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The GH/IGF axis in the sea lamprey during metamorphosis and seawater acclimation

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ABSTRACT

How the growth hormone (GH)/insulin-like growth factor (IGF) system affects osmoregulation in basal vertebrates remains unknown. We examined changes in the expression of components of the GH/IGF axis and gill ion transporters during metamorphosis and following seawater (SW) exposure of sea lamprey. During metamorphosis, increases in gill *nka* and *nkcc1* and salinity tolerance were accompanied by increases in pituitary *gh*, liver *igf1*, gill *ghr* and *igf1*, but not liver *ghr*. SW exposure of fully metamorphosed sea lamprey resulted in slight increases in plasma chloride concentrations after SW exposure, indicating a high level of SW tolerance, but no major changes in mRNA levels of gill ion transporters or components of the GH/IGF axis. Our results indicate that metamorphosis is a critical point in the lifecycle of sea lamprey for stimulation of the GH/IGF axis and is temporally associated with and likely promotes metamorphosis and SW tolerance.

1. Introduction

The sea lamprey, *Petromyzon marinus*, is an anadromous and semelparous species of lamprey with a multifaceted lifecycle (Hardisty and Potter 1971). After sea lamprey embryos hatch, the larvae (also known as ammocoetes) buries in mud and silt substrate of FW streams. This stage generally lasts from three to seven years, during which the larvae filter feeds on detritus. Upon accumulating enough mass and energy reserves they undergo metamorphosis, a time of profound morphological and physiological transformations in preparation to their marine parasitic trophic phase (Youson and Potter 1979). In addition to the outer morphological transformations, the sea lamprey also begins rearrangement of internal organs structure and function such as the gills (Peek and Youson 1979; Morris 1980; Youson 1980; Bartels and Potter 2004; Reis-Santos et al., 2008; Ferreira-Martins et al., 2016a; Shaughnessy and McCormick 2020; Sunga et al., 2020), kidney (Youson 1970, 1980, 1984; Ooi and Youson 1977, 1979; Ellis and Youson 1990; Ellis 1993), and intestine (Youson 1980, 1981, 1985; Youson and Horbert 1982; Elliott 1989; Mark Elliott and Youson 1994; Barany et al., 2020), vital for the new osmoregulatory challenges that living in SW imposes (see Ferreira-Martins et al., 2021 for a complete review).

Lampreys, together with hagfishes, are living representatives of the basal vertebrate lineage and form the jawless vertebrate group called Agnatha (Janvier 1999). Hagfishes are only found in marine environments and do not osmoregulate, whereas lampreys have evolved the capacity to live in both the FW and SW environments. To achieve this, sea lamprey maintain their internal milieu constant (about 1/3 the concentration of SW) irrespective of their external environment; thus they are hypoosmotic when in SW and hyperosmotic when in FW (Ferreira-Martins et al., 2021). This osmoregulatory strategy is the same as seen in the later evolved teleosts. To date, most of the osmoregulatory mechanisms for FW and SW osmoregulation in lamprey are analogous to those of euryhaline teleosts, a lineage from which lamprey diverged more than 500 million years ago (Janvier 1999).

When in FW, sea lamprey maintain their internal osmolality hyperosmotic compared to the external environment and thus need to balance

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the ions lost to the environment and the water gained through osmosis. To achieve this, lamprey gills contain specialized cells called FW-type ionocytes, which function to actively take up ions from the environment. In lamprey, two kinds of FW-type ionocytes have been described: one type exclusive to the larval stage and a second FW-type ionocyte that occurs in both larvae and upstream spawning migrating adults (see Ferreira-Martins et al., 2021 for a detailed review).

After migrating downstream and entering the ocean, sea lamprey face a new osmoregulatory challenge in which they need to counterbalance the gain of ions from the hyperosmotic environment and the loss of water through osmosis. To accomplish this, they drink SW from which monovalent ions are absorbed in the intestine allowing water to be absorbed through osmosis (Barany et al., 2020, 2021). Divalent ions are excreted via defecation and renal filtration and the excess of monovalent ions is actively secreted across the gill through SW-type ionocytes (see Ferreira-Martins et al., 2021 for a detailed review). The apical mechanisms for chloride secretion in teleosts is the cystic fibrosis transmembrane regulator (CFTR), though this has yet to be identified in lamprey. The basolateral membrane of SW-type ionocytes is elongated through an internal tubular system in this type of cells which are rich in a sodium-potassium/chloride cotransporter [NKCC1 (Shaughnessy and McCormick 2020)] and Na^+/K^+-ATPase [NKA (Reis-Santos et al., 2008; Ferreira-Martins et al., 2016b)]. During metamorphosis and while still in FW, juvenile sea lamprey develop the SW-type ionocytes that grant them the capacity to tolerate direct transfer to SW (Reis-Santos et al., 2008).

The GH/IGF axis in teleosts has multiple subtypes of GH receptors (GHRs), IGF receptors and IGF binding proteins that resulted from gene duplication events during the evolution of the teleost lineage (Reindl and Sheridan 2012). Growth hormone (GH) is a pluripotent hormone known to play a key role in the development of vertebrates. In fish specifically, GH is involved in many physiological processes including growth (Donaldson et al., 1979; McLean et al., 1990; Marchelidon et al., 1996), reproduction (Higgs et al., 1976; Singh et al., 1988; Van Der Kraak et al., 1990; Burzawa-Gerard and Delevallee-Fortier 1992; Le Gac et al., 1992; Singh and Thomas 1993), immune system response (Franz et al., 2016) and ionic and osmotic regulation (Clarke et al., 1977; Miwa and Inui 1985; Boeuf et al., 1990; Borski et al., 1994). GH is produced and secreted by the pituitary gland into the circulation reaching target tissues where it binds to the GH receptor (GHR), a transmembrane type 1 cytokine receptor that dimerizes upon binding. In fish, GH has been shown to promote SW acclimation, particularly in salmonids (McCormick 2001), which confers greater SW tolerance by increasing the number of SW-type ionocytes in the gills as well as NKA and NKCC levels. An increase in GH production is also associated with stimulation of insulin-like growth factor 1 (IGF1) production, mainly in the liver but also in many other tissues including gill and muscle (Le Roith et al., 2001; Wood et al., 2005; Laviola et al., 2007). Circulatory levels of IGF1 then regulate the secretion of GH and gene transcription in the pituitary via a negative feedback mechanism (Duan 1998; Reinecke et al., 2005, 2006; Wood et al., 2005; Reinecke 2010a, and reviewed by Reindl and Sheridan 2012). IGF1 has been shown to increase SW tolerance in various species of teleosts, most likely through upregulation of gill NKA and other ion transporter involved in salt secretion (McCormick 2001). Recently it was suggested that early vertebrates had a minimum of two ancestral genes that led to the emergence of distinct GH and prolactin-like genes in lampreys (Gong et al., 2022). A GH homolog has been identified in the pituitary of sea lamprey with evidence for its stimulation of an IGF homolog in the liver (Kawauchi et al., 2002). The growth hormone receptor (GHR) has also been sequenced and characterized in sea lamprey (Gong et al., 2020) with high mRNA levels in liver and heart tissues and moderate levels in the gill.

In the present study, we address the hypothesis that GH and IGF1 play a role in osmoregulation in sea lamprey, similar to what has been shown in salmonids and other teleosts. We focus on two major landmarks of the sea lamprey lifecycle: metamorphosis and the transition from FW to SW. We examined changes in metamorphosis by examining changes over time in both larvae, early-, mid- and late-metamorphosis, and post metamorphosis. As for transition from FW to SW, we acclimated late-stage metamorphic sea lamprey (stage 7) to SW and examined changes over time at 1, 3, 6, and 14 days.

2. Methods

2.1. Animal collection and maintenance

Sea lamprey, *Petromyzon marinus* (Linnaeus 1758), larvae (N = 700) and transformers (N = 545) were collected from exposed substrate at the Turners Falls canal in the Connecticut River (MA, USA) during drawdown for dam maintenance in early autumn and brought to the USGS S. O. Conte Anadromous Fish Research Center in Turners Falls, MA. All experiments were carried out in accordance with USGS-IACUC guidelines. The fish capture, rearing, and experimental procedures in this study were performed in accordance with protocols approved by the Institutional Animal Care and Use Committees at the University of Massachusetts (protocol no. 2016–0009).

2.2. Metamorphic series

For this experiment, animals that had been collected from the Turners Falls canal in September 2017 and were maintained for 8–14 months in flow-through, 1 m diameter tanks supplied with Connecticut River water (4 l min^{-1}) with a sandy, silt substrate for burrowing. Airstones were provided for each tank and oxygen levels were maintained within 90% of air-saturated levels. Larvae were exposed to natural photoperiod and fed twice a week with live yeast. A metamorphic series was obtained by sampling larvae and metamorphosing individuals on August 1st (ten larvae, and fourteen metamorphic stages 1&2), September 1st (ten larvae, and twelve metamorphic stages 3&4), October 18th (twelve larvae, and ten metamorphic stages 6&7), and November 15th (twelve larvae, and nine postmetamorphic), of 2018 (Table 1).

2.3. Salinity challenge

A salinity challenge time course was conducted using late metamorphic (stage 7) sea lampreys that had been collected from the Turners Falls canal in September. Animals were divided into four round fiberglass tanks containing dechlorinated tap water at 15 °C. After a two-week acclimation period, six animals were sampled from each tank as a zero-day control. Two of the tanks were raised to a salinity of 30 ppt by draining half of the tank and filling it back up with 60 ppt synthetic SW (Instant Ocean, Aquarium Systems Inc., Mentor, OH, USA). The same procedure was performed in the remaining two FW control tanks to mimic the draining-refilling operation. Twelve animals from each tank were sampled after 1, 6, and 14 days to account for short, mid-, and long-term SW acclimation. Animals were not fed during experimentation as they do not feed during this period of their lifecycle. Salinity was measured using a multiparameter YSI EC300 (YSI Environmental), and total ammonia content of the water was monitored regularly, and the latter did not exceed 0.5 mg/L. There were no mortalities during the experiment.

