2019

Assessing thermal adaptation using family-based association and FST outlier tests in a threatened trout species

Stephen J. Amish
University Of Montana, stephen.amish@umontana.edu

Omar Ali
University of California, Davis

Mary Peacock
University of Nevada, Reno, mpeacock@unr.edu

Michael Miller
University of California, Davis, micmiller@ucdavis.edu

Morgan Robinson
NOAA Fisheries

See next page for additional authors

Received: 24 October 2017 | Revised: 15 March 2019 | Accepted: 1 April 2019

DOI: 10.1111/mec.15100

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Assessing thermal adaptation using family-based association and F_{ST} outlier tests in a threatened trout species

Stephen J. Amish[1,2] | Omar Ali[3] | Mary Peacock[4] | Michael Miller[3] | Morgan Robinson[5] | Seth Smith[2] | Gordon Luikart[1,2] | Helen Neville[6]

[1]Conservation Genomics Group, Division of Biological Sciences, University of Montana, Missoula, Montana

[2]Flathead Biological Station, University of Montana, Polson, Montana

[3]Department of Animal Science, University of California, Davis, California

[4]Department of Biology, University of Nevada, Reno, Nevada

[5]NOAA Fisheries, Lacey, Washington

[6]Science Program, Trout Unlimited, Boise, Idaho

Correspondence
Helen Neville, Trout Unlimited, Science Program, Boise, ID.
Email: hneville@tu.org

Funding information
National Science Foundation, Grant/Award Number: DEB01258203; National Aeronautics and Space Administration, Grant/Award Number: NNX14AB84G and NNX14AC91G; National Fish and Wildlife Foundation, Grant/Award Number: 35770, -2013, NNX14AC91G, DEB01258203, -2013 and NNX14AB84G

Abstract

Discovering genetic markers associated with phenotypic or ecological characteristics can improve our understanding of adaptation and guide conservation of key evolutionary traits. The Lahontan cutthroat trout (*Oncorhynchus clarkii henshawi*) of the northern Great Basin Desert, USA, demonstrated exceptional tolerance to high temperatures in the desert lakes where it resided historically. This trait is central to a conservation hatchery effort to protect the genetic legacy of the nearly extinct lake ecotype. We genotyped full-sibling families from this conservation broodstock and samples from the only two remaining, thermally distinct, native lake populations at 4,644 new single nucleotide polymorphisms (SNPs). Family-based genome-wide association testing of the broodstock identified nine and 26 SNPs associated with thermal tolerance ($p < 0.05$ and $p < 0.1$), measured in a previous thermal challenge experiment. Genes near the associated SNPs had complex functions related to immunity, growth, metabolism and ion homeostasis. Principal component analysis using the thermotolerance-related SNPs showed unexpected divergence between the conservation broodstock and the native lake populations at these loci. F_{ST} outlier tests on the native lake populations identified 18 loci shared between two or more of the tests, with two SNPs identified by all three tests ($p < 0.01$); none overlapped with loci identified by association testing in the broodstock. A recent history of isolation and the complex genetic and demographic backgrounds of Lahontan cutthroat trout probably limited our ability to find shared thermal tolerance loci. Our study extends the still relatively rare application of genomic tools testing for markers associated with important phenotypic or environmental characteristics in species of conservation concern.

KEYWORDS

body condition, conservation hatchery adaptation, full-sibling families, genome-wide association studies, Lahontan cutthroat trout, lake life history, thermal tolerance

1 | INTRODUCTION

Genomic approaches increasingly offer powerful and novel ways to guide conservation strategies for at-risk species. For example, with the same data set one can now characterize population structure and connectivity using neutral genetic markers, while simultaneously searching for molecular signatures of local adaptation (Helyar et al., 2011; Manel et al., 2016; Narum, Buerkle, Davey, Miller, & Hohenlohe, 2013). Both aspects of variation can then be prioritized jointly to maintain evolutionary distinctiveness and assumed future adaptive potential within conservation units (Funk, McKay, Hohenlohe, & Allendorf, 2012; Kohn, Murphy, Ostrander, & Wayne, 2006; Ouborg, Pertoldi, Loeschcke, Bijlsma, & Hedrick, 2010). Typical approaches for investigating adaptation such as searching for anomalous patterns of differentiation (i.e., outlier tests, Beaumont & Nichols, 1996; Nunes, Beaumont, Butlin, & Paulo, 2011; Storz, 2005) focus on uncovering signatures assumed to reflect selection, but these patterns are rarely verified using other complementary approaches (Haasl & Payseur, 2016). Although certainly useful for exploratory purposes, these efforts often yield a suite of anonymous signals or loci not directly associated with phenotype. Studies that test specifically for genetic selection signatures associated with known phenotypic or ecological characteristics (ecomorphs, distinct life histories or physiological traits) along with annotated reference genomes can be particularly valuable (Allendorf, Hohenlohe, & Luikart, 2010; Angeloni, Wagemaker, Vergeer, & Ouborg, 2012; Manel et al., 2016). Such studies can improve conservation efforts and our understanding of adaptive processes.

Salmonids (salmon, trout and char) are excellent study species for genomic research on molecular adaptation because evidence of local and molecular adaptation is widespread in salmonids (Hendry, 2001; Primmer, 2011; Taylor, 1991). They have had a long evolutionary

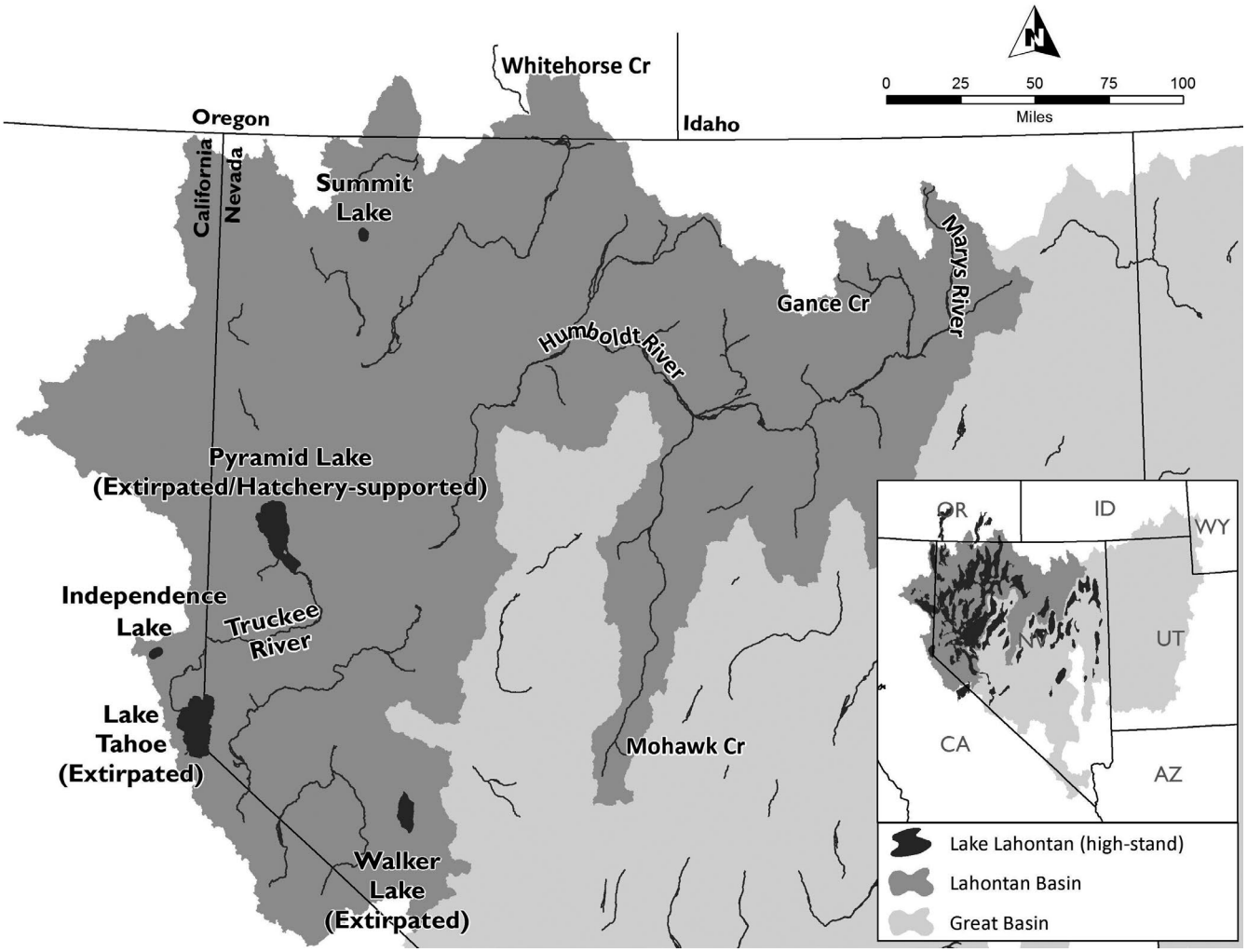

FIGURE 1 Inset map showing the location of historical "Lake Lahontan" (dark grey) within the Lahontan Basin (medium grey) and Great Basin (light grey) in the western United States. Detailed larger map showing the locations of range-wide populations used for single nucleotide polymorphism (SNP) discovery and genotyping along with extirpated lake populations. Summit and Independence lakes are the two remaining native populations representing the lake life history. Stream populations included in the SNP discovery panel included Whitehorse Creek, Gance Creek, Mary's River and Mohawk Creek. The range-wide populations were used in SNP discovery to avoid ascertainment bias, and also to evaluate SNP marker reliability (e.g., confirm F_{ST} values were similar to microsatellites)

history in heterogeneous and dynamic habitats (Waples, Pess, & Beechie, 2008), which has given rise to diverse life history strategies and polymorphisms (Kendall et al., 2014; Moore, Yeake, Peard, Lough, & Beere, 2014; Sloat et al., 2014), and their tetraploid origins have fostered a complex genetic template on which selection can act (Allendorf & Thorgaard, 1984; Conant & Wolfe, 2008). Additionally, salmonids are sensitive to environmental attributes such as temperature (with a narrow thermal niche and temperature-driven growth, Brett, 1971; Ebersole, Liss, & Frissell, 2001; Richter & Kolmes, 2005) and, despite extensive migrations, they exhibit fine-scale population structure maintained by accurate homing to their natal habitat (Dittman & Quinn, 1996; Hendry, Leonetti, & Quinn, 1995; Neville, Isaak, Dunham, Thurow, & Rieman, 2006). Finally, many salmonids are imperiled due to anthropogenic influences (Gustafson et al., 2007; Nehlsen, Williams, & Lichatowich, 1991; Waples, 1995) and are threatened by climate change (Abdul-Aziz, Mantua, & Myers, 2011; Isaak et al., 2012; Wenger et al., 2011), making the need to understand their adaptive potential increasingly pressing (Gustafson et al., 2007; Penaluna et al., 2016; Prince et al., 2017).

Few salmonids have experienced selective pressures as extreme as the Lahontan cutthroat trout (*Oncorhynchus clarkii henshawi*, LCT), for which the dramatic geological changes of the Great Basin (Figure 1) created a dynamic aquatic backdrop for evolutionary change (Madsen, Hershler, & Currey, 2002; Reheis, Sarna-Wojcicki, Reynolds, Repenning, & Mifflin, 2002; Smith & Stearley, 2018). Throughout the mid- to late Pleistocene, the habitat of LCT cycled from networks of headwater streams and smaller lakes during warmer periods to widespread inundation by the massive pluvial Lake Lahontan (Figure 1, inset), which provided LCT with an almost ocean-like environment where it persisted as an apex predator (Behnke, 1992; Grayson, 1993; Reheis et al., 2002). By around 8,000 years ago Lake Lahontan had subsided into several desert mesotrophic "terminal" lakes (lakes with no outflow) in the western region of the LCT range (i.e., Pyramid and Walker lakes, Figure 1); over time these lakes became characterized by increased alkalinity, salinity and summer temperatures compared to higher elevation stream and oligotrophic lake habitat also occupied by Lahontan cutthroat trout in these basins (Behnke, 1992; Grayson, 1993; Reheis et al., 2002). Due to this evolutionary history, lake-form (lacustrine) LCT are believed to have developed unique adaptations to tolerate the conditions in both oligotrophic and desert terminal lakes and the ability to grow to tremendous sizes as the world's largest cutthroat trout (see below, Behnke, 1992; Galat, Post, Keefe, & Bouck, 1985). The resulting phenotypic traits of this lacustrine fish—fast growth rate, large size, and a tolerance for variable temperature and alkalinity—may be especially important when considering long-term persistence of remaining or restored lake populations under global climate change. For instance, LCT in better body condition (bigger, more energy reserves) are assumed to be able to better withstand the higher metabolic costs of higher temperatures (Robinson, Gomez-Raya, Rauw, & Peacock, 2008). Additionally, due to indeterminate growth in salmonids larger females have higher fecundity, which may also contribute a demographic buffer for populations. Consequently, these important fitness traits are a major focus of the development of a conservation hatchery broodstock aimed at conserving the genetic legacy of this now federally threatened lake-form fish.

Characterizing the genes associated with these traits is an important step in understanding local adaptation and assessing evolutionary potential in a rapidly changing climate. Due to the importance of rapid growth in aquaculture, some of the physiological mechanisms and genes associated with growth have been identified and are fairly well understood (particularly those related to growth hormones and their receptors; for a review, see De-Santis & Jerry, 2007). Furthermore, growth rate and thermal tolerance have been shown to be genetically correlated in salmonids (Perry, Martyniuk, Ferguson, & Danzmann, 2005; and LCT, see below). Various studies have demonstrated clinal or geographical genetic relationships with temperature (Hand et al., 2016; Hecht, Matala, Hess, & Narum, 2015; Narum, Campbell, Kozfkay, & Meyer, 2010) and identified an underlying genetic contribution to thermal tolerance (McCairns, Smith, Sasaki, Bernatchez, & Beheregaray, 2016; Perrier, Ferchaud, Sirois, Thibault, & Bernatchez, 2017). Recent studies have also demonstrated complex associations between thermal tolerance and other biological processes in fish. For instance, because of the relationship between temperature and dissolved gases in liquids (Henry's Law) thermal and hypoxia tolerance are functionally associated in freshwater fish (Antilla et al., 2013; Garvin, Thorgaard, & Narum, 2015; but see Healy, Brennan, Whitehead, & Schulte, 2018 for a recent exception in a saltwater example). Reduced aerobic performance is a major cause of thermal stress and is predicted to be the first process driving a decline in fitness under global warming (Eliason et al., 2011; Pörtner & Knust, 2007; Sumaila, Cheung, Lam, Pauly, & Herrick, 2011). An association between temperature and immunity (Alcorn, Murray, & Pascho, 2002), such as through Major Histocompatibility Complex genes, has also been found in salmonids, suggesting a temperature-mediated selective force from pathogens and parasites (Croisetière, Bernatchez, & Belhumeura, 2010; Dionne, Miller, Dodson, Caron, & Bernatchez, 2007).

