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Osmo-respiratory compromise in the mosshead sculpin (*Clinocottus globiceps*): effects of temperature, hypoxia, and re-oxygenation on rates of diffusive water flux and oxygen uptake

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Abstract In nature, mosshead sculpins (*Clinocottus globiceps*) are challenged by fluctuations in temperature and oxygen levels in their environment. However, it is unclear how mosshead sculpins modulate the permeability of their branchial epithelia to water and O_2 in response to temperature or hypoxia stress. Acute decrease in temperature from 13 to 6 °C reduced diffusive water flux rate by 22% and $\dot{M}O_2$ by 51%, whereas acute increase in temperature from 13 to 25 °C increased diffusive water flux rate by 217% and $\dot{M}O_2$ by 140%, yielding overall Q_{10} values of 2.08 and 2.47 respectively. Acute reductions in oxygen tension from >95% to 20% or 10% air saturation did not impact diffusive water flux rates, however, $\dot{M}O_2$ was reduced significantly by 36% and 65% respectively. During 1-h or 3-h recovery periods diffusive water flux rates were

depressed while $\dot{M}O_2$ exhibited overshoots beyond the normoxic control level. Many responses differed from those seen in our parallel earlier study on the tidepool sculpin, a cottid with similar hypoxia tolerance but much smaller gill area that occupies a similar environment. Overall, our data suggest that during temperature stress, diffusive water flux rates and $\dot{M}O_2$ follow the traditional osmo-respiratory compromise pattern, but during hypoxia and re-oxygenation stress, diffusive water flux rates are decoupled from $\dot{M}O_2$.

Keywords Q_{10} · tritiated water · gill permeability · facilitated diffusion

Introduction

Marine fish such as mosshead sculpins (*Clinocottus globiceps*) that inhabit shallow tide- pools (Watters and Cech 2003; Sloman et al. 2008; Mandic et al. 2009a; Knope and Scales 2013; Blewett et al. 2022) are subject to stressful environmental conditions. In this environment, the fish are subjected to sudden and rapid diurnal fluctuations in temperature and oxygen, sometimes resulting in hypoxia, a condition of low dissolved oxygen due to high biological respiration rates (Mandic et al. 2009a; Mandic et al. 2014; Diaz and Rosenberg 2008; Richards 2011; Knope and Scales 2013; Blewett et al. 2022). These challenges are being exacerbated by climate change (Portner 2012; Breitburg et al. 2018) and nutrient enrichment-driven

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eutrophication (Diaz and Rosenberg 2008). Rapidly rising temperatures and hypoxia may both impact the physiological homeostasis of mosshead sculpins in their environment (Richards 2009; Richards 2011; Breitburg et al. 2018). In both cases the demand for tissue oxygen may become higher than what is available in the environment, limiting the ability of mitochondria to make ATP for cellular metabolic requirements (Onukwufor et al. 2016; Onukwufor et al. 2017). To survive these challenges, fish must either reduce their metabolic rates or increase their capacity for oxygen uptake. Increasing oxygen uptake rate ($\dot{M}O_2$) to meet tissue demand typically involves increasing the effective permeability of the gill (Randall and Daxboeck 1984). Although increasing effective gill permeability improves oxygen uptake, this response may also increase effective gill permeability to ions and water, resulting in unfavourable fluxes of these substances between the external environment and the blood plasma.

This tradeoff is known as the osmo-respiratory compromise (Randall et al. 1972, Wood and Eom 2021). The compromise is between physiological adjustments that are favorable to oxygen uptake such as increased gill lamellar perfusion and surface area, thinner epithelial membranes, with higher blood and water flow rates (Randall et al. 1967; Sundin and Nilsson 1997; Wood and Eom 2021) and those that are unfavorable to the diffusive fluxes of osmolytes and water, such as thicker epithelial membranes with reduced functional surface area, increased diffusion distance, and lower blood and water flows (Gonzalez and McDonald 1992; Gonzalez and McDonald 1994; Saroglia et al. 2000; Saroglia et al. 2002; Matey et al. 2008; Wu et al. 2017). The osmo-respiratory compromise phenomenon has been widely studied in freshwater fish with respect to $\dot{M}O_2$ versus ion flux rates (e.g. Randall et al. 1972; Gonzalez and McDonald 1992, 1994; Postlethwaite and McDonald 1995; Iftikar et al. 2010; Robertson et al. 2015a, b), but less so with respect to the relationship between $\dot{M}O_2$ and water fluxes (Wood et al. 2009, 2019; Ruhr et al. 2020; Giacomin et al. 2020; Onukwufor and Wood 2018; Onukwufor and Wood 2020a, b), with even fewer studies in marine fish (Giacomin et al. 2017; Wood et al. 2019; Somo et al. 2020; Onukwufor and Wood 2022).

Several of these reports have demonstrated increases in $\dot{M}O_2$ and diffusive water flux rates at high temperature and decreases in both at low temperature, in both freshwater (Onukwufor and Wood 2018; Onukwufor and Wood 2020a; Onukwufor and Wood 2020b) and seawater fish (Giacomin et al. 2017; Somo et al. 2020; Onukwufor and Wood 2022). This was attributed to the ability of temperature to modulate both metabolic rate and the effective membrane permeability of the gills. Though the overall temperature effects on $\dot{M}O_2$ and diffusive water flux rates were similar across species, their sensitivities varied greatly. For example, in freshwater rainbow trout (Onukwufor and Wood 2020a), dogfish sharks (Giacomin et al. 2017), tidepool sculpins (Somo et al. 2020) and English sole (Onukwufor and Wood 2022), the thermal sensitivities of diffusive water flux rates were higher than those of $\dot{M}O_2$. The authors suggested that it may be because diffusive water flux rates are aided, at least in part, by facilitated diffusion through aquaporins. In contrast, there are several reported cases of diffusive water flux rate having lower thermal sensitivity than $\dot{M}O_2$. For example, this occurred in Pacific sanddab (Onukwufor and Wood 2022) and zebrafish (Onukwufor and Wood 2020b). Thus, it may be that in some species, aquaporins are playing a greater role while in others they are not.

