Intraspecific variability in thermal tolerance: a case study with coastal cutthroat trout

Kara Anlauf-Dunn¹,*, Krista Kraskura² and Erika J. Eliason²

¹Oregon Department of Fish and Wildlife, 28655 Highway 34, Corvallis, OR 97333, USA
²Department of Ecology, Evolution, and Marine Biology, University of California Santa Barbara, Santa Barbara, CA 93106, USA

*Corresponding author: Oregon Department of Fish and Wildlife, 28655 Highway 34, Corvallis, OR 97333, USA.
Email: Kara.J.ANLAUF-DUNN@odfw.oregon.gov

Fish physiological performance is directly regulated by their thermal environment. Intraspecific comparisons are essential to ascertain the vulnerability of fish populations to climate change and to identify which populations may be more susceptible to extirpation and which may be more resilient to continued warming. In this study, we sought to evaluate how thermal performance varies in coastal cutthroat trout (Oncorhynchus clarki clarki) across four distinct watersheds in OR, USA. Specifically, we measured oxygen consumption rates in trout from the four watersheds with variable hydrologic and thermal regimes, comparing three ecologically relevant temperature treatments (ambient, annual maximum and novel warm). Coastal cutthroat trout displayed considerable intraspecific variability in physiological performance and thermal tolerance across the four watersheds. Thermal tolerance matched the historical experience: the coastal watersheds experiencing warmer ambient temperatures had higher critical thermal tolerance compared with the interior, cooler Willamette watersheds. Physiological performance varied across all four watersheds and there was evidence of a trade-off between high aerobic performance and broad thermal tolerance. Given the evidence of climate regime shifts across the globe, the uncertainty in both the rate and extent of warming and species responses in the near and long term, a more nuanced approach to the management and conservation of native fish species must be considered.

Key words: thermal tolerance, physiology, metabolism, intraspecific, cutthroat trout, Climate change

Editor: Dr. Steven Cooke
Received 20 July 2021; Revised 4 January 2022; Editorial Decision 2 April 2022; Accepted 15 April 2022

Cite as: Anlauf-Dunn K, Kraskura K, Eliason EJ (2022) Intraspecific variability in thermal tolerance: a case study with coastal cutthroat trout. Conserv Physiol 10(1): coac029; doi:10.1093/conphys/coac029.

Introduction

Shifts in climate regimes are projected to be associated with both changes in mean conditions as well an increase in the variability and frequency of extreme events (Bernhardt et al., 2020). The capacity for fish to cope with temperature change will be primarily mediated by their adaptive capacity and phenotypic plasticity (Seebacher et al., 2015b; Bernhardt et al., 2020). An understanding of the physiological tolerance of fish to changes in environmental temperatures is essential when trying to evaluate how climate change will influence biogeographic distributions and survival (Harada et al., 2019; Sunday et al., 2012; Walters et al., 2018). Monitoring stream temperature and evaluating components of the thermal regime (e.g. daily variability and range, duration of warm events, etc.) are valuable to understanding current and future shifts in stream temperatures (Arismendi et al., 2013). However, without complementary information relat-
ing thermal data to species thermal habitat requirements, we will be limited in our ability to make decisions about how best to manage and protect species in general, and more specifically those species most at risk (Marras et al., 2015; Zillig et al., 2021). Conservation and management of fish in a changing climate will depend on knowledge about both the rate and magnitude of change in the aquatic environment and the effects on fish physiology (Marras et al., 2015). The physiological response of fish to a changing climate provides a mechanistic explanation for population responses such as altered phenology, range shifts and biotic interactions.

Physiological performance traits (e.g. metabolic rates, locomotion, digestion, growth) of fish and other ectotherms are temperature-dependent, directly regulated by their thermal environment (Farrell, 2016; Fry, 1947; Schulte et al., 2015). The influence of temperature on the rate of physiological processes influencing metabolism leave fish vulnerable to changes in temperature but has also shaped ecological patterns and species distributions (Harada et al., 2019). Ectotherms, like fishes, have an upper thermal tolerance limit at which biological processes breakdown, leading to reduced physiological performance and eventual mortality (Farrell, 2016; Kellermann et al., 2012; Pörtner and Farrell, 2008; Schulte et al., 2015). Evidence suggests that this threshold is likely related in part to the thermal history to which species have been exposed and are adapted (Comte and Olden, 2017; Eliason et al., 2011; McKenzie et al., 2021; Olsen et al., 2021). Indeed, thermal performance has been shown to differ across salmon Oncorhynchus spp. populations of the same species (Abe et al., 2019; Chen et al., 2013; Eliason et al., 2011; Eliason et al., 2013; Stitt et al., 2014; Whitney et al., 2016), often matching the typical environmental temperatures to which they have encountered over prolonged periods. For example, Eliason et al. (2011) found that cardiorespiratory physiology varies among Fraser River sockeye salmon (Oncorhynchus nerka) populations and is related to the historical thermal conditions the species encountered during migration. In addition, on short timescales (hours to days to weeks), an individual fish can modify their physiology and morphology (i.e. acclimate) to compensate for a change in environmental temperature (Seebacher et al., 2015a). Phenotypic plasticity is expected to play a key role in a population’s resilience to climate change (Seebacher et al., 2015b); however, the capacity for phenotypic plasticity varies across species and populations (Sommer, 2020; Sparks et al., 2017). Given this variation, biologists and managers cannot assume a single thermal threshold for a species (Zillig et al., 2021). Instead, intraspecific comparisons are essential to ascertain the vulnerability of fish populations to climate change and to identify which populations may be more susceptible to extirpation and which may be more resilient to continued warming. These data can help managers adjust angling regulations and/or justify thermal reserves that are protective to native stocks.

Physiological thermal tolerance has been evaluated for many fish species (Beitinger and Bennett, 2000; McKenzie et al., 2021). The majority of this work has been done in a laboratory setting and perhaps the most common technique has focused on obtaining upper lethal temperature (CT-max, critical thermal maxima) with or without acclimation to particular temperatures (Beitinger and Bennett, 2000; Nati et al., 2020). While CT-max provides a relevant lethal index to compare relative thermal tolerance and thermal safety margins (TSMs; TSM = CT-max – maximum environmental temperature) (Pinsky et al., 2019; Sunday et al., 2014; Vinagre et al., 2019) across populations or species, it is not useful to define the functional thermal range for fitness-related performance (Farrell et al., 2009; Rodnick et al., 2004). Instead, aerobic scope (the difference between the standard and maximum oxygen consumption rate) is a measure of an organism’s aerobic capacity. While aerobic scope is commonly measured to understand optimal thermal conditions in ectotherms, including fish (Clark et al., 2013; Farrell, 2016; Schulte et al., 2015), the specific mechanisms that limit upper thermal tolerance among and across species is a subject of debate (see Jutfelt et al., 2018, and associated references and commentary). For a given species, aerobic activities (e.g. locomotion, digestion, spawning and competition) at the organism level are feasible across a range of temperatures where aerobic scope is optimal. Beyond optimal temperatures (T_{opt}), the fitness-related activities that allow an individual to thrive (e.g. locomotion, digestion) can become impaired. At a critical temperature threshold (T_{crit}), aerobic scope is zero and mortality is imminent and inevitable (Whitney et al., 2016). Functional warming tolerance (WT) calculated as the difference between the maximum environmental temperature and the upper temperature at which physiological performance ceases to be optimal to meet basic needs (T_{pejus}) can be used to evaluate the functional vulnerability of populations to current temperatures and future warming. Incorporation of management and conservation approaches that consider both the physiological and ecological constraints and optimums of native fish species must be considered given evidence of climate regime shifts across the globe, the spatial and temporal variation and uncertainty in both the rate and extent of warming and uncertainty that exists in species responses in the near and long term.

In this study, we sought to evaluate how thermal performance varies in field acclimatized coastal cutthroat trout (Oncorhynchus clarki clarki) across four distinct watersheds in OR, USA, under acute warming scenarios, not unlike an extreme heat event. Specifically, we measured CT-max and oxygen consumption rates (for the calculation of aerobic scope) in trout at three ecologically relevant temperatures (ambient, current maximum stream temperature and a novel warm intended to reflect a climate change scenario) from four streams with variable hydrologic and thermal regimes (two watersheds with a warm thermal history; two watersheds with a cooler thermal history). All experiments were conducted stream side to mimic environmentally relevant conditions and minimize transport and handling stress, and any habituation effects from the laboratory residence. Thus,
in contrast to a ‘common garden’ experiment (i.e. rearing individual fish from different populations in a common lab environment), this study design compared locally acclimatized fish, in their environmental conditions. We then used results of this study to calculate TSMs and functional WT to evaluate the degree to which coastal cutthroat trout may fare under current and future climate projections in stream temperature (∼ +3°C; Jiménez Cisneros et al., 2014). The implications of this study could challenge the use of a single thermal criterion applied to the same species across its geographic range providing both opportunities and challenges for how we think about managing thermal conditions under threat from development and climate change.