We use several approaches to uncover candidate adaptive loci related to the unique environmental tolerance of the lacustrine form of Lahontan cutthroat trout. Although genome-wide association studies (GWAS) and F_{ST} outliers between populations are commonly used in wild populations, they are rarely combined (but see Brennan et al., 2018; Jansen et al., 2017; Pfeifer et al., 2018) and related to genomic variation in measured phenotypes from families. Here, we capitalized on a previous study that tested individual thermal tolerance and quantified the heritability of thermal tolerance and body condition in full-sibling families from a conservation hatchery broodstock (Robinson et al., 2008; note that although adaptation to high total dissolved solids is also of interest in LCT conservation, we did not focus on that phenotype here as it was not measured in this previous work). Using genetic material from these same families and a panel of 4,644 newly discovered single nucleotide polymorphisms (SNPs), we used genome-wide family-based association testing to evaluate linkages between genomic variation and thermal tolerance. Next, we made F_{ST} outlier contrasts between samples from the only

two remaining native lake populations of LCT, which reside in lakes with substantial differences in temperature. We predicted that potential candidate SNP markers related to thermal tolerance in the hatchery broodstock would also be outliers in F_{ST} contrasts between these natural lake populations. Finally, we used de novo assembled sequence contigs and a recent reference genome for rainbow trout (*Oncorhynchus mykiss*) to explore the functions of putative candidate genes near our RADtag SNP loci. Our study provides confirmation for some of the assumed unique genetic characteristics of the conservation hatchery broodstock and remaining native populations of lacustrine LCT and furthers our understanding of genetic variation associated with thermal tolerance in salmonids more generally.

2 | METHODS

2.1 | Population histories and habitats

We begin with an overview of LCT that were found in the large lacustrine habitats of the Truckee River watershed, including Pyramid Lake, NV (Figure 1). Pyramid Lake is important in the evolution of lake-form LCT as the largest remnant of pluvial Lake Lahontan that had maintained its historical fish diversity from the Pleistocene (Behnke, 1992), and as the inferred origin of the current conservation broodstock central to this study (see below). It was here that LCT garnered its reputation not only for a unique physiological tolerance for both oligotrophic and saline mesotrophic lake environments but also for its ability as an apex predator to grow to unusually large size: early explorers referred to LCT in this watershed as "salmon trout" (Frémont, 1846), and Pyramid Lake generated the world record cutthroat trout in 1925 (18.6 kg, Behnke, 1992). Following decades of impacts from logging, overharvest, introduction of non-native salmonids and barriers preventing access to fluvial spawning habitat, the large, lacustrine LCT were largely extirpated by the early 1940s (LCT has since disappeared from 99% of its historical lake habitat and has been federally listed since 1970: Federal Registry Notice: Service, 1970, pp. 16,047–16,048). The genetic legacy of this lineage was thus largely thought to have been lost. However, a population of LCT discovered in the 1970s in a small stream in the Pilot Peak range of western Utah (outside the fish's historical distribution) was suspected to be a pre-extirpation transplant from Pyramid Lake. This suspicion was subsequently confirmed based on meristic commonalities (Hickman & Behnke, 1979) and a close genetic relationship with early 20th century museum samples from Pyramid Lake and its headwater system (Lake Tahoe and the Truckee River; Peacock, Hekkala, Kirchoff, & Heki, 2017).

A conservation hatchery programme was initiated from this Utah stream population in 1995 by the United States Fish and Wildlife Service (USFWS) with the goal of restoring the genetic characteristics underlying the lacustrine life history and unique physiological and metabolic adaptations of Pyramid Lake LCT. Subsequent tests of full-sibling family groups from the "Pilot Peak" hatchery strain under a thermal challenge confirmed high among-family variation in temperature tolerance, a strong correlation between temperature tolerance and body condition (0.84) and estimated the heritability of both traits (H = 0.21 and 0.28, respectively, in Table 2; Robinson et al., 2008). Efforts to recover LCT in Pyramid and other extirpated western lakes now focus largely on this conservation hatchery strain, assumed to represent the genetic adaptations of historical LCT from Pyramid Lake and to retain a possible metabolic buffer that may improve the fish's ability to persist in human-altered habitats (Robinson et al., 2008).

In addition to a focus on the Pilot Peak strain broodstock we drew inference from the only two remaining native LCT lake populations (Independence and Summit Lakes, Figure 1, which together represent <1% of the historical lake habitat for LCT; USFWS, 2009). As noted above, Pyramid Lake, at an elevation of 1,157 m, is a warmer water environment, where temperatures during summer stratification in the metalimnion range between 12 and 22°C (the upper range approaching the thermal tolerance limits for most trout; Behnke, 1992; Heredia & Budy, 2018). Its major spawning habitat and tributary, the Truckee River, is highly impacted and flow-managed, making it difficult to characterize the thermal regime spawning LCT would have experienced before extirpation, although modern summer temperatures in the river range between 20 and 26°C (Dickerson & Vinyard, 1999). The two smaller lakes mentioned above have contrasting thermal environments. Independence Lake is a contemporaneously isolated alpine lake, found high in the Truckee River watershed of the Sierra Nevada Mountains at an elevation of 2,133 m. Independence Creek, the only spawning tributary for Independence Lake, has a mean August temperature of 11.1°C. Genetic analyses suggest very little gene flow occurred historically between the LCT in Independence Lake and other populations in the Truckee River watershed. Summit Lake is an isolated terminal lake found in the high-desert steppe of northern Nevada at an elevation of 1,780 m with a mean August temperature of 14.9°C in its major spawning tributary (NorWest stream temperature data, http://www.fs.fed.us/rm/boise/AWAE/projects/NorWeST.html; Figure 1). We contrast LCT from these lakes assuming Summit Lake LCT may express some of the same adaptations to their thermal environment as Pilot Peak strain LCT putatively do, such that potentially adaptive loci uncovered in Pilot Peak fish may also be shared in contrasts between Summit Lake LCT from the colder alpine environment of Independence Lake.

2.2 | Library preparation and sequencing

Our samples included previously extracted DNA from the same individuals from the seven Pilot Peak strain families (herein referred to as PPP, or Pilot Peak Population) assessed by Robinson et al. (2008) and samples from the two natural lakes. We also included samples from several pairs of populations from contrasting (warm and cold) stream environments (Table 1, Figure 1) to enable comprehensive marker discovery and, initially, to provide broader testing of LCT across the range (but see F_{ST} outlier test between natural lake populations below, detailing that we dropped these samples ultimately from further analyses). Extracted DNA from 230 individuals was assessed using a Nanodrop spectrophotometer and double-stranded

TABLE 1 List of Lahontan cutthroat trout samples used in the RADseq single nucleotide polymorphism discovery panel, with their abbreviation codes, ecotype (or population) and sample size

Sample collection	Code	Ecotype/ population	*n*
Gance Creek	GACR	Stream	21
Mary's River, Main Stem	MSMR	Stream	15
Mary's River, West	WEMR	Stream	22
Mohawk Creek	MOCR	Stream	21
Willow Whitehorse Creek	WWCR	Stream	22
Independence Lake	INDL	Lake	21
Summit Lake	SUML	Lake	21
Pilot Peak (Pyramid lake) Family 1	PPP1	Hatchery	5
Pilot Peak (Pyramid lake) Family 2	PPP2	Hatchery	5
Pilot Peak (Pyramid lake) Family 3	PPP3	Hatchery	6
Pilot Peak (Pyramid lake) Family 4	PPP4	Hatchery	5
Pilot Peak (Pyramid lake) Family 5	PPP5	Hatchery	18
Pilot Peak (Pyramid lake) Family 6	PPP6	Hatchery	5
Pilot Peak (Pyramid lake) Family 7	PPP7	Hatchery	16

DNA concentration was estimated using Picogreen assays. DNA from 203 individuals was then selected based on DNA quality and quantity. Restriction site-associated DNA (RAD) sequencing libraries were prepared following Miller et al. (2012) using Sbf1, and normalized libraries containing up to 32 samples each (a conservative number of samples for sequencing to ensure adequate read depth) were sequenced on an Illumina HiSeq 2000 device using 100-bp paired-end reads.

2.3 | Bioinformatics

Sequence data processing, RAD locus discovery using STACKS and de novo contig assembly followed Hohenlohe et al. (2013) except where specified. Only de novo contigs mapping to one location in the rainbow trout genome were selected for our reference to exclude RAD loci from paralogous regions. We used BWA-MEM to map paired-end reads to the de novo reference. SAMTOOLS (Li, 2011; Li et al., 2009) was then used to genotype samples, using default settings. To remove low-quality genotypes from the data set, we used VCFTOOLS (Danecek et al., 2011) to filter individuals missing data at >45% of loci and loci missing >40% of their data. We chose these thresholds as a balance between ensuring data quality and retaining informative diversity among individuals or loci potentially under selection during the filtering process (Buerkle & Gompert, 2013; Huang & Knowles, 2016). To account for potential sequencing errors, singletons were removed by filtering loci with a minor allele frequency <0.5%. Loci out of Hardy–Weinberg equilibrium in more than two of seven native populations (PPP samples were not included) or with gametic disequilibrium $r^2 > 0.8$ in more than three of sesven populations were removed.

We inspected the results of our SNP filtering by examining a histogram of population F_{IS} and observed heterozygosity values to verify that SNPs discovered using the de novo reference were not paralogues or false variants due to sequencing error, clustering errors or assembly errors (Hohenlohe, Amish, Catchen, Allendorf, & Luikart, 2011). We also generated a principal components analysis (PCA) and estimated pairwise F_{ST} values to compare with previous estimates using microsatellite data (Peacock & Kirchoff, 2007). To generate unbiased estimates of population allele frequencies, we selected two individuals at random from each full-sibling family in the PPP collection, and a single random SNP from each de novo contig. Willow Whitehorse and Mohawk Creeks samples (WWCR and MOCR, Table 1) were excluded from the range-wide PCA because they were strong outliers (as also observed with microsatellites, Peacock & Kirchoff, 2007; Peacock, Neville, & Finger, 2018) and obscured the population structure among the remaining lake and stream populations (but see Figure S1). The final filtered VCF file was converted to Genetix (Belkhir, Borsa, & Chikhi, 2004) format using PGDSPIDER version 2.0.9.0 (Lischer & Excoffier, 2012). We conducted the PCA in R, using functions from the ADEGENET (Jombart, 2008) and ADE4 (Dray & Dufour, 2007) packages. Allele frequencies were centred on a mean of zero and missing data were set equivalent to the mean. We estimated pairwise F_{ST} values using GENEPOP (Rousset, 2008) for comparison with previously generated microsatellite data for some of these same populations.

2.4 | Thermal challenge genome-wide association test

To characterize the genetic variation underlying thermal tolerance in LCT, we evaluated genomic association with phenotypic variation in the seven full-sibling hatchery families using PLINK version 1.9 (http://pngu.mgh.harvard.edu/purcell/plink/). We applied the transmission disequilibrium test (TDT) to perform a family-based test of genome-wide association with individual survival time under increasing water temperatures as quantified by Robinson et al. (2008; although also quantified by Robinson et al. we did not analyse body condition as a separate phenotype here because of its demonstrated high correlation with temperature tolerance in this broodstock). In PLINK, the TDT is implemented with the QFAM function. QFAM performs a simple linear regression of phenotype on genotype after a permutation process corrects for family structure. It applies the between/within model used by Fulker, Cherny, Sham, and Hewitt (1999) and Abecasis, Cardon, and Cookson (2000), Abecasis, Cookson, and Cardon (2000), dividing genotypes into between-family and within-family components. The components are then permuted separately, and the association analysis is performed. We applied the test to both the between- and within-family components (qfam-total) using 100,000 permutations. We used a corrected *p*-value of 0.05 after applying the multiple testing correction for association studies $M_{eff\,G}$ (Gao, Starmer, & Martin, 2008).

2.5 | F_{ST} outlier test between natural lake populations

We hypothesized that LCT from pairs of regional populations (originally two pairs of streams, one pair of lakes) with contrasting thermal

regimes might share multiple F_{ST} outliers, which could then be compared to loci associated with thermal tolerance in the PPP families. Preliminary tests with BAYESCAN revealed that no F_{ST} outlier loci were detected in the two pairs of stream populations, so they were dropped from further analysis. The remaining work was done only on the Independence and Summit lake samples. Here we used F_{ST} outlier tests to search for loci with unusually high differentiation compared to the background level observed across the genome, signifying loci which deviate from neutrality and are likely to be under selection (Luikart, England, Tallmon, Jordan, & Taberlet, 2003; Storz, 2005). Because the various available outlier tests differ in their analytical approach, assumptions and resulting error (Lotterhos & Whitlock, 2014, 2015; Narum & Hess, 2011; De Villemereuil, Frichot, Bazin, Francois, & Gaggiotti, 2014) we evaluated three commonly used approaches for comparison with results from family-based association testing of the Pilot Peak broodstock.