Unlike temperature, hypoxia elicited variable effects on the osmo-respiratory compromise depending on the environment and species. In some very hypoxia-tolerant species, both $\dot{M}O_2$ and diffusive water flux rate were depressed during acute hypoxia, for example, in Amazonian oscar (Wood et al. 2009), freshwater-acclimated killifish (Wood et al. 2019; Giacomin et al. 2020), tidepool sculpins (Somo et al. 2020), English sole (Onukwufor and Wood 2022), and Pacific sanddab (Onukwufor and Wood 2022). However, in seawater-acclimated killifish, $\dot{M}O_2$ decreased but diffusive water flux rate remained unchanged (Wood et al. 2019; Giacomin et al. 2020). Others have reported increases in diffusive water flux rate in goldfish (Loretz 1979), rainbow trout (Onukwufor and Wood 2018), and zebrafish (Onukwufor and Wood 2020b) during acute hypoxia. Furthermore, the relationship between $\dot{M}O_2$ and diffusive water flux appears to change depending on both the length of hypoxia exposure and the length of the post-hypoxia re-oxygenation period (Wood et al. 2019; Somo et al. 2020; Onukwufor et al. 2020b). Therefore, it is fair to suggest that the control of both $\dot{M}O_2$ and diffusive water flux rate under acute hypoxia and post-hypoxia

recovery is complex and does not follow the canonical osmo-respiratory compromise hypothesis, suggesting that the two processes can be regulated independently.

The impact of interspecific variation in gill surface area on the regulation of gas, water, and ion permeabilities under dynamic temperature and oxygen conditions is unknown. For example, although hypoxia suppresses diffusive water flux in the tidepool sculpin *Oligocottus maculosus* (Somo et al. 2020), the unusually low gill surface area in this species (Mandic et al. 2009b) may facilitate this suppression compared to intertidal cottids with larger respiratory surfaces. In marine cottids, gill surface area generally increases with hypoxic respiratory capacity, as indexed by the critical oxygen tension for routine metabolic rate (P_{crit}) (Mandic et al. 2009b). This phenotype is epitomized by the mosshead sculpin (*Clinocottus globiceps*), which has the highest gill surface area observed in sculpins (Somo et al. 2023) and one of the lowest P_{crit} values (Mandic et al. 2009b). Although *C. globiceps* and *O. maculosus* have similar P_{crit} values, *O. maculosus* have the lowest gill surface area measured in marine sculpins (Mandic et al. 2009b; Mandic et al. 2013). How the difference in gill surface area may affect osmoregulatory function in dynamic temperature and oxygen conditions remains unknown. With this background in mind, we decided to investigate how marine mosshead sculpins (high gill area) handle temperature and hypoxia-reoxygenation challenges in their environment, with respect to relationships between diffusive water flux rates and $\dot{M}O_2$ relative to marine tidepool sculpins (low gill area) of our earlier study (Somo et al. 2020).

We first hypothesized that the larger gill surface area of mosshead sculpins would result in higher diffusive water flux rates, and more marked adjustments during temperature challenges though the overall responses would be qualitatively similar with those of tidepool sculpins. Thus, we predicted that both diffusive water flux rates and $\dot{M}O_2$ would increase with increases in temperature, with greater thermal sensitivity of the former relative to the latter. Our second hypothesis was that mosshead sculpins, like tidepool sculpins, would reduce both diffusive water flux rate and $\dot{M}O_2$ during acute hypoxia, but with time-dependent differential responses in the two parameters depending on the durations of the hypoxia and reoxygenation recovery periods.

Methods

Ethics

Mosshead sculpins were collected under Fisheries and Oceans Canada scientific license XR-239-2017. In view of conservation concerns, only a limited number of animals were collected, so N numbers (3-6) were kept to a minimum in our experiments. All experiments were done under the approved animal protocols of both Bamfield Marine Sciences Centre (BMSC) (BSMC AUP RS-17-11) and the University of British Columbia (UBC AUP A13-0309) in accordance with the Canadian Council on Animal Care guidelines.

Experimental animals

Mosshead sculpins (*Clinocottus globiceps*) weighing 3.0 ± 0.2 g (SEM) were caught by minnow trap and dipnet near BMSC, Bamfield, British Columbia, Canada (48.8355°N, 125.1355°W), and then transported to the holding facilities at the University of British Columbia, Vancouver, BC, Canada. The fish were allowed to acclimate for at least 3 months before commencement of experiments. The circulating seawater was maintained at 12.5 ± 0.5 °C, salinity 35 ppt, > 95% atmospheric oxygen saturation, and photoperiod light to dark ratio of 12h:12h. Biological filtration in the central seawater system kept ammonia, nitrite, and nitrate levels below detection; pH was 8.0 – 8.2, and PCO_2 was at air saturation. These same conditions were present during our experimental tests, except that O_2 levels were experimentally manipulated in some of them, as described below. Mosshead sculpins were fed *ad libitum* 3 times per week with commercially purchased high quality blood worms (chironomid larvae) and spirulina-loaded brine shrimp with no known detrimental effect to the fish (Hikari Sales USA, Hayward, CA, USA).

Experimental protocols

All the experimental protocols used in these investigations were based on the methods described in Onukwufor and Wood (2018) and Somo et al. (2020), with slight modifications. In brief, mosshead sculpins were fasted for 3 days before the start of all experiments. In this investigation, we consider a temperature of 13 °C

and normoxia as the control (acclimation) condition. In the first series, we measured effects of thermal stress on diffusive water flux rate and $\dot{M}O_2$ under normoxia at three different temperatures 6 °C, 13 °C and 25 °C. In the second series, we assessed the effects of two levels of hypoxia (20% air and 10% air saturation) relative to normoxia (>95% air saturation) on diffusive water flux rates and $\dot{M}O_2$ at 13 °C. For the third series, we tested the effects of short-term hypoxia (1-h 10% air saturation) with 1-h or 3-h recovery and prolonged hypoxia (3-h 10% air saturation) with 1-h or 3-h recovery on diffusive water flux rates and $\dot{M}O_2$.

