to 17 °C by the end of the season.

In ***males***, lGnRH-I concentration increased to 64.5 ± 18.7 (m ± SE) ng/brain at week 2, then declined to 15 ± 3 ng/brain at week 3 corresponding to reproductive stages III–VI with a subsequent rise and increase to 78 ± 9 ng/brain at spermiation, stages VI–VII (Fig. 3). In comparison, lGnRH-II and -III concentrations were also elevated early, 15.6 ± 0.5 and 124.7 ± 25.0 ng/brain respectively, in the season in males, dropped and then peaked in mid-season with a subsequent decline (0.2 ± 0.0 ng/brain) of lGnRH-II or increase (111.0 ± 11.0 ng/brain) of lGnRH-III at spermiation (Figs. 4 and 5). Estradiol peaked (1.4 ± 0.03 ng/ml) at stages V–VII and dropped to 0.9 ± 0.5 ng/ml at spermiation Fig. 6.

In ***females***, lGnRH-III concentration peaked at 119 ± 7 ng/brain in mid-season with a drop, 56.9 ± 9 ng/brain at ovulation while lGnRH-I remained unchanged during the season. In contrast,

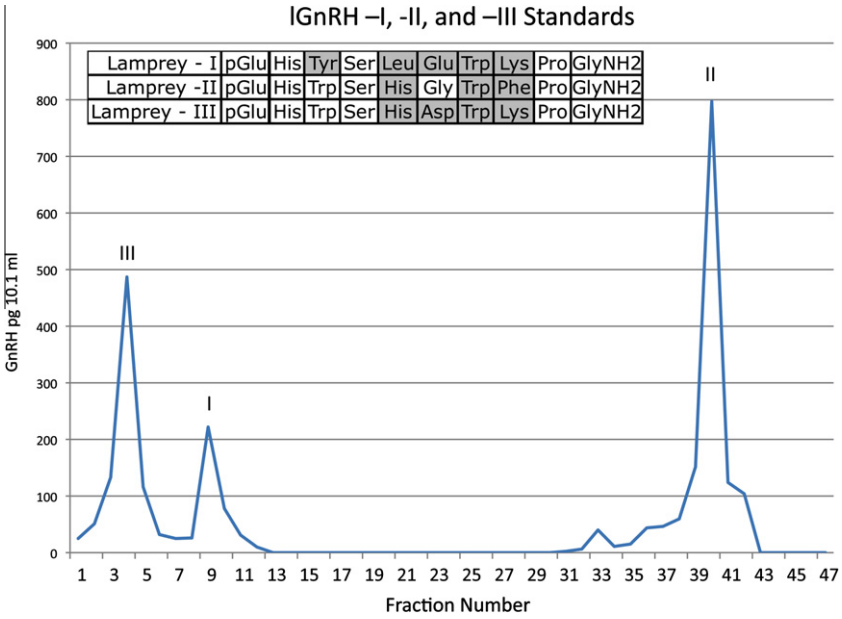

Fig. 2. HPLC chromatogram of the elution and separation of lamprey GnRH-I, -II and -III following injection of synthetic peptides. The data on lamprey GnRH-I and -III were from earlier studies (Calvin et al., 1993; MacIntyre et al., 1997).

