Comparative Biochemistry and Physiology, Part A 155 (2010) 56–63

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

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

Annual sex steroid and other physiological profiles of Pacific lampreys (*Entosphenus tridentatus*)

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ARTICLE INFO

Article history:
Received 22 April 2009
Received in revised form 31 August 2009
Accepted 19 September 2009
Available online 25 September 2009

Keywords:
Pacific lamprey
Sex steroids
Thyroid hormones
Plasma protein
Triglycerides
Plasma glucose

ABSTRACT

We documented changes in plasma levels of estradiol 17-β (E2), progesterone (P), 15α-hydroxytestosterone (15α-T), thyroxine (T4), triiodothyronine (T3), protein, triglycerides (TGs), and glucose in adult Pacific lampreys (*Entosphenus tridentatus*) held in the laboratory in two different years. Levels of E2 in both sexes ranged from 0.5 to 2 ng/mL from September to March, peaked in late April (2–4 ng/mL), and decreased in May, with levels higher in males than in females. Levels of P were low from September through April, but then increased substantially during May (2–4 ng/mL), with levels again highest in males. Levels of 15α-T in males were around 0.75 ng/mL through the winter before exceeding 1 ng/mL in April and decreasing thereafter, whereas females showed a gradual increase from 0.25 ng/mL in November to 0.5 ng/mL in April before decreasing. Thyroxine concentrations differed between fish in each year, with most having levels ranging from 0.75 to 2.5 ng/mL in the fall and winter, and only fish in 2003 showing distinct peaks (3–4 ng/mL) in early April or May. Plasma T3 was undetectable from November through mid-March before surging dramatically in April (ca. 150 ng/mL) and decreasing thereafter. Levels of protein, TGs, and glucose decreased or were stable during the fall and winter with TGs and glucose surging in late April to early May for some fish. Our study is the first to document long-term physiological changes in Pacific lampreys during overwintering and sexual maturation and increases our understanding of the life history of this unique fish.
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1. Introduction

Pacific lampreys (*Entosphenus tridentatus*) in the Columbia River Basin (CRB) have declined to only a remnant of their pre-1940s populations (Close et al., 1995). The ecological, economic, and cultural significance of this species is underestimated by most people and actions are currently being considered for their recovery in the CRB (Close et al., 1995). Identifying biological factors that may limit lamprey production in the CRB is critical for their recovery. For example, perhaps the most significant factor impacting lamprey production in the CRB is difficulty negotiating upstream fish passage facilities at dams. Recent research suggests that significant numbers of radio-tagged lampreys failed to pass fishways at dams on the Columbia and Willamette rivers, but detailed explanations for or the possible consequences of this behavior are lacking (Moser et al., 2002; Mesa et al., 2009). Although such poor performance can be attributed in part to design constraints of the fishways, the possibility of delayed tagging effects, and the relatively poor swimming ability of lampreys (Mesa et al., 2003), questions remain as to whether there may be any correlations between their physiological status, swimming performance, and motivation to migrate. Pacific lampreys are anadromous and spend most of their adult life in the ocean before migrating into freshwater in the spring of one year and spawning and dying the following spring. Thus, because Pacific lampreys do not spawn for at least a year after they enter freshwater, they may show variable motivation to migrate. For example, one reason for such an early upstream migration in Pacific lampreys may be to locate ideal areas for overwintering.

In contrast to the well-studied sea lamprey (*Petromyzon marinus*), little is known about the reproductive biology or physiology of Pacific lampreys. Knowledge of sex steroid and other physiological profiles during an annual cycle may increase our understanding of the reproductive and migratory behavior of Pacific lampreys, and would benefit recovery efforts for this species in the Pacific Northwest. Concentrations of certain steroids, such as estradiol 17-β (E2) and progesterone (P), have been correlated with reproductive stage (e.g., ovulation and spermiation) and behavior in many species of fish (see Nagahama, 1994 for review), including lampreys (reviewed in Sower, 2003). In addition, 15α-hydroxylated steroids may have a role in lamprey physiology (see Bryan et al., 2008 for review). Other hormones, such as the thyroid hormones, may also play unknown

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1095-6433/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.cbpa.2009.09.019

roles in reproductive development or migratory behavior. Documenting changes in metabolic substrates (i.e., fats, proteins, and carbohydrates) would provide insight into how Pacific lampreys accomplish an extended overwintering period followed by dramatic sexual development without feeding. Finally, information on sex steroid profiles may compliment ongoing studies of the olfactory system of Pacific lampreys (Robinson et al., 2009) because sensitivity to certain pheromone cues may be dependent on stage of sexual maturation, as previously demonstrated in sea lamprey (Li et al., 2002).

The goal of this study was to provide basic information on key physiological changes in Pacific lampreys during overwintering and final sexual maturation. To address this goal, we conducted similar laboratory studies in two different years. Specifically, we documented the profiles, over about a one year period, of levels of E2, P, 15α-hydroxytestosterone (15α-T), thyroxine (T4), tri-iodothyronine (T3), protein, triglycerides (TGs), and glucose in the plasma of Pacific lampreys held in the laboratory. Our results should provide basic information on sex steroid and other physiological profiles of these fish and will provide baseline data for exploring the possibility of an underlying physiological basis for their migratory behavior, which we hope to address in a companion paper.