Materials and Methods

Coastal cutthroat trout are widely distributed along the Western Pacific Coast, ranging from the Kenai Peninsula in Alaska to the Eel River in Northern California. They exhibit a diverse and flexible life history, residing in small headwater streams, higher gradient transition tributaries and large floodplain rivers (Trotter, 1989). This species exhibits both anadromous and stream-resident forms and vary considerably in body size, ranging from ~150 mm (mature stream resident) to over 500 mm (anadromous or sea-run). Because of this species’ expansive range and habitation of the entire river continuum (e.g. the longitudinal dimension of the stream ecosystem; Vannote et al., 1980), they are an important species across which to compare thermal physiology.

Study sites and fish sampling

Four streams from four distinct watersheds in OR, USA, were selected to conduct stream-side respirometry experiments (Fig. 1); two coastal watersheds, the Siletz and the Alsea, and two Willamette River Basin watersheds, the North Santiam (N. Santiam) and the McKenzie. We attempted to identify watersheds that had variable hydrologic regimes (defined by the magnitude of discharge and frequency, duration, timing and rate of change of flow events), which reflect interactions among many biophysical features of the ecosystem, environmental gradients and disturbance histories (Poff and Ward, 1990). We compared modelled water temperature data for each watershed using NorWeST (Isaak et al., 2017) (Table 1). Specific stream-side respirometry sites within each of the four watersheds were chosen based on presence of coastal cutthroat trout, public ownership, ease of vehicle access, adequate space for equipment and low terrace heights along the creek to enable gravity pumping of stream water into tanks.

The streams selected in the coastal watersheds (Fig. 1) are positioned in the Western Oregon Coast Range Mountains (Alsea and Siletz) at elevations below 500 m. Both streams are underlain by marine sedimentary geology, primarily sandstone and siltstone of the Tyee formation and are characterized by primarily coniferous forest and secondarily deciduous cover with shrub-scrub understory. Coastal Oregon is characterized by wet winters and dry summers with mild temperatures year-round. These systems exhibit rain-driven hydrologic regimes that are reactive and have little storage capacity. Annual precipitation ranges between 165 and 228 cm. Stream temperatures during the study (2018–2019) ranged from 16°C to 20°C and from 17°C to 20°C for Alsea and Siletz, respectively (Table 1). Coastal cutthroat trout were collected from tributaries of Fall Creek in the Alsea watershed and Little Rock Creek in the Siletz (Fig. 1a and b).

The streams located in watersheds along the western slopes of the Cascade Range in the Willamette River basin (McKenzie and N. Santiam) (Fig. 1) are both positioned above 500 m. Both streams are primarily underlain by porous volcanic geologies, allowing snow and rain runoff to filter and flow far beneath the surface. Both streams were at elevations in the rain–snow transition zones. While the McKenzie River and many of its tributaries (including the study stream) are spring fed, helping to maintain flow and constant seasonal stream temperatures, the aquifers in the upper reaches of the N. Santiam River (which includes the study stream) are variable in supply (Synder et al., 2002). Both watersheds are characterized by steep forested uplands and alluvial lowlands and include a number of impoundments (dams) lower in the watersheds. Stream temperatures during the study ranged from 10°C to 13°C and from 6°C to 10°C for McKenzie and N. Santiam, respectively (Table 1). Coastal cutthroat trout were collected from White Branch Creek in the McKenzie and the upper reaches of the N. Santiam River in the N. Santiam (Fig. 1c and d).

Acclimation temperature treatments

For all watersheds, coastal cutthroat trout [target size range, 90–150 mm fork length (FL)] were collected by electrofishing each day in the late morning or early afternoon during August and were allowed several hours to recover from capture and to adjust to the holding tank. Fish were then acclimated overnight to their treatment temperature. Eight fish were evaluated per temperature treatment, with a total of 24 fish caught per watershed throughout the duration of the experiment. The fish were placed in a 100-L fibreglass holding tank positioned immediately adjacent to the stream. Fresh water was circulated through the tank in a flow-through system using a water pump. Three large airstones were placed in the tank to ensure dissolved oxygen levels were maintained at >90% air saturation (Fig. S2). Water temperature was monitored hourly and maintained at the treatment temperature. Electrical power to run all experimental equipment was supplied by two generators (EU7000IS and EU3000iS; Honda Motor Company Ltd, Japan). All procedures were approved by University of California, Santa Barbara Institutional Animal Care and Use Committee.

The temperature treatments were selected to reflect the specific thermal conditions and to facilitate comparisons across watersheds (Table 1). The first temperature treatment
Figure 1: Reach locations (black line) within the Oregon Coast and Willamette basins where respirometry experiments occurred: (a) Little Rock Creek, Siletz (44.7206, −123.7117); (b) Fall Creek, Alsea (44.404, −123.7536); (c) N. Santiam River, N. Santiam (44.49841, −121.9837); and (d) White Branch Creek, McKenzie (44.1647, −122.0154). Fish were collected from within the reach locations, except in Fall Creek (panel b), where they were collected at locations indicated by red lines. Inset: Oregon positioned within the Northwestern USA.

Table 1: Site characteristics including elevation, NorWeST estimates of maximum weekly maximum temperature (MWMT ± SE) for both baseline (mean 2002–2011) and 2080 projected values from streams where fish were collected and study temperature treatments (ambient, maximum and climate treatments).

| Location | Stream                  | Elevation (m) | MWMT baseline | MWMT 2080 | Ambient min–max | Ambient mean (SD) | Max treatment | Climate treatment |
|----------|-------------------------|---------------|---------------|-----------|-----------------|------------------|---------------|------------------|
| Alsea    | Fall Creek Tributary    | 109           | 16.97 ± 2.53  | 18.77     | 16.6–20.0       | 18.4 (1.17)      | 19            | 22               |
| Siletz   | Little Rock Creek       | 72            | 18.87 ± 2.64  | 20.79     | 16.6–20.0       | 18.8 (0.71)      | 19            | 22               |
| McKenzie | White Branch Creek      | 642           | 15.60 ± 2.62  | 17.32     | 10.4–13.4       | 11.8 (0.90)      | 14            | 19               |
| N. Santiam | N. Santiam River    | 1127          | 15.79 ± 2.35  | 17.52     | 6.3–10.3        | 8.1 (1.31)       | 14            | 19               |

The respirometry experiments in Alsea, McKenzie and N. Santiam were conducted in August 2018 and in Siletz in August 2019. The Ambient temperatures for the Alsea were from Fall Creek where the experiment occurred.

*Isaak et al., 2017.*

reflected the ambient water temperature with the natural diurnal fluctuation (‘Ambient’ treatment). The second temperature treatment was set at the maximum summer temperature observed in the days preceding the experiment (‘Max’ treatment). The third temperature treatment was set 3°C higher than the maximum temperature and represented the novel warm conditions fish will likely have to endure to persist into the future (from here on named the ‘Climate’ treatment) (Table 1). Each acclimation period was ~18 h in duration, which was selected as ecologically relevant and representative of an acute warming event. Although full acclimation processes are anticipated to take >18 h to occur, studies have shown that metabolism can acclimate rapidly, within 1–2 days in fish species [goldfish (*Carassius auratus*); Klicka, 1965; minnows (*Umbra limi*); Hanson and Stanley, 1970; curimbatá (*Prochilodus scrofa*): Barrionuevo and Fernandes, 1998]. While the acclimation duration used in this study was relatively short, our interest was to evaluate
the thermal sensitivity and the capacity for rapid phenotypic plasticity of these fish to an acute thermal warming event similar to a potentially abrupt but brief environmental temperature shift, rather than to evaluate the full acclimation capacity of fish to chronic, extreme thermal conditions. Given that cutthroat trout live in thermally variable environments, it is ecologically relevant to understand how they can rapidly respond to changing temperatures.

For the Ambient treatment, the water temperature in the holding tank was not adjusted or heated in any way, reflecting the natural diurnal fluctuation in the stream. For the Max and Climate treatments, the water temperature of the holding tank was increased from ambient to the test temperature (by ∼1°C per hour) via an adjacent header tank that was equipped with a submerged water heater (Smart One Easy Plug Axial Heaters) powered by a generator (Fig. S2). Fish were not fed to reduce the potential that digestion processes affecting metabolism would confound the respirometry measurements.

Temperature profiles during the ambient respirometry trial (early to late August for all watersheds) were distinctly different between the coastal watersheds and Willamette basin watersheds (Table 1; Fig. S1). The two coastal watershed streams were notably warmer than those in the Willamette basin watersheds. The pattern of diurnal variation in temperature was similar across all four streams and resulted in a 2.4–3.9°C change for a 24-h period. The peak temperature for all streams occurred between 3:30 pm and 6 pm in the afternoon/evening and decreased soon thereafter (Fig. S1).

