

Allopatric and sympatric diversification within roach (*Rutilus rutilus*) of large pre-alpine lakes

Jessica M. Rieder^{1,2,3}  | Pascal Vonlanthen^{1,2,4} | Ole Seehausen^{1,2} | Kay Lucek^{1,2,5} 

¹Division of Aquatic Ecology and Evolution, Institute of Ecology and Evolution, University of Bern, Bern, Switzerland

²Eawag Swiss Federal Institute of Aquatic Science and Technology, Department of Fish Ecology and Evolution, Center of Ecology, Evolution, and Biogeochemistry, Kastanienbaum, Switzerland

³Centre for Fish and Wildlife Health, Department of Infectious Diseases and Pathobiology, University of Bern, Bern, Switzerland

⁴Aquabios GmbH, Cordast, Switzerland

⁵Department of Environmental Sciences, University of Basel, Basel, Switzerland

Correspondence

Ole Seehausen, Division of Aquatic Ecology and Evolution, Institute of Ecology and Evolution, University of Bern, Baltzerstrasse 6, 3012 Bern, Switzerland.
Email: ole.seehausen@eawag.ch

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Abstract

Intraspecific differentiation in response to divergent natural selection between environments is a common phenomenon in some lineages of northern freshwater fishes, especially salmonids and stickleback. Understanding why these taxa diversify and undergo adaptive radiations while most other fish species in the same environments do not, remains an open question. The possibility for intraspecific diversification has rarely been evaluated for most northern freshwater fish species. Here, we assess the potential for intraspecific differentiation between and within lake populations of roach (*Rutilus rutilus*)—a widespread and abundant cyprinid species—in lakes in which salmonids have evolved endemic adaptive radiations. Based on more than 3,000 polymorphic RADseq markers, we detected low but significant genetic differentiation between roach populations of two ultraoligotrophic lakes and between these and populations from other lakes. This, together with differentiation in head morphology and stable isotope signatures, suggests evolutionary and ecological differentiation among some of our studied populations. Next, we tested for intralacustrine diversification of roach within Lake Brienz, the most pristine lake surveyed in this study. We found significant phenotypic evidence for ecological intralacustrine differentiation between roach caught over a muddy substrate and those caught over a rocky substrate. However, evidence for intralacustrine genetic differentiation is at best subtle and phenotypic changes may therefore be mostly plastic. Overall, our findings suggest roach can differ between ecologically distinct lakes, but the extent of intralacustrine ecological differentiation is weak, which contrasts with the strong differentiation among endemic species of whitefish in the same lakes.

KEYWORDS

postglacial range expansion, RADseq, resource polymorphism, *Rutilus rutilus*, stable isotopes

1 | INTRODUCTION

Much of the existing species diversity of freshwater fish in northern climate zones is of recent origin, having evolved since the end of the last glaciation period ~12 kyrs ago (Hewitt, 1999; Seehausen & Wagner, 2014). During the invasion of newly available freshwater

bodies and associated range expansion, many fish species colonized a variety of different environments and as a result may have experienced competitive release that may have triggered adaptive diversification (Bolnick, Svanbäck, Araujo, & Persson, 2007; Stroud & Losos, 2016). Divergent selection between habitats frequently led to the emergence of ecologically distinct populations, ecotypes

and species. Divergence of populations may, for example, occur as a response to different predation regimes (Scharnweber et al., 2013; Walsh & Reznick, 2009), different parasites (Karvonen & Seehausen, 2012), different feeding regimes (Jonsson & Jonsson, 2001; Schluter, 1996; Svanbäck & Eklöv, 2004) or as a response to interactions among several of these and other variables (Seehausen & Wagner, 2014). The same factors when combined with intraspecific competition may also drive intraspecific diversification in sympatry (Dieckmann & Doebeli, 1999; Gavrillets, 2004; Rosenzweig, 1978; Svanbäck & Bolnick, 2007), for example within a lake where ecologically distinct individuals may occupy different niches. Such intralacustrine diversification of fish has received an ample amount of interest to study adaptive radiation (Bolnick & Fitzpatrick, 2007; Schluter, 1996; Seehausen & Wagner, 2014). Evidence for intraspecific sympatric diversification and adaptive radiation among temperate freshwater fishes is, however, restricted to relatively few taxonomic groups, particularly salmonids and a few cases of three-spined stickleback (*Gasterosteus aculeatus*) (Seehausen & Wagner, 2014). These are classic examples of adaptive radiations, that is the diversification of a single taxon into phenotypically, ecologically and genetically differentiated populations or ultimately species (Schluter, 2000). Comparatively, few studies have explored taxa beyond these classical cases to better understand why some fish taxa

form adaptive radiations, whereas others do not, and therefore, a study bias cannot be ruled out (reviewed in Seehausen & Wagner, 2014). Comparative investigations of other common taxa are consequently needed.

Cases of intralacustrine diversification in temperate freshwater fish often involve differentiation along a pelagic–benthic axis, leading to the evolution of sympatric planktivorous pelagic and benthivorous benthic species (Seehausen & Wagner, 2014). A second axis of diversification includes segregation along depth gradients such as in Arctic charr (*Salvelinus alpinus*; Jonsson & Jonsson, 2001) or whitefish (*Coregonus* sp.; Vonlanthen et al., 2009). The range and discreteness of vacant niches and available food resources in an ecosystem may determine the number of resource-specific ecotypes that can evolve (Lucek, Kristjánsson, Skulason, & Seehausen, 2016; Nosil & Sandoval, 2008; Wagner, Harmon, & Seehausen, 2014). In the case of intraspecific diversification, adaptive phenotypic differentiation may initially emerge through divergent selection on standing genetic variation (Barrett & Schluter, 2008), phenotypic plasticity or a combination of both (Lucek, Sivasundar, & Seehausen, 2014; Schluter, 2000; Smith & Skulason, 1996). Plasticity can initially promote differentiation (Pfennig et al., 2010; Snorrason & Skulason, 2004), and depending on the stability of the selective regime, divergent phenotypes may become genetically fixed through phenotypic

FIGURE 1 (a) Map of Switzerland with the sampled lakes indicated: (A) Geneva, (B) Joux, (C) Brenet, (D) Neuchatel, (E) Brienz, (F) Hallwil and (G) Walen (see Table 1 for details). (b) Roach (*Rutilus rutilus*) with the 11 morphological landmarks used: (1) anterior tip of snout, (2) anterior tip of lower lip, (3) anterior and (4) posterior point of operculum, (5) junction where the dorsolateral part of the head and body fuse, anterior insertion points of the (6) pectoral, (7) pelvic, (8) anal fin, (9) ventral and (10) dorsal junction of the caudal peduncle and tail, and (11) anterior insertion of the dorsal fin. [Correction added on 26 September 2019, after first online publication: the correct Figure 1 file has been added to this version.]