2. Materials and methods

2.1. Lamprey collection and holding

Adult Pacific lampreys were collected from the Columbia River at Bonneville Dam trapping facilities during July and August 2000 and 2002. In each year, lampreys were transported to the Columbia River Research Laboratory and 20 fish were placed in each of two artificial streams that were lined with cobble and gravel substrates and received water from the Little White Salmon River, Washington, USA. The streams were 2.1-m-long, 0.6-m-wide, and 0.6-m-deep and received water at a rate of about 2–3 L/min. Water temperatures in the artificial streams were routinely adjusted to mimic mean daily water temperatures at Bonneville Dam during the year and ranged from a low of about 5 °C in winter to 14 °C in late May. Lighting was provided by overhead incandescent lights and timers that mimicked the ambient photoperiod and produced a gradual intensity dusk and dawn. The artificial streams were surrounded by curtains to minimize outside disturbance and had screens secured to the top to prevent fish from escaping. Lampreys were not fed during our experiments, as lampreys naturally cease feeding prior to beginning the upstream migration.

2.2. Fish sampling

In both years, lampreys in the artificial streams were sampled for blood and measured for length and weight every three weeks until peak reproductive development and spawning. Some aspects of sampling differed between years and will be noted where relevant. In 2000, we alternated the sampling of all fish in each stream so that each individual was sampled once every six weeks. In 2002, we sampled half the fish from each stream every three weeks and alternated the sampling of groups so that each fish was sampled once every six weeks (all fish were uniquely identified via the insertion of tags and marks; see below). Table 1 shows the sampling dates and number of individuals sampled during both years; less than 20 fish were sampled on some dates due to mortality during the experiments, either from fish escaping from the tanks or from senescence during the later dates. On the day of sampling, the inflow to the selected artificial stream was turned off, the water level was reduced to half the original volume, and we introduced a 50 mg/L dose of buffered tricaine methanesulfonate (MS-222). After the fish became quiescent, we placed five fish in each of four 19-L buckets containing about 14 L of water and airstones connected to battery powered aerators. Two buckets (labeled one and two) contained a 75 mg/L dose of buffered MS-222 and the two other buckets (labeled three and four) contained a 50 mg/L dose of buffered MS-222. We used different doses of anesthetic to account for the length of time fish had to stay in a bucket. At this time, we also turned on the inflow to the artificial stream so it could begin re-filling. During the first sampling, we inserted Passive Integrated Transponder (PIT) tags (Biomark, Inc.) into the dorsal musculature and marked each individual with a unique pattern of fin clips for each artificial stream in case they lost the tag. Thereafter, we determined an individual's identity by scanning it for a PIT tag and recording its unique pattern of fin clips. We measured and recorded the total length (cm), weight (g), and anterior (just in front of the first dorsal fin), middle (between the two dorsal fins), and posterior (just behind the second dorsal fin) girth (all in mm) of each fish. We also noted any unique marks, scars, or evidence of injury or disease. Blood was obtained via the caudal vein using a heparinized 1 mL disposable syringe fitted with a 23-gauge needle. The blood was discharged into a 2 mL pre-heparinized centrifuge tube, placed on ice, and the fish was returned to its artificial stream. Following these procedures, we serially sampled the fish in all four buckets. When the last fish in the second bucket was being sampled, we added more buffered MS-222 to buckets three and four to increase the dose to 75 mg/L. In 2002, we followed these procedures with the following changes: (1) we added no heparin to our sample tubes; (2) we reduced the volume of water, anesthetized, and sampled fish from both streams (i.e., half of our total sample size came from each of the two streams); and (3) we used pre-heparinized Vacutainer syringes to collect the blood samples. When sampling was completed, we centrifuged the blood samples at 1500×g for 20 min at 4–6 °C. Plasma was transferred to pre-labeled tubes and stored at −80 °C for later analysis. With a crew of 5–6 people, it required about 30 min to sample 20 fish. At the end of these studies, all fish were sacrificed and their gender recorded.

Table 1
Sample dates and numbers of Pacific lampreys sampled from each artificial stream during our experiments to document physiological profiles in 2000–01 and 2002–03.

Sample date	Artificial stream	Females	Males	Total
Year 1				
9/26/00	1	7	8	15
10/17/00	2	11	9	20
11/7/00	1	7	11	18
11/29/00	2	11	9	20
12/19/00	1	7	11	18
1/9/01	2	11	9	20
1/29/01	1	7	11	18
2/20/01	2	11	9	20
3/12/01	1	7	11	18
4/3/01	2	11	9	20
4/23/01	1	6	11	17
5/15/01	2	11	9	20
5/25/01	1 and 2	5[a]	13	18
Year 2				
11/7/02	1 and 2	7	13	20
11/27/02	1 and 2	13	7	20
12/19/02	1 and 2	7	12	19
1/9/03	1 and 2	13	7	20
1/30/03	1 and 2	5	9	14
2/20/03	1 and 2	13	7	20
3/13/03	1 and 2	5	9	14
4/3/03	1 and 2	13	7	20
4/23/03	1 and 2	5	9	14
5/13/03	1 and 2	15	11	26

[a] Blood was obtained from only one of five female fish sampled.

