FISH IN HOT WATER: EFFECTS OF TAURINE ON THE THERMAL SENSITIVITY OF AEROBIC METABOLISM

BY

MATHILDE HARRING CHRISTJANSEN

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Abstract

Climate warming creates a multitude of challenges for aquatic ectotherms, both directly through heat stress and indirectly through changes to ecological dynamics – such as nutrient availability. Taurine is an essential nutrient in many aquatic ectotherms and important to physiological function and environmental stress tolerance. The present study provides insight into the role of taurine in fish thermal tolerance by examining the effects of taurine deficiency on aerobic performance in brook char (*Salvelinus fontinalis*). Taurine deficient (TD) char were created using a 5% β-alanine diet, and fish were subjected to intermittent swim tunnel respirometry under warming conditions at 2 °C/h from 15-25 °C. Routine metabolic rates ($\dot{M}O_2$) increased with temperature as was expected for an aquatic ectotherm, and temperature coefficient values (Q_{10}) did not differ between control and TD brook char (2.0 and 1.89, respectively). While a strong positive relationship between ectotherm $\dot{M}O_2$ and temperature is part of their normal physiological function, the progression of climate warming will likely elevate physiological stress and energy demands for aquatic ectotherms with potential adverse fitness consequences. Thermal sensitivity of routine $\dot{M}O_2$ was unaffected by taurine deficiency, which contrasted with previous work showing that TD brook char suffer reduced cardiovascular function without relying on anaerobic metabolism to compensate for lower oxygen circulation. It is possible that there is a profound redistribution of blood flow in TD fish that enables them to compensate for the physiological limitations imposed by taurine deficiency by lowering overall oxygen demands. Any impacts of taurine deficiency may thus only become evident when fish are exposed to a combination of stressors, such as exercise and warming simultaneously, as this would help disentangle the physiological effects of taurine from any compensatory mechanisms and provide more ecologically relevant information about how aquatic organisms may respond to the multitude of stressors arising from climate change.

Introduction

Climatic changes are increasing the frequency and severity of environmental stressors, posing significant threats to biodiversity. Freshwater systems are at particular risk due to the multitude of potential stressors accumulating from both atmospheric, riparian, and terrestrial environments (Reid *et al.*, 2019). Among climatic changes, warming constitutes a major concern for aquatic ectotherms, whose body temperatures, and thus metabolic rates, are directly linked to water temperatures (Jutfelt *et al.*, 2024). For example, fish body temperatures are constantly equilibrating with ambient conditions across highly vascularized gills equipped with large surface areas for gas exchange (Sandblom and Axelsson, 2007). In addition to increased average global temperatures, models of future climate change also predict increases in the frequency, intensity, and duration of acute warming events, such as summer heat waves (Ganguly *et al.*, 2009). Such temperature elevations can directly influence ectotherms by inducing physiological stress and compromising their abilities to perform normal aerobic biological activities, such as growth and reproduction (Dahlke *et al.*, 2020; Pandit and Nakamura, 1970). For example, Chadwick and McCormick (2017) showed that brook char (*Salvelinus fontinalis*) acclimated to 16 °C experience elevated physiological stress under warming conditions. When warmed to 22 °C, brook char plasma cortisol and heat shock protein levels increased more than 10-fold, and growth rates decreased significantly (Chadwick and McCormick, 2017).

Chronic and Acute Warming in Aquatic Systems

Chronic and acute warming events have different consequences for aquatic organisms, which through compounding effects can magnify the threat posed by climatic temperature changes (Jutfelt *et al.*, 2024). Increasing average temperatures reflect a chronic warming event, subjecting aquatic organisms to temperatures permanently elevated relative to historical values. This could pose conservation issues if environmental temperatures warm beyond the physiological optima of organisms adapted to cooler conditions, resulting in compromised fitness (Dahlke *et al.*, 2020; Eliason *et al.*, 2011; Pandit and Nakamura, 1970). For example, fitness of adult sockeye salmon (*Oncorhynchus nerka*) appears to be optimized to the average temperature of their stock-specific natal streams (Lee *et al.*, 2003). This suggests that chronic warming along migration routes might offset environmental conditions relative to fitness optima, possibly compromising homing migrations and spawning success (Farrell, 2009; Steinhausen *et al.*, 2008). Indeed, climate-change induced elevations in river and stream temperatures have already created lethal conditions for many salmon populations (Farrell *et al.*, 2008; Mathes *et al.*, 2010). Though migrating fish may be able to mitigate high temperature effects to some extent by exploiting cooler-water refugia at the expense of migration speed (Berman and Quinn, 1991; Goniea *et al.*, 2006), chronically elevated temperature conditions can also significantly impact resident populations which may be more geographically restricted in their behavioural responses to warming. In general, average temperature is a major factor affecting biogeographic distributions. For example, trout population distributions in the Appalachian Mountains are limited by temperature (Flebbe *et al.*, 2006). As a warming climate decreases the extent of suitable habitat while increasing habitat fragmentation, these species are increasingly imperiled and at greater risk of local extinctions (Flebbe *et al.*, 2006).

Short term temperature elevations reflect acute warming events, subjecting aquatic organisms to substantial changes in ambient temperature over a short period of time. Metabolic rate, and thus energy demand, increases with temperature in ectothermic organisms, so species experiencing rapid environmental temperature changes must have coping mechanisms to conserve energy and prevent tissue damage during periods of rapidly induced thermal stress (Schulte, 2015). Some species have evolved tolerance to large temperature fluctuations in response to occupying highly variable environments, such as intertidal species which must cope with large daily temperature variations (Little and Kitching, 1996; Nguyen *et al*., 2011). For example, the sea snail *Echinolittorina radiata* suppresses metabolic rate and upregulates the expression of protective heat shock proteins in response to acute thermal stress, allowing the snail to withstand short term temperature elevations without significant metabolic costs (Wang *et al*., 2023). Similarly, eurythermal longjaw mudsuckers (*Gillichthys mirabilis*) are capable of rapidly inducing the expression of genes involved in their acute heat shock response and downregulating genes for cell growth and proliferation in response to rapid warming (Buckley *et al*., 2006). This response by the mudsuckers reduces metabolic costs and increases their tolerance to acute heat stress (Buckley *et al*., 2006). In contrast, species occupying historically stable thermal environments often have more limited tolerances to acute thermal stress (Benfey *et al*., 1997; Nguyen *et al*., 2011), making them more susceptible to suffering negative consequences of expected future increases in frequency and severity of acute warming events (Ganguly *et al*., 2009). These species may lack a heat shock response (Tomanek, 2010), or it may be limited, such as in the mesothermal delta smelt (*Hypomesus transpacificus*), making them more susceptible to acute warming events (Komoroske *et al*., 2015). Though warming can affect aquatic species directly by requiring them to respond to shifting thermal environments, warming also affects aquatic species indirectly through changes to their biotic and abiotic environments, potentially further exacerbating physiological stress induced by warming temperatures.

