



Contents lists available at ScienceDirect

## Comparative Biochemistry and Physiology, Part A

journal homepage: [www.elsevier.com/locate/cbpa](http://www.elsevier.com/locate/cbpa)

# The osmorepiratory 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

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## ARTICLE INFO

## Keywords:

Tritiated water  
Allometric scaling  
Q<sub>10</sub>  
Trout  
Size  
Hypoxia  
Exercise  
Acute temperature  
Diffusive water flux  
Net sodium loss

## ABSTRACT

In the context of the osmorepiratory compromise, hypoxia and temperature have been little studied relative to exercise, and diffusive water flux rates (as assessed by <sup>3</sup>H<sub>2</sub>O efflux) have received almost no attention. We investigated the effects of fish size, hypoxia, exercise and acute temperature increase on diffusive water flux rates and net sodium loss rates in juvenile rainbow trout. Trout weighing 13–50 g were used to determine the effects of fish size under normoxia. Thereafter 25–50 g trout were selected to assess the effects of different hypoxia levels (3.15, 5.25 and 8.40 KPa), time course of hypoxia (1 h 8.40 KPa, 3 h 8.40 KPa, 1 h 8.40 KPa + 1 h normoxic recovery, and 1 h 8.40 KPa + 3 h normoxic recovery), strenuous exercise (5 min) and acute temperature challenge (transfer from 8 °C to 13 °C or to 18 °C). Small fish (13 g) had higher diffusive water flux rates than larger fish, turning over > 100% of their fractional body water pool per hour against 34% per hour for 50 g fish. Hypoxic exposure exerted a biphasic effect, increasing the diffusive water flux rate at 8.40 KPa and 5.25 KPa, while returning it to control levels at 3.15 KPa. All the levels of hypoxia increased net Na<sup>+</sup> loss. One hour hypoxia (8.40 KPa) increased diffusive water flux rate while prolonged 3 h hypoxia (8.40 KPa), and short or prolonged normoxic recovery returned diffusive water flux rates to control levels. All the treatments over the time course of hypoxia and normoxic recovery increased net Na<sup>+</sup> loss rates. Strenuous exercise increased both the diffusive water flux and net Na<sup>+</sup> loss rates. Acute temperature rise increased diffusive water flux rates, with Q<sub>10</sub> values of 4.03 for 8 to 13 °C and 2.16 for 8 to 18 °C, but the net Na<sup>+</sup> loss rate did not change. There was no significant correlation between diffusive water flux rate and net Na<sup>+</sup> loss rates at different hypoxia levels, over the course of hypoxia and normoxic recovery, or during acute temperature stress. In contrast, we observed a significant correlation between diffusive water flux and net Na<sup>+</sup> loss rates following exercise. Overall, diffusive water flux and sodium loss were regulated differently during acute temperature challenge and hypoxia, while following exercise the two parameters were regulated in a similar fashion.

## 1. Introduction

The gill of teleost fish is a multi-functional organ (Evans et al., 2005) that is used not only for respiratory gas exchange (Randall and Daxboeck, 1984; Nikinmaa, 2006) but also as a major site for ionoregulation (Marshall and Grosell, 2006; Hwang and Lee, 2007), nitrogenous waste excretion (Wood, 1993), acid-base balance (Perry and Gilmour, 2006) and water exchange (Evans, 1969; Loretz, 1979; Isaia, 1984). The latter process is perhaps the least well-studied. These functions of the gill require certain conditions for optimal performance that in many cases, differ from one another. For example, conditions enabling osmoregulation would be thick epithelial membranes with reduced functional surface area and permeability so as to minimize

adverse ion and water exchanges (Gonzalez and McDonald, 1992; Gonzalez and McDonald, 1994), whereas conditions facilitating respiratory gas exchange would include increased surface area, thin epithelial membranes, with high blood and water flow rates (Randall et al., 1967; Randall et al., 1972; Sundin and Nilsson, 1997). In order for the gill to balance between these two opposing demands, there has to be a compromise. The osmorepiratory compromise represents this trade-off (Randall et al., 1972; Nilsson, 1986).