**Field respirometry**

An eight-chamber intermittent flow-through respirometry system was constructed to measure oxygen consumption (Fig. S2). The system consists of eight clear plastic 2-L chambers that were approximately 80× the volume of the fish. Each chamber was equipped with two water pumps. A small Sicce Micra pump (∼5 L/min) flushed fresh water through the chamber and the Eheim compact pump (∼5 L/min) recirculated water continuously past a fibre-optic oxygen probe (robust Firesting O2 probe) that monitored oxygen levels in the chambers. Each oxygen probe was attached to an external optical oxygen meter (4-channel Firesting O2, PyroScience, Germany) that continuously measured dissolved oxygen levels in the chambers. Each chamber was equipped with a submerged respirometry chamber at which time measurement of oxygen consumption began and continued for 18–24 h (Eliason et al., 2020). The fish were then air-exposed for 30 s before being immediately placed in a respirometry chamber at which time measurement of oxygen consumption began and continued for 18–24 h (Eliason et al., 2008) (Fig. 2). The system operated as a closed respirometer during short measurement phases (6 min) when the chamber is closed, but between measurement phases, water in the respirometer is flushed (for 4 min) and replaced to prevent hypoxia and the build-up of metabolites (10 min measurement:flush cycles, resulting in 108–144 total measurements per trial). After 18–24 h, the fish were removed from the chambers and weighed (body mass in grammes) and measured (FL, in mm). The fish were then returned to the holding tank at their treatment temperature. If the treatment temperature was anything other than ambient, the temperature of the holding
Critical thermal maximum

Approximately 1 h after the respirometry experiment for the ambient treatment, the same fish underwent a standard critical thermal maximum (CT-max) protocol (Fangue et al., 2006). Fish were placed in a large (45.4 L) cooler with aerated fresh water. Water temperature was increased by 0.3°C per minute using a stainless-steel immersion heater coil attached to a water pump. Heating rates during the CT-max test were similar across watersheds. While we strived to heat the water 1°C every 3 min, there was some variability. The average heating rate for all locations was 0.96–0.99°C and ranged from a minimum heating rate of 0.5–0.7°C to a maximum heating rate of 1.3–1.5°C, every 3 min. When an individual fish lost equilibrium, it was immediately removed and the time and temperature was recorded. The fish was then placed in a recovery tank that was 5°C cooler than the CT-max temperature and the water temperature was gradually reduced back to ambient. At the conclusion of the CT-max test, the fish were allowed several hours to recover at ambient water temperatures before being returned to the stream.

Data and statistical analysis

We followed methods outlined in Rosewarne et al. (2016) to calculate fish oxygen consumption rate (MO2; mg O2 kg⁻¹ min⁻¹; equation 1), the final calculations yielding one MO2 value per fish per measurement phase (6 min measurement phase, 4 min flush) (Fig. 2). We corrected each measurement for background respiration that may have been caused by micro-organisms residing in the tubing or chambers:

\[ \text{MO2} = \Delta \left[ \text{O}_2 \right]_{\text{fish}} \times \left( V_{\text{chamber}} - \text{Mass} \right) - \Delta \left[ \text{O}_2 \right]_{\text{background}} \times V_{\text{chamber}} \times \text{Mass}^{-1}, \]

where MO2 is the oxygen consumption rate for each measurement phase, \( \Delta \left[ \text{O}_2 \right]_{\text{fish}} \) is the rate of decrease in oxygen content in the respirometer over the course of measurement (mg O2 L⁻¹ min⁻¹), \( \Delta \left[ \text{O}_2 \right]_{\text{background}} \) is the rate of decrease in oxygen content in the empty respirometer over the course of measurement (mg O2 L⁻¹ min⁻¹), \( V_{\text{chamber}} \) is the volume of the respirometer (L) and Mass is the body mass of the individual fish (kg). Because metabolism often scales allometrically with body size in fish (Jerde et al., 2019), we checked for body mass effects on oxygen consumption rates (MMR and SMR) in our study. We evaluated linear regression between natural log transformed raw oxygen consumption rates (ln mg O2 L⁻¹ min⁻¹) and natural log transformed body mass of each individual (ln kg) and used the regression values to confirm that allometric mass correction on our measurements was not necessary (Fig. S4; Table S9). Lastly, Fulton’s body condition factor was measured as:

\[ K = 100 \times \frac{M}{L^3}, \]

where M is the mass of the fish in grammes and L is the fork length of the fish in millimetres. Differences between body morphometrics (mass and length) and condition factor were analysed using an Analysis of Variance test (ANOVA).

SMR, MMR and aerobic scope

We evaluated several metrics to understand the extent to which oxygen consumption rate differs among individuals and across different watersheds. Standard metabolic rate (SMR; mg O2 kg⁻¹ min⁻¹) is described as the minimum maintenance metabolism or basic cost of living (i.e. fish in a resting, non-reproductive, post-absorptive state). To calculate SMR, we extracted the lowest 20% of all recorded MO2 values (N = 32–274) and calculated the mean value for each fish (SEM = 115.2 ± 4.3) (Chabot and McKenzie, 2016) (Fig. 2). For the Ambient treatment, SMR was calculated from all recorded MO2 values, even though temperature fluctuated throughout the experiment (Fig. S1). Notably, following recovery from the chase [i.e. after excess post-exercise oxygen consumption (EPOC) was complete], MO2 remained remarkably constant for an individual fish even though temperature fluctuated (Fig. S5). The corresponding temperature values for each MO2 measurement used in the SMR calculation were averaged to determine the mean temperature for SMR for each fish. Maximum metabolic rate (MMR; mg O2 kg⁻¹ min⁻¹), the upper boundary for aerobic metabolism that is achievable by an animal, was measured immediately after the 3-min chase and 30-s air exposure once fish were placed in the respirometry chambers (Little et al., 2020). For the ambient trial, the temperature at which MMR was measured did not match the SMR temperature because SMR was measured over a range of temperatures during the diurnal cycle. For the ambient treatments, the chase and air exposure occurred at approximately the same time during the day (between 9:00 and 10:00 am) for each watershed, with chase temperatures averaging 16.5 C, 18.1 C, 10.5 C and 7.4 C for the Alsea, Siletz, McKenzie and N. Santiam, respectively. Absolute aerobic scope (AAS = MMR – SMR; mg O2 kg⁻¹ min⁻¹), represents the absolute energy available to thrive in the environment (e.g. move, find food, migrate, etc.) and factorial aerobic scope (FAS = MMR/SMR) was calculated to understand whether a metabolic constraint might arise as temperatures increase (Halsey et al., 2018).

Recovery performance

We evaluated recovery performance for each individual by calculating the time it took for fish to recover to 50% of their MMR (Time [MMR0]) (Krasnare et al., 2021). This is an indication of short-term recovery from exhaustive exercise and is an estimate for the amount of recovery time necessary before the fish can resume normal activities (e.g.
swimming, foraging, etc.). Salmonids have an exceptional ability to recover, which has been demonstrated by their repeat swim performance with only 45 min of break between the consecutive swim tests (e.g. Eliason et al., 2013; Farrell et al., 2003; Farrell et al., 1998). In these studies fish decreased their oxygen consumption to 30–70% of MMR, therefore the chosen time to recover to 50% MMR is an ecologically and physiologically relevant recovery metric (Kraskura et al., 2021). Full recovery was determined as the time (h) to end of EPOC, which in this study was when MO2 values reached 20% above the SMR value. To calculate these metrics we fit a smoothed curve over all MO2 values (smooth.spline R); the time to EPOC was the time when the smoothed line intersected the 20% above SMR threshold (Fig. 2). To evaluate differences in SMR, MMR, AAS, FAS and Time [MMR50] estimates within each of the four watersheds, we used the non-parametric Kruskal–Wallis test. Where significant differences occurred (P < 0.05), the post hoc Dunn’s Multiple Comparison test was used to identify which watersheds differed.

**Routine metabolic rate and Q10**

Routine metabolic rate (RMR, mg O2 kg⁻¹ min⁻¹) was evaluated using MO2 values [N = 3–274, range; 121.0 ± 4.1 measurements, mean (SEM)] measured after the fish had fully recovered from the exhaustive chase (i.e. after EPOC was complete). Due to the diurnal temperature fluctuations during the Ambient temperature treatment, we obtained RMR measurements for fish at 4, 3, 3 and 5 different temperatures for the Alsea, Siletz, McKenzie, and N. Santiam watersheds, respectively. To be included in the RMR estimates for a given temperature, individual fish were required to have at least 3 RMR measurements at that temperature. This allowed for an examination of metabolic response at a range of temperatures experienced by individual fish. To evaluate differences in RMR values within each watershed, we developed a log-normal linear mixed model fit with RMR as the response variable and two independent fixed-effect variables: (i) temperature during the measurement (continuous variable) and (ii) basin (Alsea, McKenzie, Siletz and N. Santiam). The random intercept effects were treatment (Climate, Max and Ambient) and individual fish (lme4 package in R; Bates et al., 2015). Treatment was added as a random intercept effect to account for non-independence in these temperature treatments across basins and individual fish were included as a random effect to account for non-independence between data points across temperatures. Q10 values, which provide insight into the degree to which routine and SMRs are influenced by temperature, were calculated for all locations using following equation:

\[ Q_{10} = \left( \frac{R_2}{R_1} \right)^\frac{10}{T_2-T_1}, \]

where \( R_2 = \text{MO}_2 \) at Climate treatment temperature, \( R_1 = \text{MO}_2 \) at lowest Ambient temperature, \( T_2 = \text{temperature at } R_2 \) and \( T_1 = \text{temperature at } R_1 \). A Q10 value greater than 1 indicates that rates are increasing with temperature, less than 1 indicates that rates are decreasing with temperature and a value equal to 1 indicates that the rate is temperature independent.