Direct and Indirect Warming Effects on Aquatic Systems

Climate warming gives rise to multiple stressors for aquatic organisms due to the combination of direct and indirect warming effects on biological functions. Changing thermal environments are shifting community dynamics as species interactions shift in favour of more warm tolerant species while heat sensitive species are compromised (Colby *et al.*, 2022; Sharma *et al.*, 2011; Van Zuiden *et al.*, 2016). As an example, increasing stream temperatures in the Appalachian Mountains are compromising the competitive ability of native brook char relative to sympatric creek chub (*Semotilus atromaculatus*) by reducing feeding and aggression levels (Colby *et al.*, 2022). In turn, lower food intakes reduce growth and survival, further compromising species already imperiled by direct temperature effects on performance (Lazo *et al.*, 1998; Pandit and Nakamura, 1970). Similarly, warming can induce range shifts that enable invasive species to enter new habitats and compete with native species that may already be compromised by warming water conditions (Durhack *et al.*, 2021; Van Zuiden *et al.*, 2016). Native cool water-adapted walleye (*Sander vitreus*) populations are expected to become extirpated in many southern and central Ontario lakes as warming shifts the extent of suitable habitat. Their risk of extirpation is further enhanced by the northward range expansion by invasive smallmouth bass (*Micropterus dolomieu*), which are strong competitors of the less warm adapted walleye and will likely limit resource availability for the already heat stressed native species (Van Zuiden *et al.*, 2016).

Warming can also affect predator-prey relations. Shifting predator-prey dynamics in response to warming may arise in more than one way, including increased predation pressure from invasive species and trophic mismatch between warming effects on the life cycles of predator and prey species (Jonsson and Setzer, 2015; Sharma *et al.*, 2011). For example, 25-70% of native North American populations of cisco (*Coregonus artedii*) are expected to be extirpated by the year 2100 due to warming (Sharma *et al.*, 2011). In addition to this direct warming effect on cisco populations, invasion by non-native rainbow smelt (*Osmerus mordax*) is likely to increase predation pressure on juvenile cisco and further exacerbate the negative effects of warming on cisco populations (Sharma *et al.*, 2011). Furthermore, warming is potentially driving trophic mismatches in some predator-prey systems, such as in that of great

Arctic char (*Salvelinus umbla*) and their zooplankton prey in Lake Vättern in Sweden (Jonsson and Setzer, 2015). Warming is offsetting the developmental rates of great Arctic charr eggs relative to the peak of their zooplankton prey, causing fry to emerge at sub-optimal resource abundance. This decrease in food availability at a critical life stage is likely compromising stock recruitment through reduced fry and juvenile survival (Jonsson and Setzer, 2015).

Chronic warming is also likely to affect aquatic organisms indirectly through changes to the physical structure of aquatic habitats. Warming is expected to strengthen vertical stratification by increasing the density differences between surface and deeper waters (van de Waal *et al.*, 2010). Stratification reduces the movement of nutrients through the water column and restricts the influx of nutrient-rich deep waters to surface regions. This in turn compromises phytoplankton growth with negative consequences for higher trophic levels in terms of both energy and nutrient flow through the food web (Behrenfeld *et al.*, 2006). While warming might restrict nutrient cycling to some degree, climatic changes may also elevate the influx of certain nutrients to aquatic systems. For example, phosphorous loading in aquatic systems is expected to increase as the climate changes, as more frequent winter rainfall contributes to elevated phosphorous transfer from land to streams, and warming increases the rates of phosphorus release from the sediment (Jeppesen *et al.*, 2009). Increased nutrient loading contributes to eutrophication and is likely to drive shifts in aquatic trophic structure and impact species at each trophic level differently. For example, cyanobacterial (e.g. *Microcystis*) algal blooms are promoted by increased availability of nutrients such as phosphorous (Havens, 2008), especially when combined with warming events like summer heatwaves (Jöhnk *et al.*, 2008). Thus, warming may compromise aquatic ecosystems indirectly by promoting harmful algal blooms, which can deteriorate water quality through deoxygenation of deeper waters and toxin production, contributing to negative impacts of warming on the health and viability of aquatic organisms (Paerl *et al.*, 2001). Elevated levels of total phosphorous are also associated with decreases in the biomass ratios of zooplankton to phytoplankton and of piscivorous to planktivorous fishes, further offsetting food web structure (Jeppesen *et al.*, 2000).

Effects of Temperature on Diet

Diet composition in aquatic ectotherms generally seems to shift with temperature, with greater incorporation of plant material under warmer conditions (Jeppesen *et al.*, 2010). This trend has been observed in several different taxa, including crayfish (Carreira *et al.*, 2017), fish (Behrens and Lafferty, 2012; González-Bergonzoni *et al.*, 2012; Prejs, 1984), frogs (Carreira *et al.*, 2016), snails (Zhang *et al.*, 2020), and zooplankton (Boersma *et al.*, 2016). For example, roach (*Rutilus rutilus*) and rudd (*Scardinius erythrophthalmus*) consume more plant material at higher temperatures (Prejs, 1984). Climate warming is likely to drive shifts in fish diets towards greater omnivory and herbivory. This might be associated with changes to the nutritional value of aquatic plants at elevated temperatures (Behrens and Lafferty, 2007; Velthuis *et al.*, 2017). Cellulose can be utilized in the presence of the enzyme cellulase, which is produced by microorganisms only at temperatures of 15-42 °C and inactivated even by brief exposure to cooler conditions, thus enhancing plant nutritional value under warmer conditions and possibly explaining the dietary shift towards herbivory with rising temperatures (Vejříková *et al.*, 2016). Another potential explanation involves the positive relationship between fish metabolism and temperature, which would lead to higher food intake at elevated temperatures with resulting lower availability of animal-based food due to top-down pressures (Brown *et al.*, 2004; González-Bergonzoni *et al.*, 2012). In this case, plant-based food sources may be less limiting and thus more beneficial to consume at warmer compared to cooler temperatures, where fish metabolic rates and thus food intakes are lower and carnivory is a less limiting strategy (González-Bergonzoni *et al.*, 2012). As nutrient availability shifts in response to climate warming, some nutrients may become more abundant while others may become scarcer - meaning dietary shifts have the potential to lead to detrimental effects on individual performance. For example, while omnivorous opaleye (*Girella nigricans*) increase the proportion of plant material in their diet in response to warming (Behrens and Lafferty, 2012), this shift also compromises their cardiovascular thermal performance, thus increasing their overall sensitivity to warming conditions (Hardison *et al.*, 2021). Changing nutritional landscapes will likely favour some species over others, and limited availability of essential nutrients may compromise physiological health and performance in organisms already under stress from a warming environment.

