Do summer temperatures trigger spring maturation in Pacific lamprey, Entosphenus tridentatus?

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Do summer temperatures trigger spring maturation in Pacific lamprey, *Entosphenus tridentatus*?

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Abstract – Pacific lamprey, *Entosphenus tridentatus*, return to streams and use somatic energy to fuel maturation. Body size decreases, the lamprey mature, spawn, and then die. We predicted that warm, summer temperatures (>20 °C) would accentuate shrinkage in body size, and expedite sexual maturation and subsequent death. We compared fish reared in the laboratory at diel fluctuating temperatures of 20–24 °C (mean = 21.8 °C) with fish reared at cooler temperatures (13.6 °C). The results confirmed our predictions. Lamprey from the warm water group showed significantly greater proportional decreases in body weight following the summer temperature treatments than fish from the cool water group. A greater proportion of warm water fish sexually matured (100%) and died (97%) the following spring than cool water fish (53% sexually mature, 61% died). Females tended to mature and die earlier than males, most obviously in the warm water group.

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Key words: Agnatha; body size; starvation; metabolism; reproduction; furunculosis

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Introduction

Management practices within the commercial forest, agriculture and power generation industries in the Pacific Northwest (USA) have resulted in warming of stream temperatures to >20 °C during the summertime (Beschta et al. 1987; NRC 1996; EPA 2003; USACE 2007). The effects of these warm temperatures have been most studied in salmonids (*Oncorhynchus* spp.), where warm temperatures have been linked to elevated metabolic rates and energetic expenditures (Brett 1995; Lee et al. 2003; Rand et al. 2006), use of cool water refugia (Goniea et al. 2006; High et al. 2006), altered run times (Quinn & Adams 1996; Hodgson & Quinn 2002), susceptibility to disease (reviewed in McCullough 1999) and mortality for late migrants (Naughton et al. 2005; Keefer et al. 2007). Pacific lamprey (*Entosphenus tridentatus*) co-occur with Pacific salmon in these warming watersheds, and they have a similar life cycle to Pacific salmon (anadromy and semelparity). However, little is known about the effects of summer temperatures on maturing adult Pacific lamprey.

After ceasing their parasitic stage in the ocean, Pacific lamprey return to freshwater during the spring (April–June; Beamish 1980), and then begin their initial upstream migration during the summer (July–September; Scott & Crossman 1973), before overwintering during October–March (Scott & Crossman 1973). Like other anadromous lampreys (Kott 1971; Beamish et al. 1979; Larsen 1980), Pacific lamprey do not feed during this prolonged freshwater residency (Beamish 1980; Whyte et al. 1993) and somatic energy reserves fuel sexual maturation (Kott 1971; Beamish et al. 1979; Larsen 1980). As a result, Pacific lamprey shrink in body size (Beamish 1980; Whyte et al. 1993) prior to maturing, spawning, and then dying the following spring (April–July; Pletcher 1963, cited in Scott & Crossman 1973; Beamish 1980). Most spawning activity in Western Oregon rivers occurs

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between April and May. However, spawning can occur during late May and early June (Brumo 2006; Gunckel et al. 2006) with very little spawning (<1% of spawning population) occurring during early July in some years (Brumo 2006). Late spawning activity may be associated with high stream flows and associated cool water temperatures (Brumo 2006; Gunckel et al. 2006).

The aims of the present study were twofold: (1) to examine the effects of relatively warm, summer temperatures (>20 °C) on the maturation status (mature = spermiating or ovulating), maturation timing, survival rates and maturation characteristics (body size and general, qualitative measures of morphology) of Pacific lamprey over time (immediately after treatment and 10 months later) and (2) to ascertain whether there was evidence for a cause-and-effect relationship between body size and maturation timing. Because high temperatures increase metabolism and activity in lampreys (Johansen et al. 1973; Lewis 1980) as in other fishes, we predicted that shrinkage in body size during freshwater residency would be accentuated at relatively warm water temperatures experienced during the summer. We also predicted that if summer temperatures led to significant decreases in body size of Pacific lamprey, then a higher percentage of fish would mature early in comparison with lamprey held at cooler water temperatures. We subjected sexually immature, adult Pacific lamprey to relatively warm summer temperatures (>20 °C; 'warm water group') that mimicked the diel fluctuations of temperatures that occur in the Willamette River Basin during the summer and compared these fish with fish reared at relatively cool temperatures that occur in the upper tributaries of the Willamette River Basin (cool water group).

