UNIVERSITY OF CALIFORNIA

Santa Barbara

Distribution, thermal tolerance, and vulnerability of stream fishes in a warming world

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in Ecology, Evolution, and Marine Biology

by

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ABSTRACT

Distribution, thermal tolerance, and vulnerability of stream fishes in a warming world

by

Terra Lee Dressler

Freshwater fishes face escalating pressures as a result of habitat alteration from global climate change paired with intensified land use by humans. Habitat fragmentation, disturbances like drought and wildfire, and water pollution all contribute to reductions in the quality and quantity of available freshwater fish habitat. When a fish species inhabits a broad geographic range, however, genetically distinct populations that experience vastly different conditions can exhibit variability in tolerance to stressors such as elevated temperature. Elucidating the distributions of such fishes and the extent of the ability of individual populations to tolerate or adapt to environmental stressors is essential for understanding their vulnerability to extirpation and/or extinction from climate change.

My PhD research makes use of environmental DNA (eDNA) and streamside physiology to assess the distribution and thermal tolerance, respectively, of fishes inhabiting streams in California and Oregon. Using eDNA analysis, observed differences in biodiversity between Southern California river basins were primarily attributed to the presence of exotic species. Using streamside physiology, thermal tolerance and vulnerability to warming differed

between populations of an endangered salmonid, steelhead trout (*Oncorhynchus mykiss).* Populations with historically warmer environmental temperatures had higher thermal tolerance but were often living in temperatures closer to their upper thermal limits compared to those from cooler locations. This information allows managers to focus conservation efforts on areas that support native fish biodiversity and on specific populations of species of concern that are most vulnerable to extirpation.

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Chapter 1: Environmental DNA metabarcoding elucidates pairwise beta diversity of freshwater fish communities in Southern California

1.1 Abstract

Environmental DNA (eDNA) metabarcoding, the process of deciphering species of origin from DNA sequences shed into the environment, is a burgeoning technology that is rapidly improving the ability to assess community composition in aquatic ecosystems. This is highly advantageous in freshwater systems, where historic records of fish community composition are becoming obsolete due to rapid extirpation of native species and spread of exotic species. Here, we use eDNA metabarcoding to compare beta-diversity between the upper watersheds of four highly disturbed river basins in southern California. While we were unable to detect all the species historically expected to inhabit these areas, we uncovered key insights about biodiversity in these areas. eDNA results revealed a north-south gradient in pairwise beta-diversity between river basins, where basins located further south were more similar to one another than to basins located further north. Additionally, we found that differences in diversity of native species was primarily attributed to nestedness (differences in species richness) while differences in diversity of exotic species were primarily due to turnover (differences in species composition). We also discovered that, given a strong enough sampling effort, eDNA metabarcoding has the potential to expose finer scale biodiversity patterns within watersheds. Understanding which areas best support a diversity of native species and which areas are prone exotic species takeover can ultimately assist environmental managers with directed efforts to protect and preserve native freshwater fishes. As sampling

designs and reference databases continue to improve, so will the ability of eDNA analysis to generate high quality and up to date information on aquatic biodiversity.

1.2 Introduction

Freshwater biodiversity is in decline worldwide and continues to be threatened by pervasive and emerging threats from human impacts, including climate change and exotic species introduction (Dudgeon et al., 2006; Reid et al., 2019). In California, 83% of native freshwater fish species, most of which are endemic (Moyle 2002), are considered "critically vulnerable" or "highly vulnerable" to extinction from climate change by the year 2100 (Moyle et al., 2013). Understanding the distributions of both native and exotic freshwater fish species is crucial for conservation and restoration planning efforts. For example, such information has been used to rank catchments by their ability to support native fishes and to assess whether existing protected areas can effectively serve as buffers to native species extinction (Grantham et al., 2019). Much information on the distributions of California freshwater fishes is based on models derived from various integrated sources of past observations of fish in the wild (e.g., PISCES database; Santos et al., 2014). This technique makes use of information generated from past surveys and opportunistic observations and can be useful for predicting future habitat availability, range contractions, and extinction risk over broad geographic scales (i.e. statewide, Grantham et al., 2019; Howard et al., 2015). However, such databases become less useful as species compositions change and observational data goes out of date (Leidy & Moyle, 2021, ICUN 2022). In addition, while these databases can provide lists of fish species projected to be present at the drainage basin level (e.g., Tedesco et al., 2017) or the watershed level (e.g., Santos et al., 2014), they do not allow for a more fine-scale assessment of species distributions within watersheds.

eDNA analysis using either single species or metabarcoding approaches (Lacoursière-Roussel & Deiner 2021) provides an opportunity to assess the community composition of aquatic organisms, including those that are rare or endangered, without directly observing them (Jerde et al., 2011; Laramie et al., 2015). This technique involves analyzing DNA fragments that have been shed into the water by resident animals (Ficetola et al., 2008) by sampling water, extracting DNA from the samples, and using DNA metabarcoding and bioinformatics to obtain the sequences present in the sample and matching them to the species of origin (Thomsen et al., 2012; Simmons et al., 2015; Valentini et al., 2016; Elbrecht and Leese, 2017). This method is at least as effective as traditional sampling methods, such as electrofishing, while eliminating the need to disturb the animals within aquatic habitats (Mcelroy et al., 2020). eDNA data can then be used to calculate biodiversity metrics that can assist with conservation efforts, such as species richness and beta-diversity (Li et al., 2018), and can ultimately be analyzed alongside environmental covariates to identify drivers of changes in biodiversity and community composition (Blabolil et al., 2021; Gallego et al., 2020).

Beta-diversity, a metric that incorporates variation of species presence across sites in a given region, can enable quantification of biodiversity loss from human impacts as well as assessment of a given habitat's ability to support biodiversity at various spatial scales (Socolar et al., 2016). Pairwise beta-diversity quantifies the average dissimilarity of species presence between sampled locations (Anderson et al., 2011) and can be calculated using Sørensen or Jaccard indices that incorporate two components of beta-diversity – turnover and nestedness (Baselga and Orme, 2012). Turnover refers to differences in species composition – but not species richness – between a given pair of sampled locations (i.e., species that are

absent from one location are replaced by additional species at another location; Baselga, 2010). Nestedness refers to differences in species richness between sample locations (i.e., species that are absent from one location are not replaced by additional species; Baselga, 2010). Human impacts can lead to increases in beta-diversity because extirpation of native species and/or introduction of exotic species at a local scale can lead to greater discrepancy between sites (Socolar et al., 2016). Alternatively, human impacts can lead to decreases in beta-diversity. For example, urbanization and introductions of exotic species that are highly invasive (i.e., they outcompete native species) can lead to large scale homogenization of taxa that are supported between sites. Different conservation actions are recommended based on whether pairwise beta-diversity is increasing or decreasing and based on whether changes in pairwise beta-diversity are primarily due to turnover or nestedness (Socolar et al., 2016).

Freshwater fishes in southern California are impacted by drastic changes in habitat availability due to human population growth and land use changes (e.g. steelhead trout; Busby et al. 1996 & delta smelt; Moyle et al., 1992) as well as exotic species introductions (Marchetti et al. 2001). Herein, we pair eDNA sampling with historical records of fish species presence to answer the following questions: 1. How dissimilar are fish communities in the upper watersheds of southern California river basins? 2. Is dissimilarity between basins driven by differences in native or exotic species composition? 3. Does eDNA sampling capture the dissimilarity expected based on species distributions from historical records? 4. Can eDNA be used to compare biodiversity at a finer scale, between individual tributaries within watersheds?

1.3 Methods

1.3.1 Sample Collection & DNA Extraction

eDNA samples were collected from upper watershed areas of four different basins throughout the Los Padres and Angeles National Forests in California USA: the Los Angeles River basin, the Santa Clara River basin, the Santa Ynez River basin, and the Santa Maria River basin (Fig. 1). In each basin, between three and five 1st and 2nd order streams (1-2 sites per stream) were sampled once during fall (dry season) of either 2017 or 2018 and once during the spring (wet season) of 2018. All sites were sampled once during the dry season and once during the wet season unless the site was dry during part of the year. During each sampling event, three 1L samples were filtered on-site through a 0.22-micron glass fiber Sterivex filter capsule (MilliporeSigma, Burlington, MA, USA) using a 60 mL syringe (BD, Franklin Lakes, NJ, USA) using a protocol by Spens et al. (2017). Longmire's solution (lysis buffer;(Renshaw et al., 2015) was added to each sample before being sealed and stored at -20°C until DNA extraction. Negative controls were collected by filtering 1 L of de-ionized water through a filter capsule in the field and storing it using the same method as the eDNA samples. One negative control was collected during each day of sampling.

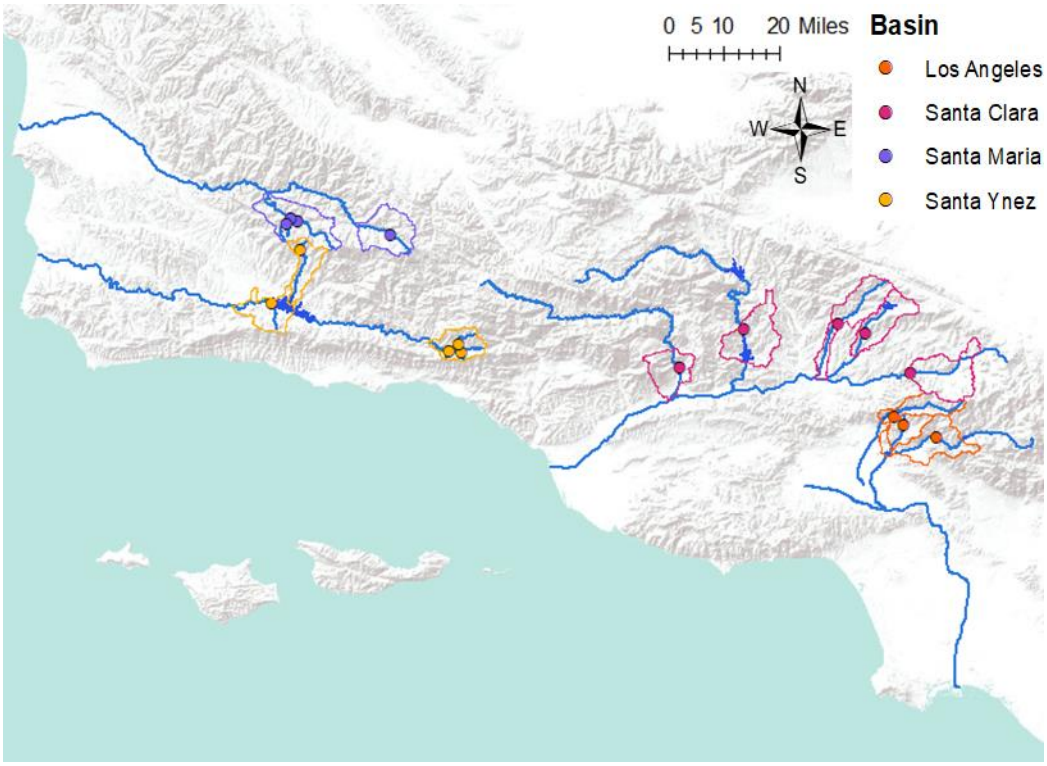

Figure 1.1. Locations of the eDNA sample sites (colored circles) and HUC12 watersheds (colored polygons; U.S. Geological Survey).

DNA extraction was conducted in a designated clean area using a 25:24:1 Phenol: chloroform: isoamyl alcohol (PCI) extraction method adapted from (Deiner et al., 2015). Briefly, proteinase K was added to each filter capsule, and samples were incubated at 56°C overnight on a rotating rack. All liquid was removed from the filter capsules using a 10 mL syringe (BD, Franklin Lakes, NJ, USA) and transferred to a labeled PCR clean microcentrifuge tube (Eppendorf, Hamburg, Germany). This step was performed under a laminar flow hood on a UV-sterilized surface. PCI was added at a 1:1 ratio of PCI:sample. Samples were then manually shaken for five minutes and centrifuged for five minutes at 10,000 RPM. The top layer was collected and added into a new labeled vial containing 24:1 Chloroform: isoamyl alcohol (CI). Samples were again shaken for five minutes and centrifuged for five minutes at 10,000 RPM. The top layer containing the DNA extract was

collected, and 5M NaCl was added to each sample at 10% of the sample volume, followed by -20°C 100% molecular grade ethanol at 200% of the sample volume to precipitate the DNA. Samples were kept at -20°C overnight and then centrifuged for 30 min at 14,000 RPM at 4°C. The ethanol was removed, and the DNA pellet was washed with 70% molecular-grade ethanol. Samples were centrifuged a second time for 30 minutes at 14,000 RPM and 4°C. The ethanol was then removed, and the samples were left to dry under the laminar flow hood until all the ethanol had evaporated. The DNA was re-dissolved in 0.25x TE buffer, incubated at 55°C for 10 min, put through a OneStep Inhibitor Removal Kit (following the manufacturer's instructions; Zymo Research, Irvine, CA USA) to filter out potential PCR inhibitors, and stored at -20°C until sequencing.

1.3.2 Amplicon PCR & High Throughput Sequencing

Amplicon PCR was conducted on each sample using 2 fish-specific primers: Ac16s and Cytb (Evans et al 2016). Primers contained adapter sequences to allow attachment of metabarcoding indexes. 50uL PCR reactions were run on a T100 PCR thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA). Each well contained 20 uL TaqMan Environmental Master Mix, 10 uL of each primer, and 10 uL of sample. Each primer set had unique cycling conditions described in Evans et al. (2016). Excess primers/dimers were separated from PCR products using a 16s magnetic bead cleanup protocol (Illumina, San Diego, CA, USA). A Nextera XT Index Kit was then used to attach unique index tags to PCR products in each well Illumina, San Diego, CA, USA. Indexes were attached by adding as per the manufacturer's instructions. Following index attachment, another PCR cleanup was done to remove excess indexes. Indexed samples were quantified on a Qubit, pooled, and submitted

to the UCSB Biological Nanostructures Lab for sequencing. Sequencing was conducted using a MiSeq v2 kit (Illumina, San Diego, CA, USA).

1.3.3 Bioinformatic Analysis, Species Assignment & Error Analysis

Bioinformatic analysis was performed following a pipeline from Mahon & Jerde (2016). For each library, FastQ files were filtered to using PRINSEQ v0.20.4. NCBI's Genbank database and BLAST tool were then used to create a custom database and find sequence matches for our samples. Sequences with over 98% matches that were at least 100bp were retained and species assignments were extracted using the Metagenome Analysis Software (Megan6). Some negative controls contained fish DNA, so error analysis was performed using a procedure outlined in (Olds et al., 2016) to set thresholds for the number of sequences required for each species to be considered present in a sample (R package: "MASS"; Ripley et al 2013). For each species that appeared in the negative controls, the number of sequences in each negative control was fit to a Poisson distribution. The mean value (lambda) for each distribution represents the expected number of DNA sequences belonging to the corresponding species in any given sample. Lambda values were plugged into probability functions to find the threshold number of sequences ≤0.1% likely to be observed in the negative controls. Samples with less than or equal to that threshold number of sequences were considered positive for the corresponding species. Positive detections were pooled to generate species lists for each river basin and for each individual stream, and these lists were pooled to obtain species lists for each sampled river basin.

1.3.4 Projected Species Composition

Lists of species expected to inhabit our sample sites were assembled using the PISCES database (Santos et al., 2014). This database utilizes past field observations from various

sources including (but not limited to) surveys conducted by the United States Forest Service, California Department of Fish and Wildlife, and the Federal Energy Regulatory Commission as well as data compiled from Moyle & Randall (1998). Based on empirical observations, the PISCES database models projected distributions of fish species throughout California and species lists are available at the United States Geological Survey Hydrologic Unit Code 12 (HUC12) watershed level. From this database, species lists were compiled from HUC12 watersheds (i.e., streams) that we collected eDNA samples from. These lists were used to generate "expected" outcomes for fish beta-diversity and basin dissimilarity.

1.3.5 Biodiversity Metrics

All biodiversity analyses were conducted in R/Rstudio. Species richness was calculated for each of the four sampled river basins by summing the total species detected. Pairwise dissimilarity between river basins was calculated via the Sørensen index (R package: betapart; (Baselga and Orme, 2012) that accounts for differences in both species turnover and nestedness between watersheds. These analyses were conducted on both eDNA results and on species lists obtained from the PISCES database. We also calculated pairwise beta-diversity using the Jaccard index that similarly accounts for turnover and nestedness. Because the total number of samples collected varied by basin (Los Angeles: 15 samples, Santa Clara: 43 samples, Santa Ynez: 39 samples, Santa Maria: 21 samples) and by stream, species accumulation curves were generated to determine the number of samples required to detect 80% and 90% of the species in the upper watersheds of these basins (R package: vegan; Oksanen et al. 2022). Results were used to determine which streams had a high enough sampling effort to be compared in a stream-level beta-diversity dissimilarity analysis.

1.4 Results

Species richness detected by eDNA was similar between the four basins (9-12 species), and within each river basin, 40-50% of detected species were native (Fig. 2A). Overall species richness was lower than expected based on species lists generated from the PISCES database, and this was largely attributed to the absence of exotic species' DNA in the eDNA samples (Fig. 2). We detected 45% of the exotic species expected to inhabit these areas and nearly all native species, except for *Entosphenus tridentata* (Pacific lamprey), which is presumed extirpated throughout much of southern California (Wang and Schaller, 2015) and *Gasterosteus aculeatus* (three-spine stickleback) that was undetectable due to lack of available 16s and Cytb sequence information for this species.

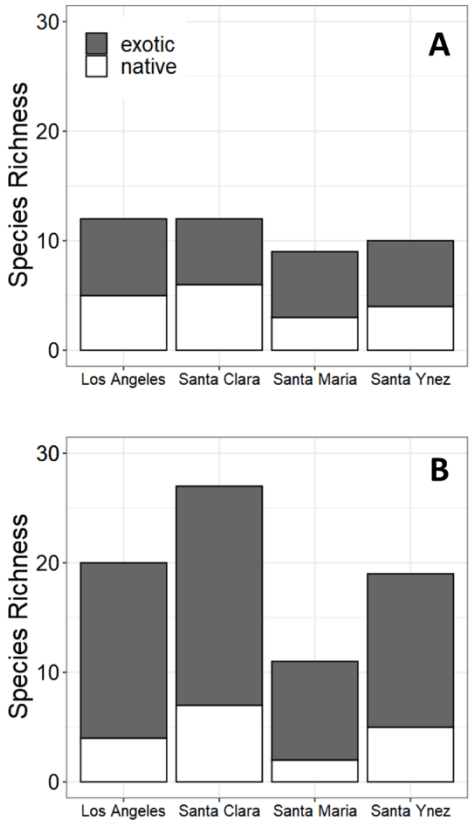

Figure 1.2. Species richness detected by eDNA (panel A) and expected from species lists generated from the PISCES database (panel B).

Species accumulation curves indicate differences in the number of samples required to maximize species detection between basins (Fig. 3). For Los Angeles and Santa Clara upper watersheds, six samples will sufficiently detect 80% of our detected fish species, and eight samples will detect 90% of our detected fish species. For the Santa Ynez basin, 26 samples are required to detect 80% of species, and 33 samples are required to detect 90%. For the Santa Maria basin, 13 samples are required to detect 80% of species, and 17 samples are required to detect 90%. Due to lower detection efficiency in the Santa Maria and Santa Ynez basins, we do not have sufficient sample size to compare beta-diversity of each individual stream sampled from these basins.

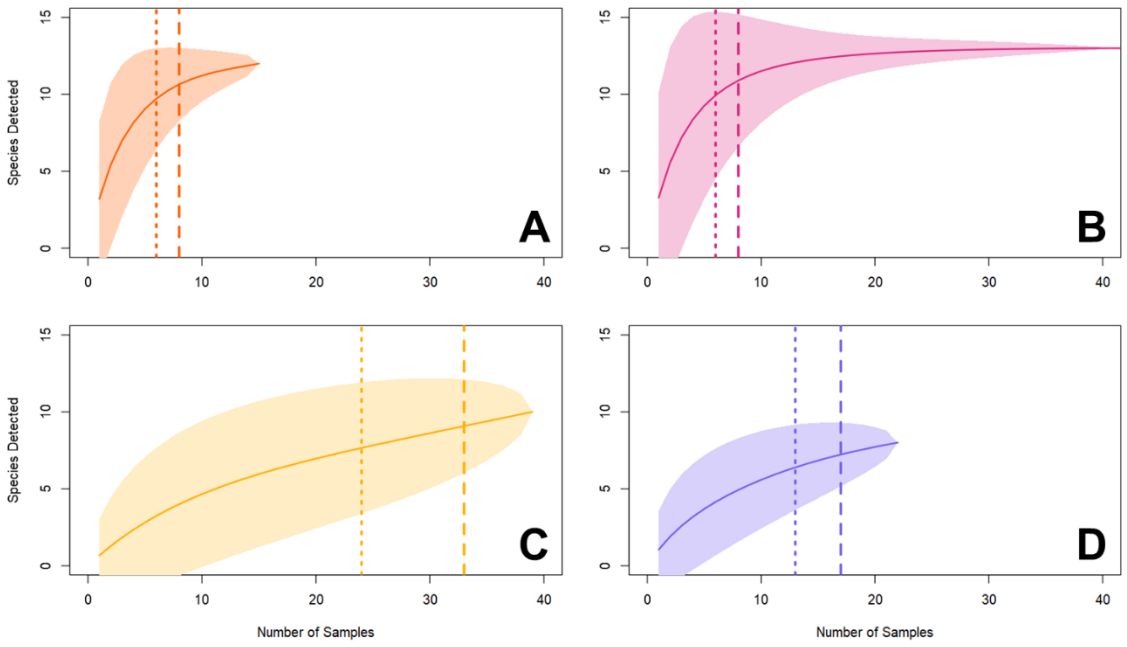

Figure 1.3. Species accumulation curves for environmental DNA sampling in each basin: Los Angeles (orange; panel A), Santa Clara (pink; panel B), Santa Ynez (yellow; panel C) and Santa Maria (purple; panel D). Solid lines represent the number of fish species predicted to be detected for a given sampling effort and shading represents the standard deviation around this prediction. Dotted lines denote the number of samples required to detect 80% of fish species and dashed lines denote the number of samples required to detect 90% of fish species.

Sørensen and Jaccard indices returned the same conclusions, so only the results from the Sørensen index are presented here. Sørensen indices reveal dissimilarity in beta-diversity at the drainage basin level using eDNA detections (Fig. 4A-C) and species lists from the PISCES database (Fig. 4D-F). Using eDNA analysis, we detected a latitudinal gradient of dissimilarity, where basins located further south (Los Angeles, Santa Clara) are more similar to one another than to basins located further north (Santa Ynez, Santa Maria; Fig. 4A). When exotic species were excluded from eDNA results, overall basin dissimilarity decreased, but the north-south dissimilarity gradient was retained (Fig. 4B). When native species were excluded, dissimilarity increased (Fig. 4C). This indicates that while both native and exotic species contribute to the differences in beta-diversity of each basin, exotic species contribute more. Contributions of turnover and nestedness to differences in beta-diversity varied based on the inclusion of exotic species. Dissimilarity in total beta-diversity and beta-diversity of exotic species detected by eDNA was primarily attributed to turnover (Fig. 5A&C), while differences in beta-diversity of native species were primarily attributed to nestedness (Fig. 5B). In other words, when native species were present in one basin and absent from another, they were typically not replaced by additional native species. Exotic species, however, were typically replaced by additional exotic species when they were absent from any given basin.

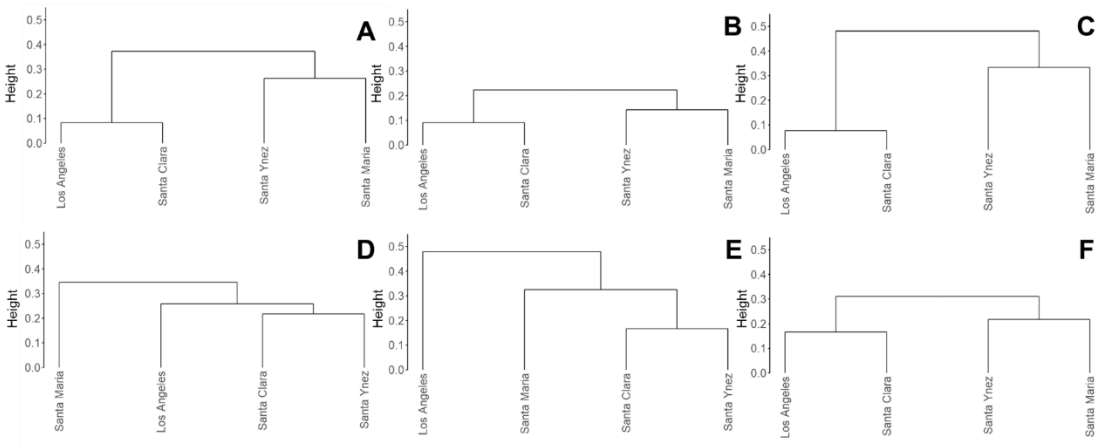

Figure 1.4. Dissimilarity (Sørensen Index) of species composition of the Los Angeles, Santa Clara, Santa Ynez, and Santa Maria watersheds detected by environmental DNA (panels A-C) and from species lists generated from the PISCES database (panels D-F). Represented are dissimilarities of beta-diversity of all fish species (panels A&D), native fish species (panels B&E), and exotic fish species (panels C&F).

