ARTICLE

Climate Ecology

Habitat fragmentation drives divergent survival strategies of a cold-water fish in a warm landscape

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Abstract

Climate change is a global phenomenon, but natural selection occurs within landscapes. Many global analyses predict how climate change will shape behavior and physiology, but few incorporate information from the landscape scales at which animals actually respond to selective pressure. We compared cold-water fish (redband trout *Oncorhynchus mykiss newberrii*) from neighboring habitats in a naturally warm, recently fragmented basin to understand how different responses to warming may arise from landscape constraints. Trout in warm, hydrologically connected Upper Klamath Lake fled summer temperatures and sought refuge in cool tributaries, while trout in an equally warm but fragmented reach of the Klamath River endured summer conditions. Trout in the river were more physiologically tolerant of high temperatures than trout in the lake across multiple metrics, including capacity for aerobic activity, recovery from exertion, and loss of equilibrium. Two independent metrics of energetic condition indicated that the behavioral strategy of trout in the lake came at a substantial energetic cost, while the physiological strategy of trout in the river was able to mitigate most energetic consequences of high temperatures. No clear genetic basis for increased tolerance was found in trout from the river, which may suggest tolerance was derived from plasticity, although our analysis could not rule out genetic adaptation. Our results show that landscape processes such as fragmentation can cause different climate survival strategies to emerge in neighboring populations. Connecting the mechanisms that favor similar survival strategies among related organisms at broad scales with mechanisms that drive landscape-scale variability within taxa should be a major goal for future predictions of biological responses to climate change.

INTRODUCTION

All animals threatened by climate change face the same simple ultimatum: "adapt, move, or die" (Aplet & McKinley, 2017; Habary et al., 2017; Narum et al., 2013). Though this wording is more concise than precise, the implication is profound—the only pathways to survival in a warming world are physiological changes (tolerance) or behavioral changes (avoidance). Understanding how these two responses will unfold across a diverse animal kingdom is a major research goal for biology and conservation. Many studies analyze broad taxonomic and spatial trends, often reaching the dire conclusion that most animals may not develop tolerance (Comte & Olden, 2017; Gunderson & Stillman, 2015; Quintero & Wiens, 2013) or avoidance (Comte & Grenouillet, 2013; Schloss et al., 2012) fast enough to survive rapid warming. Yet predictions of widespread declines may be overestimated, because such studies can rarely account for physiological and behavioral responses at finer resolutions that vary from broader patterns (Lapointe et al., 2018; Moritz & Agudo, 2013). To predict which animals will respond successfully, we must first understand how these responses operate at different spatial, temporal, and taxonomic scales.

Physiological changes such as expression of heat-resistant proteins and increased metabolic performance can allow animals to tolerate warming. These changes can occur within days through acclimatization (Seebacher et al., 2015) or may manifest in the next offspring through transgenerational plasticity (Donelson et al., 2018). Over longer timescales (many generations), genetic components of thermal tolerance may evolve through natural selection (Johnston & Bennett, 1996). Behavioral changes such as body orientation and posture allow animals to buffer their body temperature from changes in environmental temperatures, often at diel timescales (Harris et al., 2015). When buffering in place is not enough to maintain suitable body temperature, animals may avoid warming by moving to cooler locations. Movements ranging from a few meters each day to hundreds of kilometers each year can occur (Carr et al., 1996; Mundahl, 1989). Dispersal behaviors of offspring can even allow animals to avoid unfavorable temperatures over multiple generations and degrees of latitude (Perry et al., 2005). While we increasingly understand each of these responses separately, how they will interact at the individual or population level and scale up to

broader taxonomic levels remains a major knowledge gap (Donelson et al., 2019; Huey et al., 2012; Nogués-Bravo et al., 2018).

Biologists have long attempted to identify the conditions under which different responses to temperature emerge in ectotherms. The cost–benefit model (Huey & Slatkin, 1976) remains an influential contribution. The model suggests that the degree to which ectotherms respond physiologically or behaviorally depends on the energetic costs and benefits of each option. In high-level climate vulnerability analyses, costs and benefits have commonly been described using the mean difference between environmental and optimal temperatures (Kearney et al., 2009). Yet recent advancements have demonstrated that cost–benefit ratios are determined not by an average of the thermal environment, but by its finer scale landscape characteristics, including variability, configuration, and connectivity (Sears et al., 2016; Sears & Angilletta, 2015). These developments show that a landscape-level perspective of physiological and behavioral "coadaptation" to temperature (Angilletta et al., 2006) is needed to predict which ectotherms will tolerate or avoid climate change threats. However, empirical studies supporting this perspective are overwhelmingly focused on lizards, leaving implications for aquatic ectotherms unclear (Donelson et al., 2019; Huey et al., 2012). Since water transfers and stores solar energy differently than the atmosphere, how air temperature affects terrestrial ectotherms may not be comparable to how water temperature affects aquatic ectotherms (Norris & Kunz, 2012). Additionally, many freshwater systems form linear networks with ephemeral branches that move in space. Such a layout drastically changes the consequences of animal movement and increases vulnerability to fragmentation (Comte & Grenouillet, 2013). These fundamental differences raise the question of how landscape characteristics could mediate responses to warming specifically in aquatic ectotherms.

Freshwater fishes, like terrestrial ectotherms, display local variation in physiological (Eliason et al., 2011; Narum et al., 2010; Stitt et al., 2014) and behavioral (Keefer et al., 2009; Mackenzie-Grieve & Post, 2006) responses to temperature. Yet whether this variation is linked to landscape characteristics that pose energetic costs and benefits has not been investigated. Here, we determine whether the survival strategy of a cold-water fish in a naturally warm basin varies locally with landscape connectivity. We address this question in the wild

by leveraging the modification of the basin by dams. Although the landscape is naturally dotted with patches of cool water that could provide energetic benefits in summer, dam construction has increased the cost of movement and restricted access to these patches for a fraction of fish. We hypothesize that fish from the more fragmented habitat respond physiologically to summer temperatures by increasing metabolic performance, while neighboring fish in the more connected habitat respond behaviorally by moving to refuges. We also hypothesized that the physiological response incurs greater energetic costs (reflecting the increased costs imposed by fragmentation) and arises from genetic adaptation. To test these hypotheses, we characterize responses in fish from fragmented and connected habitats using multiple lines of evidence, including (1) paired body temperature and movement data, (2) tests of physiological performance, (3) metrics of energetic condition, and (4) genotypic indicators of thermal tolerance. By investigating these different aspects of survival in warm habitats, we show the importance of considering landscape-scale variability in predicting how entire taxa will respond to climate change.

