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Population Genomics Reveals Local Adaptation Related to Temperature Variation in Two Stream Frog Species: Implications for Vulnerability to Climate Warming

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ABSTRACT

Identifying populations at highest risk from climate change is a critical component of conservation efforts. However, vulnerability assessments are usually applied at the species level, even though intraspecific variation in exposure, sensitivity and adaptive capacity play a crucial role in determining vulnerability. Genomic data can inform intraspecific vulnerability by identifying signatures of local adaptation that reflect population-level variation in sensitivity and adaptive capacity. Here, we address the question of local adaptation to temperature and the genetic basis of thermal tolerance in two stream frogs (*Ascaphus truei* and *A. montanus*). Building on previous physiological and temperature data, we used whole-genome resequencing of tadpoles from four sites spanning temperature gradients in each species to test for signatures of local adaptation. To support these analyses, we developed the first annotated reference genome for *A. truei*. We then expanded the geographic scope of our analysis using targeted capture at an additional 11 sites per species. We found evidence of local adaptation to temperature based on physiological and genomic data in *A. montanus* and genomic data in *A. truei*, suggesting similar levels of sensitivity (i.e., susceptibility) among populations regardless of stream temperature. However, invariant thermal tolerances across temperatures in *A. truei* suggest that populations occupying warmer streams may be most sensitive. We identified high levels of evolutionary potential in both species based on genomic and physiological data. While further integration of these data is needed to comprehensively evaluate spatial variation in vulnerability, this work illustrates the value of genomics in identifying spatial patterns of climate change vulnerability.

1 | Introduction

One of the greatest conservation challenges of the 21st century is predicting the effects of climate change on biodiversity to inform effective conservation actions (Buckley et al. 2023; Urban et al. 2024). Many species have already been negatively impacted by increasing temperatures and associated disturbances such as heat waves, and these impacts are projected to worsen (Pigot et al. 2023; Wiens and Zelinka 2024). Within species, genetic and phenotypic diversity among populations and the distribution of populations across heterogeneous environments can foster the maintenance of intraspecific diversity that can improve the resiliency of populations in the face of changing climatic conditions (Forester et al. 2022). Identifying populations that are more or less vulnerable will be critical for focusing and planning effective conservation efforts within target species. These species-specific efforts can then be aggregated to build evidence for more widely applicable principles to identify vulnerable populations in order to improve conservation practice more broadly.

A species' vulnerability to climate change and other anthropogenic changes is determined by its exposure and sensitivity to changing conditions and is mitigated by its adaptive capacity in response (Foden et al. 2019; Thurman et al. 2020; Figure 1). While exposure quantifies the magnitude of change (i.e., departure from climatic parameters that the species has evolved with), sensitivity reflects how closely tied performance, fecundity, survival and other correlates of fitness are to those changes (Dawson et al. 2011). Given population-level exposure and sensitivity, adaptive capacity—the ability to cope with, accommodate or evolve—fundamentally determines if and how populations and species will persist or decline in response to change. Adaptive capacity is usually summarised by three processes: dispersal and colonisation abilities, phenotypic plasticity and evolutionary potential (Foden et al. 2019). Here, we define evolutionary potential as the capacity to evolve genetically based changes in traits that increase population-level fitness in response to novel or changing environmental conditions (Forester et al. 2022). This definition is analogous to the term 'adaptive potential' as used in the Convention on Biological Diversity's Global Biodiversity Framework (e.g., Hoban et al. 2023). While this climate change vulnerability framework is most commonly discussed and applied at the species level, intraspecific variation in exposure, sensitivity and adaptive capacity can play a critical role in determining overall vulnerability (e.g., Forester et al. 2023; Gervais et al. 2021; Herrando-Pérez et al. 2019). However, population-level data informing vulnerability can be challenging to collect, especially in at-risk species. Genomic data are becoming an increasingly useful tool for characterising some aspects of vulnerability, including dispersal ability, local adaptation to climatic conditions and, when physiological data are available, local adaptation of sensitivity traits such as physiological thresholds (Bay et al. 2017; Bernatchez et al. 2023; Höglund, Laurila, and Rödin-Mörch 2021; Waldvogel et al. 2020).

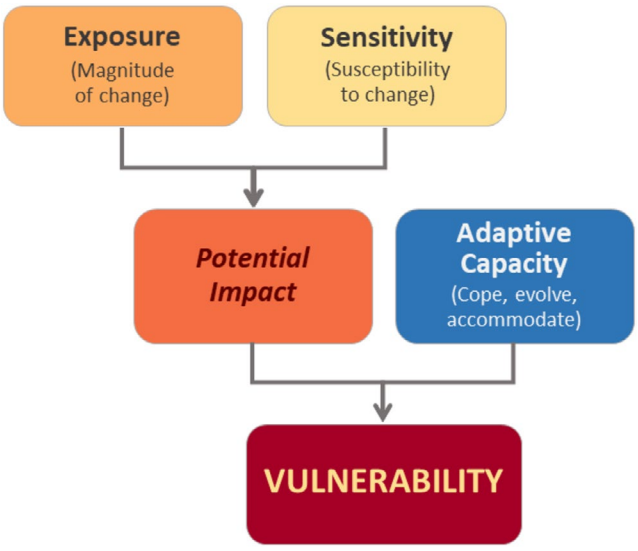

FIGURE 1 | Climate change vulnerability framework. Exposure quantifies the magnitude of change from climatic parameters the species or population has evolved with, while sensitivity reflects how closely tied fitness is to climatic conditions. Together, these factors determine the potential impact of changing climate on the species or population. Adaptive capacity summarises the ability of the species or population to respond to changing climate through plasticity (e.g., acclimation), evolutionary change or movement to track shifting conditions. Adaptive capacity can mitigate potential impacts of climate change, reducing a species' or population's overall vulnerability to climate change.

Understanding whether populations are locally adapted to climatic conditions is a key aspect of addressing spatial variation in sensitivity and adaptive capacity in response to climate change (e.g., Thomas et al. 2022). The dominant paradigm in ecological studies has been to assume that traits related to adaptive capacity, such as thermal tolerance or dispersal ability, can be characterised for a given species by sampling a single or few populations across a species' range (Beever et al. 2017; Freeman and Class Freeman 2014; Hoffmann and Sgro 2011; Isaak et al. 2016; Isaak, Wenger, and Young 2017; Sunday, Bates, and Dulvy 2012). For thermal tolerance, this leads to a prediction that the most sensitive (i.e., susceptible, Figure 1) populations are those whose critical thermal maximum temperature (i.e., CTmax, the maximum body temperature that permits performance; Angilletta 2009) is closest to current summer temperatures. These populations will have a reduced warming tolerance (i.e., the difference between CTmax and relevant metrics of summer temperature; Deutsch et al. 2008), meaning that they are susceptible to even small increases in temperatures. Under this perspective, populations at warmer, lower elevations would be predicted to be more sensitive than populations occupying cooler high-elevation environments (Figure 2a). However, from an evolutionary perspective, populations occupying divergent environments should be locally adapted to their historical temperature regime (Kawecki and Ebert 2004; Sandoval-Castillo et al. 2020). This perspective recognises population variation in traits affecting adaptive capacity, which leads to different predictions about spatial patterns of sensitivity to climate change (Valladares et al. 2014). For example, if organisms are adapted to the historical temperature regime of their local elevation, then CTmax is predicted to decrease with increasing elevation in parallel with maximum temperatures, such that warming tolerance may be similar and all populations are equally sensitive, or susceptible, to warming temperatures (Figure 2b).

Local adaptation of sensitivity traits can also influence spatial patterns of adaptive capacity and overall vulnerability. For

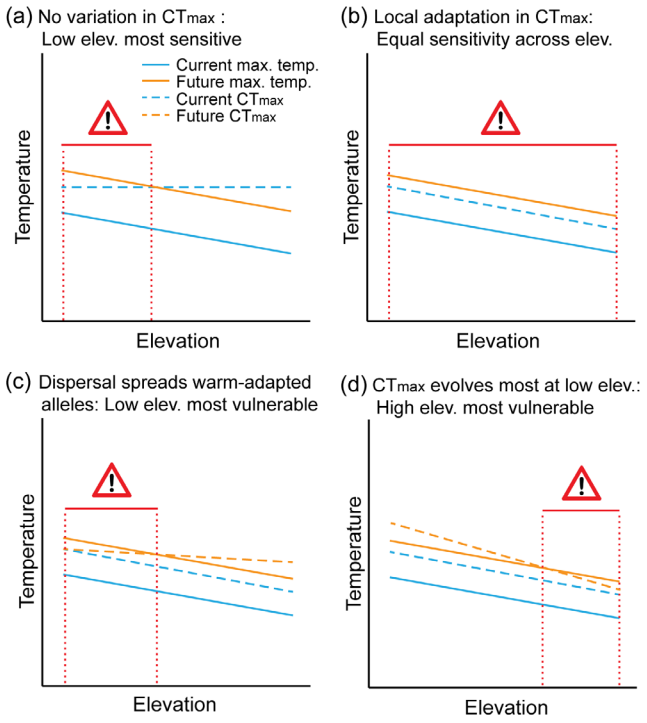

FIGURE 2 | Hypotheses of differential sensitivity (i.e., susceptibility to temperature change; a and b) and overall vulnerability (i.e., incorporating sensitivity, adaptive capacity and exposure; c and d) as a function of elevation (*x*-axes), temperature (*y*-axes), critical thermal limits (CTmax) and the potential for evolutionary responses and dispersal, both of which are components of adaptive capacity. Solid lines show current (blue) and future (orange) maximum temperatures, while dashed lines show current (blue) and future (orange) CTmax. Red lines and caution signs signify sensitive (a and b) and vulnerable (c and d) populations.

example, adaptive capacity may be reduced in populations occupying lower elevations if they have reached their upper thermal limit due to evolutionary constraints (Hoffmann, Chown, and Clusella-Trullas 2013). However, high dispersal capacity may allow these warm-tolerant alleles found in low-elevation populations to spread into higher populations via gene flow, increasing CTmax and warming tolerance at higher elevations and reducing their vulnerability, while low-elevation populations remain vulnerable (Figure 2c). Alternatively, evolutionary potential may be reduced in populations at high compared to low elevation if these populations have low additive genetic variation due to small effective population size and isolation or reduced acclimation ability (Brattstrom 1968; Funk et al. 2005; Polato et al. 2017; Figure 2d). If so, then high-elevation populations may be more vulnerable to increasing temperature than low-elevation populations, especially where higher elevations are warming more rapidly than lower elevations (Pepin et al. 2015).

While many other scenarios could be envisioned, the critical point is that populations may vary greatly in their exposure, sensitivity and adaptive capacity, and hence in their vulnerability to warming, but few studies have measured intraspecific variation in physiological traits that predict sensitivity, and even fewer have explored their genetic basis coupled with parameters relevant to estimating adaptive capacity. This is due in large part to the difficulty of accumulating these data from populations distributed across species' ranges. However, the integration of physiological and genomic data provides a powerful approach for testing for local adaptation to temperature variation (Huey et al. 2012). In addition, genomic data can provide useful proxies for dispersal ability and evolutionary potential (e.g., Rödin-Mörch et al. 2021), informing adaptive capacity in response to changing climate (Forester et al. 2022).

In this study, we address the question of whether or not populations of two species of tailed frogs are locally adapted to temperature in order to better understand spatial patterns of sensitivity and adaptive capacity in response to climate change. Tailed frogs are stream-dwelling species endemic to the mountains of the western United States and Canada and are represented by only two species: the coastal tailed frog (*Ascaphus truei*) and the Rocky Mountain tailed frog (*A. montanus*). These species are listed under Canada's Species At Risk Act as Special Concern (*A. truei*) and Threatened (*A. montanus*). They are also listed as California Priority 2 Species of Special Concern (*A. truei*), and British Columbia Imperilled and Washington State Candidate species (*A. montanus*). Tailed frogs occupy cold, fast-flowing streams and the tadpoles have relatively low thermal maxima among amphibians (Bennett et al. 2018), making them sensitive to increasing temperatures (Bury 2008; Cicchino, Shah, Forester, Dunham, et al. 2023). These species also inhabit ecoregions with different thermal regimes, with *A. truei* occupying the more temperate, coastal environments of the Klamath and Cascade Mountains and the Coast Ranges (Figure 3), while *A. montanus* occupies the continental landscapes of the northern Rocky Mountains of the United States and Canada (Figure 4). Our previous work has shown a significant amount of population-level variation in stream temperatures and CTmax in both species, with a positive relationship between maximum stream temperatures and CTmax in *A. montanus* (Cicchino, Shah, Forester, Dunham, et al. 2023). This stream temperature and CTmax covariation suggest adaptive divergence related to thermal tolerance, but common garden or genomic data are required to test this hypothesis, as variation in CTmax could be due to phenotypic plasticity. We selected CTmax because it is an ecologically meaningful index of thermal tolerance in tailed frogs and is closely related to tadpole thermal stress and mortality at temperatures predicted to be common in the near future for these populations (Cicchino, Ghalambor, and Funk 2023). Here, we build off these physiological data by applying whole-genome resequencing to individuals from four sites spanning temperature gradients and watersheds in each species. In combination with stream temperature data and individual-level estimates of CTmax, we test for a genetic signature related to CTmax variation using two genome-wide association (GWAS) methods and test for evidence of divergent selection related to temperature using two genotype–environment association (GEA) methods. To support these analyses, we develop the first annotated reference genome for the anuran family Ascaphidae (for *A. truei*), improving the taxonomic breadth of available reference genomes for anurans. Finally, we expanded the geographic scope of inferences of local adaptation using targeted capture of candidate adaptive variants from an additional 11 sites per species, providing additional evaluation of adaptive signatures. We integrate these data with our previous work in *Ascaphus* to build a more comprehensive understanding of sensitivity and adaptive capacity in these two species, providing insight into the complexity of predicting spatial patterns of climate change vulnerability that is applicable beyond our study system.

