

Maturation characteristics and life-history strategies of the Pacific lamprey, *Entosphenus tridentatus*

Benjamin J. Clemens, Stan van de Wetering, Stacia A. Sower, and Carl B. Schreck

Abstract: Lampreys (Petromyzontiformes) have persisted over millennia and now suffer a recent decline in abundance. Complex life histories may have factored in their persistence; anthropogenic perturbations in their demise. The complexity of life histories of lampreys is not understood, particularly for the anadromous Pacific lamprey, *Entosphenus tridentatus* Gairdner, 1836. Our goals were to describe the maturation timing and associated characteristics of adult Pacific lamprey, and to test the null hypothesis that different life histories do not exist. Females exhibited early vitellogenesis – early maturation stages; males exhibited spermatogonia – spermatozoa. Cluster analyses revealed an “immature” group and a “maturing–mature” group for each sex. We found statistically significant differences between these groups in the relationships between (i) body mass and total length in males; (ii) Fulton’s condition factor and liver lipids in males; (iii) the gonadosomatic index (GSI) and liver lipids in females; (iv) GSI and total length in females; (v) mean oocyte diameter and liver lipids; and (vi) mean oocyte diameter and GSI. We found no significant difference between the groups in the relationship of muscle lipids and body mass. Our analyses support rejection of the hypothesis of a single life history. We found evidence for an “ocean-maturing” life history that would likely spawn within several weeks of entering fresh water, in addition to the formerly recognized life history of spending 1 year in fresh water prior to spawning—the “stream-maturing” life history. Late maturity, semelparity, and high fecundity suggest that Pacific lamprey capitalize on infrequent opportunities for reproduction in highly variable environments.

Key words: primitive, Petromyzontiformes, life history.

Résumé : Si les lamproies (pétromyzontiformes) persistent depuis des millénaires, leur abondance est, depuis peu, à la baisse. Des cycles biologiques complexes pourraient en partie expliquer leur persistance, et des facteurs anthropiques, leur déclin. La complexité des cycles biologiques des lamproies est mal comprise, particulièrement en ce qui concerne la lamproie du Pacifique, *Entosphenus tridentatus* Gairdner, 1836, une espèce anadrome. Nos objectifs consistaient à décrire le moment des différents stades de maturation des lamproies du Pacifique adultes et les caractéristiques connexes, et à tester l’hypothèse nulle selon laquelle il n’y a pas différents cycles biologiques. Les femelles présentaient des stades de vitellogénèse précoce et de maturation précoce; les mâles, les stades de spermatogonie et spermatozoïde. Des analyses typologiques ont révélé un groupe « immature » et un groupe « en cours de maturation–mature » pour chacun des sexes. Nous avons relevé des différences statistiquement significatives entre ces groupes dans les relations entre (i) la masse corporelle et la longueur totale chez les mâles, (ii) l’indice d’embonpoint de Fulton et les lipides hépatiques chez les mâles, (iii) l’indice gonadosomatique (GSI) et les lipides hépatiques chez les femelles, (iv) le GSI et la longueur totale chez les femelles, (v) le diamètre moyen des oocytes et les lipides hépatiques et (vi) le diamètre moyen des oocytes et le GSI. Nous n’avons décelé aucune différence significative entre les groupes en ce qui concerne le lien entre les lipides musculaires et la masse corporelle. Nos analyses appuient le rejet de l’hypothèse d’un cycle biologique unique. Nos observations appuient l’existence d’un cycle biologique de « maturation océanique » dans lequel le frai a probablement lieu quelques semaines après l’entrée en eau douce, en plus du cycle biologique déjà connu comprenant une année en eau douce préalablement au frai, soit le cycle biologique de « maturation en rivière ». La maturité tardive, la semelparité et la grande fécondité de la lamproie du Pacifique donnent à penser que ce poisson mise sur des occasions de reproduction peu fréquentes dans des milieux très variables. [Traduit par la Rédaction]

Mots-clés : primitif, pétromyzontiformes, cycle biologique.

Introduction

Lampreys (Petromyzontiformes) have changed very little since they appeared in the fossil record over ~300 to 500 million years ago (Dawkins 2004; Janvier 2008; Helfman et al. 2009). Despite or perhaps because of this morphological stability, lampreys exhibit complex and diverse life histories, including variable larval periods (Potter 1980), diverse trophic ecology as adults, ranging from

nonfeeding through different types of parasitic feeding, and a wide range of body sizes, fecundities, and migration strategies. Lampreys range from very small (~100 mm in total length), low fecundity, nonfeeding, resident adults to very large (>800 mm), high fecundity, parasitic feeding, anadromous adults that return to fresh water to spawn (Beamish 1987; Gill et al. 2003; Salewski 2003; Docker 2009; Clemens et al. 2010).

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Lampreys exhibit tremendous diversity in the endocrinology of the reproductive, osmoregulatory, and metamorphic axes. This diversity in physiology can explain the distribution of freshwater resident and anadromous forms (Youson and Beamish 1991; Youson and Sower 2001). Finally, lampreys show incredible metabolic adaptations to prolonged fasting in fresh water (Larsen 1980; Whyte et al. 1993). This complexity and diversity in the behavior, ecology, and physiology of lampreys may explain why they have persisted over millennia. However, lampreys have recently declined in abundance, and anthropogenic perturbations to freshwater spawning and rearing habitats have been implicated in their demise (Renaud 1997; Jelks et al. 2008). The complexity of life histories of lampreys is not fully understood (e.g., see Docker 2009; Hess et al. 2012), particularly for the anadromous Pacific lamprey, *Entosphenus tridentatus* Gairdner, 1836 (Moyle 2002; Moyle et al. 2009).

The Pacific lamprey is a relatively large, parasitic, and anadromous fish that is found throughout river drainages that have access to the Pacific Ocean, from Baja California to Alaska and Japan. Pacific lamprey reside as burrowing, filter-feeding larvae in fluvial substrates for a few years before undergoing a metamorphosis into parasitic juveniles that emigrate to the ocean (Clemens et al. 2010). Marine-phase Pacific lamprey are estimated to spend ≤ 3.5 years in the ocean, depending on body size, where they feed as external parasites on fishes and even whales (Beamish 1980). Pacific lamprey cease feeding and return to fresh water during the spring. They begin their upstream migration during the summer. During the next spring, approximately 1 year after having entered fresh water, sexually mature Pacific lamprey spawn and then die. These migration dates can vary with latitude, being earlier in the southern portion of its range (reviewed in Clemens et al. 2010). Their prolonged freshwater residency, along with extensive migration distances (≤ 700 km) relative to other lampreys, raises many questions about the maturation characteristics and run diversity of the Pacific lamprey (Clemens et al. 2010).

Observations of different run times and different morphologies exist for Pacific lamprey, suggesting a diversity of runs or life-history types. In his review of other studies, Moyle (2002) noted, "It is possible that Pacific lampreys within one stream system have more than one run ...". And in fact, two "runs" of adult Pacific lamprey have been observed in some California rivers (Moyle et al. 2009). It is not known whether these "runs" of Pacific lamprey represent "... a spring run that spawns immediately after the upstream migration and a fall run, which holds over and spawns the following spring" (Moyle 2002), or a spring run that were migrants last year and spawners this year and a fall run that would spawn the following spring.

Native Americans note a single migration lasting from spring through fall, of which two morphotypes of Pacific lamprey were readily observed in the Umatilla River of northeast Oregon: (1) "short, brown eels" called day eels and (2) "long, dark eels" called night eels (Close et al. 2004). Similar observations have been reported by various tribes in Oregon (Miller 2012). Interviews with tribal members in the Klamath River Basin revealed the presence of a run of large, blue Pacific lamprey and small, dark Pacific lamprey (Petersen Lewis 2009). The smaller lamprey or day eels may be fish that already overwintered and spawned, whereas the larger lamprey or night eels may be recent migrants (Close et al. 2004; Petersen Lewis 2009). Alternatively, there may be two different life-history types of Pacific lamprey (Close et al. 2004). Moyle (2002) also noted, "In the Trinity River (Klamath River Basin of northern California), for example, there may be two distinct forms of Pacific lamprey, one smaller and paler than the other, that represent either separate runs or resident versus migratory individuals."

In the 177 years since they were described as a species from the Willamette Falls area of Oregon (Gairdner in Richardson et al. 1836), much remains unknown about the basic biology and run

diversity of Pacific lamprey, and this information will be essential for informing management, conservation, and tribal restoration initiatives (CRITFC 2008; Luzier et al. 2009; Clemens et al. 2010). Our first goal was to describe the maturation timing and characteristics of adult Pacific lamprey. Our second goal was to identify potential diversity of maturation times and associated characteristics; specifically to test the null hypothesis that different life histories of Pacific lamprey do not exist. To achieve these goals, we used a holistic approach, measuring several indices on individual fish: morphological, maturation status, life-history characteristics, and energetic investment. Morphological differences within Pacific lamprey, including body size and the spatial distance between dorsal fins, were measured because they can infer migration status relative to length of migration (Clemens et al. 2010) and duration of fasting (Clemens et al. 2009). The life-history characteristics of Pacific lamprey, including the relative investment of somatic versus gonad tissue were measured because of the implications for the length of the migration in fresh water "anticipated" by the fish prior to spawning (Kan 1975; Beamish et al. 1979; Whyte et al. 1993). The energetic investment (total lipid content) of Pacific lamprey was measured from different tissues because comparisons of lipid stores can be used to infer environmentally induced life-history characteristics (Meffe and Snelson 1993).