Methods for diffusive water flux rate measurements

For each experimental series, 3-6 fish were placed in 1 L of seawater labeled with 40 µCi of tritiated water (^3H_2O, Perkin Elmer, Woodbridge, ON, Canada). The container was continuously bubbled with the appropriate gases to maintain normoxia or hypoxia (see *Sections 2.7* and *2.8*), shielded with black plastic, and submerged in a water bath held at 13 °C for at least 12-h equilibration with the radioisotope. Our previous work with tidepool sculpins indicated that a 12-h period is sufficient for equilibration (Somo et al. 2020). At the end of this period, each fish was gently removed from the loading container by dipnet, quickly rinsed with ^3H_2O-free seawater to remove any ^3H_2O on the body surface, and then placed in an individual small container containing 100 ml of seawater devoid of ^3H_2O. This shielded container was similarly bubbled to maintain normoxia or hypoxia as required and was submerged in a water bath so as to maintain the required temperature (see *Section 2.6*). The diffusive water flux rate measurement was based on the appearance of ^3H_2O radioactivity from the fish into the external seawater in the first 60 min, as described below.

A 1-ml sample of seawater was taken at time 0 and at every 5-min interval for 60 min. A final 1-ml sample was taken at 12h, a time when ^3H_2O efflux was complete such that the ^3H_2O in the fish had fully equilibrated with the external seawater. The fish was then weighed and returned to its holding tank. The water samples drawn during the 0- to 60-min window were used to calculate the diffusive water flux rate. The ^3H_2O radioactivity of the sample at 12h, multiplied by the external seawater volume, plus the small amount of ^3H_2O radioactivity removed in sampling, were used to back-calculate the original dose of ^3H_2O in the mosshead sculpins at time 0h (see *Section 2.9*).

All measurements of diffusive water flux were done within the first 60 min to account for the fast equilibration rate. To this effect, for longer durations of hypoxia exposure and/or normoxic recovery, the experimental treatments were started during the ^3H_2O loading period and were continued during the 1h flux rate measurement period, as described in detail in *Section 2.8*. Following experimental treatment exposure, fish were maintained under normoxia until the final seawater sample was taken at 12h for back-calculation of the dose of ^3H_2O in the fish at time 0h.

An unavoidable limitation in this protocol (involving the transfer of fish from the loading container to the washout container) is the possibility of handling stress during the diffusive water flux measurement. Key physiological and biochemical parameters in fish such as cortisol levels, glucose and plasma ions have all been reported to be impacted by handling stress in fish (Pickering et al. 1982; López-Patiño et al. 2014). However, the impact of handling stress depends on the duration and severity of the handling (Pickering et al. 1982; López-Patiño et al. 2014). Minimal stress over short periods had no effects while longer and more severe stress produced detrimental effects on the fish. In our experiments, handling stress occurred only over a few seconds during the transfer from the loading container to the washout container, so detrimental effects on the fish were likely minimal.

Methods for respirometry

$\dot{M}O_2$ measurements were done using intermittent-flow respirometry in individual glass containers with a respirometer volume-to-body mass ratio of ~ 45x (mean ± sem = 45.5 ± 1.7) as described by Somo et al. (2020). In brief, the respirometer was submerged in a water bath kept at the target experimental temperature using a benchtop temperature regulator (model 1160S, VWR International, Radnor, PA, USA). Target temperatures were 6, 13, or 25 °C in the temperature stress experiments and 13 °C (i.e., the acclimation temperature) for all other treatments. Desired oxygen levels were maintained by bubbling nitrogen gas to deplete oxygen or bubbling air to increase oxygen depending on experimental conditions. Plastic "bubble-wrap" was placed on top of the water to reduce atmospheric O_2 ingress into the bath and maintain O_2

at the desired hypoxic level. A black plastic covering prevented visual disturbance of the fish. The O_2 level in the water bath was monitored using a handheld dissolved oxygen meter connected to a galvanic oxygen probe (model DO110, Oakton Instruments, Vernon Hills, IL, USA). The O_2 level inside the respirometers was sampled using a fiber-optic fluorescent probe in a stainless-steel housing (FOXY system, Ocean Optics, Dunedin, FL, USA) and recorded every 15 sec using Ocean Optics' NeoFox software. Magnetic stir bars below a false bottom mixed water inside the respirometers. Flushing and closed periods were automated using Aquaresp v 3.0 software (AquaResp.com) to power flush pumps through a USB power switch (model Cleware 1 USB-SwitchC IEC 16A Product no.:24-1, Cleware GmbH, Germany). At the end of each measurement, fish were weighed and returned to the holding tank.

To ensure fish in extended duration $\dot{M}O_2$ trials experienced handling similar to those in the extended duration diffusive water flux trials, fish in $\dot{M}O_2$ trials were first held in the target experimental conditions in containers of the same design used in the DWF experiments and moved into respirometers at the target conditions for the final hour of experimental treatment and $\dot{M}O_2$ measurement.

Effects of acute temperature change on diffusive water flux and oxygen uptake rates

For diffusive water flux measurements, mosshead sculpins were first loaded with ^3H_2O for 12h under standard conditions (normoxia, 35 ppt, 13 ^oC), then acutely transferred to 13 ^oC (control, $N = 3$), or acutely decreased temperature (6 ^oC, $N = 3$), or acutely increased temperature (25 ^oC, $N = 3$). For $\dot{M}O_2$ measurements, fish were acutely transferred to 13 ^oC (control, $N = 6$), or acutely decreased temperature (6 ^oC, $N = 6$), or acutely increased temperature (25 ^oC, $N = 6$). For both diffusive water flux and $\dot{M}O_2$, measurements were done for 1-h.

Effects of acute hypoxia on diffusive water flux and oxygen uptake rates

To measure the effect of acute hypoxia on diffusive water flux, mosshead sculpins were loaded with ^3H_2O for 12h under the standard conditions (normoxia, 35 ppt, 13 °C), then transferred to normoxic water that

was already set at > 95% air saturation ($N = 3$), or hypoxic water that was already set at 20% ($N = 3$) or 10% ($N = 3$) air saturation. For $\dot{M}O_2$, measurements were carried out in normoxic water ($N = 6$), or hypoxic water that was already set at 20% ($N = 6$) or 10% ($N = 6$) air saturation. For both diffusive water flux and $\dot{M}O_2$, measurements were done for 1h.

