OXFORD

Parallel shifts in trout feeding morphology suggest rapid adaptation to alpine lake environments

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Abstract

Eco-evolutionary interactions following ecosystem change provide critical insight into the ability of organisms to adapt to shifting resource landscapes. Here we explore evidence for the rapid parallel evolution of trout feeding morphology following eco-evolutionary interactions with zooplankton in alpine lakes stocked at different points in time in the Wind River Range (Wyoming, USA). In this system, trout predation has altered the zooplankton species community and driven a decrease in average zooplankton size. In some lakes that were stocked decades ago, we find shifts in gill raker traits consistent with the hypothesis that trout have rapidly adapted to exploit available smaller-bodied zooplankton more effectively. We explore this morphological response in multiple lake populations across two species of trout (cutthroat trout, *Oncorhynchus clarkii*, and golden trout *Oncorhynchus aguabonita*) and examine the impact of resource availability on morphological variation in gill raker number among lakes. Furthermore, we present genetic data to provide evidence that historically stocked cutthroat trout populations likely derive from multiple population sources, and incorporate variation from genomic relatedness in our exploration of environmental predictors of feeding morphology. These findings describe rapid adaptation and eco-evolutionary interactions in trout and document an evolutionary response to novel, contemporary ecosystem change.

Keywords: rapid adaptation, parallel evolution, gill raker, eco-evolutionary interaction

Introduction

Anthropogenically induced stressors, such as urbanization, climate change, and species introductions, are rapidly driving unprecedented ecosystem changes around the globe (Ceballos et al., 2015; Gallardo et al., 2016; Ormerod et al., 2010; Vitousek et al., 1996), presenting an urgent challenge to understand the evolutionary potential for organisms to respond to novel ecological stressors. In particular, cascading environmental change often follows invasive species introduction from human action (Ceballos et al., 2015; Clavero & García-Berthou, 2005; Gallardo et al., 2016; Miller et al., 1989; Post et al., 2008; Schindler et al., 2001; Vitousek et al., 1996). Introduced predators may reshape prey communities (Brooks & Dodson, 1965), drive trophic cascades (Knapp et al., 2005), and precipitate species extinctions or biodiversity loss (Carpenter et al., 1985; Goudswaard et al., 2008). However, few studies have explored how such ecosystem restructuring may feedback to drive the subsequent adaptation of the introduced predators (Lambrinos, 2004). When evolutionary response occurs contemporaneously with ecosystem change, it is known as an eco-evolutionary feedback loop—an effect that has been demonstrated experimentally

(Grether et al., 2001; Strauss, 2014; Turcotte et al., 2011) and has received increasing attention in recent decades (Hendry, 2016). While well-documented examples in natural systems are still limited (Lambrinos, 2004; Strauss, 2014), eco-evolutionary dynamics have been observed between alewives (*Alosa pseudoharengus*) and zooplankton in land-locked lakes (Palkovacs & Post, 2008; Palkovacs et al., 2014) and between soapberry bugs (*Jadera haematoloma*) and invasive host plants (Cenzer, 2016). These examples suggest that continued feedback between predator and prey can shape ecosystem dynamics and that interactions often arise rapidly, indicating that eco-evolutionary feedbacks may be key to understanding evolutionary change following anthropogenic interference (Palkovacs & Post, 2008). Furthermore, the predictive factors that shape rapid evolutionary responses, especially in natural systems where fewer studies have occurred, still need to be thoroughly documented and explored (Lambrinos, 2004; Moran & Alexander, 2014).

To address these outstanding questions, our current study instigates the potential for rapid evolution of trout feeding morphology following eco-evolutionary interactions between trout and zooplankton in multiple populations of golden trout

(*Oncorhynchus aguabonita*; Jordan, 1892) and cutthroat trout (*Oncorhynchus clarkii*; Richardson, 1836) stocked into naturally fishless alpine lakes in Wyoming's Wind River mountain range. We additionally explore the ecological predictors of morphological change and include genomic data from several cutthroat trout populations to characterize the population structure and provenance of historically stocked lake populations. The introduction of trout into these lakes has previously been shown to alter ecosystem trophic structure (Boyle, 2021; Knapp et al., 2001), since size-selective predation by trout drives a decrease in average zooplankton body size within the lake. Because trout rely on their gill raker morphology to capture and retain small zooplankton (Budy et al., 2005; Wright et al., 1983), variation in these morphological traits is a likely target for eco-evolutionary response in this system.

In particular, an increase in gill raker number and length or a decrease in the space between gill rakers is associated with zooplankton retention or planktivory in salmonids and several other species (Amundsen et al., 2004; Castillo-Rivera et al., 1996; Gibson, 1988; Kahilainen et al., 2011; MacNeill & Brandt, 1990; Palkovacs & Post, 2008; Roesch et al., 2013; Wright et al., 1983). Furthermore, gill raker morphology—in particular gill raker number—is heritable in trout and other salmonids (Bernatchez, 2004; Foote et al., 1999; Funk et al., 2005; Hessen et al., 1988; Leary et al., 1985; Østbye et al., 2005), suggesting that plastic responses are unlikely to fully explain shifts in feeding morphology. While trait plasticity does not preclude heritability, the heritability of gill raker traits allows us to isolate adaptive evolutionary change as the likely mechanism underlying observed morphological changes among populations.

The history of fish stocking in these lakes also presents an opportunity to document parallel evolution within trout across multiple replicate lake populations. Cases of parallel adaptation provide the opportunity to disentangle chance from natural selection (Yuan & Stinchcombe, 2020). While the parallel evolution of gill raker traits has been previously demonstrated in stickleback, whitefish, and alewives (Glazer et al., 2014; Häkli et al., 2018; Landry et al., 2007; Østbye et al., 2006; Palkovacs et al., 2008; Schluter, 2000), it has not yet been studied in trout. Furthermore, cutthroat trout populations often occur as either stream-adapted or lake-adapted ecotypes (Behnke, 2010) across their geographic range, but no studies have explored how such morphological variation might arise rapidly after colonization of new habitats.

Here, we explore evidence for the rapid adaptation of trout in relation to eco-evolutionary interactions, and thus rapid ecosystem change, across multiple alpine lakes using a combination of morphological, environmental, and genomic data. First, we explore evidence for rapid adaptation in response to changing resource landscapes by documenting differences in gill raker traits between populations that differ in their histories of stocking. Specifically, we contrast historically stocked lakes, where there have been generations of reproduction since stocking, with recently stocked lakes and hatchery populations, where there has not been the opportunity for selection to act over multiple generations. While drift may also lead to phenotypic shifts within small populations, observing parallel shifts in multiple populations would rule out drift alone as the most likely mechanism of phenotypic change. Secondly, we expect competitive fitness landscapes and resource availability to shape the strength of selection. Thus, we explore the relationship between morphological change (i.e., number of gill rakers) and environmental and dietary variables that serve as broad proxies for intraspecific competition and resource availability to further examine factors that may shape rapid adaptive change. We expect greater population density to increase both intraspecific competitive pressure and morphological variability available for selection and expect this relationship to mediate dietary trends in populations, which may in turn shape selection. Lastly, we incorporate genomic data to investigate relatedness among several cutthroat trout populations and account for population genetic structure among lakes in our exploration of environmental predictors of feeding morphology. In sum, this study provides an example of parallel local adaptation in two species of trout and integrates information from morphological, environmental, and genomic data to shed light on the complex dynamics of evolution in an ecosystem context.

Materials and methods

Description of study system and data collection

Study system

We collected cutthroat trout (*O. clarkii*) and golden trout (*O. aguabonita*) from 18 high-elevation, alpine lakes across the Wind River Range in Wyoming (Figure 1, Supplementary Table S1). For cutthroat trout, we targeted lakes stocked with Yellowstone cutthroat trout (*O. c. bouvieri*) although some contained Snake River fine-spotted cutthroat trout (*O. c. behnkei*) or may have contained both subspecies historically (see Supplementary Table S2). Lakes in which we detected hybridization with golden or rainbow (*O. mykiss*) trout were excluded from this study as hybridization may affect values of meristic counts (Leary et al., 1985; Ostberg et al., 2011). Lakes populated with nontarget species were also excluded from the analyses.

All lakes were originally fishless (Wiley, 2003), but some have been recurrently or recently stocked, whereas others were stocked historically (i.e., early to mid-1900s) and persist naturally. Surveys of fishless lakes in the region suggest these alpine lakes naturally contained large-bodied zooplankton prior to fish introduction (Boyle, 2021). Since stocking, size-selective predation of zooplankton by fish has restructured the zooplankton communities and driven a shift to smaller-bodied zooplankton species (Boyle, 2021), similar to observations elsewhere following fish introduction into alpine lakes (Anderson, 1980; Bradford et al., 1998; Carlisle & Hawkins, 1998; Donald et al., 2001; Knapp et al., 2001; Schabetsberger et al., 2009; Stoddard, 1987).