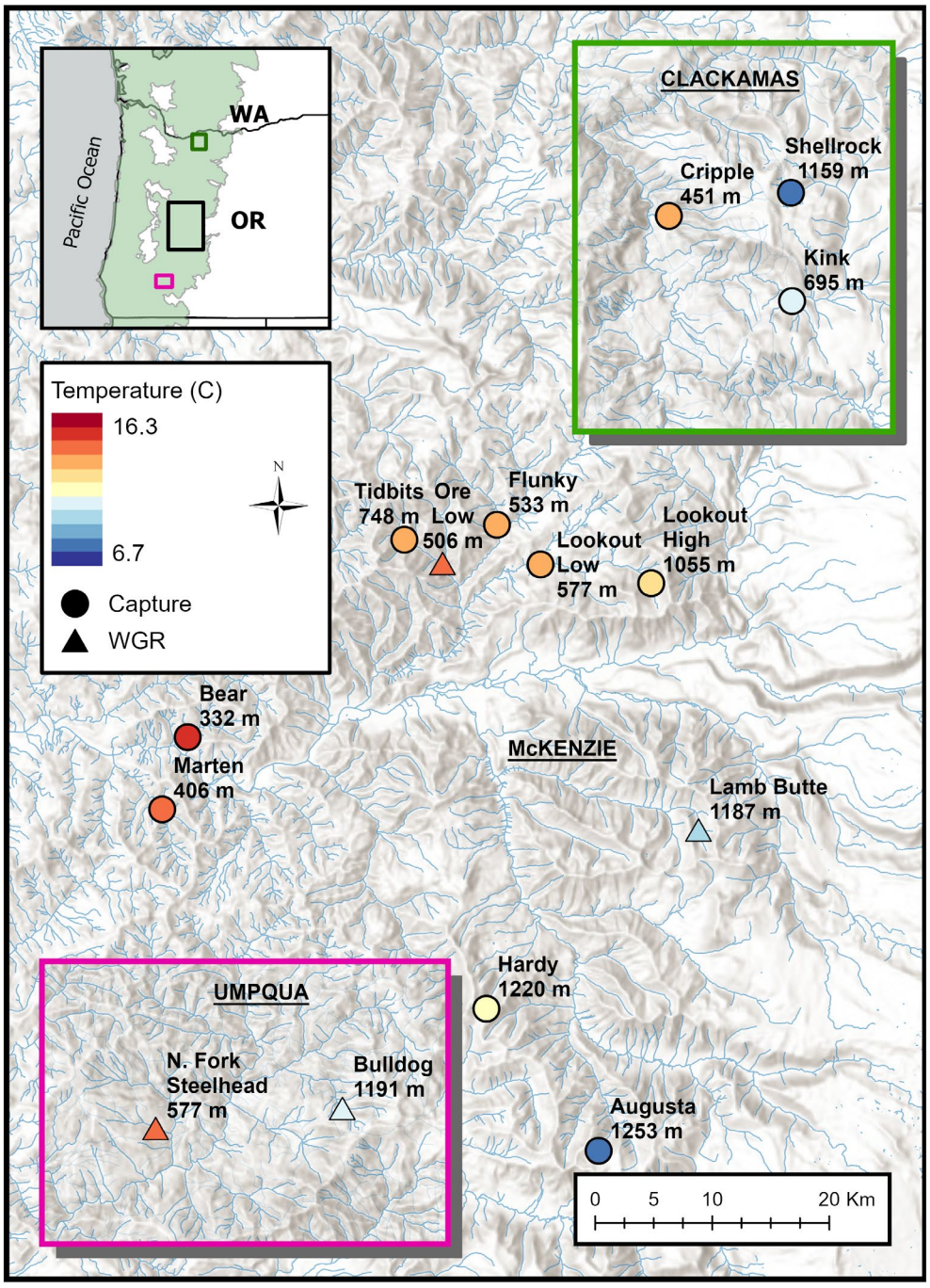

FIGURE 3 | Sampling locations for *Ascaphus truei* in Oregon across three watersheds: Clackamas in the north (green inset), McKenzie in the centre (main map) and Umpqua in the south (pink inset). Site names include elevation and are colour coded by mean August stream temperature. Triangles are four whole-genome resequencing sites (WGR, 40 individuals total) and circles are 11 capture sites (100 individuals total). Basemap: ESRI World Hillshade.

2 | Methods

2.1 | Field Sampling

We sampled 15 stream reaches (~100 m) across three watershed basins in the summers of 2017 and 2018 in each of Oregon and Montana (Figures 3 and 4; Table S1). We captured tadpoles by shifting and brushing benthic substrate while holding an aquarium net downstream. We held tadpoles in 2-L insulated containers with stream water in the field with frequent water changes to ensure they stayed cold. All tadpole collection was performed under the following permits: Oregon Department of Fish and Wildlife permit numbers 114-18 and 110–17; Montana Department of Fish, Wildlife and Parks permit number 2017-060-W; and Colorado State University Institutional Animal Care and Use Committee protocol 16-6667AA.

2.2 | Temperature Data

We used a combination of August mean temperature data from the NorWeST summer stream temperature model

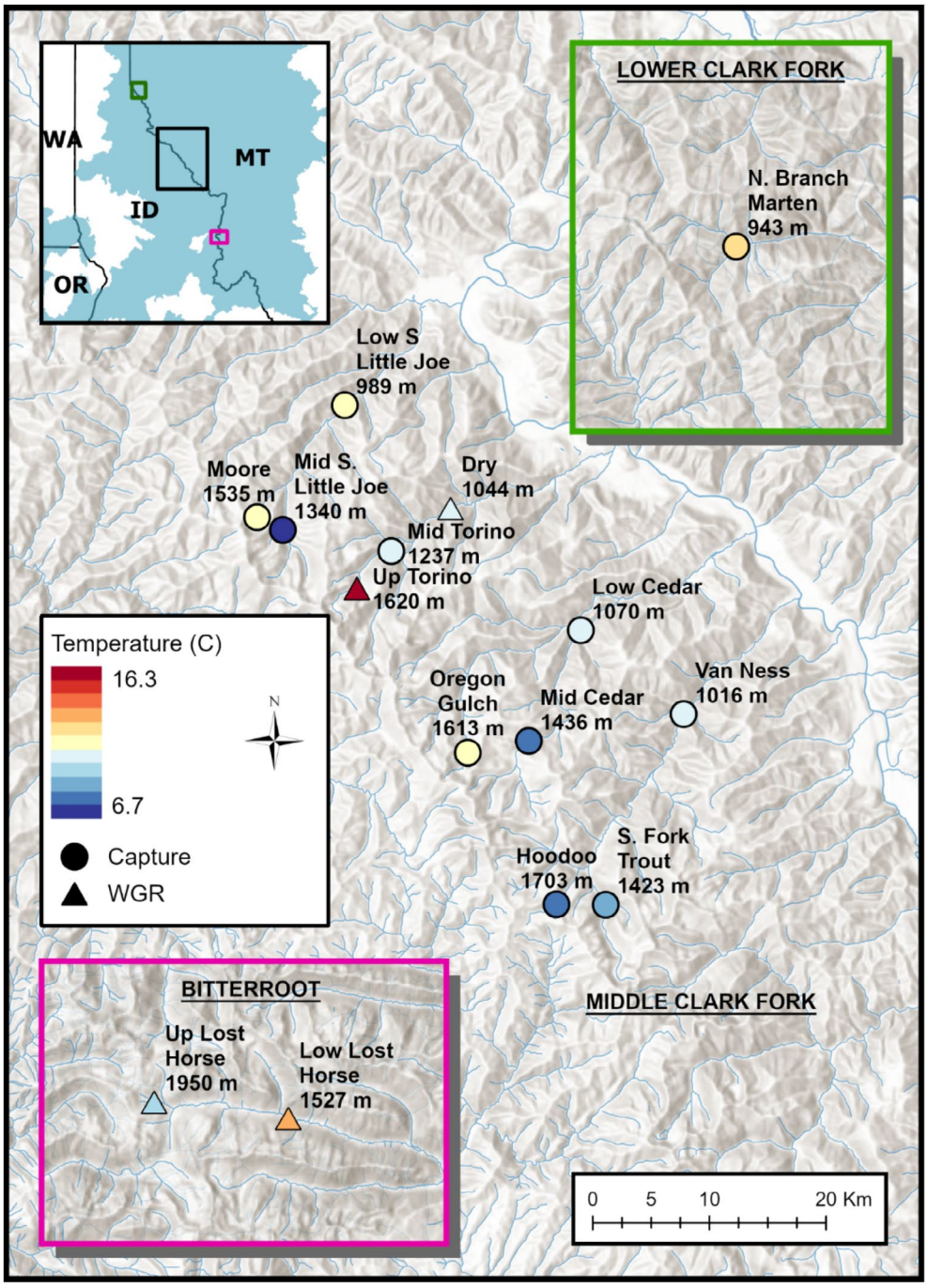

FIGURE 4 | Sampling locations for *Ascaphus montanus* in Montana across three watersheds: Lower Clark Fork in the north (green inset), Middle Clark Fork in the centre (main map) and Bitterroot in the south (pink inset). Site names include elevation and are colour coded by mean August stream temperature. Triangles are four whole-genome resequencing sites (WGR, 40 individuals total) and circles are 11 capture sites (108 individuals total). Basemap: ESRI World Hillshade.

(Isaak et al. 2017) and data collected directly from sampled stream reaches. In these streams, August is the month when annual maximum temperatures are most likely to be realised, as well as the month for which most field data are available for fitting regional models of stream temperature (Dunham et al. 2005). For in-stream data collection, we installed two temperature loggers (Onset Hobo Pendants UA-001-064) at most sites (14 of 15 sites per species) at the upstream and downstream ends of the stream reach using rebar pounded into the substrate (Table S1). We protected temperature loggers from debris and sunlight using a PVC tube and drilled holes to allow water flow. Water temperature was recorded every 4 h. We plotted the time-series temperature data to detect obvious errors and screened the data using standard deviation time plots (Dunham et al. 2005). We evaluated in-stream temperature data availability for August 2017 and 2018 and averaged upstream and downstream loggers when available. We compared in-stream August mean temperature data with NorWeST data, which represents August mean temperature averaged from 1993 to 2011. In cases where in-stream August mean temperatures were within 1°C of NorWeST

estimates for that stream reach, we used the NorWeST value, otherwise, we used the in-stream data. The 30-year averages provided in the NorWeST data were preferred in this study as they represent long-term environmental conditions, and therefore longer-term selection pressures, on *Ascaphus* populations. We also evaluated NorWeST projections of stream temperature at all sites for 2080 (average of 2070–2099), which are derived from the Intergovernmental Panel on Climate Change's Special Report on Emissions Scenarios (SRES) A1B Scenario (representing a mid- to high-emissions scenario; Isaak et al. 2017). Unless otherwise noted, all statistical analyses for temperature and the following data used R version 4.0.3 (R Core Team 2021).

2.3 | Critical Thermal Maximum and Length Data

We collected critical thermal maximum (CTmax) data at all sites using temperature ramping experiments as described in Cicchino, Shah, Forester, Dunham, et al. (2023). Briefly, tadpoles were held at 8°C for 3 days in oxygenated and recirculated natal stream water without food. To measure CTmax, we transferred tadpoles to mesh containers in the experimental tank containing natal stream water at 8°C. We held tadpoles for 3 min to acclimate to the chamber conditions before ramping the temperature at a rate of 0.3°C/min. We defined CTmax as the temperature at which tadpoles no longer responded to a tactile stimulus. Upon reaching CTmax, we returned tadpoles to an 8°C holding container and allowed them to recover for up to an hour. Following experiments, tadpoles were euthanised using a 20% benzocaine solution. We then photographed each tadpole laterally with a ruler before taking a small tissue sample from the tail, preserved in 95% ethanol. We used ImageJ (Rasband 2018) to measure the length of each tadpole (tip of snout to tip of tail) from the lateral photographs; each tadpole was measured twice and the values were averaged. For each species, we used linear mixed-effects models to test relationships between individual CTmax estimates and August mean temperatures, while controlling for tadpole length and sampling site. Individual data are compiled in Table S2.