2.4. Sampling

Animals were euthanized by an lethal dose of buffered ethyl-m-benzoate 160 mL MS-222/L water (400 mg/L buffered with $NaHCO_3$, pH 7.0; Argent Chemical Laboratories, Redmond, WA, USA). The stage of each metamorphic sea lamprey was recorded according to Youson and Potter (1979). Each animal was weighed (±0.01 g) and measured to the nearest mm (total length). Blood was collected by cutting the tail and collecting blood into heparinized capillary tubes. Capillary tubes were then spun at 5000×*g* for 5 min at room temperature to obtain plasma.

Table 1
Length, mass and tank water temperature, date and sampling size for larval and metamorphosing sea lamprey sampled for metamorphic series experiment. Data are mean ± SEM.

Time	Life stage	Sampling size (n)	Length (cm)	Weight (g)	Temperature (°C)
August 1st	Larvae	10	12.4 ± 0.3	2.9 ± 0.2	24.0
	Metamorphic stages 1&2	14	14.0 ± 0.3	4.2 ± 0.3	
September 1st	Larvae	10	12.6 ± 0.2	2.8 ± 0.1	24.3
	Metamorphic stages 3&4	12	13.4 ± 0.3	4.2 ± 0.3	
October 18th	Larvae	12	13.1 ± 0.2	2.9 ± 0.1	13.0
	Metamorphic stages 6&7	10	13.8 ± 0.3	4.1 ± 0.3	
November 15th	Larvae	12	12.4 ± 0.2	2.5 ± 0.2	5.0
	Postmetamorphic	9	13.8 ± 0.6	3.8 ± 0.5	

Samples of the gill, liver, brain, pituitary, kidney, anterior and posterior intestine, and plasma were collected. For gill Na^+/K^+-ATPase activity measurement, filamental gill tissue was dissected according to McCormick (1993). All samples were immediately frozen on dry ice after sampling and kept at −80 °C until further processing.

2.5. RNA extraction, quantification, quality control and cDNA synthesis

RNA was extracted, quantified and purity assessed from gill, liver, and pituitary according to (Shaughnessy and McCormick 2020). A total of 0.5 μg RNA was treated with DNase prior to cDNA synthesis using a Promega DNase Treatment kit (Madison, WI, USA). The cDNA was synthesized using high capacity cDNA reverse transcriptase kit (Applied Biosystems, Foster City, CA, USA) and a PTC-100™ Programmable Thermal Control thermo cycler (MJ Research, Inc. Watertown, MA, USA), and stored at −20 °C.

2.6. Real-time RT-PCR

Oligo probes for quantification of mRNA abundance of *igf*, *ghr, gh, nka,* and *nkcc1,* were developed and optimized as well as housekeeping genes (*gapdh*, *ef1α*). Real-time PCR was conducted on cDNA isolated from the gill, liver, and pituitary using SYBR Select Master Mix (Applies Biosystems, Foster City CA) in a StepOnePlus™ Real-Time PCR System (Applied Biosystems). A 10 μL reaction was set according to the manufacturer's instructions with 4 μL of 10 fold diluted cDNA and 6 μL of a Master Mix containing 150 nM of each primer pair. Oligo primer information is shown in Table 2. Real-Time RT-PCR consisted in enzyme activation at 50 °C for 2 min, initial denaturation at 95 °C for 2 min and 40 cycles of denaturing, annealing and extension at 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s, respectively. Specificity of the assays was checked using a melt curve and efficiency of assays using a dilution series was prepared from a pool of all samples.

Table 2
Sea lamprey, *Petromyzon marinus*, specific oligo probes (sense and anti-sense) for Real-Time RT PCR with annealing temperature, original gene ascension number, and amplicon size. Primers with an asterisk (*) were designed with Primer3 [http://bioinfo.ut.ee/primer3-0.4.0/(Untergasser et al., 2012)] and reference sources are given for the remainder.

Oligo Primer	Sequence (5′-3′)	Annealing Temp (°C)	GenBank Ascension #	Reference
Pm_gapdh_F	TGCAAAGCACGTCATCATCTC	60	AY578058	(Shifman et al., 2009)
Pm_gapdh_R	TTCTCGTGGTTTACTCCCATCA			
Pm_ef1a_F*	GTGGGTCGTGTTGAGACTGG	60	KU726618	
Pm_ef1a_R*	GGTCGTTCTTGCTGTCAC			
Pm_igf_F	AAGCGAAACCTCTGTGAGGG	60	AB081462	(Kawauchi et al., 2002)
Pm_igf_R	TGGGTCACCGTTTGCTTTCT			
Pm_gh_F	TCTACAATGAAAGGAGGCTCTC	60	AB081461	(Kawauchi et al., 2002)
Pm_gh_R	GGTACAAGTCAGAAGCACG			
Pm_ghr_F*	CTCCTCGCCACGCTACTG	60		
Pm_ghr_R*	TCATGCGGTTGAACTCCTCC			
Pm_nkcc1_F	GAGAGGTTTCGCGACAAGAC	60		(Ferreira-Martins et al., 2016a)
Pm_nkcc1_R	CGCTCACGAGTAGAACGTCA			
Pm_nka_F	CGTGGAATCGTCATCAACAC	60		(Ferreira-Martins et al., 2016a)
Pm_nka_R	GCGACAGGATGAAGAAGGAG			

2.7. Chloride quantification in plasma

Chloride concentration was measured in plasma samples from the salinity challenge by titration using a digital chloridometer (Chloride Analyzer 925; Corning, Halstead, UK).

2.8. Measurement of gill Na^+/K^+-ATPase activity

Gill Na^+/K^+-ATPase (NKA) activity was measured according to McCormick (1993). Samples were thawed and homogenized in SEID buffer (SEI with 0.1% deoxycholate) and centrifuged at 3200×*g* for 5 min at 4 °C. The supernatant was removed and used for the assay. Two duplicate sets, one with 0.5 mmol l^{-1} ouabain added to inhibit NKA activity and one without, were run using 10 μl of homogenate. Protein was measured using a bicinchoninic acid protein assay (BCA) with bovine serum albumin used as standard (Pierce™ BCA Protein Assay Kit, Thermo Fisher Scientific). Measurements of ouabain-sensitive and ouabain-insensitive NKA activities were expressed in μmol ADP mg^{-1} protein h^{-1}.

2.9. Statistical analyses

Data were statistically analyzed using one-way or two-way ANOVA tests followed by suitable post hoc test using SigmaPlot 11.0 (Systat Software, Inc.) Data are shown as means ± SEM. The fiducial limit was set at 0.05.

3. Results

3.1. Metamorphic series

3.1.1. Quantification of mRNA levels

Pituitary *gh* mRNA levels were low in both larvae and metamorphosing sea lamprey in August and September, but increased substantially in both groups in October and November (Fig. 1A). There were no differences in pituitary *gh* mRNA between larvae and metamorphic sea lamprey at any of the time points examined. Pituitary *gh* mRNA levels of metamorphosing individuals were 7.0-fold higher in November than they were in August.