We first used the Bayesian approach in BAYESCAN 2.0, which estimates the probability that each locus is subject to selection by decomposing F_{ST} coefficients into a population-specific component (beta) shared by all loci and a locus-specific component (alpha) shared by the populations (Foll & Gaggiotti, 2008). We ran 20 pilot runs (5,000 iterations in length) followed by a burn-in of 50,000 iterations and a final run of 100,000 iterations. Second, we used the coalescent FDIST2 approach of Beaumont and Nichols (1996) as implemented in LOSITAN (Antao, Lopes, Lopes, Beja-Pereira, & Luikart, 2008). LOSITAN used 50,000 iterations, neutral mean F_{ST}, forced mean F_{ST}, the infinite alleles mutation model, 99.5% confidence intervals and a conservative false discovery rate (FDR) of 0.05. A locus was considered a putative outlier under positive selection if simulated F_{ST} values were less than the true sample F_{ST} in >99.5% of simulations (P-Sim >0.995). Finally, BAYPASS 2.1 (Gautier, 2015) was also used to search for a signature of adaptive divergence among the two remaining lake populations based on the *XtX* differentiation measure (Günther & Coop, 2013) using default parameters. This statistic represents an SNP-specific "F_{ST}" corrected by the scaled covariance among population allele frequencies. We then used a pseudo-observed data (POD) analysis procedure which simulates allele count data based on the observed scaled population covariance matrix to estimate a decision criterion (i.e., a 5% threshold *XtX* value) for selection (Gautier, 2015). Observed mean *XtX* values were normalized based on the POD threshold so that 1% values were equal to 1 for the F_{ST} outlier test comparison.

2.6 | Annotation and further analysis of significant SNPs from TDT and F_{ST} outlier tests

Two sources of data were used to annotate the SNPs uncovered by the family-based TDT and F_{ST} outlier tests for possible biological function, with the expectation that many of these would be related to thermal and/or anoxia tolerance, immunity and growth. Gnomon gene predictions (Sourorov, Tatusover, & Lipman, 2004) were drawn from the recently annotated NCBI Omyk 1.0 reference genome sequence (GenBank RefSeq assembly accession GCF_002163495.1) with overlapping mapping coordinates extracted for each SNP. In addition, de novo contigs for the filtered SNP data were aligned to *Salmo salar* (Atlantic salmon) and *O. mykiss* sequences in the NCBI Nucleotide Collection (nr/nt) database using the BLASTN algorithm and default alignment parameters (Expected threshold = 10, word size = 11, Match Score = 2, Mismatch score = −3, Gap Cost = Existence: 5, Extension: 2). To remove BLASTN alignments with low sequence similarity an *e*-value threshold of 1e-13 was used. For each sequence, the Gnomon annotation was used as the primary source except where the mapping CIGAR string suggested poor alignment, in which case the BLASTN hit with the lowest *e*-value was selected as the primary annotation. Gene names were extracted from either the Gnomon or NCBI annotations and queried against the UniProt Protein knowledgebase (UniProtKB) to further illuminate their biological function.

We ran a PCA using only the loci identified as related to thermal tolerance by the TDT (i.e., potentially adaptive loci) to explore whether the wild lacustrine populations from different thermal environments aligned with the broodstock families with the highest and lowest temperature tolerance (PPP5 vs. PPP7 as indicated by survival under a thermal challenge in Robinson et al., 2008); alignment would suggest that similar genes or regions of the genome were associated with thermal tolerance in both the wild populations and the broodstock families. We hypothesized that the warmer lake (Summit) would sort with the best-performing, most temperature-tolerant family (PPP5). We used a relaxed *p*-value (corrected $p < 0.1$) in the TDT to identify a larger set of potentially informative loci for this exploratory analysis. Based on clear differentiation between PPP and the lake forms at these potentially adaptive loci, we tested within this potentially adaptive locus set for loci with extreme discriminatory contributions (i.e., outliers) using the PCADAPT R package (Luu, Basin, & Blum, 2017). Outlier SNPs were identified based on the multidimensional Mahalanobis distance (computed from the mean and covariance matrix between axis *z*-scores), with *p*-values scaled using the genomic inflation factor, for each principal component (Luu et al., 2017). The *p*-values were then converted to *q*-values in the R package QVALUE (Bass, Dabney, & Robinson, 2015) using an FDR of 0.1.

Finally, we compiled all significant loci from the TDT analysis of the PPP broodstock and the native lake F_{ST} outlier tests to look for SNPs common to the two general approaches. Loci significant for both types of test would include those associated with thermal tolerance in the family-based genome-wide association test which also have significantly different allele frequencies in the two native lake populations, and would provide additional confirmation of parallel adaptive pathways (Perrier, Bourret, Kent, & Bernatchez, 2013). To identify candidate loci for further testing, we used a *p*-value of 0.05 or lower for each test.

3 | RESULTS

3.1 | RAD loci discovery

Each of the 203 individuals sequenced had an average of 1.9 million reads after removal of PCR duplicates and quality filtering. FASTQC

histograms revealed average base quality scores >30 up to the 94th base in the 100-bp reads. For discovery of RAD loci, we chose data from a panel of 28 individuals from seven range-wide populations, represented by >1,000,000 reads each. After requiring that each locus be genotyped in at least one individual per population, 30,248 RAD loci were chosen for de novo reference assembly. Using the paired-reads from our discovery panel samples, CAP3 assembled consensus sequence contigs for 28,433 loci (94%). The assembled consensus sequences (contigs) were based on an average of 418 read-pairs and had a mean length of 338 nucleotides. The consensus sequences for 22,225 de novo contigs (85%) mapped to the rainbow trout genome. After choosing contigs mapping only to a single location in the rainbow trout genome, 19,324 de novo contigs were identified for the reference assembly.

3.2 | Genotyping with de novo reference, SNP filtering and population structure

After quality filtering, a total of 4,644 variable SNPs was identified and genotyped in 203 focal individuals from range-wide population samples. The number of SNPs discovered is similar to other conservation studies, and unsurprising given the species' history

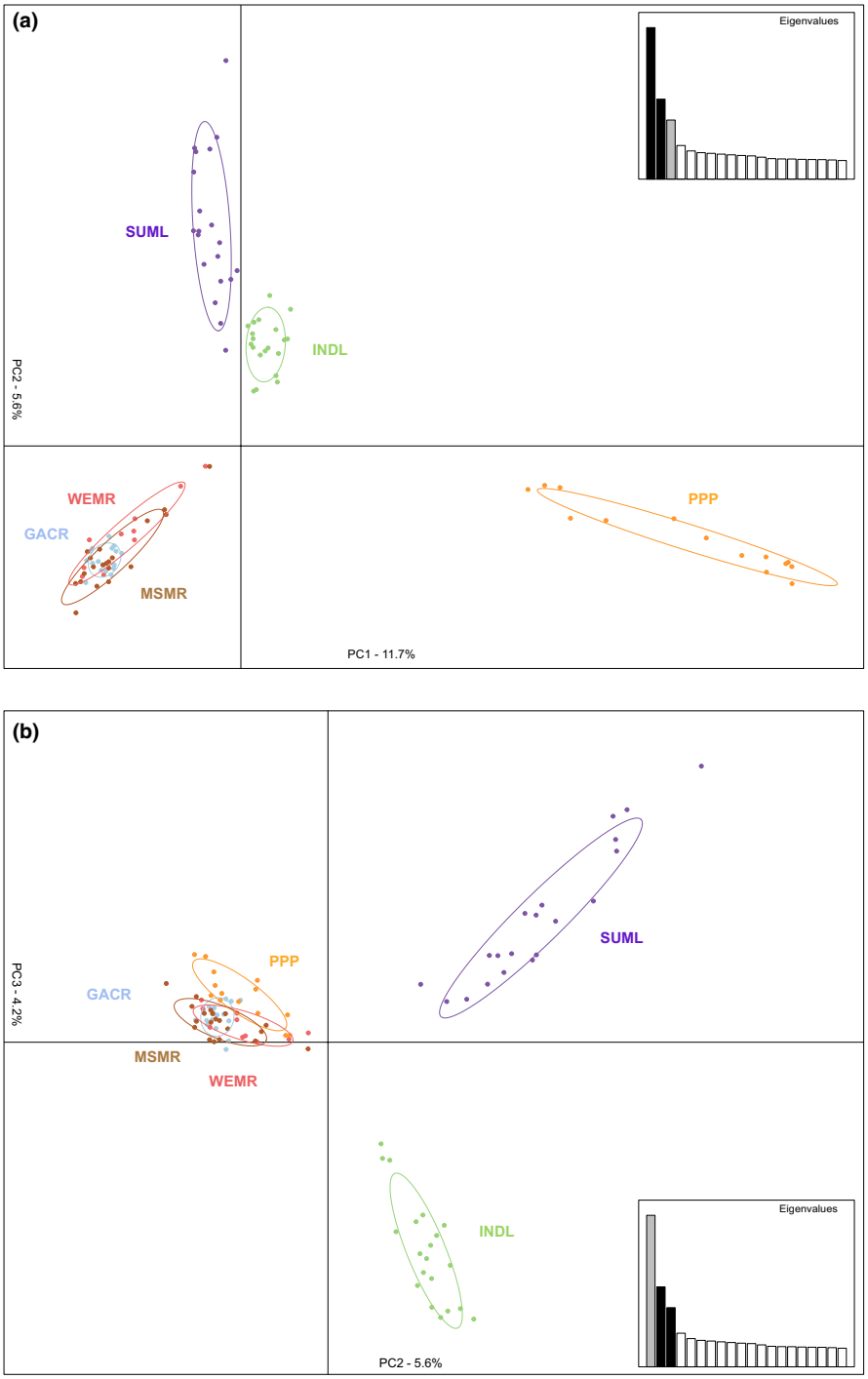

FIGURE 2 (a) Principal components analysis (PCA) of range-wide populations with PC1 (*x*-axis; 11.65% variation) showing the divergence of the Pilot Peak Population broodstock (PPP) from both native lake populations (Summit Lake, SUML; and Independence Lake, INDL) and the fluvial stream populations (GACR, WEMR, MSMR). PC2 (*y*-axis; 5.60%) further differentiates the native lake populations from all others. (b) PC2 is now the *x*-axis, and PC3 (*y*-axis; 4.19%) differentiates the native lake populations (SUML and INDL) from each other. GACR points plotted in light blue are obscured in both plots by the MSMR and WMR points and labels. The inset bar graph shows the Eigenvalue loadings for the axes relevant to each graph [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Single nucleotide polymorphisms (SNPs) found to be significant outliers in the family-based transmission disequilibrium association test when using thermal tolerance as the individual phenotype variable

	RBT genome location				qFAM association test		Annotation Information				
RAD contig	Chromosome	SNP position	Ref	Alt	*p*	Rank	Gene name	Key biological functions	Sequence name	NCBI reference	*e*-Value
chr115114_2	omy02	16,036,303	G	A	0.0014	15	SNRNP48	Small nuclear ribonucleoprotein particle part of the minor U11/12-dependent spliceosome protein assembly process	U11/U12 small nuclear ribonucleoprotein 48 kDa protein-like	XM_021558683.1	NA
chr37010_3	omy03	31,282,860	G	T	0.0006	7	MAN1A1	Protein glycosylation, modulates cellular function including cell adhesion	Mannosyl-oligosaccharide 1,2-alpha-mannosidase IA-like	XM_021582754.1	NA
chr95001_2	omy03	35,627,535	C	T	0.0003	2	MACF1	Protein which plays a role in cross-linking actin to other cytoskeletal proteins and also binds to microtubules, plays a key role in wound healing and epidermal cell migration	Microtubule-actin cross-linking factor 1-like	XM_021596128.1	NA
chr24140_1	omy04	43,290,110	T	G	0.0006	7	SLC39A7	Cellular zinc ion homeostasis and transmembrane zinc importer	*Oncorhynchus mykiss* zinc transporter SLC39A7.B	HM208332.1	2.00E-167
chr64659_1	omy05	49,456,674	G	A	0.0001	1	NA	NA	NA	NA	NA
chr124336_3	omy06	53,688,751	C	A	0.0004	4	DHCR7	Production of cholesterol by reduction, which is part of steroid biosynthesis; involved in multicellular organism growth	7-Dehydrocholesterol reductase-like	XM_021607019.1	NA
chr83645_1	omy06	69,205,763	T	A	0.0016	19	TRIM66	Negative regulation of transcription	Tripartite motif-containing protein 66	XM_021607619.1	NA
chr24829_2	omy07	34,792,378	C	A	0.0015	16	NA	NA	NA	NA	NA
chr3491_1	omy07	35,686,088	C	G	0.0013	14	NA	NA	NA	NA	NA
chr117331_1	omy07	42,727,262	T	G	0.0016	18	CADPS	Calcium-binding protein involved in exocytosis of vesicles filled with neurotransmitters and neuropeptides	Calcium-dependent secretion activator 1	XM_021611130.1	NA
chr11163_2	omy08	47,511,064	A	G	0.0018	25	SLC35A3	Carbohydrate transport, defective SLC35A3 causes arthrogryposis, mental retardation, and seizures	UDP-*N*-acetylglucosamine transporter-like	XM_021612860.1	NA
chr65235_3	omy08	63,361,025	G	T	0.0017	23	NA	NA	NA	NA	NA
chr101068_2	omy09	14,757,100	A	G	0.0016	19	TYRP1	Pigment biosynthesis	5,6-Dihydroxyindole-2-carboxylic acid oxidase-like	XM_021615090.1	NA
chr61206_2	omy10	31,494,493	G	T	0.0005	6	RNF121	Endoplasmic reticulum unfolded protein response, ubiquitin-dependent protein degradation	RING finger protein 121	XM_021618231.1	NA

(Continues)

TABLE 2 (Continued)