<u>Taurine in Fish Physiological Function</u>

One nutrient that may become limiting for some species as aquatic systems warm is taurine. Taurine is a sulfur-containing non-proteinogenic β-amino acid and an important nutrient for fish physiological function and stress tolerance (Brosnan and Brosnan, 2006; Dixon *et al.*, 2023). Fish species differ in their capacity to synthesize taurine endogenously, and some species must acquire it through their diet (Goto *et al.*, 2001; Goto *et al.*, 2003; Kim *et al.*, 2005). While taurine is an abundant amino acid in animal tissues, plants contain low or negligible amounts insufficient to support physiological demand (Brosnan and Brosnan, 2006; Gaylord *et al.*, 2006; Spitze *et al.*, 2003). As warming is likely to drive dietary shifts towards greater reliance on plant material (Jeppesen *et al.*, 2010), fish requiring dietary taurine may become deficient, increasing their risk of suffering the negative consequences of taurine deficiency. This might especially impact fish that predominantly are carnivorous and thus more likely to rely on dietary sources of taurine (Kotzamanis *et al.*, 2020). Taurine is involved in a multitude of physiological functions in fish, functioning as a potent regulator of cardiovascular function and the most important osmolyte in fish cardiomyocytes (Dixon *et al.*, 2023; Vislie and Fugelli, 1975). Osmolytes are organic molecules involved in regulating cell osmotic pressure and volume, thereby helping to preserve homeostasis and prevent physiological stress (Burg and Ferraris, 2008). Taurine deficiency is likely to be detrimental to performance, with negative effects on energy metabolism (Dixon *et al.*, 2023), growth (Kotzamanis *et al.*, 2020), heart function (Dixon *et al.*, 2023; Pansani *et al.*, 2012), osmoregulation (Fugelli and Vislie, 1982), and stress tolerance (Banerjee *et al.*, 2019; Dixon *et al.*, 2023; Henry and MacCormack, 2018). For example, a 20% cardiac taurine deficiency in brook char significantly affects whole animal sensitivity to acute thermal and hypoxic stress (Dixon *et al.*, 2023), while a taurine rich diet improves recovery from anoxia in killifish (*Fundulus heteroclitus*) cardiac muscle (Henry and MacCormack, 2018). With warming aquatic environments, fish may become more vulnerable to heat stress if their diets are lacking in taurine. Higher taurine levels in tiger puffer (*Takifugu rubripes*) increase thermal tolerance (Shi *et al.*, 2020), and shifts towards more plant-based diets with insufficient levels of taurine may compromise the ability of some fish species to cope with warming conditions. Further, changing environmental conditions, including exposure to hypoosmotic or acidic water (Fugelli and Vislie, 1982; Vislie, 1980), or heat stress may induce taurine depletion (Liu *et al.*, 2019), further exacerbating the negative effects of warming on fish relying on dietary taurine.

As taurine is important for fish cardiovascular function, taurine deficiency may compromise aerobic performance under heat stress. The ability of a fish to maintain aerobic performance under thermal stress is dependent upon its capacity to increase cardiac output proportionally with temperature (Eliason and Anttila, 2017). Cardiac output is compromised in taurine deficient (TD) brook char both under severe hypoxia and under acute thermal stress (Dixon *et al.*, 2023; El, 2023), and thermal stress might have a similar effect on aerobic performance given the role of taurine in both cardiovascular function and environmental stress tolerance, including hypoxia and temperature (Dixon *et al.*, 2023). Indeed, taurine has been found to support elevated critical swim speeds and performance in European seabass (*Dicentrarchus labrax*) by protecting against oxidative stress under sustained swimming (Ceccotti *et al.*, 2019), and TD mice exhibit impaired exercise performance (Seidel *et al.*, 2019). During exercise, mismatches between tissue demands for oxygen and the capacity of the cardiovascular system to supply oxygen limit aerobic function at high temperatures (Pörtner and Knust, 2007). If taurine deficiency compromises cardiovascular function, we might expect taurine deficiency to compromise aerobic function during thermal stress.

Purpose of the Present Study

Here, I aim to provide insight into the effects of taurine on fish aerobic performance during thermal stress by comparing aerobic metabolic rates of healthy and taurine deficiency brook char under optimal and suboptimal thermal conditions, focusing on heat stress. Taurine plays an important role in supporting cardiac function, and thus oxygen delivery, and environmental stress tolerance in brook char, although the extent to which it affects aerobic scope is not known (Dixon *et al.*, 2023). Given that taurine supports cardiac function and oxygen delivery under thermal stress in brook char (Dixon *et al.*, 2023; El, 2023), and that maximum aerobic performance is dependent on cardiac output (Eliason and Anttila, 2017), taurine deficiency is expected to 1) decrease maximum aerobic performance over a range of temperatures, 2) cause more rapid declines in performance above the thermal maximum, and 3) reduce thermal tolerance during exercise under warming conditions. Thus, this work will provide insight into the physiological mechanisms underlying aerobic performance and environmental stress tolerance in fish.

Materials and Methods

Animal Housing Conditions

Brook char were acquired from Fraser’s Mills Fish Hatchery (Fraser’s Mills, NS, Canada) and transported to the Harold Crabtree Aqualab at Mount

Allison University. Fish were housed in groups in 100 L tanks receiving partially recirculated, filtered, and aerated freshwater at 15±0.5 °C (total system volume of 1300 L) under natural photoperiod for at least 6 months prior to experimentation. Brook char aerobic scope is maximized around 15-16 °C (Durhack *et al.*, 2021; Graham, 1949). Control (mean±s.e.m. body mass 274.1±1.2 g) and taurine-deficient (303.3±2.1 g) fish were housed separately and fed one of two experimental diets once daily to satiation: control brook char were fed 4 mm Optiline RC extruded salmonid feed (Skretting, NB, Canada) while TD fish received the same feed spray-coated with 5% by mass β-alanine and dehydrated prior to use (Allo *et al.*, 1997). All fish received their experimental diet for at least 4 weeks prior to experimentation. Experiments were performed in accordance with the guidelines provided by the Canadian Council on Animal Care and approved by the Mount Allison University Animal Care Committee (Protocol No. 103144).