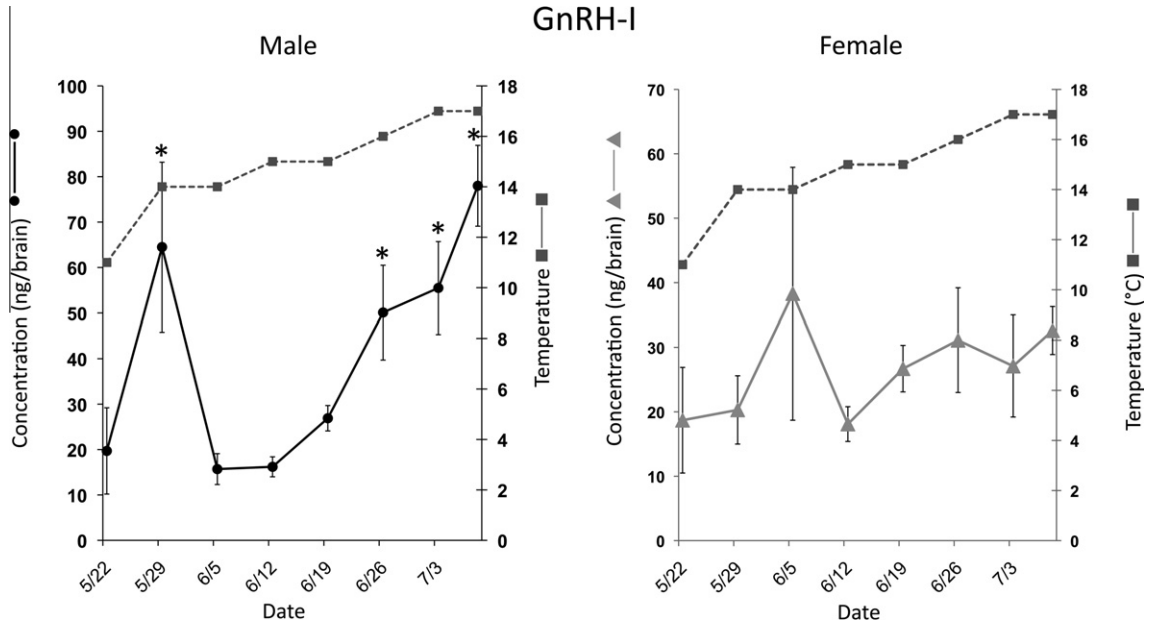

Fig. 3. Water temperature and brain GnRH-I in male (left) and female (right) sea lamprey. Temperature profile is shown by the dotted line. Each point (except temperature) represents the mean ± SEM ($n = 10$). *Represents a significant difference ($P < 0.05$).

lGnRH-II concentrations in females were elevated (16.2 ± 0.6 ng/brain) at the beginning of the season and then dropped and by the third week remained below 2 ng/brain during the rest of the season. Estradiol increased from 0.010 ± .004 ng/ml in week 1 to 0.65 ± 0.008 ng/ml in week 2 and remained elevated during the rest of the season.

4. Discussion

Recently, a novel GnRH, lamprey GnRH-II (lGnRH-II), was identified in lampreys and demonstrated to activate the pituitary–gonadal axis in adult lampreys (Kavanaugh et al., 2008). In the present study, we provide new evidence that brain lamprey GnRH-II along with lGnRH-I and -III undergo dynamic changes during the final reproductive period in adult male and female lampreys. These data support the evidence that lampreys are the earliest evolved vertebrates for which there are demonstrated functional roles for three GnRHs that act as neurohormones (Kavanaugh et al., 2008; Sower et al., 2009). Generally, gnathostomes have one or two GnRHs that act as hypothalamic hormones, two pituitary gonadotropins luteinizing hormone, LH, and follicle stimulating hormone, FSH, and one gonadal FSH receptor and one LH receptor compared to the lamprey that have three hypothalamic GnRHs, only one known pituitary gonadotropin and one gonadal glycoprotein receptor (Freamat et al., 2006; Freamat and Sower, 2008; Sower et al., 2009; Sower et al., 2006). How three GnRHs in lamprey differentially regulate the one known gonadotropin during reproduction is unknown at this time. When the identification of the lamprey gonadotropin is completed (Sower et al., 2009), future studies will be able to examine the effects of each of the three lamprey GnRH peptides on GTH synthesis and/or release.

Temperature is considered an important and critical environmental factor for reproduction in lampreys (Sower, 1998; Sower, 2003; Sower and Kawauchi, 2001). Upstream spawning sea lampreys kept at temperatures below 15.5 °C will not ovulate or spermiate unless the temperature is elevated close to their optimal spawning temperatures of 21 °C (Sower, 2003; Sower et al., 1985). Despite the unusually and exceptionally cold water temperature (temperature did not rise above 17 °C) that occurred in the 2008 reproductive season reported here, the results from the present study showed progressive changes in hormonal levels over time that occurred in a changing temperature environment. These data support earlier studies in which increases of brain GnRH and/or plasma estradiol correlated with an increase of water temperatures in adult male or female lampreys prior to spawning (Bolduc and Sower, 1992; Fahien and Sower, 1990; Gazourian et al., 1997; MacIntyre et al., 1997).