For each of the watersheds, one temperature treatment, 19°C, was shared. For the Coastal watersheds this represented the Max temperature treatment; in the Willamette basin watersheds, 19°C represented the Climate temperature treatment. Using the same analytical approach (Kruskal–Wallis test, Dunn’s test), we also evaluated whether SMR, MMR, AAS, FAS and Time [MMR50] estimates varied at 19°C across the four watersheds. All analyses were conducted using R studio version 1.0.143. Significance level for all statistical tests was \( \alpha = 0.05 \).

**Evaluating climate implications**

Metabolic rate is a temperature-dependent performance trait that can influence species distributions and vulnerability (Schulte et al., 2011). When trying to understand species response to a changing climate, considering the interaction between exposure and thermal limits is important. We calculated two measures to understand species resiliencies to increasing stream temperatures. First, TSM was calculated as the difference between an individual’s CT-max and the maximum temperature of the habitat (environmental temperature). This measure can provide an approximation of the amount of environmental warming an organism can tolerate before death is imminent. As TSM values get smaller, there is less capacity to deal with warming (or higher probability of death) (Pinsky et al., 2019; Sunday et al., 2014; Vinagre et al., 2019; Walters et al., 2018).

Second, we calculated functional WT. WT was calculated as the difference between an organism’s \( T_{pejus} \) and the maximum temperature of that organism’s habitat. The definition for \( T_{pejus} \) is expected vary across species and even the life cycle, as the physiological requirements to thrive differ. We define \( T_{pejus} \) for coastal cutthroat trout as the temperature at which an individual’s FAS is equal to 3 (Eliason et al., 2022). We specifically chose a FAS threshold rather than an AAS threshold because FAS can indicate when a metabolic constraint is developing: the metabolic floor is a much greater concern than the metabolic ceiling in this system (Eliason et al., 2022). Cutthroat trout at this life stage are highly unlikely to routinely use MMR and require their full, maximal AAS. Thus, setting \( T_{pejus} \) as a high AAS threshold has little ecological relevance in this system (Farrell, 2016). In contrast, FAS can help us infer how much of the energetic capacity is being allocated to simple maintenance costs. As temperatures increase, SMR costs increase, and accordingly a greater proportion of the energy intake must be allocated simply to support maintenance metabolism. Any energy allocated to SMR is not being allocated to other critical activities such as growth or reproduction. Higher maintenance costs may require the fish to spend more time foraging and eating to support the increased metabolic demand and also have sufficient
energy to meet other activities (e.g. growth) requirements, which may confer lost opportunity costs (territory, mates) and increase susceptibility to predation. In addition, in order to thrive, cutthroat trout must have the energetic capacity to digest a meal. Rainbow trout double their metabolism during digestion of a moderate sized meal (2% of body mass, Eliason et al., 2008), thus an FAS of 2 is necessary for digestion (Farrell, 2016). However, to thrive, a fish must also be able to digest a meal and have sufficient scope remaining for other activities (e.g. swimming, defending territory, remaining vigilant, growth, reproduction; Jutfelt et al., 2021). Thus, we propose an FAS threshold of 3 for a subadult salmonid to be able to thrive (Eliason et al., 2022). For a different species and life stage, different criteria will be appropriate. For example, for migrating adult sockeye salmon populations maximally swimming hundreds of kilometres up the Fraser River, BC, Canada, to reach distant spawning grounds are expected to have a T_{pejus} threshold of 90% of AAS (Eliason et al., 2011).

All told, the WT measure can provide an approximation of the amount of environmental warming an organism can tolerate before performance declines. Small WT values indicate that the species is living close to their thermal edge and small amounts of warming will likely result in decreases in performance.

To calculate T_{pejus}, we developed linear models between FAS and average study temperatures for each watershed (Table S3). We then used the equation to predict the temperature at which FAS is estimated to be ~3. When calculating both TSM and WT, we used the maximum weekly maximum temperature (MWMT) estimates available from NorWeST (Isaak et al., 2017) to represent the maximum environmental temperature. Kruskal–Wallis tests were used to evaluate whether the distributions of both WT and TSM were statistically different among watersheds. Where differences were detected for either WT or TSM, the post hoc Dunn’s Multiple Comparison test was used to identify differences between watersheds.

**Results**

Across all locations and treatments mean individual body mass ranged from 17.5 to 29.8 g (22.8 ± 1.00 g; mean ± SEM), individual fork lengths ranged from 100 to 181 mm (130.2 ± 1.92 mm; mean ± SEM) and condition factor ranged from 0.93 to 1.13 (1.00 ± 0.02; mean ± SEM) (Fig. S4; Table S1). Body mass, length and condition factor did not differ across temperature treatments within a watershed.
Figure 4: A maximum likelihood mixed model was used to describe how RMR depends on temperature in coastal cutthroat trout. The fitted equation is $\ln(\text{RMR}) \sim 1.076(\text{Temperature}) + □(\text{unique to watershed}) + \text{error}$. Coefficients $(□)$ for each watershed were as follows: Alsea, $-0.5749$; McKenzie, $-0.4784$; N. Santiam, $-0.4042$; and Siletz, $-0.5926$. Treatment and individual fish unique to each watershed were non-independent factors. Treatment has positive effect on these slopes with Climate $>$ Max $>$ Ambient. See Table S2 for model output.

or across watersheds (ANOVA, $P > 0.05$; Fig. S4; Table S1). All coastal cutthroat trout in the Willamette (McKenzie, N. Santiam) are resident fish, but the coastal trout (Alsea, Siletz) could be a mix of sea-run and resident fish.

Critical thermal maxima

The Siletz watershed had the highest mean CT-max (30.375 $°C$ SE 0.059) followed by the Alsea (Table S2). The mean CT-max for the coastal watersheds were significantly higher (by over 2.5 $°C$) compared with the sample of fish from the Willamette basin watersheds (Table S2). Of note specifically in the McKenzie watershed, two fish lost equilibrium $\sim$10 min before the rest of the fish. The remaining fish lost equilibrium within 40 s of each other, which was similar to the results seen in the other watersheds, with a minimum of 1 s to maximum of 44 s.

SMR and MMR

SMRs increased with an increase in temperature for coastal cutthroat trout from all watersheds (Fig. 3; Table S5): Alsea ($\chi^2 = 14.591, P < 0.001$), Siletz ($\chi^2 = 14.04, P < 0.001$), McKenzie ($\chi^2 = 6.952, P = 0.030$) and N. Santiam ($\chi^2 = 17.615, P < 0.001$). In all watersheds the SMR in the Climate treatment was significantly higher than in the Ambient treatments (Dunn’ post hoc $P < 0.05$), and in all watersheds except the Alsea, the SMR during the Climate treatment was significantly higher than the Max treatment (Dunn’s post hoc $P < 0.05$; Fig. 3A; Table S5). The coastal cutthroat trout in the N. Santiam watershed had the lowest ambient SMR values, which were also associated with the lowest water temperatures observed across the four watersheds (Fig. 3D; Table S5).

MMRs increased with an increase in temperatures though the specific pattern varied across watersheds (Fig. 3). MMR values were statistically different across treatments in all watersheds: Alsea ($\chi^2 = 7.417, P = 0.024$), Siletz ($\chi^2 = 9.690, P = 0.007$), McKenzie ($\chi^2 = 8.757, P = 0.012$) and N. Santiam ($\chi^2 = 12.99, P = 0.001$). In the Alsea, there was a significant decrease in MMR during the Climate trial (Dunn’s post hoc $P < 0.05$; Fig. 3A; Table S5). Q10 values (comparing Ambient with Climate temperatures) for each location were 3.25, 2.02, 1.17 and 2.01 for Alsea, Siletz, McKenzie and N. Santiam, respectively, indicating that there was variable temperature sensitivity across watersheds.

At the shared 19°C temperature, the Alsea and the N. Santiam coastal cutthroat trout had higher SMR than the Siletz and the McKenzie ($\chi^2 = 20.643, P < 0.001$) (Fig. 7A; Table S7). The MMR values were significantly higher in the Alsea compared with the fish in the other watersheds ($\chi^2 = 13.636, P = 0.003$) (Fig. 7B; Table S7).

Aerobic scope

There were three distinct patterns for AAS with temperature: both the N. Santiam and McKenzie watersheds showed a significant increase in AAS with an increase in temperature (respectively, $\chi^2 = 9.642, P = 0.008, \chi^2 = 6.952, P = 0.031$) (Table S6; Fig. 5C and D); the Alsea showed a significant decrease in AAS at 22°C, the climate treatment ($\chi^2 = 8.834, P = 0.012$) (Table S6; Fig. 5A); and the Siletz displayed no change in AAS with temperature (Table S6; Fig. 5B).
FAS decreased with increasing water temperature in most of the watersheds: Alsea ($\chi^2 = 11.209$, $P = 0.003$), Siletz ($\chi^2 = 9.966$, $P = 0.006$) and N. Santiam ($\chi^2 = 10.052$, $P = 0.006$). In contrast, FAS was unaffected by temperature in the McKenzie watershed (Fig. 6; Table S6). The N. Santiam watershed had the most precipitous decline (48%) in FAS across temperature (Mean ± SEM): Ambient = 12.025 ± 2.250, Max = 9.462 ± 0.841, Climate = 6.224 ± 0.274), (Fig. 6D). When comparing at 19°C, AAS and FAS differed across watersheds (Fig. 7C and D, Table S6). Specifically, AAS was significantly higher in the Alsea compared with the Siletz ($\chi^2 = 10.432$, $P = 0.015$; Table S7; Fig. 7C), while FAS differed between the Siletz and N. Santiam ($\chi^2 = 16.102$, $P = 0.001$; Table S7; Fig. 7D).

