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Article

Juvenile sea lamprey (*Petromyzon marinus*) have a wide window of elevated salinity tolerance that is eventually limited during springtime warming

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Abstract

The present study examined changes in biometric characteristics, osmoregulatory capacity, and seawater (SW) tolerance of juvenile sea lamprey (*Petromyzon marinus*) throughout the varying thermal changes from late autumn to late spring. Body length, mass, and condition factor were maintained until April, when significant declines in mass and condition factor were observed to correspond with increases in temperature. Nearly 100% survival in SW was maintained through April. In May, after river and estuarine temperatures had increased, significant mortality in SW (up to 50%) was observed. After SW acclimation, plasma chloride was maintained at an elevated set point, and gill Na^+/K^+-ATPase activity was elevated. Neither parameter appeared to be affected during springtime warming. Together, these results provide a first characterization of the sustained osmoregulatory performance of juvenile sea lamprey after metamorphosis and show that the window of increased hypo-osmoregulatory performance for SW entry lasts for at least 5 months but may ultimately be limited by increases in river water temperatures in late spring.

Key words: sea lamprey, osmoregulation, salinity, migration, temperature

Introduction

Sea lamprey (*Petromyzon marinus*) is an anadromous fish of North America and Europe. Like all anadromous fishes, sea lamprey make a freshwater (FW)-to-seawater (SW) migration as juveniles to access the greater food resources and potential for growth in the ocean, and then return to FW to spawn. As larvae (also referred to as "ammocoetes"), sea lamprey live burrowed in the soft substrate of riverbeds, filter-feeding on suspended organic matter. After 4–6 years of relatively slow growth in larval life, sea lamprey undergo a months-long, larvae-to-juvenile metamorphosis lasting from mid-summer to late autumn, characterized by dramatic morphological and physiological changes to allow parasitism and survival in SW (Beamish and Potter 1972; O'Boyle and Beamish 1977; Beamish et al. 1978; Youson 1979, 1980; Richards and Beamish 1981; Reis-Santos et al. 2008; Barany et al. 2020, 2021*a*, 2021*b*; Shaughnessy and McCormick 2020; Shaughnessy et al. 2020). Although morphological changes such as the appearance of eyes and teeth occur early in the metamorphosis of anadromous lamprey (stages 1–4) (Youson 1979), the critical development of SW tolerance occurs in the later stages (stages 5–7) (Richards and Beamish 1981; Reis-Santos et al. 2008; Barany et al. 2020, 2021*a*, 2021*b*; Shaughnessy and McCormick 2020; Shaughnessy et al. 2020; Sunga et al. 2020).

At each life stage (larvae, juvenile, and adult), sea lamprey serve a unique and important ecological role, and as such, the shrinking size of some populations is of conservation concern. Due to the relatively high biomass they contribute to streams in which they are present (Hansen and Hayne 1962; Smith and McLain 1962), the physical and geochemical impacts of their burrowing activity (Shirakawa et al. 2013), and their filtering of the water column (Moore and Mallatt 1980), larval lamprey are important in the nutrient processing and cycling of riverine ecosystems. Downstream migrating juveniles are an important prey item for many FW and estuarine fishes and birds (Kelly and King 2001). The semelparous life history of anadromous sea lamprey is also of ecological importance. Following the scent of pheromone signals released by larvae (Fine et al. 2004), prespawning adults migrating upstream in the spring bring with them (and deposit after their natural, postspawning senescence) an abundance of marine-derived nutrients at an important time of the year when riverine habitats tend to be nutrient-poor (Nislow and Kynard 2009; Weaver et al. 2016). Although the sea lamprey is considered a species of "least concern" (IUCN 2022), there are many vulnerable native populations of anadromous sea lamprey, particularly along the southern coast of Europe (Maitland et al. 2015). For instance, sea lamprey populations on the Iberian Peninsula, Italy, and the eastern Adriatic Sea are

expected to substantially diminish in size under predicted climate change scenarios (Lassalle et al. 2008).

For anadromous fishes to survive SW entry, they must be able to maintain ion and water homeostasis despite transitioning between environments with drastically different salinities. The transformation of the gill epithelium from a site of active ion uptake to active ion secretion is critical in surviving SW entry (Marshall and Grosell 2006) and can only be carried out by a minority of fish species (~4%) that are amphihaline (Schultz and McCormick 2013). The cellular mechanism by which lamprey accomplish branchial salt secretion appears to be similar to that of bony fishes (Shaughnessy and Breves 2021), involving the paracellular transport of Na^+ and the transcellular transport of Cl^- facilitated by electrogenic gradients produced by the activity of Na^+/K^+-ATPase (NKA) in the gill ionocytes (Reis-Santos et al. 2008; Ferreira-Martins et al. 2016, 2021; Shaughnessy and McCormick 2020). Much like the parr-to-smolt transformation in salmonids (McCormick 2013), increases in NKA activity and abundance during the lamprey metamorphosis correspond to increases in SW tolerance (Beamish et al. 1978; Reis-Santos et al. 2008; Shaughnessy and McCormick 2020).

