Seawater acclimation affects cardiac output and adrenergic control of blood pressure in rainbow trout (Oncorhynchus mykiss)—implications for salinity variations now and in the future

Erika Sundell1,*, Daniel Morgenroth1, Jeroen Brijs2, Andreas Ekström1, Albin Gräns2 and Erik Sandblom1

1Department of Biological and Environmental Sciences, University of Gothenburg, PO: Box 115, 405 30, Gothenburg, Sweden
2Department of Animal Environment and Health, Swedish University of Agricultural Sciences, PO: Box 115, 405 30, Uppsala, Sweden

*Corresponding author: Erika Sundell, Department of Biological and Environmental Sciences. Tel: +46 738 27 45 61.
Email: erika.sundell@hotmail.com

Greater salinity variations resulting from ongoing climate change requires consideration in conservation management as this may impact on the performance of aquatic organisms. Euryhaline fish exhibit osmoregulatory flexibility and can exploit a wide range of salinities. In seawater (SW), they drink and absorb water in the intestine, which is associated with increased gastrointestinal blood flow. Yet, detailed information on other cardiovascular changes and their control across salinities is scant. Such knowledge is fundamental to understand how fish are affected during migrations between environments with different salinities, as well as by increased future salinity variability. We used rainbow trout (Oncorhynchus mykiss) as a euryhaline model species and determined dorsal aortic blood pressure, cardiac output and systemic vascular resistance in vivo after chronic freshwater—or SW-acclimation. We also assessed α-adrenergic control of blood pressure using pharmacological tools. Dorsal aortic blood pressure and systemic vascular resistance were reduced, whereas cardiac output increased in SW. α-Adrenergic stimulation with phenylephrine caused similar dose-dependent increases in resistance and pressure across salinities, indicating unaltered α-adrenoceptor sensitivity. α-Adrenergic blockade with prazosin decreased resistance and pressure across salinities, but the absolute reduction in resistance was smaller in SW. Yet, both pressure and resistance after prazosin remained consistently lower in SW. This shows that SW-acclimation lowers systemic resistance through reduced vascular α-adrenergic tone, along with other unknown vasodilating factors. The marked changes in adrenergic regulation of the vasculature across salinities discovered here may have implications for cardiovascular and aerobic performance of fishes, with possible impacts on fitness-related traits like digestion and exercise capacity. Moreover, the evolution of more complex circulatory control systems in teleost fishes compared with elasmobranchs and cyclostomes may have been an important factor in the evolution of euryhalinity, and may provide euryhaline teleosts with competitive advantages in more variable salinity environments of the future.

Key words: α-Adrenergic, arterial blood pressure, vascular resistance, water salinity, euryhalinity, climate change

Editor: Steven Cooke
Received 7 May 2018; Revised 9 October 2018; Editorial Decision 28 October 2018; accepted 29 October 2018

Cite as: Sundell E, Morgenroth D, Brijs J, Ekström A, Gräns A, Sandblom E (2018) Seawater acclimation affects cardiac output and adrenergic control of blood pressure in rainbow trout (Oncorhynchus mykiss)—implications for salinity variations now and in the future. Conserv Physiol 6 (1): coy061; doi:10.1093/conphys/coy061.
Introduction

Global climate change is predicted to affect the salinity and its variability in aquatic environments (Kultz, 2015; Seebacher and Franklin, 2012). For example, exacerbated transient reductions in salinity may be predicted for shallow coastal areas due to increases in precipitation and freshwater (FW) run off (Meier et al., 2012), whereas increases in salinity has been postulated for semi-arid regions due to lower precipitation and increased evaporation (Jeppesen et al., 2015). Such changes in salinity can be challenging and constrain the performance of fish. Euryhaline fishes possess physiological traits that allow them to inhabit a wide range of salinities. Osmoregulatory capacity may, therefore, be of added importance for the ability of fish to cope with future environmental changes (Kultz, 2015), and understanding of the physiological constraints and mechanisms underlying euryhalinity is important to predict future resilience of fish populations and inform management efforts (Cooke et al., 2013; Kultz, 2015; Seebacher and Franklin, 2012).

FW generally has a volume loading effect on fish (i.e. gain of water and loss of ions), whereas seawater (SW) has a volume depleting effect (i.e. loss of water and gain of ions; Olson, 1992; Smith, 1932). These passive effects of water salinity are counteracted by a range of active physiological and behavioural modifications (Marshall and Grosell, 2006). In FW, fish take up ions from the surrounding water via specialized cells in the gills and excrete dilute urine (Evans et al., 2005; Perry et al., 2003; Wood and Bucking, 2010). In contrast, in SW, they actively drink and create an inward directed flow of water through solute-linked water absorption mechanisms in the intestine (Bath and Eddy, 1979; Sundell and Sundh, 2012). Excess ions are then actively excreted across the gills and kidneys (Evans et al., 2005; Grosell et al., 2010), and water is conserved by maintaining low urine volumes (Linhart et al., 1999; Smith, 1930). Collectively, these changes allow euryhaline teleosts to maintain osmotic homeostasis with a constant plasma osmolality of ~300 mOsm across environmental salinities (Evans, 2008; McCormick and Saunders, 1987; McCormick et al., 1998).