2.3. Assays and data analysis

Plasma samples were assayed for levels of E2, P, T4, total protein, total TGs, and glucose in 2000–2001 and for those constituents plus 15α-T and T3 in 2002–2003. Concentrations of E2 and P were

measured by a radioimmunoassay previously validated for use with lamprey plasma (Sower et al., 1985; Linville et al., 1987). Levels of 15α-T were measured as in Bryan et al. (2003). Levels of T4 and T3 were measured by Dr. Richard Ewing of Biotech Research and Consulting, Inc., Corvallis, Oregon, USA, using enzyme immunoassays that were also validated for use with lamprey plasma. Plasma protein concentrations were measured using a bicinchoninic acid procedure (Smith et al., 1985) and total triglyceride and glucose concentrations were measured using commercial kit assays (Sigma Diagnostics, St. Louis, MO, USA) that we modified for use with microwell plates.

For our work in 2000–2001, where we alternated sampling of fish in the two artificial streams, we calculated mean (and SD) values for all plasma constituents by gender and compared them within a time period using two-sample *t*-tests. We plotted the mean values over time, serially joining results from the two artificial streams for graphical purposes, and compared values among sample dates within each gender and sample group (i.e., fish from artificial stream one or two) using Analysis of Variance (ANOVA). When the *F*-test was significant ($P \leq 0.05$), we compared the mean of the first sample period for each group to all subsequent means (except the last one) using Dunnett's multiple comparisons procedure. Because the last sample in each year was comprised of all remaining individuals, we compared the final mean to that from fish in the first two samples within each gender using a two-sample *t*-test. For 2002–2003 data, where we sampled fish from both artificial streams each time, we first compared mean values from each gender between artificial streams using two-sample *t*-tests to validate pooling data for further analyses. Out of 160 paired comparisons, only 20 (12.5%) means were found to be significantly different. Of these, seven comparisons had at least one group with a sample size of only two or three fish. Based on these results, we felt justified in pooling data from fish of each sex in the different streams for each sample period. We again plotted means over time and, within a sample date, compared the means from male and female fish using two-sample *t*-tests. We again used ANOVA followed by Dunnett's procedure to compare mean values of the same groups of fish over time. All analyses were conducted using GraphPad Prism 5.02 software and the accepted level of significance was $P \leq 0.05$, unless otherwise indicated.

3. Results

3.1. Sex steroid profiles

In both years, E2 levels in male and female lampreys were similar from November through mid-February, ranging from about 0.75–2.0 ng/mL (Fig. 1). Concentrations of E2 started to increase during March of each year, peaked at the end of April in 2001 and the beginning of April in 2003, and declining thereafter. In both years, peak levels of E2 in females were around 2 ng/mL and significantly higher than initial values in 2003 only ($P < 0.05$). In males, peak levels of E2 were close to 4 ng/mL and were significantly higher than initial values in both years ($P < 0.001$). During the time of maximum