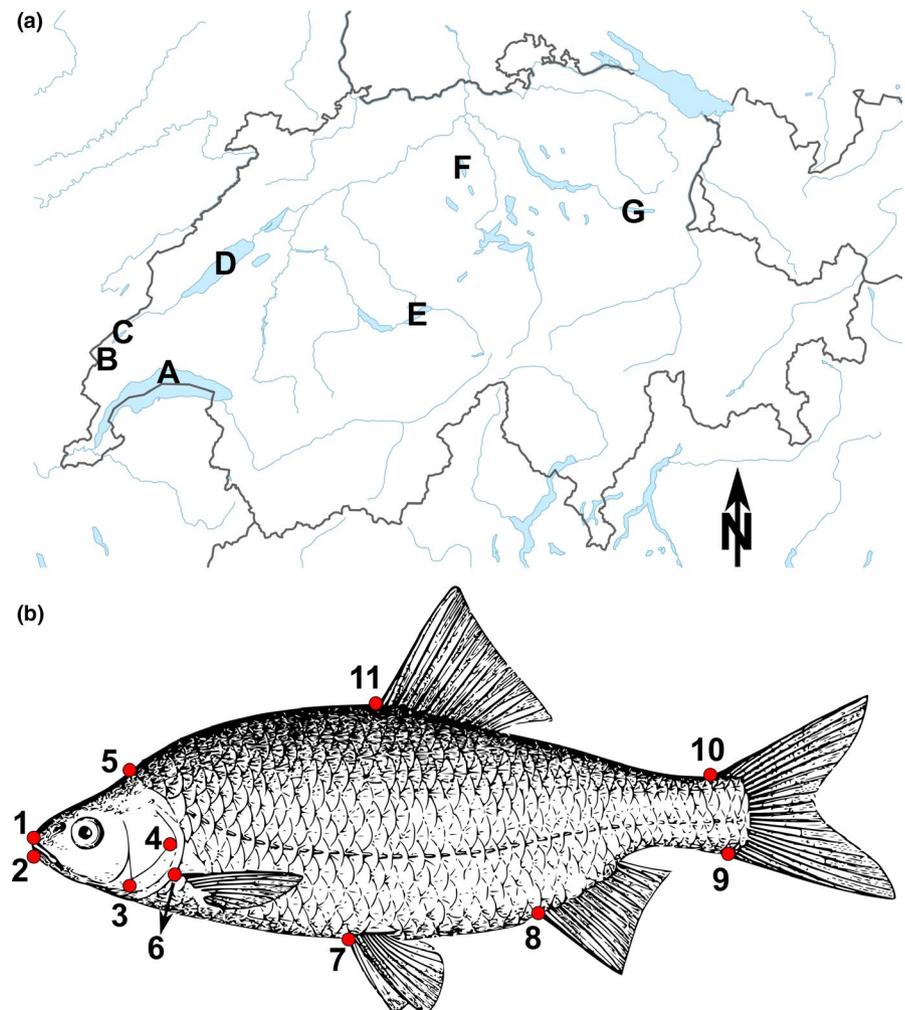


TABLE 1 Characteristics of each sampled lake and the sample size of each data set. For each lake, the coordinates, the trophic status based on dissolved phosphate (parts per million—ppm), its elevation and maximal depth are given. In addition to the sampling date, depth range where individuals were sampled is indicated. Samples sizes for morphology, genomics and stable isotopes are provided. Phosphate levels are based on measurements taken in 2002

Lakes	Geographic coordinates		Trophic status			Numbers of samples					
	Latitude	Longitude	Phosphate (ppm)	Trophic level	Elevation (m)	Maximum depth (m)	Sampled	Depth range (m)	Morphology	Genomics	Stable isotopes
Brienz	47°48'E	45°49'N	3	Oligotrophic	564	260	Sept 2011	1.0–12.0	190	41	28
Brenet	6°19'E	46°40'N	29	Eutrophic	100	18	Sept 2011	1.9–20.0	342	-	-
Hallwil	8°12'E	47°17'N	16	Mesotrophic	449	28	Oct 2012	1.9–20.0	94	10	13
Joux	6°17'E	46°38'N	16	Mesotrophic	100	32	Sept 2011	1.1–15.0	257	-	-
Geneva	6°33'E	46°26'N	23	Mesotrophic	372	310	Sept 2012	0.5–42.0	102	9	12
Neuchâtel	6°55'E	46°59'N	6	Oligotrophic	429	152	Sept 2011	1.2–37.0	208	10	15
Walen	9°12'E	47°07'N	4	Oligotrophic	419	151	Oct 2012	1.1–27.0	30	10	14

canalization, genetic assimilation or genetic accommodation (Crispo, 2008; Thibert-Plante & Hendry, 2011). On the other hand, plasticity may shield the genome from the effects of selection and prevent genetic fixation (Ghalambor, McKay, Carroll, & Reznick, 2007; Price, Qvarnström, & Irwin, 2003). If reproductive isolation cannot evolve, adaptive variation may sometimes be maintained by intraspecific resource polymorphisms either through adaptive phenotypic plasticity (Pfennig et al., 2010) or frequency-dependent selection (Svanbäck & Bolnick, 2007).

Here, we test for the presence of intraspecific differentiation and diversification in a widespread and abundant fish species of postglacial lakes—the roach (*Rutilus rutilus*). Roach are often considered to be generalist feeders (Persson, 1983), but may specialize on part of the food spectrum, such as zooplankton, to avoid predation and/or interspecific competition (Faulks, Svanbäck, Eklöv, & Östman, 2015; Svanbäck, Eklöv, Fransson, & Holmgren, 2008). Roach have also been shown to, in some cases, undergo ontogenetic dietary shifts, for example from zooplankton to macrophytes or mussels (Prejs, Lewandowski, & Stańczykowska-Piotrowska, 1990; Vejříková et al., 2017). Roach represent an ideal candidate to test for intraspecific diversification, given: (a) its broad dietary niche providing the ecological opportunity to explore a wide range of the available niche space and thus to potentially adapt to one or more niches, (b) its wide distribution across Europe (Kottelat & Freyhof, 2007), (c) its ability to inhabit an array of different environments (including streams and the pelagic and littoral zones of lakes (Svanbäck et al., 2008; Faulks et al., 2015), (d) its large population sizes, and (e) its modest economic importance, resulting in little to no direct management. Additionally, the roach in this study (Figure 1) often coexist with adaptive radiations of whitefish and are ecologically similar to some of the shallow water whitefish species (Doenz, Bittner, Vonlanthen, Wagner, & Seehausen, 2018; Hudson, Vonlanthen, & Seehausen, 2011; Vonlanthen et al., 2012), thus providing the potential for ecological niche shifts of roach in response to interspecific interactions, as has been shown for other fish species (Braband, 1985; Faulks et al., 2015; Persson, 1983).