Various cardiovascular adjustments are also important for euryhaline fishes when responding and acclimating to water salinity (Brijs et al., 2015, 2016, 2017). Gastrointestinal blood flow increases at least 2-fold in chronically SW-acclimated rainbow trout due to a combination of increased cardiac output (mediated by increased stroke volume) and an increased proportion of blood flow directed to the gastrointestinal tract (Brijs et al., 2016). This elevation in gastrointestinal blood flow is believed to be essential for the convection of absorbed ions and water, as well as for supplying oxygen and nutrients to metabolically active gastrointestinal tissues (Brijs et al., 2015, 2016). In theory, the elevated gastrointestinal blood flow of trout in SW could be caused either by an elevated arterial blood pressure and/or a reduced gastrointestinal vascular resistance (Olson, 2011). However, the dorsal aortic blood pressure ($P_{DA}$) decreased by 11–21% in FW-acclimated trout acutely exposed to SW for 24 hours (Maxime et al., 1991), or short-term acclimated to SW for 2 weeks (Olson and Hoagland, 2008). Nonetheless, knowledge gaps remain as no study has determined the effects of salinity on systemic vascular resistance ($R_{SYS}$, the sum of the gastrointestinal and the somatic vascular resistances), and it is largely unknown how the hemodynamic status of fish is affected after more chronic SW exposure. Moreover, the only previous study that recorded blood pressure responses to short-term acclimation to SW used rainbow trout with surgically opened pericardia (Olson and Hoagland, 2008), which is known to negatively affect cardiac performance and blood pressure dynamics (Farrell et al., 1988; Sandblom et al., 2006).

Baseline systemic vascular resistance in teleost fishes is to a great extent determined by the $\alpha$-adrenergic tone on the resistance vasculature, which is primarily mediated by adrenergic neuronal activity (Sandblom and Axelsson, 2011; Sandblom and Gräns, 2017; Smith, 1978; Smith et al., 1983). Thus, it could be hypothesized that the elevated gastrointestinal blood flow in SW-acclimated trout is mediated by a reduced $\alpha$-adrenergic vasomotor tone on the gastrointestinal resistance vasculature. Indeed, the gastrointestinal vasculature is under $\alpha$-adrenergic control because gastrointestinal blood flow is markedly reduced following injection of $\alpha$-adrenergic agonists (Axelsson and Fritsche, 1991; Axelsson et al., 1989, 2000; Sandblom et al., 2012; Seth, 2010), and changes in gastrointestinal blood flow and gastrointestinal vascular resistance with feeding, exercise and hypoxia are at least partially due to changes in $\alpha$-adrenergic vasomotor tone (Axelsson and Fritsche, 1991; Seth and Axelsson, 2010; Seth et al., 2008). Interestingly, hypertensive trout fed a high salt diet had a decreased dorsal aortic $\alpha$-adrenoceptor mRNA expression along with a blunted $P_{DA}$ response to exogenous catecholamines (Chen et al., 2007). While this shows that vascular $\alpha$-adrenoceptor density and vascular adrenergic sensitivity can be dynamically regulated in trout, it is unknown how the adrenergic control of blood pressure is affected by acclimation to different water salinities. Thus, there is a need for simultaneous measurements of arterial pressure and flow to resolve how $R_{SYS}$ and $P_{DA}$ changes with salinity in euryhaline fishes. This information is of importance to understand how cardiovascular and aerobic performance traits of fishes are affected by transient and chronic salinity changes, which may have implications for dispersal and fitness of estuarine and migratory fish species now and in a future with more pronounced salinity variations.
along with $P_{DA}$ to calculate $R_{SYS}$ in vitro in chronically FW- and SW-acclimated rainbow trout; with the hypothesis that SW-acclimated trout would exhibit reduced $R_{SYS}$ and $P_{DA}$. Further, we examined whether differences in $R_{SYS}$ and $P_{DA}$ across salinities could be explained by altered resistance vessel sensitivity to $\alpha$-adrenergic stimulation or through changes in intrinsic $\alpha$-adrenergic tone by using specific $\alpha$-adrenergic pharmacological tools.

**Methods**

**Experimental animals**

Rainbow trout (O. mykiss) were obtained from a local hatchery (Vänneåns fiskodling, Sweden; see Table 1 for mass and length) and kept in a 1000-l tank with aerated recirculating FW (salinity 0–1 ppt) at 10.5 ± 1.0°C for at least 2 weeks. A subset of 30 fish was subsequently randomly assigned for transfer to another identical 1000-l tank with recirculating aerated SW (salinity 30–33 ppt) at the same temperature (10.5 ± 1.0°C). The fish were then acclimated to their respective salinity treatment for a minimum of 6 weeks prior to experimentation. During the holding and acclimation periods, they were fed three times per week with dry commercial trout pellets (9 mm Protec Trout pellets, Skretting, Stavanger, Norway), but fasted for 1 week prior to experimentation. During the holding and acclimation periods, they were fed three times per week with dry commercial trout pellets (9 mm Protec Trout pellets, Skretting, Stavanger, Norway), but fasted for 1 week prior to experimentation. Animal handling and surgical procedures were performed in accordance with ethical permit #165-2015, approved by the ethical committee in Gothenburg.

