© 2019. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2019) 222, jeb207522. doi:10.1242/jeb.207522

RESEARCH ARTICLE

Hemodynamic responses to warming in euryhaline rainbow trout: implications of the osmo-respiratory compromise

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ABSTRACT

In seawater, rainbow trout (*Oncorhynchus mykiss*) drink and absorb water through the gastrointestinal tract to compensate for water passively lost to the hyperosmotic environment. Concomitantly, they exhibit elevated cardiac output and a doubling of gastrointestinal blood flow to provide additional O_2 to the gut and increase convective flux of absorbed ions and water. Yet, it is unknown how warming waters, which elevate tissue O_2 demand and the rate of diffusion of ions and water across the gills (i.e. the osmo-respiratory compromise), affects these processes. We measured cardiovascular and blood variables of rainbow trout acclimated to freshwater and seawater during acute warming from 11 to 17°C. Relative to freshwater-acclimated trout, cardiac output was 34% and 55% higher in seawater-acclimated trout at 11 and 17°C, respectively, which allowed them to increase gastrointestinal blood flow significantly more during warming (increases of 75% in seawater vs. 31% in freshwater). These adjustments likely served to mitigate the impact of warming on osmotic balance, as changes in ionic and osmotic blood composition were minor. Furthermore, seawater-acclimated trout seemingly had a lower tissue O_2 extraction, explaining why trout acclimated to freshwater and seawater often exhibit similar metabolic rates, despite a higher cardiac output in seawater. Our results highlight a novel role of gastrointestinal blood perfusion in the osmo-respiratory compromise in fish, and improve our understanding of the physiological changes euryhaline fishes must undergo when faced with interacting environmental challenges such as transient warming events.

KEY WORDS: Cardiovascular, Gastrointestinal blood flow, *Oncorhynchus mykiss*, Osmoregulation, Warming

INTRODUCTION

Euryhaline fishes can tolerate wide variations in environmental salinity. This allows them to exploit resource-rich littoral (e.g. the intertidal zone) and estuarine habitats (Hampel and Cattrijsse, 2004), as well as to undertake migrations to increase food availability and reproductive output (i.e. diadromous fish) during their life cycle (Gross et al., 1988). Euryhalinity is a relatively rare trait in fish as it depends on substantial physiological modifications in order to maintain osmotic, ionic and acid–base balance across water salinities (Edwards and Marshall, 2013; Evans et al., 2005; Kültz, 2015). For example, teleost fish migrating from freshwater (FW) to seawater (SW) require a complete reversal in osmoregulatory strategies. In FW, they are hyper-osmotic relative to the environment, facing a continuous passive entry of water and loss of ions. To compensate, they produce dilute urine, while reducing renal ion excretion and increasing dietary and branchial ion uptake (Edwards and Marshall, 2013; Wood and Bucking, 2011). In contrast, marine teleosts hypo-osmoregulate to counter dehydration and entry of ions. Their gills and kidneys become a site for active ion secretion and glomerular filtration rate is reduced, resulting in the production of urine isosmotic to the plasma (Edwards and Marshall, 2013; Marshall and Grosell, 2005). Moreover, maintenance of the hydric balance is achieved via drinking and intestinal water absorption, the latter largely driven by enterocyte Na^+/K^+-ATPases creating an osmotic gradient between the lumen and the lateral intercellular space (Grosell, 2006).

In salmonids, transition from FW to SW is typically characterized by a transient period of osmotic imbalance, followed by compensatory upregulation of branchial and intestinal ion pump expression and activity (Madsen et al., 1996; Seidelin et al., 2000). Concomitant with this upregulation, a series of cardiovascular changes takes place. Rainbow trout acutely exposed to seawater exhibit a stroke volume-mediated elevation in cardiac output (Maxime et al., 1991), where a substantial proportion of blood flow is directed to the celiacomesenteric artery perfusing the gut, which doubles gastrointestinal blood flow (GBF) (Brijs et al., 2015). This mechanism is thought to sustain an elevated demand for nutrients and O_2 in the gastrointestinal tissues as osmoregulatory function increases, as well as to transport absorbed ions to their respective sites of excretion at the gills and kidneys (Brijs et al., 2015, 2016). Chronically SW-acclimated trout also have an elevated stroke volume and cardiac output (Brijs et al., 2016, 2017; Sundell et al., 2018), which is at least partially due to increased end-diastolic cardiac filling (Frank–Starling mechanism), as indicated by an elevated central venous blood pressure in SW (Brijs et al., 2017).

Global warming is expected to result in both increasing average temperatures, and more extreme and frequent transient heat waves (Frölicher et al., 2018; Meehl et al., 2018). As whole organism metabolism and O_2 demand increase with warming (Ekström et al., 2017; Eliason and Anttila, 2017; Fry and Hart, 1948; Sandblom et al., 2016), ventilation, cardiac output and/or tissue O_2 extraction also increase (Brijs et al., 2018; Clark et al., 2008b). Moreover, while ecophysiological studies often focus on how single environmental drivers (e.g. changes in temperature) affect physiological performance to explain ecological trends, organisms may face multiple and interacting environmental changes in nature, and the response to individual drivers might be very different from the response when several drivers are combined (Todgham and Stillman, 2013). For example, the interacting effect of changes in temperature with other environmental drivers such as salinity remains largely unexplored.

Interestingly, hemodynamic conditions of the gills that benefit branchial gas exchange during warming, such as lamellar recruitment

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Received 21 May 2019; Accepted 31 July 2019

