SUPPORTING INFORMATION

Species divergence under competition and shared predation

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CONTENTS

For Supplementary Tables S1-S9, see the accompanying Excel Workbook

Supplementary Methods

Supplementary Methods detail the Methods in the main paper

Study lakes and populations

While stickleback lakes with and without sculpin have been described previously (Vamosi 2003; Ormond et al. 2011; Ingram et al 2012; Miller et al. 2015), we also identified lakes with sculpin but without stickleback for this study. First, we searched for lake records in the same geographic region in the Fish Inventories Data of the Ministry of Environment of British Columbia (http://a100.gov.bc.ca/pub/fidq/viewFdisProjects.do). We then combined this information with our own experience surveying respective lakes or knowledge communicated to us by other researchers. Finally, we confirmed the presence/absence of stickleback and sculpin in all study lakes through extensive minnow trapping, and by snorkeling each lake along the shoreline looking for stickleback and sculpin in their expected habitats.

When choosing our study lakes, we considered the following three aspects. (i) We aimed for lakes of the three types to be interspersed geographically to minimize systematic differences among them other than in the presence/absence of sculpin and stickleback (Figure 1). The major axis of a principal component analysis of ten physical and chemical characteristics (PC1; 42.6% percent variance explained) did not separate sympatric lakes from either allopatric sculpin or from allopatric stickleback lakes. Allopatric sculpin lakes and allopatric stickleback lakes tended to separate along PC1 despite clear overlap (see Supplementary Analysis 2 and Table S1 for further details), but we do not compare populations from these two types of lakes. (ii) With one exception, our study lakes are in different watersheds. North Lake (sympatric) and Klein Lake (allopatric stickleback) are connected by a steep, tiny creek with several physical barriers between them. Fish migration between these two lakes is unlikely, and their stickleback populations do not share mtDNA haplotypes (Miller et al. 2019). We therefore propose that our study populations have evolved independently since they were established sometime after the retreat of the ice sheets of the last Pleistocene glaciation ~12,000 years ago (Bell & Foster 1994; Dennenmoser et al. 2015; Miller et al. 2019). (iii) According to our own extensive sampling and the Fish Inventories Data, we ensured that our study lakes have no other small fish species that could share trout as a predator together with sculpin and/or stickleback.

Fish sampling

We sampled stickleback (> 30 mm standard length) and sculpin (> 40 mm standard length) between May and July in 2015 (and some in 2016) using unbaited minnow traps and occasional dip netting (permits: MRSU15-170469, MRSU16-229407). Traps were set from shore or kayak between a depth of 0.5 and 5 meters. Traps were set during the day for less than 3.5 hours when possible, although some sculpins were caught by overnight trapping. When too many fish were caught, we haphazardly subsampled fish while balancing the representation of each sex per population and species. Sample sizes per analysis were generally well balanced among populations (Tables S2 & S3). Representation of the two sexes was generally also well balanced within populations. Hence, variation in sex ratio plays a minimal role in differences among populations in trait means, our unit for analysis (see below).

Fish were euthanized using an overdose of buffered MS-222. We measured the standard length of all fish immediately with calipers. We then pinned freshly killed sculpin specimen left-side-up in a natural position and with its anal, caudal, and dorsal fins spread out on a standardized background. We photographed each specimen with a digital camera on a tripod and then preserved each specimen in 95% ethanol. To preserve stomach contents more quickly, the abdominal cavities of larger sculpin were opened from the anus towards the head. Stickleback were immediately preserved in 95% ethanol, brought to the lab, stained with Alizarin Red to better visualize bony armor traits, and photographed on a flat surface with their right lateral side facing up.

Details of antipredator and foraging trait measurements

For antipredator traits, we measured traits predicted to provide protection from trout predation. In stickleback, we measured: (i) averaged length of the first and second dorsal spines from joint to tip; (ii) length of the left pelvic spine from joint to tip; (iii) maximal anterior-posterior length of the pelvic girdle; and (iv) number of left-side lateral bony plates (including anterior plates). In sculpin, we measured eight traits that we expect should be selected by sculpin to better defend against or escape attacks from large-gaped (trout) predators: (i) length of the left pre-opercular spine from base to tip; (ii) length of the spiny fin ray (i.e., fifth ray) of the first dorsal fin; (iii) length of the pelvic spine (fused with the first ray of the pelvic fin); (iv) ventral left-right body width between the pre-opercular spines; (v) peduncle depth; and the total area of the splayed (vi) second dorsal fin, (vii) anal fin, and (viii) caudal fin. Sculpin trait measurements i-iv were taken from preserved specimens using hand-held calipers, while traits v-viii were measured from lateral photographs using ImageJ (Schneider et al. 2012).

For foraging traits, we measured the same four traits in both species: (i) number of gill rakers (counted on the left ceratobranchial of the main gill arch); (ii) mean gill raker length (average length of the second, third, and fourth gill raker from the epibranchial-ceratobranchial joint on the ceratobranchial); (iii) gape width (mouth closed); and (iv) premaxilla length. Measurements were taken directly from preserved specimens, except premaxilla length, which was measured from lateral photographs using ImageJ (Schneider et al. 2012).

Size correction of morphological traits and diet scores

We followed Berner (2011) to remove unwanted effects of variation in size (standard length, SL) of individuals on trait values in our analyzes. We applied the same size correction procedure to 'benthic diet proportions' and stable isotope data (see below).

For each trait within each species, we used an ANCOVA with 'population' and log-transformed 'SL' as predictors of log-transformed 'trait' (e.g., gill raker length), as implemented with the following syntax in *R* (*R* Development Core Team 2021): *lm(log(trait) ~ log(SL) + population)*. Using these models, individual residuals from the common within-group slope were added to the predicted trait value for each population at the mean size of all individuals (Reist 1986). This yielded size-corrected individual trait data, which were then averaged per population to obtain size-corrected population means. For reporting size-corrected population trait means in original and hence meaningful trait units (i.e., in 'mm' or counts), we took the antilog of the size-corrected trait values before averaging per population (see Tables S6 & S7). We note that conducting size correction with alternative procedures (e.g., following Paccard et al. 2020) or analyzing individual trait values using a statistical model with 'size' included as a covariate (see Table S8 for details) yielded very similar results, thereby supporting identical conclusions.

Body shape analysis

Body shape of fish often evolves in response to variation in predation and/or competition (Langerhans & Reznick 2010). Thus, we asked whether shifts in body shape in the species reflect the effects of shared predation and competition we detected in the analyses of specific antipredator and foraging traits. We quantified body shape shifts with geometric morphometrics based on 21 (stickleback) and 19 (sculpin) fixed landmarks digitized on lateral body-shape photographs from all populations using tpsDig2 (Rohlf 2018) (see Fig. S3). Because ethanol preservation of stickleback led to bending of some specimens, we removed this unwanted shape variation with the 'Unbend specimens' module in tpsUtil (Rohlf 2009) by using four landmarks placed along the anterioposterior axis of each fish. Sculpin were photographed prior to ethanol preservation, hence, there were no obvious bending artifacts.

Separately for each species, we superimposed landmark coordinates to obtain Procrustes coordinate scores and calculated centroid size, using the *geomorph R*-package (Adams et al. 2017). To remove possible influences of size on shape variation, we performed a linear regression of Procrustes coordinates onto centroid size using all individuals per species and retained the residuals. With the residual values, we then performed linear discriminant analysis (LDA) on all specimens per species with 'population' (but not 'lake type') as the grouping variable and kept the LD1 scores for analysis (following e.g., Miller et al. 2015). We preferred LDA over PCA to reduce landmark data dimensionality because LDA reduces effects of technical variation in geometric morphometric analyses (e.g., due to measurement errors or inconsistent positioning of specimens during photography (Tabachnick and Fidell 2013)), and because our interest was in variation *among* rather than *within* populations. For each species, we averaged individual LD1 scores per population and tested for a difference in population means between lake types using permutation (Manly 2007).

Stomach content analyses

We analyzed stomach contents of ethanol-preserved fish from all 35 study populations to test for ecological shifts between allopatric and sympatric populations in the species. We dissected stomachs and examined their contents under a dissection microscope. Prey items were identified referencing the taxonomic resolution of prey classification from Bolnick et al. (2010) but including categories that were not previously defined (stickleback: 36 categories, sculpin: 35, including an ‘unknown’ category; see Tables

S4 & S5 for details). We excluded individuals with empty stomachs or only nonidentifiable digested contents (13% of stickleback and 26% of sculpin). We collapsed prey categories into broader taxonomic groups reflecting functionally similar prey to facilitate interspecific comparison (Tables S4 & S5). The average sample size of fish with non-empty stomachs per population was 16 for stickleback (total N = 279) and 15 for sculpin (total N = 254). The median trap soak time for those stickleback and sculpin was 1.5 and 2 hours, respectively. Soak times did not differ between allopatric and sympatric populations within the species (t-test P-values > 0.1).