**Surgery and instrumentation**

Individual rainbow trout were anesthetized in FW containing Tricaine methanesulphonate (MS-222, 150 mg l⁻¹) buffered with NaHCO₃ (300 mg l⁻¹). Length and weight were determined before placing the fish dorsally on water-soaked foam on a surgical table. The gills of the fish were continuously irrigated with recirculating aerated FW (i.e. for both FW- and SW-acclimated fish) at 10°C containing MS-222 (75 mg l⁻¹) buffered with NaHCO₃ (150 mg l⁻¹) throughout the surgery. FW was used as anaesthetic solvent for both acclimation groups since earlier attempts of using SW as solvent for the SW-acclimated group resulted in impaired post-surgical recovery, possibly due to impaired drinking during the anesthetized state. The dorsal aorta was cannulated with a custom-made PE-50 catheter using a steel wire guide (Sandblom and Axelsson, 2006; Smith and Bell, 1964). The cannula was inserted dorsally at a ~45° angle, between the second and third pair of gill arches inside the mouth cavity (Axelsson and Fritsche, 1994). The catheter was filled with heparinized (100 IU ml⁻¹) 0.9% saline and exteriorized through the snout and locked in place by a bubble on the catheter (Sovio et al., 1975). The fish was then placed on its side and the operculum and the gill arches were lifted to expose the opercular cavity (Sandblom and Axelsson, 2006). The ventral aorta was gently dissected free without damaging nearby nerves and vessels. A Transonic 2.5PSL flow probe (factory calibrated to 10°C; Transonic systems, Inc., Ithaca, NY, USA) was placed around the aorta with the help of a silk suture (size 4–0). Finally, the probe lead and the catheter were attached to the skin with several silk sutures. After surgery, the fish were immediately placed in the experimental setup, which consisted of individual opaque holding tubes with a volume of ~3 l, floating in a 120-l tank receiving a continuous supply of aerated FW or SW (11 ± 1°C) depending on the acclimation salinity. All fish were allowed a recovery time of at least 40 h before experiments were initiated.

**Experimental protocol**

Baseline recordings of cardiac output, heart rate and $P_{DA}$ were first performed for a minimum of 2 h at the start of each experiment. When stable baseline conditions had been confirmed, four dosages of the $\alpha$-adrenergic agonist phenylephrine (10, 30, 60 and 100 μg kg⁻¹) and saline (0.9%) as a control, were injected into the dorsal aortic catheter, followed by 0.3–0.4 ml saline (0.9%) to clear the catheter dead space. The administration of phenylephrine dosages was randomized, and an additional 0.3–0.4 ml saline (0.9%) was injected when the peak $P_{DA}$ response had leveled off to ensure that all traces from the previous injection was cleared from the catheter. Last, one dosage of the $\alpha$-adrenergic antagonist prazosin (1 mg kg⁻¹) was injected in the same way to obtain a complete $\alpha$-adrenergic blockade. All injections were administered in volumes of 1 ml kg⁻¹ body mass ($M_b$). While the administration order of phenylephrine and saline injections was randomized for each fish, prazosin was always administered last. Before a new injection was administered, care was taken to allow all cardiovascular variables to return to stable baseline levels. The time for this varied among individuals and injections but was typically never longer than one hour. After the experiments, the fish were killed with a sharp blow to the head.

**Data acquisition and analysis**

The dorsal aortic catheter was connected to a pressure transducer (pib Mediinteknik, Kirchseen, Germany) that was calibrated against a static water column with the water level in the experimental tank serving as the zero reference. The signal from the pressure transducer was amplified using a 4ChAmp pre-amplifier (Somedic, Hörby, Sweden). The Transonic flow probe was connected to a Transonic flow meter (Transonic systems, Inc., Ithaca, NY, USA). The signals

| Table 1: Morphological characteristics of freshwater- and seawater-acclimated rainbow trout (Oncorhynchus mykiss) |
|--------------------------------------------------|------------------|------------------|
| **Body mass (g)** | **Freshwater** | **Seawater** |
| 331.4 ± 14.0 | 318.9 ± 14.9 |
| **Fork length (cm)** | 31.3 ± 0.4 | 31.1 ± 0.4 |
| **Condition factor** | 1.08 ± 0.02 | 1.06 ± 0.02 |

Data are presented as means ± SEM (n = 11–13). No significant differences were found between acclimation groups for any of the variables.
from the flow meter and pressure transducer were relayed to a 16SP PowerLab system (ADIInstruments, Castle Hill, Australia) that was connected to a computer with LabChart pro data acquisition software (7.3.2, ADInstruments). Heart rate was determined from the pulsatile blood flow or pressure recordings using the blood pressure module in LabChart pro. \( R_{SYS} \) was calculated from \( P_{DA} \) and cardiac output as:

\[
R_{SYS} = \frac{P_{DA}}{\text{cardiac output}}
\]

Stroke volume was calculated as:

\[
\text{stroke volume} = \frac{\text{cardiac output}}{\text{heart rate}}
\]

Mean values for baseline \( P_{DA} \), cardiac output and heart rate for each individual were obtained from representative calm periods, which were taken at the end of the initial 2 h baseline recording period. To analyze the responses to \( \alpha \)-adrenergic stimulation with the different dosages of phenylephrine, mean values for all cardiovascular variables were taken at the peak blood pressure response after each drug injection. The order of administration for the different dosages of phenylephrine was randomized for each fish. Cardiovascular variables after complete \( \alpha \)-adrenergic blockade with prazosin were obtained approximately two hours after the drug had been administered and the blood pressure had reached a new steady state. All mean values were typically calculated as 30 s means.

