Journal of Comparative Physiology B (2020) 190:205–217
https://doi.org/10.1007/s00360-020-01259-4

ORIGINAL PAPER

Reverse translation: effects of acclimation temperature and acute temperature challenges on oxygen consumption, diffusive water flux, net sodium loss rates, Q_{10} values and mass scaling coefficients in the rainbow trout (*Oncorhynchus mykiss*)

John O. Onukwufor[1,2] · **Chris M. Wood**[2]

Received: 1 May 2019 / Revised: 17 December 2019 / Accepted: 9 January 2020 / Published online: 21 January 2020

Abstract

Our understanding is limited on how fish adjust the effective permeability of their branchial epithelium to ions and water while altering O_2 uptake rate (MO_2) with acute and chronic changes in temperature. We investigated the effects of acclimation temperature (8 °C, 13 °C and 18 °C) and acute temperature challenges [acute rise (acclimated at 8 °C, measured at 13 °C and 18 °C), acute drop (acclimated at 18 °C, measured at 8 °C and 13 °C) and intermediate (acclimated at 13 °C, measured at 8 °C and 18 °C)] on routine MO_2, diffusive water flux, and net sodium loss rates in 24-h fasted rainbow trout (*Oncorhynchus mykiss*). In the temperature challenge tests, measurements were made during the first hour. In acclimated trout at all temperatures, allometric mass scaling coefficients were much higher for diffusive water flux than for MO_2. Furthermore, the diffusive water flux rate was more responsive (overall $Q_{10}=2.75$) compared to MO_2 ($Q_{10}=1.80$) over the 8–18 °C range, and for both, Q_{10} values were greater at 8–13 °C than at 13–18 °C. The net Na^+ flux rates were highly sensitive to acclimation temperature with an overall Q_{10} of 3.01 for 8–18 °C. In contrast, very different patterns occurred in trout subjected to acute temperature challenges. The net Na^+ flux rate was temperature-insensitive with a Q_{10} around 1.0. Both MO_2 and diffusive water flux rates exhibited lower Q_{10} values than for the acclimated rates in response to either acute increases or decreases in temperature. These results fit Pattern 5 of Precht (undercompensation, reverse effect) and more precisely Pattern IIB of Prosser (reverse translation). These inverse compensatory patterns suggest that trout do not alter their rates very much when undergoing acute thermal challenges (diurnal fluctuations, migration through the thermocline). The greater changes seen with acclimation may be adaptive to long-term seasonal changes in temperature. We discuss the roles of aquaporins, spontaneous activity, and recent feeding in these responses.

Keywords Acute · Chronic · Inverse temperature compensation · Tritiated water · MO_2 · Net Na^+ flux rate · Adaptation · Osmorespiratory compromise

Introduction

The effects of acute temperature challenge and long-term temperature acclimation on aerobic metabolic rate, as assessed by oxygen consumption (MO_2), have been widely studied in aquatic ectotherms (e.g. Fry et al. 1942; Fry and Hart 1948; Peterson and Anderson 1969; Fry, 1971; Brett and Groves 1979; Schurman and Steffensen 1997; Rodnick et al. 2004; Gollock et al. 2006; Perez-Cassanova et al. 2008; Clark et al. 2011; Healy and Schulte 2012; Norin et al. 2014; Chen et al. 2015; Hvas et al. 2017). In most fish, Q_{10} values are close to or above 2.0, with values for acute temperature challenge tending to be higher than those for acclimation.

Communicated by B. Pelster.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00360-020-01259-4) contains supplementary material, which is available to authorized users.

✉ John O. Onukwufor
john_onukwufor@urmc.rochester.edu

[1] Present Address: Department of Anesthesiology and Perioperative Medicine, University of Rochester Medical Center, Rochester, NY 14642, USA

[2] Department of Zoology, University of British Columbia, Vancouver, BC V6T 1Z4, Canada

Clearly, the stimulatory effects of increased temperature (or inhibitory effects of decreased temperature) on MO_2 are greater than can be explained by actions on simple diffusion and chemical reaction rates alone, where Q_{10} values are close to 1.0. Rather, they must depend on biologically mediated processes (e.g. active transport and facilitated diffusion) where Q_{10} values are close to 2.0, and often above. Increases in MO_2 with temperature are probably linked to the effects of temperature on tissue O_2 demand due in large part to increases in mitochondrial respiration rate (Onukwufor et al. 2015, 2017) accompanied by increases in the effective permeability of the respiratory epithelial membranes, and increases in the convective processes (matching changes in ventilatory flow and cardiac output) which transport oxygen (Sidell et al. 1973; Hazel and Prosser 1974; Cossins and Prosser 1978; Brett and Groves 1979). Furthermore, the higher Q_{10} values for acute temperature challenges indicate that the immediate effects are generally greater than those of acclimation to the same temperatures. Thus longer term homeostatic responses tend to minimize the changes in metabolic rate and accompanying processes.

There are far fewer studies on diffusive water flux rate (measured with tritiated water) than on MO_2, but the modern view is that this occurs mainly through the transcellular route in the respiratory epithelia (gills), mediated in large part by channels (aquaporins) which facilitate the diffusion of water (Tingaud-Sequiera et al. 2010; Cerda and Finn 2010; Madsen et al. 2015). Note that diffusive water flux is not the same as osmotic water flux, which can only be measured indirectly from changes in body weight and urine flow. While both are functions of gill permeability, the relationship between the two is complex (see Potts et al. 1967; Franz, 1968; Motais et al. 1969; Loretz 1979; Isaia 1984; Evans et al. 2005; Kwong et al. 2013). Diffusive water fluxes are unidirectional, very similar in the influx and efflux directions, and very high, while osmotic water fluxes are net fluxes which are much lower (e.g. < 1% of unidirectional diffusive water fluxes). It is impossible to precisely calculate the net flux rate from the difference of unidirectional flux rates. Thus, the latter can be measured in either direction; the efflux method, as used in the present study, is easier, more accurate, and non-destructive. The relatively few measurements of Q_{10} values for diffusive water flux rates in acclimated fish (Evans 1969; Isaia 1972; Loretz 1979; Giacomin et al. 2017, 2019; Onukwufor and Wood 2018) appear to be similar to those for MO_2 (i.e. close to 2.0, supporting the view that they are biologically mediated, probably by aquaporins), while under temperature challenges, these fluxes tend to increase with Q_{10} values slightly higher than those for MO_2. Possible causes could include those also affecting MO_2 (e.g. convective changes, increases in the effective permeability of the respiratory membranes for simple diffusion [i.e. increases in perfused and ventilated branchial surface area, decreases in mean blood-to-water diffusion distance, changes in membrane structure], as well as increases in facilitated diffusive permeability [e.g. aquaporin function]). Unlike MO_2 and diffusive water flux rates that show temperature sensitivity, the few available measurements of temperature effects on net sodium flux rate indicate rather minimal effects (Isaia 1972; Motais and Isaia 1972; Onukwufor and Wood 2018). This difference could be because net Na^+ flux rate is a function of both active uptake and passive loss processes (e.g. Gonzalez and McDonald 2000).

In view of the very limited knowledge on the simultaneous effects on net sodium loss rate, diffusive water flux rate, and MO_2, our objective was to probe the effects of acute and chronic temperatures on the three parameters together in rainbow trout (*Oncorhynchus mykiss*). We reasoned that for MO_2 to increase in response to elevated temperatures, the effective branchial membrane permeability to water exchange and ion loss would also increase as a trade-off for high MO_2 uptake by the gill. This trade-off is termed the osmorespiratory compromise (Randall et al. 1972; Nilsson 1986; Wood and Randall 1973a, b; Gonzalez and McDonald 1992, 1994; Giacomin et al. 2017; Onukwufor and Wood 2018). Factors that could contribute to changes in the effective branchial membrane permeability include functional surface area, mean diffusion distance, simple diffusive permeability, facilitated diffusive permeability, and gill water and blood flow rates and distributions. We have used two complementary schemes that have stood the test of time to interpret our results. Precht's (1958) scheme considers changes between two different temperatures, while Prosser's (1958) scheme considers changes over a wide range of temperatures by looking at rapid (acute) and prolonged changes (acclimation).

With this background in mind, our first prediction was that the sensitivity of diffusive water flux rate to temperature should be similar to that of MO_2 (i.e. similar Q_{10} values). Our second was that the changes observed in acclimated fish should be less (i.e. lower Q_{10} values) than changes in the acutely challenged fish. Our final prediction was that acclimation to different temperatures would not greatly alter the net sodium flux rate as the acclimated fish should regain approximate ionic balance, but that due to the osmorespiratory compromise, much larger changes in this parameter (i.e. higher Q_{10} values) would occur in fish exposed to acute temperature challenges.