Using species lists from the PISCES database, watershed dissimilarity was comparable to the eDNA results but did not follow the latitudinal gradient indicated by the eDNA results (Fig 4D-F). Instead, the Santa Clara and Santa Ynez watersheds were most similar, followed by Los Angeles and Santa Maria. When exotic species were excluded, this pattern remained, and overall dissimilarity increased. When native species were excluded, however, dissimilarity decreased, and the latitudinal gradient returned. According to lists of species expected to be present in the upper watersheds of these river basins, native species should contribute more to dissimilarity between basins. PISCES database species lists indicate a greater contribution of nestedness than turnover in all cases (Fig. 5D-F), meaning that in the absence of any given fish species, it was most often not replaced by another species.

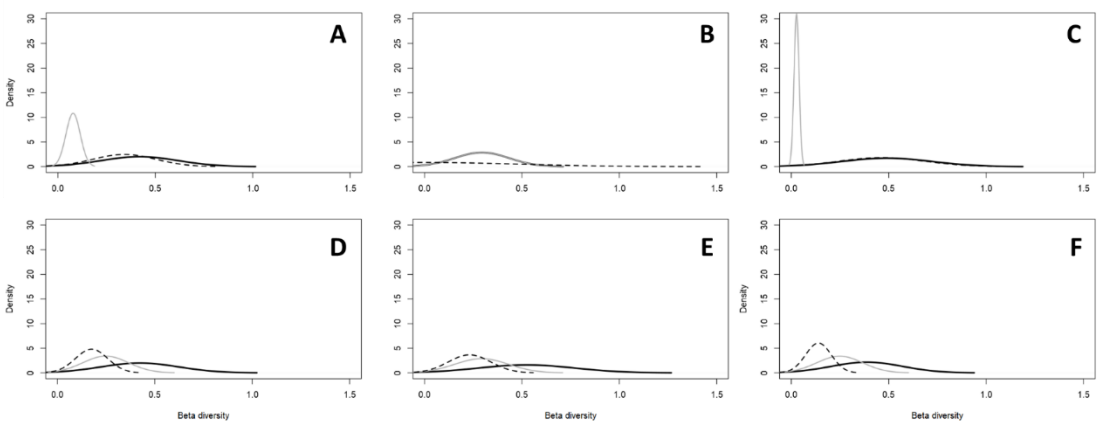

Figure 5. Contributions of turnover (dashed line) and nestedness (gray line) to beta-diversity (solid black line) calculations from eDNA results (panels A-C) and from the PISCES database (panels D-F) for all fish species (A&D), native species (B&E), and exotic species (C&F).

For streams within the Los Angeles and Santa Clara River basins where ≥6 eDNA samples were taken, we were able to detect dissimilarity in beta-diversity between some streams, primarily attributed to nestedness, although three of the four Santa Clara River steams were not significantly dissimilar (Fig. 6). Beta-diversity between individual streams was therefore primarily due to differences in species richness, although more samples are needed for a more thorough comparison of fish diversity in individual upper watershed streams in southern California.

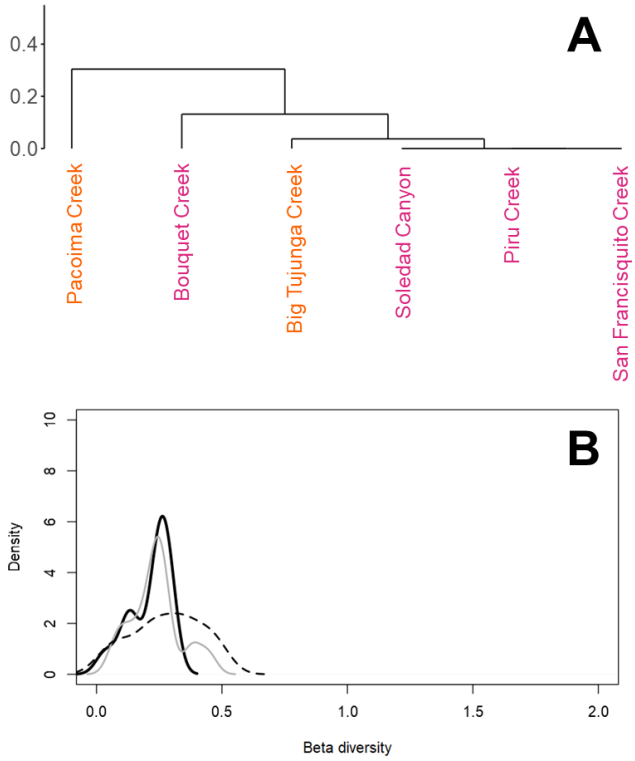

Figure 6. Dissimilarity (Sørensen Index) of beta-diversity (panel A) between streams within the Los Angeles (orange) and Santa Clara (pink) River basins where ≥6 environmental DNA samples were collected and contributions of turnover (dashed line) and nestedness (gray line) to beta-diversity in these streams (panel B).

1.5 Discussion

Here we demonstrate the potential for eDNA metabarcoding to elucidate patterns of biodiversity within and between freshwater basins. To our knowledge, this is the first study to use eDNA analysis to compare the biodiversity of streams using pairwise beta-diversity. Even with a limited number of eDNA samples and despite detecting fewer species than expected (Fig. 2, Table 1), we were able to detect key species of conservation concern (*Oncorhynchus mykiss* (steelhead trout), *Catostomus santaannae* (Santa Ana sucker)) and differences in beta-diversity between basins. Given a large enough sampling effort, eDNA analysis has the potential to reveal fine-scale species composition and biodiversity patterns within watersheds (Berger et al., 2020; Li et al., 2018) that are more difficult to obtain using traditional sampling methods (seining, electrofishing surveys) and opportunistic observations (Mcelroy et al., 2020).

In lotic systems, eDNA sampling effort required to maximize fish species detections varies based on fish biomass, species richness, river geomorphology, or eDNA degradation rates (Bylemans et al., 2018, Jo et al., 2019; Shogren et al., 2017). Unsurprisingly, we found that our sample locations differed in the sampling effort required to maximize species detections (Fig. 3). Based on our species accumulation curves, we recommend 6-8 samples for sites within Los Angeles and Santa Clara River basins and at least 13 samples for sites within the Santa Maria River basin in order to detect the DNA of >80% of the fish species at these locations (Fig.3). For sites within the Santa Ynez River basin, we recommend a larger sampling effort, as 26 samples were required to detect the DNA from 80% of the fish species in this community. However, some sample sites within the Santa Ynez River basin (Alder Creek, Juncal Creek, Fox Creek) had recently been impacted by a wildfire and debris flow

when sampling occurred, so it is possible that fish eDNA will become more abundant as fish populations recover from this disturbance and that it will be possible to detect these species with a more modest sampling effort. We did not have enough eDNA sample replicates from our sites to draw broad conclusions about how beta-diversity varies between individual streams or HUC12 watersheds, but we have verified that eDNA analysis can be used for this purpose. Based on our results, it appears that the sampled areas in the Santa Clara River basin have the same beta-diversity (with the exception of Bouquet Creek) and that these streams differ in beta-diversity to the sampled locations in the Los Angeles River basin, primarily due to nestedness (Fig. 6).

We found that basins located further south (Los Angeles, Santa Clara) were more similar to one another than to basins located further north (Santa Ynez, Santa Maria) and vice versa. This makes intuitive sense but differs from dissimilarity patterns expected based on distribution information from the PISCES database (Fig. 4). There a few possible explanations for this discrepancy between expected and measured dissimilarity. First, the PISCES database contains empirical observations of fish species throughout California and projected species distributions have been modeled based on those empirical observations (Santos et al. 2014). It is possible that these predicted distributions are imprecise or that some species have been extirpated since they were empirically observed (e.g. *Entosphenus tridentata*, Pacific lamprey). It is also possible that some species are present at low biomasses such that they were not detectable by our experimental design (Jo et al., 2019; Jo and Yamanaka, 2022).

Another possible explanation is that our eDNA reference database did not contain sufficient 16s and Cytb sequences to detect all species that inhabit these areas, resulting in an

artificially low number species being detected by eDNA. Indeed, some DNA sequences in our samples could only be identified to the family or genus level. For example, many sequences assigned to Cyprinidae family were indistinguishable between *Cyprinus carpio* (common carp) and *Carrasius auratus* (goldfish). Both of these invasive species were expected to be present at many of our sample sites and they are also able to hybridize with one another. Since we cannot be certain whether these sequences belong to common carp, goldfish, or hybrids, these sequences were included in our analysis as 1 species. However, all except 1 undetected exotic species (*Menidia beryllina*; inland silverside) had genetic sequences for 16s and/or Cytb available in Genbank during the time the bioinformatics was conducted, so this is not likely to have substantially affected our results. Fortunately, genetic sequences are continually added the NCBI Genbank database, and this common challenge for eDNA studies will continue to diminish over time (Jerde et al., 2021; Mahon et al., 2023; Marques et al., 2021).

It is also possible that undetected exotic species are present elsewhere in the Hydrologic Unit Code 12 (HUC12) watersheds that the PISCES database species lists are based on. In some cases, the HUC12 watersheds cover more area than our eDNA samples. For example, we sampled at the upstream end of the corresponding HUC12 areas at Big Tujunga Creek, Piru Creek, and Cachuma Creek and in the middle of HUC12 areas at Sisquoc River, Manzana Creek, and Bouquet Creek (Fig. 1). Undetected exotic species could exist further downstream in these areas and patterns of dissimilarity between basins could vary depending on precise sample location. In fact, many of these exotic species such as sunfishes, carps, and catfish are expected to inhabit higher order (lower watershed) (Barila et al., 1981; Harrel et al., 1967). Partitioning of fish species to different stream orders (and thus different flow

speeds, temperatures, dissolved oxygen, and food resources) is expected given the variation in habitat requirements between freshwater fish species, and these patterns can be elucidated using eDNA (Berger et al., 2020; Cech et al., 1990; García-Machado et al., 2022; Moyle and Vondracek, 1985; Zbinden and Matthews, 2017). For a more complete snapshot of fish biodiversity in these upper watersheds, future eDNA efforts should collect samples from more locations throughout individual streams and/or HUC12 watersheds and utilize updated reference databases to ensure maximum detectability of fish species.

A key takeaway from our eDNA analysis is that exotic species contributed more to basin-level dissimilarity than native species. This is unexpected not only because it is inconsistent with PISCES database predictions (Fig. 4), but also because introductions of exotic fish species have generally caused California freshwater fish communities to be more homogenous (not more distinct) than in the past (Martchetti et al. 2001). This finding that exotic species turnover contributed most to the dissimilarity between the upper watersheds of the Los Angeles, Santa Clara, Santa Ynez, and Santa Maria river basins has important implications for conservation in these areas. This indicates that the primary reason beta-diversity differs between basins is that there are different invasive species inhabiting each basin. Because exotic species contribute heavily to beta-diversity in these areas, streams with the highest beta-diversity should not be assumed to be thriving. This is in agreement with previous studies that have found that high beta-diversity in fish communities can occur in streams that have experienced anthropogenic disturbance and species (Dala-Corte et al., 2019; Qiao et al., 2022; Trovillion et al., 2023, but see Daga et al., 2015). Dissimilarity between native fish species assemblages in our sampled basins was primarily driven by nestedness (differences in native species richness). In this case, it is recommended that

conservationists focus on preserving locations with the highest species richness and prioritize such areas over places with lower species richness (Socolar et al. 2016). We emphasize that if this approach is being taken, managers should consider places with high native species richness rather than highest overall species richness, as exotic species are prevalent. However, taxonomic diversity does not necessarily indicate functional diversity, as different species can fill similar niches within the same habitat (Colin et al., 2018; Milardi et al., 2019; Zhang et al., 2021). Future studies should consider assessing beta-diversity of functional groups to generate more telling information on what these habitats have and what they are lacking (Aglieri et al., 2020; Dala-Corte et al., 2019).

The present study has validated that eDNA metabarcoding can be used to measure pairwise beta-diversity of fish communities at different special scales in freshwater environments. Moving forward, it is imperative that eDNA sampling and analysis techniques continue to evolve along with the rapid advancements in methodology that continue to improve this method. In many cases, this entails standardization of methods across studies to broaden the inferences and implications made possible by this work (Kelly et al. 2023). In other cases, it is beneficial to consider the characteristics of specific landscapes and methodological adjustments that maximize the probability of species detections in those landscapes (e.g. environments with high versus low species richness; Cilleros et al., 2019; García-Machado et al., 2022). This study represents just one example of many potential applications for eDNA analysis in the fields of ecology and conservation biology.

Basin	Stream	Sample Location	Number of Samples	Seasons sampled	Cottus asper	Catostomus fumeiventris	Catostomus santaanae	Entosphenus tridentata	Gasterosteus aculeatus	Gila orcutti	Oncorhynchus mykiss	Rhinichthys osculus
Los Angeles	Big Tujunga Creek	34.52494, -118.44282	6	dry, wet	U		E			E	E	E
	Little Tujunga Creek	34.32819, -118.34144	3	wet								
	Pacoima Creek	34.34584, -118.36575	6	dry, wet								
Santa Clara	Bouquet Creek	34.52494, -118.44282	9	dry, wet	E	E	E			E	E	U
	Piru Creek	34.53348, -118.75800	9	dry, wet								
	San Francisquito Creek	34.54596, -118.51365	9	dry, wet								
	Sespe Creek	34.45070, -118.92500	3	wet								
	Soledad Canyon	34.44011, -118.324644	6	dry, wet								
Santa Maria	Davey Brown Creek	34.77130, -119.94448	6	dry, wet	E					E	E	
	Manzana Creek	34.76598, -119.92399	6	dry, wet								
	Munch Creek	34.75871, -119.95502	3	wet								
	Sisquoc River	34.73708, -119.68392	3	wet								
Santa Ynez	Alder Creek	34.48341, -119.49626	6	dry, wet	U		U			E	E	
	Cachuma Creek	34.70258, -119.91731	12	dry, wet								
	Fox Creek	34.48544, -119.52936	6	dry, wet								
	Hilton Creek	34.58847, -119.99115	12	dry, wet								
	Juncal Creek	34.50123, -119.50439	3	wet								

Table 1.1. Environmental DNA (eDNA) detection results for native fish species in the Los Angeles, Santa Clara, Santa Maria, and Santa Ynez basins, with E symbols denoting positive eDNA detections that were expected and U symbols denoting eDNA detections that were unexpected. Shaded cells indicate species that were undetected by eDNA samples but were expected to be present according to the PISCES database (Santos et al. 2014).

Basin	Stream	Sample Location	Number of Samples	Seasons sampled	Ameiurus catus	Ameiurus melas	Ameiurus nebulosus	Cyprinus carpio OR Carassius auratus	Cyprinella lutrensis	Dorosoma petenense	Gambusia affinis	Ictalurus furcatus	Ictalurus punctatus	Lepomis cyanellus	Lepomis macrochirus	Lepomis microlophus	Menidia beryllina	Micropterus salmoides	Notemigonus crysoleucas	Percina Macrolepida	Pimephales promelas	Pomoxis nigromaculatus	Pomoxis annularis	Salmo trutta	Tridentiger bifasciatus
Los Angeles	Big Tujunga Creek	34.52494, -118.44282	6	dry, wet		E	E	E		E				E	E			E							
	Little Tujunga Creek	34.32819, -118.34144	3	wet																					
	Pacoima Creek	34.34584, -118.36575	6	dry, wet																					
Santa Clara	Bouquet Creek	34.52494, -118.44282	9	dry, wet		E	E	E		E				E	E			E							
	Piru Creek	34.53348, -118.75800	9	dry, wet																					
	San Francisquito Creek	34.54596, -118.51365	9	dry, wet																					
	Sespe Creek	34.45070, -118.92500	3	wet																					
	Soledad Canyon	34.44011, -118.324644	6	dry, wet																					
Santa Maria	Davey Brown Creek	34.77130, -119.94448	6	dry, wet				E		E			E	E	E							E			
	Manzana Creek	34.76598, -119.92399	6	dry, wet																					
	Munch Creek	34.75871, -119.95502	3	wet																					
	Sisquoc River	34.73708, -119.68392	3	wet																					
Santa Ynez	Alder Creek	34.48341, -119.49626	6	dry, wet		E				E			E	E	U						U	E			
	Cachuma Creek	34.70258, -119.91731	12	dry, wet																					
	Fox Creek	34.48544, -119.52936	6	dry, wet																					
	Hilton Creek	34.58847, -119.99115	12	dry, wet																					
	Juncal Creek	34.50123, -119.50439	3	wet																					

Table 2: environmental DNA (eDNA) detection results for exotic fish species in the Los Angeles, Santa Clara, Santa Maria, and Santa Ynez basins, with E symbols denoting positive eDNA detections that were expected and U symbols denoting eDNA detections that were

unexpected. Shaded cells indicate species that were undetected by eDNA samples but were expected to be present according to the PISCES database (Santos et al. 2014).

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Chapter 2: Thermal tolerance and vulnerability to warming differ between populations of wild *Oncorhynchus mykiss* near the species' southern range limit

2.1 Abstract

Fish habitat temperatures are increasing due to human impacts including climate change. For broadly distributed species, thermal tolerance can vary at the population level, making it challenging to predict which populations are most vulnerable to warming. Populations inhabiting warm range boundaries may be more resilient to these changes due to adaptation or acclimatization to warmer temperatures, or they may be more vulnerable as temperatures may already approach their physiological limits. We tested functional and critical thermal tolerance of two populations of wild Oncorhynchus mykiss near the species' southern range limit and, as predicted, found population-specific responses to temperature. Specifically, the population inhabiting the warmer stream, Piru Creek, had higher critical thermal maxima and higher functional thermal tolerance compared to the population from the cooler stream, Arroyo Seco. Arroyo Seco O. mykiss are more likely to experience a limitation of aerobic scope with warming. Piru Creek O. mykiss, however, had higher resting metabolic rates and prolonged exercise recovery, meaning that they could be more vulnerable to warming if prey or dissolved oxygen become limited. Temperature varies widely between streams near the O. mykiss southern range limit and populations will likely have unique responses to warming based on their thermal tolerances and metabolic requirements.

2.2. Introduction

As global temperatures continue to rise due to climate change, aquatic ectotherms are projected to undergo increased local extinctions at warm range boundaries, critically altering the distribution and population dynamics of vital fish stocks (Hickling *et al.*, 2006; Chen *et al.*, 2011; Sunday, Bates and Dulvy, 2012). Poleward range shifts have already been observed in both marine (Sanford *et al.*, 2019) and freshwater (Comte and Grenouillet, 2013) fish species, as populations inhabiting extreme conditions at warm range boundaries are likely to experience physiologically limiting temperatures (Rijnsdorp *et al.*, 2009; Mcdonnell and Chapman, 2015; Yu *et al.*, 2018). However, exposure to such conditions over generations can lead to local adaptation, allowing these fringe populations to be better equipped to withstand temperature extremes (Fangue, Hofmeister and Schulte, 2006; Schulte, 2007; Barrett *et al.*, 2011; Gracey, 2022). To predict species range shifts and to effectively manage populations at range edges, it is important to understand their vulnerability to current conditions, and their potential resiliency to ongoing climate-induced temperature change.

Vulnerability and resiliency to current and future habitat temperatures can be assessed by measuring functional and critical thermal limits. Functional thermal tolerance limits refer to temperatures where key fitness-related performance traits become restricted. At these temperatures, fish do not die but are limited in their ability to grow, compete, evade predators, and/or reproduce (Claireaux and Lefrançois, 2007; Farrell, 2009; Schulte, 2015; Fry, 1971). Functional thermal tolerance is often approximated by measuring aerobic capacity across a range of temperatures because many of these essential performance traits are dictated by aerobic metabolism (Fry, 1971; Fry and Hart, 1948). Optimal and limiting

temperatures are often mediated by the amount of oxygen fish have available after accounting for baseline requirements, which tend to increase exponentially with rising temperature (Fry, 1947; Farrell, 2016). Aerobic capacity, or aerobic scope, can be quantified by calculating the difference (absolute aerobic scope, AAS) or the ratio (factorial aerobic scope, FAS) between oxygen consumption rates of individual fish at rest (resting metabolic rate, RMR) and during or immediately after maximal exercise (maximum metabolic rate, MMR). This concept of oxygen and capacity limited thermal tolerance (OCLTT) (Pörtner and Knust, 2007; Pörtner and Farrell, 2008; Pörtner, 2001) is disputed and the relationships between temperature, aerobic scope, and other whole animal performances can vary between species and temperature regimes (Clark, Sandblom and Jutfelt, 2013; Jutfelt *et al.*, 2018; Norin, Malte and Clark, 2014; Gräns *et al.*, 2014). Nevertheless, optimum and limiting temperatures for aerobic scope have been linked to fish range limits (Payne *et al.*, 2016). In addition, the ability to recover from exhaustive exercise is crucial for wild fish as they frequently use anaerobic burst swimming (e.g. foraging, avoiding predators, interacting with fisheries, competing with conspecifics for space or mates). Prolonged recovery could lead to lost opportunities (e.g. food, space, mates) or increased vulnerability (e.g. predators, disease). The effect of temperature on the speed of recovery from MMR and on the amount of oxygen required to recover from MMR (excess post-exercise oxygen consumption; EPOC) can therefore also be used as a metric of functional thermal tolerance (Farrell, 2016).

Critical thermal limits are temperatures that are lethal to fish when temperature is increased or decreased rapidly. Critical thermal maximum (CT_{MAX}) tests approximate upper lethal temperatures by measuring loss of equilibrium (Beitinger, Bennett and Mccauley, 2000). Fish are unlikely to survive loss of equilibrium in the wild, especially in the presence

of predators (Beitinger and Lutterschmidt, 2011). There is an apparent latitudinal gradient in fish CT_{MAX} where species occupying warmer habitats at lower latitudes tend to have the highest CT_{MAX} (Sunday *et al.*, 2020), but CT_{MAX} can also vary within species at the population level and can change at the individual level depending on holding or habitat temperature (i.e., acclimation/acclimatization), time of day, and heating rate (Beitinger, Bennett and Mccauley, 2000; Healy and Schulte, 2012; Illing *et al.*, 2020; McKenzie *et al.*, 2021). CT_{MAX} are used to calculate thermal safety margins (TSMs), or the difference between the maximum habitat temperature and the upper lethal temperature (Sunday *et al.*, 2014; Pinsky *et al.*, 2019). Because temperature tends to limit fish performance at temperatures below CT_{MAX}, however, TSMs often overestimate the amount of warming that a fish population can withstand before facing declines (Farrell, 2009; Rodnick *et al.*, 2004; Eliason, Van Wert and Schwieterman, 2022). Therefore, functional thermal tolerance limits are used to calculate functional warming tolerance (FWT), or the difference between maximum habitat temperature and the temperature where fish performance begins to decline, and this can be used to understand the relative vulnerabilities of fish populations to habitat warming (Anlauf-Dunn, Kraskura and Eliason, 2022; Eliason, Van Wert and Schwieterman, 2022).

Fishes that inhabit broad geographic ranges often consist of genetically distinct populations that experience vastly different thermal conditions. Acclimatization or local adaptation to a range of thermal habitats leads to interpopulation variability in thermal tolerance (Eliason *et al.*, 2011; Narum *et al.*, 2013; Fangue, Hofmeister and Schulte, 2006; Barrett *et al.*, 2011; Zillig *et al.*, 2021). In warm environments, specific adaptive or acclimation strategies include enhanced heat shock protein production (Healy and Schulte,

2012; Madeira *et al.*, 2012; Narum *et al.*, 2013), increased surface area of gill lamellae (Sollid, Weber and Nilsson, 2005; Mcbryan *et al.*, 2016), enhanced cardiac capacity (due to increased heart size, compact myocardium thickness and capillary density) (Eliason *et al.*, 2011; Tepolt and Somero, 2014; Chen *et al.*, 2018; Klaiman *et al.*, 2011; Anttila *et al.*, 2015), and increased mitochondrial capacity (Chung and Schulte, 2015; Chung *et al.*, 2017). These responses, whether plastic or adaptive, can boost a population's ability to tolerate temperature extremes (Fangue, Hofmeister and Schulte, 2006; Barrett *et al.*, 2011). They also enable physiological processes, particularly those dictated by aerobic metabolism, to be optimized at habitat-specific temperatures (Eliason *et al.*, 2011; Chen *et al.*, 2018; Verhille *et al.*, 2016). However, maintaining adaptations and mounting acclimatory responses are energetically costly and can have fitness consequences for warm-exposed fish populations (Peterson, Hilborn and Hauser, 2014). These costs also frequently result in a tradeoff between upper thermal tolerance and thermal plasticity where populations with the ability to tolerate high temperatures have restricted capacity for thermal acclimation and vice versa (Comte and Olden, 2017; Scheuffele, Rubio-Gracia and Clark, 2021).

Wild Pacific salmon, of the genus *Oncorhynchus*, are of critical ecological, cultural, and economic importance and many species have already experienced declines due to increased water temperature combined with habitat degradation and increased frequency and intensity of drought (Busby *et al.*, 1996; Grant, MacDonald and Winston, 2019; Crozier *et al.*, 2021). Declines are especially prominent near the warm range boundaries for these species (Ford *et al.*, 2011). *Oncorhynchus mykiss* (steelhead/rainbow trout), *Oncorhynchus kisutch* (coho salmon), and *Oncorhynchus tshawytscha* (chinook salmon), for example, are considered endangered or threatened in coastal California under the United States Federal Endangered

Species Act. Pacific salmonid populations currently persisting at these range limits may already experience physiologically limiting temperatures and are likely vulnerable to further declines and extirpation should temperatures continue to increase. We studied wild *O. mykiss*, also known as steelhead (anadromous phenotype) or rainbow trout (freshwater resident phenotype) that inhabit an extraordinarily broad native distribution, extending along the west coast of North America from Baja California to Alaska, and across to the Kamchatka Peninsula in eastern Russia (Page and Burr, 2011). Although these fish are most often reported to occupy cold-water habitats, *O. mykiss* populations inhabit a wide range of thermal conditions across their latitudinal distribution. Thermal tolerance has been studied in this species in the central part of its range (e.g., Northern California, Myrick and Cech, 2000; Central California, Verhille *et al.*, 2016), but thermal tolerance has never been studied in wild *O. mykiss* inhabiting their southern range limit. We predicted that *O. mykiss* inhabiting their southern range limit have distinctive adaptations to cope with warm temperatures due to selection pressures associated with their habitat conditions.