**Recovery**

Overall, the fish recovered rapidly from the exhaustive chase protocol; mean Time [MMR50] ranged from 10 to 24 min (Fig. 8; Table S8). Recovery time increased significantly with warming for fish in two of the watersheds (Alsea and N. Santiam; Fig. 8A and D; Table S8). In contrast, the McKenzie and Siletz fish maintained a stable recovery duration across all temperatures (Fig. 8B and C; Table S8). When comparing across watersheds at the shared 19°C temperature, there were no differences in Time[MMR50] (Table S8).

**Routine metabolic rates**

RMRs for coastal cutthroat showed similar patterns across all watersheds, increasing exponentially with an increase in water temperature (Fig. 4, Table S4). Q10 values (comparing lowest Ambient and Climate temperature treatments) for each watershed were 3.20, 1.93, 1.48 and 1.48 for Alsea (17–22°C), Siletz (18–22°C), McKenzie (10–19°C) and N. Santiam watersheds (6–19°C), respectively.

**Climate implications: Functional WT and TSM**

WT values were similar across the watersheds. The N. Santiam and Alsea had similar WT values though the underlying Tpejus and baseline MWMT varied (Fig. 9; Table S2). We were unable to calculate WT for the fish in the McKenzie watershed given the stability of FAS across temperatures and the inability to define Tpejus. Fish from the Alsea had the highest mean TSM (12.805 ± 0.0115°C) followed by the McKenzie (11.652 ± 0.938°C) where there was the greatest variability.
between individuals, which makes sense given the variation in CT-max (Mckenzie CT-max range, 22.6–29.4) (Table S2). The N. Santiam and Siletz had the same mean TSM (11.5°C) though the fundamental values differed; the Siletz had the highest CT-max and a baseline MWMT that was nearly 3 degrees higher than the N. Santiam. The N. Santiam had the lowest CT-max and one of the lowest baseline MWMT (Fig. 9; Table 1). Across all watersheds, future 2080 projections of maximum weekly maximum temperatures resulted in lower WT and TSM values (Fig. 9; Table S2).

**Discussion**

In this study, we discovered considerable intraspecific variation in physiological performance and thermal tolerance across coastal cutthroat trout from four distinct watersheds in Oregon. While the diurnal variability was similar across the watersheds (range, 2.4°C in Siletz to 3.9°C in the N. Santiam), the extent of warming (e.g., min, max) did differ. Fish from the cooler, more stable, spring-fed system (McKenzie) had the most variable CT-max values while also having the most stable FAS values across all temperature treatments. Fish from the warmest thermal regimes (e.g., Siletz) had higher CT-max values and had less variability in AAS. Conversely, fish from cooler thermal regimes exposed to novel warm conditions (e.g. Alsea and N. Santiam) had the only decrease in MMR during the Climate trial (Alsea) and the most precipitous decline in FAS (N. Santiam). Thermal history appeared to be more descriptive of the thermal metabolic response than watershed. However, fish from all watersheds appear to be at low risk of acute thermal stress because they all maintained high aerobic capacity and recovered rapidly from exhaustive exercise, even when tested at 3°C above current maximum temperatures. Below, we discuss the management and conservation implications of these results.

**Coastal cutthroat trout in Oregon differ in thermal tolerance**

We found compelling evidence that fish from different watersheds (McKenzie, N. Santiam, Alsea, Siletz) differed in physiological performance and thermal tolerance. The fish from the coastal watersheds had higher thermal tolerance (2–3°C higher CT-max) compared with the two watersheds in the Willamette River basin, but fish clearly differed in physio-

---

**Figure 6:** Boxplots displaying factorial aerobic scope (FAS = MMR/SMR) for coastal cutthroat in the Alsea (A), Siletz (B), McKenzie (C) and N. Santiam (D) watersheds. The lower and upper boundaries of the boxplot represent the interquartile range (25th and 75th percentiles), the darker centre line delineates the median and the whiskers indicate the minimum and maximum. The dashed horizontal line indicates the estimated FAS needed to thrive for this life stage (i.e. FAS = 3). Significant pairwise differences (Dunn’s post hoc test; \(P < 0.05\)) in means within a location are noted by lower-case grouping letters. Different letters indicate significant pairwise differences.
Figure 7: Boxplots displaying SMR (A), MMR (B), AAS (C) and FAS (D) for coastal cutthroat trout at the shared temperature of 19°C. The lower and upper boundaries of the boxplot represent the interquartile range (25th and 75th percentiles), the darker centre line delineates the median and the whiskers indicate the minimum and maximum. The dashed horizontal line indicates the estimated FAS needed to thrive for this life stage (i.e. FAS = 3). Significant pairwise differences (Dunn’s post hoc test; P < 0.05) in means within a location are noted by lower-case grouping letters. Different letters indicate significant pairwise differences

Strong intraspecific variability is beneficial for species resilience to climate change and provides important contributions for humans as well (Des Roches et al., 2021). Similar to the current study, intraspecific variability in physiological performance and thermal tolerance has been reported in numerous salmonid species including sockeye salmon (Lee et al., 2003; Eliason et al., 2011; Chen et al., 2013; Whitney et al., 2016; Anttila et al., 2019), chum salmon (Abe et al., 2019), Chinook salmon (Zillig et al., 2021) and brook trout (Stitt et al., 2014). Our study presents a unique approach by contrasting fish from different hydrologic regimes and thermal histories. Further, understanding this variability for a species with such varied life history, diverse habitat use and broad spatial extent is novel and helps us understand the persistence of phenotypic variation that contributes to the adaptive potential of a population; high variation may enable populations to adapt and persist under novel, new conditions (Hoffmann et al., 2017). Further, phenotypic diversity enables the portfolio effect, where detrimental changes in environmental conditions are buffered by phenotypic variation across populations such that the aggregated overall population remains stable (Brennan et al., 2019; Schindler et al., 2010). Thus, across systems, intraspecific variation should

Logical performance on a finer scale, even between coastal watersheds. Fish from the Alsea watershed clearly demonstrated a decrease in performance with warming: MMR, AAS, FAS and recovery time (Time[MMR50]) all decreased to 60–80% of maximum performance in the climate change scenario compared with current ambient temperature conditions. In contrast, fish in the other coastal watershed, Siletz, displayed an increase in performance with warming: AAS and MMR were maximal at the highest test temperature (i.e. Climate treatment). Notably, Alsea had a 23% higher AAS then Siletz but a 1.5°C lower Tpejus, which suggests there may have been a trade-off between aerobic scope and thermal tolerance. It is possible that fish in the Alsea require a greater aerobic scope and the cost could be reduced thermal breadth for performance. For the Willamette River basin watersheds, the McKenzie fish performed exceptionally well at the climate change temperature, displaying maximal performance for all traits (MMR, AAS, FAS, recovery time). In contrast, the N. Santiam fish did have high AAS and MMR at the warmest test temperature, but FAS and recovery duration were clearly impaired. These results suggest that there are different metabolic responses to acute thermal challenges for fish across these watersheds.
be evaluated, maintained and restored to support healthy and resilient populations.

Coastal cutthroat trout in Oregon are at low risk of thermal stress

We found no evidence that coastal cutthroat trout from any of the watersheds suffered from a substantial reduction in performance across the temperature range tested here. The temperatures tested were meant to reflect currently observed diurnal variability, maximum temperature and climate warming. In this way we attempted to summarize and describe not only the physiological demands associated with daily thermal cycling but also how phenotypic traits that characterize metabolism operate under thermal stress (Halsey et al., 2018). However, we found no evidence of a major metabolic constraint as temperatures warm. AAS did not decrease below 70% of maximum levels at any of the test temperatures for any of the watersheds (Fig. S3). While FAS did decrease in the Climate treatment for most of the watersheds, a high FAS (i.e. >3) was still maintained across temperatures, which suggests fish would have had ample energy available to be able to digest a meal, swim, escape predators, etc. (Eliason et al., 2008; Eliason et al., 2022; Farrell, 2016). This finding is supported by the recovery performance. Across all temperatures, fish rapidly recovered from the chase treatment and were back to 50% of their MMR level within 10–25 min. Although recovery duration did significantly increase with the Climate treatment, functionally, this only amounted to an additional ∼5 min of recovery time. This suggests the fish would be able to resume normal activities within 10–15 min of an exhaustive exercise event, across any of the temperatures tested here. Similarly, maintenance metabolism did not become a loading factor on aerobic scope as temperature warmed. Specifically, while RMR did increase as temperatures warmed, it did so at a modest rate (i.e. Q10 values were below 3.5). Other studies have similarly found that some salmonids can maintain high physiological performance across the range of encountered temperatures, including Chinook salmon (Poletto et al., 2017) and rainbow trout Oncorhynchus mykiss (Verhille et al., 2016).

