

INTRAGUILD PREDATION DRIVES EVOLUTIONARY NICHE SHIFT IN THREESPINE STICKLEBACK

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Intraguild predation—competition and predation by the same antagonist—is widespread, but its evolutionary consequences are unknown. Intraguild prey may evolve antipredator defenses, superior competitive ability on shared resources, or the ability to use an alternative resource, any of which may alter the structure of the food web. We tested for evolutionary responses by threespine stickleback to a benthic intraguild predator, prickly sculpin. We used a comparative morphometric analysis to show that stickleback sympatric with sculpin are more armored and have more limnetic-like body shapes than allopatric stickleback. To test the ecological implications of this shift, we conducted a mesocosm experiment that varied sculpin presence and stickleback population of origin (from one sympatric and one allopatric lake). Predation by sculpin greatly increased the mortality of allopatric stickleback. In contrast, sculpin presence did not affect the mortality of sympatric stickleback, although they did have lower growth rates suggesting increased nonpredatory effects of sculpin. Consistent with their morphology, sympatric stickleback included more pelagic prey in their diets, leading to depletion of zooplankton in the mesocosms. These findings suggest that intraguild prey evolution has altered food web structure by reducing both predation by the intraguild predator and diet overlap between species.

KEY WORDS: Character displacement, *Cottus asper*, *Gasterosteus aculeatus*, geometric morphometrics, mesocosm experiment, omnivory.

Food web interactions, such as predation and resource competition, are important agents of natural selection that can drive evolutionary change (e.g., Schluter 1994; Reznick et al. 1997;

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Langerhans et al. 2004; Grant and Grant 2006; Nosil and Crespi 2006). This evolution results in the modification of trophic interactions, which mesocosm experiments have suggested can have effects at the food web or ecosystem level (Harmon et al. 2009; Palkovacs et al. 2009; Bassar et al. 2010; Palkovacs et al. 2011). These ecological consequences of one species' evolution may alter selective pressures on it or other species, leading to eco-evolutionary feedbacks (Post and Palkovacs 2009; Schoener 2011). A full understanding of food web structure and dynamics

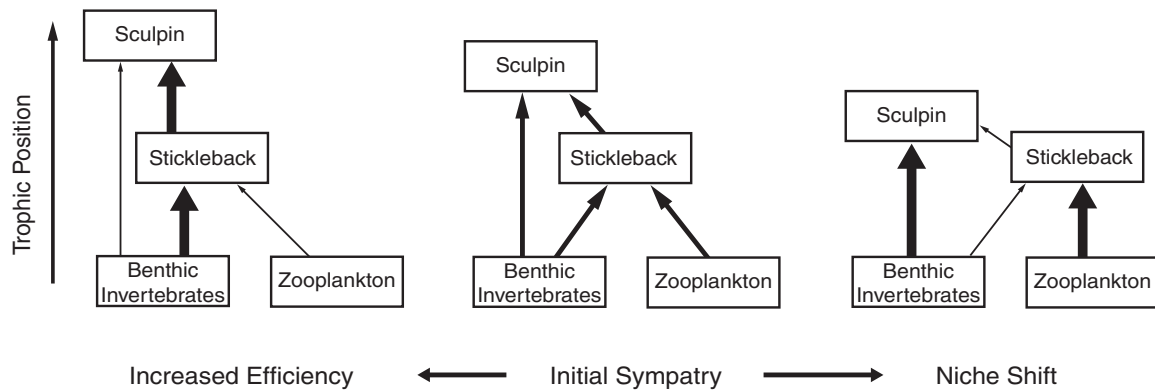


Figure 1. Hypothesized evolutionary responses of stickleback to intraguild predation and their ecological consequences. Vertical positions of species or trophic groups indicate their trophic position (a continuous measure of trophic level), and arrow widths indicate relative strength of feeding interactions. Following initial sympatry with sculpin, stickleback may become superior competitors for their shared benthic invertebrate resources (increased efficiency), likely leading to higher predation rates by sculpin on stickleback. Alternatively, stickleback may undergo a niche shift to rely more on the alternative resource (zooplankton), likely reducing predation and thus the trophic position of sculpin.

will therefore require knowledge of how trophic interactions both cause and are altered by evolution.

Intraguild predation is a widespread trophic interaction in which one species both feeds on a second species and competes with it for a shared resource (Polis et al. 1989; Polis and Holt 1992; Arim and Marquet 2004). This double threat of competition and predation can have substantial negative demographic effects on the intraguild prey (Polis et al. 1989; Diehl 1995), and may result in strong natural selection. Although the direction of selection resulting from intraguild predation has not been studied, we can derive some hypotheses from theoretical predictions addressing the conditions under which intraguild prey can persist (Holt and Polis 1997; Vandermeer 2006). If traits of the intraguild prey that favor persistence also confer higher fitness on individuals and are genetically variable, natural selection may result in an evolutionary response by the intraguild prey that enhances its persistence in the food web.

Intraguild prey species are more likely to persist if the competitive and/or predatory impacts of the intraguild predator are weakened (Amarasekare 2007; Daugherty et al. 2007; Kondoh 2008). A classic prediction that has been supported by empirical work is that the intraguild prey can often persist if it is a better competitor for the shared resource than the intraguild predator (Holt and Polis 1997; Morin 1999; Vance-Chalcraft et al. 2007; Kondoh 2008). A corresponding “increased efficiency” hypothesis predicts that the intraguild prey will evolve traits that increase its ability to consume the shared resource (Fig. 1). Alternatively, the intraguild prey may persist if it is subsidized by resources that are not utilized by the intraguild predator (Daugherty et al. 2007; Holt and Huxel 2007). A second possible evolutionary response is a “niche shift” that increases reliance on these alternative resources and thereby reduces diet overlap with the intraguild

predator (Fig. 1). Either increased efficiency on a shared resource (character convergence) or a niche shift to an alternative resource (character displacement) may occur under interspecific competition alone (Abrams 1987), and should reduce the competitive impact of the intraguild predator on the intraguild prey. Additional evolutionary responses may reduce the predatory impact of the intraguild predator through inducible or constitutive defensive traits (Kratina et al. 2010). Interestingly, a niche shift by the intraguild prey that includes a spatial habitat shift may reduce both competition and predation (Polis and Holt 1992; Finke and Denno 2006).

Any evolutionary response by the intraguild prey has the potential to rearrange the food web. Intraguild predation varies along a continuum between a tritrophic food chain and simple resource competition (Vandermeer 2006), meaning that the “increased efficiency” and “niche shift” hypotheses predict different food chain lengths (Fig. 1). Under the increased efficiency model, the competitively inferior intraguild predator is itself more likely to persist if it increases its consumption of the intraguild prey (Holt and Polis 1997). In contrast, both a niche shift and the evolution of antipredator traits by the intraguild prey may increase the intraguild predator’s reliance on the shared resource. If its trophic interactions with other species are unchanged, the intraguild predator’s trophic position (a continuous measure of trophic level) is thus expected to increase if the intraguild prey evolves increased efficiency, and to decrease if it undergoes a niche shift. A niche shift may also cause the intraguild prey to derive its energy from another channel in the food web. These rearrangements of the food web have the potential to alter food web functioning and stability by changing food web compartmentalization and the strength of top-down control (Pace et al. 1999; Rooney et al. 2008).