In this study, we aimed to determine whether wild *O. mykiss* near their warm range boundary exhibit interpopulation variability in thermal tolerance and whether these populations currently experience physiologically limiting temperatures or if they could withstand additional warming. We measured aerobic capacity, exercise recovery, and CT_{MAX} in two genetically distinct (Abadía-Cardoso *et al.*, 2016) populations of wild *O. mykiss* that inhabit distinctly different thermal habitats near the southern range limit for the species (Fig 1). We conducted all experiments streamside using ecologically relevant diurnally cycling temperatures to mimic natural environmental conditions (Anlauf-Dunn, Kraskura and Eliason, 2022). In addition, we collated stream temperature data from 11 trout-

bearing streams in the Los Padres National Forest in Southern California to assess temperature regimes experienced by trout at their range limit. We predicted that the population from the warmer stream, Piru Creek, would have a higher upper thermal tolerance and a reduced capacity to rapidly acclimate to elevated temperatures than the population from the cooler stream, Arroyo Seco. We also predicted that the Piru Creek population would have reduced TSM and FWT due to higher habitat temperatures and would thus be less capable of withstanding future warming than the Arroyo Seco population.

2.3. Methods

2.3.1 O. mykiss Populations

Experiments were conducted from August 21-25, 2019 at Piru Creek (34.61691, -118.74427; Castaic, California, USA) and from September 9-13, 2019 at Arroyo Seco (36.11914, -121.46904; Greenfield, California, USA; Figure 1) when temperatures were anticipated to be near peak levels for both populations. Both streams contain robust *O. mykiss* populations and are located near the southern latitudinal limit of this species' native range. Both the Piru Creek and Arroyo Seco populations have coastal steelhead genetic lineage but have resided for many generations behind man-made barriers to ocean migration (Abadía-Cardoso *et al.*, 2016). These populations have minimal evidence of genetic introgression with hatchery rainbow trout despite a history of widespread stocking of hatchery rainbow trout in California (Abadía-Cardoso *et al.*, 2016).

Piru Creek, a tributary to the Santa Clara River, is located further south and is characterized by high and variable temperatures (~9-25 °C; Fig. 2.1 & Fig. A3). The Piru Creek *O. mykiss* population is known as being one of the most southern wild populations that continues to thrive despite warm temperatures, manipulated habitat, introduction of

predatory invasive species (e.g., largemouth bass), heavy recreational use, and frequent wildfires. This population is located between two reservoirs (Figure 1). Downstream, a 61 m dam near the confluence with the mainstem river has blocked this population from ocean migration since 1955. Upstream, a 118 m dam separates this population from the headwaters. This reach is perennially wetted due to continuous outflow from the upstream reservoir.

Arroyo Seco, a tributary to the Salinas River, is located ~300 km north of Piru Creek. We sampled fish from a spring-fed, perennially wetted reach near the headwaters. This population typically experiences cooler temperatures (~5-23 °C; Fig. 2.1) than Piru Creek and is located in a more pristine, unmanipulated habitat. Still, this population has been earmarked for conservation due to inhabiting "stochastic" conditions relative to the rest of the range (Boughton *et al.*, 2009; NMFS 2012). This population coexists with invasive species (e.g., Sacramento pikeminnow and brown trout) and this area is at high risk for wildfire. In fact, the Dolan Fire (Aug. 18-Dec. 31, 2020) and subsequent debris flow in 2021 prevented us from retrieving our temperature loggers, so we do not have year-round temperature data for this site (Fig. 2.1). There are no year-round complete barriers to anadromy for this population, but there are six barriers that partially or temporally block upstream migration to our sample site (Fig. 2.1). On the upstream end is a natural chute where high flows are required for fish to ascend. Further downstream there are three road crossings, a diversion dam, and a section of the stream that has sustained damage from gravel mining. Fish passage from the ocean is only possible during high flow events. These barriers, combined with typical low flows due to groundwater extraction in the lower watershed, make steelhead migration extremely unlikely for this population.

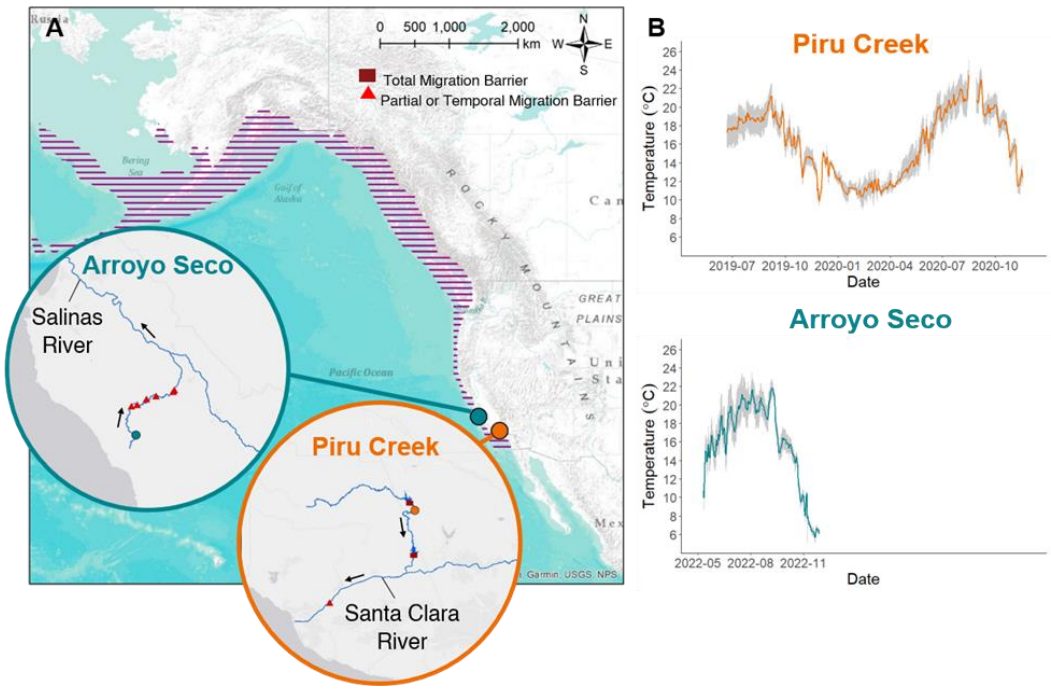

Figure 2.1. Location of study populations (panel A), Arroyo Seco (blue) and Piru Creek (orange), and temperatures at each site (panel B). Circles indicate each study site location. Purple hatched shading shows the full range of anadromous *O. mykiss* in North America ('AquaMaps', 2019). Dark red rectangles indicate impassable barriers to fish migration (dams) and red triangles indicate partial or temporal barriers to migration (e.g., road crossings and other human-constructed or natural features that require high flow for fish passage). Stream temperatures were recorded at 15-minute intervals using Onset Pendant loggers. Orange and blue lines indicate mean daily stream temperatures and gray shading represents minimum and maximum daily temperatures.

2.3.2 Field Methodology

All experiments were conducted streamside no more than 3.2 km from fish collection sites. A temporary tank system was constructed at each site. Water was pumped directly from the creek into 2 header tanks (531 L and 102 L) equipped with Smart One Easy Plug Axial Heaters (Pentair Aquatic Eco-Systems, Inc., Minneapolis, USA) to heat water to test temperatures. Water was pumped from the header tanks into an acclimation tank (102 L) and respirometry tanks (six tanks, 102 L each). Power was supplied from two portable inverter generators (EU7000IS and EU3000IS; Honda Motor Company Ltd, Japan).

All experimental procedures were approved by the University of California Santa Barbara Institutional Animal Care and Use Committee and experiments were performed in accordance with the relevant guidelines and regulations. All experiments were non-lethal and all fish were returned to the stream upon completion.

Juvenile *O. mykiss* (Piru: n = 32, body mass = 23.8 ± 2.45 g; Arroyo Seco 2019: n = 34, body mass = 27.4 ± 4.08 g) were collected via electrofishing using settings specific to the water conductivity at each location. Typically, fish thermal tolerance studies are conducted by assessing performance of fish either acutely exposed or acclimated to two or more static temperatures. We instead allowed fish to experience natural diurnal fluctuations that they typically experience in their environment. Temperature logger data revealed diurnal temperature fluctuations between 3 °C and 5 °C at both of our study sites during experiments (Piru: 17.5-22.5 °C; Arroyo Seco: 14.5-18 °C), making it less ecologically relevant to test fish held at static temperatures. Instead, we allowed our holding and respirometry tank temperatures to fluctuate along with natural diurnal temperature cycles in order to best simulate each population's natural thermal habitat and to mimic what an increase in stream temperature would most likely look like for these fish. Fish were held at one of three temperature treatments: 1: ambient stream temperature, 2: 2-3 °C above ambient stream temperature (+3 °C), and 3: 5 °C above ambient stream temperature (+5 °C) for 19-23 hours before respirometry (see Table 1). This fairly brief overnight temperature exposure duration was selected to represent a short-term, ecologically relevant thermal stress for the fish

Fish were always collected the day before their experiment began. After collection, fish were held overnight at their treatment temperature before respirometry experiments began

(mean duration: 20 h). For temperature treatments above ambient, fish were placed in the acclimation tank at ambient stream temperature and ramped up to their treatment temperature at a rate of 2 °C per hour. Dissolved oxygen in the acclimation tank was maintained at above 90% saturation at all times. During the day, temperature and dissolved oxygen were monitored using an OxyGuard Handy Polaris Dissolved Oxygen Meter (OxyGuard, Denmark). Temperatures were continuously recorded in the stream and acclimation tanks using HOBO Waterproof Bluetooth Pendant Temperature Data Loggers (Onset Computer Corporation, Bourne, USA). Fish were not fed during acclimation to ensure that they were in a post-absorptive state during experiments and the holding and respirometry tanks were covered with fine mesh shade cloth and situated underneath shade canopies so that food items could not enter the tanks from above.

Once fish completed the overnight ~20 h temperature treatment, oxygen uptake rate (MO_2) measurements were taken using intermittent flow respirometry beginning the following morning. We used twelve respirometry chambers (volumes 1.4 L, 1.8 L, and 2.1 L) constructed from airtight plastic containers (Lock & Lock, Seoul, South Korea) and tested 1 fish in each chamber. Within each chamber, we aimed for a water volume: fish mass ratio of 20:1 but fish were smaller than anticipated and the mean ratio was 87:1 (assuming 1 kg fish = 1 L water). Two respirometers were placed in each of the six respirometry tanks. Each chamber was fitted with a FireStingO2 robust oxygen probe (PyroScience, Germany) on a recirculation loop that moved water through the chamber at all times using a CompactON 300-L h^{-1} pump (Eheim, Germany). Each chamber was also connected to a Universal 300-L h^{-1} pump (Eheim, Germany) that intermittently flushed oxygenated water through the chamber from the surrounding tank to ensure that the fish never experienced

dissolved oxygen levels of less than 70% air saturation. At Piru, MO_2 was measured over 4-minute measurement cycles, separated by 6-minute flush cycles. At Arroyo Seco, MO_2 was measured over 5-12-minute measurement cycles, followed by 10-minute flush cycles.

Maximum metabolic rate (MMR) was measured first, followed by exercise recovery, followed by resting metabolic rate (RMR) and standard metabolic rate (SMR). To induce (MMR), fish were chased individually for 3 minutes in a bucket using quick hand movements and gentle caudal fin contact to induce burst swimming. Fish were then immediately netted and exposed to air for 1 minute (Little *et al.*, 2020). This method has been found to elicit the same MMR results as other chase methods (e.g. a chase to exhaustion) while having greater statistical power (Little *et al.*, 2020). Fish were then placed in respirometers and MO_2 measurements were taken continuously over 20 hours. Shade cloth was placed over the respirometer tanks to minimize disturbance and direct sun exposure during MO_2 measurements. For five fish at Piru, MMR measurements were compromised and MMR was re-measured the following day.

After 20 hours of respirometry, fish were removed from the chambers, weighed, and measured for fork length and standard length. Bacterial respiration was measured for 1 hour by measuring MO_2 in each chamber after the fish were removed and was determined to be negligible.

Upper thermal tolerance was assessed using CT_{MAX} tests conducted post-respirometry. At this point, the fish had been exposed to their treatment temperatures for ~40 hours. To account for possible effects of diurnal light cycle on CT_{MAX} (Healy and Schulte, 2012), all fish were tested at the same time of day. Fish were transferred to an aerated cooler containing water at their corresponding holding temperature for the time of day of the

experiment (see Table 1). Dissolved oxygen in the cooler remained >90% throughout CT_{MAX} testing. Water temperature was increased by 0.3°C per minute (Myrick and Cech, 2000) by circulating heated water through a stainless-steel coil and dipping the coil in and out of the water. Temperature at the moment each fish lost equilibrium was recorded, at which point the individual fish were immediately transferred to a bucket and recovered back to ambient stream temperature. After CT_{MAX} testing and recovery, fish were released back to their collection site.

2.3.3 Data Analysis and Statistics

All data analysis was conducted using RStudio version 1.4.423. All statistical tests used a significance level of $\alpha = 0.05$. First, decreases in dissolved oxygen from each MO_2 measurement cycle were plotted and inspected visually for linearity. For MMR measurements, regressions with an R^2 of ≥0.8 were included in our analysis (Piru: n = 25, Arroyo Seco 2019: n = 23). For all other measurements, regressions with an R^2 of less than 0.75 were excluded from further analysis. Fish with ≥75% of regressions with an R^2 above 0.75 were included in RMR analysis (Piru: n = 19, Arroyo Seco: n = 18). MO_2 ($mgO_2\ kg^{-1}\ min^{-1}$) for each measurement cycle was calculated using the slope of each regression using the following equation: $MO_2 = (\text{slope} * (v_R - m))/m * (m/0.03)^{(1\text{-scaling exponent})}$, where v_R is the respirometer volume and m is the fish body weight in kg. All MO_2 measurements were scaled to a common body size of 30 g using data-generated scaling exponents of 0.79 for MMR and 0.67 for all other MO_2 measurements. These exponents were generated by fitting linear regressions to the log-log relationship between body size and MO_2 (Fig. A2).

MMR was calculated using the steepest 120 s slope from the first measurement cycle (taken immediately after the chase; Little *et al.*, 2020). All MMR measurements occurred

during the first measurement cycle post-chase. Both SMR and RMR are used to quantify the oxygen uptake of a fish at rest. We define SMR as the minimum oxygen uptake rate across the 20-hour respirometry period (Chabot, Steffensen and Farrell, 2016). Standard metabolic rate (SMR) was calculated by averaging the lowest ten MO_2 measurements (after excluding the five absolute lowest (Chabot, Steffensen and Farrell, 2016). RMR includes all MO_2 measurements taken while the fish is at rest post-exercise recovery (Fig. 2.3). All measurements taken after MO_2 reached 20% of SMR (after EPOC was complete, typically ~5-8 hours after MMR) were considered "post-recovery" and included in the RMR calculations (Fig. A1). By defining RMR this way, we were able to measure the oxygen uptake rate of at-rest fish at each temperature the fish experienced during their respective diurnal temperature cycles to account for possible effects of this natural acute temperature change on resting metabolism.

Due to the diurnal temperature fluctuations, we obtained RMR measurements for fish at eight different temperatures at Piru and six different temperatures at Arroyo Seco (with temperatures rounded to the nearest 1 °C). To be included in the RMR estimates for a given temperature, individual fish were required to have at least 3 RMR measurements taken at that temperature. In order for a given temperature to be included in our RMR analysis, at least three fish had to have RMR estimates at that temperature. The effect of temperature on RMR and on *ln*(RMR) was modeled for each population using the R package lme4 (Bates *et al.*, 2015) to fit linear mixed effects models with acclimation treatment group included as an additional fixed effect and individual fish ID included as a random effect. The best fit models were selected using BIC. Type III ANOVAs (R package: "car"; Fox and Weisberg, 2018) revealed that the interaction between the fixed effects was insignificant for both

populations, so the interaction term was dropped and type II ANOVAs were used to test these relationships. The effect of acclimation treatment was also found to be insignificant, so this effect was dropped from the final models. Student's t-tests were used to compare RMR measurements at each of the four common temperatures between populations.

We calculated aerobic scopes for each fish using RMR measurements taken at temperatures as close as possible to MMR temperatures. While we are aware that using RMR instead of standard metabolic rate (SMR) could underestimate aerobic scope, fish in the present study recovered quickly and were very calm in the chambers with minimal spontaneous activity. RMR values were thus very close to SMR values, and underestimates of aerobic scope are likely negligeable. Absolute aerobic scope (AAS) was calculated by subtracting RMR from MMR. Factorial aerobic scope (FAS) was calculated by dividing MMR by RMR. MMR, AAS, and FAS results were analyzed using one-way ANOVAs to compare across temperature treatments for each population. CT_{MAX} results were analyzed using one-way ANOVAs and post-hoc Tukey's tests to compare between acclimation treatments for each population and using Mann-Whitney U tests to compare between populations for two common temperature treatments (Piru Ambient & Arroyo Seco +3°C; Piru +3°C & Arroyo Seco +5°C).

MO_2 measurements taken before fish reached 20% of SMR were considered part of each fish's recovery period (Fig. A1). We first assessed exercise recovery by quantifying the duration and magnitude of EPOC. In other words, we measured the time it took for each fish to reach 20% of SMR after MMR was measured and the amount of oxygen consumed during this time using methods described in Zhang *et al.* (2018). EPOC magnitude and duration were compared between acclimation groups for each population using one-way

ANOVAs and between populations using Student's t-tests. We then assessed MO_2 as a percent of MMR (%MMR) over the first hour of exercise recovery at 10, 20, 30, 40, 50, and 60 minutes after the chase. Changes in %MMR over time were assessed using a linear mixed effect model with the variables time, population, and temperature treatment as fixed effects and fish ID as a random effect. A type III ANOVA revealed that there were no significant interactions between fixed effects, so the interaction terms were dropped and a type II ANOVA was used to analyze this relationship. There was also no significant effect of temperature treatment on changes in %MMR over time, so this effect was dropped from the final model. Post-hoc analysis was conducted using least-squares means (R package: "emmeans"; Lenth *et al.*, 2019).

2.3.4 Habitat Temperature Data

Stream temperature was recorded using HOBO Waterproof Bluetooth Pendant Temperature Data Loggers (Onset Computer Corporation, Bourne, USA), HOBO Dissolved Oxygen Loggers (Onset Computer Corporation, Bourne, USA), and miniDOT Loggers (Precision Measurement Engineering, Vista, USA). All loggers recorded water temperature measurements continuously every 10 minutes (miniDOT Loggers) or 15 minutes (HOBO Pendants and Dissolved Oxygen Loggers) minutes for the duration of their deployment. Temperature data were obtained from Piru Creek from June 2019-June 2022. Initial loggers placed at Arroyo Seco during the time of this study were destroyed in a fire and subsequent debris flow. Therefore, temperature data was obtained from Arroyo Seco from May-November 2022. To compare Piru Creek to other streams within the Santa Clara River watershed, we collected temperature data from Lion Creek (34.54338 °N, -119.16372 °W) and Piedra Blanca Creek (34.58515 °N, -119.16543 °W) in 2021-2022 and we obtained

open access daily temperature summary data from the NorWesT Reigonal Database (Chandler *et al.*, 2016) for Santa Paula Creek (34.42763 °N, -119.09089 °W) from 2008-2011 and Sespe Creek (34.44492 °N, -118.92715 °W) from 2008-2013. Additionally, we collected temperature data from 10 additional trout-bearing streams throughout the Los Padres National Forest between June and October of 2019 to compare thermal regimes with Piru Creek and Arroyo Seco. Temperature maxima and minima were considered the maximum and minimum daily stream temperatures that occurred during 3 or more days per sample year during the summer months (June-September). All temperature measurements were rounded to the nearest degree for this analysis.

2.4. Results

2.4.1 Critical Thermal Maximum

CT_{MAX} was higher, but less plastic for *O. mykiss* from Piru Creek compared to the Arroyo Seco. At Arroyo Seco, mean CT_{MAX} ranged from 27.5-29.8 °C and increased significantly with acclimation temperature (Fig. 2.2, panel B; Table A1). At Piru Creek, CT_{MAX} averaged 31.0 °C and did not differ among acclimation treatments (Fig. 2.2, panel A; Table A1). When comparing common acclimation treatments between populations, Piru Creek *O. mykiss* had a higher CT_{MAX} than Arroyo Seco *O. mykiss* (Table A2).

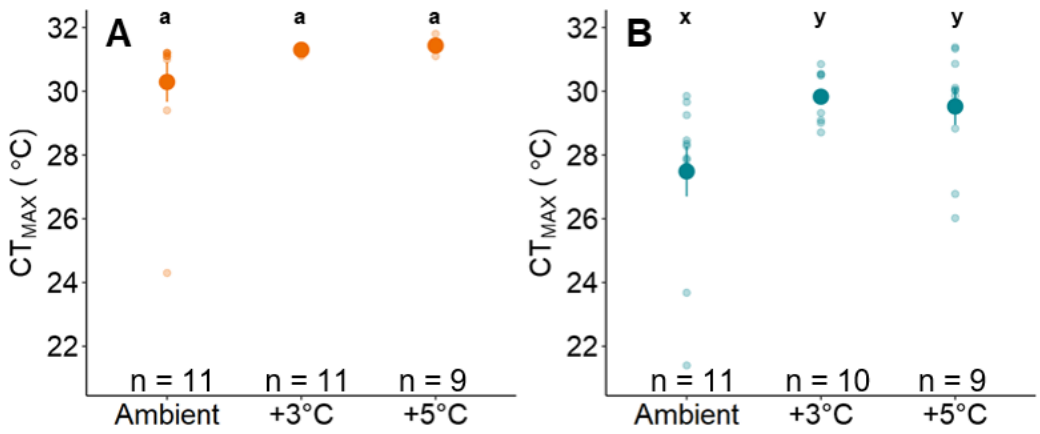

Figure 2.2. Critical thermal maximum (CT_{MAX}) for each temperature treatment at Piru Creek (panel A) and Arroyo Seco (panel B). Bold lines indicate median CT_{MAX} for each treatment. Differing letters indicate statistically significant differences within populations (one-way Anova; $p<0.05$; Piru Creek: a,b; Arroyo Seco: x,y).

2.4.2 Oxygen Uptake Rate

Following the chase, all fish exhibited a similar oxygen uptake rate (MO_2) profile characterized by a rapid drop in MO_2 after maximum metabolic rate (MMR) followed by a slow decline in MO_2 to a stable, baseline level. Within acclimation treatments, resting metabolic rate (RMR) remained stable despite fluctuating temperatures (Fig. 2.3).

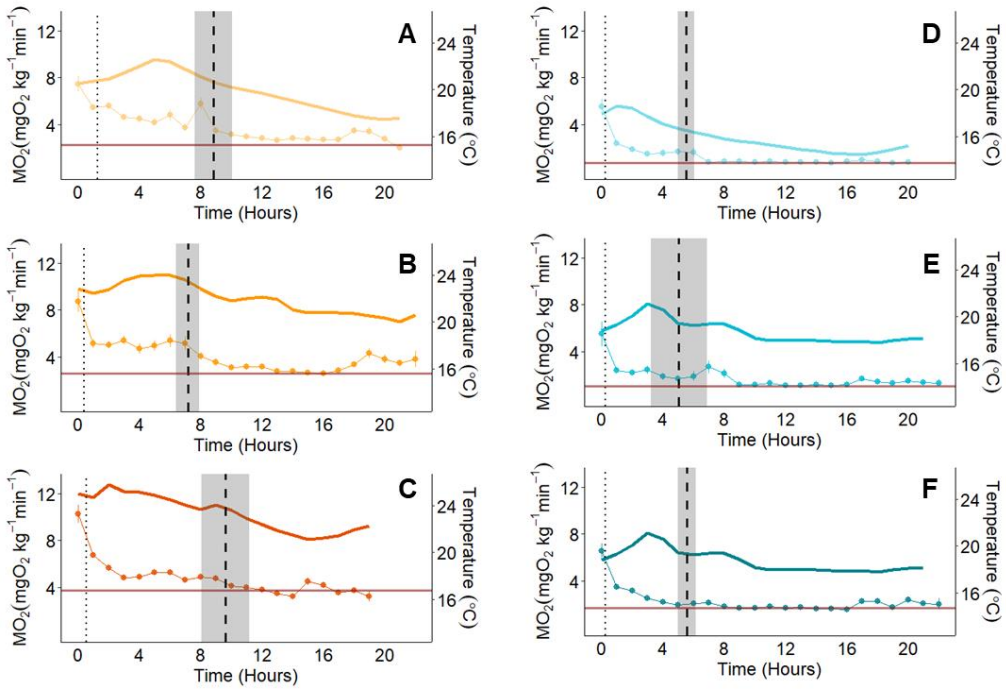

Figure 2.3. Mean ± SEM hourly oxygen uptake rate (MO_2) and temperature over the duration of the experiment for each temperature treatment (panels A and D: Ambient, panels B and E: +3°C, panels C and F: +5°C) and population (Piru: orange, Arroyo Seco: blue). The temperature profile that occurred during the experiment is indicated by the solid-colored lines. Each point represents the mean ± SEM hourly MO_2 for the fish in each respective group. The data point at time 0 was taken immediately after the chase and represents Maximum Metabolic Rate (MMR). Mean standard metabolic rate (SMR; red horizontal lines), mean time to reach 50% of MMR (MMR50; vertical dotted lines) and the mean ± SEM time to reach 20% of SMR (duration of recovery, i.e., duration of EPOC; vertical dashed lines) are indicated.