Methods

Adult Pacific lamprey were randomly collected by hand from the Willamette River at the base of Willamette Falls near Oregon City (OR, USA) on 14 June 2006. Willamette Falls is 43 river kilometres upstream of the confluence of the Willamette and Columbia Rivers, and 206 river kilometres from the Pacific Ocean. Pacific lamprey congregate at the falls prior to ascending during May through October (Mesa et al. 2007) and migrating upstream to spawning grounds. The fish were transported in aerated coolers (66 l) to the Fish Performance and Genetics Laboratory at Oregon State University, where they were transferred to a holding tank (4.9 m long × 0.8 m wide × 0.3 m deep, ~1000 l) with flow-through, pathogen-free well water. Holding tank temperatures averaged ~13.5 °C. The tank was covered to prevent escapement and to reduce light levels as lamprey are photophobic (Hardisty & Potter 1971). Nontransparent, plastic pipes (~5 cm in diameter and 0.6–0.9 m in length) were provided for refuge for the lamprey, and they were frequently observed using the pipes.

During 10 July to 11 July of 2006, 72 healthy-looking fish were selected from a larger group of fish. The fish were anaesthetised with 50 $mg \cdot l^{-1}$ of tricaine methanesulfonate (MS–222) buffered with 125 $mg \cdot l^{-1}$ of $NaHCO_3$. Fish were then tagged with Passive Integrated Transponder tags (32 mm long), which were inserted into the body cavity a few millimetres anterior to the cloaca. Six fish were randomly assigned to each of six warm water tanks and six cool water tanks for a total of 36 fish per experimental group. At the start of the experiment, none of the animals showed signs of secondary sexual characteristics that would enable identification of sex, as described by Hardisty & Potter (1971).

Experimental tanks were circular, with a diameter of 0.8 m, a depth of 0.5 m and volume of 295 l. Two plastic, nontransparent pipes (~5 cm in diameter and 0.6 m in length) were provided in each tank for lamprey to use as cover. The tanks were covered to reduce light levels. Tanks were supplied with flow-through well water at an average flow rate of 1.25 $l \cdot min^{-1}$ at different temperatures and the tank temperatures were recorded with automated temperature loggers (Hobos® by Onset, Bourne, MA, USA).

We examined temperature patterns in the Willamette River Basin and used these patterns to guide our warm water and cool water temperature treatments. The gradient of mean summer temperatures in the mainstem Willamette River ranged from 16.5 °C in the upper river to 21.9 °C in the lower river (Table 1). We also examined the range of temperatures in tributaries to the Willamette River with a focus on moderately warm and moderately cold temperature reaches. The mean range of summertime temperatures for moderately warm tributaries = 22.1–22.8 °C, whereas moderately cool tributaries had a mean range of 11.5–14.5 °C (data for years 2001 and 2002 provided by the Oregon Department of Environmental Quality). Lastly, we examined the range of diel fluctuations in summertime temperatures across the mainstem

Table 1. Mean water temperatures for the mainstem Willamette River for July–September.

Willamette river kilometre	Mean °C
29	21.7
64	21.9
143	20.4
213	18.9
291	16.5

Data are for year 2001 or 2002 and were provided by the Oregon Department of Environmental Quality.

Willamette River, which were 1.0–3.8 °C (data for the years 2001–2004 provided by the Oregon Department of Environmental Quality). Based on these temperature patterns in the Willamette River Basin, we subjected warm water tanks to a target mean temperature that mimicked the mean summertime temperatures of moderately warm tributaries at ~22 °C with a diel temperature fluctuation resembling the maximum temperature fluctuation of ~4 °C (minimum temperature during nighttime = 20 °C; maximum temperature during the daytime = 24 °C). Cool water tanks were subjected to a mean temperature of 13.6 °C, which is within the range of temperatures occurring in moderately cool tributaries (see above) and also the mean annual temperature in the Willamette Basin (13.3 °C; Stanford et al. 2005) (Fig. 1). No diel temperature fluctuation was provided for fish subjected to the cool water treatment.