Journal of Experimental Biology (2019) 222, jeb207522. doi:10.1242/jeb.207522

List of symbols and abbreviations

FW	freshwater
GBF	gastrointestinal blood flow
MCHC	mean corpuscular hemoglobin concentration
Pa	dorsal aortic blood pressure
Pa_{O_2}	partial pressure of arterial O_2
Pv	central venous blood pressure
Pv_{O_2}	partial pressure of venous O_2
SW	seawater

and reduced blood–water diffusion distance also result in greater osmoregulatory challenges, as they also favor diffusion of water and ions. The balance between these processes is known as the osmo-respiratory compromise (Sardella and Brauner, 2007). For example, elevated branchial blood perfusion in response to exercise or warming in FW results in increased influx of water and loss of ions across the gills (Gonzalez and McDonald, 1992; Isaia, 1972; Onukwufor and Wood, 2018), while in SW the same challenges lead to dehydration and elevated plasma ion concentration (Gallaugher et al., 2001; Isaia, 1972; Motais and Isaia, 1972). Thus, to compensate for the exacerbated diffusive loss of water through the gills in SW at elevated temperatures, it could be hypothesized that SW-acclimated fish must increase the rate of intestinal water absorption proportionally more, which would necessitate a proportionally greater increase in GBF in SW compared to FW. Alternatively, failure of SW-acclimated fish to sufficiently elevate GBF with warming could result in disturbed osmotic and ionic blood composition (e.g. high osmolality, hypernatremia), possibly compromising cardiac function. Indeed, such homeostatic imbalances have been shown to impair cardiac contractility in amphibians (Hillman, 1984; Hillman and Withers, 1988), and have been suggested as a possible underlying cause for the reduced swimming performance observed in fish acutely exposed to increased salinity (Brauner et al., 1992; McKenzie et al., 2001). In fact, in a previous study comparing cardiovascular responses to moderate warming from 10 to 16.5°C, Brijs et al. (2017) noted a reduced stroke volume in SW-acclimated rainbow trout (*Oncorhynchus mykiss*) at the elevated temperature, indicating cardiac impairment, but the mechanism underlying this effect is unknown. In salmonids, the inner spongy myocardium relies solely on luminal venous O_2 supply while the outer compact myocardium is also perfused by coronary arteries (Farrell et al., 2012). The partial pressure of venous O_2 (Pv_{O_2}) commonly decreases with warming, which may constrain stroke volume as Pv_{O_2} drives the diffusion of O_2 into the spongy myocardium (Clark et al., 2008b; Ekström et al., 2016). Another possibility is, therefore, that the reduced cardiac stroke volume observed in SW-acclimated fish during warming (Brijs et al., 2017) is caused by greater reductions in Pv_{O_2} in SW, perhaps as a result of increased O_2 extraction from the osmoregulating gut tissues at elevated temperatures.

Despite its crucial role for osmoregulation, the effect of warming on GBF dynamics in fish acclimated to different salinities has not been analyzed. Furthermore, it is currently unknown how GBF dynamics relates to blood osmotic homeostasis across acclimation salinities during warming. To test this, we recorded cardiac output, blood pressures and gastrointestinal blood flow, along with sequential blood sampling to determine hemoglobin concentration as a measure of blood O_2-carrying capacity, as well as plasma ion composition and osmolality, during a standardized acute warming protocol (11 to 17°C) in FW- and SW-acclimated rainbow trout. We specifically hypothesized that SW-acclimated trout would exhibit greater increases in GBF during warming to counter an increased osmotic loss of body water and to sustain an elevated metabolic demand of the gut in SW. Furthermore, by measuring Pv_{O_2} and plasma ion composition across salinities and temperatures, we tested the hypotheses that decreased Pv_{O_2} or disturbed plasma osmotic homeostasis could explain previously observed reductions in cardiac contractility during warming in SW-acclimated trout (Brijs et al., 2017).

MATERIALS AND METHODS

Fish and holding conditions

Rainbow trout (*Oncorhynchus mykiss* Walbaum 1792) of mixed sex were obtained from a commercial fish farm (EM-Lax AB, Västervik, Sweden) and acclimated in FW for at least 2 weeks. Subsequently, half of the fish were transferred to SW (salinity 32±2 ppt, $[K^+]$: 9.6 mmol l^{-1}, $[Na^+]$: 521 mmol l^{-1}, $[Cl^-]$: 469 mmol l^{-1}; source of the sea salt: Aquaforest, Brzesko, Poland) and allowed to acclimate for a minimum of 4 weeks prior to the experiments. FW- and SW-acclimated fish were maintained at ∼11°C under a 12 h:12 h light:dark photoperiod in 2000 l tanks supplied with recirculating aerated water. The fish were fed 7 mm pellets (Protec Trout pellets, Skretting, Stavanger, Norway) twice weekly until the start of the experiments, and were fasted for 1 week prior to the start of experiments. The study was covered by ethical permit 165-2015, approved by the regional ethical committee in Gothenburg.

Surgery and instrumentation

The study consisted of two experimental series using two differently instrumented groups of fish that were subjected to the same experimental protocol (see below for details). In the first experiment, perivascular flow probes were used to measure GBF and cardiac output, and to determine heart rate and stroke volume. In the second experiment, fish were cannulated to measure dorsal aortic (*Pa*), central venous blood pressures (*Pv*) and heart rate, along with blood sampling at regular intervals to determine hematocrit, hemoglobin concentration, Pa_{O_2} (partial pressure of arterial O_2), Pv_{O_2}, blood ion composition and osmolality.

For all surgical protocols and acclimation groups, fish were anesthetized in FW containing 150 mg l^{-1} MS222 (ethyl-3-aminobenzoate methanesulphonic acid, Sigma-Aldrich Inc., St Louis, MO, USA) buffered with 300 mg l^{-1} $NaHCO_3$ (see Sundell et al., 2018). Once the opercular movements ceased, body length and mass were determined and the fish was placed on a surgical table covered with wet foam. A continuous flow of FW at 10°C containing maintenance anesthesia (100 mg l^{-1} MS222 buffered with 200 mg l^{-1} $NaHCO_3$) was delivered over the gills.

Surgical protocol 1: Cardiac output and gastrointestinal blood flow

The fish was placed laterally on its left side and the celiacomesenteric artery was accessed via a lateral incision ∼3 mm above the pectoral fin. The artery originates from the dorsal aorta and supplies the swim bladder, gonads, spleen, stomach, intestine and liver (Olson, 2011). The vessel was carefully dissected free using blunt dissection and a Perspex cuff (diameter 1.6 to 1.8 mm depending on the size of the vessel) with an integrated 20 MHz Doppler flow crystal (Iowa Doppler products, Iowa City, IA, USA) was placed around the vessel, taking care not to restrict vessel blood flow or causing damage to nearby nerves. The incision was closed with interrupted 3-0 silk sutures. Following this procedure, the ventral aorta was accessed via an incision in the isthmus in the opercular cavity, and the vessel was dissected free using blunt dissection, thus

avoiding damaging surrounding vessels, nerves or the pericardium. A 2.5-mm Transonic transit-time blood flow probe (L type; Transonic Systems, Ithaca, NY, USA) was placed around the ventral aorta. The probe was secured to the skin with two silk sutures, one inside the opercular cavity and one to the skin just outside the opercular cavity. Finally, the leads from the two flow probes were secured in front of the dorsal fin with a single anchor suture. As temperature can affect the probe readings, each Transonic flow probe was bench calibrated at 11°C, 13°C, 15°C and 17°C with known flow rates, following the methods specified in the user manual.