Threespine stickleback (*Gasterosteus aculeatus*) in lakes in southwestern British Columbia naturally occur with and without intraguild predators (Vamosi 2003), making them an appropriate system in which to test for evolutionary responses to intraguild predation. Stickleback repeatedly colonized small lakes from the ocean at the end of the last ice age (about 12,000 years ago). In contrast to a few lakes in which benthic and limnetic specialist species pairs have evolved, most lakes contain a single “solitary” stickleback population that feeds on both benthic invertebrates in the littoral zone and zooplankton in the open water. We focus here on two types of solitary populations: “sympatric” stickleback that occur in lakes with prickly sculpin (*Cottus asper*), a putative intraguild predator, and “allopatric” stickleback from lakes without sculpin. Sculpin prey on stickleback eggs, fry, and adults up to 60% of their body length (Moodie 1972; Pressley 1981). Sculpin also feed on many of the same benthic invertebrate resources as stickleback, making them likely intraguild predators. Although direct competitive effects between the two species have yet to be measured, a field enclosure experiment indicated that the presence of sculpin can reduce foraging success and decrease the breadth of diet items used by individual stickleback (Bolnick et al. 2010). We do not currently know the colonization history of these two species: if stickleback colonized first, sympatric populations may be derived from an allopatric-like ancestor, whereas if sculpin arrived prior to or concurrently with stickleback, sympatric populations may instead have retained ancestral traits. For concreteness, we discuss our results and interpretation in terms of character shifts in sympatry, but the predictions based on sculpin presence apply in either case.

Based on the scenarios we have outlined above, we predicted that stickleback sympatric with sculpin would either evolve increased efficiency on the shared resource (benthic invertebrates), or undergo a niche shift to rely more on nonbenthic resources (e.g., zooplankton). We tested these outcomes as well as antipredator morphological adaptations by comparing the phenotypes of wild and laboratory-reared stickleback from lakes with and without sculpin. To investigate ecological changes predicted to accompany morphological shifts, we used one population of each type in a mesocosm experiment to (1) compare the effects of sculpin addition on the fitness and resource use of sympatric and allopatric stickleback, and (2) measure the food web consequences of stickleback character shifts. Both our comparative and experimental data support the hypothesis that stickleback have undergone a niche shift in the presence of their intraguild predator.

Methods

COMPARATIVE ANALYSIS OF BODY SHAPE

We analyzed geometric body shapes of stickleback from 24 populations in southwestern British Columbia to test for morphological

changes in response to the intraguild predator. We sampled five populations sympatric with prickly sculpin and 10 allopatric populations from lakes without sculpin. Although densities of sculpin have not been measured in the sympatric lakes, sculpin are frequently observed in the littoral zone and readily enter minnow traps (T. Ingram and R. Svanbäck, unpubl. data). All lakes also contain cutthroat trout (*Oncorhynchus clarkii*), a predator of stickleback in open water habitats. To provide context for the comparison between populations occurring with and without sculpin, we also sampled benthic and limnetic stickleback species from three lakes containing species pairs, as well three anadromous marine populations representing the heavily armored ancestral form (Walker and Bell 2000). The marine population from Little Campbell River is represented by two samples, one collected from the wild and the other raised in freshwater ponds at the University of British Columbia. Almost all populations occupy different watersheds and derive from separate colonizations of freshwater (Table S1), so we treat them as statistically independent replicates. Lakes with and without sculpin have similar average depth (analysis of variance [ANOVA] of log-transformed depth by population type: $F_{1,13} = 0.01$, $P = 0.92$) and elevation ($F_{1,13} = 0.83$, $P = 0.38$). There was a nonsignificant tendency for lakes containing sculpin to have larger surface areas (20.9 ± 4.05 SE) than lakes without sculpin (12.5 ± 3.90 SE; $F_{1,13} = 3.62$, $P = 0.08$), with considerable overlap in surface area between lakes with and without sculpin.

Fish collected from each population were euthanized with MS-222 (Argent Chemical Laboratories, Redmond, WA), preserved in 10% formalin for at least two weeks, stained with alizarin red to highlight bone, and stored in 37% isopropyl alcohol. We took digital photographs of the left side of each specimen and digitized 22 landmarks representing the positions of bony elements of the jaw, head and spines, and insertion points of the fins (Fig. S1). These landmarks are similar to those used in previous shape analyses, are known to vary among stickleback populations, and in many cases have known functional significance (Walker 1997; Walker and Bell 2000; Spoljaric and Reimchen 2007; Albert et al. 2008). Landmark positions on each photograph were digitized with tpsDig 1.40 software (Rohlf 2005a), centered and scaled to unit size using tpsRelw 1.44 (Rohlf 2005b), and rotated to align with the average shape by minimizing the sum of squared distances over all homologous landmarks (Zelditch 2004).

These procedures resulted in 22 x and 22 y coordinates for each fish. Because there were many more traits (44) than populations or population types, we did not feel that a test based on all 44 traits as response variables was justified. Instead, we reduced the dimensionality of the data by using a linear discriminant analysis (LDA), implemented in the MASS package (Venables and Ripley 2002) in the R environment (R Development Core Team 2009), which identified major axes of shape variation among

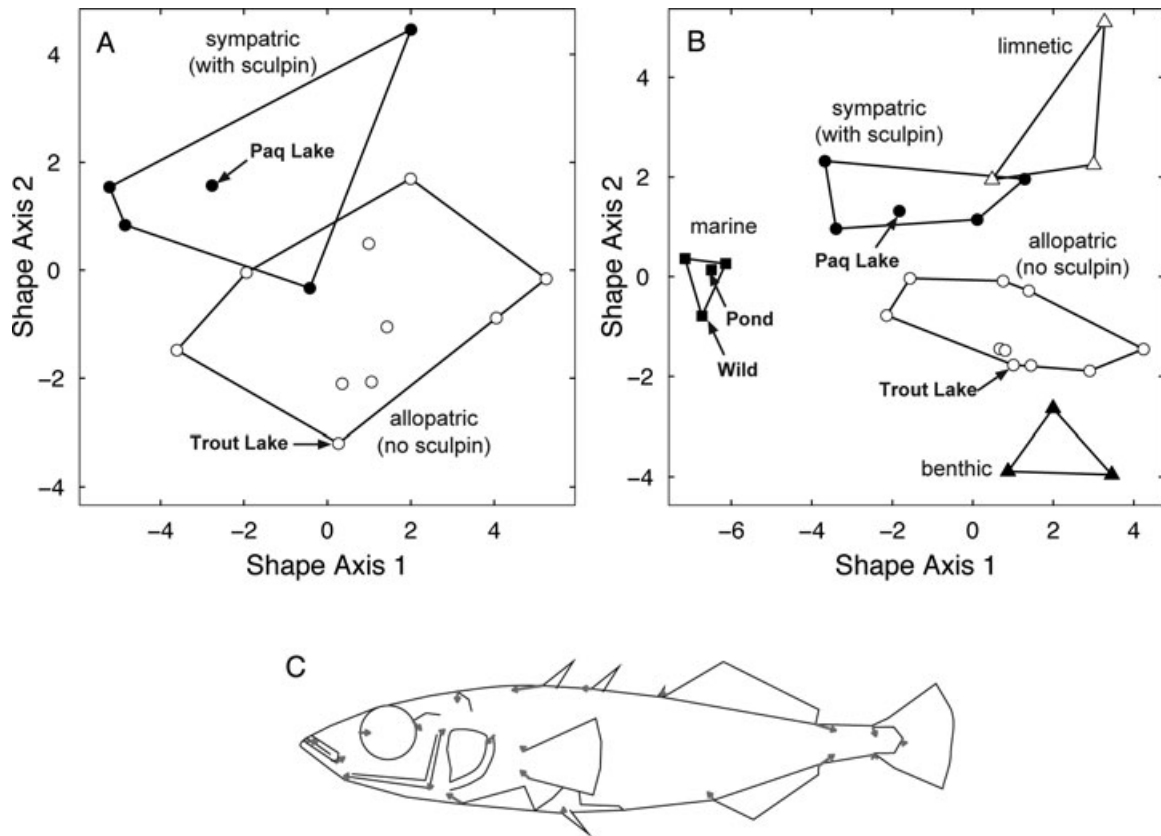


Figure 2. Body shape differences among the different types of stickleback populations. Population means are shown along the first two major shape axes from linear discriminant analyses separating (A) solitary populations only and (B) populations of all types. In each case, the LDA separated populations without reference to population type. Symbols and convex hulls identify different types of stickleback populations, and arrows identify the two populations used in the mesocosm experiment. In (B), arrows also indicate wild-caught and pond-reared marine stickleback from the Little Campbell River marine population. (C) Differences in mean landmark positions between solitary populations in lakes without sculpin (base of arrow) and lakes with sculpin. Arrow lengths are multiplied by three for greater visibility.