Fish were acclimated to the warm water treatment from a baseline temperature of ~13.6 °C to 20–24 °C during 12 July to 15 July of 2006 by an increasing surge-like temperature regime. For example, heated water was supplied to warm water tanks on 12 July allowing a slow rise in temperature from 13 °C to 16 °C before the water heater was turned off and then again on 13 July from 13 °C to 18 °C before being turned off. This acclimation regime continued with 2 °C increases above the previous day's maximum temperature until 16 July whereupon the temperature was left to fluctuate between 20 and 24 °C. The warm water treatment was terminated on 1 October 2006 at 7.20 hours. In this way, fish in the warm water group experienced an average water temperature of 21.8 °C with a mean diel fluctuation of ~4 °C for 94 days, totalling 2049 temperature units during the summer period, compared with fish in the cool water group, which experienced an average water temperature of 13.6 °C, totalling 1278 temperature units over the same time period. Upon termination of the warm water treatment on 1 October 2006, both groups of fish were subjected to ambient well water averaging 12.8 °C (range: 11.5–13.8 °C) for 220 days for a total of 2800 temperature units through 9 May 2007. Six fish in one warm water tank experienced ambient temperatures when the inflow of heated water for this tank failed for approximately 3–4 weeks; the data from these fish were omitted from the analyses.

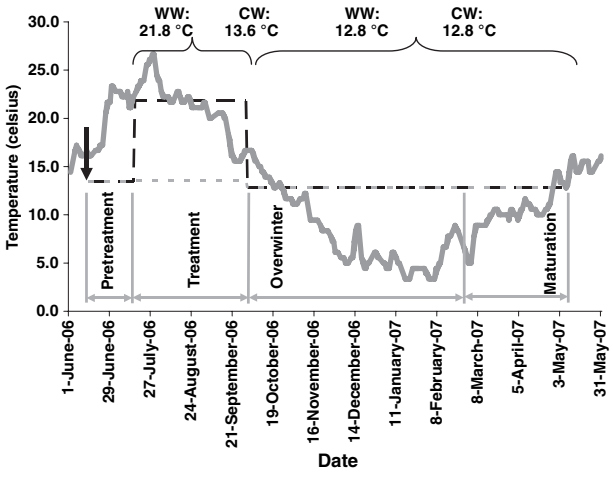

Fig. 1. Temperature profile of the Willamette River at Willamette Falls (grey line; data from Oregon Department of Fish and Wildlife: http://www.dfw.state.or.us/fish/fish_counts/willamette%20falls.asp), where the experimental lamprey were collected. The black, vertical arrow indicates the date when lamprey were collected at the falls (14 June 2006). Also shown are the mean temperature profiles experienced by the warm water ('WW'; black, long-dashed line) and cool water ('CW'; grey, short-dashed line) lamprey in the laboratory. Mean temperature values experienced by WW and CW lamprey are shown above the brackets for the summer treatment period and overwinter holding to spring maturation periods.

The fish were transferred to holding tanks for the posttreatment, overwinter to maturation period (mid-October 2006–May 2007). The holding tanks (1.8 m × 0.6 m × 0.6 m, 648 l) were supplied with flow-through well water at an average rate of 4.0 $l \cdot min^{-1}$. All tanks had cobble substrate (~360–1920 cm^3) for refuge. Mortalities during the summer treatment period resulted in fewer than the original 36 fish per treatment group (see Results). Therefore, 11–14 fish were held per tank, and two tanks held fish from the cool water group for a total of 24 fish between two tanks and two tanks held fishes from the warm water group for a total of 27 fish between two tanks. Aquarium pumps circulated water between each of two tanks in the warm water group and each of two tanks in the cool water group at a mean flow rate of 0.4–0.5 $l \cdot min^{-1}$. Inflow rates per tank were approximately 0.75 $l \cdot min^{-1}$. This exchange of water was done so that mating pheromones and potentially pheromones that induce sexual maturation (see Li 2005) would be similar for all individuals of an experimental group during the spring maturation period.