Materials and methods

Fish collection

Willamette Falls, Oregon City, Oregon, USA, is a natural 12 m high falls on the Willamette River, at about 205 km from the Pacific Ocean. The falls are flanked by a hydroelectric dam on one side of the river. The falls are the source of the largest tribal harvest of Pacific lamprey in the state of Oregon (Kostow 2002), and quite probably the world, rivaled only by the Klamath River (Petersen Lewis 2009). Pacific lamprey congregate at Willamette Falls en route to spawning locations (Clemens et al. 2012a). Lamprey were collected weekly to monthly during April–September of 2007 and 2008 and less frequently during 2009 (April, May, and July).

During the spring, fish were collected from a trap in the fish ladder of the dam on a weekly to biweekly frequency. When river discharge decreased in the summer, we were able to access the base of the falls to collect lamprey by hand on a monthly basis. Because it is not known when Pacific lamprey enter fresh water and how long they have been in fresh water, we used recent migrants collected at the interface of the Pacific Ocean and the Klamath River as a baseline comparison. Recent migrants were collected from this location for two reasons: (1) there is no established and reliable means of collecting adult lamprey at the mouth of the Columbia and (2) there is an established and reliable means of collecting adult lamprey at the mouth of the Klamath River (i.e., tribal fishers).

Recent-migrant lamprey entering the Klamath River mouth from the Pacific Ocean (river kilometer 0) at Requa, California, USA, were collected and used as a comparison for fish collected from Willamette Falls. In collaboration with the Yurok and Karuk tribes, we collected lamprey alive with traditional fishing spears (described in Petersen Lewis 2009). We collected from this location on four separate occasions, during April 2007, June 2007, April 2008, and April 2009. The timing of these collections was based upon the availability of fish and tribal members to catch them.

Measurements and tissue sampling

A number of morphological and physiological indices were measured on Pacific lamprey. These included morphology, reproductive status, female fecundity, and lipid reserves.

Table 1. Sample size (*N*) by sex, collection location, sampling years, and types of analyses done on the maturation characteristics and diversity of Pacific lamprey, *Entosphenus tridentatus*.

Sex	Location	Years	Analyses	<i>N</i>
Females	WF*	2007–2009	Descriptive‡	175; 1
	RM†	2007–2009	Descriptive‡	18; 3
	WF*	2007–2008	Statistics§	118
	RM†	2007–2008	Statistics§	8
Males	WF*	2007–2009	Descriptive‡	196; 6
	RM†	2007–2009	Descriptive‡	20; 3
	WF*	2007–2008	Statistics§	126
	RM†	2007–2008	Statistics§	12

*WF is Willamette Falls, the inland target population for monitoring.

†RM is recent migrants from the ocean, used as a baseline comparison. Further details can be found in the Materials and methods.

‡Total sample size for descriptions of maturation proportions (see Figs. 3, 6). The second number in the sample size column indicates the number of fish used for measurements of gut morphology (Fig. 1).

§Empirical tests (cluster analyses and subsequent linear models; see Figs. 4, 7A–12B).

Sampling procedure, comprehensive indices, 2007–2008

Up to 50 lamprey were collected per month and they were immediately placed in aerated river water (66–132 L aerated bins) until processed. The fish were then euthanized by an overdose of tricaine methanesulfonate (MS-222), buffered with NaHCO₃. The carcasses were processed for morphology, lipids, fecundity, and gonadal histology.

Sampling procedure, supplementary indices, 2009

Approximately 10 fish were collected from the Klamath River mouth during April and from Willamette Falls during May and July. These fish were sampled for gonadal histology and gut morphology (more details below). The results of the gonadal histology were used to supplement those from comprehensive sampling of fish (see above). Accordingly, these supplementary data from 2009 were used for descriptive purposes only and were not part of the statistical analyses of the comprehensive sampling.

Morphology

2007–2008

Fish mass was measured to the nearest 0.1 g; total body length (TL) was measured to the nearest millimetre and the internal organs removed by dissection. Livers and ovaries were weighed to the nearest 0.1 g. A few of the fish (1.7% of all sampled) were missing tails and so these lamprey were omitted from body morphology analyses.

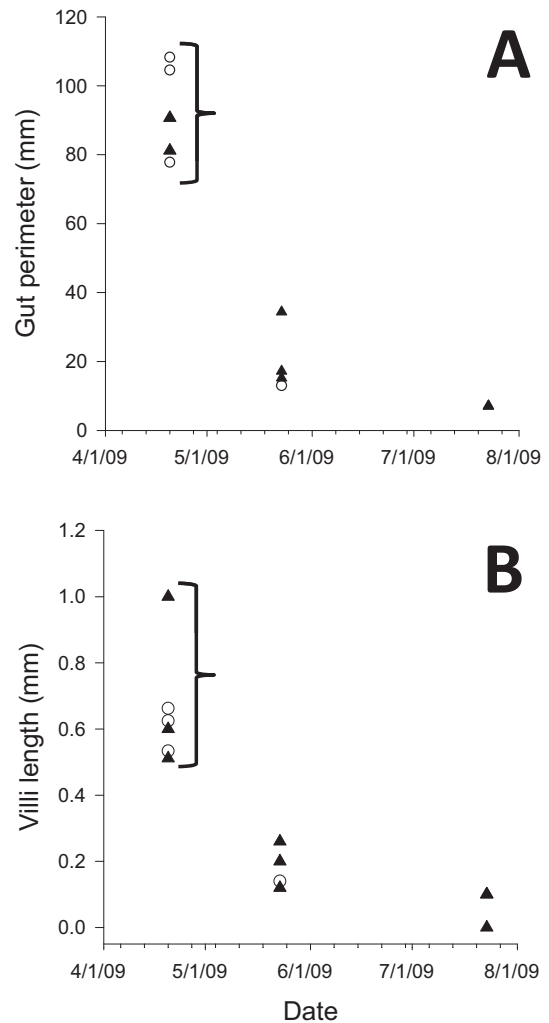
Body mass and TL measurements were used to calculate Fulton's condition factor, or the relative robustness of the fish: body mass \times (TL³ \times 100 000)⁻¹ (Anderson and Neumann 1996). Ovary and body mass measures were used to calculate the gonadosomatic index (GSI): ovary mass \times (body mass – ovary mass)⁻¹. Liver and body mass measures were used to calculate the hepatosomatic index (HSI): liver mass \times (body mass – liver mass)⁻¹.

We measured the distance between the two dorsal fins of the lamprey (herein, "dorsal-fin gap") because prior evidence suggested that this metric is a useful proxy for sexual maturation (Clemens et al. 2009). Digital images of each fish were taken with a Pentax Optio W30 camera against a measuring scale (millimetres) background. The images were used to assess the straight-line distance of the dorsal-fin gap to the nearest 0.1 mm via a digital software program (VistaMetrix by SkillCrest). The digital scale was ground-truthed against the measurement scale in the image with the fish.

2009

To estimate the general time since last feeding, we measured characteristics of the gut morphology of the lamprey. A small

Fig. 1. Measures of gut morphology of Pacific lamprey, *Entosphenus tridentatus*. (A) Gut perimeter; (B) gut villi length. Solid triangles are males and open circles are females. The braces indicate recent migrants entering the Klamath River mouth from the Pacific Ocean during April. All other fish (May and July) were collected from Willamette Falls, Oregon, USA. The recent migrants had larger gut perimeters and villi lengths than the other fish, suggesting that they had only recently ceased feeding in preparation for their spawning migrations. Date format on the x axis is month, day, and year.



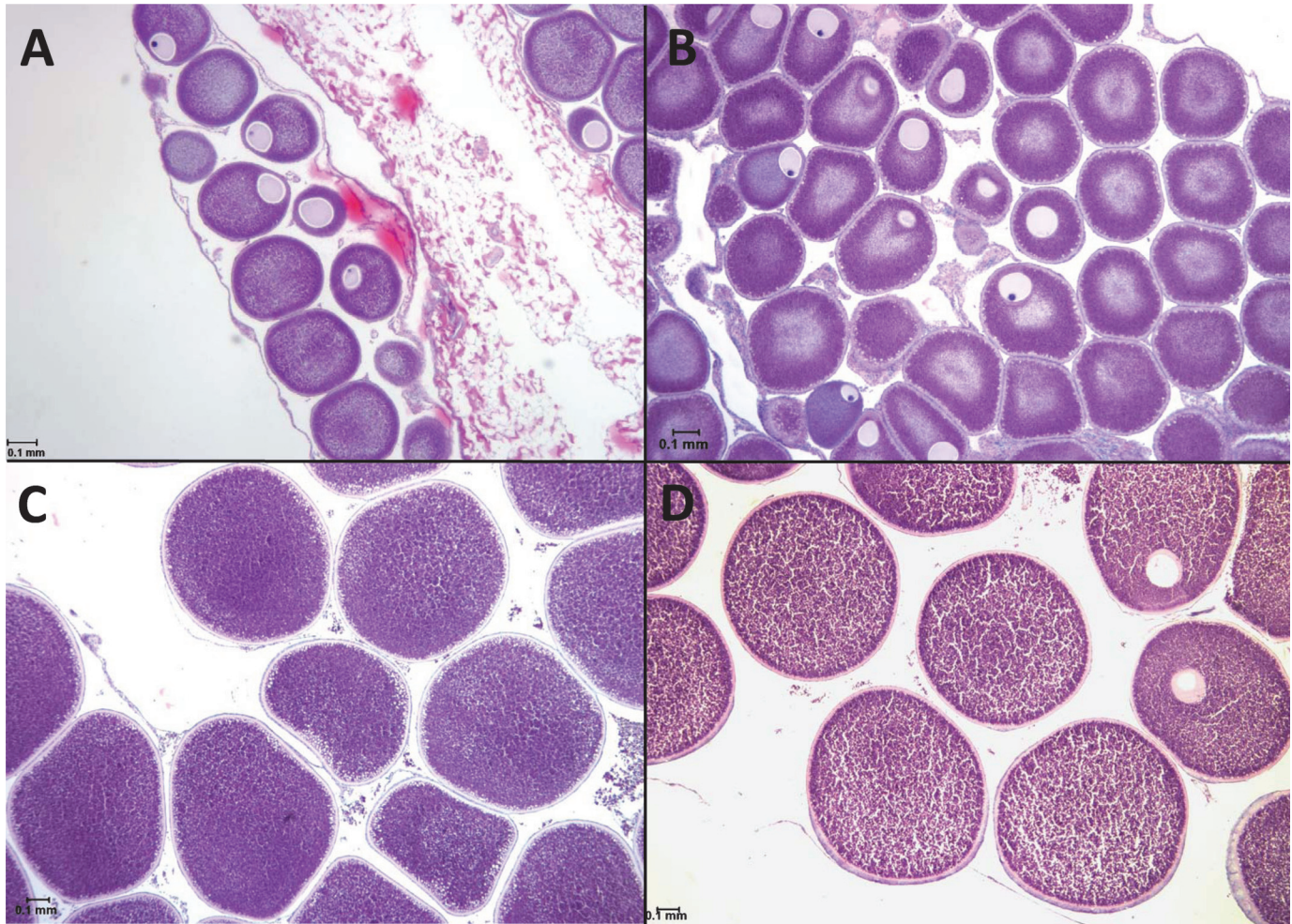
section of the gut of the lamprey was removed from behind the posterior tip of the liver by dissection and placed in 10% buffered formalin. Each sample was processed as a cross section for histology to a thickness of 7–10 μ m, stained with hematoxylin and eosin, and later viewed under a compound light microscope (Leica DMLB). Images were taken with a digital camera (SPOT, Diagnostic Instruments, Inc.). The internal gut perimeter was measured to the nearest 0.1 mm, via the SPOT computer software, to trace the inner surface of the gut, including around the villi lining the gut via the software. In a similar fashion, the maximum length of the gut villi was measured as the straight-line distance between the surface lining of the gut and the distal tip of individual villi for 4–6 villi, and then averaged.