Surgical protocol 2: Arterial and venous blood pressure and blood sampling

The sinus venosus was non-occlusively cannulated using a polyethylene catheter (PE-50, Clay Adams; Becton Dickinson and Co, Sparks, MD, USA) filled with heparinized (100 IU ml^{-1}) 0.9% NaCl as described by Sandblom et al. (2006). Briefly, the ductus of Cuvier was accessed inside the opercular cavity by lifting the operculum and the gill arches. An incision was made parallel to the fifth branchial arch and a 4-0 silk suture was tied to the lateral surface of the ductus to enable lifting the vessel. A small incision was made in the vessel and a catheter was inserted and advanced towards the sinus venosus. A suture secured the catheter inside the ductus and two more sutures secured the catheter to the skin of the fish in front of the dorsal fin. The fish was then placed dorsally and a heparinized PE-50 catheter was inserted into the dorsal aorta using the method described by Soivio et al. (1975) and modified by (Kiessling et al., 1995). Blood was withdrawn to verify correct placement, then the catheter was flushed and filled with heparinized saline, exteriorized through a hole pierced in the snout and attached with the same dorsal suture as the venous catheter.

Experimental protocol

The instrumented fish were transferred into individual 12.5 l PVC holding tubes and allowed to recover for a minimum of 40 h prior to the start of the experimental protocol. The water flow through the tubes was ∼10 liters min^{-1} with supplementary aeration to ensure appropriate water air saturation (>90%) across temperatures. After connecting the fish to the recording equipment in the morning, baseline values at 11°C were recorded for 2 h or until steady state resting values had been attained. Blood samples were taken from the cannulated fish from both the arterial and venous catheters at this time point. Blood was collected for P_{O_2} analyses (100 µl from each catheter) using an airtight 250 µl Hamilton syringe and immediately analyzed. Another >0.3 ml of arterial and venous blood was then withdrawn using a 1 ml tuberculin syringe and centrifuged for 5 min at 5000 ***g*** and the plasma was kept frozen at −80°C for later analyses of plasma osmolality and concentrations of Na^+, K^+ and Cl^-. Hematocrit and [Hemoglobin] was also determined using blood from the venous cannula prior to centrifugation. After the plasma sampling at 11°C, the red blood cells were gently resuspended in an equivalent volume of 0.9% saline and reinfused into the fish to minimize loss of red blood cells. The temperature was then increased in steps of 2°C per 30 min, maintaining each target temperature stable for at least 30 min, which resulted in a temperature increase of 2°C h^{-1}. Once the final temperature of 17°C was reached, the fish were left undisturbed for a minimum of 1 h at this temperature. Finally, arterial and venous blood was sampled again from each cannulated fish as described above. Fish were euthanized with a sharp blow to the head upon termination of the experiment.

Data acquisition and analyses

The Transonic flow probe was connected to a three-channel Transonic blood flow meter (model T206; Transonic Systems, Ithaca, NY, USA). The Doppler flow probe was connected to a Doppler flow meter (model 545C-4, Iowa Doppler products, IA, USA). The venous and arterial catheters were connected to pressure transducers (model DPT-6100, pvb; Medizintechnik, Kirchseeon, Germany) and the signal was amplified using a 4ChAmp amplifier (Somedic, Hörby, Sweden). The pressure transducers were calibrated against a static water column at the start of each experimental protocol. The blood for P_{O_2} measurements was transferred to a MC100 Microcell Respirometer (Strathkelvin Instruments Ltd., Motherwell, UK) modified to fit a 3 mm Firesting O_2 optode (https://skfb.ly/6AMFz), connected to a FireSting O_2 system (PyroScience, Aachen, Germany) and calibrated at each temperature using two-point calibration. All signals where recorded using a 16SP PowerLab system (ADInstruments, Castle Hill, Australia) at a sampling rate of 10 Hz using LabChart pro data acquisition software (version 7.3.2, ADInstruments, Castle Hill, Australia).

Hematocrit was determined by spinning the blood in microhematocrit tubes for 5 min at 10,000 ***g*** and measuring the resulting fraction of red blood cells. [Hemoglobin] was measured using a handheld Hb 201^+ analyzer (Hemocue, Ängelholm, Sweden), and the values were calibrated for fish blood according to Clark et al. (2008a). Plasma [Na^+], [K^+] and [Cl^-] were determined with an electrolyte analyzer (Convergys® ISE Comfort, Convergent Technologies, Coelbe, Germany). Plasma osmolality was determined using an Advanced Model 3320 micro-osmometer (Advanced Instruments, Norwood, MA, USA).

Calculations

Condition factor was calculated by using the formula:

$$\text{Condition factor} = \left(\text{body mass}/\text{length}^3\right) \times 100. \quad (1)$$

Depending on the instrumentation, heart rate was determined from the pulsating ventral aortic blood flow or the blood pressure traces. Stroke volume was calculated as:

$$\text{Stroke volume} = \text{cardiac output}/\text{heart rate}. \quad (2)$$

The relative changes in GBF were estimated from the changes in blood velocity compared to the values at 11°C, which was set to 100%. To further interpret the relative changes in GBF, the absolute GBF was estimated using the average diurnal basal GBF obtained in a previous study on similar sized trout instrumented with Transonic flow probes at 11°C (Brijs et al., 2016). Accordingly, the basal GBF at 11°C in the present study was set to 4.0 ml min^{-1} kg^{-1} for FW trout and 8.6 ml min^{-1} kg^{-1} for SW trout. This meant that the relative changes in flow with increasing temperature recorded here with Doppler flow probes could be converted to absolute flow values (see Results).

Q_{10} for rate-dependent variables (i.e. cardiac output, heart rate and GBF) was calculated using values at 11 and 17°C as:

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{10/(t_2-t_1)}, \quad (3)$$

where R_1 and R_2 are the rates measured at 11 and 17°C, respectively.