**Statistical analysis**

Statistical analyses were conducted using SPSS Statistics 24 (IBM Corp., Armonk, NY, USA). Independent \( t \)-tests were used for all comparisons between acclimation groups containing one dependent factor, including all baseline and prazosin treatment analyses, as well as all analyses of the absolute changes induced by prazosin. To assess the general effect of the different dosages of phenylephrine within each acclimation group, as well as the general effect between acclimation groups, a repeated measures ANOVA was used with individuals as subject variables and the dose of phenylephrine as the repeated variable. In the model, we included dose of phenylephrine (0, 10, 30, 60, 100 µg kg\(^{-1}\)), acclimation group and their interactions as fixed effects. To meet the assumptions of statistical tests, a logarithmic transformation was applied for cardiac output, \( R_{SYS} \) and \( P_{PULSE} \), and a square root transformation was applied for \( P_{DA} \). When the assumption of sphericity in the general linear model analyses was not met, we used Greenhouse–Geisser corrections to interpret if the results were significant. Values are presented as means ± SEM and statistical significance was accepted at \( P \leq 0.05 \).

**Results**

There were no obvious behavioural differences between acclimation groups as fish from both groups generally remained calm in the experimental setup throughout the recording period. There were no differences in body mass, length or condition factor between acclimation groups (\( P < 0.05 \); Table 1).

**Effects of salinity on baseline cardiovascular variables**

The SW-acclimated rainbow trout had a significantly higher cardiac output compared to FW-acclimated trout (26.3 ± 4.1 versus 15.7 ± 1.9 ml min\(^{-1}\) kg\(^{-1}\); \( T_{16} = 2.853, P = 0.012 \); Fig. 1A). The elevated cardiac output in SW was associated with a significantly higher stroke volume (0.44 ± 0.06 versus 0.28 ± 0.03 ml beat\(^{-1}\); \( T_{16} = 2.340 P = 0.033 \); Fig. 1C), whereas no significant difference in heart rate between the two acclimation groups was observed (\( T_{22} = 1.423, P = 0.169 \); Fig. 1B). Despite the elevated cardiac output, the SW-acclimated trout exhibited a significantly lower \( P_{DA} \) (20.7 ± 1.5 cm H\(_2\)O) than FW-acclimated trout (30.8 ± 1.6 cm H\(_2\)O; \( T_{22} = 4.337 P < 0.001 \); Fig. 1D). Consequently, the lower \( P_{DA} \) of SW-acclimated trout was explained by a significantly reduced \( R_{SYS} \) (0.90 ± 0.15 versus 2.13 ± 0.28 cm H\(_2\)O min\(^{-1}\) ml; \( T_{16} = 4.147, P = 0.001 \); Fig. 1E). The decreased \( P_{DA} \) in SW was also reflected in significant reductions in dorsal aortic diastolic (\( T_{22} = 4.130 P < 0.001 \); Fig. 2A), systolic (\( T_{22} = 4.820 P < 0.001 \); Fig. 2B) and pulse (\( T_{22} = 3.984 P = 0.001 \); Fig. 2C) pressures.

**Cardiovascular effects of \( \alpha \)-adrenergic drugs in FW- and SW-acclimated trout**

Intra-arterial injection of the \( \alpha \)-adrenergic agonist phenylephrine caused dose-dependent increases in \( R_{SYS} \) and \( P_{DA} \) in both acclimation groups, but both variables were consistently lower in SW-acclimated trout (Fig. 3A, B). However, when analyzing the absolute changes in \( R_{SYS} \) and \( P_{DA} \) from baseline values with each dosage of phenylephrine (data not shown), there were no significant differences between acclimation groups indicating that the responsiveness to \( \alpha \)-adrenergic stimulation was unchanged across salinity acclimation groups (\( F_{1} = 2.184, P = 0.159 \) and \( F_{1} = 0.219, P = 0.645 \), respectively).

Phenylephrine injections significantly affected cardiac output (\( F_{3} = 2.974, P = 0.026 \)) and stroke volume (\( F_{3} = 16.144, P < 0.001 \)) in both acclimation groups (Table 2). Further, administration of phenylephrine typically reduced heart rate in both SW and FW, indicating a barostatic reflex (\( F_{2,44} = 13.059, P < 0.001 \); Table 2). Although not statistically tested, cardiac output appeared to increase at the lower dosages of phenylephrine due to an increased stroke volume, whereas it decreased at higher dosages of phenylephrine as stroke volume reached an upper limit while heart rate continued to decrease (Table 2).
After complete \(\alpha\)-adrenoceptor blockade with prazosin, R\(\text{SYS}\) remained consistently lower in SW \((T_{14} = 2.442\) and \(P = 0.028\); Fig. 1E), but the absolute reduction in R\(\text{SYS}\) with prazosin was significantly greater in FW- compared to SW-acclimated rainbow trout \((T_{14} = 2.895, P = 0.019;\) Fig. 4). P\(\text{DA}\) was also consistently lower in SW-acclimated trout after prazosin treatment \((T_{20} = 2.689, P = 0.014;\) Fig. 1D), although the absolute change in P\(\text{DA}\) from baseline with prazosin was not statistically different between acclimation groups. All other blood pressure variables also showed a similar magnitude in the absolute change after prazosin across acclimation groups, but again were consistently lower in the SW-acclimated trout (Fig. 2).

While prazosin induced a significantly greater heart rate increase in FW-acclimated trout compared to SW-acclimated trout (data not shown), there was still no significant difference in heart rate between acclimation groups after prazosin \((T_{20} = 1.579, P = 0.130;\) Fig. 1B). The magnitude of the absolute changes in stroke volume and cardiac output with prazosin were not significantly different between acclimation groups. However, the clear and significant differences in baseline values for these variables that were observed in untreated trout disappeared with prazosin treatment (cardiac output: \(T_{14} = 0.840, P = 0.415\) and stroke volume: \(T_{14} = 1.317, P = 0.209;\) Fig. 1A, C).