Reproductive status

Gonadal histology

Gonadal histology was used to determine the maturation stage of each lamprey, as described in Clemens et al. (2012b).

Fig. 2. Histological images of ovaries from adult females of the Pacific lamprey, *Entosphenus tridentatus*. (A) Early vitellogenesis; (B) mid-vitellogenesis; (C) late vitellogenesis; (D) early maturation (pre-ovulation). Scale bars = 0.1 mm.



Determination of maturation stage for males was done by averaging the results of stages across four random areas of each testes, as per Fahien and Sower (1990).

Female fecundity

A subsample of the ovary, weighing ~1 g, was removed from the middle of the ovary and preserved in 10% buffered formalin. These samples were later transferred to 70% ethanol. Individual oocytes were carefully dissected away from the connective tissue. The oocytes were spread along the bottom of a petri dish and the “photocopy method” (Smith and Marsden 2007) was used to enumerate them. All samples were counted twice and averaged if the oocyte counts differed by <10%. If the counts differed by ≥10%, a third count was done and averaged with the count that was within <10%. Egg counts were extrapolated to estimate total fecundity, using the equation: ovary mass × (subsample egg count × subsample ovary mass⁻¹).

Lipid reserves

Subsamples of trunk muscle (~0.1 to 0.2 g), ovary tissue (~1 to 2 g), and whole livers (~2 to 6 g) were sampled from lamprey for the purpose of estimating lipid contents. An approximately 3 cm² piece of lateral trunk musculature was removed with a razor blade from below the base of the second dorsal fin. A subsample of ovary tissue was removed from the middle of the ovary. All samples were placed in airtight plastic bags on ice before storing at -20 °C. Tissue samples were thawed, dried, and homogenized, and

then total lipids were extracted from the tissues via a Soxhlet apparatus following the protocol of Anthony et al. (2000). Total lipid mass was estimated as the difference in mass between the dried, homogenized sample before extraction minus the mass of the same sample after extraction. Total lipids were expressed as the percentage of the dry mass of the tissues.

Statistics

To determine the diversity of maturation phenotypes of the adult Pacific lamprey, separate cluster analyses were conducted for each sex. One male intersex from 2007 was omitted from the analyses and is reported elsewhere (Clemens et al. 2012b). The two-step cluster analysis (log-likelihood dissimilarity measure) procedure (SPSS statistical package version 18.0.0; SPSS Inc., Chicago, Illinois, USA) was used, with gonad stage as the categorical variable to construct pre-clusters. For males, the continuous variables used to refine the final clusters were Fulton’s condition factor, HSI, and dorsal-fin gap (the distance between the two dorsal fins). For females, the same continuous variables were used to refine the final clusters, with the addition of GSI. Only fish for which these full suite of indices were available were used. A larger number of fish were used for maturation staging via gonadal histology.

The continuous variables were adjusted for time by regressing each against ordinal date in individual, simple linear regressions, and then adding the residuals from each regression to the mean

Table 2. Means, standard errors (SE), and sample size of each of two clusters (immature and maturing–mature) for each sex of the Pacific lamprey, *Entosphenus tridentatus*.

Metric	Immature			Maturing–mature		
	Mean	SE	N	Mean	SE	N
Females						
Body mass (g)	355.9	8.6	67	314.6	8.1	59
Total length (mm)	595.5	5	67	561.9	5.1	59
Fulton's condition factor	0.167	0.002	67	0.177	0.003	59
Dorsal-fin distance (mm)	17.7	0.8	67	14.5	0.9	59
Gonad stage*	MVT	EVT	67	LVT	EMT	59
Relative fecundity [†]	414	23	10	397	13	30
Absolute fecundity	148 240	10 770	10	127 178	6 099	30
GSI (% body mass) [‡]	3.9	0.1	67	10.5	1	59
Oocyte diameter (mm)	0.4	0.0	67	0.7	0	59
Oocyte lipids (%) [§]	44.5	1.4	12	34.3	1	30
Muscle lipids (%) [§]	29.1	3.1	12	26.6	1.6	29
Liver lipids (%) [§]	58.3	1.5	26	33.8	3.6	32
HSI (% body mass)	1.3	0.0	67	1.3	0	59
Males						
Body mass (g)	308.6	7.4	113	319.1	13	25
Total length (mm)	571	4	113	568	7	25
Fulton's condition factor	0.163	0.002	113	0.175	0.007	25
Dorsal-fin distance (mm)	16.1	0.5	113	10.1	1.4	25
Gonad stage*	SG–SC	SG–SC	113	SZ	SG–SC	25
Muscle lipids (%) [§]	30.0	2.8	30	28.1	1.7	15
Liver lipids (%) [§]	65.1	0.8	46	60	1.8	16
HSI (% body mass)	1.4	0.0	113	1.5	0.1	25

*The modal and range of gonad stages are represented. MVT, mid-vitellogenesis; EVT, early vitellogenesis; LVT, late vitellogenesis; EMT, early maturation; SG–SC, spermatogonia and spermatocytes; SZ, spermatozoa.

[†]Relative fecundity is expressed as number of oocytes·body mass⁻¹.

[‡]Gonadosomatic index.

[§]Lipids are expressed as percentage of dry mass.

^{||}Hepatosomatic index (HSI).

value for each variable. The data were sorted randomly to minimize any effects of chronological or other types of data ordering. Pearson product moment correlations were used to determine the strength of associations among indices. Indices with a $r \geq 0.5$ were examined further within each cluster for each sex. The data were tested for normal distribution and equal variances, and then the nature of the morphological and physiological interactions were examined using linear models.

Results

Sample sizes for different analyses are reported in Table 1. In comparison with lamprey collected from Willamette Falls, recent migrants had large gut perimeters with well-developed villi, indicating that they had only recently finished their parasitic feeding (Figs. 1A, 1B).

Cluster analysis identified two clusters for females and two clusters for males. For females, the two clusters were “immature fish” (early to mid-vitellogenesis) and “maturing–mature fish” (late vitellogenesis to early maturation) gonad stages (Figs. 2A–2D, Table 2). For males, the two clusters were “immature fish” (spermatogonia–spermatocytes) and “maturing–mature fish” (spermatocytes, spermatids, or spermatozoa) (Figs. 5A–5D, Table 2). For both sexes, lamprey in the “immature” cluster occurred within the same collection dates as “maturing–mature” (MM) fish, indicating that maturation cohorts or runs of fish were intermixed in time and space (e.g., see Fig. 4).

Females

Fish from Willamette Falls showed four maturation stages: (1) early, (2) mid-, and (3) late vitellogenesis, and (4) early maturation (Figs. 2A–2D). Recent migrants from the Klamath River mouth primarily showed the first two of these stages, early and mid-vitellogenesis. However, a small proportion of fish were in the late vitellogenic stage (maturing–mature group): 1 out of 15

recent migrants from comprehensive sampling in 2007 and 2008. Another 1 out of 3 recent migrants from descriptive sampling were also in the late vitellogenic stage during 2009 (Table 1, Fig. 3). These data suggest that some recent migrants in the Klamath River may spawn in the same year that they enter the river.

The maturation stages varied within and across dates. Maturation occurred during April–May, when a large proportion of fish were in the late vitellogenic or early maturation stages (Fig. 3) and the GSI was highest (Fig. 4). Fish collected between June and September were approximately evenly divided between mid- and late vitellogenic fish, with a small percentage of fish being less mature (early vitellogenic fish; Fig. 3). Recent migrants in the Klamath River mouth were less mature, being primarily early and mid-vitellogenic fish—with the exception of the late vitellogenic fish noted above. The GSI was measured on one of these two late vitellogenic, recent migrants from the Klamath River mouth, and it was 5.5%. Four of the recent migrants that were early vitellogenic had GSIs of 1.2%–2.8%. The other four that were mid-vitellogenic had GSIs of 2.3%–3.4% (Fig. 4). No ovulating or spawned fish were observed.