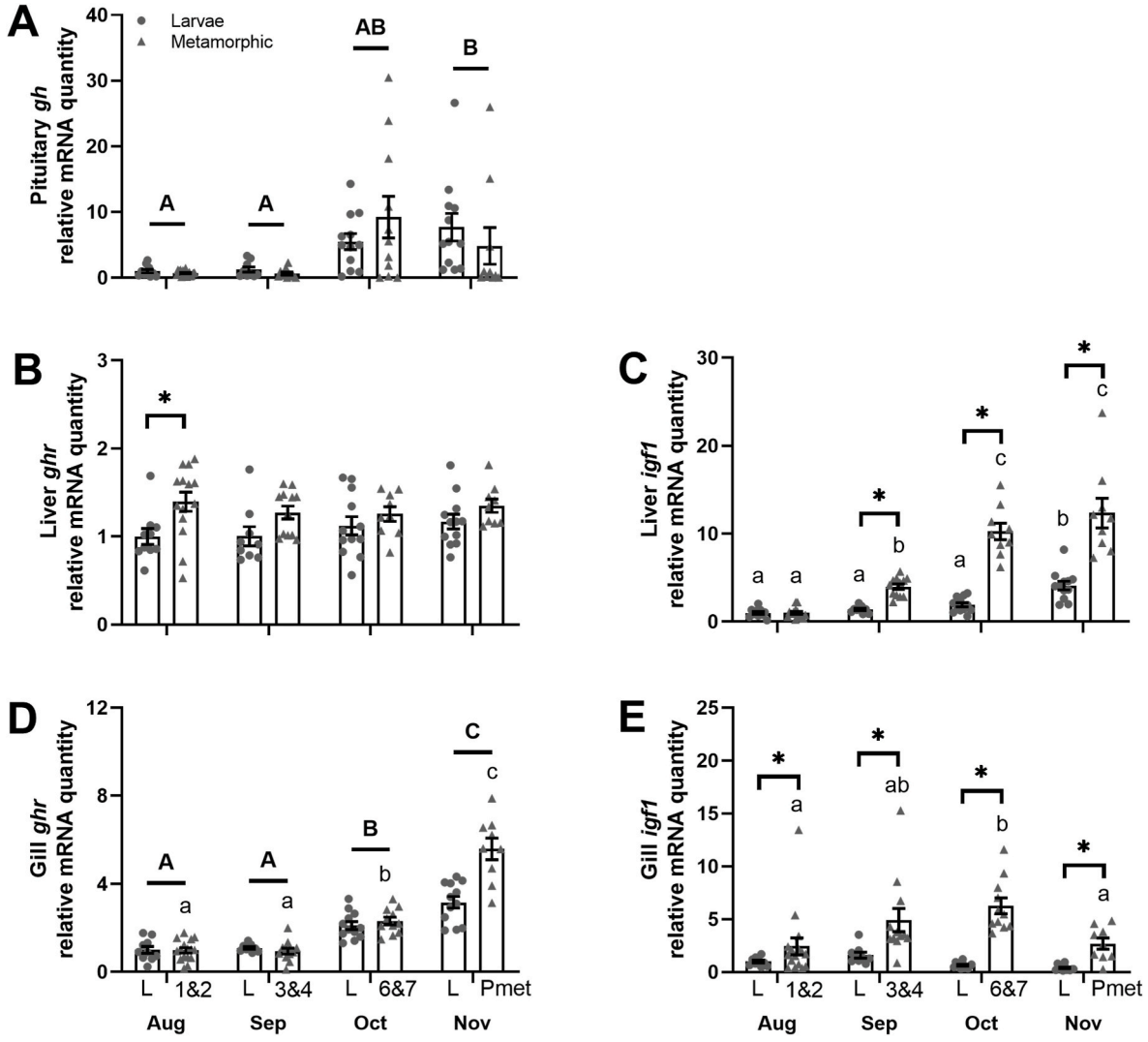

Fig. 1. Relative mRNA quantity of pituitary growth hormone; *gh* (**A**), liver and gill growth hormone receptor; *ghr* (**B** and **D**, respectively), liver and gill insulin-like growth factor 1; *igf1* (**C** and **E**, respectively). All groups were kept in freshwater. Groups and group sizes are: larvae; L (n = 10 sampled in August and September, n = 12 in October and November), and metamorphic stages: 1 and 2 sampled in August; 1&2 Aug (n = 14), 3 and 4 in September; 3&4 Sep (n = 12), 6 and 7 in October; 6&7 Oct (n = 10), and post metamorphic juveniles in November; Pmet Nov (n = 9). Values are relative to the FW larvae control in August. Values are shown as means ± SEM. Different upper-case letters indicate significant differences between season irrespective of stage. Different lower-case letters denote significant differences between season within developmental stages. Analysis was performed using a two-way ANOVA followed by a Student-Newman-Keuls method test, $p < 0.05$. Differences between metamorphic stages within same season are represented by an asterisk (*).

Liver *ghr* mRNA levels remained mostly unaltered and a difference was only found between early metamorphic stages (1&2) compared to larvae sampled at same time in August (Fig. 1B). Liver *igf1* mRNA levels were highest in larvae in November compared to August, September and October (Fig. 1C). In metamorphosing individuals, there were progressive, large increases in liver *igf1* mRNA levels, with levels in fully metamorphosed individuals 12.2-fold higher that they were in larvae and early metamorphic stages (1&2).

Gill *ghr* mRNA levels in larvae remained unaltered, although there was a clear tendency to increase in October and November compared to August and September. On the other hand, gill *ghr* mRNA levels in metamorphosing individuals were 2.4- and 5.7-fold higher in late metamorphic stages (6&7) and postmetamorphic, respectively, compared to early metamorphic stages (1&2) (Fig. 1D). Gill *igf1* mRNA levels remained unaltered in larvae. In contrast, gill *igf1* mRNA levels in metamorphosing sea lamprey were 2.6-fold higher in late metamorphic stages (6&7) compared to early metamorphic stages (1&2). Following this peak, gill *igf1* mRNA levels in postmetamorphic stage returned to similar levels found in early metamorphic stages (1&2) (Fig. 1E).

Gill *nka* mRNA levels remained unaltered in larvae whilst in metamorphosing sea lamprey in late metamorphic stages (6&7) were 9.0-fold higher compared to early metamorphic stages (1&2). The highest gill *nka* mRNA levels were found in postmetamorphic juveniles, which were 13.9-fold higher than observed in early metamorphic stages (1&2) (Fig. 2A). Finally, gill *nkcc1* mRNA levels increased through metamorphosis. Gill *nkcc1* mRNA level were 36.7-fold higher in late metamorphic stages (6&7) compared to early metamorphic stages (1&2), and remained high in postmetamorphic juveniles (Fig. 2C).

3.1.2. Gill NKA activity

In the metamorphic series, low levels of gill NKA activity were found in larvae and early (1&2) and mid-stages (3&4) of metamorphosis (Fig. 2B). Large increases in gill NKA activity were found in late metamorphic stages (6&7) and in postmetamorphic juveniles ($p < 0.001$) showing 13.5- and 36.3-fold higher gill NKA activity, respectively, compared to early metamorphic stages (1&2).

3.2. Salinity exposure

3.2.1. Plasma chloride levels

In the salinity time course experiment, plasma chloride concentrations where higher ($p < 0.001$) in juvenile sea lamprey after acclimation to SW for 1, 3, 6, and 14 days when compared to respective time control groups in FW (Fig. 3A).

3.2.2. Gill NKA activity

No differences were found in gill Na^+/K^+-ATPase activity during the salinity time course (Fig. 3B).

3.2.3. Quantification of mRNA levels

Pituitary *gh* mRNA levels remained mostly unaltered through the salinity time course in FW- and SW-acclimated sea lamprey postmetamorphic juveniles. At 3 days, lower pituitary *gh* mRNA levels were found in SW acclimated postmetamorphic sea lamprey compared to those in FW (Fig. 4A). No changes were observed in liver *igf1* mRNA levels at any time point or following SW exposure (Fig. 4B). Gill *igf1* mRNA levels remained generally unaltered, though at 6 days *igf1* mRNA levels were higher in postmetamorphic sea lamprey acclimated to SW compared to those in FW (Fig. 4C). Similarly, gill *nka* mRNA levels remained mostly unaltered through the salinity time course, except at day 1 during which *nka* mRNA levels here higher in SW-acclimated postmetamorphic lamprey compared to those in FW (Fig. 4D).

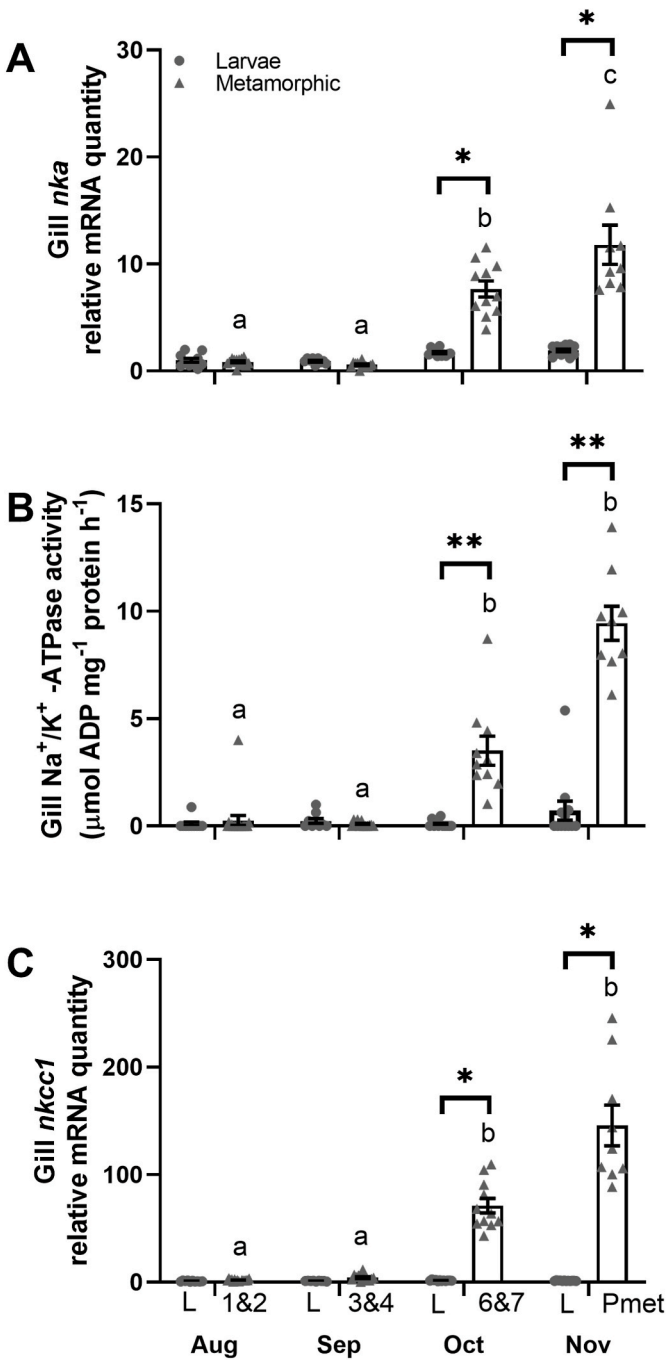

Fig. 2. Relative mRNA quantity of gill Na^+/K^+-ATPase α subunit; *nka* (**A**) and $Na^+:K^+:2Cl^-$ cotransporter type 1; *nkcc1* (**C**), and Na^+/K^+-ATPase activity in µmol ADP mg^{-1} protein h^{-1} (**B**) in the gill of different sea lamprey. All groups were kept in freshwater. Groups and group sizes are: larvae; L (n = 10 in August and September, n = 12 in October and November), and metamorphic stages: 1 and 2 in August; 1&2 Aug (n = 14), 3 and 4 in September; 3&4 Sep (n = 12), 6 and 7 in October; 6&7 Oct (n = 10), and post metamorphic juveniles in November; Pmet Nov (n = 9). Values for gill *nka* and *nkcc1* mRNA levels are relative to the FW larvae control in August. Analysis was performed using a two-way ANOVA followed by a Student-Newman-Keuls method test; $p < 0.05$. Different lower-case letters denote significant differences between season within developmental stages. Differences between metamorphic stages within same season are represented by an asterisk (*).