Figure 1: Cardiovascular variables in freshwater- (FW, 0–1 ppt, open bars) and seawater- (SW, 30–33 ppt, closed bars) acclimated rainbow trout (Oncorhynchus mykiss). The variables are (A) cardiac output (CO, \(n = 8\) SW; 9 FW), (B) heart rate (HR, \(n = 10\) SW; 13 FW), (C) stroke volume (SV, \(n = 8\) SW; 9 FW), (D) dorsal aortic blood pressure (P\(\text{DA}\), \(n = 10\) SW; 13 FW) and (E) systemic vascular resistance (R\(\text{SYS}\), \(n = 8\) SW; 9 FW) during baseline conditions and after \(\alpha\)-adrenoreceptor blockade with prazosin (1 mg kg\(^{-1}\)). Data are presented as means \(\pm\) SEM. Asterisks (*) denote significant effect of acclimation salinity \((P \leq 0.05)\).
**Discussion**

**Hemodynamic status in FW and SW and possible implications for aerobic performance traits**

The present findings demonstrate that $P_{DA}$ and $R_{SYS}$ are significantly reduced in chronically SW-acclimated rainbow trout. Moreover, our results confirm previous observations of significantly elevated cardiac output in SW-acclimated trout (Brijs et al., 2015, 2016). These fundamental cardiovascular changes in response to salinity open up a range of important questions of how aerobic performance traits such as digestion and swimming capacity are affected by salinity in euryhaline fishes. For example, it is unknown if the elevated cardiac output in SW affects the scope for cardiac output, or whether the cardiovascular system possesses sufficient phenotypic plasticity to compensate across salinities; e.g. by increasing the maximal cardiac output in SW to maintain cardiac scope. While acclimation to different salinities generally has negligible impacts on the maximum swimming capacity of euryhaline fishes (Beamish, 1978; Christensen et al., 2016, 2017), we are not aware of any study comparing maximum cardiac performance during sustained swimming at different acclimation salinities. Thus, while our findings require consideration in conservation management of fish populations that are exposed to varying environmental salinities in their natural habitats, they also highlight the need for further experiments on cardiorespiratory responses to exercise in euryhaline fishes across salinities.

Our data strongly suggest that the elevated gastrointestinal blood flow previously observed in SW-acclimated trout (Brijs et al., 2015, 2016) is caused by a reduced gastrointestinal vascular resistance, since the driving pressure for gastrointestinal blood flow (i.e. $P_{DA}$) was markedly reduced in SW and therefore cannot explain the elevated blood flow. However, it is not possible to conclude if a dilation of somatic vascular beds also contributed to the overall reduction in $R_{SYS}$, or if somatic vascular resistance increased to aid blood flow distribution to the gastrointestinal tract in SW. Forced feeding of FW and SW-acclimated rainbow trout increased gastrointestinal blood flow with the same absolute amount, which shows that the baseline difference in gastrointestinal blood flow persists after feeding (Brijs et al., 2016). However, whether the elevated gastrointestinal blood flow and decreased $\alpha$-adrenergic tone on the gastrointestinal resistance vasculature of unfed SW fish constrains the ability to redistribute blood flow away from the gastrointestinal tract to supply swimming muscles during exercise represents another interesting topic to explore in the future.

The reduced $P_{DA}$ in SW-acclimated trout was most likely an effect of the reduced $R_{SYS}$, which the marked rise in cardiac output was unable to compensate for. However, there are also a few other factors that may have contributed to the decreased $P_{DA}$ in SW. The total circulating blood volume is typically reduced in SW, which could possibly contribute to the reduced blood pressure (Olson, 1992; Olson and Hoagland, 2008). It is also possible that branchial vascular resistance increases with SW-acclimation, which would also reduce the down-stream $P_{DA}$. To fully resolve these possibilities, simultaneous measurements of ventral aortic blood pressure and gastrointestinal blood flow in FW- and SW-acclimated trout, along with the cardiac output and $P_{DA}$ measurements performed here, are required.
Seawater acclimation alters the α-adrenergic control of cardiovascular function

Reduced $R_{\text{SYS}}$ can result either from elevated vasodilatory and/or reduced vasoconstrictory stimulation of the resistance vasculature (Nilsson, 1994; Olson and Farrell, 2003). The present study examined the α-adrenergic vasomotor tone and found that SW-acclimation of trout leads to reduced α-adrenergic constriction of the systemic resistance vasculature. This decreased α-adrenergic vasoconstriction can either be due to a reduced vascular α-adrenergic sensitivity (e.g. via down-regulation of vascular α-adrenoceptors), or a reduced intrinsic α-adrenergic neurohumoral tone (Chen et al., 2007;...
Likely vasodilating candidates include natriuretic peptides, such as atrial natriuretic peptide, which has a strong vasodilating effect on the gastrointestinal resistance vasculature, as well as indirect vasodilatory effects by stimulating nitric oxide production (Bohlen, 1998; Levine et al., 1978; Steenbergen and Bohlen, 1993; Zani and Bohlen, 2005). Interestingly, an increased activity of neuronal nitric oxide synthase was found in the anterior intestine of rainbow trout after 7 days of exposure to SW (25 ppt; Gerber et al., 2018). This indicates a role of nitric oxide in SW-acclimation and as a possible mediator of gastrointestinal vasodilation.