2.4 | Reference Genome and Annotation

We developed a reference genome for *A. truei* using high-molecular-weight DNA extracted as described in Session et al. (2016) from whole blood from a single male frog (Kermit) collected by Cherie Mosher in British Columbia, Canada (UTM Zone 9T, 558268 E, 6048350 N). A reference genome sequence was then assembled by integrating linked short reads (108× nominal sequencing coverage in 150 bp paired-end reads from a 10x Genomics Chromium Genome library, sequenced with Illumina HiSeq X) with single-molecule, real-time continuous long reads (SMRT CLR, 15× coverage in reads of mean length 5427 bp sequenced with Pacific Biosciences Sequel). Long-range linkages were obtained through high-throughput chromatin conformation capture (Hi-C) paired ends (42× sequence coverage in 150 bp paired ends from Dovetail Genomics Hi-C library prepared from liver, sequenced with Illumina HiSeq4000 and NextSeq). Data are summarised in Table S3

We assembled the reference genome hierarchically following a previously described strategy used for the Túngara frog *Engystomops pustulosus* (Bredeson et al. 2024). First, linked reads were assembled with Supernova (v. 2.0.1; Weisenfeld et al. 2017). Next, the Supernova assembly was scaffolded with PacBio CLR data using DBG2OLC (v. Jun 11, 2015; Ye et al. 2016), and the resulting assembly was error corrected two times with BLASR (commit 4323a52; Chaisson and Tesler 2012) and PBDAGCon (pitchfork commit 1a2f1e79; Chin et al. 2013) using the map4cns pipeline (v. 0.2.0-20-gdd89f52; https://bitbucket.org/rokhsar-lab/map4cns). Error-corrected scaffolds were organised into chromosomes by aligning the Hi-C reads to the assembly with Juicer (v. 1.5.4-71-gd3ee11b; Durand, Robinson, et al. 2016), with initial scaffolds longer than 10 kb ordered and oriented by 3D-DNA (commit 745779b; Dudchenko et al. 2017) followed by manually reordering, reorienting and linking using Juicebox (v. 1.9.0; Durand, Shamim, et al. 2016). Gaps were closed by aligning PacBio reads to the Hi-C-based assembly using BWA (v. 0.7.17) (Li and Durbin 2009) and filling spanned gaps with PBJelly (PBSuite v15.8.24) (English et al. 2012). After a second round of manual review of Hi-C linkages with Juicer and Juicebox, the assembly was then error corrected with the custom script LoReM (v. 1.0; https://github.com/abmudd/Assembly), which flags and breaks regions with potential mis-assemblies based on low spanning coverage and high number of read terminals (starts and ends) from linked read and long read data aligned with BWA. Following mis-assembly detection, the Hi-C data were again aligned to the assembly with Juicer and the chromosome structure was ordered and oriented using Juicebox. Finally, the assembled sequence was polished with two rounds of error correction with linked read data. Linked reads were adapter trimmed with trim_10X.py (v. 1.0; https://github.com/abmudd/Assembly) and aligned to the assembly with BWA. Variants called by FreeBayes (v. 1.1.0-54; Garrison and Marth 2012) with a read depth within 2 standard deviations of the Gaussian fit were corrected using the script ILEC in the map4cns pipeline (v. 0.2.0-20-gdd89f52; https://bitbucket.org/rokhsar-lab/map4cns). Remaining gaps were resized with the PacBio data, and closure was attempted with the adapter-trimmed 10x genomics data using Platanus (v. 1.2.1; Kajitani et al. 2014).

We extracted RNA from tissue samples from four individuals (Table S4): the primary sequencing of *A. truei* male (Kermit); a tadpole (Tadpole2) collected by Cherie Mosher in British Columbia, Canada (UTM Zone 9T, 558388 E, 6048031N); a second *A. truei* male (Hum2) collected by Justin M. Garwood, Ryan Bourque, Matt Kluber, William Devenport and Kristina Zabierek in California, USA (UTM Zone 10T, 422811 E, 4527864 N); and a female *A. montanus* (MTF2) collected by Richard Honeycutt and Blake Hossack in Montana, USA (UTM Zone 12T, 272115 E, 5210575 N). All samples were washed twice with PBS, homogenised in TRIzol reagent and centrifuged, followed by flash freezing of the supernatant. RNA was isolated using the TRIzol Reagent User Guide (Pub. No. MAN0001271 Rev. A.0) protocol.

To annotate protein-coding genes and repetitive elements, we developed a catalogue of *A. truei* repetitive sequences using RepeatModeler (v. 1.0.11; Smit and Hubley 2015) and combined it with similarly determined repeat libraries from a set of diverse pipanuran genomes (Bredeson et al. 2024) as well as ancestral frog repeats from RepBase (v. 23.12; Bao, Kojima, and Kohany 2015). This composite library was mapped to the

A. truei reference sequence with RepeatMasker (v. 4.0.7; Smit, Hubley, and Green 2015). We then inferred the location and exon structure of protein-coding genes by combining direct transcriptome sequencing from diverse *A. truei* samples with homology modelling using the latest *Xenopus tropicalis* gene set (Bredeson et al. 2024). We obtained *A. truei* transcriptome sequences from 23 diverse tissue samples of both tadpoles and adults (Illumina TruSeq Stranded mRNA Sample Prep LS Protocol (Part #15031058 Rev. E), sequenced with Illumina HiSeq 4000; Table S3). We annotated exon structures using Gene Model Mapper (v. 1.6; Keilwagen et al. 2016) with aligned *A. truei* mRNA sequences, mapped *X. tropicalis* annotations and soft-masked repeats as input evidence. Functional annotation was performed using InterProScan (v. 5.34-73.0; Jones et al. 2014).

2.5 | Whole-Genome Resequencing

We selected 80 individual tadpoles for whole-genome resequencing using the Illumina Nextera DNA Flex Library Prep kit. We selected four sites per species (10 individuals per site), including a high- and low-elevation population from two of the three sampled watersheds for each species (Figures 3 and 4). We used Qiagen DNeasy Blood and Tissue Kits for DNA extraction, adding 4 μL of RNase after tissue digestion. We quantified DNA using Qubit dsDNA assays and verified quality in a subset of samples using agarose gel electrophoresis. We used 500 ng of DNA for each library preparation, following the Nextera protocol (Illumina Document # 1000000025416 v07, May 2019). We pooled unique, dual-indexed libraries at equal quantities (60 ng) before sequencing on two S4 flow cells of an Illumina NovaSeq 6000 (paired-end, 150 bp reads) with Novogene Corporation. Species and populations were split across libraries and sequencing lanes to control for library and lane effects.

For bioinformatics, we first used Trim Galore! (v. 0.6.4; Krueger 2019), a wrapper for Cutadapt (v. 2.5; Martin 2011) and FastQC (v. 0.11.8; Andrews 2019), to quality trim sequences (Phred score < 20) and remove Nextera transposase adapters (stringency = 6). We then reran Trim Galore! to remove over-represented sequences, as identified by FastQC. We mapped retained reads to the reference genome using the Burrows–Wheeler Aligner algorithm BWA_MEM (v. 0.7.17; Li 2013). We then removed PCR duplicates using Picard's MarkDuplicates (v. 2.20.6; Broad Institute 2019). To prepare a set of known variants for base quality recalibration in GATK (v. 3.8-1; McKenna et al. 2010), we called variants with both GATK HaplotypeCaller and bcftools (v. 1.9; Li 2011) mpileup using the following settings: calculating genotype likelihoods (-pairHMM) using logless caching (GATK); and a coefficient for downgrading mapping quality (-adjust-MQ) of 50 (bcftools). We filtered the output from each variant caller using the following filters in vcftools (v. 0.1.17 (Danecek et al. 2011)): retain only biallelic SNPs, minimum genotype equality of 20, minimum depth of 10, maximum amount of missing data of 0.75 and minor allele frequency threshold of 5%. We then intersected these filtered variant files using bedtools (v. 2.28.0; Quinlan and Hall 2010) intersect and used the overlapping variants for the known variants input to BaseRecalibrator. We applied the recalibration using PrintReads and then ran a final round of HaplotypeCaller on the recalibrated BAM files.

We split the variant files by species (40 individuals per species) and removed invariant sites and sites with missing data in more than 10 of 40 individuals. We imputed missing values within each species using Beagle (v. 4.1; Browning and Browning 2016), setting model scale = 1.5 and niterations = 25 based on recommendations from Pook et al. (2020). We used an effective population size (N_e) estimate of 600 to parameterise Beagle based on the average of N_e point estimates (excluding infinite values, see Table S5) calculated for 45 *A. montanus* populations (microsatellite data from Metzger et al. 2015). We used NeEstimator (v. 2.01; Do et al. 2014) to calculate N_e based on the 13 microsatellite loci from Metzger and colleagues, using a minor allele frequency threshold of 0.05. We used bcftools to filter the imputed VCFs by removing low-quality variants (QUAL < 30), singletons and invariant sties and retaining only biallelic SNPs and indels. We subsampled the imputed, filtered data sets to ~100,000 SNPs for each species and evaluated heterozygote miscall rates within watersheds (pooling individuals, i.e., $n = 20$ in each watershed) using the whoa package version 0.0.2.999 (Anderson 2019).

2.6 | Identification of Candidate Adaptive Variants

We identified candidate adaptive variants using two GEA tests: partial redundancy analysis (pRDA; Capblancq and Forester 2021; Forester et al. 2018) and latent factor mixed models (LFMM-lasso; Frichot et al. 2013) based on August mean temperature at each site, and two GWAS tests: pRDA and GEMMA (Zhou and Stephens 2012) based on individual CTmax measurements. Our sampling design was developed to maximise power to detect adaptive differentiation by sampling across the extremes of the temperature gradient within drainages while accounting for potential population structure across drainages (Forester et al. 2018; Lotterhos and Whitlock 2015). For pRDA, we corrected for population structure using the first principal component derived from the LD-pruned genotype matrices (plink --indep-pairwise 50 5 0.2, v. 1.90 b6.15; Chang et al. 2015). We retained one principal component to control for population structure for both species based on the minimum average partial test (Shriner 2011). Similarly, we used $K = 2$ in LFMM and the centred genotype matrix in GEMMA. For the genotype–phenotype analyses (pRDA and GEMMA), we included tadpole length as a covariate to control for tadpole size. We identified candidate adaptive markers for pRDA and LFMM based on loadings and *z*-scores (respectively) that were larger than 2.5 standard deviations from the mean loading/*z*-score. For GEMMA, we used an analogous *p*-value cutoff of $p < 0.012$ using the likelihood ratio test *p*-value.

We compiled all candidate adaptive variants and annotated them using snpEFF v. 4.3t (Cingolani et al. 2012). For downstream analysis of variants identified by each GEA and GWAS method, we retained variants located in all identified genes and the top 5000 modifiers for each species. We matched identified genes against protein names in the UniProtKB database (The UniProt Consortium 2021) for *Xenopus tropicalis* and *Homo sapiens*. We identified a subset of candidate adaptive variants for a targeted capture design. For each GEA and GWAS method and each species, we selected: (1) the top 500 detections (i.e., highest loadings and lowest p-values, which could be genes or modifiers); (2) a small set of candidate genes; (3) a small set of cross-species detections;

and (4) all genes within each species that were in the top 500 detections by both GEA or GWAS methods. For candidate genes, we identified protein names that contained the following terms: heat, hsp, hsf, HMGB1 or cold (i.e., related to thermal tolerance, Hoffmann, Sørensen, and Loeschcke 2003; Narum et al. 2013; Somero 2005); hypoxia, egln, HIF, NFAT, calcineurin or GSK3 (i.e., related to hypoxia and anoxia, Al-Attar and Storey 2018; Yang, Qi, and Fu 2016); mhc or immune (i.e., related to immune response); and stress (i.e., related to stress response). All identified candidate variants were evaluated for targeted capture design by, Ann Arbor, MI). We used RDA to evaluate the contributions of these identified variants to explaining variation in site-specific stream temperature and individual CTmax.

2.7 | Genetic Diversity, Population Structure and Effective Population Sizes

We evaluated genetic diversity and population structure in each species using putatively neutral variants. These neutral data sets were generated by removing repeat regions, gene regions and candidate adaptive variants. We calculated observed and expected heterozygosity, F_{IS}, and mean pairwise F_{ST} using vcftools. Before calculating F_{IS}, we pruned for linkage disequilibrium with bcftools using a window size of 25 variants and LD threshold of 0.5 (Kardos, Luikart, and Allendorf 2015). We used PCA to visualise population structure in vegan (v. 2.5-7; Oksanen et al. 2020). To estimate N_e, we selected a random subset of ~6000 variants from each species' putatively neutral markers for analysis with NeEstimator, after removing singletons. We used this subset of markers distributed across chromosomes to avoid pseudoreplication while maximising precision (i.e., precision in N_e does not increase much after using a few thousand SNPs, Waples 2024).