FIGURE 1 Map of the Klamath River Basin, showing relevant bodies of water (blue), dams (black circles), United States political boundaries (gray lines), and the three reaches corresponding to sampling groups in this study, as detailed in *Methods* (sections of water labeled in green). UKL, Upper Klamath Lake.

METHODS

Study setting

We explored landscape influences on responses to temperature in redband rainbow trout (*Oncorhynchus mykiss newberrii*), a cold-water fish, in the Klamath River Basin, Oregon, USA. The basin is predominantly warm in summer (relative to the broader range of redband trout), but scattered with patches of perennially cool water due to the region's porous geology and numerous groundwater features (Gannett et al., 2007). Six dams fragmented the Klamath River beginning in 1918 (Hamilton et al., 2016), including J.C. Boyle Dam, which was built just upstream of the largest known groundwater spring complex in the mainstem river (Figure 1). The 8-km segment of flowing river above the dam, known as the Keno Reach, exhibits some of the hottest temperatures in the basin (>25°C; PacifiCorp, 2013) yet still supports redband trout. Although J.C. Boyle Dam has passage facilities (i.e., fish ladders), fish movements past the dam site decreased dramatically following its construction (Jacobs et al., 2006). Thus, connectivity to the major inflow of cool water downstream has likely been impaired by dams, so the cost of movement from the Keno Reach to cool water appears to be high. However, the assumption that no cool water is available within the Keno Reach is based on remote mapping of surface temperatures (Watershed Sciences, 2002)
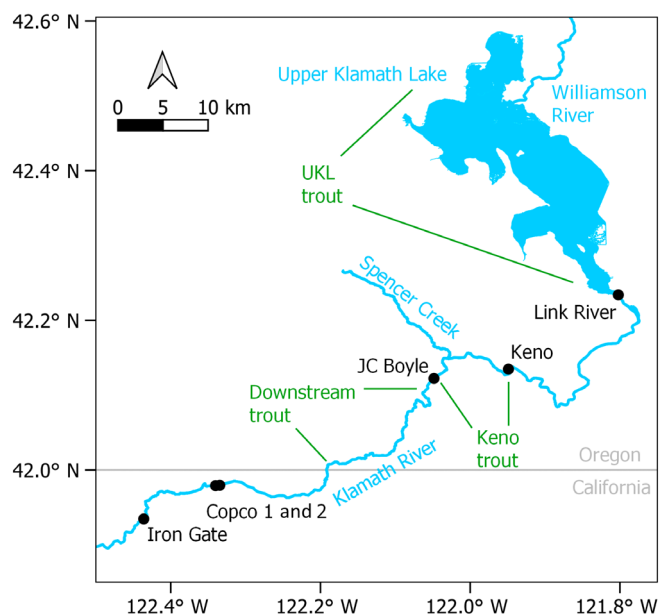
and is yet to be validated. Other warm areas of the basin, such as shallow, hypereutrophic Upper Klamath Lake (UKL), have retained more of their historical connectivity. Though UKL is only 15 km (33 river km upstream) from the Keno Reach and features a very similar thermal regime, the pathways from UKL into its cool tributaries remain free of dams. Therefore, we compared responses to summer temperatures in trout from the fragmented Keno Reach ("Keno trout") with those in trout from the more connected UKL ("UKL trout") to compare responses under greater and lesser costs of movement, respectively.

Behavior

We used radio telemetry to monitor movement behavior in Keno and UKL trout. We implanted individually identifiable, temperature-sensing radio tags in Keno trout in 2017 (282–476 mm fork length [FL]; $n = 14$) and in UKL trout in 2016 and 2017 (444–760 mm FL; $n = 91$). All fish were tagged in warm habitats (i.e., within UKL and the Keno Reach) during the spring, approximately 5–8 km from the nearest known cool habitat to maintain similar expected costs of travel for thermoregulation. Detailed tagging and tracking procedures followed Hahlbeck et al. (2022) except that for Keno trout we used a smaller tag with an 8-month battery life (model MST-930, Lotek, Newmarket, ON) to maintain the same relative tag

burden as in the generally larger fish from UKL. To monitor potential behavioral thermoregulation, between 2016 and 2018, we conducted ~300 opportunistic mobile tracking surveys distributed throughout UKL, its major tributaries, and the Keno Reach. We also placed 24-h stationary radio receivers at the boundaries of the three largest cool spring-fed habitats connected to UKL. We obtained detections from all fish except one UKL trout, which we suspect was because of a nonfunctional tag. Behavioral thermoregulation was assessed by comparison of tag temperature with the dominant thermal habitat (i.e., UKL and the mainstem Keno Reach). Daily mean temperatures for the Keno Reach and UKL were obtained from fixed monitoring sites operated by the U.S. Geological Survey (USGS, 2022). Tag temperatures obtained near the end of battery life can be unreliable, so as a precaution, we stopped tracking approximately four months before the expected end of battery life for each tag.

Physiology

We used stream-side respirometry to evaluate the thermal tolerance of UKL and Keno trout in August 2018. UKL trout were sampled from the Williamson River, a major tributary of UKL (42.663777° N, 121.846298° W). Keno trout were sampled from Spencer Creek, the lone spawning tributary to the Keno Reach (42.156299° N, 122.027346° W). Redband trout suitable for the size of the respirometry chamber (UKL trout 22.4 g ± 5.9 g, Keno trout 20.5 g ± 10.1 g, mean ± SD, $n = 24$ each group) were captured by electrofishing and placed in a holding tank. Fish were held overnight without feeding to ensure they were postabsorptive during respirometry and to allow recovery from capture and adjustment to the tank.