	RBT genome location				qFAM association test		Annotation Information				
RAD contig	Chromosome	SNP position	Ref	Alt	*p*	Rank	Gene name	Key biological functions	Sequence name	NCBI reference	*e*-Value
chr68988_2	omy11	6,714,681	C	A	0.0003	2	PIEZO1	Cation transport, cellular response to mechanical stimulus, positive regulation of integrin and cell adhesion, regulation of membrane potential	*Salmo salar* piezo-type mechanosensitive ion channel component 1-like	XM_014126607.1	5.00E-30
chr82655_1	omy17	65,019,506	A	C	0.0011	10	KCND3	Membrane repolarization, potassium ion transport, regulation of heart rate and transmembrane ion transport	Potassium voltage-gated channel subfamily D member 3	XM_021569984.1	NA
chr61824_4	omy18	3,244,519	T	C	0.0012	11	IGFBP-5A	Regulation of cell growth	*Oncorhynchus mykiss* insulin-like growth factor binding protein 5 paralogue A	gi|461489514	6.02E-51
chr98443_1	omy18	33,019,570	C	T	0.0004	4	PVRL	Immune response, cell adhesion, spermatid development, viron attachment to host cell	Poliovirus receptor-like	XM_021571983.1	NA
chr90531_1	omy22	18,361,279	C	T	0.0012	11	SLC27A2	Fatty acid and lipid metabolism	*Salmo salar* very long-chain acyl-CoA synthetase-like	XM_014186043.1	2.00E-91
chr28213_2	omy23	8,024,047	T	G	0.0015	16	PSD	Phospholipid binding, signal transduction	PH and SEC7 domain-containing protein 1-like	XM_021580347.1	NA
chr41701_1	omy24	10,346,432	T	C	0.0018	25	MARK4	Serine/threonine-protein kinase, plays a role in cell cycle, cell division, mitosis, and in energy homeostasis by regulating satiety and metabolic rate	MAP/microtubule affinity-regulating kinase 4-like	XM_021582205.1	NA
chr63694_1	omyUn2	49,143,536	T	A	0.0012	11	CALCOCO1	Probably functions as a component linking cellular metabolism with other core functions including protein synthesis and degradation, calcium signaling and cell growth	*Salmo salar* coiled-coil transcriptional coactivator b (Kiaa1536)	gb|EU025717.1	3.00E-83
chr4049_2	omyUn2	63,082,685	T	C	0.0017	22	NA	NA	NA	NA	NA
chr117307_1	omyUn3	38,309,621	A	C	0.0016	19	NA	NA	NA	NA	NA
chr36636_1	omyUn3	48,789,667	T	G	0.0017	23	NA	NA	NA	NA	NA
chr15995_1	omyUn3	81,004,640	C	T	0.0008	9	CACNA2D4	Regulates calcium current density and activation/inactivation kinetics of the calcium channel	*Salmo salar* voltage-dependent calcium channel subunit alpha-2/delta-4-like	XM_014187683.1	4.00E-69

Note: Mapping location in the Omyk1.0 genome and annotation from NCBI BLAST database (if *e*-value is shown), the corrected *p*-value from the association test and the rank of the *p*-value among all annotated SNPs are shown; the top nine ranked SNPs were significant at a table-wide *p*-value of 0.05 (*p* value corrected for multiple tests <0.00094) while all 26 SNPs were significant at a corrected *p*-value of 0.10 (*p* value corrected for multiple tests <0.00189). The gene name and key biological functions of the gene are given, the reference sequence and *e*-value when appropriate

and recent population dynamics, including population fragmentation and declines (see Narum et al., 2013: table 1). Summary plots of within-population variation demonstrated the effectiveness of the filters for removing potential paralogous loci, errors during the RAD locus discovery process or de novo contig assembly (see Hohenlohe et al., 2011 for general approach). A histogram of F_{IS} values for loci confirmed the distribution was centred near zero, with most values between −0.5 and 0.5. In addition, a histogram of observed heterozygosity values showed a single peak near zero (i.e., there was no indication of a bimodal distribution which might signal the presence of homeologues; Hohenlohe et al., 2011).

The range-wide PCA showed similar patterns of population-level structuring as previous microsatellite-based studies of native LCT, supporting the reliability of our SNP genotyping (Peacock & Kirchoff, 2007; Peacock et al., 2018). The first axis strongly separated the PPP hatchery broodstock from stream-form populations found in more eastern basin sites, with the two native lacustrine populations (Independence and Summit) falling in-between (Figure 2). The second and third axes further separated the two native lacustrine populations from the stream populations and the PPP broodstock. In addition, pairwise F_{ST} estimates showed the expected levels (0.02–0.6) of divergence based on the history and fragmented nature of the remaining populations, in agreement with microsatellite data (F_{ST} = 0.01–0.55; Peacock & Kirchoff, 2007).

3.3 | Thermal challenge genome-wide association test

The family-based TDT evaluated individuals in each family for SNPs associated with thermal tolerance. Nine SNPs were significant at a *p*-value of 0.05 (critical value corrected for multiple association tests <0.00094) and 26 SNPs were significant at a *p*-value of 0.10 (critical value <0.00189; Table 2). Of the nine SNPs identified at the highest stringency, two were mapped to chromosome omy03, with a single SNP mapped to chromosomes omy04, omy05, omy06, omy10, omy11, omy18 and omyUn (Figure 3). Of the 26 outlier SNPs identified under more lenient stringency ($p < 0.1$), 19 were annotated using Gnomon annotation from the rainbow trout genome and BLASTN results with an *e*-value less than 1e-13 (Table 2). Gnomon and BLASTN annotations were almost always in agreement, except where BLASTN results had low similarity to the query sequence or where the contig sequence aligned poorly read mapping quality score (MAPQ < 20) with multiple insertions or deletions to the rainbow trout genome.

Key biological functions and genes associated with the 19 annotated loci included immune response, metabolic pathways or processes, growth, protein assembly, cardiac function, calcium and ion homeostasis, and anaerobic resiliency (Table 2, see Discussion for further detail). As specific examples, the available annotations for the SNPs with the four lowest *p*-values (adjusted critical $p < 0.0005$) included: MACF1, a protein involved in cell structure, cell migration and wound healing; PIEZO1, involved in cation transport, regulation of membrane potential and cellular response to mechanical stimulus; DHCR7, an enzyme involved in the production of cholesterol, steroid biosynthesis and growth; and polio virus receptor-like (PVRL), involved in immune response and spermatid development (Table 2).

The PCA using 26 SNPs identified by the TDT (using a corrected *p*-value of 0.10) showed only slight differentiation between the PPP individuals with the highest and the lowest survival time in the thermal challenge (PPPH and PPPL, respectively; Figure 4). PPP individuals were strongly differentiated from the wild lake populations along the first axis, which explained 17.9% of the variation (Figure 4). No additional differentiation between the PPP individuals and the wild lake populations was evident on the second axis. Additionally, the Summit and Independence lake populations were only slightly differentiated from each other and did not align with the PPP groups as predicted (i.e., Summit Lake did not align with PPPH, the most

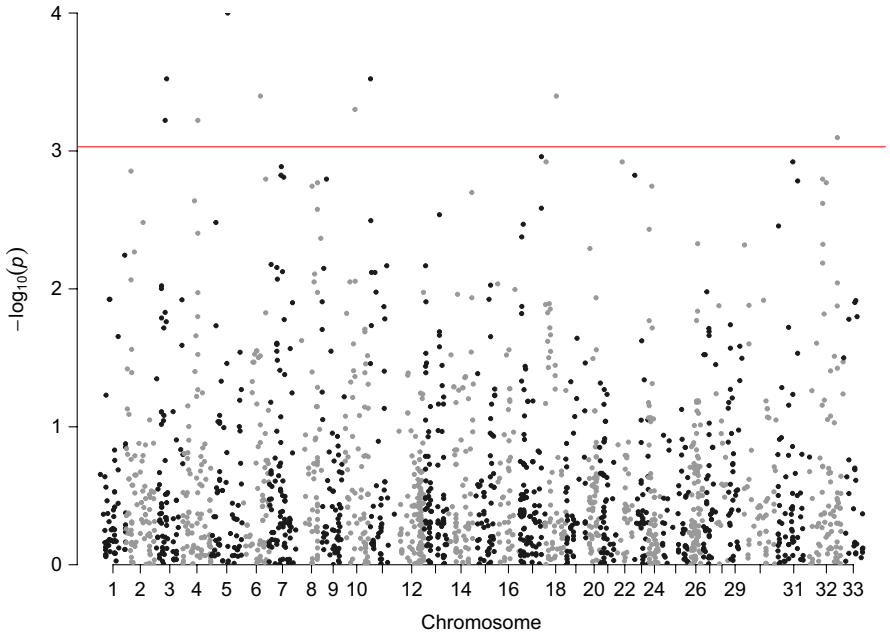

FIGURE 3 Manhattan plot of the family-based transmission disequilibrium association test (TDT) empirical *p* values after correction for multiple tests. Nine significant outliers were mapped to the rainbow trout genome and are shown above the red line (corrected $p = 0.05$). Loci mapping to sequences on the unordered chromosome were plotted as four additional chromosomes (30–33). Annotation for the single nucleotide polymorphisms (SNPs) is given in Table 2 [Colour figure can be viewed at wileyonlinelibrary.com]

temperature-tolerant individuals; Figure 4). Given the observed allele frequency differences between both lake populations and the PPP individuals, we plotted the loadings for axis 1 and identified five SNPs that were significant outliers (FDR 0.1; *q*-val < 0.00002). Three SNPs (*omy07_35686088*, *omy07_34792378* and *omy05_49456674*) had no available annotation based on mapping and BLASTN results. SNP *omy18_33019570* (*r* = 0.458; *q*-val = 3.18 E-09) was located in the annotated region for the PVRL gene involved in immune response, cell adhesion and spermatid development. SNP *omy06_69205763* (*r* = 0.271; *q*-val = 1.5 E-03) was located in the annotated region for the TRIMM66 gene involved in the negative regulation of transcription (Table 2).

3.4 | F_{ST} outlier tests between natural lake populations

The BAYESCAN algorithm identified four SNPs as significant outliers under putative divergent selection with high posterior probabilities and an FDR of 0.05 (Figure S2). Sequences containing these SNPs were mapped to the rainbow trout genome, with three of them located in chromosome omy05 (*omy5_14122662*, *omy5_76852970*, *omy5_77686103*), and one of them localized to chromosome omy12 (*omy12_51549483*; Table 3). LOSITAN identified 42 loci putatively under directional selection (*p* = 0.01). These high F_{ST} outliers included the four noted above as significant in BAYESCAN (Table 2; Figure S2). Four pairs of high F_{ST} outliers <1 million bp apart were observed on four different chromosomes. These included two high F_{ST} outliers detected in the BAYESCAN analysis on chromosome 5 (*omy05_76852970*, *omy05_77686103*) and a pair of SNPs on each of chromosome 18 (*omy18_27629677*, *omy18_27689738*), chromosome 21 (*omy21_47126944*, *omy21_47637540*) and chromosome 23 (*omy23_45754879*, *omy23_46297342*). Forty-one SNPs were significant outliers (0.01 threshold) in the BAYPASS *XtX* distribution (*XtX* > 6.69, *SD* = 1) using the POD analysis (Figure S3). These loci included SNP *omy05_76852970*, which was identified as a high F_{ST} outlier by both BAYESCAN and LOSITAN, along with 14 additional SNPs that were also identified as high F_{ST} outliers with LOSITAN (Table 3). Sequences containing the high *XtX* outlier SNPs were mapped to the rainbow trout genome, with 18 of 29 chromosomes represented and three or more SNPs located in chromosomes omy02, omy05, omy08, omy09, omy10, omy13, omy20 and omyUn. Among the remaining SNPs, a wide array of genes and proteins were represented. We constrained further detailed investigation (below) to the subset of loci shared among multiple tests.

3.5 | Shared SNPs from TDT and F_{ST} outlier tests

We tested if the same loci associated with thermal tolerance in the PPP broodstock were also F_{ST} outliers in the wild lake populations with different thermal regimes. No SNPs that were significant in the TDT of the PPP families were also identified as significant in any of the F_{ST} outlier tests between the native populations. However, one pair of SNPs, where each SNP was identified by one of the two tests, mapped to the same chromosome and were *c*. 115 kb apart (*omy08_63245323*, *omy08_63361025*). Based on BLAST annotation, SNP *omy08_63361025* was similar to the PHTF2 gene, involved in transcription regulation (Table 3). No annotation was found for SNP *omy08_632453* (Table 2). The remaining loci were all >1 Mb apart, with most being >25 Mb apart.

Eighteen loci were shared among two or three of the F_{ST} outlier tests (Table 3). SNP *omy05_76852970* was highly significant in all three F_{ST} outlier tests, but no annotated genes were found in

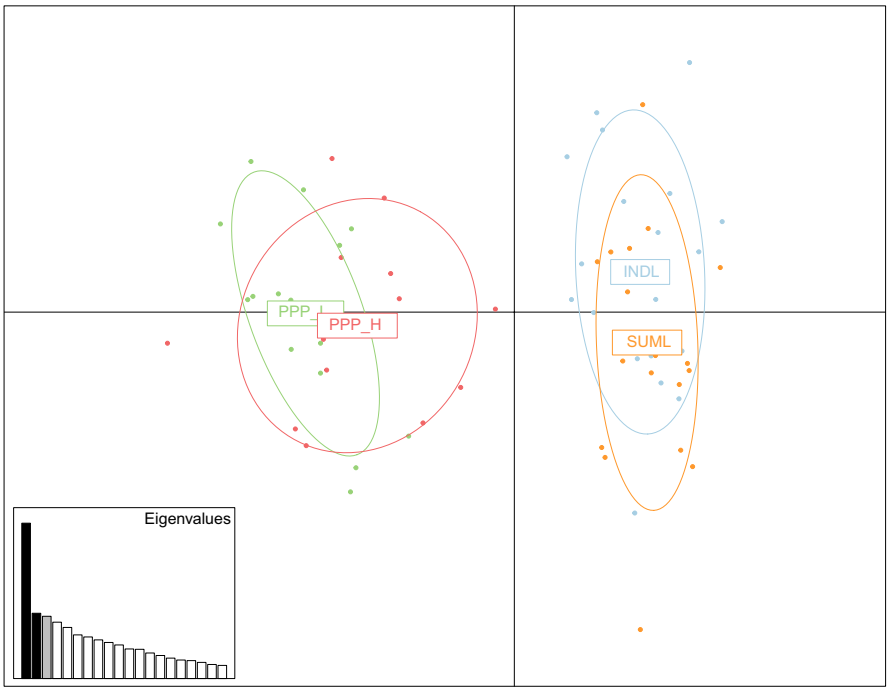

FIGURE 4 Principal components analysis (PCA) using 26 single nucleotide polymorphisms (SNPs) from the transmission disequilibrium test (TDT) (more liberal corrected *p* < 0.10) to examine whether similar axes of genetic variation explain differences between the thermal challenge families and wild populations with different thermal regimes. The Pilot Peak individuals most tolerant to high temperatures (PPPH) and the least tolerant family (PPPL) are plotted along with the samples from a "warm" wild population from Summit Lake and a "cold" wild population from Independence Lake. The first axis explains 17.9% of the genetic variation and separates the native lake populations from the Pilot Peak individuals. Axis 2 explains little additional variation among the groups [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 SNP loci significant ($p < 0.05$) for three F_{ST} outlier tests (BAYESCAN, BAYPASS and LOSITAN) between the wild lake populations