The 68% increase in cardiac output in SW-acclimated trout was primarily mediated via an increased stroke volume, as heart rate remained unchanged across salinities (Brijs et al., 2015, 2016, 2017). Nonetheless, the reduction in heart rate induced by phenylephrine in both acclimation groups reveals a functional cardiac baroreflex response at both salinities (Sandblom and Axelsson, 2005). While the increased stroke volume has previously been attributed to a reduced venous capacitance and an increased central venous pressure with SW-acclimation (Brijs et al., 2017), the present study also indicates that the elevated venous pressure may be due to the reduced $R_{SYS}$. Interestingly, the significant elevation in baseline cardiac output and stroke volume in SW-acclimated trout was abolished following the prazosin treatment. This could be due to an increased central venous pressure following prazosin treatment in FW-acclimated trout due to altered trans-vascular fluid shifts, as observed in previous in vivo studies (see Sandblom and Gräns, 2017).

**Conclusions and perspectives**

The present study emphasizes that profound cardiovascular changes occur during acclimation to different salinities in a euryhaline teleost fish. These changes are likely important for maintaining osmotic homeostasis but may impact on aerobic performance traits, which requires future research attention and consideration in conservation management. While the present findings suggest that previous observations of increased gastrointestinal blood flow in SW are due to a reduced $\alpha$-adrenergic tone on the gastrointestinal resistance vasculature, our data also indicate that other vasoactive factors are important for mediating these responses. Thus, deciphering the apparently complex interplay between the various neural and hormonal cardiovascular control systems at play during FW to SW transition represents another challenging avenue for further research. From an evolutionary perspective, it could be speculated that the evolution of more complex control systems involving both neural and hormonal vascular control systems in teleost fishes, that are not present in elasmobranchs and cyclostomes (Nilsson, 1983, 1997). Other possible vasodilator candidates include nitric oxide and nitric oxide derivatives that act as general vasodilators in the vasculature of teleost fish (Olson and Donald, 2009; Sandblom and Gräns, 2017). In mammals, intestinal hyperosmolarity, as would be expected with SW drinking in fish, have both direct vasodilatory effects on the gastrointestinal resistance vasculature, as well as indirect vasodilatory effects by stimulating nitric oxide production (Bohlen, 1998; Levine et al., 1978; Steenbergen and Bohlen, 1993; Zani and Bohlen, 2005).
1994; Sandblom and Axelsson, 2011; Sandblom and Gräns, 2017), has been an important prerequisite for the evolution of euryhalinity in this diverse group of vertebrates. This evolutionary transition has undoubtedly equipped many teleost species with the physiological machinery necessary to tolerate large acute and chronic salinity changes, as well as the ability to exploit and undertake long-distance migrations across environments with highly contrasting salinities. In fact, this capacity may provide euryhaline teleosts with competitive advantages allowing them to better cope with greater salinity variations in the future resulting from climate change and other anthropogenic perturbations.

Funding
This work was supported by the Swedish Research Council (Vetenskapsrådet) [2011–04786] and the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (Svenska Forskningsrådet Formas) [2016–00729].

Author contributions
E.Sa. conceived and designed the study; E.Su. and D.M. performed the experiments with technical assistance from A.E. and J.B.; E.Su., E.Sa. and A.G. analyzed the data and performed the statistical analysis; E.Su. and E.Sa. wrote the manuscript, with all co-authors providing input on the written text.

Competing interests
The authors declare no competing interests.

References
Axelsson M, Fritsche R (1991) Effects of exercise, hypoxia and feeding on the gastrointestinal blood flow in the Atlantic cod Gadus morhua. J Exp Biol 158: 181–198.

Axelsson M, Fritsche R (1994) Cannulation techniques. In Mommsen TP, Hochachka PW, eds. Analytical Techniques, vol. 3. Elsevier Science, Amsterdam, pp 17–36.

Axelsson M, Driedzic WR, Farrell AP, Nilsson S (1989) Regulation of cardiac output and gut blood flow in the searaven, Hemitripterus americanus. Fish Physiol Biochem 6: 315–326.

Axelsson M, Thorarensen H, Nilsson S, Farrell AP (2000) Gastrointestinal blood flow in the red Irish lord, Hemilepidotus hemilepidotus: long-term effects of feeding and adrenergic control. J Comp Physiol 170: 145–152.

Bath RN, Eddy FB (1979) Salt and water balance in Rainbow Trout (Salmo Gairdneri) rapidly transferred from fresh water to sea water. J Exp Biol 83: 193–202.

Beamish FWH (1978) Swimming capacity. In Hoar WS, Randall DJ, eds. Fish physiology, vol. 7. New York, San Fransisco. Academic Press Ltd, London, pp 101–187.

Bohlen H (1998) Mechanism of increased vessel wall nitric oxide concentrations during intestinal absorption. Am J Physiol Heart Circ Physiol 44: 542–550.

Brijs J, Axelsson M, Gräns A, Pichaund N, Olsson C, Sandblom E (2015) Increased gastrointestinal blood flow: An essential circulatory modification for euryhaline rainbow trout (Oncorhynchus mykiss) migrating to sea. Sci Rep 5: 10430. doi:10.1038/srep10430.

Brijs J, Gräns A, Ekström C, Olsson C, Axelsson M, Sandblom E (2016) Cardiorespiratory upregulation during seawater acclimation in rainbow trout: effects on gastrointestinal perfusion and postprandial responses. Am J Physiol Regul Integr Comp Physiol 310: 858–865.

Brijs J, Sandblom E, Dekens E, Näslund J, Ekström A, Axelsson M (2017) Cardiac remodeling and increased central venous pressure underlie elevated stroke volume and cardiac output of seawater-acclimated rainbow trout. Am J Physiol Regul Integr Comp Physiol 312: 31–39.