Individuals were assigned to one of three respirometry trials at different initial temperatures ($n = 8$ fish per trial). The temperatures represented ambient stream conditions, maximum summer conditions, and climate warming conditions. Since UKL trout were sampled from a cool tributary, whereas Keno trout were sampled from a warm tributary, different temperatures representing ambient, maximum, and warming conditions were chosen for each group of trout (UKL: 11, 18, 22°C, respectively; Keno: 14, 22, 25°C, respectively). For trials above ambient stream temperature, the holding tank was slowly warmed (~1°C h^{-1}) to the test temperature and then held constant for 18 h prior to the trial. At the start of each trial, fish were chased to exhaustion following standard protocols (Little et al., 2020; Norin & Clark, 2016) and air-exposed for 30 s. Immediately afterward, fish were transferred to chambers that measured the rate of metabolic oxygen consumption (MO$_2$) for 18–24 h (see Appendix S1 and

Anlauf-Dunn et al., 2022 for more detailed methods). Temperature was continuously monitored and allowed to fluctuate naturally in the ambient trial, which began in the morning (Appendix S1: Figure S1), but kept constant in the other two trials. At the end of this measurement period, length (in millimeters) and weight (in grams) were recorded. One hour after the ambient trial, a standard assessment of critical thermal maximum (CT$_{max}$) (Becker & Genoway, 1979) was conducted using all fish from the ambient temperature trial simultaneously. Water temperature was continuously increased by the recommended ramping rate of 0.3°C min^{-1}, and the temperature at loss of equilibrium was recorded for each fish. All fish survived the assessment. Further details are provided in Appendix S1.

We compared the thermal tolerance of UKL and Keno trout using several metrics from the respirometry trials in addition to CT$_{max}$. Maximum metabolic rate (MMR; in milligrams of O$_2$ per liter per minute) was determined from MO$_2$ immediately following exhaustion for 6 min (Little et al., 2020). We then calculated standard metabolic rate (SMR; in milligrams of O$_2$ per liter per minute) as the 10% quantile of all subsequent MO$_2$ measurements (Chabot et al., 2016). From these two metrics, we determined absolute aerobic scope (AAS = MMR − SMR) and factorial aerobic scope (FAS = MMR/SMR), indicators of metabolic capacity for activities (swimming, foraging, etc.) beyond meeting basal demands. We also estimated the recovery time fish would need to resume these activities after exhaustion. We used the time elapsed before MO$_2$ fell below 50% of MMR, a level at which other salmonids are able to fully recover their swimming ability (Eliason et al., 2013; Kraskura et al., 2021). Background respiration was negligible for both experiments, and the data were not adjusted to account for background respiration (Appendix S1). Nine individual MO$_2$ profiles did not decrease smoothly as expected during recovery from exercise. In two cases, this was associated with pump malfunction, and in seven cases possibly indicated that the overnight acclimatization period was insufficient for recovery from capture. Two mortalities were also observed during the climate trial in the Keno Reach. These individuals were excluded from MO$_2$ analysis, yielding a final sample size of 4, 4, and 6 Keno trout and 7, 8, and 8 UKL trout (for ambient, maximum, and climate trials, respectively). At least 77 and an average of 128 MO$_2$ measurements were used to determine metabolic rates for each of these fish.

We conducted one-sided Mann–Whitney U tests to test our hypothesis that Keno trout exhibited higher performance than UKL trout for each metric (i.e., greater MMR, AAS, FAS, and CT$_{max}$, and lower recovery time and SMR). For metrics that varied with temperature (not CT$_{max}$), tests compared UKL trout data at the shared

temperature of 22°C with Keno trout data at or above 22°C. This approach maximized the statistical power of our small sample size in a conservative way. (Since performance in terms of these metrics is known to decrease on the warm side of optimum temperature, Keno trout performance above 22°C would be lower than UKL trout performance at 22°C under the null hypothesis of identical thermal tolerance.) We also followed analysis methods recently used to infer warm tolerance from SMR, MMR, and AAS in another *O. mykiss* population (Chen et al., 2015). Specifically, we fitted SMR with a quadratic model using linear least squares regression and fitted MMR and AAS with three-parameter Gaussian models using nonlinear least squares regression. To ensure model fitting was realistic, the mean parameter (the location of the peak) was constrained to <30°C and the other two parameters (SD and height of the peak) were constrained to be positive in Gaussian models. All analyses were conducted in R (R Core Team, 2019).

Seasonal energetics

We used two complementary indices to characterize the energetic condition by nonlethal methods. Fulton's condition factor coarsely describes fish shape (plumpness) using the ratio of fish mass to the cube of its length (Ricker, 1975). Phase angle, an electrical characteristic of fish tissue, reflects the ratio of water within versus between cells, which is higher while fish energy stores are accumulating and lower while they are being consumed (Cox & Heintz, 2009). Thus, Fulton's condition factor approximates cumulative growth or depletion of energy stores, whereas phase angle approximates the recent rate of growth or depletion.

We captured adult UKL trout ($n = 227$) and Keno trout ($n = 67$) by hook-and-line in 2018 to measure changes in these energetic performance indices over the summer. A similar assortment of artificial baits was tried regularly in both locations to minimize size bias of sampling (within our target range, i.e., adult fish). Trout were anesthetized with Aqui-S (AquaTactics, Lower Hutt, New Zealand), measured (FL; nearest millimeters), and weighed (nearest 5 g). Resistance and reactance, which are used to calculate phase angle, were measured using a commercial handheld unit (Seafood Analytics, Juneau, AK). The electrodes were placed externally along the lateral line just behind the operculum while the fish was in a nonconductive PVC tray. As a cautionary measure to ensure sampling remained nonlethal, trout were not sampled when mean daily temperatures exceeded 20°C. We also collected a subset of data to reduce handling time where needed. Thus, we obtained condition data for both UKL and Keno trout only just before summer (mid-May

to mid-Jun) and at the end of summer (mid-Aug to mid-Sep), while we continued to sample UKL trout in cool tributaries throughout the period in between. Final sample sizes for the overlapping sampling periods are shown in Figure 4.

Though phase angle and condition factor are useful to describe energetic condition within populations, both metrics can be affected by length and shape differences between populations (Blackwell et al., 2000; Cox & Heintz, 2009). To make a more even comparison, we standardized each condition metric by population group (UKL or Keno trout), such that the new values reflected a percent difference from the pre-summer median. We used two-sided Mann–Whitney U tests to assess the statistical significance of any difference between the beginning and the end of summer for each group and condition metric, as well as a length difference between the two groups. We also examined the effect of within-group length variation on condition. We first fitted linear regressions on FL to each condition metric using data from all time periods. Then, we subtracted the predicted effect of length from each condition value and redid the analysis using these new values (i.e., the residuals).

