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Larval Metamorphosis of Individual Pacific Lampreys Reared in Captivity

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Abstract.—This study is one of the first to follow individual Pacific lampreys *Lampetra tridentata* through the process of metamorphosis. Readily observable external changes were described for 13 individual Pacific lampreys undergoing metamorphosis. Changes occurred to the mouth, eyes, and branchial region from July to at least November. During metamorphosis, Pacific lampreys also exhibited asymmetric growth, including an increase in snout depth, that had not previously been reported in the literature. The order of the morphological changes and the patterns of asymmetric growth in the Pacific lamprey closely matched those reported for another *Lampetra* species, the American brook lamprey *L. appendix*, but exhibited unexpected variations from those reported in other species of lampreys. Excepting one catastrophic event, under captive rearing conditions 96.4% of the ammocoetes survived and the maximum growth rates of 0.040–0.071 mm/d were within the range of those estimated for ammocoetes rearing naturally in stream environments. Supplemental feeding improved larval growth but did not influence the incidence of metamorphosis in captively reared animals. To develop effective conservation strategies for Pacific lampreys in the Columbia River basin, it would be prudent to consider that metamorphosis, a time when the animals are relatively vulnerable, may last from July to December.

The Pacific lamprey *Lampetra tridentata* is found around the Pacific Rim from Japan to southern California and into northern Mexico (see Hart 1973; Moyle 1976, 2002). Evidence has begun to accumulate suggesting that the numbers of Pacific lampreys spawning in the United States, particularly in the Columbia River basin, are in decline (Hammond 1979; Beamish and Northcote 1989; Claire 2003; Moser and Close 2003). As a result, Pacific lampreys have become a species of concern for federal and state agencies, Native American tribes, and the local public (see Close et al. 2002; Claire 2003). The U.S. Fish and Wildlife Service was recently petitioned to list the Pacific lamprey as a threatened or endangered species (USFWS 2004), and management efforts have begun to focus on the conservation of this species. Although the Pacific lamprey has been studied for many years (see Pletcher 1963; Beamish 1980; Moser et al. 2002; Meeuwig et al. 2006), managers still lack critical information about its life history and basic biology that is necessary to develop effective conservation strategies (USFWS 2004).

In lampreys, the transformation from larva (ammocoete) to juvenile (macropthalmia) is a true metamorphosis wherein most or all organ systems seem to undergo reorganization (Potter 1980). This larval metamorphosis (henceforth "metamorphosis"; Youson 1980) is a critical developmental interval during which the lampreys do not feed and are vulnerable and easily influenced by external factors (Purvis 1980; Morman 1987; Youson et al. 1993; Holmes and Youson 1994). For certain species, such as the sea lamprey *Petromyzon marinus*, metamorphosis has been studied extensively. Protocols have been developed for collecting ammocoetes from streams and rearing them in captivity (see Hanson et al. 1974), which has, in turn, allowed the evaluation of metamorphosis under controlled conditions. The numerous specific stages that lampreys exhibit during metamorphosis have been described (see Manion and Stauffer 1970; Potter et al. 1978; Youson and Potter 1979; Youson 2003), and criteria have been developed to predict which ammocoetes will transform (see Holmes and Youson 1994; Holmes et al. 1994). It has been suggested that the sequence of external changes, which has been well documented for sea lampreys (Potter et al. 1978), presents a seven-stage model that is suitable to follow for all lampreys. However, while metamorphosis has been relatively well described for some lampreys (particularly the sea lamprey; Potter et al. 1982), the process can vary between species (Beamish and Thomas 1984).

Relatively little has been reported on metamorphosis in the Pacific lamprey. While changes in body proportions have been associated with different stages of Pacific

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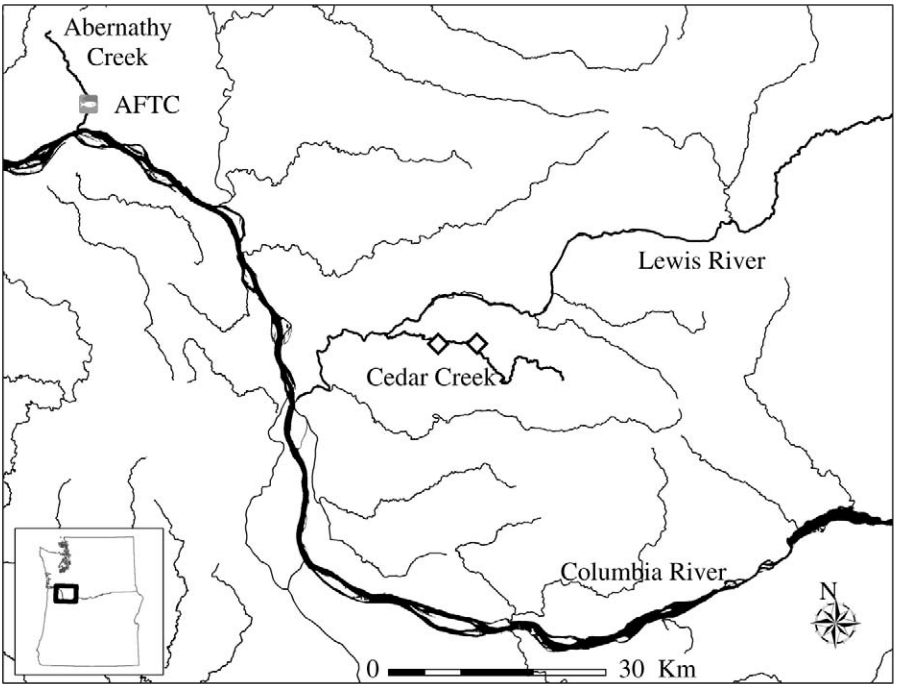

