OPEN ACCESS | Research Article

Determining the upper thermal tolerance of Athabasca Rainbow Trout (*Oncorhynchus mykiss*) across naturally varying stream temperatures

Sarah Hnytka[a,b], **Jordan Rosenfeld**[c], **and Eva C. Enders**[d]

[a]Freshwater Institute, Fisheries and Oceans Canada, Winnipeg, Manitoba R3T 2N6, Canada; [b]Institute of Oceans and Fisheries, Vancouver, BC V6T 1Z4, Canada; [c]Ministry of Environment, Vancouver, Vancouver, BC, Canada; [d]Institut national de la recherche scientifique, Quebec City, Quebec G1K 9A9, Canada

Corresponding author: **Sarah Hnytka** (email: sarah.hnytka@dfo-mpo.gc.ca).

Abstract

Stream temperature is a key driver of physiological function in ectothermic fish, and fish have clear upper and lower limits to thermal habitat use. Stream temperature increases from climate change are a major threat to coldwater taxa like Athabasca Rainbow Trout (*Oncorhynchus mykiss*), listed as Endangered under Canada's *Species at Risk Act*. To better understand their vulnerability to climate change and prioritize recovery locations, Athabasca Rainbow Trout were collected in August 2021 from three streams in the upper Athabasca River, Alberta, Canada, across a gradient of thermal regimes ranging from cold to warm. Individual thermal tolerance was measured using two physiological metrics: agitation temperature (T_{ag}) and critical thermal maxima (CT_{max}) using a portable streamside laboratory. T_{ag} is a behavioural indicator of a thermal avoidance threshold, whereas CT_{max} is a physiological response that can be interpreted as the upper thermal limit where long term survival is reduced. Results provide strong evidence that CT_{max} is a plastic metric in Athabasca Rainbow Trout; however, T_{ag} is not. This suggests that Athabasca Rainbow Trout demonstrate some thermal plasticity in terms of CT_{max}, yet the lack of plasticity in T_{ag} indicates potential limitations in their adaptability to warming stream temperatures.

Key words: critical thermal maxima, agitation temperature, thermal physiology, Athabasca Rainbow Trout, streamside laboratory, climate change

Introduction

Climate change can affect aquatic ecosystems by altering thermal and hydrologic regimes, negatively impacting coldwater fish species, which are especially sensitive to temperature change (Ficke et al. 2007; Döll and Zhang 2010). Fish are ectotherms, with body temperature and all subsequent physiological and biochemical processes governed by surrounding thermal conditions, thereby inextricably linking fish physiology and behaviour to water temperature (McNab 2002; Pörtner 2004; Volkoff and Rønnestad 2020). The challenge for fish, as with all organisms, is to maintain body temperature within a range that is optimal for performance. Climate change is creating a warmer and more variable aquatic environment, threatening to push fish species past their physiological limits (McCullough et al. 2009; Pörtner and Peck 2010; Horton et al. 2016). When fish experience temperatures outside of their optimal range, behavioural responses (e.g., avoidance or active habitat selection) or physiological plasticity (e.g., stress response, thermal adaptation) are designed to return fish to homeostasis as efficiently as possible (Habary et al. 2017; Alfonso et al. 2021). However, the suite of behavioural and physiological responses that best maintain homeostasis and maximize fitness will depend on antecedent (acclimation) temperatures, as well as the magnitude and duration of thermal stress and the ecological strategy adopted by any given species (e.g., low vs. high plasticity; avoiders vs. adapters). The ability of different fish species to handle future environmental shifts will likely depend on their ability to mitigate temperature changes either by *moving* (i.e., occupying or migrating into thermally suitable habitats) or *adapting* (i.e., altering their fixed physiological tolerances or scope for phenotypic plasticity (Habary et al. 2017).

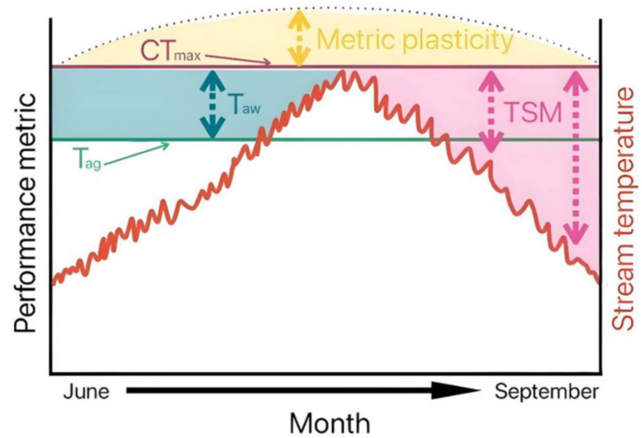
Upper thermal limits in fish are characterized by metrics like agitation temperature (T_{ag}) and critical thermal maximum (CT_{max}), which are typically estimated using nonlethal, acute temperature experiments (Jeffries et al. 2018). T_{ag} is thought to be an ecologically relevant behavioural metric indicating the initiation threshold for thermally-induced avoidance behaviour, when fish abandon their current habitat in search for a thermally suitable alternative (e.g., thermal refugia; McDonnell and Chapman 2015). Water temperatures above T_{ag} often exceed optimal temperatures, where sublethal effects begin to occur (Bear et al. 2007; Jeffries et al. 2018). In contrast, CT_{max} describes the upper temperature limit where physiological dissociation and equilibrium loss occur (Becker and Genoway 1979). Fish can shift their behavioural, physiological, and morphological traits in response to the surrounding environment, thereby maintaining a higher level of fitness across a larger range of conditions (Angilletta 2009; Gamperl et al. 2020). Thermal traits will also be influenced by recent thermal exposure, generally referred to as acclimation temperature (i.e., recent set of thermal conditions an organism has experienced). In general, laboratory experiments indicate greater thermal tolerance following higher acclimation temperatures in fish (Comte and Olden 2017; Potts et al. 2021); however, the impacts of naturally variable stream conditions, like fluctuating temperatures and exposure to extreme heat, are less well understood.

Athabasca Rainbow Trout are found in the cold, unproductive headwaters of the Athabasca River in northwestern Alberta, Canada, and their distribution is strongly influenced by water temperature and elevation (COSEWIC 2014; DFO 2018). This ecotype of Rainbow Trout is listed as Endangered under the Canadian *Species at Risk Act* due to significant range contraction and threats associated with habitat loss, competition with invasive species, and genetic introgression. Climate change will further impact habitat quantity and quality as stream temperatures are predicted to increase by 2.0–7.4 °C during summer months in the Athabasca River watershed by 2100 (Du et al. 2019). This study is intended to help fill some of the knowledge gaps in their thermal physiology and adaptability of Athabasca Rainbow Trout, providing important information to guide management decisions for this endangered ecotype.

Thermal tolerance of Athabasca Rainbow Trout has been previously inferred from other Rainbow Trout populations using a variety of physiological metrics, however, it is unclear to what extent these results are transferrable to an ecotype at the northern extent of the Rainbow Trout distribution. The upper critical temperature limits and thermal plasticity of adult Athabasca Rainbow Trout was assessed by measuring T_{ag} and CT_{max} from different source creeks across a natural gradient of acclimation temperatures. The agitation window was also analyzed, representing the difference between CT_{max} and T_{ag} ($T_{aw} = CT_{max} - T_{ag}$; Fig. 1), which provides a measure of the thermal buffer between the initiation of an agitation response and the ability to escape from critical conditions that may ultimately lead to organism death (Wells et al. 2016; Firth et al. 2021). T_{aw}, although likely subject to acclimation, is primarily a selected phenotypic attribute as both CT_{max} and T_{ag} are subject to local adaptation (i.e., among populations or species; McDonnell et al. 2021). The thermal safety margin (TSM; maximum stream temperature–CT_{max}; Potts et al. 2021) was used to characterize the divergence between a population's phenotypic tolerance and the site-specific thermal regime at a given location. TSM may be interpreted as representing the thermal risk associated with a particular site or temperature regime, given a particular phenotype.