In immunohistochemical studies, lamprey GnRH-II similar to gnathostome type II GnRH, was widely distributed and expressed in a variety of tissues as demonstrated by RT-PCR (Kavanaugh et al., 2008; von Schalburg et al., 1999; White and Fernald, 1998). In the adult lamprey brain, lGnRH-II nerve fibers were shown to originate from cells in the arc-shaped hypothalamic/preoptic areas ending at the neurohypophysis and proposed to form the preopticohypophysial tract (Kavanaugh et al., 2008). The distribution of lamprey GnRH-II neurons was quite similar to distributions of lamprey GnRH-I and -III neurons, which were studied previously in the sea lamprey brain (Nozaki et al., 2000). These immunohistochemical studies along with the biological studies, suggested that lGnRH-II acts as a third hypothalamic GnRH (Kavanaugh et al., 2008). In the present study, the peak of lGnRH-II during mid-season further supports a key role of this isoform of GnRH in male reproduction. This peak corresponded to the increase of sperm being present in the testes. In contrast, lGnRH-II had a major decline in female lampreys at the beginning of the season and did not change during the reproductive season. This was an unexpected result, since previously lGnRH-II was shown to stimulate plasma estradiol in adult female lamprey (Kavanaugh et al., 2008). However, the reduced concentrations of lGnRH-II could reflect a rapid release rate of this brain hormone and not necessarily a lack of function. Until the synthesis, storage and release rates of each brain GnRH are known, the elevated or depressed concentrations of GnRH-I, -II, or -III in the brains of lampreys can only be correlated with reproductive stages. Another important aspect about the GnRH system to consider is the anatomy of the hypothalamic-pituitary axis in agnathans (lampreys and hagfish). In contrast to gnathostomes, lampreys have a "diffusional median eminence" which means that GnRH travels to the pituitary by diffusion across the connective tissue and/or via the third ventricle rather than via the portal system of the median eminence in tetra-