Experimental Setup

The experimental setup consisted of three 10 L large swim tunnels (Loligo Systems, Tjele, Denmark, Product No. SW10110) with aerated freshwater from the Harold Crabtree Aqualab, recirculating through an insulated 350 L reservoir. Baseline water temperature in the swim tunnels was regulated by adjusting temperature in the reservoir using a 20 L refrigerated circulator (Polyscience, Product No. AP20R-30-A11B) with stainless steel heating coils (see Thermal Stress Exposure for details on temperature regulation). Swim tunnels were used for intermittent flow respirometry using AutoResp v2 software (Loligo Systems). Water velocities in the swim tunnels were regulated using DAQ-BT instruments (Loligo Systems, Product No. SW10600) calibrated with a two-point standard curve in AutoResp using a handheld flowtherm NT flow probe (Höntzsch flow measuring technology). Oxygen saturation and temperature measurements were obtained via a Witrox 4 instrument (Loligo Systems, Product No. OX11875). Fiber optic oxygen probes were calibrated in aerated freshwater and sodium sulfite solution. Intermittent respirometry was achieved using a NETIO4 power bar (Loligo Systems) to control inflow of aerated water to the respirometer chamber via integrated pumps. Water in both swim tunnels and the reservoir was replaced between trials to avoid buildup of waste products in the system.

Anesthetic Exposure and Recovery

Control (n = 9) and TD (n = 9) brook char were briefly exposed to 100 mg/L SYNCAINE tricaine methanesulfonate (MS-222) (Syndel Product No. 02168510) and 200 mg/L $NaHCO_3$ in freshwater until respiration ceased. Body dimensions and mass were obtained, and fish were then transferred into separate experimental swim tunnels and allowed to recover overnight in aerated freshwater at 14±0.5 °C under low light conditions, with low water flow of approximately 0.5 body lengths per second (BL/s) to support water mixing and respiration without triggering swimming behaviour.

Thermal Stress Exposure

Water temperature in the swim tunnels was initially regulated by controlling the temperature in the reservoir with the end goal of achieving a 2 °C/h heating rate from 15-25 °C. However, solely using the refrigerated circulator for temperature regulation was insufficient to achieve the desired rate. The addition of an immersion heating rod (Ulanet, Columbus, IN) enabled greater temperature rates and increasing the ambient temperature from 22 to 25 °C during trials helped compensate for radiant heat loss from the recirculating system. Any combination of fixed settings between the two heaters resulted in non-linear temperature rates, and a linear rate was ultimately achieved by manually adjusting the heater settings at specific water temperatures throughout the trial. Temperature settings were adjusted in comparable intervals between trials to ensure consistency in heating rates, and rates were verified in real time digitally using Witrox 4 and AutoResp and confirmed with an analog alcohol thermometer during experimental trials.

Following overnight recovery, brook char were subjected to intermittent respirometry (90 s flush, 30 s wait, 180 s measure) under increasing temperature from 15-25 °C at a rate of 2±0.1 °C/h (mean ± s.e.m.). Water velocities were maintained at approximately 0.5 BL/s. Rates of oxygen consumption ($\dot{M}O_2$, mg O_2/kg/h) were recorded in AutoResp v2. Following experimentation, fish were immediately removed from the swim tunnels and returned to the Harold Crabtree Aqualab for recovery at 15±0.5 °C. Trials with control and TD fish were evenly distributed between the three swim tunnels (3 fish per treatment per tunnel), and all fish were used only once in experimental trials.

Critical Thermal Maximum for Swimming (CT_{swim})

Efforts were made to quantify CT_{swim} as thermal tolerance during exercise is considered more ecologically relevant than loss of equilibrium at the critical thermal maximum (CT_{max}) due to the higher physiological sensitivity to extreme thermal when not at rest (Blasco *et al.*, 2020). Following overnight recovery, control and TD brook char were challenged to swim at increasing water velocities until fatigue to determine their critical swimming speed (U_{crit}). Water velocities were increased stepwise every 5 min starting at 0.5 BL/s. The endpoint was defined as the transition from steady aerobic swimming to unsteady anaerobic swimming, whereby the fish would use rapid tailbeats to propel itself forward and then coasting back, referred to as "burst and coast" swimming, which relies on fast glycolytic muscles (Marras *et al.*, 2013; Webb, 1998). When a fish fatigued, it would fail to maintain position against the current and fall back against the rear screen in the swim tunnel, and water velocity was then quickly reduced to 0.5 BL/s so the fish could recover. The intended CT_{swim} tests required brook char to swim at 85% of their predetermined U_{crit} under continuously increasing water temperatures at a rate of 2 °C/h. The response variable for CT_{swim} was the temperature at which the fish refused to swim and fell back against the rear screen in the swim tunnel (Blasco *et al.*, 2020). Upon reaching the test end point, fish were removed from the swim tunnel and returned to the Harold Crabtree Aqualab for recovery at 15±0.5 °C. CT_{swim} determinations were unsuccessful and not pursued further due to large fractional errors in water velocity based on the sizes of the animals and the dimensions of the swim tunnels (Bell and Terhune, 1970), for reasons discussed in greater detail in the results. Instead, measurements of routine metabolic rate from 15-25 °C for control and TD brook char were obtained to gain further insight into the effects of taurine on thermal tolerance, though this omitted the exercise component that would be part of CT_{swim} assessments.

Statistical Analysis

Data management was performed mainly in R (version 4.3.2; R Core Team, 2023) using the *tidyverse* package (version 2.0.0; Wickham *et al.*, 2019). Temperature rates were analysed using simple linear regressions. The three $\dot{M}O_2$ measurements closest to 15, 17.5, 20, 22.5, and 25°C for all fish were extracted, and the two most similar values were averaged to obtain one measurement per temperature per fish. For one fish, both the highest and lowest $\dot{M}O_2$ measurements deviated from the median value by 22%, so in this case all three values were averaged prior to statistical analysis. Temperature coefficients (Q_{10}) were calculated for each fish and then averaged by treatment using the formula by Prosser (1991):

$$Q_{10} = \left(\frac{k_2}{k_1}\right)^{\frac{10}{T_2 - T_1}}$$

where k_1 is the rate of the biological reaction, in this case this case $\dot{M}O_2$, at the lower temperature, T_1, and k_2 is the rate of the biological reaction at the higher temperature, T_2. Mean Q_{10} values between control and TD brook char were compared using a two-sample t-test. Data met parametric assumptions as assessed using Levene's test for homogeneity of variances and the Shapiro-Wilk normality test with the *car* package (version 3.1.2; Fox and Weisberg, 2019).