Previous allozyme studies implicate that genetic differentiation in roach occurs predominantly between, but not within, drainage systems as a result of different colonization events following the last glaciation period (Hänfling, Durka, & Brandl, 2004; Laroche, Durand, & Bouvet, 1999). Roach from lakes with distinct colonization histories often differ in body shape, potentially as a response to different predation regimes (Scharnweber et al., 2013) or varying levels of intra- (Svanbäck et al., 2008) or interspecific competition (Faulks et al., 2015). However, in cases where genetic data were available, phenotypic differentiation showed only minor association with the level of genetic differentiation, suggesting that plasticity may often underlie phenotypic differences among roach populations (Faulks et al., 2015; Scharnweber et al., 2013). The aforementioned studies were, however, conducted in relatively shallow lakes, which might not provide the same ecological opportunities for genetic and adaptive differentiation as large, deep and oligotrophic lakes do (Seehausen & Wagner, 2014). In addition, studies of lacustrine populations

compared different drainages that were likely independently colonized, potentially resulting in different evolutionary contingencies (Faulks et al., 2015; Scharnweber et al., 2013; Svanbäck et al., 2008). By integrating phenotypic data of roach from seven large pre-alpine lakes with genomic and ecological (i.e. stable isotopic) data of five of these, we assess to which degree allopatric populations from lakes within the same drainage system that are connected by rivers differ from each other. We further test for intralacustrine differentiation of roach caught over different substrates within Lake Brienz. As Brienz is the most pristine lake that we studied (Figure 1, Table 1), it is also the most likely lake to reveal whether intralacustrine diversification evolved in roach as a response to local ecological opportunities. This is because the fish fauna of this lake experienced relatively little human impact, that is did not undergo a phase of eutrophication and re-oligotrophication during the second part of the 20th century like many other Swiss lakes (Vonlanthen et al., 2012), and is one of the few pre-alpine lakes that still hosts its whole adaptive radiation of whitefish (Doenz et al., 2018; Hudson et al., 2011; Vonlanthen et al., 2012).

2 | MATERIALS AND METHODS

2.1 | Study area and sampling

We sampled 1,223 roach from seven pre-alpine Swiss lakes between September 2011 and October 2012 (Figure 1a; Table 1). All lakes belong to the Aare/Rhine drainage except for Lake Geneva, which belongs to the Rhone drainage. However, former biogeographic work found that *R. rutilus* from the Rhone drainage formed a genetic cluster with specimens from the Rhine drainage (Larmuseau, Freyhof, Volckaert, & Van Houdt, 2009), potentially reflecting human translocations and/or natural drainage crossings, which has been observed for other fish species between the Aare/Rhine drainage and Lake Geneva (Gousskov & Vorburger, 2016; Vonlanthen et al., 2007). All specimens were collected during *Project Lac*, a large fish diversity assessment of pre-alpine lakes that aimed to probe all available littoral substrates and depth-related habitats for each lake using a standardized gillnet approach (reviewed in Alexander et al., 2015a, 2015b). Briefly, for each lake the littoral habitats (<5 m deep) were classified based on substrate composition and particle size, macrophyte morphology and density, and proximity to an inwardly or outwardly flowing watercourse. Fishing was subsequently performed using a combination of two different gillnet protocols that combined different mesh sizes to reduce size-selective catch biases (described in detail in Alexander et al., 2015a). Nets were set in a randomized way within the available area of both benthic and littoral habitats. Netting effort reflected the relative abundance of each habitat with a minimum number of three nets per habitat (Alexander et al., 2015a, 2015b). Following capture, the total length of each specimen was measured and each sample was photographed on the left side for further morphological analyses. From a subset of specimens, muscle tissue samples were collected for further genetic and stable isotopic analyses (Table 1).

2.2 | Assessing phenotypic differentiation

We quantified individual shape phenotypes based on 11 landmarks (Figure 1b) in TPSDIG2 (Rohlf, 2006) and subsequently conducted a Procrustes fit on the obtained shape data in MORPHOJ 1.05e (Klingenberg, 2011) for (a) all lake populations, (b) Lake Brienz specimens, and (c) roach used in the genetic and stable isotopic analyses (see Table 1). We corrected Procrustes coordinates for size by performing a regression against standard length, retaining the residuals. To identify the major axes of phenotypic variation, we performed a principal component (PC) analysis on each size-corrected dataset. The scores along the second and third PC axes in the overall data set were tested for differentiation among lake populations using ANOVAs with post hoc Tukey–Kramer tests. PC1 of size-corrected landmarks (accounting for 25.5%, 23.9% and 25.3% of the total variance for the overall data set, the Lake Brienz data set and the genetic/isotopic data set, respectively) was not analysed because it was driven by the bending of the fish and, therefore, represented a nonbiological artefact (Figure S1). We calculated pairwise Mahalanobis distances among lake populations as well as substrate types within Lake Brienz, and estimated their significances with 10,000 bootstrap replicates. To further assess the degree of phenotypic differentiation among lake populations, we calculated P_{ST} following Kaeuffer, Peichel, Bolnick, and Hendry (2012). P_{ST} is a unit-less and scale-free proportional measurement of pairwise difference, here using the scores of PC2 and PC3. For each P_{ST} , we established the 95% confidence interval using a resampling approach with 1,000 replicates following the procedure by Lucek, Sivasundar, Roy, and Seehausen (2013). Finally, we tested for an association between pairwise P_{ST} and F_{ST} , and between P_{ST} and pairwise differences in phosphate levels of lakes (Table 1), using Mantel tests in R 3.1.1 with 10,000 bootstrap replicates to determine significance.