4. Discussion

The GH/IGF axis has been thoroughly studied in mammals and

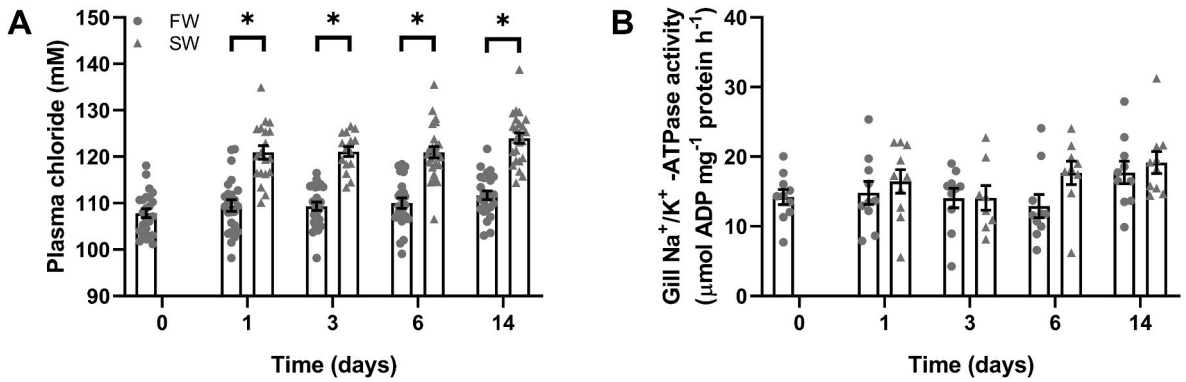

Fig. 3. (**A**) Plasma ion concentration in post metamorphic juvenile sea lampreys acclimated to freshwater (FW) and seawater (SW). Group sizes are FW 0, 1, 3 and 14 days (n = 24), FW 6 days (n = 23), SW 1 day (n = 19) and SW 3 days (n = 15), and (**B**) Na^+/K^+-ATPase activity in μmol ADP mg^{-1} protein h^{-1} in postmetamorphic juvenile sea lampreys acclimated to freshwater (FW) and seawater (SW). Groups and group sizes are: FW 0, 1, 3, 6 and 14 days and SW 1 and 14 days (n = 10), SW 3 and 6 days (n = 8). Significant differences between treatments at same experimental time are represented by an asterisk (*), $p < 0.05$.

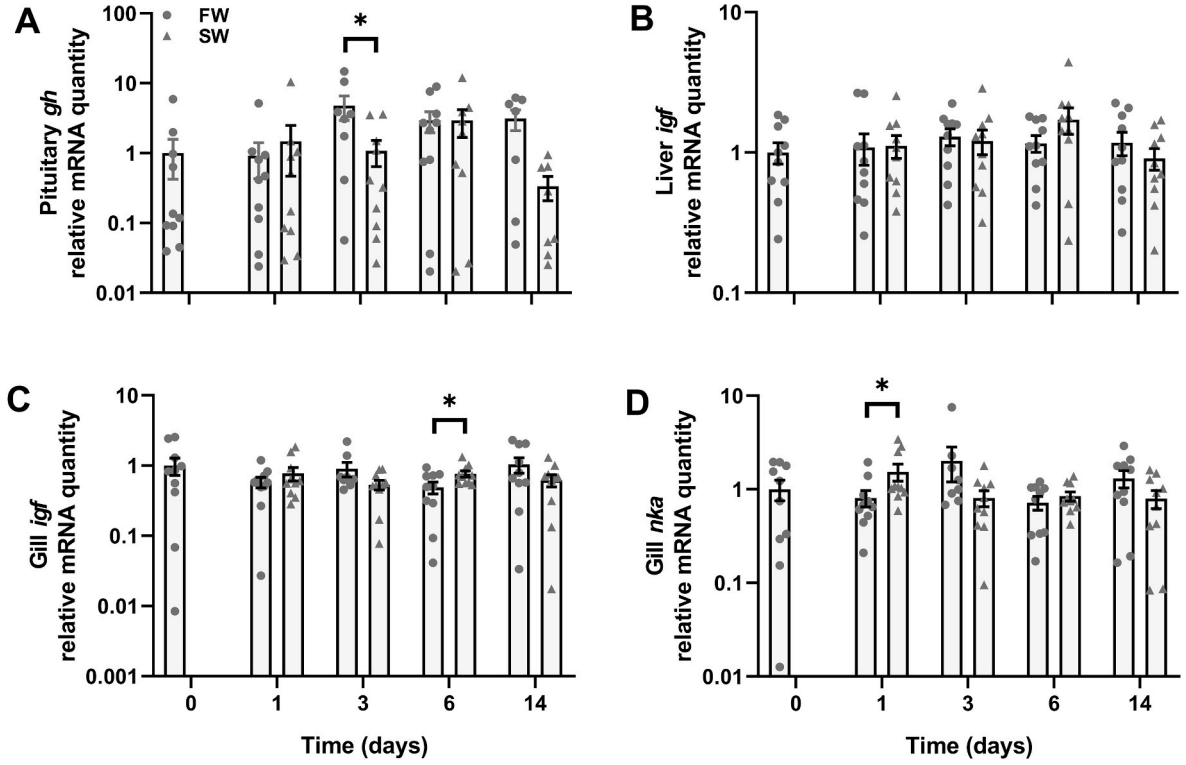

Fig. 4. Relative mRNA quantity of pituitary growth hormone; *gh* (**A**), liver and gill insulin-like growth factor 1; *igf1* (**B** and **C**, respectively), and gill Na^+/K^+-ATPase α subunit; *nka* (**D**) of post metamorphic juvenile sea lampreys acclimated to freshwater (FW) and seawater (SW) for 0, 1, 3, 6 and 14 days (all time points n = 10 except pituitary and gill FW day 3 and were n = 8 and 7, respectively, and pituitary SW day 6 and 14 were n = 9 and 8, respectively). Significant differences between treatments at same experimental time are represented by an asterisk (*), $p < 0.05$.

teleost fishes, but to our knowledge no studies have been conducted to assess the function of the GH/IGF axis in lamprey, a living representative of the base of the vertebrate lineage (Fig. 5B). With this study on anadromous sea lamprey, we have shed light on changes in the GH/IGF axis during critical life history events (Fig. 5A). To determine this, we have focused on two remarkable stages of the sea lamprey's lifecycle, the metamorphosis that occurs in FW in preparation for downstream migration, and the SW entry that marks the start of the marine trophic phase. We found seasonal increases in pituitary GH mRNA expression, remarkable increases in liver and gill IGF1 mRNA expression during metamorphosis that are accompanied by elevated mRNA expression of gill ion transporters and the acquisition of salinity tolerance.

In the present study, there were no differences in pituitary *gh* mRNA levels between larval and metamorphosing juveniles, but there were significant increases in late fall in both groups. Previous work has shown that the number of 'GH-like' cells in the pituitary of sea lamprey increases during metamorphosis (Nozaki et al., 2008). Liver *ghr* mRNA levels were significantly elevated early in metamorphosis, whereas gill *ghr* mRNA levels were elevated late in metamorphosis. These modest changes in *gh* and *ghr* transcription contrast to the large and sustained increases in *igf1* mRNA levels in the liver and gill that occur during metamorphosis. Gill *igf1* transcription increases early and declines late in metamorphosis, whereas liver *igf1* transcription starts later and remains elevated. The liver is assumed to be the major source of circulating IGF1 in fish, so the elevation of liver and gill *igf1* mRNA suggests that both endocrine and paracrine actions of this hormone are important