For both populations, MMR increased with increasing temperature (Table 1). MMR was not measured at common temperatures between populations so we compared MMR measurements between similar temperatures: Arroyo Seco at 19 °C and 21 °C and Piru Creek at 20 °C. There were no significant differences in MMR between populations at these temperatures (df = 2, F = 0.068, p = 0.935).

RMR increased exponentially with increasing temperature at both populations (Fig. 2.4; model selection Table A3). RMR was measured at four common temperatures between the two populations (18 °C, 19 °C, 20 °C, and 21 °C; Fig. 2.5, Table A4). At Piru Creek, RMR

of *O. mykiss* was 1.6-2.3 times greater than RMR in trout from Arroyo Seco at all common temperatures (Table A4; Fig. 2.5). Notably, between 18 °C and 21 °C, RMR of Arroyo Seco *O. mykiss* had a greater Q_{10} (3.95) compared to Piru Creek (2.03) signifying that RMR of Arroyo Seco trout is more temperature sensitive than RMR of Piru Creek trout.

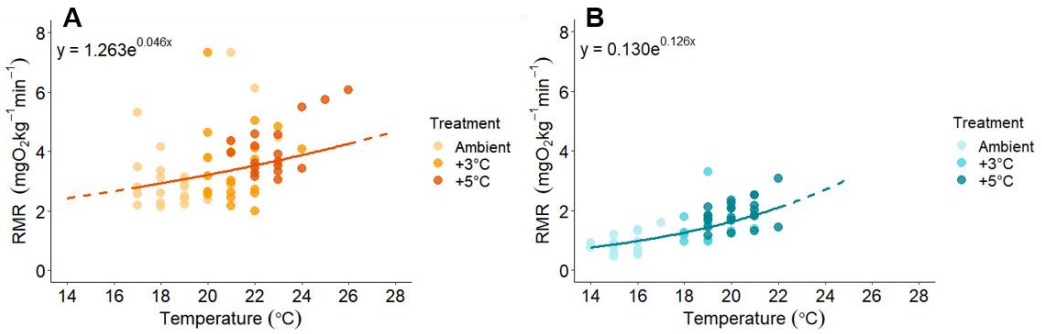

Figure 2.4. Resting metabolic rate (RMR) measurements for each temperature at Piru Creek (panel A) and Arroyo Seco (panel B). Solid lines indicate predicted values within the range of measured RMR temperatures and dashed lines indicate predicted values outside of the range of measured RMR temperatures.

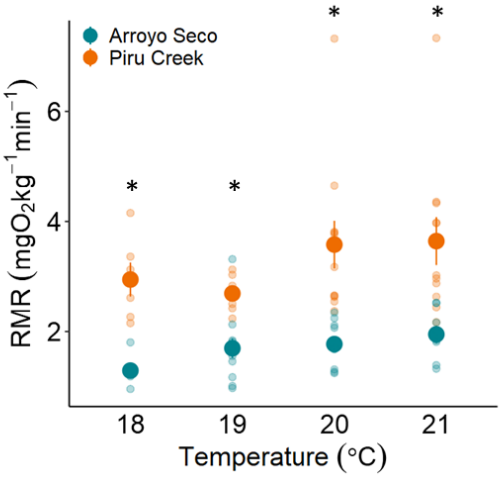

Figure 2.5. Resting metabolic rate (RMR) for each population at 4 common temperatures. Asterisks indicate statistically significant differences between populations at each temperature (t-test or Welch's t-test; $p<0.05$).

Absolute aerobic scope (AAS) was not significantly different across test temperatures for either population (Piru Creek: df = 2, F = 1.652, p = 0.227; Arroyo Seco: df = 2, F =

1.742, p = 0.214; Table 1). The relationship between temperature and factorial aerobic scope (FAS), however, was population dependent. At Piru Creek, FAS did not vary significantly with temperature. At Arroyo Seco, FAS declined significantly with increasing temperature (Table 1, Fig. 2.6). At all test temperatures, FAS remained above 3 at both populations. Temperatures where FAS ≤ 3 are thought to be associated with feeding and growth limitations (Eliason, Higgs and Farrell, 2008; Farrell, 2016; Adams *et al.*, 2022; Eliason, Van Wert and Schwieterman, 2022), because RMR tends to increase by 2-3 times during digestion. We therefore aimed to identify the temperatures where FAS = 3 for each population. Based on a linear model fit from these data, we predict that FAS would reach 3 for the Arroyo Seco population at ~23 °C (Fig. 2.6). This metric could not be estimated for the Piru Creek population because we were unable to fit a model describing the relationship between FAS and temperature.

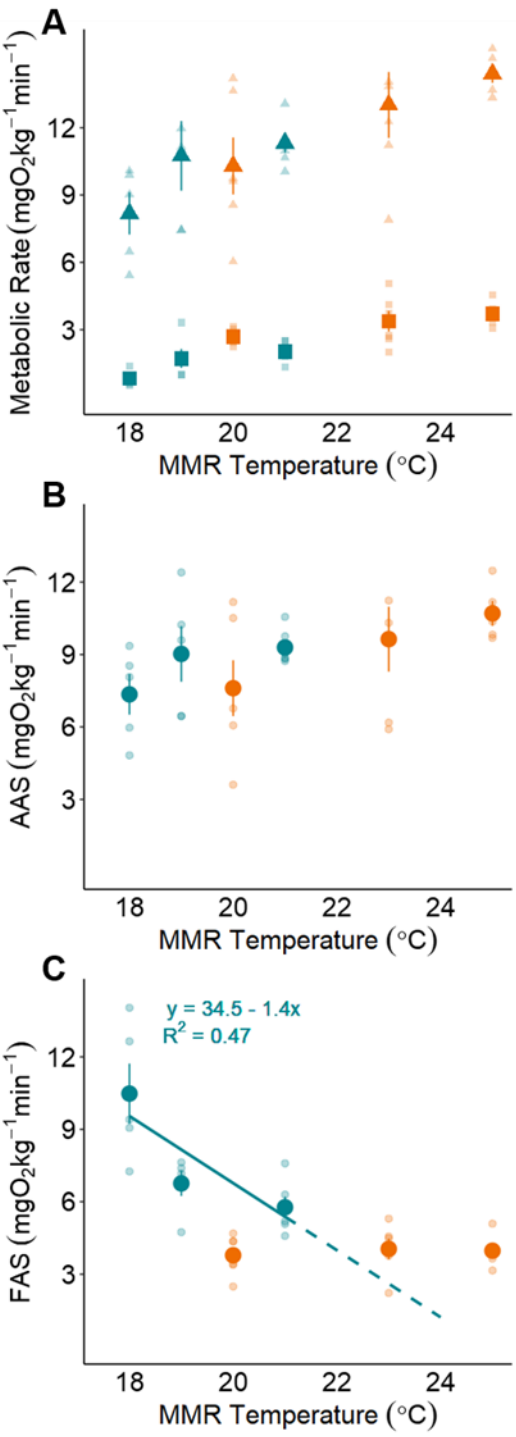

Figure 2.6. Absolute aerobic scope (AAS; panel A) and factorial aerobic scope (FAS; panel B) for individual fish (small points) and mean ± SEM FAS for each temperature treatment (large points) for Piru Creek (orange symbols) and Arroyo Seco (blue symbols). Modeled relationship between temperature and FAS for Arroyo Seco is represented by the line and the corresponding equation.

2.4.3 Recovery

The magnitude and duration of excess post-exercise oxygen consumption (EPOC) were higher for the Piru Creek population than for the Arroyo Seco population (magnitude: t = -5.47, df = 22.337, p < 0.001; duration: t = -3.54, df = 30.58, p = 0.001). EPOC magnitude averaged 416.97 ± 35.56 mg O_2 kg^{-1} for Arroyo Seco *O. mykiss* and 1066.07 ± 111.83 mg O_2 kg^{-1} for Piru Creek *O. mykiss*. EPOC duration averaged 517.0 ± 44.3 min for Piru Creek and 325.53 ± 31.1 min for Arroyo Seco (Fig. 2.3). There was no significant difference in EPOC duration or magnitude between temperature treatment groups for either population (Table 2). During the first hour of recovery, MO_2 decreased significantly during the first 20 minutes of recovery and then plateaued for the next 40 minutes of recovery (Fig. 2.7). At all post-MMR time points (10, 20, 30 ,40, 50 and 60 min), MO_2 for Arroyo Seco *O. mykiss* was at a lower %MMR than Piru Creek (i.e., Arroyo Seco trout recovered their aerobic capacity more quickly than Piru Creek trout; Table A5).

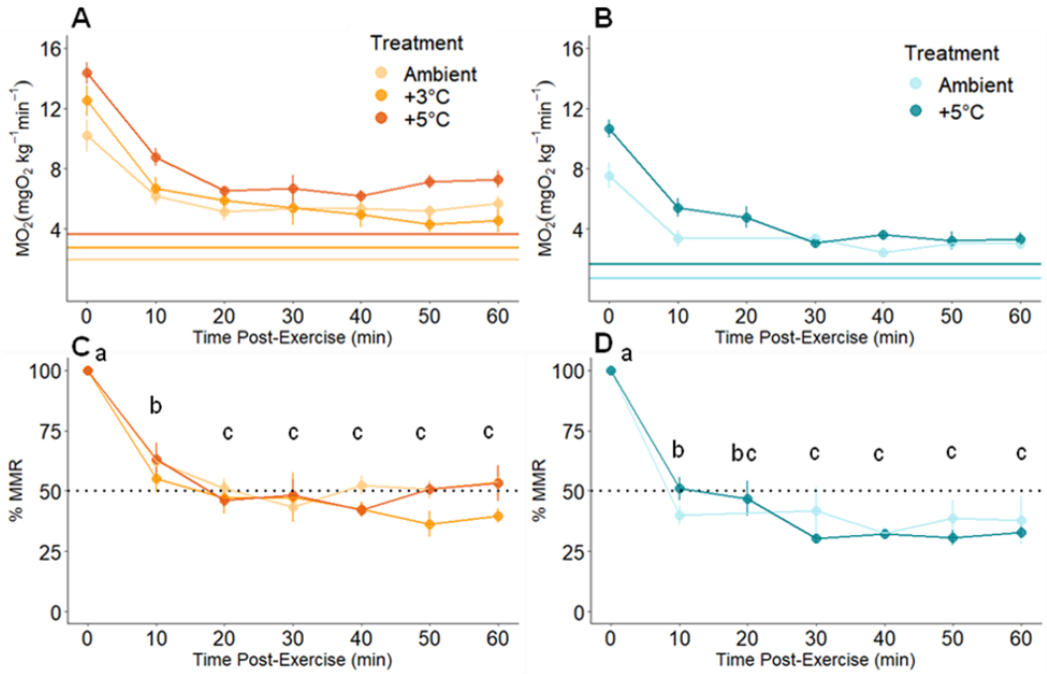

Figure 2.7. Oxygen uptake rates (MO_2) over the first hour of recovery from exhaustive exercise for Piru Creek (A and C) and Arroyo Seco (B and D) fish. Panels A and B show MO_2 values with horizontal lines indicating the mean standard metabolic rate (SMR) for each temperature treatment. Panels C and D show MO2 as a percentage of maximum metabolic rate (MMR). Data is presented as mean ± SEM, lowercase letters indicate statistical differences between time points (repeated measures Anova; $p < 0.05$).

2.4.4 Stream Temperatures, Thermal Safety Margins and Functional Warming Tolerance

From 2019 to 2022, we measured a maximum stream temperature of 25 °C at Piru Creek. *O. mykiss* Piru Creek therefore had a TSM of 6 °C. We cannot calculate FWT for Piru Creek *O. mykiss* because their FAS never got below 3 for any of our treatments and we could not fit a linear regression to the data to approximate the FAS=3 temperature. We do not have continuous temperature data for Arroyo Seco during the time that we conducted these experiments because a fire and debris flow washed away our temperature loggers. During the experiments in September of 2019, the maximum stream temperature was 18 °C. After this fire and debris flow, the stream habitat is now shallower with less riparian cover and temperatures are expected to be warmer than in 2019 (stream temperatures reached 23 °C in September of 2022). In the summer of 2022, we measured a maximum stream temperature of 23 °C at Arroyo Seco. Based on the mean CT_{MAX} measured for the 19-24°C treatment, Arroyo Seco *O. mykiss* have a TSM of 6.5 °C. Based on the modeled FAS = 3 temperature for this population, we estimate that these fish have FWT of 0 °C, meaning that they currently experience temperatures at the edge of their functional thermal limits. More temperature data will be required to determine if Arroyo Seco will consistently reach or exceed 23 °C in future years.

Water temperature regimes are highly variable within and between watersheds in the Los Padres National Forest (Fig. 2.8 & S3, Table A6). Based on long term temperature data from our data loggers and from open access data collected in the past, stream temperatures can get as low as 5°C during the winter and up to 30°C in the summer throughout the forest (Fig. 2.8). In the summer, some streams remain stable throughout the day with daily temperature fluctuations of 0.3-3 °C (e.g., Lion Creek, Fig. 2.8 & S3; Davy Brown Creek, Fig. A3; Bear Creek, Fig. A3) while others undergo daily temperature fluctuations of up to 10°C (e.g., Santa Paula Creek, Fig. 2.8; Matilija Creek, Fig. A3).

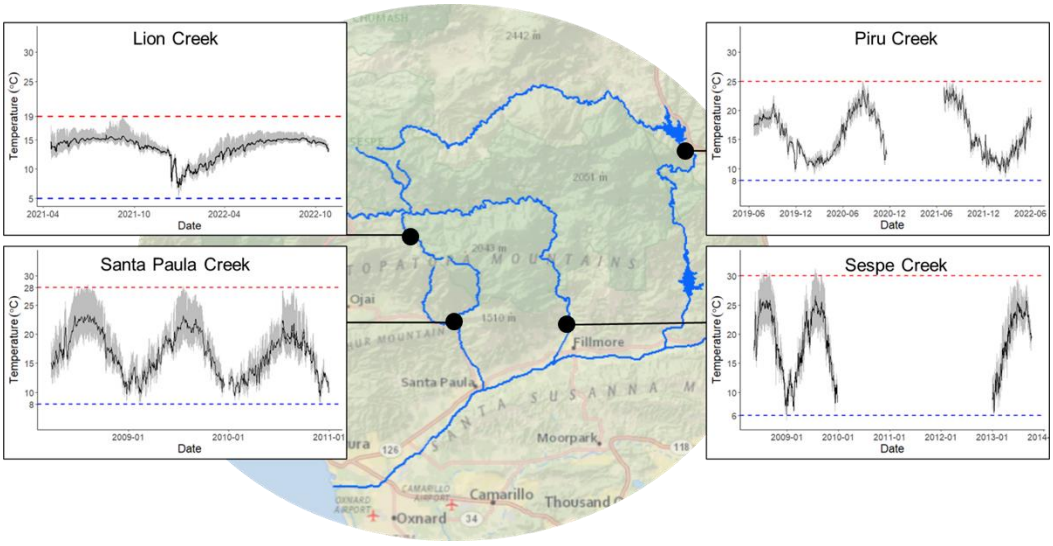

Figure 2.8: Locations of temperature data loggers in the Santa Clara River watershed with temperature data plotted for each location. In the temperature plots, black lines indicate mean daily temperatures, gray shading represents daily temperature ranges, red dashed lines indicate maximum stream temperatures, and blue dashed lines indicate minimum stream temperatures during the time data was recorded.

2.5. Discussion

In this study we tested the functional and critical thermal tolerance of two genetically distinct (Abadía-Cardoso *et al.*, 2016) wild *O. mykiss* populations near the southern limit of their native range (Fig. 2.1). Southern California trout populations experience high temperatures and have undergone local declines from habitat loss and degradation

(Boughton *et al.*, 2005). We discovered that *O. mykiss* populations differ in both functional and critical thermal tolerance over a brief time scale of thermal exposure, and current local maximum temperatures exceed functional limits for the Arroyo Seco River population. Moreover, water temperatures in trout-bearing streams throughout Southern California can reach exceptionally high temperatures (e.g., 25-30°C), and trout in this part of their range are likely confined to a limited number of thermal refugia (e.g. Lion Creek, Davey Brown Creek; Figure S3).

Southern *O. mykiss* have unique thermal physiology. We specifically chose a brief overnight temperature exposure duration representing a short-term thermal stress for these fish because it is ecologically relevant given the natural stochasticity and speed of temperature change in these systems. Full physiological acclimation to a new temperature regime can take several weeks (Stewart *et al.*, 2023) and thus maximal acclimation capacity could not be assessed here. Even still, there is evidence that salmonids can acclimate rapidly on timescales that are similar to rates of temperature change in their wild habitats (e.g., brown trout swimming performance, 48 hours; Macnutt *et al.*, 2004; Arctic char heartrate, 3 days; Gilbert, Adams and Farrell, 2022). Given the rapid temperature changes experienced in these fringing aquatic environments, fish in the wild would rarely have the time and exposure conditions (i.e. several weeks under a particular thermal condition) to fully physiologically acclimate. In agreement with our hypotheses, we found evidence of population specificity of thermal tolerance, where the population from the warmer stream (Piru Creek) has higher thermal tolerance compared to the population from the historically cooler stream (Arroyo Seco). This is unsurprising given that hatchery strains of this species have exhibited elevated thermal tolerance when exposed to warm temperatures over

generations (Chen *et al.*, 2015; Adams *et al.*, 2022). Piru Creek *O. mykiss* have a higher, but less plastic upper lethal temperature limit (CT_{MAX}) compared to the Arroyo Seco population. Moreover, we estimated that Arroyo Seco *O. mykiss* would reach a functional thermal threshold (FAS = 3) at ~23 °C, while Piru Creek *O. mykiss* did not show evidence of a decline in FAS at temperatures up to 25 °C, indicating a higher functional thermal limit for the Piru Creek population. Piru Creek *O. mykiss* also appear to have a higher functional thermal tolerance compared to a warm-adapted population inhabiting the California Central Valley where FAS = 2 at 23 °C (Verhille *et al.*, 2016) and compared to a hatchery strain that has undergone generations of selection for high thermal tolerance in Western Australia, where FAS ≤ 1.8 at 25 ºC (Chen *et al.*, 2015). Both *O. mykiss* populations in the present study have high functional thermal tolerance compared to hatchery-raised *O. mykiss* in more northern parts of their wild range. Myrick & Cech (2000) observed diminished growth rates above 19 °C in two strains of *O. mykiss* from Northern California. Many more studies have been conducted on hatchery *O. mykiss* in British Columbia, Canada and at similar latitudes in Europe. When acutely exposed to elevated temperatures, Anttila et al. (2013) found optimal aerobic and cardiac performance at 19 °C and a significant decline in performance at 23 °C in *O. mykiss*. Heath & Hughes (1973) detected diminished heart function and venous oxygen deficiency in *O. mykiss* acutely exposed to 24-25 °C. Following acclimation, Jain & Farrell (2003) observed impaired exercise recovery at 15 °C, Farrell et al. (1996) found diminished cardiac function at 18 °C and 22 °C, and Taylor et al. (1996) detected reduced aerobic swimming capacity at 18 °C. There appears to be a latitudinal gradient of functional thermal tolerance for *O. mykiss* and that Piru Creek exhibit the highest functional thermal limits of any measured population.

RMR of Piru Creek and Arroyo Seco *O. mykiss* did not change significantly based on time of day or diurnal temperature (Fig. 2.3). In other words, the baseline oxygen requirements of these fish remained stable throughout daily temperature fluctuations of 4-5 ºC. This contrasts with other salmonid species such as Atlantic salmon (Tunnah, Currie and MacCormack, 2017) and coastal cutthroat trout (Anlauf-Dunn, Kraskura and Eliason, 2022) that show clear fluctuations in RMR during ecologically relevant temperature fluctuations. These fish likely possess adaptations that allow them to withstand daily temperature fluctuations without major changes in oxygen requirements. This could be advantageous because it allows for unrestricted aerobic scope (and therefore a better ability to flee predators, hunt for food, increase growth rates and fecundity, etc.) during the warmest times of day. The consistently higher Piru Creek RMR (almost double that of Arroyo Seco fish) was an unexpected result given that warm-adapted fish populations tend to display lower RMR due to thermal compensation (Sandblom *et al.*, 2016; Healy and Schulte, 2012; Mcbryan *et al.*, 2016). This suggests that the high thermal tolerance of the Piru Creek population is conferred by strategies that are energetically costly to maintain. Since peak temperatures are stochastic and relatively short-lived in this system (Fig. 2.1), it is possible that temperature-based selection is acting on pathways involved in acute thermal tolerance (e.g., heat shock protein production). Future studies should investigate the mechanisms for this apparent tradeoff in thermal tolerance and resting oxygen requirements.

Anaerobic activity (i.e., maximal exercise) is common in juvenile salmonids, as they continuously avoid predators and compete for space and food. Fast recovery allows them to resume their normal functions quickly after these encounters and the fast recovery times observed in this study (~10-20 minutes) are likely advantageous for this life stage. Although

exercise recovery was unimpacted by test temperatures in the present study, all of our recovery metrics indicate that Piru Creek *O. mykiss* have greater costs associated with exercise recovery compared to Arroyo Seco *O. mykiss*. After MMR, the Arroyo Seco population recovered a greater proportion of their aerobic capacity in a shorter period of time than the Piru Creek population (Fig. 2.7). The Arroyo Seco population also had a lower EPOC magnitude and duration compared to the Piru Creek population.

CT_{MAX} experiments were conducted after fish had been exposed to their respective temperature regimes for ~40 hours (i.e. 20 h holding period plus 20 h respirometry perdio). Arroyo Seco *O. mykiss* CT_{MAX} increased with increasing temperatures, indicating that these fish are able to adjust their upper thermal limits rapidly. Prologued exposure to these temperature regimes may confer a different response, potentially further increasing CT_{MAX} if the acclimation response was incomplete after 40 h or potentially even decreasing CT_{MAX} if prolonged high temperature exposure had detrimental impacts on fish performance. Even still, this study demonstrates the novel result these fish are capable of rapidly adjusting their thermal tolerance in response to acute temperature changes in this system. By contrast, Piru Creek *O. mykiss* did not adjust their CT_{MAX} based on test temperatures. This indicates that the Arroyo Seco population has greater plasticity in upper critical thermal limits compared to the Piru Creek population. Piru Creek CT_{MAX} matches the maximum CT_{MAX} measured consistently for this species (31 °C; Recsetar *et al.*, 2012; McKenzie *et al.*, 2021), indicating that this population has reached its proverbial "concrete ceiling" for upper thermal tolerance (Sandblom *et al.*, 2016). These results are consistent with the commonly observed tradeoff between upper thermal tolerance and thermal plasticity (Comte and Olden, 2017; Scheuffele, Rubio-Gracia and Clark, 2021).

Our results suggest that these two populations will be impacted by rising temperatures in different ways. While Piru Creek *O. mykiss* showed no evidence of limited AAS, FAS, or recovery metrics with increasing temperatures, this population will require sufficient food and oxygen to support their relatively high oxygen requirements for baseline metabolism and exercise recovery. This population will likely be more vulnerable to extirpation from warming if warming coincides with food limitation or low oxygen availability (Auer *et al.*, 2020; Auer *et al.*, 2015; Marcek *et al.*, 2020). Southern California streams are known to exhibit substantial changes in water levels and invertebrate assemblages during drought years (Cooper, Kristan and John, 2021), so Piru Creek and nearby populations may very well be at risk of experiencing these stressors. But, the reduced Q_{10} of RMR across temperatures allows the Piru Creek population to maintain a steady sufficiently high FAS (≥ 3) across a broad range of temperatures (20 ºC to 25 ºC). On the other hand, the Arroyo Seco population is more likely to face a limitation of FAS with increasing temperatures and likely reach a critical threshold of FAS = 3 around 23 °C (Fig. 2.6), a temperature that they now experience after a recent fire and debris flow (Fig. 2.1). Notably, we did not push either population to their functional thermal limits with our experimental temperatures in this study (it was a priority to release all the fish back to the wild and thus test temperatures were intentionally kept below hazardous levels). Further, we do not know whether population differences are due to local adaptation, developmental plasticity and/or acclimatization to local environmental conditions because we did not conduct a controlled, common garden type experiment.

Piru Creek *O. mykiss* have a TSM of 6 °C and maintained a high FAS (>3) at 25 °C, suggesting they still had sufficient aerobic capacity to thrive at maximum habitat

temperatures and are not at immediate risk of extirpation from warm temperatures. The warmest temperature we measured in this stream was 25 °C, and the stream only reached this temperature during the hottest time of day and dropped back down at night. However, it is important to note that this does not necessarily reflect the status of populations in other parts of this watershed (Fig. 2.8). Piru Creek is located directly below a reservoir and receives constant cold-water inflow year-round. In Santa Paula Creek, a creek in the Lower Santa Clara River watershed within the same subbasin as Piru Creek, *O. mykiss* habitat temperatures have been measured up to 33 °C, exceeding our measured CT_{MAX} temperatures (Sloat and Osterback, 2013). Sloat and Osterback (2013) observed *O. mykiss* feeding up to 29 °C, but they were absent in the same pools when temperatures exceeded 30 °C. In Sespe Creek, another tributary in this watershed, trout have been observed over-summering in isolated pools that reach 28 °C during the hottest time of day, although thermal stratification provides cool refuges from warm surface waters (Matthews and Berg, 1997). In another case study in this watershed (Piedra Blanca Creek), *O. mykiss* were observed dead in a drying pool that measured 28 °C (Desforges *et al.*, 2023), while upstream temperatures reached a maximum of 22 °C from June-October of the same year and never fluctuated by more than 2.5 °C per day. This indicates that although Piru Creek *O. mykiss* do not appear to be in imminent danger of extirpation from warming, the same is not guaranteed for other populations in the same watershed.