FIGURE 1.—Map of the study area showing the locations of the sites in Cedar Creek at which Pacific lampreys were collected (diamonds) as well as Abernathy Fish Technology Center (AFTC).

lamprey metamorphosis (Richards 1980), the stages that have been used to describe this metamorphosis have largely been assigned a priori using information developed from other species (see Hammond 1979; Richards 1980; Richards and Beamish 1981; van de Wetering 1998; Claire 2003). As a result, current research often uses criteria initially developed for the sea lamprey to judge the extent of transformation in the Pacific lamprey (see Mueller et al. 2006). While the types of changes that occur when Pacific lampreys transform are similar to those in sea lampreys (Richards 1980), it is not clear whether the pattern of changes is similar in both species. In addition, the specific timing (i.e., onset and completion) of Pacific lamprey metamorphosis is uncertain.

Existing information on metamorphosis in the Pacific lamprey is largely from periodic observations of the occurrence of individuals in streams (Hammond 1979; Richards 1980; Richards and Beamish 1981; van de Wetering 1998). To our knowledge, there have been no attempts to follow the progression of individual Pacific lampreys over the entire developmental process. The goal of this study was to examine the process of metamorphosis in Pacific lampreys by following the development of individuals under controlled conditions. We hypothesized that Pacific lampreys would exhibit the same pattern of metamorphic changes as sea lampreys (Youson and Potter 1979) and follow the seven-stage model commonly used to evaluate metamorphic stages in numerous lamprey species (for example, see Holmes et al. 1999). Our objectives were to use previously described metamorphic stages (see Youson and Potter 1979) and document their timing, sequence, and duration as well as to identify the beginning, end, and duration of metamorphosis in the Pacific lamprey. To achieve these objectives wild ammocoetes were captured and reared in captivity. In an effort to assess the adequacy of the rearing conditions, we also evaluated whether supplemental feeding influenced the growth, survival, and rate of metamorphosis in Pacific lamprey ammocoetes. Since stream conditions and sampling logistics prevented us from following the natural development of individuals in the stream, we did not compare captively reared and naturally rearing lampreys. However, previous comparisons have suggested that the process of metamorphosis is similar between captively reared and naturally rearing lampreys (see Youson and Potter 1979).

Methods

Animals and collection.—On 23 and 24 June 2004, ammocoetes of unknown age were collected from Cedar Creek, Washington, near river kilometers 8.5 and 15.0 (measuring from the confluence of the creek and the Lewis River; Figure 1). Both Pacific lampreys

TABLE 1.—The eight metamorphic stages of development used in this study. Stages were evaluated by external examination of the eyes, mouth, and branchial region. For stages 1–7, the external feature reflects a change that is characteristic of that stage. For reference, these stages and their characteristics which have been well described by Manion and Stauffer (1970), Youson and Potter (1979), and Beamish and Thomas (1984) are also given; the references to figures pertain to those articles.

Stage	External feature		
	Mouth	Eyes	Branchial region
0 (ammocoete; lamprey is brown with a dark dorsal region, a light ventral region, and a reddish branchial region)	Anterior end consists of an oral hood	Eyes are present as dark spots, incompletely developed and not yet distinct from the skin's surface	Branchiopores are connected by a longitudinal groove
1		Distinct, thin, light-colored periphery around an enlarged, dark eye spot but not a distinct pupil	
2	Anterior cirrhi of the mouth area enlarged, papillae-like projections		
3		Distinct iris and pupil	
4			Branchiopores no longer connected by a longitudinal groove
5	Lingual laminae present in the oral area		
6	Initial teeth white, soft, and blunt		
7 (defined as completion of metamorphosis)	Final teeth, hard, sharp and yellowing		

and western brook lampreys *L. richardsoni* are present in the Cedar Creek watershed. We used information on the distribution of Pacific lampreys in Cedar Creek (Stone and Barndt 2005) to focus our collections in areas we expected to find Pacific lampreys. Using guidelines for the identification of Pacific lamprey ammocoetes (Richards et al. 1982; Meeuwig et al. 2006), we determined the experimental fish were Pacific lampreys. In addition, all ammocoetes that transformed during this experiment were confirmed as Pacific lampreys. The ammocoetes were collected by electrofishing (see Stone et al. 2006) and then transported for approximately 4 h until reaching the U.S. Fish and Wildlife Service's Abernathy Fish Technology Center (AFTC), Longview, Washington. Once at AFTC, ammocoetes were anesthetized with MS-222 (tricaine methanesulfonate) at a concentration of 50 mg/L. The total length (TL) in mm and total weight (TW) in g of each individual were measured. Initially, none of the ammocoetes showed external signs of metamorphosis. For individual identification, each fish was marked with a subcutaneous injection of orange, red, green, or yellow elastomer at a specific location on its body (see Stone et al. 2006).

Rearing conditions.—Lampreys were randomly assigned to 12 plastic tanks (41 cm wide × 61 cm long × 20 cm deep) at a density of 12 individuals per tank (215 ammocetes/m^3) following Morman (1987). Each tank held 5–7 cm of river sand with a particle size less than 0.5 mm in diameter. Approximately 0.013 m^3 of the tank held water. To allow water to circulate through the tanks, 5-cm-diameter holes (four in each tank) were cut 4 cm from the top of the tank and 4 cm from each corner of the sides, then covered by 1-mm-diameter mesh. Two tanks were placed in each of six troughs (42 cm wide × 155 cm long × 36 cm deep) with the original location being selected randomly. The location of tanks within each trough was alternated every 2 or 3 weeks. A constant 1.5 L/min (Manion and Stauffer 1970) of unfiltered Abernathy Creek water flowed through each trough as well as into and out of each tank providing a natural temperature regime that ranged from 18.8°C (25 July) to 6.3°C (19 November). Ammocoetes were held on a natural photoperiod regime using both natural and artificial light sources. During the middle of the daylight period, average light intensity was approximately 2,500 lx. On September 29, once transforming lampreys reached stage 3 of metamorphosis, rocks with a mean pebble count of 49.6 mm (Wolman 1954) were gathered from a dry Abernathy Creek streambed. To provide structure for nonburrowed lampreys, nine rocks were added to each of the tanks on the substrate surface.