The concurrent increase in gill NKA activity and SW tolerance during the sea lamprey larvae-to-juvenile metamorphosis is akin to similar preparatory physiological adjustments made during the parr–smolt transformation of anadromous salmonids, such as Atlantic salmon (*Salmo salar*). In Atlantic salmon, peak gill NKA activity and SW tolerance are maintained for a relatively short period, beginning in March or April, typically reaching a maximum in May before rapidly declining to parr levels by June (McCormick 2013). The width of the smolt window is decreased by elevated temperatures; elevated temperatures have only a moderate effect on the onset of smolting but lead to an earlier and more rapid decrease in gill NKA activity and SW tolerance at the end of the smolt window (McCormick et al. 1999). The rapid onset of a postsmolt decline in osmoregulatory performance likely limits the optimal window for SW entry in Atlantic salmon to only ~2–4 weeks, corresponding to the observed downstream migratory behavior for this species (McCormick et al. 1998, 2014; Zydlewski et al. 2014). Whether a similarly transient window of osmoregulatory performance exists for sea lamprey juveniles has not been investigated before the present study.

The timing of the downstream migration made by juvenile sea lamprey appears to be bimodal. Studies in North American and European sea lamprey populations typically report migration occurring in either late autumn to early winter (November–January) or in early spring (March–April) and is thought to correspond to periods of increased flows in rivers and streams (Potter 1980; Kelly and King 2001). Although several studies have examined the timing of emigration from natal tributaries, the timing of SW entry of juvenile sea lamprey is largely unknown. As lamprey stop filter-feeding after the onset of metamorphosis and only resume feeding as newly parasitic juveniles once at sea (Richards and Beamish 1981; Sunga et al. 2020), whether sea lamprey out-migrate in early winter or overwinter and out-migrate the following spring may have important physiological consequences. That is, due to the dramatic change in feeding behavior, delaying a seaward migration until spring would result in a prolonged period of fasting lasting many months (Potter 1980; Youson 1980; Richards and Beamish 1981).

Although it has been shown several times that anadromous lampreys develop high SW tolerance beginning in the later stages of metamorphosis in late autumn, the osmoregulatory capacity and SW tolerance of sea lamprey remaining in FW after this period have not been examined. We hypothesized that the high SW tolerance of juvenile sea lamprey would be transient, eventually declining as fish remained in FW. The aims of the present study were to (*i*) examine changes in biometric characteristics of postmetamorphic juvenile sea lamprey through late spring and evaluate the relationship of biometrics to thermal history and (*ii*) examine whether and for how long SW tolerance is maintained after metamorphosis.

Materials and methods

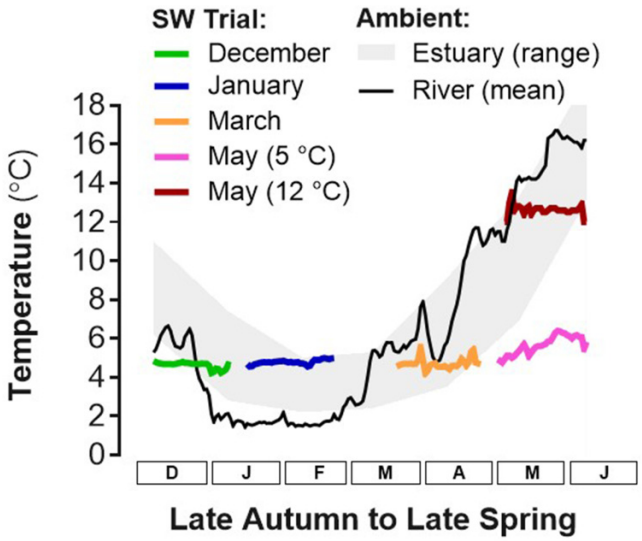
Animal collection and care

For all experimentation, mid-metamorphic sea lamprey were collected via electrofishing in September from the power canal adjacent to the Conte Anadromous Fish Research Laboratory (US Geological Survey) located on the Connecticut River in Turners Falls, Massachusetts (USA). After collection from the wild, sea lamprey were brought back to the laboratory and reared through the remainder of their metamorphosis in 1.5 m diameter flow-through tanks in filtered and UV-treated river water (FW) at 4 $L \cdot min^{-1}$ under ambient river temperatures and natural photoperiod. The bottoms of rearing tanks were laid with 10 cm of sandy substrate for voluntary burrowing. All rearing and experiments were carried in accordance with the US Geological Survey and Conte Lab institutional guidelines and an approved IACUC review (SP-09078).

Biometric monitoring

Sea lamprey were individually color-marked so that we could follow changes in weight and length of individuals over time. To apply the color tag, sea lamprey were lightly anesthetized using a nonlethal dose of MS-222 (100 $mg \cdot L^{-1}$ buffered by $NaHCO_3$, pH 7.4) and individually color-coded by injecting acrylic paint between rays along the caudal fin. For the duration of growth monitoring, these individually marked sea lamprey were held in duplicate 1.5 m diameter tanks maintained in flow-through Connecticut River water at ambient river temperatures (Fig. 1) in conditions equivalent to the rearing conditions described above. Approximately monthly from December to June, each individual sea lamprey was again lightly anesthetized and measured for body length and mass. The dates for these measurements were 10 December, 22 January, 3 March, 15 April, and 14 June. After measurements were taken, anesthetized sea lamprey were monitored in a 20 L recovery tank until they regained consciousness and normal behavior. All sea lamprey recovered from anesthetization. The relationships between changes in length, mass, or condition factor and the degree-days between measurements were assessed.

Fig. 1. Water temperatures and experimental design. Temperature traces of ambient Connecticut River estuary (gray area), ambient Connecticut River rearing water (black line), and 30-day experimental seawater (SW) exposures (thick colored lines). SW exposures were carried out at 5 °C, with an additional, isothermal 12 °C SW trial in May only. Estuary temperature data were obtained from the National Oceanic and Atmospheric Administration Center for Operational Oceanographic Products and Services (https://tidesandcurrents.noaa.gov).