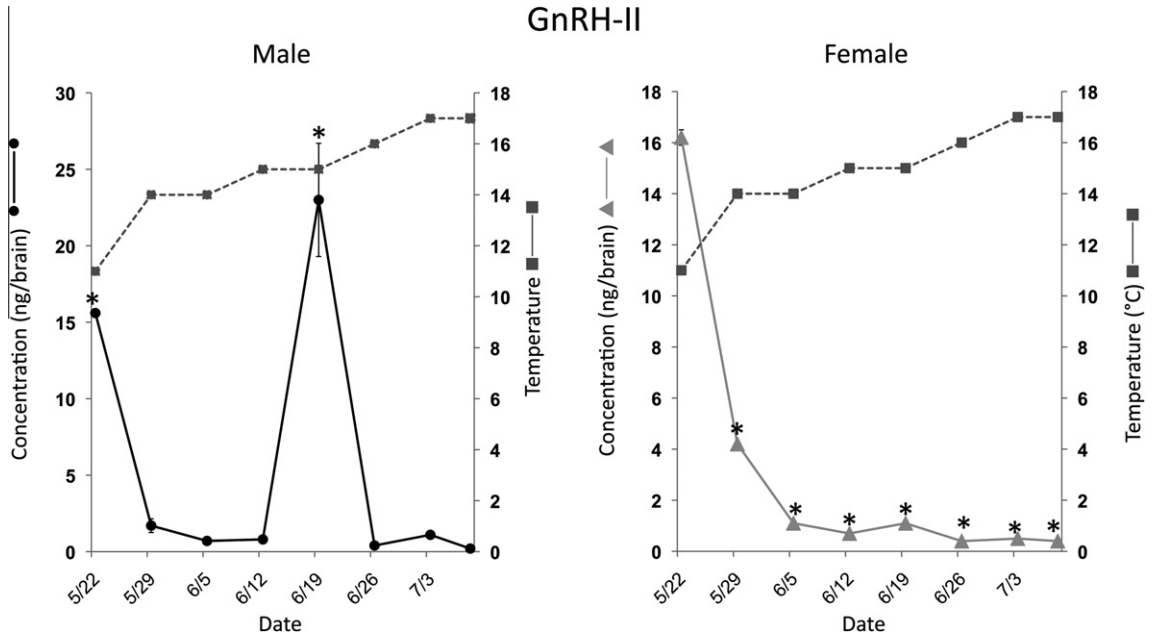

Fig. 4. Water temperature and brain GnRH-II in male (left) and female (right) sea lamprey. Temperature profile is shown by the dotted line. Each point (except temperature) represents the mean ± SEM ($n = 10$). *Represents a significant difference ($P < 0.05$).

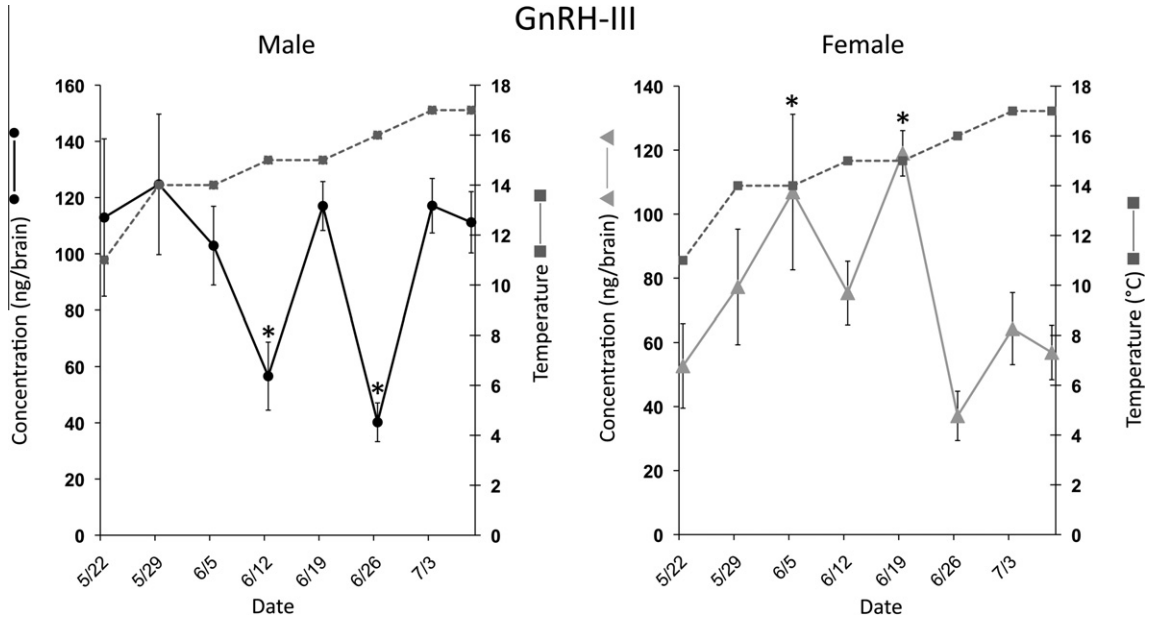

Fig. 5. Water temperature and brain GnRH-III in male (left) and female (right) sea lamprey. Temperature profile is shown by the dotted line. Each point (except temperature) represents the mean ± SEM ($n = 10$). *Represents a significant difference ($P < 0.05$).

pods or direct innervation of the anterior pituitary in teleosts (Nozaki et al., 1994). In addition, the concentrations of brain GnRH occur at higher concentrations than seen in other vertebrates (Bolduc and Sower, 1992; Fahien and Sower, 1990), suggesting that there may be a higher production and/or metabolism of each of the GnRHs compared to gnathostomes. Further studies on the metabolism of lamprey GnRHs and its receptors in relation to pituitary GTH will be needed to help clarify the functions of each GnRH.