The overall aims of this study were to (i) evaluate the effect of naturally fluctuating acclimation temperatures on upper thermal tolerance of Athabasca Rainbow Trout; (ii) to assess whether Athabasca Rainbow Trout, as an ecotype at the northern periphery of the Rainbow Trout range, have a unique (e.g., lower) CT_{max} than other Rainbow Trout populations; and (iii) to compare T_{ag} in Athabasca Rainbow Trout with previously published studies to expand our understanding of variation in T_{ag} among fish species.

Fig. 1. Conceptual illustration of the measured physiological performance indicators, including critical thermal maxima (CT_{max}), agitation temperature (T_{ag}), agitation window (T_{aw}), and thermal safety margin (TSM). Dotted line indicates the scope for potential plasticity of CT_{max} or other metrics in relation to stream temperature (solid red line) throughout the summer months.



Methods

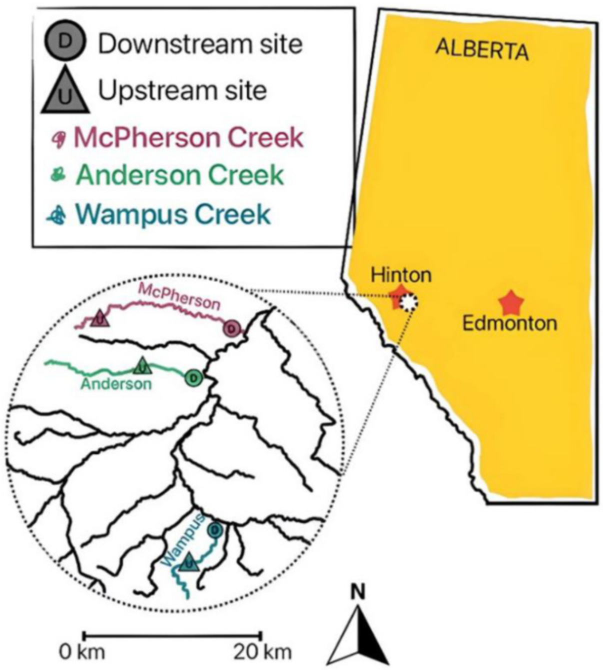
Study sites

Three focal creeks were selected in the McLeod River, a tributary in the upper Athabasca River watershed, Alberta, Canada (Fig. 2), to encompass the natural thermal variability of creeks occupied by Athabasca Rainbow Trout. Anderson Creek (AN) was selected as a cold (14.4 °C maximum August temperature in 2021), Wampus Creek (WP) as intermediate (18.7 °C), and McPherson Creek (MP) as a warm (20.3 °C) creek. An upstream (U) and downstream (D) site were selected on each creek for within-stream comparison, and water temperature was recorded every 30 min at each of the six sites using temperature loggers (HOBO Pendant, MX2202, Onset, Bourne, MA, USA). Because tributaries are relatively close with no intervening barriers, it is assumed that there is sufficient gene flow to ensure a single homogenous population, and that any differences in thermal tolerance reflect either developmental plasticity or acclimation to different thermal environments.

Study fish

Research permits were approved by DFO's Freshwater Institute Animal Care Committee following the Canadian Council on Animal Care guidelines (Animal Use Protocol: FWI-ACC-2021-52), Alberta Fish and Wildlife Division (Research and Collection Permit: 21-317), and the DFO Species at Risk

Fig. 2. Location of sites within the three study creeks (Anderson, Wampus, and McPherson) in the Athabasca River watershed, Alberta, Canada. Colour represents thermal regime (blue = cold, green = intermediate, red = warm water creek) with upstream (U) and downstream (D) sites represented by triangles and circles, respectively.



Program (SARP) who issued a permit under Section 73 of the *Species at Risk Act* (SARA Permit: 21-PCAA-00032). A total of 120 wild Athabasca Rainbow Trout were collected between 4 and 15 August 2022 across the six sampling sites using an LR-24 backpack electrofisher (Smith Root, Vancouver, WA, USA; settings: 290 V, 35 Hz, 13%). Fish length did not differ significantly among sites (average total length = 147 mm ± 36 mm and wet body mass = 38.8 ± 29.1 g). Young-of-the-year fish were excluded from this study, which focused on thermal tolerance of adults or sub-adults. Fish were placed in an opaque instream flow-through holding bag (where feeding was assumed to be reduced) for up to 24 h prior to the start of each experiment.

Experimental setup for measuring upper thermal tolerance

Upper thermal tolerance experiments were conducted in a mobile streamside laboratory trailer to determine CT_{max} and T_{ag}. Large heating tanks were filled to a predetermined volume with stream water ensuring the experimental starting temperature reflected resident thermal conditions. Throughout the experiment, fish were individually housed in small acrylic chambers with mesh ends ($n = 6$). Individual chambers functioned to reduce social and physical interactions between study fish while permitting continuous water exchange between the chamber and tank. Water temperature in the tank was controlled by five submersible heaters (TH-0300S titanium heaters, Finnex, Chicago, IL, USA) to gradually increase the temperature at a standardized heating rate of 0.3 °C/min (Becker and Genoway 1979) for both CT_{max} and T_{ag} trials, with temperature monitored to ensure an accurate heating rate. Two circulation pumps and air stones were also placed in the tank to ensure uniform water temperature and saturated dissolved oxygen levels. Two experimental trials were conducted daily using identical replicate tank systems to increase fish throughput.

Experimental procedure

On the morning of each experiment, six fish were removed from the instream holding bags where they were placed the day before and moved into individual chambers within the heating tank. Fish were acclimated for 2 h at a stable temperature to reduce potential stress associated with handling and a new environment. Once the acclimation period at ambient stream temperature was complete, the dynamic heating period began. Fish movement was carefully monitored for behavioural cues that define the onset of agitation. T_{ag} was always observed before the onset of CT_{max} and was defined as 5 s of consistent burst swimming or aggressive turning inside individual confinement chambers (McDonnell and Chapman 2015). CT_{max} was defined by a loss of equilibrium (inability to remain upright) and/or disorganized locomotory movement (Beitinger et al. 2000). Fish were immediately removed from the warm experimental tank when CT_{max} was observed and placed in a cool recovery tank. Fork length (in mm) and wet body mass (in g) measurements were recorded, and a fin clip was taken from the left pelvic fin of each fish for genetic analysis ($n = 120$). Once fish had regained equilibrium and recovered, they were released back into the stream at the site of capture.

Genetic analysis

Rainbow Trout of hatchery origin were historically stocked throughout the Athabasca River watershed (COSEWIC 2014). To ensure that all fish used in the data analysis were genetically pure, fin clips from all assayed fish were genetically analyzed for introgression with stocked Rainbow Trout. All protocols closely followed those of Geraldes and Taylor (2021). Tissue samples were digested, and DNA was extracted and amplified to estimate the ancestry coefficient between wild and hatchery Rainbow Trout groups, expressed as the proportion of the genome of each fish that was of wild origin (i.e., Q_H). Although five tissue samples had insufficient genetic material for analysis, the remaining 115 samples were all genetically pure with an introgression coefficient $Q_i > 0.95$, indicating genetic introgression was rare in experimental fish caught in McPherson, Anderson, and Wampus creeks. Based on these results, it is reasonable to infer that all experimental fish ($n = 120$) were genetically pure Athabasca Rainbow Trout.