Seawater exposure trials

Artificial SW (35‰) was made using a commercial sea salt formula (Crystal Sea Salt, Baltimore, MD, USA) dissolved in dechlorinated municipal water. For each SW trial, lamprey were transferred from holding in ambient river water to 5 °C SW (all trials) or 12 °C SW (one May trial) and held in SW for 30 days; lamprey were sampled at day 0 (FW), day 2 (SW), and day 30 (SW). A total of five SW trials were conducted from late autumn to spring; the dates and ambient river temperature at the start of trial were as follows: 9 December, 5.2 °C; 20 January, 1.6 °C; 22 March, 5.3 °C; 3 May, 11.5 °C (Fig. 1). We chose an SW temperature of 5 °C that represented a typical temperature of the Connecticut River/Long Island Sound estuary in winter and early spring (Fig. 1). However, due to increases in river and estuary temperatures in late spring, a 12 °C SW trial in May was added to provide an isothermal SW transfer.

Sampling

Sea lamprey were euthanized in a lethal dose of MS-222 (200 mg·L^{-1} buffered by $NaHCO_3$, pH 7.4) and measured for length and mass. Blood was collected via caudal severance into heparinized capillary tubes. Hematocrit values and blood plasma were obtained by centrifugation of whole blood sample. After centrifugation, hematocrit was measured using a microcapillary reader and blood plasma was collected as the liquid (noncellular) phase of the whole blood sample. Gill tissues (filaments only, no cartilaginous arch) were excised and placed in ice-cold SEI buffer (250 mmol·L^{-1} sucrose, 10 mmol·L^{-1} EDTA, 50 mmol·L^{-1} imidazole, pH 7.3). After gill sampling, the remaining whole bodies were blotted dry, weighed (wet mass), then frozen at −80 °C for later analysis of whole-body water content. To determine whole-body water content, bodies were dried for 2 days at 60 °C. Dried whole-body samples were reweighed (dry mass), and the difference between "wet mass" and "dry mass" was used to calculate values for whole-body water content (% water). Blood plasma and gill samples were immediately flash frozen and stored at −80 °C. Plasma chloride concentration was measured using a standard chloridometer (Haake Buchler Instruments Inc., USA).

Gill Na^+/K^+-ATPase activity

Gill Na^+/K^+-ATPase activity was measured as previously described (McCormick 1993). Gill tissue was homogenized in SEID buffer (SEI + 0.1% deoxycholate) using an electronic handheld microcentrifuge tube homogenizer (Fisherbrand, Fisher Scientific, USA). The homogenized samples were centrifuged at 2000*g* for 5 min and the supernatant was used in the assay. The ATPase activity specific to NKA was measured by exploiting the ouabain inhibition of NKA activity in an enzyme-linked assay that connected in a 1:1 ratio the production of ADP to the reduction of NADH to NAD^+. Gill homogenates were run in duplicate without and with 0.5 mmol·L^{-1} ouabain. Absorbance of NADH (340 nm) was measured every 20 s throughout a 10 min kinetic assay, and the difference between the rate of NADH reduction (i.e., ADP production) under uninhibited and ouabain-inhibited conditions was considered the rate of ADP production by NKA. Protein concentration of each sample was determined spectrophotometrically (BCA Protein Assay Kit, Pierce, USA). Enzyme activity is reported as μmol ADP·(mg protein^{-1})·h^{-1}. Activity and protein assays were run on a ThermoMax microplate reader using SoftMax software (Molecular Devices, San Jose, CA, USA).

Calculations and statistics

The parameter "degree-days", also known as "thermal time", is a quantification of the cumulative interaction of temperature and time for evaluating the biology of ectothermic species (Trudgill et al. 2005). As an independent variable metric, a degree-day is calculated as the difference between the average daily temperature for that day and a chosen baseline (or threshold) temperature, which is typically set at a standard value (0, 5, 10, or 15 °C; Bonhomme 2000; Chezik et al. 2014*a*, 2014*b*); cumulative degree-days are the sum of non-negative degree-days across some span of time of interest. Cumulative degree-days have been widely used to assess the impact of temperature exposure on many aspects of fish physiology, including evaluating salmon smolt physiology (Saunders et al. 1985; Sigholt et al. 1998; McCormick et al. 1999; Handeland et al. 2004; Zydlewski et al. 2014), which is similar in many ways to the physiology of juvenile lamprey (Shaughnessy and Breves 2021). In the present study, the baseline temperature for calculating a degree-day was set to 0 °C, and cumulative degree-days (also referred to simply as

"degree-days") were calculated from the date the first biometric measurements were taken on 9 December.

Condition factor was calculated as Fulton's condition factor (K; Heincke 1908; Ricker 1975): (mass ÷ length^3) × 1000, with mass in g and length in cm. Changes in (Δ) biometric data were fit by linear or quadratic regression. For each biometric parameter, the extra sum-of-squares F test was used to statistically determine the model (linear or quadratic) that best fits the data. Whole-body water content (% water) was calculated as [(wet mass − dry mass)/wet mass] × 100. All group data are presented as mean ± standard error. Normality and homogeneity of variance assumptions were tested using Shapiro–Wilk and Levene's tests. Respective statistical analyses (analysis of variance (ANOVA) or t test), post hoc analyses, and P values are described where appropriate in results, figures, and figure captions. An α value of 0.05 was used to demarcate significance in all statistical analyses.