Males

Fish from Willamette Falls showed four maturation stages: (1) spermatogonia–spermatocytes (SG–SC), (2) spermatocytes (SC), (3) spermatids (SD), and (4) spermatozoa (SZ) (Figs. 5A–5D). Recent migrants from the Klamath River mouth primarily showed the first stage, SG–SC. However, a small proportion of fish were in the SC stage (maturing–mature group): 1 out of 17 recent migrants from comprehensive sampling in 2007 and 2008. Another 2 fish out of the remaining 19 recent migrants from descriptive sampling (all years, 2007–2009) were also in the SC stage (Table 1, Fig. 6). These data suggest that some recent migrants in the Klamath River may spawn in the same year in which they enter the river.

Fig. 3. Percentage of female Pacific lamprey, *Entosphenus tridentatus*, at each of four stages of maturity, as determined by gonadal histology. Data from all years (2007–2009) combined. “Recent migrants” were fish collected at the interface of the Pacific Ocean and the Klamath River mouth, as they entered the river; all other fish are from Willamette Falls. Numbers in italic type above each month indicate the sample size from which the proportions were determined.

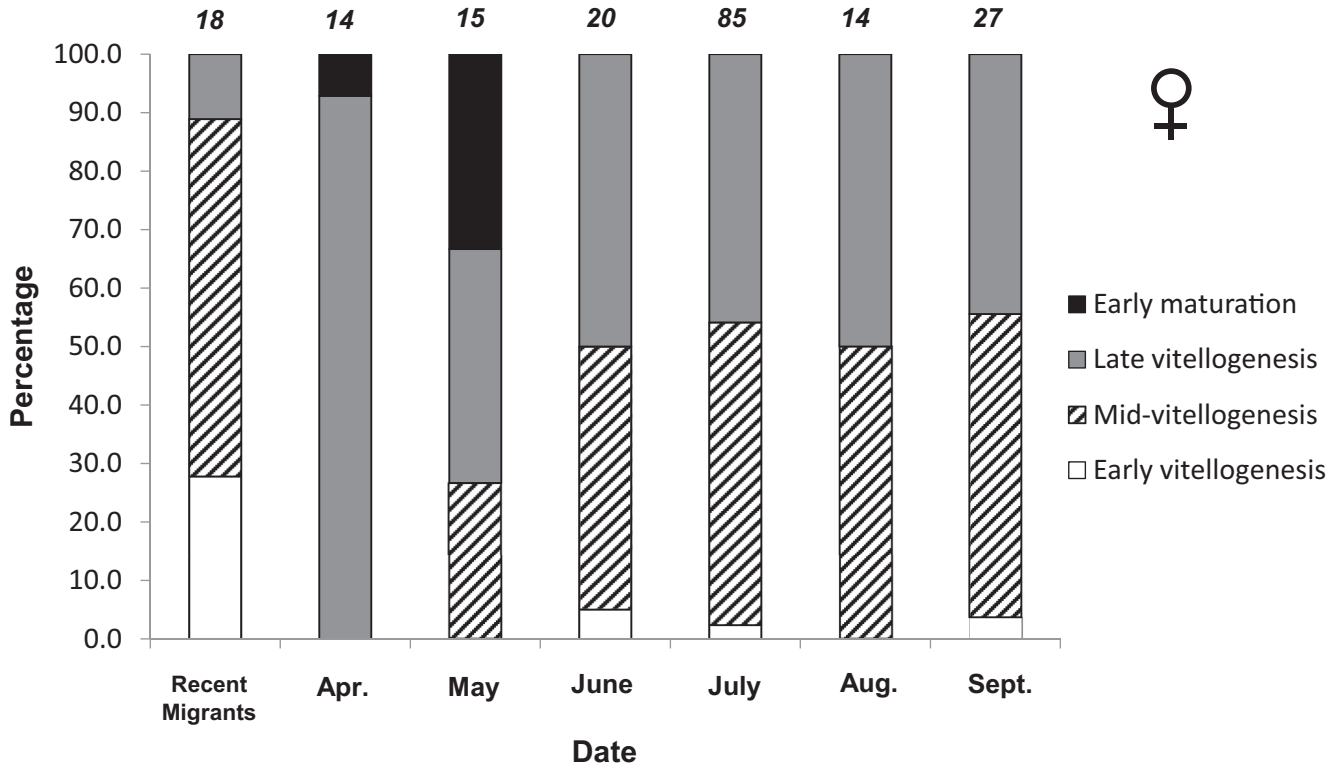
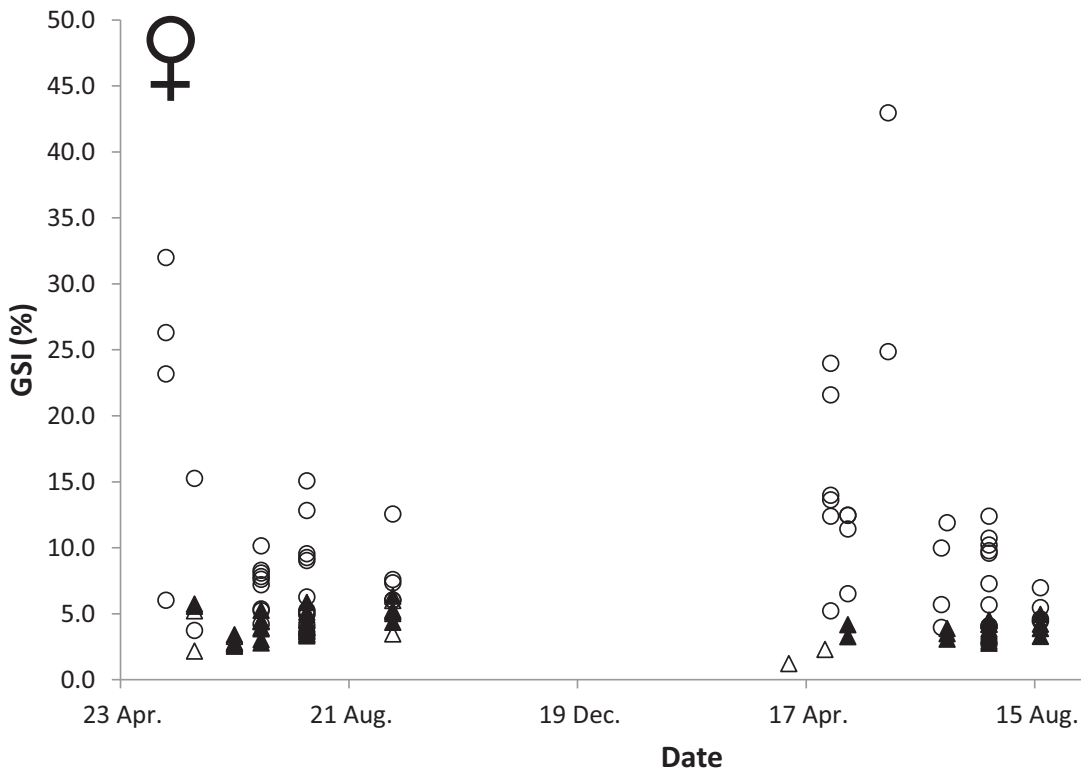
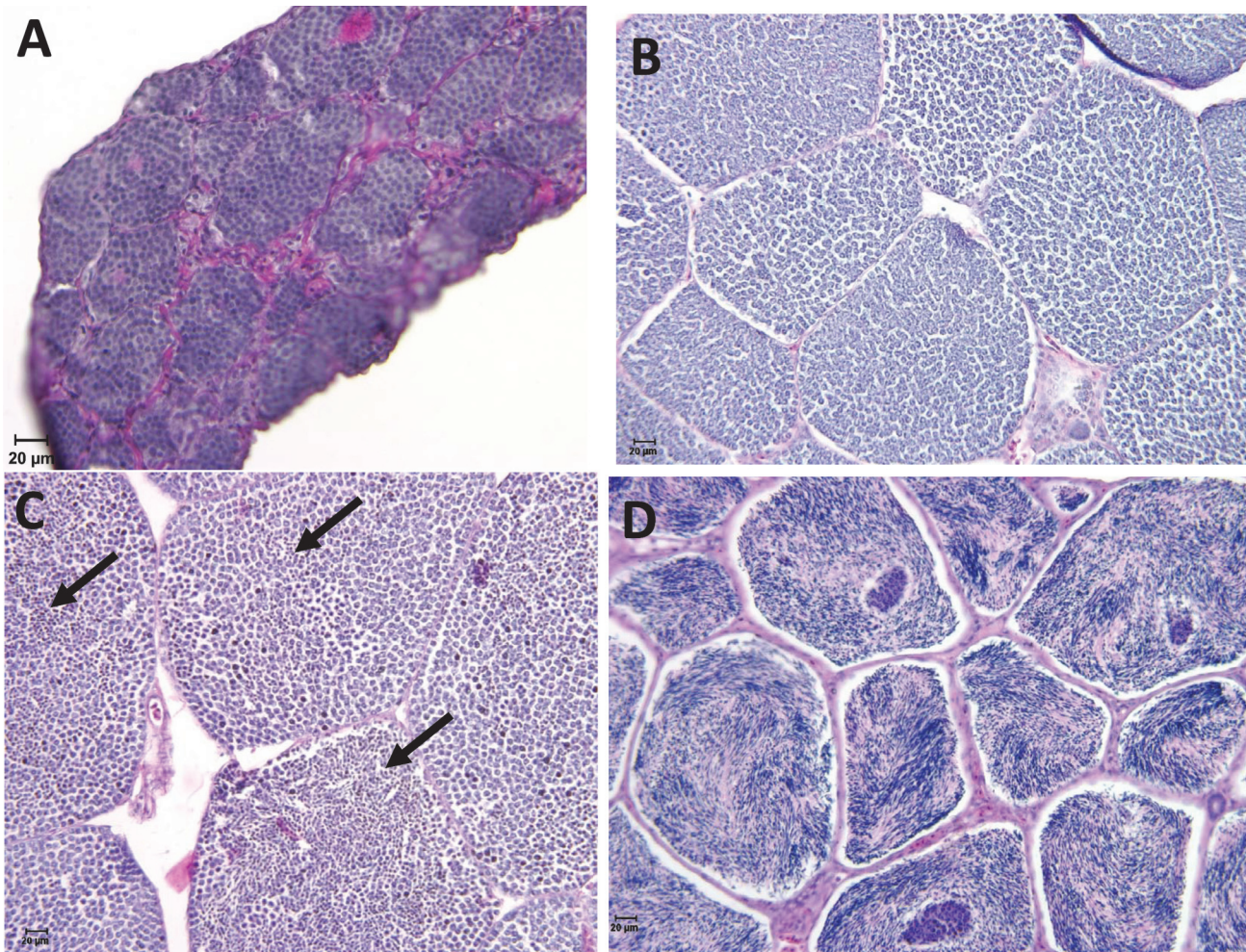