Data and statistical analyses

Long-term acclimation temperature prior to measurement of thermal tolerance calculated as the mean water temperature at each site for 7 days preceding the experiment were

Table 1. Regression equations for agitation temperature (T_{ag}), critical thermal maxima (CT_{max}), agitation window (T_{aw}), and thermal safety margin (TSM).

Response variable	Equation: (Response = (Slope * Predictor) + Intercept)
T_{ag}	T_{ag} = (−0.110 * Acclimation) + (0.014 * Fork length) + 21.563
CT_{max}	CT_{max} = (0.314 * Acclimation) + (−0.009 * Fork length) + 25.180
T_{aw}	T_{aw} = (0.419 * Acclimation) + (−0.022 * Fork length) + 3.734
TSM	TSM = (−1.196 * Acclimation) + (−0.010 * Fork length) + 26.842

based on observed temperature logger data. However, 2021 temperature data were missing from the upstream site in McPherson Creek and was subsequently inferred based on the water temperature relationship between up and downstream sites of McPherson Creek measured the following summer.

Data from thermal tolerance experiments were excluded if behavioural observations were deemed inaccurate or ambiguous. Three T_{ag} data points were excluded when fish either expressed agitated behaviour from the start of the experiment, or if fish failed to express any agitated behaviour prior to CT_{max}. Five CT_{max} observations were excluded when fish demonstrated full recovery of equilibrium and activity immediately after being placed in the recovery tank, as this indicates a behavioural misinterpretation of loss of equilibrium, and that fish were prematurely removed from the experiment. The raw experimental data for this research is available from Dryad, located at https://doi.org/10.5061/dryad.5qfttdzh5.

We first modelled CT_{max} as a one-way ANOVA treating site as a fixed effect to investigate differences in mean performance among sites ($n = 6$). To derive more generalizable models (Table 1), site mean values for the response variables T_{ag}, CT_{max}, T_{aw}, and TSM were also calculated and modelled as a function of acclimation temperature and fork length as independent predictor variables using a linear mixed effect (lme) model with site as a random effect, using a Kenward-Roger degree of freedom approximation. All database manipulations and statistical analyses were performed in R version 3.6.3 (R Core Team 2020).

Literature review on thermal metric variation

To determine the scale of variation in published CT_{max} among Rainbow Trout populations, a literature search was conducted using Google Scholar. The search included a term for the experimental metric (CT_{max}, critical thermal maxima/maximum, or upper thermal tolerance) and the species name (Rainbow Trout, *Oncorhynchus mykiss*, or the obsolete *Salmo gairdneri*). Studies were excluded if they included additional treatments looking at the effect of social interaction (e.g., dominant vs. subordinate) on thermal tolerance, performed surgery on test fish, or tested multiple stressors at the same time as temperature (e.g., hypoxia in combination with temperature), as confounding variables make it challenging to establish a clear link between temperature and physiological thresholds. Thermal habitat use studies (e.g., thresholds inferred from visual observation of trout leaving warm habitat) were excluded to ensure comparable methodologies across studies. Data was extracted from the 24 studies that met these criteria (Table 2, Supplemental material) from tabular data or using WebPlotDigitizer (https://automeris.io/WebPlotDigitizer/) to extract data from graphs. CT_{max} was then modelled using a mixed effect model as a function of acclimation temperature and study as a random effect.

A second literature review was conducted to compare T_{ag} in Athabasca Rainbow Trout to values reported for other fish species. Search terms included agitation temperature, avoidance temperature, and T_{ag}. However, because T_{ag} is a relatively new metric only 10 studies were identified (Table 3, Supplemental material). Acclimation temperature, species order (Cypriniformes, Cichliformes, Perciformes, Salmoniformes), and study as a random effect ($n = 11$, including data from this study) were then used as factors to predict T_{ag} in a mixed effect model.

Results

Assessment of upper thermal tolerance

Acclimation temperatures (7-day average stream temperature prior to thermal experiments) ranged from 7.4 to 16.3 °C across the six sampling sites and differed significantly among streams ($F_{2,116} = 203.2$, $p < 0.001$). Agitation temperature varied greatly among individual fish, ranging from 16.0 to 27.5 °C, with a mean of 22.0 ± 2.6 °C (Fig. 3*a*). T_{ag} increased significantly with fish size ($F_{1,113.3} = 4.05$, $p = 0.047$, slope = 0.014, Table 1), with larger fish becoming agitated at higher temperatures. CT_{max} ranged from 24.1 to 30.8 °C, with an overall average of 28.0 ± 1.5 °C across sites. The upstream site on the coldest creek (AN–U) had the lowest mean CT_{max} at 26.4 °C, whereas the downstream site on the warm creek (MP–D) had the highest mean CT_{max} at 29.4 °C (Fig. 3*b*). Both acclimation temperature ($F_{1,\,4.2} = 23.81$, $p = 0.007$, slope = 0.314) and fork length ($F_{1,116.1} = 7.94$, $p = 0.006$, slope = −0.009) were significant predictors of CT_{max}. A post hoc test revealed the coldest site (AN–U) and warmest site (MP–D) had significantly different CT_{max} from the intermediate sites (Tukey HSD, $p < 0.05$). Agitation window was extremely variable among individual fish ranging from 0.4 to 13.4 °C with an overall average of 6.0 ± 3.1 °C (Fig. 3*c*). Fork length was the only significant predictor and had a negative relationship with T_{aw} ($F_{1,112.4} = 9.21$, $p = 0.003$, slope = −0.022). The TSM ranged between 2.2 and 15.5 °C, averaging 10.0 ± 3.3 °C across all sites (Fig. 3*d*). Acclimation temperature was the most significant predictor of TSM ($F_{1,4.1} = 70.19$, $p < 0.001$, slope = −1.196), with warmer acclimation temperatures negatively effecting the thermal safety margin (slope = −1.12). TSM

Fig. 3. Upper thermal tolerance metrics for Athabasca Rainbow Trout in in the upper McLeod River watershed with acclimation temperatures increasing along a site gradient from left (8.5 °C) to right (16.9 °C). The six site locations encompass three creeks (AN = Anderson, WP = Wampus, MP = McPherson) with an upstream (U) and downstream (D) location. Panels indicate (*a*) agitation temperature, T_{ag}; (*b*) critical thermal maxima, CT_{max}; (*c*) agitation window, T_{aw}; and (*d*) thermal safety margin, TSM. Coloured boxes represents the 50% interquartile range, whiskers represent minimum and (25th percentile −1.5 * interquartile range), maximum values (75th percentile + 1.5 * interquartile range) and black circles (•) represent outliers. Open circles (°) represent means, whereas black horizontal lines represent medians. Different letters represent statistically significant differences among sites.