We calculated interspecific diet similarly between sympatric populations and between allopatric populations of sculpin and stickleback using the 'Proportional Similarity' (PS) index for diet (Schoener 1968; Bolnick et al. 2002):

$$PS_{i,j} = \sum_k \min\,(p_{i,k}, p_{j,k})$$

where $p_{i,k}$ is the proportion of the *k*th prey type in *i*'s diet, and $p_{j,k}$ is the proportion of the *k*th prey type in *j*'s diet. To obtain population-level diets, we first converted individual diets to proportions by prey category and then calculated the mean across all individuals per population. To test whether interspecific diet overlap is reduced in sympatry, we focused on the 13 main prey categories that were found in at least one sympatric and one allopatric population of both sculpin and stickleback (Tables S4 & S5). Swallowed fish eggs were not included as it was often unclear whether they constituted stickleback or sculpin eggs and because we were interested in diet shifts driven by competition (we note, however, that retaining the 'fish eggs' diet category for analysis affected the results only marginally). We compared diet overlap of all pairs of sympatric stickleback and sculpin populations to that of all pairs of allopatric stickleback with allopatric sculpin populations. We used a permutation test to infer statistical significance of this pattern (Manly 2007). That is, we randomly re-assigned the population labels within each species and calculated the difference between the mean diet overlap in all pairwise population comparisons of sympatric stickleback and sculpin and the mean diet overlap in all pairwise population comparisons of allopatric stickleback and sculpin for each such permutation. In total, we ran 10,000 permutations. We then calculated a two-tailed P-value based on how many times the absolute difference in these permutations was greater than the absolute difference observed in the real data. Importantly, the calculation of diet overlap for each permutation and the real data was identical, thus accounting for a possible bias of pseudoreplication in this test (because each population was used multiple times for calculating pairwise interspecific diet comparisons) when testing the statistical significance of the pattern. We also ran an unconstrained correspondence analysis (CA) on the combined diet data set from the two species to examine multivariate diet divergence between allopatric and sympatric populations along the main axis of diet variation.

Stable isotope diet analyses

Stomach contents provide only a snapshot of an individual's diet and are thus sensitive to how and when individuals are caught. We thus also quantified the diet of all 35 study populations using stable isotope ratios of muscle tissue, which integrates diet of an individual over recent months (Pinnegar & Polunin 1999; Post 2002). We analyzed the ratio of nitrogen isotopes ^{15}N to ^{14}N ($\delta^{15}N$) from pulverized dorsolateral muscle tissue. Generally, higher $\delta^{15}N$ values indicate foraging prey from a higher trophic level (Post 2002), whereas in stickleback, we know that higher $\delta^{15}N$ values indicate increased foraging on (predatory) open-water zooplankton relative to benthic macroinvertebrates (Matthews et al. 2010; Kaeuffer et al. 2012; Ravinet et al. 2013; Arnegard et al. 2014; Østbye et al. 2016). In sculpin, higher $\delta^{15}N$ values may indicate increased predation on stickleback (i.e., stickleback adults, fry or eggs) and/or increased foraging of other (benthic) prey from a higher trophic level in the food web (Post 2002).

To estimate the $\delta^{15}N$-baseline of each lake we used either all soft tissue from a snail, the foot tissue of a mussel, or the average of both if available. A snail and/or a mussel sample were obtained from 21 of our 26 study lakes (15 populations per species) at the same time as fish collection. We did not find a difference in $\delta^{15}N$ between snails and mussels within the six lakes with both a snail and a mussel sample (paired t-test; $t = 0.33$, $P = 0.754$), indicating that they can be used interchangeably to estimate baseline $\delta^{15}N$ in a lake (in accordance with Matthews et al. 2010). While $\delta^{15}N$ is often complemented with carbon isotope ratios ($\delta^{13}C$) to estimate the proportion of feeding from littoral versus pelagic food chains, the lack of both a littoral reference (snail) and a pelagic reference (mussel) for most lakes prevented us from correcting for differences in $\delta^{15}N$ signatures of base resources among lakes (Post 2002) and thus from making meaningful among-population comparisons of $\delta^{13}C$. At least in stickleback, however, $\delta^{15}N$ is known to correlate negatively with $\delta^{13}C$ (see e.g., Figure

2A in Ravinet et al. 2013), suggesting that the information content of $\delta^{13}C$ and $\delta^{15}N$ is largely redundant.

We quantified $\delta^{15}N$ from sculpin (average N per population = 16.5, range: 10 – 25, N total = 280) and stickleback (N = 10 per population, N total = 180) from each study population. We haphazardly chose individuals for this analysis, although we made sure to balance the representation of the two sexes and to leave out individuals that were extremely small or large (notably, there was no systematic size difference between allopatric and sympatric populations within the species, see Fig. S8). Within populations, $\delta^{15}N$ was positively associated with size (SL) for both stickleback and sculpin (both $P < 0.001$). Because we wanted to compare the populations' diets irrespective of the size of the examined individuals, we size-corrected individual $\delta^{15}N$ values within each species using the same approach as applied to the morphological data (see above).

Within each species, we used an ANCOVA to test for an effect of lake type on mean population $\delta^{15}N$ while controlling for baseline $\delta^{15}N$. In this paper, we always used Type III sums of squares for calculating F-statistics using the *R*-package *car* (Fox & Weisberg 2019) and visualized the effect of interest, here that of 'lake type', using the *R*-package *visreg* (Breheny & Burchett 2017). We also tested the prediction that diet niche width of sympatric populations should be reduced compared to allopatric populations because of niche partitioning of the species in sympatric lakes. For this test, we used the among-individual stable isotope variance within a population as a proxy for a population's niche width (Bearhop et al. 2004), as calculated from size-corrected $\delta^{15}N$ values for all 35 study populations from the 26 lakes. We found no effect of $\delta^{15}N$ sample size on within-population $\delta^{15}N$ variance in sculpin ($P > 0.5$), and sample size of $\delta^{15}N$ did not vary among stickleback populations. Yet, within-population $\delta^{15}N$ variance was positively correlated with population mean $\delta^{15}N$ in sculpin, but not in stickleback. Thus, we included mean population $\delta^{15}N$ as a covariate when testing for an effect of lake type on within-population $\delta^{15}N$ variance using ANCOVA. Variances do often not follow normal distributions, which was also true in this case; thus, before running these linear models, we used square root transformation (for stickleback) and log transformation (for sculpin) to normalize the data. This decision was based on a visual inspection of histograms of residuals and QQ-plots.

Finally, we evaluated whether the detected foraging trait variation matches with diet variation across populations. We used population means of $\delta^{15}N$ to predict population means of each foraging trait and of PC1 values from a PCA of all foraging traits (see above) with linear models. Baseline $\delta^{15}N$ of lakes was added as a covariate.

All data handing, statistical analyses and plotting for this paper was done in *R* (R Development Core Team 2021).

Supplementary Analyses

Supplementary Analysis 1. Experimental confirmation that sculpin competition causes stickleback to shift to open-water foraging

Rationale: Both stomach contents and stable isotope data analyses suggested that stickleback in lakes with sculpin prey on more open-water zooplankton (see Figures 5c and 5d). While a previous cattle tank experiment found that allopatric stickleback *surviving* several weeks of exposure to sculpin had a more limnetic diet than stickleback from cattle tanks without sculpin (Ingram et al. 2012), this experiment used large sculpin (up to 16 cm SL), thus making it difficult to evaluate whether direct competition or realized sculpin predation on the more benthic stickleback explained the observed diet shift. Based on our stomach content analysis of sympatric sculpin, however, sculpin are not a frequent predator of adult stickleback (Supplementary Discussion), suggesting that sculpin competition could be the main reason why stickleback shift to more open-water foraging in sympatric lakes. We thus sought to test experimentally whether sculpin competition alone, irrespective of other interactions, can drive stickleback to forage less from benthic prey.

Methods: We reanalyzed data from a field experiment conducted previously by Bolnick et al. (2010). Notably, this experiment tested for an influence of both sculpin as well as trout on niche widths (but not explicitly on diet shifts) in stickleback. While our prime interest in re-analyzing this experiment was in whether and how sculpin competition affects stickleback diet, we used the 'trout competition'-treatment from this experiment as a control treatment to compare the results from the 'sculpin competition'-treatment against (see below). Testing for a possible influence of trout competition on stickleback is also interesting as trout is the only other potential fish competitor of stickleback present in all study lakes. If stickleback showed diet shifts in response to sculpin competition but not in response to trout competition, then this would further support the primary importance of sculpin in driving diet shifts as seen in wild stickleback.

The experiment by Bolnick et al. (2010) was conducted as follows: Twenty seine-net enclosures (each 10 m^2) were arranged in five blocks of four enclosures in Blackwater Lake, one of our sympatric study lakes. Enclosures had open bottoms and were set in 1-2 meters deep water. Each enclosure was stocked with 40 random, wild-caught adult stickleback that were collected near the enclosures. This fish density within enclosures is slightly above natural densities (Svanbäck & Bolnick 2007; Bolnick et al. 2010). Per block, one enclosure was left unmanipulated and thus only contained stickleback ('only stickleback'); in a second enclosure, four small sculpin were added ('sculpin competition'); in a third enclosure, seven small cutthroat trout were added ('trout competition'); and in a fourth enclosure, four small sculpin and seven small trout were added ('full competition'). Notably, the mean SL of the added sculpin and trout was 62.1 mm (sd = 18.1) and 42.5 mm (sd = 16.0) respectively, thus making it highly unlikely that these fish preyed on adult stickleback (which had, on average, a SL of 51.5 mm (sd = 8.4) in the experiment). The experiment ran undisturbed for 15 days, after which stickleback were removed, anaesthetized, and preserved in 10% buffered formalin. In the lab, the standard length of each fish was measured, and prey items were dissected from each recaptured stickleback, sculpin, or trout and identified to the lowest feasible taxonomic level and counted (SA1 Table 1).