	RBT genome location		Alleles		BAYESCAN			BAYPASS			LOSITAN		TDT				
RAD contig	chr	SNP pos	Ref	Alt	$\log_{10}$ PO	1 − *Q*-val	F_{ST}	Mean *XtX*	std *XtX*	*SD XtX*	F_{ST}	*p*	*p*	Gene	NCBI ref.	Key biological functions	*e*-value
137738_1	omy02	10,204,766	A	G	−0.202	0.724	0.322	8.87	1.33	4.07	0.81	0.001	NA	ASCC3	XM_021625959.1	Cell proliferation, DNA repair and DNA duplex unwinding	NA
34094_1	omy02	60,624,004	T	C	−0.934	0.882	0.22	8.77	1.31	2.94	0.74	0.002	0.484	PPP6R1	XM_014148458.1	Transcription regulation	NA
28463_1	omy03	2,426,896	C	T	−0.952	0.115	0.225	6.88	1.03	2.61	0.44	0.000	NA	NUB1	XM_014181350.1	Protein modification, immune response	9.00E-128
90031_1	omy05	14,122,662	T	C	1.433	0.014	0.53	1.59	0.24	1.46	0.97	0.000	NA	ZFYVE16	110,523,341	Endosome membrane regulation, scaffold protein in the transforming growth factor-beta signalling pathway	2.46E-40
99232_2	omy05	76,852,970	G	T	2.744	0.002	0.62	18.59	2.78	4.68	1.00	0.000	0.469	NA	NA	NA	NA
108911_1	omy05	77,686,103	T	G	2.355	0.003	0.61	2.14	0.32	2.06	0.97	0.000	NA	PHLPP1	110,523,018	Akt and PKC signal regulation, development and function of T cells	NA
110651_1	omy08	63,245,323	T	A	−0.852	0.334	0.231	8.30	1.24	3.07	0.71	0.002	0.052	PHTF2	XM_014166697.1	May play a role in transcription regulation	1.00E-31
3583_1	omy10	43,616,089	C	A	−0.856	0.312	0.232	8.09	1.21	3.35	0.67	0.004	NA	NRXN2	XM_021617221.1	Neuron cell–cell adhesion, signal transduction	NA
38416_1	omy11	14,526,200	T	A	−0.895	0.149	0.228	7.00	1.05	2.98	0.62	0.003	0.699	MLLT10	XM_021620388.1	Probably involved in transcriptional regulation	NA
18761_4	omy11	70,564,322	T	C	−0.950	0.115	0.225	11.55	1.73	3.98	0.67	0.004	NA	CYLD	XM_014123306.1	Involved in cell cycle and innate immune response	5E-17
89753_1	omy12	51,549,483	A	G	0.851	0.041	0.49	1.18	0.18	1.30	0.92	0.000	0.487	ABLIM3	110,537,795	Regulation mitochondrial protein targeting, RNA polymerase II transcription regulation, DNA transcription	NA
5172_1	omy16	31,162,568	C	T	−0.873	0.238	0.232	8.25	1.23	2.99	0.62	0.001	NA	MORN1	XM_021565656.1	May play a role in cell structure	NA

(Continues)

TABLE 3 (Continued)

RAD contig	RBT genome location		Alleles		BAYESCAN			BAYPASS			LOSITAN		TDT				
	chr	SNP pos	Ref	Alt	$\log_{10}$ PO	1 − *Q*-val	F_{ST}	Mean *XtX*	std *XtX*	*SD XtX*	F_{ST}	*p*	*p*	Gene	NCBI ref.	Key biological functions	*e*-value
36817_1	omy19	27,278,953	C	T	−0.864	0.295	0.231	7.61	1.14	3.33	0.68	0.003	0.500	FLRT2	XM_021573897.1	Developmental protein involved in cell adhesion, axon guidance and heart morphogenesis	NA
34895_1	omy20	2,358,493	G	T	−0.233	0.692	0.315	12.22	1.83	3.51	0.77	0.001	NA	GAD1	XM_014140923.1	Glutamate catabolic process, locomotor exploratory behaviour, cerebral palsy	9.00E-84
106030_2	omy25	61,403,799	A	G	−0.843	0.353	0.234	8.02	1.20	3.08	0.58	0.001	NA	MNAT1	XM_021584958.1	Cell cycle, transcription, transcription regulation, DNA repair, adult heart morphogenesis	NA
58856_1	omy28	10,003,294	C	T	−0.973	0.110	0.224	6.78	1.01	2.79	0.62	0.003	NA	LOC110508496	XM_021589051.1	Unknown	NA
54881_2	omyUn1	62,999,612	C	T	−0.917	0.124	0.226	7.07	1.06	2.77	0.44	0.000	NA	NA	NA	NA	NA
37607_1	omyUn3	39,021,533	A	T	−1.003	0.103	0.218	7.40	1.11	3.32	0.48	0.002	NA	PCDH15	XM_014179327.1	Calcium-dependent protein involved in cell adhesion and hearing	2.00E-22

Note: The mapping location for each SNP to the rainbow trout genome, the gene name and the biological function of the NCBI omyV6 genome or BLASTN annotation are listed. In addition, the details for the F_{ST} outlier tests and TDT *p*-value are shown, including the $\log_{10}$ of the posterior odds, the Q value, F_{ST} (adjusted by BAYESCAN for population structure), the BAYPASS population structure corrected normalized mean *XtX* (*SD* XtX 0.01 critical threshold = 1), and *p*-value for the TDT association test before correcting for multiple tests.
PVRL, polio virus receptor-like; SNP, single nucleotide polymorphism; TDT, transmission disequilibrium test.

the rainbow trout genome within 5 Mb and no similar sequences were identified with BLASTN. A second SNP <1 million bp away, *omy05_77686103*, was a significant outlier in both the BAYPASS and LOSITAN tests. SNP *omy05_77686103* is located in the PHLPP1-like gene of the rainbow trout genome, associated with Akt and protein kinase C network signal regulation and the function of T-cells. Fifteen significant outliers were shared between the LOSITAN and BAYPASS tests ($p < 0.01$), with the remaining three outliers detected using both BAYESCAN and LOSITAN. Functions were broadly associated with cellular growth and development, transcription, immune response and heart morphogenesis (see key biological functions in Table 3). Outlier loci were located throughout the genome, but multiple SNPs were significant on chromosomes omy02 (two), omy05 (three) and omy11 (two).

4 | DISCUSSION

Our study adds to the growing application of genomic tools testing for adaptive markers associated with important traits in nonmodel organisms and natural populations, including organisms of conservation concern. Here we had the still-rare opportunity to move beyond an anonymous genome scan approach by associating genetic variation in known siblings (families) with a measured and heritable phenotype of adaptive importance. Ideally, we would have been able to relate the outlier loci uncovered in the natural lake populations directly to measured thermotolerance. Several recent studies linking information from selection scans to measured traits in natural populations and integrating outlier and association tests have uncovered important evolutionary findings (Brennan et al., 2018; McGirr & Martin, 2016; Pfeifer et al., 2018). Our more challenging situation, where small population size and threatened status made it impossible to measure the phenotype of interest, is common in species of conservation concern. Despite this, our contrast between the native lake populations uncovered a suite of potentially adaptive loci associated with intuitive functions, which merit further study. More clearly, our family-based analysis identified a diverse suite of nine loci associated significantly with individual temperature resiliency, a trait of interest in restoring the lake ecotype of a desert trout that is now largely extinct in the wild.

4.1 | Other studies of thermal adaption

Although complex genomic architecture probably underlies thermal tolerance and body size (or condition), other studies using various breeding and genetic techniques have discovered genetic associations. Thermal tolerance quantitative trait loci (QTL) have been identified in hatchery rainbow trout using backcross families, where two QTL associated with upper temperature tolerance accounted for between 9% and 13% of the phenotypic variance (Jackson et al., 1998); similar findings were reported for upper temperature tolerance and body condition in Arctic char (Reid, Szanto, Glebe, Danzmann, & Ferguson, 2005; Somorjai, Danzmann, & Ferguson, 2003). Everett and Seeb (2014) used a line of haploid Chinook salmon to map loci which were then related to QTL for temperature tolerance and body size—up to 64% of the observed variation in thermal tolerance was explained by QTL on three linkage groups. Overlapping (shared) QTL related to thermotolerance, weight, length and body condition have been observed in sockeye salmon, and may be due QTL hotspots (Larson et al., 2015).

Thermal adaptation in salmonid fishes has been shown to involve many biological processes, with a relatively clear understanding of direct physiological effects of temperature on growth, immune response and hypoxia. Growth rate in salmonids is directly linked to temperature (Brett, 1971; Brett, Shelbourn, & Shoop, 1969), and immune responses have been shown to be temperature-related as well (Alcorn et al., 2002; Holt, Amandi, Rohovec, & Fryer, 1989; Sanders, Pilcher, & Fryer, 1978). Aerobic pathways are also important in response to thermal stress (Gamperl et al., 2002; Garvin et al., 2015; Portner, 2002; but see Healy et al., 2018), and energy conservation may also be an important response to hypoxia (e.g., ion channel arrest, Hochachka, 1986). For example, variation in temperature tolerance in Atlantic salmon has been shown to be associated with hypoxia tolerance, ventricle size and myoglobin levels (Anttila et al., 2013). Transcriptomic research in salmon has uncovered a similar set of complex biological processes associated with thermal adaptation. Stressful anoxic conditions and high water temperatures have been associated with the up-regulation of genes involved in cell redox homeostasis, inflammation/immunity, calcium and ion homeostasis, and metabolism (Jeffries et al., 2012; Jeffries, Hinch, Sierocinski, Pavlidis, & Miller, 2013; Narum & Campbell, 2015; Tomalty et al., 2015).

4.2 | Family-based testing for thermal tolerance in LCT

Our findings echo the complex biological processes detected in previous work on thermal tolerance in salmonids. The SNPs we found associated with thermal tolerance phenotypes have functions including immune response, metabolism, growth, cardiac function, calcium and ion homeostasis, and anaerobic resiliency (Table 2). The varied biological associations uncovered here may be explained in part by physiological efforts to balance and maintain energy during hypoxia. For example, metabolic arrest and stabilization of membrane ion and electrical potential are effective strategies for extending tolerance to hypoxia (Hochachka, 1986). We found thermal tolerance was putatively associated with six SNPs involved in cation transport and energy homeostasis or metabolism (PIEZO1, DHCR7, IGFBP-5A, CALCOCO1, SLC27A2, MARK4). The link between PIEZO1, calcium influx and ATP release may be another biological pathway important for energy conservation through its regulation of microvascular tone (Cinar et al., 2015). In addition, the discovery of SNPs associated with growth and metabolism support the genetic correlation observed by Robinson et al. (2008) between body condition and thermal tolerance (0.84) in these LCT families and suggest a possible physiological link to their historically large size. However, additional

future research (e.g., with more loci) is needed to better understand the pathways and genetic basis of these thermal tolerance phenotypes in Pyramid Lake LCT.

4.3 | F_{ST} tests versus family-based tests

Our F_{ST} outlier tests discovered distinct sets of outlier loci differentiating the native lake populations with contrasting thermal regimes (and potentially other ecological, demographic and genetic differences, see below). Outlier loci had functions putatively related to processes such as cellular development, proliferation and growth, transcription, immune response, and heart morphogenesis (Table 3). A subset of these were significant across multiple tests and models, with BAYPASS and LOSITAN sharing the most overlap. Among the shared outliers, one SNP (*omy05_76852970*) was the most significant locus for all three methods, and a neighbouring SNP <1 million bp away (*omy05_77686103*; PHLPP1) was highly significant using the BAYPASS and LOSITAN tests. Detection of multiple outlier SNPs in the same region, using multiple tests, provides some evidence of differential selection or local adaptation between the two remaining wild lake populations.

Despite the fact that the native lake environments clearly differ in temperature, we cannot relate these outlier patterns directly to temperature tolerance; assumedly LCT in these waters have been shaped by other differences in environment and population histories as well (see below). However, if thermotolerance was under strong selection in the lake populations, and if the PPP and native lake LCT followed similar genetic pathways in evolving thermal adaptations, we expected to find some overlap between the loci associated with the thermotolerance phenotype measured in the hatchery broodstock and the outlier loci identified in contrasts of the two wild lake populations. In the end the TDT and F_{ST} outlier tests showed no shared loci. One pair of SNPs (with each identified by one of the two tests) only *c*. 115 kb apart did map to chromosome omy08, potentially suggesting a common region associated with the thermotolerance phenotype and differentiation in the wild populations. While we lack linkage disequilibrium (LD) estimates for LCT because no linkage map or genome assembly exists for LCT, this distance is in keeping with distances encompassing significant LD in other fish. In outbred natural populations of rainbow trout, LD typically spans thousands of bases (<10 kb, Chen, Farrell, Matala, Hoffman, & Narum, 2018), and average LD in natural European eel (*Anguilla Anguilla*) populations suggest a similar distance, with LD = 10–20 kb (Pujolar et al., 2014). Genome-wide LD analysis on an inbred hatchery line of rainbow trout revealed much stronger LD ($r^2 \geq 0.25$) spanning on average over 1 Mb across the genome (Vallejo et al., 2018). The close mapping distance of the two significant markers observed here presents a target for future research.

Our PCA ordination using significant SNPs associated with temperature tolerance in the families found little evidence of differentiation between the native lake populations at these loci. Instead, we observed five thermotolerance loci identified in the PPP broodstock which had significantly *different* frequencies in the native populations. Two of these five SNPs are related to immune response. One immune-related SNP mapped to the PVRL gene on the rainbow trout genome, which in humans is involved in the humoral immune response (Maier et al., 2007). A second mapped to the tripartite motif-containing protein 66 (TRIM66) gene involved in innate immunity to viruses in humans, and silencing of TRIM66 suppresses cell proliferation, invasion and migration in some cancer cells (Ma, Dai, Zhang, & Zhao, 2017). Previous work has found adaptation to hatchery conditions involved genes associated with wound healing, immunity and metabolism (Christie, Marine, Fox, French, & Blouin, 2016). This suggests a need for future research into the genetic differences between the remaining wild populations of LCT and the hatchery broodstock being managed for recovery of the lake-form ecotype.

4.4 | Why were different loci discovered by different tests?