Mean corpuscular hemoglobin concentration (MCHC) was calculated as:

$$\text{MCHC} = [\text{hemoglobin}]/\text{hematocrit} \times 100. \quad (4)$$

Journal of Experimental Biology (2019) 222, jeb207522. doi:10.1242/jeb.207522

Table 1. Body mass and condition factor of rainbow trout (*Oncorhynchus mykiss*) acclimated to freshwater (FW) and seawater (SW)

	FW	SW
Experimental series 1	*n*=10	*n*=10
Body mass (g)	862.5±38.0	823.9±41.6
Condition factor	1.04±0.03	1.05±0.03
Experimental series 2	*n*=9	*n*=9
Body mass (g)	740.8±135.7	748.8±83.5
Condition factor	1.13±0.02	1.10±0.02

Values are means±s.e.m. There were no significant differences between acclimation groups or experimental series.

Blood P_{O_2} was converted to kPa from recorded $\%O_2$ saturation values taking the atmospheric pressure and temperature-dependent water vapor pressure into consideration.

Statistical analyses

SPSS statistics 24 (IBM Corp., Armonk, NY, USA) was used for all statistical analyses. Differences in condition factor, body mass and Q_{10} data for cardiac output, heart rate and GBF were analyzed using independent-samples *t*-tests or Mann–Whitney *U*-tests depending on whether data was normally distributed or not, and, if the variances were unequal, Welch *t*-test was used. To analyze the hemodynamic variables throughout the temperature challenge, a linear mixed model was used. Individual fish were included as subject variable, temperature (within-subject factor), treatment (between-subject factor), sampling site (venous and arterial, within-subject factor) and the interaction between temperature and treatment as fixed effects and cardiovascular variables as dependent variables. For relative GBF, the same analysis was performed but only at three temperatures (13, 15 and 17°C), as all measurements at 11°C where adjusted to 100%. The calculated absolute GBF values were not subjected to statistical analysis. A first-order autoregressive (AR1) covariance structure, indicating that recordings that were close in time were also more dependent than temporally distant recordings, provided the best fit to the data (as indicated by the Akaike information criterion, AIC) for variables analyzed at four temperatures (11, 13, 15 and 17°C) and an unstructured covariance structure for variables measured at two temperatures (11 and 17°C). To correct for body mass differences, body mass was initially included as a covariate in the model. However, since no significant effects of body mass were found, the final data analysis excluded body mass as a covariate. Homoscedasticity and normal distribution of data was assessed by visual inspection of the residual plots. Data for $P_{V_{O_2}}$ and osmolality were transformed to their natural logarithms to meet the assumptions of this model. When significant effects were found, these were further explored with between- and within-treatments pair-wise comparisons, where the confidence intervals were adjusted for multiple testing using Bonferroni correction. Statistical significance was accepted at $P<0.05$. All data are presented as means±s.e.m.

RESULTS

Morphological features of the experimental fish

Condition factor and body mass did not significantly differ between acclimation groups in the first and second experimental series. All morphological information on the fish used in this study is summarized in Table 1.

Cardiovascular responses to warming across salinities

At 11°C, cardiac output was 20.4±1.7 and 15.2±2.1 ml min^{-1} kg^{-1} in SW- and FW-acclimated fish, respectively, and was significantly higher in SW-acclimated trout when analyzed across test temperatures (Fig. 1A). This was due to a significantly greater stroke volume in SW fish (0.60±0.08 vs. 0.34±0.04 ml kg^{-1}, Fig. 1B), while heart rate was similar between the two groups (45.4±2.9 beat min^{-1} in FW vs. 39.6±2.9 beat min^{-1} in SW, Fig. 1C). During the acute rise in temperature, cardiac output of both FW and SW fish increased significantly (Q_{10} of 1.7±0.2 and 2.2±0.2, respectively, salinity effect: $t_{16}=-1.825$, $P=0.087$) through increases in heart rate (Q_{10} of 2.0±0.1 in FW vs. 2.3±0.2 in SW, salinity effect: $t_{13.6}=-1.598$, $P=0.133$; Fig. 1A,C), whereas stroke volume remained unchanged across temperatures (Fig. 1B).

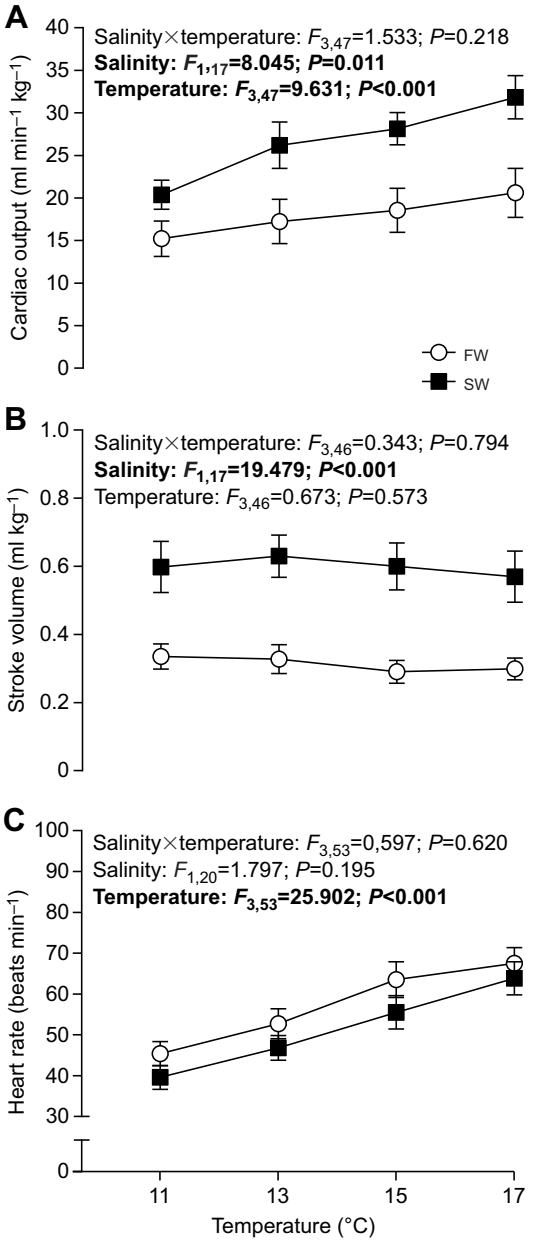

Fig. 1. The effects of warming on cardiovascular variables in rainbow trout (*Oncorhynchus mykiss*). (A) Cardiac output, (B) stroke volume and (C) heart rate of trout acclimated to freshwater (FW; open circles, *n*=10) and seawater (SW; filled squares; *n*=8–10). The results from the mixed model for the respective variables are presented in each panel. Statistical significance ($P<0.05$) is indicated by bold text. Values are means±s.e.m.