To assess phenotypic differentiation within Lake Brienz, we calculated Mahalanobis distances between individuals from different substrates using PC2 scores. Based on the observed clustering of phenotypes (see Results 2.1), we combined individuals from different substrates into broader substrate categories, that is rocky (boulders, cobble) and muddy (ledge, inlet/outlet, vegetation). We subsequently performed an ANOVA on individual PC2 and PC3 scores to test for a difference between individuals from these broader substrate categories.

2.3 | Genomics

We prepared two restriction site-associated (RAD) genomic libraries using *SbfI* restriction sites following Lucek, Keller, Nolte, and Seehausen (2018). Libraries contained DNA from 42 and 50 individually barcoded specimens, respectively. Each library was single-end sequenced on one lane of an Illumina HiSeq 2000 platform together with ~10% bacteriophage PhiX genomic DNA (Illumina Inc.) to increase complexity at the first 10 sequenced base pairs. Reads without the complete *SbfI* recognition sequence

were subsequently discarded. Using the FASTX toolkit (http://hannonlab.cshl.edu/fastx_toolkit/), we removed any reads with at least one base with a Phred quality score <10 or more than 5% of base pairs with quality <30. This approach yielded 102.6 million high quality reads for analysis.

Given the lack of a reference genome for roach, we generated a de novo assembly using all filtered reads for all individuals having more than 250k reads with USTACKS (Catchen, Amores, Hohenlohe, Cresko, & Postlethwait, 2011). The following settings were used: minimum stack size of 75 reads, allowing a maximum of two base pairs of difference for stacks to be merged and excluding loci with unusually high coverage to avoid repetitive regions. The de novo assembly consisted of 49,772 contigs and was used to map reads for each individual with BWA MEM 0.7.17 (Li, 2013). We also aligned raw sequencing reads against the PhiX 174 reference genome (accession: NC_001422; Sanger et al., 1977) masking known variants. We then used the PhiX-alignments to create a base quality score recalibration table for each library using BASERECALIBRATOR from GATK v. 3.7-0 (McKenna et al., 2010). We subsequently recalibrated the base quality scores of each roach alignment to remove potential library effects with the GATK tool PRINTREADS. We called genotypes with UNIFIEDGENOTYPE implemented in GATK v. 3.7-0, considering only bases with a mapping quality >20. Using VCFTOOLS v. 0.1.14 (Danecek et al., 2011), we filtered the resulting VCF file, where we set genotypes with quality <28 or depth <6 to missing. We further applied a minor-allele frequency cut-off of 0.03 considering only biallelic SNP positions with ≤20% missing data. Following all filtering steps, a total of 3,865 polymorphic SNPs were available for the subsequent analyses comprising all lakes and 4,721 polymorphic SNPs for the Lake Brienz dataset.

We estimated the level of pairwise genetic differentiation between roach populations from different lakes using pairwise locus-by-locus F_{ST} in GENODIVE v. 2.0b27 (Meirmans & Van Tienderen, 2004). Significances were assessed with 10,000 permutations, applying a Bonferroni correction for the pairwise comparisons. To calculate the probability of each individual to be assigned to its sample population, we employed a discriminant function analysis on principal components (DAPC) with ADEGENET (Jombart, Devillard, & Balloux, 2010) in R based on the first ten PC axes and the four leading discriminant axes. We further used ADEGENET to calculate the observed heterozygosity (H_O) for each roach population. SNPRELATE (Zheng et al., 2012) was used to perform a PC analysis based on the genomic data.

We used RAXML 8.2.11 (Stamatakis, 2014) to test for genetic differentiation among individuals from different lakes as well as among individuals caught over different substrates within Lake Brienz. In both cases, we implemented a generalized time-reversible (GTR) model with optimized substitution rates and a gamma model of rate heterogeneity. We then applied an ascertainment bias correction for each dataset to account for the fact that we only used polymorphic SNPs. Significances were assessed using 1,000 bootstrap replicates. We also tested for intralacustrine genetic differentiation between individuals caught over a muddy or rocky substrate within Lake

Brienz using DAPC based on the 20 leading PC axes accounting for 80% of the total variation and also calculated the average locus-by-locus genetic differentiation (F_{ST}) between individuals from the two substrates in GENODIVE.

2.4 | Stable isotope analysis

We obtained the stable isotopes signature of individuals using muscle tissue for 12–28 individuals per lake (Table 1). In fish, differences in $^{13}C/^{12}C$ ratios fall along a gradient where low values indicate a diet dominated by plant and algae matter, whereas increased values reflect a shift towards higher trophic levels (Post, 2002). To further obtain isotopic baseline values, we collected snails (*Lymnaeidae* sp. and *Planorbidae* sp.) at the time each lake was sampled (Table 1), except for lakes Neuchatel and Geneva, where we collected baseline material in September 2013. All samples were dried at 55°C for 48 hr. Dry mass (0.5–1.0 mg) was subsequently analysed with internal reference standards (18 Sucrose [IAEA-CH-6], 18 IAEA-N2 and 18 caffeine [IAEA-600]). The remaining uncertainty as estimated by the standards was 0.08% (VPDB). The stable isotopic signature was used to: (a) test for differences in the stable isotopic signature among populations from different lakes with an ANOVA followed by a Tukey's HSD *post hoc* decomposition, (b) to test for an association between morphology and diet across all lake populations by regressing the baseline corrected $\delta^{13}C$ values against the scores of the second and third phenotypic PC axes, respectively, (c) to determine whether the trophic status as measured by the phosphate level of a lake (Table 1) affected the diet of the respective roach population by using an ANOVA and lastly, and (d) to test for ecotypic differentiation based on stable isotopes between individuals caught over a rocky or muddy substrate within Lake Brienz using a Mann-Whitney *U* test.