Materials and methods

Experimental animals

All experimental procedures were approved by the University of British Columbia (UBC) Animal Care Committee

(Certificate A14-0251) in accordance with the Canadian Council on Animal Care guidelines. Rainbow trout (*Oncorhynchus mykiss*) weighing 103 ± 3 g (SEM, $N = 100$) were obtained from Miracle Springs Inc., Fraser Valley, BC, Canada; at the hatchery, the fish are raised in natural stream water where temperature fluctuates both diurnally and seasonally, and feeding is ad libitum. At UBC, the fish were held initially at 13 °C in dechlorinated Vancouver tapwater containing in mg/L $CaCO_3$ alkalinity, 3.0; hardness 3.3; and in mM: K^+, 0.004, Mg^{2+}, 0.007, Ca^{2+}, 0.03, Cl^-, 0.05, Na^+, 0.06, pH 7.0. Fish were acclimated for a minimum of 2 weeks to either 8 °C, 13 °C or 18 °C prior to experiments. Each acclimation group (approximately 30 fish) was held in a 500-L tank. Acclimation to 13 °C was done in a flow-through tank. Acclimations to 8 and 18 °C were done in static systems with daily exchange of 80% of the water with aged water. During this period, fish were fed at a ration of 1% of their body weight daily with commercial diet (EWOS, Surrey, BC, Canada). Trout used for experiments were sampled from their acclimation tanks prior to the daily feeding and, therefore, had been fasted for 24 h. The photoperiod was maintained at 12L:12D throughout the experimental periods.

Experimental protocol

Experimental procedures were performed as described in detail by Onukwufor and Wood (2018). Briefly, to avoid visual disturbance, all containers were shielded in black plastic and fitted with sampling ports and aeration devices. For all procedures, tanks were placed in a table-trough that served as constant-temperature bath set to the appropriate temperature (8, 13, or 18 °C), and normoxic conditions (> 80% air saturation) were maintained throughout. In each trial, 4–5 trout were placed together in a 2-L loading bath of water labelled with 40 µCi of tritiated water (^3H_2O, Perkin Elmer, Woodbridge, ON, Canada), and maintained at the acclimation temperature. Our pilot study showed that equilibration was complete within 6 h. Following 6 h of equilibration, trout were netted individually out of the loading bath, rinsed in clean water to remove any ^3H_2O on the body surface, and transferred to individual containers containing 1 l of clean water at the test temperature. The air exposure period was < 10 s. Fish equilibrate very quickly upon transfer to a new temperature, with 90–95% thermal equilibration occurring within 1–5 min (Crawshaw and Hammel 1974; Crawshaw 1979; Moffitt and Crawshaw 1983). Therefore, water samples (5 ml) for ^3H_2O analysis were taken at time zero and thereafter every 5 min for 60 min. Additional water samples (10 ml) for Na^+ analysis were taken at 0 and 60 min. After the 60-min water sampling, a further 6 h was allowed to ensure that ^3H_2O washout was complete before taking the final water sample. The fish were then weighed.

For MO_2 measurements, our goal was to determine routine MO_2 under conditions as close as possible to those used for diffusive water and Na^+ flux measurements (i.e. a similar degree of handling disturbance). Separate fish were used. Briefly, 4–5 trout were sampled from the holding tank and confined in 2 l of water for 6 h at the acclimation temperature (i.e. sham ^3H_2O loading), then individually netted, rinsed, and transferred to the individual experimental containers containing 1 l of water. Fish were allowed 30 min in the container after which the aeration device was removed, and the initial water PO_2 was recorded using a portable dissolved oxygen (DO) probe and meter (YSI Model 55, Yellow Spring, OH, USA). The DO probe was removed, and the container was then sealed for 10 min, after which a final PO_2 measurement was made. This short measurement period ensured that water PO_2 remained > 50% air saturation at all experimental temperatures. Blank tests demonstrated that there was no detectable microbial respiration, even at the highest temperature, so no blank corrections were required.

Effects of acclimation temperature on diffusive water, net Na^+ flux rates and MO_2

Fish were loaded with ^3H_2O as outlined above, at their respective acclimation temperatures of 8 °C ($N = 9$), 13 °C ($N = 9$) and 18 °C ($N = 9$), and then the experiment (diffusive water and Na^+ flux rates) was carried out in the standard fashion at this same temperature. The MO_2 measurement was also done in the standard fashion for separate fish ($N = 9$) at each acclimation temperature.

Effects of acute temperature challenge on diffusive water, net Na^+ flux rates and MO_2

In 8 °C-acclimated fish (for acute temperature challenges), trout were first loaded with ^3H_2O in the standard fashion at 8 °C. They were then acutely transferred to either 8 °C [acclimation control ($N = 10$)], 13 °C ($N = 10$), or 18 °C ($N = 10$) for diffusive water and Na^+ flux measurements in the standard fashion. For 13 °C-acclimated fish, trout were first loaded with ^3H_2O in the standard fashion at 13 °C and then acutely transferred to 13 °C [acclimation control ($N = 10$)], 8 °C ($N = 10$) or 18 °C ($N = 10$) for flux measurements in the standard fashion. For 18 °C-acclimated fish, trout were first loaded with ^3H_2O in the standard fashion at 18 °C. Fish were then acutely transferred to 18 °C [acclimation control ($N = 10$)], 13 °C ($N = 10$) or 8 °C ($N = 10$) for flux measurements in the standard fashion. In all these acute temperature challenge experiments, MO_2 measurements were performed in the standard fashion using separate fish.

Analytical techniques and calculations

The concentration of ^3H_2O in water samples was analyzed using a scintillation counter (LS6500, Beckman Coulter, Fullerton, CA, USA) as described by Onukwufor and Wood (2018). Briefly, 10 ml of Optiphase 3 fluor (Perkin-Elmer, Wellesley, MA, USA) was added to the 5-ml water sample. Quenching was constant as demonstrated in our internal standardization tests so there was no need for correction. The rate constant of ^3H_2O efflux, expressed as a decimal fraction of the total exchangeable body water pool per hour (h^{-1}), which is approximately exponential with time (Evans 1967), was calculated by determining the rate of decline in the total tritiated water content in the fish:

$$k = \left(\ln \mathrm{CPM}_1 - \ln \mathrm{CPM}_2\right)/\left(\mathrm{time}_1 - \mathrm{time}_2\right), \quad (1)$$

where k is the rate constant of the efflux (in h^{-1}), CPM_1 = total ^3H_2O radioactivity (in cpm) in the fish at $time_1$ (in h), and CPM_2 = total ^3H_2O radioactivity (in cpm) in the fish at $time_2$ (in h). The product of $k \times 100\%$ yields the percentage of body water turned over per hour. ^3H_2O efflux rates were calculated by regressing the natural logarithm of CPM measurements against time over the 1-h measurement period to yield the slope k.

By measuring the ^3H_2O radioactivity in the water after 6 h, when the fish had completely equilibrated with the known volume of water, we were able to calculate the total amount of ^3H_2O radioactivity (CPM_{total}) in the system. By additionally taking into account the total amount of ^3H_2O removed in each water sample, we were able to accurately back-calculate the ^3H_2O CPM in the fish at each time point of the trial.

To obtain the actual diffusive water flux rates (ml h^{-1}), the rate constants of water efflux (k) were used with the assumption that 80% of the body mass of fish is occupied by exchangeable water (Holmes and Donaldson 1969; Isaia 1984; Olson 1992).

$$\text{Diffusive water flux rate } \left(\text{ml h}^{-1}\right) = M \times k \times 0.8, \quad (2)$$

where M = fish weight, k = rate constant, and 0.8 = fractional water pool.

In order to take into account the possible influence of body mass, the logarithm of diffusive water flux (Y = ml h^{-1}) was regressed against the logarithm of fish weight (M), with intercept (a) and slope (b), the latter representing the allometric mass scaling coefficient:

$$Y = aM^b \quad (3)$$

The allometric mass scaling coefficient (b) was used to correct the observed flux rate of an individual fish ($X_{obs,}$ ml h^{-1}) of mass M_{obs} (g) to that (X_{corr}) of a 100-g fish (mid-range of the fish used in the experimental tests):

$$X_{corr} = X_{obs} \times 10^{b \times \log(100g/Mobs)} \quad (4)$$

To obtain the flux rate in ml g^{-1} h^{-1} the value was then divided by 100 g.

PO_2 values were converted to µmol O_2 l^{-1} using oxygen solubility coefficients at the appropriate temperature from Boutilier et al. (1984). MO_2 (µmol h^{-1}) was calculated from changes in O_2 concentration, factored by time and chamber volume, which was corrected for the volume of the fish. The logarithm of MO_2 was regressed against that of fish weight to account for body mass using Eq. 3 above, and the data were allometrically scaled to 100 g using Eq. 4. Scaling coefficients are reported in the "Results".

The concentration of Na^+ was determined using flame atomic absorption spectrophotometry (AAnalyst 800, Perkin-Elmer, Wellesley, MA, USA). Certified reference materials (CRM) BURTAP-05 (Environment Canada, Burlington, ON) and blanks were analyzed together with water samples, with a recovery rate of 93–102%. The limit of quantification was 0.5 µmol l^{-1}. As the results demonstrated that allometric scaling was not appropriate for net Na^+ flux rate (J^{Na}_{net}, µmol g^{-1} h^{-1}), it was simply calculated from changes in Na^+ concentration, factored by time, body weight, and chamber volume.

The temperature coefficients (Q_{10} values) for MO_2, diffusive water flux and net Na^+ flux rates for temperature-acclimated fish, and for fish subjected to acute temperature transfers, were calculated for the temperature ranges 8–13 °C, 13–18 °C and 8–18 °C using the van't Hoff equation:

$$Q_{10} = \left(R_2/R_1\right)^{[10/(T_2-T_1)]}, \quad (5)$$

where R_2 and R_1 represent MO_2, diffusive water flux, or net Na^+ flux rates at two temperatures T_2 and T_1, and where $T_2 > T_1$. As different fish were used in each trial, we used mean values in calculating Q_{10} for both temperature-acclimated fish, and acutely temperature-challenged fish.