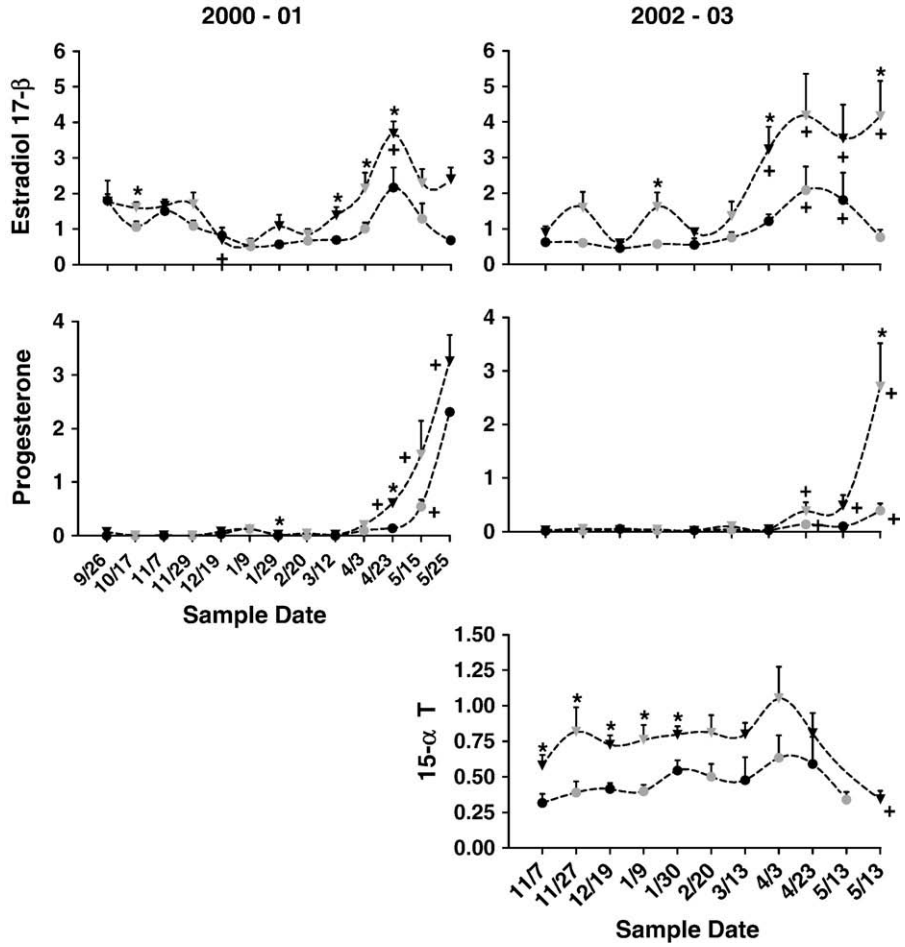

Fig. 1. Mean (and SD) plasma estradiol 17-β (E2), progesterone (P), and 15α-hydroxytestosterone (15α-T) concentrations from female (circles) and male (triangles) Pacific lampreys sampled in our laboratory during 2000–01 (left panels) and 2002–03 (right panels). Within each gender, the alternating symbol colors represent repeated samples from the same group of individuals. For example, the black circles were means derived from the same individuals over time. An asterisk denotes a significant difference in means between the sexes and a cross denotes a difference between that value and the initial value for that group. Sample size for each date is given in Table 1.

reproductive development, from about mid-March to late May, concentrations of E2 were often significantly higher in males than in females ($P<0.05$). During this time of E2 increase, the development of secondary sexual characteristics, such as ventral ridges and cloacal swelling, proceeded rapidly.

In both years, levels of P in both sexes were almost undetectable from mid-September through April (Fig. 1). Thereafter, levels of P increased dramatically in male fish, with maximal values (about 3.25 ng/mL) occurring in mid to late May. In both years, on or after 3 April, levels of P in males and females were sometimes significantly higher than initial values ($P<0.05$). However, concentrations of P in males were not always significantly higher than those in females because of high variability in the samples (Fig. 1). Because of the dramatic reproductive development of females in mid to late May and lack of blood, we were unable to obtain blood samples from all females except one in 2001. This fish showed a dramatically elevated level of P relative to earlier samples.

In 2002–03, concentrations of 15α-T were low (<1.0 ng/mL) and generally stable in both sexes from early November to mid-March (Fig. 1). During this time, males had mean levels of 15α-T that stayed around 0.75 ng/mL, whereas values for females were always about half that value. Concentrations of 15α-T were significantly higher in males than in females from November through February (Fig. 1). In early April, levels of 15α-T in males rose to 1.0 ng/mL and decreased thereafter. This trend was less evident in female fish. Across time, the only sample that was significantly different from initial values was for males on 13 May ($P<0.05$).

3.2. Thyroid hormones

Trends in circulating levels of T4 were variable and differed somewhat between males and females and between years (Fig. 2). Statistically significant differences between the sexes occurred only in early February in both years. In 2000–01, males had low levels of T4 at the start of the experiment (about 0.75 ng/mL) and showed a gradual increase to a level more than twice the initial value by late February (about 2.25 ng/mL). The mean concentration of T4 in males on 29 January was significantly higher than the initial value ($P<0.05$). Thereafter, T4 concentrations declined and varied somewhat around a mean of about 1.5 ng/mL. In females, during most of the experiment, mean levels of T4 generally varied from about 1 to 1.5 ng/mL. From late December through early January, levels of T4 were maximal at about 2.0 ng/mL. Across time, levels of T4 in females never differed significantly.

In 2002–03, levels of T4 were generally stable in both sexes from early November to mid-March, ranging from about 1.0–1.5 ng/mL (Fig. 2). Thereafter, both sexes showed a distinct peak in T4 concentrations followed by a rapid decrease. In females, maximal values of about 3.0 ng/mL occurred in April and were significantly higher than initial values in November. In males, the highest levels of T4 occurred in April, with a peak of around 4.0 ng/mL, and these concentrations differed significantly from the initial values. Also in 2002–03, concentrations of T3 were low in both sexes from November through mid-March, but did differ significantly between males and females in early November and late February (Fig. 2). In early April, both sexes showed a highly significant ($P<0.001$) almost 150-fold increase in T3 levels followed by a precipitous decline.