3 | RESULTS

3.1 | Differentiation among roach from different lakes

Roach differed phenotypically between lakes along both the second ($F_{6,1216} = 45.19, p < .001$) and third PC axes ($F_{6,1216} = 16.55, p < .001$), accounting for 16.7% and 13.1% of the overall shape variance, respectively (Figure 2, Table S1). The *post hoc* decomposition suggests that individuals from lakes Brienz and Brenet account for most of the variation captured by the two PC axes (Table S2). Variation along PC2 was driven by differences in the position of the mouth (landmarks 1 & 2) and the position of the pelvic and pectoral fins (landmarks 6 & 7). In contrast, PC3 was mainly driven by differences in the position of the dorsal fin (Table S1). This resulted in a group of specimens from lakes Walen, Neuchatel, Hallwil, Joux, Geneva and Brenet with a terminal mouth and a more anterior dorsal fin and a second group consisting of roach from Lake Brienz, which had a

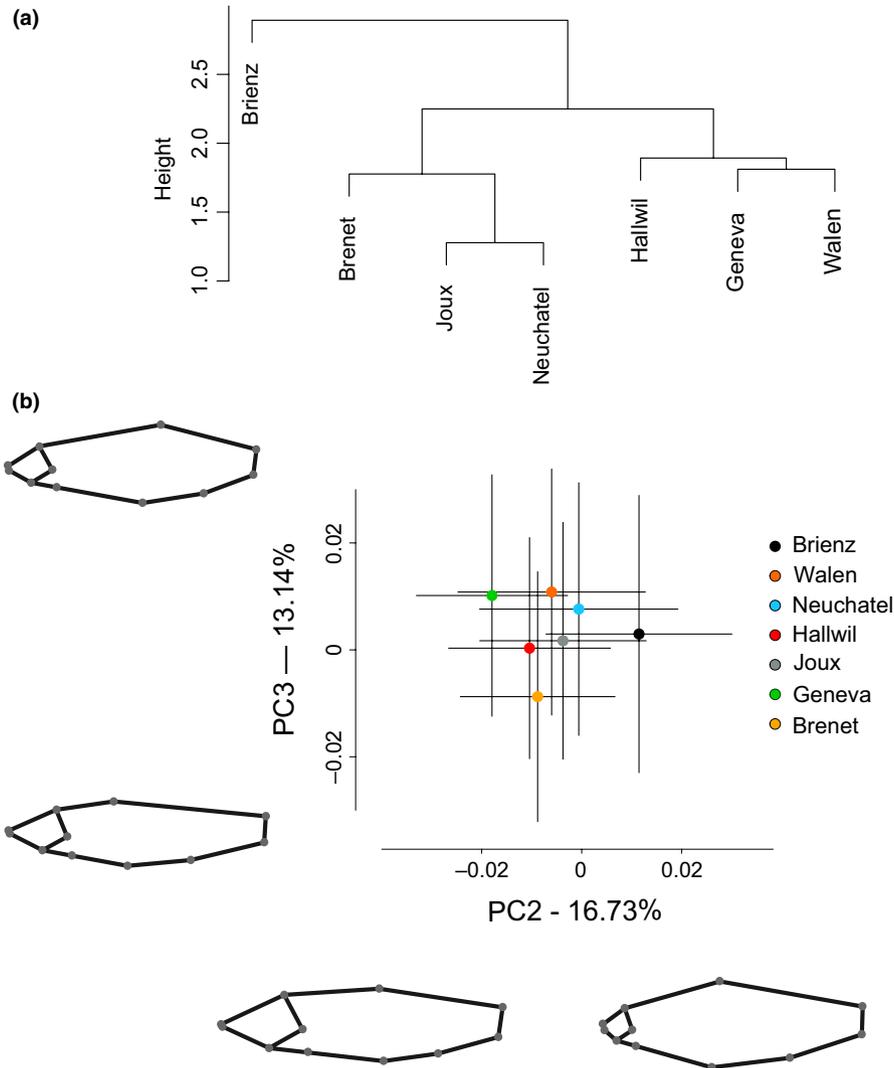


FIGURE 2 Phenotypic relationships across lake populations. (a) Mahalanobis distance dendrogram. (b) Principal component (PC) analysis of body shape for all seven-lake populations. Shown are the mean values across the second and third PC axes with the 95% confidence interval for each population. Changes in body shape are further indicated

TABLE 2 Observed heterozygosity (H_o) of each lake population as well as the pairwise genetic differentiation (F_{ST}) among populations. F_{ST} values are presented in the lower triangle and Bonferroni corrected significance levels in the upper triangle

	H_o	Brienz	Hallwil	Geneva	Neuchatel	Walen
Brienz	0.265		<0.001	<0.001	<0.001	<0.001
Hallwil	0.257	0.032		<0.001	<0.001	<0.001
Geneva	0.257	0.026	0.032		<0.001	<0.001
Neuchatel	0.264	0.025	0.025	0.005		<0.001
Walen	0.265	0.038	0.036	0.030	0.026	

compact head, a subterminal mouth and a posteriorly placed dorsal fin (Figure 2).

Consistent with a single colonizing lineage, the degree of pairwise genetic differentiation among lake populations was generally low ($F_{ST} \leq 0.040$) but significant (Table 2). The low level of genetic differentiation between the roach populations from Lake Neuchatel and Geneva is consistent with a recent drainage crossing (Larmuseau et al., 2009) and/or human translocations.

Despite the low level of genetic differentiation, 99% of all individuals were correctly assigned to their lake of origin by DAPC (Figure 3a). The genetic PC analysis showed a clustering of ultraoligotrophic (Brienz and Walen) and mesotrophic lake populations (Hallwil, Geneva, and Neuchatel) along PC1, accounting for 2.75% of the total genetic variation (Figure 3b). Our phylogenomic reconstruction showed a clustering similar to the DAPC assignment (Figure 3c), where individuals from Brienz seemed

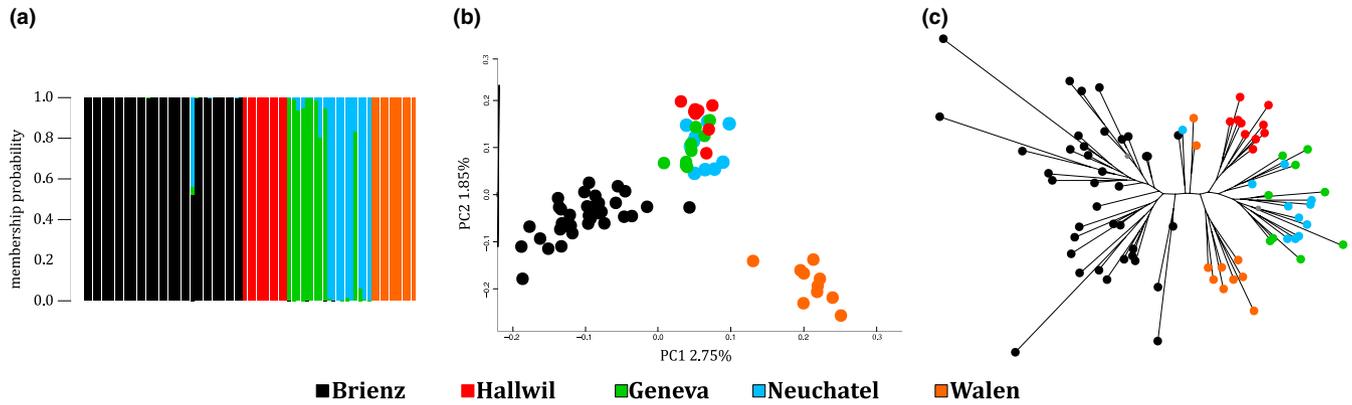


FIGURE 3 Population genomic structure across different lake populations. (a) Individual-based assignment probabilities based on a discriminant function analysis of PC components (DAPC). (b) Principal component analysis based on 3,865 polymorphic SNPs. (c) RAxML phylogeny tree depicting the genetic relationship of all roach (no significant bootstrap support except two nodes with >50% support highlighted by a.)