Genetics

We sought to determine whether potential phenotypic differences in thermal tolerance were reflected in genotypic differences between Keno and UKL trout. However, thermal tolerance is controlled by many genes. A difference in any one thermal tolerance gene could reflect thermal adaptation through natural selection but could instead reflect neutral variation due to genetic drift (Chen et al., 2018). The degree of spawning sympatry between these groups before dam construction is unknown, so we could not directly account for the likelihood of differences due to genetic drift. Instead, we analyzed genotypes of additional trout from the next mainstem fragment downstream of the Keno Reach (downstream trout), between J.C. Boyle and Copco 1 Dams (Figure 1). Here, like in UKL, connectivity to cool springs remains unimpeded by dams. But unlike UKL trout, downstream trout were known to spawn sympatrically with Keno trout at the time of dam construction (Jacobs et al., 2006). Evidence from microsatellites (neutral genetic markers) suggests that since then, minimal drift between Keno and downstream trout has occurred (Pearse et al., 2011). Therefore, downstream trout can act as a reference group in the case of a genetic difference between UKL and Keno trout at markers associated with thermal tolerance. Congruence among UKL and downstream genotypes (which have access to known cool habitats) would favor thermal adaptation as the more likely

driver, while congruence among Keno and downstream genotypes (which remain similar at neutral markers) would favor genetic drift.

Adfluvial adult UKL trout (i.e., >425 mm; $n = 61$) were sampled in portions of UKL and the Williamson River mainly using hook-and-line, while a subset of samples were collected from post-spawn carcasses. Adult Keno trout ($n = 86$) and downstream trout ($n = 104$) were sampled by hook-and-line in two locations within their respective reaches (Figure 1). We collected a small piece of caudal fin tissue and extracted its DNA. Using GT-seq (Campbell et al., 2015), a high-throughput sequencing method, we obtained genotypes for each DNA sample at a panel of genetic markers (single nucleotide polymorphisms, SNPs). The panel, developed by Columbia River Inter-Tribal Fish Commission (Collins et al., 2020), includes 251 neutral markers and seven markers associated with thermal tolerance in Columbia River redband trout *O. mykiss gairdneri* (Appendix S1: Table S1). We employed several quality control measures to ensure that genotype data used for analysis were well-represented and met key assumptions for analysis (Appendix S1).

We used the ratio of genetic variance between and within each pair of groups (F_{ST}) as a point estimate of differentiation (Weir & Cockerham, 1984). We calculated separate F_{ST} estimates for neutral and thermal tolerance markers to characterize background and adaptive patterns of differentiation. We used principal components analysis (PCA) to visualize differentiation across individual markers. We also conducted a discriminant analysis of principal components (DAPCs) to determine the number of genetically distinct groups best supported by the observed patterns of differentiation (Jombart, 2008). All analyses were conducted in R (R Core Team, 2019; see Appendix S1 for package details).

RESULTS

Behavior

Telemetry results indicated behavioral thermoregulation is a widespread response to summer heat stress among UKL trout. By the start of July, when lake temperatures began reaching peak values of 26–27°C, all but one tagged UKL trout had moved into tributaries or zones of groundwater influence near the lake perimeter. During the 2 months that followed, 90% of transmitted body temperatures were at least 4°C cooler than dominant environmental conditions, suggesting that behavioral thermoregulation in summer is widespread among UKL trout. In the Keno Reach, daily mean and extreme temperatures were nearly identical to those of UKL throughout that timeframe (90% of each differed by ≤1°C), yet Keno trout exhibited minimal movement and body temperatures that closely matched dominant conditions (Figure 2). All tagged individuals detected were located within the Keno Reach, and 90% of body temperatures were not measurably (0.1°C) cooler than the mainstem, even though among other years, 2017 summer water temperatures were relatively warm (Appendix S1: Figure S2). Thus, we concluded that behavioral thermoregulation in summer is negligible among Keno trout.

Physiology

Our full suite of physiological indicators aligned in suggesting that Keno trout are more heat-tolerant than UKL trout. Differences between UKL trout at 22°C and Keno trout at 22–25°C were not significant for SMR ($p > 0.05$) and just outside the significance range for
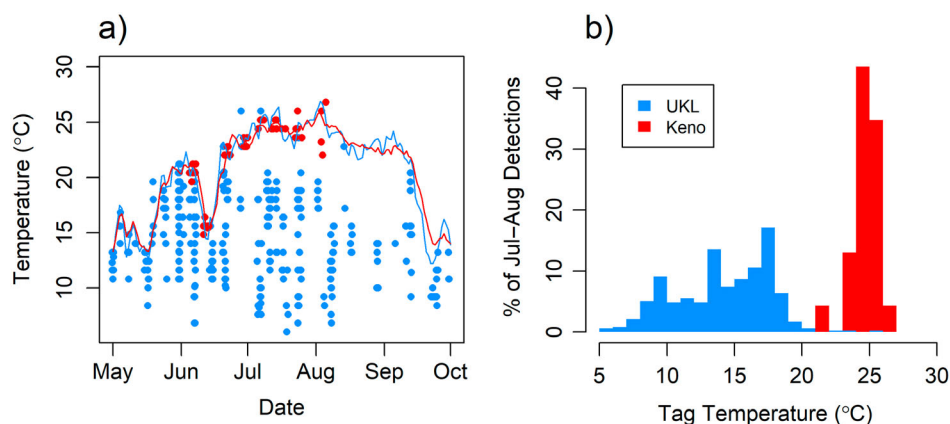

FIGURE 2 Temperatures selected by Upper Klamath Lake (UKL) and Keno trout. (a) Body temperature from detections of active tagged UKL trout (blue circles; $n = 407$ detections) and Keno trout (red circles; $n = 63$) obtained during daytime tracking in 2017, shown against daily maximum temperature in UKL (blue line) and the Keno Reach (red line). (b) Histogram of body temperature from detections of active tagged UKL trout (blue; $n = 486$) and Keno trout (red; $n = 23$) during July and August across all years of tracking.

MMR ($p = 0.06$). However, it is more likely that there are significant differences undetected by the tests because differences were significant for all other metrics (AAS, FAS, and recovery time: $p < 0.01$; CT_{max}: $p < 0.001$), including those calculated directly from SMR and MMR (i.e., AAS and FAS, below). The regression models also suggested that there were differences between Keno and UKL trout for SMR and MMR, further contradicting both significance tests (Table 1 and Figure 3a). Fitted SMR was lower for Keno trout than that for UKL trout across the entire temperature range, and the 95% CIs for the SMR models did not overlap at warmer temperatures. Similarly, MMR in UKL trout peaked at 18.1°C and decreased with warmer temperatures, whereas MMR in Keno trout increased across the temperature range and peaked at the upper constraint (30.0°C) according to the models. While it is unlikely that MMR truly peaks at 30°C in Keno trout, this result suggested it is also unlikely that MMR at warm temperatures truly does not differ between Keno and UKL trout (despite the p value marginally above 0.05).