2.8 | Targeted Capture

Our final capture array was designed to bind sequences from 1433 candidate adaptive variants in *A. truei* and 1486 in *A. montanus*. We extracted DNA and quantified samples for whole-genome resequencing samples. DNA was sheared to ~200 bp using a Covaris E220 focused ultrasonicator at the University of California-Davis DNA Technologies Core. We used 230–1000 ng (average ~ 900 ng) of DNA for each library, following the NEBNext Ultra II DNA Library Prep Kit for Illumina. Each individual was labelled with a unique dual-index primer pair and we used five PCR amplification cycles. Libraries were target enriched and PCR amplified using the MyBaits standard protocol after a 24-h hybridisation. Libraries were pooled and the resulting pool was sequenced two times on a HiSeq 4000 (paired-end, 150 bp reads) with Novogene Corporation. We sequenced 124 individual *A. truei* and 123 *A. montanus*.

For bioinformatics, we used AfterQC for quality control, preliminary data filtering and error profiling (Chen et al. 2017), and identified and removed overrepresented sequences. We used Trim Galore! to quality trim sequences (Phred score < 20) and remove Illumina adapters (stringency = 6). We mapped retained reads to the reference genome using BWA_MEM and removed PCR duplicates using Picard's MarkDuplicates. We then called variants with GATK HaplotypeCaller as above. We pulled the targeted variants, split the variant files by species and imputed missing values within each species using Beagle, as above. Finally, we used bcftools to filter the imputed VCFs by removing low-quality variants (QUAL < 30), singletons and invariant sites and variants with > 70% missing data, and removed samples genotyped at fewer than 1000 variants. We used RDA to evaluate the contributions of retained variants to explaining variation in stream temperature and CTmax.

3 | Results

3.1 | Temperature and CTmax

For sites where we had both temperature logger and NorWeST data, in-stream August mean temperatures (hereafter 'summer temperatures') were within 1°C of the 1993–2011 NorWeST mean for half of our sites. In these cases, we used NorWeST 30-year averages as they are representative of local temperature conditions and will better reflect long-term selection pressures on *Ascaphus* populations. Sites that had larger deviations from the NorWeST August means likely reflect complex topographic and hydrological features contributing to thermal diversity that are not captured by the NorWeST model (e.g., Leach and Moore 2019; Schultz et al. 2017). In these cases, we use in-stream summer temperature data, as these will best reflect local selection pressures on populations. For example, in Montana, we had multiple high-elevation sites that were outlets of lakes (which were warm during summer months) and where summer temperatures were much warmer than predicted by the NorWeST model (Figure 4; Table S1; Moore Creek, Oregon Gulch, Upper Torino Tributary). These warmer, high-elevation sites contributed to a lack of correlation between summer temperatures and elevation in Montana (Pearson's correlation = 0.005, $p = 0.987$). By contrast, summer temperatures in Oregon were significantly correlated with elevation (Pearson's correlation = −0.830, $p = 0.0001$). While summer temperatures were not statistically different between states (*t*-test, $t = -1.725$, df = 27.98, $p = 0.10$), Montana streams had nearly 2 degrees more range in summer temperatures (minimum = 6.72°C, maximum = 16.30°C, range = 9.58°C) compared to Oregon streams (minimum = 7.66°C, maximum = 15.27°C, range = 7.61°C), reflecting their continental versus coastal climatic influences, respectively. NorWeST projections for 2080 under the SRES A1B climate change scenario showed an average shift of 2.14°C (range 1.91°C–2.32°C) for sites in Oregon and an average shift of 2.08°C (range 1.88°C–2.33°C) for sites in Montana (Table S6). On average, this represents a shift in summer temperatures from 11.65°C to 13.80°C in Oregon and 9.65°C to 11.73°C in Montana.

After excluding one outlier *A. truei* individual with a spurious CTmax estimate (> 9 standard deviations), we retained CTmax data from 162 *A. truei* and 163 *A. montanus* tadpoles. In both species, tadpole length had a significant negative effect on CTmax ($p < 0.001$, Table S7), while only *A. montanus* CTmax showed a significant (positive) relationship with August mean stream temperatures after accounting for tadpole length and site (*A. montanus* $p = 0.0035$, *A. truei* $p = 0.145$, Figure 5, Table S7, see also Cicchino, Shah, Forester, Dunham, et al. 2023). Both species showed variability in CTmax values within sites (*A. truei* median of ranges = 1.1°C, *A. montanus* median of ranges = 1.3°C).

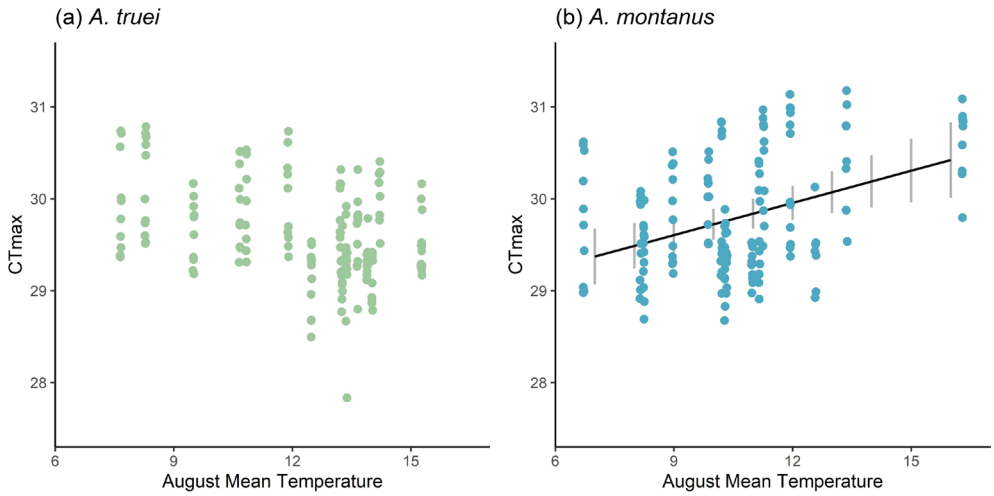

FIGURE 5 | Relationships between critical thermal maximum (CTmax) and August mean stream temperatures for (a) *A. truei* ($n = 162$) and (b) *A. montanus* ($n = 163$). Prediction slope (black line) and 95% confidence intervals (grey lines) estimated by the linear mixed-effects model are shown for *A. montanus* ($p = 0.0035$).

3.2 | Reference Genome and Annotation

The *A. truei* reference genome sequence is 3.10 billion base pairs (Gbp) long, with half of the assembled sequence in 8 scaffolds longer than 76.7 Mbp and 4717 contigs longer than 155.4 kbp. The longest 22 scaffolds are identified with chromosomes (total 1.98 Gbp), and range in length from 11.2 to 330.6 Mbp. Slightly more than half (55.9%) of the assembled genome sequence was characterised as repetitive, including the bulk of subchromosomal scaffolds. Annotation identified 16,254 high-confidence protein-coding genes, 94.5% of which could be assigned a putative functional annotation based on sequence similarity. These annotated genes possessed an average of 7.38 exons per gene (median exon size 124 bp and median intron size 1055 bp), with median coding sequence length of 1017 bp (339 amino acids), comparable to other recently annotated frog genomes. *A. truei* assembly and protein-coding annotations were deposited in NCBI under GenBank accession GCA_036426205.1.

3.3 | Whole-Genome Resequencing

The two NovaSeq S4 lanes produced ~1 TB of data, with 10–12 GB representing each of the 80 individuals. Mapping quality was high, with an average of 98% of reads mapping across both lanes and species (Table S8). The average percentage of reads mapped was slightly lower in *A. montanus* (98.2%) than *A. truei* (98.4%). Average read depth was 6.1× (range 4.8× − 9.0×) across lanes and was lower in *A. montanus* (5.8×) than *A. truei* (6.4×). Slightly lower read mapping and read depth in *A. montanus* may be due to mapping to the *A. truei* reference genome (e.g., Bohling 2020). The known variants input for GATK BaseRecalibrator included 114,407 SNPs. After filtering the recalibrated and imputed VCFs, we retained 39,825,878 variants for *A. truei* (34,339,610 SNPs and 5,486,268 biallelic indels) and 33,709,071 variants for *A. montanus* (28,980,064 SNPs and 4,729,007 biallelic indels). Heterozygote miscall rates were low: for *A. montanus*, the Dry and Upper Torino drainage (Middle Clark Fork) had a mean miscall rate of 0.020 (95% CIs: 0.019–0.022), while the Upper and Lower Lost Horse drainage (Bitterroot) had a mean miscall rate of 0.012 (95% CIs: 0.011–0.014); for *A. truei*, the Bulldog and North Fork Steelhead drainage (Umpqua) had a mean miscall rate of 0.054 (95% CIs: 0.053–0.055), while the Lamb and Ore drainage (McKenzie) had a mean miscall rate of 0.058 (95% CIs: 0.057–0.059).

3.4 | Genetic Diversity, Population Structure and Effective Population Sizes

We calculated observed and expected heterozygosity and pairwise F_{ST} for the eight whole-genome resequencing sites using 10,671,372 and 9,450,199 putatively neutral variants in *A. truei* and *A. montanus*, respectively. For F_{IS}, we used an LD-pruned subset of these variants totalling 4,089,856 and 3,458,884 loci in *A. truei* and *A. montanus*, respectively. Observed and expected heterozygosity were slightly lower in *A. truei* than *A. montanus*, while F_{IS} values were slightly higher (Table 1), which may reflect relatively higher isolation and reduced gene flow in *A. truei*. Pairwise F_{ST} values were low within watersheds (average of 0.011 for *A. truei* and −0.004 for *A. montanus*) and higher across watersheds (average of 0.115 for *A. truei* and 0.089 for *A. montanus*; see Table S9 for full results). These results were reflected in the PCAs for both species, with PC1 splitting sites across watersheds and PC2 identifying minor substructures within watersheds (Figure S1). Lower within-species F_{ST} for *A. montanus* may be a result of mapping to the *A. truei* genome (Bohling 2020), although other studies have found similarly low-to-moderate intraspecific divergence among *A. montanus* populations (Metzger et al. 2015; Spear and Storfer 2010). For all sites, point N_e estimates were negative and confidence intervals were infinity, suggesting genetic drift is negligible within sites and that N_e values are large (Waples 2024).

3.5 | Identification of Candidate Adaptive Variants

We identified candidate adaptive markers for pRDA and LFMM based on loadings and *z*-scores (respectively) that were larger than 2.5 standard deviations from the mean loading/*z*-score. For

GEMMA, we used an analogous p-value cutoff of $p < 0.012$. The two GEA methods identified a total of 1,131,492 unique candidate variants in *A. truei* and 566,375 in *A. montanus*. The two GWAS methods identified a total of 748,450 unique candidate variants in *A. truei* and 523,658 in *A. montanus*. We analysed a reduced set of these detections by pulling all genes and the top 5000 modifiers identified by each method. A large number of genes and variants related to temperature (GEA methods) and CTmax (GWAS methods) were identified in each species, with some overlap across methods within species (*A. truei* and *A. montanus* rows in Table 2). There were gene annotations identified in both species related to temperature and CTmax; however, overlap in the specific variants identified was low ('Overlap across species' row in Table 2).

We identified a subset of these variants for a targeted capture panel, including 2382 variants in *A. truei* and 2275 variants in *A. montanus* (Table S10). Candidate variants in this subset identified by GEA explained 31% of the variance in stream temperature across four sites in both *A. truei* and *A. montanus* ($p = 0.001$ for both species; Table 3). Similarly, candidate variants in the subset identified by GWAS explained 37% of the variance in CTmax across four sites and 40 individuals in *A. truei* and 32% of the variance in CTmax in *A. montanus* ($p = 0.001$ for both species; Table 3). Plots of RDAs including both predictors provide an integrative visualisation of these results for *A. truei* (Figure 6a) and *A. montanus* (Figure 6b), where individuals are located in the multivariate space as a function of their multilocus genotypes at the candidate adaptive variants. Each individual tadpole's point is colour coded to reflect the summer stream temperatures they experience, and the size of the point is scaled to the value of their CTmax. In both species, individuals on the right-hand sides of these plots have more candidate adaptive alleles related to higher temperatures and higher CTmax, while individuals on the left-hand sides have more alleles related to lower temperatures and lower CTmax. This visualisation reflects potential local adaptations of populations to stream temperature (or other environmental parameters correlated with stream temperature) and a potential genetic basis to CTmax variability across populations in both species.

TABLE 1 | Observed (H_o) and expected (H_e) heterozygosity and F_{IS} for whole-genome resequencing sites (10 individuals/site) for *Ascaphus truei* and *A. montanus*.