The golden trout sampled in this study trace their strain origins to the Kern River drainage in California; however, the cutthroat source populations historically used to stock these lakes are largely unknown (Supplementary Table S2). Many lakes were likely stocked with cutthroat from nearby riverine populations or from eggs or fry obtained from hatcheries around Yellowstone Lake (Wyoming Game and Fish Department [WGFD], personal communication). Some populations we sampled may contain progeny from historically stocked populations as well as recently stocked fish from hatchery broodstocks (e.g., Windy Lake, Kagevah Lake; Supplementary Tables S1 and S2). We estimated time since the last stocking event for each lake from a combination of WGFD stocking records and personal communication with U.S. Fish and Wildlife Service (USFWS).
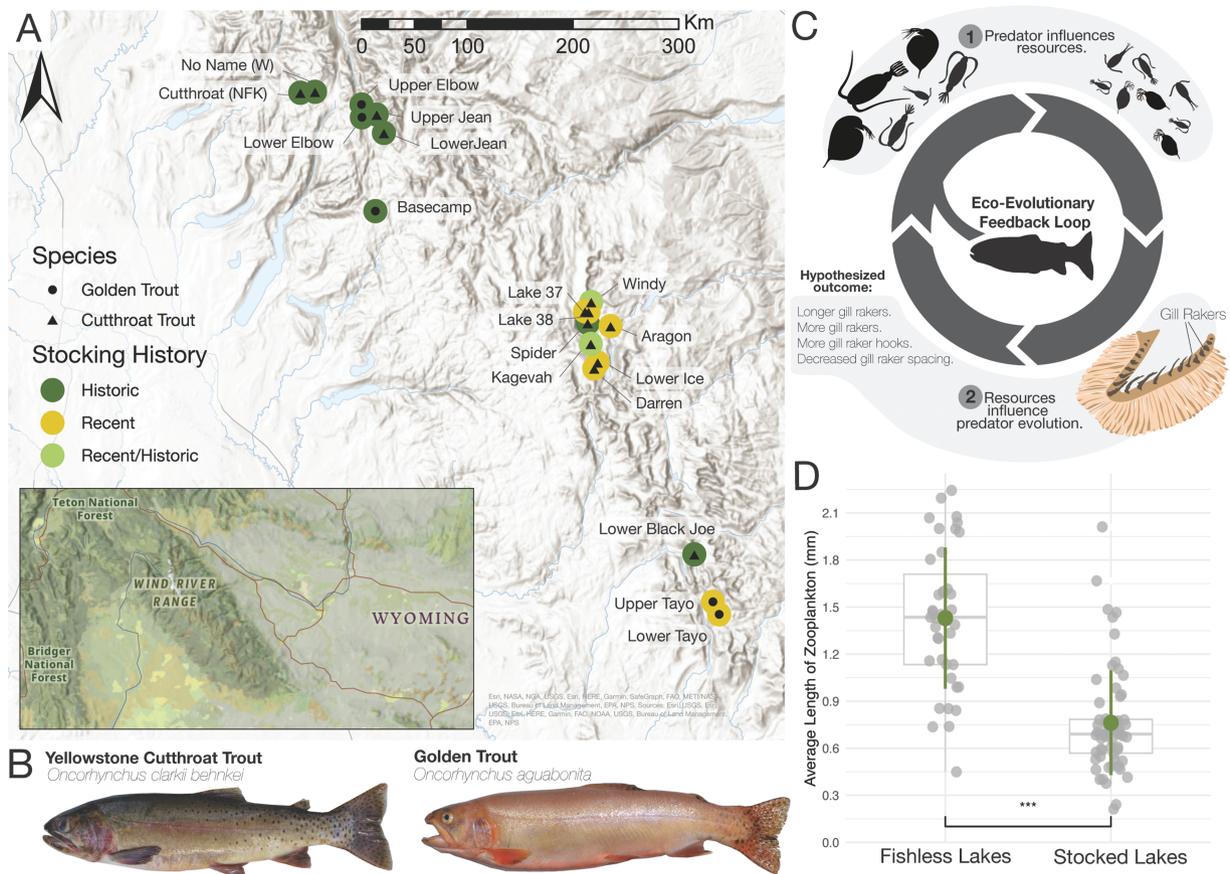
Figure 1. (A) Map of the Wind River Range (Wyoming) and sampled lakes. (B) Photographs of focal species. (C) Conceptual diagram of hypothesized eco-evolutionary feedback loop and predicted outcomes of predator feeding morphology. (D) Average interspecific zooplankton lengths within lakes sampled throughout the Wind River Range. Data from Boyle (2021).

Field and environmental data collection

Field data and specimen collection took place during the 2018–2021 summer field seasons. We collected fish with gill-nets set directly perpendicular to shore (Supplementary Table S4). We collected zooplankton samples from the deepest point of each lake via two vertical 15-m tows with a conical plankton net and preserved individuals in 70% ethanol for further laboratory analysis including taxon identification, body size measurements, and density counts. For a complete description of zooplankton collecting procedures and laboratory analysis, see Boyle (2021). We calculated average interspecific zooplankton body size within each lake and obtained biomass data for four zooplankton taxa frequently identified in fish stomachs (i.e., *Daphnia mendotae*, *Daphnia pulex*, *Leptodiaptomus minutus*, and *Hesperodiaptomus shoshone*) from Boyle (2021).

We obtained lake depth using either a Vexilar LPS-1 Hand-held Digital Sonar Depth Finder, when available, or a marked rope and drop weight. After capture, fish were sacrificed with an overdose of MS-222 under IACUC protocol 20190816CW00387-02 from the University of Wyoming. We collected fish total length and weight and photographed each specimen prior to taking a fin clip, a muscle sample, and extracting stomachs for later diet analysis. Trout heads were removed and placed in 10% formalin for preservation and further morphological analysis in the laboratory. Specimens and associated photographs and data are prepared for cataloging at the University of Wyoming Museum of Vertebrates.

We also obtained 4- to 16-month-old golden trout and cutthroat trout during the summer of 2020 from several current hatchery broodstocks (see Supplementary Table S1). The current Yellowstone cutthroat trout brood stock originates from a population of mixed fluvial- and lacustrine-oriented trout that spawn upstream from the LeHardy Rapids near the outlet of Yellowstone Lake in Yellowstone National Park, WY (Burningham et al., 2002). Hatchery fish are fed dried pelleted fish food that is substantially larger than zooplankton and most aquatic insects. Because large quantities of pelleted food were available to trout, it is unlikely that natural selection acts much on foraging traits among hatchery fish.

Because resource availability and intraspecific competition may shape selective pressure within lake populations (Bolnick, 2004; Schluter, 2003), we collected additional data on lake area and lake elevation from lake shape polygons drawn in Google Earth (Google Earth Pro, v7.3.4, accessed 2022) and quantified the degree of alpine vegetation (normalized difference vegetation index, NDVI) surrounding each lake using Sentinel-2 QA multispectral image data in Google Earth Engine (Gorelick et al., 2017). See supporting information for additional details.

Morphological data collection

To examine evidence for shifts in feeding morphology among stocked populations, we collected fish heads from all specimens captured in the field. We fixed tissue in a 10% formalin solution before transferring all samples to 70% ethanol for

long-term storage. We then extracted the first gill arch from the right side of each specimen and dyed it for visibility using an Alizarin Red and potassium hydroxide (KOH) solution (Krueger, 1988). We took images of the anterior and posterior sides of each gill arch using a Leica Microsystems microscope outfitted with a camera and counted the number of mature gill rakers on the anterior side and all the gill rakers on the posterior side. We then obtained measurements for gill raker morphology from each image using ImageJ (Schneider et al., 2012). Arch length was defined as the composite sum of distances between each gill raker from the first gill raker to the last, and the length of the longest gill raker was obtained by measuring the distance from the base of the gill raker to the tip of the gill raker. Average raker widths were calculated by averaging the widths of the two gill rakers adjacent to the central gill raker, which nests in the elbow of the gill arch, on both the top and bottom arch. Gill raker hooks (also termed branchiospinules, Castillo-Rivera et al., 1996) were counted along a 1-mm portion of the edge of the two gill rakers next to the gill rakers adjacent to the central raker, and these measurements were combined to calculate an average for each specimen (see Supplementary Figure S1). See Supplementary Material for photos of gill arches, further measurement details, and arch staining procedure.

Dietary data collection

We collected total counts of prey items in fish stomachs for all historically stocked and one recently stocked cutthroat trout population to include dietary data in our analysis of predictors of feeding morphology (Supplementary Table S1). Contents were emptied into a petri dish and examined under a Leica microscope. We identified prey items to the lowest taxonomic level possible and recorded counts of items within each taxonomic group. We used the WTdMC() and Eindex() functions in the RInSp (Zaccarelli et al., 2013) package in R to calculate measures of total niche width (TNW) and individual specialization (E) for each lake from raw prey count data, respectively. TNW is calculated from the Shannon–Weaver index of a matrix of diet item prey counts per individual following the equations and suggestions by (Roughgarden, 1974) for discrete count data. E value estimates the degree of interindividual diet specialization in a population(see Araújo et al., 2008 for theory and development) and scales from 0 to 1, where values of 0 indicate no variation in diet among individuals and larger values indicate greater levels of dietary specialization (or individual dietary differences) among individuals in the population. We used the default Saramaki clustering index and included a jackknife estimate of variance (Araújo et al., 2008; Zaccarelli et al., 2013).