Experimental treatments.—Ammocoetes were reared under three different feeding regimes randomly assigned to each trough. Three tanks did not receive supplemental food (no feed), three tanks were fed 1.00 g baker's yeast per larva (Holmes et al. 1999) (high feed), and four tanks were fed a combination of 0.27 g baker's yeast plus 0.03 g Biokyowa (see Polkinghorne

TABLE 1.—Extended.

Stage	Manion and Stauffer (1990)	Youson and Potter (1979)	Beamish and Thomas (1984)
0 (ammocoete; lamprey is brown with a dark dorsal region, a light ventral region, and a reddish branchial region)	Larvae	Large ammocoete (see Figures 1A, 2A)	Ammocoete (see Figures 1, 2)
1	Stage 1	Stage 2 (see Figure 1C)	Stage 2 (see Figure 2)
2	Stage 1	Stage 2 (see Figure 2C)	Stage 2 (see Figure 1)
3	Stage 2	Stage 3 (see Figure 1D)	Stage 4 (see Figure 2)
4	Stage 4	Stage 4 (see Figure 1E)	Stage 7 (see Figure 2)
5	Not well defined	Stage 5 (see Figures 1F, 1G)	Stage 4 (see Figure 1)
6	Stage 3	Stage 6 (see Figure 2G)	Stage 6 (see Figure 1)
7 (defined as completion of metamorphosis)	Newly metamorphosed	Stage 7 (see Figure 2H)	Stage 7 (see Figure 1)

et al. 2001) fish food per larva (low feed). Two nonexperimental tanks each held 12 lampreys that were used to maintain experimental rearing densities by replacing any mortalities in the other 10 tanks. Twelve lampreys were randomly assigned to each tank. For the lampreys in the three no-feed tanks, the mean (SE) TLs were 118 (6.6), 111 (9.8), and 111 (5.4) mm. For the lampreys in the three high-feed tanks, the mean (SE) TLs were 118 (16.2), 117 (8.1), and 117 (6.1) mm. For the lampreys in the four low-feed tanks, the mean (SE) TLs were 110 (6.4), 118 (5.9), 121 (8.7), and 114 (4.5) mm. Ammocoetes were fed once each week at the amounts described above. For the first 3 weeks of the study, water was turned off for 24 h immediately after feeding (as in Holmes et al. 1994). In subsequent weeks, the water remained on. While lampreys remained in the tank, residual food and any fungus were scraped from the top layer of substrate once every 2 weeks. On alternate weeks, while lampreys were removed for measurements, all the substrate from each tank was vigorously cleaned by mixing and rinsing with water.

Data collection.—We anticipated that metamorphosis would occur between July and November (see Richards 1980). Thus, between 24 June and 8 December 2006, lampreys were examined every 2 or 3 weeks. Lampreys were captured and anesthetized with MS-222 (50 mg/L), and individuals were identified by mark. Survival was evaluated during each sampling event. To examine growth, measurements of TL (rounded to the highest millimeter) and TW (measured to the nearest hundredth of a gram) were obtained from all individuals during each sampling event. To evaluate the onset, duration, and sequence of metamorphic stages (as discussed in Youson 1980), external examinations focused on the eyes, mouth, and branchial groove. Based on the original work of Manion and Stauffer (1970), refined by Youson and Potter (1979) and Potter et al. (1982), and used by Beamish and Thomas (1984), we recorded features based on existing descriptions of morphological changes (Table 1). To track changes in body proportions associated with metamorphic development, digital photographs were taken of a subset of the ammocoetes we anticipated would transform (those longer than 120 mm TL and heavier than 3.0 g TW, see Holmes and Youson 1997). Images were analyzed using the ImageJ program available from the National Institutes of Health. Total length, snout length, prebranchial length, branchial length, first and second dorsal fin height, and eye diameter were measured (Figure 2) as in Potter et al. (1978) and Manion and Stauffer (1970). Condition factor was calculated as $(TW/TL^3) \times 10^6$ (see Holmes et al. 1999). In addition, snout depth was measured as the height of the snout at the midpoint of, and perpendicular to, the prebranchial axis (Figure 2). While the frequency of examination was adequate for the objectives of the study, this design did not allow for the detection of changes that may have occurred on shorter time scales (e.g., days).

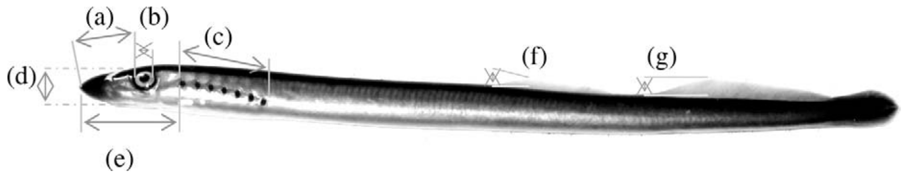

FIGURE 2.—Image of a typical Pacific lamprey macropthalmia showing the regions used to measure body proportions: (a) snout length, (b) eye diameter, (c) branchial length, (d) snout depth, (e) prebranchial length, (f) first dorsal fin height, and (g) second dorsal fin height.