Species	Site	H_o	H_e	F_{IS}
A. truei	Ore	0.23	0.27	0.040
	Lamb	0.23	0.27	0.044
	North Fork Steelhead	0.23	0.27	0.075
	Bulldog	0.23	0.27	0.069
A. montanus	Dry	0.27	0.30	−0.035
	Upper Torino	0.27	0.30	−0.013
	Lower Lost Horse	0.26	0.30	0.008
	Upper Lost Horse	0.26	0.30	0.043

3.6 | Targeted Capture

After filtering, we retained 1052 variants and 100 individuals for *A. truei* and 1269 variants and 108 individuals for *A. montanus*. While this reduced set of variants explained a much smaller proportion of the variance in stream temperatures and CTmax across 11 additional sites in each species, the results were significant (e.g., $p < 0.05$) in all tests except CTmax in *A. truei* (Table 2), illustrating a consistent relationship with temperature adaptation in both species. Plots of RDAs using both predictors for this smaller set of capture data are provided for *A. truei* (Figure 6c) and *A. montanus* (Figure 6d). Again, individuals are located in the multivariate space as a function of their multilocus genotypes at the genotyped candidate adaptive variants and points are colour coded and scaled to the individual's summer stream temperature and CTmax, respectively. In *A. truei*, the temperature relationship is strong, with most individuals from warmer sites located in the top half of the plot, and individuals from cooler sites in the bottom (Figure 6c). By contrast, the CTmax signature in the capture data for *A. truei* is relatively weak, with no clear pattern of circle size related to CTmax. These patterns

TABLE 2 | Unique gene annotations and variants across species and detection methods (two genotype–environment association or GEA methods and two genome-wide association or GWAS methods) for a subset of candidate adaptive variants identified from four sites per species, with 10 individuals per site. 'Overlap' refers to duplicate gene annotation or variant detections across GEA and GWAS methods.

Species	Identification method	Unique gene annotations	Unique variants
A. truei	GEA	6233	19,213
	GWAS	5408	14,287
	Overlap	1881	475
A. montanus	GEA	4493	12,067
	GWAS	3963	9967
	Overlap	1406	895
Overlap across species	GEA	1403	18
	GWAS	1165	21
	Overlap	263	0

TABLE 3 | RDA results for the full set of candidate adaptive markers identified for targeted capture from whole-genome resequencing (WGR) data and the final targeted capture data.

Data set	Species	Variants related to	Number of individuals	Number of sites	Number of variants	Adjusted r^2	p
WGR, capture candidates	*A. truei*	Temperature	40	4	1145	0.310	0.001
		CTmax	40	4	1202	0.373	0.001
	A. montanus	Temperature	40	4	1192	0.307	0.001
		CTmax	40	4	1083	0.323	0.001
Targeted capture	*A. truei*	Temperature	100	11	476	0.017	0.001
		CTmax	100	11	562	0.001	0.210
	A. montanus	Temperature	108	11	662	0.003	0.004
		CTmax	108	11	599	0.002	0.011

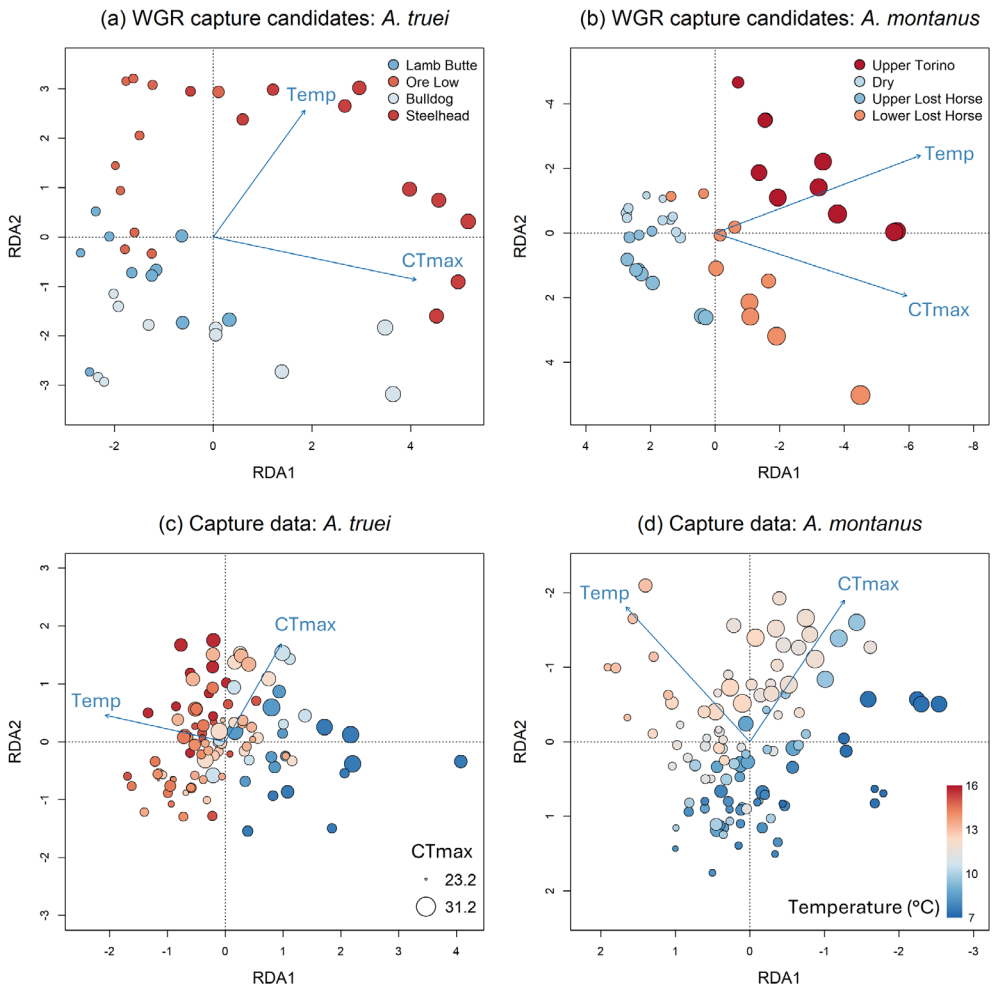

FIGURE 6 | Redundancy analysis (RDA) plots for *A. truei* (left, a and c) and *A. montanus* (right, b and d) showing capture candidates from whole-genome resequencing at four sites (10 individuals per site per species; top, a and b, labelled by site) and targeted capture data at 11 sites (100 individuals total for *A. truei* and 108 individuals total for *A. montanus*; bottom, c and d). Circles represent individual tadpoles, which are positioned in the multivariate space by their multilocus genotype as a function of the two predictors, August mean stream temperature (Temp) and CTmax, shown as blue vectors. In all plots, each individual's circle is colour coded by their stream's August mean temperature (legend in d), while the size of each circle is scaled to that individual's CTmax (legend in c). A plot of capture data by site is available in Figure S2.

correspond with the statistical results for *A. truei* across these two predictors (i.e., a significant relationship with temperature and nonsignificant result with CTmax; Table 3) and can be contrasted with the significant relationships for *A. truei* related to both predictors in the WGR capture candidates (Figure 6a; top row of Table 3). Results across these candidate adaptive data

sets are more consistent for *A. montanus*. Capture data show strong relationships between multilocus genotypes and both predictors, with individuals sorting more clearly in the ordination space along the two predictor vectors (Figure 6d), reflecting the significant statistical results for *A. montanus* (Table 3) and in agreement with the RDA of capture candidates (Figure 6b).

4 | Discussion

We combined genomic data with temperature and physiological data to provide strong evidence for local adaptation to temperature variation in two, cold-water stream frog species. The correlation of genetically based adaptive variation with temperature and thermal tolerance indicates that tailed frog populations vary in sensitivity to increasing stream temperatures, and have the potential to evolve in response. Below we discuss these results in more depth, as well as their implications for sensitivity, evolutionary potential and overall vulnerability to climate change.

4.1 | Integrative Evidence for Local Adaptation to Temperature Variation

For *A. montanus*, both physiological and genomic data provide strong evidence for local adaptation to temperature (Figures 5b and 6b,d). Previous work found that CTmax is positively related to maximum stream temperatures in *A. montanus*, but that study was unable to distinguish between plasticity and evolutionary adaptation as drivers of the observed patterns (Cicchino, Shah, Forester, Dunham, et al. 2023). Our study provides evidence for a genetic basis for CTmax, with a large number of variants significantly related to individual-level CTmax across four sites in two different watersheds (Figure 6b; Tables 2 and 3). Sequencing of a subset of these variants across 11 additional sites distributed across three watersheds confirmed a significant signature of local adaptation of CTmax to average August stream temperatures (Figure 6d; Table 3). The large number of variants identified points to a polygenic basis that was expected given that complex physiological traits such as CTmax are expected to have a polygenic architecture (Bernatchez 2016; Healy et al. 2018; Rose et al. 2018). GEA tests revealed a large number of variants significantly related to summer temperature variation across populations, indicating adaptive divergence related to temperature variability across the sampled range (Figure 6b,d; Table 3). Importantly, most of the variants and gene annotations identified by GWAS and GEA tests did not overlap (Table 2), indicating that there are other traits in addition to CTmax that are involved in temperature adaptation, or parameters correlated with temperature, across the range of *A. montanus*.

By contrast, *A. truei* shows more complexity in the relationships among CTmax, temperature variation and local adaptation (Figures 5a and 6a,c). Previous work found that CTmax was not significantly related to maximum stream temperatures in *A. truei*, perhaps due to lower variation in temperatures among sites when compared to *A. montanus* (Cicchino, Shah, Forester, Dunham, et al. 2023). Despite this, our genomic data provide evidence for a polygenic genetic basis for CTmax in *A. truei*. Similarly to our findings in *A. montanus*, we identified a large number of variants significantly related to individual-level CTmax across four sites in two different watersheds (Figure 6a; Tables 2 and 3). However, unlike *A. montanus*, sequencing of a subset of these variants across 11 additional sites distributed across three watersheds did not show evidence of a significant relationship with CTmax (Figure 6c; Table 3). This may be due to less variation in CTmax across populations of *A. truei* relative to *A. montanus*, although reduced power with the subset of variants sequenced could also have contributed. In both species, however, there is substantial variation in CTmax among individuals within populations (Figure 5), indicating that there is available phenotypic variation for selection to act upon; if this variation is heritable, these populations could have potential for a more rapid adaptive response to rising temperatures. Finally, GEA results in *A. truei* uncovered similar relationships as *A. montanus*, with a large number of variants significantly related to population-level stream temperature variation across the sampled species range, indicating adaptive divergence related to temperature variability (Figure 6a; Table 3). Variants and gene annotations identified by GWAS and GEA tests also showed low overlap. In both species, these signatures of adaptive divergence related to temperature variability point to potential local adaptation to the thermal environment and/or other covarying environmental parameters, such as stream flow regimes, stream productivity, food quality or biotic interactions. Additionally, we identified more candidate variants in *A. truei* relative to *A. montanus*, which may reflect the relatively higher population differentiation in *A. truei* (detected in our F_{ST} analyses and previous studies, Spear and Storfer 2008, 2010), potentially facilitating greater adaptive differentiation at more loci among populations in *A. truei*, although this hypothesis will need further evaluation. Finally, we found moderate levels of overlap in gene annotations and variants for GEA and GWAS tests across species (Table 2), suggesting that different genes and variants may be involved in adaptation to temperature regimes in these species, a result consistent with their long evolutionary divergence (i.e., late Miocene and early Pliocene, Nielson, Lohman, and Sullivan 2001) as well as differences in climatic regimes across their ranges.

4.2 | Implications for Sensitivity to Increasing Temperatures

Climate change vulnerability assessments commonly assume or imply that a single estimate of thermal tolerance is representative of a species' sensitivity, often due to a focus on exposure, limited intraspecific data availability across the species' range or a presumption that intraspecific variation is negligible relative to interspecific variation (Butt et al. 2016; Foden et al. 2013, 2019; Herrando-Pérez et al. 2019). This assumption leads to the conclusion that populations in the warmest parts of a species' range (e.g., low latitude or low elevation) will likely be the most sensitive to warming from climate change, as these will be the first populations where environmental temperatures will exceed thermal tolerance (Hoffmann and Sgro 2011; Sunday, Bates, and Dulvy 2012; e.g., Figure 2a). Our results add to growing evidence (e.g., Gervais et al. 2021; Herrando-Pérez et al. 2019; Jackson et al. 2024; Nati et al. 2021) that intraspecific variability in thermal tolerance and local adaptation to temperature regimes can result in more complex

spatial patterns of sensitivity across species' ranges than predicted by the assumption of invariant thermal tolerance.

In *A. montanus*, combined evidence for local adaptation to temperature variation suggests that all populations may have similar sensitivity to warming (i.e., consistent warming tolerance; Figure 2b) since populations that currently occupy warmer streams show higher CTmax. By contrast, conclusions about sensitivity for *A. truei* depend on which data are being considered. Based on physiological data alone, the assumption that populations in warmer environments are more sensitive (i.e., lower warming tolerance than population in cooler environments; Figure 2a) is supported, given that *A. truei* populations do not vary in their CTmax across stream temperatures. This conclusion supports the one made in Cicchino, Shah, Forester, Dunham, et al. (2023) that physiological vulnerability decreases with elevation among population of this species (which is unsurprising given the data used here are a subset of the physiological data used in that study). However, genomic data show signatures of local adaptation in both CTmax and temperature variability across sites, indicating that, at least based on some metrics of thermal sensitivity, all populations will be similarly sensitive to changing conditions. These different conclusions regarding sensitivity in *A. truei* highlight the importance of collecting multiple data types and lines of evidence, where possible, to create a more informed assessment of patterns of sensitivity (Huey et al. 2012).