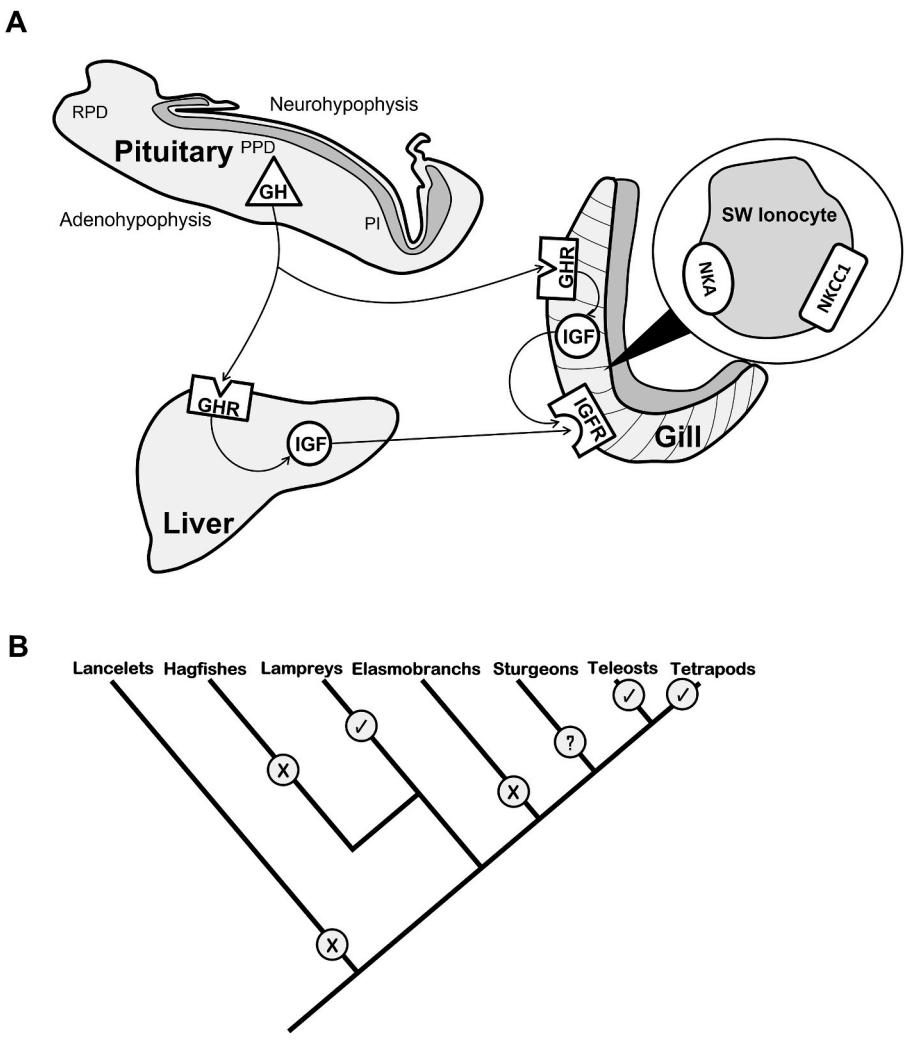

Fig. 5. (**A**) Proposed GH/IGF mechanism in the sea lamprey, *Petromyzon marinus* pituitary, liver, and gill. PPD, proximal pars distalis; RPD rostral pars distalis; PI pars intermedia; GH, growth hormone; GHR, growth hormone receptor; IGF, insulin-like growth factor; IGFR, insulin-like growth factor receptor; NKA, Na^+/K^+-ATPase; NKCC1, $Na^+:K^+:2Cl^-$ cotransporter 1. Arrows represent product in circulation. (**B**) Basic cladogram illustrating the vertebrate lineage and emphasizing major fish taxa. Cross symbolizes taxas in which true osmoregulation in seawater does not occur (e.g. they are isosmotic in ocean and estuarine environments). Tick means osmoregulation occurs and GH/IGF axis has been shown to regulate ion homeostasis. Question mark symbolizes taxa in which osmoregulation occurs but GH/IGF axis has not been shown to play a direct role in ion homeostasis.

in regulating metamorphic changes (Fig. 1).

The observed changes in activation of the GH/IGF axis during sea lamprey metamorphosis are similar to what has been observed in anadromous salmon that undergo similar increases in salinity tolerance prior to downstream migration (Hoar 1976; McCormick et al., 2013). Increased liver and gill *igf1* mRNA levels (Stefansson et al., 2012) and circulating levels of IGF1 (McCormick et al., 2007) have been shown to occur during smolting of Atlantic salmon, coincident with increased transcription and abundance of gill NKA and NKCC and whole organism salinity tolerance. Furthermore, treatment of Atlantic salmon with exogenous GH and IGF1 increase salinity tolerance, NKA and NKCC transcription (Takei and McCormick 2013). Upregulation of NKA and NKCC1 has been associated with the development of SW tolerance in sea lamprey (Reis-Santos et al., 2008; Ferreira-Martins et al., 2016b; Shaughnessy and McCormick 2020). It seems likely that the observed activation of the GH/IGF axis in sea lamprey is causal to the increased transcription of *nka* and *nkcc1* (Fig. 2), though manipulations of GH and IGF1 levels will be necessary to demonstrate this directly. It should also be noted that there are a number of other organ systems that change during metamorphosis of sea lamprey (Youson 1980), and it will be of interest to see if transcription of *igf1* increases in these tissues as well as in the gill.

Thyroid hormones play an important role in metamorphosis of sea lamprey (Youson 1997). Unlike in other vertebrates such as flounder and amphibians in which increases in thyroid hormones stimulate metamorphoses (Gavlik et al., 2002; Denver 2013), it is a decrease in thyroid hormones that appears to be a major triggering event for metamorphosis of sea lamprey (Youson 1997). Our results clearly indicate that the GH/IGF axis is activated during metamorphosis, and it will be important for future studies to establish whether there are interactions between the thyroid and GH/IGF endocrine axes.

It has recently been shown that 11-deoxycortisol is the main corticosteroid in sea lamprey and that it has an important role in stimulation of salt secretory mechanisms (including nka and nkcc1) during metamorphosis (Close et al., 2010; Shaughnessy et al., 2020). In teleosts, the GH/IGF axis and cortisol interact to promote SW tolerance (reviewed by Takei and McCormick 2013). Some of the mechanisms for this interaction have been examined in salmonids that undergo similar preparatory adaptations for seawater entry as occur in sea lamprey. Exogenous GH treatment increases the sensitivity of the interrenal tissue to ACTH, resulting in elevated plasma cortisol for any given level of ACTH (Young 1988). Shrimpton and McCormick (1998) found that GH treatment of salmon increased the abundance of cortisol receptors in the gill, which increases the capacity of cortisol to increase NKA activity in the gill (Shrimpton and McCormick 1999). In a parallel pathway, cortisol upregulates the mRNA expression of the GH and IGF1 receptors in the gill of Atlantic salmon, potentiating the effect of the GH/IGF axis (Tipsmark and Madsen 2009). 11-deoxycortiol and its receptor increase during the later stages of sea lamprey metamorphosis (Shaughnessy et al., 2020), after the activation of the GH/IGF axis that we have seen in the present study. We hypothesize that similar pathways of interaction between GH/IGF1 and cortisol that exist for teleosts may also be present

in sea lamprey, and that the increase in 11-deoxycortisol and its receptor may be causally related to prior activation of the GH/IGF axis. Future studies conducted in sea lamprey using GH, IGF1 and 11-deoxycortisol treatments are needed to assess the potential synergistic role in the upregulation of SW ionoregulatory mechanisms.

Sea lamprey do not feed during or immediately after metamorphosis, thus undergoing an extended period of 'endogenous' starvation that can last several months (Hardisty and Potter 1971). In several teleost species it has been shown that hepatic *ghr* mRNA levels in the liver decrease during fasting (Deng et al., 2004; Fukada et al., 2004; Saera-Vila et al., 2005; Small et al., 2006; Norbeck et al., 2007; Picha et al., 2008; Peterson and Waldbieser 2009) and in the gills (Norbeck et al., 2007). Liver and plasma *igf1* mRNA levels decrease, and as a result of reduced negative feedback, plasma GH decreases (Reinecke 2010b). In our study, *ghr* mRNA levels in the liver did not change and gill *ghr* mRNA levels increased, while liver and gill *igf1* expression increased. None of these responses are typical of starvation in teleosts, and thus we surmise that the observed changes are not a function of endogenous food withdrawal but rather are associated with metamorphosis itself. Since GH has been implicated in regulating appetite in fish (Johnsson and Björnsson 1994), it would be of interest see how the GH/IGF axis is altered during the initiation of parasitic feeding in sea lamprey.

In this study, plasma chloride concentrations increased only slightly one day after exposure to SW, indicating a high level of salinity tolerance and that a new physiological set point had been established after exposure to SW (see reviews by Bartels and Potter 2004; Ferreira-Martins et al., 2021). The mRNA levels of key ion secretory mechanisms (*nka* and *nkcc1*) in the gill remained largely unaltered after SW exposure, especially in comparison to the order of magnitude changes that occur during metamorphosis. Similarly, there were only small increases in pituitary *gh* mRNA levels and gill *igf* mRNA levels at 6 days in response to salinity. This limited salinity response of the GH/IGF axis is similar to that seen in smolting salmonids (Nilsen et al., 2008) and is likely due to the prior development of high salinity tolerance and attendant small changes in ion perturbations after exposure to seawater. In other teleost species that do not have preparatory increases in salinity tolerance, response of the GH/IGF axis to increased salinity is more dramatic, as in some non-salmonid species such as killifish, *F. heteroclitus* (Mancera and McCormick 1998); tilapia *O. mossambicus* (Borski et al., 1994; Yada et al., 1994; Vijayan et al., 1996; Morgan et al., 1997); and striped bass, *M. saxatilis* (Madsen et al., 1996).

Our GH/IGF axis pathway model (Fig. 5A) proposes that GH is produced and released by the pituitary of sea lamprey. Following this, GH acts in two parallel pathways. In one pathway, the GH in circulation binds to the GHR in the liver, where it promotes the production and then release of IGF1 into circulation. The circulatory IGF1 then binds to the IGFR in the gill, promoting the up regulation of ion secretory mechanisms (NKA and NKCC1). In a second pathway, GH in circulation binds to the GHR in the gill of sea lamprey, where it also promotes the up regulation of the ion secretory mechanisms (NKA and NKCC1), either directly or through the production of IGF1.

To date, very little is known about the GH/IGF axis at the base of the vertebrate lineage (Fig. 5B). GH- and IGF1-like peptides have been identified in the basal chordate lancelets (Shu Jin Chan et al., 1990; Fang et al., 2008; Guo et al., 2009; Li et al., 2017; Li 2022). The sea lamprey belongs to the cyclostome superclass (jawless vertebrates) which encompasses the Myxiniformes (hagfishes) and the Petromyzontiformes (lampreys) (Hardisty 1982). At present, molecular studies indicate that hagfishes and lamprey form a monophyletic group (Stock and Whitt 1992; Delarbre et al., 2002). Nevertheless, lamprey physiology and morphology is very different from the hagfishes (Hardisty 1982; Forey and Janvier 1993), which supports the hypothesis that these two cyclostome groups have been separated for a long time (Janvier 1999). This divergence makes lamprey a key group for the understanding of the evolution of vertebrates, especially in regard to osmoregulatory physiology, since hagfish are exclusively marine and do not osmoregulate (Martini 1998). Future studies with GH and IGF1 treatments in hagfish could help elucidate whether some ion regulatory function of the GH/IGF axis emerged before or after the divergence of sea lamprey and hagfish that occurred about 470–390 Mya. While elasmobranchs (sharks, rays, and skates) do not osmoregulate in SW, they do secrete Na^+ and Cl^- through specialized rectal glands, but a role in for the GH/IGF axis in its development or function has not been established. Chondrosteans (sturgeons and paddlefishes) osmoregulate in FW and SW and to our knowledge there is no direct evidence for a role of GH in osmoregulation in this clade. As noted throughout the discussion, the GH/IGF axis has been shown to promote seawater tolerance in a number of teleosts (Takei and McCormick 2013). In tetrapods, the GH/IGF axis has a role in renal development and a role in ion and water balance (Rabkin and Schaefer 2004). Our results with sea lamprey indicate that the role of the GH/IGF axis in osmoregulation may have evolved early in vertebrate evolution.