Journal of Experimental Biology (2019) 222, jeb207522. doi:10.1242/jeb.207522

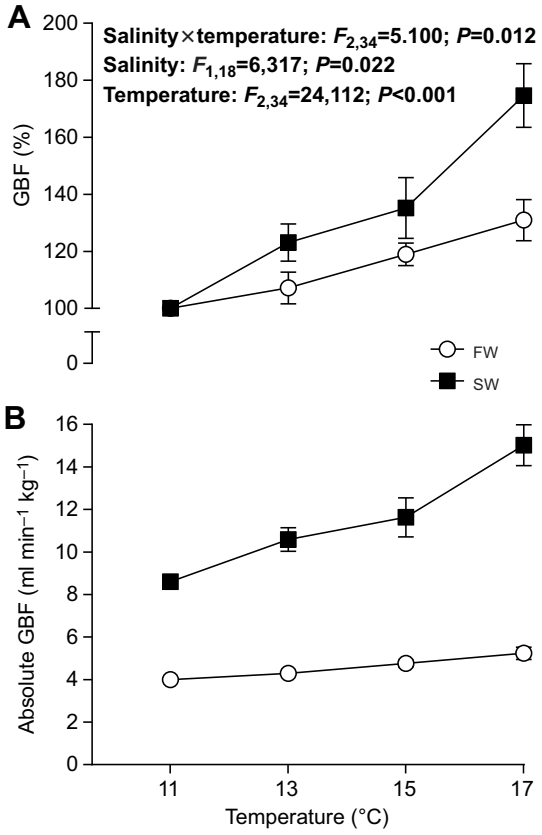

Fig. 2. Gastrointestinal blood flow in rainbow trout during acute warming. (A) Relative gastrointestinal blood flow (GBF) and (B) estimated absolute GBF calculated using the average diurnal basal GBF obtained by Brijs et al., (2016) of trout acclimated to freshwater (FW; open circles) and seawater (SW; filled squares). Statistical analyses generated via mixed model for each variable are presented in each figure with significance (P<0.05) in bold. Values are means±s.e.m. (n=9 for FW and n=10 for SW rainbow trout).

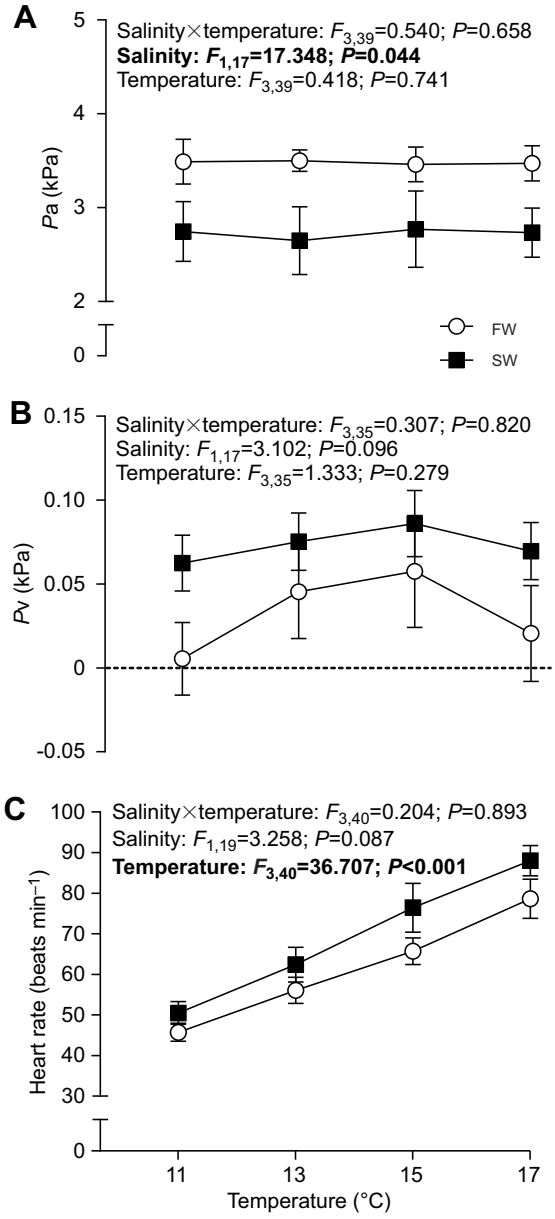

Fig. 3. Cardiovascular variables of rainbow trout during acute warming. (A) Dorsal aortic blood pressure (Pa), (B) Central venous blood pressure (Pv) and (C) heart rate of trout acclimated to freshwater (FW; open circles) and seawater (SW; filled squares). Statistical analyses generated via mixed model for each variable are presented in each figure with significance (P<0.05) in bold. Values are means±s.e.m. (n=7–9 for FW and n=6–9 for SW rainbow trout).

The relative GBF increased significantly in both FW and SW fish during the acute increase in water temperature (Fig. 2A). However, the two salinity treatment groups responded markedly different, as the rate of increase in GBF with warming was much greater in SW (temperature effect$_{SW}$: $F_{2,34}$=26.221, P<0.001) compared with FW (temperature effect$_{FW}$: $F_{2,34}$=4.152, P=0.024). Accordingly, the Q_{10} value for GBF was significantly higher in SW (2.6±0.3) compared with FW trout (1.6±0.1, salinity effect: $t_{13.3}$=−3.161, P=0.007). Indeed, SW trout had a significantly higher relative GBF throughout the temperature protocol (salinity effect: $F_{1,18}$=6.317, P=0.022; Fig. 2A), but since the GBF measurements are relative and the effects of temperature and salinity are interacting the effects of salinity cannot be statistically separated from the effects of temperature. However, when recalculated to absolute values, the GBF at 17°C was 5.2 ml min^{-1} kg^{-1} in FW and nearly threefold higher (15.0 ml min^{-1} kg^{-1}) in SW (Fig. 2B). This suggests that the relative measurements underestimated the increase in GBF in SW-acclimated trout, and that the stimulatory effect of increased salinity on GBF is a general pattern across temperatures (Fig. 2B).