Based on stream temperatures during this study (14-18 °C in 2019) Arroyo Seco *O. mykiss* appeared to be buffered against reaching the FAS= 3 threshold of 23 °C. Unfortunately, the Dolan Fire (2020) subsequent debris flow has since altered the habitat in this stream and temperatures reached 23 °C during the summer of 2022 (Fig. 2.1).

Accordingly, Arroyo Seco *O. mykiss* currently have a TSM of 6.5 °C and a FWT of 0 °C, indicating that they experienced functionally limiting temperatures in 2022. However, vulnerability is a combination of temperature exposure, physiological sensitivity, and adaptive capacity. Here, we tested the physiological sensitivity of these fish under ecologically relevant temperature exposures, but their full acclimation capacity remains uncertain due to our short exposure times. It is possible these fish could improve thermal tolerance and aerobic performance through physiological acclimation processes if they experience these temperatures for longer time periods (i.e., days or weeks). We are also uncertain about the capacity of this population to adapt to these conditions over generations. Both acclimation and adaptation could protect this population from adverse effects of habitat warming.

Stream environments in Southern California are notoriously stochastic and subjected to stressors such as drought and wildfire and *O. mykiss* populations are at risk for potential co-occurring stressors such as hypoxia (Matthews and Berg, 1997), food limitation (Boughton *et al.*, 2007), and predation by non-native species (Katz *et al.*, 2013). While populations such as Piru Creek are not in immediate danger of experiencing their thermal limits, they may be at risk of experiencing these other stressors, which can interact with temperature to constrain growth, summer survival, and life history expression (Grantham *et al.*, 2012; Myrvold and Kennedy, 2015; Benjamin *et al.*, 2013). Additionally, other southern *O. mykiss* populations risk exceeding upper functional thermal limits in the wild and rely on small pockets of refugia from lethal temperatures and complete habitat drying. Our temperature data from the summer of 2019 indicate that there are cool, stable refugia within all four watersheds that we sampled (Fig. A3). However, manmade barriers and extreme seasonal

drying often prevent fish from moving between tributaries of the same watershed, especially in the summer (Boughton *et al.*, 2009). There is thus a limited ability for fish to behaviorally select their environment. Indeed, genetic analysis of southern *O. mykiss* populations has indicated that tributaries within the same watershed (including Piru Creek, Lion Creek, Sespe Creek, and Santa Paula Creek from the Santa Clara River watershed) are genetically distinct from one another and it is unlikely that they have interbred with one another in the recent past. Our results reinforce the need to integrate population-specific thermal physiology with habitat temperature data in order to understand which populations are most in need of management interventions to improve summer survival (e.g., riparian vegetation restoration to reduce water temperatures, protection from angling, and invasive species removal). Overall, very little is known about the stream temperature heterogeneity, thermal tolerance, or movement for *O. mykiss* at their range limit. It is critical that future studies continue to collect this information for this species as well as other species of conservation concern in order to most effectively protect and restore valuable fish stocks in a changing climate.

The present study tested the thermal tolerance of two populations of wild *O. mykiss* inhabiting their southern range limit by measuring aerobic performance and upper critical thermal tolerance of fish held and tested at diurnally fluctuating temperatures closely mimicking their natural environment. Our data revealed population-specific functional thermal limitations. The population inhabiting the historically cooler stream (Arroyo Seco) already encounters temperatures that limit aerobic scope. The population inhabiting the warmer stream (Piru Creek) appears to be more resilient to temperature increase, but will need to consume enough food and oxygen to maintain high resting oxygen uptake

requirements. Beyond these two populations, several other trout-bearing streams throughout Southern California routinely exceed 25 °C during summer months, indicating that other nearby populations may be more susceptible to warming compared to the Piru Creek population. Taken together, these results reveal population-specific mechanisms of vulnerability to climate change and potential for increased resiliency to thermal stress at the southern range limit. As stream temperatures continue to warm, the survival of fishes inhabiting their range limits will depend on the thermal properties of individual streams and the ability of populations within to physiologically adjust. This highlights the need for population-specific conservation and management strategies, especially broadly distributed fish species that occupy a range of thermal habitats.

Population	*Group*	*Holding Temperature Range (°C)*	*MMR Temperature (°C)*	*n (MMR)*	*MMR*	*n (SMR, RMR, AAS, FAS)*	*SMR*	*RMR Temperature (°C)*	*RMR*	*AAS*	*FAS*
Piru Creek	Ambient	17-22	20	8	10.19 ± 1.06^{a}	6	2.37 ± 0.16^{a}	19	2.69 ± 0.15	7.60 ± 1.16	3.78 ± 0.34
	+3°C	20-24	23	9	12.71 ± 1.04^{ab}	6	2.42 ± 0.29^{a}	22	3.38 ± 0.46	9.64 ± 1.34	4.05 ± 0.453
	+5°C	21-26	25	8	14.34 ± 0.72^{b}	5	3.23 ± 0.16^{b}	23	3.70 ± 0.26	10.70 ± 0.51	3.97 ± 0.32
Arroyo Seco	Ambient	14-19	18	10	7.43 ± 0.67^{x}	5	0.76 ± 0.11^{x}	16	0.83 ± 0.15	7.35 ± 0.84	10.48 ± 1.24^{x}
	+3°C	17-21	19	7	10.25 ± 1.15^{y}	5	1.62 ± 0.38^{xy}	19	1.72 ± 0.43	9.03 ± 1.15	6.76 ± 0.52^{y}
	+5°C	19-24	21	8	10.64 ± 0.59^{y}	6	1.94 ± 0.30^{y}	21	2.03 ± 0.19	9.29 ± 0.29	5.77 ± 0.44^{y}

Table 2.1. Oxygen uptake rate data [Maximum metabolic rate (MMR); Standard Metabolic Rate (SMR); Resting Metabolic Rate (RMR) at aerobic scope temperatures; Absolute Aerobic Scope (AAS), Factorial Aerobic Scope (FAS)] for each temperature treatment and population, all values are presented as mean ± SEM. Differing letters indicate statistically significant differences within populations (one-way Anova; $p < 0.05$; Piru Creek: a, b; Arroyo Seco: x, y).

Population	*Acclimation Group*	*n (EPOC)*	*EPOC duration (min)*	*df*	*F-value*	*p-value*	*EPOC magnitude (mgO_2kg^{-1})*	*df*	*F-value*	*p-value*
Piru Creek	Ambient	6	53.1 ± 22.0	2	0.976	0.398	958.82 ± 222.52	2	0.292	0.75
	+3°C	6	20.9 ± 4.9				1048.62 ± 206.55			
	+5°C	7	23.0 ± 5.8				1172.96 ± 180.00			
Arroyo Seco	Ambient	5	14.6 ± 4.4	2	0.14	0.871	358.57 ± 62.84	2	1.741	0.217
	+3°C	4	24.7 ± 3.0				361.02 ± 89.79			
	+5°C	6	22.0 ± 3.4				502.93 ± 51.31			

Table 2.2. Excess post exercise oxygen consumption (EPOC) duration and magnitude (mean ± SEM) from each population as well as one-way ANOVA results ($p < 0.05$) testing for differences between temperature treatment groups within each population.

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Chapter 3: Beyond Latitude: Thermal tolerance and Vulnerability of a Broadly Distributed Salmonid Across a Habitat Temperature Gradient

3.1 Abstract

Salmonid fishes are a focal point of conservation physiology due to their high value to humans and ecosystems and their susceptibility to decline from climate change. A significant challenge in conserving these fishes is that populations of the same species can be locally adapted to vastly different habitats within their wild ranges, and can therefore have unique tolerance or vulnerability to environmental stressors within those habitats. Within the state of Oregon, USA, summer steelhead (Oncorhynchus mykiss) inhabit both cool, coastal waters most typically associated with Pacific salmonids as well as arid, inland environments where temperatures are more extreme. Here, we utilize streamside physiological experiments paired with habitat temperature monitoring to assess the thermal tolerance and vulnerability of 4 populations of summer steelhead from distinct thermal habitats. All populations had unique responses of critical thermal maximum, aerobic scope, and exercise recovery to temperature. Despite populations from warm habitats exhibiting higher thermal tolerance than populations from cooler habitats, summer steelhead from warm habitats appear to be more vulnerable to the physiological consequences of warming based on the extreme temperatures they already experience during the summer. These results demonstrate an example of thermal physiology varying between populations within the same portion of their latitudinal range and highlight the need for habitat-specific conservation strategies for this species.

3.2 Introduction

Aquatic environments face intensifying pressures from the effects of global climate change including increases in average water temperatures, daily and seasonal thermal variability, and the magnitude and frequency of episodic heat waves (Ficke, Myrick and Hansen, 2007; Rijnsdorp et al., 2009; Kaushal et al., 2010; Reid et al., 2019). Warming directly challenges the survival and fitness of fishes inhabiting these environments due to physiological disruptions at the biochemical, tissue/organ, and whole organism levels (Schulte, Healy and Fangue, 2011; Whitney et al., 2016; Little, Loughland and Seebacher, 2020). Physiological thermal tolerance limits and habitat temperature patterns can be used to elucidate the vulnerability of fish species to climate warming. When a species inhabits a broad geographic range, however, genetically distinct populations can experience vastly different thermal conditions and exhibit interpopulation variability in thermal tolerance (Fangue, Hofmeister and Schulte, 2006; Barrett et al., 2011; Eliason et al., 2011; Narum and Campbell, 2015; Zillig et al., 2021). This makes it challenging to understand which populations are most vulnerable to warming and to decide where more active management actions should be taken.

Numerous studies have detected interpopulation variation in thermal tolerance within species of fish, where populations occupying warmer habitats can withstand higher temperatures than populations inhabiting cooler habitats (McKenzie et al., 2021). In some cases, this variation follows a latitudinal gradient, such as in Atlantic killifish (Fundulus heteroclitus) where a subspecies in the warmer, southern portion of the species' range has a higher critical thermal maximum (CTMAX) and mitochondrial oxygen binding capacity than a subspecies inhabiting the cooler, northern part of the range (Fangue, Richards and

Schulte, 2009; Chung et al., 2017). However, intraspecific variation in thermal tolerance can exist on an even finer scale given large enough differences in habitat temperatures with limited gene flow between habitats. Adult sockeye salmon (Oncorhynchus nerka) populations have differing optimal temperature windows for aerobic and cardiac function that closely correspond with their natal stream temperatures within a single watershed (Fraser River) in British Columbia (Eliason et al., 2011). Embryo and juvenile O. nerka in the same system have different optimal rearing temperatures, swimming performance temperatures, and critical thermal limits based on temperatures of their rearing habitats (Chen et al., 2013; Whitney, Hinch and Patterson, 2013; Eliason et al., 2017). European perch (Perca fluviatilis) inhabiting a chronically warm enclosure near a power plant exhibit thermal compensation of resting oxygen uptake and heart rates as well as increased mitochondrial capacities compared to a reference population inhabiting cooler temperatures (Sandblom et al., 2016; Pichaud et al., 2019). Redband trout (Oncorhynchus mykiss gairdneri) from a desert population have been found to have a higher upper thermal limit, broader optimum temperature window for aerobic scope, higher maximum heart rate, and reduced heat shock protein expression following exposure to diel thermal stress compared to a montane population when reared in common garden conditions (Narum et al., 2013; Chen et al., 2018).

Intraspecific variation in thermal tolerance can be assessed using both critical and functional thermal limit tests, each of which has benefits and limitations. Critical thermal maximum (CTMAX), the temperature where fish lose equilibrium when temperature is increased rapidly, acts as a proxy for lethal thermal limits (Beitinger and Lutterschmidt, 2011). Habitat temperature maximums can be subtracted from CTMAX to determine

thermal safety margins (TSMs) for each population (Sunday et al., 2014; Pinsky et al., 2019). CTMAX tests are relatively easy and quick to perform and have been conducted on countless fish species to date, facilitating comparisons across and within species. However, many essential and fitness-enhancing functions become limited at temperatures below CTMAX (Rodnick et al., 2004; Farrell, 2009; Eliason, Van Wert and Schwieterman, 2022).

Functional thermal tolerance can be assessed by determining the upper thermal threshold when key physiological performance metrics become impaired. Fish require energy for maintenance (e.g. circulation, respiration, nervous function, protein turnover), growth (tissue biosynthesis) and for functions such as feeding, digestion and predator evasion that are essential for long term survival and fitness (Fry, 1971; Claireaux and Lefrançois, 2007; Farrell, 2009; Eliason, Van Wert and Schwieterman, 2022). Recovery from exhaustive exercise is an ecologically important factor for fishes, given that many fish rely on anaerobic exercise to catch prey, escape predators and compete for conspecifics (Birnie-Gauvin et al., 2023), yet are vulnerable (e.g. to predation, disease) and may miss opportunities (e.g. feeding, mating) during the recovery period. Both the energetic cost and duration of recovery can increase with warming (Kraskura et al., 2020). Absolute aerobic scope (AAS) is energetic capacity to support activities beyond maintenance at a given temperature and is calculated by subtracting a fish's oxygen uptake rate at rest (resting metabolic rate, RMR) from its maximum capacity for oxygen uptake (maximum metabolic rate, MMR; Farrell 2009). Factorial aerobic scope (FAS = MMR/RMR) is the factor by which an individual can increase metabolism above maintenance levels to support the costs of physiological functions (e.g. digestion, locomotion; Careau, Killen and Metcalfe, 2014). FAS can indicate when a metabolic constraint begins to develop. AAS and FAS tend to decrease at high

temperatures because RMR tends to increase exponentially with temperature while MMR typically cannot increase past a certain temperature (Fry, 1947; Farrell, 2016; Eliason, Van Wert and Schwieterman, 2022). O. mykiss require an FAS of at least 2 (the ability to double RMR) in order to digest a moderate-sized meal and it is estimated that they require an FAS of at least 3 (the ability to triple RMR) to be able to perform other functions during digestion (Eliason, Higgs and Farrell, 2008; Adams et al., 2022; Eliason, Van Wert and Schwieterman, 2022). The difference between the temperature where FAS = 3 (TFAS3) and the maximum stream temperatures can be used to calculate the functional warming tolerance (FWT) for a given trout population (Anlauf-Dunn, Kraskura and Eliason, 2022; Eliason, Van Wert and Schwieterman, 2022). This represents the amount of warming a stream can undergo before the fish experience functional limitations, which is useful for informing management actions such as habitat restoration and angling restriction.

Herein, we determine the thermal tolerance and vulnerability of four populations of a broadly distributed fish species, Oncorhynchus mykiss, occupying different thermal environments within the state of Oregon, USA. O. mykiss, also known as steelhead (anadromous phenotype) or rainbow trout (freshwater resident phenotype) naturally occur along the west coast of North America from southern California to Alaska and inhabit a wide range of thermal conditions both between and within latitudes (Page and Burr, 2011). In Oregon, O. mykiss populations inhabit cool, coastal watersheds as well as arid, inland watersheds. This study is focused on summer-run steelhead (populations where adults migrate from the ocean during the summer as opposed to during the winter) because this ecotype is listed as “threatened” under the Endangered Species Act in many watersheds throughout Oregon and there are active management efforts to conserve and protect them

(ODFW 2010, ODFW 2014, ODFW 2022). We focused on juvenile O. mykiss that remain in tributaries for the entirety of this life stage and must be able to survive summer temperatures in order to reach adulthood. The goals of this study were 1. to compare thermal tolerance between populations of juvenile summer-run steelhead inhabiting different thermal regimes within the same part of their latitudinal range and 2. to pair thermal tolerance and habitat temperature data to determine which populations are currently most vulnerable to decline or extirpation from rising temperatures. We hypothesized that populations of summer-run steelhead from warm habitats have higher critical and functional thermal tolerance compared to populations from cool habitats. We also hypothesized that populations from warm habitats are more vulnerable to warming (i.e. have lower TSMs and FWT) because they currently experience temperatures are closer to their thermal limits during the summer.

3.3 Methods

We tested the functional and critical thermal tolerance of wild juvenile summer-run *O. mykiss* from four watersheds located throughout the state of Oregon during July and August of 2021 and 2022. All experiments were conducted streamside and each population was tested in its natal water. Streamside experiments are advantageous because they allow for close mimicking of natural temperature conditions, minimization of transport stress, and release of fish back into the wild after testing. In this study, wild-caught fish were acclimatized to the local field conditions and each population was expected to be genetically distinct given that they are from different (Arciniega *et al.*, 2016), though genetic analysis was not conducted to confirm. Accordingly, any differences between watersheds may be due to a combination of genetic differentiation (e.g. local adaptation) and plasticity (e.g.

developmental plasticity, parental effects, acclimatization). All methods were approved by the University of California Santa Barbara Institutional Animal Care and Use Committee.

3.3.1 Life History

We studied *O. mykiss* in locations that are known to specifically support summer-run steelhead and not winter-run steelhead. Winter-run and summer-run steelhead are physically indistinguishable but, in most cases, there is natural run differentiation and genetic differences between the 2 ecotypes (Papa *et al.*, 2007; Arciniega *et al.*, 2016). Winter-run steelhead cannot access our selected study locations due to high flow conditions at the times that they migrate. Given the vastly different geographies of our study locations, the life history and phenological timings are variable by basin. In general, adults migrate to their freshwater spawning grounds after 2-4 years at sea between May and October each year. Peak migration timing varies depending on the location with peaks occurring May through July in western Oregon basins (Siletz and North Umpqua), and July and August for eastern Oregon basins (e.g. Lower Deschutes and John Day). Adult steelhead hold in freshwater until spawning which occurs between January and May. Peak spawning timing varies by basin with earlier peaks seen in the western basins. Juvenile summer steelhead rear in freshwater for 1-3 years. Smolt outmigration occurs between January and June and peaks during late spring. Experiments were conducted on juvenile fish that were likely 1-2 years old.

3.3.2 Populations

Lower Deschutes: Buckhollow Creek is a fourth-order tributary of the Deschutes River in the eastern portion of the Lower Deschutes basin (Fig. 1). The land ownership and management of the Lower Deschutes is primarily private with federal (BLM) lands along

river and the stream corridors. The watershed is characterized by Columbia River basalt flows and has a semi-arid climate. Summer temperatures are relatively warm in Buckhollow Creek, ranging from ~16-25°C (Table 1, Fig. 1).

John Day: Bridge Creek is a fourth-order tributary of the John Day River in the Lower John Day basin in eastern Oregon (Fig. 1). While surrounded by volcanic lithologies, Bridge Creek is primarily overlain by sedimentary and plutonic lithologies, residing within the Calarno unit which contains fossil bearing rock formations (Bestland *et al*., 2002). In the winter, much of the precipitation arrives as snow. Summer temperatures are warm in Bridge Creek, ranging from ~9-27°C (Table 1, Fig. 1).

North Umpqua: Steamboat Creek is a third-order tributary of the North Umpqua River in Western Oregon (Fig. 1). The Steamboat creek watershed is under federal (USFS) land ownership and management and is underlain by volcanic lithology. Precipitation arrives primarily in the form of snow. Summer temperatures are intermediate in Steamboat Creek, ranging from ~8-21°C (Table 1, Fig. 1).

Siletz: Gravel Creek is a third-order tributary of the Siletz River in Western Oregon (Fig. 1). The Gravel creek watershed has private industrial timber as the primary land ownership/management and is underlain by sandstone and basalt. The climate is highly influenced by the climate patterns of the Pacific Ocean and the majority of the precipitation falls as rain in the winter months. Summer temperatures are relatively cool in Gravel Creek, ranging from ~8-16°C (Table 1, Fig. 1).

3.3.3 Habitat temperature monitoring

We deployed Onset HOBO TidbiT MX temperature data loggers in each stream to measure continuous water temperature once per hour. Data loggers were deployed in the

stream before the start of the experiment, measured continuously for the duration of the experiment, for many months following the experiment with the goal of capturing the maximum temperatures, as well as the daily temperature variability, that each population experiences during the summer. The John Day logger was washed away during winter storms, so temperature metrics were calculated from the data recorded prior to and during our experiments. John Day temperature data plotted in Figure 1 is from a gauge deployed by the United States Geological Survey located ~13 miles downstream of our study location.

3.3.4 Experimental Setup and Holding Temperatures

At each site, we constructed a temporary partially recirculating tank system pumping water from the stream through a series of tanks used for temperature exposure and respirometry. Juveniles from each *O. mykiss* population (Lower Deschutes: n = 33, body mass = 15.60 ± 1.89g; John Day: n = 39, body mass = 38.7 ± 2.34g; North Umpqua: n = 43, body mass = 17.49 ± 1.06g; Siletz: n = 40, body mass = 24.01 ± 1.27g) were captured via electrofishing and exposed to 1 of 3 or 4 fluctuating temperature treatments for 20 hours prior to physiological testing (Fig. A4). While some aspects of thermal acclimation can occur rapidly in fishes (Klicka, 1965; Barrionuevo and Fernandas, 1998; Macnutt *et al.*, 2004; Gilbert *et al.*, 2022), this 20-hour exposure is relatively acute and is likely not enough time for the fish to complete a full acclimation response (Stewart et al. 2023). Due to the stochastic nature of temperature in these systems and the speed at which temperature can increase during heat waves (Fig.1), this acute exposure was more ecologically relevant than allowing the fish weeks to acclimate, as is typical in lab studies. Fish were not fed during holding to ensure that they would not be digesting during thermal tolerance experiments, as digestion introduces additional energetic costs (McCue, 2006; Eliason, Higgs and Farrell,

2008). Holding and respirometry tanks were covered with mesh cloth and shade canopies to ensure that food items did not fall into the tanks.

Temperature fluctuates diurnally during the summer months for all 4 populations, between 4-5°C for the Deschutes, Siletz, and North Umpqua populations, and up to 14°C for the John Day population. We therefore used fluctuating treatments in our experiments to ensure that we were mimicking the fish's natural environment as closely as possible (Fig. A4). For the Siletz population, temperature treatments included ambient (15-18°C), 18-22°C, 20-24°C, and 23-26°C. For the North Umpqua population, treatments included ambient (16-20°C), 18-22 °C, 20-24°C, and 23-26°C. For the Lower Deschutes population, treatments included ambient (18-22°C), 20-24°C, and 23-27°C. For the John Day population, treatments included ambient (14-27°C), 20-24°C, and 23-27°C. Thus, there was one common temperature treatment for all populations (i.e. 20-24°C).

3.3.5 Functional Thermal Tolerance: Aerobic Scope and Exercise Recovery

We used intermittent flow respirometry to measure oxygen uptake rates (MO_2) during rest and immediately after exercise. To measure MO_2, individual fish were placed in an airtight plastic container (Lock & Lock, Seoul, South Korea) fitted with a FireStingO2 robust oxygen probe (PyroScience, Germany) to measure dissolved oxygen and a Universal 300-L h^{-1} aquarium pump (Eheim, Germany) to circulate water throughout the chamber. A MICRA Compact 90 GPH aquarium pump (SICCE, Italy) flushed oxygenated water through each chamber from the surrounding tank between MO_2 measurements so that the fish never experienced dissolved oxygen levels below 80% air saturation. During each trial, one chamber was left empty to measure bacterial respiration, which was found to be negligible in all cases.

MO_2 was measured for 20 hours to obtain resting metabolic rate (RMR) measurements for each temperature during the diurnal cycle. All trials used a 6-minute measurement period followed by a 4-minute flush cycle for RMR measurements with 2 exceptions: extra time was added to RMR measurement cycles for the ambient treatments at Lower Deschutes and at North Umpqua to ensure a sufficient decrease in O_2 for adequate MO_2 measurements (Lower Deschutes: 1 minute added; North Umpqua: 4 minutes added). Maximum metabolic rate (MMR) was measured the following morning after 20 hours of RMR measurements. Fish were transferred into a bucket, chased by hand for 3 minutes, exposed to air for 1 minute (Little *et al.*, 2020), and placed back into respirometers for 1 hour to measure MMR and exercise recovery.

3.3.6 Critical Thermal Maximum

CT_{MAX} tests were used to assess upper thermal tolerance and were conducted immediately after respirometry when fish had been held at test temperatures for ~40 hours. CT_{MAX} start temperatures were always within 1 °C of the chase temperature for each treatment (Table 2). To obtain CT_{MAX} measurements, fish were first placed into an aerated cooler and given 10 min to adjust to their surroundings. Then, water temperature was increased at a rate of 0.3 °C min^{-1} (Beitinger, Bennett and Mccauley, 2000) by pumping heated water through a stainless-steel coil and dipping the coil in and out of the water. The temperature at loss of equilibrium (CT_{MAX}) was recorded for each individual fish. When fish lost equilibrium, they were immediately netted and placed into an aerated bucket to recover. Fish were slowly brought back down to ambient stream temperatures and were released back into the wild.