These trends in MMR and SMR allowed Keno trout to maintain capacity for activity under warming. The regression models indicated that AAS declined at temperatures warmer than 14.2°C in UKL trout but remained stable across the temperature range in Keno trout (Figure 3b). The mean parameter for the Keno trout AAS model was the upper constraint of 30.0°C (like the MMR model), further indicating that AAS did not decline at the warm temperatures tested. All fish maintained FAS above 2, which has been proposed as the limit for digestive function because specific dynamic action (the energy cost of digestion) approximately doubles the total metabolic rate in a fish otherwise resting at SMR (Farrell, 2016). Even at 25°C, all Keno trout maintained FAS above 3, which has been proposed as a threshold to

thrive, accounting for extra capacity needed for other activities while digesting a meal (Anlauf-Dunn et al., 2022). In contrast, at 22°C, a majority of UKL trout were below the threshold to thrive and approaching the limit for digestive function (Figure 3c). Recovery in the warmer trials was much faster for Keno trout at warm temperatures (Figure 3d), though two mortalities were observed at 25°C. Keno trout also exhibited greater tolerance at very short time scales, with a CT_{max} of 31.3 ± 0.1°C (mean $\pm$ SE), over 2°C higher than UKL trout (28.9 ± 0.3°C).

Seasonal energetics

Condition factor results revealed that UKL trout condition declined significantly ($p < 0.001$) by a median of 7.2% over the summer, twice the significant decline observed in Keno trout ($p < 0.05$; Figure 4a). Phase angle in UKL trout also decreased significantly ($p < 0.01$; median = 14%) from the onset of summer to the end, corroborating the energy loss suggested by condition factor during the period of tributary use (Figure 4b). However, phase angle did not significantly decrease over the summer in Keno trout ($p > 0.05$; median = 1.2%), which did not reflect the energy loss suggested by condition factor. Since condition factor and phase angle measure the amount and rate of change in energy stores, respectively (see *Methods*), the discrepancy between the two metrics may suggest that Keno trout were in a state of energetic recovery (but had not yet recovered) by the time of our end-of-summer sampling. FL distributions of sampled fish indicated that Keno trout were significantly shorter than UKL trout ($p < 0.01$; Keno trout: 355 ± 48 mm; UKL trout: 547 ± 84 mm), which contributed to generally higher condition factor and lower phase angle before

TABLE 1 Regression equations for each group of redband trout and physiological metric (response variable), where T = temperature in degrees Celsius (predictor variable).

Group	Metric	Equation	Parameter estimate (SE)		
			a	**b**	**c**
UKL	SMR	$SMR = aT^2 + bT + c$	0.01 (0.03)	0.05 (0.93)	−0.10 (7.13)
	MMR	$MMR = ae^{-0.5[(T-b)/c]^2}$	16.37 (2.00)	18.18 (5.16)	13.75 (14.79)
	AAS	$AAS = ae^{-0.5[(T-b)/c]^2}$	13.27 (1.74)	14.21 (3.15)	10.46 (6.85)
Keno	SMR	$SMR = aT^2 + bT + c$	0.06 (0.03)	−2.04 (1.08)	18.24 (9.89)
	MMR	$MMR = ae^{-0.5[(T-b)/c]^2}$	21.35 (11.27)	30.00 (49.57)	20.05 (46.94)
	AAS	$AAS = ae^{-0.5[(T-b)/c]^2}$	15.59 (10.09)	30.00 (175.17)	34.16 (280.05)

Note: Equations are plotted in Figure 3.

Abbreviations: AAS, absolute aerobic scope; MMR, maximum metabolic rate; SMR, standard metabolic rate; UKL, Upper Klamath Lake.
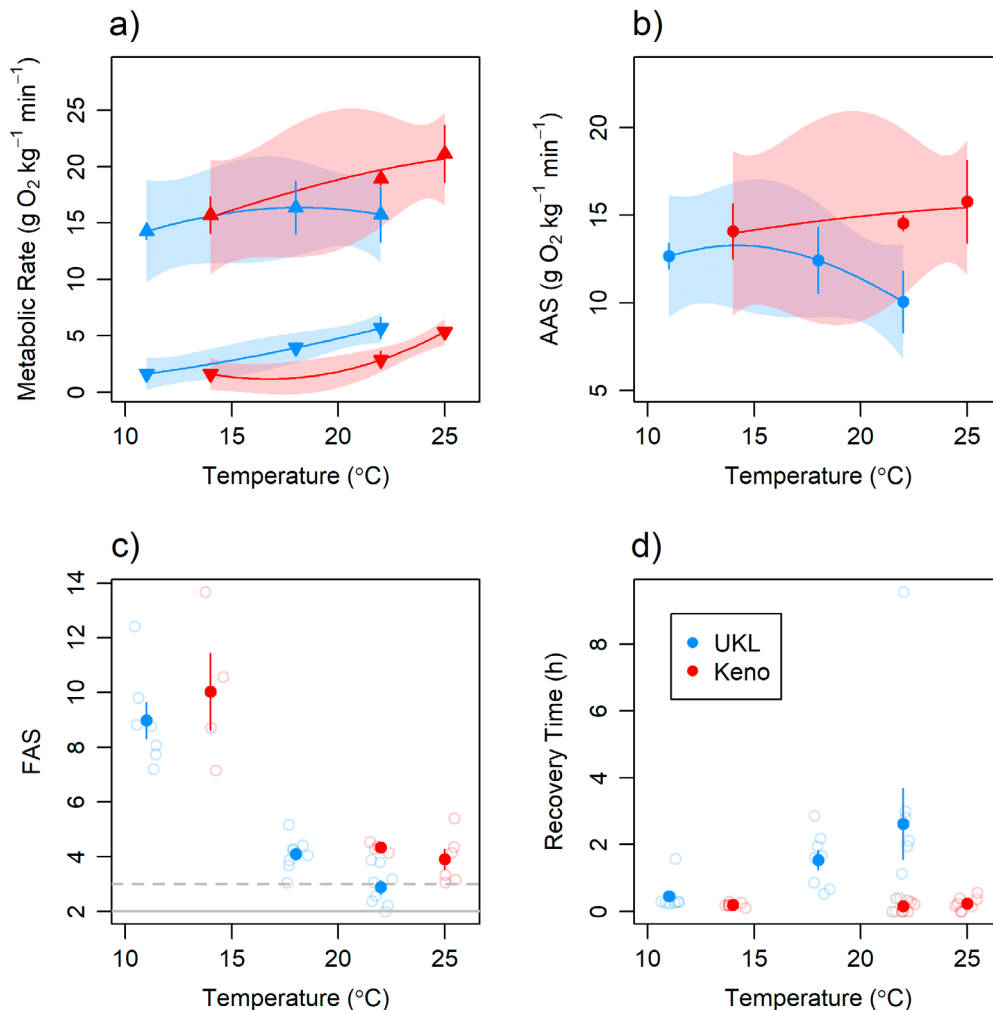
FIGURE 3 Physiological characteristics of Upper Klamath Lake (UKL) trout (blue) and Keno trout (red) at ambient, maximum, and climate warming temperature treatments. For all panels, solid symbols represent the mean at each temperature with lines extending to ±1SE. Results from ambient trials are displayed at their starting temperatures, at which maximum metabolic rate (MMR) was measured (11 and 14°C). (a) MMR (upward-pointing triangles) and standard metabolic rate (SMR; downward-pointing triangles). (b) absolute aerobic scope (AAS; equivalent to MMR − SMR). Panels (a) and (b) also show the predicted mean (solid lines) and 95% CI (shaded regions) of the corresponding regression model (Table 1). (c) factorial aerobic scope (FAS; equivalent to MMR/SMR). The gray lines represent thresholds relevant to organism function; at the dashed line, organisms may no longer thrive (i.e., perform normal activities aerobically), while at the solid line, organisms may no longer digest properly (Anlauf-Dunn et al., 2022; Farrell, 2016). (d) Recovery time. In panels (c) and (d), faded open symbols represent individual data, to which a small amount of noise has been added on the *x*-axis to improve visibility of overlapping points.