Statistical analyses

All data have been expressed as the mean ± SEM (N). One-way ANOVA (with temperature as the independent variable) was applied in all series, followed by Tukey's post hoc test to identify means that were significantly different from one another. Prior to test, data were checked for normality and homogeneity of variances, and where data failed the test, transformations were applied. Some data passed after square root transformation, and the remaining failing data were analyzed using Kruskal–Wallis One Way Analysis of Variance on Ranks. A significance level of $p < 0.05$ was used

throughout. SigmaPlot 11 (Systat Software, CA, USA) was employed for all linear regression, curve fitting, and statistical analyses.

Results

Allometric scaling of diffusive water flux rates, MO_2, and sodium net loss rates

Mean fractional water turnover rates (k, Eq. 1) increased substantially with acclimation temperature ($p < 0.05$) from 0.462 ± 0.011 h^{-1} at 8 °C to 0.798 ± 0.031 at 13 °C and 1.212 ± 0.059 at 18 °C (all $N = 10$). When k values were converted to diffusive water flux rates by Eq. 3, there were significant log-linear relationships (Fig. 1a–c) at all three temperatures. The latter yielded the allometric scaling coefficients which were 0.990 at 8 °C, 1.054 at 13 °C, and 0.875 at 18 °C. These values did not differ significantly ($p > 0.05$). For MO_2, there were also significant log-linear relationships (Fig. 2a–c) at all three temperatures. The latter yielded much lower allometric scaling coefficients which were 0.38 at 8 °C, 0.57 at 13 °C and 0.30 at 18 °C, and again there were no significant differences ($p > 0.05$) among these values. The scaling coefficients for diffusive water flux were significantly ($p < 0.05$) higher than those of MO_2. In each case the allometric coefficients were used to scale the experimental data to those of a 100-g fish by Eq. 4. These same coefficients (based on the temperature of acclimation) were used to scale the data for the temperature challenge experiments. For example, for an 8 °C acclimated trout acutely transferred to 18 °C, a scaling coefficient of 0.990 was used for water flux and 0.38 for MO_2. None of the relationships between J^{Na}_{net} and body weight were significant at any temperature (data not shown).

Effect of acclimation temperature on diffusive water flux rates, MO_2 and Na^+ net flux rates

Diffusive water flux rates increased significantly with acclimation temperature with significantly different rates at all three temperatures with an overall Q_{10} (8–18 °C) of 2.75 (Fig. 3a). MO_2 also increased significantly with acclimation temperature (Fig. 3b), but the relative increases were lower than those of diffusive water flux, with an overall Q_{10} (8–18 °C) of 1.80. For both, increases were greater in the 8–13 °C range than in the 13–18 °C range. Net Na^+ flux rates were slightly negative at all three temperatures, and loss rates increased significantly with acclimation temperature (Fig. 3c), in a similar pattern to that of diffusive water flux rates, though Na^+ net loss rates were not significantly different at 8 versus 13 °C. Q_{10} values for Na^+ net loss rates (3.01–3.03) were high and very stable across the temperature range. As net Na^+ flux rates and diffusive water efflux rates were measured on the same animals, we tested whether there were any correlations between these two parameters, but none were significant ($p > 0.05$) at any of the acclimation temperatures.

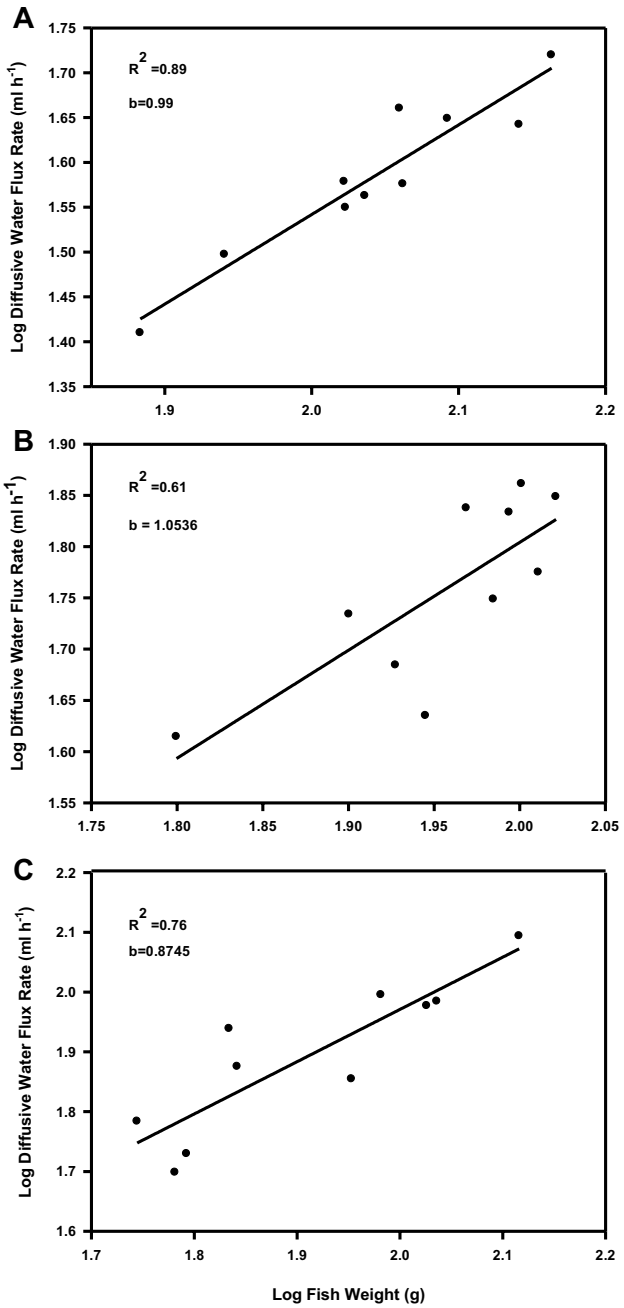

Fig. 1 Log diffusive water flux rates (ml h^{-1}) vs log fish weight (g) plots are shown in **a** 8 °C, **b** 13 °C, and **c** 18 °C ($N = 10$). (Note that the slopes from these regressions were used to calculate the allometric scaling coefficients for both acclimation experiments and acute temperature challenge experiments; see "Materials and methods" for details)

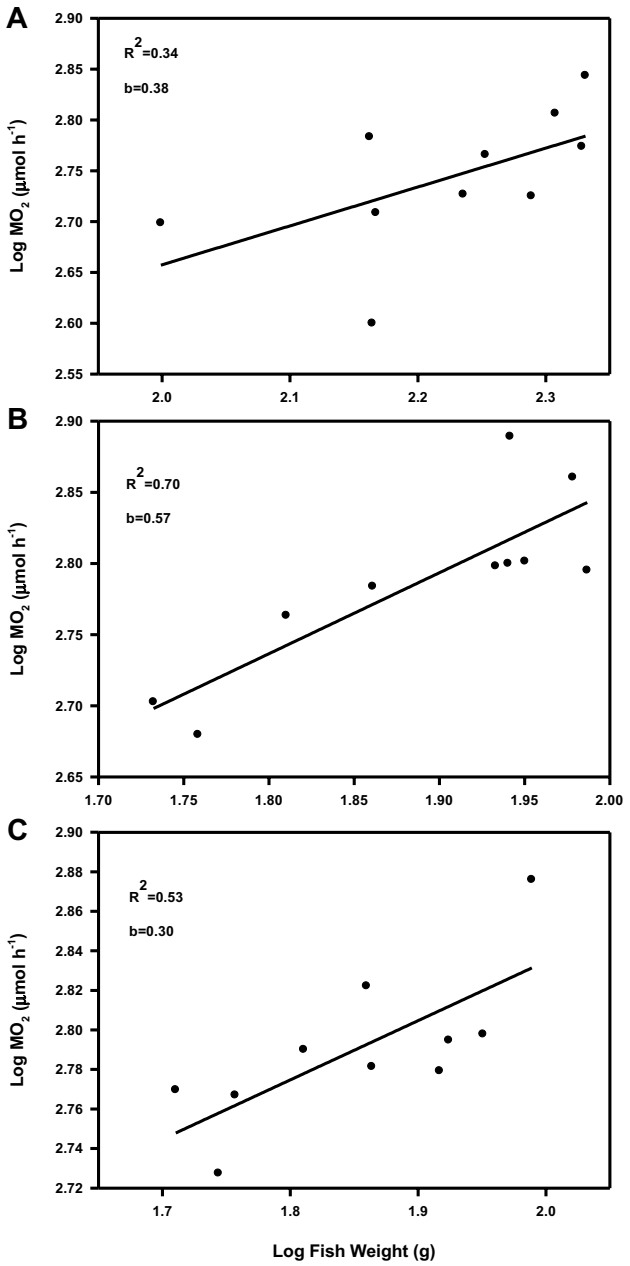

Fig. 2 Log MO_2 (µmol O_2 h^{-1}) vs log fish weight (g) plots are shown in **a** 8 °C, **b** 13 °C, and **c** 18 °C ($N=10$). (Note that the slopes from these regressions were used to calculate the allometric scaling coefficients for both acclimation experiments and acute temperature challenge experiments; see "Materials and methods" for details)