Analysis.—Due to early and complete mortality in the high-feed treatments, these lampreys were not included in analyses. The proportions of lampreys that survived in captivity as well as the proportions of lampreys initiating metamorphosis were compared between low-feed and no-feed treatments by means of Fisher's exact test (Rohlf and Sokal 1995; Sokal and Rohlf 1995). Given that the proportions in the replicate tanks were not different from each other, treatment proportions were pooled together. Since lampreys going through metamorphosis are not generally considered to feed (Moore and Potter 1976), growth during the experimental period was only evaluated for ammocoetes. Overall growth (June to December) as well as whether maximum daily growth rates were positive were analyzed by means of paired-comparison *t*-tests. Daily growth rate for individual lampreys was calculated as (change in TL) / (days associated with the change). Individual growth rates were used to calculate the mean daily growth rate for each tank. To describe the timing, duration, and sequence of metamorphosis, the morphometric characteristics each lamprey exhibited were recorded each time it was examined. The relationships between body proportions (measured the first time a given stage appeared in each transforming individual) and metamorphic stage (see Youson 1980) were evaluated by means of Kendall's correlation analysis (Sokal and Rohlf 1995). Some of the lampreys that were photographed did not go through metamorphosis and the quality of some photographs was inadequate (e.g., poor focus) to allow accurate measurements to be made on all characteristics for every lamprey. Thus, a variable number of animals represented each metamorphic stage in the analysis of body proportions. For all statistical comparisons, significance was assigned when $P < 0.05$.

Results

Survival

Thirty-nine ammocoetes died during the course of this study. Mortality between late June and late July was 30.0% ($n = 36$) and occurred only in tanks under the high feeding regime, during a time when water was shut off (just before introducing food into the tanks) and left off for 24 h. During this event, all ammocoetes in the high-feed treatment died presumably due to circumstances that led to anoxic conditions. After this mortality event, water was left on at all times to ensure that the surviving animals were not subject to the same conditions as those that died. Mortality between late July and early November, after water was left on during feeding, was 3.6% ($n = 3$). Survival did not vary significantly between the low- and no-feed treatments (Fisher's exact test: df = 1, $P < 0.05$).

Growth

At the beginning of the study (23 and 24 June), the mean TL in the no-feed treatments ranged from 109.9 to 117.7 mm (Figure 3) and that in the low-feed treatments from 109.4 to 120.9 mm (Figure 4). At the end of the study (8 December), the mean TL in the no-feed treatments ranged from 109.5 to 116.2 mm and that in the low-feed treatments from 112.9 to 121.3 mm. The mean TL of the surviving ammocoetes in three of the four low-feed tanks increased significantly (3.6, 3.5, and 4.4 mm) from June to December (paired

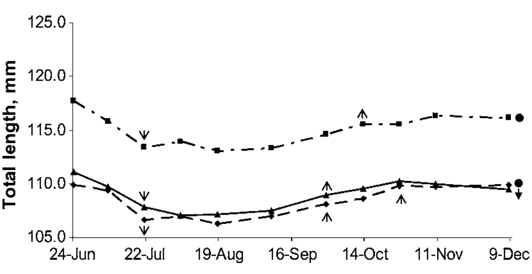

FIGURE 3.—Growth of larval Pacific lampreys in the no-feed treatment group. Each line represents one of three replicate treatment tanks ($n = 10$, 11, and 12 lampreys). Length is the mean total length of all individuals in a given tank. Open arrowheads pointing downward represent significant decreases in length during the experiment, open arrowheads pointing upward significant increases in length. The downward-pointing solid arrowhead indicates that the lampreys were shorter at the end of the experiment than they were at the beginning; solid circles indicate that they were the same length at the end of the experiment.

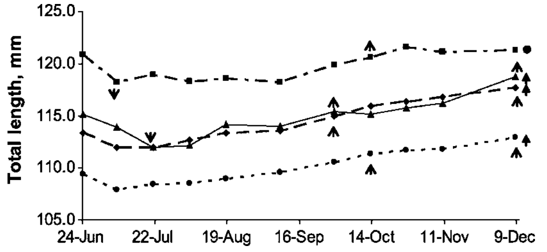

FIGURE 4.—Growth of larval Pacific lampreys in the low-feed treatment group. Each line represents one of four replicate treatment tanks ($n = 9$, 7, 10, and 9 lampreys). The upward-pointing solid arrowheads indicate that the lampreys were longer at the end of the experiment than they were at the beginning. See Figure 3 for an explanation of the other symbols.

t-tests: df = 6, 8, and 8; $P < 0.05$). The mean TL of the ammocoetes in one low-feed tank and two no-feed tanks was not significantly different (0.4, 0.0, and −1.5 mm) in June and December (paired *t*-tests: df = 9, 10, and 9; $P \geq 0.05$). The mean TL of the surviving ammocoetes in one of the three no-feed tanks decreased significantly (−1.6 mm) from June to December (paired *t*-test: df = 11, $P < 0.05$).

From June to December, the mean ammocoete growth rate for each tank ranged from −0.01 to 0.03 mm/d. In the no-feed tanks, the lampreys decreased in length through 21 July (Figure 3), the mean ammocoete growth rate ranging from −0.16 to −0.12 mm/d. In the low-feed tanks, the lampreys typically decreased in length through 7 July (Figure 4), the mean ammocoete growth rate ranging from −0.21 to −0.10 mm/d. The period of maximum growth occurred most often during September. Mean growth rates attained significantly positive values for all tanks (paired *t*-tests: df = 10 or 11, $P < 0.05$), tank maximums ranging from 0.06 to 0.18 mm/d (Table 2).