populations relative to variation within populations. Importantly for our analysis, we used population as our classification variable rather than population type, so the LDA did not directly differentiate the population types. We used LDA instead of alternatives such as principal components analysis (PCA) because LDA isolates those characters that vary among populations by identifying axes that maximize variation among relative to within populations (Tabachnick and Fidell 1996). In contrast, PCA can be heavily influenced by within-population variation, including measurement error and artifacts such as upward or downward bending of specimens. We visualized vectors of shifts in individual landmarks (Figs. 2, S1) after first using PCA to remove specimen bending effects following Albert et al. (2008).

We conducted two linear discriminant analyses: (1) our primary analysis including only solitary populations with and without sculpin (again, without identifying them by population type), and (2) an additional analysis that included solitary, benthic, limnetic, and marine populations (LDA loadings given in Table S2).

Our subsequent shape analyses treated population as the level of replication. We tested for differences among population types using multivariate and univariate ANOVAs on the population means of the first two discriminant functions arising from each analysis. To ensure that the process of maximizing separation of populations using LDA did not introduce bias toward detecting differences among population types, we used two randomization procedures to generate null distributions of the test statistics. In the first, we randomized population means among population types and repeated the discrimination and multivariate analysis of variance in each iteration. This analysis preserves the association of individual fish with population but randomizes their association with type. In the second test, we randomized assignment of individual fish to both populations and types and then repeated the same steps. This analysis breaks down all differences between populations and population types under the null hypothesis.

To correct for possible effects of variation in body size, we repeated all analyses after adjusting each landmark to the average

size of all fish using analysis of covariance (ANCOVA). This analysis adjusted each trait separately using populations as groups, size (standard length) as the covariate, and a common slope for all populations.

MESOCOSM EXPERIMENT

Our experiment crossed two treatments in a factorial design: sculpin addition (control vs. one sculpin added) and stickleback population of origin (from a lake containing sculpin vs. a lake without sculpin). This design allowed us to validate ecological inferences made from observed character shifts by testing for overall differences between populations and for differential responses to sculpin presence.

We established 36 experimental mesocosms in 1136 L plastic cattle tanks 1 m deep and 2 m wide. We randomly assigned eight tanks to each of our four primary treatments, and left the remaining four tanks free of all fish to test for overall fish effects. Tanks were filled with water and seeded with 10 L of benthic mud as well as filtrate from 80 L of water from nearby experimental ponds, containing a variety of benthic and pelagic invertebrates and their propagules. We added 0.05 g KH_2PO_4 and 1.0 g NaNO_3 to each cattle tank to stimulate primary production, then left the tanks for two weeks before adding fish. To provide stickleback with shade and refuge from predation, we suspended a 25 cm diameter open-ended cylinder of black 7 mm mesh (DuPont Vexar, Wilmington, DE) below the water surface.

We used minnow traps to collect 128 “allopatric” stickleback from Trout Lake, 128 “sympatric” stickleback from Paq Lake, and 16 sculpin from Sakinaw Lake, all in different watersheds on the Sechelt Peninsula. Trout and Paq Lakes are similar in productivity (summer surface chlorophyll a : 1–4 $\mu\text{g L}^{-1}$; T. Ingram, unpubl. data), surface area and depth (Table S1), and have stickleback with representative allopatric and sympatric body shapes (Fig. 2). We observed that stickleback in Trout Lake grew to a larger maximum size than in Paq Lake, so we selectively excluded some of the largest fish from our collection.

Fish were transported to the University of British Columbia, and housed for two days in outdoor holding tanks. Eight stickleback were assigned to each mesocosm; each was anaesthetized with 0.1 g L^{-1} MS-222, then individually marked by subcutaneous injection of a two color combination of elastomer dye (Northwest Marine Technology, Shaw Island WA). Stickleback were then weighed to 0.01 g, allowed to recover, and added to the mesocosms in early May 2010. There were no differences in initial size between stickleback added to mesocosms from Paq (range 0.42–2.19 g, mean 0.88 g) and Trout Lake (range 0.33–1.99 g, mean 0.91 g; ANOVA: $F_{1,254} = 0.34$, $P = 0.56$). Sculpin were weighed and systematically assigned to the two sculpin addition treatments to ensure similar size distributions (allopatric: 13.90 ± 4.33 g; sympatric: 13.86 ± 3.64 g; mean \pm SD). All sculpin were

large enough (9–16 cm standard length) to ingest the majority of the stickleback (3–5.5 cm standard length) in the tanks (Pressly 1981). Sculpin were added to tanks two days after the stickleback were introduced.

We surveyed tanks after two, four, and six weeks to assess survivorship of stickleback and growth of stickleback and sculpin. We placed minnow traps in the tanks for two days, checking traps frequently, and removing them at night to prevent predation by sculpin within the traps. Each recaptured fish was weighed again to measure its growth, and then returned it to its tank. To maintain approximately constant stickleback densities, we replaced fish that were not recaptured with additional fish from Paq and Trout Lakes. Because of differences in mortality (see Results), the number of replacement fish varied among treatments (allopatric control: 35; allopatric + sculpin: 77; sympatric control: 60; sympatric + sculpin: 62). Replacement fish were tagged and weighed, then distributed to return the densities in each tank to eight fish (first round of replacements) and seven fish (second round), although some densities were temporarily higher (up to 11 in one tank) because fish were missed during a round of recaptures. Fish collected from the two lakes did not differ in size during the first round of replacements (Paq Lake: range 0.39–2.16 g, mean 0.92 g; Trout Lake: range 0.44–1.58 g, mean 0.92 g; ANOVA: $F_{1,118} = 0.003$, $P = 0.95$), although in the second round replacement sympatric stickleback were smaller on average (Paq Lake: range 0.23–1.57 g, mean 0.55 g; Trout Lake: range 0.37–1.32 g, mean 0.76 g; ANOVA: $F_{1,112} = 19.9$, $P < 0.001$). Any effect of this size difference is expected to increase predation rates on sympatric stickleback relative to allopatric stickleback in tanks with sculpin; our findings in the opposite direction are therefore conservative (see Results).

During the third and final round of recaptures, all stickleback and sculpin were removed from the tanks, weighed, euthanized with MS-222, and frozen. To ensure exhaustive sampling of fish, we trapped for three days then swept a large net through each tank multiple times. All 16 sculpin were recovered; one had died a few days before the experiment ended, so we calculated its growth rate based on its weight two weeks earlier. Stickleback reproduction occurred in many tanks, so we visually surveyed tanks for the presence of fry periodically during the experiment and five weeks after it ended. At this point, we collected approximately 25 stickleback fry from each of six cattle tanks without sculpin (three from each population) and transported them to the laboratory (see below).