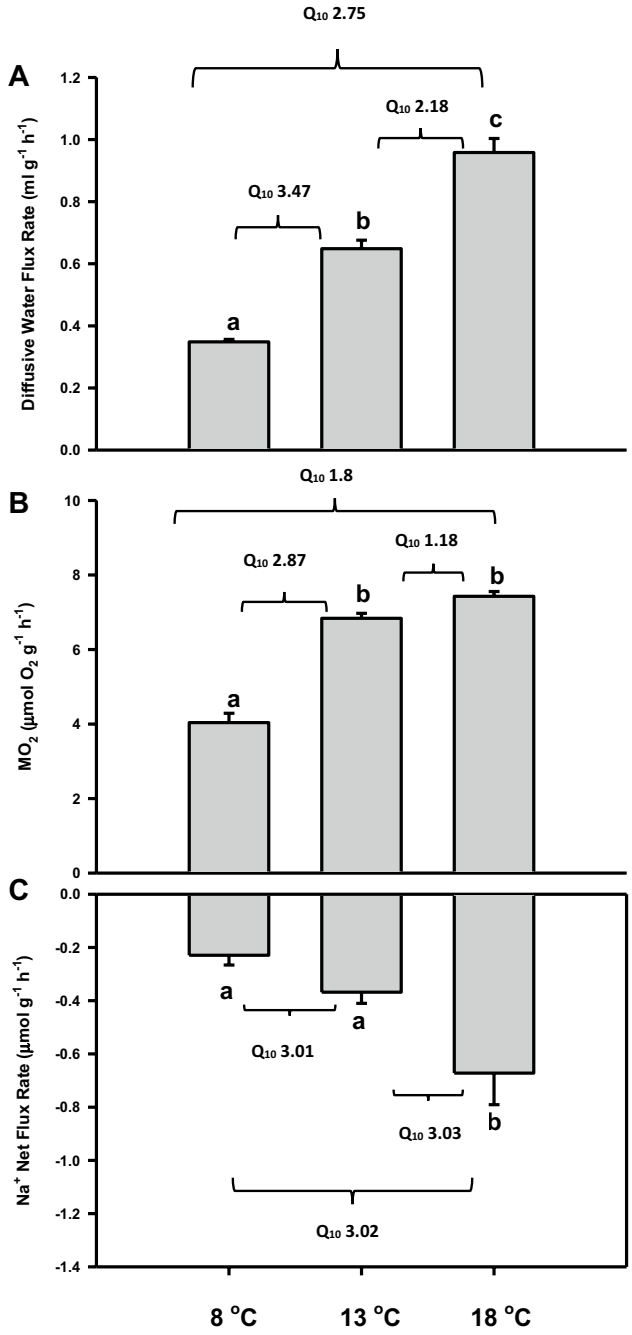

Fig. 3 Effects of acclimation temperature on **a** diffusive water flux rates (ml g^{-1} h^{-1}) and mean Q_{10} values, **b** MO_2 (µmol O_2 g^{-1} h^{-1}) and mean Q_{10} values, and **c** net Na^+ loss rates (µmol g^{-1} h^{-1}) and mean Q_{10} values. Rainbow trout were acclimated to 8 °C ($N=9$), 13 °C ($N=9$) and 18 °C ($N=9$). Different sets of fish were used for each treatment (see "Materials and methods"). Values are means ± SEM. Means not sharing the same letter are significantly different from one another at $p<0.05$

Effects of acute temperature challenge on diffusive water flux rates, MO_2 and net Na^+ loss rates

Data for the acute temperature challenge tests of each of the acclimation groups have been plotted in Figs. 4, 5, 6, using the same format as in Fig. 3. This facilitates display of the Q_{10} values and comparisons of patterns among the three parameters (diffusive water flux, MO_2, and net Na^+ flux rates). In Supplementary Figs S1–S3, the same acute temperature challenge data have been plotted for all treatment groups as a function of each parameter, facilitating comparisons among acclimation groups.

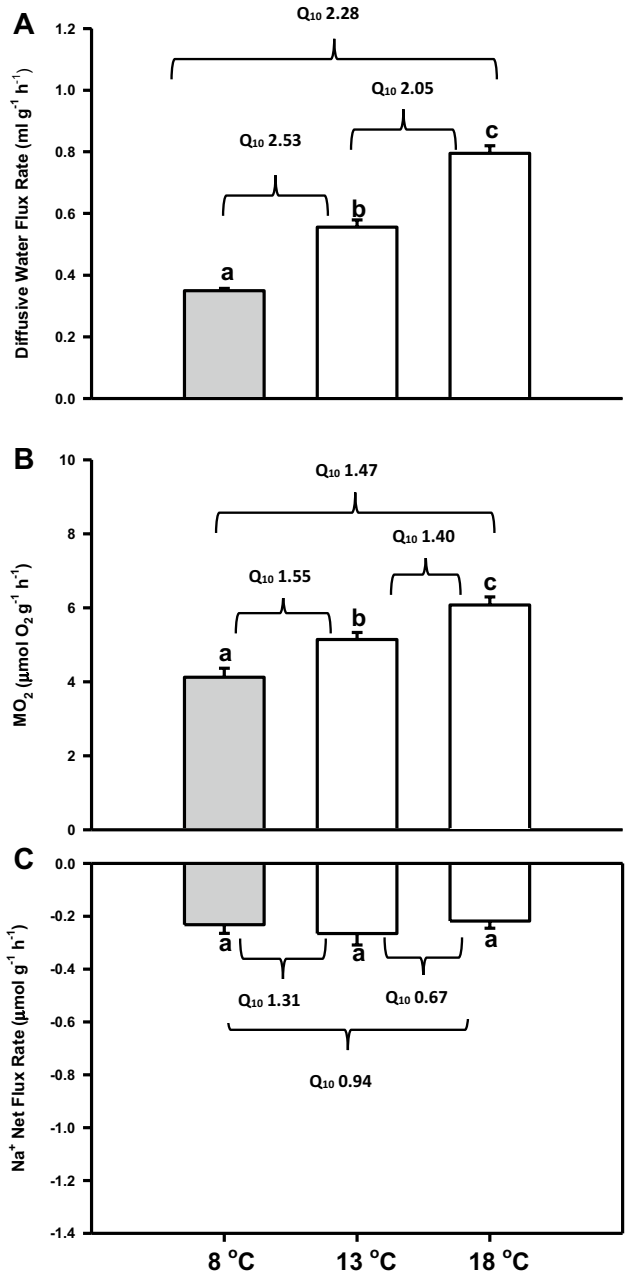

Fig. 4 Effects of acute temperature increases on **a** diffusive water flux rates (ml g^{-1} h^{-1}) and mean Q_{10} values, **b** MO_2 (µmol O_2 g^{-1} h^{-1}) and mean Q_{10} values, and **c** net Na^+ loss rates (µmol g^{-1} h^{-1}) and mean Q_{10} values. Rainbow trout were acclimated (gray bars) to 8 °C ($N = 10$), and acutely challenged (white bars) with 13 °C ($N = 10$) or 18 °C ($N = 10$). Different sets of fish were used for each treatment (see "Materials and methods"). Values are means ± SEM. Means not sharing the same letter are significantly different from one another at $p < 0.05$

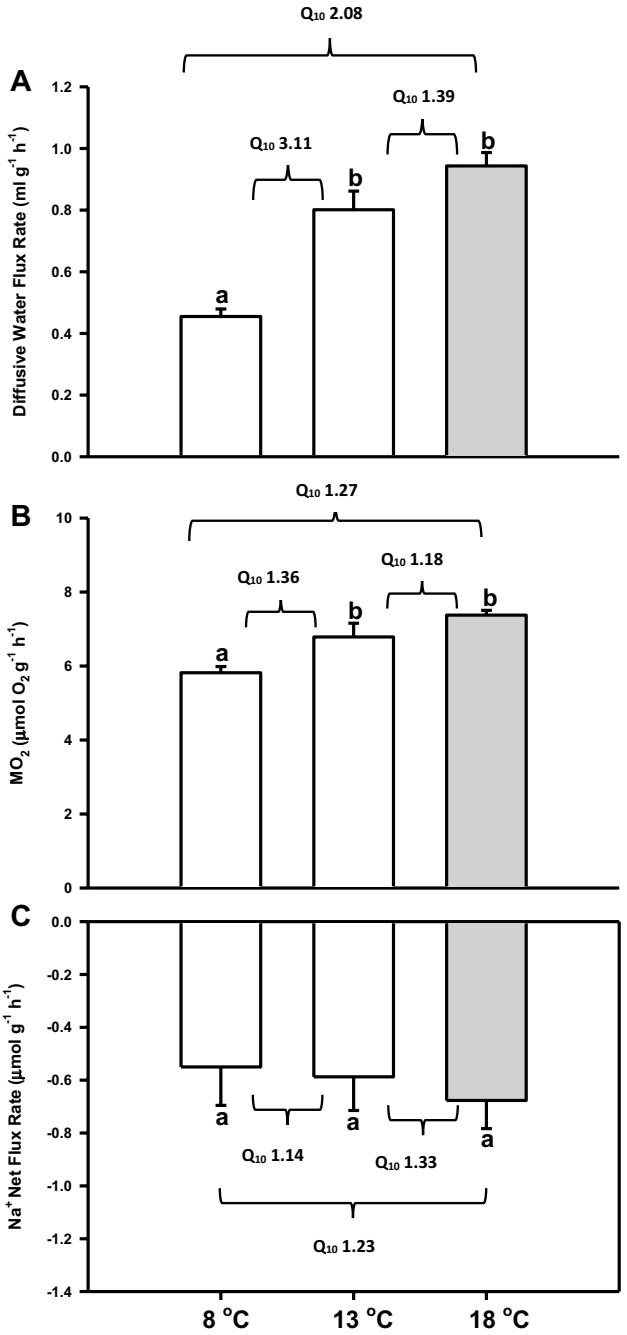

Fig. 5 Effects of acute temperature decreases on **a** diffusive water flux rates (ml g^{-1} h^{-1}) and mean Q_{10} values, **b** MO_2 (µmol O_2 g^{-1} h^{-1}) and mean Q_{10} values, and **c** net Na^+ loss rates (µmol g^{-1} h^{-1}) and mean Q_{10} values. Rainbow trout were acclimated (gray bars) to 18 °C ($N = 10$), and acutely challenged (white bars) with 13 °C ($N = 10$) or 8 °C ($N = 10$). Different sets of fish were used for each treatment (see "Materials and methods"). Values are means ± SEM. Means not sharing the same letter are significantly different from one another at $p < 0.05$