At 11°C, the Pa was 3.3±0.2 kPa in FW trout and 2.7±0.3 kPa in SW trout, and there was a significant salinity effect across temperatures (Fig. 3A). However, the Pv at 11°C was 0.01 kPa ±0.02 in FW and 0.06±0.02 kPa in SW, but there was no significant salinity acclimation effect (Fig. 3B). There were no changes with warming in either Pa (Fig. 3A) or Pv (Fig. 3B). With heart rates of 45.7±2.2 beats min^{-1} in FW and 50.5±2.8 beats min^{-1} in SW at 11°C, the fish of the second experimental series showed no significant differences in heart rate between salinity acclimation groups either, and both groups also responded very similarly to warming as fish in the first experimental series (Q_{10} of 2.6±0.3 in FW vs. 2.7±0.3 in SW fish; salinity effect: U=46, P=0.666; Fig. 3C).

Hematological responses to warming across salinities

Hematocrit and [hemoglobin] were lower in the SW-acclimated trout at both 11 and 17°C (salinity effect: $F_{1,15}$=10.918, P=0.005 and $F_{1,15}$=6.286, P=0.024, respectively; Table 2) and decreased

Journal of Experimental Biology (2019) 222, jeb207522. doi:10.1242/jeb.207522

Table 2. Hematological variables of FW and SW rainbow trout

Measured variables		FW	SW
Hematocrit (%)	11°C	25.4±0.8	20.6±1.1*
	17°C	23.6±0.7‡	19.0±1.4‡,*
[Hemoglobin] (g l^{-1})	11°C	70.6±3.2	58.9±3.1*
	17°C	64.9±4.0‡	52.8±3.8‡,*
MCHC (g l^{-1})	11°C	279.4±9.4	285.3±8.9
	17°C	274.8±10.4	277.3±9.8

MCHC, mean corpuscular hemoglobin content. Values are means±s.e.m. (FW, *n*=8; SW, *n*=9). *Significant salinity effect; ‡Significant temperature effect. Statistical significance at *P*<0.05.

with temperature in both salinity treatments (temperature effect: $F_{1,15}$=13.096, *P*=0,003 and $F_{1,15}$=14.217, *P*=0.002, respectively). However, MCHC was not significantly affected by salinity acclimation (salinity effect: $F_{1,15}$=0.202, *P*=0.659) and temperature (temperature effect: $F_{1,15}$=0.384, *P*=0.545; Table 2).

When not accounting for sampling site (i.e. arterial and venous blood), acclimation to SW generally resulted in higher plasma osmolality (salinity effect: $F_{1,16}$=73.734, *P*<0.001), [K^+] (salinity effect: $F_{1,16}$=21.632, *P*<0.001), [Na^+] (salinity effect: $F_{1,16}$=49.756, *P*<0.001) and [Cl^-] (salinity effect: $F_{1,16}$=54.227, *P*<0.001; Table 3) when measured at 11°C. Moreover, increasing temperatures elevated [K^+] (temperature effect: $F_{1,16}$=9.280, *P*=0.008; Table 3) across salinities. However, [Na^+] and [Cl^-] were affected differently by temperature depending on acclimation salinity (salinity×temperature effect: $F_{1,16}$=17.867, *P*=0.001; $F_{1,15}$=8.221, *P*=0.012 for [Na^+] and [Cl^-], respectively), as the 6°C increase resulted in a significant decrease in [Na^+] (temperature $effect_{FW}$: $F_{1,15}$=5.636, *P*=0.032) in FW-acclimated fish, while in SW-acclimated trout both [Na^+] (temperature $effect_{SW}$: $F_{1,17}$=11.735, *P*=0.003) and [Cl^-] (temperature $effect_{SW}$: $F_{1,16}$=8.440, *P*=0.011; Table 3) increased with warming. There was no statistically significant general effect of temperature on osmolality (temperature effect: $F_{1,16}$=2.647, *P*=0.124, Table 3). Moreover, there were relatively minor differences in plasma ion composition between arterial and venous blood, but [K^+] was significantly higher in arterial blood of freshwater-acclimated trout at 17°C and at 11°C in seawater-acclimated trout (sampling site effect: $F_{1,16}$=13.302, *P*=0.002, Table 3). Osmolality was significantly lower in arterial blood at 11°C in FW trout (sampling site effect: $F_{1,13}$=9.268, *P*=0.009, Table 3).

Pa_{O_2} was significantly higher in FW fish at 11°C (16.6 kPa±0.4 vs. 13.6±1.2 kPa; salinity effect 11°C: t_{10}=2.423, *P*=0.036), and this difference was maintained at the highest temperature (salinity effect: $F_{1,16}$=8.181, *P*=0.011, Fig. 4). Pv_{O_2} was 5.6±0.7 kPa for FW and 5.7±0.3 kPa for SW trout at 11°C and did not significantly differ between salinity acclimation treatments at any of the temperatures (salinity effect: $F_{1,16}$=0.130, *P*=0.723; Fig. 4). Nonetheless, both Pa_{O_2} and Pv_{O_2} decreased significantly with warming, where Pa_{O_2} was 15.0±0.5 kPa in FW and 12.0±1.0 kPa in SW and Pv_{O_2} was 5.2±0.6 in FW and 4.2±0.2 in SW at 17°C (temperature effect: $F_{1,15}$=16.995, *P*=0.001; $F_{1,15}$=7.911, *P*=0.013 for Pa_{O_2} and Pv_{O_2}, respectively, Fig. 4).

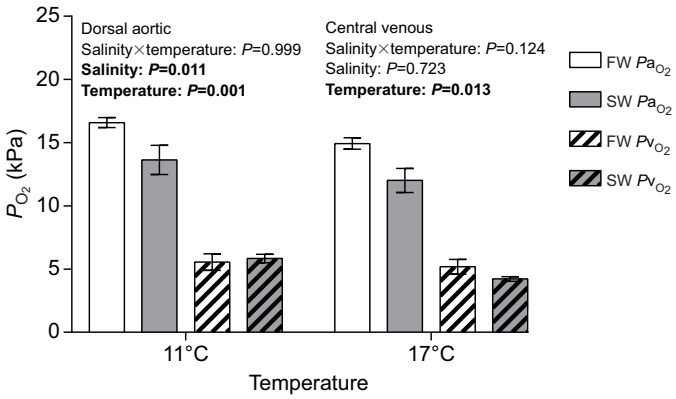

Fig. 4. Arterial and venous P_{O_2} of rainbow trout during acute warming. Dorsal aortic (Pa_{O_2}) and central venous partial pressure of O_2 (Pv_{O_2}) of trout acclimated to freshwater (FW) and seawater (SW). Statistical analyses generated via mixed model for each variable are presented in each figure with significance (*P*<0.05) in bold. Values are means±s.e.m. (*n*=8–9 for both FW and SW rainbow trout).