Stages of Metamorphosis

Six stages of metamorphosis were observed during the study. All lampreys were in stage 0 when they were collected on 23 and 24 June. Stage 1 individuals appeared from 7 July to 18 August (Figure 5). Stage 2 individuals appeared from 4 August to 8 September. Stage 3 individuals appeared from 4 August to 29 September. Stage 4 individuals appeared from 8 September to 13 October. Stage 5 individuals appeared from 8 September to 27 October. Stage 6 individuals appeared from 27 October through at least 10 November, the end of the observational period. Stage 7 individuals did not appear between 23 June and 10 November, the period of this study when metamorphic stages were recorded.

Incidence of Metamorphosis

Thirteen lampreys initiated metamorphosis. Overall, the incidence of metamorphosis was 15.7% (13 of 83). In the no-feed group, 8.3% (3 of 36) of ammocoetes went through metamorphosis. In the low-feed group, 21.3% (10 of 47) ammocoetes went through metamorphosis. The number of metamorphic animals did not vary significantly between treatments (Fisher's exact test: df = 1, $P \geq 0.05$).

Onset, Timing, and Duration of Metamorphosis

One lamprey (22LPG) was observed in stage 1 of metamorphosis starting on 4 August. This lamprey remained in stage 1 through 10 November, the date when observations were scheduled to be terminated. To confirm whether this particular lamprey continued through metamorphosis, we examined the eyes of each transformer on 8 December. By 8 December, lamprey 22LPG had developed a distinct iris and pupil and was in stage 3 of metamorphosis. Given this extended and atypical duration of stage 1, information from this individual was excluded from analysis associated with onset, timing and duration.

The onset of metamorphosis, or stage 1, appeared between 7 July and 18 August (Figure 5). Lampreys that had not begun metamorphosis by 18 August did not initiate metamorphosis for the duration of the experiment. By 10 November, all metamorphic lampreys ($n = 12$) had exhibited the characteristics of stages 1, 2, 3, 4,

TABLE 2.—Mean maximum growth rates observed in Pacific lamprey ammocoetes in two treatments. All maximum growth rates were significantly positive.

Treatment	Tank	*N*	Maximum growth rate (mm/d)	SE	Period of maximum growth
No feed	21	10	0.066	0.017	8 Sep–13 Oct
	22	11	0.062	0.010	29 Sep–27 Oct
	62	12	0.060	0.010	8 Sep–13 Oct
Low feed	41	9	0.040	0.014	4 Aug–13 Oct
	42	7	0.071	0.027	27 Oct–8 Dec
	51	10	0.069	0.017	8 Sep–13 Oct
	52	9	0.067	0.016	8 Sep–13 Oct

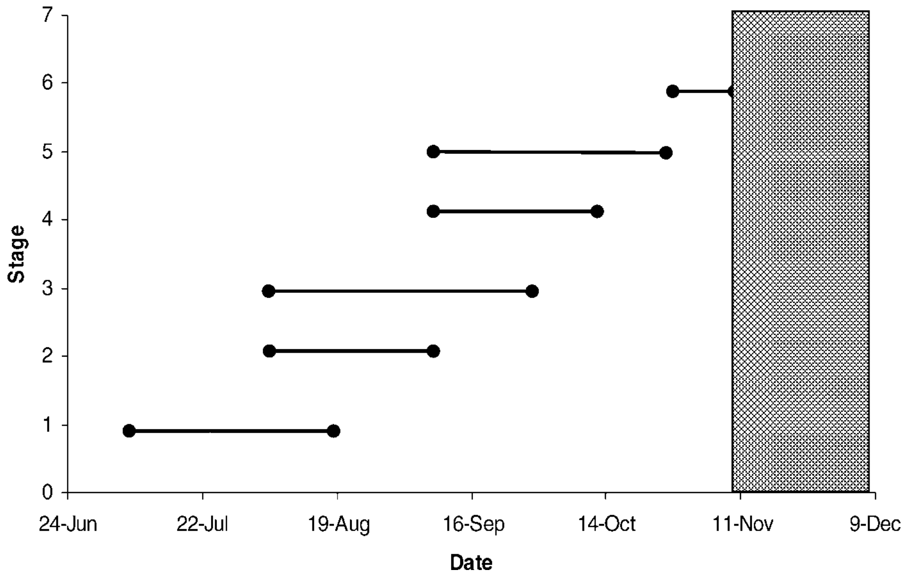

FIGURE 5.—Dates on which the various stages of metamorphosis (see text) appeared in Pacific lampreys. Stage 7 lampreys were not observed during the experiment; no monitoring took place from 10 November to 9 December.

and 5 and seven lampreys had exhibited the characteristics of stage 6. Stage 2 appeared an average of 25 d after stage 1, but the diagnostic features for these two stages appeared concurrently in one lamprey (Table 3). Overall, stage 3 appeared an average of 1 d after stage 2, but the diagnostic features for stage 3 appeared concurrently with ($n = 4$) or before ($n = 2$) the diagnostic features for stage 2 in six lampreys. Stage 4 appeared an average of 39 d after stage 3, but the diagnostic features for these two stages appeared concurrently in one lamprey. Stage 5 appeared an average of 15 d after stage 4, but the diagnostic features for these two stages appeared concurrently in four lampreys. As minimums, lampreys were observed in stage 5 for an average of 30 d and there was an average of 106 d between stage 1 and stage 6 (Table 3).