most distinct, whereas individuals from Geneva and Neuchatel clustered together. However, bootstrapping yielded no significant node support, suggesting substantial levels of gene flow. Levels of H_O differed marginally among lake populations (Table 2), and this variation was negatively correlated with the phosphate levels (see Table 1) observed in each lake (Pearson correlation: $\rho = .958$; $t_{1,3} = 5.78$, $p = .010$). Pairwise F_{ST} s were neither correlated with differences in phosphate levels (Mantel test: $r_M = .114$, $p = .600$) nor with pairwise phenotypic (P_{ST}) differentiation among lake populations (PC2: $r_M = .088$, $p = .466$; PC3: $r_M = -.113$, $p = .690$). P_{ST} was likewise not correlated with differences in phosphate among lakes (PC2: $r_M = .367$, $p = .165$; PC3: $r_M = -.151$, $p = .613$).

Stable isotopes indicate significant trophic differentiation of roach among lakes ($F_{4,77} = 47.49$, $p < .001$), where all but two post hoc comparisons (Neuchatel–Geneva and Neuchatel–Walen) were significant. Stable isotopes range from a more herbivorous diet in Lake Brienz ($\delta^{13}C$ of -22.63 ± 1.80) to a more omnivorous diet within Lake Hallwil ($\delta^{13}C$ of -29.72 ± 1.16 ; Figure 4). However, the stable isotopic values were neither correlated with individual scores along the second ($F_{1,80} = .01$, $p = .990$) or third ($F_{1,80} = 0.19$, $p = .665$) phenotypic PC axes, nor were they correlated with differing phosphate levels ($F_{1,3} = 1.14$, $p = .365$).

3.2 | Diversification within Lake Brienz

Pairwise Mahalanobis distances suggested phenotypic clustering of individuals caught over “rocky” (boulder, cobbles) versus “muddy” (ledge, vegetation) substrates (Figure 5c; Table S3). Individuals caught close to the inlet or outlet clustered with the muddy substrate group and were subsequently included in this substrate category (Figure 5). Consistent with this clustering, we found significant phenotypic differentiation between individuals caught over muddy and rocky substrates along the second ($F_{1,81} = 12.77$, $p < .001$) but not third ($F_{1,81} = 0.01$, $p = .902$) PC axes. Variation along PC2 was driven by morphological differences in the position of the dorsal, caudal and pelvic fin (landmarks 11, 9 and 7), whereas PC3 was

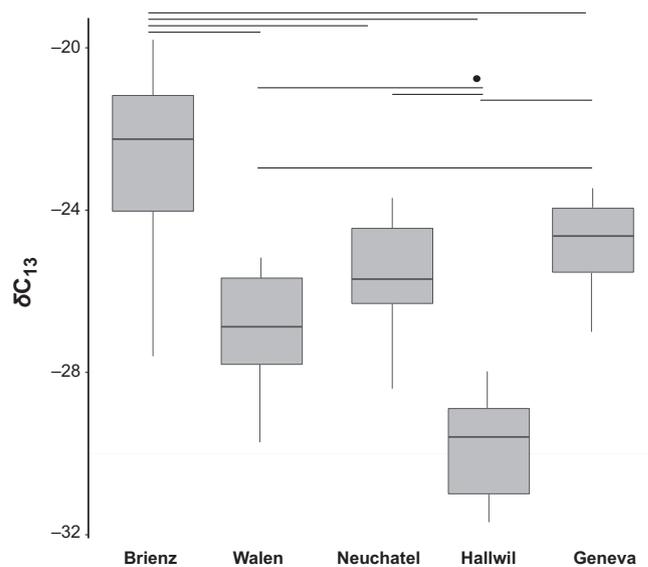


FIGURE 4 Boxplot summarizing the variance in $\delta^{13}C$ among roach from different lakes. Horizontal bars indicate significant comparisons ($p < .05$) after a post hoc Tukey–Kramer ANOVA decomposition (see main text for details)

driven by the placement of the dorsal (landmark 11) and pectoral fin (landmark 6) and the position of the mouth (landmarks 1 & 2). The two phenotypic clusters did not differ in their diet assessed by stable isotopes ($W = 61.5$, $p = .540$).

Our phylogenomic reconstruction did not yield any significant clustering by substrate (Figure 5b). Concordantly, there was no genome-wide differentiation between individuals caught over muddy or rocky substrate ($F_{ST} = -0.001$, $p = .759$). When using a discriminant function analysis that maximizes the differentiation among substrates, a bimodal distribution occurred along the discriminant axis, supporting some genetic differentiation (Figure 5a). Indeed, we found five SNPs among the total of 4,721 polymorphic SNPs within Lake Brienz that showed a $F_{ST} > 0.3$, each belonging to a different contig (Table S4). To identify potential genes involved in