Besides the GTH and GnRH receptors, there are several other important brain neurohormones/factors that have been shown to stimulate/modulate GnRH and/or gonadotropin synthesis and/or release in vertebrates. In some teleosts, those neurohormones/factors that have been clearly identified to have significant functions, besides the major neurohormone of GnRH, are dopamine, neuropeptide Y, g-aminobutyric acid (GABA) and more recently gonadotropin-inhibitory hormone (GnIH) and kisspeptin (KiSS). In lampreys, GABA and neuropeptides Y have been shown to be involved with brain GnRH and reproduction (Conlon et al., 1994; Root et al., 2004, 2005). In 2000 and 2003, two new brain hormones were identified called GnIH and Kisspeptin, respectively, that act on the hypothalamic-pituitary axis (Seminara et al., 2003; Tsutsui et al., 2000). GnIH is a dodecapeptide first identified in quail and shown to inhibit the synthesis and release of gonadotropins (Tsutsui et al., 2000). Subsequently, GnIH which belongs to the LPXRF-amide family of peptides has been described in fish but its actions on the hypothalamic-pituitary axis have not been eluci-

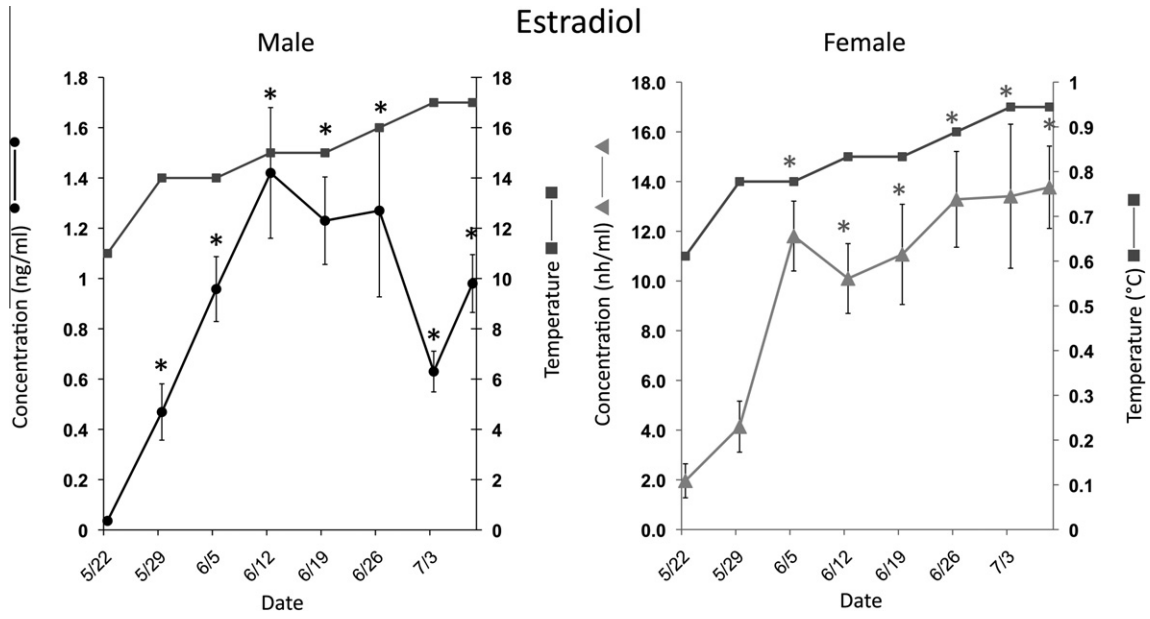

Fig. 6. Water temperature and plasma estradiol in male (left) and female (right) sea lamprey. Temperature profile is shown by the dotted line. Each point (except temperature) represents the mean ± SEM ($n = 10$). *Represents a significant difference ($P < 0.05$).

dated (Tsutsui et al., 2007). The family of LPXRF0 amide peptides has now been identified in lampreys but the functions of these peptides are currently unknown (Tsutsui, Sower, unpublished). The actions and interactions of these various neurohormones/neuromodulators are not known in lampreys and whether one or a combination of these hormones exert a stimulatory or inhibitory effect on the GnRH system is likely highly dependent on the hormonal milieu as well as the fish's reproductive and developmental stage and environmental factors including photoperiod and water temperature in which the fish live. Further studies will be required to gain an understanding of the complexity of the hypothalamic-pituitary axis in controlling reproduction in lampreys.