We calculated proportions of each prey item per stickleback individual by dividing each prey count by the total number of prey in the stomach of every individual. Separately for sculpin and trout, we obtained a population-level estimate of diet composition by calculating the average of individual proportions of each prey item. Based on the same metric used to calcualte diet overalp between populations of wild-caught fish (PS, see Methods in the main paper), we quantified diet overlap between between every individual stickleback and the average diet compositions of sculpin ($PS_{sculpin}$), as well as trout (PS_{trout}). First, we used ANCOVA to test for the effects of 'sculpin', 'trout', and a 'trout x sculpin' interaction on $PS_{sculpin}$ as well as PS_{trout}, separately. Standard length (of stickleback) and block were added as co-variates. Secondly, according to the analysis from natural populations, we also matched prey categories from this experiment to the prey categories found in natural stickleback populations and classified prey items as either benthic or limnetic (SA1 Table 1). We then calculated the proportion of benthic prey in the diet of each stickleback from the experiment and tested for a benthic-limnetic niche shift between stickleback from enclosures with vs. without sculpin, using an ANCOVA to test for an effect of 'sculpin' on 'benthic prey proportion'. 'Trout', 'block' and 'size' (standard length) were added as covariates. Finally, we determined the extent to which taxonomic groups in stickleback diets shifted in parallel in the experiment and in the natural populations in response to sculpin. We thus determined the five prey categories with the highest mean frequency across all natural populations, as well as the five prey categories with the highest frequency across all stickleback from the field enclosure experiment, and compared mean shifts in these prey categories between natural allopatric vs. sympatric populations and between all stickleback from enclosures without vs. with sculpin from the experiment.

Results: We found that individual diet overlap between stickleback (N = 599) and the average diet of small sculpin (N = 14) was substantial ($PS_{sculpin} = 0.253$, SD = 0.195), especially when compared to the overlap between stickleback and the average diet of small trout (N = 20) ($PS_{trout} = 0.085$, SD = 0.101; PS_{trout} vs. $PS_{sculpin}$ using Welch's $t = -18.7$, $P < 0.001$). The 'trout presence' x 'sculpin presence' interaction was not significant for $PS_{sculpin}$ ($P = 0.19$), so we dropped this interaction from the model. Importantly, the reduced model indicated that the presence of sculpin significantly lowered $PS_{sculpin}$ ($P < 0.001$; SA1 Table 2), with stickleback shifting toward more limnetic prey in the presence of sculpin ($P = 0.001$; SA1 Table 2). This experimental result mirrors the foraging shifts between wild sympatric and allopatric stickleback populations along the benthic-limnetic axis. In contrast, stickleback diet overlap with trout – which appear to only weakly compete with stickleback – was insensitive to the presence or absence of trout, sculpin, or an interaction of these (SA1 Table 2).

When investigating shifts of specific prey species, we found that for all five prey categories that were most frequent across all natural stickleback populations (i.e., chironomids, chydoridae, limnetic cladocerans, gammarus, fish eggs) the foraging shift was in the same direction between wild allopatric stickleback vs. sympatric stickleback and between stickleback from enclosures without vs. with sculpin (SA1 Figure 1). Similarly, for three out of the five prey categories that were most frequent across all experimental stickleback, the direction of the foraging shift matched that seen in the natural populations (note that only the shift on ephemeroptera nymphs clearly differed between the experimental stickleback and natural populations, while the relatively abundant trichoptera larvae in the experiment was found only very rarely in the natural populations and thus showed no shift at all) (SA1 Figure 1).

Conclusion: Overall, these experimental results suggest that competition with sculpin (but not with trout) for benthic prey causes stickleback to shift to more open-water foraging, and that this diet shift of stickleback matches remarkably well with the diet shift observed between wild stickleback from lakes with and without sculpin.

SA1, Table 1

Supplementary Analysis 1, Table 1. Diet categories in experimental stickleback. The leftmost column gives prey categories as they were originally described during data collection by Bolnick et al. (2010). The second column gives the respective general category from stomach contents data from natural populations (see Table S4). Categories with NA in this column could not be matched clearly to such general categories. The third column indicates whether a prey category was treated *a priori* as limnetic or benthic when testing whether stickleback in enclosures with sculpin have a more benthic diet than stickleback in enclosures without sculpin.

Prey categories as recorded by Bolnick et al. (2010)	**Corresponding general diet category** (see Table S4)	**Habitat**
Anisoptera.larvae	anisoptera.nymph	benthic
Aphid	aphid	NA
Bosmina	limnetic.cladoceran	limnetic
Calanoid	copepod	limnetic
Ceratopogonid.larvae	ceratopogonidae.larva	benthic
Chironomid.larvae	chironomid	benthic
Chydorus	chydoridae	NA
Collembola	NA	benthic
Cyclopoid	copepod	limnetic
Daphnia.Macrothricidae	limnetic.cladoceran	limnetic
Diptera.adult	NA	NA
Diptera.pupae	other.diptera.larva	NA
Ephemeroptera	ephemeroptera.nymph	benthic
Gammarus	gammarus	benthic
Harpacticoid	NA	limnetic
Stickleback.larvae	NA	NA
Hemiptera	NA	NA
Hydracarina	NA	limnetic
Mussel	bivalve	benthic
Nematode	NA	benthic
Ostracod	ostracod	limnetic
Polyphemus	limnetic.cladoceran	limnetic
Snail	gastropod	benthic
Stickleback.eggs	eggs	benthic
Tabanidae.larvae	NA	benthic
Trichoptera	trichoptera.larva	benthic
Zygoptera.larvae	zygoptera.nymph	benthic

SA1, Table 2

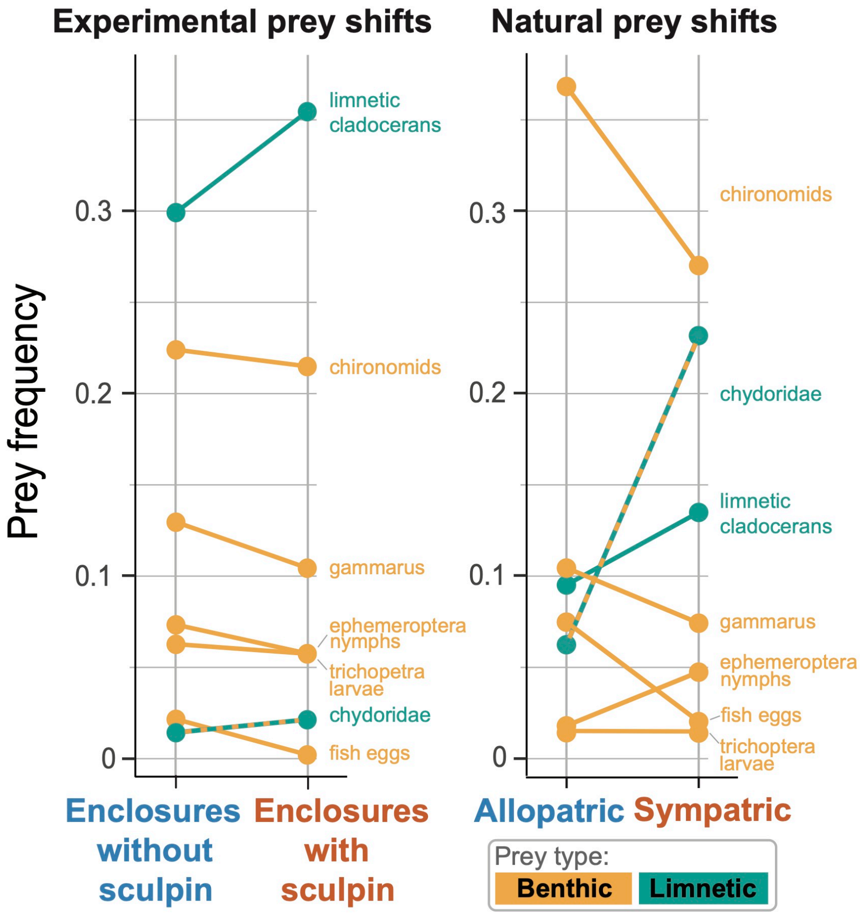
Supplementary Analysis 1, Table 2. Effects of small sculpin and small trout on stickleback diet in a field enclosure experiment. We added, besides 'Trout' and 'Sculpin', 'Block' and 'SL' (standard length) into the ANCOVA to test for effects on **(a)** PS_{trout} (trout x sculpin interaction P-value = 0.183), **(b)** $PS_{sculpin}$ (trout x sculpin interaction P-value = 0.191), and **(c)** the proportion of benthic prey. The syntax of each model is specified below. In conclusion, we find that in response to sculpin, stickleback shift to a more limnetic diet, thus reducing diet overlap with sculpin (see bolded results in (b) and (c)). In contrast, small trout have no obvious effect on stickleback. Note that PS_{trout} (a), $PS_{sculpin}$ (b), and the proportion of benthic prey (c) were all increased for larger stickleback individuals.

(a) PS_{trout}	D.F.	Sum. Sq.	F-value	p-value
Trout	1	0.021	2.12	0.146
Sculpin	1	0.003	0.319	0.572
SL	1	0.146	14.515	< 0.001
Block	4	0.016	0.397	0.811
Residuals	591	5.923		
Model syntax: lm(PSi_trout ~ Trout + Sculpin + SL + Block)				

(b) $PS_{sculpin}$	D.F.	Sum. Sq.	F-value	p-value
Trout	1	0.032	1.006	0.316
Sculpin	**1**	**0.379**	**11.982**	**< 0.001**
SL	1	3.754	118.757	< 0.001
Block	4	0.087	0.689	0.6
Residuals	591	18.68		
Model syntax: lm(PSi_sculpin ~ Trout + Sculpin + SL + Block)				

(c) Benthic prey	D.F.	Sum. Sq.	F-value	p-value
Sculpin	**1**	**1.292**	**10.512**	**0.001**
Trout	1	0.004	0.034	0.938
SL	1	16.123	131.139	< 0.001
Block	4	0.792	1.589	0.176
Residuals	591	72.660		
Model syntax: lm(Prop.benthic.prey ~ Sculpin + Trout + SL + Block)				

SA1, Figure 1



Supplementary Analysis 1, Figure 1. Parallel shifts in benthic and limnetic prey types between experimental enclosures with vs. without sculpin competition and natural populations from lakes with vs. without sculpin. Depicted are the frequency shifts for the five most common prey categories across all stickleback from the experiment as well as across all natural populations (three of these categories overlap: chironomids, chydoridae, gammarus). Prey was classified into benthic and limnetic, although a clear such *a priori* classification was difficult for chydoridae (see Table S4 for details). Note that the direction of shifts in consumed benthic and limnetic prey types are mostly congruent between experimental stickleback from enclosures with sculpin and wild-caught stickleback from lakes with sculpin.