4.3 | Integrative Evidence for Evolutionary Potential

The available data support a conclusion of generally high evolutionary potential in *Ascaphus* populations included in this study. In both species, we see high variability in CTmax within sites, and, in *A. montanus*, among sites, indicating the maintenance of trait variation across scales (Figure 5). GWAS tests identified a polygenic genetic basis to this CTmax variability, pointing to local adaptation of *A. montanus* populations (i.e., based on both whole-genome and targeted capture results), with weaker signatures in *A. truei* (i.e., based on whole-genome data only; Figure 6). This polygenic architecture may confer increased evolutionary potential in CTmax across these populations and allow for more consistent evolutionary responses to natural selection (Kardos and Luikart 2021). GEA tests identified additional (mostly non-overlapping) signatures of local adaptation to temperature variation in both species, suggesting evolutionary potential related to additional traits involved in thermal tolerance, or other traits or environmental characteristics that are correlated with temperature variability. Finally, in agreement with previous work (Metzger et al. 2015; Spear, Crisafulli, and Storfer 2012; Spear and Storfer 2008, 2010) we found high genetic diversity and large effective population sizes at four sites in both species (Table 1), with low population structure within drainages. These genetic data suggest a high capacity to retain genetic variation over time and high efficacy of selection within populations with minimal negative impacts from genetic drift, although we note that some *A. truei* populations at the species' northern range boundary (not included in this study) show reduced genetic diversity due to historical range expansion (Mosher, Johnson, and Murray 2022). While these results point to robust evolutionary potential for both species across the sampled portions of their ranges, it is unclear how and to what extent these characteristics will have the potential to reduce population- and species-level vulnerability to increasing temperatures.

4.4 | Implications for Vulnerability

A comprehensive climate change vulnerability assessment involves integration of intraspecific data on exposure, sensitivity and all three components of adaptive capacity: dispersal capacity, phenotypic plasticity and evolutionary potential. In our case, evidence for local adaptation to temperature based on physiological and genomic data in *A. montanus* and genomic data in *A. truei* suggests similar levels of sensitivity among populations (Figure 2b), regardless of stream temperature, but relatively invariant CTmax across temperatures in *A. truei* suggests that populations occupying warmer streams may be most sensitive to warming (Figure 2a). For *A. truei*, this points to the importance of reducing additional stressors such as timber harvest (which reduces canopy cover and increases siltation, impacting larval biomass; Wahbe and Bunnell 2003), in populations occupying warmer streams. Additionally, some *A. montanus* populations currently experience maximum summer temperatures that are close to temperatures associated with mortality over a 3-day period (Cicchino, Ghalambor, and Funk 2023; Cicchino, Shah, Forester, Dunham, et al. 2023). For these populations, similar reductions in stressors that drive increasing stream temperature will likely be important. In terms of adaptive capacity, the low genetic differentiation within watersheds in both species confirms results from previous studies showing relatively high gene flow and dispersal potential for both *Ascaphus* species (Metzger et al. 2015; Spear, Crisafulli, and Storfer 2012; Spear and Storfer 2008, 2010), improving both colonisation abilities and the potential for adaptive gene flow (Figure 2c). As noted by previous studies in both species, maintaining intact forest cover both within and between riparian areas is a key factor for sustaining gene flow and population connectivity (Spear and Storfer 2008, 2010). Additionally, all available data suggest high levels of evolutionary potential in response to temperature variation: variability in CTmax both within (in both species) and across (in *A. montanus*) sites combined with substantial genomic evidence, high genetic diversity and large effective population sizes supports evolutionary potential related to temperature and thermal tolerance. This evidence for high dispersal capacity and evolutionary potential in response to temperature change is important given that previous work in *Ascaphus* has shown relatively low acclimation capacity of CTmax across populations in both species, which will limit the role of thermal tolerance plasticity in improving adaptive capacity (Cicchino, Shah, Forester, Dunham, et al. 2023). Furthermore, most streams lack significant microclimatic spatial variation in temperature, making behavioural thermoregulation and the use of thermal refuges less likely options under future warmer conditions (Cicchino et al. 2024). Despite this, movement of warm-tolerant genotypes among populations, especially within watersheds, is one potential mechanism to reduce vulnerability as stream temperatures

continue to change over time, with NorWeST projections indicating at least 2°C shifts in average summer stream temperatures by 2080 (e.g., Figure 2c, Table S6). Additional work to measure the sublethal effects of warming on sensitivity could contribute to the growing body of evidence suggesting that exposure to heat well below CTmax can have detrimental impacts on organisms (Harvey et al. 2023; Shah et al. 2023; Li et al. 2013). Finally, further integration of these data would allow for a more comprehensive evaluation of spatial variation in vulnerability within species in response to shifting temperatures and changes in stream flow, for example, through the use of individual-based eco-evolutionary simulation models (e.g., Bay et al. 2017; Forester et al. 2023).

5 | Conclusions

Predicting responses to climate warming is a challenging problem because it requires integrating organismal and population responses over different time scales (Huey et al. 2012). Genomic data, in combination with at-site measurement of climatic conditions, physiology and individual-based modelling can provide a holistic and powerful approach for predicting spatial patterns of climate change vulnerability over short (i.e., physiological responses) and longer (i.e., evolutionary) time scales. These efforts require large amounts of data, however, as well as controlled experiments, which are not possible in the majority of species of conservation concern. The use of proxies for population-level sensitivity and adaptive capacity traits will therefore be critical to inform these assessments in at-risk species (e.g., Forester et al. 2022; Huey et al. 2012). Compiling data in species that are more amenable to study, such as tailed frogs, can not only provide data to inform and validate proxies but can also help guide efforts for data-poor cases and ultimately identify trends and general principles (Foden et al. 2013, 2019). This study is part of that effort to provide insights into the complexity of predicting spatial patterns of climate change vulnerability more broadly.

Author Contributions

A.S.C., A.A.S., B.R.F., C.K.G., E.L.L., J.B.D. and W.C.F. contributed to conceptualisation of the research and methods development. A.S.C. and B.R.F. conducted lab and field work. A.S.C., B.R.F., E.C.A. and W.C.F. contributed to sequencing study design and bioinformatics. A.S.C., B.R.F. and W.C.F. conducted the statistical analyses and interpreted the data. A.S.C., B.R.F. and E.L.L. contributed to data visualisation. A.J.C. and B.W.M. coordinated field work and contributed to development of the reference genome. A.B.M., J.V.B. and D.R. conducted all lab and bioinformatics work to develop the reference genome. B.R.F. and W.C.F. wrote the paper. All authors edited and provided feedback on subsequent versions.

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Conflicts of Interest

DR is a member of the Scientific Advisory Board of, and a minor shareholder in, Dovetail Genomics LLC, which provides as a service the high-throughput chromatin conformation capture (Hi-C) technology used in this study.

Data Availability Statement

Ascaphus truei assembly and protein-coding annotations are available in NCBI under GenBank accession GCA_036426205.1. The raw data for the reference genome, including genomic data for assembly, RNA-seq for 23 tissue samples and annotations, can be found in NCBI under BioProject accession PRJNA550271. Raw, demultiplexed sequencing data are available on the NCBI Sequence Read Archive under BioProject accession PRJNA1199690 (Forester et al. 2025). Filtered VCFs and metadata are available on Dryad (Forester et al. 2024, https://doi.org/10.5061/dryad.2280gb638 in review).

Benefit-Sharing Statement

Benefits from this research accrue from the sharing of our data and results on public databases as described above, contributing to the conservation of biodiversity.

References

Al-Attar, R., and K. B. Storey. 2018. "Effects of Anoxic Exposure on the Nuclear Factor of Activated T Cell (NFAT) Transcription Factors in the Stress-Tolerant Wood Frog." *Cell Biochemistry and Function* 36, no. 8: 420–430. https://doi.org/10.1002/cbf.3362.

Anderson, E. C. 2019. "whoa: Evaluation of genotyping error in genotype-by-sequencing data (Version R package version 0.0.2.999) [Computer software]." https://CRAN.R-project.org/package=whoa.

Andrews, S. 2019. "FastQC: A Quality Control Tool for High Throughput Sequence Data (0.11.8) [Computer Software]." http://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

Angilletta, M. J. 2009. *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford, UK: Oxford University Press. https://doi.org/10.1093/acprof:oso/9780198570875.001.1.

Bao, W., K. K. Kojima, and O. Kohany. 2015. "Repbase Update, a Database of Repetitive Elements in Eukaryotic Genomes." *Mobile DNA* 6, no. 1: 11. https://doi.org/10.1186/s13100-015-0041-9.

Bay, R. A., N. H. Rose, C. A. Logan, and S. R. Palumbi. 2017. "Genomic Models Predict Successful Coral Adaptation if Future Ocean Warming Rates Are Reduced." *Science Advances* 3, no. 11: e1701413. https://doi.org/10.1126/sciadv.1701413.

Beever, E. A., L. E. Hall, J. Varner, et al. 2017. "Behavioral Flexibility as a Mechanism for Coping With Climate Change." *Frontiers in Ecology and the Environment* 15, no. 6: 299–308. https://doi.org/10.1002/fee.1502.

Bennett, J., P. Calosi, S. Clusella-Trullas, et al. 2018. "GlobTherm, a Global Database on Thermal Tolerances for Aquatic and Terrestrial Organisms." *Scientific Data* 5: 180022. https://doi.org/10.1038/sdata.2018.22.

Bernatchez, L. 2016. "On the Maintenance of Genetic Variation and Adaptation to Environmental Change: Considerations From Population Genomics in Fishes." *Journal of Fish Biology* 89, no. 6: 2519–2556. https://doi.org/10.1111/jfb.13145.

Bernatchez, L., A.-L. Ferchaud, C. S. Berger, C. J. Venney, and A. Xuereb. 2023. "Genomics for Monitoring and Understanding Species Responses to Global Climate Change." *Nature Reviews Genetics* 25: 165–183. https://doi.org/10.1038/s41576-023-00657-y.

Bohling, J. 2020. "Evaluating the Effect of Reference Genome Divergence on the Analysis of Empirical RADseq Datasets." *Ecology and Evolution* 10, no. 14: 7585–7601. https://doi.org/10.1002/ece3.6483.

Brattstrom, B. H. 1968. "Thermal Acclimation in Anuran Amphibians as a Function of Latitude and Altitude." *Comparative Biochemistry and Physiology* 24, no. 1: 93–111. https://doi.org/10.1016/0010-406X(68)90961-4.

Bredeson, J. V., A. B. Mudd, S. Medina-Ruiz, et al. 2024. "Conserved Chromatin and Repetitive Patterns Reveal Slow Genome Evolution in Frogs." *Nature Communications* 15, no. 1: 579. https://doi.org/10.1038/s41467-023-43012-9.

Broad Institute. 2019. "Picard Toolkit (2.20.6) [Computer Software]. Broad Institute, GitHub Repository." http://broadinstitute.github.io/picard/.

Browning, B. L., and S. R. Browning. 2016. "Genotype Imputation With Millions of Reference Samples." *American Journal of Human Genetics* 98, no. 1: 116–126. https://doi.org/10.1016/j.ajhg.2015.11.020.

Buckley, L. B., E. Carrington, M. E. Dillon, et al. 2023. "Characterizing Biological Responses to Climate Variability and Extremes to Improve Biodiversity Projections." *PLOS Climate* 2, no. 6: e0000226. https://doi.org/10.1371/journal.pclm.0000226.

Bury, R. B. 2008. "Low Thermal Tolerances of Stream Amphibians in the Pacific Northwest: Implications for Riparian and Forest Management." *Applied Herpetology* 5: 63–74.

Butt, N., H. P. Possingham, C. De Los Rios, et al. 2016. "Challenges in Assessing the Vulnerability of Species to Climate Change to Inform Conservation Actions." *Biological Conservation* 199: 10–15. https://doi.org/10.1016/j.biocon.2016.04.020.

Capblancq, T., and B. R. Forester. 2021. "Redundancy Analysis: A Swiss Army Knife for Landscape Genomics." *Methods in Ecology and Evolution* 12: 2298–2309. https://doi.org/10.1111/2041-210X.13722.

Chaisson, M. J., and G. Tesler. 2012. "Mapping Single Molecule Sequencing Reads Using Basic Local Alignment With Successive Refinement (BLASR): Application and Theory." *BMC Bioinformatics* 13, no. 1: 238. https://doi.org/10.1186/1471-2105-13-238.

Chang, C. C., C. C. Chow, L. C. Tellier, S. Vattikuti, S. M. Purcell, and J. J. Lee. 2015. "Second-Generation PLINK: Rising to the Challenge of Larger and Richer Datasets." *GigaScience* 4, no. 1: 7. https://doi.org/10.1186/s13742-015-0047-8.

Chen, S., T. Huang, Y. Zhou, Y. Han, M. Xu, and J. Gu. 2017. "AfterQC: Automatic Filtering, Trimming, Error Removing and Quality Control for Fastq Data." *BMC Bioinformatics* 18, no. 3: 80. https://doi.org/10.1186/s12859-017-1469-3.

Chin, C.-S., D. H. Alexander, P. Marks, et al. 2013. "Nonhybrid, Finished Microbial Genome Assemblies From Long-Read SMRT Sequencing Data." *Nature Methods* 10, no. 6: 563–569. https://doi.org/10.1038/nmeth.2474.