We also calculated the proportion of pelagic prey and the total prey count for each individual and averaged these values within lakes to obtain lake-level estimates of zooplankton reliance and total prey abundance as proxies for resource abundance. Here, total prey count in fish stomachs is assumed to broadly reflect the relative abundance of prey available to trout. Prey were categorized as pelagic if generally found in the pelagic zone, following guidelines outlined in Schluter and McPhail (1992). For a visualization of diet composition, see Supplementary Figures S3 and S4.

Genomic data collection

Fin clips were collected from all samples, but only some historically stocked lakes were selected for DNA extraction and sequencing (Supplementary Table S1). Our goal was to generate genotyping-by-sequencing data for lakes for which the origins of historically stocked individuals were uncertain in order to (a) gain insight into possible origins of historic populations, (b) examine their similarity to available hatchery and reference populations, and (c) understand how relatedness among historic populations may underlie similarity in feeding morphology. We also sought to rule out the possibility that all historic cutthroat trout lakes were stocked only with trout from Yellowstone Lake, since some populations of cutthroat trout associated with Yellowstone Lake may already have morphology consistent with adaptation to a lake environment, including a larger number of anterior and posterior gill rakers than stream-derived populations (Behnke, 2010). As comparative reference populations, we included individuals from two Wyoming hatcheries, including the current Yellowstone cutthroat trout LeHardy Rapids broodstock and the Snake River cutthroat trout broodstock, and individuals from an additional population of stream-dwelling Yellowstone cutthroat trout from South Fork Owl Creek located in central Wyoming to the northeast of the Wind River Range.

DNA extraction and sequencing protocols, as well as assembly methods, follow those detailed in Rosenthal et al. (2022) and Mandeville et al. (2019). Variable genetic sites (i.e., single-nucleotide polymorphisms; SNPs) were identified using SAMtools mpileup (v1.8; Li, 2011). We then used VCFtools (v0.1.14; Danecek et al., 2011) to retain only loci with data in at least 50% of individuals and loci with a minimum minor allele frequency of 0.03. Individuals that were missing data for 50% or more of these sites were removed from the final data set.

Following this protocol, we generated two VCF files for downstream analysis. The first included all cutthroat trout individuals in our genomic data set to explore divergence between populations via PCA and F_{ST} estimates (n = 236 before filtering). For the second, we included individuals from populations for which we had corresponding morphological data (i.e., excluding South Fork Owl Creek) and included two golden trout populations, Upper and Lower Tayo Lake, to serve as outgroups in the construction of a phylogenetic tree (n = 203 before filtering). We called SNPs independently for these two sets of individuals.

Exploring variation in gill raker morphology

To explore evidence for morphological shifts that are consistent with adaptation in stocked populations, we sought to describe variation in feeding morphology (a) by comparing differences between populations that share similar stocking histories and (b) by comparing morphological differences across lakes. Following the example of previous trout studies (Behnke, 2010; Forbes & Allendorf, 1991; Marnell et al., 1987), we combined counts of the posterior and anterior gill rakers together into a total gill raker count and excluded samples with missing gill rakers from the final analyses. We also excluded undeveloped anterior gill rakers from the total raker counts. In cutthroat trout, as in many other fishes (Foote et al., 1999), gill raker number and the number of gill raker hooks do not increase with increasing fish length (Supplementary Figures S5 and S6), and thus does not require correction for body length and supports previous observations that gill raker number remains stable throughout a trout's life span (Leary et al., 1985). Gill raker length and spacing increase proportionally with fish total length and were thus size corrected for body length

(Kahilainen & Østbye, 2006). Space between gill rakers was calculated by combining values of gill raker width, arch length, and number of gill rakers to estimate the average space between gill rakers for each specimen (Palkovacs & Post, 2008). See Supplementary Material for size-correction equations.

After data cleaning, we used *t*-tests to examine the differences in feeding morphology between populations that were recently stocked, historically stocked, and raised in the hatchery (Figure 2). We classified populations as "Historic" if the most recent stocking event occurred several decades ago (i.e., 47–89 years ago) and populations persist naturally, "Recent" if the stocking of hatchery fish is ongoing (i.e., populations were stocked two to three times between 2000 and 2020) with no evidence for successful natural reproduction and no evidence that previously stocked populations have persisted, or "Hatchery" if obtained directly from a hatchery (Supplementary Table S2). We excluded lakes with uncertain stocking histories, retaining a total *n* = 241 for cutthroat trout and *n* = 128 for golden trout. We performed a Welch's two-sample *t*-test to test differences between each group for both species for four morphological variables that have been associated with increased planktivory: (a) gill raker number, (b) gill raker spacing, (c) number of gill raker hooks, and (d) gill raker length.

Next, we further explored variation in morphological traits among lakes with golden trout and cutthroat trout using one-way ANOVAs and follow-up Tukey's HSD tests (Figure 3). This analysis, focused on individual lakes rather than lakes grouped according to similar stocking histories as above, aimed to examine variation in predicted response among particular populations. We chose to focus these analyses on total gill raker number because it is heritable (Bernatchez, 2004; Glazer et al., 2014; Palkovacs et al., 2014), nonplastic (Behnke, 2010; Glazer et al., 2014), has repeatedly evolved in parallel in lacustrine fish populations (Häkli et al., 2018; Landry et al., 2007; Østbye et al., 2006), and has clearly been linked to feeding efficiency and planktivory (Hessen et al., 1988; Kahilainen & Østbye, 2006; Roesch et al., 2013; Wright et al., 1983). Thus, variation in this trait is likely to represent an adaptive morphological response. For all tests, significance was assessed at an alpha level of *p* = .05. For these analyses, we included lakes with uncertain stocking histories and retained *n* = 264 cutthroat trout (4–48 individuals across 15 populations) and *n* = 128 golden trout (7–29 individuals across 6 populations).

Exploring predictors of gill raker number

We examined the impact of our environmental and dietary predictor variables on our primary response variable of interest—gill raker number—with linear mixed-model (LMM) regression using an AICc-based model averaging approach to examine the impact of predictors on overall model fit (Burnham & Anderson, 1998, Figure 5). We expected time since stocking and variables associated with increased population size, density, or intraspecific competition to predict higher gill raker number since population size and competition favor directional selection and minimize stochasticity from drift (Schluter, 2000). We further hypothesized that
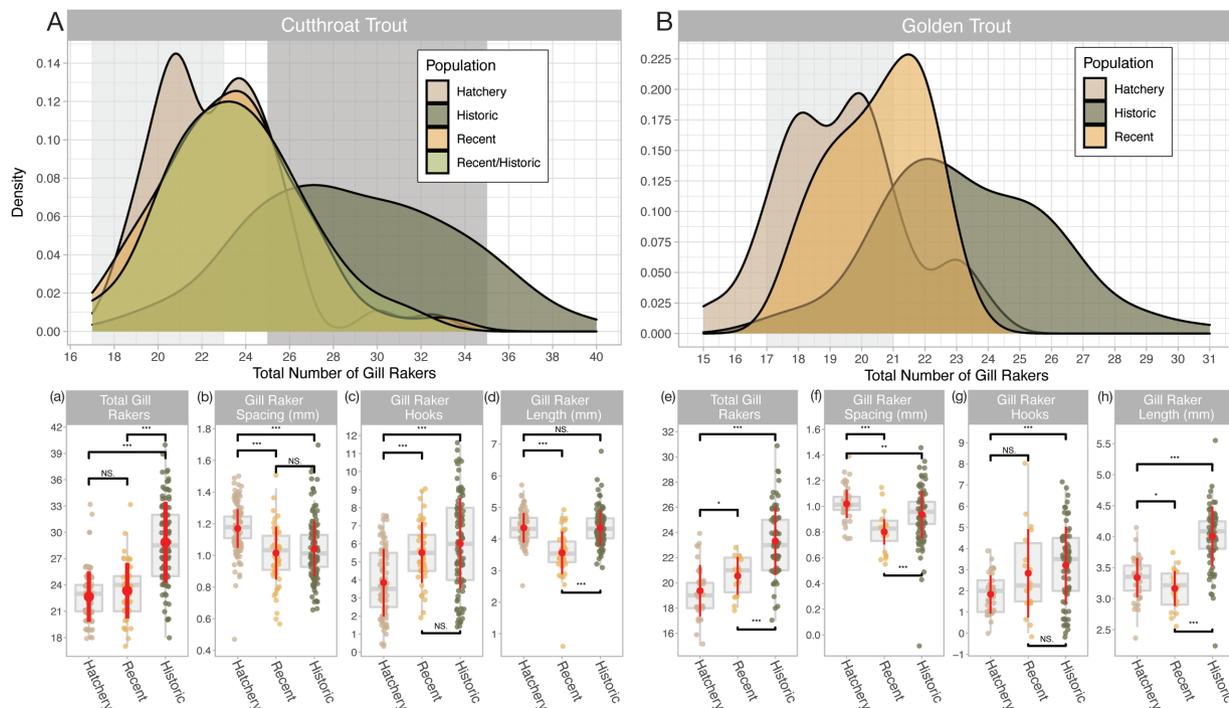