Effects of short-term or prolonged hypoxia and recovery on diffusive water flux and oxygen uptake rates

Here the effects short (1-h) or prolonged (3-h) hypoxia and recovery periods on diffusive water flux and $\dot{M}O_2$ of mosshead sculpins were assessed. We made measurements under normoxia (>95 air saturation, N=3-6), short (1-h) hypoxia (10%, $N = 3$-6), 1-h hypoxia (10%) + 1-h normoxic recovery ($N = 3$), and 1-h hypoxia (10%) + 3-h normoxic recovery ($N = 3$-6). For prolonged hypoxia and recovery, we assessed 3-h hypoxia (10%, N = 3-6), 3-h hypoxia (10%) + 1-h of normoxic recovery ($N = 3$-6) and 3-h hypoxia (10%) + 3-h of normoxic recovery (N=3-6). For $\dot{M}O_2$ we made measurements under normoxia (>95 air saturation, N=3-6), short (1-h) hypoxia 10% ($N = 3$-6), 1-h hypoxia (10%) + 1-h normoxic recovery (N = 3-6), and 1-h hypoxia (10%) + 3-h normoxic recovery ($N = 3$-6). For prolonged hypoxia and recovery, we assessed 3-h of hypoxia (10%, N =3-6), 3-h hypoxia (10%) + 1-h of normoxic recovery and 3-h hypoxia (10%) + 3-h of normoxic recovery (N =3-6). For both diffusive water flux and $\dot{M}O_2$, measurements were done for 1h.

For diffusive water flux measurements, in the 3-h hypoxia treatment, the first 2h of hypoxia exposure were done within the loading container after the end of the 12-h loading period. The fish were then transferred to the 100 ml of ^3H_2O -free seawater, which was also maintained under hypoxia, for flux measurements during the 3rd h of hypoxia exposure. For the 1-h hypoxia + 1-h normoxia recovery treatment, 12-h loading under normoxia + 1-h of hypoxia were done in the loading container, with the 1-h normoxic recovery period used for diffusive water flux measurement. For the 1-h hypoxia + 3-h normoxia recovery period experiment, the 12-h loading under normoxia + 1-h hypoxia + 2-h normoxic recovery were all done in the loading container and the final 1-h of normoxic recovery was used for the diffusive water flux

measurement. For the 3-h hypoxia and 1-h normoxic recovery period experiment, the 12-h loading under normoxia + 3-h of hypoxia were all done in the loading container and then the 1-h of normoxic recovery was used for diffusive water flux measurement. For the 3-h of hypoxia + 3-h of normoxic recovery period experiment, the 12-h of loading under normoxia + 3-h of hypoxia + 2-h of normoxic recovery were done in the loading container and the final 1-h of normoxic recovery was used to measure the diffusive water flux. Therefore, in all the treatments, the diffusive water flux measurements were performed during the 1-h period when the fish was first transferred to the 100 ml of ^3H_2O -free seawater.

Analytical techniques and calculations

The concentration of ^3H_2O in seawater samples was measured using a scintillation counter (LS6500, Beckman Coulter, Fullerton, CA, USA) as described by Onukwufor and Wood (2018). Briefly, 2 ml of Optiphase 3 fluor (Perkin-Elmer, Wellesley, MA, USA) were added to the 1-ml water sample and vortexed prior to loading into the scintillation counter. Quenching correction was not needed, since our internal standardization tests revealed that quenching was constant. The rate constant k (h^{-1}) of ^3H_2O efflux was calculated by first determining the initial amount of ^3H_2O in the fish. This was done using the final water sample at the end of 12h of the washout period when the fish water pool was at equilibrium with the external water pool. The ^3H_2O radioactivity of the sample at 12h multiplied by the external seawater volume, plus the small amount of ^3H_2O radioactivity removed in sampling, yielded the original dose of ^3H_2O in the mosshead sculpins at time 0h. Knowing the initial amount of ^3H_2O in the fish, it was possible to calculate the amount of ^3H_2O in the fish at every sample time from 0 to 60 min. We then regressed the natural logarithm (ln) of the total ^3H_2O in the fish against time (h) on a linear scale. The slope represented the fractional rate constant k (h^{-1}) for water turnover. The percent body turnover per hour was obtained by multiplying the rate constant k by 100. The diffusive water flux rate in ml/h was obtained by multiplying percent body turnover per hour by 0.8 ml/g which is the total exchangeable water pool of fish (Holmes and Donaldson 1969; Isaia 1984; Olson 1992).

$\dot{M}O_2$ calculations followed the procedure described by Somo et al. (2020). In brief, oxygen partial pressure values were first converted to O_2 concentrations (in µmol/L) using salinity- and temperature-dependent solubility coefficients (Boutilier et al. 1984). Following that, the change in O_2 concentration was multiplied by respirometer volume and divided by time to yield $\dot{M}O_2$ in µmol O_2/h.

In order to assess the effects of body mass, the logarithms of $\dot{M}O_2$ and diffusive water flux rate were regressed against the logarithms of fish weight, resulting in allometric coefficients of b = 1.17 ± 0.52 for $\dot{M}O_2$ and 0.9543 ± 0.0800 for diffusive water flux rate. In view of the fairly narrow weight range and this finding that the slopes for $\dot{M}O_2$ and diffusive water flux rate were not significantly different from 1.0 and from each other, we used the data without allometric scaling. Therefore, for both, rates per fish were divided by the fish weight to yield values in ml/g/h for diffusive water flux rate and µmol O_2/g/h for $\dot{M}O_2$.

Temperature coefficients (Q_{10} values) for diffusive water flux and $\dot{M}O_2$ over the ranges 6-13 °C, 13-25 °C and 6-25 °C were calculated as described by Onukwufor and Wood (2020a):

$$Q_{10} = \left(\frac{R_2}{R_1} \right)^{[10/(T2-T1)]}$$

where R2 and R1 are the rates at the higher temperature (T2) and lower temperature (T1) respectively. Since different fish were used in each trial, we employed mean values of diffusive water flux rates and $\dot{M}O_2$ at each temperature for these calculations.

Statistical analysis

All data have been expressed as means ± 1 SEM (N). For statistical comparisons, data were first subjected to normality and homogeneity of variance tests, and all data passed these tests. Data were analyzed using One-Way ANOVA plus Tukey's *post hoc* test (multiple comparisons), with temperature, hypoxia, normoxia, and recovery periods as independent variables. Significance was accepted at p < 0.05. Statistical analyses, linear regressions, and curve fitting were done using GraphPad™ Prism v7 (GraphPad Software, San Diego, CA, USA).