Experimental and holding tanks were checked for mortalities at least three times per week, and dead fish were removed and identified by tag number. The dead fish were dissected to identify sex and determine maturation status (mature = ovulating or spermiating). Body size of dead fish was not recorded as we had determined previously that the body size of dead fish changes following death (B. Clemens, unpublished data). To assist in interpretation of the cause of death and to monitor fish health, we screened a subset of lamprey from a spring 2006 collection (fresh fish not held in the laboratory) and also from the spring 2007 maturation period (experimental fish held in the laboratory between June 2006 and May 2007), for common fish pathogens, including *Aeromonas salmonicida*, the aetiological

agent of furunculosis (Bernoth 1997a; Cipriano & Bullock 2001). Potential key infection sites for *A. salmonicida* were assayed, including kidney and hemorrhagic tissue (Hiney et al. 1997). Samples were placed on tryptic soy agar (TSA) plates and observed for growth after 4–8 days at room temperature. Assays were recorded as positive for *A. salmonicida* if brown-pigmented (chromagenic) colonies grew on the TSA and a brown, diffusible pigment was observed in the medium. This is the classic method for diagnosing presumptive *A. salmonicida*, although false-negatives (i.e., atypical or achromatic *A. salmonicida*) and false-positives (i.e., chromagenic bacteria other than *A. salmonicida*) are minor possibilities with this technique (Bernoth 1997b). Two isolates were collected from lamprey taken from Willamette Falls during May 2006 and these isolates were examined for characteristics of presumptive positive *A. salmonicida*, as described by Shotts (1994).

Six weekly or bi-weekly maturation checks were made on the adult lampreys during the maturation period. During the first two maturation checks, all specimens were examined, and 17–31% of all fish were checked thereafter on the remaining four dates to minimise handling stress on individual fish. Fish within each experimental group were handled identically and all collection gear was disinfected and rinsed thoroughly prior to handling fish from the other experimental group. The experiment was terminated on 9 May 2007.

Body weights and total lengths (TLs) were measured (1) on anaesthetised fish before they were stocked into the experimental tanks immediately prior to initiation of the summer temperature treatment (July 2006), (2) on anaesthetised fish following summer temperature treatment (October 2006) and (3) at the termination of the experiment (May 2007). To minimise handling stress during the spring maturation period, fish were not anaesthetised during March of 2007, and only body weight was measured. At the end of the experiment, all fish were killed and dissected to identify sex and to determine the maturation status. Fish were noted as mature if the males were spermiating or if the females were ovulating.

Statistical analyses

We tested for significant tank effects ($P < 0.05$) within treatment groups for differences in body size, survival and sex ratio during the experimental temperature period (July–October 2006). Body size was tested with one-way ANOVAs. The proportion of fish remaining alive, per tank was tested with chi-square at the end of the temperature treatment. Finally, the sex ratio of each tank was tested with chi-square. Because fish were subjected to the milieu of all remaining fish in their treatment group during the overwinter holding to spring maturation periods (October 2006–May 2007), either by cohabitation in the same tank or by recirculation of water from an adjacent tank containing fish from the same group, tank effects were controlled for. As previously mentioned this was done so that pheromones would be similar for all individuals of an experimental group during the spring maturation period.

A repeated measures ANOVA was conducted on the body weights of warm water and cool water fish during July 2006–March 2007, when sufficient numbers of both groups of fish remained alive.

Relative body size (body weight and TL) reductions over time were calculated as proportional reductions in the initial body size from July 2006 to the end of the summer temperature treatment (October 2006) and July 2006 to the early spring maturation period (March 2007). Separate MANOVAs were conducted for body weight and TL for each time period on arcsine-transformed proportions of initial body size. 'Treatment type', 'sex' and the interaction between treatment type and sex were included as model factors in the MANOVA.

We tested whether individuals that were initially large during the summer (July 2006; pretreatment phase) lived longer. The initial body weight during July 2006 was used as a dependent factor and 'treatment type', 'sex', 'status' (dead or alive) and the interactions of these terms were factors in MANOVA models for each of two time periods: (1) the treatment period (July–October 2006) and (2) the early maturation period (March 2007). Suggestive trends ($P < 0.10$; Ramsey & Schafer 2002) were further explored within each experimental group with MANOVAs, one-way ANOVAs, and unpaired *t*-tests, although the effects were not considered significant unless $P < 0.05$. The MANOVAs included July 2006 body weights as the dependent factors and 'sex', 'status' (dead or alive) and the interaction of sex and status as model factors.