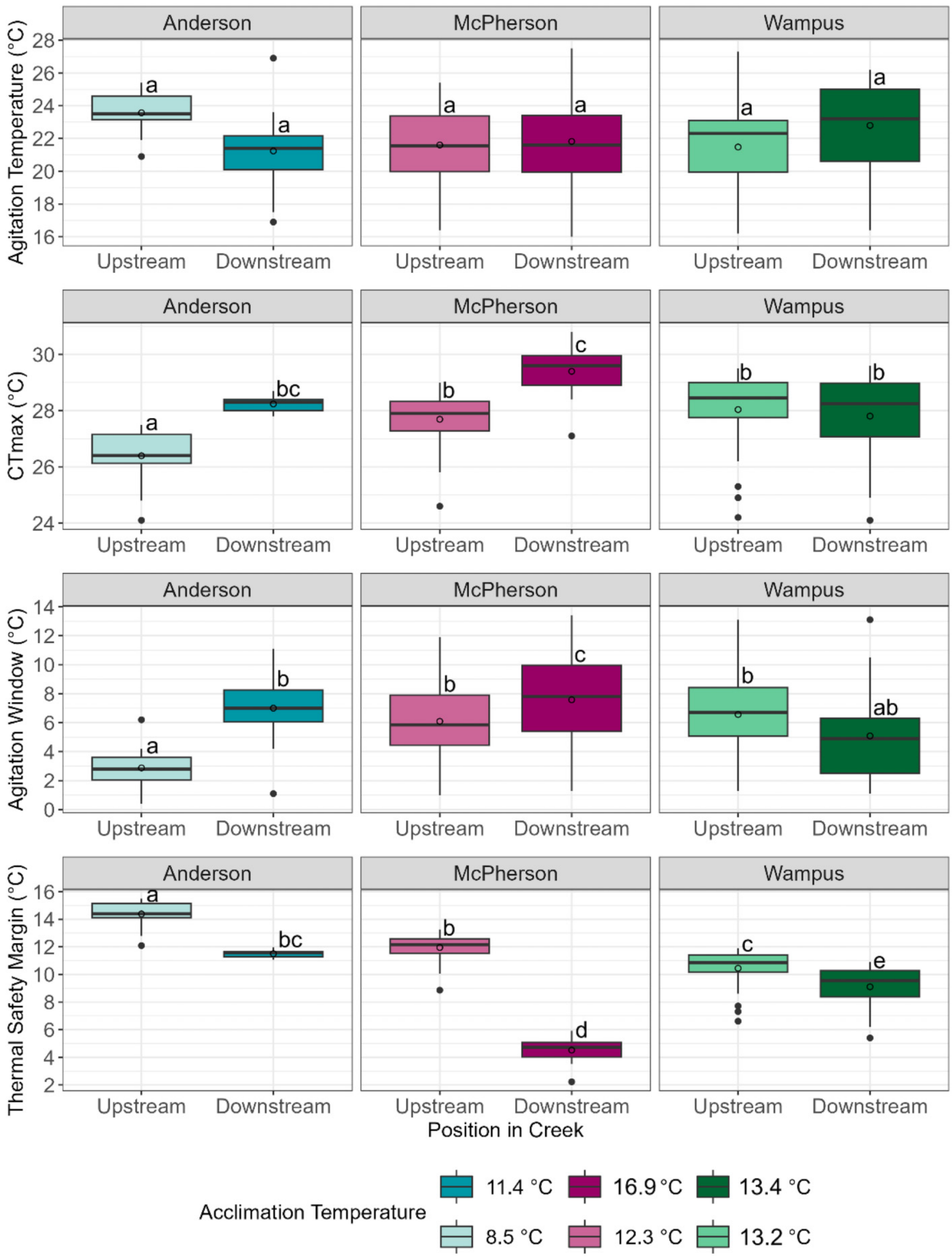
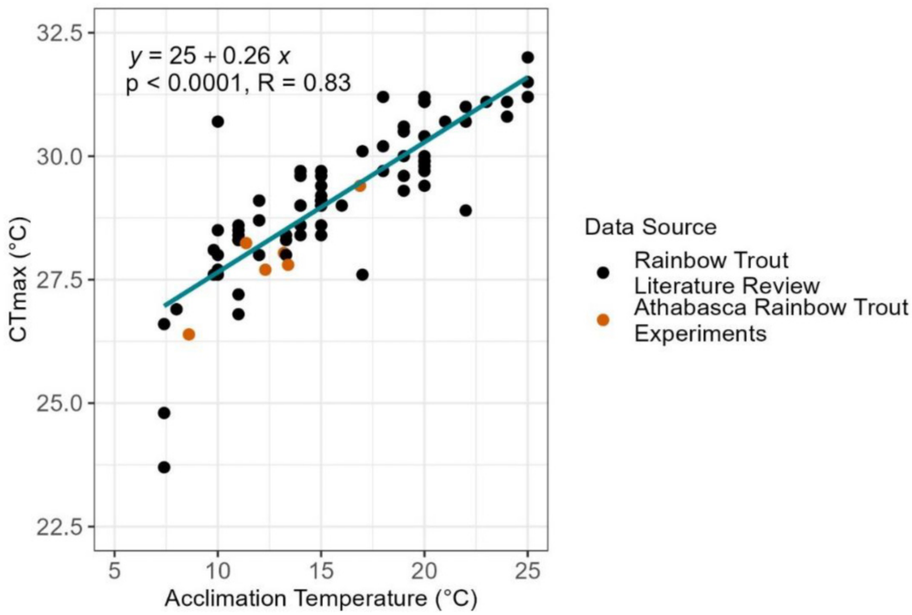


Fig. 4. Relationship between critical thermal maxima (CT_{max}) and acclimation temperature among multiple Rainbow Trout studies ($n = 24$). Black data points indicate CT_{max} values of Rainbow Trout populations from published studies, red data points indicate Athabasca Rainbow Trout CT_{max} estimates from this study.



also decreased with fork length ($F_{1,113.9} = 10.24$, $p = 0.002$, slope $= -0.010$).

Literature review of thermal metric variation

Regression analysis of literature review data showed that acclimation temperature had a significant positive effect on CT_{max} across multiple RBT populations ($F_{1,83} = 131.59$, $p < 0.001$; Fig. 4). No meaningful difference in CT_{max} was apparent between Athabasca Rainbow Trout and the predicted Rainbow Trout average, with CT_{max} values for Athabasca Rainbow Trout well within the range of other Rainbow Trout populations (Fig. 4), providing no support for the assumption of reduced thermal tolerance in Athabasca Rainbow Trout.

The relationship between T_{ag} and acclimation temperature in salmonids is poorly documented in the literature because T_{ag} is a relatively new metric, resulting in a limited sample size. However, this sample size was sufficient to determine that acclimation temperature was a significant positive predictor of T_{ag} ($F_{1,29} = 654.11$, $p < 0.001$, Fig. 5*a*). Fish order was also a significant predictor of T_{ag} ($F_{3,29} = 62.01$, $p < 0.001$), with highest T_{ag} in cichlids, followed by cyprinids, percids, and salmonids. Literature source also accounted for significant variation in T_{ag} ($F_{6,29} = 19.63$, $p < 0.001$).