Results

Acute temperature change alters both diffusive water flux rates and $\dot{M}O_2$ in mosshead sculpins

Acute temperature change had significant effects on diffusive water flux rates ($p = 0.0001$) in mosshead sculpins (Fig. 1A). Here decreasing the temperature from 13 to 6 °C resulted in a non-significant decrease in the rate of diffusive water flux rate by 22%. However, an acute increase in temperature from 13 to 25 °C substantially increased the diffusive water flux rate by 217%. The overall Q_{10} from 6 to 25 °C was 2.08 with a low Q_{10} over 6-13 °C of 1.42 and a high Q_{10} over 13-25 °C of 2.62 (Table 1). The $\dot{M}O_2$ values were also significantly ($p = 0.0001$) affected by acute changes in temperature (Fig. 1B). An acute decrease in temperature from 13 to 6 °C reduced $\dot{M}O_2$ by 57% while an acute increase from 13 to 25 °C increased the rate by 140%. The overall Q_{10} for $\dot{M}O_2$ from 6 to 25 °C was high with a value of 2.47. The Q_{10} value of 3.35 was higher at the low temperature range (6-13 °C), with a lower value of 2.07 for the high temperature range (13-25 °C), an opposite pattern than for diffusive water flux rate (Table 1).
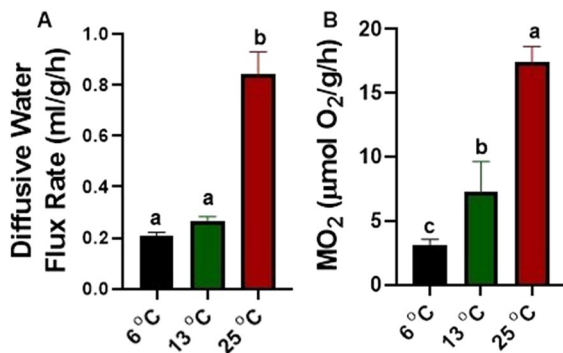
Table 1 The mean Q_{10} values of mosshead sculpins (*Clinocottus globiceps*) for diffusive water flux rates and $\dot{M}O_2$ during expose to acute temperature challenges from the acclimation temperature of 13 °C to 6 °C and from 13 °C to 25 °C. Data are mean Q_{10} values for N = 3-6 different animals at each temperature

Treatment	Q_{10} 6-13 °C	Q_{10} 13-25 °C	Q_{10} 6-25 °C
Diffusive Water Flux Rate (ml/g/h)	1.42	2.62	2.08
$\dot{M}O_2$ (μmol O_2/g/h)	3.35	2.07	2.47

Divergent effects of acute hypoxia on diffusive water flux rates and $\dot{M}O_2$ in mosshead sculpins

Acute hypoxia had no significant effect on diffusive water flux rates ($p = 0.43$) in mosshead sculpins with non-significant increases of only 2.7% and 8.5% at 20% or 10% air saturation respectively relative to the normoxic control value (Fig. 2A). In marked contrast, $\dot{M}O_2$ declined significantly ($p < 0.05$) under acute hypoxia with reductions of 36% and 65% at 20% and 10% air saturation respectively (Fig. 2B).
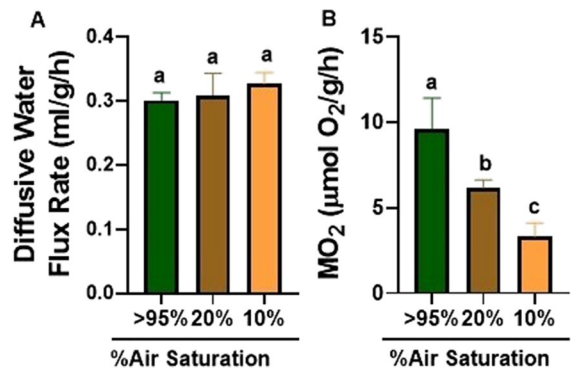

Fig. 1 Effects of acute temperature changes on mosshead sculpins (*Clinocottus globiceps*) (**A**) diffusive water flux rate (ml/g/h) ($N = 3$), and (**B**) $\dot{M}O_2$ (μmol O_2/g/h) ($N = 6$). Measurements were done under normoxia at the acclimation temperature of 13 °C, 35 ppt, and after acute downward transfer from 13 °C to 6 °C and acute upward transfer from 13 °C to 25 °C using separate fish for each treatment. Data are means ± S.E.M. Means with different letters are significantly different (p < 0.05)

Fig. 2 Effects of transfer of mosshead sculpins (*Clinocottus globiceps*) from normoxia (PO_2 > 95% air saturation) to two levels of acute hypoxia (PO_2 = 20% and 10% air saturation). (**A**) Diffusive water flux rate (ml/g/h) ($N = 3$), and (**B**) $\dot{M}O_2$ (μmol O_2/g/h) ($N = 6$). Measurements were all done at the acclimation temperature of 13 °C, 35 ppt using separate fish for each treatment. Data are means ± S.E.M. Means with different letters are significantly different (p < 0.05)

Effects of different durations of hypoxia and normoxic recovery on diffusive water flux rates and $\dot{M}O_2$ in mosshead sculpins

Experimental variations in the duration of hypoxia and recovery periods in mosshead sculpins produced overall significant differences ($p = 0.0001$) in diffusive water flux rates (Fig. 3A). An acute decrease in environmental O_2 from >95 to 10% air saturation for 1-h resulted in a non-significant increase of 8.2% in diffusive water flux rate (Fig. 3A). Following 1-h exposure to 10% air saturation with short (1-h) or longer (3-h) normoxic recovery periods resulted in depressed diffusive water flux rates of 19% ($p = 0.001$) and 15% ($p = 0.007$) respectively, relative to the normoxic value. Longer exposure (3-h) to 10% air saturation resulted in a non-significant increase of 2.2% of diffusive water flux rate relative to the normoxic >95% air saturation value. However, 1-h and 3-h normoxic recovery periods following 3-h of hypoxia resulted in marginally significant (P=0.07) or significant (P=0.003) reductions by 10% and 21% respectively

in diffusive water flux rate relative to the normoxic control value (Fig. 3A).