3.3.7 Data and Statistical Analysis

All data analysis was conducted in RStudio version 2022.07.2 with a significance level of $\alpha = 0.05$ for statistical tests. All MO_2 data were inspected for linearity and regressions with $R^2 < 0.9$ were discarded. MO_2 values were then obtained from each regression using the following equation: $MO_2 = (\text{slope} * (v_R - m))/m * (m/0.025)^{(1\text{-scaling exponent})}$, where v_R is the respirometer volume and m is the fish body weight in kg (R package: AnalyzeResp). Scaling exponents (0.74 for MMR and 0.72 for RMR) were obtained from linear regressions fitted to the log-log relationship between body mass and raw MO_2 values across all populations and temperatures, and including 2 additional steelhead populations from California (Fig. A5). Data were scaled to a common body mass of 25g, the average body mass for all fish tested in this study. Fish with more than 25% of MO_2 regressions with $R^2 < 0.9$ were excluded entirely from RMR analysis. To calculate RMR, the first 240 min data was discarded for each fish to ensure that they had recovered from handling stress. The mean temperature during each MO_2 measurement was then rounded to the nearest degree and MO_2 values were averaged at each temperature during the diurnal fluctuation to represent the RMR at those temperatures. RMR calculations comprised of $n<3$ MO_2 measurements for an individual fish were not included in statistical analysis. Due to fluctuating temperature treatments, we obtained RMR measurements from 4-5 temperatures for each fish, with the exception of the John Day ambient treatment, where we obtained RMR from 14 different temperatures. The effect of temperature on RMR and *ln*(RMR) was determined for each population with linear mixed models using both temperature and treatment as fixed effects and fish ID as a random effect (R package: lme4; Bates *et al*., 2015). Results were obtained using Type II and Type III ANOVAs (R package: "car", Fox and Weisberg, 2018) and BIC was used to determine the best fit models.

To calculate MMR, we used the steepest 120 s slope from the measurement cycle where MMR occurred (Little *et al*., 2020). In most cases this was the first measurement cycle post-chase, except for 4 fish where MMR occurred later in the recovery process. MMR always occurred post-chase and never during RMR trials. MMR was compared between temperatures within populations using one-way ANOVAs.

Aerobic scopes were calculated using the RMR measured at the temperature that each fish was chased at (RMR_{chase}). Absolute aerobic scope (AAS) was calculated by subtracting RMR_{chase} from MMR. Factorial aerobic scope (FAS) was calculated by dividing MMR by the RMR_{chase}. Both AAS and FAS were compared between treatments within populations using one-way ANOVAs. Second-order polynomials were fit to AAS data when possible and optimal AAS temperatures (T_{OPT}) as well as pejus temperatures (T_{PEJ}) were calculated from these curves. T_{OPT} represents the temperature corresponding with the highest AAS and T_{PEJ} represents the range of temperatures where fish have at least 80% of their peak AAS available to them (Clark, Sandblom and Jutfelt, 2013; Farrell, 2016). Linear regressions were fit to assess the relationship of FAS and temperature to determine the temperature where FAS = 3 (T_{FAS3}) for each population.

To assess the impact of temperature on exercise recovery, we examined how temperature influences the time it takes for each of our study populations to recover to a rate of oxygen consumption where they have 80% of their AAS available to them ($Time_{AAS80}$) and until they have an FAS of 3 available to them ($Time_{FAS3}$). $Time_{AAS80}$ and $Time_{FAS3}$ serve as additional metrics of functional thermal tolerance. MO_2 was measured every ~10 minutes for 50-60 minutes after fish were chased. Biexponential decay models were fit to describe the decrease in MO_2 over time for each treatment and temperature (Scarabello *et al*., 1991).

These models had the following formula: $MO_2(t) = Ae^{\alpha t} + Be^{\beta t} + RMR$ where t is time, α and A are the slope and y-intercept, respectively, of the first exponential decay, β and B are the slope and y-intercept, respectively, of the second exponential decay, and RMR is the average RMR_{chase} for each corresponding population and temperature. These models describe the average decay of MO_2 over time for each population and temperature. To solve for $Time_{AAS80}$ and $Time_{FAS3}$, we used the RMR_{chase} of each individual fish in these models and found the time point (rounded to the nearest 0.1s) where MO_2 was equal to 80% of the fish's AAS (for $Time_{AAS80}$) and where MMR divided by MO_2 was equal to 3 (i.e. time to recovery to FAS = 3, $Time_{FAS3}$).

CT_{MAX} was compared between temperature treatments using one-way ANOVAs, with the exception of the John Day population where treatments were compared using a student's t-test (CT_{MAX} was only measured for 2 of the 3 treatments at this site). CT_{MAX} was compared between populations at the common temperature treatment of 20-24°C using a one-way ANOVA.

3.4 Results

3.4.1 Habitat temperature characteristics

Habitat temperatures indicate that all four summer steelhead populations experience distinct thermal regimes in their respective habitats (Table 1, Fig. 1). The John Day and Lower Deschutes reached the warmest temperatures during the summer months with maximum temperatures of 24.9°C (July 2022) and 27.1°C (July 2021), respectively. North Umpqua reached intermediate temperatures, with a maximum summer temperature of 21.6°C (July 2022). Siletz remained the coolest during the summer with a maximum temperature of 15.9°C (June 2021). Daily variability during the summer months (June, July,

and August) ranged from 3-13°C at John Day, 1-7°C at Lower Deschutes, 1-6°C at North Umpqua, and 0.4-4°C at Siletz. All temperatures approached freezing during the winter months, but exact temperature minimums are uncertain due to our data loggers having unreliable readings at temperatures <4°C.

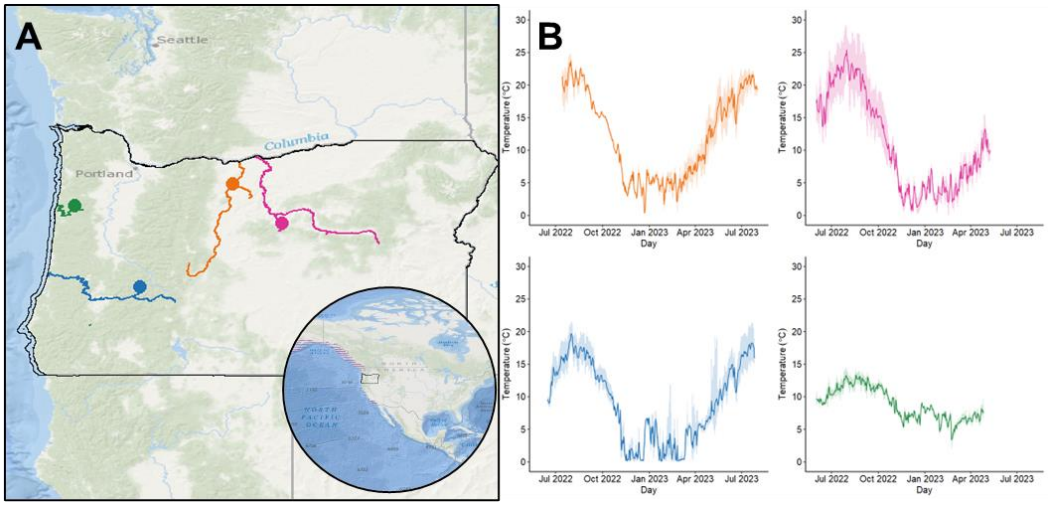

Figure 3.1: Map of the study area (panel A) with study streams shown as colored lines and exact study locations represented by circular points (Lower Deschutes: orange; John Day: pink; North Umpua: blue; Siletz: green) and continuous temperature data collected from each study location between June 2022 and July 2023 (panel B; John Day data source: United States Geological Survey) with lines indicating mean daily temperatures and shaded areas indicating daily temperature range.

3.4.2 Critical Thermal Maximum

CT_{MAX} ranged from 27.4-32.5°C, and varied in magnitude and plasticity between summer steelhead populations (Table 1, Figure 2). *O. mykiss* from the Siletz has increasing CT_{MAX} with increasing holding temperatures (ANOVA, $p < 0.001$) while *O. mykiss* from the North Umpqua and John Day showed no change in CT_{MAX} with increasing holding temperatures (ANOVA, $p = 0.967$ & t-test, $p = 0.725$ respectively). Lower Deschutes *O. mykiss* had slightly decreased CT_{MAX} at temperatures above ambient (ANOVA, $p < 0.001$). At common starting temperatures of 19°C and 22°C, the Lower Deschutes population had significantly higher CT_{MAX} than the Siletz and North Umpqua populations (Kruskal-Wallis

tests, $p < 0.001$ for both 19°C and 22°C). At 19°C CT_{MAX} of the John Day population was higher than the North Umpqua and Siletz populations but lower than the Lower Deschutes population, with the caveat that during this treatment the John Day population experienced a much wider range of temperatures (14-27°C) compared to the other populations (18-22°C).

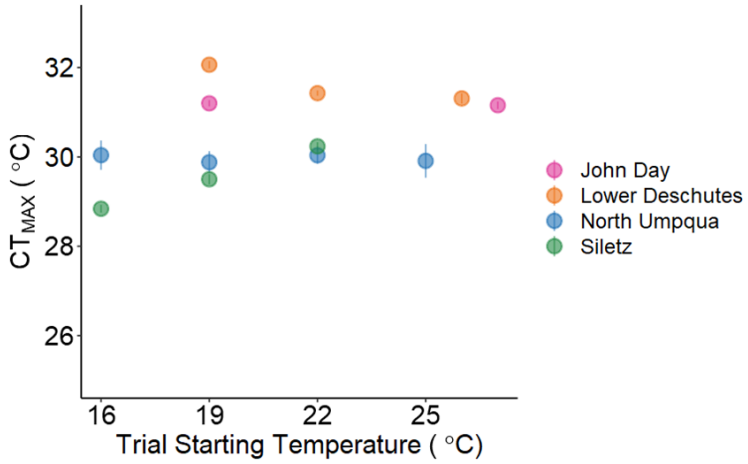

Figure 3.2: CT_{MAX} for all populations and temperatures (mean ± SEM).

3.4.3 Metabolic Rate

RMR increased exponentially with temperature for all populations and was influenced by both acute temperatures during the diurnal fluctuations and by holding temperature treatments (Fig. 3). For the Lower Deschutes and North Umpqua populations, there were significant effects of acute temperature and treatment, but not their interaction, on RMR (Table A7). For the Siletz population, there were significant effects of acute temperature, treatment, and their interaction on RMR (Table A7). For the John Day population, there were significant effects of acute temperature and the interaction between acute temperature and treatment but no effect of treatment itself (Table A7). At the common temperature treatment of 20-24°C, John Day and Lower Deschutes *O. mykiss* had 20-50% higher RMR at all temperatures compared to Siletz and North Umpqua *O. mykiss* (Fig. A6).

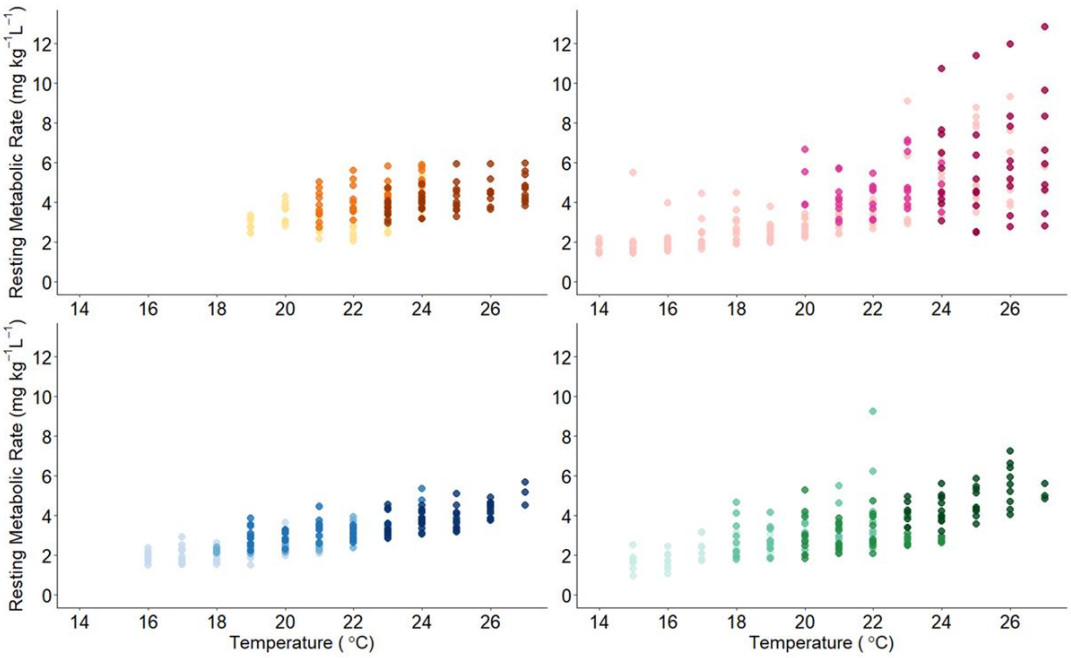

Figure 3.3: Resting Metabolic Rate (RMR) for all treatments and populations (Deschutes: orange; John Day: pink; North Umpqua: blue; Siletz: green). Points indicate mean RMR for individual fish at each temperature.

Overall, steelhead MMR was not strongly affected by temperature (Table 2, Fig. 4). MMR did not change with temperature for John Day, North Umpqua, and Siletz populations (ANOVAs, p = 0.185, 0.721, & 0.125 respectively). For the Lower Deschutes population, MMR increased between 19°C and 22°C but did not differ between 22°C and 26°C (ANOVA, p = 0.009). The effect of temperature on AAS, however, was population dependent. AAS did not change between test temperatures for the Lower Deschutes population (Table 1, Fig. 5, ANOVA p = 0.261). Polynomial curves were fit to the relationship between AAS and temperature for the John Day, North Umpqua, and Siletz populations. T_{OPT} ranged from 17-21°C and T_{PEJ} between 21-23°C for these 3 populations, and in all cases, there was a significantly lower AAS at the "climate" test temperature (Table 2, Fig. 5). AAS also varied between populations when tested at common temperatures. The Siletz and North Umpqua populations were tested at all the same temperatures and the Siletz

population had a higher AAS at all temperatures except 25°C where AAS was not significantly different (ANOVA,). At 19°C, the John Day and Siletz populations had a higher AAS compared to the Lower Deschutes and North Umpqua populations (Fig. 6). At 22°C, the John Day population had a significantly higher AAS than the North Umpqua population, but all other pairwise AAS comparisons were not significantly different (Fig. 6).

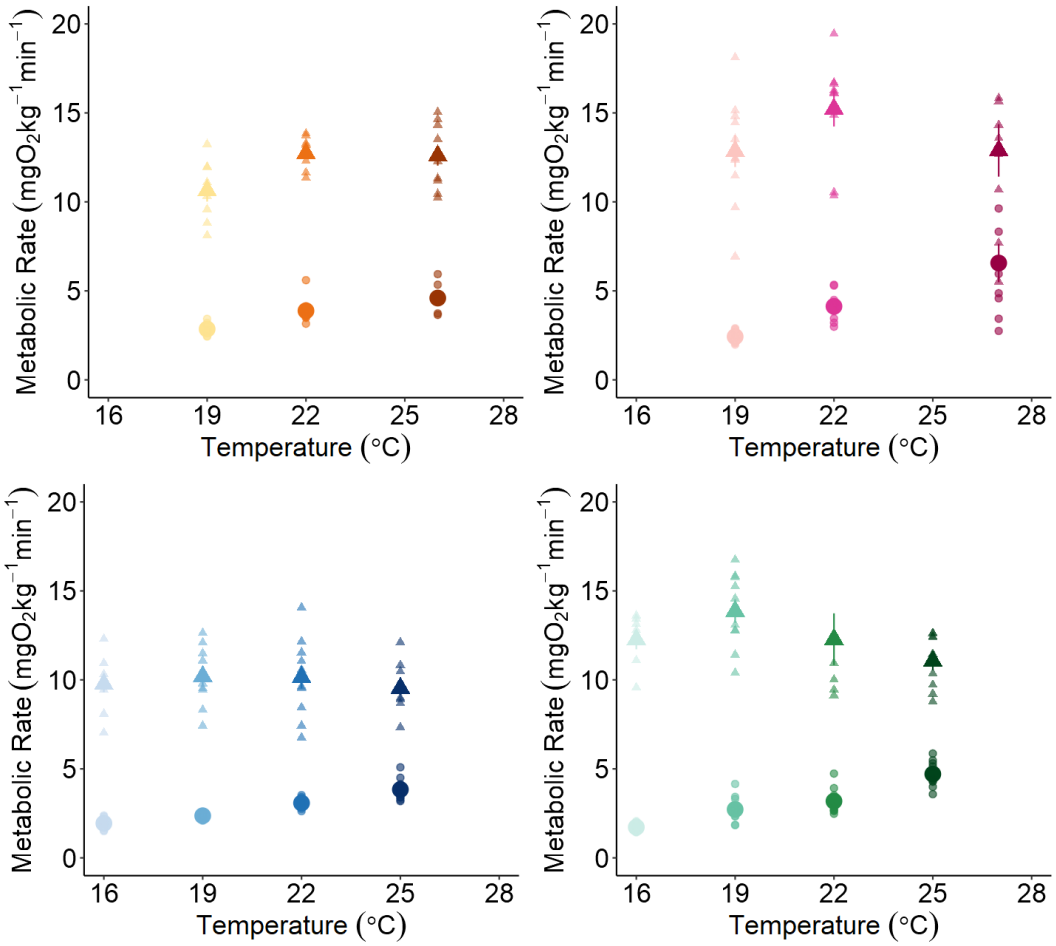

Figure 3.4: Maximum metabolic rate (MMR; triangles) and resting metabolic rate (RMR; circles) for all populations (Deschutes: orange; John Day: pink; North Umpqua: blue; Siletz: green). Large filled points indicate mean ± SEM MMR and RMR at each temperature and small points represent individual measurements of MMR and RMR.

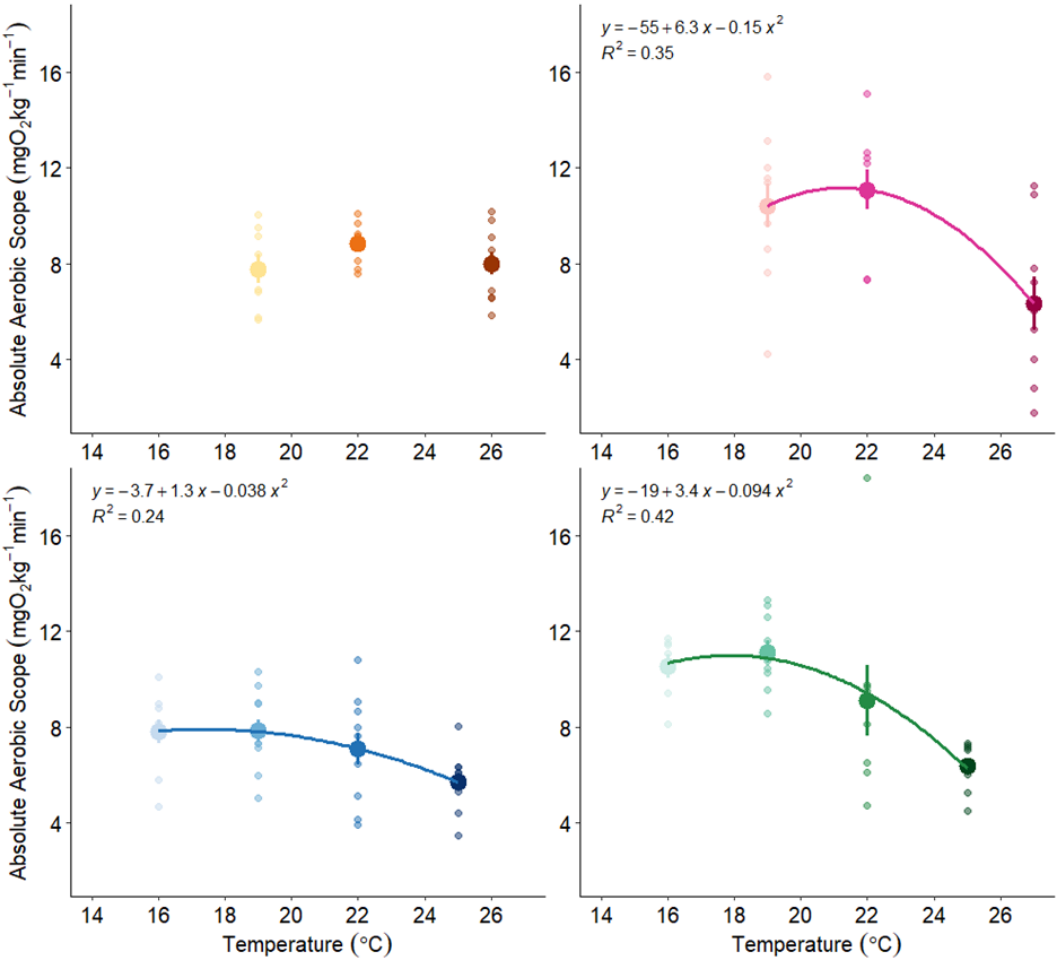

Figure 3.5: Absolute aerobic scopes (AAS) for all populations (Deschutes: orange; John Day: pink; North Umpqua: blue; Siletz: green). Large filled points indicate mean ± SEM AAS at each temperature. Curves and equations represent quadratic polynomial functions fitted to describe the relationship between AAS and temperature where possible.

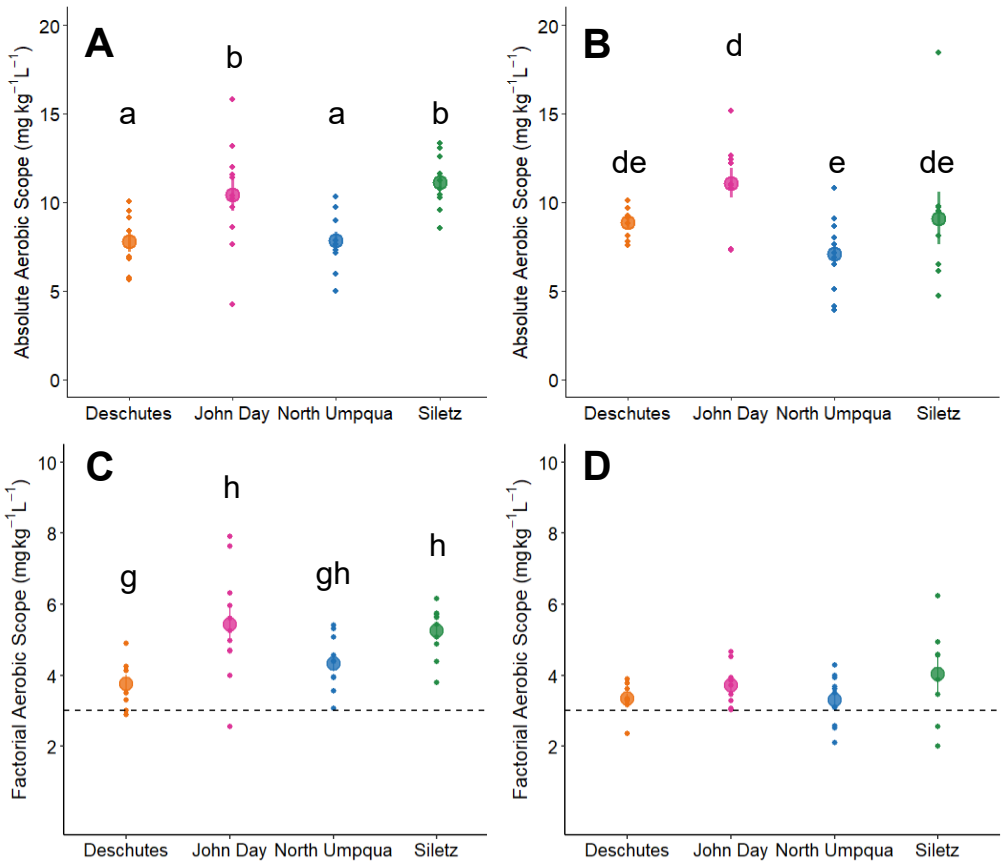

Figure 3.6. Absolute aerobic scope (AAS; panels A&B) and factorial aerobic scope (FAS; panels C&D) for the Lower Deschutes (orange), John Day (pink), North Umpqua (blue), and Siletz (green) populations at common temperatures. Small points indicate AAS or FAS of individual fish and large points indicate mean ± SEM AAS or FAS for each population. Lowercase letters indicate statistically significant differences between populations (ANOVA, $p<0.05$).

FAS decreased linearly with increasing temperatures for all populations (Table 2, Fig. 7). Model selection confirmed that the best fit included a unique slope and y-intercept for each population rather than an average of all 4 populations. The regression for the Lower Deschutes population is shallower (slope = 0.14) compared to the others (slopes = 0.3-0.46). T_{FAS3} temperatures ranged from 23.8-24.9°C (Table 2).