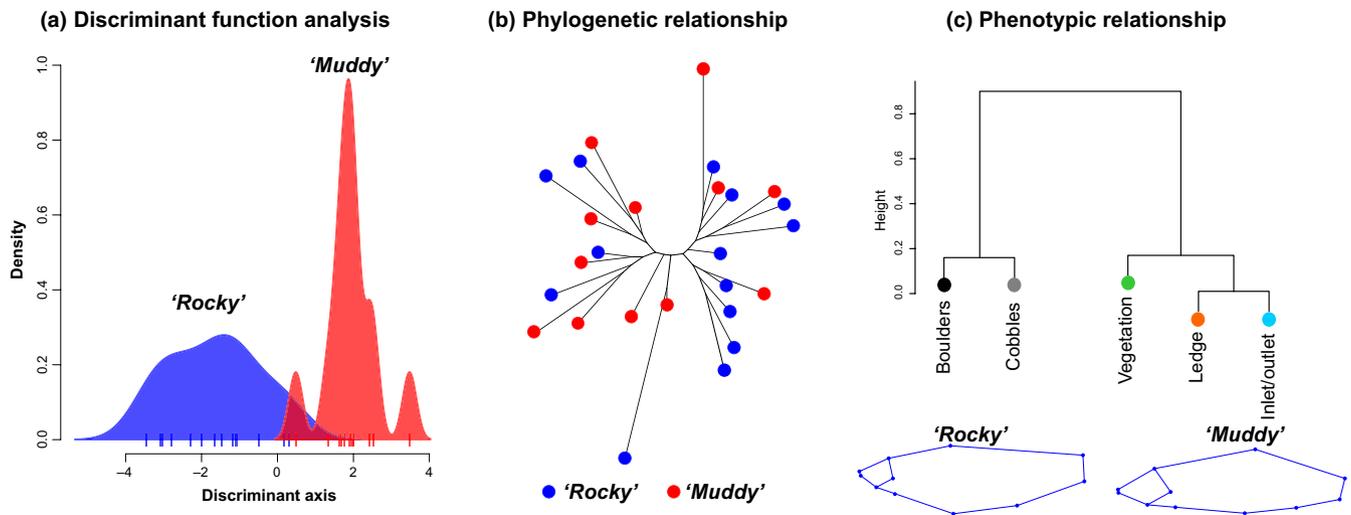


FIGURE 5 Differentiation of roach within Lake Brienz based on: (a) discriminant function analysis of genetic data comparing individuals assigned to different substrate groups (rocky vs. muddy). (b) RAxML phylogeny tree depicting the genetic relationship of Brienz roach (no significant bootstrap support). (c) Morphological relationship based on Mahalanobis distances between different substrates. Morphological differences between individuals caught over rocky (boulders and cobble) and muddy (ledge, inlet/outlet and vegetation) substrates are indicated

substrate-related differentiation, we further matched each contig against the NCBI nucleotide collection on the 26 October 2018 using megablast (Boratyn et al., 2013). Of the five contigs, two overlapped with known genes: (a) *FSTL5: Follistatin-related protein 5*, and (b) *PCSK5: Proprotein convertase subtilisin/kexin type 5*—a gene involved in neuromast deposition within the lateral line system in zebrafish, where a deficiency resulted in reduced spatial awareness and sensing of the environment (Chitramuthu et al., 2010).

4 | DISCUSSION

4.1 | Postglacial diversification of roach

Understanding why some species undergo diversification, while others do not, remains a conundrum. Evidence for species diversification among temperate freshwater fish comes from a small range of taxa, whereas intraspecific diversification remains unassessed for most other fish species (Seehausen & Wagner, 2014). Roach have a broad geographic distribution in Europe and occur in a variety of habitats—including deep and ultraoligotrophic lakes that provide a wide range of potential niches to diversify, making roach a good candidate to look for diversification (Faulks et al., 2015; Svanbäck et al., 2008). We found evidence for intraspecific differentiation between roach populations from ultraoligotrophic lakes and lakes with a higher trophic level, as well as some diversification within the ultraoligotrophic Lake Brienz.

Former studies suggested that roach often adapt to their local environment and become phenotypically differentiated, for example along a littoral–pelagic axis as a result of intra- and interspecific competition (Faulks et al., 2015; Svanbäck et al., 2008) or predation (Scharnweber et al., 2013), and this phenotypic differentiation

has often been attributed to phenotypic plasticity (Faulks et al., 2015; Svanbäck et al., 2008). Studying roach from pre-alpine lakes in Switzerland, we found subtle yet significant genetic differentiation among populations from different pre-alpine lakes, where populations from ultraoligotrophic lakes are genetically more distinct (Figure 3b). This is consistent with recent colonization, potentially combined with ongoing gene flow within the Aare/Rhine drainage. Alternatively, the effective population size may be too large for drift to become a dominant factor (Gillespie, 2001). We also found roach from Lake Geneva to cluster closely with individuals from the nearby Aare/Rhine system (Figure 3). This suggests a common origin, potentially due to historical connectivity as observed for other fish species (Gousskov & Vorburger, 2016; Vonlanthen et al., 2007) or supplementary human translocations. Despite their low level of genetic differentiation (Table 2), roach differed both phenotypically (Figure 2b, Table S1) and ecologically (Figure 4) between lakes. Individuals differed predominantly in their head shape, with the population from Lake Brienz being most distinct, showing a slender head and more subterminal orientation of the mouth (Figure 2). The observed phenotypic changes among roach from different lakes hint towards a functional and potentially adaptive response related to feeding regimes that differ between lakes (Wainwright & Barton, 1995). Phenotypic changes in head and body shape similar to the ones observed here were indeed found to occur in response to differences in resource use in other fish (Anker, 1974; Barel, 1983; Pfaender, Schliewen, & Herder, 2009). Given the lack of an association between the degree of phenotypic and genetic differentiation, the observed phenotypic changes likely represent a plastic response to varying environmental pressures, as has been proposed for other roach populations (Faulks et al., 2015; Scharnweber et al., 2013).

4.2 | Intralacustrine diversification in Lake Brienz

Habitat-dependent divergent selection can lead to the evolution of distinctly adapted ecotypes within a system (Nosil, 2012; Schluter, 2000). When combined with intra- and interspecific competition, divergent selection can lead to differences in prey utilization between individuals from structurally contrasting environments. These factors are common drivers of diversification among postglacial freshwater fishes (Dieckmann & Doebeli, 1999; Gavrillets, 2004; Rosenzweig, 1978; Svanbäck & Bolnick, 2007). Both intra- and interspecific competition, such as with perch (*Perca fluviatilis*), have been shown to drive resource polymorphism in roach from Swedish lakes (Faulks et al., 2015; Svanbäck et al., 2008). This may similarly apply for roach in Lake Brienz where perch are the most abundant fish species caught, followed by roach and whitefish. Roach were moreover restricted to depths <3 m, overlapping with perch and part of the whitefish species, thus providing the potential for interspecific competition (Alexander et al., 2015a; Doenz et al., 2018).

