Experimental Evidence of an Eco-evolutionary Feedback during Adaptive Divergence

Highlights
- The ecosystem effects of organisms can evolve rapidly
- Ecosystem modifications by organisms can persist across generations
- Adults can influence selection pressures on juveniles via habitat modification
- Eco-evolutionary feedbacks may occur during adaptive divergence between populations

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In Brief
The traits governing how organisms modify their environments can evolve rapidly during adaptation. In an experiment, Matthews et al. found that stickleback-mediated modifications on their aquatic environment influenced selection pressures on a subsequent generation. This suggests feedbacks between ecosystem modification and evolutionary change.
Experimental Evidence of an Eco-evolutionary Feedback during Adaptive Divergence

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SUMMARY

Differences in how organisms modify their environment can evolve rapidly and might influence adaptive population divergence [1, 2]. In a common garden experiment in aquatic mesocosms, we found that adult stickleback from a recently diverged pair of lake and stream populations had contrasting effects on ecosystem metrics. These modifications were caused by both genetic and plastic differences between populations and were sometimes comparable in magnitude to those caused by the presence/absence of stickleback. Lake and stream fish differentially affected the biomass of zooplankton and phytoplankton, the concentration of phosphorus, and the abundance of several prey (e.g., copepods) and non-prey (e.g., cyanobacteria) species. The adult-mediated effects on mesocosm ecosystems influenced the survival and growth of a subsequent generation of juvenile stickleback reared in the same mesocosms. The prior presence of adults decreased the overall growth rate of juveniles, and the prior presence of stream adults lowered overall juvenile survival. Among the survivors, lake juveniles grew faster than co-occurring stream juveniles, except in mesocosm ecosystems previously modified by adult lake fish that were reared on plankton. Overall, our results provide evidence for reciprocal interactions between ecosystem dynamics and evolutionary change (i.e., eco-evolutionary feedbacks) in the early stages of adaptive population divergence.

RESULTS AND DISCUSSION

In the early stages of adaptive population divergence, ecologically differentiated populations (i.e., ecotypes) can evolve contrasting effects on community structure and ecosystem functions [2–6]. However, very little is known about the identity [7], plasticity [8], and evolutionary rates of the traits underlying the ecosystem effects of organisms [2]. If the contrasting ecosystem effects of organisms are sufficiently large and cause persistent environmental differences during adaptive divergence, then an eco-evolutionary feedback may emerge where organism-mediated environmental modifications become an important agent of selection that influences fitness relationships between the diverging populations [9].

Eco-evolutionary feedbacks have a long history in ecology and evolution and have been formulated (and reformulated) in many different ways [10–14]. In very general terms, an eco-evolutionary feedback occurs when the ecosystem effects (biotic and abiotic) of a population of organisms reciprocally influences fitness variation in the population, selection pressures, and/or evolutionary responses [9, 15]. Feedback loops can emerge from the effects of individuals on population-, community-, and ecosystem-level processes [16, 17].

We tested for an eco-evolutionary feedback using a two-phase mesocosm experiment. In phase 1, we quantified differences in the modification of mesocosm ecosystems caused by two recently diverged (<150 years) and ecologically differentiated (i.e., lake and stream ecotypes) populations of stickleback [18] (Supplemental Experimental Procedures). In this population pair, both phenotypic plasticity and standing genetic variation are implicated in ecotype formation [19]. Prior to phase 1, we reared individuals of each ecotype to adulthood on a typical resource from their own native habitat and from the habitat of the other ecotype, allowing us to test how interactions between genetic background and phenotypic plasticity might affect mesocosm ecosystems. In phase 2, we replaced the adults in each mesocosm with a mixture of laboratory-reared juveniles from both ecotypes (reared in common garden) in order to test how modifications of mesocosm ecosystems affected the growth and survival of co-occurring juveniles of the same ecotypes.