These same experimental treatments produced very different patterns in $\dot{M}O_2$, though again there were highly significant effects ($P=0.0001$) of treatment (Fig. 3B). Acute exposure of mosshead sculpins to 10% air saturation for 1-h, resulted in a significant 58% decrease ($p=0.002$) in $\dot{M}O_2$. However, exposure to 10% air saturation for 1-h followed by 1-h of normoxic recovery resulted in a significant 71% overshoot ($p = 0.0001$) of $\dot{M}O_2$ above the normoxic control level. This declined with a longer 3-h normoxic recovery period to about 45% higher than the normoxic control level but remained significant ($p = 0.02$) (Fig. 3B). Exposure to 10% air saturation for 3h induced a significant 70% reduction ($p = 0.0002$) in $\dot{M}O_2$ when compared to the normoxic rate. Exposure to 10% air saturation for 3-h followed by 1-h normoxic recovery, resulted in a significant overshoot of $\dot{M}O_2$ ($p = 0.0001$) to 78% higher than the normoxic control, whereas after 3-h of recovery, the overshot was reduced to 35% and was no longer significant ($p = 0.14$) relative to the control (Fig. 3B).
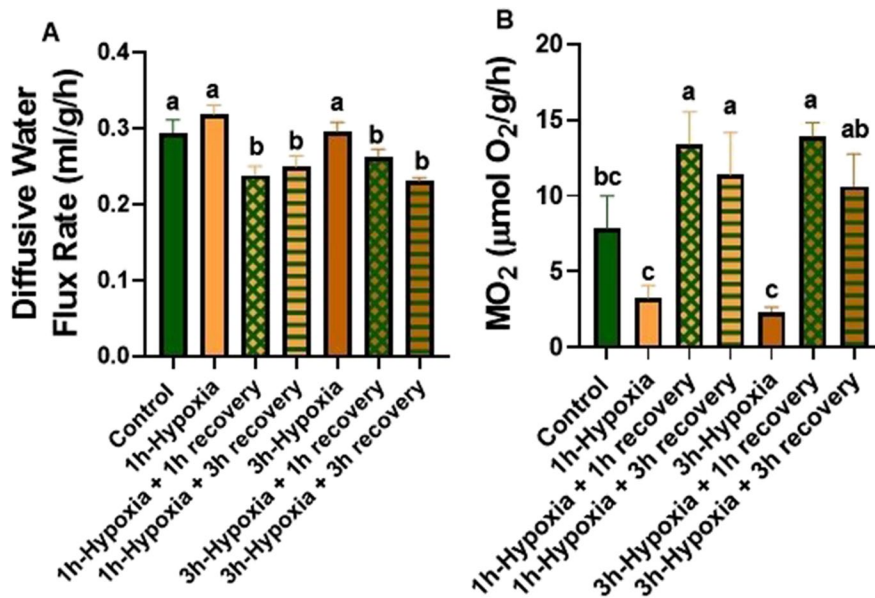

Fig. 3 Effects of durations of hypoxia and normoxic recovery on mosshead sculpins (*Clinocottus globiceps*) diffusive water flux and $\dot{M}O_2$. Fish were exposed to 10% hypoxia for 1-h, 10% hypoxia 1-h + 1-h recovery, 10% hypoxia 1-h + 3-h recovery, 10% hypoxia 3-h, 10% hypoxia 3-h + 1-h recovery and 10% hypoxia 3-h + 3-h recovery. (**A**) Diffusive water flux rate (ml/g/h) ($N = 3$), and (**B**) $\dot{M}O_2$ (μmol O_2/g/h) ($N = 6$). Measurements were all done at the acclimation temperature of 13 °C, 35 ppt using separate fish for each trial. Data are means ± S.E.M. Means with different letters are significantly different ($p < 0.05$)

Discussion

General overview and comparison of mosshead sculpins versus tidepool sculpins

As discussed in detail subsequently, our results support the view that the traditional osmo-respiratory compromise applies during thermal stress in the mosshead sculpins, such that $\dot{M}O_2$ and diffusive water flux rates vary more or less in parallel during acute temperature challenges. However, during hypoxia and re-oxygenation recovery, there is a clear decoupling of diffusive water flux rates from $\dot{M}O_2$, in agreement with the emerging view that the traditional osmo-respiratory compromise may often not apply under these conditions.

With respect to our first hypothesis, we had predicted that the larger gill surface area of mosshead sculpins would result in higher diffusive water flux rates than in tidepool sculpins (Somo et al. 2020). This was not supported: diffusive water flux rates at the acclimation temperature of 13 °C were actually slightly lower in mosshead sculpins than in tidepool sculpins, despite almost identical $\dot{M}O_2$ values in the two species. We had also predicted that mosshead sculpins would exhibit more marked adjustments during temperature challenges, but with a qualitatively similar pattern to that in tidepool sculpins. This was partially true. The patterns were qualitatively similar and the diffusive water flux rate in mosshead sculpins exhibited a much higher sensitivity ($Q_{10} = 2.62$) to an acute upward temperature challenge (13 °C to 25 °C) than seen in tidepool sculpins ($Q_{10} = 1.90$) (Fig. 1, Table 1; Somo et al. 2020). This correlated with a much greater rise in $\dot{M}O_2$ ($Q_{10} = 2.07$), relative to the low response ($Q_{10} = 1.31$) of the tidepool sculpin over the same range. However, the differential sensitivity was reversed with respect to acute low temperature challenge. Here the mosshead sculpins exhibited rather low temperature sensitivity in diffusive water flux ($Q_{10} = 1.42$) relative to tidepool sculpins ($Q_{10} = 3.97$), with the opposite pattern for $\dot{M}O_2$ ($Q_{10} = 3.35$ versus $Q_{10} = 2.21$ respectively). Over the entire temperature range of 6 °C to 25 °C, mosshead sculpins had a slightly lower sensitivity for diffusive water flux rates ($Q_{10} = 2.08$) than for $\dot{M}O_2$ ($Q_{10} = 2.47$), which did not agree with our original prediction.