Figure 2. Gill raker number and length values in historically stocked populations suggest adaptation to planktivory. Density distributions of total gill raker number are shown for (A) cutthroat and (B) golden trout. Light gray shading represents ranges reported for typical populations (Behnke, 2010; Gold, 1977). Dark gray shading represents ranges for some lake-adapted populations of Yellowstone cutthroat trout (Behnke, 2010). Morphological differences are shown between pooled hatchery, historically stocked, and recently stocked populations for four morphological variables: number of gill rakers (a, e), gill raker spacing (b, f), number of gill raker hooks (c, g), and length of the longest gill raker (d, h). Red circles and lines represent means and standard deviations. Brackets show significance levels from a Welch's two-sample *t*-test. * Significant difference < .05, **significant difference < .01, ***significant difference < .001.
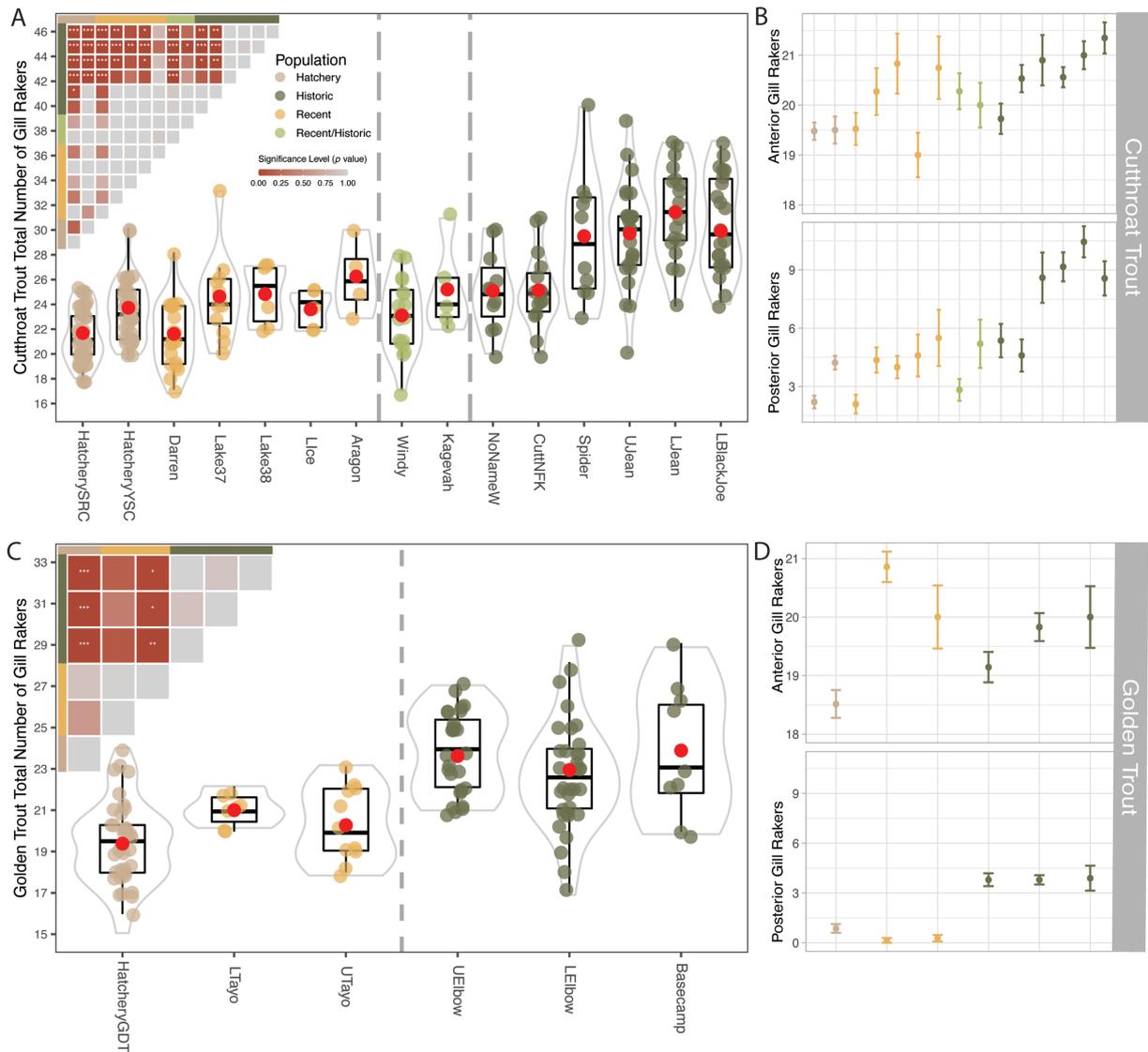
Figure 3. Morphological values in total gill raker count vary among historically stocked lakes and arise from different patterns in anterior and posterior gill raker counts. Among-population differences in total gill raker number are shown for (A) cutthroat trout and (C) golden trout. Red circles indicate population mean values. Results from a Tukey's HSD post hoc analysis on total gill raker count (see Exploring variation in gill raker morphology in Methods) among populations are shown in heatmap format. Populations occur in the same order shown for boxplots. Differences in anterior and posterior gill rakers are also shown for each population (B and D) and underlie the shifts in total gill raker count. *Significant difference < .05, **significant difference < .01, ***significant difference < .001.

the biomass of potentially important zooplankton prey taxa would be positively associated with elevated gill raker counts and expected variables describing alternative sources of prey or resource abundance to negatively predict gill raker number.

Because data for different categories of our predictor variables (i.e., lake-specific environmental differences, zooplankton availability, and population diet composition) were not available for all the lakes in our data set, we subset our data into groups of lakes for which data were available for inclusion in separate LMMs. Data subsets are hereafter referred to as the environmental ($n = 172$ across 13 lakes), dietary ($n = 122$ across 8 lakes), and zooplankton ($n = 150$ across 11 lakes) data sets, respectively (see Supplementary Table S1 for additional information on data availability for each population). For each subset, we excluded highly correlated variables (Pearson correlation coefficient $r > .80$) and log-transformed right-skewed variables to improve the

linearity of the relationship to the response (Gelman & Hill, 2006). Since lake area was an order of magnitude larger than other variable values, it was rescaled using scale() in the base package in R (R Core Team, 2012). See supporting information for additional details.

We fit LMMs for each subset with the lmer() function from the lme4 package in R (Bates et al., 2015) and included all uncorrelated predictor variables as fixed effects and lake as a random effect to account for the grouping of fish into lakes. For all subsets, we visually confirmed that our primary response variable, total number of gill rakers (i.e., anterior + posterior gill rakers), was normally distributed, and we checked model assumptions by visually examining the normality and homogeneity of the fitted model residuals and fit all models with a normal distribution (additional details in supporting material). Using the MuMIn package (Barton, 2012), we then performed Akaike information criterion-based (AICc) model selection to test every combination
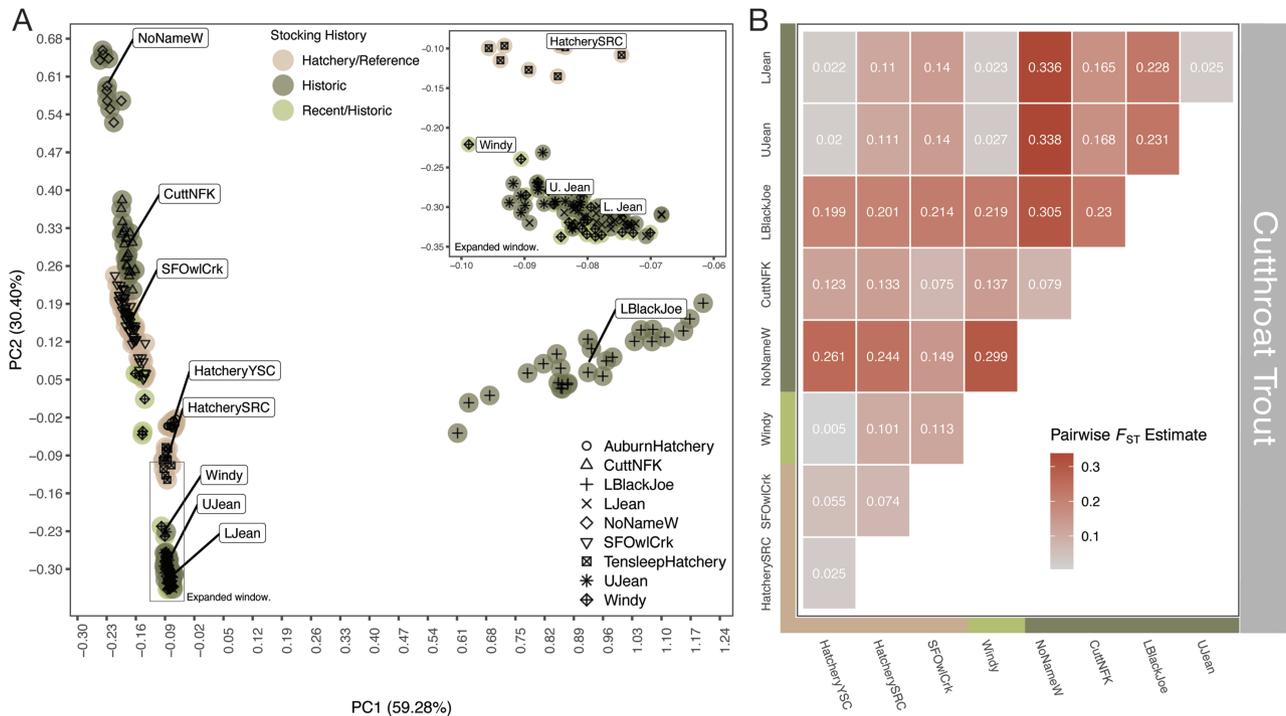
Figure 4. Cutthroat trout populations sampled in the Wind River Range demonstrate population structure and evidence for divergence from several possible source populations. (A) A principal component analysis of SNPs obtained from genotyping-by-sequencing data for all populations where genomic data were available. (B) A heatmap of genomic distance (Reich–Patterson F_{ST} estimates) calculated between all pairs of populations. Yellowstone cutthroat (YSC) and Snake River cutthroat (SRC) hatchery broodstocks along with trout from South Fork Owl Creek (SFOwlCrk), a stream-dwelling population near the Wind River Range, were included as possible source populations.