When 8 °C acclimated trout were acutely challenged with higher temperatures, diffusive water exchange rates increased progressively, with an overall Q_{10} (8–18 °C) of 2.28 (Fig. 4a). MO_2 increased with acute temperature rise, with an overall Q_{10} (8–18 °C) of 1.47 (Fig. 4b). Overall, the relative changes with temperature were less than in the acclimation series, and increases in the 13–18 °C range were

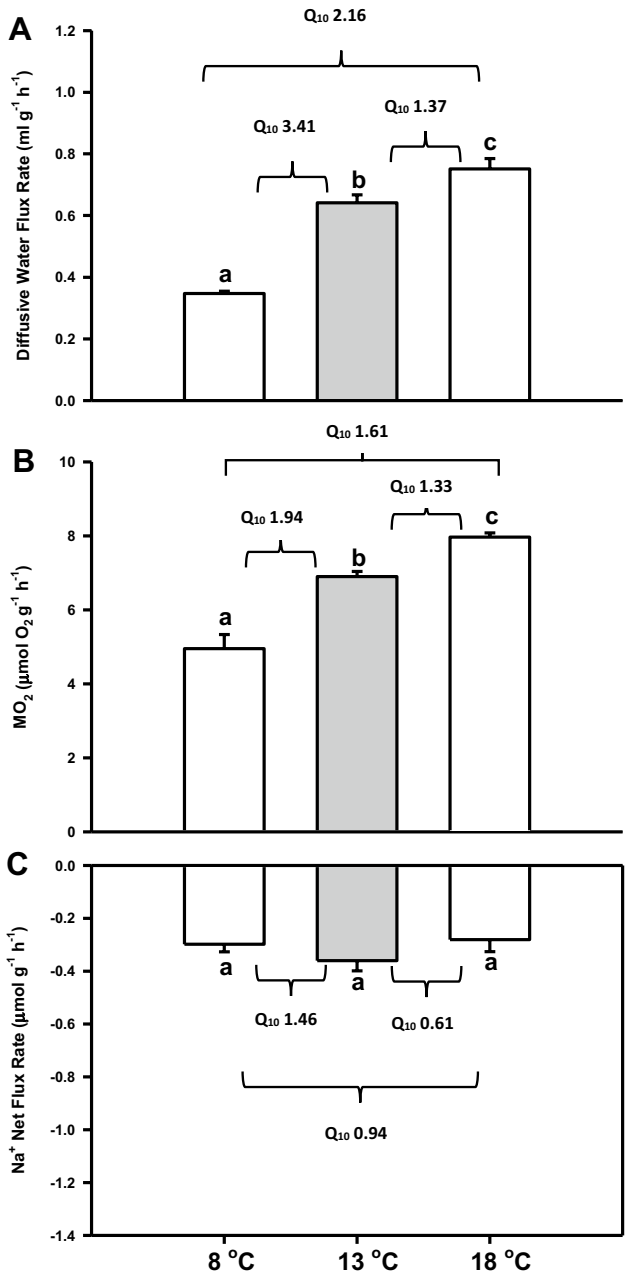

Fig. 6 Effects of acute temperature decreases and increases on **a** diffusive water flux rates (ml g^{-1} h^{-1}) and mean Q_{10} values, **b** MO_2 (µmol O_2 g^{-1} h^{-1}) and mean Q_{10} values, and **c** net Na^+ loss rates (µmol g^{-1} h^{-1}) and mean Q_{10} values. Rainbow trout were acclimated (gray bars) to 13 °C ($N = 10$) and acutely challenged (white bars) with 8 °C ($N = 10$) or 18 °C ($N = 10$). Different sets of fish were used for each treatment (see "Materials and methods"). Values are means ± SEM. Means not sharing the same letter are significantly different from one another at $p < 0.05$

lower than in the 8–13 °C range. The net Na^+ flux rates did not change significantly ($p > 0.05$) during acute temperature increases, with Q_{10} values staying close to 1.0 (Fig. 4c), in contrast to the high values (> 3.0) in the acclimation series. (cf. Fig. 3c).

When 18 °C acclimated trout were acutely challenged with lower temperatures (Fig. 5a), diffusive water exchange rates decreased progressively, with an overall Q_{10} (18–8 °C) of 2.08. MO_2 decreased only slightly with acute decreases in temperature, with an overall Q_{10} (18–8 °C) of 1.27 (Fig. 5b). Again, the relative changes were smaller than in the acclimation series, with greater sensitivity over the lower temperature range. Similar to the acute temperature increase series (Fig. 4c), the net Na^+ flux rate did not change significantly ($p > 0.05$) with acute decreases in temperature, so the Q_{10} stayed close to 1.0 (Fig. 5c).

When the acute temperature challenges were performed on trout acclimated to the intermediate temperature (13 °C), diffusive water exchange decreased at low (8 °C) and increased at high temperatures (18 °C) with an overall Q_{10} of 2.16. MO_2 followed the same pattern as that of diffusive water flux, with a decrease at low (8 °C) and increase at high temperatures (18 °C), with an overall Q_{10} of 1.61 (Fig. 6b). Again relative decreases in the 13–8 °C range exceeded the increases in the 13–18 °C range, and all changes were lower than in the acclimation series. Again the net Na^+ flux rate did not change significantly ($p > 0.05$) with acute increase or decrease in temperature with an overall Q_{10} value that was close to 1 (Fig. 6c).

As with acclimated trout ("Effects of acute temperature challenge on diffusive water flux rates, MO_2 and net Na+ loss rates"), there were no significant relationships (i.e. $p > 0.05$) between net Na^+ flux rates and diffusive water efflux rates within any of the temperature challenge treatment groups.

Discussion

Overview

Measurements of the rates of diffusive water flux, MO_2, and net Na^+ flux in both acclimated and acutely temperature-challenged rainbow trout revealed some surprising differences from previous studies. Notably, all three of our predictions based on these previous studies (see "Introduction") were disproven. In contrast to our first prediction, diffusive water flux rate was more sensitive than MO_2 to temperature in both acclimated fish and fish subjected to acute temperature challenges. This may reflect the differential role of aquaporins in both diffusive water fluxes and MO_2. Perhaps our most important finding was the disproval of our second prediction. Rather than seeing that temperature effects were lower in acclimated fish, for all three flux rates, there was in fact less temperature sensitivity of both MO_2 and diffusive water flux (i.e. lower Q_{10} values) when the fish were

acutely challenged with altered temperature. With respect to the frameworks of Precht (1958) and Prosser (1958), this result indicates that all three rates followed Pattern 5 ("under compensation") of Precht (1958) and Pattern IIB ("reverse translation") of Prosser (1958), as illustrated in Fig. 7. Contrary to our final prediction, net Na^+ loss rates were not independent of acclimation temperature but rather increased greatly with temperature, whereas the opposite was true with respect to acute temperature challenges that had minimal effects on net Na^+ balance. Thus again, in our protocol, the rates during acute temperature challenge were set more by the temperature of acclimation than by the temperature of challenge.

Allometric scaling of diffusive water flux rates and MO_2

When the allometric scaling coefficients at the three acclimation temperatures (i.e. 8, 13, and 18 °C) were compared (Fig. 1a–c), there were no significant differences among the values, suggesting temperature-independence. Our scaling coefficient values for diffusive water flux rates at the three acclimation temperatures were within the range reported for brown trout (*Salmo trutta*) and Mozambique tilapia (*Tilapia mossambica*) acclimated at 20 °C (Potts et al. 1967; Evans 1969). Thus scaling coefficients, at least at warmer acclimation temperatures, are similar across different fish species.

The MO_2 scaling coefficients (Fig. 2a–c) were much lower than the canonical value of 0.75 at all three acclimation temperatures (Schmidt-Nielsen 1984; Clarke and Johnston 1999; Savage et al. 2004). Others have also noted much lower scaling exponents in MO_2 (Barlow 1961; Clarke and Johnston 1999) suggesting that the 0.75 scaling exponent is not universal (Glazier 2006; Killen et al. 2010; Carey et al. 2013, 2014). Variability in scaling may arise from differences in fish size, nutrition, experimental condition, and temperature (Glazier 2005; Carey et al. 2014). As a cautionary, we note that the sample sizes were relatively small, and size–stress interactions may have occurred, both of which may have influenced the calculated scaling coefficients. Regardless, the observed low scaling coefficients for MO_2 at the three temperatures indicate that body size has less effect on absolute MO_2 (and more effect on mass-specific MO_2) than it does on the comparable metrics for diffusive water flux rate. The net Na^+ flux did not correlate with body mass at the three temperatures (results not shown). This agrees with our earlier findings on rainbow trout under acute temperature stress as did the lack of significant relationships between net Na^+ flux rates and diffusive water flux rates in the individual fish (Onukwufor and Wood 2018).