The high level of genetic divergence, different genetic backgrounds, and varied demographic and genetic histories all probably influenced our ability to detect selection signatures in these natural and propagated populations (Haasl & Payseur, 2016; Jensen, Foll, & Bernatchez, 2016). Summit Lake is a small habitat that has been isolated for over 8,000 years (Curry & Melhorn, 1990). Independence Lake is within the same watershed as Pyramid Lake, but is relatively distinct based on these SNPs and other neutral genetic markers (e.g., microsatellites; Peacock et al., 2018). Recruitment into the Independence Lake LCT population has been decimated by the presence of non-native brook trout (*Salvelinus confluentus*) over the last few decades (Scoppettone, Rissler, Shea, & Somer, 2012), which probably contributed to drift (given low N_e) in this population. The amount of genetic variability retained in the modern Pilot Peak broodstock is also certainly reduced compared to the historical Pyramid Lake population. Transplanted to a small mountain tributary for decades and later subsampled to initiate the current broodstock, it has been bottlenecked twice recently. In addition, hatchery propagation can dramatically affect genetic variation and gene expression (Araki, Cooper, & Blouin, 2007; Christie et al., 2016; Christie, Marinea, French, & Blouin, 2011). The full magnitude of divergence between the PPP broodstock and the wild populations was not apparent before this study. Small effective population size increases genetic drift and may have produced different alleles and genetic backgrounds in the wild versus hatchery populations (Messer & Petrov, 2013; Perrier et al., 2017), limiting our ability to find shared loci across populations and tests. The limited number of outliers detected by BAYESCAN in the two wild lake populations (and in previous analyses of stream populations) probably reflects this. Furthermore, for polygenic traits (see below), divergent genetic backgrounds will limit discovery of functional (or associated) loci depending on the effect size of the loci involved.

Additionally, low LD or varying LD structure (blocks) along chromosomes can cause different loci to be discovered when using different tests and populations. For instance, a thermal tolerance gene

(or genome region) might not have been detected if no SNP markers were in LD with a functional gene of major effects (Johnston et al., 2014). LD along chromosomes is likely to be high in both the hatchery and the wild lake populations due to their relatively small effective size and bottleneck history, and so we probably have reasonable power to detect genes with major effects if they exist. Nonetheless, our 4,000 SNP loci might not provide high power in all genome regions for detecting associations or F_{ST} outlier loci. Future studies with additional loci and with links mapping might detect more functional gene associations. Increasing the number of loci has led to the discovery of genes in related studies (e.g., compare Johnston et al., 2014 and Barson et al., 2015).

Selection or association signals can also be missed when many loci of small effect are responsible for a polygenic trait. This may be a common source of genetic variation underlying many traits (Harrisson, Pavlova, Telonis-Scott, & Sunnucks, 2014; Stephan, 2016; De Villemereuil et al., 2014), although recent evidence also suggests the influence of loci of large effect can be important in salmonid evolution (Hess, Zendt, Matala, & Narum, 2016; Prince et al., 2017). In many cases, uncertainty about the architecture of adaptation and the trajectory of future selective pressures make it prudent to consider the importance of genome-wide variation in driving evolutionary potential and population persistence (Harrisson et al., 2014; Miller & Hedrick, 1991; Vrijenhoek & Leberg, 1991). Thus, while our study uncovers temperature tolerance-related variants associated with complex physiological and biological attributes such as growth, anaerobic resiliency and immunity in the PPP broodstock, and other variants potentially under selection in the native lake forms, any active management targeting specific genes to conserve adaptive diversity (Funk et al., 2012; Hemmer-Hansen, Therkildsen, Meldrup, & Nielsen, 2013; Hudson, Vonlanthen, & Seehausen, 2014) should consider impacts to genome-wide variation that may be vital for future adaptive responses in a rapidly changing environment.

5 | CONCLUSIONS

Studies combining experimental research and field studies of natural populations are rare but valuable. Our use of experimental family-based association testing, multiple outlier loci tests in natural populations, and functional gene annotation allowed us to identify candidate genes related to immune response, growth, and thermal and anaerobic resiliency. This provides an initial step toward discovery of potentially adaptive variation in LCT (Jensen et al., 2016; Manel et al., 2016; Rellstab, Gugerli, Eckert, Hancock, & Holderegger, 2015). Importantly, the loci we identified are not likely to be representative of the entire genetic architecture for thermal tolerance and other important phenotypic traits because, for example, limited LD might lead to low statistical power to detect associations and outliers, as is true for any studies in nonmodel species. Future studies with high-density mapped loci and known LD are needed to ensure high power to detect functional or adaptive genes. Nonetheless, our work identified SNP loci and gene regions to guide future research and complements a growing body of studies on the genetic basis of fitness traits and adaptive variation in trout and other nonmodel organisms.

ACKNOWLEDGEMENTS

This work was funded by a National Fish and Wildlife Foundation grant (35770) to H.N. at Trout Unlimited, with minor support to H.N. from the Ecological Forecasting programme of NASA-Roses-2013 (NNX14AC91G). G.L. and S.A. were partially supported by a grant from NSF (DEB01258203) and by a separate grant from NASA-Roses-2013 (NNX14AB84G). We thank the Summit Lake Paiute Tribe (Summit Lake), the Nature Conservancy and California Department of Fish and Wildlife (Independence Lake), and the US Fish and Wildlife Service's Lahontan National Fish Hatchery Complex (Pilot Peak broodstock) for original genetic samples provided to M.P. and M.R. Thanks to Ryan Kovach and Marty Kardos and the anonymous reviewers for providing comments and suggestions on a previous version of the manuscript.

AUTHOR CONTRIBUTION

H.N., M.P., S.J.A. and G.L. designed the study, with samples provided by M.P., M.R. and H.N. M.M. and O.A. conducted sequencing laboratory work and contributed to initial data analysis, while S.J.A. performed bioinformatics, final data analyses and annotation research. S.S. performed annotation research and data analysis. H.N. and S.J.A. drafted the manuscript, with edits and revisions from M.P., M.R., M.M., S.S. and G.L.

DATA AVAILABILITY STATEMENT

Sampling locations, a list of the RAD loci used in the analysis, as well as individual phenotype data from the Robinson et al. (2008) study of thermal tolerance are posted on Dryad. Raw data are deposited in NCBI SRA repository under BioProject PRJNA539940.

ORCID

Helen Neville https://orcid.org/0000-0001-9848-4565

REFERENCES

Abdul-Aziz, O. I., Mantua, N. J., & Myers, K. W. (2011). Potential climate change impacts on thermal habitats of Pacific salmon (*Oncorhynchus* spp.) in the North Pacific Ocean and adjacent seas. *Canadian Journal of Fisheries and Aquatic Sciences*, *68*, 1660–1680.

Abecasis, G., Cardon, L., & Cookson, W. (2000). A general test of association for quantitative traits in nuclear families. *The American Journal of Human Genetics*, *66*, 279–292. https://doi.org/10.1086/302698

Abecasis, G. R., Cookson, W. O., & Cardon, L. R. (2000). Pedigree tests of transmission disequilibrium. *European Journal of Human Genetics*, *8*, 545–551. https://doi.org/10.1038/sj.ejhg.5200494

Alcorn, S. W., Murray, A. L., & Pascho, R. J. (2002). Effects of rearing temperature on immune functions in sockeye salmon (*Oncorhynchus nerka*). *Fish & Shellfish Immunology*, *12*, 303–334. https://doi.org/10.1006/fsim.2001.0373

Allendorf, F. W., Hohenlohe, P. A., & Luikart, G. (2010). Genomics and the future of conservation genetics. *Nature Reviews Genetics*, *11*, 696–709. https://doi.org/10.1038/nrg2844

Allendorf, F. W., & Thorgaard, G. H. (1984). Tetraploidy and the evolution of salmonid fishes. In B. J. Turner (Ed.), *Evolutionary genetics of fishes: Monographs in evolutionary biology* (pp. 53). New York, NY: Springer.

Angeloni, F., Wagemaker, N., Vergeer, P., & Ouborg, J. (2012). Genomic toolboxes for conservation biologists. *Evolutionary Applications*, *5*, 130–143. https://doi.org/10.1111/j.1752-4571.2011.00217.x

Antao, T., Lopes, A., Lopes, R. J., Beja-Pereira, A., & Luikart, G. (2008). LOSITAN: A workbench to detect molecular adaptation based on a Fst-outlier method. *BMC Bioinformatics*, *9*, 323.

Anttila, K., Dhillon, R. S., Boulding, E. G., Farrell, A. P., Glebe, B. D., Elliott, J. A. K., … Schulte, P. M. (2013). Variation in temperature tolerance among families of Atlantic salmon (*Salmo salar*) is associated with hypoxia tolerance, ventricle size and myoglobin level. *Journal of Experimental Biology*, *216*, 1183–1190. https://doi.org/10.1242/jeb.080556

Araki, H., Cooper, B., & Blouin, M. S. (2007). Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. *Science*, *318*, 100–103. https://doi.org/10.1126/science.1145621

Barson, N. J., Aykanat, T., Hindar, K., Baranski, M., Bolstad, G. H., Fiske, P., … Primmer, C. R. (2015). Sex-dependent dominance at a single locus maintains variation in age at maturity in salmon. *Nature*, *528*, 405. https://doi.org/10.1038/nature16062

Bass, A. J., Dabney, A., & Robinson, D. (2015). *qvalue: Q-value estimation for false discovery rate control. R package*.Retrieved from https://rdrr.io/bioc/qvalue/

Beaumont, M. A., & Nichols, R. A. (1996). Evaluating loci for use in the genetic analysis of population structure. *Proceedings of the Royal Society London Series B*, *263*, 1619–1626.

Behnke, R. J. (1992). *Native Trout of Western North America*. Bethesda, MD: American Fisheries Society.

Belkhir, K., Borsa, P., & Chikhi, L. (2004). *GENETIX, logiciel sous Windows™ pour la génétique des populations. Laboratoire génome, populations, interactions CNRS UMR 5000*. Montpellier, France: Université de Montpellier II.

Brennan, R. S., Healy, T. M., Bryant, H. J., La, M. V., Schulte, P. M., & Whitehead, A. (2018). Integrative population and physiological genomics reveals mechanisms of adaptation in killifish. *Molecular Biology and Evolution*, *35*, 2639–2653. https://doi.org/10.1093/molbev/msy154

Brett, J. R. (1971). Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). *American Zoologist*, *11*, 99–113.

Brett, J. R., Shelbourn, J. E., & Shoop, C. T. (1969). Growth rate and body composition of fingerling sockeye salmon, *Oncorhynchus nerka*, in relation to temperature and ration size. *Journal of the Fisheries Research Board of Canada*, *26*, 2363–2394.

Buerkle, A. C., & Gompert, Z. (2013). Population genomics based on low coverage sequencing: How low should we go? *Molecular Ecology*, *22*, 3028–3035. https://doi.org/10.1111/mec.12105

Chen, Z., Farrell, A. P., Matala, A., Hoffman, N., & Narum, S. R. (2018). Physiological and genomic signatures of evolutionary thermal adaptation in redband trout from extreme climates. *Evolutionary Applications*, *11*, 1686–1699. https://doi.org/10.1111/eva.12672

Christie, M. R., Marine, M. L., Fox, S. E., French, R. A., & Blouin, M. S. (2016). A single generation of domestication heritably alters the expression of hundreds of genes. *Nature Communications*, *7*, 10676. https://doi.org/10.1038/ncomms10676

Christie, M. R., Marinea, M. L., French, R. A., & Blouin, M. S. (2011). Genetic adaptation to captivity can occur in a single generation. *Proceedings of the National Academy of Sciences USA*, *109*, 238–242. https://doi.org/10.1073/pnas.1111073109

Cinar, E., Zhou, S., DeCourcey, J., Wang, Y., Waugh, R. E., & Wan, J. (2015). Piezo1 regulates mechanotransductive release of ATP from human RBCs. *Proceedings of the National Academy of Sciences USA*, *112*, 11783–11788. https://doi.org/10.1073/pnas.1507309112

Conant, G. C., & Wolfe, K. H. (2008). Turning a hobby into a job: How duplicated genes find new functions. *Nature Reviews. Genetics*, *9*, 938.

Croisetière, S., Bernatchez, L., & Belhumeura,, (2010). Temperature and length-dependent modulation of the MH class II geneexpression in brook charr (*Salvelinus fontinalis*) by a cis-acting minisatellite. *Molecular Immunology*, *47*, 1817–1829.

Curry, B. B., & Melhorn, W. N. (1990). Summit Lake landslide and geomorphic history of Summit Lake Basin, Northwestern Nevada. *Geomorphology*, *4*, 1–17. https://doi.org/10.1016/0169-555X(90)90023-J

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., … Durbin, R. (2011). The variant call format and VCFtools. *Bioinformatics*, *27*, 2156–2158. https://doi.org/10.1093/bioinformatics/btr330

De Villemereuil, P., Frichot, E., Bazin, E., Francois, O., & Gaggiotti, O. (2014). Genome scan methods against more complex models: When and how much should we trust them? *Molecular Ecology*, *23*, 2006–2019. https://doi.org/10.1111/mec.12705

De-Santis, C., & Jerry, D. R. (2007). Candidate growth genes in finfish—Where should we be looking? *Aquaculture*, *272*, 22–38. https://doi.org/10.1016/j.aquaculture.2007.08.036

Dickerson, B. R., & Vinyard, G. L. (1999). Effects of high chronic temperatures and diel temperature cycles on the survival and growth of Lahontan cutthroat trout. *Transactions of the American Fisheries Society*, *128*, 516–521. https://doi.org/10.1577/1548-8659(1999)128<0516:EOHCTA>2.0.CO;2

Dionne, M., Miller, K. M., Dodson, J. J., Caron, F., & Bernatchez, L. (2007). Clinal variation in MHC diversity with temperature: Evidence for the role of host–pathogen interaction on local adaptation in Atlantic salmon. *Evolution*, *61*, 2154–2164. https://doi.org/10.1111/j.1558-5646.2007.00178.x

Dittman, A. H., & Quinn, T. P. (1996). Homing in Pacific salmon: Mechanisms and ecological basis. *Journal of Experimental Biology*, *199*, 83–91.

Dray, S., & Dufour, A. B. (2007). The ade4 package: Implementing the duality diagram for ecologists. *Journal of Statistical Software*, *22*, 1–20.