of predictor variables on model fit (Burnham & Anderson, 1998; Kisel & Barraclough, 2010; Wagner et al., 2012). We then obtained relative importance (RI) scores for each predictor, which is calculated by taking the sum of the Akaike weights (i.e., normalized likelihoods) for each model in which the predictor was included and calculating a proportion that scales from 0 (low predictive power) to 1. AICc scores were compared for models fit by a maximum likelihood estimation (Zuur et al., 2009). Supplementary Table S3 provides a list of top component models derived from the global model for each data subset. All analyses and data visualization were performed in R (R Core Team, 2012) and RStudio (Allaire, 2009).

Exploring genetic population structure and its relationship to morphological variation among lakes

Analysis of population structure

We visually explored population structure using a principal component analysis (PCA) of our sequence data (Figure 4). After filtering (described in the Genomic data collection section), we retained *n* = 220 individuals across 9 populations. We first calculated a genotype covariance matrix of similarity between individuals following the methods described in Klobucar et al. (2021) and Junker et al. (2020) and then performed a PCA on the genotype covariance matrix using the prcomp function in the R stats package (R Core Team, 2012). We quantified genomic variation among populations by calculating pairwise F_{ST} values between each population included in the PCA (Figure 4B). We used the Reich–Patterson F_{ST} estimator (Reich et al., 2009), since it is unbiased for small sample sizes and performs well when

sample sizes are unbalanced (Willing et al., 2012) as is the case with our data. We used 100 bootstrap replicates to estimate 95% confidence intervals for the F_{ST} estimates (Figure 4B; Supplementary Table S5). These methods are similar to those outlined by Junker et al. (2020) and code for this analysis can be obtained from https://github.com/jessicarick/reich-fst.

Accounting for phylogenetic structure and genomic relatedness in Bayesian LMMs

To account for correlation among populations due to shared ancestry, we further tested the relationship between the top predictors from our initial LMM analysis and gill raker number in a series of Bayesian linear mixed models via MCMCglmm (Hadfield, 2010), which included both a phylogenetic tree and a genomic distance matrix in the model as random effects, following methods discussed in Joly et al. (2019). This analysis aimed to complement our initial LMM results by further assessing the relationship between various predictors and feeding morphology after accounting for spurious correlation from relatedness among populations. First, we generated a maximum likelihood phylogenetic tree using RAxML (Stamatakis, 2014), incorporating all populations and individuals for which we had genomic data. We used the Lewis ascertainment bias correction to account for the lack of invariant sites in our SNP data (Leaché et al., 2015; Lewis, 2001) and assessed confidence in this topology with 100 replicates of RAxML's rapid bootstrap algorithm (Stamatakis, 2014). Using the best ML topology from RAxML, we made this tree ultrametric using chronopl() with the lambda parameter set to 0 in the R package ape (Paradis et al., 2019). Next, we pruned
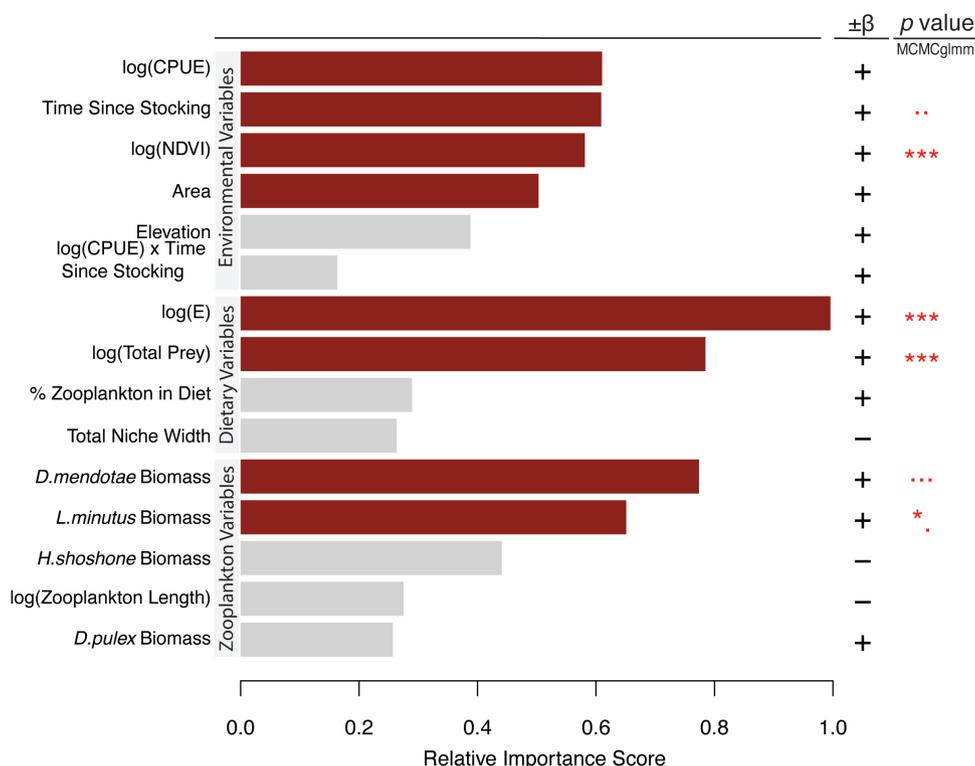
Figure 5. Some environmental and dietary variables predict elevated gill raker number among historically stocked cutthroat trout populations. Environmental variables (*n* = 13 lakes), dietary variables (*n* = 8), and zooplankton abundance variables (*n* = 11) were grouped into separate data subsets based on data availability. For each subset, we specified a global LMM and then performed model selection based on AICc scores and calculated relative importance (RI) based on model weights for each predictor. Predictors with RI > 0.5 were incorporated into Bayesian mixed models (MCMCglmm) to examine their association after correcting for population structure and relatedness. An asterisk (*) is present for each case where the predictor was significant in one of the three repeated Bayesian model runs (MCMCglmm), and a period (.) is present when marginally significant (.05 ≤ *p* ≤ .10). CPUE = catch per unit effort, a proxy for population density. NDVI = normalized difference vegetation index, a proxy lake shore vegetation. *E* = population degree of dietary specialization.

the tree so that each tip represents a population present in our morphological data set. We randomly sampled one individual per population and retained only that individual as a representative of the population. For lakes that were recently stocked with hatchery fish—but for which individually matched genomic data were not available—we randomly sampled an individual from Ten Sleep hatchery in our genomic data set as a representative substitute for that lake. Since not all populations were monophyletic, we repeated the random sampling of individuals three times to generate three distinct pruned trees for each data subset to capture variation that may result from the random selection of different individuals on the original tree. Each tree was incorporated into a separate MCMCglmm run to examine variation in the parameter estimates. For each subset, we also generated a distance matrix between individuals from the available SNP data. We converted the VCF to a DNAbin object with the ape package in R (Paradis et al., 2019) and retained only individuals that matched those in our morphological data set (thus, *n* = 145 for 11 lakes in environmental subset, *n* = 102 for 7 lakes in dietary subset, and *n* = 133 for 9 lakes in zooplankton subset). Again, for recently stocked individuals for which genomic data were not available, we randomly sampled individuals from Ten Sleep hatchery in our genomic data set as representative substitutes. We calculated the distance matrix using the dist.snp() function with the "GENPOFAD" option from the pofadinr package in R (Joly, 2016; Joly et al., 2015,

2019) and scaled it by subtracting the maximum distance value from each value in the matrix (Joly et al., 2019). We then performed a single value decomposition of the matrix to obtain disentangled, uncorrelated values as outlined in Stone et al. (2011) and Joly et al. (2019).