STICKLEBACK SURVIVAL, GROWTH, AND REPRODUCTION

We used two approaches to quantify stickleback survival. First, we performed a survival analysis using only the 256 stickleback present at the start of the experiment. We measured survival time

as the number of days until a stickleback was last recaptured, and used the mean survival time in each tank as input for a parametric survival analysis with log-normal error structure, using the “surv” and “psm” functions in R (Crawley 2007). We modeled the proportion of mesocosm populations predicted to become extinct over time as a function of sculpin addition, stickleback population of origin, and their interaction, using likelihood ratio tests to assess significance of each term. As an alternative method allowing stickleback added later in the experiment to be included, we categorized fish as “survivors” if they were ever recaptured (i.e., survived at least one two-week census period), and “non-survivors” otherwise. We quantified the survival rate in each tank as the proportion of survivors, assuming that recapture probabilities were constant across treatments. Unless otherwise noted, we transformed response variables when appropriate to meet the assumptions of linear models, and modeled them as a function of sculpin addition, stickleback population of origin, and the sculpin \times population interaction using a linear model in R.

We estimated the growth rate of each stickleback and sculpin as the total change in its mass between initial addition and final recapture, divided by its residence time in the enclosure. We modeled individual stickleback growth rate as a function of population, sculpin addition, and their interaction using a linear mixed effect model with tank as a random effect. Mass change may involve reproductive status as well as somatic growth, so we repeated analyses after removing 53 females that we judged to be gravid based on a visibly distended abdomen during any recapture. We used initial mass as a covariate to account for any relationship between fish size and growth rate. There were no significant interactions between initial mass and treatment affecting growth rate ($P > 0.14$), so the relationship between initial mass and growth rate was assumed to be constant across treatments. For sculpin, we tested whether growth rate differed between tanks with sympatric versus allopatric stickleback, again using initial mass as a covariate.

We also tested whether sculpin affected the probability of stickleback successfully reproducing (i.e., producing fry), and whether any such effect differed between populations. We used log-linear analyses to test whether sculpin addition, stickleback population, or their interaction impacted the probability of successful reproduction.

STICKLEBACK DIET AND IMPACT ON PREY COMMUNITIES

We quantified diet by examining the stomach contents of all stickleback and sculpin recovered at the end of the experiment. We counted and identified diet items, and classified them as benthic (insect larvae, molluscs, and stickleback eggs) or pelagic (zooplankton, chironomid pupae, and surface insects). We used average lengths of diet items and published length-dry weight

regression formulae (McCauley 1984; Sample et al. 1993) to estimate the average mass of each item, and multiplied masses by prey counts to estimate the biomass of benthic and pelagic resources in each stomach. We then calculated the proportion of pelagic items by biomass in each stomach, and averaged this value within each tank. Four tanks with no stickleback alive at the end of the experiment were excluded from the statistical analysis of diet.

To test for differences in invertebrate biomass among treatments, we sampled zooplankton and benthic invertebrates directly from each mesocosm before the final removal of fish. We filtered 12 L of water from approximately 10 cm below the surface through a 64 μm sieve, then stained and preserved zooplankton with two to three drops of acid Lugol's solution. Zooplankton samples were identified to functional groups (usually genera) and counted under a dissecting microscope. Body lengths of up to 10 specimens per taxon per sample were measured with an ocular micrometer, and then used to estimate the biomass of zooplankton resources available to stickleback in each tank (McCauley 1984). Occasional benthic taxa (chironomid larvae) present in zooplankton samples, and small taxa not consumed by adult stickleback (copepod nauplii and rotifers) were excluded from these biomass calculations.

To sample benthic invertebrates, a small dipnet was used to collect two 120 cm^2 scoops of benthic substrate (mud and decomposing leaves) from each tank. The substrate was rinsed through a 500 μm sieve and searched for live organisms for 15 min. Invertebrates were stored in 95% ethanol for approximately one week, then counted and identified to functional groups. We measured benthic invertebrate biomass directly by drying samples for 24 h at 60°C and weighing them to 0.1 mg. We used the dry mass of the substrate sampled from each tank as a covariate to account for a strong relationship between substrate mass and benthic invertebrate biomass ($F_{1,27} = 59.7$, $P < 0.001$). Substrate mass did not vary among treatments (ANOVA: all $P > 0.4$) and there were no substrate mass by treatment interactions affecting benthic invertebrate mass (all $P > 0.15$), so the effect of substrate mass was assumed to be constant across treatments.

We estimated the proportional biomass of pelagic versus benthic resources in the tank as the zooplankton biomass divided by the combined zooplankton and benthic invertebrate biomass. We estimated these values by extrapolating our sampled biomass based on the fraction of the tank sampled (approximately 12 of 1136 L for zooplankton and 240 of 31,400 cm^2 for benthos), after first using a linear regression to adjust benthic invertebrate biomass to account for variation in the mass of substrate sampled.

We also tested for overall top-down control of zooplankton and benthic invertebrate biomasses, and of the proportion of benthic biomass. We used linear models contrasting the four mesocosms without fish with the 32 mesocosms containing fish. These tests are orthogonal to the contrasts in the main linear models, and

are presented separately so that the main analyses are consistent among response variables.

STICKLEBACK MORPHOLOGY

We characterized additional morphological traits of stickleback from Paq and Trout Lake to confirm that they differ in multiple traits associated with antipredator defense and pelagic foraging. We measured standard length and six ecomorphological traits from 30 wild-caught stickleback sampled from each lake concurrently with the experimental fish. We measured four defensive armor traits: the number of lateral plates on the left side of the body, and the lengths of the first and second dorsal spine and the left pelvic spine (Reimchen 1994). We also recorded the number and maximum length of the gill rakers on the first gill arch, both of which are associated with increased plankton feeding (Lavin and McPhail 1986). We log-transformed gill raker length and left the remaining traits in their natural units to achieve homogeneity of variances across populations and body sizes.

To test for a genetic basis of differences between populations, we reared juvenile stickleback from each population born in the experimental tanks without sculpin. These fish were reared in laboratory aquaria on mixed diets of brine shrimp nauplii, frozen *Daphnia*, and frozen bloodworms (i.e., both benthic and pelagic prey items). At approximately nine months age, we sacrificed 20 fish from each population and measured the same traits as for the wild-caught fish. As these fish were collected after hatching, we cannot exclude paternal or maternal effects. However, given the absence of predator cues early in life, and the low likelihood that juvenile fish foraged with adult stickleback, we feel that if divergent phenotypes were maintained in the laboratory it would provide strong evidence for genetically based differences between populations.

To test for differences between populations, we first fit ANCOVAs modeling each of the six traits as a function of population of origin, rearing environment, and standard length as an overall size measure, including all two- and three-way interactions. Where there was no evidence for different allometric slopes among groups (i.e., $P > 0.1$ for interactions that included standard length), these terms were deleted from the model. We used the reduced ANCOVAs to test for significance of population (indicating overall phenotypic differences), rearing environment, and population \times rearing environment interactions (indicating that the magnitude of the difference between populations changed due to a plastic response to the laboratory environment; Day et al. 1994).