Supplementary Analysis 2. Can variation in known abiotic factors or in the presence of predatory fish species explain shifts in foraging or antipredator morphology in sculpin or stickleback?

Our test of the effects of shared predation and competition is largely based on comparing eco-morphological shifts in replicate allopatric and sympatric populations of wild-caught stickleback and sculpin (but see the SA1 for experimental evidence that sculpin drive a foraging niche shift in stickleback). Here, we test the robustness of our comparative investigation by testing for an influence of several possible confounding biotic and abiotic factors in our analyses. That is, to what extent are these replicate lakes?

Abiotic variation among study lakes

We investigated whether abiotic variation among study lakes could explain our main study results. We characterized abiotic variation by measuring 10 physical and chemical variables from our study lakes (see Table S1 for details). These data were then used in a principal component analysis (PCA) to characterize overall abiotic habitat variation among the study lakes. We found that neither allopatric sculpin lakes nor allopatric stickleback lakes differed clearly from sympatric lakes along 'abiotic PC1', which explained 42.6% of the total abiotic variation (see SA2 Figure 1). Despite considerable overlap, both allopatric stickleback lakes and allopatric sculpin lakes differed from sympatric lakes along 'abiotic PC2' in the same direction (see SA2 Figure 1 and Table S1). This pattern was mainly driven by a lower average Ca concentration and pH of sympatric lakes compared to allopatric lakes (SA2 Figure 1, Table S1). Since allopatric stickleback lakes and allopatric sculpin lakes differed from sympatric lakes in the *same* direction, and only along 'abiotic PC2', abiotic variation seems unlikely to explain the phenotypic shifts in *opposite* direction between the species when comparing populations from sympatric and allopatric lakes (Figures 2 and 3). Still, we aimed to further test whether abiotic differences between lake types could be a main cause for phenotypic divergence of allopatric and sympatric population in the species.

If phenotypic differences between sympatric and allopatric populations was potentially confounded by abiotic differences between lake types, we would expect this abiotic variation to also affect phenotypic variation among populations within lake types. We tested this using linear models with the *R*-syntax *lm(trait.PC ~ lake.type + abiotic.PC),* considering the two major PC axes of abiotic variation ('abiotic PC1' and 'abiotic PC2', see SA2 Figure 1) and the major PC axes of phenotypic variation (Figure 2). Significant effects of abiotic variation in these models would suggest potentially confounding environmental correlates.

For stickleback, we found that neither 'abiotic PC1' nor 'abiotic PC2' affected antipredator or foraging trait variation (all P > 0.12). 'Lake type' remained a strong and highly significant predictor of 'antipredator PC1' and 'foraging PC1' between study lakes in all these models; indeed, the effect of 'lake type' was often statistically better supported in these models compared to models without added abiotic covariates (always P < 0.005). The same held true when we added both 'abiotic PC1' and 'abiotic PC2' as covariates.

For sculpin, abiotic variation did not show significant effects in any of the models (all P > 0.3), except for an effect of 'abiotic PC1' on 'antipredator PC1' (P = 0.018). Importantly, in this model controlling for abiotic variation, evidence for the effect of 'lake type' on 'antipredator PC1' was even stronger (effect of 'lake type' without vs. with 'abiotic PC1' included was t = -2.55 (P = 0.022) vs. t = -3.84 (P = 0.002)). Furthermore, neither 'abiotic PC2' nor 'lake type' showed a significant effect when they were both added as covariates of 'antipredator PC1'. This result came not unexpected given the partial overlap of 'lake type' and variation along 'abiotic PC2' (see SA2 Figure 1), which makes it challenging to test statistically for an independent contribution of 'lake type' and 'abiotic variation' on antipredator trait variation. Yet, there was no obvious correlation between 'abiotic PC2' and 'antipredator PC1' among sculpin populations *within* each lake type (sympatric lakes: Pearson's *r* = 0.18 (P = 0.635), allopatric sculpin lakes: Pearson's *r* = 0.43 (P = 0.291)), suggesting that differences *between* sympatric and allopatric sculpin populations along antipredator PC1 are unlikely to be caused by abiotic variation along 'abiotic PC2'. Finally, when we added both 'abiotic PC1' and 'abiotic PC2' in the sculpin models, the 'lake effect' remained significant for the model explaining 'antipredator PC1' (P = 0.047), and nearly significant (P = 0.061) in the model explaining 'foraging PC2'.

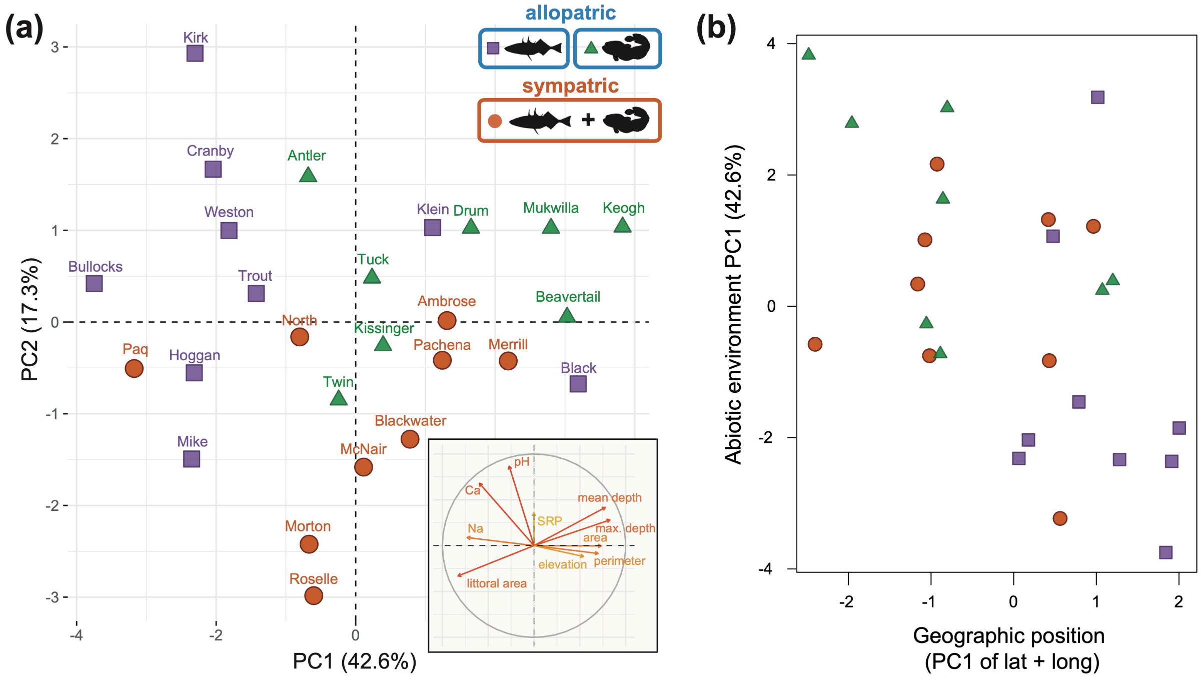
Overall, we conclude that known abiotic variation among study lakes is unlikely to explain main differences in antipredator and foraging traits between allopatric and sympatric populations in sculpin and stickleback.

Biotic variation among study lakes

Can known or possible variation in the presence/absence of other piscivorous fish species besides the omnipresent cutthroat trout explain variation in antipredator traits? An analysis tackling this question revealed that (possible) such predator variation among study lakes cannot easily explain observed shifts in antipredator traits in stickleback and sculpin (see SA2 Figure 2 for details).

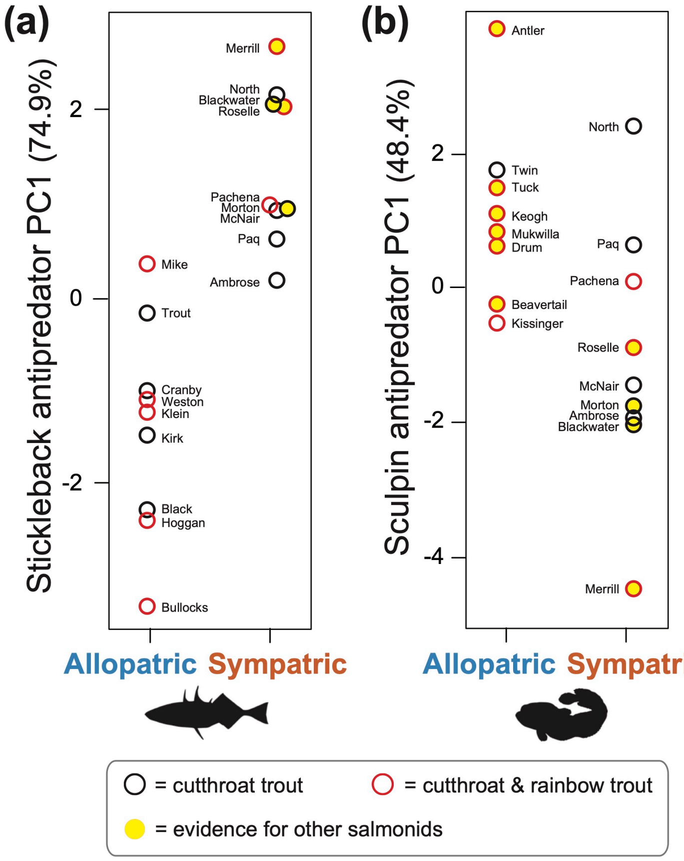
While under some theoretical considerations (e.g., apparent competition) the availability of multiple prey species to a predator could result in a higher density of the predator and thus generally increased predation (see the Introduction of the paper), we have no reason to believe that lakes of the different types differ *per se* (intrinsically) in trout density and thus in total predation pressure. In fact, if trout predation was generally higher in sympatric lakes, then we would expect to see antipredator traits to be increased in both prey species in sympatry. Evidently, this is not what we found (see Figures 2 and 3).