Next, we fit a separate MCMCglmm (Hadfield, 2010) model for each predictor with a Relative Importance score of >0.5 from the LMMs. By incorporating both the phylogenetic tree and the distance matrix as random effect structures, we could explore the relationship between the predictor and response after accounting for trait variance that may result from phylogenetic structure and relatedness (Joly et al., 2019; Stone et al., 2011). We specified uninformative and diffuse inverse-Wishart priors for the fixed and random effects, respectively, and ran the models for 110,000 iterations with an appropriate burn-in of 10,000. We sampled the chain every 30 generations and assessed model convergence by visual inspection of convergence plots.

Results

Variation in gill raker morphology

First, we examined morphological differences across populations classified as recently stocked, historically stocked, or hatchery-raised to assess evidence that meets our expectation for adaptive shifts, especially in historically stocked populations. After data cleaning and filtering, we retained 241 cutthroat

trout (n = 92, 102, and 47 in the hatchery, historic, and recently stocked categories, respectively) and 128 golden trout (n = 37, 73, and 18 in the hatchery, historic, and recently stocked categories). In cutthroat trout, we observed strong evidence to support our expectation for adaptation since we observed increased gill raker number in the historic populations when compared to both the hatchery (Δμ = 6.2, p < .001) and recently stocked populations (Δμ = 5.6, p < .001; Figure 2, Table 1). We also found evidence that gill raker spacing and number of gill raker hooks differed between historic and hatchery populations in the direction we expect for adaptive shifts (Δμ = –0.13 mm, p < .001 and Δμ = 2.16, p < .001, respectively); however, there was no evidence that these traits differed between recently stocked and historic populations (Δμ = –0.02 mm, p = .4 and Δμ = 0.05, p = .2, respectively). Furthermore, there was no evidence that raker length differed between hatchery and historic populations (Δμ = 0 mm, p = 1.0), but we found evidence that raker length was longer in historically stocked populations when compared only to the recently stocked populations, which was again partly consistent with our expectations for adaptation (Δμ = 0.79 mm, p < .001; Figure 2, Table 1).

In golden trout, we again found strong evidence, consistent with our expectations for adaptation, that gill raker number was higher in the historic populations when compared with both recently stocked (Δμ = 2.7, p < .001) and hatchery (Δμ = 3.9, p < .001) populations (Figure 2; Table 1). However, while we observed shifts in both gill raker spacing (Δμ = –0.08 mm, p = .004) and gill raker hooks (Δμ = 1.37, p < .001) between hatchery and historic populations that were consistent with our predictions, gill raker spacing was greater (Δμ = 0.14 mm, p < .001) and the number of raker hooks was higher (Δμ = 0.38, p = .5) in historic populations compared with recently stocked populations. Finally, we found strong evidence that historically stocked golden trout have substantially longer gill rakers than those from hatchery (Δμ = 0.66 mm, p < .001) or recently stocked populations (Δμ = 0.84 mm, p < .001; Figure 2; Table 1), which again was consistent with our expectations for adaptation to planktivory in alpine lakes.

We also examined the difference among all populations sampled in the field with one-way ANOVAs to further examine variation among lakes. For this analysis, we included all lake and hatchery populations (also including lakes with uncertain stocking histories) and retained 264 cutthroat trout (4–48 individuals per population across 15 populations) and 128 golden trout (7–29 individuals per population across 6 populations), and found significant differences between groups for both cutthroat trout $F(14, 211)$ = 18.9, p < .001 and golden trout $F(5,428)$ = 14.9, p < .001, consistent with our expectations for adaptive shifts. Results from a Tukey's HSD test for multiple comparisons between groups are summarized in Figure 3. We also found notable variation among historic cutthroat populations (Figure 3A) since Lower Black Joe Lake, Lower Jean Lake, Upper Jean Lake, and Spider Lake all exhibit an increase in total gill raker number while No Name (W) Lake and Cutthroat (NFK) Lake do not follow this trend.

Predictors of gill raker number

In our model, selection-based evaluation of environmental predictors on gill raker number (environmental variables subset; n = 172 with 4–25 individuals across 13 lakes), all predictors averaged across component models were positively associated with gill raker number (Table 2). Several of the top component models were indistinguishable from one another (ΔAIC < 2; Burnham & Anderson, 1998), suggesting weak power to determine key predictors from only one top model. We calculated RI scores for all predictors and found that catch per unit effort (CPUE), time since stocking, NDVI, and lake area had the highest RI scores and were thus the most frequently associated with the top-ranking models (Figure 5).

Within the subset of lakes where we examined dietary data (dietary variables subset; n = 122 with 5–25 individuals across 8 lakes), individual diet specialization (E) and total prey count emerged as the predictors with the highest RI scores (Table 2; Figure 5).

Table 1. Summarized values and Welch's two-sample t-test results for morphological comparison between hatchery, recently stocked, and historically stocked populations.

	Hatchery		Recent		Historic		T-test p-values	T-test p-values	T-test p-values
	Mean	SD	Mean	SD	Mean	SD	Ha.–Re.	Re.–Hi.	Ha.–Hi.
Cutthroat trout									
Gill rakers	22.7	2.87	23.3	3.17	28.9	4.6	.2	<.001***	<.001***
Gill raker spacing	1.17	0.12	1.02	0.17	1.04	0.18	<.001***	.4	<.001***
Gill raker hooks	3.85	1.87	5.51	1.68	6.01	2.51	.0007***	2	<.001***
Gill raker length	4.35	0.47	3.56	0.67	4.35	0.56	<.001***	<.001***	1
Golden trout									
Gill rakers	19.4	2.05	20.6	1.5	23.3	2.7	.02*	<.001***	<.001***
Gill raker spacing	1.02	0.11	0.8	0.1	0.94	0.18	<.001***	.001***	.004**
Gill raker hooks	1.84	0.91	2.83	2.1	3.21	1.82	0.07	.5	<.001***
Gill raker length	3.34	0.32	3.16	0.28	4	0.48	0.05*	<.001***	<.001***

Note. Ha. = Hatchery; Hi. = Historic; Re. = Recent. For each population, sample mean and standard deviation (*SD*) for total gill raker number are shown.
* Significant difference < .05,
** significant difference < .01,
*** significant difference < .001.

Table 2. Model averaged coefficients and diagnostics.

Model predictors	LMM parameters and diagnostics				MCMCglmm diagnostics	
	RI	β	SE	*p*-value	DIC	*p*-value
Environmental variables						
Log(CPUE)	0.61	0.836	1.62	0.61	784.0 (784.3–784.5)	.816 (.814–.819)
Time since stocking	0.61	0.034	0.028	0.23	785.0 (784.9–785.0)	.092 (.079–.101)
Log(NDVI)	0.58	5.770	4.00	0.15	780.1 (780.1–780.1)	.013 (.013–.014)[*]
Lake area	0.50	1.270	0.954	0.19	789.9 (789.5–790.4)	.744 (.730–.760)
Lake elevation	0.39	0.004	0.027	0.87	—	—
Log(CPUE) × time since stocking	0.16	0.039	0.026	0.15	—	—
Dietary variables						
Individual specialization (*E*)	1.00	27.12	4.441	<0.001[***]	143.0 (140.4–145.9)	.002 (.002–.003)[**]
Log(total prey count)	0.78	0.456	0.205	0.028[*]	189.1 (175.3–202.7)	.003 (.002–.003)[**]
% pelagic prey consumed	0.29	0.638	1.085	0.56	—	—
Population total niche width	0.26	–0.350	0.975	0.72	—	—
Zooplankton abundance variables						
D. mendotae biomass	0.77	19.53	8.176	0.018[*]	685.2 (685.1–685.3)	.08 (.076–.085)
L. minutus biomass	0.65	1.14	0.585	0.053	684.0 (678.8–691.9)	.096 (.022–.177)
H. shoshone biomass	0.44	–2.11	1.595	0.19	—	—
Log(lake averaged zooplankton length)	0.28	–0.41	2.485	0.87	—	—
D. pulex biomass	0.26	0.005	1.15	1.00	—	—

Note. For the LMMs, reported conditional averages for model parameter estimates (β), standard errors (*SE*), and *p*-values were calculated across all models where the predictor is present. RI scores were calculated for each predictor from model weights based on AICc scores. For all models, the response variable is the number of total gill rakers. Separate global models were specified for environmental, dietary, and zooplankton variables based on data availability, and model selection was performed for each data subset (*n* = 13, 8, and 11 lakes, respectively). Additional MCMCglmm analyses were performed for each predictor with an RI score > 0.50. A phylogenetic tree pruned to the correct number of populations and a genomic relatedness matrix calculated from genome-wide SNPs were included for each MCMCglmm run as random effects to account for phylogenetic structure and genomic relatedness. MCMCglmm runs were repeated three times for each predictor with a different randomly sampled phylogenetic tree to capture variation within the tree. Mean *p*-value and DIC values are reported first followed by the estimated parameter range in parentheses. CPUE = catch per unit effort, a proxy for population density; DIC = deviance information criterion; LMM = linear mixed-model; NDVI = normalized difference vegetation index; RI = relative importance.
[*] Significant difference < .05
[**] significant difference < .01
[***] significant difference < .001.