standardization to early-summer values. However, these differences applied similarly to all seasons and no conclusions changed when the effect of length was removed from either condition metric.

Genetics

Poor sequencing success (<90% of individuals) occurred at a portion of genetic markers, including three thermal tolerance markers. Similarly, poor sequencing success (<90% of markers) occurred in a portion of individuals.

Markers and individuals that did not meet these data quality standards or other assumptions were dropped from further analysis (Appendix S1). Ultimately, we used 203 neutral markers and four thermal tolerance markers to analyze genetic differentiation among 34 UKL trout, 76 Keno trout, and 81 downstream trout.

Keno and downstream trout differed much more from UKL trout than from each other at both neutral and thermal tolerance markers (Table 2). However, the relatively weak differentiation between Keno and downstream trout was four times stronger at thermal tolerance markers ($F_{ST} = 0.04$) than at neutral markers ($F_{ST} = 0.01$). The
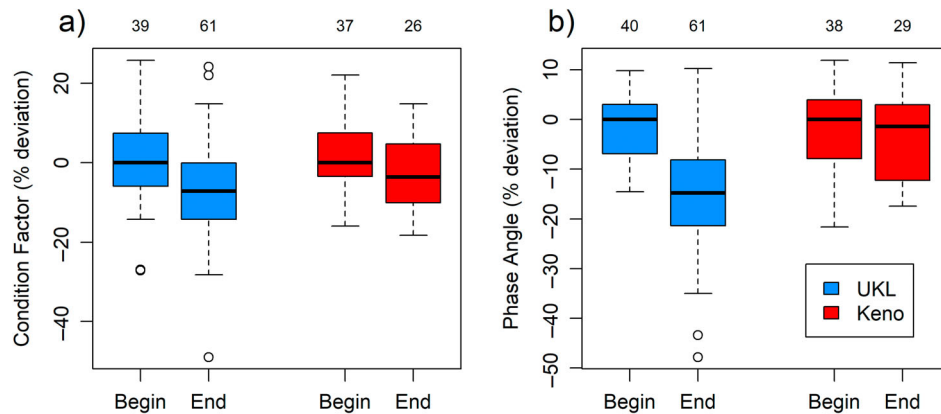
FIGURE 4 Metrics of energetic condition at the beginning and end of summer (mid-May to mid-June and mid-August to mid-September respectively) for Upper Klamath Lake (UKL) and Keno trout (blue and red respectively). Boxplots show the median (dark line), middle 50% (box), and range (capped at 1.5 times the interquartile range; whiskers) of the data, as well as outliers (circles). (a) Fulton's condition factor *K*. (b) Phase angle. Values are standardized to indicate percent difference from the beginning-of-summer median. Sample sizes are provided above each box.

TABLE 2 Pairwise F_{ST} estimates (Weir & Cockerham, 1984) measuring genetic differentiation between three groups of redband trout, with 95% CIs in parentheses.

Group	UKL	Keno	Downstream
UKL		0.28 (0.11–0.37)	0.31 (0.17–0.36)
Keno	0.34 (0.30–0.39)		0.04 (0.00–0.09)
Downstream	0.34 (0.30–0.38)	0.01 (0.00–0.02)	

Note: Values above the diagonal are based on markers associated with thermal tolerance (four markers). Values below the diagonal are based on variation at presumably neutral markers (203 markers). All estimates are statistically significant (Keno and downstream trout at thermal tolerance markers: $p < 0.05$; all other estimates: $p < 0.01$).

Abbreviation: UKL, Upper Klamath Lake.

PCA and DAPC yielded similar results. Only one PCA was meaningful, and it distinguished Keno and downstream trout from UKL trout (Figure 5). Interestingly, two Keno trout clustered with the UKL trout, suggesting a potential low level of unknown movement between the two habitats or remnant genetics from historical movement before dam construction. The DAPC model of differentiation best supported by bayesian information criterion (BIC) was based on these two groups, but the BIC value for a three-group model was quite similar (Appendix S1: Table S3).