SA2, Figure 1



Supplementary Analysis 2, Fig. 1. Abiotic variation of study lakes and its relationship with geographic proximity. (a) The first two axes of a principal component analysis (PCA) of ten chemical and physical characteristics of all study lakes (see Table S1 for details). Because PCA does not accept missing data, we first imputed missing information (indicated as NA in Table S1) by using the *estim_ncpPCA()* function of the *R*-package *missMDA*. On the right bottom corner of (a), the direction (arrow orientation) and strength (arrow length and heat-map color) of each abiotic variable's loading onto PC1 and PC2 is indicated. Although allopatric sculpin lakes and allopatric stickleback lakes differ along PC1 (resampling P = 0.012), critically, neither lakes with only sculpin nor lakes with only stickleback differ from sympatric lakes along PC1 (P = 0.157 and P = 0.108, respectively). When considering each abiotic characteristic separately, the only clear difference between sympatric vs. allopatric lakes was in Calcium (Ca) concentration and pH (notably, pH and Ca concentration are expected to depend directly on one another and thus were strongly correlated across lakes; Pearson's $r = 0.8$), with sympatric lakes having, on average, lower pH and a lower Ca concentration than either lakes with only stickleback or only sculpin (Table S1). Furthermore, water from allopatric stickleback lakes had lower sodium (Na) concentration than sympatric and allopatric sculpin lakes (see also Miller et al. 2019; Table S1). **(b)** Geographically more close-by lakes, as quantified by the first principal component values of a PCA of latitude and longitude coordinates of all lakes (in decimal degrees), are more similar in abiotic characteristics (Pearson's r between abiotic PC1 and geographic PC1 values = -0.5, P = 0.007).

SA2, Figure 2



Supplementary Analysis 2, Fig. 2. Allopatric-sympatric divergence in antipredator morphology in stickleback and sculpin is not explained by the presence of different predatory salmonid species between lake types. (a) There are fisheries records for the presence of rainbow trout and cutthroat trout in both allopatric stickleback as well as sympatric study lakes, and the additional presence of rainbow trout does not appear to be associated with variation in the strength of antipredator morphology of stickleback populations between or within lake types. We also found some historical records for the presence of additional salmonids in some sympatric lakes (see Table S1 for details) but not in allopatric-stickleback study lakes. However, this does not explain the difference in antipredator morphology between allopatric and sympatric populations because there are several sympatric lakes that have no evidence for additional salmonid species (e.g., North, McNair, and Pachena Lakes) but that exhibit more increased armor than any surveyed allopatric stickleback population. **(b)** There is evidence for rainbow trout and cutthroat trout in all but one allopatric sculpin study lake (Twin Lake), and there are online records for additional salmonids in all but two allopatric sculpin study lakes. However, several of the sympatric study lakes also contain rainbow trout and cutthroat trout, and there is evidence in four sympatric study lakes for the presence of additional salmonid species. Notable, for example, is Twin Lake – an allopatric sculpin lake without rainbow trout or any other salmonids besides cutthroat trout, in which sculpin exhibit the second most strongly developed antipredator traits among all sculpin populations. Furthermore, there are some fisheries records that indicate the presence of rainbow trout as well as other salmonid species in the sympatric Merrill Lake. However, the sculpin population in that lake has the least developed antipredator traits. Finally, sculpin in the sympatric North Lake have the most strongly developed antipredator traits of any sympatric sculpin population, although there is no evidence for any other predatory salmonid species besides cutthroat trout in that lake.

Overall, we conclude that the possible presence/absence of other salmonid predators of stickleback and sculpin besides native cutthroat trout cannot explain the shifts in antipredator traits between allopatric vs. sympatric populations of stickleback and sculpin.

Supplementary Discussion

Can direct sculpin predation explain why sympatric stickleback have increased antipredator traits?

Several lines of evidence suggest that direct sculpin predation is unlikely to be the main reason for why stickleback in sympatric lakes have increased antipredator traits.

First, stomach contents revealed little current sculpin predation on stickleback even though sculpin were collected during the breeding season of stickleback when bottom-nesting stickleback would likely be most vulnerable to sculpin predation. That is, of the nine sympatric sculpin populations investigated, five populations had sculpin individuals that had eaten eggs (putatively from stickleback) or freshly-hatched stickleback fry (12 of the 124 sympatric sculpin specimens with non-empty stomachs). Three other sculpin had eaten small and unidentifiable fish. Three sympatric populations contained sculpin that had eaten adult stickleback (5 of the 124 sculpin). The average soak time of the traps that caught these five fish was high (11.2 hours) compared to the median of 1.5 hours from all traps, suggesting that predation might have happened artificially in traps. Indeed, two of the swallowed stickleback were, upon visual inspection, very little digested. Two further stickleback had digested to some degree but were still well-recognizable stickleback bodies. The last sculpin individual only had stickleback armor in its stomach. Thus, most of the few adult stickleback that we found in sculpin stomachs likely represented opportunistic predation by sculpin in long-soaking minnow traps, rather than an indication of a currently frequent natural phenomenon (see also Broadway & Moyle 1978; Brown et al. 1995). Predation of stickleback eggs or fry also cannot select for increased armor in adult stickleback. Indeed, similar evidence comes from analyzing $\delta^{15}N$ of muscle tissue: If sculpin predation on adult stickleback were common, sympatric sculpin should occupy a higher trophic level than sympatric stickleback, but they do not (Figure 5c). In fact, also in small sculpin (standard length < 65 mm), which are clearly unable to prey on larger stickleback, we found $\delta^{15}N$ to be higher in sympatric than allopatric sculpin ($N = 66$; $P = 0.026$). Hence, elevated $\delta^{15}N$ of sympatric sculpin relative to allopatric sculpin most likely stems, if at all, from predation on stickleback eggs or fry, which cannot select for increased armor in adult stickleback.

Furthermore, frequent sculpin predation on adult stickleback would likely favor adaptations for fast-start swimming of sympatric sculpin to better capture mobile stickleback (Norton 1991), but the opposite was true (see Figures 2b, 3, 4). Also, sympatric sculpin did not have a clearly increased gape width as expected if they were frequent predators of adult stickleback (Figure 3). Finally, neither of two previous experiments found sculpin predation to select for increased defensive traits in stickleback (MacColl & Chapman 2011; Miller et al. 2017). Considered together, we conclude that although large sculpin can certainly be opportunistic predators of stickleback (Moodie 1972; Pressley 1981, MacColl & Chapman 2011), sculpin predation alone is unlikely to explain the increased armor of stickleback in lakes with sculpin.