Chen X, Moon TW, Olson KR, Dombkowski RA, Perry SF (2007) The effects of salt-induced hypertension on α1-adrenoreceptor expression and cardiovascular physiology in the rainbow trout (Oncorhynchus mykiss). Am J Physiol 293: 1384–1392.

Christensen EAF, Illing B, Iversen NS, Johansen JL, Domenici P, Steffensen JF (2018) Effects of salinity on swimming performance and oxygen consumption rate of shiner perch Cymatogaster aggregata. J Exp Mar Biol Ecol 504: 32–37.

Cooke SJ, Sack L, Franklin CE, Farrell AP, Beardall J, Wikelski M, Chown SL (2013) What is conservation physiology? Perspectives on an increasingly integrated and essential science. Conserv Physiol 1: 1–23.

Cousins KL, Farrell AP (1996) Stretch-induced release of atrial natriuretic factor from the heart of rainbow trout (Oncorhynchus mykiss). Can J Zool 74: 380–387.

Evans DH (2008) Teleost fish osmoregulation: what have we learned since August Krogh, Homer Smith, and Ancel Keys. Am J Physiol Regul Integr Comp Physiol 295: 704–713.

Evans DH, Piermarini PM, Choe KP (2005) The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. Physiol Rev 85: 97–177.

Farrell AP, Olson KR (2000) Cardiac natriuretic peptides: a physiological lineage of cardioprotective hormones? Physiol Biochem Zool 73: 1–11.

Farrell AP, Johansen JA, Graham MS (1988) The role of the pericardium in cardiac performance of the trout (Salmo gairdneri). Physiol Zool 61: 213–221.

Gerber LB, Jensen FBS, Madsen SS (2018) Dynamic changes in nitric oxide synthase expression are involved in seawater acclimation of rainbow trout Oncorhynchus mykiss. Am J Physiol Regul Integr Comp Physiol 314: 552–562.
Conservation Physiology

Grosell M, Farrell AP, Brauner CJ (2010) The role of the gastrointestinal tract in salt and water balance. In Grosell M, Farrell AP, Brauner CJ, eds. The Multifunctional Gut of Fish. Academic Press, San Diego, pp 135–164.

Jeppesen E, Bruet C, Naselli-Flores L, Papastergiadou E, Stefanidis K, Nõges T, Nõges P, Attayde JL, Zohary T, Coppens J, et al. (2015) Ecological impacts of global warming and water abstraction on lakes and reservoirs due to changes in water level and related changes in salinity. Hydrobiologia 750: 201–227.

Kaiya H, Takei Y (1996a) Changes in plasma atrial and ventricular natriuretic peptide concentration after transfer of eels from freshwater and seawater or vice versa. Gen Comp Endocrinol 104: 337–345.

Kaiya H, Takei Y (1997) Interaction of osmotic and volaemic regulation of atrial and ventricular natriuretic peptide secretion in conscious eels. J Endocrinol 149: 441–447.

Kaiya H, Takei Y (1996b) Osmotic and volaemic regulation of atrial and ventricular natriuretic peptide secretion in conscious eels. J Endocrinol 107: 322–326.

Kultz D (2015) Physiological mechanisms used by fish to cope with salinity stress. J Exp Biol 218: 1907–1914.

Levine SE, Granger DN, Brace RA, Taylor AE (1978) Effect of hyperosmolality on vascular resistance and lymph flow in the cat ileum. Am J Physiol Heart Circ Physiol 3: 14–20.

Linhart O, Walford J, Sivaloganathan B, Lam TJ (1999) Effects of osmotic and ionic factors on the motility of stripped and testicular sperm of freshwater- and seawater-acclimated tilapia, Oreochromis mossambicus. J Fish Biol 55: 1344–1358.

Marshall WS, Grosell M (2006) Ion transport, osmoregulation and acid–base balance. In Evans DH, Claiborne JB, eds. The physiology of fishes. CRC Press, Boca Raton, pp 177–231.

Maxime V, Pennec JP, Peyraud C (1991) Effects of direct transfer from freshwater to seawater on respiratory and circulatory variables and acid–base status in rainbow trout. J Comp Physiol B 161: 557–568.

McCormick SD, Saunders RL (1987) Preparatory physiological adaptations for marine life of salmonids: osmoregulation, growth, and metabolism. Am Fish Soc Symp 1: 211–229.

McCormick SD, Hansen LP, Quinn TP, Saunders RL (1998) Movement, migration, and smoltling of Atlantic salmon (Salmo salar). Can J Fish Aquat Sci 55: 77–92.

Meier M, Andersson HC, Arheimer B, Blencckner T, Chubarenko B, Donnelly C, Eilola K, Gustafsson BG, Hansson A, Havenhand J, et al. (2012) Comparing reconstructed past variations and future projections of the Baltic sea ecosystem first results from multi model ensemble simulations. Environ Res Lett 7: 1–8.

Nelson JA, Tang Y, Boutiller RG (1996) The effects of salinity change on the exercise performance of two Atlantic cod (Gadus morhua) populations inhabiting different environments. J Exp Biol 199: 1295–1309.

Nilsson S (1983) Autonomic nerve function in the vertebrates. Springer Verlag, Berlin, Heidelberg, New York.

Nilsson S (1994) Evidence for adrenergic nervous control of blood pressure in teleost fish. Physiol Zool 67: 1347–1359.