DISCUSSION

We found that within the Upper Klamath Basin, redband trout cope with warm summer temperatures in contrasting ways. In the more fragmented Keno Reach, we detected no evidence of behavioral thermoregulation, but multiple indicators of physiological tolerance at high temperatures: greater capacity for aerobic activity, faster recovery from exertion, and higher critical maximum temperature. In more connected UKL, we observed lower physiological tolerance, but a population-wide expression of thermoregulatory behavior via movement to cooler habitats. The behavioral response we observed in UKL trout is expressed by rainbow trout and other *Oncorhynchus* species in another thermally connected portion of the basin much further downstream, from Iron Gate Dam (67 km downstream of J.C. Boyle Dam) to the mouth of the Klamath River (Brewitt & Danner, 2014; Strange, 2011; Sutton et al., 2007). In other words, trout from opposite ends of the basin are more similar to each other than trout from the middle in terms of their strategies for surviving warm temperatures. This counterintuitive pattern does not match spatial variation in climate (Perry et al., 2011), nor taxonomic similarity shaped by countless generations of evolution (Pearse et al., 2011). Instead, this pattern matches connectivity within landscapes, structured by human modifications only decades old. Like terrestrial ectotherms (Sears et al., 2016; Sears & Angilletta, 2015), how aquatic ectotherms ultimately respond to temperature—behaviorally or physiologically—is apparently determined by costs and benefits of fine-scale landscape characteristics, rather than coarse-scale mean characteristics of environments and taxa.

A similar study that compared thermal tolerance in human-modified and unmodified landscapes proposed a framework for responses to climate warming in fishes (Sandblom et al., 2016). This framework hypothesized that upper physiological limits such as CT_{max} and MMR are "concrete ceilings" with limited plasticity, whereas resting processes like SMR are "plastic floors" that account for most of the flexibility in thermal tolerance. In
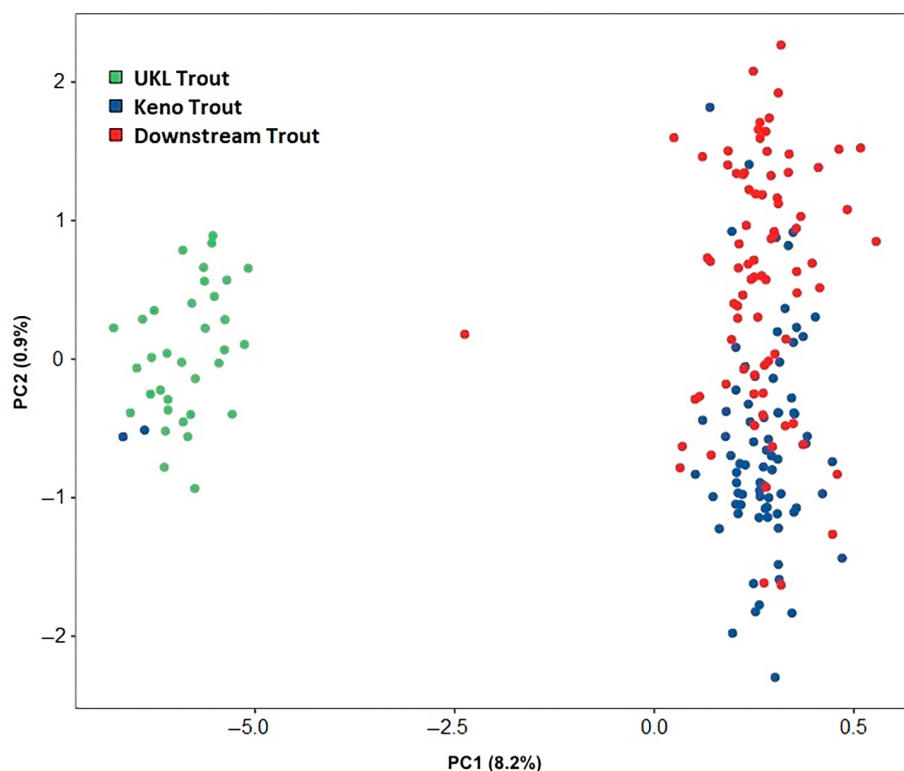
FIGURE 5 Plot of the two primary axes from a principal components analysis (PCA) using all 207 genetic markers. The percent of variation explained by each axis is provided in parentheses. The key shows the sampling group to which each individual trout belongs. UKL, Upper Klamath Lake.

contrast, the balance of evidence in our study suggested that flexibility in both ceilings and floors was critical to the extended thermal tolerance range of Keno trout. Keno trout maintained lower SMR at higher temperatures compared with UKL trout. However, Keno trout were also able to maintain an increasing MMR up to 25°C, whereas MMR of UKL trout began to decline just above 18°C. These two trends together yielded a consistent AAS and more gradually declining FAS under increasing temperature, such that Keno trout could probably sustain activities like feeding and digestion even at 25°C (Farrell, 2016). Our findings agree with growing evidence that MMR is a plastic rather than concrete ceiling in salmonids, especially in *O. mykiss* (Adams, 2020; McKenzie et al., 2020; Verhille et al., 2016) and also in species generally considered less heat-tolerant (Guzzo et al., 2019; Poletto et al., 2017; Raby et al., 2016).

Unlike MMR, CT_{max} does not measure "functional thermal tolerance"—the range of temperatures in which aerobic scope can sustain key activities (Whitney et al., 2016). Yet, like MMR, CT_{max} is a useful physiological ceiling for comparing populations, and the difference between Keno trout and UKL trout (2.4°C) is striking in the context of our study system. Generally, CT_{max} exhibits a small amount of plasticity with a large change

in acclimation temperature (Pörtner & Peck, 2010). *O. mykiss* is among the most studied fish species with respect to many physiological metrics, and many estimates of CT_{max} under a variety of acclimation temperatures are available (Chen et al., 2015). A regression meta-analysis using this suite of available data suggested that the capacity for acclimation in the species is limited to a highest possible CT_{max} of 31.2°C (McKenzie et al., 2020). Our estimate in Keno trout (31.3°C) is already at this proposed limit and among the highest values recorded for *O. mykiss*, typically achieved under extreme acclimation in the laboratory (≥25°C, but see Recsetar et al., 2012). Yet Keno trout were acclimatized to an ambient thermal regime that did not exceed 23°C and was below 20°C for two thirds of the daily cycle. This discrepancy supports the recent discovery that fluctuating acclimation temperatures confer greater tolerance than the equivalent mean (Salinas et al., 2019). While the general principle of diminishing returns remains well-supported for acclimation to warm temperatures, our results highlight the possibility that laboratory studies have underestimated the degree of plasticity provided by realistic acclimation conditions.