DISCUSSION

Seawater acclimation exacerbates GBF responses to warming

Prior to this study, only a few other studies had examined the effect of temperature on GBF dynamics in fish (Brijs et al., 2018; Gräns et al., 2009, 2013), but none at different salinities and in combination with indicators of osmoregulatory function. Indeed, the O_2 demand of gastrointestinal tissues increases during warming, which is met by elevations in GBF in both freshwater (Brijs et al., 2018; Gräns et al., 2009) and marine fishes (Gräns et al., 2013). Consistent with these previous studies, GBF increased with warming across salinity acclimation groups of the present study. However, in accordance with our hypothesis, the relative increase in GBF in response to warming was markedly greater in SW, as SW-acclimated trout increased relative GBF by 75% during warming from 11°C to 17°C (Q_{10} of ∼2.6), whereas the GBF increased by only 31% in FW (Q_{10} of ∼1.6). The significance of this finding is even clearer when converting our relative values to absolute numbers using the routine GBF for FW- and SW-acclimated trout reported by Brijs et al. (2016). They showed

Table 3. Ion composition and osmolality of arterial and venous plasma of FW and SW rainbow trout during acute warming

		Freshwater		Seawater	
	Sampling site	11°C	17°C	11°C	17°C
[K^+] (mmol l^{-1})	Arterial	2.3±0.1	2.7±0.2‡,§	2.9±0.1*,§	3.1±0.2
	Venous	2.2±0.1	2.3±0.2	2.7±0.1*	3.1±0.2*,‡
[Na^+] (mmol l^{-1})	Arterial	153.4±2.4	150.6±2.8	183.1±4.0*	191.1±5.4*,‡
	Venous	156.5±2.9	152.9±2.5	186.7±5.2*	188.2±5.3*
[Cl^-] (mmol l^{-1})	Arterial	123.9±2.5	121.9±4.2	154.1±3.8*	161.6±5.0*,‡
	Venous	127.2±3.8	124.4±2.3	155.8±4.8*	156.6±5.0*
Osmolality (mOsm kg^{-1})	Arterial	298.2±2.3§	298.8±4.3	355.3±6.9*	359.6±12.1*
	Venous	303.4±2.7	303.2±2.8	356.3±7.2*	365.4±9.1*,‡

The variables were obtained from the dorsal aorta (arterial) and sinus venosus (venous) at 11°C and 17°C. All values are means±s.e.m. (*n*=7–9). *Significant salinity effect; ‡significant temperature effect; §significant differences between arterial and venous blood. Statistical significance at *P*<0.05.

that the GBF in FW rainbow trout at 10°C was 4 ml min^{-1} kg^{-1} and more than twofold in SW-acclimated trout (8.6 ml min^{-1} kg^{-1}). Thus, combining these routine values with the relative changes in GBF recorded here during acute warming, it can be estimated that the absolute GBF at 17°C may be up to three times greater in SW-acclimated trout (15.0 vs. 5.2 ml min^{-1} kg^{-1}) compared with FW trout.

There are several possible reasons for this striking difference in GBF response to warming across salinities. The osmoregulatory role of the gastrointestinal tract in FW is mostly related to fluxes of ions and water during digestion (Bucking and Wood, 2006a,b), whereas in marine fish, the gut is constantly processing imbibed SW to replenish water passively lost to the environment (Shehadeh and Gordon, 1969). Moreover, consistent with the osmo-respiratory compromise, increases in gill ventilation and branchial blood perfusion during warming can be expected to exacerbate diffusional loss of water in marine fish. This should be compensated for by increased intestinal water absorption (Evans, 1969), which is powered by the osmotic gradient created through active transport of ions from the intestinal lumen into the lateral interspace between enterocytes (Grosell, 2006). However, such elevated rates of ion and water absorption may not only require increased blood flow to sustain elevated O_2 demands (i.e. for ATP production), but also for the convective transport of absorbed water, as well as ions and acid–base equivalents resulting from the processing of SW to their respective sites of excretion. While the SW-acclimated trout in this study showed significant increases in plasma [Cl^-] and [Na^+] with warming, which is consistent with an osmo-respiratory compromise, these changes were relatively minor and within range of steady state values for teleosts (Evans, 1993), suggesting sufficient osmoregulatory function within the temperature range examined here.

Vascular resistance dictates gastrointestinal blood flow across salinities and temperatures

The reduced *P*a and increased cardiac output in SW-acclimated fish prior to warming in the two experimental series of the present study suggests a reduced systemic vascular resistance in SW. This, corroborates the findings of Sundell et al. (2018) who measured arterial pressure and cardiac output in the same fish at different acclimation salinities. Moreover, using the calculated absolute GBF at 11°C, it appears that a substantially higher proportion of cardiac output was directed to the gastrointestinal tract in SW fish (42% of cardiac output) compared to FW trout (25% of cardiac output). Taking into account the reduced blood pressure and systemic vascular resistance in SW-acclimated trout (Sundell et al., 2018), this suggests that reduced gastrointestinal vascular resistance and not elevated arterial blood pressure explains the elevated gastrointestinal blood flow of SW trout. However, the lower *P*a in SW-acclimated trout may also be partly attributed to reduced blood volume, as previously reported by Olson and Hoagland (2008).

Fish at both salinities responded to temperature increase by elevating cardiac output and GBF with no significant changes in *P*a, indicating that gastrointestinal vascular resistance was reduced further at the elevated temperature. Indeed, at 11°C, cardiac output was 34% higher in SW-acclimated trout and this difference increased to 55% at 17°C. Consequently, when the proportion of cardiac output allocated to the gut is estimated using the calculated absolute GBF values, it remained relatively stable at both salinities and across temperatures, being 25% in FW and 47% in SW at 17°C. Generally, cardiac output increases with warming until temperatures approach the critical thermal maximum of the fish (Ekström et al., 2014). It is currently unknown whether SW acclimation has any effect on maximum cardiac output or the temperature where cardiac output fails at critically high temperatures in fish. Even so, if maximum cardiac output is similar across salinities, and fish in SW require larger increases in cardiac output to support the greater elevations in GBF with warming, it can be speculated that their cardiac scope is reduced at a faster pace. While this would ultimately lead to an inability to further elevate GBF with increasing temperatures and negatively influence osmotic homeostasis of fish in SW, future studies on maximum cardiac performance across salinities and at critically high temperatures would be instrumental to resolve these questions. In fact, evidence for a correlation between aerobic capacity, intestinal artery blood flow (branches from the celiacomesenteric artery) and osmoregulatory function is given by two studies on chinook salmon (*Oncorhynchus tshawytscha*) chronically trained at different water flow intensities in SW (Gallaugher et al., 2001; Thorarensen et al., 1993). Fish trained at high water velocities had a higher maximum oxygen consumption rate and could sustain GBF up to higher swimming speeds, which could explain why they were able to better maintain plasma osmolality (Gallaugher et al., 2001; Thorarensen et al., 1993).