Olson KR (1992) Blood and extracellular fluid volume regulation: role of the renin angiotensin system, kallikrein-kinin system, and atrial natriuretic peptides. In Hoar WS, Randall DJ, Farrell AP, eds. Fish physiology, vol. 12B. Academic Press, San Diego, New York, London, pp 135–254.

Olson KR (2011) Circulatory system design: roles and principles. In Farrell AP, ed. Encyclopedia of Fish Physiology. Academic Press, San Diego, pp 977–983.

Olson KR, Meisheiri KD (1989) Effects of atrial natriuretic factor on isolated arteries and perfused organs of trout. Am J Physiol 256: 10–18.

Olson KR, Farrell AP (2005) The cardiovascular system. In Evans DH, Claiborne JB, eds. The Physiology of Fishes, 3rd edn. CRC Press, Boca Raton, pp 119–142.

Olson KR, Hoagland TM (2008) Effects of freshwater and saltwater adaptation and dietary salt on fluid compartments, blood pressure, and venous capacitance in trout. Am J Physiol Regul Integr Comp Physiol 294: 1061–1067.

Olson KR, Donald JA (2009) Nervous control of circulation—the role of gasotransmitters, NO, CO, and H2S. Acta Histochem 111: 244–256.

Perry SF, Shahsvarani A, Georgalis T, Bayaa M, Furimsky M, Thomas SLY (2003) Channels, pumps and exchangers in the gill and kidney of freshwater fishes: their role in ionic and acid-base regulation. J Exp Zool A 300A: 53–62.

Quinn TP, Myers KW (2004) Anadromy and the marine migrations of Pacific salmon and trout: Rounsefell revisited. Rev Fish Bio Fisher 14: 421–442.

Sandblom E, Axelsson M (2005) Baroreflex mediated control of heart rate and vascular capacitance in trout. J Exp Biol 208: 821–829.

Sandblom E, Axelsson M (2006) Adrenergic control of venous capacitance during moderate hypoxia in the rainbow trout (Onchorhyncus mykiss): role of neural and circulating catecholamines. Am J Physiol Reg Integr Comp Physiol 291: 711–718.

Sandblom E, Axelsson M (2011) Autonomic control of circulation in fish: a comparative view. Auton Neurosci 165: 127–139.

Sandblom E, Gräns A (2017) Form function and control of the vasculature. In Gamperl K, Gillis TE, Farrell AP, Brauner CJ, eds. Fish physiology, vol. 36. Academic press, London, pp 369–433.

Sandblom E, Axelsson M, Farrell AP (2006) Central venous pressure and mean circulatory filling pressure in the dogfish Squalus acantias: adrenergic control and role of the pericardium. Am J Physiol 291: 1465–1473.

Sandblom E, Davison W, Axelsson M (2012) Cold physiology: postprandial blood flow dynamics and metabolism in the Antarctic fish
Pagothenia borchgrevinki. PLoS One 7: e33487. doi.org/10.1371/journal.pone.0033487.

Seebacher F, Franklin CE (2012) Determining environmental causes of biological effects: the need for a mechanistic physiological dimension in conservation biology. Phil Trans R Soc B 367: 1607–1614.

Seth H (2010) On the regulation of Postprandial Gastrointestinal Blood Flow in Teleost Fish. PhD thesis, University of Gothenburg, Gothenburg, Sweden.

Seth H, Axelsson M (2010) Sympathetic, parasympathetic and enteric regulation of the gastrointestinal vasculature in rainbow trout (Oncorhynchus mykiss) under normal and postprandial conditions. J Exp Biol 213: 3118–3126.

Smith HW (1930) The absorption and excretion of water and salts by marine teleosts. Am J Physiol 93: 480–505.

Smith HW (1932) Water regulation and its evolution in the fishes. Q Rev Biol 7: 1–26.

Smith LS, Bell GR (1964) A technique for prolonged blood sampling in free-swimming salmon. J Fish Res 21: 711–717.

Smith MP, Takei Y, Olson KR (2000) Similarity of vasorelaxant effects of natriuretic peptides in isolated blood vessels of salmonids. Physiol Biochem Zool 73: 494–500.

Soivio A, Nynolm K, Westman K (1975) A technique for repeated sampling of the blood of individual resting fish. J Exp Biol 63: 207–217.

Steenbergen J, Bohlen H (1993) Sodium hyperosmolarity of intestinal lymph causes arteriolar vasodilation in part mediated by EDRF. Am J Physiol 34: 323–328.

Sundell K, Sundh H (2012) Intestinal fluid absorption in anadromous salmonids: importance of tight junctions and aquaporins. Front Physiol 3: 1–12.

Tang Y, Boutilier RG (1988) Correlation between catecholamine release and degree of acidic stress in trout. Am J Physiol Reg Integr Comp Physiol 255: 395–399.

Wagner GN, Kuchel LJ, Lotto A, Patterson DA, Shrimpton JM, Hinch SG, Farrell AP (2006) Routine and active metabolic rates of migrating adult wild sockeye salmon (Oncorhynchus nerka Walbaum) in seawater and freshwater. Physiol Biochem Zool 79: 100–8.

Wood CM, Bucking C (2010) The role of feeding in salt and water balance. In Grosell M, Farrell AP, Brauner CJ, eds. Fish Physiology, vol. 30. Academic Press, London, pp 165–212.

Zani BG, Bohlen HG (2005) Sodium channels are required during in vivo sodium chloride hyperosmolarity to stimulate increase in intestinal endothelial nitric oxide production. Am J Physiol Heart Circ Physiol 288: 89–95.