Changes in Body Proportions during Metamorphosis

Relative to total body length, snout length ranged from 3.9% to 10.3%, prebranchial length from 7.5% to 17.5%, the height of the first dorsal fin from 0.68% to 1.82%, the height of the second dorsal fin from 1.36% to 3.20%, eye diameter from 1.04% to 3.51%, and snout depth from 3.37% to 6.54% (Figure 6). Each of these body proportions was positively related to metamorphic stage (Kendall's τ: $P < 0.05$). Branchial length ranged from 11.3% to 15.9% of the total body length and condition factor ranged from 1.34 to 1.79. Both of these proportions were unrelated to metamorphic stage (Kendall's τ: $P > 0.05$).

Discussion

This study is one of the first to follow individual Pacific lampreys through the process of metamorphosis. While numerous lamprey studies have examined the development of captive individuals (see Manion and Stauffer 1970; Potter et al. 1978; Beamish and Thomas 1984; Beamish and Medland 1988; Holmes and Youson 1993, 1997), few have focused on Pacific lampreys (Richards and Beamish 1981). Instead, the majority of studies on Pacific lamprey metamorphosis relied on repeated field surveys (see Pletcher 1963; Kan 1975; Richards 1980; Beamish and Levings 1991;

TABLE 3.—Interval between metamorphic stages in individual Pacific lampreys. When stages appeared concurrently, the minimum is 0. When the higher-numbered stage appeared before the lower-numbered stage, the minimum is negative. The symbol U1 indicates that the higher-numbered stage was not observed in all lampreys and that the mean is a minimum; the symbol U2 indicates that the higher-numbered stage was not observed in any lamprey.

Stage interval	Duration of interval (d)		
	95% CI around mean	Minimum	Maximum
1–2	25 ± 14	0	63
2–3	1 ± 12	−14	35
3–4	39 ± 11	14	70
4–5	15 ± 9	0	35
5–6 (U1)	≥30 ± 7	≥15	≥63
6–7 (U2)	Unknown	Unknown	Unknown
1–6 (U1)	≥106 ± 7	≥85	≥126
1–7 (U2)	Unknown	Unknown	Unknown

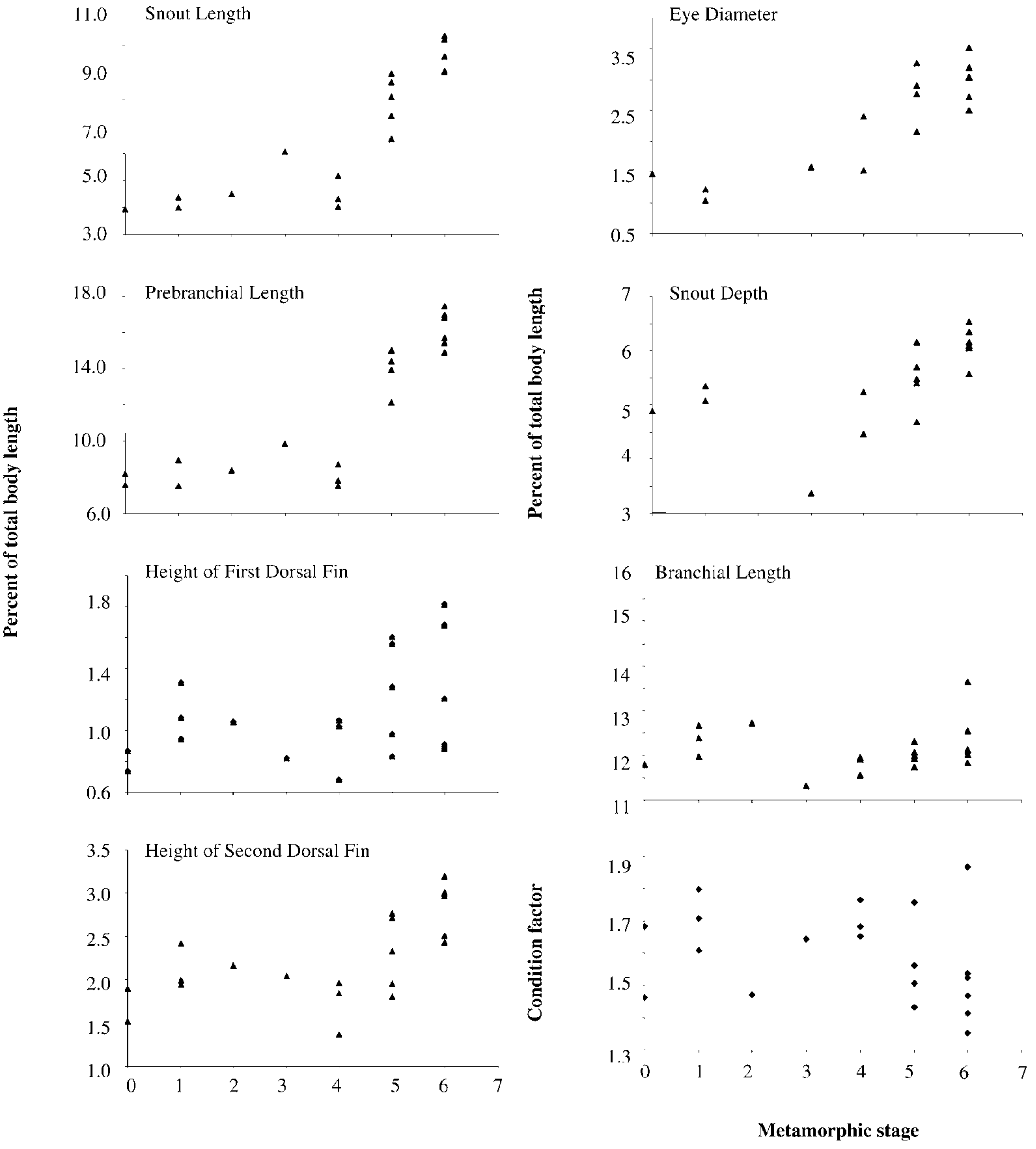

FIGURE 6.—Body proportions (percent of total length) and condition factors associated with the metamorphic stages of individual Pacific lampreys. All relationships were positive and significant except those for branchial length and condition factor (Kendall's $\tau = 0.82$ for snout length, 0.71 for prebranchial length, 0.34 for the height of the first dorsal fin, 0.53 for the height of the second dorsal fin, 0.65 for eye diameter, 0.65 for snout depth, 0.06 for branchial length, and −0.21 for condition factor).