The proportion of fish remaining alive was calculated for warm water and cool water fish over time. These proportions were arcsine-transformed and analysed with two ANCOVA models, one for the summer temperature treatment period (July–October 2006) and another for the spring maturation period (March–May 2007). 'Day' was included as the covariate (continuous variable) and 'treatment type' (warm water/cool water) and the interaction between treatment type and day were independent factors in each of these ANCOVA models.

Results

There was no statistically significant tank effect within treatment groups on the body size, survival or sex ratio

of Pacific lamprey. The data for all warm water fish were pooled and compared with the pooled data from all cool water fish.

Changes in body size

There was a suggestive, but insignificant trend for warm water fish to decrease in body weight to a greater extent than cool water fish throughout the experiment (July 2006–March 2007; repeated measures ANOVA, $P = 0.0679$) (Fig. 2).

During the summer treatment period (July–October 2006), warm water fish had significantly greater proportional decreases in body weight than cool water fish (MANOVA, $P = 0.0067$) (Fig. 3a). There was no significant difference between warm water and cool water fish with regards to proportional decreases in TL during the summer treatment period (July–October 2006; MANOVA, $P = 0.2295$ (Fig. 3b). Throughout the experiment (July 2006–March 2007), there was no significant difference in proportional decreases in body weight between warm and cool water fish (MANOVA, $P = 0.9669$) (Fig. 3a).

Body size, sex and death in cool water fish

Male lampreys from the cool water group were significantly smaller than females immediately prior to initiation of the summer temperature treatment (July 2006; one-way ANOVA, $P = 0.0247$) (Fig. 2).

Cool water fish that died by May 2007 tended to have initial body weights smaller than fish that remained alive (MANOVA, $P = 0.0074$). The small fish that died were males (MANOVA, $P = 0.0040$). Weights of cool water males that died by May 2007 were significantly lower than cool water males that remained alive (unpaired t-test, $P = 0.0358$). Similarly, there was a suggestive, but insignificant trend for small, cool water females to die by May 2007 whereas large females remained alive (unpaired t-test, $P = 0.0845$).

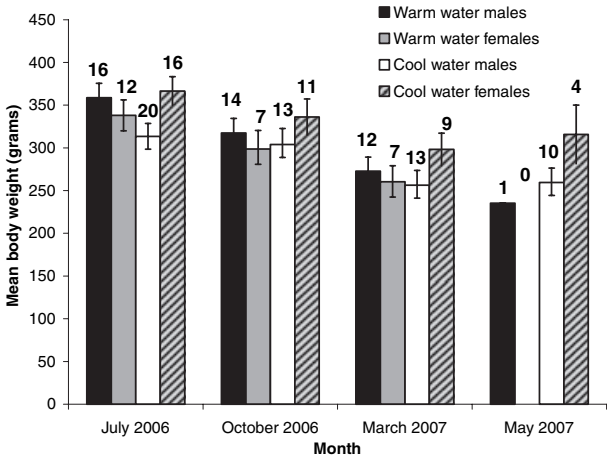

Fig. 2. Mean body weight (±SE) of adult Pacific lamprey. Numbers of fish remaining alive, and on which measurements were made, are shown above the bars.

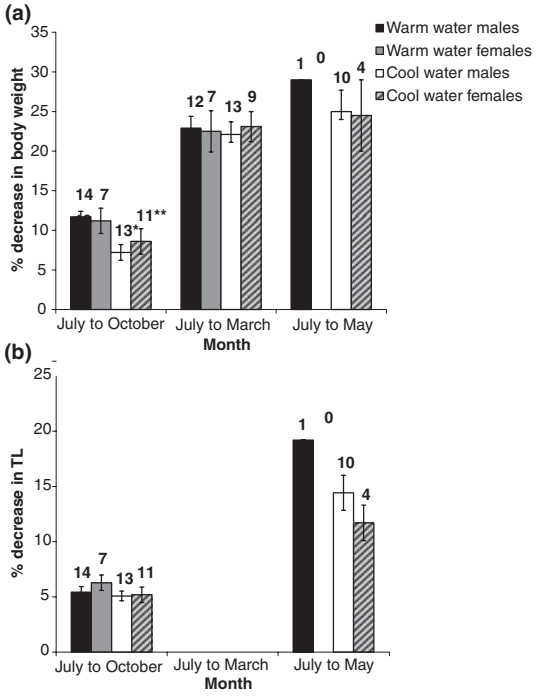

Fig. 3. Mean per cent decreases in body weight (±SE) (a) and total length (±SE) (b) of adult Pacific lamprey, between July 2006 and May 2007. Numbers of fish remaining alive, and on which measurements were made, are shown above the bars. No measurements were made on TL during March (see Methods). Asterisks indicate the statistical similarity/dissimilarity amongst the fish in that period only (see Results for details).