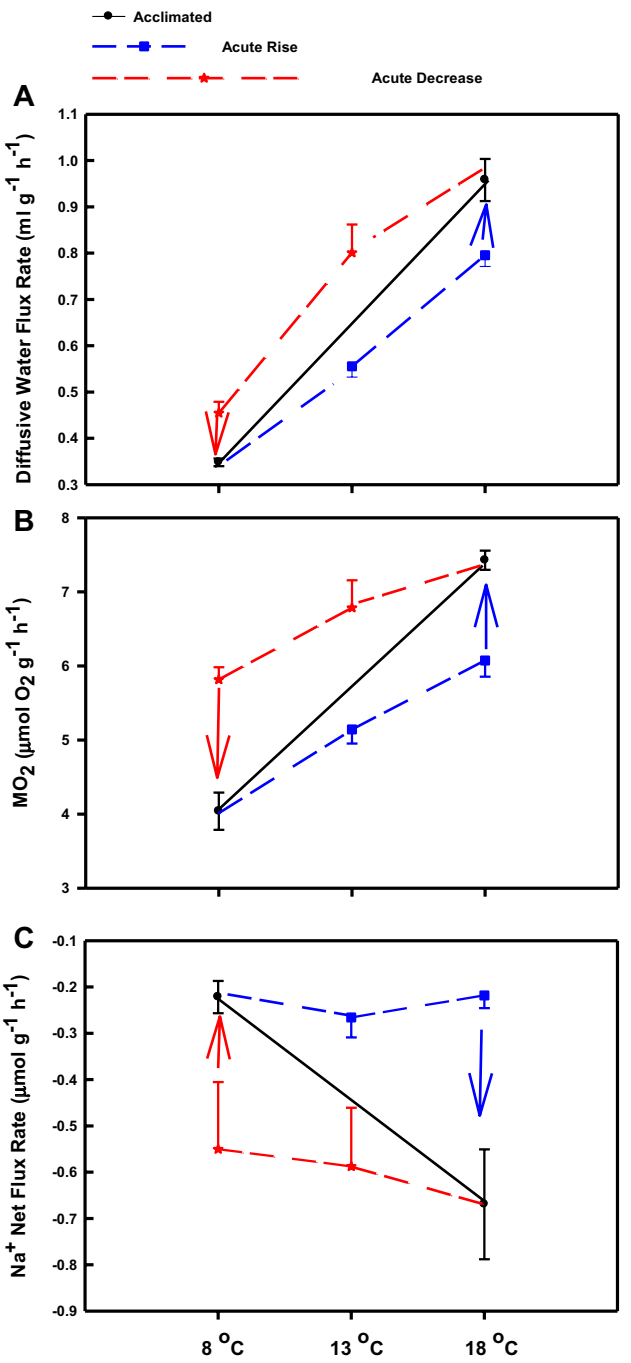

Fig. 7 A summary comparison of the mean rates of **a** diffusive water flux (ml g^{-1} h^{-1}), **b** MO_2 (µmol O_2 g^{-1} h^{-1}), and **c** net Na^+ loss rates (µmol g^{-1} h^{-1}) in trout acclimated to the three temperatures (8 °C, 13 °C, 18 °C, solid black lines) versus trout (dotted blue lines) acutely transferred from acclimation temperature 8 °C to challenge temperatures 13 °C and 18 °C, versus trout (dotted red lines) acutely transferred from acclimation temperature 18 °C to challenge temperatures 13 °C and 8 °C. The arrows on the dotted lines indicate the direction of change over time. The transfer data for the 13 °C-acclimated trout have been omitted for clarity

Effects of acclimation temperature on diffusive water flux, MO_2 and net Na^+ loss rates

Our data for routine MO_2 as a function of temperature are generally similar to those of other studies on salmonids (reviewed by Fry 1971; Brett and Groves 1979; Clarke and Johnston 1999), though comparable studies of temperature effects on routine diffusive water flux rates and net Na^+ flux rates in salmonids are lacking. We clearly showed that acclimation to higher temperatures results in greater MO_2, and greater diffusive water flux rates, as well as greater Na^+ net loss rates (Fig. 3a–c). As explained in "Introduction", the first two were expected based on the basic effects of temperature on mitochondrial-driven metabolic demand and membrane permeability, with both resulting in the osmorespiratory compromise. Relative to previous reports on a range of species summarized in the Introduction, the overall Q_{10} for diffusive water flux (2.75; Fig. 3) for our acclimated trout was somewhat higher, and the overall Q_{10} for MO_2 (1.80) was somewhat lower (Fig. 3). This greater sensitivity of water flux rate (higher Q_{10}) than MO_2 was contrary to our first prediction, and probably resulted from the involvement of aquaporins, protein channels that facilitate the diffusion of water (Agre et al. 2002; King et al. 2004; Tingaud-Sequiera et al. 2010; Cerda and Finn 2010; Madsen et al. 2015). As the osmotic gradient from water to blood was likely invariant in our experiments, and osmotic water flux is very small relative to diffusive water flux (see "Introduction"), this would have made negligible contribution. Aquaporin function is highly regulated under variable environmental conditions such as pH (Zeuthen and Klaerke 1999; Sutka et al. 2005), salinity (Cutler and Cramb 2002; Watanabe et al. 2005; Tipsmark et al. 2010), and temperature (Azad et al. 2004; Ionenko et al. 2010). The exact mechanism(s) responsible for temperature-dependence of aquaporin function are not clear, but could involve the following: (i) changes in the lipid boundary layer, altering the available space for water movement (Azad et al. 2004; Ionenko et al. 2010); (ii) changes in the protonation and deprotonation of the gated pores (Zeuthen and Klaerke 1999; Sutka et al. 2005); (iii) allosteric effects on the subunits resulting in conformational changes in aquaporin protein structure (Hazel and Prosser 1974), and (iv) protein expression levels of aquaporins could be up or downregulated (Cutler and Cramb 2002). Regardless, there is as yet no evidence that O_2 moves similarly by facilitated diffusion through comparable membrane pores. Overall, our results suggest that diffusive water flux and MO_2 were regulated through different pathways with different temperature sensitivities.

For both diffusive water flux and MO_2, the acclimation Q_{10} values between 13 and 18 °C were much lower than those between 8 and 13 °C. While 13–16 °C is close to the optimal temperature for rainbow trout (Fry 1971; Brett and Groves 1979), 18 °C is starting to approach the temperature (20 °C) where negative effects on appetite, growth, and metabolism have been observed in rainbow trout (e.g. Linton et al. 1998). There are similar reports of higher Q_{10} values at lower temperatures in a variety of species (Roberts 1964; Crawshaw 1984; Walsh et al. 1983; Lemons and Crawshaw 1985).

Contrary to our third prediction, the net Na^+ flux rates became more and more negative with increasing acclimation temperature, with a high Q_{10} (Fig. 3c). Gonzalez and McDonald (2000) observed similar patterns in rainbow trout. While these authors did not measure net Na^+ flux rates, they reported that Na^+ influx rate was approximately constant across acclimation temperatures, whereas Na^+ efflux rate increased greatly with acclimation temperature. This pattern could well explain our present results (Fig. 3c). We had expected that after long-term acclimation, the fish would be in approximate ionic balance at each temperature. It is possible that the observed pattern was due to temperature effects on the fluidity of branchial cell membranes and/or the activity of membrane-bound transport enzymes in a differential fashion (Hazel 1969, Cossins and Prosser 1978). In addition, temperature could alter the protein structure or composition of the tight junctions (Hazel and Prosser 1974). Any or all of these mechanisms could have altered the balance between active uptake and passive diffusive loss of Na^+, resulting in more negative flux rates at higher acclimation temperature, though it is difficult to see how this would contribute to ionic homeostasis. An alternative explanation for the high-temperature dependence could be feeding, which is a significant source of ions in freshwater fish (Wood and Bucking 2011). Our fish had been fasted only 24 h, and so were likely still absorbing Na^+ from the food. Digestive processing is faster at higher temperature, so the excretion of the excess ingested salt in the food would happen faster. Overall, many studies (Evans 1969; Motais and Isaia 1972; Onukwufor and Wood 2018) have noted that temperature had minimal effects on the net Na^+ flux rates; however, these studies were done either with minimal acclimation, acute temperature challenge, and/or a prolonged fasting regime. Future studies could clarify these issues by examining the responses of the unidirectional influx and efflux rates of Na^+ separately, in both fed and fasted trout subjected to acute temperature challenges and long-term temperature acclimation.