Results

COMPARATIVE ANALYSIS OF BODY SHAPE

The LDA of shape coordinates revealed considerable shape differences between stickleback populations sympatric with sculpin

and allopatric populations (Fig. 2A; multivariate ANOVA using population means as replicates: Wilks $\lambda = 0.39$, $F_{1,13} = 9.4$, $P = 0.003$). Randomization tests confirmed that these differences were not an artifact of the LDA used to delineate shape axes: differences between population types were substantially larger than expected whether we randomized populations among population types ($P = 0.005$) or randomized individuals among population types prior to the LDA ($P < 0.001$). We obtained similar results when we adjusted each landmark coordinate for body size prior to these analyses (results not shown).

The second LDA showed variation among all five types of stickleback populations along the first two shape axes (Fig. 2B; multivariate ANOVA using population means as replicates: Wilks $\lambda = 0.030$, $F_{4,20} = 22.9$, $P < 0.0001$). This result remained highly significant when we used randomization tests or size correction as described above (results not shown). The first axis clearly separated ancestral marine stickleback from the four derived freshwater population types, which are less streamlined with a larger head, larger eyes, and a more posterior first dorsal spine (Fig. S1). The second axis separated the benthic and limnetic species, representing the extreme body shapes found in fresh water. Limnetic-like stickleback have narrower bodies and larger eyes, jaws and dorsal, and anal fins than benthic-like stickleback (Fig. S1). Among solitary populations, the shape of stickleback sympatric with sculpin differed from allopatric stickleback on both of these axes. This divergence was greatest on the second, benthic-limnetic axis ($F_{1,13} = 48.9$, $P < 0.0001$, $R^2 = 0.79$), but was also evident on the first, marine-freshwater axis ($F_{1,13} = 5.2$, $P = 0.04$, $R^2 = 0.29$).

Compared with allopatric stickleback from lakes without sculpin, sympatric stickleback showed a conspicuous anterior shift in the first dorsal spine, a more slender body, more extensive dorsal and anal fins, and a larger ectocoracoid (the insertion point of the deep adductor muscle that powers pectoral fin swimming; Fig. 2B). These shape differences are typically associated with increased predation and with sustained swimming and foraging in the pelagic habitat of lakes (Walker 1997; Walker and Bell 2000; Hendry et al. 2011). This finding suggests that in the presence of an intraguild predator stickleback have undergone a morphological transition associated with increased use of zooplankton, supporting the niche shift hypothesis.

STICKLEBACK SURVIVAL, GROWTH, AND REPRODUCTION

We detected strong differences in the effect of sculpin addition on the fitness of sympatric and allopatric stickleback in the mesocosm experiment. Parametric survival analysis revealed a strong interaction whereby sculpin addition only reduced the survival of the allopatric stickleback population (sculpin \times population interaction: $df = 1$, $G = 7.65$, $P = 0.006$; Fig. S2). Similarly, a lower proportion of allopatric stickleback survived between census

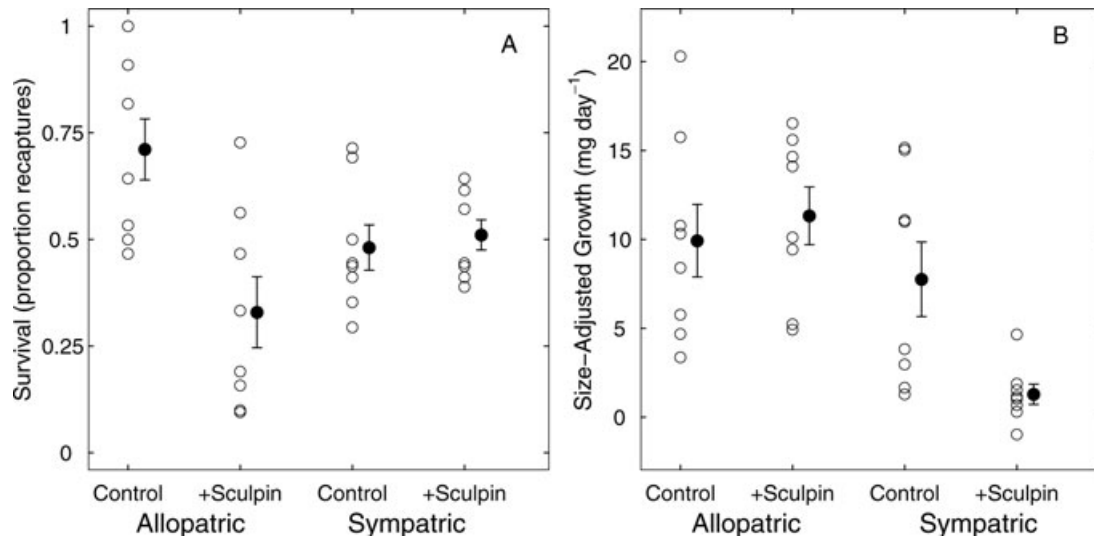


Figure 3. Survival and growth rates of “sympatric” and “allopatric” stickleback populations (from a lake with sculpin and a lake without sculpin, respectively), in experimental mesocosms with (+Sculpin) and without sculpin (Control). (A) Proportion of stickleback in each treatment surviving for at least one 14-day census period. (B) Average growth rates between the addition and final recapture of stickleback in each treatment, after removing gravid females and adjusting growth rates to the mean initial mass. Open symbols represent individual mesocosms, and error bars indicate treatment mean \pm SE.

periods in the presence (0.33 ± 0.08 ; mean \pm SE) versus absence (0.71 ± 0.07) of sculpin, whereas the survival rates of sympatric stickleback were similar in the presence (0.51 ± 0.04) and absence (0.48 ± 0.05) of sculpin (Fig. 3A; sculpin effect: $F_{1,28} = 7.7$, $P = 0.01$; population effect: $F_{1,28} = 0.15$, $P = 0.7$; sculpin \times population interaction: $F_{1,28} = 10.5$, $P = 0.003$). Sculpin growth rate was nearly threefold higher in mesocosms with allopatric stickleback (92.3 ± 14.0 mg day⁻¹) than with sympatric stickleback (31.3 ± 7.8 mg day⁻¹; $F_{1,13} = 14.7$, $P = 0.002$), and was unrelated to initial mass ($F_{1,13} = 1.1$, $P = 0.31$). This provides further evidence that sympatric stickleback experienced reduced predation by sculpin.

Initial analysis of stickleback growth rate indicated higher growth rates in allopatric stickleback (linear mixed-effects model: $F_{1,28} = 3.6$, $P = 0.067$) and in the absence of sculpin ($F_{1,28} = 4.2$, $P = 0.049$). There was a nonsignificant sculpin \times population interaction ($F_{1,28} = 1.8$, $P = 0.19$; see below) and a negative relationship between initial mass and growth rate ($F_{1,195} = 4.1$, $P = 0.044$). Some of the recorded changes in mass were attributable to reproductive status, as 53 females were visibly gravid at some point in the experiment. The frequency of gravid females was higher among sympatric than allopatric stickleback (average 1.5 vs. 0.81 per tank; two-way ANOVA on square root-transformed data: $F_{1,28} = 4.8$, $P = 0.038$; no significant effects of sculpin or interaction: $P > 0.35$). When we repeated the analysis after excluding these gravid females, we found a stronger difference between populations ($F_{1,28} = 12.2$, $P = 0.002$), a weaker effect of sculpin ($F_{1,28} = 2.4$, $P = 0.13$), and a negative effect of initial mass ($F_{1,158} = 4.0$, $P = 0.046$). There was also a

marginally significant sculpin \times population interaction ($F_{1,28} = 3.9$, $P = 0.058$). With gravid females excluded, sympatric stickleback had lower size-corrected growth rates when sculpin were present (1.3 ± 0.6 mg day⁻¹) than when sculpin were absent (7.8 ± 2.1 mg day⁻¹), whereas allopatric stickleback had comparably high growth rates in the control (9.9 ± 2.0 mg day⁻¹) and sculpin addition treatments (11.3 ± 1.6 mg day⁻¹).