In the context of other studies, plasticity appears to have been a likely pathway to physiological tolerance in

Keno trout. However, it is worth noting that our study does not rule out local genetic adaptation (e.g., Eliason et al., 2011). Among all the genetic comparisons we made, differentiation between Keno and downstream trout was relatively weak, matching patterns of genetic drift (Pearse et al., 2011) more closely than the physiological patterns we observed. Yet, interestingly, differentiation was nearly four times stronger at just four thermal tolerance markers than at 203 neutral markers. Thermal tolerance is governed by many interacting genes, and only some (or none) may have been represented in the panel we used. A recent analysis covering a much larger fraction of the redband trout genome identified strong association with thermal tolerance within a chromosomal region not included here (Chen & Narum, 2021). Additionally, the thermal tolerance markers we used were originally identified in a different subspecies of redband trout, *O. mykiss gairdneri* (Chen et al., 2018), so the significance of these markers could be different in *O. mykiss newberrii*. The fact that three of the seven thermal tolerance markers genotyped too poorly for analysis could suggest that subspecies differences were a confounding factor. Overall, our study did not yield evidence for thermal adaptation in Keno trout but should not be interpreted as evidence against thermal adaptation given its limited power. Though only decades have passed since dam construction in the Keno Reach, mounting evidence in other systems suggests that may be enough time for fishes to adapt genetically to warming (Chen et al., 2015; Nyboer et al., 2020; Smith et al., 1983).

The surprising lesser decline in condition we observed in Keno trout may be additional evidence of an undetected genetic basis for adaptation, as many plastic responses are thought to have high energy costs (Narum et al., 2013, but see Murren et al., 2015). Alternatively, our evaluation of energetic performance may have been unable to capture the full costs of thermal tolerance. For example, increased physiological performance at one end of the temperature spectrum can result in decreased performance at the opposite end (Pörtner et al., 2006; Schou et al., 2015). In cooler seasons when UKL trout performance is optimized, Keno trout performance may be suboptimal; likewise, winter temperatures may prove more stressful for Keno trout than UKL trout. Carryover effects, impacts of events in one season that are realized in a later season (O'Connor et al., 2014), could also delay the appearance of tolerance costs. Additionally, extensions of the cost–benefit framework we used have asserted that while the original model (Huey & Slatkin, 1976) quantified costs of tolerance in energetic terms, these units overlook broader fitness costs (e.g., predation risk, infection, and fecundity) that may be even more critical, especially at warm temperatures

(Alford & Lutterschmidt, 2018; Vickers et al., 2011). For example, though body size distributions of our focal groups before dam construction are unknown, the stark difference in FL we observed could reflect a fitness cost of physiological tolerance in Keno trout. Fish that cope with warming generally achieve smaller adult body sizes (Audzijonyte et al., 2019), and the reproductive output of smaller fish is often disproportionately less than that of larger fish (Barneche et al., 2018). Lacking a more definitive understanding of the basis and fitness costs of tolerance in Keno trout, we conclude that while some salmonids may change their thermal physiology at magnitudes and rates more relevant to climate change than previously thought, the implications for other key aspects of life history are uncertain.

Whether genetic or plastic, the capacity to adjust thermal physiology is fundamentally linked to the capacity for behavior, which is in turn set by the landscape (Muñoz & Bodensteiner, 2019). Such complex interactions lie at the heart of debate surrounding the adaptability of salmonids under climate change. Some researchers point to examples of low physiological adaptability and highlight risks of catastrophic loss (Muñoz et al., 2015a). Others point to examples of high behavioral adaptability and stress that outcomes from extirpation to expansion will occur in different populations (Mantua et al., 2015). To resolve the debate, joint consideration of behavior and physiology in redband trout was proposed as a productive research direction (Muñoz et al., 2015b). Our findings in redband trout acknowledge the relevance of how intrinsically adaptable salmonids are, but show that a more fruitful perspective may ask how adaptable the landscape allows salmonids to be. For example, UKL is susceptible to severe and frequent hypoxia, whereas physical features of the Keno Reach appear to partially buffer hypoxic events (PacifiCorp, 2013). Hypoxia tolerance and thermal tolerance are intertwined in *O. mykiss* (Garvin et al., 2015; Zhang et al., 2018). Therefore, physiological adaptability to warming in redband trout may be restricted by oxygen availability in UKL but expressed in the equally warm but better oxygenated Keno Reach. In another example, the behavioral adaptability exhibited by UKL trout was shown to depend critically on the rich foraging available in the lake (Hahlbeck et al., 2022). Food is also abundant in the Keno Reach (Addley et al., 2005), which may likewise support the physiological adaptability of Keno trout, as increased feeding can augment some aspects of warm tolerance in salmonids (Dodrill et al., 2016; Zillig et al., 2021). A more holistic understanding of salmonid adaptability to climate change will require research that integrates oxygen, food, connectivity, and the many other landscape costs and benefits that determine how that intrinsic adaptability can be expressed.

The Upper Klamath Basin is emblematic of changing aquatic habitats in temperate zones throughout the world. Current conditions here (a warm dominant temperature regime, deteriorated water quality, and disconnected habitats) are the future conditions expected in many other temperate ecosystems. Some of these conditions will likely intensify with climate change in the Klamath ecosystem as well. Even with the upcoming removal of four of the six dams, annual mean river temperatures are predicted to be 1–2°C above the historical average by 2061 (Perry et al., 2011) and accelerating groundwater depletion from irrigation pumps not considered by the model could exacerbate that trend (Gannett et al., 2012). How fish survive in this environment thus has far-reaching implications for climate change, but in a paradoxical sense—we demonstrated that wholesale prediction of climate change responses at broad spatial, temporal, and taxonomic scales may fail, by overlooking the processes within populations and landscapes that shape those responses. Such processes operate at the intersection of physiology and ecology (Boult & Evans, 2021). If we are to bridge rather than blur the two perspectives to improve climate change predictions (Jutfelt et al., 2018; Pörtner et al., 2017), our study suggests that at this moment there is more to gain from broader investigation of variability rather than broader assumption of similarity (Des Roches et al., 2021). The effectiveness of conservation and restoration fundamentally depends on questions of variability at landscape scales. Will trout in the Keno Reach exploit lower costs of behavioral thermoregulation once J.C. Boyle Dam is removed? Or will the benefits of their physiological tolerance continue to prevail? Our work establishes a baseline for finding these answers in the Klamath Basin and illustrates a need for asking these questions in other landscapes if we are to truly understand how animals will respond to climate change.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data and code (Hahlbeck, 2023) are available at Zenodo: https://doi.org/10.5281/zenodo.8072288.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.