Supplementary Figures

Figure S1

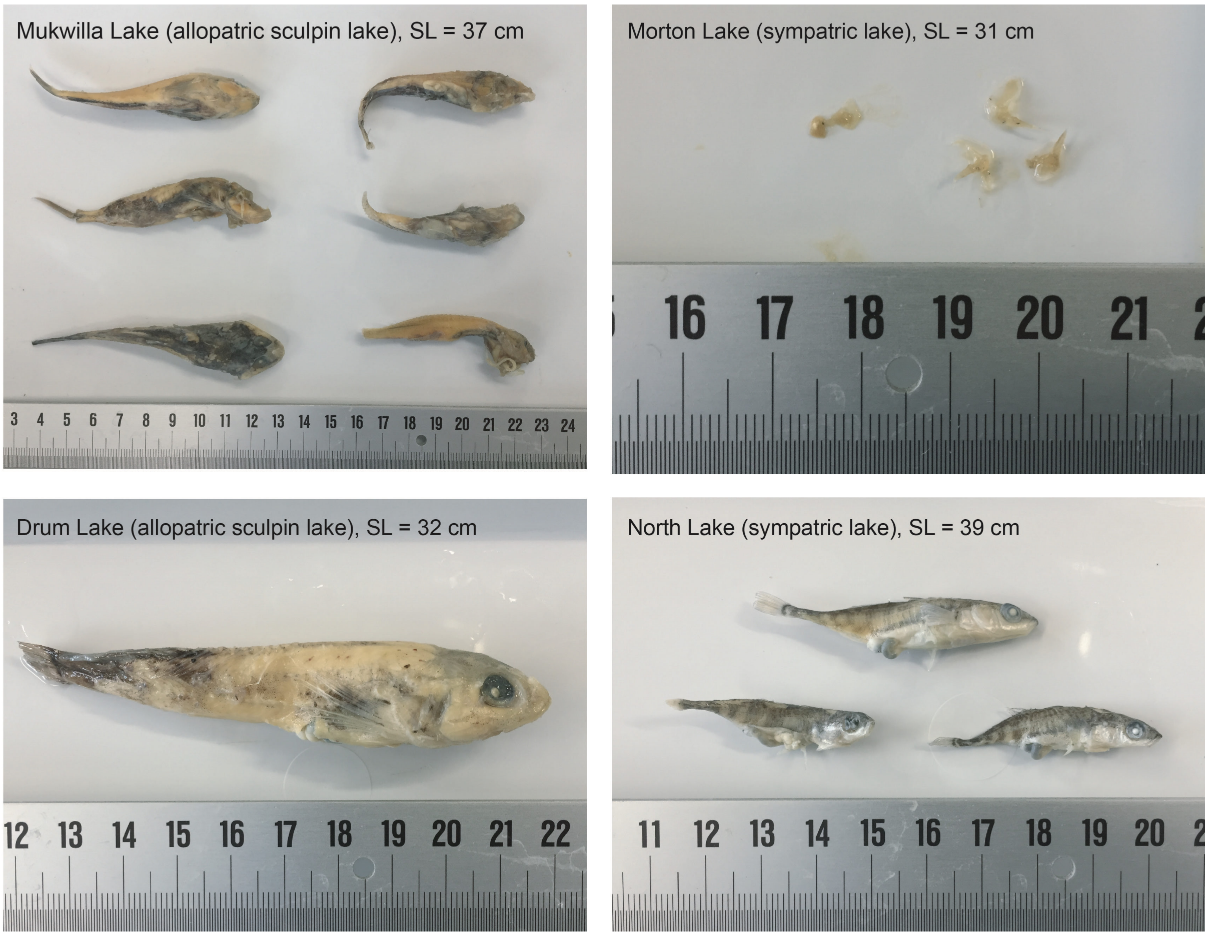
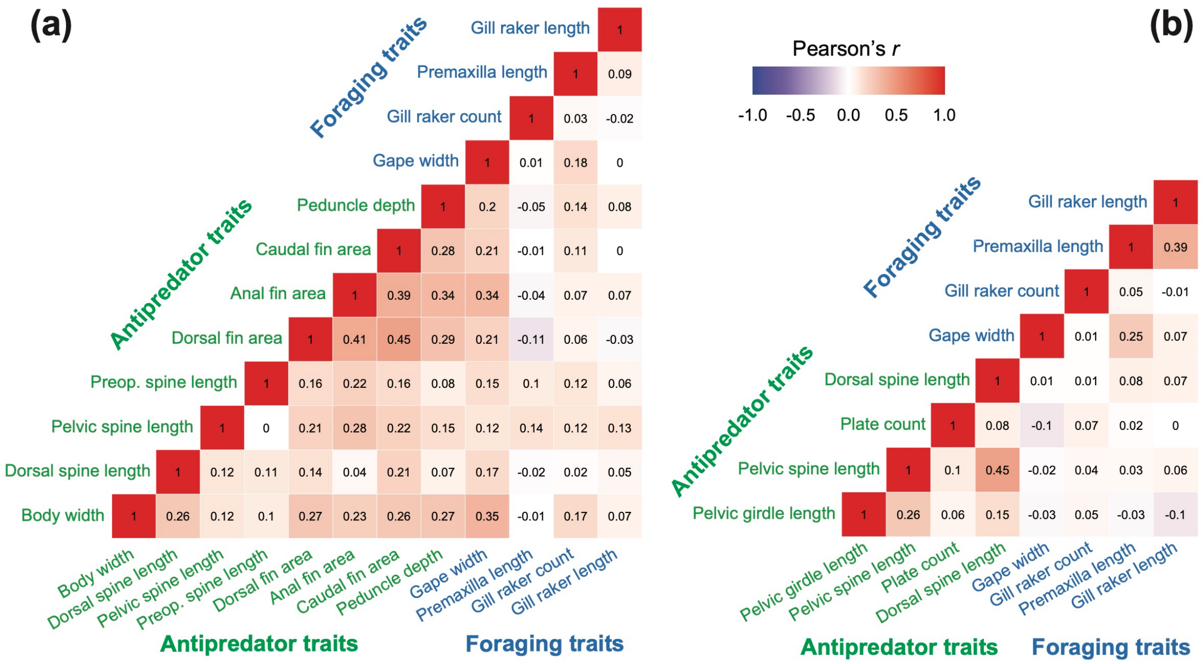


Fig. S1.: Cutthroat trout is a major predator of both sculpin and stickleback. In some study lakes, we used ground pole angling (bait: Berkley PowerBait® Trout Bait) or spin fishing to verify the presence of trout predators, and to test for the absence of other large fish predators (Table S1). Some of the captured cutthroat trout not only made delicious meals, but we also checked their stomach contents (see Table S9 for details). Overall, we found evidence for ample trout predation on both sculpin and stickleback in several lakes. The exemplary pictures show stickleback (right pictures) or sculpin (left pictures), or their remainders (i.e., the top-right picture shows stickleback armor), found within a single cutthroat trout specimen. The standard length (SL) of the respective cutthroat trout and the lake it was caught in is indicated within each picture.

Figure S2



(a)

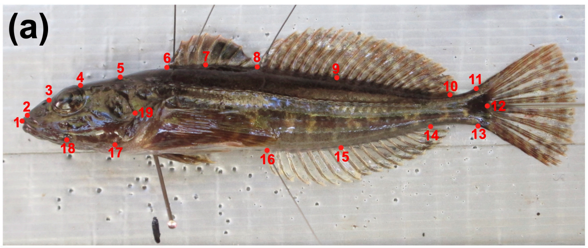
	Body width	Dorsal spine length	Pelvic spine length	Preop. spine length	Dorsal fin area	Anal fin area	Caudal fin area	Peduncle depth	Gape width	Premaxilla length	Gill raker count	Gill raker length
Gill raker length												1
Premaxilla length										1		0.09
Gill raker count											1	0.03	-0.02
Gape width									1	0.01	0.18	0
Peduncle depth								1	0.2	-0.05	0.14	0.08
Caudal fin area							1	0.28	0.21	-0.01	0.11	0
Anal fin area						1	0.39	0.34	0.34	-0.04	0.07	0.07
Dorsal fin area					1	0.41	0.45	0.29	0.21	-0.11	0.06	-0.03
Preop. spine length				1	0.16	0.22	0.16	0.08	0.15	0.1	0.12	0.06
Pelvic spine length			1	0	0.21	0.28	0.22	0.15	0.12	0.14	0.12	0.13
Dorsal spine length		1	0.12	0.11	0.14	0.04	0.21	0.07	0.17	-0.02	0.02	0.05
Body width	1	0.26	0.12	0.1	0.27	0.23	0.26	0.27	0.35	-0.01	0.17	0.07

(b)

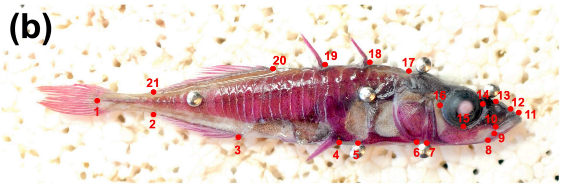
	Pelvic girdle length	Pelvic spine length	Plate count	Dorsal spine length	Gape width	Gill raker count	Premaxilla length	Gill raker length
Gill raker length								1
Premaxilla length							1	0.39
Gill raker count						1	0.05	-0.01
Gape width					1	0.01	0.25	0.07
Dorsal spine length				1	0.01	0.01	0.08	0.07
Plate count			1	0.08	-0.1	0.07	0.02	0
Pelvic spine length		1	0.1	0.45	-0.02	0.04	0.03	0.06
Pelvic girdle length	1	0.26	0.06	0.15	-0.03	0.05	-0.03	-0.1

Fig. S2: Trait correlations for (a) sculpin and (b) stickleback. Pearson's *r* was first calculated for all pairwise trait combinations within each population per species using size-corrected trait values. Population-specific trait correlations were then averaged per species. Note that trait correlations are generally low, and, importantly, close to zero between putative foraging and antipredator traits. This suggests that the measured traits, and particularly antipredator and foraging traits, have the potential to respond independently from one another to selection.

Figure S3



Landmark ID	Description
1	Mid-anterior upper lip
2	Anterior maxilla endpoint
3	Interorbital pore
4	Posterior end of eye vs. body intersection
5	End of cranium
6	Anterior insertion of first dorsal fin
7	Base of 5th ray in first dorsal fin (counted from anterior)
8	Anterior insertion of second dorsal fin
9	Base of 9th ray in the second dorsal fin (counted from anterior)
10	Posterior insertion of second dorsal fin
11	Upper base of caudal fin
12	Posterior extent of caudal peduncle
13	Lower base of caudal fin
14	Posterior insertion of anal fin
15	Base of 8th anal fin ray (counted from anterior)
16	Anterior insertion of anal fin
17	Ventral-distal intersection of the pre-operculum and the operculum
18	Posterior end of premaxilla
19	Distal end (tip) of pre-opercular spine



Landmark ID*	Description
1 (1)	Posterior extent of caudal peduncle
2 (2)	Posterior insertion of anal fin
3 (3)	Anterior insertion of anal fin
4 (4)	Insertion point of pelvic spine into the pelvic girdle
5 (6)	Posterior extent of ectocorocoid
6 (8)	Anterior extent of ectocorocoid
7 (13)	Posterioventral extent of preopercular
8 (14)	Anterioventral extent of preopercular
9 (15)	Posterior extent of premaxilla
10 (16)	Posterior extent of maxilla
11 (17)	Anterior extent of maxilla
12 (18)	Anterior extent of nasal
13 (19)	Lachrymal at nasal capsule
14 (20)	Anterior extent of orbit
15 (21)	Ventral extent of orbit
16 (22)	Posterior extent of orbit
17 (23)	Posterior extent of supraoccipital
18 (24)	Anterior insertion of first dorsal spine
19 (25)	Anterior insertion of second dorsal spine
20 (26)	Anterior insertion of third dorsal spine
21 (27)	Posterior insertion of dorsal fin

** The landmark numbers in brackets correspond to the landmark ID's in Albert et al. 2008 (Evolution). Notably, we excluded landmarks from Albert et al. 2008 that only marked antipredator structures because we aimed to capture general body shape differences using geometric morphometrics, while we measured antipredator traits separately.*

Fig. S3.: Lateral body shape landmarks set on photographs taken from (a) freshly killed sculpin and (b) alizarin-stained stickleback. These landmarks were used for geometric morphometrics. Pictures of specimens were also used to measure some foraging and antipredator traits, while other such traits were measured directly from the specimens. See Methods for further details.