Proportion alive

The proportion of fish remaining alive declined during the summer temperature treatment (July 2006–October 2006; ANCOVA, $P < 0.0001$); however, there was no difference in the proportions of fish remaining alive between warm water and cool water fish during this period (ANCOVA, $P = 0.8209$) (Fig. 4). None of the 19 fish (12 cool water + 7 warm water) that died during July 2006–October 2006 were mature. No mortalities occurred during the overwinter period (November 2006–February 2007), a time in which the fish were inactive and resided under the cobble substrate.

The proportion of warm water fish remaining alive during March–May 2007 declined at a greater rate than cool water fish (ANCOVA, $P < 0.0001$) (Fig. 4), with a 60% spring mortality for warm water fish versus 25% spring mortality for cool water fish. One hundred per cent of the warm water fish were sexually mature (i.e., were spermiating or ovulating) during the

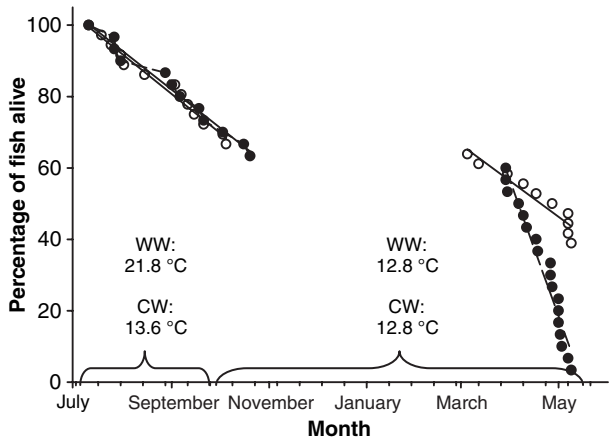

Fig. 4. Percentage of adult Pacific lamprey from the warm water treatment ('WW' = filled circles) and cool water treatment ('CW' = open circles) remaining alive during July–October 2006 and March–May 2007. No mortalities occurred during the November 2006–February 2007 period, a time in which the fish were inactive and residing within the cobble substrate. All dead fish during the spring period were sexually mature (i.e., ovulating or spermiating).

Table 2. Percentages of males (M) to females (F) of adult Pacific lamprey remaining alive at the start (July 2006) and termination (October 2006) of the temperature treatment, and during the maturation period in May 2007.

	Warm water group	Cool water group
July 2006	57% (M):43% (F)	56% (M):44% (F)
October 2006	63% (M):37% (F)	54% (M):46% (F)
May 2007	100% (M):0% (F)	71% (M):29% (F)

spring, compared with 53% of cool water fish, and all spring mortalities were mature. Overall, 97% of the warm water fish and 61% of the cool water fish died by the end of the experiment in May 2007. Males survived for a longer duration than females (Table 2).

Aeromonas salmonicida

We detected *A. salmonicida* from the fresh, pretreatment (spring 2006) collection of adult Pacific lamprey from Willamette Falls and also from the group of experimental fish collected from this same location and held in our laboratory between June 2006 and May 2007. Eight of 20 immature mortalities (two from the warm water group and six from the cool water group) during the late summer and early fall of 2006 showed gross symptoms consistent with furunculosis (bleeding in the body cavity, and from the gills or anus), as described for other fishes by Hiney et al. (1997), Bernoth (1997b), Cipriano & Bullock (2001). We have also observed an association between this type of internal hemorrhaging and a positive assay for *A. salmonicida* in adult Pacific lamprey (B. Clemens et al., unpublished data), which further suggests that the eight mortalities described above likely had furunculosis, although we did not assay these particular fish for *A. salmonicida*.