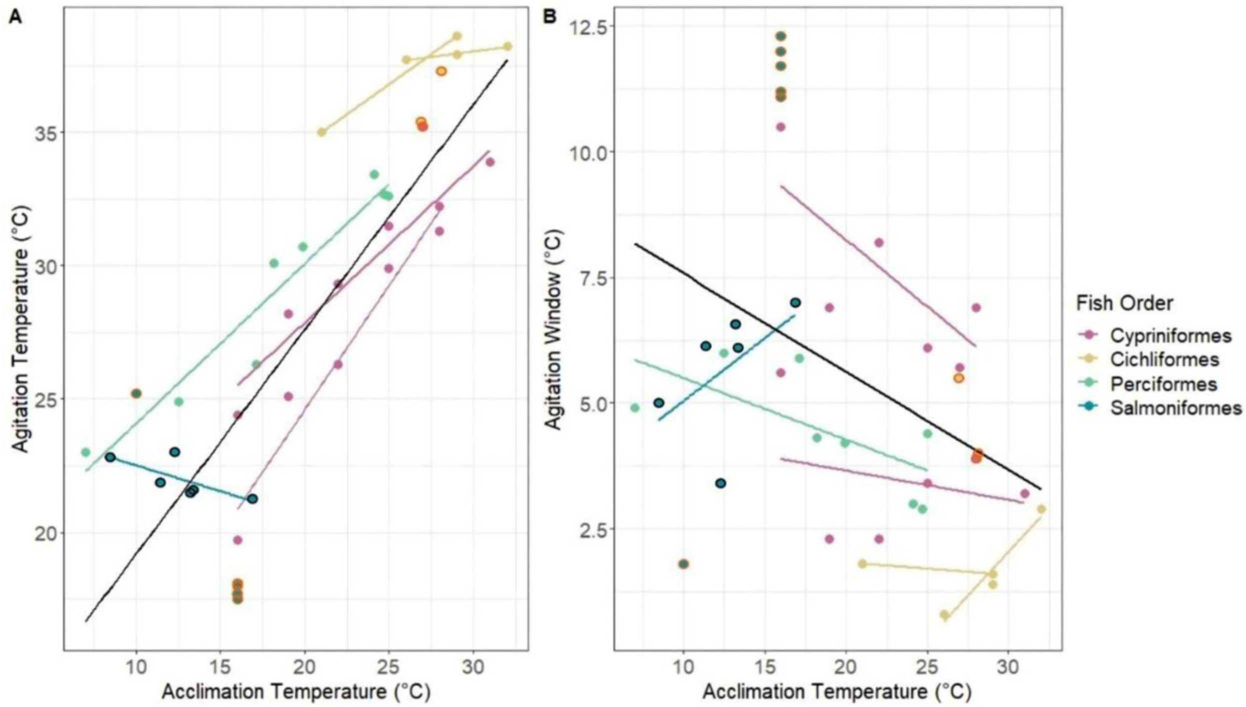
The relationship between T_{aw} and acclimation temperature varied among individual studies. A significant overall negative relationship between T_{aw} and acclimation temperature suggests that upwards plasticity in the initiation of thermally induced agitation temperature exceeds plasticity in CT_{max} ($F_{1,29} = 36.75$, $p < 0.001$, Fig. 5*b*). Fish order ($F_{3,29} = 28.37$, $p < 0.001$) and literature source ($F_{6,29} = 28.60$, $p < 0.001$) were also significant factors in the model, with salmonids having the largest average T_{aw}, followed by cyprinids, percids, and then cichlids.

Discussion

Athabasca Rainbow Trout will likely experience changes in the quantity and distribution of thermally suitable habitat in the future due to increasing stream temperatures associated with climate change (COSEWIC 2014; Habary et al. 2017). To better understand their potential response (i.e., move vs. adapt), we tested four quantitative metrics of performance under a natural gradient of varying stream temperatures. These included behavioural indicators associated with the onset of thermal stress (T_{ag}, T_{aw}) and physiological responses associated with extreme thermal stress (CT_{max}, TSM).

Avoidance behaviours characterized by metrics like T_{ag} are selected responses that serve as cues to minimize harm from conditions that exceed physiological limits. For example, water temperatures above T_{ag} or low dissolved oxygen concentrations beyond tolerance thresholds can trigger an agitation response in fish (Petersen and Petersen 1990), where fitness related activities like foraging and predator avoidance are negatively impacted (McDonnel and Chapman 2015). T_{ag} is a relatively new metric that has previously been described only twice for salmonids, with Athabasca Rainbow Trout values falling above the range reported for Brook Trout (*Salvelinus fontinalis*; 17.5–18.1 °C, Wells et al. 2016) and below for Westslope Cutthroat Trout (*Oncorhynchus clarkii lewisi*; 25.2 °C, Enders and Durhack 2022). In contrast to these studies, Athabasca Rainbow Trout showed no detectable acclimation plasticity in this metric, suggesting diversity in adaptive strategies. However, this could also be a consequence of differences in experimental methodology; the natural

Fig. 5. Relationships between agitation temperature (T_{ag}, panel A) and agitation window (T_{aw}, panel B) with acclimation temperature for various fish orders reported in the literature. Point colouration "•" represent various fish categories classified by phylogenetic order, and coloured trendlines represent individual experiments. Data points with a red outline represent studies with a single acclimation temperature, therefore, only included in the overall linear regression analysis (black line). Data points with a black outline represent Athabasca Rainbow Trout from this study.



acclimation regime in this study exposed fish to considerable diurnal variation in acclimation temperature, unlike laboratory studies of T_{ag}, potentially decreasing the benefits of elevating T_{ag} at higher mean acclimation temperatures.

An increase in CT_{max} at higher acclimation temperatures is a commonly observed physiological response to environmental warming (McDonnell and Chapman 2015). As expected, CT_{max} was plastic in relation to acclimation temperature in Athabasca Rainbow Trout, following the trend observed for other native and hatchery Rainbow Trout populations. Furthermore, CT_{max} values for the Athabasca Rainbow Trout ecotype were similar to other Rainbow Trout populations, providing no support for the inference that Athabasca Rainbow Trout are more cold-adapted (i.e., have reduced upper thermal tolerance) than more southerly populations near the core of the species' distribution.

The agitation window can provide insight into how quickly fish sense and react to temperature changes in the environment. A larger T_{aw} (lower T_{ag}) will provide a greater buffer and warning zone for impending thermal stress; however, this will disrupt normal behaviours earlier thereby reducing the range of suitable habitat (i.e., it entails greater lost opportunity costs). T_{aw} for Athabasca Rainbow Trout varied substantially among individuals, averaging 6.0 °C in our study, comparatively less than observed for other salmonids in the literature (average of 10.3 °C, $n = 7$). It appears that Athabasca Rainbow Trout may have a smaller buffer zone than other salmonids, allowing them to continue using warmer thermal habitats before the onset of T_{ag}, although we would recommend a larger sample size to confirm these findings. More controlled studies looking at the repeatability of T_{aw} among individuals, populations, and species would provide greater insight into whether and how individuals and species adopt different thermal strategies, and how this is affected by thermal regime.

Sources of variation in agitation temperature

Differences in thermal performance metrics among individuals, populations, and species may reflect different ecological and physiological strategies for optimizing fitness under thermal stress. Variation in T_{ag} may provide insight into potential selection on avoidance behaviour, where agitation behavior leading to emigration reduces thermal stress. However, interpreting the ecological significance of differences in T_{ag} is complicated by experimental or sampling artifacts that may create divergence in performance metrics driven by methodology rather than biology.