Conditions that favour increased oxygen uptake and/or demand such as hypoxia, exercise and temperature stress resulted in an increase in unfavourable net fluxes of osmolytes (e.g. Randall et al., 1972; Wood and Randall, 1973a; Wood and Randall, 1973b; Gonzalez and McDonald, 1992; Gonzalez and McDonald, 1994; Postlethwaite and

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McDonald, 1995; Iftikar et al., 2010; Robertson et al., 2015a; Robertson et al., 2015b; Giacomini et al., 2017). Most of these studies have involved salmonids such as the rainbow trout, and the majority have focused on gas and ion exchange. The evidence for effects on water exchange is far less extensive than that for ion fluxes, which is somewhat surprising considering that water constitutes about 80% of the body of the fish (Isaia, 1984), and the gills account for about 90% of water exchange (Motaïs et al., 1969; Haywood et al., 1977). Stevens (1972), using tilapia, and Wood and Randall (1973c) and Hofmann and Butler (1979) both using rainbow trout, all concluded, based on measurements of urine flow rate and/or body weight changes, that net water uptake increased with O<sub>2</sub> uptake during exercise in freshwater fish. Loretz (1979) reported an increase in <sup>3</sup>H<sub>2</sub>O flux rate with increase in temperature in goldfish, while Giacomini et al. (2017) observed a decreased <sup>3</sup>H<sub>2</sub>O flux rate at an acutely reduced temperature (7.5 °C) and an increased <sup>3</sup>H<sub>2</sub>O flux rate at an acutely elevated temperature (22 °C) in seawater spiny dogfish. Additionally, Loretz (1979) demonstrated that <sup>3</sup>H<sub>2</sub>O efflux increased during moderate hypoxia, while Giacomini et al. (2017) reported that changes in <sup>3</sup>H<sub>2</sub>O efflux paralleled those in O<sub>2</sub> uptake during temperature challenges.

Unidirectional water flux rates (often called “diffusive water flux”), as measured by <sup>3</sup>H<sub>2</sub>O fluxes, are approximately 100-fold greater than net water flux rates (often called “osmotic water flux”) as estimated from body weight changes and urine flow, and the relationship between the two is complex (see review by Franz, 1968). In part this is because diffusive water flux is a direct measurement, whereas osmotic water flux is an indirect calculation (Loretz, 1979). Furthermore, osmotic water flux is a net flux dependent on the differences between osmotic (and hydrostatic) pressures between external and internal environments, while diffusive water flux is unidirectional. Additionally, diffusive water exchange probably occurs mainly through the cell membrane, at least in part via aquaporins (Evans et al., 2005) in freshwater teleosts, whereas osmotic water flux may additionally involve bulk flow through paracellular pathways (Potts et al., 1967; Evans, 1969; Motaïs et al., 1969; Loretz, 1979; Isaia, 1984). Diffusive water flux and net water flux may not necessarily change in parallel. For example, in the Amazonian oscar, a teleost with exceptional hypoxia tolerance that shows an unusual decrease in gill ion fluxes during severe hypoxia (Wood et al., 2007; Wood et al., 2009; De Boeck et al., 2013; Robertson et al., 2015b), osmotic permeability to water also declined, but the fall in net water flux (30%) was less than that (70%) in diffusive water permeability (Wood et al., 2009). Interestingly, while ion fluxes decreased during hypoxia, they increased during exercise in oscars (Robertson et al., 2015a), though water fluxes were not measured in the exercise study. This suggests that oscars employed different approaches in dealing with the osmorepiratory compromise during exercise versus hypoxia.

With this background in mind, we used <sup>3</sup>H<sub>2</sub>O to measure diffusive water flux rates across the gills of juvenile rainbow trout (*Oncorhynchus mykiss*). Our first objective was to determine if the size of the fish affects their water flux rate under resting normoxic control conditions, before addressing the possible influences of experimental treatments. Therefore, our first hypothesis was that diffusive water flux would scale with body mass in a similar fashion to that traditionally found for O<sub>2</sub> consumption (i.e. a mass scaling allometric coefficient in the range of 0.6–0.9; Clarke and Johnson, 1999). This proved not to be the case. After sorting out the issues of fish size, our second objective was to determine the effects of acute temperature stress, hypoxia and exercise on both diffusive water flux and net sodium loss rates. These treatments were designed to invoke the osmorepiratory compromise. The trout is an energetic, highly aerobic species, living in a variable habitat (Hardy, 2002), and it is constantly under threat by such environmental stressors. In the present experiments, the effect of these treatments on net Na<sup>+</sup> loss rates was also examined, to assess the component that has been traditionally measured in osmorepiratory compromise studies. Our second hypothesis was that all three treatments would increase

both net sodium loss and diffusive water flux rates. Our third hypothesis was that diffusive water flux rates and net sodium loss rates would be correlated on an individual basis, as they are likely affected in the same manner by changes in gill permeability.