Results

The ambient temperature of the river changed seasonally, reaching a low of ~2 °C in January and February and a high of ~16 °C by the end of all experimentation in mid-June (Fig. 1). In the FW holding tank (kept at ambient river temperature) from which juvenile sea lamprey were transferred for the SW trials, there was only a single mortality (out of ~150 individuals) between December and May. In early winter, some juvenile sea lamprey in this holding tank remained burrowed in the substrate, while others had emerged and were freely swimming or attached via suction to the sides of the tanks. Gradually, by late spring, every juvenile lamprey had emerged from the sediment.

Biometric monitoring

Initial body length of sea lamprey in December was 15.7 ± 0.3 cm and decreased to 14.5 ± 0.4 cm by June (Fig. 2A). Initial body mass was 4.6 ± 0.3 g and remained relatively stable until April (4.4 ± 0.4 g), but by June had decreased significantly to 3.2 ± 0.4 g (Fig. 2B). Initial condition factor (1.18 ± 0.02) was also maintained through April (1.2 ± 0.3) until decreasing to 1.02 ± 0.04 in June (Fig. 2C). Changes in body length, body mass, and condition factor were significantly associated with cumulative degree-days. The cumulative degree-days from the initial biometric measurement in December were as follows: January, 171; March, 244; April, 473; June, 1296 (Figs. 2D–2F). By the measurements taken in June, body length, body mass, and condition factor had declined by 6%, 27%, and 16%, respectively (Figs. 2D–2F). The relationship between ΔLength (y-axis) and degree-days (x-axis) was equally well fit by linear and quadratic regression ($F_{[1,42]} = 1.65$; $P = 0.206$), so the simpler of the two models (linear regression) is shown, $y = mx + b$: $m = -0.004$, $b = 0.26$. The relationships between ΔMass, and ΔCondition (y-axis), and degree-days (x-axis) were best fit by quadratic regression, $y = Ax^2 + Bx + C$: ΔMass ($A = -1.47 \times 10^{-5}$, $B = -2.02 \times 10^{-3}$, $C = -0.02$; $F_{[1,42]} = 10.18$; $P = 0.003$); ΔCondition ($A = -1.27 \times 10^{-5}$, $B = -3.81 \times 10^{-3}$, $C = -0.38$; $F_{[1,42]} = 6.74$; $P = 0.013$).

Seawater exposure trials

Tank temperature of each 30-day SW acclimation trials was maintained within ±1 °C at the target temperatures of either 5 or 12 °C (Fig. 1). Body length and mass of FW (time = 0) controls of each respective salinity trial declined from December to May (Table 1). Condition factor fluctuated between December and May, though did not significantly decline during this time; the substantial declines in condition factor began after the May trial time = 0 sampling. Together, the timing and magnitude of the declines in body length, mass, and condition factor data from the FW (time = 0) controls of the salinity trials were similar to the changes observed from the biometric monitoring experiment (Fig. 2). Whole-body water content of the FW (time = 0) control sea lamprey increased from ~75% water in December to ~82% water in May (Table 1), whereas hematocrit values decreased over this time, from ~27% red blood cells (RBC) in December to ~16% RBC in May (Table 1). Plasma chloride (~110–115 mmol·L^{-1}) and gill NKA activity (~19–22 μmol ADP·(mg protein^{-1})·h^{-1}) did not significantly differ among the FW (time = 0) controls from December to May (Table 1).

Survival was nearly 100% for the first three SW trials, from December through April (Fig. 3A). However, significant mortality was observed in both May trials (Fig. 3A). The survival curves for the May SW trials were determined to be significantly different than the survival curves of the earlier three SW trials ($X^2 = 31.4$; Mantel–Cox test). In May, transfer from ambient FW (12 °C) to 5 °C SW caused over 50% mortality within the first 3 days, then no further mortality was observed among the surviving individuals. When lamprey were transferred from ambient FW (12 °C) to 12 °C SW, less initial mortality was observed (~10% within 3 days), but significant mortality (50%) occurred by 30 days.

Condition factor (~1.1–1.2) fluctuated among the FW (time = 0) controls, but did not significantly differ among them (Fig. 3B). A significant reduction in condition factor was observed in the 12 °C SW trial in May (reduced from 1.16 to 1.01). However, no significant reduction in condition factor was observed in the concurrent 5 °C SW trial. In an analysis comparing FW (time = 0) and 30-day SW values for each trial, only an effect of trial ($P_{trial} = 0.049$) on condition factor was detected; there was no effect of salinity ($P_{salinity} = 0.285$) or interaction effect ($P_{interaction} = 0.053$; two-way ANOVA; trial × salinity).

Plasma chloride in FW remained consistent (~115 mmol·L^{-1}) throughout winter and spring, and exposure to SW resulted in the elevation of plasma chloride to ~130–140 mmol·L^{-1} (Fig. 3C). In an analysis comparing FW (time = 0) and 30-day SW values for each trial, effects of salinity ($P_{salinity} < 0.001$) and trial ($P_{trial} = 0.022$) on plasma chloride were detected; there was no interaction effect ($P_{interaction} = 0.283$; two-way ANOVA; trial × salinity).