Effects of acute temperature challenge versus temperature acclimation on diffusive water Flux rates, MO_2 and net Na^+ loss rates

Our findings on the acute temperature sensitivity did not align with the general patterns observed by others on diffusive water flux rates (Evans 1969; Isaia 1972; Loretz 1979), MO_2 (Schurman and Steffensen 1997; Rodnick et al. 2004; Gollock et al. 2006; Clark et al. 2011; Hvas et al. 2017), and net Na^+ flux rates (Evans 1969; Motais and Isaia

1972) where acute temperature challenges resulted in larger changes than the acclimation temperature effects. In contrast, we show that acute challenges cause smaller changes (i.e. lower Q_{10} values) than acclimation temperature effects (i.e. higher Q_{10} values). Thus, when trout were acclimated at higher temperature and measured after acute transfer to lower temperature, their rates were higher than when they were acclimated to this lower temperature. Conversely, when they were acclimated at lower temperature and measured after acute transfer to high temperature, their rates were lower than when they were acclimated to this higher temperature. This can be seen by comparing the Q_{10} values in Figs. 4, 5, and 6 versus Fig. 3, or by comparing the acclimation rates with temperature-challenge rates in Supplementary Figs S1, S2, and S3. The overall pattern is illustrated by Fig. 7 and clearly fits Pattern 5 of Precht (1958) (undercompensation, reverse effect) and more precisely Pattern IIB of Prosser (1958) (reverse translation). In common parlance, this is often termed inverse compensation.

Fry (1971) has emphasized that the importance of spontaneous activity is often overlooked in temperature versus metabolism studies and pointed to the seminal work of Peterson and Anderson (1969) who measured both routine MO_2 and spontaneous activity immediately after temperature challenges, in fish in boxes in the laboratory. They reported that when 6 °C-acclimated salmon were challenged with higher temperature, there was a small increase in spontaneous activity in a time course corresponding to our measurements (i.e. first hour) which eventually stabilized at an even higher level of spontaneous activity. In contrast, when 18 °C-acclimated salmon were challenged with lower temperature, there was a large increase in spontaneous activity in the first hour, but this eventually stabilized at a much reduced level. Routine MO_2 tended to track these events. These different spontaneous activity responses caused by temperature challenges in opposite directions would result in exactly the pattern of reverse translation in MO_2 that we observed, but would have been missed in most studies where the acutely challenged fish are given more time to stabilize before the measurements are made.

Perspectives

We have demonstrated that as temperature changed acutely and then chronically, for all three parameters (MO_2, diffusive water exchange rate, and net Na^+ loss rate), trout followed Pattern 5 ("under compensation") of Precht (1958) and Pattern IIB ("reverse translation") of Prosser (1958). Thus, for example, as temperature increased, the rate rose by only a moderate amount immediately, but would increase further as acclimation occurred. Conversely, as temperature fell, the rate fell moderately, but would fall to a greater extent as acclimation occurred (Fig. 7). These patterns were most clear when the starting acclimation temperature was one of the extremes (8 °C or 18 °C), and somewhat less clear when the starting temperature was intermediate (13 °C) where the challenges caused a rotation response. Nevertheless, the rates during acute temperature challenge were set more by the temperature of acclimation than by the temperature of challenge, and the greater quantitative separation of steady-state rates seen in acclimated fish took some time to develop. In terms of adaptive significance, these patterns mean that trout do not change their rates very much immediately when undergoing acute thermal challenges. This would be homeostatic for quick diel migrations through the thermocline for feeding (Brett 1971; Brett and Groves 1979) and would also be homeostatic when the fish are living in lakes and streams with big diurnal thermal cycles. The greater changes seen with acclimation would be adaptive to long-term seasonal changes in temperature. Why have these patterns been seen in our fish, but rarely in previous studies? It may be that the experimental treatment used in our trials was more representative of real-world conditions. The trout had been fed only 24 h previously, in contrast to extended fasting that is often used to achieve "standard metabolic conditions". Furthermore, rather than recording rates at zero activity level, we elected to work with spontaneously active fish and made measurements during the first hour after temperature challenge, necessitated by the requirements for measuring diffusive water flux rates.

Acknowledgements Supported by an NSERC Discovery grant (Grant RGPIN-2017-03843) to CMW. We thank two anonymous reviewers whose constructive comments improved the MS.

References

Agre P, King LS, Yasui M, Guggino WB, Ottersen OP, Fujiyoshi Y, Engel A, Nielsen S (2002) Aquaporin water channels-from atomic structure to clinical medicine. J Physiol 542:3–16

Azad AK, Sawa Y, Ishikawa T, Shibata H (2004) Phosphorylation of plasma membrane aquaporin regulates temperature-dependent opening of tulip petals. Plant Cell Physiol 45:608–617

Barlow GW (1961) Intra- and interspecific differences in rate of oxygen consumption in gobiid fishes of the genus *Gillichthys*. Biol Bull 121:209–229

Boutilier RG, Heming TA, Iwama GK (1984) Appendix: Physicochemical parameters for use in fish respiratory physiology. In: Hoar WS, Randall DJ (eds) Fish physiology: gills-anatomy, gas transfer, and acid-base regulation: part A, vol 10. Academic Press, London, pp 404–430

Brett JR (1971) Energetic response of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon *(Oncorhynchusnerka)*. Am Zool 11:99–113

Brett JR, Groves TDD (1979) Physiological energetics. In: Hoar WS, Randall DJ, Brett JR (eds) Fish physiology: bioenergetics and growth, vol 8. Academic Press, London, pp 280–352

Carey N, Sigwart JD, Richards JG (2013) Economies of scaling: More evidence that allometry of metabolism is linked

to activity, metabolic rate and habitat. J Exp Mar Biol Ecol 439:7–14
Carey N, Dupont S, Lundve B, Sigwart JD (2014) One size fits all: stability of metabolic scaling under warming and ocean acidification in echinoderms. Mar Biol 161:2131–2142
Cerda J, Finn RN (2010) Piscine aquaporins: an overview of recent advances. J Exp Zool 313A:623–650
Chen Z, Snow M, Lawrence CS, Church AR, Narum SR, Devlin RH, Farrell AP (2015) Selection for upper thermal tolerance in rainbow trout (*Oncorhynchus mykiss* Walbaum). J Exp Biol 218:803–812
Clark TD, Jeffries KM, Hinch SG, Farrell AP (2011) Exceptional aerobic scope and cardiovascular performance of pink salmon (*Oncorhynchus gorbuscha*) may underlie resilience in a warming climate. J Exp Biol 214:3074–3081
Clarke A, Johnston NM (1999) Scaling of metabolic rate with body mass and temperature in teleost fish. J Anim Ecol 68:893–905
Cossins AR, Prosser CL (1978) Evolutionary adaptation of membranes to temperature. Proc Natl Acad Sci USA 75:2040–2043
Crawshaw LI (1979) Responses to rapid temperature change in vertebrate ectotherms. Amer. Zool. 19:225–237
Crawshaw LI (1984) Low temperature dormancy in fish. Am J Physiol 246:R479–R486
Crawshaw LI, Hammel HT (1974) Behavioral regulation of internal temperature in the brown bullhead *Ictalurus nebulosus*. Comp Biochem Physiol 47A:51–60
Cutler CP, Cramb G (2002) Branchial expression of an aquaporin 3 (AQP-3) homologue is downregulated in the European eel *Anguilla anguilla* following seawater acclimation. J Exp Biol 205:2643–2651
Evans DH (1967) Sodium, chloride and water balance of the intertidal teleost, *Xiphister atropurpureus*. III. The roles of simple diffusion, exchange diffusion, osmosis and active transport. J Exp Biol 47:525–534
Evans DH (1969) Studies on the permeability of water of selected marine, freshwater and euryhaline teleosts. J Exp Biol 50:689–703
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
Franz TJ (1968) On the diffusion of tritiated water through skin. J Invest Dermatol 50:260–261
Fry FEJ (1971) The effect of environmental factors on the physiology of fish. In: Hoar WS, Randall DJ (eds) Fish physiology, vol 6. Academic Press, New York, pp 1–98
Fry FEJ, Hart JS (1948) Cruising speed of goldfish in relation to water temperature. J Fish Res Board Can 7:169–175
Fry FEJ, Brett JR, Clawson GH (1942) Lethal limits of temperature for young goldfish. Rev Can Biol 1:50–56
Giacomin M, Schulte PM, Wood CM (2017) Differential effects of temperature on oxygen consumption and branchial fluxes of urea, ammonia, and water in the dogfish shark (*Squalus acanthias suckleyi*). Physiol Biochem Zool 90:627–637
Giacomin M, Eom J, Schulte PM, Wood CM (2019) Acute temperature effects on metabolic rate, ventilation, diffusive water exchange, osmoregulation, and acid-base status in the Pacific hagfish (*Eptatretus stoutii*). J Comp Physiol B 189:17–35
Glazier DS (2005) Beyond the "3/4 power law": variation in the intra and interspecific scaling of metabolic rate in animals. Biol Rev 80:611–662
Glazier DS (2006) The 3/4 power law is not universal: evolution of isometric, ontogenetic metabolic scaling in pelagic animals. Bioscience 56:325–332
Gollock MJ, Currie S, Petersen LH, Gamperl AK (2006) Cardiovascular and haematological responses of Atlantic cod (*Gadus morhua*) to acute temperature increase. J Exp Biol 209:2961–2970
Gonzalez RJ, McDonald DG (1992) The relationship between oxygen consumption and ion loss in a freshwater fish. J Exp Biol 163:317–332
Gonzalez RJ, McDonald GD (1994) The relationship between oxygen uptake and ion loss in fish from diverse habitats. J Exp Biol 190:95–108
Gonzalez RJ, McDonald GD (2000) Ionoregulatory responses to temperature change in two species of freshwater fish. Fish Physiol Biochem 22:311–317
Hazel JR (1969) The effect of thermal acclimation upon brain cholinesterase activity of *Carassius auratus* and *Fundulus heteroclitus*. Life Sci 8:775–784
Hazel JR, Prosser CL (1970) Interpretation of inverse acclimation to temperature. Z Vgl Physiol 67:217–228
Hazel JR, Prosser CL (1974) Molecular mechanisms of temperature compensation in poikilotherms. Physiol Rev 54:620–677
Healy TM, Schulte PM (2012) Thermal acclimation is not necessary to maintain a wide thermal breadth of aerobic scope in the common killifish (*Fundulus heteroclitus*). Physiol Biochem Zool 85:107–119
Hochachka PW (1966) Lactic dehydrogenases in poikilotherms: Definition of a complex isozyme system. Comp Biochem Physiol 18:261–269
Holmes WN, Donaldson EM (1969) Body compartments and distribution of electrolytes. In: Hoar WS, Randall DJ (eds) Fish physiology, vol 1. Academic Press, New York, pp 1–89
Hvas M, Folkedal O, Imsland A, Oppedal F (2017) The effect of thermal acclimation on aerobic scope and critical swimming speed in Atlantic salmon, *Salmo salar*. J Exp Biol 220:2757–2764
Ionenko IF, Anisimov AV, Dautova NR (2010) Effect of temperature on water transport through aquaporins. Biol Plant 54:488–494
Isaia J (1972) Comparative effects of temperature on the sodium and water permeabilities of the gills of a stenohaline freshwater fish *(Carassius auratus)* and a stenohaline marine fish *(Serranus scriba cabrilla)*. J Exp Biol 57:359–366
Isaia J (1984) Water and nonelectrolyte permeability. In: Hoar WS, Randall DJ (eds) Fish physiology, vol 10B. Academic Press, San Diego, pp 1–38
Kent J, Prosser CL (1980) Effects of incubation and acclimation temperatures on incorporation of U-[^{14}C] glycine into mitochondrial protein of liver cells and slices from green sunfish *Lepomis cyanellus*. Physiol Zool 53:293–304
Killen SS, Atkinson D, Glazier DS (2010) The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature. Ecol Lett 13:184–193
King LS, Kozono D, Agre P (2004) From structure to disease: the evolving tale of aquaporin biology. Nat Rev Mol Cell Biol 5:687–698
Kwong RW, Kumai Y, Perry SF (2013) The role of aquaporin and tight junction proteins in the regulation of water movement in larval zebrafish (*Danio rerio*). PLoS ONE 8:e70764. https://doi.org/10.1371/journal.pone.0070764
Lemons DE, Crawshaw LI (1985) Behavioral and metabolic adjustments to low temperatures in the largemouth bass (*Micropterus salmoides*). Physiol Zool 58:175–180
Linton TK, Morgan IJ, Walsh PJ, Wood CM (1998) Chronic exposure of rainbow trout (*Oncorhynchus mykiss*) to simulated climate warming and sublethal ammonia: a year-long study of their appetite, growth, and metabolism. Can J Fish Aquat Sci 55:576–586
Loretz AC (1979) Water exchange across fish gills: the significance of tritiated-water flux measurements. J Exp Biol 79:147–162