Ebersole, J. L., Liss, W. J., & Frissell, C. A. (2001). Relationship between stream temperature, thermal refugia and rainbow trout *Oncorhynchus mykiss* abundance in arid-land streams in the northwestern United States. *Ecology of Freshwater Fish*, *10*, 1–10. https://doi.org/10.1034/j.1600-0633.2001.100101.x

Eliason, E. J., Clark, T. D., Hague, M. J., Hanson, L. M., Gallagher, Z. S., Jeffries, K. M., … Farrell, A. P. (2011). Differences in thermal tolerance among sockeye salmon populations. *Science*, *332*, 109–112. https://doi.org/10.1126/science.1199158

Everett, M. V., & Seeb, J. (2014). Detection and mapping of QTL for temperature tolerance and body size in Chinook salmon (*Oncorhynchus tshawytscha*) using genotyping by sequencing. *Evolutionary Applications*, *7*, 480–492.

Foll, M., & Gaggiotti, O. (2008). A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: A Bayesian perspective. *Genetics*, *180*, 977–993. https://doi.org/10.1534/genetics.108.092221

Frémont, J. C. (1846). *Narrative of the exploring expedition to the Rocky Mountains, in the year 1842, and to Oregon and North California, in the years 1843–44*. London, UK: Wiley and Putnam.

Fulker, D. W., Cherny, S. S., Sham, P. C., & Hewitt, J. K. (1999). Combined linkage and association sib-pair analysis for quantitative traits.

The American Journal of Human Genetics, *64*, 259–267. https://doi.org/10.1086/302193

Funk, C. W., McKay, J. K., Hohenlohe, P. A., & Allendorf, F. W. (2012). Harnessing genomics for delineating conservation units. *Trends in Ecology & Evolution*, *27*(9), 489–496. https://doi.org/10.1016/j.tree.2012.05.012

Galat, D. L., Post, G., Keefe, T. J., & Bouck, G. R. (1985). Histological changes in the gill, kidney and liver of Lahontan cutthroat trout, Salmo clarki henshawi, living in lakes of different salinity-alkalinity. *Journal of Fish Biology*, *27*, 533–552. https://doi.org/10.1111/j.1095-8649.1985.tb03200.x

Gamperl, A. K., Rodnick, K. J., Faust, H. A., Venn, E. C., Bennett, M. T., Crawshaw, L. I., … Li, H. W. (2002). Metabolism, swimming performance, and tissue biochemistry of high desert redband trout (*Oncorhynchus mykiss* ssp.): Evidence for phenotypic differences in physiological function. *Physiological and Biochemical Zoology*, *75*, 413–431.

Gao, X., Starmer, J., & Martin, E. R. (2008). A multiple testing correction method for genetic association studies using correlated single nucleotide polymorphisms. *Genetic Epidemiology*, *32*, 361–369. https://doi.org/10.1002/gepi.20310

Garvin, M. R., Thorgaard, G. H., & Narum, S. R. (2015). Differential expression of genes that control respiration contribute to thermal adaptation in redband trout (*Oncorhynchus mykiss gairdneri*). *Genome Biology and Evolution*, *7*, 1404–1414. https://doi.org/10.1093/gbe/evv078

Gautier, M. (2015). Genome-wide scan for adaptive divergence and association with population-specific covariates. *Genetics*, *201*, 1555–1579. https://doi.org/10.1534/genetics.115.181453

Grayson, D. K. (1993). *The desert's past: A natural history of the Great Basin*. Washington, DC: Smithsonian Institution.

Günther, T., & Coop, G. (2013). Robust identification of local adaptation from allele frequencies. *Genetics*, *195*(1), 205–220. https://doi.org/10.1534/genetics.113.152462

Gustafson, R. G., Waples, R. S., Myers, J. M., Weitkamp, L. A., Bryant, G. J., Johnson, O. W., & Hard, J. J. (2007). Pacific salmon extinctions: Qantifying lost and remaining diversity. *Conservation Biology*, *21*, 1009–1020.

Haasl, R. J., & Payseur, B. A. (2016). Fifteen years of genomewide scans for selection: Trends, lessons and unaddressed genetic sources of complication. *Molecular Ecology*, *25*, 5–23. https://doi.org/10.1111/mec.13339

Hand, B. K., Muhlfeld, C. C., Wade, A. A., Kovach, R. P., Whited, D. C., Narum, S. R., … Luikart, G. (2016). Climate variables explain neutral and adaptive variation within salmonid metapopulations: The importance of replication in landscape genetics. *Molecular Ecology*, *25*, 689–705. https://doi.org/10.1111/mec.13517

Harrisson, K. A., Pavlova, A., Telonis-Scott, M., & Sunnucks, P. (2014). Using genomics to characterize evolutionary potential for conservation of wild populations. *Evolutionary Applications*, *7*, 1008–1025. https://doi.org/10.1111/eva.12149

Healy, T. M., Brennan, R. S., Whitehead, A., & Schulte, P. M. (2018). Tolerance traits related to climate change resilience are independent and polygenic. *Global Change Biology*, *24*, 5348–5360. https://doi.org/10.1111/gcb.14386

Hecht, B. C., Matala, A. P., Hess, J. E., & Narum, S. R. (2015). Environmental adaptation in Chinook salmon (*Oncorhynchus tshawytscha*) throughout their North American range. *Molecular Ecology*, *24*(22), 5573–5595.

Helyar, S. J., Hemmer-hansen, J., Bekkevold, D., Taylor, M. I., Ogden, R., Limborg, M. T., … Nielsen, E. E. (2011). Application of SNPs for population genetics of nonmodel organisms: New opportunities and challenges. *Molecular Ecology Resources*, *11*, 123–136. https://doi.org/10.1111/j.1755-0998.2010.02943.x

Hemmer-Hansen, J., Therkildsen, N. O., Meldrup, D., & Nielsen, E. E. (2013). Conserving marine biodiversity: Insights from life-history trait candidate genes in Atlantic cod (*Gadus morhua*). *Conservation Genetics*, *15*, 213–228. https://doi.org/10.1007/s10592-013-0532-5

Hendry, A. P. (2001). Adaptive divergence and the evolution of reproductive isolation in the wild: An empirical demonstration using introduced sockeye salmon. *Genetica*, *112–113*, 515–534.

Hendry, A. P., Leonetti, F., & Quinn, T. P. (1995). Spatial and temporal isolating mechanisms - the formation of discrete breeding aggregations of sockey salmon (*Oncorhynchus nerka*). *Canadian Journal of Zoology*, *73*, 339–352.

Heredia, N. A., & Budy, P. (2018). Trophic ecology of Lahontan cutthroat trout: Historical predator–prey interaction supports native apex predator in a unique desert lake. *Transactions of the American Fisheries Society*, *147*, 842–854. https://doi.org/10.1002/tafs.10066

Hess, J. E., Zendt, J. S., Matala, A. R., & Narum, S. R. (2016). Genetic basis of adult migration timing in anadromous steelhead discovered through multivariate association testing. *Proceedings of the Royal Society B: Biological Sciences*, *283*, 20153064. https://doi.org/10.1098/rspb.2015.3064

Hickman, T. J., & Behnke, R. J. (1979). Probable discovery of the original Pyramid Lake cutthroat trout. *The Progressive Fish-Culturist*, *41*, 135–137. https://doi.org/10.1577/1548-8659(1979)41[135:PDOTOP]2.0.CO;2

Hochachka, P. W. (1986). Defense strategies against hypoxia and hypothermia. *Science*, *231*, 234–241. https://doi.org/10.1126/science.2417316

Hohenlohe, P. A., Amish, S. J., Catchen, J. M., Allendorf, F. W., & Luikart, G. (2011). Next-generation RAD sequencing identifies thousands of SNPs for assessing hybridization between rainbow and westslope cutthroat trout. *Molecular Ecology Resources*, *11*, 117–122. https://doi.org/10.1111/j.1755-0998.2010.02967.x

Hohenlohe, P. A., Day, M. D., Amish, S. J., Miller, M. R., Kamps-Hughes, N., Boyer, M. C., … Luikart, G. (2013). Genomic patterns of introgression in rainbow and westslope cutthroat trout illuminated by overlapping paired-end RAD sequencing. *Molecular Ecology*, *22*, 3002–3013. https://doi.org/10.1111/mec.12239

Holt, R. A., Amandi, A., Rohovec, J. S., & Fryer, J. L. (1989). Relation of water temperature to bacterial cold-water disease in Coho salmon, Chinook salmon, and rainbow trout. *Journal of Aquatic Animal Health*, *1*, 94–101. https://doi.org/10.1577/1548-8667(1989)001<0094:ROWTTB>2.3.CO;2

Huang, H., & Knowles, L. L. (2016). Unforeseen consequences of excluding missing data from next-generation sequences: Simulation study of RAD sequences. *Systematic Biology*, *65*, 357–365. https://doi.org/10.1093/sysbio/syu046

Hudson, A. G., Vonlanthen, P., & Seehausen, O. (2014). Population structure, inbreeding and local adaptation within an endangered riverine specialist: The nase (*Chondrostoma nasus*). *Conservation Genetics*, *15*, 933–951. https://doi.org/10.1007/s10592-014-0590-3

Isaak, D. J., Muhlfeld, C. C., Todd, A. S., Al-chokhachy, R., Roberts, J., Kershner, J. L., … Hostetler, S. W. (2012). The past as prelude to the future for understanding 21st-Century climate effects on Rocky Mountain trout. *Fisheries*, *37*, 542–556. https://doi.org/10.1080/03632415.2012.742808

Jackson, T. R., Ferguson, M. M., Danzmann, R. G., Fishback, A. G., Ihssen, P. E., O'Connell, M., & Crease, T. J. (1998). Identification of two QTL influencing upper temperature tolerance in three rainbow trout (*Oncorhynchus mykiss*) half-sib families. *Heredity*, *80*, 143–151. https://doi.org/10.1046/j.1365-2540.1998.00289.x

Jansen, M., Geerts, A. N., Rago, A., Spanier, K. I., Denis, C., De Meester, L., Orsini, L. (2017). Thermal tolerance in the keystone species *Daphnia magna*—A candidate gene and an outlier analysis approach. *Molecular Ecology*, *26*, 2291–2305.

Jeffries, K. M., Hinch, S. G., Sierocinski, T., Clark, T. D., Eliason, E. J., Donaldson, M. R., … Miller, K. M. (2012). Consequences of high temperatures and premature mortality on the transcriptome and blood physiology of wild adult sockeye salmon (*Oncorhynchus nerka*). *Ecology and Evolution*, *2*, 1747–1764.

Jeffries, K. M., Hinch, S. G., Sierocinski, T., Pavlidis, P., & Miller, K. M. (2013). Transcriptomic responses to high water temperature in two species of Pacific salmon. *Evolutionary Applications*, *7*, 286–300. https://doi.org/10.1111/eva.12119

Jensen, J. D., Foll, M., & Bernatchez, L. (2016). The past, present and future of genomic scans for selection. *Molecular Ecology*, *25*, 1–4. https://doi.org/10.1111/mec.13493

Johnston, S. E., Orell, P., Pritchard, V. L., Kent, M. P., Lien, S., Niemelä, E., … Primmer, C. R. (2014). Genome-wide SNP analysis reveals a genetic basis for sea-age variation in a wild population of Atlantic salmon (*Salmo salar*). *Molecular Ecology*, *23*, 3452–3468.

Jombart, T. (2008). a R package for the multivariate analysis of genetic markers. *Bioinformatics*, *24*, 1403–1405.

Kendall, N. W., McMillan, J. R., Sloat, M. R., Buehrens, T. W., Quinn, T. P., Pess, G. R., … Zabel, R. W. (2014). Anadromy and residency in steelhead and rainbow trout *Oncorhynchus mykiss*: A review of the processes and patterns. *Canadian Journal of Fisheries and Aquatic Sciences*, *72*, 319–342.

Kohn, M. H., Murphy, W. J., Ostrander, E. A., & Wayne, R. K. (2006). Genomics and conservation genetics. *Trends in Ecology & Evolution*, *21*, 629–637. https://doi.org/10.1016/j.tree.2006.08.001

Larson, W. A., McKinney, G. J., Limborg, M. T., Everett, M. V., Seeb, L. W., & Seeb, J. E. (2015). Identification of multiple QTL hotspots in sockeye salmon (*Oncorhynchus nerka*) using genotyping-by-sequencing and a dense linkage map. *Journal of Heredity*, *107*, 122–133.

Li, H. (2011). A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics*, *27*, 2987–2993. https://doi.org/10.1093/bioinformatics/btr509

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N. … 1000 Genome Project Data Processing Subgroup. (2009). The sequence alignment/map (SAM) format and SAMtools. *Bioinformatics*, *25*, 2078–2079.

Lischer, H. E. L., & Excoffier, L. (2012). PGDSpider: An automated data conversion tool for connecting population genetics and genomics programs. *Bioinformatics*, *28*, 298–299. https://doi.org/10.1093/bioinformatics/btr642

Lotterhos, K. E., & Whitlock, M. C. (2014). Evaluation of demographic history and neutral parameterization on the performance of F_{ST} outlier tests. *Molecular Ecology*, *23*, 2178–2192.

Lotterhos, K. E., & Whitlock, M. C. (2015). The relative power of genome scans to detect local adaptation depends on sampling design and statistical method. *Molecular Ecology*, *24*, 1031–1046. https://doi.org/10.1111/mec.13100

Luikart, G., England, P. R., Tallmon, D., Jordan, S., & Taberlet, P. (2003). The power and promise of population genomics: From genotyping to genome typing. *Nature Reviews Genetics*, *4*, 981–994. https://doi.org/10.1038/nrg1226

Luu, K., Basin, E., & Blum, M. G. B. (2017). pcadapt: An R package to perform genome scans for selection based on principal component analysis. *Molecular Ecology Resources*, *17*, 67–77.

Ma, Y., Dai, H.-Y., Zhang, F., & Zhao, D. (2017). TRIM66 expression in non-small cell lung cancer: A new predictor of prognosis. *Cancer Biomarkers*, *20*, 309–315. https://doi.org/10.3233/CBM-170207

Madsen, D. B., Hershler, R., & Currey, D. R. (2002). Introduction. In R. Hershler, D. B. Madsen & D. R. Currey (Eds.), *Great Basin Aquatic Systems History* (pp. 1–10).