The elevated gill NKA activity in FW that occurs during metamorphosis was maintained at ~20 μmol ADP·mg^{-1}·h^{-1} throughout the spring (Fig. 3D). In the January, March, and the 12 °C May trials, gill NKA activity increased by 50% after 30 days acclimation to SW, but gill NKA activity did not increase after SW acclimation in the December or 5 °C May

Fig. 2. Biometric monitoring of individually tagged juvenile sea lamprey (*Petromyzon marinus*). Body length, body mass, and condition factor (A–C) are presented for individually color-tagged, juvenile sea lamprey in fresh water. Light gray lines and symbols represent individual data, and dark brown lines and symbols represent mean data (mean ± standard error; $n = 9$). In panels A–C, different letters indicate significant differences between groups (one-way ANOVA, Tukey's). In panels D–F, % change (Δ) in respective biometric data (*y*-axis) is calculated relative to the initial measurements in December (set to 0) and plotted against the respective cumulative degree-days over that time (*x*-axis). Dark brown lines represent line of best fit. See text for additional details for calculations and analyses.

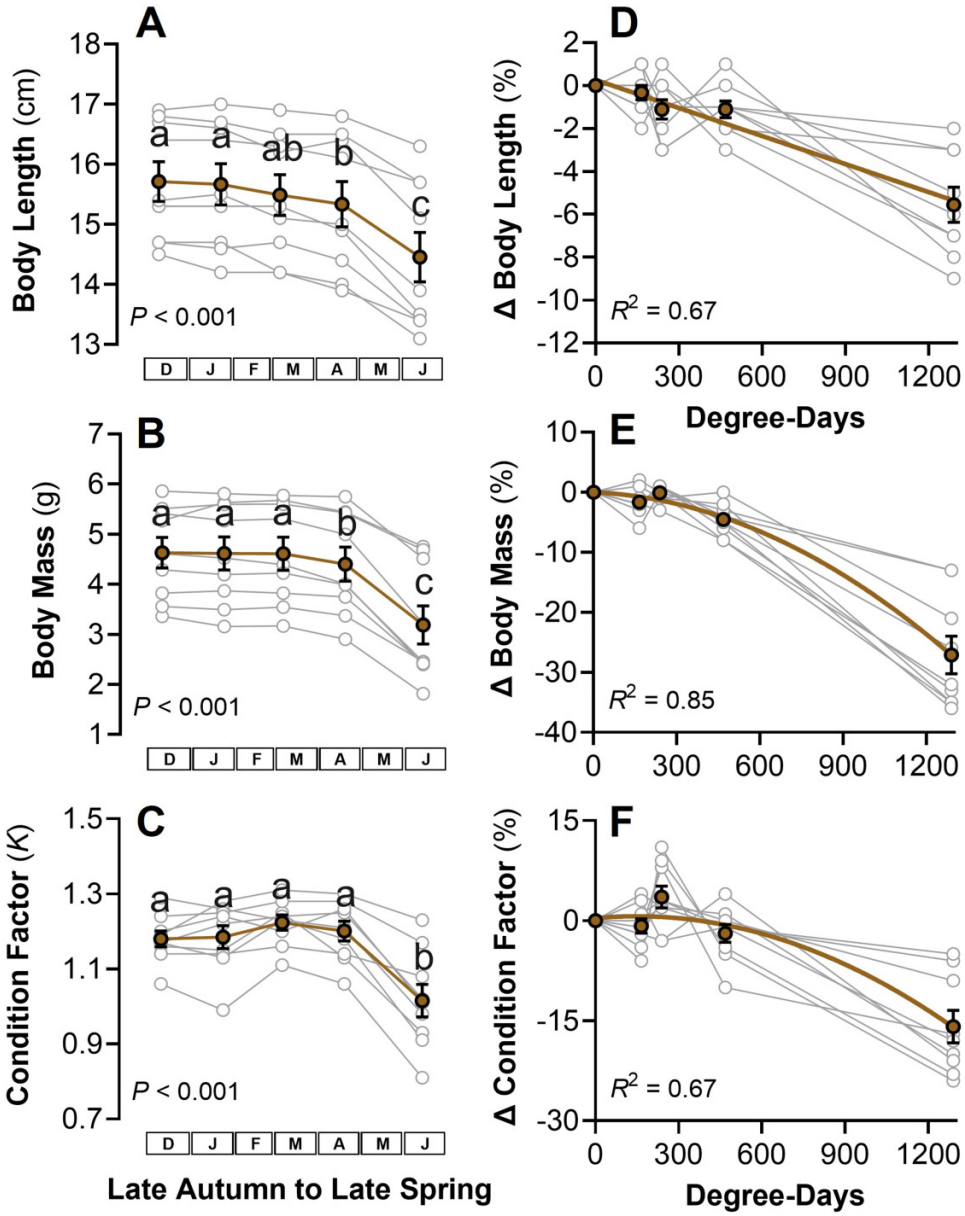
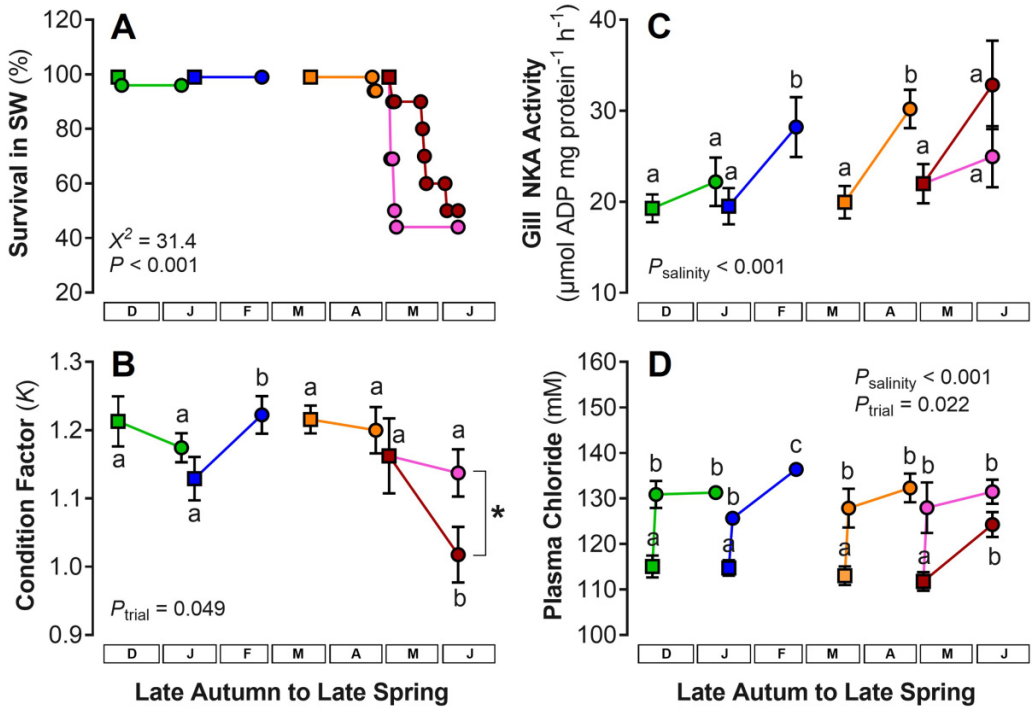