Figure S4

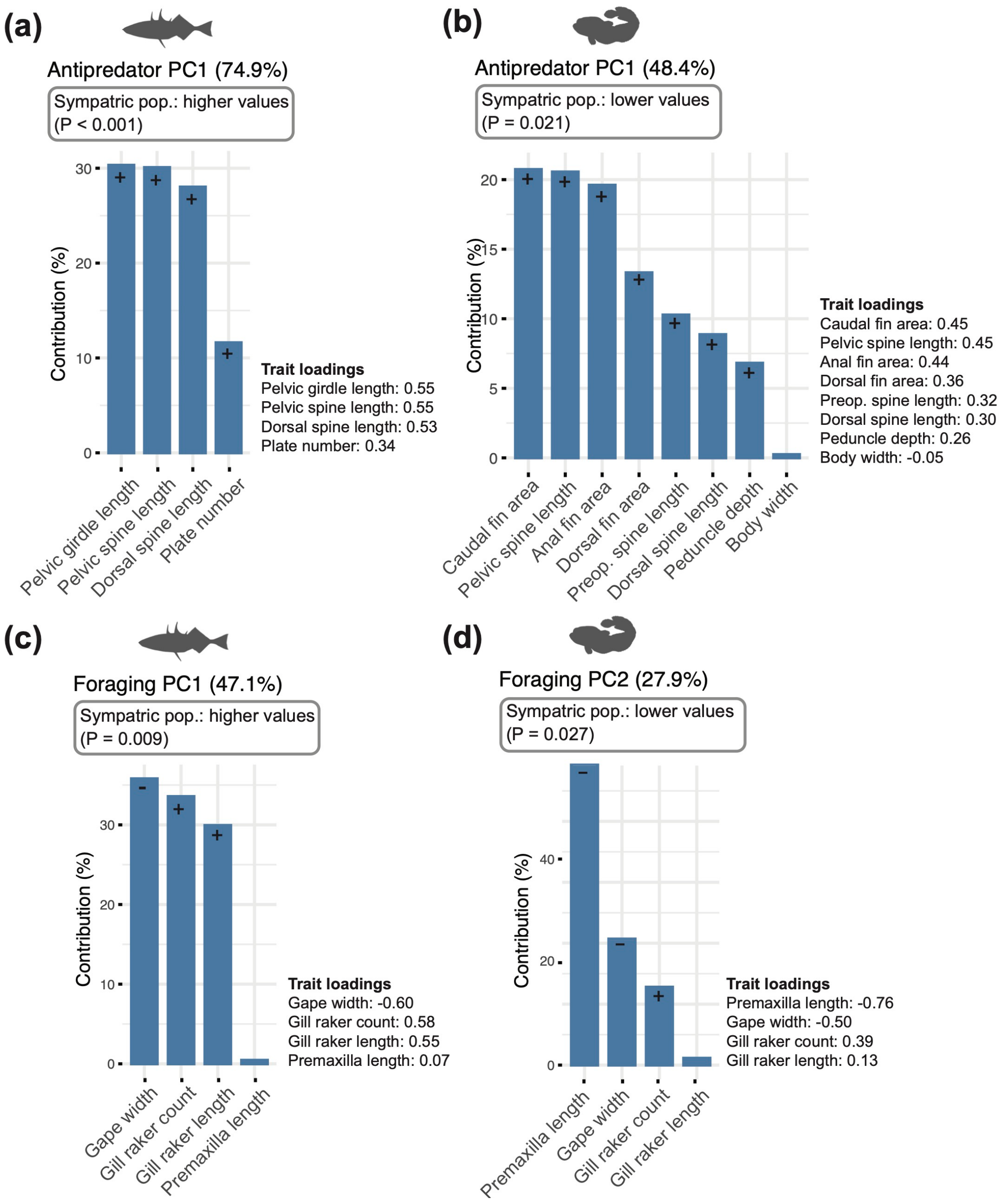


Fig. S4: Trait loadings on principal component axes from independent PCAs of foraging and antipredator straits for sculpin and stickleback. See Figures 2a and 2b and the Results section for details. Shown here are the loadings for the first or second PC axis whenever sympatric and allopatric populations differ with a statistical significance of P < 0.05 along this axis. Within gray boxes, P-values of the difference between allopatric and sympatric populations are indicated, as well as whether sympatric populations have, on average, higher or lower values along the respective PC axis. These axes include: **(a)** PC1 of antipredator traits in stickleback, **(b)** PC1 of antipredator traits in sculpin, **(c)** PC1 of foraging traits in stickleback, and **(d)** PC2 of foraging traits in sculpin. Bars represent the contribution in percentage of each trait on the respective axis, as inferred by the *fviz_contrib()* function from the *R*-package factoextra (Kassambara & Mundt 2020). If this contribution is greater than 5%, the direction of the contribution is indicated by either a 'plus' symbol (i.e., the trait loads positively on the respective PC axis) or a 'minus' symbol (the trait loads negatively on the respective PC axis). Next to each bar plot, conventional values for trait loadings are given as insert tables. Note that except for foraging traits in sculpin (where allopatric and sympatric populations differ along PC2), allopatric and sympatric populations always differ along PC1.

Figure S5

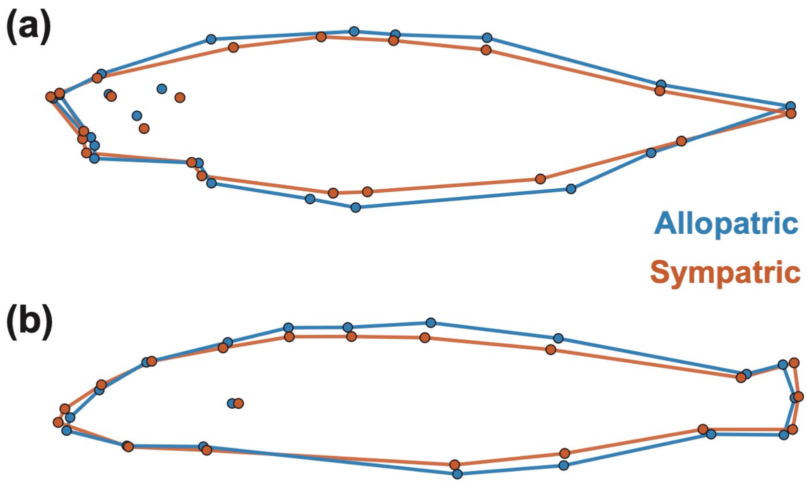


Fig. S5: Comparison of the average shape of allopatric and sympatric (a) stickleback and (b) sculpin. Comparison of the mean shape across all sympatric and all allopatric (a) stickleback and (b) sculpin is based on size-corrected Procrustes coordinates calculated from landmark data (see Fig. S3 and Methods for details). Coordinates were plotted using the *plotRefToTarget()* function of the *geomorph R*-package (Adams et al. 2017), magnifying shape differences two-fold.

Figure S6

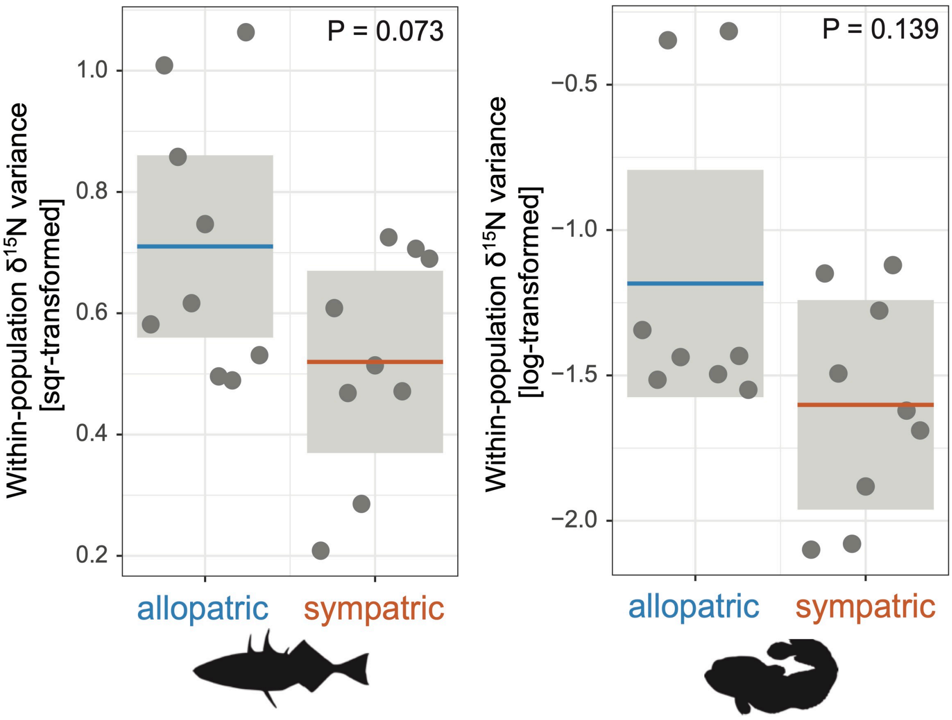


Fig. S6. Within-population niche widths tend to be wider in allopatric than sympatric populations, especially in stickleback. Dots give partial residuals of δ^{15}N variances within each study population (N total = 35) and blue lines show the effect of lake-type on within-population δ^{15}N variance per species, surrounded by 95% confidence bands as visualized by the *R*-package *visreg*.