van de Wetering 1998). While these studies on Pacific lampreys provide important insights, their level of inference is limited to the presence or absence of observed metamorphic stages in a stream environment. To conclude that individuals in a given stage of metamorphosis are absent from a stream, it is generally necessary to know the probability of capturing one of these individuals (see Peterson et al. 2004). Since previous studies on Pacific lamprey metamorphosis did not account for capture probability, they were unable to distinguish whether lampreys in a given metamorphic stage were not present because they had completed that part of their development or because they were simply not captured. In addition, these studies could not determine whether lampreys in a given metamorphic stage were present in the stream, but had left the survey area. For example, Richards (1980) documented the occurrence of various developmental stages and described metamorphosis primarily from lampreys that he was able to capture over time at one sample site near the mouth of the Chemainus River, an area where almost all downstream migrants would pass. Alternatively, by rearing Pacific lampreys in captivity, this study was able to directly follow the metamorphic development of specific individuals. Although some caution is warranted because only 13 lampreys initiated metamorphosis, these individuals provided valuable information about the timing, sequence, and duration of the process as well as individual variability. It is not uncommon for relatively small sample sizes to be useful for describing aspects of metamorphosis (see Beamish and Thomas 1984; Holmes et al. 1999), especially when assessing individuals (see Manion and Stauffer 1970). Captive-rearing studies can be an important adjunct to field studies.

As Pacific lampreys proceed through metamorphosis, the pattern of external changes varies among individuals. This was evidenced in both the sequence of stage appearance and the length of time between stages. For example, in some individuals, characteristics of stage 2 preceded those of stage 3 whereas in other individuals the opposite pattern was observed. In addition, some individuals exhibited stage 4 characteristics as little as 14 d after stage 3 whereas other individuals exhibited stage 4 characteristics up to 70 d after stage 3. Individual variability in metamorphosis is somewhat contrary to much of the literature, which suggests that modifications in each individual character tend to occur at similar times and rates in members of any one population (Youson and Potter 1979) and that a high degree of synchrony in the timing of metamorphosis (Potter et al. 1978) is characteristic of all lampreys (Hardisty and Potter 1971). In contrast to this notion, Beamish and Thomas (1984) found evidence that in southern brook lamprey *Ichthyomyzon gagei*, while the sequence of changes in external morphology and the overall duration of metamorphosis were extremely consistent each year, the timing of each stage was not precise within a population and there was significant overlap in the time when various (particularly sequential) metamorphic stages appeared. *Ichthyomyzon* and *Lampetra* (also see Percy and Potter 1977) species may inherently exhibit more variation in the timing of changes than do other species, such as those from the genus *Petromyzon*. Alternatively, as in this study, it may be necessary to follow individual animals to detect individual variability in the timing of changes. Since changes to various organ systems (e.g., both eyes and mouth) happen concurrently (see Youson and Potter 1979), to precisely assess the metamorphic progression of individual Pacific lampreys it may be useful to focus on a specific organ system (e.g., either eyes or mouth) where developmental changes must occur sequentially.

Metamorphosis in Pacific lampreys occurs over an extended period of time that encompasses multiple seasons. All individuals began metamorphosis during the summer, often in July, and no individual initiated the process after August. This supports the suggestion that the onset of metamorphosis for holarctic lampreys is restricted to summer (see Potter et al. 1978) and corroborates previous reports from field observations that documented the onset of Pacific lamprey metamorphosis near the month of July (Pletcher 1963; Hammond 1979; Richards 1980; van de Wetering 1998). Lampreys at various metamorphic stages were present throughout the fall, and no individual had completed metamorphosis by the middle of November. Thus, when the lampreys completed metamorphosis and the duration of the entire process could not be determined empirically. Based on the literature, the time at which the Pacific lamprey completes metamorphosis is somewhat ambiguous (Pletcher 1963; Kan 1975; Hammond 1979; Beamish 1980; Richards 1980; Richards and Beamish 1981; Beamish and Levings 1991; van de Wetering 1998; Claire 2003). This study suggests that November may be the earliest that some populations of Pacific lampreys complete metamorphosis. The duration of metamorphosis is species-specific (Beamish and Medland 1988) and all available information would suggest that individual Pacific lampreys in this study required a minimum of 105–150 d to complete the process, which is within the range reported for the genus *Lampetra* (Poltorykhina 1971; Bird and Potter 1979a, 1979b). In addition, as with other species of lampreys (see Hardisty and Potter 1971; Youson 1980), it is probable that physiological, behavioral, or microscopic changes in morphology occurred before and after macroscopic changes were expressed. When information on Pacific lamprey metamorphosis is considered as a whole, July to December may be the minimum period when metamorphic Pacific lampreys could be present in Columbia River basin streams.