Maier, M. K., Seth, S., Czeloth, N., Qiu, Q., Ravens, I., Kremmer, E., … Bernhardt, G. (2007). The adhesion receptor CD155 determines the magnitude of humoral immune responses against orally ingested antigens. *European Journal of Immunology*, *37*, 2214–2225. https://doi.org/10.1002/eji.200737072

Manel, S., Perrier, C., Pratlong, M., Abi-Rached, L., Paganini, J., Pontarotti, P., & Aurelle, D. (2016). Genomic resources and their influence on the detection of the signal of positive selection in genome scans. *Molecular Ecology*, *25*, 170–184. https://doi.org/10.1111/mec.13468

McCairns, R. J. S., Smith, S., Sasaki, M., Bernatchez, L., & Beheregaray, L. B. (2016). The adaptive potential of subtropical rainbowfish in the face of climate change: Heritability and heritable plasticity for the expression of candidate genes. *Evolutionary Applications*, *9*, 531–545. https://doi.org/10.1111/eva.12363

McGirr, J. A., & Martin, C. H. (2016). Novel candidate genes underlying extreme trophic specialization in Caribbean pupfishes. *Molecular Biology and Evolution*, *34*, 873–888.

Messer, P. W., & Petrov, D. A. (2013). Population genomics of rapid adaptation by soft selective sweeps. *Trends in Ecology & Evolution*, *28*, 659–669. https://doi.org/10.1016/j.tree.2013.08.003

Miller, M. R., Brunelli, J. P., Wheeler, P. A., Liu, S., Rexroad, C. E., Palti, Y., … Thorgaard, G. H. (2012). A conserved haplotype controls parallel adaptation in geographically distant salmonid populations. *Molecular Ecology*, *21*, 237–249. https://doi.org/10.1111/j.1365-294X.2011.05305.x

Miller, P. S., & Hedrick, P. W. (1991). MHC polymorphism and the design of captive breeding programs: Simple solutions are not the answer. *Conservation Biology*, *5*, 556–558. https://doi.org/10.1111/j.1523-1739.1991.tb00369.x

Moore, J. W., Yeake, J. D., Peard, D., Lough, J., & Beere, M. (2014). Life-history diversity and its importance to population stability and persistence of a migratory fish: Steelhead in two large North American watersheds. *Journal of Animal Ecology*, *83*, 1035–1046. https://doi.org/10.1111/1365-2656.12212

Narum, S. R., Buerkle, C. A., Davey, J. W., Miller, M. R., & Hohenlohe, P. A. (2013). Genotyping-by-sequencing in ecological and conservation genomics. *Molecular Ecology*, *22*, 2841–2847. https://doi.org/10.1111/mec.12350

Narum, S. R., & Campbell, N. R. (2015). Transcriptomic response to heat stress among ecologically divergent populations of redband trout. *BMC Genomics*, *16*, 103. https://doi.org/10.1186/s12864-015-1246-5

Narum, S. R., Campbell, N. R., Kozfkay, C. C., & Meyer, K. A. (2010). Adaptation of redband trout in desert and montane environments. *Molecular Ecology*, *19*, 4622–4637. https://doi.org/10.1111/j.1365-294X.2010.04839.x

Narum, S. R., & Hess, J. E. (2011). Comparison of F_{ST} outlier tests for SNP loci under selection. *Molecular Ecology Resources*, *11*(Suppl. 1), 184–194.

Nehlsen, W., Williams, J. E., & Lichatowich, J. A. (1991). Pacific salmon at the crossroads: Stocks at risk from California, Oregon, Idaho, and Washington. *Fisheries*, *16*, 4–21. https://doi.org/10.1577/1548-8446(1991)016<0004:PSATCS>2.0.CO;2

Neville, H. M., Isaak, D. J., Dunham, J. B., Thurow, R. F., & Rieman, B. E. (2006). Fine-scale natal homing and localized movement as shaped by sex and spawning habitat in Chinook salmon: Insights from spatial autocorrelation analysis of individual genotypes. *Molecular Ecology*, *15*, 4589–4602. https://doi.org/10.1111/j.1365-294X.2006.03082.x

Nunes, V. L., Beaumont, M. A., Butlin, R. K., & Paulo, O. S. (2011). Multiple approaches to detect outliers in a genome scan for selection in ocellated lizards (*Lacerta lepida*) along an environmental gradient. *Molecular Ecology*, *20*, 193–205. https://doi.org/10.1111/j.1365-294X.2010.04936.x

Ouborg, N. J., Pertoldi, C., Loeschcke, V., Bijlsma, R., & Hedrick, P. W. (2010). Conservation genetics in transition to conservation genomics. *Trends in Genetics*, *26*, 177–187. https://doi.org/10.1016/j.tig.2010.01.001

Peacock, M. M., Hekkala, E. R., & Kirchoff, V. S. , &., & Heki, L. G. (2017). Return of a giant: DNA from archival museum samples helps to identify a unique cutthroat trout lineage formerly thought to be extinct. *Royal Society Open Science*, *4*(11), 171253. https://doi.org/10.1098/rsos.171253

Peacock, M. M., & Kirchoff, V. (2007). *Analysis of genetic variation and population genetic structure in Lahontan cutthroat trout (*Oncorhynchus clarkii *henshawi) extant populations* (pp. 109). Reno, NV: U.S. Fish and Wildlife Service.

Peacock, M. M., Neville, H., & Finger, A. J. (2018). The Lahontan Basin evolutionary lineage of cutthroat trout. In P. Trotter, P. Bisson, L. Schultz, & B. Roper (Eds.), *Cutthroat trout evolutionary biology and taxonomy* (pp. 231–260). Bethesda, MD: American Fisheries Society.

Penaluna, B. E., Abadía-Cardoso, A., Dunham, J. B., García-Dé León, F. J., Gresswell, R. E., Luna, A. R., … Fausch, K. D. (2016). Conservation of native pacific trout diversity in western North America. *Fisheries*, *41*(6), 286–300. https://doi.org/10.1080/03632415.2016.1175888

Perrier, C., Bourret, V., Kent, M. P., & Bernatchez, L. (2013). Parallel and nonparallel genome-wide divergence among replicate population pairs of freshwater and anadromous Atlantic salmon. *Molecular Ecology*, *22*, 5577–5593. https://doi.org/10.1111/mec.12500

Perrier, C., Ferchaud, A.-L., Sirois, P., Thibault, I., & Bernatchez, L. (2017). Do genetic drift and accumulation of deleterious mutations preclude adaptation? Empirical investigation using RADseq in a northern lacustrine fish. *Molecular Ecology*, *26*, 6317–6335. https://doi.org/10.1111/mec.14361

Perry, G. M. L., Martyniuk, C. M., Ferguson, M. M., & Danzmann, R. G. (2005). Genetic parameters for upper thermal tolerance and growth-related traits in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, *250*, 120–128. https://doi.org/10.1016/j.aquaculture.2005.04.042

Pfeifer, S. P., Laurent, S., Sousa, V. C., Linnen, C. R., Foll, M., Excoffier, L., … Jensen, J. D. (2018). The evolutionary history of Nebraska deer mice: Local adaptation in the face of strong gene flow. *Molecular Biology and Evolution*, *35*, 792–806. https://doi.org/10.1093/molbev/msy004

Portner, H. O. (2002). Climatic variations and the physiological basis of temperature- ependent biogeography: Systemic to molecular hierarchy of thermal tolerance in animals. *Comparative Biochemistry and Physiology*, *132A*, 739–761.

Pörtner, H. O., & Knust, R. (2007). Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science*, *315*(5808), 95–97. https://doi.org/10.1126/science.1135471

Primmer, C. R. (2011). Genetics of local adaptation in salmonid fishes. *Heredity*, *106*, 401–403. https://doi.org/10.1038/hdy.2010.158

Prince, D. J., O'Rourke, S. M., Thompson, T. Q., Ali, O. A., Lyman, H. S., Saglam, I. K., … Miller, M. R. (2017). The evolutionary basis of premature migration in Pacific salmon highlights the utility of genomics for informing conservation. *Science Advances*, *3*(8), e1603198–https://doi.org/10.1126/sciadv.1603198

Pujolar, J. M., Jacobsen, M. W., Als, T. D., Frydenberg, J., Munch, K., Jónsson, B., … Hansen, M. M. (2014). Genome-wide single-generation signatures of local selection in the panmictic European eel. *Molecular Ecology*, *23*, 2514–2528. https://doi.org/10.1111/mec.12753

Reheis, M. C., Sarna-Wojcicki, A. M., Reynolds, R. L., Repenning, C. A., & Mifflin, M. D. (2002). Pliocene to Middle Pleistocene lakes in the western Great Basin: Ages and connections. In R. Hershler, D. B. Madsen, & D. R. Currey (Eds.), *Great Basin Aquatic Systems History* (pp. 405). Washington, DC: Smithsonian Institution.

Reid, D., Szanto, A., Glebe, B., Danzmann, R., & Ferguson, M. (2005). QTL for body weight and condition factor in Atlantic salmon (*Salmo salar*): Comparative analysis with rainbow trout (*Oncorhynchus mykiss*) and Arctic charr (*Salvelinus alpinus*). *Heredity*, *94*, 166–172. https://doi.org/10.1038/sj.hdy.6800590

Rellstab, C., Gugerli, F., Eckert, A. J., Hancock, A. M., & Holderegger, R. (2015). A practical guide to environmental association analysis in landscape genomics. *Molecular Ecology*, *24*, 4348–4370. https://doi.org/10.1111/mec.13322

Richter, A., & Kolmes, S. A. (2005). Maximum temperature limits for Chinook, coho, and chum salmon, and steelhead trout in the Pacific northwest. *Reviews in Fisheries Science*, *13*, 23–49. https://doi.org/10.1080/10641260590885861

Robinson, M. L., Gomez-Raya, L., Rauw, W. M., & Peacock, M. M. (2008). Fulton's body condition factor K correlates with survival time in a thermal challenge experiment in juvenile Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*). *Journal of Thermal Biology*, *33*, 363–368. https://doi.org/10.1016/j.jtherbio.2008.05.004

Rousset, F. (2008). GENEPOP'007: A complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources*, *8*, 103–106. https://doi.org/10.1111/j.1471-8286.2007.01931.x

Sanders, J. E., Pilcher, K. S., & Fryer, J. L. (1978). Relation of water temperature to bacterial kidney disease in Coho salmon (*Oncorhynchus kisutch*), Sockeye salmon (*O. nerka*), and steelhead trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada*, *35*, 8–11.

Scoppettone, G. G., Rissler, P. H., Shea, S. P., & Somer, W. (2012). Effect of brook trout removal from a spawning stream on an adfluvial population of Lahontan cutthroat trout. *North American Journal of Fisheries Management*, *32*, 586–596. https://doi.org/10.1080/02755947.2012.675958

Sloat, M. R., Fraser, D. J., Dunham, J. B., Falke, J. A., Jordan, C. E., McMillan, J. R., & Ohms, H. A. (2014). Ecological and evolutionary patterns of freshwater maturation in Pacific and Atlantic salmonines. *Reviews in Fish Biology and Fisheries*, *24*, 689–707. https://doi.org/10.1007/s11160-014-9344-z

Smith, G. R., & Stearley, R. F. (2018). The fossil record of cutthroat trout: Implications for evolution and conservation. In P. Trotter, P. Bisson, L. Shultz, & B. Roper (Eds.), *Cutthroat trout: Evolutionary biology and taxonomy*. Bethesda, MD: American Fisheries Society.

Somorjai, I. M., Danzmann, R. G., & Ferguson, M. M. (2003). Distribution of temperature tolerance quantitative trait loci in Arctic charr (*Salvelinus alpinus*) and inferred homologies in rainbow trout (*Oncorhynchus mykiss*). *Genetics*, *165*, 1443–1456.

Sourorov, A., Tatusover, T., & Lipman, D. (2004). Genome annotation with Gnomon-a multi-step combined gene prediction tool. Intelligent Systems for Molecular Biology Proceedings: 125.

Stephan, W. (2016). Signatures of positive selection: From selective sweeps at individual loci to subtle allele frequency changes in polygenic adaptation. *Molecular Ecology*, *25*, 79–88. https://doi.org/10.1111/mec.13288

Storz, J. F. (2005). Using genome scans of DNA polymorphism to infer adaptive population divergence. *Molecular Ecology*, *14*, 671–688.

Sumaila, U. R., Cheung, W. W., Lam, V. W., Pauly, D., & Herrick, S. (2011). Climate change impacts on the biophysics and economics of world fisheries. *Nature Climate Change*, *1*, 449. https://doi.org/10.1038/nclimate1301

Taylor, E. B. (1991). A review of local adaptation in Salmonidae, with particular reference to Pacific and Atlantic salmon. *Aquaculture*, *98*, 185–207.

Tomalty, K. M. H., Meek, M. H., Stephens, M. R., Rincón, G., Fangue, N. A., May, B. P., & Baerwald, M. R. (2015). Transcriptional response to acute thermal exposure in juvenile chinook salmon determined by RNAseq. *G3: Genes|genomes|genetics*, *5*, 1335–1349. https://doi.org/10.1534/g3.115.017699

USFWS (2009). *Lahontan cutthroat trout (*Oncorhynchus clarkii henshawi*) 5-year review: Summary and evaluation* (pp. 199). Reno, NV: U.S. Fish and Wildlife Service.

Vallejo, R. L., Silva, R. M. O., Evenhuis, J. P., Gao, G., Liu, S., Parsons, J. E., … Palti, Y. (2018). Accurate genomic predictions for BCWD resistance in rainbow trout are achieved using low-density SNP panels: Evidence that long-range LD is a major contributing factor. *Journal of Animal Breeding and Genetics*, *135*, 263–274. https://doi.org/10.1111/jbg.12335

Vrijenhoek, R. C., & Leberg, P. L. (1991). Let's not throw the baby out with the bathwater: A comment on management for MHC diversity in captive populations. *Conservation Biology*, *5*, 252–254. https://doi.org/10.1111/j.1523-1739.1991.tb00131.x

Waples, R. S. (1995). Evolutionarily significant units and the conservation of biological diversity under the Endangered Species Act. *American Fisheries Society*, *17*, 8–27.

Waples, R. S., Pess, G. R., & Beechie, T. (2008). Evolutionary history of Pacific salmon in dynamic environments. *Evolutionary Applications*, *1*, 189–206.

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

SUPPORTING INFORMATION

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

How to cite this article: Amish SJ, Ali O, Peacock M, et al. Assessing thermal adaptation using family-based association and F_{ST} outlier tests in a threatened trout species. *Mol Ecol*. 2019;28:2573–2593. https://doi.org/10.1111/mec.15100