In this study, we have demonstrated stimulated molecular response of the GH/IGF axis to life history change in sea lamprey, a living representative of the base of the vertebrate lineage. We have focused on two major landmarks in the sea lamprey lifecycle, metamorphosis and ocean entry. Metamorphosis is a period of major morphological and physiological transformation, and here we have shown that pituitary *gh* expression and that *igf* expression in the liver and gill increase in metamorphosing sea lamprey. We have also shown an increase in transcription of *nka* and *nkcc1* in the gill. Increases in these critical ion transporting genes are tightly connected to the development of seawater tolerance, a key adaptive feature for downstream migration and entry into a SW environment. Along with this mRNA evidence, a sharp increase in NKA activity further supports this hypothesis of establishment of hypoosmoregulatory mechanisms in the sea lamprey prior to the beginning of its marine trophic phase. Our results also indicate that a role of the GH/IGF axis in development, metamorphosis and osmoregulation developed in the vertebrate lineage prior to the divergence between the agnathan and gnathostomes lineages.

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CRediT authorship contribution statement

Diogo Ferreira-Martins: Conceived of the project, Conducted live animal experimentation, Performed assays and data analyses, Writing – original draft, Writing – review & editing. **Emily Walton:** Performed assays and data analyses, Writing – original draft. **Rolf O. Karlstrom:** Conceived of the project, Writing – review & editing. **Mark A. Sheridan:** Conceived of the project, Funding acquisition, Writing – review & editing. **Stephen D. McCormick:** Conceived of the project, Funding acquisition, Conducted live animal experimentation, Writing – review & editing, Supervision.

Declaration of competing interest

The author declares there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Data availability

Data will be made available on request.

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References

Barany, A., Shaughnessy, C.A., Fuentes, J., Mancera, J.M., McCormick, S.D., 2020. Osmoregulatory role of the intestine in the sea lamprey (*Petromyzon marinus*). Am. J. Physiol. Regul. Integr. Comp. Physiol. 318, R410–R417. https://doi.org/10.1152/AJPREGU.00033.2019.

Barany, A., Shaughnessy, C.A., Pelis, R.M., Fuentes, J., Mancera, J.M., McCormick, S.D., 2021. Tissue and salinity specific Na+/Cl− cotransporter (NCC) orthologues involved in the adaptive osmoregulation of sea lamprey (*Petromyzon marinus*). Sci. Rep. 11, 1–13. https://doi.org/10.1038/s41598-021-02125-1.

Bartels, H., Potter, I.C., 2004. Cellular composition and ultrastructure of the gill epithelium of larval and adult lampreys: implications for osmoregulation in fresh and seawater. J. Exp. Biol. 207, 3447–3462. https://doi.org/10.1242/jeb.01157.

Boeuf, G., Prunet, P., Le Bail, P.Y., 1990. Un traitement à l'hormone de croissance peut-il stimuler la smoltification du saumon atlantique? Comptes Rendus de l'Academie Des Sciences - Serie III 310, 75–80.

Borski, R.J., Yoshikawa, J.S.M., Madsen, S.S., Nishioka, R.S., Zabetian, C., Bern, H.A., Grau, E.G., 1994. Effects of environmental salinity on pituitary growth hormone content and cell activity in the euryhaline tilapia, *Oreochromis mossambicus*. Gen. Comp. Endocrinol. 95, 483–494. https://doi.org/10.1006/gcen.1994.1148.

Burzawa-Gerard, E., Delevallee-Fortier, B., 1992. Growth hormone implication during experimental induction of vitellogenesis by 17β-estradiol in the female silver eel (*Anguilla anguilla* L.). Comptes Rendus de l'Academie Des Sciences - Serie III 314, 411–416.

Chan, Shu Jin, Cao, Q.P., Steiner, D.F., 1990. Evolution of the insulin superfamily: cloning of a hybrid insulin/insulin-like growth factor cDNA from amphioxus. Proc. Natl. Acad. Sci. U. S. A 87, 9319–9323. https://doi.org/10.1073/pnas.87.23.9319.

Clarke, W.C., Farmer, S.W., Hartwell, K.M., 1977. Effect of teleost pituitary growth hormone on growth of Tilapia mossambica and on growth and seawater adaptation of sockeye salmon (*Oncorhynchus nerka*). Gen. Comp. Endocrinol. 33, 174–178. https://doi.org/10.1016/0016-6480(77)90241-6.

Close, D.A., Yun, S.-S., McCormick, S.D., Wildbill, A.J., Li, W., 2010. 11-Deoxycortisol is a corticosteroid hormone in the lamprey. Proc. Natl. Acad. Sci. USA 107, 13942–13947. https://doi.org/10.1073/pnas.0914026107.

Delarbre, C., Gallut, C., Barriel, V., Janvier, P., Gachelin, G., 2002. Complete mitochondrial DNA of the hagfish, *Eptatretus burgeri*: the comparative analysis of mitochondrial DNA sequences strongly supports the cyclostome monophyly. Mol. Phylogenet. Evol. 22, 184–192. https://doi.org/10.1006/mpev.2001.1045.

Deng, L., Zhang, W.M.M., Lin, H.R.R., Cheng, C.H.K., 2004. Effects of food deprivation on expression of growth hormone receptor and proximate composition in liver of black seabream *Acanthopagrus schlegeli*. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 137, 421–432.

Denver, R.J., 2013. Neuroendocrinology of Amphibian metamorphosis. In: Current Topics in Developmental Biology. Academic Press, pp. 195–227. https://doi.org/10.1016/B978-0-12-385979-2.00007-1.

Van Der Kraak, G., Rosenblum, P.M., Peter, R.E., 1990. Growth homone-dependent potentiation of gonadotropin-stimulated steroid production by ovarian follicles of the goldfish. Gen. Comp. Endocrinol. 79, 233–239. https://doi.org/10.1016/0016-6480(90)90108-X.

Donaldson, E.M., Fagerlund, U.H.M., Higgs, D.A., McBride, J.R., 1979. Hormonal enhancement of growth. In: Hoar, W.S., Randall & JR Brett, D.J. (Eds.), Fish Physiology Vol VIII. Academic Press, New York, pp. 456–597.

Duan, C., 1998. Nutritional and developmental regulation of insulin-like growth factors in fish. J. Nutr. 128, 306S–314S. https://doi.org/10.1093/jn/128.2.306s.

Elliott, W.M., 1989. Development of the Endocrine Pancreas and Related Parts of the Alimentary Canal during Metamorphosis in the Lamprey. *Petromyzon marinus* L. University of Toronto, Toronto, Canada.

Ellis, L.C., 1993. An Ultrastructural Investigation of the Pronephric Kidney of the Sea Lamprey, *Petromyzon marinus* L., throughout the Life Cycle. University of Toronto, Canada.

Ellis, L.C., Youson, J.H., 1990. Pronephric regression during larval life in the sea lamprey. *Petromyzon marinus* L. *Anatomy and Embryology* 182, 41–52. https://doi.org/10.1007/BF00187526.

Fang, Y., Weng, Y., Song, H., 2008. Distribution of α-MSH-like immunoreactive cells in the nervous system, Hatschek's pit and other tissues of amphioxus, *Branchiostoma belcheri*. Prog. Nat. Sci. 18, 463–468. https://doi.org/10.1016/j.pnsc.2007.11.015.

Ferreira-Martins, D., McCormick, S.D., Campos, A., Lopes-Marques, M., Osório, H., Coimbra, J., Castro, L.F.C.C., Wilson, J.M., 2016a. A cytosolic carbonic anhydrase molecular switch occurs in the gills of metamorphic sea lamprey. Sci. Rep. 6, 33954 https://doi.org/10.1038/srep33954.

Ferreira-Martins, D., Coimbra, J., Antunes, C., Wilson, J.M., 2016b. Effects of salinity on upstream-migrating, spawning sea lamprey, *Petromyzon marinus*. Conserv. Physiol. 4, cov064. https://doi.org/10.1093/conphys/cov064.

Ferreira-Martins, D., Wilson, J.M., Kelly, S.P., Kolosov, D., McCormick, S.D., 2021. A review of osmoregulation in lamprey. J. Great Lake. Res. 47, S59–S71. https://doi.org/10.1016/j.jglr.2021.05.003.

Forey, P., Janvier, P., 1993. Agnathans and the origin of jawed vertebrates. Nature 361, 129–134. https://doi.org/10.1038/361129a0.

Franz, A.C., Faass, O., Kollner, B., Shved, N., Link, K., Casanova, A., Wenger, M., D'cotta, H., Baroiller, J.F., Ullrich, O., et al., 2016. Endocrine and local IGF-I in the bony fish immune system. Biology 5, 9. https://doi.org/10.3390/biology5010009.

Fukada, H., Ozaki, Y., Pierce, A.L., Adachi, S., Yamauchi, K., Hara, A., Swanson, P., Dickhoff, W.W., 2004. Salmon growth hormone receptor: molecular cloning, ligand specificity, and response to fasting. Gen. Comp. Endocrinol. 139, 61–71. https://doi.org/10.1016/j.ygcen.2004.07.001.

Le Gac, F., Ollitrault, M., Loir, M., Le Bail, P.Y., 1992. Evidence for binding and action of growth hormone in trout testis. Biol. Reprod. 46, 949–957. https://doi.org/10.1095/biolreprod46.5.949.