Data were also analysed for differences between control and taurine-deficient brook char $\dot{M}O_2$ across temperatures using a multivariate repeated measures ANOVA following assessments of multivariate homoscedasticity by Box's M test for homogeneity of covariance matrices, multivariate normality by visual inspection of Mahalanobis distances, and Mauchly's test for sphericity using the car, *heplots* (version 1.6.0; Friendly *et al.*, 2024), and *RVAideMemoire* (version 0.9.83.7; Herve, 2023) packages. Greenhouse-Geisser corrections for statistical significance were used for departure from sphericity.

Results

Thermal Stress Exposure

Initial temperature ramps were regulated using fixed heater settings throughout 6 trials (A-F), with the goal of achieving a linear rate of 2 °C/h from 15-25 °C. Solely using fixed heater settings resulted in heating rates that were either too high or too low, and generally non-linear based on visual inspection (**Figure 1**).

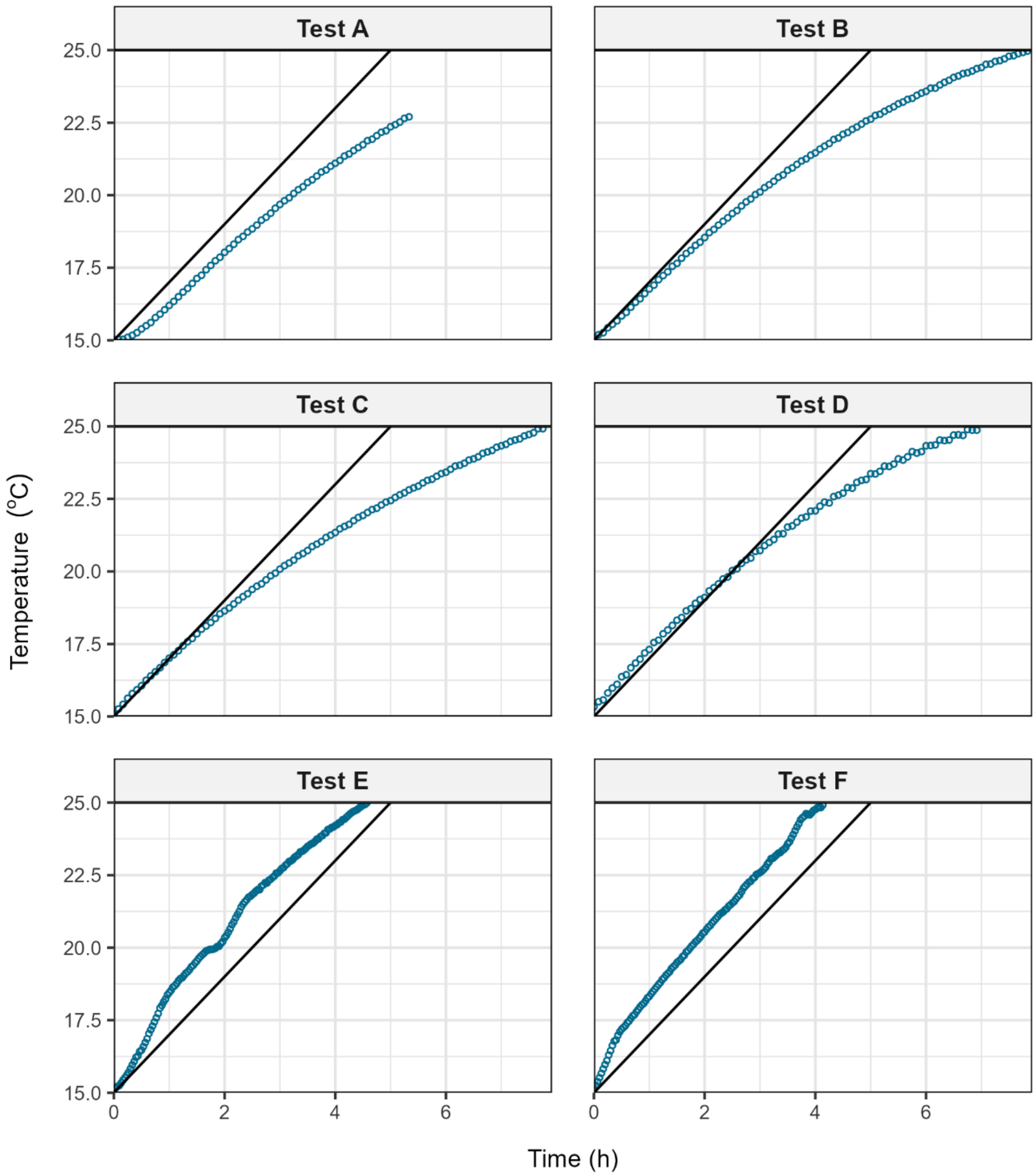

Figure 1 Swim tunnel water heating rates for tests of temperature *versus* time using fixed heater settings throughout each trial (A-F). All tests resulted in non-linear rates, of which some were also too high or too low for an average of 2 °C/h. Black lines represent perfectly linear slopes of 2 °C/h for visual comparison against test results. Test A was terminated before reaching 25 °C as the heating rate was identified as too low at the 5.5-hour mark.

The most consistent linear rates of 2 °C/h were achieved through the combined use of the refrigerated circulator and immersion heating rod, with manual adjustment of the heating rod temperature setting at fixed temperature intervals between 15-25 °C and changing the ambient temperature from 22 °C to 25 °C at the beginning of the heating test (**Figure 2**). The test rate was 2.07 °C/h (linear regression: $r^2 = 0.996$; $F_{1,157} = 3.6 \times 10^4$, $p < 0.001$). The same heater settings were then applied to all subsequent trials for brook char routine metabolic rate from 15-25 °C for consistency between trials.