Cicchino, A. S., A. A. Shah, B. R. Forester, et al. 2023. "Acclimation Capacity of Critical Thermal Maximum Varies Among Populations: Consequences for Estimates of Vulnerability." *Ecosphere* 14, no. 11: e4691. https://doi.org/10.1002/ecs2.4691.

Cicchino, A. S., A. A. Shah, B. R. Forester, J. B. Dunham, C. K. Ghalambor, and W. C. Funk. 2023. "Multi-Scale Relationships in Thermal Limits Within and Between Two Cold-Water Frog Species Uncover Different Trends in Physiological Vulnerability." *Freshwater Biology* 68, no. 7: 1267–1278. https://doi.org/10.1111/fwb.14102.

Cicchino, A. S., C. K. Ghalambor, and W. C. Funk. 2023. "Linking Critical Thermal Maximum to Mortality From Thermal Stress in a Cold-Water Frog." *Biology Letters* 19, no. 6: 20230106. https://doi.org/10.1098/rsbl.2023.0106.

Cicchino, A. S., C. K. Ghalambor, B. R. Forester, J. B. Dunham, and W. C. Funk. 2024. "Greater Plasticity in CTmax With Increased Climate Variability Among Populations of Tailed Frogs." *Proceedings of the Royal Society B: Biological Sciences* 291, no. 2034: 20241628. https://doi.org/10.1098/rspb.2024.1628.

Cingolani, P., A. Platts, L. L. Wang, et al. 2012. "A Program for Annotating and Predicting the Effects of Single Nucleotide Polymorphisms, SnpEff: SNPs in the Genome of *Drosophila melanogaster* Strain w1118; iso-2; iso-3." *Fly* 6, no. 2: 80–92. https://doi.org/10.4161/fly.19695.

Danecek, P., A. Auton, G. Abecasis, et al. 2011. "The Variant Call Format and VCFtools." *Bioinformatics* 27, no. 15: 2156–2158. https://doi.org/10.1093/bioinformatics/btr330.

Dawson, T. P., S. T. Jackson, J. I. House, I. C. Prentice, and G. M. Mace. 2011. "Beyond Predictions: Biodiversity Conservation in a Changing Climate." *Science* 332, no. 6025: 53–58. https://doi.org/10.1126/science.1200303.

Deutsch, C. A., J. J. Tewksbury, R. B. Huey, et al. 2008. "Impacts of Climate Warming on Terrestrial Ectotherms Across Latitude." *Proceedings of the National Academy of Sciences of the United States of America* 105, no. 18: 6668–6672. https://doi.org/10.1073/pnas.0709472105.

Do, C., R. S. Waples, D. Peel, G. M. Macbeth, B. J. Tillett, and J. R. Ovenden. 2014. "NeEstimator v2: Re-Implementation of Software for the Estimation of Contemporary Effective Population Size (Ne) From Genetic Data." *Molecular Ecology Resources* 14, no. 1: 209–214. https://doi.org/10.1111/1755-0998.12157.

Dudchenko, O., S. S. Batra, A. D. Omer, et al. 2017. "De Novo Assembly of the *Aedes aegypti* Genome Using Hi-C Yields Chromosome-Length Scaffolds." *Science* 356, no. 6333: 92–95. https://doi.org/10.1126/science.aal3327.

Dunham, J., G. Chandler, B. Rieman, and D. Martin. 2005. *Measuring Stream Temperature With Digital Data Loggers: A User's Guide (Gen. Tech. Rep. RMRS-GTR-150WWW)*, 1–18. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. https://doi.org/10.2737/RMRS-GTR-150.

Durand, N. C., J. T. Robinson, M. S. Shamim, et al. 2016. "Juicebox Provides a Visualization System for Hi-C Contact Maps With Unlimited Zoom." *Cell Systems* 3, no. 1: 99–101. https://doi.org/10.1016/j.cels.2015.07.012.

Durand, N. C., M. S. Shamim, I. Machol, et al. 2016. "Juicer Provides a One-Click System for Analyzing Loop-Resolution Hi-C Experiments." *Cell Systems* 3, no. 1: 95–98. https://doi.org/10.1016/j.cels.2016.07.002.

English, A. C., S. Richards, Y. Han, et al. 2012. "Mind the Gap: Upgrading Genomes With Pacific Biosciences RS Long-Read Sequencing Technology." *PLoS One* 7, no. 11: e47768. https://doi.org/10.1371/journal.pone.0047768.

Foden, W. B., B. E. Young, H. R. Akçakaya, et al. 2019. "Climate Change Vulnerability Assessment of Species." *Wiley Interdisciplinary Reviews: Climate Change* 10, no. 1: e551. https://doi.org/10.1002/wcc.551.

Foden, W. B., S. H. M. Butchart, S. N. Stuart, et al. 2013. "Identifying the World's Most Climate Change Vulnerable Species: A Systematic Trait-Based Assessment of All Birds, Amphibians and Corals." *PLoS One* 8, no. 6: e65427. https://doi.org/10.1371/journal.pone.0065427.

Forester, B. R., A. S. Cicchino, A. A. Shah, et al. 2024. "Data From: Population Genomics Reveals Local Adaptation Related to Temperature Variation in Two Stream Frog Species: Implications for Vulnerability to Climate Warming. *Dryad, Dataset*." https://doi.org/10.5061/dryad.2280gb638.

Forester, B. R., A. S. Cicchino, A. A. Shah, et al. 2025. "Data From: Population Genomics Reveals Local Adaptation Related to Temperature Variation in Two Stream Frog Species: Implications for Vulnerability to Climate Warming. NCBI, Datasets, GenBank Accession GCA_036426205.1 and BioProject Accessions PRJNA550271 and PRJNA1199690."

Forester, B. R., C. C. Day, K. Ruegg, and E. L. Landguth. 2023. "Evolutionary Potential Mitigates Extinction Risk Under Climate Change in the Endangered Southwestern Willow Flycatcher." *Journal of Heredity* 114: 341–353. https://doi.org/10.1093/jhered/esac067.

Forester, B. R., E. A. Beever, C. Darst, J. Szymanski, and W. C. Funk. 2022. "Linking Evolutionary Potential to Extinction Risk: Applications and Future Directions." *Frontiers in Ecology and the Environment* 20, no. 9: 507–515. https://doi.org/10.1002/fee.2552.

Forester, B. R., J. R. Lasky, H. H. Wagner, and D. L. Urban. 2018. "Comparing Methods for Detecting Multilocus Adaptation With Multivariate Genotype–Environment Associations." *Molecular Ecology* 27: 2215–2233. https://doi.org/10.1111/mec.14584.

Freeman, B. G., and A. M. Class Freeman. 2014. "Rapid Upslope Shifts in New Guinean Birds Illustrate Strong Distributional Responses of Tropical Montane Species to Global Warming." *Proceedings of the National Academy of Sciences of the United States of America* 111, no. 12: 4490–4494. https://doi.org/10.1073/pnas.1318190111.

Frichot, E., S. D. Schoville, G. Bouchard, and O. François. 2013. "Testing for Associations Between Loci and Environmental Gradients Using Latent Factor Mixed Models." *Molecular Biology and Evolution* 30, no. 7: 1687–1699. https://doi.org/10.1093/molbev/mst063.

Funk, W. C., M. S. Blouin, P. S. Corn, et al. 2005. "Population Structure of Columbia Spotted Frogs (*Rana luteiventris*) is Strongly Affected by the Landscape." *Molecular Ecology* 14, no. 2: 483–496. https://doi.org/10.1111/j.1365-294X.2005.02426.x.

Garrison, E., and G. Marth. 2012. "Haplotype-Based Variant Detection From Short-Read Sequencing (arXiv:1207.3907). arXiv." https://doi.org/10.48550/arXiv.1207.3907.

Gervais, C. R., C. Huveneers, J. L. Rummer, and C. Brown. 2021. "Population Variation in the Thermal Response to Climate Change Reveals Differing Sensitivity in a Benthic Shark." *Global Change Biology* 27, no. 1: 108–120. https://doi.org/10.1111/gcb.15422.

Harvey, J. A., K. Tougeron, R. Gols, et al. 2023. "Scientists' Warning on Climate Change and Insects." *Ecological Monographs* 93, no. 1: e1553.

Healy, T. M., R. S. Brennan, A. Whitehead, and P. M. Schulte. 2018. "Tolerance Traits Related to Climate Change Resilience Are Independent and Polygenic." *Global Change Biology* 24, no. 11: 5348–5360. https://doi.org/10.1111/gcb.14386.

Herrando-Pérez, S., F. Ferri-Yáñez, C. Monasterio, et al. 2019. "Intraspecific Variation in Lizard Heat Tolerance Alters Estimates of Climate Impact." *Journal of Animal Ecology* 88, no. 2: 247–257. https://doi.org/10.1111/1365-2656.12914.

Hoban, S., M. W. Bruford, J. M. da Silva, et al. 2023. "Genetic Diversity Goals and Targets Have Improved, but Remain Insufficient for Clear Implementation of the Post-2020 Global Biodiversity Framework." *Conservation Genetics* 24: 181–191. https://doi.org/10.1007/s10592-022-01492-0.

Hoffmann, A. A., and C. M. Sgro. 2011. "Climate Change and Evolutionary Adaptation." *Nature* 470, no. 7335: 479–485. https://doi.org/10.1038/nature09670.

Hoffmann, A. A., J. G. Sørensen, and V. Loeschcke. 2003. "Adaptation of Drosophila to Temperature Extremes: Bringing Together Quantitative and Molecular Approaches." *Journal of Thermal Biology* 28, no. 3: 175–216. https://doi.org/10.1016/S0306-4565(02)00057-8.

Hoffmann, A. A., S. L. Chown, and S. Clusella-Trullas. 2013. "Upper Thermal Limits in Terrestrial Ectotherms: How Constrained Are They?" *Functional Ecology* 27, no. 4: 934–949. https://doi.org/10.1111/j.1365-2435.2012.02036.x.

Huey, R. B., M. R. Kearney, A. Krockenberger, J. A. M. Holtum, M. Jess, and S. E. Williams. 2012. "Predicting Organismal Vulnerability to Climate Warming: Roles of Behaviour, Physiology and Adaptation." *Philosophical Transactions of the Royal Society, B: Biological Sciences* 367, no. 1596: 1665–1679. https://doi.org/10.1098/rstb.2012.0005.

Höglund, J., A. Laurila, and P. Rödin-Mörch. 2021. "Population Genomics and Wildlife Adaptation in the Face of Climate Change." In *Population Genomics: Wildlife*, edited by P. A. Hohenlohe and O. P. Rajora, 333–355. Cham: Springer International Publishing. https://doi.org/10.1007/13836_2019_69.

Isaak, D. J., M. K. Young, C. H. Luce, et al. 2016. "Slow Climate Velocities of Mountain Streams Portend Their Role as Refugia for Cold-Water Biodiversity." *Proceedings of the National Academy of Sciences of the United States of America* 113, no. 16: 4374–4379. https://doi.org/10.1073/pnas.1522429113.

Isaak, D. J., S. J. Wenger, and M. K. Young. 2017. "Big Biology Meets Microclimatology: Defining Thermal Niches of Ectotherms at Landscape Scales for Conservation Planning." *Ecological Applications* 27, no. 3: 977–990. https://doi.org/10.1002/eap.1501.

Isaak, D. J., S. J. Wenger, E. E. Peterson, et al. 2017. "The NorWeST Summer Stream Temperature Model and Scenarios for the Western U.S.: A Crowd-Sourced Database and New Geospatial Tools Foster a User Community and Predict Broad Climate Warming of Rivers and Streams." *Water Resources Research* 53, no. 11: 9181–9205. https://doi.org/10.1002/2017WR020969.

Jackson, S. A., K. N. Lucas, K. E. Wehrly, and K. M. Alofs. 2024. "Intraspecific Variation in Metabolic Responses of a Cool Water Fish to Increasing Temperatures." *Canadian Journal of Fisheries and Aquatic Sciences* 81: 1621–1633. https://doi.org/10.1139/cjfas-2023-0377.

Jones, P., D. Binns, H.-Y. Chang, et al. 2014. "InterProScan 5: Genome-Scale Protein Function Classification." *Bioinformatics* 30, no. 9: 1236–1240. https://doi.org/10.1093/bioinformatics/btu031.

Kajitani, R., K. Toshimoto, H. Noguchi, et al. 2014. "Efficient De Novo Assembly of Highly Heterozygous Genomes From Whole-Genome Shotgun Short Reads." *Genome Research* 24: 1384–1395. https://doi.org/10.1101/gr.170720.113.

Kardos, M., and G. Luikart. 2021. "The Genetic Architecture of Fitness Drives Population Viability During Rapid Environmental Change." *American Naturalist* 197, no. 5: 511–525. https://doi.org/10.1086/713469.

Kardos, M., G. Luikart, and F. W. Allendorf. 2015. "Measuring Individual Inbreeding in the Age of Genomics: Marker-Based Measures Are Better Than Pedigrees." *Heredity* 115, no. 1: 63–72. https://doi.org/10.1038/hdy.2015.17.