Fig. 4. Gonadosomatic index (GSI) for female Pacific lamprey, *Entosphenus tridentatus*. Open circles are maturing–mature Pacific lamprey from Willamette Falls, solid triangles are immature lamprey from Willamette Falls, and open triangles are immature recent migrants collected from the Klamath River mouth.



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Fig. 5. Histological images of testes from male adults of the Pacific lamprey, *Entosphenus tridentatus*. (A) Spermatogonia; (B) spermatocytes; (C) spermatocytes and spermatids (examples of spermatids indicated by arrows); (D) mature spermatozoa. Scale bars = 20 μ m.



The maturation stages varied within and across dates. However, the proportions for each maturation stage were such that there was evidence of maturation during April–June, when a large proportion of fish had SD and SZ. Fish collected between July and September were mostly SG–SC (Fig. 6).

Character interactions

All data tested in the following linear models met assumptions for normality and homoscedasticity.

Morphology and lipid reserves

There was no significant difference in body mass between MM and immature female lamprey (MANOVA, $p = 0.3786$), after controlling for TL (Fig. 7A). By contrast, there was suggestive but inconclusive evidence for a greater body mass for immature male lamprey in comparison with MM fish (MANOVA, $p = 0.0623$), after controlling for TL (Fig. 7B, Table 2).

The percentage of lipids in the muscle was lower in smaller females. There was no significant difference in muscle lipids between MM and immature female lamprey (MANOVA, $p = 0.7887$; Fig. 8A, Table 2). Similarly, there was no significant difference in muscle lipids between MM and immature male lamprey (MANOVA, $p = 0.1182$; Fig. 8B, Table 2).

There was a negligible increase in Fulton's condition factor with decreases in liver lipids for both sexes. There was no significant difference in Fulton's condition factor between MM and immature female lamprey (MANOVA, $p = 0.2144$), after controlling for

liver lipids (Fig. 9A). By contrast, there was a significant difference in Fulton's condition factor between MM and immature male lamprey (MANOVA, $p = 0.0284$), after controlling for liver lipids (Fig. 9B). Liver lipids generally decreased with maturation stage for both sexes (Table 2; for females see also Fig. 10).

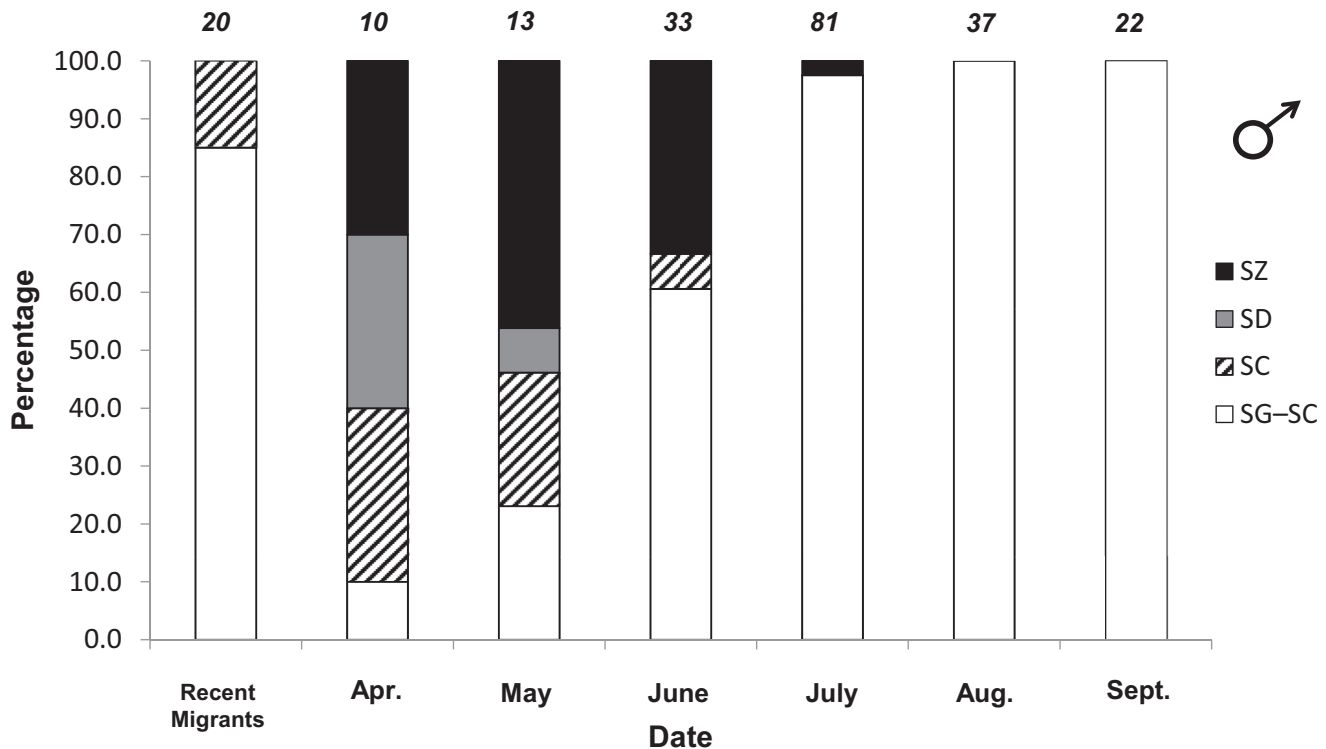
There was a highly significant difference in GSI between MM and immature female lamprey (MANOVA, $p < 0.0000$): the GSI of MM females increased with exponential decreases in liver lipids, whereas the GSI of immature females showed a negligible change in GSI in relation to liver lipids (Fig. 10, Table 2).

Morphology, ovary characters, lipid reserves

There was a highly significant difference in the GSI between MM and immature females (MANOVA, $p < 0.0000$): the GSI of MM females showed an exponential increase per unit decrease in TL, whereas immature females showed a weak linear increase (Fig. 11, Table 2).

Maturing–mature female lamprey showed significantly greater mean oocyte diameters than immature fish (MANOVA, $p < 0.0000$; Table 2). The mean oocyte diameter of MM fish showed an increase per exponential decrease in liver lipids, whereas the mean oocyte diameter of immature females showed little change per unit decrease in liver lipids (Fig. 12A, Table 2). Oocyte diameter increased with logarithmic increases in GSI for both MM and immature fish, although this relationship was significantly different

Fig. 6. Percentage of male Pacific lamprey, *Entosphenus tridentatus*, at each of four stages of maturity, as determined by gonadal histology. Data from all years (2007–2009) combined. “Recent migrants” were fish collected at the interface of the Pacific Ocean and the Klamath River mouth, as they entered the river; all other fish are from Willamette Falls. Numbers in italic type above each month indicate the sample size from which the proportions were determined. SZ, spermatozoa; SD, spermatids; SC, spermatocytes; SG–SC, a mixture of spermatogonia and spermatocytes.



between MM and immature female lamprey (MANOVA, $p < 0.0000$; Fig. 12B, Table 2).

There was no significant difference in fecundity between MM and immature female lamprey after controlling for body mass (MANOVA, $p = 0.2393$; Table 2). The relationship between fecundity and body mass can be expressed as: fecundity = $329.78 \times (\text{body mass}) + 23\,202$ ($r^2 = 0.5295$). Ovary lipids increased exponentially with decreases in oocyte diameter with the function: ovary lipids = $64.566^{-0.872(\text{oocyte diameter})}$ ($r^2 = 0.7268$; Table 2).