TSMs and functional WT

One actionable approach to understanding species thermal needs and risk is to evaluate thermal tolerance in relation to...
Figure 9: Displayed for each watershed, the mean critical thermal maximum (purple), the temperature at which \( T_{\text{pejus}} \) occurs as modelled (blue; see Table S3), baseline modelled maximum weekly maximum temperatures (NorWeST) from the streams where fish were collected (orange; Isaak et al., 2017), future (2080) projected maximum weekly maximum temperatures (NorWeST) from the streams where fish were collected (grey; Isaak et al., 2017) and WT values (red; see Table S2).

Environmental temperatures. We calculated TSM using CT-max values together with stream temperature data to get a depiction of relative tolerances for fish across these four watersheds. TSM values were highest for the Alsea fish when compared with fish in the other watersheds. The fish in the McKenzie watershed, which reside in streams that currently have cool, stable thermal regimes, had the second highest and most variable TSM values, which contrast with their overall metabolic performance. Research by Sandblom et al. (2016) may explain this discrepancy. They found that while basal energy requirements and resting cardiorespiratory functions are thermally plastic, maximum capacities and upper critical heat tolerances are much less flexible and will limit the adaptive capacity of fish as the climate warms. Using 2080 projections of stream temperature, all TSMs decreased as predicted, but most still showed a strong buffer before lethality (3–5°C).

Evaluating TSM values can provide an approach for managers to identify and broadly categorize populations that may be at risk (Walters et al., 2018). However, individuals will experience significant thermal stress at temperatures below their thermal maximum, which will inhibit performance and productivity (Blasco et al., 2020). WT values, the difference between \( T_{\text{pejus}} \) and environmental temperatures, can provide a more actionable metric for managers as it is more ecologically relevant. Here, we propose a new threshold for \( T_{\text{pejus}} \) for subadult salmonids based on a threshold of FAS (FAS = 3). FAS can indicate when a metabolic constraint is developing for fish, specifically when baseline metabolism costs become prohibitively high (see Material and Methods for thorough description). The Siletz fish had the highest \( T_{\text{pejus}} \) reflecting the inherently warm conditions they experienced. The Alsea and N. Santiam fish had similar WT (≈ 7.5°C) despite different \( T_{\text{pejus}} \) and thermal histories. As such, these fish in these watersheds do not appear to be at immediate threat from thermal stress. Additionally, these species appear well adapted to their local conditions while also have similar ‘buffers’ to climate change warming. We sampled low elevation, coastal and moderate to higher elevation headwater streams to display the breadth of thermal variability coastal cutthroat trout experience across their range in Oregon. Whether we could apply similar WT values across their full geographical range (i.e. Alaska to Northern California) based on their thermal history is still an open question. Exploring \( T_{\text{pejus}} \) variability given potentially similar underlying environmental conditions influencing thermal regimes and contrasting \( T_{\text{pejus}} \) for species across distributions could elucidate how species are able to tolerate and buffer the short-term consequences of temperature increases.

Data needed to move the field forward and assist managers

The present study found compelling evidence that acute thermal sensitivity varies across watersheds and matches thermal history; however, we cannot wholly conclude that these differences are due to local adaptation. While we took tissue samples from the fish for potential future genetic analysis, the genetic variation within or between watersheds is currently unknown. Furthermore, a common garden approach is necessary to examine adaptive variation, which was not used here. It is possible that the results obtained here could be primarily due to phenotypic plasticity (i.e. field acclimatization processes experienced by the fish in their local environments). However, given the strong evidence of local adaptation among populations of salmonids (Adkison, 1995; Fraser et al., 2011), including cutthroat trout (Drinan et al., 2012), it is also possible that a genetic x environment interaction is an important mechanism underlying our trends. Future interdisciplinary work examining the adaptive capacity of cutthroat trout to climate change is warranted.

More broadly, improvements in physiological thermal sensitivity data across life stages and range of freshwater fish species will provide a strong understanding of their ability to tolerate climatic variation and provide managers clues on how to mitigate climate thermal stress. Many studies have shown that knowledge of life stage and life history traits (e.g. reproductive rates, dispersal abilities, physiological tolerances, etc.) that inform sensitivity can be as useful for understanding of taxonomy and distribution (Dahlke et al., 2020; Pacifici et al., 2015; Willis et al., 2015).

Finally, understanding current thermal exposure for stream-dwelling species is challenging given the lack of continuous stream temperature data that captures and characterized stream habitats at spatial (river basins, streams, coastal to inland, etc.) and temporal scales (daily, seasonal, annual) (Fitz Gerald et al., 2021). Improvements in the consistency, spatial and temporal interval of sampling and access to stream temperature data will improve the accuracy of WTs.
Advantages and limitations of the approach

Stream-side respirometry provides a direct link between phenotypic trait expression and environmental temperatures. Stressors are reduced (fish handling and transportation are minimized, natal water source is utilized) and fish are returned to the same location in the stream where they were captured. We are able to evaluate metabolic trait expression under naturally fluctuating conditions throughout a diel cycle, which is not typically possible in a laboratory setting. There are, however, trade-offs to field-versus laboratory-based respirometry and some issues to consider when evaluating field-based results. One logistical constraint with the field respirometry method can be reliable and consistent daily access to fish. In general, our fish were similarly sized and most (>80%) were less than 152 mm though, it is possible that some individuals were at different stages of sexual reproduction, which may have contributed to some of the variability in our data. However, we found no differences in any of the metabolic traits evaluated as a result of length or mass. Further, it is also conceivable that some of the fish from the coastal watersheds were migrants (versus residents). This may mean that metabolic costs were partitioned differently and may have contributed to increased variation in MO2 in those watersheds.

A second constraint is related to fish capture in a field setting. In our study, we used electrofishing to capture fish. Unfortunately, there is not a huge body of literature on recovery dynamics from electrofishing. We certainly would anticipate that electrofishing would have behavioural and physiological impacts on the fish immediately after capture. For example, Schreer et al. (2004) demonstrated that rainbow trout were behavioural impaired for up to 1 h after electroshock and cardiac function took 2–3 h to return to resting levels. A study with cutthroat trout found that plasma lactate and cortisol increased in response to electroshocking but returned to baseline levels by 6 h (Mesa and Schreck, 1989). Given these two studies, we expect the fish were likely recovered by the time the experiments began, but we cannot be certain.

A third consideration in this study is that fish were only exposed to ecologically relevant, brief overnight thermal acclimation periods, so full thermal acclimation processes were likely incomplete; however, fish were already acclimatized to summer temperatures (experiments were conducted in August). Thus, caution must be used when comparing these results with other laboratory-based studies where fish were laboratory acclimated for many weeks to a thermal regime. Fish may not have been entirely postprandial when the experiment began (18 h after capture) if they had consumed a meal immediately before capture and this could have influenced the results with slightly higher metabolic costs for fish still digesting.

Despite the constraints highlighted above, these data have wide applicability from helping to define parameters in bioenergetics models (e.g. optimal temperatures at which aerobic scope is maximized), to providing an understanding of movement and survival strategies among individuals from fragmented habitats (Armstrong et al., 2021; Hahlbeck et al., 2021). Utilizing field data describing both stream temperatures and species physiological tolerances improves the accessibility of these data for managers who want to make use of the best available science but need translatable measures on which to base decisions and actions.

Summary and conclusions

This study provides a promising and hopeful outlook for Coastal cutthroat trout in terms of their vulnerability to climate changes that will likely alter thermal regimes. Coastal cutthroat trout displayed considerable intraspecific variability in physiological performance and thermal tolerance across the four watersheds we examined. Thermal tolerance matched the historical experience: the coastal watersheds experiencing warmer ambient temperatures had higher critical thermal tolerance compared with the interior, cooler Willamette watersheds. Physiological performance varied across all four watersheds. There was evidence of a trade-off between high aerobic performance and broad thermal tolerance. The fish with the highest aerobic scope (Alsea) displayed a decline in aerobic scope with warming and lower Tpjus, while fish from a neighbouring watershed (Siletz) maintained a consistent, albeit lower aerobic scope across the full range of test temperatures and higher Tpjus. This high intraspecific variability is anticipated to confer strong adaptive capacity for the species. Notably, all these Coastal cutthroat trout populations appear to be at low risk of thermal stress. Ambient water temperature could warm a further 5–7°C before functional performance is expected to become impaired. Finally, this study provides a practical framework for future studies on intraspecific variability in freshwater fishes using a novel stream-side respirometry system.

Data Availability Statement

Data are not yet provided but will be available upon acceptance of this manuscript. R code to analyse metabolic rate performance metrics will be open source on Github (all links to source code and complementary documentation will be provided after revisions). No other novel code was used. Data will be permanently archived here: https://github.com/kjadunn/Coastal-Cutthroat-Trout-Resp/.