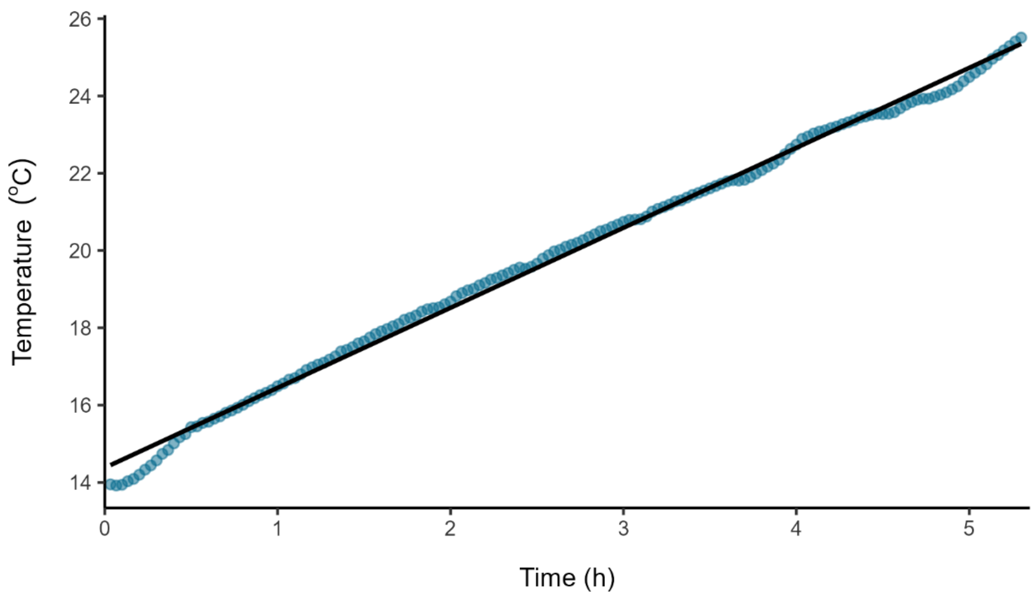

Figure 2 Relationship between system water temperature and time with manual adjustment of temperature settings. Data were fit with a linear regression: $\beta = 2.07$ °C/h, $r^2 = 0.996$, $F_{1,157} = 3.6 \times 10^4$, $p < 0.001$.

Water temperature rates were linear over time for trials of brook char routine metabolic rates from 15-25 °C. The mean rate across trials (1-6) was 1.98 ± 0.05 °C/h (mean ± s.e.m., $n = 6$), as assessed through linear regressions of trial water temperatures over time (**Figure 3**).

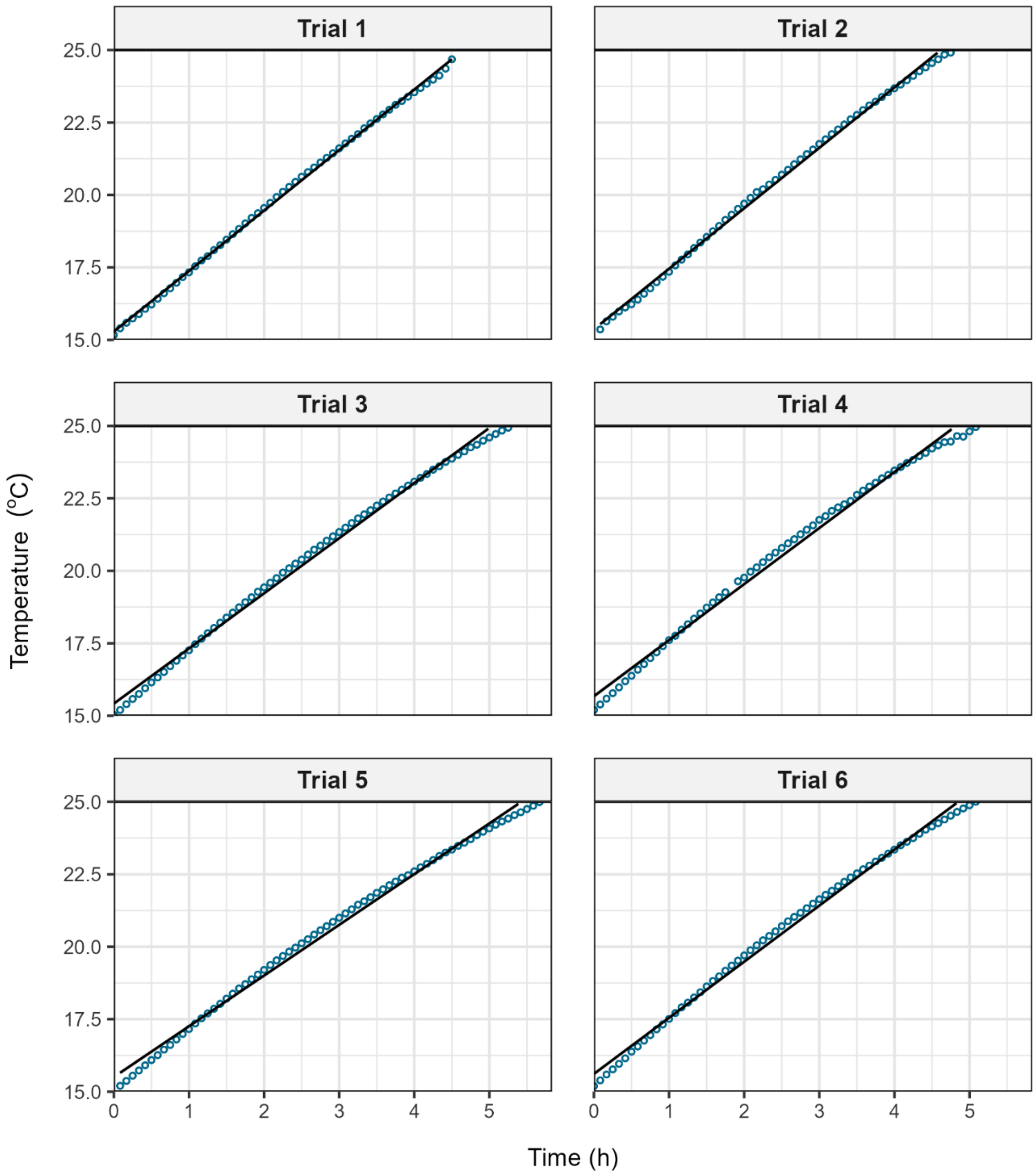

Figure 3 Heating rates from brook char thermal stress exposure trials using manually adjusted heater settings throughout each trial (1-6) to achieve a linear temperature rate of 1.98 ± 0.05 °C/h (mean ± s.e.m., n = 6). Solid black lines represent linear regressions fit to the data for each trial; Trial 1: $r^2 = 0.999$, $p < 0.001$, $\beta = 2.105$ °C/h; Trial 2: $r^2 = 0.996$, $p < 0.001$, $\beta = 2.098$ °C/h. Trial 3: $r^2 = 0.995$, $p < 0.001$, $\beta = 1.915$ °C/h; Trial 4: $r^2 = 0.993$, $p < 0.001$, $\beta = 1.964$ °C/h; Trial 5: $r^2 = 0.994$, $p < 0.001$, $\beta = 1.779$ °C/h; Trial 6: $r^2 = 0.994$, $p < 0.001$, $\beta = 1.989$ °C/h.