Discussion

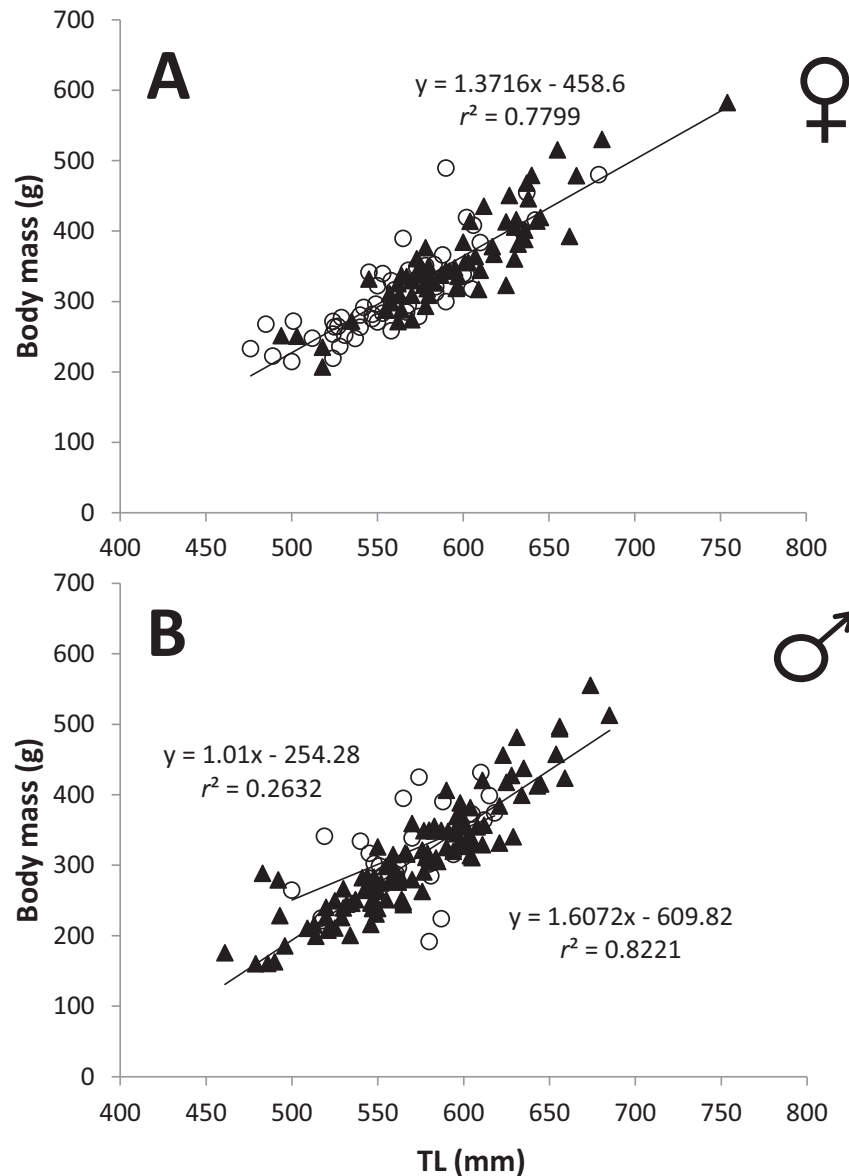
Our goals were to describe the maturation timing and associated characteristics of adult Pacific lamprey and to test the null hypothesis that different life histories of Pacific lamprey do not exist. Our data are suggestive of two distinct maturation cohorts of Pacific lamprey, comprising a maturing–mature (MM) run and an immature run. The maturing–mature run included one recent-migrant male from the Klamath River mouth. Additional lamprey from the Klamath River mouth were used for descriptions of maturation stage, four of which had maturation stages consistent with the maturing–mature group: two females in the late vitellogenic stage and two males in the spermatocyte stage. In total, 11% of females (2 out of 18 fish) collected from the Klamath River mouth were in the late vitellogenic stage and 8% of males (3 out of 36 fish) collected from the Klamath River mouth were in the spermatocyte stage. These data represent some evidence that, at least in the Klamath River, a maturation cohort of Pacific lamprey consists of recent migrants that spawn within several weeks of entering fresh water, another cohort that matures and spawn at least 1 year later (the expected life history of Pacific lamprey; see Beamish 1980), and old migrants (arrived in fresh water last year and spawn during the current year). Accordingly, we reject the

null hypothesis that different life histories of Pacific lamprey do not exist.

We hypothesize that the MM recent migrants that would likely spawn within several weeks of entering fresh water may be analogous to a winter or “ocean-maturing” steelhead, *Oncorhynchus mykiss* (Walbaum, 1792), that optimizes feeding and growth opportunities in the open ocean for a few years before entering fresh water during the late winter or early spring and spawning relatively low in the river system shortly afterwards (Quinn 2005). Alternatively, ocean-maturing lamprey may be similar to coastal cutthroat trout, *Oncorhynchus clarkii* (Richardson, 1836), that feed and grow in the coastal areas of the ocean for a few months before entering fresh water to spawn (Behnke 2002). There could be other less apparent explanations as well. We also hypothesize that the lamprey, which would likely spawn within about 1 year of entering fresh water, are analogous to a summer or “stream-maturing” steelhead that foregoes feeding and growth opportunities, enters fresh water during the summer–fall, and accesses spawning grounds to spawn at specific temperatures that would promote fitness the following spring. Based on the foregoing rationale, we adopt the following terminology for the Pacific lamprey: “ocean-maturing” lamprey are those that are likely to spawn within several weeks of entering fresh water and “stream-maturing” lamprey are those that fit the more conventional definition for the life history of lamprey (sensu Beamish 1980), spending about 1 year in fresh water prior to spawning.

We hypothesize that stream-maturing Pacific lamprey forego continued feeding and growth opportunities in the ocean to enter fresh water where they will mature and spawn. And there is evidence that, like stream-maturing steelhead, the spawning date of Pacific lamprey must be optimized, as the time-to-hatch and normal

Fig. 7. Body mass versus total body length (TL) for Pacific lamprey, *Entosphenus tridentatus*, collected from Willamette Falls and the Klamath River mouth (recent migrants). (A) Females; (B) males. Open circles are maturing–mature fish and solid triangles are immature fish. The lines are least squares simple linear regressions. Because there was no significant difference in body mass relative to TL for females of either maturation cohort (further details in Results), this relationship is expressed as one regression model. There was suggestive but inconclusive evidence for a greater body mass for immature male lamprey compared with maturing–mature fish (further details in Results); therefore, these two maturation cohorts are expressed as separate linear regression models.



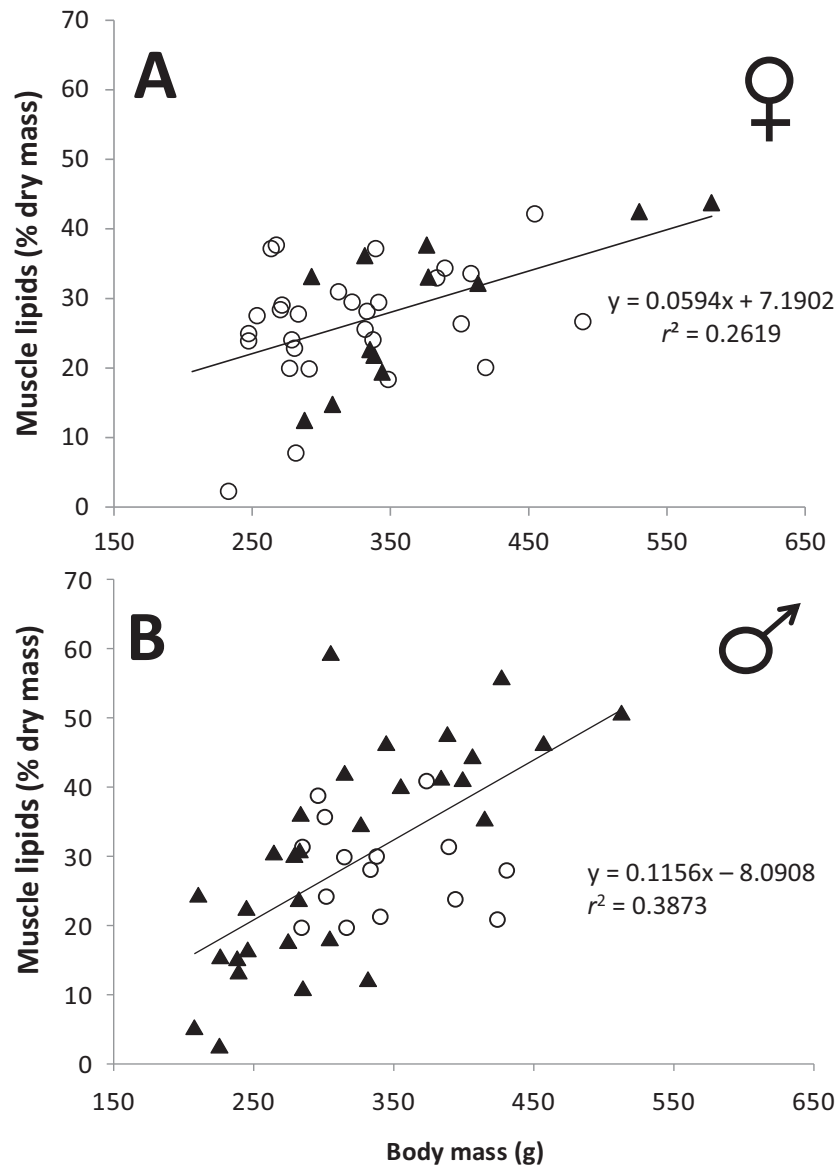
development of their zygotes strongly correlates with ambient water temperatures (e.g., see Meeuwig et al. 2005).

Relative to sea lamprey, *Petromyzon marinus* L., 1758, the process of sexual maturation in Pacific lamprey occurs over a more protracted period. For example, our Pacific lamprey females showed early stages of vitellogenesis, whereas anadromous sea lamprey, collected in fresh water, show only the later stages of vitellogenesis through maturation and spawning (Bolduc and Sower 1992). Similarly, our Pacific lamprey males showed a mix between spermatogonia and spermatocytes, whereas anadromous sea lamprey males in fresh water show only later stages of maturation, from beginning meiosis with spermatocytes onward (Fahien and Sower 1990). This protracted maturation process by the Pacific lamprey occurs in conjunction with their prolonged, sometimes extensive spawning migrations (Clemens et al. 2010). And it may explain why the muscle lipids for both sexes of our Pacific lamprey was

higher than for sea lamprey—averaging around 30% (Table 2) vs. $\leq 8\%$ for anadromous sea lamprey (Beamish et al. 1979) and $\leq 13\%$ for landlocked sea lamprey (Kott 1971)—higher muscle lipid contents may be necessary to fuel the extensive migrations.