With respect to our second hypothesis, we had predicted that mosshead sculpins, like tidepool sculpins

(Somo et al. 2020), would reduce both diffusive water flux rate and $\dot{M}O_2$ during acute hypoxia. Again, this was not supported: mosshead sculpins exhibited no significant changes in diffusive water flux rates during either 1-h or 3-h of hypoxia despite marked reductions in $\dot{M}O_2$ (Figs. 2 and 3). In tidepool sculpins the depression of diffusive water flux rate during acute hypoxia generally persisted during short and prolonged recovery periods (Somo et al. 2020). However, in mosshead sculpins, the unchanged diffusive water flux rates during acute hypoxia transformed into significant depressions during short and prolonged recovery periods (Fig. 3A). Furthermore, in tidepool sculpins, reduced $\dot{M}O_2$ during acute hypoxia returned to pre-normoxic control levels during both recovery periods with no overshoot (Somo et al. 2020). On the other hand, in mosshead sculpins the decreases in $\dot{M}O_2$ during hypoxia were followed by pronounced overshoots relative to the pre-normoxic control levels during both short and prolonged recovery (Fig. 3B). Note that the 10% oxygen saturation level used in both studies was slightly below the mean P_{crit} of both the tidepool and mosshead sculpins (Mandic et al. 2009b). Overall, marked species-specific differences exist between the tidepool and mosshead sculpins in their $\dot{M}O_2$ and diffusive water flux rates following stress induced by temperature, hypoxia and hypoxia-oxygenation periods.

Both diffusive water flux rates and $\dot{M}O_2$ correlate with acute temperature change but with different sensitivities, suggesting distinct regulatory mechanisms

Acute temperature change directly modulates whole-organism metabolic oxygen demand (Hochachka and Somero 2002), and the fish cardiorespiratory system responds to match oxygen supply to the new demand, resulting in a positive correlation between $\dot{M}O_2$ and temperature. Oxygen supply capacity at the gill increases through increased ventilation, increases in total lamellar perfusion and lamellar perfusion pressure, thinning of the perfused lamellar epithelium, and decreasing water-to-blood diffusion distances; together these functional responses increase effective gill permeability to enhance oxygen uptake across the gill (Randall et al. 1972; Nilsson 1986). These same functional responses which increase the permeability of the gill to oxygen with warming may also increase gill

permeability to water and ions, as the osmo-respiratory compromise predicts. Several studies have shown that acute temperature stress greatly influences gill epithelial membrane permeability in both freshwater and seawater fish (Evans 1969; Loretz 1979; Giacomin et al. 2017; Somo et al. 2020; Onukwufor and Wood 2018; Onukwufor and Wood 2020a, b, 2022). In mosshead sculpins both diffusive water flux rate and $\dot{M}O_2$ increased with an increase in temperature and decreased with a decrease in temperature, in accord with the commonly observed pattern (Fig. 1). Indeed, temperature effects on epithelial membrane permeability may be almost universal across multiple fish species. Although the functional responses to warming alter gill permeability to oxygen, water, and ions, other factors, such as the temperature sensitivity of hemoglobin-O_2 binding dynamics, may also contribute to the thermal sensitivity of whole-organism $\dot{M}O_2$, resulting in divergent thermal sensitivities of diffusive water flux and whole-organism $\dot{M}O_2$, as observed here (Table 1).

Unlike $\dot{M}O_2$, diffusive water flux rates may be strongly influenced by aquaporins that have been implicated in playing an important role in facilitating water movement in gill epithelia (Tingaud-Sequiera et al. 2010; Cerda and Finn 2010; Madsen et al. 2015; Ruhr et al. 2020). In contrast with tidepool sculpins, (Somo et al. 2020) and a number of other freshwater and seawater species (see Introduction) but in agreement with recent reports on Pacific sanddab (Onukwufor and Wood 2022) and zebrafish (Onukwufor and Wood 2020b), mosshead sculpins exhibited lower overall thermal sensitivity for diffusive water flux than for $\dot{M}O_2$ (Table 1). It may be that in some species, aquaporins are playing a greater role while in others they are not, and/or that the aquaporins differ among species in their thermal sensitivity. Regardless, the fact that temperature elicited a strong positive correlation between diffusive water flux rate and $\dot{M}O_2$ (Fig. 4A) is an indication of its overall control on the two parameters. This is in general agreement of temperature modulating effects on biological processes (Hazel and Prosser 1974; Cossins and Prosser 1978).

Differential effects of acute hypoxia on diffusive water flux rates and $\dot{M}O_2$

The original concept of the osmo-respiratory compromise is that during stress, increases in the effective
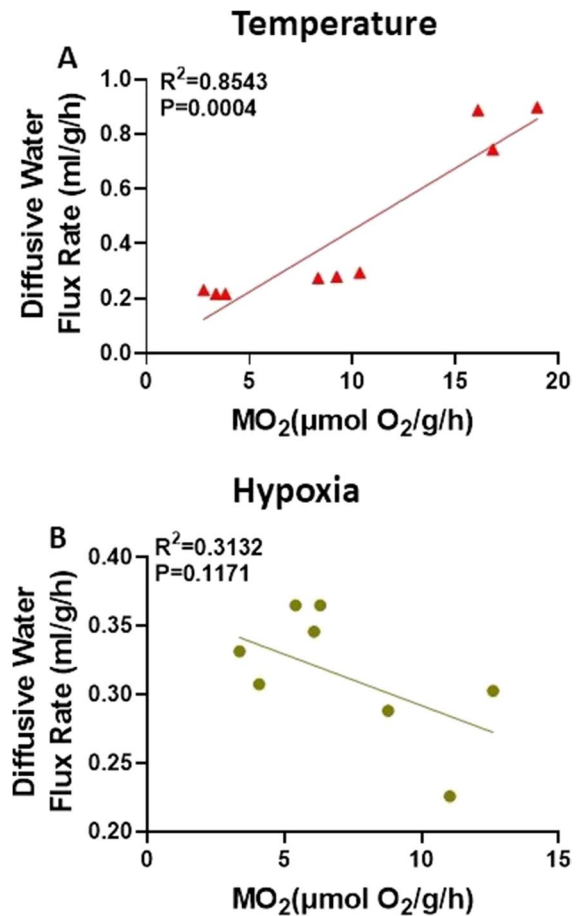