Madsen SS, Engelund MB, Cutler CP (2015) Water transport and functional dynamics of aquaporins in osmoregulatory organs of fishes. Biol Bull 229:70–92

Moffitt BP, Crawshaw LI (1983) Effects of acute temperature changes on metabolism, heart rate and ventilation frequency in carp *Cyprinus carpio* L. Physiol Zool 56:397–403

Motais R, Isaia J (1972) Temperature-dependence of permeability to water and sodium of the gill epithelium of the eel *Anguilla Anguilla*. J Exp Biol 56:587–600

Motais R, Isaia J, Rankin JC, Maetz J (1969) Adaptive changes of the water permeability of the teleostean gill epithelium in relation to external salinity. J Exp Biol 51:529–546

Nilsson S (1986) Control of gill blood flow. In: Nilsson S, Holmgren S (eds) Fish physiology: recent advances. Croom Helm, London, pp 87–101

Norin T, Malte H, Clark TD (2014) Aerobic scope does not predict the performance of a tropical eurythermal fish at elevated temperatures. J Exp Biol 217:244–251

Olson KR (1992) Blood and extracellular fluid volume regulation. In: Hoar WS, Randall DJ, Farrell AP (eds) Fish physiology, vol 12B. Academic Press, San Diego, pp 135–254

Onukwufor JO, Wood CM (2018) The osmorespiratory compromise in rainbow trout (*Oncorhynchus mykiss*): The effects of fish size, hypoxia, temperature and strenuous exercise on gill diffusive water fluxes and sodium net loss rates. Comp Biochem Physiol A 219–220:10–18

Onukwufor JO, Kibenge F, Stevens D, Kamunde C (2015) Modulation of cadmium-induced mitochondrial dysfunction and volume changes by temperature in rainbow trout. Aquatic Toxicol 158:75–87

Onukwufor JO, Stevens D, Kamunde C (2017) Combined effect of cadmium, temperature and hypoxia-reoxygenation on mitochondrial function in rainbow trout (*Oncorhynchus mykiss*). Aquatic Toxicol 182:129–141

Pérez-Casanova JC, Afonso LOB, Johnson SC, Currie S, Gamperl AK (2008) The stress and metabolic responses of juvenile Atlantic cod *Gadus morhua* L. to an acute thermal challenge. J Fish Biol 72:899–916

Peterson RH, Anderson JM (1969) Influence of temperature change on spontaneous locomotor activity and oxygen consumption of Atlantic salmon, *Salmo salar*, acclimated to two temperatures. J Fish Board Can 26:93–109

Potts WTW, Foster MA, Rudy PP, Parry Howells G (1967) Sodium and water balance in the cichlid teleost, *Tilapia mossambica*. J Exp Biol 47:461–470

Precht H (1958) Concepts of the temperature adaptation of unchanging reaction systems of cold-blooded animals. In: Prosser CL (ed) Physiological adaptation. American Physiological Society, Washington, pp 50–78

Prosser CL (1958) General summary: the nature of physiological adaptation. In: Prosser CL (ed) Physiological adaptation. American Physiological Society, Washington, DC, pp 167–180

Randall DJ, Baumgarten D, Malyusz M (1972) The relationship between gas and ion transfer across the gills of fishes. Comp Biochem Physiol 41A:629–637

Reich AJ, Belli JA, Blaskovics NE (1960) Oxygen consumption of whole animals and tissues in temperature acclimated amphibians. Proc Soc Exp Biol 103:436–439

Roberts JL (1964) Metabolic responses of freshwater sunfish to seasonal photoperiods and temperatures. Helgol Wiss Meeresuntersuch 9:459–473

Rodnick KJ, Gamperl AK, Lizars KR, Bennett MT, Rausch RN, Keeley ER (2004) Thermal tolerance and metabolic physiology among redband trout populations in south-eastern Oregon. J Fish Biol 64:310–335

Savage VM, Gillooly JF, Brown JH, Charnov EL (2004) Effects of body size and temperature on population growth. Am Nat 163:429–441

Schmidt-Nielsen K (1984) Scaling, why is animal size so important?. Cambridge University Press, Cambridge

Schurmann H, Steffensen JF (1997) Effects of temperature, hypoxia and activity on the metabolism of juvenile Atlantic cod. J Fish Biol 50:1166–1180

Sidell BD, Wilson FR, Hazel J, Prosser CL (1973) Time course of thermal acclimation in goldfish. J Comp Physiol 84:119–127

Sutka M, Alleva K, Parisi M, Amodeo G (2005) Tonoplast vesicles of *Beta vulgaris* storage root show functional aquaporins regulated by protons. Biol Cell 97:837–846

Tingaud-Sequeira A, Calusinska M, Finn RN, Chauvigne F, Lozano J, Cerda J (2010) The Zebrafish genome encodes the largest vertebrate repertoire of functional aquaporins with dual paralogy and substrate specificities similar to mammals. BMC Evol Biol 10:38. https://doi.org/10.1186/1471-2148-10-38

Tipsmark CK, Sorensen KJ, Madsen SS (2010) Aquaporin expression dynamics in osmoregulatory tissues of Atlantic salmon during smoltification and seawater acclimation. J Exp Biol 213:368–379

Walsh PJ, Foster GD, Moon TW (1983) The effects of temperature on metabolism of the American eel *Anguilla rostrata* (LeSueur): compensation in the summer and torpor in the winter. Physiol Zool 56:532–540

Watanabe S, Kaneko T, Aida K (2005) Aquaporin-3 expressed in the basolateral membrane of gill chloride cells in Mozambique tilapia *Oreochromis mossambicus* adapted to freshwater and seawater. J Exp Biol 208:2673–2682

Wood CM, Bucking C (2011) The role of feeding in salt and water balance. In: Grosell M, Farrell AP, Brauner CJ (eds) Fish physiology: the multifunctional gut of fish, vol 30. Academic Press, San Diego, pp 165–211

Wood CM, Randall DJ (1973a) The influence of swimming activity on sodium balance in the rainbow trout (*Salmo gairdneri*). J Comp Physiol 82:207–233

Wood CM, Randall DJ (1973b) Sodium balance in the rainbow trout (*Salmo gairdneri*) during extended exercise. J Comp Physiol 82:235–256

Zeuthen T, Klaerke DA (1999) Transport of water and glycerol in aquaporin 3 is gated by H^+. J Biol Chem 274:21631–21636