Concentrations of plasma estradiol (E2) and progesterone have been used as measures of reproductive development and gonadal activity and considered to be a major reproductive hormone in both male and female lampreys. In sea and Japanese river (*Lampetra japonica*) lampreys, E2 concentrations increased during spermiation (Fahien and Sower, 1990; Fukayama and Takahashi, 1985; Sower et al., 1985) and decreased during ovulation (Bolduc and Sower, 1992; Linville et al., 1987; Sower et al., 1985). In the first reported study examining sex steroid profiles in the Pacific lamprey (*Entosphenus tridentatus*) during overwintering and sexual maturation, E2 levels were usually higher in males than in females and increases coincided with the development of secondary sex characteristics (Mesa et al., 2010). In the present study, there were higher plasma concentrations of estradiol in females compared to males. In both sexes, plasma estradiol significantly increased as the season progressed correlating with a temperature increase that is in general agreement with these earlier studies. In males, higher estradiol concentrations corresponded to males that have mature sperm as shown in maturing lampreys (Fukayama and Takahashi, 1985; Linville et al., 1987; Sower et al., 1985) and are consistent with the presence of an estrogen receptor in the male testes (Ho et al., 1987). In females, it has been noted that there is generally a significant drop of estradiol at ovulation corresponding to increasing temperature (Bolduc and Sower, 1992; Sower et al., 1985). In the current study, there was not a decrease of estradiol in females at ovulation as had been previously shown. Although in one of the years tested in Bolduc and Sower (1992), there were low estradiol concentrations correlated with low water temperatures, which is similar to the data from this study in which there were exceptionally low water temperatures during the season. While estradiol is considered to be a major steroid involved in reproductive processes, the precise function(s) of estradiol in both male and females lampreys needs to be elucidated. There are still many questions remaining as to the type of steroids that are synthesized and respective functions (reviewed in Bryan et al., 2008).

In summary, the dynamics and significant correlations of brain GnRH-I, -II, -III and estradiol correlated with temperature showed differential patterns between male and female adult lampreys. In females, lamprey GnRH-I concentrations did not change significantly during the reproductive season, whereas lGnRH-III underwent significant increases during the same period similar to earlier studies that showed higher lGnRH-III concentrations compared to lGnRH-I in female (MacIntyre et al., 1997). The current study was the first to document lamprey GnRH-II in lampreys during this reproductive phase. As stated earlier, lamprey GnRH-II was elevated in females at the beginning of the season with a subsequent major decline. While these data provide further evidence that lamprey GnRH-III may be the major GnRH controlling reproduction in adult female lampreys, more information is required on the modes of action of GnRH at the pituitary. Three GnRH receptors have been identified and shown to be selective for one of the three lamprey GnRH peptides (Aquilinia-Beck A., MacDonald C., Freamat M., Kavanaugh S.I., and Sower S.A., unpublished). A key to our understanding of the role of each of the GnRHs is how they differentially stimulate the synthesis or release of one pituitary glycoprotein (GTH) hormone (Sower et al., 2009). The differential control of the three GnRHs on one gonadotropin is likely dependent on the hormonal milieu as well as the fish's reproductive and developmental stage and environmental factors including photoperiod and water temperature in which the fish live. Further studies will be required to gain an understanding of the complexity of the hypothalamic-pituitary axis in controlling reproduction in lampreys.

Acknowledgments

This project was funded by NSF grants 0421923, 0849569 and a REU Supplement to SAS. "This is Scientific Contribution Number

2423 from the New Hampshire Agricultural Experiment Station". Thanks to members (past or present) of the Sower Lab/Center including Caryn MacDonald, Kristen Gazda, Geoff Bushold, Lisa Merrill, Bernadine Schultz, Christina Stark, Crescentia Anne Healy, Mike Wilmot, Mihael Freamat, Takayoshi Kosugi, Joey Miseirvitch, Cee Messineo-Dimou, Ciara Kazakis, Erica Spater, Kerrie Sakowski and the UNH Vet Diagnostics Laboratory.

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