In our experiment, we tested the following four predictions. We predicted that recently diverged lake and stream ecotypes of stickleback would have contrasting effects on mesocosm ecosystems (prediction 1) that were caused by a combination of both genetic background and plasticity (prediction 2) [3, 8, 19]. We also predicted that modification of mesocosm ecosystems by adults could persist long enough (e.g., via prey depletion or habitat modification) so as to influence the resource environment of a subsequent generation of juveniles (prediction 3). Finally, we predicted that the adult-mediated modification of mesocosm ecosystems could influence either (1) the survival of juvenile stickleback or (2) the differential growth and survival of juvenile ecotypes (prediction 4). Either of these outcomes could increase the likelihood of feedbacks between ecosystem modification and fitness variation during adaptive population divergence.
Experimental Design

Phase 1 of our experiment (April 22 to August 18, 2011) was a complete randomized block design with eight replicate blocks of five mesocosms, consisting of one mesocosm with no fish (NF treatment) and four mesocosms with factorial treatment combinations (LB, LP, SB, and SP) to represent lake (L) and stream (S) fish reared on either plankton (P) or benthic (B) food for 1 year (Supplemental Experimental Procedures). Although we used separate sexes in each block, we did not test for effects of sex because of unintended reproduction in two tanks.

Phase 2 of our experiment (September 13 to November 23, 2011) began after we removed the adult fish and introduced 32 juveniles (16 L, 16 S) to each of 20 mesocosms, consisting of four replicate blocks of the same treatment combination from phase 1 (i.e., NF, LB, LP, SB, and SP). We analyzed variation in growth and survival of juveniles using linear mixed-effects models with tank nested within block. Each mesocosm received juveniles from multiple families (i.e., full and half siblings co-occurred within tanks), but we neither measured nor controlled for variation in family specific survival and growth (Supplemental Experimental Procedures).

During phase 1, we measured a wide range of ecosystem metrics (Table S1) and tested for the effects of fish presence/absence (F), as well as for the effects of genetic background of stickleback (G), rearing environment in the lab (E), and their interaction (GxE). We used log response ratios (LRRs) [20] to compare the magnitude of treatment (i.e., G, E, and GxE collectively) and fish effects across multiple ecosystem metrics (Supplemental Experimental Procedures). This approach allows us to compare the relative importance of recent phenotypic evolution and the
Effects on Mesocosm Ecosystems

At the end of phase 1, we measured 59 ecosystem metrics and found 24 significant fish effects and nine significant treatment effects (i.e., at least one of the G, E, or GxE effects was significant). The fish effects were widespread among our ecosystem metrics (Figure 1, filled-in circles; Table S1) and had greater multivariate effect sizes (MVESs, measured as centroid distances from a non-metric multidimensional scaling analysis) than the treatment effects (MVES \(_F\) = 0.83 [SD = 0.61], MVES \(_G\) = 0.21 [0.21], MVES \(_E\) = 0.25 [0.22], MVES \(_GxE\) = 0.14 [0.23]; all paired t tests comparing fish and treatment effects had \(p < 0.05\); Table S2). However, there were also several significant treatment effects (Figure 1), including those on zooplankton biomass (G), phytoplankton biomass (GxE), dissolved nutrients (GxE), and abundances of both zooplankton (cyclopoids, E; ostracods, G) and phytoplankton (Cyanobacteria, GxE; Chlorophyceae, G; Zygnematophyceae, E).

Treatment effects varied both in their magnitude and significance during phase 1 (Figure 2; Table S1) and often occurred in the absence of significant fish effects (e.g., large open circles in Figures 1 and S2). There was no correlation between the relative magnitudes of fish and treatment effects (Figure S2). For example, tanks with fish always had a smaller average size of zooplankton and lower abundance of certain species (e.g., Daphnia, Calanoid copepods, and Bosmina) compared to tanks without fish (Figure S3), but the significance of treatment effects varied both among and within zooplankton species over time (Figure S3). The frequency (albeit low), magnitude, and independent occurrence of significant treatment effects relative to fish effects (Figures 1, 2, S2, and S3) suggests that considering both the origin (e.g., genetics or plasticity) and amount (e.g., degree of trait divergence) of recent phenotypic divergence might help us explain variation in community structure and ecosystem conditions that is unaccounted for by fish presence/absence [14, 21, 22].