Sculpin addition inhibited stickleback reproduction, as only four of 16 tanks with sculpin contained fry, compared to 13 of 16 in tanks without sculpin (log-linear analysis; $df = 2$, $G^2 = 14.52$, $P = 0.0007$; stickleback population and interactive effects were nonsignificant).

STICKLEBACK DIETS AND IMPACTS ON PREY COMMUNITIES

Diet of stickleback in the mesocosms, as revealed by their stomach contents at the end of the experiment, differed between populations but was not strongly affected by sculpin addition (Fig. 4). The proportion of pelagic prey (zooplankton) biomass in the diet was higher in the sympatric population (0.34 ± 0.043) than in the allopatric population (0.21 ± 0.033 ; $F_{1,24} = 5.7$, $P = 0.025$), in agreement with the morphological evidence for a niche shift to the pelagic habitat. The proportion of pelagic biomass in diets was not significantly affected by sculpin ($F_{1,24} = 1.5$, $P = 0.24$) or a sculpin \times population interaction ($F_{1,24} = 1.0$, $P = 0.33$), although allopatric stickleback had slightly more pelagic diets with sculpin present. One sculpin stomach contained an allopatric stickleback at the time of capture; otherwise, sculpin stomachs contained

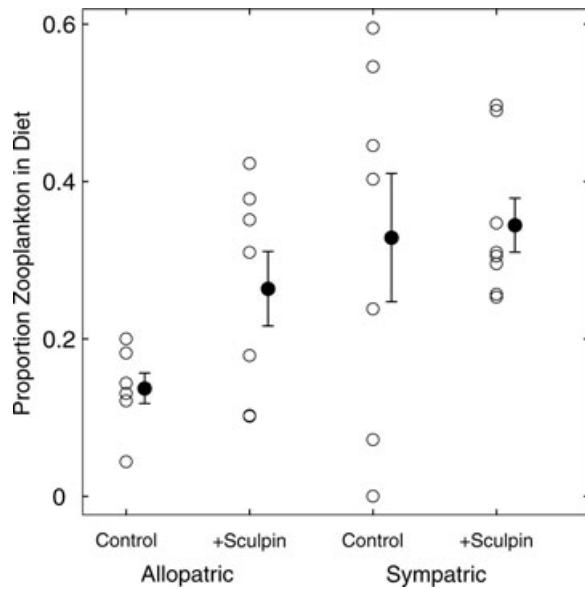


Figure 4. Diets of stickleback recaptured at the end of the six-week mesocosm experiment. Values represent the estimated proportion of pelagic diet items (by biomass) in the stomachs, averaged within each mesocosm. Open symbols represent individual mesocosms, and error bars indicate treatment mean \pm SE.

greater than 99% benthic invertebrates by mass, including many diet items shared with stickleback.

The observed niche shift by stickleback toward increased zooplanktivory affected the biomass of their resources. Consistent with their more pelagic diet and morphology, sympatric stickleback reduced zooplankton biomass ($6.4 \pm 3.4 \text{ mg L}^{-1}$) much more than did allopatric stickleback ($15.4 \pm 5.4 \text{ mg L}^{-1}$), whether

sculpin were present or not (population: $F_{1,28} = 13.7$, $P < 0.001$; sculpin \times population interaction: $F = 0.2$, $P = 0.9$; Fig. 5A). In contrast, allopatric stickleback caused a slightly greater depletion of benthic invertebrate biomass than sympatric stickleback, but only in tanks without sculpin (population: $F_{1,27} = 0.5$, $P = 0.5$; sculpin \times population interaction: $F_{1,27} = 3.3$, $P = 0.08$; Fig. 5B). Sculpin addition led to an increase in zooplankton biomass ($F_{1,28} = 5.6$, $P = 0.025$), but did not result in a detectable depletion of benthic invertebrate biomass ($F_{1,27} = 0.8$, $P = 0.39$). By strongly suppressing zooplankton biomass and weakly enhancing benthic invertebrate biomass, sympatric stickleback reduced the pelagic proportion of the total invertebrate biomass in the mesocosm (0.0097 ± 0.005) relative to allopatric stickleback (0.036 ± 0.01 ; ANOVA on logit-transformed proportions: $F_{1,28} = 21.9$, $P < 0.0001$). Sculpin addition increased the pelagic proportion of biomass ($F_{1,28} = 4.5$, $P = 0.043$), whereas there was no sculpin \times population interaction ($F_{1,28} = 1.5$, $P = 0.22$).

Both zooplankton and benthic invertebrates were subject to top-down control in the mesocosms, with higher biomass in the four fish-free tanks than in tanks with fish (zooplankton biomass: $F_{1,34} = 4.2$, $P = 0.048$; benthic invertebrate biomass: $F_{1,33} = 7.0$, $P = 0.01$). There was no significant difference in the proportion of pelagic biomass in tanks with and without fish ($F_{1,34} = 1.0$, $P = 0.31$).

STICKLEBACK MORPHOLOGY

We detected strong phenotypic differences between sympatric Paq Lake stickleback and allopatric Trout Lake stickleback, which persisted in the laboratory (Fig. 6). Stickleback from Paq Lake had longer dorsal and pelvic spines, more lateral plates and longer

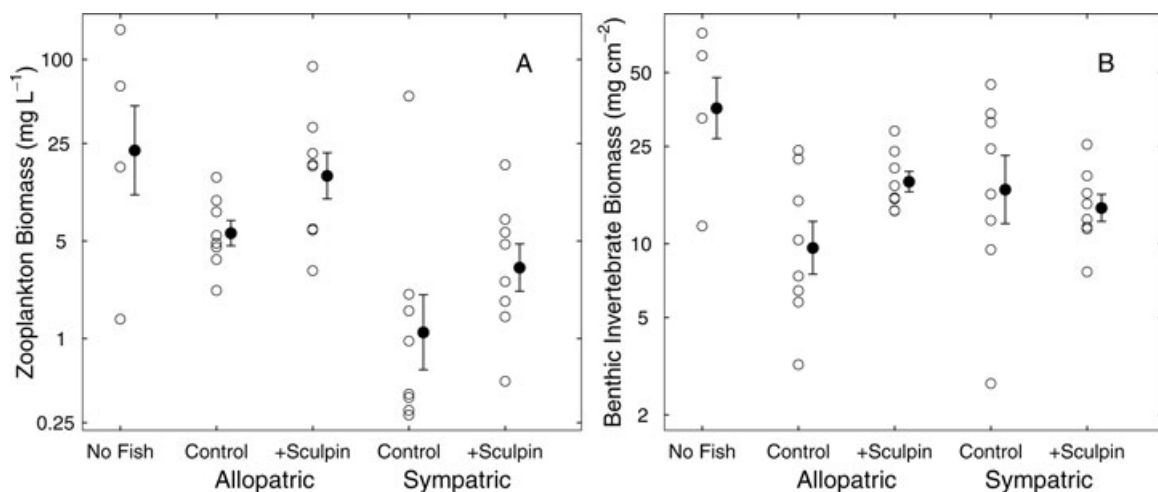


Figure 5. Biomass of pelagic and benthic invertebrates in mesocosms at the end of the six-week experiment. (A) Zooplankton biomass estimated from abundances, length measurements, and length-dry weight regressions. (B) Dry mass of invertebrates collected from benthic substrate, corrected to remove a relationship with substrate mass. Open symbols represent individual mesocosms, and error bars indicate treatment mean \pm SE. Treatments are as in Figures 3 and 4, with the addition of four mesocosms without fish to test for overall top-down control.