3.3. Nutritional factors

In 2000–01, plasma protein levels were similar between genders (except in early February, when the means differed significantly) and showed a gradual decline from mid-September (about 45 mg/mL) through early April (about 35 mg/mL; Fig. 3). Thereafter, protein levels in females declined rapidly, reaching a low of about 10 mg/mL in the single female we sampled at the end of May. In males, after early April, protein levels were stable before decreasing to about 25 mg/mL at the end of May. Several mean values in both sexes after January were significantly ($P<0.05$) lower than initial values in September and October. In 2002–03, plasma protein levels were similar between the sexes and ranged from 35 to 45 mg/mL from November through mid-April. Protein levels dropped about 10 mg/mL during our last sample in mid-May. Only females from our second sampling group had mean values that were significantly lower than the initial value (Fig. 3). Prior to March, trends in total plasma TGs differed between fish in each year, but after March, the trends were similar (Fig. 3). From November to March, levels of plasma TGs in lampreys from 2000 to 2001(range of 1.6–4.5 mM/L) were higher than those of fish from 2002 to 2003 (range = 0.5–1.8 mM/L). For fish in both years, levels of TGs started to increase in mid-March, reached a peak near the end of April (range = 2.6–4.1 mM/L), and declined thereafter. Only males had mean levels of TGs that differed significantly from initial values—three samples from fish in 2001 and the highest value recorded for fish in 2003 (Fig. 3). The only significant differences between the sexes occurred in 2000–01, when levels of TGs in females were higher than males in early April. Trends in plasma glucose levels were similar between the sexes and between fish in each year, typically ranging from about 2.0 to 3.5 mM/L (Fig. 3). One exception, however, was that fish in 2000–01, but not those in 2002–03, showed a distinct increase (significant in males only) in glucose levels in May that ranged from 3.3 to 5.1 mM/L. Significant differences in levels of the nutritional factors between the sexes occurred only in 2000–01 during December and late April.

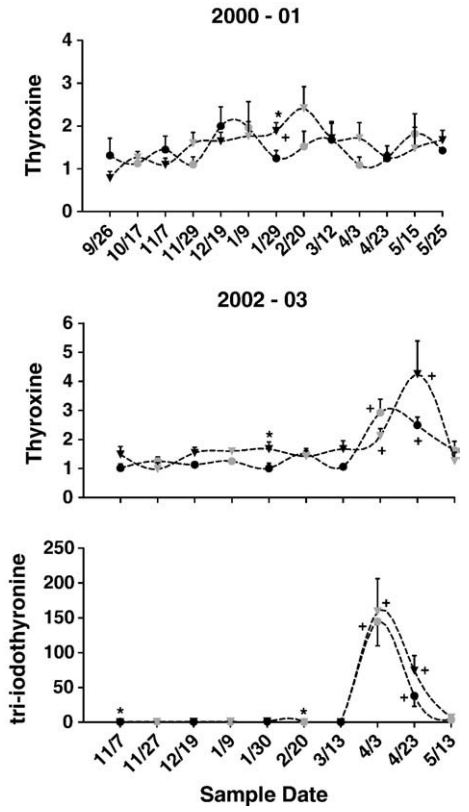

Fig. 2. Mean (and SD) plasma thyroxine (T4) and triiodothyronine (T3) concentrations from female (circles) and male (triangles) Pacific lampreys sampled in our laboratory during 2000–01 (the top panel—T4 only) and 2002–03 (the middle and lower panels). Within each gender, the alternating symbol colors represent repeated samples from the same group of individuals. For example, the black circles were means derived from the same individuals over time. An asterisk denotes a significant difference in means between the sexes and a cross denotes a difference between that value and the initial value for that group. Sample size for each date is given in Table 1.