Brook char routine metabolic rates across increasing temperatures from 15-25 °C were assessed by calculating and comparing Q_{10} between control and TD fish, and with a multivariate repeated measures ANOVA (**Figure 4**). Metabolic rates for both control and TD fish increased with temperature ($F_{4,13}$ = 29.46, $p < 0.001$), with Q_{10} values of 2.0 ± 0.1 (n = 9) and 1.89 ± 0.1 (n = 9) for control and TD fish, respectively. There were no statistically significant differences in metabolic rates between control and TD fish at any temperature ($F_{1,16}$ = 0.15, p = 0.706), and no interaction between temperature and treatment ($F_{4,13}$ = 1.07, p = 0.409). Q_{10} also did not differ significantly between treatment groups (t_{16} = -0.63, p = 0.535).

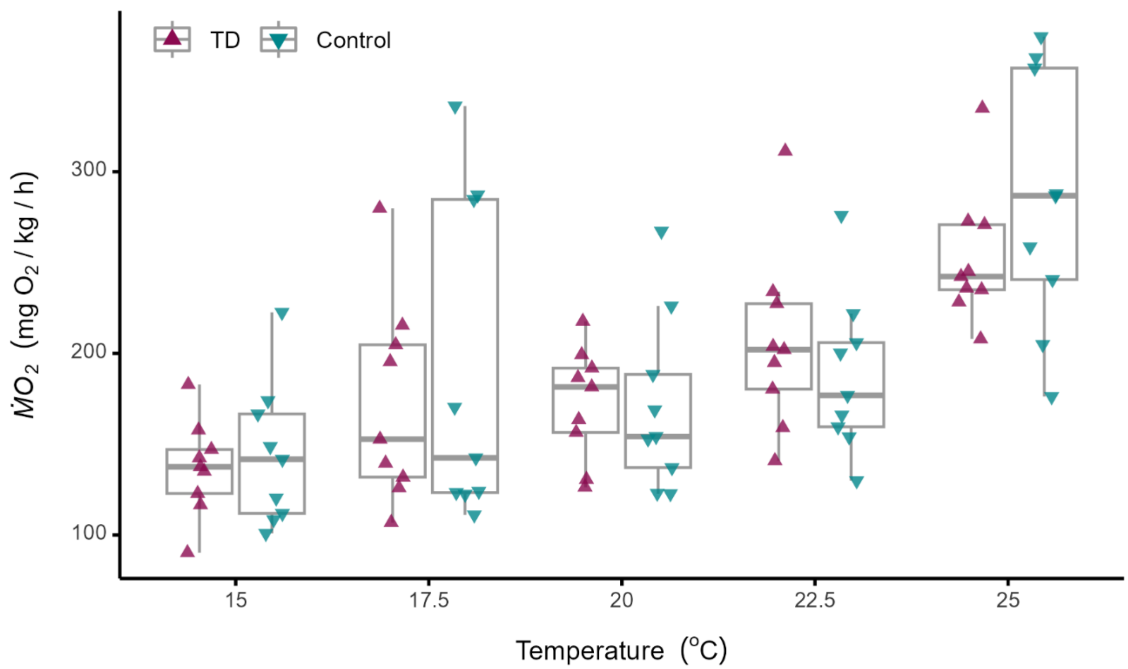

Figure 4 Brook char routine metabolic rates for increasing water temperatures from 15-25 °C for control and taurine deficient (TD) fish. Fish metabolic rate increased with temperature ($p < 0.001$, $F_{4,13}$ = 29.46), while no statistically significant difference was observed between treatment groups ($F_{1,16}$ = 0.15, p = 0.706). Boxplots represent interquartile ranges (boxes and whiskers), median values (solid lines within boxes). Points represent average routine metabolic rate for individual fish at each temperature.

Critical Thermal Maximum for Swimming (CT_{swim})

A significant amount of time was required to fully commission the swim tunnel system and develop a protocol to achieve consistent linear increases in water temperature during trials. At the point of completion, brook char had reached body dimensions too large relative to the sizes of the swim chambers to exhibit normal swimming behaviour in the chambers, precluding identification of a behaviour-based endpoint for CT_{swim} tests. As such, it was not possible to assess the effects of taurine deficiency on aerobic performance or thermal tolerance during exercise. Differences in water velocity based on the sizes of the fish relative to the dimensions of the swim chambers resulted in fractional errors in water velocity above 100% due to compression of water flow between walls of the chamber and the fish' bodies, dramatically offsetting perceived water velocities relative to setpoint velocities during swim trials (Bell and Terhune, 1970). Instead of initiating swimming behaviour as water velocities increased, brook char would brace themselves against the water current with their pectoral and pelvic fins on the bottom of the swim tunnel and their tail against the back grate. Most fish never attempted to swim, and the only indication of reaching a "maximum water velocity" was failure to maintain upright position in the swim tunnel, whereby the fish would start bending laterally and end up curled against the back grate of the chamber, at which point the water velocity would quickly be reduced to minimize stress to the fish. Some brook char attempted to swim, but most of these individuals appeared to initially try swimming upwards, as if trying to enter the water column after resting against the bottom. Because the fish were large compared to the swim tunnels, this resulted in them bumping against the top cover of the swim tunnel, and in some cases the front as well, without being able to achieve continued swimming in the chamber. As fish could not exhibit normal swimming behaviour inside the tunnels due to their large body sizes relative to the chamber dimensions, CT_{swim} tests were not pursued further.

Discussion

The present work aimed to provide further insight into the physiological role of taurine in fish thermal tolerance by examining the effects of taurine deficiency on brook char aerobic metabolism. The effects of taurine on fish aerobic metabolic rate during acute thermal stress were assessed in brook char using intermittent swim tunnel respirometry. Warming significantly increased routine metabolic rates in both TD and control fish, while there was no clear effect of taurine deficiency on routine metabolic rate. Implications and possible explanations of the present findings are discussed in further detail below.

Effects of Temperature on Metabolic Rate

Brook char routine metabolic rates increased with temperature during the 15-25 °C ramp, which was expected based on the general positive relationship between ectotherm metabolic rate and temperature (Jutfelt *et al.*, 2024). In fish, routine metabolic rates generally double or triple with a 10 °C increase in temperature (Reid *et al.*, 1997), which is consistent with Q_{10} values reported here. The Q_{10} for the 15-25 °C ramp for both control and TD brook char were also comparable to values reported for other salmonids (Hvas *et al.*, 2017; Onukwufor and Wood, 2018; Tunnah *et al.*, 2017). While a strong link between environmental temperature and metabolic rate is part of normal physiological function in ectotherms, it poses a concern given climate warming and the anticipated increases in both acute and chronic warming events (Ganguly *et al.*, 2009; Jutfelt *et al.*, 2024). With the progression of climate change, more frequent and prolonged exposures to higher temperatures will increase metabolic rates, resulting in elevated energy demands and possibly greater physiological stress (Chadwick and McCormick, 2017). While brook char aerobic scope was not determined in this work, temperature-induced increases in metabolic rate are likely to shift the energy budgets of aquatic ectotherms with negative fitness consequences like compromised growth and reproductive output (Farrell, 2009; Pandit and Nakamura, 1970; Steinhausen *et al.*, 2008). Brook char and other salmonids are already experiencing the negative consequences of climate warming through compromised spawning behaviour and increasing habitat fragmentation (Farrell *et al.*, 2008; Flebbe *et al.*, 2006; Warren *et al.*, 2012).