We have provided evidence of linear relationships between somatic deterioration and gonadal development indicative of shrinkage in body size to fuel maturation. Accordingly, we agree with Larsen's hypothesis for lamprey that "[sexual maturation] may depend on a metabolic signal related to starvation" (Larsen 1980), at least for stream-maturing Pacific lamprey. These relationships include increases in GSI and oocyte diameter in tandem with decreases in total length, dorsal fin gaps, and liver lipids and concomitant increases in condition factor (Clemens 2011 and present paper). Liver lipids decreased with later maturation stages, which is interesting given that the liver supplies lipids to developing oocytes in the form of vitellogenin (Youson 1981). Shrinkage

Fig. 8. Muscle lipids versus total body mass in Pacific lamprey, *Entosphenus tridentatus*, collected from Willamette Falls and from the estuary (recent migrants). (A) Females; (B) males. Open circles are maturing–mature fish and solid triangles are immature fish. The lines are least squares simple linear regressions. Because there was no significant difference in muscle lipids relative to body mass for either sex of either maturation cohort (further details in Results), this relationship is expressed as one regression model for each sex.



in body length of Pacific lamprey during fasting, to fuel gonadal maturation (Larsen 1980; Clemens et al. 2009), would explain an increase in condition factor. Our data support these morphological trends with maturation. We were surprised to find that oocyte lipids decreased with maturation stage. However, this may simply be due to the immature oocytes being smaller and more densely packed and therefore containing a greater lipid content per dry mass than large, mature oocytes that had more fluid space around them.

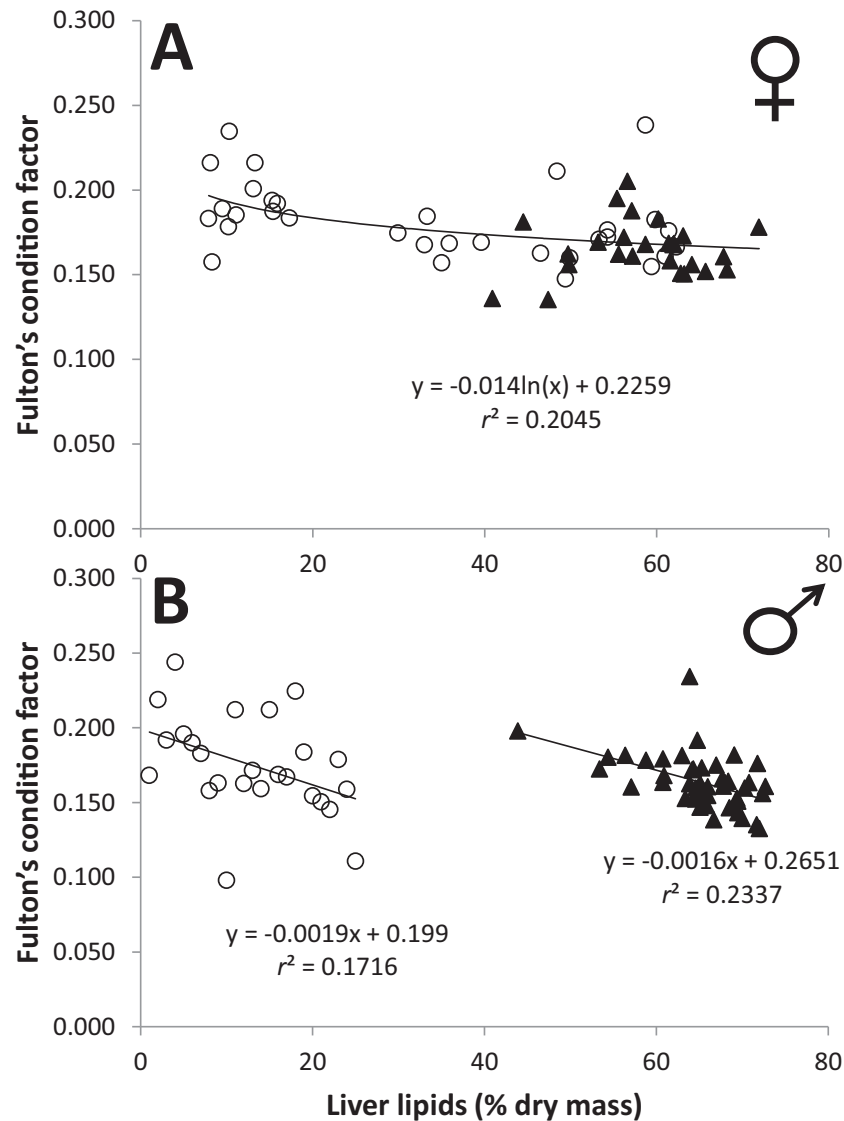
Several of the indices that we have used in our cluster analyses overlapped between the two maturation cohorts. The level of genetic or environmental inducement on these characters, including body shrinkage and consequent maturation, is unknown. However, the evidence that we present may suggest a genetic component to maturation timing for the ocean-maturing lamprey, whereas thermal history experienced by each lamprey likely has more of an effect on body morphology, bioenergetics, and

maturation timing for stream-maturing lamprey (e.g., see Clemens et al. 2009).

Life-history strategies of the Pacific lamprey

Evolution has favored late maturity in Pacific lamprey of about 8–13 years (3–8 years as ammocoetes + ~4 years as parasitic adults + ~1 year during the spawning migration; Clemens et al. 2010), semelparity, and incredibly high fecundity. This fecundity is ~13 to 150 times greater than the fecundity of steelhead (fecundities ~2000 to 12 000 oocytes; Bulkley 1967; Quinn et al. 2011), the fish of which we have compared with run diversity of Pacific lamprey. This late maturity and high fecundity by the Pacific lamprey resembles a “periodic” life-history strategy, similar to sturgeons (Acipenseriformes), although sturgeons are repeat spawners; and unlike sharks (Carcharhiniformes) and guppies (Cyprinodontiformes). Sharks have high survival, low fecundity, and late maturation; guppies have low survival, low fecundity, and early

Fig. 9. Fulton's condition factor versus liver lipids for Pacific lamprey, *Entosphenus tridentatus*, collected from Willamette Falls and from the estuary (recent migrants). (A) Females; (B) males. Open circles are maturing–mature fish and solid triangles are immature fish. The lines are least squares simple linear regressions. Because there was no significant difference in Fulton's condition factor relative to liver lipids for females of either maturation cohort (further details in Results), this relationship is expressed as one regression model. By contrast, there was a significant difference in this relationship for male lamprey (further details in Results), and so the two maturation cohorts are expressed as separate linear regression models.



maturation (Winemiller and Rose 1992). Pacific lamprey have low survival, high fecundity (this paper), and late maturity. Periodic strategists—like the Pacific lamprey—produce massive quantities of small offspring to capitalize on infrequent opportunities for favorable reproduction in environments with large spatial or seasonal variation (Winemiller and Rose 1992).

We have described the maturation characteristics of Pacific lamprey and identified two life histories: ocean-maturing and stream-maturing. Two useful areas for research on these life histories of the Pacific lamprey will be to (1) assess their geographical distribution and temporal persistence and (2) ascertain the selection processes leading to their expression, particularly with regards to the population cycles of these periodic strategists.

Prospectus on conservation, research, and monitoring

The relative information level on the reproductive biology of the Pacific lamprey was recently characterized as low (Clemens et al. 2010) and this paper has advanced understanding in this

area. Information from our trait interactions can be used to predict the relative level of maturity of particular lamprey, and therefore inform selection efforts of wild brood stock for new aquaculture practices and ongoing translocation of adults. Both aquaculture and translocation are being used by Native American tribes to preserve and restore populations of the Pacific lamprey. Translocation is the practice of collecting adults from low in the Columbia River Basin and moving them past barriers (dams) and into regions in the upper watersheds to reintroduce them into formerly occupied streams (e.g., see Ward et al. 2012).

This paper also provides a prospectus on the research and monitoring of lamprey populations. In research designed to assess dam passage efficiency through the use of radio-tagged lamprey, often (always?) mature fish (those with well-developed, obvious secondary sexual characteristics) have either been precluded from tagging, not observed, or not reported (e.g., see Moser et al. 2002, Robinson and Bayer 2005, Mesa et al. 2010, Clemens et al. 2012a,

Fig. 10. Gonadosomatic index (GSI) versus liver lipids for female Pacific lamprey, *Entosphenus tridentatus*, collected from Willamette Falls and from the Klamath River mouth (recent migrants). Open circles are maturing–mature fish and solid triangles are immature fish. The lines are least squares simple linear regressions. Maturing–mature females had a significantly different relationship between GSI and liver lipids than immature fish (further details in Results).