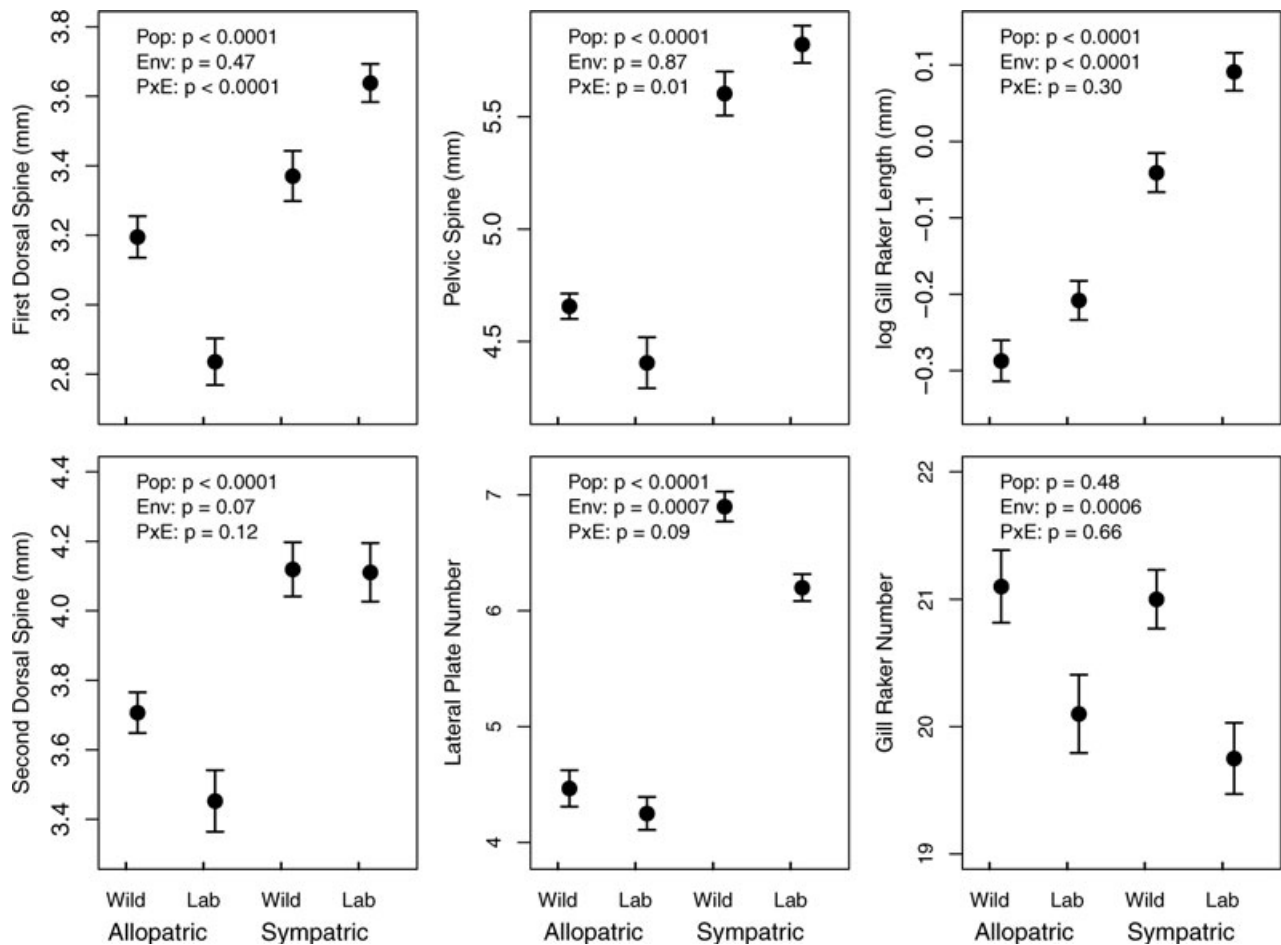


Figure 6. Morphology of wild-caught and laboratory-reared stickleback from the allopatric (Trout Lake) and sympatric (Paq Lake) populations used in the experiment (mean \pm SE). Linear measurements (spine and gill raker lengths) are size corrected to the median standard length of 43.8 mm. $N = 30$ for wild-caught and 20 for laboratory-reared fish per lake. ANOVA significance levels are given for population effects (Pop), rearing environment effects (Env), and their interaction (PxE).

gill rakers, consistent with higher predation risk, and a more pelagic habitat and diet (Lavin and McPhail 1986; Reimchen 1994; Matthews et al. 2010). All traits showed highly significant differences between populations ($P < 0.0001$) except for gill raker number ($P = 0.48$; for statistical results refer to Table S3). There were no differences in allometric slopes between groups (all $P > 0.1$), so we tested for trait differences among populations and rearing environments assuming a common slope.

These differences appear to be genetically based, as the fish reared in the laboratory showed as much phenotypic differentiation between populations as the wild-caught fish (Fig. 6). Laboratory-reared fish had longer gill rakers and fewer lateral plates and gill rakers, although some of this effect may be because different individuals measured these fish close to a year apart. However, any measurement bias should be consistent between populations, making the tests for population and population \times rearing environment effects valid. There were population \times rearing environment interactions for first dorsal spine

and pelvic spine lengths, but in these cases differences between populations were stronger in laboratory-reared than in wild fish. These patterns indicate that the morphological differences between these populations have a largely genetic basis.

Discussion

We have presented several lines of evidence that stickleback have evolved in response to a putative intraguild predator, prickly sculpin. The morphological comparison suggested that the presence of the intraguild predator leads to changes in stickleback body shape associated with enhanced defenses and a shift to a more pelagic habitat. The mesocosm experiment indicated that a sympatric population that evolved in the presence of sculpin is less vulnerable to predation and more planktivorous than an allopatric population that evolved in the absence of sculpin. Overall, our results show that the response of stickleback conforms to the “niche shift” rather than the “increased efficiency” hypothesis.

Our data should be considered in light of several important caveats. First, the morphological shifts represent observational data, meaning that other differences between lakes with and without sculpin may contribute to differences between stickleback populations. For example, the marginally higher average surface area of lakes with sculpin may correlate with greater availability of pelagic prey or abundance of avian predators. Second, our mesocosm experiment needs to be replicated with additional populations to confirm that the ecological shifts we report are consistently associated with the presence or absence of sculpin. This concern is somewhat lessened by the consistency of the comparative and experimental patterns, but we cannot yet generalize all of our results to all lakes with and without sculpin. Third, the use of mesocosms allows greater experimental replication, but is necessarily associated with reduced ecological realism. The relatively small scale of the mesocosms is likely to have increased the encounter rate between stickleback and sculpin, and increased the importance of nutrient subsidy from fish excretion or decomposition. These issues of scale are common to most mesocosm studies, and can best be addressed by conducting complementary studies at multiple spatial scales where possible.