Acknowledgments

Several ODFW staff helped with the data collection, including Staci Stein, Treyan Cornwell, Emily Loose, Dan Coffman and Courtney Zambory. Finally, we are very appreciative of the external and anonymous reviews that significantly improved this manuscript.
Funding

This work was funded by the Oregon Department of Fish and Wildlife (ODFW). We also recognize NSF Santa Barbara Coastal LTER for providing support to K.K. and the Hellman Family Fellows Fund and University of California, Santa Barbara for providing support to E.J.E.

Supplementary material

Supplementary material is available at Conservation Physiology online.

References

Abe TK, Kitagawa T, Makiguchi Y, Sato K (2019) Chum salmon migrating upriver adjust to environmental temperatures through metabolic compensation. J Exp Biol 222: 1–10.

Adkison MD (1995) Population differentiation in Pacific salmons: local adaptation genetic drift, or the environment? Can J Fish Aquat Sci 52: 2762–2777.

Anttila K, Farrell AP, Patterson DA, Hinch SG, Eliason EJ (2019) Cardiac SERCA activity in sockeye salmon populations: an adaptive response to migration conditions. Can J Fish Aquat Sci 76: 1–5.

Arismendi I, Johnson SL, Dunham JB, Haggerty ROY (2013) Descriptors of natural thermal regimes in streams and their responsiveness to change in the Pacific northwest of North America. Freshw Biol 58: 880–894.

Armstrong JB, Fullerton AH, Jordan CE, Ebersole JL, Bellmore JR, Arismendi I, Penaluna BE, Reeves GH (2021) The importance of warm habitat to the growth regime of cold-water fishes. Nat Clim Change 11: 354–361.

Barionuevo WR, Fernandes MN (1998) Time-course of respiratory metabolic adjustments of a south American fish, Prochilodus scrofa, exposed to low and high temperatures. J Appl Ichthyol 14: 37–41.

Barros DJ, Dokken KJ, Mach MD, Mastrandrea TE, Bilir M, Chatterjee KL, Ebi YO, Estrada RC, Genova B, Girma ES et al. Mastrandrea LN, White, ed. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 229–269.

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67: 1–48. https://10.18637/jss.v067.i01.

Beitinger TL, Bennett WA (2000) Quantification of the role of acclimation temperature in temperature tolerance of fishes. Environ Biol Fishes 58: 237–275.

Bernhardt JR, O’Connor MI, Sunday JM, Gonzalez A (2020) Life in fluctuating environments. Philos Trans R Soc B 375: 20190454.

Blasco FR, Taylor EW, Leite CA, Monteiro DA, Rantin FT, McKenzie DJ (2020) Tolerance of an acute warming challenge declines with body mass in Nile tilapia: evidence of a link to capacity for oxygen uptake. BioRxiv.

Brennan SR, Schindler DE, Cline TJ, Walsworth TE, Buck G, Fernandez DP (2019) Shifting habitat mosaics and fish production across river basins. Science 364: 783–786.

Chabot DJ, McKenzie FC (2016) Metabolic rate in fishes: definitions, methods and significance for conservation physiology. J Fish Biol 88: 1–9.

Chen Z, Anttila K, Wu J, Whitney CK, Hinch SG, AP (2013) Optimum and maximum temperatures of sockeye salmon (Oncorhynchus nerka) populations hatched at different temperatures. Can J Zool 91: 265–274.

Clark TD, Sandblom E, Jutfelt F (2013) Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. J Exp Biol 216: 2771–2782.

Comte L, Olden JD (2017) Evolutionary and environmental determinants of freshwater fish thermal tolerance and plasticity. Glob Chang Biol 23: 728–736.

Dahlke FT, Wohlrab S, Butzin M, Pörtner HO (2020) Thermal bottlenecks in the life cycle define climate vulnerability of fish. Science 369: 65–70.

Des Roches S, Pendleton LH, Shapiro B, Palkovacs EP (2021) Conserving intraspecific variation for nature’s contributions to people. Nat Ecol Evol 5: 574–582.

Drinan DP, Zale AV, Webb MAH, Taper ML, Shepard BB, Kalinowski ST (2012) Evidence of local adaptation in Westslope cutthroat trout. Trans Am Fish Soc 141: 872–880.

Durack T (2020) Comparing metabolic rate estimates of two similar salmonids: Salvelinus confluentus and Salvelinus fontinalis [unpublished dissertation]. University of Manitoba.

Eliason EJ, Clark TD, Hague MJ, Hanson LM, Gallagher ZS, Jeffries KM, Gale MK, Patterson DA, Hinch SG, Farrell AP (2011) Differences in thermal tolerance among sockeye salmon populations. Science 332: 109–112.

Eliason EJ, Clark TD, Hinch SG, Farrell AP (2013) Cardiorespiratory collapse at high temperature in swimming adult sockeye salmon. Conserv Physiol 1.

Eliason EJ, Higgs DA, Farrell AP (2008) Postprandial gastrointestinal blood flow, oxygen consumption and heart rate in rainbow trout (Oncorhynchus mykiss). Comp Biochem Physiol A Mol Integr Physiol 149: 380–388.

Eliason EJ, Van Wert, Schwieterman GD (2022) Chapter 4: Applied Aspects of the Cardiorespiratory System. In SJ Cooke, NA Fangue, EJ Eliason, eds, Fish Physiology Conservation Physiology for the Anthroponcne: A Systems Approach Part A. Academic Press, Cambridge, MA, ISBN: 9780128242667.

Fangue NA, Hofmeister M, Schultz PM (2006) Intraspecific variation in thermal tolerance and heat shock protein gene expression in common killifish, Fundulus heteroclitus. J Exp Biol 209: 2859–2872.
Farrell AP (2016) Pragmatic perspective on aerobic scope: peaking, plummeting, pejus and apportioning. *J Fish Biol* 88: 322–343.

Farrell AP, Eliason EJ, Sandblom E, Clark TD (2009) Fish cardiorespiratory physiology in an era of climate change. *Can J Zool* 87: 835–851.

Farrell AP, Gamperl AK, Birtwell IK (1998) Prolonged swimming, recovery and repeat swimming performance of mature sockeye salmon *Oncorhynchus nerka* exposed to moderate hypoxia and pentachlorophenol. *J Exp Biol* 201: 2183–2193.

Farrell AP, Lee CG, Tierney K, Hodaly A, Clutterham S, Healey M, Hinch S, Lotto A (2003) Field-based measurements of oxygen uptake and swimming performance with adult Pacific salmon using a mobile respirometer swim tunnel. *J Fish Biol* 62: 64–84.

FitzGerald AM, John SN, Apgar TM, Mantua NJ, Martin BT (2021) Quantifying thermal exposure for migratory riverine species: phenomenology of Chinook salmon populations predicts thermal stress. *Glob Chang Biol* 27: 536–549.

Fry FEJ (1947) Effects of the environment on animal activity Publications of the Ontario Fisheries Research Laboratory. 68: 1–62.

Gilbert MJH, Harris LN, Malley BK, Schimnowski A, Moore JS, Farrell AP (2020) The thermal limits of cardiorespiratory performance in anadromous Arctic char (*Salvelinus alpinus*): a field-based investigation using a remote mobile laboratory. *Conserv Phys Ther* 8.

Hahbeck N, Tinniswood WR, Sloat MR, Ortega JD, Wyatt MA, Hereford M, Ramirez BS, Crook DA, Anlauf-Dunn KJ, Armstrong JB (2021) Contribution of warm habitat to cold-water fisheries. *Conserv Biol*. https://doi.org/10.1111/cobi.13857.

Halsey LG, Killen SS, Clark TD, Norin T (2018) Exploring key issues of aerobic scope interpretation in ectotherms: absolute versus factorial. *Rev Fish Biol Fish* 28: 405–415.

Hanson RC, Stanley JG (1970) The effects of hypophysectomy and temperature acclimation upon the metabolism of the central mudminnow, *Umbrá limí* (Kirtland). *Comp Biochem Physiol* 33: 871–879.

Harada AE, Healy TM, Burton RS (2019) Variation in thermal tolerance and its relationship to mitochondrial function across populations of *Tigriopus californicus*. *Front Physiol* 10: 213.

Hoffmann AA, Sgró CM, Kristensen TN (2017) Revisiting adaptive potential, population size, and conservation. *Trend Ecol Evol* 32: 506–517.

Isaak D, Wenger S, Peterson E, Ver Hoef, Nagel D, Luce C, Hostetler S, Dunham J, Roper B, Wollrab S et al. (2017) The NorWest summer stream temperature model and scenarios for the western U.S.: a crowd-sourced database and new geospatial tools foster a user community and predict broad climate warming of rivers and streams. *Water Resour Res* 53: 9181–9205.

Jerde CL, Kraskura K, Eliason EJ, Csik SR, Stier AC, Taper ML (2019) Strong evidence for an intraspecific metabolic scaling coefficient near 0.89 in fish. *Front Physiol* 10: 1166.

Jiménez Cisneros BE, Oki T, Arnell NW, Benito G, Cogley JG, Döll P, Jiang T, Mwakalila SS (2014) Freshwater resources. In *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects*. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change Field, C.B., V.R.