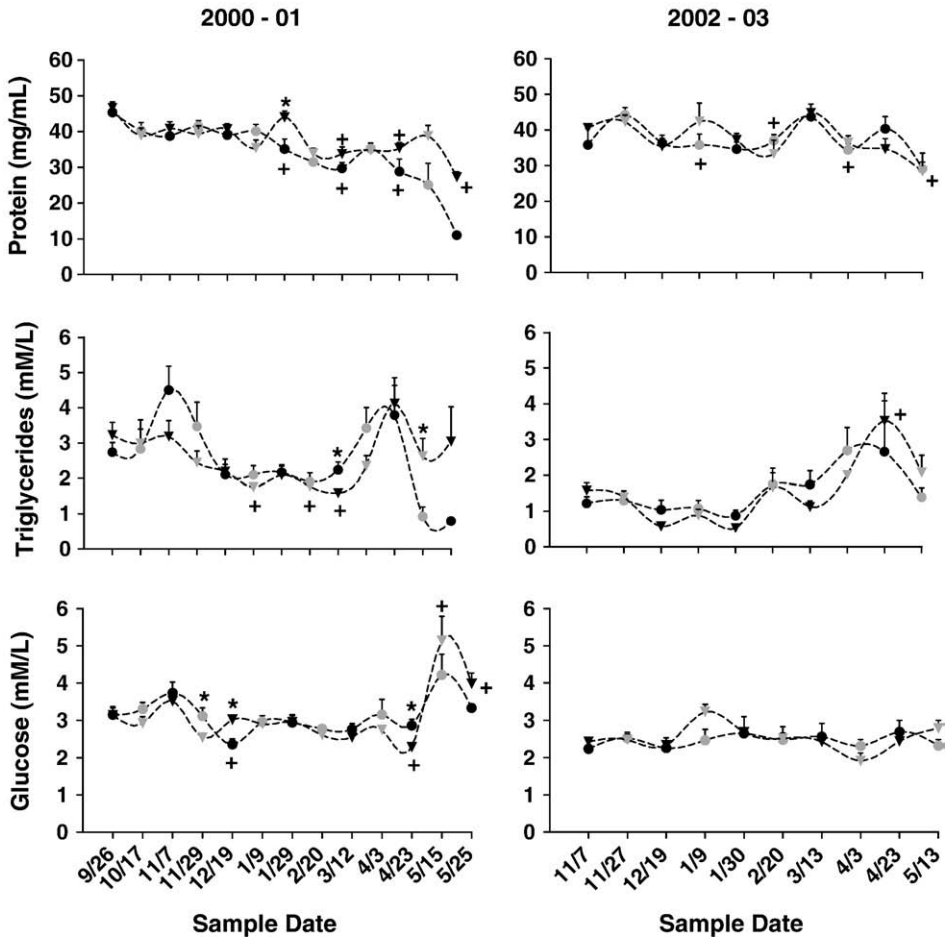

Fig. 3. Mean (and SD) plasma protein, triglycerides (TG), and glucose concentrations from female (circles) and male (triangles) Pacific lampreys sampled in our laboratory during 2000–01 (left panels) and 2002–03 (right panels). Within each gender, the alternating symbol colors represent repeated samples from the same group of individuals. For example, the black circles were means derived from the same individuals over time. An asterisk denotes a significant difference in means between the sexes and a cross denotes a difference between that value and the initial value for that group. Sample size for each date is given in Table 1.

4. Discussion

The Pacific lamprey has a remarkable life history. After spending 4–6 years as filter-feeding larvae in freshwater, juveniles undergo a metamorphosis and migrate downstream to the ocean. Adults spend from 12 to 40 months in the ocean as a free swimming parasite before migrating upstream in the spring of one year, overwintering, and spawning in the spring of the following year (Hardisty and Potter, 1971). Our descriptions of sex steroid and other physiological profiles in these fish are the first reported in this species of lamprey, help define their life history and reproductive development, and provide insight into their metabolism during overwintering and final sexual maturation.

Levels of E2 and P are measures of reproductive development and gonadal activity. In sea and Japanese river (*Lampetra japonica*) lampreys, E2 concentrations increase during spermiation (Fukayama and Takahashi, 1985; Sower et al., 1985; Fahien and Sower, 1990) and decrease during ovulation (Sower et al., 1985; Linville et al., 1987; Bolduc and Sower, 1992). Despite these general trends, the precise function of E2 during final reproductive development in lampreys is still not well defined. In our fish, E2 levels were usually higher in males than in females and increases coincided with the development of secondary sex characteristics. Higher levels of this steroid in males during final maturation generally agrees with work on the sea lamprey and the Japanese river lamprey and are consistent with the presence of an estrogen receptor in the male testis (Fukayama and Takahashi, 1985; Sower et al., 1985; Linvillle et al., 1987; Sower and Gorbman, 1998; Ho et al., 1987). However, unlike these other studies, our data are somewhat equivocal regarding an increase in E2 during spermiation. In 2001, during the presumed time of spermiation in our fish (i.e., May), E2 levels in males were decreasing, but in 2003, levels of E2 remained high during this time. Possible reasons for this discrepancy include inaccuracy in our presumption that spermiation occurred from mid to late May, alterations in reproductive development and behavior of fish in different years due to the laboratory rearing environment, or our three-week sampling regime was not enough to resolve differences.

In both years of our study, during the time of increasing E2 levels, fish progressively developed secondary sex characteristics, including dorsal and ventral ridges and swelling in the cloacal region. Also during this time, females had very swollen abdomens indicative of substantial gonad development and ongoing vitellogenesis. Peak ripeness, evident by extrusion of gametes via easy palpation of the abdomen, occurred during mid to late May in most of our fish. The presence of E2 has been linked to the development of secondary sex characteristics and vitellogenesis in other species of lampreys (Larsen, 1974; Pickering, 1976; Fukayama and Takahashi, 1985) and our results concur.

Notably, the peak in E2 levels coincided with the surge in T3 levels from our fish in 2003. Such a response is different than that seen in teleosts, where E2 is known to lower the sensitivity of the thyroid to TSH and lower the blood thyroid hormone levels (Leatherland, 1985;

Cyr et al., 1988; Leatherland et al., 1990). The correspondence between E2 and T3 in our fish in 2003 is also different from results of other studies using lampreys, which could be due to species differences such as the timing of maturation, differences in sampling, or some other unknown reason. Sower et al. (1985) found no clear correlation between levels of E2 and T3 in the sea lamprey and Leatherland et al. (1990) reported a lowering of T4 concentrations and a rise in T3:T4 ratios in the southern hemisphere lamprey *Geotria australis* given various doses of E2. The dramatic surge in T3 levels seen in our fish, coincident with peak levels of E2, suggests that T3 may be involved with final reproductive development in Pacific lampreys, but further research will be necessary to confirm this notion.