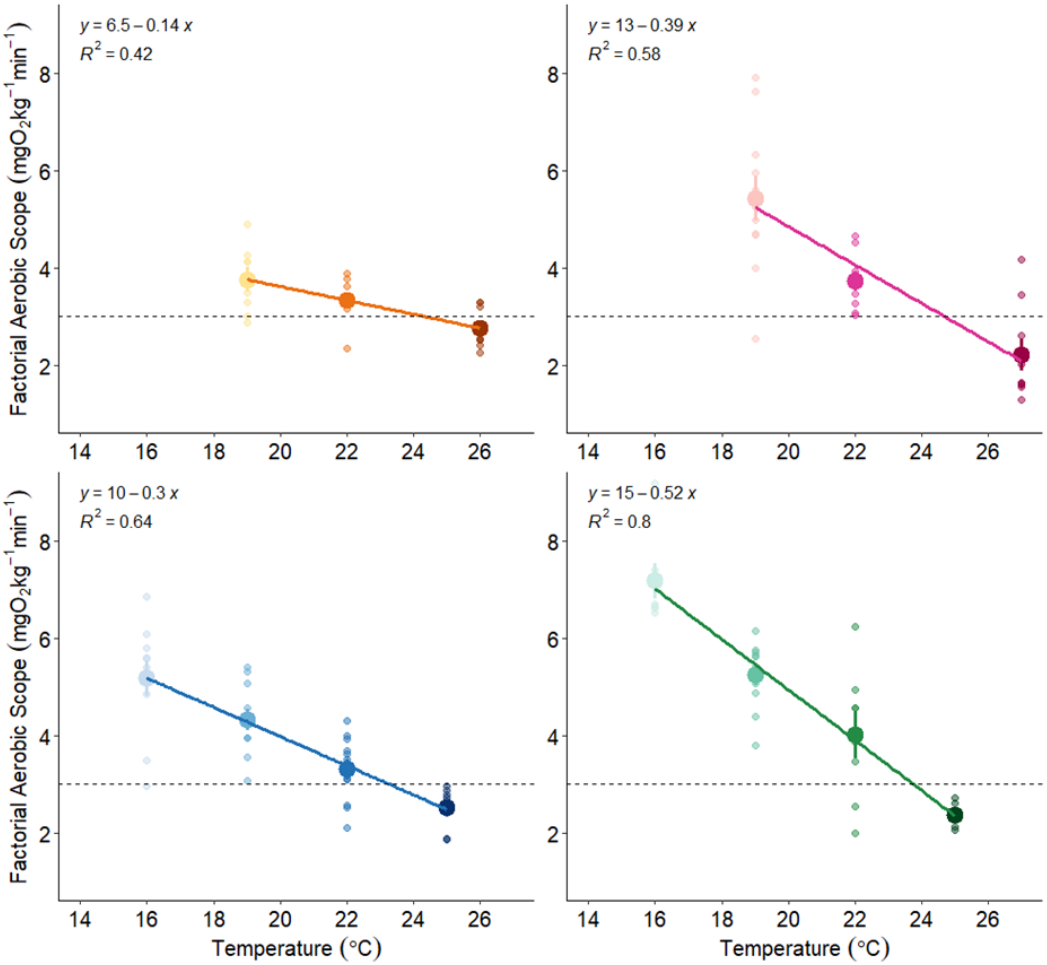

Figure 3.7: Factorial aerobic scopes (FAS) for all populations (Deschutes: orange; John Day: pink; North Umpqua: blue; Siletz: green). Large filled points indicate mean ± SEM FAS at each temperature. Solid lines and equations represent linear models fitted to describe the relationship between FAS and temperature where possible. Dashed lines indicate FAS=3.

3.4.4 Exercise Recovery

After MMR, MO_2 decreased in a biexponential decay pattern for the entirety of the 50-60min recovery period with the first, steeper decay occurring between time 0-20 after MMR and the second, shallower decay occurring between 20-60min after MMR (Fig. 8). Temperature had a significant impact on recovery timing, with higher temperatures resulting in prolonged recovery (higher $Time_{AAS80}$ and $Time_{FAS3}$) for the John Day, Siletz, and North Umpqua populations (Fig. 8&9, Table 3). For the John Day population, recovery was

impaired between 22°C and 27°C (Fig. 8&9, Table 3). For the Siletz and North Umpqua populations, recovery was impaired between 19°C and 22°C (Fig. 8&9, Table 3). Temperature did not impact recovery timing for the Lower Deschutes population but Time $_{FAS3}$ is significantly higher than the other populations (Table 3). At common temperatures of 19°C and 22°C, the John Day population had a significantly lower $Time_{AAS80}$ and $Time_{FAS3}$ compared to the other populations and therefore had the fastest exercise recovery (Table 3). The Lower Deschutes population had the highest T_{FAS3} (and therefore the slowest recovery of FAS) at 19°C and a higher $Time_{FAS3}$ than the John Day and Siletz populations at 22°C.

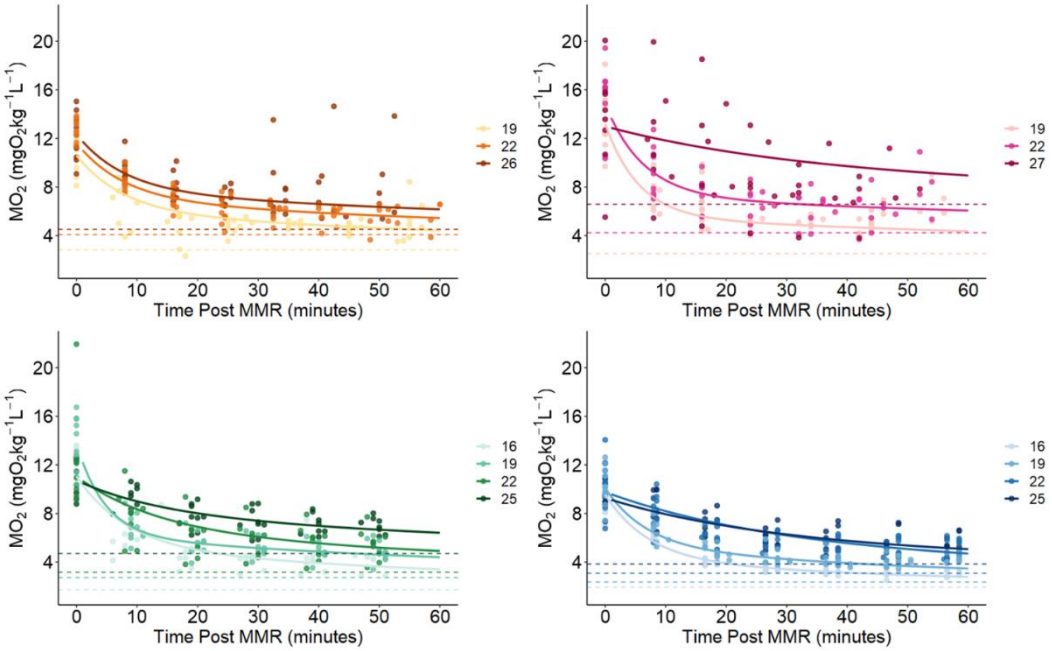

Figure 3.8: Oxygen uptake rate (MO_2) over time during 1 hour of exercise recovery with MMR at time = 0 for each population (Deschutes: orange; John Day: pink; North Umpqua: blue; Siletz: green) and temperature. Points represent MO_2 measurements for individual fish, curves plot the biexponential decay function fit to each population and temperature, and dashed lines indicate RMR for each population and temperature.

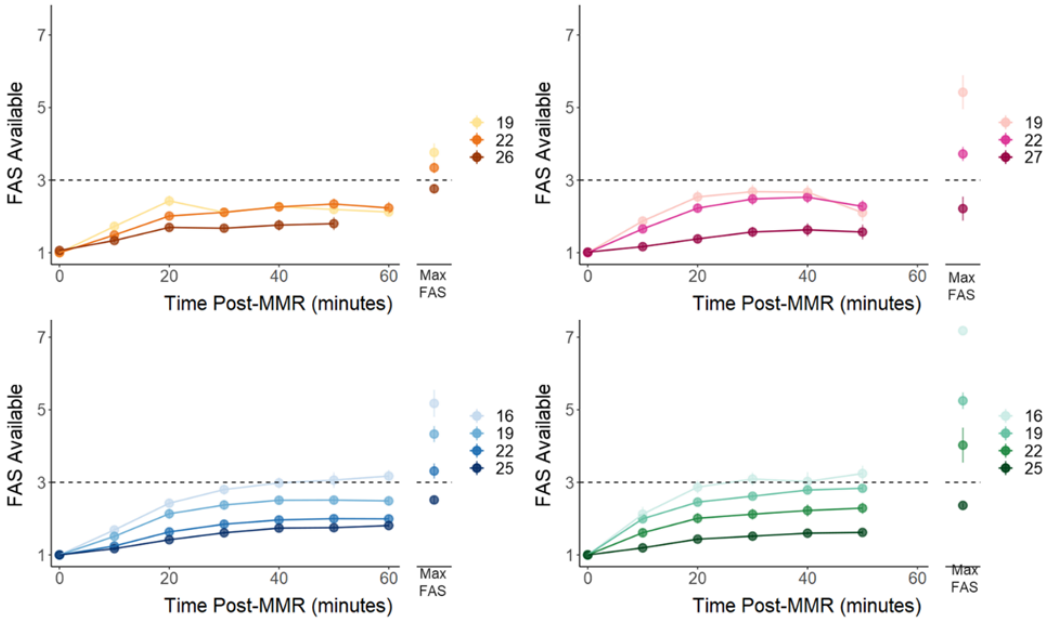

Figure 3.9: Factorial aerobic scope (FAS) available to the fish during 1 hour of exercise recovery with MMR at time = 0 for each population (Deschutes: orange; John Day: pink; North Umpqua: blue; Siletz: green) and temperature. Points represent mean ± SEM FAS available for each population and temperature every 10 minutes post-MMR.

3.5 Discussion

Here we measured aerobic scope, exercise recovery, and CT_{MAX} of four populations of juvenile summer-run steelhead trout exposed to acute, ecologically relevant temperature increases. We found clear intraspecific differences in thermal performance across populations. As predicted, the thermal tolerance of this species varies across a gradient of habitat temperature conditions rather than latitude, highlighting the need for population-specific management strategies for this species and ecotype. While we cannot identify the mechanism underlying these intraspecific differences (i.e. whether these differences were due to long term thermal acclimatization, parental effects, or local adaptation), it is clear that the populations currently experiencing the warmest temperatures are living close to their

thermal limits and are likely to face physiological challenges if temperatures continue to increase.

3.5.1 Thermal Safety Margins differed across populations

CT_{MAX} values were all within the range previously measured for *O. mykiss* (24-32°C, McKenzie *et al*., 2021), and the populations from the warmest locations, John Day and Lower Deschutes, were at the upper end of this range (i.e. 30.3-32.5°C). As expected, the John Day and Lower Deschutes populations had higher CT_{MAX} than the Siletz and North Umpqua (cooler locations) populations and most other previously studied *O. mykiss* with the exception of two warm-adapted hatchery strains in Western Australia and Arizona, USA and a wild population at the southern end of the species' native range in California, USA (Fig. 2; Recsetar *et al.*, 2012; Adams *et al.*, 2022; Dressler *et al.*, 2023). The John Day population experienced ambient temperature swings of 14-27°C during experiments and CT_{MAX} of fish exposed to this swing and tested at 19°C was the same as fish exposed to just the upper end of this swing (24-27°C) and tested at 27°C. In this case, CT_{MAX} appears to be associated with the warm end of this diurnal temperature swing with limited plasticity. The other warm-acclimatized population (Lower Deschutes) similarly displayed a high CT_{MAX} overall, but no improvement with high acclimation exposure. In contrast, the population from the coldest habitat, Siletz, had the lowest CT_{MAX} in ambient conditions, but CT_{MAX} displayed rapid plasticity, increasing with acclimation exposure to warmer temperatures (Fig. 2, Table 2). Results from the John Day, Siletz, and Lower Deschutes populations provide evidence of a tradeoff in magnitude and plasticity of CT_{MAX}, meaning that upper thermal limits of warm-dwelling summer steelhead are unlikely to be able to acclimate if temperatures continue to increase. However, the North Umpqua population had a lower CT_{MAX} than the John Day and

Lower Deschutes populations at common temperatures and CT_{MAX} did not exhibit rapid acclimation (Fig. 2, Table 2). This population also had more interindividual variability than any of the other populations (Table 2). It could be that this population takes longer than 40 hours to start acclimating and the inter-individual variability is an artefact of some individuals beginning to acclimate faster than others. Another possible explanation is that the North Umpqua population relies more on local adaptation than phenotypic plasticity for adjusting their upper thermal limits in response to warming. Regardless, managers should be aware that while summer steelhead in the North Umpqua may have the capacity to increase their upper thermal limits, they are not able to do so over a rapid timescale characteristic of heat waves in this area.

While these warm-dwelling *O. mykiss* populations had high critical thermal limits, they also had lower TSMs compared to populations from cooler habitats. The John Day population had the lowest TSM (4.1°C), followed by Lower Deschutes (7.6°C), North Umpqua (8.4°C), and Siletz (12.9°C). In other words, ambient temperatures would only have to increase by ~4°C for the John Day population to reach its CT_{MAX} and it is unlikely this population would be able to acclimate given the fast rate of temperature change in this system and the observed lack of plasticity of CT_{MAX}. The other 3 populations are well buffered against warming by comparison and the Siletz population is exceptionally well buffered given its high TSM combined with the rapid plasticity of CT_{MAX} (Fig. 2).

3.5.2 Energetic costs and Functional Warming Tolerance differed across populations

Based on differences in RMR between populations at a common temperature treatment of 20-24ºC, the populations that experience warmer summer temperatures (Lower Deschutes, John Day) likely need to eat more to keep up with their metabolic costs. The

Lower Deschutes and John Day population had 20-50% higher RMR than the North Umpqua and Siletz populations. This result is uncommon, as prolonged warm exposure tends to result in reduced RMR (e.g Healy and Schulte, 2012; Mcbryan *et al.*, 2016; Sandblom *et al.*, 2016; Railsback, 2022), but is consistent with a similar study on *O. mykiss* populations in California (Dressler *et al.*, 2023). We cannot be certain that these differences in RMR are consistent across all temperatures, but it is noteworthy that the North Umpqua and Siletz populations have a lower RMR at their T_{PEJ} and T_{FAS3}, which fall within the range of this common temperature treatment (Table 4). A high RMR indicates that the fish have higher costs for maintenance metabolism, and thus a greater amount of the energy consumed by these fish is allocated first to ensure basic baseline function before excess energy can be allocated to fitness-enhancing performances such as swimming and digestion.

The population specificity of responses of AAS to temperature reveals a tradeoff between magnitude and thermal sensitivity of aerobic scope that seems to correlate with habitat temperature regimes. As habitat temperature gets warmer, summer steelhead populations appear to sacrifice the magnitude of peak AAS in favor of reduced thermal sensitivity of AAS. The Siletz population experiences the coolest temperatures (Fig. 1) and has a higher peak AAS (AAS at $T_{OPT,}$ 11.74 $mgO_2kg^{-1}L^{-1}$), a higher AAS at common temperatures below T_{PEJ}, and a narrower T_{OPT} window (9.7°C) compared to the North Umpqua population (peak AAS: 7.41 $mgO_2kg^{-1}L^{-1}$, T_{OPT} window: 12.8°C) that experiences intermediate temperatures (Fig. 1). The Lower Deschutes population experiences high temperatures (Fig. 1) and displayed a low AAS and an extremely broad T_{OPT} window, such that this population had the same AAS at 19°C, 22°C, and 26°C, similar to a southern California population in Dressler *et al*. (2023). At 26°C, the Lower Deschutes population

has a higher AAS compared to the Siletz and North Umpqua populations at 25°C, representing the payoff of having low thermal sensitivity. However, reduced AAS at more intermediate temperatures indicates that this population is likely to have a reduced capacity for functions like growth and predator evasion.

John Day summer steelhead were the exception to this trend of trading off peak AAS and thermal sensitivity. However, this population inhabits a stream that has unique thermal characteristics compared to the others. This stream reaches the warmest peak temperatures of all of our study locations but was also the most variable, fluctuating by up to 13°C daily. This population has a similar peak AAS (11.14 $mgO_2kg^{-1}L^{-1}$) as the Siletz population and a slightly narrower T_{OPT} window (7.8°C). The AAS curve for this population was right-shifted and therefore had a higher T_{OPT} and upper T_{PEJ} compared to the Siletz and North Umpqua populations. Daily variability of habitat temperatures can therefore also lead to population differences in thermal tolerance. While the John Day and Lower Deschutes populations both experience warm maximum summer temperatures, it is possible that the John Day population does not invest in acclimation to these temperatures because they only occur for a brief period of time during the day. It is also worth noting that population differences in AAS can also be related to other selective factors including migration distance, flow rates and gradient, and presence of predators or competitors (e.g. Eliason *et al*., 2011). John Day *O. mykiss* have the longest migration of the four populations (Table 1) and Siletz *O. mykiss* compete with coastal cutthroat trout in the tributary where we obtained the fish. These factors may contribute to these two populations having higher peak AAS than the others.

As hypothesized, the increased thermal tolerance of the populations from warm habitats was not enough to confer a substantial buffer to warming. FAS was least temperature-

sensitive (i.e. slope of the decline was shallowest) by for Lower Deschutes and T_{AAS80} did not change between 19-26°C, reaffirming that this population is the least temperature sensitive. T_{FAS3} did not vary as much as expected (23-25°C), but FWT varied greatly between populations. John Day summer steelhead the highest T_{FAS3} and recovery was not prolonged until 27°C, but had the lowest FWT of -2.2°C, indicating that current temperatures exceed the functional thermal limits for these fish. Lower Deschutes had a FWT of -0.5, indicating that habitat temperatures reach the functional thermal limits for this population. The North Umpqua population has a slight buffer against warming (FWT = 1.7°C), but recovery was impaired at 22°C (0.4°C from the maximum measured stream temperature), suggesting that this population may soon experience physiological limitations from temperature. One caveat is that we do not have information on thermal heterogeneity in these tributaries, and therefore cannot be sure whether or not thermal refugia are available to these fish. However, projected decreases in streamflow and increases in water temperatures are predicted to cause existing thermal refugia to shrink and not support as many individuals in the near future (Mantua, Tohver and Hamlet, 2010). The Siletz population had the largest FWT of 7.9°C, a substantial buffer against warming. While coastal summer steelhead populations such as the Siletz should still be monitored to track trends in temperature, it is unlikely that temperature will be a physiological limitation for these fish. In contrast, inland populations will require more active management efforts as well as further studies linking physiology with trends in behavior and food resources (e.g., Hahlbeck *et al*., 2023)

3.5.3 Exercise recovery timing varied between populations and metrics

Here, we quantified exercise recovery using two novel metrics ($Time_{AAS80}$ & $Time_{FAS3}$) to approximate the time it took each fish to reach a level of recovery where they could

resume normal activities. Exercise recovery is often quantified using a 3-phase curve fit between the time of MMR and the time that standard metabolic rate (SMR) is reached. The area under the curve is used to calculate the amount of oxygen consumed by the fish during the recovery period (excess post-exercise oxygen consumption; EPOC; Zhang et al. 2018). It can take up to 12 hours for a fish to fully recover to SMR, during which metabolites and stress hormones are restored to baseline levels (Scarabello, Heigenhauser and Wood, 1991; Lee, 2003; MacNutt *et al.*, 2006; Eliason *et al.*, 2013; Zhang *et al.*, 2018). However, salmonids can resume aerobically challenging activities after partial recovery (Farrell *et al*., 1998, Jain *et al*., 1998, Lee *et al*., 2003, MacNutt *et al*., 2006, Eliason *et al*., 2013, Eliason and Farrell, 2016) and fish are unlikely to have multiple hours to rest and recover in the wild. We opted instead to measure recovery over 1 hour to capture the initial phase of rapid recovery and part of the plateau phase. In general, summer steelhead took much longer to reach $Time_{FAS3}$ compared to $Time_{AAS80}$ at temperatures of 19°C and above (Table 3). Since FAS $\geq$ 3 is needed for feeding and digestion, this metric is likely more relevant to this juvenile life stage than $Time_{AAS80}$, which may be more relevant for migratory life stages. $Time_{FAS3}$ was significantly higher for the Lower Deschutes population, even at non-stressful temperatures, than any of the other populations meaning that while this population is more resistant to incurring higher energetic costs at warm temperatures, costs of recovering from aerobic efforts are high all the time. This means that Lower Deschutes summer steelhead could be more susceptible to mortality from predator evasion or catch-and-release fishing. In contrast, the John Day population recovered extremely quickly at non-stressful temperatures (Table 3). This is likely advantageous given the large daily temperature swings these fish

encounter. Energetic costs incurred during the brief time of that temperatures are hot can likely be quickly recuperated once temperatures start to cool.

3.5.4 Conclusions

The present study documents intraspecific differences in thermal tolerance between populations of summer steelhead inhabiting distinct thermal environments located within Oregon, USA. While warm exposure appears to confer elevated functional and critical thermal tolerance, this does not guarantee reduced vulnerability to climate warming. In fact, warm-dwelling summer steelhead populations appear to be at the greatest risk of experiencing physiologically limiting temperatures. Therefore, managers should focus active conservation efforts such as habitat restoration on warm-dwelling, inland populations for this species. Population-specific management strategies, particularly for broadly distributed species like steelhead, will be crucial for mitigating the impact of climate change on fishes.

	Deschutes (Buckhollow Creek)	John Day (Bridge Creek)	North Umpqua (Steamboat Creek)	Siletz (Gravel Creek)	Source
Watershed Size (km^2)	512.8	697.32	588.51	24.89	n/a
Steelhead Distribution Area (km)	115.59	62.02	82.67	9.82	n/a
Migrating Distance (km)	428.29	561.32	260.06	107.71	n/a
Estimated Mean Annual Discharge (m^3s^{-1})	71.8	35	77	11.7	Liang et al. 1994
30-Year Annual Precipitation range (mm)	312-341	303-839	1239-2050	2008-3825	Daley et al. 1994
2021 Annual Estimate of Precipitation (mm)	219-240	185-590	987-1720	2288-4306	Daley et al. 1994
Elevation (m)	229	1930	580	211	n/a
Annual Temperature Range	~1-25°C	~1-27°C	~1-21°C	~2-16°C	Present study
Maximum Diurnal Temperature Range	7°C	13°C	6°C	4°C	Present study

Table 3.1: Environmental characteristics for each study location.

Population	Holding Temperature Fluctuation (°C)	Chase Temperature (°C)	MMR ($mgO_2kg^{-1}L^{-1}$)	RMR ($mgO_2kg^{-1}L^{-1}$)	AAS ($mgO_2kg^{-1}L^{-1}$)	FAS	CT_{MAX} Start Temperature (°C)	CT_{MAX} (°C)
Lower Deschutes	18-22	19	10.62 ± 0.54^{a}	2.85 ± 0.12^{a}	7.78 ± 0.61	3.76 ± 0.25^{a}	20	32.06 ± 0.08^{a}
	20-24	22	12.72 ± 0.29^{b}	3.88 ± 0.24^{b}	8.84 ± 0.29	3.34 ± 0.15^{ab}	22	31.43 ± 0.06^{b}
	23-27	26	12.59 ± 0.56^{b}	4.60 ± 0.21^{b}	7.99 ± 0.47	2.76 ± 0.12^{b}	26	31.31 ± 0.11^{b}
John Day	14-27	19	12.85 ± 0.89	2.42 ± 0.10^{d}	10.42 ± 0.91^{d}	5.42 ± 0.47^{d}	21	31.20 ± 0.07
	20-24	22	15.23 ± 0.99	4.13 ± 0.28^{d}	11.09 ± 0.83^{d}	3.72 ± 0.20^{e}	n/a	not measured
	23-27	27	12.89 ± 1.74	6.56 ± 1.08^{e}	6.32 ± 1.11^{e}	2.21 ± 0.33^{f}	26	31.16 ± 0.08
Siletz	15-19	16	12.27 ± 0.55	1.72 ± 0.09^{g}	10.55 ± 0.50^{g}	7.17 ± 0.36^{g}	16	28.84 ± 0.09^{g}
	18-22	19	13.86 ± 0.66	2.72 ± 0.23^{h}	11.14 ± 0.49^{g}	5.25 ± 0.23^{gh}	20	29.50 ± 0.11^{h}
	20-24	22	12.28 ± 1.45	3.19 ± 0.28^{h}	9.10 ± 1.49^{g}	4.02 ± 0.48^{h}	22	30.24 ± 0.09^{i}
	23-26	25	11.06 ± 0.52	4.70 ± 0.26^{i}	6.36 ± 0.33^{h}	2.36 ± 0.07^{i}	n/a	not measured
North Umpqua	16-19	16	9.76 ± 0.46	1.94 ± 0.09^{j}	7.82 ± 0.49^{j}	5.17 ± 0.37^{j}	18	30.04 ± 0.33
	18-22	19	10.20 ± 0.47	2.37 ± 0.05^{j}	7.83 ± 0.48^{j}	4.33 ± 0.22^{j}	19	29.88 ± 0.25
	20-24	22	10.15 ± 0.64	3.08 ± 0.09^{k}	7.07 ± 0.64^{jk}	3.31 ± 0.21^{k}	22	30.03 ± 0.25
	23-26	25	9.54 ± 0.38	3.84 ± 0.18^{l}	5.70 ± 0.35^{k}	2.52 ± 0.11^{k}	25	29.91 ± 0.38

Table 3.2. Oxygen update rates (Maximum metabolic rate (MMR); Resting Metabolic Rate (RMR) at aerobic scope temperatures; Absolute Aerobic Scope (AAS), Factorial Aerobic Scope (FAS)) and CT_{MAX} for each temperature treatment and population. All values are presented as mean ± SEM. Differing letters indicate statistically significant differences between temperature treatments within populations (one-way Anova or Kruskal-Wallis test; $p<0.05$; Deschutes River: a,b; John Day River: d,e,f; Siletz River: g,h,i; North Umpqua River: j,k,l).

Population	Holding Temperature Fluctuation (°C)	Chase Temperature (°C)	$Time_{AAS80}$ (minutes)	$Time_{FAS3}$ (minutes)
Lower Deschutes	18-22	19	71.0 ± 13.6^{a}	162.1 ± 0.12^{a}
	20-24	22	50.0 ± 2.6^{a}	156.6 ± 0.24^{a}
	23-27	26	60.0 ± 4.9^{a}	n/a
John Day	14-27	19	11.2 ± 0.5^{d}	12.1 ± 1.0^{d}
	20-24	22	12.1 ± 0.7^{d}	24.5 ± 4.3^{e}
	23-27	27	34.1 ± 4.3^{e}	n/a
Siletz	15-19	16	41.5 ± 3.9^{g}	39.0 ± 4.5^{g}
	18-22	19	37.7 ± 3.5^{g}	51.7 ± 6.1^{gh}
	20-24	22	66.2 ± 9.2^{h}	88.7 ± 21.3^{h}
	23-26	25	83.5 ± 4.4^{h}	n/a
North Umpqua	16-19	16	29.2 ± 3.3^{j}	36.0 ± 8.8^{j}
	18-22	19	40.7 ± 3.4^{j}	77.5 ± 16.6^{j}
	20-24	22	66.4 ± 4.5^{k}	125.8 ± 19.9^{k}
	23-26	25	64.2 ± 3.1^{k}	n/a

Table 3.3: Exercise recovery metrics for each temperature treatment and population including the time to have 80% of absolute aerobic scope available ($Time_{AAS80}$) and the time to have and factorial aerobic scope of 3 available ($Time_{FAS3}$) after being chased. All values are presented as mean ± SEM. Differing letters indicate statistically significant differences between temperature treatments within populations (one-way Anova or Kruskal-Wallis test; $p<0.05$; Deschutes River: a,b; John Day River: d,e,f; Siletz River: g,h,i; North Umpqua River: j,k,l).