The sequence of changes in Pacific lampreys varied somewhat from the seven-stage model of metamorphosis (Youson and Potter 1979; Potter et al. 1982; Beamish and Thomas 1984). This model, originally described in sea lampreys, is often used as a template to evaluate the metamorphic stage of lampreys (Richards and Beamish 1981; Beamish and Thomas 1984; Beamish and Medland 1988; Holmes et al. 1999), including Pacific lampreys (Mueller et al. 2006). As expected, Pacific lampreys in this study progressed through substantial changes in the morphology of their eyes, mouth, and branchial region, similar in nature to what has been described for other lamprey species (see Manion and Stauffer 1970; Youson and Potter 1979; Beamish and Thomas 1984; Beamish and Medland 1988, for examples). Unexpectedly, the Pacific lampreys in this investigation expressed a distinct iris and pupil almost concurrently with readily observable changes in the anterior cirrhi of the mouth and exhibited the loss of the branchial groove before the expression of rudimentary or final teeth. The sequence observed for Pacific lampreys appeared to be almost identical to that reported for American brook lampreys by Holmes et al. (1999). While the differences between the seven-stage model and these two *Lampetra* studies are relatively minor, this information may reflect a slightly different organization of the development process between some species (or perhaps genera) and helps to emphasize the importance of developing species-specific information.

Evaluating asymmetric growth may allow a quantitative description of Pacific lamprey metamorphosis. Asymmetric growth in Pacific lampreys from this study was evidenced by growth in all body regions examined during metamorphosis excepting the branchial region and the length–weight relationship. This included asymmetric growth in snout depth, which had not been previously reported. Potter et al. (1978) suggested that many of the increases in the anterior region of the lamprey were associated with imminent changes to their feeding behavior. It is reasonable to speculate that this would also be true for the increase in snout depth observed in Pacific lampreys during this study. The relative length of the branchial region is generally believed to shorten during metamorphosis in all lampreys (see Beamish and Thomas 1984). In contrast to this notion, such a change was not observed for the Pacific lampreys in this study. While Richards (1980) did report a shortening of the branchial region during metamorphosis in Pacific lampreys from the Chemainus River, Holmes et al. (1999) did not find this pattern in American brook lampreys, another *Lampetra* species. Although the condition factor of premetamorphic lampreys has been shown to have utility as a predictor of which ammocoetes will transform (Youson et al. 1993), it does not appear to have a consistent quantitative relationship with metamorphic stage. Beamish and Medland (1988) were not able to detect changes in condition factor between metamorphic stages 2 and 8 in mountain brook lampreys *I. greeleyi*. However, in sea lampreys, Potter et al. (1978) reported an increase in condition factor between metamorphic stages 1 and 3, followed by a decrease through stage 7. The condition factor of Pacific lampreys in this study was not clearly related to metamorphic stage. Alternatively, certain characteristics, such as increasing eye diameter, appear to be consistent among all lamprey species during metamorphosis (see Manion and Stauffer 1970; Beamish and Thomas 1984; Beamish and Medland 1988; Holmes et al. 1999), including the Pacific lampreys in this study.

Pacific lamprey ammocoetes are able to survive and grow well in captivity. This was apparent when, excepting one catastrophic event, essentially all ammocoetes survived during the investigation. Relatively high survival was consistent with previous attempts to rear ammocoetes in captivity (Mallatt 1983; Morman 1987; Youson et al. 1993; Holmes and Youson 1994; Holmes et al. 1999; Meeuwig et al. 2005). In addition, Pacific lamprey ammocoetes in this study expressed growth rates well within the range of those estimated for ammocoetes rearing naturally in stream environments (see Pletcher 1963; Lowe et al. 1973). It can be difficult to obtain relatively natural growth rates in captive environments (Murdoch et al. 1991; Meeuwig et al. 2005). However, data from this study support the suggestions that natural growth rates can be obtained under the conditions of relatively low rearing density (Mallatt 1983; Morman 1987; Murdoch et al. 1991), naturally long days and warm water (Mallatt 1983; Holmes et al. 1994), and the presumed presence of food items in unfiltered stream water (Morman 1987). Thus, it appears that Pacific lamprey ammocoetes can be brought into captivity and reared in continuously flowing water in a manner that promotes survival and natural growth and allows for evaluations of metamorphosis.

For captively reared Pacific lampreys, supplemental feeding improved ammocoete growth. Ammocoetes that received supplemental food expressed the highest growth rates observed and these were the only ammocoetes to exhibit net positive growth over the 5-month period. This is consistent with the notion that food resources can be limiting (Mallatt 1983) and that supplemental food can improve growth when rearing lampreys in captivity (Mallatt 1983; Youson et al. 1993). However, ammocoetes that did not receive supplemental food also expressed positive growth rates. This is similar to the reports of Manion and Stauffer (1970) as well as Morman (1987) and suggests that, in a captive environment, unfiltered stream water may provide items on which ammocoetes can feed.

Supplemental feeding did not influence the incidence of metamorphosis. Holmes et al. (1994) also found that food deprivation did not influence the proportion of sea lampreys going through metamorphosis. Since lampreys were collected for this study in late June and some began to transform in July, it is reasonable to presume that those that ultimately transformed had committed to the process of metamorphosis before being collected. If lampreys are collected near the onset of metamorphosis, supplemental feeding may not be necessary for the transformation to occur, as it is thought that lampreys do not feed during metamorphosis (Moore and Potter 1976; Beamish et al. 1979).

Conservation efforts for the Pacific lamprey could benefit by considering the specific time when metamorphic Pacific lampreys are present in streams and, to the extent possible, should be based on information specific to that species. Although it appears that lamprey metamorphosis is a synchronized process within a population, this study suggests that there can be considerable variation in timing among individuals within a population. While the changes that occur during the metamorphosis of Pacific lampreys are similar to those that have been described for other species, it appears that there are some species-specific or population-specific differences. When considered along with other reports, the data from this study suggest that Pacific lampreys going through metamorphosis may be found in Columbia River basin streams throughout the summer and fall and perhaps into the winter.

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