Jutfelt F, Norin T, Åsheim ER, Rowsey LE, Andreassen AH, Morgan R, Clarkand TD, Speers-Roesch B (2021) ‘Aerobic scope protection’ reduces ectotherm growth under warming. *Funct Ecol* 35: 1397–1407.

Jutfelt F, Norin T, Ern R, Overgaard J, Wang T, McKenzie DJ, Lefevre S, Nilsson GE, Metcalfe NB, Hickey AJ et al. (2018) Oxygen- and capacity-limited thermal tolerance: blurring ecology and physiology. *J Exp Biol* 221: 1–2.

Kellermann V, Overgaard J, Hoffmann AA, Flejaigard C, Svenning JC, Loeschcke V (2012) Upper thermal limits of drosophila are linked to species distributions and strongly constrained phylogenetically. *Proc Natl Acad Sci* 109: 16228–16233.

Klicka J (1965) Temperature acclimation in goldfish: lack of evidence for hormonal involvement. *Physiol Zool* 38: 177–199.

Kraskura K, Jerde CL, Eliason EJ (2021) Active and resting metabolic rate scaling relationships in fishes across ecologies, salinity, and body shapes. *Integr Comp Biol* 61: E482–E483.

Lee CG, Farrell AP, Lotto A, Hinch AG, Healey MC (2003) Excess post-exercise oxygen consumption in adult sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon following critical speed swimming. *J Exp Biol* 206: 3253–3260.

Little AG, Dressler T, Kraskura K, Hardison E, Hendriks B, Prystay T, Farrell AP, Cooke SJ, Patterson DA, Hinch SG et al. (2020) Maxed out: optimizing accuracy, precision, and power for field measures of maximum metabolic rate in fishes. *Physiol Biochem Zool* 93: 243–254.

Macnaughton CJ, Durhac TK, Mochnacz NJ, Enders EC (2021) Metabolic performance and thermal preference of westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and non-native trout across an ecologically relevant range of temperatures. *Can J Fish Aquat Sci* 78: 1247–1256.

Marras S, Cucco A, Antognarelli F, Azzurro E, Milazzo M, Bariche M, Butterón M, Kay S, Di Bitetto M, Quattruccio G et al. (2015) Predicting future thermal habitat suitability of competing native and invasive fish species: from metabolic scope to oceanographic modelling. *Conserv Physiol* 3.

McKenzie DJ, Zhang Y, Eliason EJ, Schulte PM, Claireaux G, Blasco FR, Nati JJ, Farrell AP (2021) Intraspecific variation in tolerance of warming in fishes. *J Fish Biol* 98: 1536–1555.

Mesa MG, Schreck CB (1989) Electrofishing mark–recapture and depletion methodologies evoke behavioral and physiological changes in cutthroat trout. *Trans Am Fish Soc* 118: 644–658.

Nati JJH, Svendsen MBS, Marras S, Killen SS, Steffensen JF, McKenzie DJ, Domenici P (2020) Intraspecific variation in thermal tolerance differs between tropical and temperate fishes. *bioRxiv* .
Norin T, Clark TD (2016) Measurement and relevance of maximum metabolic rate in fishes. *J Fish Biol* 88: 122–151.

Olsen T, Shelton JM, Dallas HF (2021) Does thermal history influence thermal tolerance of the freshwater fish *Galaxias zebratus* in a global biodiversity hotspot? *J Therm Biol* 97: 1–10.

Pacifici M, Foden WB, Visconti P, Watson JE, Butchart SH, Kovacs KM, Scheffers BR, Hole DG, Martin TG, Akçakaya HR et al. (2015) Assessing species vulnerability to climate change. *Nat Clim Change* 5: 215–224.

Pinsky ML, Eikeset AM, McCauley DJ, Payne JL, Sunday JM (2019) Greater vulnerability to warming of marine versus terrestrial ectotherms. *Nature* 569: 108–111.

Poff NL, Ward JV (1990) Physical habitat template of lotic systems: recovery in the context of historical pattern of spatiotemporal heterogeneity. *Environ Manag* 14: 629–645.

Poletto JB, Cocherell DE, Baird SE, Nguyen TX, Cabrera-Stango V, Farrell AP, Fangue NA (2017) Unusual aerobic performance at high temperatures in juvenile Chinook salmon, Oncorhynchus tshawytscha. *Conserv Physiol* 5.

Pörtner HO, Farrell AP (2008) Physiology and climate change. *Science* 322: 690–692.

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

Rosewarne PJ, Wilson JM, Svendsen JC (2016) Measuring maximum and standard metabolic rates using intermittent-flow respirometry: a student laboratory investigation of aerobic metabolic scope and environmental hypoxia in aquatic breathers. *J Fish Biol* 88: 265–283.

Sandblom E, Clark TD, Gräns A, Ekström A, Brijs J, Sundström LF, Odelström A, Adill A, Aho T, Jutfelt F (2016) Physiological constraints to climate warming in fish follow principles of plastic floors and concrete ceilings. *Nat Commun* 7: 1–8.

Schindler DE, Hilborn R, Chasco B, Boatright CP, Quinn TP, Boatright CP, Quinn TP, Rogers LA, Webster MS (2010) Population diversity and the portfolio effect in an exploited species. *Nature* 465: 609–612.

Schreer JF, Cooke SJ, Connors KB (2004) Electrofishing-induced cardiac disturbance and injury in rainbow trout. *J Fish Biol* 64: 996–1014.

Schulte PM, Healy TM, Fangue NA (2011) Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. *Integr Comp Biol* 51: 691–702.

Schulte PM, Podrabsky JE, Stillman JH, Tomanek L (2015) The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. *J Exp Biol* 218: 1856–1866.

Seebacher F, Ducret V, Little AG, Adriaenssens B (2015a) Generalist-specialist trade-off during thermal acclimation. *R Soc Open Sci* 2: 140251–140251.

Seebacher F, White CR, Franklin CE (2015b) Physiological plasticity increases resilience of ectotherm animals to climate change. *Nat Clim Change* 5: 61–66.

Sommer RJ (2020) Phenotypic plasticity: from theory and genetics to current and future challenges. *Genetics* 215: 1–13.

Sparks MM, Westley PA, Falke JA, J.A. and T.P. Quinn. (2017) Thermal adaptation and phenotypic plasticity in a warming world: insights from common garden experiments on Alaskan sockeye salmon. *Glob Chang Biol* 23: 5203–5217.

Stitt BC, Burness G, Burgomaster KA, Currie S, McDermid JL, Wilson CC (2014) Intraspecific variation in thermal tolerance and acclimation capacity in brook trout (*Salvelinus fontinalis*): physiological implications for climate change. *Physiol Biochem Zool* 87: 15–29.

Sunday JM, Bates AE, Dulvy NK (2012) Thermal tolerance and the global redistribution of animals. *Nat Clim Change* 2: 686–690.

Sunday JM, Bates AE, Kearney MR, Colwell RK, Dulvy NK, Longino JT, Huey RB (2014) Thermal-safety margins and the necessity of thermoregulatory axes across latitude and elevation. *Proc Natl Acad Sci* 111: 5610–5615.

Synder KJ, Sullivan TJ, Raymond RB, Moore D, Gilbert E (2002) North Santiam River watershed assessment. E&S Environmental Chemistry, Inc. http://northsantiam.org/wp-content/uploads/assessment-reports/2002-06-NORTH-SANTIAM-RIVER-WATERSHED-ASSESSMENT.pdf.

Trotter PC (1989) Coastal cutthroat trout: a life history compendium. *Trans Am Fish Soc* 118: 463–473.

Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE (1980) The river continuum concept. *Can J Fish Aquat Sci* 37: 130–137.

Verhille CE, English KK, Cocherell DE, Farrell AP, Fangue NA (2016) High thermal tolerance of a rainbow trout population near its southern range limit suggests local thermal adjustment. *Conserv Physiol* 4.

Vinagre C, Dias M, Cereja R, Abreu-Afonso F, Flores AA, Mendonça V (2019) Upper thermal limits and warming safety margins of coastal marine species—indicator baseline for future reference. *Ecol Indic* 102: 644–649.

Walters AW, Mandeville CP, Rahel FJ (2018) The exposure of warming and warming tolerance determines fish species vulnerability to warming stream temperatures. *Biol Lett* 14: 1–5.

Whitney JE, Al-Chokhachy R, Bunnell DB, Caldwell CA, Cooke SJ, Eliason EJ, Rogers M, Lynch AJ, Paukert CP (2016) Physiological basis of climate change impacts on North American inland fishes. *Fisheries* 41: 332–345.

Willis SG, Foden W, Baker DJ, Belle E, Burgess ND, Carr JA, Doswald N, Garcia RA, Hartley A, Hof C et al. (2015) Integrating climate change vulnerability assessments from species distribution models and trait-based approaches. *Biol Conserv* 190: 167–178.

Zillig KW, Lusardi RA, Moyle PB, Fangue NA (2021) One size does not fit all: variation in thermal eco-physiology among Pacific salmonids. *Rev Fish Biol Fish* 31: 95–114.