Gavlik, S., Albino, M., Specker, J.L., 2002. Metamorphosis in summer flounder: manipulation of thyroid status to synchronize settling behavior, growth, and development. Aquaculture 203, 359–373. https://doi.org/10.1016/S0044-8486(01)00624-X.

Gong, N., Ferreira-Martins, D., McCormick, S.D., Sheridan, M.A., 2020. Divergent genes encoding the putative receptors for growth hormone and prolactin in sea lamprey display distinct patterns of expression. Sci. Rep. 10, 1–11. https://doi.org/10.1038/s41598-020-58344-5.

Gong, N., Ferreira-Martins, D., Norstog, J.L., McCormick, S.D., Sheridan, M.A., 2022. Discovery of prolactin-like in lamprey: Role in osmoregulation and new insight into the evolution of the growth hormone/prolactin family. Proc. Natl. Acad. Sci. U.S.A. 119, e2212196119 https://doi.org/10.1073/pnas.2212196119.

Guo, B., Zhang, S., Wang, S., Liang, Y., 2009. Expression, mitogenic activity and regulation by growth hormone of growth hormone/insulin-like growth factor in *Branchiostoma belcheri*. Cell Tissue Res. 338, 67–77. https://doi.org/10.1007/s00441-009-0824-8.

Hardisty, M.W., 1982. Lampreys and hagfishes: analysis of cyclostome relationships. In: H, M.W., Potter, I.C. (Eds.), The Biology of Lampreys. Academic Press, London, pp. 165–259.

Hardisty, M.W., Potter, I.C., 1971. The Biology of Lampreys, vol. 1. Academic Press.

Higgs, D.A., Donaldson, E.M., Dye, H.M., McBride, J.R., 1976. Influence of bovine growth hormone and L-thyroxine on growth, muscle composition, and histological structure of the gonads, thyroid, pancreas, and pituitary of coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Board Can. 33, 1585–1603. https://doi.org/10.1139/f76-199.

Hoar, W.S., 1976. Smolt transformation: evolution, behavior, and physiology. J. Fish. Res. Board Can. 33, 1233–1252. https://doi.org/10.1139/f76-158.

Janvier, P., 1999. Catching the first fish. Nature 402, 21–22. https://doi.org/10.1038/46909.

Johnsson, J.I., Björnsson, B.T., 1994. Growth hormone increases growth rate, appetite and dominance in juvenile rainbow trout, *Oncorhynchus mykiss*. Anim. Behav. 48, 177–186. https://doi.org/10.1006/anbe.1994.1224.

Kawauchi, H., Suzuki, K., Yamazaki, T., Moriyama, S., Nozaki, M., Yamaguchi, K., Takahashi, A., Youson, J., Sower, S.A., 2002. Identification of growth hormone in the sea lamprey, an extant representative of a group of the most ancient vertebrates. Endocrinology 143, 4916–4921. https://doi.org/10.1210/en.2002-220810.

Laviola, L., Natalicchio, A., Giorgino, F., 2007. The IGF-I signaling pathway. Curr. Pharmaceut. Des. 13, 663–669. https://doi.org/10.2174/138161207780249146.

Li, M., 2022. The origination of growth hormone/insulin-like growth factor system: a story from ancient basal chordate amphioxus. Front. Endocrinol. 13, 1–8. https://doi.org/10.3389/fendo.2022.825722.

Li, M., Jiang, C., Zhang, Y., Zhang, S., 2017. Activities of amphioxus GH-like protein in osmoregulation: insight into origin of vertebrate GH family. Int. J. Endocrinol. https://doi.org/10.1155/2017/9538685.

Madsen, S.S., RS, N., Bern, H., 1996. Seawater acclimation in the anadromous striped bass, *Morone saxatilis*: strategy and hormonal regulation. In: McCormick, S., Sheridan, M., Patino, R., MacKinlay, D. (Eds.), The Physiology of Migratory Fish: Symposium Proceeding International Congress on the Biology of Fishes. American Fisheries Society, San Francisco, CA: Bethesda, MD, pp. 167–174.

Mancera, J.M., McCormick, S.D., 1998. Evidence for growth hormone/insulin-like growth factor I axis regulation of seawater acclimation in the euryhaline teleost *Fundulus heteroclitus*. Gen. Comp. Endocrinol. 111, 103–112. https://doi.org/10.1006/gcen.1998.7086.

Marchelidon, J., Schmitz, M., Houdebine, L.M., Vidal, B., Belle, N Le, Dufour, S., Le Belle, N., Dufour, S., 1996. Development of a radioimmunoassay for European eel growth hormone and application to the study of silvering and experimental fasting. Gen. Comp. Endocrinol. 102, 360–369. https://doi.org/10.1006/gcen.1996.0079.

Mark Elliott, W., Youson, J.H., 1994. Alterations to the subepithelial layers of the larval alimentary tract during metamorphosis in the sea lamprey *Petromyzon marinus*, with emphasis on tissue interactions. Cell Tissue Res. 276, 503–513. https://doi.org/10.1007/BF00343947.

Martini, F.H., 1998. The ecology of hagfishes. In: The Biology of Hagfishes. Springer Netherlands, Dordrecht, pp. 57–77. https://doi.org/10.1007/978-94-011-5834-3_5.

McCormick, S.D., 1993. Methods for nonlethal gill biopsy and measurement of Na + , K + -ATPase activity. Can. J. Fish. Aquat. Sci. 50, 656–658. https://doi.org/10.1139/f93-075.

McCormick, S.D., 2001. Endocrine control of osmoregulation in teleost fish. Am. Zool. 41, 781–794. https://doi.org/10.1093/icb/41.4.781.

McCormick, S.D., Shrimpton, J.M., Moriyama, S., Björnsson, B.T., 2007. Differential hormonal responses of Atlantic salmon parr and smolt to increased daylength: a possible developmental basis for smolting. Aquaculture 273, 337–344. https://doi.org/10.1016/j.aquaculture.2007.10.015.

McCormick, S.D., Regish, A.M., Christensen, A.K., Björnsson, B.T., 2013. Differential regulation of sodium–potassium pump isoforms during smolt development and seawater exposure of Atlantic salmon. J. Exp. Biol. 216, 1142–1151. https://doi.org/10.1242/jeb.080440.

McLean, E., Donaldson, E.M., Dye, H.M., Souza, L.M., 1990. Growth acceleration of coho salmon (*Oncorhynchus kisutch*) following oral administration of recombinant bovine somatotropin. Aquaculture 91, 197–203. https://doi.org/10.1016/0044-8486(90)90188-S.

Miwa, S., Inui, Y., 1985. Effects of l-thyroxine and ovine growth hormone on smoltification of amago salmon (*Oncorhynchus rhodurus*). Gen. Comp. Endocrinol. 58, 436–442. https://doi.org/10.1016/0016-6480(85)90116-9.

Morgan, J.D., Sakamoto, T., Grau, E.G., Iwama, G.K., 1997. Physiological and respiratory responses of the Mozambique tilapia (*Oreochromis mossambicus*) to salinity acclimation. Comp. Biochem. Physiol. A Physiol. 117, 391–398. https://doi.org/10.1016/S0300-9629(96)00261-7.

Morris, R., 1980. Blood composition and osmoregulation in ammocoete larvae. Can. J. Fish. Aquat. Sci. 37, 1665–1679. https://doi.org/10.1139/f80-214.

Nilsen, T.O., Ebbesson, L.O., Kiilerich, P., Björnsson, B.T., Madsen, S.S., McCormick, S.D., Stefansson, S.O., 2008. Endocrine systems in juvenile anadromous and landlocked Atlantic salmon (*Salmo salar*): seasonal development and seawater acclimation. Gen. Comp. Endocrinol. 155, 762–772. https://doi.org/10.1016/j.ygcen.2007.08.006.

Norbeck, L.A., Kittilson, J.D., Sheridan, M.A., 2007. Resolving the growth-promoting and metabolic effects of growth hormone: differential regulation of GH–IGF-I system components. Gen. Comp. Endocrinol. 151, 332–341. https://doi.org/10.1016/j.ygcen.2007.01.039.

Nozaki, M., Ominato, K., Shimotani, T., Kawauchi, H., Youson, J.H., Sower, S.A., 2008. Identity and distribution of immunoreactive adenohypophysial cells in the pituitary during the life cycle of sea lampreys, *Petromyzon marinus*. Gen. Comp. Endocrinol. 155, 403–412. https://doi.org/10.1016/j.ygcen.2007.07.012.

Ooi, E.C., Youson, J.H., 1977. Morphogenesis and growth of the definitive opisthonephros during metamorphosis of anadromous sea lamprey. *Petromyzon marinus* L. *Development* 42, 219–235. https://doi.org/10.1242/dev.42.1.219.

Ooi, E.C., Youson, J.H., 1979. Regression of the larval opisthonephros during metamorphosis of the sea lamprey, *Petromyzon marinus* L. Am. J. Anat. 154, 57–79. https://doi.org/10.1002/aja.1001540105.

Peek, W.D., Youson, J.H., 1979. Transformation of the interlamellar epithelium of the gills of the anadromous sea lamprey, *Petromyzon marinus* L., during metamorphosis. Can. J. Zool. 57, 1318–1332. https://doi.org/10.1139/z79-168.

Peterson, B.C., Waldbieser, G.C., 2009. Effects of fasting on IGF-I, IGF-II, and IGF-binding protein mRNA concentrations in channel catfish (*Ictalurus punctatus*). Domest. Anim. Endocrinol. 37, 74–83. https://doi.org/10.1016/j.domaniend.2009.03.004.