A lack of consistent criteria for defining T_{ag} could be one contributing factor to reported variation in T_{ag} among species, in particular the use of different endpoint criteria. T_{ag} was initially defined as the temperature where fish initiate "swimming around the confines of the tank in a quick, agitated manner" (McDonnell and Chapman 2015). T_{ag} has subsequently been reported as a sustained escape response behaviour >5 s (Enders and Durhack 2022) or as the first observed agitation period lasting >40 s (Potts et al. 2021);

however, the difference in temperature threshold associated with 5 s versus 40 s of agitated behaviour may be substantial. Thus, apparent differences among and within species may reflect different methodologies (i.e., threshold criteria) rather than differences in intrinsic biology. This may be further compounded by subjective observer assessments of what constitutes agitation behavior. Reporting T_{ag} values using a standardized metric definition and criteria for agitated behaviour is essential for comparing across studies. To this end, we recommend that T_{ag} be defined as a 5 s sustained escape response to reflect the onset of temperature avoidance behaviour. Alternatively, researchers could measure temperatures associated with both 5 s and 40 s of agitation behavior (or other duration) to allow cross-calibration across studies.

Stability of acclimation temperatures is another potential driver of variation in thermal thresholds. Upper thermal tolerance metrics including T_{ag} and CT_{max} are frequently measured in laboratory settings following long periods of stable acclimation temperature; however, the effect of naturally fluctuating diurnal stream temperatures on thermal tolerance remains unclear. Similarly, the relevance of acclimation measured under constant temperature to thermal acclimation in the wild where diurnal temperature fluctuations are the norm is also uncertain. Variable acclimation temperature experiments with Chinese Bream (*Parabramis pekinensis*) and Fathead Minnow (*Pimephales promelas*) showed that fish exposed to fluctuating acclimation temperatures had higher CT_{max} values relative to control fish experiencing stable temperatures (Peng et al. 2014; Salinas et al. 2019). This trend has also been observed in salmonids with higher CT_{max} values observed in wild Rainbow Trout, Brook Trout, and Brown Trout (*Salmo trutta*) populations exposed to diurnally fluctuating temperatures relative to hatchery populations raised at stable temperature (Carline and Machung 2001). However, it is unclear in this example whether the relatively higher thermal tolerance observed in wild populations is due to selection for altered thermal tolerance in hatchery fish, or acclimation to a stable hatchery thermal regime. Increased thermal tolerance associated with fluctuating acclimation temperatures is consistent with Jensen's inequality, where extreme temperatures have a stronger influence than mean values (Bernhardt et al. 2018). Wild fish populations from fluctuating environments are in reality experiencing higher acclimation temperatures compared to fish raised at the same mean temperature under stable laboratory conditions and might therefore be expected to have a higher CT_{max}. A better quantitative understanding of the effects of stable versus fluctuating acclimation temperature on CT_{max} would allow better extrapolation of CT_{max} measured under stable laboratory temperatures to more ecologically realistic field conditions.

Effects of exposure duration on thermal physiology

While acclimation temperatures in the laboratory are generally well defined, acclimation conditions for wild fish (i.e., their thermal exposure history prior to temperature stress) may be highly variable, which almost certainly affects their subsequent thermal performance. A better understanding of how acclimation temperatures in the laboratory relate to acclimation (antecedent) temperature in the wild is essential if laboratory thermal tolerance thresholds are to be applied to real world prediction of response to climate change. Fundamental to this is understanding how frequency and duration of antecedent thermal conditions (i.e., heat events) affect subsequent thermal tolerance of fish exposed to temperature fluctuations in the wild. Exposure length and frequency during the pre-measurement period in an experiment–or during antecedent thermal conditions for a wild fish before a thermal event–is consequential for subsequent performance, and wild fish can be exposed to any combination of short exposures to extreme temperatures, repeated exposures, or long sustained exposures, all of which can impact thermal tolerance differently (Healy and Schulte 2012; Pandey et al. 2021; O'Sullivan et al. 2023).

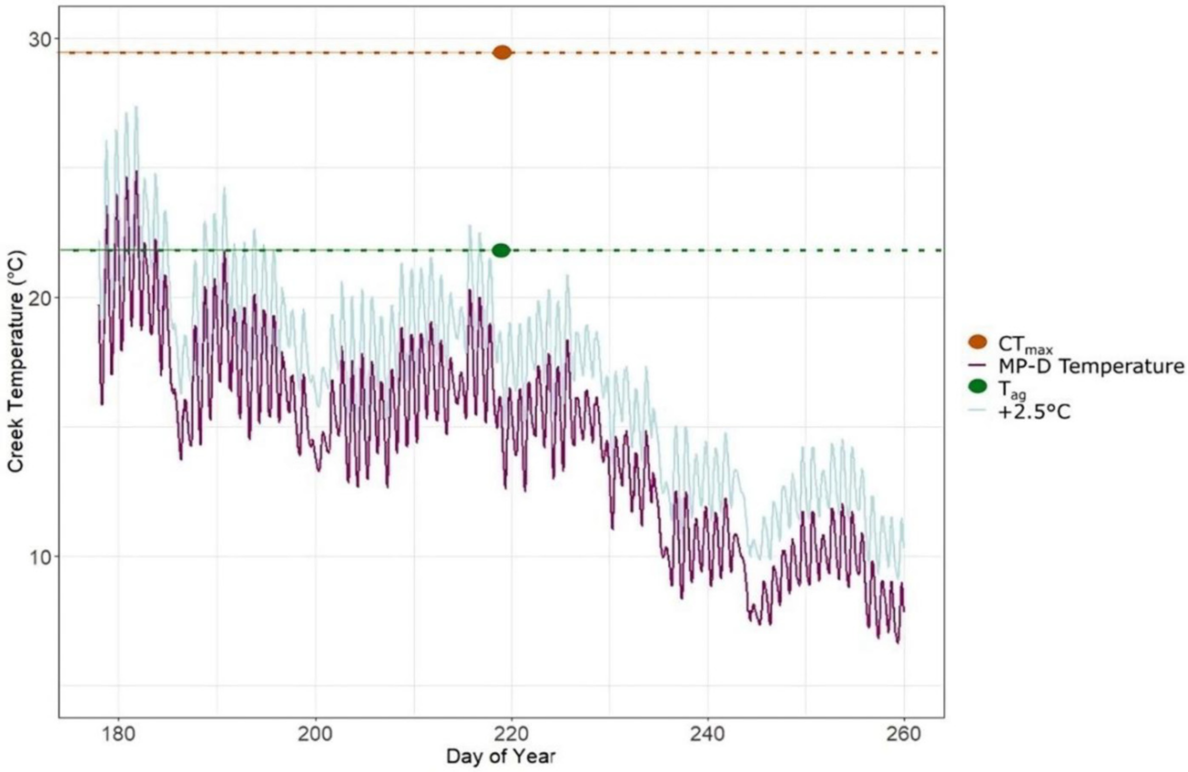
Vulnerability of Athabasca Rainbow Trout to climate change

If future climate projections are available, T_{ag} and CT_{max} metrics can directly inform potential sensitivity to increasing stream temperatures and extreme events (Habary et al. 2017). To evaluate the vulnerability of Athabasca Rainbow Trout to climate change, the warmest study site (MP–D) was selected using temperature data collected during a heat dome event that occurred between 25 June and 1 July 2021, when air temperatures reached 41.2 °C near our field sites (Jasper Alberta; Government of Canada 2023). Stream temperatures at MP–D site reached a maximum of 24.9 °C during this event, surpassing Athabasca Rainbow Trout T_{ag} by ~3 °C (Fig. 6). This implies that Athabasca Rainbow Trout likely experienced some degree of thermal stress at this site during this time period, unless they were able to access thermal refugia. However, stream temperatures before and after the heat wave were below T_{ag} indicating thermal stress was neither prolonged nor repeated numerous times throughout the summer. Throughout the heatwave, CT_{max} remained a minimum of 4.5 °C above stream temperature, indicating a relatively large buffer (i.e., TSM). However, climate modeling for the Athabasca River watershed predicts stream temperatures will increase up to 2.5 °C during summer months by the second half of the 21st century (Morales-Marín et al. 2019). Temperature increases of this magnitude would sharply increase the frequency and duration of thermal stress while reducing the TSM to ~2 °C. This hypothetical scenario would create major concerns for long-term habitat suitability in warmer streams like McPherson Creek. In contrast, the remaining five sampling sites all had large TSMs ranging from 9.1 to 14.4 °C. While these cooler creeks imply a higher degree of local resilience and thermal habitat suitability, range contraction as warmer sites become unsuitable seems certain, although the potential scope for range expansion in habitats that are currently too cold is unclear.