Although statistical significance (P-values) of 'lake type' on 'within-population δ^{15}N variance' is above 0.05 (see P-values indicated within the plots), within-population δ^{15}N variance tends to be increased in allopatric than sympatric populations of *both* species. This pattern matches a common prediction of ecological release, where increased ecological opportunity in the absence of a competitor leads to an increased niche width in another species. The statistical model used to obtain these plots separately for each species was: *lm(within.pop.*δ^{15}*N.variance ~ lake.type + mean.pop.*δ^{15}*N, data=d*). Data were first normalized before running these models, using log-transformation for sculpin and sqr-transformation for stickleback. Visualized is the effect of 'lake type'.

Figure S7

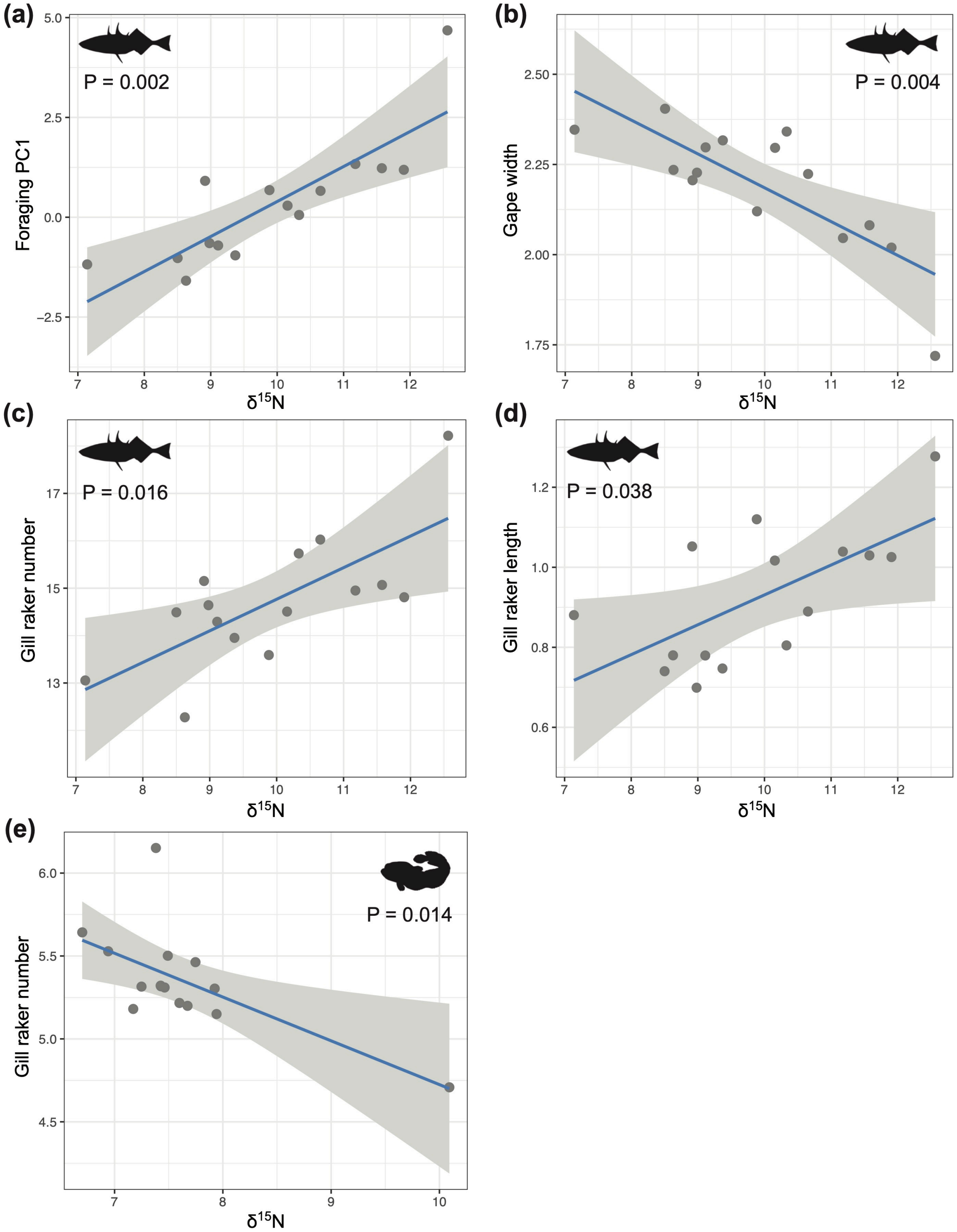


Fig. S7: Higher $\delta^{15}N$ is associated with a more limnetic foraging morphology in stickleback (a-d), and fewer gill rakers in sculpin (e). We first calculated $\delta^{15}N$ population means from size-corrected individual $\delta^{15}N$ values. These $\delta^{15}N$ population means where then used, while controlling for baseline $\delta^{15}N$ of lakes (as calculated from mussel and/or snail tissue; see Methods for details), to test for an effect on foraging PC1 or PC2, as well as on every individual foraging trait using linear models.

We found a clear positive association between $\delta^{15}N$ and **(a)** stickleback foraging PC1, whereby the more limnetic sympatric stickleback populations had higher PC1 values than the more benthic allopatric stickleback populations (Fig. 2b). Accordingly, higher $\delta^{15}N$ values were negatively associated with **(b)** a narrower gape and positively associated with **(c)** more and **(d)** longer gill rakers in stickleback. The only foraging trait of stickleback not showing any clear association with $\delta^{15}N$ was premaxilla length ($P > 0.5$). In sculpin, in contrast, the only trait or foraging principal component axis associated with $\delta^{15}N$ was gill raker length **(e).** The P-value provided within each panel gives the statistical significance of the effect of $\delta^{15}N$ on the respective trait. The dots are partial residuals of population means, and shading indicates 95% confidence bands as calculated and plotted by the *R*-package *visreg*.

Figure S8

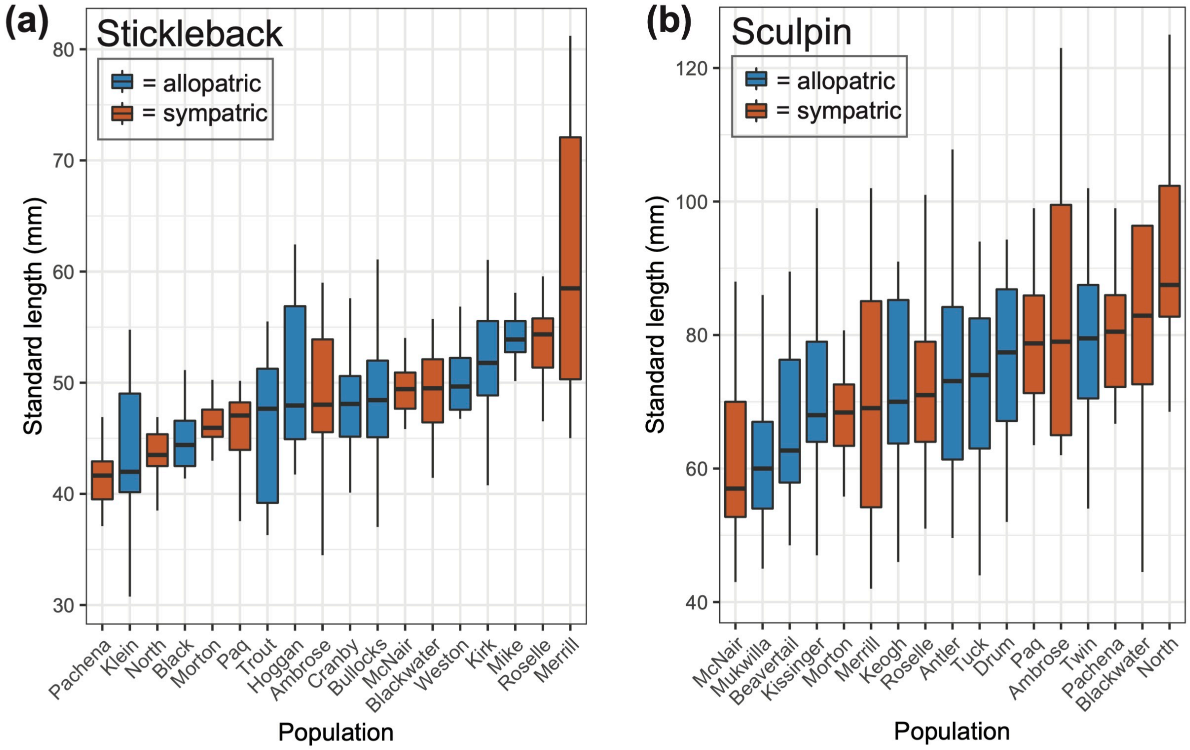


Fig. S8.: Body size of sampled fish per population. Shown is the standard length of all **(a)** stickleback individuals and **(b)** sculpin individuals randomly sampled from natural populations and analyzed in this study.

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