Fig. 4 A) The relationship between diffusive water flux rate (ml/g/h) and $\dot{M}O_2$ (µmol O_2/g/h) for temperature challenges 13 °C to 7 °C and 13 °C to 25 °C (N= 9), in mosshead sculpins (*Clinocottus globiceps*). R^2 and significance levels are given for the regression, with the following equation for temperature: Y = 0.04523*X -0.002320, where Y is diffusive water flux rate (ml/g/h) and X is $\dot{M}O_2$ (µmol O_2/g/h). **B**) The relationship between diffusive water flux rate (ml/g/h) and $\dot{M}O_2$ (µmol O_2/g/h) for hypoxia challenges (PO$_2$ > 95% to 20 or 10% air saturation, $N = 9$) in mosshead sculpins (*Clinocottus globiceps*). R^2 and significance levels are given for the regression, with the following equation for hypoxia: Y = -0.007486*X +0.3665, where Y is diffusive water flux rate (ml/g/h) and X is $\dot{M}O_2$ (µmol O_2/g/h)

permeability of the gills for $\dot{M}O_2$ will result in corresponding increases in osmolyte and water permeability. As with warming, during hypoxia exposure, teleosts make many modifications of gill physiology which increase effective gill permeability to O_2. As observed in our study (Fig. 2), $\dot{M}O_2$ usually falls, but not by as much as if these adjustments to ventilation

and gill permeability had not been implemented (reviewed by Randall 1982; Farrell and Richards 2009). Similarly, in the tidepool sculpin, a close relative of the mosshead sculpin, fish respond to hypoxia exposure by exploiting cardiorespiratory adjustments to maximize oxygen uptake capacity, with $\dot{M}O_2$ falling as a consequence of environmental oxygen limitation (Somo et al. 2022). Therefore, although $\dot{M}O_2$ fell during hypoxia exposure in mosshead sculpins, the fish were likely increasing effective gill permeability to enhance oxygen extraction capacity in the oxygen-depleted environment, though future studies should investigate ventilatory and physiological adjustments at the gill to support this contention.

Under hypoxia, the traditional osmo-respiratory compromise predicts increased diffusive water flux commensurate with the increase in effective gill permeability. Instead, in mosshead sculpins, diffusive water flux remained unchanged during acute hypoxia compared with control conditions (Fig. 2), resulting in no significant correlation between diffusive water flux and $\dot{M}O_2$ (Fig. 4B). Many less hypoxia-tolerant species including goldfish (Loretz 1979), trout (Iftikar et al. 2010; Robertson et al. 2015b; Onukwufor and Wood 2018), and zebrafish (Robertson et al. 2015b; Onukwufor and Wood 2020b), show increases in diffusive water or ion fluxes during hypoxia exposure, in accord with the traditional osmo-respiratory compromise. In contrast, the effect of hypoxia on diffusive water flux in mosshead sculpins is more similar to that observed in a growing number of hypoxia-tolerant species, such as the closely-related tidepool sculpin (Somo et al. 2020), Pacific sanddab and English sole (Onukwufor and Wood 2022), as well as in freshwater Amazonian oscar and tambaqui (Wood et al. 2009; De Boeck et al. 2013; Robertson et al. 2015b) and killifish (Wood et al. 2019; Giacomin et al. 2020), which reduce or do not change flux rates in hypoxia or post-exposure reoxygenation. This selective uncoupling of water and ion permeability from effective oxygen permeability in hypoxia-tolerant species is thought to contribute to whole-organism hypoxia tolerance. This would reduce the energy cost of osmoregulation, at a time of limited aerobic ATP production capacity and high ventilatory costs (Wood et al. 2009). Thus, during acute hypoxia exposure and recovery, $\dot{M}O_2$ and diffusive water flux rates appear to be under the regulatory control of different mechanisms, thereby decoupling effective water

permeability from effective O_2 permeability. Future studies should analyze the impact of hypoxia exposure on ventilation, respiratory factors at the gill (e.g.,Scott et al. 2008), short-term gill morphological responses to hypoxia (e.g., Wood et al. 2009; Matey et al. 2008; Matey et al. 2011) as well as long-term gill morphological responses to hypoxia (e.g. Saroglia et al. 2000; Saroglia et al. 2002; Wu et al. 2017), and the regulation of aquaporins (e.g., Ruhr et al. 2020) and other protein channels to determine the mechanistic basis of the decoupling of oxygen and water permeability.

Reoxygenation elicited opposite responses on diffusive water flux rates and $\dot{M}O_2$

Earlier, Somo et al. (2020) reported that in tidepool sculpins, $\dot{M}O_2$ recovered to normoxic control levels following acute (1 h) and prolonged (3 h) hypoxia exposure, while diffusive water flux either remained depressed or returned to control levels, depending on the duration of hypoxia exposure. We expected a similar response pattern in mosshead sculpins, which inhabit the same oxygen- and temperature-dynamic intertidal environment (Mandic et al. 2009b). However, we saw a rather different response pattern (Fig. 3). In contrast to tidepool sculpins, mosshead sculpins appear to have accrued an O_2 debt during hypoxia exposure as evidenced by significant overshoots in $\dot{M}O_2$ during hypoxic recovery relative to normoxic controls (Fig. 3B). At the same time, diffusive water flux rates were depressed (Fig. 3A).

Although mosshead and tidepool sculpins have identical P_{crit} (Mandic et al. 2009b; Somo 2022), mosshead sculpins have a lower time to loss of equilibrium (Mandic et al. 2013), perform aquatic surface respiration at a higher PO_2 (Mandic et al. 2009a), and have a higher routine $\dot{M}O_2$ (Mandic et al. 2009b). Despite the identical P_{crit} values, overall, these observations suggest a lower hypoxia tolerance in mosshead sculpins and possibly a greater reliance on anaerobic ATP production during hypoxia, necessitating overshoots in $\dot{M}O_2$ during the recovery period to pay off the "oxygen debt". The mosshead response is partially similar to that observed in both freshwater- and seawater acclimated killifish, which had depressed diffusive water flux during short- and long-term re-oxygenation periods, but this occurred in the absence of elevated $\dot{M}O_2$ (Wood et al. 2019).

The mosshead sculpin may use this strategy to reduce the cost of osmoregulation during recovery from hypoxia, a time of unavoidably high ventilatory costs. This cost-saving measure could contribute to the persistence of this species in the temperature- and oxygen-variable intertidal environment by limiting the metabolic load of osmoregulation and preserving metabolic capacity to support ecologically-relevant performances. The molecular mechanisms underpinning these processes in the gills remain unknown but will be an exciting area for future study.

Declarations

Ethical approval Mosshead sculpins were collected under Fisheries and Oceans Canada scientific license XR-239-2017. All experiments were done under the approved animal protocols of both Bamfield Marine Sciences Centre (BMSC) (BSMC AUP RS-17-11) and the University of British Columbia (UBC AUP A13-0309) in accordance with the Canadian Council on Animal Care guidelines

Competing interests All authors declare no competing interest

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