Substrate-related phenotypic differentiation is common among freshwater fishes, where adaptive phenotypic changes often occur in head shape, as a response to different feeding regimes (Caldecutt & Adams, 1998; McGee, Schluter, & Wainwright, 2013), and in fin position or body shape in response to different swimming regimes (Hendry, Hudson, Walker, Räsänen, & Chapman, 2011; Walker, 1997). Within Lake Brienz, we found roach to show evidence for such substrate-related intralacustrine phenotypic diversification, as individuals fell into two phenotypic clusters (Figure 5). Individuals caught over muddy substrates showed a more caudal position of the dorsal fin, consistent with adaptation to more active swimming in cyprinid fish (Felley, 1984). This, together with an elongated snout and a more terminal mouth (Figure 5), could reflect feeding on more pelagic prey as has been found for other lake-dwelling roach populations (Faulks et al., 2015; Svanbäck et al., 2008). In contrast, individuals caught over a rocky substrate had a more anterior dorsal fin, consistent with increased manoeuvrability in structured environments such as between rocks. The compact head and subterminal mouth of fish from a rocky substrate is also often associated with a predominantly benthic feeding strategy (Wainwright & Barton, 1995). To which degree these phenotypic differences are associated with selective feeding strategies, for example if and to which extent fish caught over a muddy vegetated substrate feed on macrophytes, remains unknown as we relied solely on stable isotope data. With the latter, we found no association between phenotypes and resource use. This, however, contrasts with the increased range of stable isotope values found for roach in Lake Brienz (Figure 4) and could reflect limited power to distinguish differences in microhabitats given our restricted sample sizes. However, stable isotopes represent a long-term average diet, and the observed phenotypic segregation shown here may be seasonal (Post, 2002).

Average genome-wide differentiation between the two substrate-related phenotypic groups was absent (i.e. $F_{ST} = -0.001$), and no apparent clustering occurred in our phylogenetic reconstruction (Figure 5).

This is also consistent with plasticity acting as the main driver for the observed phenotypic differentiation. However, a discriminate function analysis that captured the differences between the two groups suggests a bimodal distribution of individuals (Figure 5a). Among the five markers that showed the highest degree of genetic differentiation between substrates (Table S4), one occurred within the gene *PCSK5* that is involved in lateral line development (Chitramuthu et al., 2010). The lateral line organ is important for spatial awareness and sensing of the environment, and the observed genetic differences could suggest divergent selection between the two substrates that differ in their complexity, being consistent with the detected differences in body shape (Figure 4). Genomic differentiation at only few target loci is consistent with a very early stage of divergence-with-gene-flow, where further differentiation depends on the evolution of barriers to gene flow (Nosil, 2012). The absence of significant genomic differentiation could also reflect a limited resolution given the restricted number of polymorphic SNPs available for our analyses (Wagner et al., 2013).

The slight differentiation of roach of different habitats contrasts with the co-occurring adaptive radiation of whitefish, which had a similar time span as roach to evolve in Lake Brienz, that is since the retreat of the glaciers ~12 kyrs ago. Within Lake Brienz, there are a total of four genetically differentiated whitefish species, segregated along the water depth and pelagic–benthic axes, which are distinct in their morphology, including the gill rakers (Doenz et al., 2018), thus suggesting adaptation to different trophic niches (Roesch, Lundsgaard-Hansen, Vonlanthen, Taverna, & Seehausen, 2013). Given the abundances of perch and whitefish in Lake Brienz (Alexander et al., 2015a; Doenz et al., 2018), the limited degree of diversification in roach could be a result of different factors: (a) Interspecific competition may have constrained roach from diversifying, (b) If the observed phenotypic differentiation (Figure 5) is primarily due to phenotypic plasticity, the latter could have constrained diversification by shielding the genome from selection, thus decreasing the potential for genetic divergence (Ghalambor et al., 2007; Price et al., 2003), (c) The fundamental niche of roach may be narrower than that of whitefish, preventing roach to explore otherwise available niche space. For example, roach prefer warmer water and are therefore restricted to the shallow zones of lakes, whereas whitefish can tolerate colder water, allowing them to explore the deeper sections of lakes (Coutant, 1977; Kottelat & Freyhof, 2007), and (d) Recent genomic work suggests that adaptive diversification in stickleback and whitefish often occurs from standing genetic variation in genomic regions that show structural changes, including inversions (Jones et al., 2012; Marques et al., 2016) or chromosomal rearrangements (Dion-Côté et al., 2016). Such structural genomic rearrangements may then facilitate diversification through coupling of co-adapted alleles (Butlin & Smadja, 2018). Given the limited evidence for genetic differentiation in roach (Figure 5, Table S4), such genomic features may be lacking, which may constitute a genetic constraint that impedes diversification and the build-up of genetic barriers to gene flow (Seehausen et al., 2014).

5 | CONCLUSIONS

Intraspecific differentiation in response to habitat-dependent divergent selection is thought to be a major driver of diversification and adaptive radiation in freshwater fish, yet evidence comes from only a few taxonomic groups (Seehausen & Wagner, 2014). Combining phenotypic, ecological and genomic data, we show differentiation between lake populations of roach from ultraoligotrophic lakes and lakes with a higher trophic level within the same drainage system, potentially in response to different abiotic and biotic factors. In one ultraoligotrophic lake, we also found evidence for intralacustrine diversification with different phenotypes being associated with distinct substrates. However, given the lack of genetic differentiation, phenotypic changes are likely to be mostly plastic, where the lack of diversification may also reflect genomic constraints. This needs to be investigated in the future. Taken together, our study reveals striking differences in the degree of phenotypic and genetic differentiation between this lineage of roach and the lineage of whitefish that has undergone impressive adaptive radiations in the same lakes. However, our study also indicates the potential for more subtle intraspecific differentiation and diversification in a widespread and abundant freshwater fish species, especially in ultraoligotrophic lakes. This may similarly apply to other fish species and highlights the importance to study both an ecologically and a geographically broad range of populations within a species to assess cryptic biodiversity (Bickford et al., 2007).

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DATA ACCESSIBILITY

BAM files with aligned de-multiplexed and base quality score recalibrated reads are available through the short read archive (www.ncbi.nlm.nih.gov/sra). BioProject ID: PRJNA533015. Phenotypic and stable isotopic data are available through Zenodo: 10.5281/zenodo.3270671.

ORCID

Jessica M. Rieder  <https://orcid.org/0000-0001-8922-9925>

Kay Lucek  <https://orcid.org/0000-0002-2253-2556>

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SUPPORTING INFORMATION

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

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