Picha, M.E., Turano, M.J., Tipsmark, C.K., Borski, R.J., 2008. Regulation of endocrine and paracrine sources of Igfs and Gh receptor during compensatory growth in hybrid striped bass (*Morone chrysops* X *Morone saxatilis*). J. Endocrinol. 199, 81–94. https://doi.org/10.1677/JOE-07-0649.

Rabkin, R., Schaefer, F., 2004. New concepts: growth hormone, insulin-like growth factor-I and the kidney. Growth Hormone IGF Res. 14, 270–276. https://doi.org/10.1016/j.ghir.2004.02.001.

Reindl, K.M., Sheridan, M.A., 2012. Peripheral regulation of the growth hormone-insulin-like growth factor system in fish and other vertebrates. Comp. Biochem. Physiol. Mol. Integr. Physiol. 163, 231–245. https://doi.org/10.1016/j.cbpa.2012.08.003.

Reinecke, M., 2010a. Insulin-like growth factors and fish reproduction. Biol. Reprod. 82, 656–661. https://doi.org/10.1095/biolreprod.109.080093.

Reinecke, M., 2010b. Influences of the environment on the endocrine and paracrine fish growth hormone-insulin-like growth factor-I system. J. Fish. Biol. 76, 1233–1254. https://doi.org/10.1111/j.1095-8649.2010.02605.x.

Reinecke, M., Björnsson, B.T., Dickhoff, W.W., McCormick, S.D., Navarro, I., Power, D. M., Gutiérrez, J., 2005. Growth hormone and insulin-like growth factors in fish: where we are and where to go. Gen. Comp. Endocrinol. 142, 20–24. https://doi.org/10.1016/j.ygcen.2005.01.016.

Reinecke, M., Zaccone, G., Kapoor, B.G., 2006. Insulin-like growth factor I and II in fish. Fish Endocrinol. 1, 87–130.

Reis-Santos, P., McCormick, S.D., Wilson, J.M., 2008. Ionoregulatory changes during metamorphosis and salinity exposure of juvenile sea lamprey (*Petromyzon marinus* L.). J. Exp. Biol. 211, 978–988. https://doi.org/10.1242/jeb.014423.

Le Roith, D., Bondy, C., Yakar, S., Liu, J.-L.L., Butler, A., 2001. The somatomedin hypothesis: 2001. Endocr. Rev. 22, 53–74. https://doi.org/10.1210/edrv.22.1.0419.

Saera-Vila, A., Calduch-Giner, J.-A., Pérez-Sánchez, J., 2005. Duplication of growth hormone receptor (GHR) in fish genome: gene organization and transcriptional regulation of GHR type I and II in gilthead sea bream (*Sparus aurata*). Gen. Comp. Endocrinol. 142, 193–203. https://doi.org/10.1016/j.ygcen.2004.11.005.

Shaughnessy, C.A., McCormick, S.D., 2020. Functional characterization and osmoregulatory role of the Na+-K+-2Cl- cotransporter in the gill of sea lamprey (*Petromyzon marinus*), a basal vertebrate. Am. J. Physiol. Regul. Integr. Comp. Physiol. 318, R17–R29. https://doi.org/10.1152/AJPREGU.00125.2019.

Shaughnessy, C.A., Barany, A., McCormick, S.D., 2020. 11-Deoxycortisol controls hydromineral balance in the most basal osmoregulating vertebrate, sea lamprey (*Petromyzon marinus*). Sci. Rep. 10, 1–13. https://doi.org/10.1038/s41598-020-69061-4.

Shifman, M.I., Yumul, R.E., Laramore, C., Selzer, M.E., 2009. Expression of the repulsive guidance molecule RGM and its receptor neogenin after spinal cord injury in sea lamprey. Exp. Neurol. 217, 242–251. https://doi.org/10.1016/j.expneurol.2009.02.011.

Shrimpton, J.M., McCormick, S.D., 1998. Regulation of gill cytosolic corticosteroid receptors in juvenile Atlantic salmon: interaction effects of growth hormone with prolactin and triiodothyronine. Gen. Comp. Endocrinol. 112, 262–274. https://doi.org/10.1006/gcen.1998.7172.

Shrimpton, J.M., McCormick, S.D., 1999. Responsiveness of gill Na+/K+-ATPase to cortisol is related to gill corticosteroid receptor concentration in juvenile rainbow trout. J. Exp. Biol. 202, 987–995. https://doi.org/10.1242/JEB.202.8.987.

Singh, H., Thomas, P., 1993. Mechanism of stimulatory action of growth hormone on ovarian steroidogenesis in spotted seatrout, *Cynoscion nebulosus*. Gen. Comp. Endocrinol. 89, 341–353. https://doi.org/10.1006/gcen.1993.1042.

Singh, H., Griffith, R.W., Takahashi, A., Kawauchi, H., Thomas, P., Stegeman, J.J., 1988. Regulation of gonadal steroidogenesis in *Fundulus heteroclitus* by recombinant salmon growth hormone and purified salmon prolactin. Gen. Comp. Endocrinol. 72, 144–153. https://doi.org/10.1016/0016-6480(88)90190-6.

Small, B.C., Murdock, C.A., Waldbieser, G.C., Peterson, B.C., 2006. Reduction in channel catfish hepatic growth hormone receptor expression in response to food deprivation and exogenous cortisol. Domest. Anim. Endocrinol. 31, 340–356.

Stefansson, S.O., Haugland, M., Björnsson, B.T., McCormick, S.D., Holm, M., Ebbesson, L. O.E., Holst, J.C., Nilsen, T.O., 2012. Growth, osmoregulation and endocrine changes in wild Atlantic salmon smolts and post-smolts during marine migration. Aquaculture 362–363, 127–136. https://doi.org/10.1016/j.aquaculture.2011.07.002.

Stock, D., Whitt, G., 1992. Evidence from 18S ribosomal RNA sequences that lampreys and hagfishes form a natural group. Science 257, 787–789. https://doi.org/10.1126/science.1496398.

Sunga, J., Wilson, J.M., Wilkie, M.P., 2020. Functional re-organization of the gills of metamorphosing sea lamprey (*Petromyzon marinus*): preparation for a blood diet and the freshwater to seawater transition. J. Comp. Physiol. B 190, 701–715. https://doi.org/10.1007/s00360-020-01305-1.

Takei, Y., McCormick, S.D., 2013. Hormonal control of fish euryhalinity. In: McCormick, S.D. (Ed.), *Fish Physiology*, Pp 69–123, AP Farrell & CJ Brauner. Academic Press. https://doi.org/10.1016/B978-0-12-396951-4.00003-7.

Tipsmark, C.K., Madsen, S.S., 2009. Distinct hormonal regulation of Na+,K+-atpase genes in the gill of Atlantic salmon (*Salmo salar* L.). J. Endocrinol. 203, 301–310. https://doi.org/10.1677/JOE-09-0281.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B.C., Remm, M., Rozen, S. G., 2012. Primer3-new capabilities and interfaces. Nucleic Acids Res. 40 https://doi.org/10.1093/nar/gks596 e115–e115.

Vijayan, M.M., Morgan, J.D., Sakamoto, T., Grau, E.G., Iwama, G.K., 1996. Food-deprivation affects seawater acclimation in tilapia: hormonal and metabolic changes. J. Exp. Biol. 199, 2467–2475. https://doi.org/10.1242/JEB.199.11.2467.

Wood, A.W., Duan, C., Bern, H.A., 2005. Insulin-like growth factor signaling in fish. Int. Rev. Cytol. 243, 215–285. https://doi.org/10.1016/S0074-7696(05)43004-1.

Yada, T., Hirano, T., Grau, E.G., 1994. Changes in plasma levels of the two prolactins and growth hormone during adaptation to different salinities in the euryhaline tilapia, *Oreochromis mossambicus*. Gen. Comp. Endocrinol. 93, 214–223. https://doi.org/10.1006/gcen.1994.1025.

Young, G., 1988. Enhanced response of the interrenal of coho salmon (*Oncorhynchus kisutch*) to ACTH after growth hormone treatment in vivo and in vitro. Gen. Comp. Endocrinol. 71, 85–92. https://doi.org/10.1016/0016-6480(88)90298-5.

Youson, J.H., 1970. Observations on the opisthonephric kidney of the sea lamprey of the Great Lakes, *Petromyzon marinus* L., at various stages during the life cycle. Can. J. Zool. 48, 1313–1316. https://doi.org/10.1139/z70-223.

Youson, J.H., 1980. Morphology and physiology of lamprey metamorphosis. Can. J. Fish. Aquat. Sci. 37, 1687–1710. https://doi.org/10.1139/f80-216.

Youson, J.H., 1981. The alimentary canal. In: Hardisty, M.W., Potter, I.C. (Eds.), The Biology of Lampreys. Academic Press, London, pp. 95–189.

Youson, J.H., 1984. Differentiation of the segmented tubular nephron and excretory duct during lamprey metamorphosis. Anat. Embryol. 169, 275–292. https://doi.org/10.1007/BF00315633.

Youson, J.H., 1985. Organ development and specialization in lamprey species. In: Evolutionary Biology of Primitive Fishes. Springer US, Boston, MA, pp. 141–164. https://doi.org/10.1007/978-1-4615-9453-6_9.

Youson, J.H., 1997. Is lamprey metamorphosis regulated by thyroid hormones? Am. Zool. 37, 441–460. https://doi.org/10.1093/icb/37.6.441.

Youson, J.H., Horbert, W.R., 1982. Transformation of the intestinal epithelium of the larval anadromous sea lamprey *Petromyzon marinus* L. during metamorphosis. J. Morphol. 171, 89–117. https://doi.org/10.1002/jmor.1051710107.

Youson, J.H., Potter, I.C., 1979. A description of the stages in the metamorphosis of the anadromous se lamprey, *Petromyzon marinus* L. Can. J. Zool. 57, 1808–1817. https://doi.org/10.1139/z79-235.