Watershed	T_{OPT}	T_{PEJ}	T_{FAS3}	CT_{MAX}	Max Stream Temp	TSM	FWT
Lower Deschutes	-	-	24.4	32.1	24.9	7.2	-0.5
John Day	21.3	17.4, 25.2	24.9	31.2	27.1	4.1	-2.2
North Umpqua	17.4	11.0, 23.8	23.3	30.0	21.6	8.4	1.7
Siletz	17.9	13.0, 22.7	23.8	28.8	15.9	12.9	7.9

Table 3.4: Thermal vulnerability metrics for each population including optimal (T_{OPT}) and pejus (T_{PEJ}) temperaures for absolute aerobic scope, temperatures where factorial aerobic scope = 3 (T_{FAS3}), average ambient critical maxima (CT_{MAX}), maximum stream temperatures, thermal safety margins (TSM) and functional warming tolerance (FWT). All values are in °C.

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Appendix

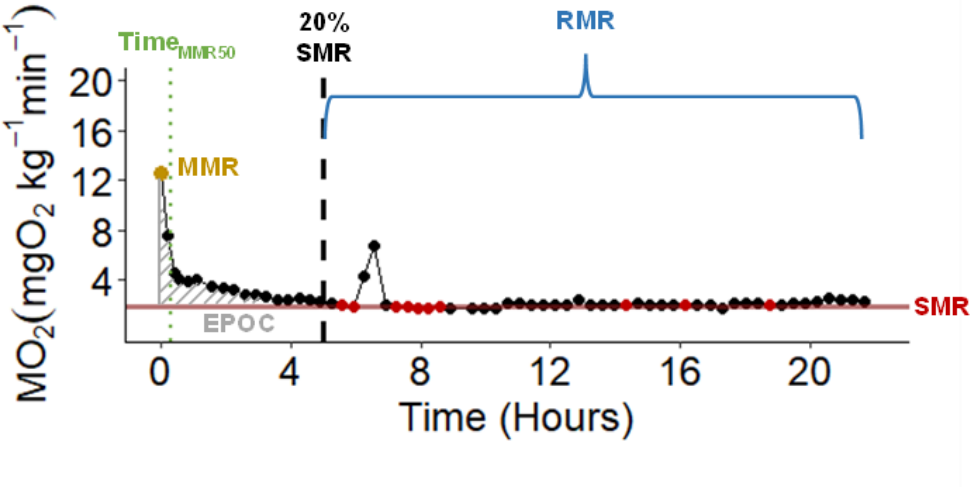

Figure A1. A representative MO_2 trace of a single fish over the duration of a respirometry trial. Circular points represent MO_2 measurements. The large yellow point at time 0 represents maximum metabolic rate (MMR). The green dotted line represents the duration to

reach 50% of MMR ($Time_{MMR50}$). The red horizontal line represents standard metabolic rate (SMR) and the red points represent the values that were averaged to calculate SMR. The bold dashed line represents the time that MO2 reached 20% of SMR. All points before the 20% SMR line were considered part of the fish's recovery period. The area under the curve between MMR and 20% SMR, shown in gray, was considered excess post-exercise oxygen consumption (EPOC). All points after the 20% SMR line were considered measurements of resting metabolic rate (RMR).

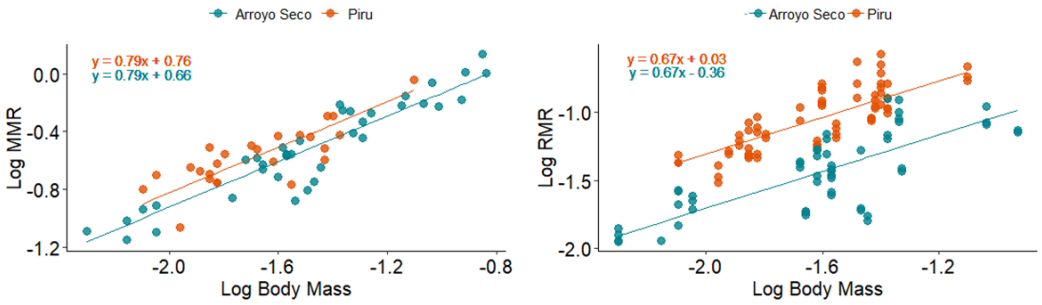

Figure A2. Log-log plots of the relationship between body mass and MMR (panel A) and RMR (panel B) with Piru Creek trout shown in orange and Arroyo Seco trout shown in blue.

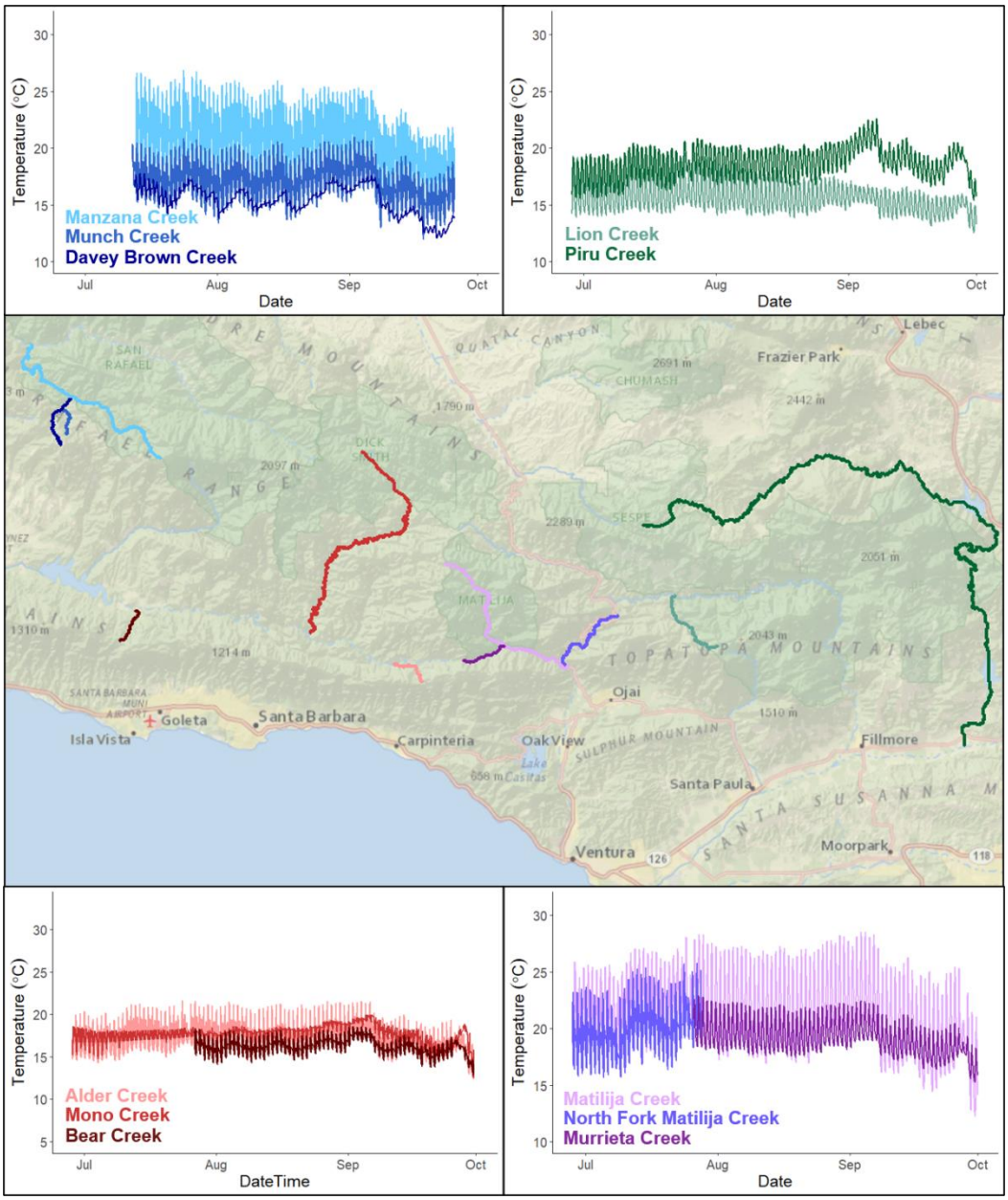

Figure A3: Temperature data (collected every 15 minutes) and stream locations for HOBO Dissolved Oxygen and HOBO Pendant loggers placed in 4 separate watersheds during the summer of 2019 (Santa Maria River: blue, Santa Clara River: green, Santa Ynez River: red, Ventura River, purple).

Population	*Temperature Treatment*	*Temperature Range (°C)*	*CT_{MAX} Start Temperature (°C)*	*Mean ± SEM CT_{MAX}*	*Body mass (g)*	*n*	*df*	*F-value*	*p-value*
Piru Creek	Ambient	17-22	20	30.29 ± 0.62[a]	30.5 ± 2.36	11	2	2.680	0.086
	+3°C	20-24	24	31.30 ± 0.02[a]	23.5 ± 5.87	11			
	+5°C	22-26	25	31.43 ± 0.06[a]	15.3 ± 1.86	9			
Arroyo Seco	Ambient	14-19	16	27.49 ± 0.78[x]	29.0 ± 3.05	11	2	4.711	**0.017**
	+3°C	17-21	19	29.83 ±0.24[x]	34.3 ± 13.02	10			
	+5°C	19-24	22	29.52 ± 0.57[y]	24.3 ± 4.27	10			

Table A1. Critical Thermal Maximum (CT_{MAX}) for each population and temperature treatment. All values are presented as mean ± SEM. Differing letters indicate statistically significant differences within populations (one-way Anova; $p<0.05$; Piru Creek: a,b; Arroyo Seco: x,y).

Temperature Treatment	*Population*	*CT_{MAX} Start Temperature (°C)*	*Mean ± SEM CT_{MAX}*	*Body mass (g)*	*n*	*W*	*p-value*
17-22°C	Piru Creek	20	30.29 ± 0.62[a]	30.5 ± 2.36	11	92	**0.007**
	Arroyo Seco	19	29.83 ±0.24[b]	34.3 ± 13.02	10		
20-24°C	Piru Creek	24	31.30 ± 0.02[x]	23.5 ± 5.87	11	4.711	**0.013**
	Arroyo Seco	22	29.52 ± 0.57[y]	24.3 ± 4.27	10		

Table A2. Critical Thermal Maximum (CT_{MAX}) for each population at each common temperature treatment. All values are presented as mean ± SEM. Differing letters indicate statistically significant differences between populations (Mann-Whitney U; $p<0.05$).

Population	*Dependent Variable*	*Fixed Effect*	X^2	*Df*	*p-value*	*AIC*	*BIC*
Piru Creek	RMR	Temperature	3.955	1	**0.047**	188.37	205.76
		Treatment Group	1.121	2	0.571		
		Temperature: Treatment Group	1.225	2	0.542		
	RMR	Temperature	6.346	1	**0.012**	185.58	198.62
		Treatment Group	1.829	2	0.401		
	RMR	Temperature	14.855	1	**<0.001**	183.38	192.08
	ln(RMR)	Temperature	4.676	1	**0.031**	0.56	17.95
		Treatment Group	0.645	2	0.724		
		Temperature: Treatment Group	0.560	2	0.756		
	ln(RMR)	Temperature	6.578	1	**0.010**	-2.88	10.16
		Treatment Group	3.517	2	0.172		
	ln(RMR)	Temperature	15.045	1	**<0.001**	-3.50	5.20
Arroyo Seco	RMR	Temperature	3.725	1	0.054	61.63	77.24
		Treatment Group	2.235	2	0.327		
		Temperature: Treatment Group	2.633	2	0.268		
	RMR	Temperature	10.630	1	**0.001**	60.05	71.76
		Treatment Group	3.567	2	0.168		
	RMR	Temperature	33.491	1	**<0.001**	59.44	67.25
	ln(RMR)	Temperature	9.228	1	**0.002**	18.35	33.96
		Treatment Group	4.890	2	0.087		
		Temperature: Treatment Group	4.272	2	0.118		
	ln(RMR)	Temperature	9.928	1	**0.002**	18.20	29.91
		Treatment Group	4.807	2	0.090		
	ln(RMR)	Temperature	30.345	1	**<0.001**	18.71	26.52

Table A3. Statistical outputs from each of the linear mixed models fitted to the relationship between resting metabolic rate (RMR) and temperature. Temperature refers to the test temperature at the time of each MO_2 measurement. Treatment Group refers to holding temperature regimes (Ambient, +3°C, +5°C). Best fit models are highlighted in grey.

Temperature (°C)	*Population*	*Mean ± SEM RMR*	*n*	*df*	*t-value*	*p-value*
18	Piru Creek	2.95 ± 0.31	6	9	-4.54	**0.001**
	Arroyo Seco	1.29 ± 0.14	5			
19	Piru Creek	2.69 ± 0.15	6	16	-3.92	**0.002**
	Arroyo Seco	1.70 ± 0.18	12			
20	Piru Creek	3.58 ± 0.43	11	11.77	-4.00	**0.002**
	Arroyo Seco	1.77 ± 0.13	10			
21	Piru Creek	3.64 ± 0.44	11	12.56	-3.65	**0.003**
	Arroyo Seco	1.95 ± 0.16	8			

Table A4. Resting Metabolic Rate (RMR) of *O. mykiss* from each of the Piru Creek and Arroyo Seco populations measured at 4 common temperatures. Represented are mean and standard error values and t-test results comparing populations at each temperature.

Dependent Variable	*Fixed Effect*	*X^2*	*Df*	*p-value*	*BIC*
Percent MMR	Time post-MMR	0.781	5	0.974	34.67
	Population	10.950	1	**<0.001**	
	Temp Treatment	3.04	2	0.165	
	Time: Population	3.185	5	0.674	
	Time: Temp Treatment	12.345	10	0.246	
	Population: Temp Treatment	0.815	1	0.400	
	Time: Population: Temp Treatment	3.624	4	0.433	
Percent MMR	Time post-MMR	37.712	5	**<0.001**	-119.42
	Population	49.393	1	**<0.001**	
	Temp Treatment	3.900	2	0.083	
Percent MMR	Time post-MMR	37.812	5	**<0.001**	-133.76
	Population	45.626	1	**<0.001**	

Table A5. Statistical outputs from linear mixed models fitted to the relationship between percent maximum metabolic rate (MMR) and time post-MMR. The best fit model is highlighted in grey.

Stream Name	Watershed	Location	Maximum Summer Temperature	Minimum Summer Temperature	Mean Summer Temperature	Summer Diurnal Fluctuation Range	Years Measured
Arroyo Seco	Salinas River	36.11914, -121.46904	23°C	13°C	19°C	1-5°C	2022
Lion Creek	Santa Clara River	34.54338, -119.16372	20°C	13°C	15°C	1-6°C	2019, 2021, 2022
Piedra Blanca Creek	Santa Clara River	34.58515, -119.16543	22°C	13°C	18°C	0-2°C	2021
Piru Creek	Santa Clara River	34.62655, -118.74397	25°C	15°C	20°C	1-4°C	2019, 2020, 2021
Sespe Creek	Santa Clara River	34.44492, -118.92715	31°C	17°C	24°C	2-11°C	2008, 2009, 2013
Santa Paula Creek	Santa Clara River	34.42763, -119.09089	28°C	15°C	21°C	1-10°C	2008, 2009, 2010
Davey Brown Creek	Santa Maria River	34.73486, -119.96602	18°C	12°C	15°C	0-2°C	2019
Manzana Creek	Santa Maria River	34.76897, -119.93607	27°C	13°C	20°C	6-11°C	2019
Munch Creek	Santa Maria River	34.74594, -119.92533	21°C	12°C	17°C	3-6°C	2019
Alder Creek	Santa Ynez River	34.48329, -119.49618	22°C	14°C	17°C	2-6°C	2019
Bear Creek	Santa Ynez River	34.5257 -119.86739	18°C	14°C	16°C	0-4°C	2019
Mono Creek	Santa Ynez River	34.54117 -119.62472	20°C	15°C	18°C	0-6°C	2019
Matilija Creek	Ventura River	34.52345, -119.40136	28°C	16°C	21°C	3-10°C	2019
Murrieta Creek	Ventura River	34.50331, -119.38805	23°C	17°C	19°C	3-5°C	2019
North Fork Matilija Creek	Ventura River	34.50606, -119.27772	25°C	17°C	20°C	4-8°C	2019
Upper North Fork Matilija Creek	Ventura River	34.51057, -119.38292	29°C	14°C	21°C	2-14°C	2019

Table A6. Summary of all temperature data collected throughout the Los Padres National Forest. Temperatures shown represent the maximum, minimum, and average temperatures for each location during the summer months (June-September) during the years each logger was deployed.

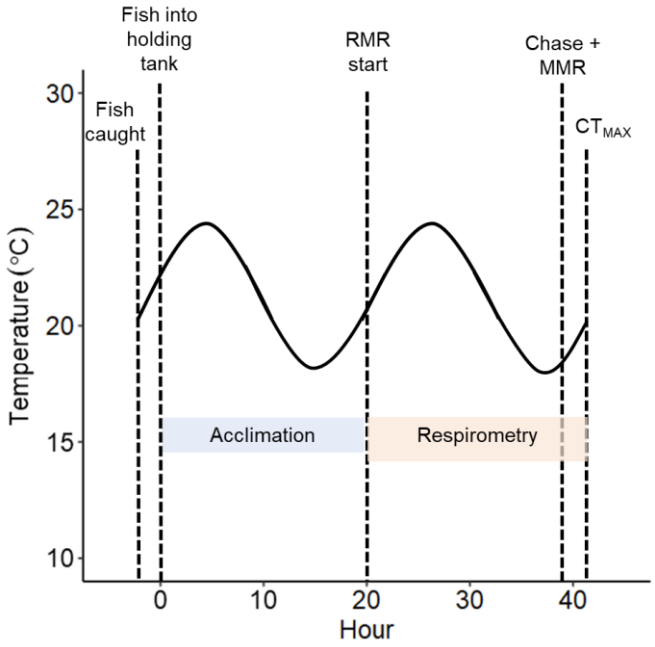

Figure A4: Conceptual diagram of the timing of temperature treatments, respirometry, and CT_{MAX}.

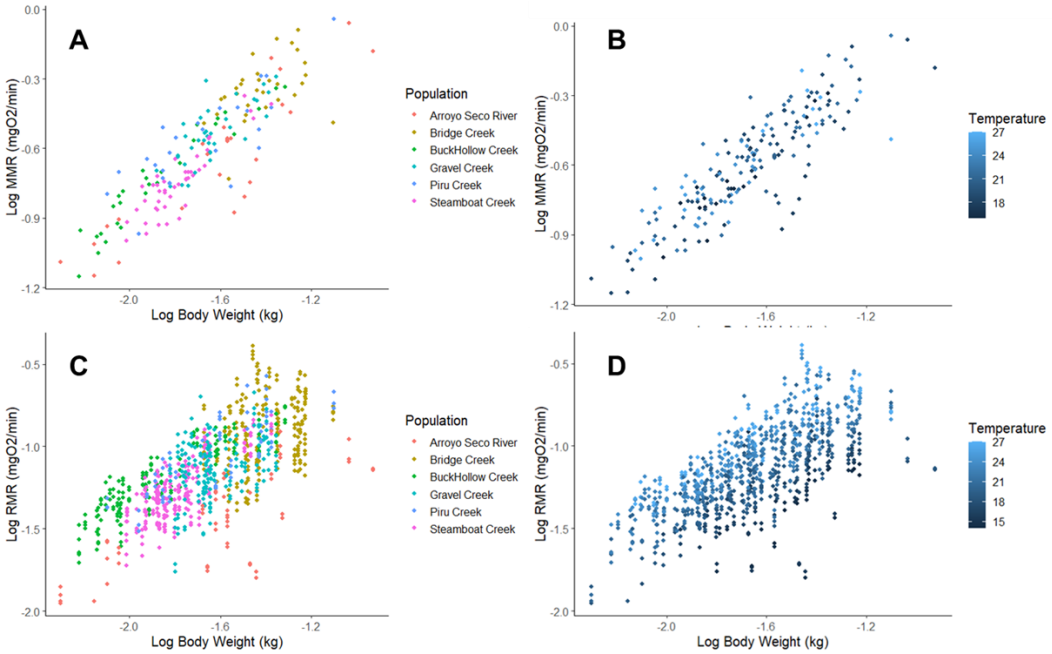

Figure A5: Log-log relationships between body weight and Maximum Metabolic Rate (MMR; panels A and B) and between body weight and Resting Metabolic Rate (RMR; panels C and D). In panels A and C, color indicates *O. mykiss* population. In panels B and D, shading represents the temperature at which each metabolic rate measurement was taken, with lighter shading indicated warmer temperaures and darker shading indicating cooler temperaures.

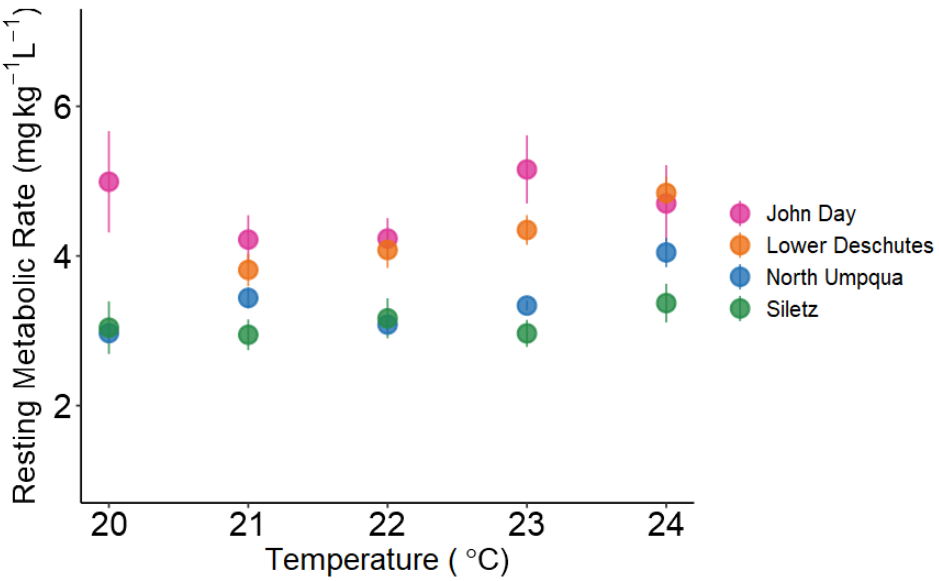

Figure A6. Resting metabolic rate (RMR) for each temperature within a common temperature treatment of 20-24°C.

Population	*Dependent Variable*	*Fixed Effect*	X^2	*Df*	*p-value*	*BIC*
Lower Deschutes	RMR	Acute Temperature	26.7078	1	**<0.001**	269.41
		Treatment	6.9115	2	**0.032**	
		Acute Temperature: Treatment	10.3037	2	**0.006**	
	ln(RMR)	Acute Temperature	24.7388	1	**<0.001**	-131.77
		Treatment	2.6732	2	0.263	
		Acute Temperature: Treatment	4.9332	2	0.085	
	ln(RMR)	Acute Temperature	79.379	1	**<0.001**	-137.04
		Treatment	15.680	2	**<0.001**	
John Day	RMR	Acute Temperature	276.8700	1	**<0.001**	708.11
		Treatment	3.8573	2	0.145	
		Acute Temperature: Treatment	3.1066	2	0.212	
	RMR	Acute Temperature	274.0674	1	**<0.001**	700.41
		Treatment	1.6324	2	0.442	
	RMR	Acute Temperature	287.31	1	**<0.001**	691.22
	ln(RMR)	Acute Temperature	569.3172	1	**<0.001**	2.86
		Treatment	2.7799	2	0.249	
		Acute Temperature: Treatment	13.0620	2	**0.001**	
North Umpqua	RMR	Acute Temperature	22.6364	1	**<0.001**	295.05
		Treatment	5.7491	3	0.124	
		Acute Temperature: Treatment	6.5360	3	0.089	
	RMR	Acute Temperature	132.080	1	**<0.001**	285.35
		Treatment	16.844	3	**<0.001**	
	ln(RMR)	Acute Temperature	36.1010	1	**<0.001**	-188.08
		Treatment	5.4436	3	0.142	
		Acute Temperature: Treatment	3.2050	3	0.361	
	ln(RMR)	Acute Temperature	131.406	1	**<0.001**	-201.04
		Treatment	24.544	3	**<0.001**	
Siletz	RMR	Acute Temperature	11.3632	1	**<0.001**	425.51
		Treatment	18.3869	3	**<0.001**	
		Acute Temperature: Treatment	22.5578	3	**<0.001**	
	ln(RMR)	Acute Temperature	41.000	1	**<0.001**	-35.64
		Treatment	10.429	3	**0.015**	
		Acute Temperature: Treatment	12.633	3	**0.006**	

Table A7: Model selection for relationships between resting metabolic rate (RMR), acute temperature, and temperature treatment. Best fit models are highlighted in gray.