## 2. Materials and methods

### 2.1. Experimental fish

Rainbow trout (*Oncorhynchus mykiss*) were obtained from Miracle Springs Inc. (Fraser Valley, BC, Canada), and held for a minimum of two weeks prior to experiments in a 500-L flow-through holding tank at 8 °C. Dechlorinated Vancouver tap water (in mM: Na<sup>+</sup>.006; Cl<sup>-</sup>, 0.05; Ca<sup>2+</sup>, 0.03; Mg<sup>2+</sup>, 0.007; K<sup>+</sup>, 0.004; and in mg/L CaCO<sub>3</sub>, alkalinity, 3.0; hardness 3.3; pH 7.0) was used for acclimation and all experiments. Fish were maintained in 12 L:12D photoperiod and fed every day at 1% of their body weight with commercial pellets (EWOS, Surrey, BC, Canada). On the day of experiment, fish were first netted individually from the holding tank and put through the tritiated water loading protocol as described in Section 2.2. The remaining fish in the holding tank were then fed as described above, so experimental fish had been fasted for about 24 h. All experimental procedures were approved by the University of British Columbia Animal Care Committee (certificate A14-0251) in accordance with the Canadian Council on Animal Care guidelines. None of the fish died as a result of the experimental treatments.

### 2.2. Experimental protocol

All procedures were carried out in covered containers which were shielded in black plastic to minimize visual disturbance and fitted with aeration devices and sampling ports. Containers were submerged in a table-trough that served as a water bath, maintained at 8 °C by a flow-through of Vancouver dechlorinated tap water. In order to avoid the stress of injection, fish were loaded with tritiated water (<sup>3</sup>H<sub>2</sub>O, Perkin Elmer, Woodbridge, ON, Canada) by external incubation for 6 h; preliminary experiments demonstrated that equilibration was complete within this time. Adequate measures (tank shielding, minimal movement by the experimenter, quiet) were ensured so that fish were not stressed before and during the experimental period as stress has been shown to affect rainbow trout sodium loss rates (Postlethwaite and McDonald, 1995). For each experimental run, 5 fish were loaded simultaneously in a 2-L water volume labelled with 40 µCi of <sup>3</sup>H<sub>2</sub>O. After the equilibration period, fish were individually netted from the loading container, quickly rinsed with dechlorinated water to remove any external <sup>3</sup>H<sub>2</sub>O on the body surface, and then gently transferred to the experimental containers. Each held 1 L of dechlorinated water devoid of <sup>3</sup>H<sub>2</sub>O. Then 5 mL of water was sampled at time zero and thereafter every 5 min for 60 min, with a final sample taken after 6 h when washout was complete. The fish was weighed at this time, and then returned to its holding tank. The 0–60 min samples were used for diffusive water flux measurements, and the 6-h sample was used to calculate the original dose of <sup>3</sup>H<sub>2</sub>O in the fish at time 0 (see Calculations). Additional 10-mL samples were taken at 0 min and 60 min for net Na<sup>+</sup> flux rates, but some samples for Na<sup>+</sup> determinations were lost, so net Na<sup>+</sup> flux rates were measured in most but not all individuals. Note that because the efflux of <sup>3</sup>H<sub>2</sub>O from the fish is rapid, it was necessary to make all experimental measurements during the first 1-h period after the fish was removed from the loading medium. Therefore, in the prolonged hypoxia experiments, it was necessary to start the hypoxia treatment during the loading period, as outlined below. In the acute hypoxia trials, the fish were added directly to water already set to the desired O<sub>2</sub> level (e.g. 8.40 kPa), and this was always the case during <sup>3</sup>H<sub>2</sub>O efflux trials, whereas in the longer-term hypoxia pre-exposures the water PO<sub>2</sub> was brought to the desired PO<sub>2</sub> over 1–2 min. Fluctuations in PO<sub>2</sub> during hypoxia were about ± 0.4 kPa. This fluctuation in

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