Table 1. Body length, body mass, condition factor (CF; Fulton's, *K*), whole-body water content, hematocrit (Hct), plasma chloride, and gill Na^+/K^+-ATPase (NKA) activity of juvenile sea lamprey (*Petromyzon marinus*) are presented for the freshwater (time = 0) controls of the series of seawater trials from December to May.

Date	Length (cm)	Mass (g)	CF (*K*)	Water content (%)	Hct (% RBC)	Plasma chloride ($mmol \cdot L^{-1}$)	Gill NKA activity
9 Dec.	15.4 ± 0.4a	4.5 ± 0.3a	1.21 ± 0.04a	75.4 ± 0.7a	27.2 ± 0.8a	115.1 ± 2.4a	19.3 ± 1.5a
20 Jan.	15.2 ± 0.2ab	4.0 ± 0.2ab	1.13 ± 0.03a	78.5 ± 0.8b	22.3 ± 1.1ab	114.7 ± 1.7a	19.5 ± 2.0a
22 Mar.	14.7 ± 0.4ab	3.9 ± 0.3ab	1.22 ± 0.02a	79.6 ± 0.7b	19.6 ± 1.6bc	113 ± 2.1a	20.0 ± 1.8a
3 May	14.5 ± 0.2b	3.5 ± 0.2b	1.16 ± 0.05a	82.1 ± 0.8c	16.1 ± 1.6c	111.8 ± 2.1a	22 ± 2.2a

Note: Data presented as mean ± standard error ($n = 10$). Different letters indicate significant differences between groups (one-way ANOVA, Tukey's post hoc). Gill NKA activity is expressed as $\mu mol\ ADP \cdot (mg\ protein^{-1}) \cdot h^{-1}$.

Fig. 3. Seawater (SW) performance of juvenile sea lamprey (*Petromyzon marinus*). Data from five separate SW trials lasting 30 days each. All SW trials (differentiated with different colors) were run at 5 °C except one May trial (dark red line), which was run at 12 °C. See Fig. 1 for colors and water temperature data corresponding to each trial. Squares represent data from ambient-temperature freshwater (FW; time = 0) samplings, and circles represent data from SW exposed fish. (A) Survival curves for each SW trial (survival curves compared using Mantel–Cox test). (B) Fulton's condition factor at 0 (FW) and 30 days of SW trials. (C) Gill NKA activity at 0 (FW) and 30 days of SW trials. (D) Plasma chloride concentrations at 0 (FW), 2, and 30 days of SW trials. Different letters indicate significant differences between groups within respective SW trial (two-way ANOVA, Tukey's). Asterisk indicates significant difference between May trials. In panels B–D, data are presented as mean ± standard error (n = 5–15). P values derived from a two-way ANOVA (trial × salinity) using only the FW and 30-day SW data.



trials. In an analysis comparing FW (time = 0) and 30-day SW values for each trial, no effect of trial (P_{trial} = 0.092) or the interaction ($P_{interaction}$ = 0.204) on gill NKA activity was detected, but a significant effect of salinity ($P_{salinity}$ < 0.001) was detected (two-way ANOVA; trial × salinity).

Discussion

The poor SW tolerance of larval sea lamprey precedes the high SW tolerance of juvenile sea lamprey, which are capable of surviving direct transfer to SW with minimal osmotic perturbations (Reis-Santos et al. 2008; Barany et al. 2020; Shaughnessy and McCormick 2020). The present study demonstrated that this high level of SW tolerance in sea lamprey juveniles is maintained from December (immediately after metamorphosis) well into late spring (~5 months). This lengthy window of high osmoregulatory capacity in juvenile sea lamprey should allow great flexibility in the timing of SW entry of seaward migrants. The loss of salinity tolerance in late spring, based on the reduced SW survival rates, corresponded to the loss of mass and condition during this time. Although no mechanism to explain the loss of SW tolerance in late spring could be firmly established in the present study, we discuss the influence of elevated temperatures in late spring and other factors that may cause the end to their prolonged window of SW tolerance.