In our final model, which incorporated zooplankton density variables as predictors (zooplankton variables subset: *n* = 150 with 5–25 individuals across 11 lakes), the biomass of the two smaller-bodied zooplankton species in our data set (*D. mendotae* and *L. minutus*) emerged as stronger predictors of elevated gill raker number than the two relatively larger-bodied species (*D. pulex* and *H. shoshone*). Again, several top models in this model subset were closely ranked (see Supplementary Table S3).

Analysis of population structure

We found evidence for substantial genetic divergence among the populations of cutthroat trout (after filtering *n* = 220 with 11–58 individuals across 9 populations). Most populations within our data set separated into distinct clusters by lake or by hatchery on both PC1 and PC2 which explained 59.28% and 30.40% of the variation, respectively (Figure 4A). Several historic populations, including Lower Black Joe Lake, No Name (West) Lake, and Cutthroat (NFK) Lake, were highly divergent from both each other and from the Yellowstone cutthroat trout and Snake River cutthroat trout hatchery populations that we included as references (Figure 4A). Lower Black Joe Lake was especially divergent from all other populations. Notably, Windy Lake—which has been stocked on multiple

occasions with fish from different origins (Supplementary Table S2, M. M. Mazur, U.S. Fish & Wildlife Service Fish Biologist, personal communication)—had substantial differentiation among individuals within the population, with some specimens clustering with the South Fork Owl Creek population, some with the Upper and Lower Jean Lakes, and some in closer proximity to the Ten Sleep Hatchery brood stock (Figure 4A). No Name (W) Lake and Cutthroat (NFK) Lake also clustered more closely to Yellowstone cutthroat trout reference samples from South Fork Owl Creek than the Ten Sleep Hatchery fish (i.e., the LeHardy Rapids broodstock).

The F_{ST} estimates calculated between all pairs of populations in our data set further supported the distinctiveness of each population and the strong differentiation between historically stocked populations (Figure 4B; Supplementary Table S5). Again, Lower Black Joe Lake was strongly differentiated from other populations, and our pairwise F*ST* estimates between all populations—except between Windy Lake (recently stocked) and the Yellowstone cutthroat trout hatchery population—were significantly different from zero (Supplementary Table S5), with population mean estimates of F_{ST} ranging from 0.005 to 0.336. Notably, we also observed significant differences between the two subspecies of trout, Yellowstone cutthroat trout (i.e., the LeHardy Rapids brood stock from Ten Sleep Hatchery), and Snake

River cutthroat trout from Auburn Hatchery (0.025, 95% CI: 0.02–0.03).

Accounting for phylogenetic structure and genomic relatedness in Bayesian LMMs

After incorporating variance from phylogenetic structure and genomic relatedness, we found evidence that NDVI explains variation in gill raker number (mean p-value = .013, mean deviance information criterion [DIC] = 780.1; Table 2; Figure 5). We also found some evidence that time since stocking drives elevated gill raker number, since time since stocking was marginally significant across runs (mean p-value = .092, mean DIC = 785.0; Table 2; Figure 5). We found strong evidence that E value (i.e., the degree of among individual specialization in the population) and total prey counts predict gill raker number (mean p-value = .002, mean DIC = 143.0 and mean p-value = .003, mean DIC = 189.1, respectively; Table 2; Figure 5). Finally, we found some evidence that the presence of *D. mendotae* and *L. minutus* was associated with increased gill raker number (mean p-value = .080 and .096, mean DIC = 685.2 and 684.0, respectively; Table 2) since these variables were marginally significant across model runs. However, there was no evidence that lake area or CPUE (mean p-value = .744 and .816, mean DIC = 789.9 and 784.0, respectively; Table 2; Figure 5) predicts gill raker number in these models. We again used environmental (n = 145 with 4–22 individuals per lake across 9 lakes), dietary (n = 102 with 5–22 individuals per lake across 7 lakes), and zooplankton (n = 133 with 7–23 individuals per lake across 9 lakes) data subsets, but for these analyses retained only individuals for which we could evaluate both genomic and phenotype data, as described above (see Supplementary Table S1).

Discussion

Global rates of anthropogenic disruption make it critical to document evolutionary responses to changing ecosystems. Here we document such a response in trout after they are stocked into a novel environment where dynamic ecological interactions with prey likely shape selective pressure. We find that naturally reproducing populations of both golden and cutthroat trout in historically stocked lakes have higher numbers of gill rakers when compared to hatchery or recently stocked populations. We also find that gill rakers are longer in historically stocked golden trout populations. Since there is clear evidence that trout introduction causes major shifts in alpine lake prey resources in this system of alpine lakes (Boyle, 2021) and in many others (Anderson, 1980; Bradford et al., 1998; Carlisle & Hawkins, 1998; Donald et al., 2001; Knapp et al., 2001; Schabetsberger et al., 2009; Stoddard, 1987), we view this as a likely example of an eco-evolutionary feedback, with rapid adaptation driven by the introduction of trout themselves.

The case for rapid adaptive evolutionary change

Our interpretation that these morphological changes are an example of rapid adaptive evolution rests on several assumptions, including that (a) gill raker traits are heritable, (b) morphological differences arose within lakes after stocking, (c) morphological changes are adaptive and driven by predator-induced shifts in prey availability, and (d) morphological

shifts cannot be explained by drift alone but arise from selective pressure within lake populations. We explore these assumptions in this system below.

Adaptation requires that the variation in gill raker traits is heritable. Although we did not directly test heritability, gill raker number is heritable in many fishes, including salmonids (Bernatchez, 2004; Foote et al., 1999; Funk et al., 2005; Glazer et al., 2014; Hagen, 1973; Hessen et al., 1988; Leary et al., 1985; Østbye et al., 2005; Palkovacs et al., 2014; Schluter, 2000). Gill raker number also remains constant throughout the trout life span, suggesting that plasticity does not extensively shape variation in this trait. Thus, while plasticity does not preclude heritability, these observations suggest that morphological shifts in this system are likely due to the evolution of heritable gill raker traits rather than plastic responses to environmental differences.

Second, trait adaptation requires that morphological shifts occurred in situ after stocking rather than reflecting the morphology of introduced stocks. This is unambiguous for golden trout, since the Wind River populations trace their origins directly to the Kern River in California and display elevated gill raker number and length in comparison to current broodstocks (Behnke, 2010; Gold, 1977). For cutthroat, this is difficult to address because stocking sources are poorly documented and cutthroat stocks originating from Yellowstone Lake—which may exhibit elevated gill raker counts—were common in the early to mid-20th century (WGFD, personal communication; Behnke, 2010). However, the current LeHardy Rapids cutthroat broodstock, which exists in close and unobstructed geographic proximity to Yellowstone Lake, does not have elevated gill raker counts (Burningham et al., 2002). Thus, not all populations of trout associated with Yellowstone Lake exhibit lake-adapted morphology even if they were historically stocked directly from the lake. Further investigating this variation will require extensive genetic and morphological sampling of the Yellowstone Lake population and geographically proximate populations.

We also observe large genetic divergences among historic populations that suggest at least three distinct population sources. In our PCA, Lower and Upper Jean Lakes have close genetic affinity to the LeHardy Rapids broodstock, which presumably resembles the Yellowstone Lake population genetically. No Name (W) and Cutthroat (NFK) Lake, which do not exhibit elevated gill raker counts, cluster more closely to stream-dwelling cutthroat from South Fork Owl Creek. And Lower Black Joe Lake, the most highly divergent population, likely represents progeny from a third unsampled source. Finis Mitchell—an iconic guide in the Wind River Range during the early-to-mid 20th century—was the first to stock this lake around 1930 (Mitchell, 1975, p. 10). While the source of these fish is unknown, it is possible that he at times used Colorado cutthroat trout (*Oncorhynchus clarki pleuriticus*) as well as Yellowstone cutthroat trout from local hatcheries (Mitchell, 1975; M. M. Mazur, personal communication). Colorado cutthroat trout, which are native to the nearby Green River drainage, generally exhibit similar gill rakers counts as stream-dwelling Yellowstone cutthroat trout (Trotter, 2008) and are often difficult to distinguish from them phenotypically. Future genetic work incorporating Colorado cutthroat trout samples will be useful in better understanding the origins of this interesting population. Lastly, it is notable that the difference between many of these historic populations is greater than that

between the Yellowstone and Snake River cutthroat trout broodstocks, which are currently classified as separate subspecies (Behnke, 2010).

Third, our conclusion that morphological shifts connote adaptive evolution requires that these shifts aid in the retention of small planktonic prey, which may increase foraging efficiency and fitness in alpine lakes. Although we are not aware of studies investigating planktivory and feeding morphology in cutthroat or golden trout, increased gill raker number and length have been associated with planktivory in lake trout (*Salvelinus namaycush*, Martin & Sandercock, 1967), and gill raker number has been linked to feeding efficiency in other salmonid species (Amundsen et al., 2004; Hessen et al., 1988; Kahilainen et al., 2011; Langeland & Nøst, 1995; Roesch et al., 2013).