Kawecki, T. J., and D. Ebert. 2004. "Conceptual Issues in Local Adaptation." *Ecology Letters* 7, no. 12: 1225–1241. https://doi.org/10.1111/j.1461-0248.2004.00684.x.

Keilwagen, J., M. Wenk, J. L. Erickson, M. H. Schattat, J. Grau, and F. Hartung. 2016. "Using Intron Position Conservation for Homology-Based Gene Prediction." *Nucleic Acids Research* 44, no. 9: e89. https://doi.org/10.1093/nar/gkw092.

Krueger, F. 2019. "Trim Galore! (0.6.4) [Computer Software]." http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/.

Leach, J. A., and R. D. Moore. 2019. "Empirical Stream Thermal Sensitivities May Underestimate Stream Temperature Response to Climate Warming." *Water Resources Research* 55, no. 7: 5453–5467. https://doi.org/10.1029/2018WR024236.

Li, H. 2011. "A Statistical Framework for SNP Calling, Mutation Discovery, Association Mapping and Population Genetical Parameter Estimation From Sequencing Data." *Bioinformatics* 27, no. 21: 2987–2993. https://doi.org/10.1093/bioinformatics/btr509.

Li, H. 2013. "Aligning Sequence Reads, Clone Sequences and Assembly Contigs With BWA-MEM. *arXiv:1303.3997 [q-Bio]*." http://arxiv.org/abs/1303.3997.

Li, H., and R. Durbin. 2009. "Fast and Accurate Short Read Alignment With Burrows–Wheeler Transform." *Bioinformatics* 25, no. 14: 1754–1760. https://doi.org/10.1093/bioinformatics/btp324.

Li, Y., J. M. Cohen, and J. R. Rohr. 2013. "Review and Synthesis of the Effects of Climate Change on Amphibians." *Integrative Zoology* 8, no. 2: 145–161.

Lotterhos, K. E., and M. C. Whitlock. 2015. "The Relative Power of Genome Scans to Detect Local Adaptation Depends on Sampling Design and Statistical Method." *Molecular Ecology* 24, no. 5: 1031–1046. https://doi.org/10.1111/mec.13100.

Martin, M. 2011. "Cutadapt Removes Adapter Sequences From High-Throughput Sequencing Reads." *EMBnet.Journal* 17, no. 1: 10–12. https://doi.org/10.14806/ej.17.1.200.

McKenna, A., M. Hanna, E. Banks, et al. 2010. "The Genome Analysis Toolkit: A MapReduce Framework for Analyzing Next-Generation DNA Sequencing Data." *Genome Research* 20, no. 9: 1297–1303. https://doi.org/10.1101/gr.107524.110.

Metzger, G., A. Espindola, L. P. Waits, and J. Sullivan. 2015. "Genetic Structure Across Broad Spatial and Temporal Scales: Rocky Mountain Tailed Frogs (*Ascaphus montanus*; Anura: Ascaphidae) in the Inland Temperate Rainforest." *Journal of Heredity* 106, no. 6: 700–710. https://doi.org/10.1093/jhered/esv061.

Mosher, C. M., C. J. Johnson, and B. W. Murray. 2022. "Reduced Genetic Diversity Associated With the Northern Expansion of an Amphibian Species With High Habitat Specialization, *Ascaphus truei*, Resolved Using Two Types of Genetic Markers." *Ecology and Evolution* 12, no. 3: e8716. https://doi.org/10.1002/ece3.8716.

Narum, S. R., N. R. Campbell, K. A. Meyer, M. R. Miller, and R. W. Hardy. 2013. "Thermal Adaptation and Acclimation of Ectotherms From Differing Aquatic Climates." *Molecular Ecology* 22, no. 11: 3090–3097. https://doi.org/10.1111/mec.12240.

Nati, J. J. H., M. B. S. Svendsen, S. Marras, et al. 2021. "Intraspecific Variation in Thermal Tolerance Differs Between Tropical and Temperate Fishes." *Scientific Reports* 11, no. 1: 21272. https://doi.org/10.1038/s41598-021-00695-8.

Nielson, M., K. Lohman, and J. Sullivan. 2001. "Phylogeography of the Tailed Frog (*Ascaphus truei*): Implications for the Biogeography of the Pacific Northwest." *Evolution* 55, no. 1: 147–160. https://doi.org/10.1111/j.0014-3820.2001.tb01280.x.

Oksanen, J., F. G. Blanchet, M. Friendly, et al. 2020. "vegan: Community Ecology Package (Version R Package Version 2.5-7) [Computer Software]." http://CRAN.R-project.org/package=vegan.

Pepin, N., R. S. Bradley, H. F. Diaz, et al. 2015. "Elevation-Dependent Warming in Mountain Regions of the World." *Nature Climate Change* 5, no. 5: 424–430. https://doi.org/10.1038/nclimate2563.

Pigot, A. L., C. Merow, A. Wilson, and C. H. Trisos. 2023. "Abrupt Expansion of Climate Change Risks for Species Globally." *Nature Ecology & Evolution* 7, no. 7: 1060–1071. https://doi.org/10.1038/s41559-023-02070-4.

Polato, N. R., M. M. Gray, B. A. Gill, et al. 2017. "Genetic Diversity and Gene Flow Decline With Elevation in Montane Mayflies." *Heredity* 119, no. 2: 107–116. https://doi.org/10.1038/hdy.2017.23.

Pook, T., M. Mayer, J. Geibel, et al. 2020. "Improving Imputation Quality in BEAGLE for Crop and Livestock Data." *G3: Genes, Genomes, Genetics* 10, no. 1: 177–188. https://doi.org/10.1534/g3.119.400798.

Quinlan, A. R., and I. M. Hall. 2010. "BEDTools: A Flexible Suite of Utilities for Comparing Genomic Features." *Bioinformatics* 26, no. 6: 841–842. https://doi.org/10.1093/bioinformatics/btq033.

R Core Team. 2021. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. http://www.R-project.org.

Rasband, W. 2018. "ImageJ (v. 1.52a) [Computer Software]. U. S. National Institutes of Health." https://imagej.nih.gov/ij/.

Rose, N. H., R. A. Bay, M. K. Morikawa, and S. R. Palumbi. 2018. "Polygenic Evolution Drives Species Divergence and Climate Adaptation in Corals." *Evolution* 72, no. 1: 82–94. https://doi.org/10.1111/evo.13385.

Rödin-Mörch, P., H. Palejowski, M. Cortazar-Chinarro, et al. 2021. "Small-Scale Population Divergence Is Driven by Local Larval Environment in a Temperate Amphibian." *Heredity* 126, no. 2: 279–292. https://doi.org/10.1038/s41437-020-00371-z.

Sandoval-Castillo, J., K. Gates, C. J. Brauer, S. Smith, L. Bernatchez, and L. B. Beheregaray. 2020. "Adaptation of Plasticity to Projected Maximum Temperatures and Across Climatically Defined Bioregions." *Proceedings of the National Academy of Sciences of the United States of America* 117, no. 29: 17112–17121. https://doi.org/10.1073/pnas.1921124117.

Schultz, L. D., M. P. Heck, D. Hockman-Wert, et al. 2017. "Spatial and Temporal Variability in the Effects of Wildfire and Drought on Thermal Habitat for a Desert Trout." *Journal of Arid Environments* 145: 60–68. https://doi.org/10.1016/j.jaridenv.2017.05.008.

Session, A. M., Y. Uno, T. Kwon, et al. 2016. "Genome Evolution in the Allotetraploid Frog *Xenopus laevis*." *Nature* 538, no. 7625: 336–343. https://doi.org/10.1038/nature19840.

Shah, A. A., S. Hotaling, A. B. Lapsansky, et al. 2023. "Warming Undermines Emergence Success in a Threatened Alpine Stonefly: A Multi-Trait Perspective on Vulnerability to Climate Change." *Functional Ecology* 37, no. 4: 1033–1043.

Shriner, D. 2011. "Investigating Population Stratification and Admixture Using Eigenanalysis of Dense Genotypes." *Heredity* 107, no. 5: 413–420. https://doi.org/10.1038/hdy.2011.26.

Smit, A., and R. Hubley. 2015. "RepeatModeler Open-1.0 [Computer Software]." http://www.repeatmasker.org.

Smit, A., R. Hubley, and P. Green. 2015. "RepeatMasker Open-4.0 [Computer Software]." http://www.repeatmasker.org.

Somero, G. N. 2005. "Linking Biogeography to Physiology: Evolutionary and Acclimatory Adjustments of Thermal Limits." *Frontiers in Zoology* 2, no. 1: 1. https://doi.org/10.1186/1742-9994-2-1.

Spear, S. F., and A. Storfer. 2008. "Landscape Genetic Structure of Coastal Tailed Frogs (*Ascaphus truei*) in Protected vs. Managed Forests." *Molecular Ecology* 17, no. 21: 4642–4656. https://doi.org/10.1111/j.1365-294X.2008.03952.x.

Spear, S. F., and A. Storfer. 2010. "Anthropogenic and Natural Disturbance Lead to Differing Patterns of Gene Flow in the Rocky Mountain Tailed Frog, *Ascaphus montanus*." *Biological Conservation* 143, no. 3: 778–786. https://doi.org/10.1016/j.biocon.2009.12.021.

Spear, S. F., C. M. Crisafulli, and A. Storfer. 2012. "Genetic Structure Among Coastal Tailed Frog Populations at Mount St. Helens Is Moderated by Post-Disturbance Management." *Ecological Applications* 22, no. 3: 856–869. https://doi.org/10.1890/11-0627.1.

Sunday, J. M., A. E. Bates, and N. K. Dulvy. 2012. "Thermal Tolerance and the Global Redistribution of Animals." *Nature Climate Change* 2, no. 9: 686–690. https://doi.org/10.1038/nclimate1539.

The UniProt Consortium. 2021. "UniProt: The Universal Protein Knowledgebase in 2021." *Nucleic Acids Research* 49, no. D1: D480–D489. https://doi.org/10.1093/nar/gkaa1100.

Thomas, L., J. N. Underwood, N. H. Rose, et al. 2022. "Spatially Varying Selection Between Habitats Drives Physiological Shifts and Local Adaptation in a Broadcast Spawning Coral on a Remote Atoll in Western Australia." *Science Advances* 8, no. 17: eabl9185. https://doi.org/10.1126/sciadv.abl9185.

Thurman, L. L., B. A. Stein, E. A. Beever, et al. 2020. "Persist in Place or Shift in Space? Evaluating the Adaptive Capacity of Species to Climate Change." *Frontiers in Ecology and the Environment* 18, no. 9: 520–528. https://doi.org/10.1002/fee.2253.

Urban, M. C., J. Swaegers, R. Stoks, et al. 2024. "When and How Can We Predict Adaptive Responses to Climate Change?" *Evolution Letters* 8, no. 1: 172–187. https://doi.org/10.1093/evlett/qrad038.

Valladares, F., S. Matesanz, F. Guilhaumon, et al. 2014. "The Effects of Phenotypic Plasticity and Local Adaptation on Forecasts of Species Range Shifts Under Climate Change." *Ecology Letters* 17, no. 11: 1351–1364. https://doi.org/10.1111/ele.12348.

Wahbe, T. R., and F. L. Bunnell. 2003. "Relations Among Larval Tailed Frogs, Forest Harvesting, Stream Microhabitat, and Site Parameters in Southwestern British Columbia." *Canadian Journal of Forest Research* 33: 1256–1266.

Waldvogel, A.-M., B. Feldmeyer, G. Rolshausen, et al. 2020. "Evolutionary Genomics Can Improve Prediction of Species' Responses to Climate Change." *Evolution Letters* 4, no. 1: 4–18. https://doi.org/10.1002/evl3.154.

Waples, R. S. 2024. "Practical Application of the Linkage Disequilibrium Method for Estimating Contemporary Effective Population Size: A Review." *Molecular Ecology Resources* 24: e13879. https://doi.org/10.1111/1755-0998.13879.

Weisenfeld, N. I., V. Kumar, P. Shah, D. M. Church, and D. B. Jaffe. 2017. "Direct Determination of Diploid Genome Sequences." *Genome Research* 27, no. 5: 757–767. https://doi.org/10.1101/gr.214874.116.

Wiens, J. J., and J. Zelinka. 2024. "How Many Species Will Earth Lose to Climate Change?" *Global Change Biology* 30, no. 1: e17125. https://doi.org/10.1111/gcb.17125.

Yang, W., Y. Qi, and J. Fu. 2016. "Genetic Signals of High-Altitude Adaptation in Amphibians: A Comparative Transcriptome Analysis." *BMC Genetics* 17, no. 1: 134. https://doi.org/10.1186/s12863-016-0440-z.

Ye, C., C. M. Hill, S. Wu, J. Ruan, and Z. Ma (Sam). 2016. "DBG2OLC: Efficient Assembly of Large Genomes Using Long Erroneous Reads of the Third Generation Sequencing Technologies." *Scientific Reports* 6, no. 1: 31900. https://doi.org/10.1038/srep31900.

Zhou, X., and M. Stephens. 2012. "Genome-Wide Efficient Mixed-Model Analysis for Association Studies." *Nature Genetics* 44, no. 7: 821–824. https://doi.org/10.1038/ng.2310.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.