Conclusion

Physiological stress from climate change and associated habitat shifts will continue to impact Athabasca Rainbow Trout and other salmonids throughout their global distri-

Fig. 6. Summer stream temperatures in McPherson Creek (site MP–D) compared to quantitative metrics of performance (T_{ag}, CT_{max}, TSM) for Athabasca Rainbow Trout at this site. Dark purple line indicates the measured creek temperatures in 2021, whereas light blue presents a hypothetic situation where creek temperatures are 2.5 °C higher under future climate scenario. Filled circles "•" indicate when the metric was calculated, with the dashed lines extending for metric comparison. T_{ag}, agitation temperature; CT_{max}, critical thermal maxima; TSM, thermal safety margin.



bution (Wenger et al. 2011; DFO 2018). The threat of thermal niche constriction compounded by competition from invasive species, genetic introgression, and habitat loss are predicted to create challenges for this endangered ecotype (Fisheries and Oceans Canada 2020). The outcome for Athabasca Rainbow Trout will depend on their ability to adapt to climate change, either by exploiting thermal refuges or potentially altering their physiology. Understanding thresholds for sublethal avoidance (T_{ag}) and lethal temperatures (CT_{max}) is essential for understanding future habitat suitability and distribution. This study indicates that Athabasca Rainbow Trout show physiological plasticity in upper thermal tolerance, but not for behavioural avoidance thresholds, indicating a potentially lower resilience to sublethal effects associated with increasing stream temperature. Assessing climate related temperature risks for endangered fish populations is critical for guiding conservation efforts to protect critical habitats, and to predict the ecological impacts of increasing stream temperatures.

Acknowledgements

We would like to express our gratitude to all the staff in the Riverine Ecology Lab who contributed to this project, including Tyana Rudolfsen, Travis Durhack, Matt Teillet, and Amanda Caskenette for their field research support. We also extend our thanks to Eric Taylor and his team for conducting the genetic analysis of the fin samples. This project was made possible through the financial support of Fisheries and Oceans Canada.

Article information

Editor(s)

Maud C.O. Ferrari, Marco Rodriguez

History dates

Received: 15 January 2024
Accepted: 27 November 2024
Version of record online: 16 May 2025

Notes

This paper is part of a collection entitled Progress and Priorities for the Recovery of Aquatic Species at Risk in Canada.

Copyright

Data availability

The raw experimental data for this research are available from Dryad, located at https://doi.org/10.5061/dryad.5qfttdzh5.

Author information

Author ORCIDs

Sarah Hnytka https://orcid.org/0009-0001-1950-8272
Jordan Rosenfeld https://orcid.org/0000-0002-5827-9740

Author notes

Eva C. Enders served as Subject Editor at the time of manuscript review and acceptance; peer review and editorial decisions regarding this manuscript were handled by Maud Ferrari and Marco Rodriguez.

Author contributions

Conceptualization: SH, ECE
Data curation: SH
Formal analysis: SH
Funding acquisition: SH, ECE
Investigation: SH
Methodology: SH
Project administration: SH
Resources: ECE
Supervision: JR, ECE
Validation: JR, ECE
Visualization: SH
Writing – original draft: SH
Writing – review & editing: JR, ECE

Competing interests

The authors declare they have no competing interests related to this manuscript.

Supplementary material

Supplementary data are available with the article at https://doi.org/10.1139/facets-2024-0241.

References

Alfonso, S., Gesto, M., and Sadoul, B. 2021. Temperature increase and its effects on fish stress physiology in the context of global warming. Journal of Fish Biology, **98**(6): 1496–1508. doi:10.1111/jfb.14599. PMID: 33111333.

Angilletta, M.J. 2009. Thermal adaptation: a theoretical and empirical synthesis. Oxford University Press, Oxford, United Kingdom.

Bear, E.A., McMahon, T.E., and Zale, A.V. 2007. Comparative thermal requirements of Westslope Cutthroat Trout and Rainbow Trout: implications for species interactions and development of thermal protection standards. Transactions of the American Fisheries Society, **136**(4): 1113–1121. doi:10.1577/T06-072.1.

Becker, C.D., and Genoway, R.G. 1979. Evaluation of the critical thermal maximum for determining thermal tolerance of freshwater fish. Environmental Biology of Fishes, **4**(3): 245–256. doi:10.1007/BF00005481.

Beitinger, T.L., Bennett, W.A., and McCauley, R.W. 2000. Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. Environmental Biology of Fishes, **58**(3): 237–275. doi:10.1023/A:1007676325825.

Bernhardt, J.R., Sunday, J.M., Thompson, P.L., and O'Connor, M.I. 2018. Nonlinear averaging of thermal experience predicts population growth rates in a thermally variable environment. Proceedings of the Royal Society B: Biological Sciences, **285**(1886): 20181076. doi:10.1098/rspb.2018.1076. PMID: 30209223.

Carline, R.F., and Machung, J.F. 2001. Critical thermal maxima of wild and domestic strains of trout. Transactions of the American Fisheries Society, **130**(6): 1211–1216. doi:10.1577/1548-8659(2001)130%3c1211:CTMOWA%3e2.0.CO;2.

Comte, L., and Olden, J.D. 2017. Evolutionary and environmental determinants of freshwater fish thermal tolerance and plasticity. Global Change Biology, **23**(2): 728–736. doi:10.1111/gcb.13427. PMID: 27406402.

COSEWIC. 2014. COSEWIC assessment and status report on the Rainbow Trout Oncorhynchus mykiss in Canada. Committee on the Status of Endangered Wildlife in Canada. xi+60p. Available from www.registrelep-sararegistry.gc.ca/default_e.cfm.

DFO. 2018. Recovery Potential Assessment of Rainbow Trout, Oncorhynchus mykiss (Athabasca River Populations). Canadian Science Advisory Secretariat Science Advisory Report. 28p.

Döll, P., and Zhang, J. 2010. Impact of climate change on freshwater ecosystems: A global-scale analysis of ecologically relevant river flow alterations. Hydrology and Earth System Sciences, **14**(5): 783–799. doi:10.5194/hess-14-783-2010.

Du, X., Shrestha, N.K., and Wang, J. 2019. Assessing climate change impacts on stream temperature in the Athabasca River Basin using SWAT equilibrium temperature model and its potential impacts on stream ecosystem. Science of the Total Environment, **650**: 1872–1881. doi:10.1016/j.scitotenv.2018.09.344. PMID: 30286353.

Enders, E.C., and Durhack, T.C. 2022. Metabolic rate and critical thermal maximum CT_{max} estimates for Westslope Cutthroat Trout, *Oncorhynchus clarkii lewisi*. Conservation Physiology, **10**(1). doi:10.1093/conphys/coac071. PMID: 36570737.