It has been observed that the increase in gill and intestine NKA activity and protein abundance during sea lamprey metamorphosis corresponds to the increase in SW tolerance (Beamish et al. 1978; Reis-Santos et al. 2008; Shaughnessy and McCormick 2020; Shaughnessy et al. 2020; Barany et al. 2021*a*). As expected, juvenile sea lamprey in the present study exhibited gill NKA activity that was 10- to 15-fold higher than what has been reported for premetamorphic larvae (Reis-Santos et al. 2008; Shaughnessy et al. 2020), and acclimation to SW tended to further increase gill NKA. These elevated gill NKA activity levels were maintained throughout the winter and spring, corresponding to the prolonged window of elevated hypo-osmoregulatory capacity and SW tolerance observed in the present study.

This long window (~5 months) of elevated SW tolerance that we observed in the juvenile sea lamprey draws contrast to the quite short window (~2–4 weeks) of SW tolerance in anadromous salmon smolts (McCormick 2013). In salmonids, the regression of hypo-osmoregulatory capacity after their relatively short window for SW entry is interesting to consider in comparison to the correspondingly long window in sea lamprey that is described in the present manuscript. The

reversibility of the osmoregulatory preparations for SW entry, as exhibited in the postsmolt life-stage in salmonids, may be adaptive. For instance, it would be adaptive to reverse preparations for marine life if a migratory fish seasonally loses access to the sea due to reasons either zoogenic (e.g., damming) or environmental (e.g., drought) or a combination of the two (Kemp et al. 2012; Lokteff et al. 2013; Malison et al. 2014). It has been shown that over evolutionary time, populations of anadromous salmonids landlocked by anthropogenic damming have adapted to lessen their osmoregulatory preparations for marine life (McCormick et al. 2019). The case of the landlocked sea lamprey of the Great Lakes of North America is interesting in this regard. It has not been shown whether osmoregulatory preparations for marine life occur during the metamorphosis of these landlocked populations. If they were to still make these osmoregulatory preparations during metamorphosis, it is presumable that these landlocked sea lamprey must at some point reverse such osmoregulatory preparations, similar to the physiology of a salmon postsmolt, to maintain osmoregulatory homeostasis in the FW environment of the lakes. If so, it is possible that the window of high SW tolerance is shorter in the landlocked sea lamprey versus the anadromous sea lamprey. However, the development and timing of hypo-osmoregulatory preparation in landlocked sea lamprey are not well-studied.

Unlike the highly synchronized timing of lamprey metamorphosis (Youson 2003), the timing of the downstream migration made by juvenile lamprey within a population can vary considerably, from October to April (Evans et al. 2021). An important distinction must be made between when juvenile lamprey out-migrate from natal streams and when they subsequently enter SW. Many studies have reported that the downstream migrations of lamprey populations from natal streams corresponds to periods of high discharge and occurs either in late winter, early spring, or is bimodally distributed between those two windows, but it is largely unknown when they enter SW immediately after leaving their natal streams (see review by Dawson et al. 2015). Understanding that newly metamorphosed juvenile sea lamprey exhibit rapid growth after initiation of feeding behavior begins in an estuary (Silva et al. 2013) and that remaining upstream means many more months of fasting, it is presumable that early winter out-migrants have the advantage of greater adult growth, and thus reproductive potential. However, in a comparison between early- and late-season out-migrating juvenile sea lamprey in the Great Lakes, no difference in adult growth was detected (Swink and Johnson 2014). Therefore, it could be that the prolonged maintenance of hypo-osmoregulatory capacity and SW tolerance observed in the present study is an adaptive trait in anadromous sea lamprey—a larger window for out-migration allows for a greater selection of conditions favorable for SW entry.

In this study, juvenile sea lamprey body condition and SW survival was eventually reduced in late spring, corresponding to the timing of increases in river temperatures, defining an end to their long window of hypo-osmoregulatory capacity. A low metabolic rate determined by low activity and cold-water temperatures between December and April (<6°C) is a likely explanation for the apparent maintenance of body mass and condition over the first several months of fasting observed in the present study. An increase in metabolic rate during the ~8°C increase in river temperatures between April and June, in association with the prolonged fasting during the juvenile life stage, might explain the significant decline in body mass and condition that occurred during this time. In landlocked larval sea lamprey, it has been shown that lipid and carbohydrate metabolism decline with temperature beginning in September and remain at very low levels over winter until March (O'Boyle and Beamish 1977). Likewise, when temperatures increase in spring, increases in lipid and carbohydrate metabolism were observed (O'Boyle and Beamish 1977). By regressing degree-days with the concurrent changes in biometric indices, it was clear that the acceleration of thermal time in late spring negatively correlated with changes in body length, mass, and condition.