Cardiac function is maintained in SW-acclimated trout across temperatures

Fish acutely transferred from FW to SW may experience a transient decrease in Pa_{O_2}, followed by a recovery to original values within 6 to >30 h (Larsen and Jensen, 1993; Madsen et al., 1996; Maxime et al., 1991; Stagg et al., 1989). This is thought to result from the dehydration of the branchial tissues impairing O_2 diffusion and subsequent rehydration upon acclimation to SW (Madsen et al., 1996). The trout used in this study had been acclimated to SW for a minimum of 4 weeks; it is therefore unlikely that the observed lower Pa_{O_2} was due to the dehydrating effect of SW on the gills. Nevertheless, despite Pa_{O_2} being initially lower in SW-acclimated trout in the present study, both acclimation groups showed a similar Pv_{O_2}. As the SW-acclimated fish had a significantly lower [hemoglobin] and higher cardiac output, this likely reflects a decreased tissue O_2 extraction in SW trout. This could also explain why some studies have reported little or no change in O_2 consumption with SW acclimation (Brijs et al., 2015; Ern et al., 2014), despite substantial increases in cardiac output (Maxime et al., 1991; Sundell et al., 2018). To better substantiate this hypothesis, simultaneous measurements of arterial and venous O_2 content of fish in both salinities would be required. Another possibility is that fish in SW have a lower venous blood hemoglobin O_2 affinity. SW acclimation is associated with blood acidosis in fish, as it is partly reliant on the endogenous production of HCO_3^- and consequent liberation of protons (Genz et al., 2008; Grosell and Taylor, 2007; Wilson and Grosell, 2003). The HCO_3^- is secreted into the intestinal lumen in exchange for Cl^- and contributes to the osmotic gradient that drives water absorption across the intestine (Grosell, 2006). Therefore, a drop in blood pH in SW would result in a right shift of the hemoglobin O_2 dissociation curve (Bohr effect), explaining why Pv_{O_2} is similar across salinities despite the fact that Pa_{O_2} is initially lower in SW. FW- and SW-acclimated trout in this study also showed decreases in Pv_{O_2} during warming, a well-known response to warming in fish (Clark et al., 2008b). At 17°C, the Pv_{O_2} of FW- and SW-acclimated trout in this study were 5.2 and 4.2 kPa, respectively, which are within previously reported resting Pv_{O_2} values for salmonids in normoxia (Clark et al., 2008b; Steinhausen et al., 2008) and well above the estimated threshold at which cardiac function is impaired (Steffensen and Farrell, 1998). During warming, fish at both salinities displayed

tachycardia-driven elevations in cardiac output, while stroke volume remained unchanged. These cardiovascular responses are in line with previous observations in rainbow trout in both FW and SW (Brijs et al., 2017), as well as in other fish species during acute warming (Ekström et al., 2016; Gollock et al., 2006; Steinhausen et al., 2008). However, contrary to the results of Brijs et al. (2017), the SW-acclimated trout in the present study showed no signs of cardiac impairment and they maintained a higher cardiac output and stroke volume than their FW-acclimated conspecifics across test temperatures. Thus, the initial hypothesis that increased O_2 extraction from osmoregulatory processes could lead to greater reductions in $P_{V_{O_2}}$ in SW and impair cardiac function through limitations in luminal O_2 supply to the heart was clearly not supported, at least not within the temperature range analyzed in this study.

Conclusions and future perspectives

This is the first study examining how acclimation to different salinities affects GBF dynamics and osmotic homeostasis in response to warming in a fish. Our results indicate that the GBF dynamics of the rainbow trout are remarkably tuned to compensate for osmotic imbalances resulting from acute environmental warming. While the gills have been much more extensively studied in the context of the osmo-respiratory compromise because of their important role in the exchange of gases, water and osmolytes, the present study is a novel addition, as the role of the gastrointestinal tract and its blood supply in maintaining osmotic balance has so far received much less attention. Rainbow trout (i.e. steelhead strain) naturally undertake migrations between salinities as part of its life cycle, facing not only osmotic challenges but also acute fluctuations in temperature (e.g. while moving across the thermocline or along the way to their in-river spawning grounds), the severity of which are exacerbated by global warming. Therefore, the results presented here improve the understanding of the physiological mechanisms salmonids, and perhaps euryhaline fishes in general, employ at different salinities to cope with an environmental driver that is becoming increasingly detrimental to both wild and farmed fish populations as average water temperatures and the incidence of heatwaves increase. Feeding status (i.e. post absorptive vs. postprandial), degree of activity or life stage may have additional implications for the distribution of gastrointestinal blood flow and consequently osmotic balance. Studies of combined environmental drivers at different physiological states are therefore essential areas of future research to better understand the ecological implications of global warming and its associated environmental changes in aquatic habitats.

Acknowledgements

We gratefully acknowledge Jeroen Brijs for his mentorship throughout the initial stage of the study.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: E.S.; Methodology: D.M., E.S.; Validation: D.M.; Formal analysis: D.M., A.G.; Investigation: D.M., A.E., P.H.; Resources: D.M., A.E., M.A., E.S.; Data curation: D.M., A.G.; Writing - original draft: D.M.; Writing - review & editing: D.M., A.E., P.H., A.G., M.A., E.S.; Visualization: D.M.; Supervision: A.G., M.A., E.S.; Project administration: D.M., E.S.; Funding acquisition: D.M., E.S.

Funding

This work was supported by the Swedish Research Council (Vetenskapsrådet; 2011–04786 to E.S.), the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (Svenska Forskningsrådet Formas; 2016-00729 to E.S.), Helge Ax:son Johnsons Foundation (D.M.) and the Wilhelm and Martina Lundgren Research foundation (D.M.).

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