Two warm water fish and 17 cool water fish were assayed during the end of the experiment in May 2007. All of the warm water fish that were assayed were males because the females had all died earlier (Table 2), whereas 12 of the 17 cool water fish were males. Both warm water fish tested positive for *A. salmonicida*. Only two (1 male + 1 female) of the 17 cool water fish tested positive for *A. salmonicida.* The female that tested positive also exhibited symptoms of furunculosis (nodules/furuncules), as described for other fishes by Hiney et al. (1997), Bernoth (1997b) and Cipriano & Bullock (2001). All fish that tested positive were sexually mature (ovulating or spermiating), whereas all fish that tested negative were sexually immature.

Maturation characteristics

When palpated, mature females did not emit eggs unless the fish were dead or moribund. Mature males, however, almost always emitted sperm when palpated. Eggs appeared on the bottom of tanks holding warm water fish during mid-April, suggesting that some spawning activity may have occurred during this time.

Spermiating and ovulating lamprey showed a decrease in the space between their two dorsal fins to the point where the fins touched. These mature fish also showed secondary sexual characteristics (Hardisty & Potter 1971) and a liver coloration (Kott 1970) similar to previous descriptions for other lampreys. Approximately 1/3 of all fish were examined for liver colour and we found that the liver of immature fish was typically orange in colouration in both sexes. Immature fish less often had a brown, purple or grey liver colour. During April–May 2007, the liver of mature fish was typically dark green in both sexes and less frequently a light orange or grey. Changes in somatic tissue were correlated with the maturation status of the lamprey. In general, maturing lamprey had a light pink-to-white colouration in the trunk musculature and a relatively thin body wall, whereas immature fish had a red coloration in the trunk musculature and a relatively thick body wall.

Discussion

Because high temperatures can exponentially increase routine metabolism and motor and ventilatory activity in lampreys (Johansen et al. 1973; Lewis 1980) as in other fishes, we predicted that shrinkage in body size would be accentuated at relatively warm water

temperatures experienced during the summer. We also predicted that if summer temperatures led to significant decreases in body size of Pacific lamprey, then maturation would be expedited (i.e., a relatively high proportion of fish would mature the following spring) in comparison with lamprey held at cool water temperatures. Our results supported these predictions.

European river lamprey, *Lampetra fluviatilis*, subjected to a warm range (12–17 °C) of seasonally – varying temperatures in the lab matured and died earlier (mid-March to early April) *within* season than those subjected to a cool range (7–11 °C; matured and died late April to mid-May) (Larsen 1965). Pacific lamprey subjected to relatively warm temperatures during the summer mature the following spring (this study).

Our Pacific lamprey matured between March and May and females tended to mature and die earlier than males, most obviously in the warm water group. The warm water group (and several in the cool water group) matured within the seasonal spawning period of wild fish, which typically occurs April–early June (Brumo 2006; Gunckel et al. 2006). The timing of maturation of our lamprey was also identical with the March–May maturation period of Pacific lamprey raised in the laboratory under seasonally varying temperatures in another study (Mesa & Bayer 2005). Whereas we do not know whether the remainder of the cool water group would have matured later within the year – the fish were killed in May so that we could measure and compare body size, maturation status and the presence of the pathogen, *A. salmonicida* before all of the warm water fish died – it seems unlikely that the immature, cool water lamprey would have matured during 2007 for two reasons. First, the vast majority of spawning activity occurs in April and terminates by late May or early June in Western Oregon Rivers (Brumo 2006; Gunckel et al. 2006). Second, our immature lamprey did not show morphological/anatomical signs of impending sexual maturation [apparent approximately a few weeks to 1 month before sexual maturity in Pacific lamprey (B. J. Clemens, personal observation) and in *L. fluviatilis* (Larsen 1965)]. However, the idea that cool water may prolong immaturity of Pacific lamprey for >1 year in freshwater remains to be explored. Wild fish may have different energy demands than lamprey in the laboratory, which should be considered when attempting to extrapolate our results to nature. For example, wild fish could experience greater energy demands than those held in the laboratory via high river flows against which they would have to maintain station or swim against. Alternatively, wild lamprey could experience lower energy demands than those held in the laboratory during the cool overwintering period (e.g., compare the winter temperature profile of our laboratory fish with that of the Willamette River at Willamette Falls in Fig. 1).