Effects of Taurine Deficiency on Thermal Tolerance

Taurine was expected to affect brook char thermal sensitivity due to its role in fish cardiovascular function and environmental stress tolerance (Dixon *et al.*, 2023; Vislie and Fugelli, 1975). Control and TD brook char routine metabolic rates did not differ significantly across the thermal range of 15-25 °C, which contrasted with the expectation that TD brook char would exhibit lower routine metabolic oxygen demands during thermal stress compared to control fish. The similarity in oxygen demands between TD and control fish suggests that cardiac outputs are also the same between treatment groups, as cardiac output must increase with elevated metabolic rates to support higher oxygen demands (Eliason and Anttila, 2017), however, this is not the case. Cardiac output is lower in TD brook char during thermal stress, with stroke volume being relatively temperature-insensitive and heart rates generally lower across temperatures in TD fish compared to controls (Dixon *et al.*, 2023; El, 2023). If aerobic metabolic rates are the same but with lower cardiac output in TD fish, then TD brook char would generally be expected to rely at least partially on anaerobic metabolism to compensate for lower oxygen circulation. Activating anaerobic metabolism would result in accumulating lactate levels (Driedzic and Gesser, 1994; Eliason and Anttila, 2017). However, neither control or TD brook char exhibit elevated heart or plasma lactate levels following exhaustive exercise or acute thermal stress (Dixon *et al.*, 2023; El and MacCormack, unpublished), suggesting that activation of anaerobic metabolism cannot explain the differences in cardiac output without differences in oxygen consumption during thermal stress between TD and control fish.

It is possible that there is a profound redistribution of blood flow in TD fish to help compensate for the physiological limitations imposed by taurine deficiency. Many fish species are capable of redirecting blood flow in response to hypoxia (Gamperl and Driedzic, 2009), and it is possible that other factors generating sub-optimal physiological conditions, like taurine deficiency, can induce such changes as well. If blood flow is redirected from peripheral systems to more vital organs, TD fish may be better able to support similar aerobic metabolic rates as control fish during temperature stress under resting conditions while also maintaining lower cardiac output, without accumulating lactate from anaerobic metabolism. For example, some fish species can reduce blood flow to the gastrointestinal system during exercise or hypoxia stress (Axelsson and Fritsche, 1991; Gamperl and Driedzic, 2009; van Leeuwen *et al.*, 2016). During locomotion, blood flow is directed away from the digestive system in fish like Atlantic cod (*Gadus morhua*) and common carp (*Cyprinus carpio*)(Axelsson and Fritsche, 1991). This ability is also present in other taxa like snakes and crabs (McGaw, 2007; Secor and White, 2010). Heat-stressed TD fish may be able to compensate for impaired heart function by restricting blood flow to the digestive system, thereby reducing overall oxygen demands at the expense of digestive function. Redirecting blood flow from digestive organs to locomotory muscles might enable TD fish to perform similarly to control fish during shorter term exposure to environmental stress like exercise, hypoxia, or warming. However, directing blood flow away from digestive organs might impact digestive efficiency and compromise growth under prolonged exposure to environmental stress like chronic warming, thereby reducing fitness (Thorarensen *et al.*, 1993). This potential trade-off would only be evident in longer term studies using repeated or continuous stress exposure and might be a useful approach to better understanding the effects of taurine on fish thermal stress tolerance.

It is also likely that blood flow is redirected away from locomotory muscle in TD brook char to compensate for reduced cardiac function. Hypoxia stress causes blood flow to be redirected away from more peripheral muscles in snapper (*Pagrus auratus*)(Janssen *et al.*, 2010), and this response might also occur during thermal stress. If TD brook char experience reduced blood flow to locomotory muscle tissue, then their aerobic swimming performance might be impaired under thermal stress due to lower oxygen delivery to working muscle tissue. This could compromise fitness in wild animals by impairing critical activities like spawning migrations and predator escape responses.

The possibility of TD brook char redistributing blood flow reemphasizes the relevance of further exploring how taurine influences their aerobic performance during thermal stress. While TD brook char oxygen consumption does not differ from that of control fish under increasing temperatures at resting conditions, forcing the fish to swim during heating might reveal bigger differences in aerobic metabolic rates if blood flow is redirected towards vital organs within the body. The CT_{swim} protocol may be particularly relevant in this case, as it combines the stress of continuous heating with that of continuous swimming in an ecologically relevant test of thermal performance (Blasco *et al.*, 2020). While it was not possible to assess CT_{swim} for TD and control brook char in this study, pursuing CT_{swim} tests in future work would be helpful in teasing apart the underlying physiological effects of taurine in fish. This would be particularly informative as previous work on rainbow trout has revealed no differences in aerobic scope or critical swimming speed (U_{crit}) in TD and control fish (Gates *et al.*, 2022), neither of which assess performance under increasing temperatures during sustained exercise.

Conclusions

Further work is required to better understand how taurine impacts fish aerobic metabolic rate under thermal stress. Taurine deficiency had no clear effect on brook char routine metabolic rate and it remains unclear why cardiac output is lower in TD fish at elevated temperatures without corresponding changes in stroke volume or increased reliance on anaerobic metabolism. As the climate warms, aquatic organisms may experience lower dietary taurine availability and elevated taurine depletion from physiological stress responses to environmental changes (Jeppesen *et al.*, 2010; Liu *et al.*, 2019). As the present work only addressed the effects of taurine on routine metabolic rate, TD fish may employ physiological responses such as redistributing blood flow to compensate for compromised heart function, thereby masking potential impacts of taurine deficiency on metabolic rate. Any impacts of taurine deficiency on metabolic rate may only become obvious when fish are exposed to a combination of stressors, such as exercise and warming simultaneously. The CT_{swim} approach would enable the determination of how taurine deficiency affects aerobic performance with the added benefit of providing ecologically relevant insight into the aerobic performance of brook char under acute warming (Blasco *et al.*, 2020). This would provide valuable insight into how aquatic organisms might respond to the multitude of stressors created by climate change and be useful for the conservation of aquatic ecosystems as a whole as the environment continues to change.

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