We observed significant declines in SW survival in late May after fish had experienced large increases in river temperatures corresponding with declines in body mass and condition factor. Gill NKA activity in FW in May remained high, gill NKA had increased in SW, and plasma chloride values in SW remained well-regulated, all of which are good indicators that these fish maintained a high capacity for hypo-osmoregulation. Considering this, the reduction of SW survival in May might not be fully explained by osmoregulatory failure alone. However, it should be noted, that SW gill NKA activity and plasma chloride values were taken from fish that had survived in SW for 30 days, and thus were those able to acclimate to SW. It is likely that the individuals that were unable to tolerate SW in May did indeed exhibit hypo-osmoregulatory failure, such as a lack of ability to upregulate gill NKA activity or regulate plasma chloride in SW. It is also interesting to consider the changes in whole-body water content that were observed—water content increased significantly from March to May. This could indicate that juvenile sea lamprey have readjusted their homeostatic set point for water content in FW over time, or increased their water content due to the loss of lipid that is likely to have occurred due to lack of feeding. However, neither interpretation of the changes in water regulation serves to explain the loss of SW tolerance during this time.

Though it has not been explicitly tested in the present manuscript, it should be considered that SW tolerance in juvenile sea lamprey is related to loss of mass or condition due to prolonged lack of feeding. Some information can be gleaned from the two SW trials in May that were carried out at different temperatures, wherein condition factor declined sharply over the 30 days in SW in the 12 °C trial and yet remained relatively stable in the 5 °C trial (Fig. 3). First, the differences between the 5 and 12 °C trials indicate that the elevated temperatures in late spring, and not solely fasting, likely contributed to the loss of body condition in late spring that was also observed in our color-tagged fish in FW. Second, the similar outcomes in mortality between the 5 and 12 °C SW trials, despite the difference in changes in condition factor over this time, indicates that a decline in condition factor is not solely responsible for the reduced SW tolerance observed. Indeed, previous work relating size and

osmoregulation in sea lamprey has yielded mixed results (Beamish 1980).

The SW trials in May also demonstrated that temperature had an effect on the nature of SW survival in late spring. Mortality among lamprey transferred from ambient (~12 °C) FW to 5 °C SW was immediate (i.e., in the first few days), whereas mortality among lamprey transferred from ambient 12 °C FW to 12 °C SW occurred much more gradually (over several weeks). A similar finding of more rapid mortality occurring in a low temperature SW transfer has been observed in Atlantic salmon smolts (Sigholt and Finstad 1990; Handeland et al. 2000). In these previous studies, it was suggested that cellular and metabolic adjustments that are immediately required upon SW entry are slowed at lower temperatures, resulting in mortality that is more immediate in nature in the low temperature SW exposures. However, such an explanation does explain the results of the present study, as little to no immediate mortality was observed in the three preceding SW trials, which were all carried out at an equally low temperature (5 °C). Although the present manuscript is not able to elucidate a mechanism for the effect of temperature on SW tolerance, the difference in SW survival between our 5 and 12 °C SW trials in May indicates an effect of temperature on SW tolerance does exist, and future studies on this effect are warranted.

It should be noted that the net temperature change during transfer from ambient river water to SW was much greater in the 5 °C SW trial in May (a change of ~7 °C) than that of the preceding SW trials (~0–3 °C). It is possible that experiencing simultaneous thermal and salinity stressors contributed to the immediate mortality in the 5 °C SW trial in May. When we transferred lamprey to 12 °C SW in May, which removed any simultaneous thermal stress (and was more ecologically relevant considering the ~12 °C temperature of the estuary in May), mortality was spread across the 30-day SW trial rather than concentrated at the beginning. From both trials, the conclusion could be the same: SW entry as late in spring as May (after river and estuary temperatures have risen to ~10 °C) is beyond the window for optimal osmoregulatory performance in sea lamprey. Further research is needed to mechanistically explain the springtime reduction in SW tolerance in the juvenile life stage of sea lamprey, for which empirical investigations are lacking (Evans et al. 2021). We propose that such investigations should focus on the impacts of prolonged fasting, temperature-driven increases in metabolism, and their interaction as possible explanations. Finally, it may also be important to consider how the newly developed parasitic lifestyle of the juvenile sea lamprey may determine the nature and timing of exposure to changes in salinity and temperature. It is known that Atlantic salmon often move through estuaries within a single tidal cycle and that this is among the most mortality-inducing events in their natural life history (McCormick et al. 1998; Thorstad et al. 2012) Given that lamprey are known to begin attaching to potential hosts in the estuary (Silva et al. 2013), they may not always be in control of when and how quickly they are exposed to changes in salinity and temperature. Thus, it may be critical that juvenile sea lamprey arrive in an estuary and begin feeding before their salinity tolerance has begun to decline in late spring.

In conclusion, the present study provides an important insight into the critical downstream migratory life stage of anadromous sea lamprey that has received relatively little attention (Evans et al. 2021). We show that postmetamorphic juvenile sea lamprey have a large window of SW tolerance lasting from November to May, which is much larger than the 2- to 4-week window of SW tolerance in salmon smolts (McCormick et al. 1998). Further, we showed that reduced SW survival occurs in late spring, corresponding to the timing of substantial increases in ambient temperatures. We propose that increases in temperatures may determine the end of the window of opportunity for SW entry in late-season out-migrating juvenile sea lamprey, although further research is needed to offer such mechanistic insights. Our findings may help explain the vulnerability of the sea lamprey populations in the southern European range, which inhabit rivers with early spring temperatures that reach well over 10 °C (Kamarianakis et al. 2016) and which are considered vulnerable to warming trends posed by climate change (Mateus et al. 2012). Such sensitivities to temperature of this life stage could be beneficial to consider in management and conservation efforts throughout the North American and European range for populations of sea lamprey where warming river or estuarine temperatures are of concern.

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Data availability

The authors confirm that the relevant data supporting the findings of this study are available within the article text and figures.

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Competing interests

The authors declare no competing or financial interests. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US Government.

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