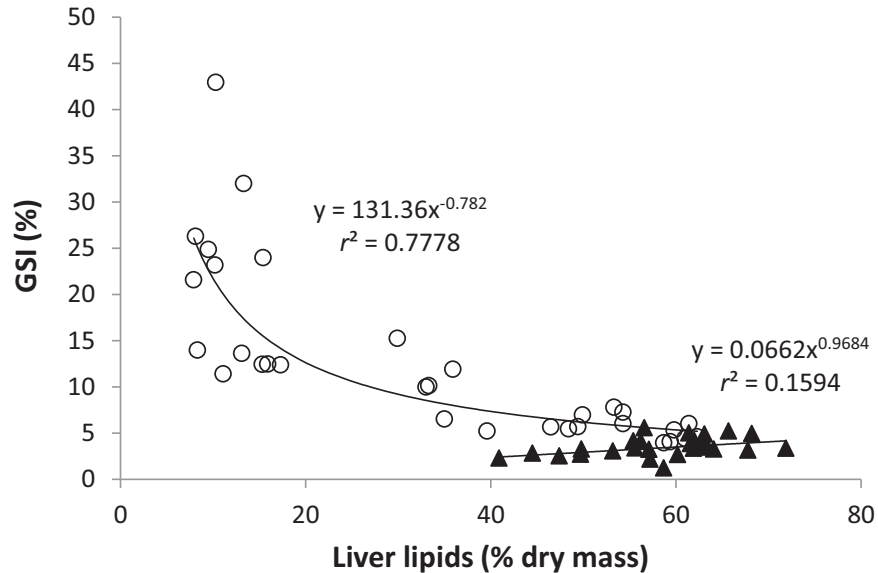
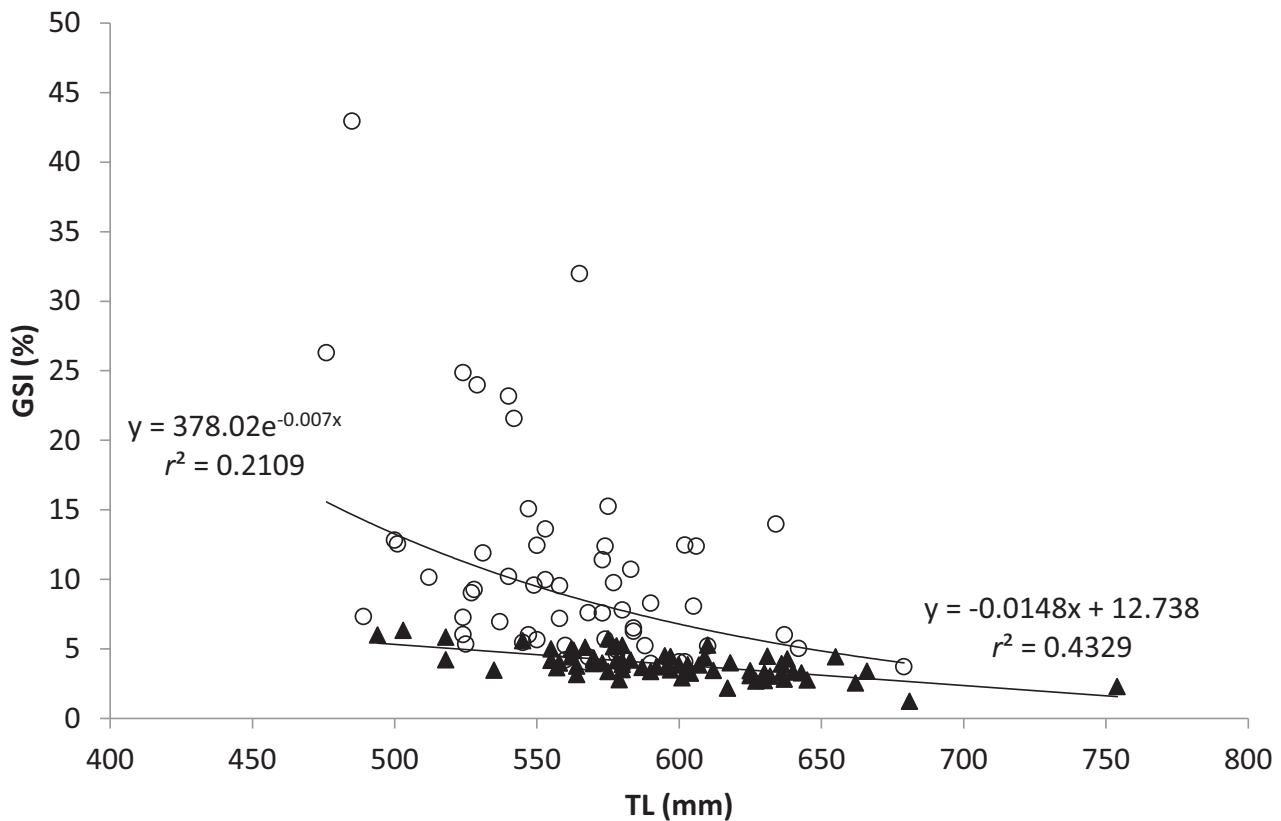


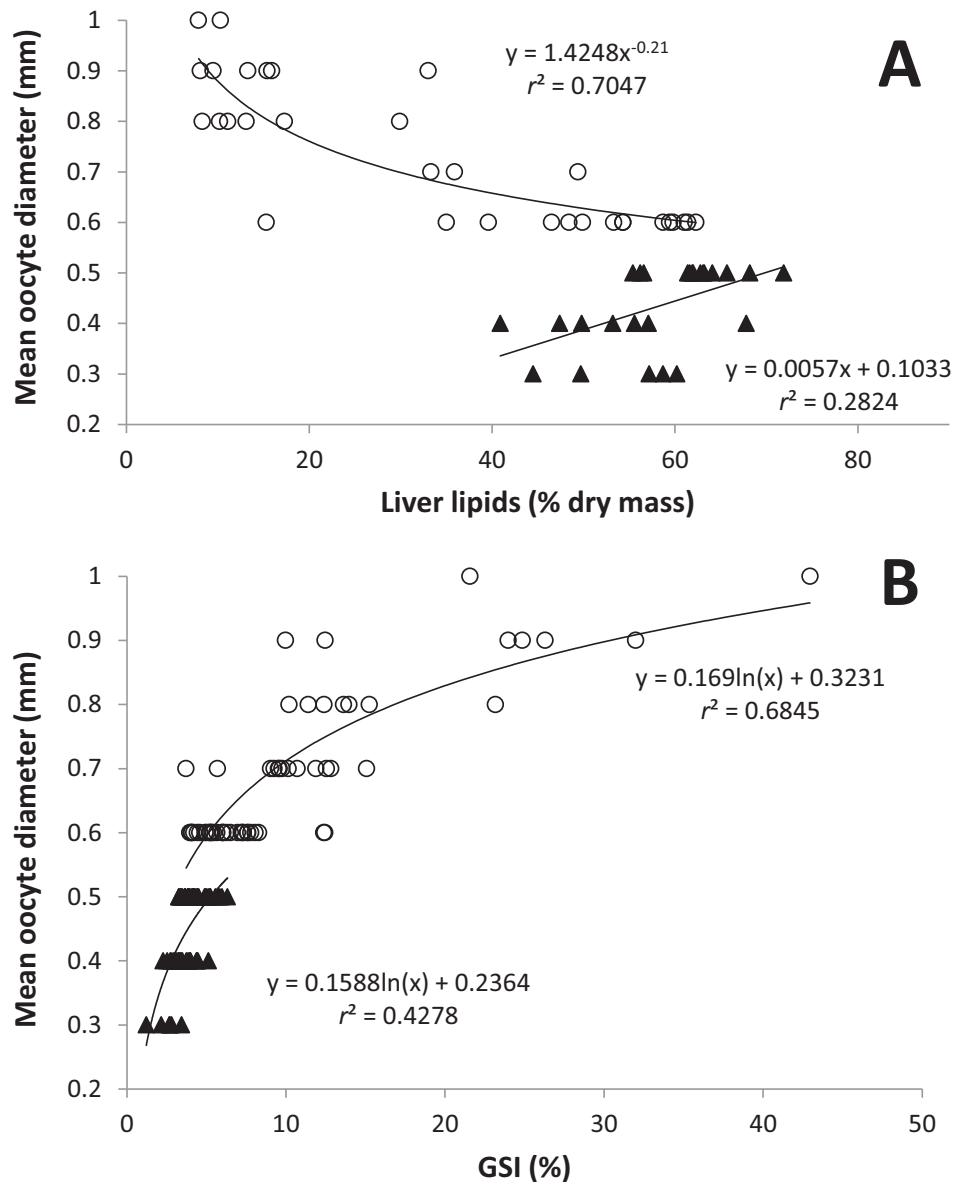
Fig. 11. Gonadosomatic index (GSI) versus total body length (TL) for female Pacific lamprey, *Entosphenus tridentatus*, collected from Willamette Falls and from the Klamath River mouth (recent migrants). Open circles are maturing–mature fish and solid triangles are immature fish. The lines are least squares simple linear regressions. Maturing–mature females had a significantly different relationship between GSI and TL (further details in Results).



Keefe et al. 2013), thereby potentially biasing passage and other migratory behavior in significant, albeit unknown ways. To fully understand how Pacific lamprey respond to challenges in dam passage across both sexes, all size ranges, and all stages of sexual

maturity, a tagging procedure should include all individuals. Second, abundance monitoring of adults has focused on counting adult passage at dam viewing windows. If ocean-maturing Pacific lamprey occur in river systems, then one wonders whether these

Fig. 12. (A) Mean oocyte diameter versus liver lipids and (B) mean oocyte diameter versus gonadosomatic index (GSI) for female Pacific lamprey, *Entosphenus tridentatus*, collected from Willamette Falls and from the Klamath River mouth (recent migrants). Open circles are maturing–mature fish and solid triangles are immature fish. The lines are least squares simple linear regressions. Maturing–mature females had a significantly different relationship between oocyte diameter and liver lipids than immature fish, as well as between oocyte diameter and GSI (further details in Results).



fish might occasionally enter and spawn below the dam(s) doing the counting, and thereby lead to lower than expected counts at that dam.

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Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. This study was approved by Institutional Animal Care and Use Committee of Oregon State University (ACUP No. 3569).

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