Ficke, A.D., Myrick, C.A., and Hansen, L.J. 2007. Potential impacts of global climate change on freshwater fisheries. Reviews in Fish Biology and Fisheries, **17**: 581–613. doi:10.1007/s11160-007-9059-5.

Firth, B.L., Drake, D.A.R., and Power, M. 2021. Seasonal and environmental effects on upper thermal limits of Eastern Sand Darter (*Ammocrypta pellucida*). Conservation Physiology, **9**(1). doi:10.1093/conphys/coab057. PMID: 35928053.

Fisheries and Oceans Canada. 2020. Recovery Strategy for the Rainbow Trout (*Oncorhynchus mykiss*) in Canada (Athabasca River populations). Species at Risk Act Recovery Strategy Series. vii+90p.

Gamperl, A.K., Ajiboye, O.O., Zanuzzo, F.S., Sandrelli, R.M., Ellen de Fátima, C.P., and Beemelmanns, A. 2020. The impacts of increasing temperature and moderate hypoxia on the production characteristics, cardiac morphology and haematology of Atlantic Salmon (*Salmo salar*). Aquaculture, **519**: 734874. doi:10.1016/j.aquaculture.2019.734874.

Geraldes, A., and Taylor, E.B. 2021. Site C Clean Energy Project, Fish Genetics Study. Annual report to British Columbia Hydro: Construction Year 6. 52p. Available from https://www.sitecproject.com/sites/default/files/Fish-Genetics-Study-2020-Annual-Report.pdf.

Government of Canada. 2023. Daily data report for June 2021 [online]. Available from https://climate.weather.gc.ca/climate_data/daily_data_e.html?StationID=10223&timeframe=2&StartYear=1840&EndYear=2023&Day=7&Year=2021&Month=6.

Habary, A., Johansen, J.L., Nay, T.J., Steffensen, J.F., and Rummer, J.L. 2017. Adapt, move or die–how will tropical coral reef fishes cope with ocean warming? Global Change Biology, **23**(2): 566–577. doi:10.1111/gcb.13488. PMID: 27593976.

Healy, T.M., and Schulte, P.M. 2012. Factors affecting plasticity in whole-organism thermal tolerance in common killifish (*Fundulus heteroclitus*). Journal of Comparative Physiology B, **182**: 49–62. doi:10.1007/s00360-011-0595-x.

Horton, R.M., Mankin, J.S., Lesk, C., Coffel, E., and Raymond, C. 2016. A review of recent advances in research on extreme heat events. Current Climate Change Reports, **2**: 242–259. doi:10.1007/s40641-016-0042-x.

Jeffries, K.M., Fangue, N.A., and Connon, R.E. 2018. Multiple sub-lethal thresholds for cellular responses to thermal stressors in an estuarine fish. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, **225**: 33–45.

McCullough, D.A., Bartholow, J.M., Jager, H.I., Beschta, R.L., Cheslak, E.F., Deas, M.L., et al. 2009. Research in thermal biology: burning questions for coldwater stream fishes. Reviews in Fisheries Science, **17**(1): 90–115. doi:10.1080/10641260802590152.

McDonnell, L.H., and Chapman, L.J. 2015. At the edge of the thermal window: effects of elevated temperature on the resting metabolism, hypoxia tolerance and upper critical thermal limit of a widespread African cichlid. Conservation Physiology, **3**(1):cov050. doi:10.1093/conphys/cov050.

McDonnell, L.H., Mandrak, N.E., Kaur, S., and Chapman, L.J. 2021. Effects of acclimation to elevated water temperature and hypoxia on thermal tolerance of the threatened Pugnose Shiner (*Notropis anogenus*). Canadian Journal of Fisheries and Aquatic Sciences, **78**(9): 1257–1267. doi:10.1139/cjfas-2020-0362.

McNab, B.K. 2002. The physiological ecology of vertebrates: A view from energetics. Cornell University Press, Ithaca, New York. 576p.

Morales-Marín, L.A., Rokaya, P., Sanyal, P.R., Sereda, J., and Lindenschmidt, K.E. 2019. Changes in streamflow and water temperature affect fish habitat in the Athabasca River basin in the context of climate change. Ecological Modelling, **407**: 108718.

O'Sullivan, A.M., Corey, E.M., Collet, E.N., Helminen, J., Curry, R.A., MacIntyre, C., et al. 2023. Timing and frequency of high temperature events bend the onset of behavioural thermoregulation in Atlantic Salmon (*Salmo salar*). Conservation Physiology, **11**(1). doi:10.1093/conphys/coac079.

Pandey, A., Rajesh, M., Baral, P., Sarma, D., Tripathi, P.H., Akhtar, M.S., et al. 2021. Concurrent changes in thermal tolerance thresholds and cellular heat stress response reveals novel molecular signatures and markers of high temperature acclimation in Rainbow Trout. Journal of Thermal Biology, **102**: 103124. doi:10.1016/j.jtherbio.2021.103124.

Peng, J., Cao, Z.D., and Fu, S.J. 2014. The effects of constant and diel-fluctuating temperature acclimation on the thermal tolerance, swimming capacity, specific dynamic action and growth performance of juvenile Chinese Bream. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, **176**: 32–40.

Petersen, J.K., and Petersen, G.I. 1990. Tolerance, behaviour and oxygen consumption in the Sand Goby, *Pomatoschistus minutus (Pallas)*, exposed to hypoxia. Journal of Fish Biology, **37**(6): 921–933. doi:10.1111/j.1095-8649.1990.tb03596.x.

Pörtner, H.O. 2004. Climate variability and the energetic pathways of evolution: the origin of endothermy in mammals and birds. Physiological and Biochemical Zoology, **77**(6): 959–981. doi:10.1086/423742.

Pörtner, H.O., and Peck, M.A. 2010. Climate change effects on fishes and fisheries: towards a cause-and-effect understanding. Journal of Fish Biology, **77**(8): 1745–1779. doi:10.1111/j.1095-8649.2010.02783.x.

Potts, L.B., Mandrak, N.E., and Chapman, L.J. 2021. Coping with climate change: phenotypic plasticity in an imperiled freshwater fish in response to elevated water temperature. Aquatic Conservation: Marine and Freshwater Ecosystems, **31**(10): 2726–2736. doi:10.1002/aqc.3620.

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Salinas, S., Irvine, S.E., Schertzing, C.L., Golden, S.Q., and Munch, S.B. 2019. Trait variation in extreme thermal environments under constant and fluctuating temperatures. Philosophical Transactions of the Royal Society B: Biological Sciences, **374**(1768):20180177. doi:10.1098/rstb.2018.0177.

Volkoff, H., and Rønnestad, I. 2020. Effects of temperature on feeding and digestive processes in fish. Temperature, **7**(4): 307–320. doi:10.1080/23328940.2020.1765950.

Wells, Z.R., McDonnell, L.H., Chapman, L.J., and Fraser, D.J. 2016. Limited variability in upper thermal tolerance among pure and hybrid populations of a cold-water fish. Conservation Physiology, **4**(1):cow063. doi:10.1093/conphys/cow063.

Wenger, S.J., Isaak, D.J., Luce, C.H., Neville, H.M., Fausch, K.D., Dunham, J.B., et al. 2011. Flow regime, temperature, and biotic interactions drive differential declines of trout species under climate change. Proceedings of the National Academy of Sciences, **108**(34): 14175–14180. doi:10.1073/pnas.1103097108.