However, the increase in gill raker number that we documented is largely due to an increase in the posterior gill rakers, and their position casts doubt as to whether they function as direct sieves for zooplankton. In other studies where planktivory is associated with morphological evolution, the species are often obligate planktivores (e.g., Palkovacs et al., 2014), and only anterior gill raker counts are observed or recorded (Amundsen et al., 2004; Häkli et al., 2018; Palkovacs et al., 2014). In contrast, trout are dietary generalists and have short, sparse gill rakers. However, trout taxonomists routinely consider posterior gill rakers in their classification of trout subpopulations and they likely play an important—although understudied—role in feeding, local adaptation, and prey retention (Behnke, 2010; Leary et al., 1985; Ostberg et al., 2011). Posterior gill rakers, and other bony protrusions such as basibranchial teeth, are also elevated in lake-adapted populations of cutthroat (Behnke, 2010; Marnell et al., 1987), suggesting that a general increase in bony protuberances throughout the buccal cavity may aid with retention of small prey. This alternate retention mechanism may explain why we did not observe shifts in gill raker spacing in historic lakes, as has been previously documented in planktivorous species (e.g., Palkovacs et al., 2014). These findings mirror those by Langeland and Nøst (1995) who also conclude that gill raker spacing does not underlie zooplankton retention in salmonids and may suggest alternate paths to adaptive response in trout species compared to planktivorous fishes.

Fourth and finally, our conclusions require that morphological shifts arose from selective pressure rather than phenotypic drift alone. Documenting elevated numbers of gill rakers in multiple historically stocked populations for both cutthroat trout and golden trout strongly suggests parallel evolution rather than random variation due to drift. Although there is some question as to whether shifts in some cases for cutthroat trout may be related to the source populations rather than in situ shifts; in golden trout the case for independent origins is stronger and we find support for not only gill raker number, but also gill raker length.

The role of environmental predictors

Although the overall trend among historically stocked cutthroat trout lakes is for evolutionary shifts to increased gill raker number, responses varied and not all lakes demonstrated this pattern. When we examined predictors of this variation, we found some evidence that NDVI, the degree of dietary specialization within the population (*E*), and total prey counts

predicted gill raker number even after incorporating variation from population structure in our models. We also found some evidence that the time since stocking and the biomass of *D. mendotae* and *D. minutus* play a role in this variation.

Several of these variables suggest that competition or resource availability may shape selection in these lakes (Bolnick, 2004; Schluter, 2003). NDVI broadly quantifies the amount of primary productivity of alpine vegetation around the lake (Carlson et al., 2017; Testolin et al., 2020) and likely serves as a proxy for increased aquatic biomass, nutrient availability, and prey abundance. Although this link is speculative, NDVI frequently serves as a proxy for terrestrial biomass and productivity and has been linked to lake photosynthetic efficiency when lake surface reflectance is measured with handheld devices (Peñuelas et al., 1993; Wang et al., 2004). Lakeshore development and vegetation have also been used in other contexts to infer the productivity of resident fish and invertebrates (Peterlin & Urbanič, 2013; Schindler et al., 2000). Thus, lakes with high NDVI may harbor populations that have greater access to primary resources and are thus more resilient to environmental stressors and population persistence across time. This connection between resource use and stability (approximated very broadly here by NDVI) may underlie the capacity for reciprocal evolution between interacting zooplankton and fish communities. More broadly, this finding may illuminate the delicate connection between the strength of selection (strong enough to precipitate change but not strong enough to drive extirpation) and substrate required for natural selection to take place (adequate abundance of zooplankton prey species and sufficient trout population size). However, we emphasize that this connection is tenuous and requires further study.

Furthermore, *E* value measures the tendency for individuals to subdivide a population's total available resources and may thus reflect important differences in the level of intraspecific competition, resource utilization, or resource availability among populations (Bolnick et al., 2002, 2003, 2007). If a sizeable portion of individuals in a population of specialists relies heavily on zooplankton to reduce intraspecific competition, then natural selection acting on those individuals may drive stronger positive selection for morphological adaptation throughout the population than would be the case in a population composed of generalists with flexible dietary patterns. Indeed, the historic lakes that do not show elevated raker counts have lower values of *E* (*E* = 0.64–0.66) than historic lakes that do exhibit elevated gill raker counts (*E* = 0.66–0.77). Thus, individual specialization may arise in some well-established and naturally reproducing populations as a means to reduce intraspecific competition (Bolnick et al., 2003) and may drive selection for increased gill raker number. However, it is additionally possible that specialization arose only after selection for increased gill raker number and, while we cannot disentangle cause from correlation at present, we highlight this as a promising direction for future study. Lastly, we found evidence that total prey count was positively associated with elevated gill raker number. As with the relationship between gill raker number and NDVI, this may suggest that ecosystems with abundant prey (or successful predators) are more likely to have experienced adaptive shifts associated with eco-evolutionary interactions.

Although we found limited evidence that population density (i.e., CPUE) explained variation in gill raker number, it

is possible that past oscillation in population density—rather than present population sizes—have shaped morphological variation over time. Similarly, although time since stocking predicted morphological variation in the LMM analysis, we found less evidence for time since stocking as a strong predictor of gill raker number after incorporating variation from phylogenetic structure. In these lakes, shifts to elevated gill raker numbers have occurred in some populations after only 47 years (e.g., Spider Lake) and not in others, even after 88 years (e.g., Cutthroat NFK Lake). This suggests that factors shaping selection are likely more complex than the direct relationship between time since stocking and gill raker number or that selection on feeding morphology is not strong in some lakes. Lastly, we note that our follow-up MCMCglmm analysis of high RI predictors included a reduced subset of data than our initial mixed-model analysis since we only included individuals for which we could incorporate genomic data. Thus, the difference in results between these analyses may reflect the mismatch in data availability.

Significance and future directions

In summary, we find some evidence for the adaptation of trout feeding morphology to a novel, resource-poor alpine ecosystem. We have also discussed the results of our study in the context of a complex and dynamic ecosystem and documented an example of predator evolution putatively shaped by predator-induced ecosystem restructuring (Brooks & Dodson, 1965; Matthews et al., 2016; Palkovacs & Post, 2008; Strauss, 2014). A relevant contemporary question in conservation biology is whether organisms can adapt rapidly enough to cope with anthropogenically induced habitat changes (Catullo et al., 2019), especially within small, isolated populations (Lo Cascio Sætre et al., 2017). It is also unclear how frequently rapid evolution is essential to the structure and function of natural ecosystems (Ellner et al., 2011; Reznick et al., 2019). By documenting likely adaptive shifts in multiple small, isolated, stocked populations, we provide further insights into these questions and present evidence that eco-evolutionary interactions play a critical role in ecosystem ecology.

We have also documented morphological shifts consistent with evolution toward a lake-dwelling ecotype of cutthroat trout in some stocked populations (Behnke, 2010; Foote et al., 1999). Yellowstone cutthroat trout naturally occur as both stream-adapted (17–23 total gill rakers) and lake-adapted ecotypes (25–37 total gill rakers) across their geographic range (Behnke, 2010), suggesting that local adaptation may actively shape these foraging traits and that morphological shifts may have arisen in parallel as isolated populations colonized novel lakes. To our knowledge, previous studies have not sought to examine such evolutionary transitions within contemporary timescales. Future studies are thus needed to explore whether other lake-adapted ecotypes of trout have rapidly developed elsewhere after stocking and how natural variation within locally adapted source populations provides the standing genetic or morphological variation for subsequent rapid adaptation in the face of novel environmental pressures.

Finally, these findings have important implications for understanding the capacity of even small founding populations to respond to novel ecological and evolutionary pressures in the face of rapid environmental change. Furthermore, the complexity of our observations provides insight into the pattern and timing of adaptive change that follows from anthropogenic interference and eco-evolutionary dynamics and informs our understanding of the role that evolutionary rescue may play in facilitating adaptation to dynamic, resource-poor environments.

Supplementary material

Supplementary material is available online at *Evolution* (https://academic.oup.com/evolut/qpad059).

Data availability

Associated data and scripts have been made available from the Dryad Digital Repository at https://doi.org/10.5061/dryad.8931zcrv4 (Combrink et al., 2023). Sequence data have been submitted to the GenBank databases under BioProject accession PRJNA906988.

Author contributions

L.L.C., C.E.W., A.W.W., and A.C.K. designed the research study. L.L.C., C.E.W., A.W.W., E.G.M., and A.C.K. obtained funding. L.L.C., L.J.B., A.W.W., A.C.K., C.E.W., J.A.R., E.G.M., and W.C.R. assisted with field work, data collection, and laboratory analysis. L.L.C. analyzed the data. L.L.C. wrote the article with input from all authors.

Conflict of interest: The authors declare no conflict of interest. Editorial processing of the manuscript was done independently of CEW, who is an associate editor of Evolution.

Acknowledgments

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