The role of P during final reproductive development in lampreys is even less well defined than E2. Linville et al. (1987) reported that levels of P: (1) did not change significantly at different stages of maturity in female sea lampreys; (2) were not correlated with various reproductive behaviors in either sex; and (3) were, on average, higher in males during final reproductive development. In contrast, Bolduc and Sower (1992) reported elevated levels of P in female sea lampreys at the time of spawning and, in comparing their data to that of Fahien and Sower (1990), noted that levels of this steroid were higher in females. However, Bolduc and Sower (1992) also noted that because levels of P in their sea lampreys were low, specific actions for this steroid have not been proposed. In both years of our study, we noted essentially undetectable levels of P in both sexes from mid-September through early April. Thereafter, levels of this steroid started to rise and showed a dramatic surge in the month of May, with highest levels measured in males and during the time of peak ripeness. We should note, however, that we were only able to obtain blood from one of five female fish on 25 May, 2001. When compared to results of the studies cited previously, our fish showed a different trend in levels of P and had mean values during the final stages of maturation that were 2–3 times higher. Interestingly, the surge of P shown by our fish is similar to that of 17α-20β-dihydroxy-4-pregnen-3-one (DHP) in salmonids detected at ovulation (Young et al., 1983; Fitzpatrick et al., 1986; Slater et al., 1994). The role of DHP in a number of teleosts is to trigger final maturation by inducing final meiotic maturation (Scott and Canario, 1987; Nagahama and Yamashita, 1989). As suggested by Bolduc and Sower (1992), whether P has precise roles or is merely a precursor to active forms in lamprey reproductive physiology needs further research. Because there is a lack of evidence for in vitro synthesis of P by lamprey gonadal and adrenocortical tissues (see Bryan et al., 2004) and concentrations of P are relatively low, it may be that lampreys use a hormone other than P during the final stages of reproductive development (Kime and Rafter, 1981). Indeed, the recent work of Bryan et al. (2004, 2006) and Young et al. (2007) suggests that 15α-hydroxyprogesterone may play a significant role in the reproduction of lampreys.

Evidence suggests that lampreys produce gonadal steroids differing from those of other vertebrates by having an extra hydroxyl group at the C15 position (Kime and Rafter, 1981; Kime and Callard, 1982; Lowartz et al., 2003; Bryan et al., 2003, 2004, 2006, 2008). These authors suggest that these 15-hydroxylated steroids may be the functional hormones in lampreys. Plasma 15α-T concentrations rise in male Pacific lamprey from a mean 0.6 to 1.5 ng/ml after injection of gonadotropin-releasing hormone (GnRH) III, but not GnRH I (Bryan et al., 2006). To further explore whether 15α-T might have a physiological role in reproduction in Pacific lampreys, we measured levels of 15α-T in our fish during 2002–2003 and attempted to correlate our findings with reproductive development. Our results showed that levels of this steroid were fairly low (often less than 1 ng/mL on average), stable, and always higher in males than in females. Only males showed a distinct, but fairly small, peak in levels of 15α-T during final maturation in April. The testes of sea lamprey are able to convert testosterone to 15α-T in high yield and 15α-T is present in their blood plasma (Bryan et al., 2003). However, the concentrations reported for sea lamprey, which ranged from a mean of 62 pg/mL in ovulating females to 275 pg/mL in pre-spermiating males, were about ¼ to ½ the levels measured in our fish. Although possible arguments have been presented for the hypothesis that 15α-T is a functional hormone in lampreys (Bryan et al., 2003; Young et al., 2007), the biological significance of this steroid awaits determination.

Thyroxine levels in our laboratory study nearly tripled in males from mid-September to mid-February in 2001 and surged from April to May in 2003. During all other sample periods, T4 concentrations were relatively constant, generally varying around a mean of 1.5 ng/mL. In 2001, T4 levels were not clearly correlated with any other factor we measured, but in 2003, the surge in T4 levels in April and May (which was not evident in fish from 2001) were coincident with or preceded increases in E2, T3, and plasma triglycerides. Except for a transient decline during the early months of the upstream migration, Leatherland et al. (1990) also noted that T4 concentrations in *G. australis* were maintained at relatively constant levels. Interestingly, *G. australis* and *E. tridentatus* have unusually long adult freshwater residency, lasting from 12 to 16 months. One possible reason for the maintenance of T4 levels could be to provide adequate substrate over a long period of time for the hepatic monodeiodination of T4 to the more physiologically active tri-iodothyro (T3). Indeed, Leatherland et al. (1990) noted a progressive decrease in serum T3 concentrations during the upstream migration of *G. australis* and suggested that such change may be linked to the altered metabolic needs of an animal undergoing prolonged starvation. In contrast to these results, Sower et al. (1985) reported significant changes in plasma concentrations of T4 and T3 during final maturation and spawning of the sea lamprey. Notably, at the time of spermiation, they observed a significant increase in T4 and a decrease in T3, and during ovulation, their fish showed significant decreases in both hormones. Further, some of their data was correlated with changes in plasma fatty acids and protein indicative of the widely-reported metabolic role for these hormones in numerous species.

In 2003, we assayed T3 levels in Pacific lampreys during the time of overwintering and final maturation to clarify our understanding of thyroid hormones in this species. As described above, the results of Leatherland et al. (1990) and Sower et al. (1985) were inconsistent regarding T3 dynamics in the sea lamprey, perhaps in part due to the fact that sea lampreys did not overwinter in freshwater streams. Our results produced yet another different trend, with T3 levels being virtually undetectable from November through March before showing a dramatic surge in April and rapidly declining thereafter. This surge in T3 did coincide with peaks in E2, 15α-T, and, in females only, T4. It also occurred during the time of increasing TG levels. Because we are unaware of any specific roles for thyroid hormones in lamprey reproductive physiology, the elevations in T3 and T4 in our fish in April and May probably facilitated mobilization of metabolic substrates for final sexual maturation and spawning. Indeed, thyroid function may have evolved in concert with endocrine control of reproduction and be associated primarily with gonadal maturation (Norris, 1985).