A final caveat to consider is the relative contribution of evolutionary change and phenotypic plasticity to the differences between wild-caught sympatric and allopatric stickleback. Morphological differences between our focal populations persist in the laboratory (Fig. 6), and thus do not appear to result from plasticity. Rearing and transplant experiments (Fig. 2; Spoljaric and Reimchen 2007), line-cross analyses (Schluter et al. 2004; Berner et al. 2011), and genetic mapping studies (Albert et al. 2008), all indicate that body shape differences among stickleback populations typically have a genetic basis. Both genetic differences and early life experience can contribute to antipredator and foraging behavior of stickleback populations (Day et al. 1994; Dingemans et al. 2009), so experiments with laboratory-reared fish will be required to estimate the contribution of evolutionary change to the differences between populations from lakes with and without sculpin.

Our results add to a growing literature on the evolutionary effects of antagonistic interspecific interactions (e.g., Langerhans et al. 2004; Grant and Grant 2006). Although most of this research has focused on the effects of competition or predation in isolation, by studying intraguild predation, we can investigate the combined competitive and predatory effects of a single antagonist. This opportunity also presents the challenges of identifying how competition and predation interact, and how much each contributes to the niche shift in sympatric stickleback populations. Habitat segregation between stickleback and sculpin may result from predator avoidance and produce a diet shift as a byproduct, or the shift in diet may be the result of character displacement due to interspecific competition for benthic resources. Whether com-

petition, predation or both are the primary drivers of stickleback shifts in sympatry, the net consequences for the food web will likely be mediated by the ensuing changes to both the strength of predation and the degree of resource sharing between sculpin and stickleback.

Our data suggest that the evolution of an intraguild prey species can reduce the predatory impact of its intraguild predator. Enhanced morphological defenses, such as spines and lateral plates, reduce the vulnerability of stickleback to gape-limited piscivores (Reimchen 1994), and are likely to contribute to the reduction in predation rate seen in our experiment. Unmeasured traits, including antipredator behaviors, are also likely to contribute to the decreased vulnerability of sympatric stickleback. Stickleback that have evolved alongside or been exposed to predators may also have enhanced predator inspection and avoidance behaviors (Bell and Sih 2007; Dingemans et al. 2009). Additionally, the niche shift to the open water habitat may be a form of antipredator behavior, as it should reduce encounter rates with the intraguild predator (Finke and Denno 2006).

In contrast to their higher survivorship in the presence of sculpin, the sympatric stickleback population appears to show reduced growth rates in the presence of sculpin. This suggests that the nonpredatory effects of sculpin may have increased when their direct impact via predation decreased. Nonpredatory effects may include behavioral responses to predator cues as well as interference or resource competition. One explanation for these effects is a foraging-predation risk trade-off that results in sympatric stickleback feeding less frequently or on less profitable resources in the presence of sculpin (Milinski and Heller 1978; Werner and Hall 1988). However, sympatric stickleback appeared to maintain a relatively high reliance on zooplankton prey whether sculpin were present or not, which may contribute to their lower overall growth rates in the mesocosm. An alternative explanation is that sympatric stickleback experience stronger resource competition in the presence of sculpin, which is counterintuitive given that they feed more on pelagic resources than allopatric stickleback. Allopatric stickleback may not have experienced a comparable growth decline in the presence of sculpin if direct predation decreased density and hence intraspecific competition between censuses. On the other hand, sympatric stickleback still consumed 66% benthic invertebrates in the mesocosms (Fig. 4), and may have experienced stronger resource competition from sculpin that were unable to feed on stickleback. Additional work will be needed to clarify the mechanisms by which sculpin presence affects stickleback growth rate, and to ascertain whether this effect is seen in other stickleback populations and in larger systems with more pelagic habitat available. In either case, the presence of sculpin has the potential to make benthic habitats less profitable for stickleback even if direct predation is reduced.

Our results also shed some light on the effect of interspecific interactions on speciation in threespine stickleback. The evolution of benthic and limnetic species pairs has been driven in part by resource competition between stickleback (Schluter 1994; Pritchard and Schluter 2001), and may have been facilitated by predation from cutthroat trout (Vamosi and Schluter 2002; Rundle et al. 2003). In contrast to these individual effects of competition and predation, the presence of an intraguild predator appears to inhibit speciation, as species pairs have not evolved in any lakes containing sculpin (Vamosi 2003). Both predation and competition from sculpin may reduce the profitability of the benthic niche, limiting the potential for persistent divergent selection. The strong negative effects of sculpin presence on stickleback reproduction detected in this study may alter reproductive behaviors such as courtship and nesting (Pressly 1981), which contribute to assortative mating in lakes with species pairs (McPhail 1994). Further investigations of stickleback and other species may reveal whether intraguild predation generally has an inhibitory effect on speciation.

We have focused on the effects of sculpin on stickleback rather than the reverse, but intraguild predators are also likely to evolve in response to the presence or evolution of intraguild prey. Our data suggest that stickleback evolution has reduced predation rates and thus lowered the trophic position of sculpin. Stickleback trophic positions typically vary between 3.4 and 4 (Matthews et al. 2010), whereas many benthic invertebrates are herbivorous (i.e., trophic position 2), so sculpin that no longer feed on stickleback may be at least a full trophic level closer to the base of the food web. The threefold lower growth of sculpin in tanks with sympatric stickleback suggests that this dietary change has negative fitness consequences for the sculpin. A possible consequence is that natural selection would favor sculpin that are more effective piscivores. This hypothesis could be tested by measuring selection on sculpin introduced to experimental ponds with and without stickleback. Sculpin do not generally occur in lakes without stickleback, but systems in which intraguild predators occur in the presence and absence of intraguild prey (e.g., Trinidadian killifish and guppies; Palkovacs et al. 2009) would be useful for identifying evolutionary responses of intraguild predators to intraguild prey.

Our results contribute to a growing body of studies suggesting that trait differences between populations can have consequences for food webs (Harmon et al. 2009; Palkovacs and Post 2009; Bassar et al. 2010). By depleting zooplankton, sympatric stickleback shifted the balance of biomass toward a more benthic-dominated food web (although sculpin presence partially compensated for this effect). As the population effect of zooplankton was consistent with their stomach contents and independent of sculpin presence, it is likely to be a result of differences in foraging behavior rather than changes in density or activity levels. If an evolutionary response by stickleback has reduced predation by

sculpin, this has likely shortened benthic food chains by removing or weakening an intermediate trophic link (Post and Takimoto 2007). In contrast, the niche shift to more pelagic habitats is likely to increase encounters with predatory cutthroat trout, which may lengthen pelagic food chains and contribute to the observed increase in bony armor (Vamosi and Schluter 2002). As stickleback become more planktivorous, benthic and pelagic channels of the lake food web may also become partially decoupled (Fig. 1). Food chain length and energy channel coupling are important determinants of food web dynamics and stability (Pace et al. 1999; Rooney et al. 2008), suggesting that an evolutionary response to intraguild predation may have broad ecological consequences.

We have provided some of the first empirical evidence for the direction of evolution in response to intraguild predation. Niche shifts, instead of increased efficiency, may be a common evolutionary response of intraguild prey, especially when alternative resources are spatially segregated from the intraguild predator. By altering the competitive and predatory components of the interaction, evolutionary responses to intraguild predation can result in a dynamic restructuring of natural food webs.

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Supporting Information

The following supporting information is available for this article:

Figure S1. Landmark coordinates and shape shifts in threespine stickleback.

Figure S2. Parametric survival analysis of stickleback stocked at the beginning of the experiment.

Table S1. Lake morphology and sample sizes of stickleback populations sampled for geometric body shape analysis.

Table S2. Description of landmarks and analyses of body shape.

Table S3. Effects of size, population and phenotypic plasticity to trait values of stickleback^a.

Supporting Information may be found in the online version of this article.

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