Maturation and mortality were related to a previously existing small body size in cool water fish and also to significant proportional decreases in body size in warm water fish. This suggests that a minimum, threshold body size exists at which maturation must occur or sufficient energy reserves will not be available for reproduction. Our conclusion seems to agree with Larsen's (1980) conclusion that sexual maturation in *L. fluviatilis* was related to '…a metabolic signal related to starvation'.

Early maturation timing of adult lamprey exposed to relatively warm summer temperatures raises questions about influences on fitness. Early maturation could minimise the length of time that adult lamprey would be exposed to predation during freshwater residency, which could increase the number of lamprey available for spawning. However, for Pacific lamprey experiencing warm temperatures in the lower river, early maturation timing could uncouple spawn timing with optimal habitat characteristics in the upper watershed for spawning, embryonic development and larval emergence, rearing and growth. Clearly, there are many facets to the ecology of maturation timing in Pacific lamprey, and more research is needed to ascertain how warm summer temperatures affect reproductive fitness.

Body size

Indirect estimates of body shrinkage suggest that Pacific lamprey shrink by ∼18% to 30% in body length between the start of their upstream migrations and spawning (Kan 1975; Beamish 1980; Chase 2001). The direct estimates of our fish, tracked over the course of 10 months, fall within this range of shrinkage in body length for maturing fish. For example, four spermiating male lamprey, including one fish from the warm water group and three from the cool water group, showed maximum reductions in body length of 19–22% between July 2006 and May 2007. The overall reduction in body length between the time these fish first entered freshwater until the end of our experiment was undoubtedly >19–22%.

Through laboratory breeding trials, Beamish & Neville (1992) suggested that a difference in TL > ∼20% precluded successful reproduction between the paired species, river (*L. ayresi*) and brook (*L. richardsoni*) lampreys, which led them to suggest that such differences in body length were an isolating factor for reproduction. Taken together with estimated shrinkage of 18–30% for Pacific lamprey, we wonder whether reductions in TL > 20% would preclude spawning with larger, more recent migrants from the ocean that might mature without overwintering and

shrinking substantially in body size. Our question assumes that ocean-maturing races of Pacific lamprey exist; we do not yet have evidence supporting or refuting this hypothesis.

Aeromonas salmonicida and furunculosis

Warm water temperatures (15–20 °C) have been correlated with proliferation of *A. salmonicida*, outbreaks of furunculosis, and increased mortality in salmonids (Wedemeyer 1996; Pickering 1997). In addition to warm water, furunculosis outbreaks have been attributed to hydrographic features that can aggregate infected fish, such as at the base of waterfalls (Mackie et al. 1930, cited in Johnsen & Jensen 1994). Pacific lamprey can be found in aggregations at temperatures >20 °C at the base of Willamette Falls (B. Clemens, unpublished data), and we have detected *A. salmonicida* in fresh collections of adult Pacific lamprey from this location, and in our experimental fish, which were also taken from this location. Sexual maturation appeared to be associated with incidence of *A. salmonicida*, although we only directly assayed a subset of mortalities (including euthanized fish at the end of the experiment) during spring of 2007 (mortalities were examined for gross symptoms during 2006). Our research raises questions that warrant further exploration of the nature of the association of *A. salmonicida* with sexual maturation in Pacific lamprey. For example, does *A. salmonicida* proliferate as a result of the sexual maturation process? Does this pathogen kill lamprey before they spawn?

Maturation characteristics

Maturation characteristics have been described for other lampreys (Kott 1970; Hardisty & Potter 1971), and yet no comparable descriptions have been published for Pacific lamprey. In Pacific lamprey, we found that the lack of space between the two dorsal fins (i.e., the fins touch) occurs as a result of body shrinkage, and it appears to be a consistent indicator of sexual maturation in Pacific lamprey (this study) and in other lampreys (Hardisty & Potter 1971). We also observed a correlation between sexual maturity and liver colour – immature fish generally had orange-coloured livers whereas mature fish generally had dark green-coloured livers. This finding is consistent with work by Kott (1970) on Great Lakes sea lamprey (*Petromyzon marinus*): early-migrating fish had orange livers, and late-migrating, mature fish had green-coloured livers. The green colouration arises from the accumulation of the bile pigment, biliverdin, in the liver and an associated degeneration of the liver (Kott 1970).

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