Annual cycles in the gonads and thyroid gland and their interactions have been described for numerous species of vertebrates (Sage, 1973; Sower et al., 1984). Likewise, in lampreys, there are some known correlations between the gonads and thyroid gland during metamorphosis and in the final reproductive processes (reviewed in Youson and Sower, 2001). Numerous activities, such as migration, metamorphosis, and reproduction, are organized and coordinated via the neuroendocrine axis. In lampreys, recent data suggests the existence of a primitive, overlapping yet functional hypothalamic-pituitary-gonadal and hypothalamic-pituitary-thyroid endocrine system (Freamat and Sower, 2008; Sower et al., 2009). To date, there is only one pituitary glycoprotein hormone (Sower et al., 2009) and two glycoprotein hormone receptors (Freamat et al., 2006; Freamat

and Sower, 2008) in lampreys as opposed to three or four glycoprotein hormones interacting specifically with three receptors in Gnathostomes. From our study, it also seems that there are correlations between the thyroid and gonadal systems, suggesting the occurrence of coordinated reproductive hormones with the dynamics of the thyroid and metabolic systems during migration and reproduction.

Trends in levels of the nutritional factors we assayed were fairly similar between fish in the two years. The gradual decreases in levels of plasma protein and TGs and the relative stability of glucose levels seen in our fish probably reflect metabolic demands associated with overwinter maintenance. The increase in levels of TGs and, in 2001 only, glucose probably help fuel processes associated with final sexual maturation and are mediated by some of the hormone changes described above. The trends in plasma protein and TGs are probably conservative indications of energy use in Pacific lampreys since we essentially removed a large contributor to energy use—the swimming activity associated with migration. Nevertheless, similar findings have been reported for other species of lampreys. For example, Sower et al. (1985) reported decreases of greater than 50% in plasma protein levels in *P. marinus* during the upstream spawning migration. Sower et al. (1985) also showed that plasma protein levels were much lower in female than in male lampreys at the time of spawning, which we observed in our fish in 2001. We concur with their speculation that the lower plasma protein levels in females probably reflect the higher metabolic demands of gonad development relative to that of males.

Lipids are thought to be an important energetic substrate in lampreys during the spawning migration and for gonad development (Beamish et al., 1979; Plisetskaya, 1980; Sower et al., 1985). Our data support this notion for at least three reasons. First, plasma TGs decreased, although mildly, in both sexes during the period from mid-September to February. As mentioned earlier, it is possible that this decrease is smaller than what might be observed from actively migrating fish in the wild. Second, we noted a substantial increase in plasma TGs during the time of maximum gonad development, suggesting an increased demand for energy during this time. Like *G. australis*, Pacific lampreys are sexually immature for much of their adult freshwater residency and show rapid ovarian development during the months just prior to spawning. No doubt that much of the energy mobilized during this period went for the process of vitellogenesis. Finally, at the time of spawning, plasma TGs dropped in both sexes, with females showing a more severe decrease than males. All of these findings suggest that lipids are used for routine metabolism and final sexual maturation and energetic demands are highest in females.

The relative stability of plasma glucose levels in our fish during a prolonged period of fasting indicates that gluconeogenesis is occurring (Larsen, 1980). Larsen (1976) noted that the stability of glucose levels and the rise in levels seen at spermiation and ovulation seem to be physiologically well-regulated. In our fish in 2001, the rise in glucose levels occurred during mid-May, which was the time when gametes could be easily expressed by palpation. We did not observe such an increase in glucose levels in our fish in 2003. As suggested by Larsen (1980), the dynamics of glucose during the period of final maturation probably reflects the high activity in all metabolic processes of lampreys in this phase.

In summary, our study documented changes in sex steroids, thyroid hormones, and nutritional factors in adult Pacific lampreys during overwintering and sexual maturation, a period of about eight months. Such basic information on their physiology and reproductive biology increases our understanding of the life history of Pacific lampreys. In a companion paper, we will compare levels of physiological variables from fish passing dams on the Columbia River to levels from fish in our studies reported here. We hope to explore the potential correlations between physiological status and the tendency to migrate in these fish.

Acknowledgements

We thank Mary Moser of NOAA Fisheries and Darren Ogden of the Pacific States Marine Fisheries Commission for capturing and sampling fish at Bonneville Dam and for providing data summaries; Dena Gadomski, Susan Imholt, Mike Meeuwig, Becky Reiche, Craig Robinson, Brian Sharp, and Lisa Weiland for laboratory and editorial assistance; Dick Ewing for the thyroid hormone assays; and Mike Langeslay and David Clugston of the U. S. Army Corps of Engineers for administering this project. Reviews by Alec Maule and Mary Moser improved the manuscript. Mention of trade names does not imply endorsement by the U. S. Geological Survey.

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