The independent occurrence of significant treatment and fish effects (Figures S2 and S3) makes it difficult to disentangle the mechanisms underlying the effects of genetic and plastic differences between lake and stream populations on mesocosm ecosystems. For example, in the first five sampling weeks of phase 1, the G effects on phytoplankton biomass (Figure 2A) might have resulted from the treatment effects on zooplankton biomass and copepod density observed during the same period (Figures 2B and 2C). However, this G effect was transient, and a new GxE effect on phytoplankton emerged at the end of phase 1 (Figure 2A), along with a significant G effect but insignificant fish effect on zooplankton biomass (Figure 2B). Variation in the occurrence of significant treatment and fish effects through time (Figure 2) could reflect weak, transient, or attenuating presence/absence of stickleback for explaining variation in community and ecosystem structure in mesocosms [14, 21, 22].
effects of top predators on lower trophic levels. Alternatively, it could reflect predators having a combination of direct and indirect effects on material cycling and biogeochemical processes in ecosystems through a wide variety of mechanisms [23], including both trophic and non-trophic interactions [24]. Regardless of the mechanisms driving ecosystem modifications, our results support our first two predictions: mesocosm ecosystems differed among treatments at the end of phase 1 due to a combination of genetic effects, rearing environment effects (i.e., plasticity), and their interaction (Figures 1 and S2).

To test our third prediction, we investigated whether the effects on mesocosm ecosystems of adults (phase 1; Figures 1 and 2) influenced three indicators of the resource environment experienced by juveniles in the next generation (phase 2; Figure 3), specifically, zooplankton communities, Dreissena abundance, and macroinvertebrates associated with macrophytes (Figure 3; Table S1).

First, at the end of phase 1, zooplankton biomass was lowest in tanks with stream fish reared on benthic food (GxE effect: $F_{1,10} = 12.53, p < 0.001$) but did not differ between tanks with and without fish (fish effect: $F_{1,16} = 0.40, p = 0.53$). In contrast, by the end of phase 2, there was a clear fish effect but no treatment effects on zooplankton biomass (Figure 3A; fish effect: $F_{1,16} = 5.62, p = 0.018$; treatment effects: all $p > 0.05$), suggesting a legacy effect of fish presence during phase 1 on resources available for juveniles in phase 2 (Figure 3A).

Second, we found that the fish effects on Dreissena differed between phases. In phase 1, we saw no difference in Dreissena abundance in mesocosms with or without fish (Figure 3B; fish effects, phase 1: $F_{1,16} = 0.40, p = 0.53$), but in phase 2, we observed more Dreissena in those mesocosms that previously had fish during phase 1 (phase 2: $F_{1,16} = 9.47, p = 0.002$). Dreissena were common in our mesocosms (Figure 3B; phase 1: mean = 7/tank, SD = 5.5; phase 2: mean = 30/tank, SD = 17) and can be very important grazers of plankton communities in natural ecosystems, reaching densities of >100 individuals/m$^2$ and feeding rates up to 100 ml/hr [25]. Dreissena can have strong effects on zooplankton and phytoplankton communities [26], but further experiments would be necessary to test for competition between Dreissena and juvenile stickleback.

Third, we found a GxE effect (caused by adults in phase 1) on the biomass of macroinvertebrates associated with macrophytes at the end of phase 2 (Figure 3C; $F_{1,10} = 3.93, p = 0.047$). The interactive effects of genetic background and plasticity on trait variation (e.g., body shape and morphology) [19, 27] might account for the treatment effects of adults on resources in phase 2. However, the link between trait variation and feeding behavior is highly uncertain [27], and it is difficult to predict divergence in ecosystem effects from morphological and shape divergence between populations [1, 28–30]. In general, we need a better understanding of the functional significance of morphological variation [31] over a range of timescales (e.g., lab foraging trials and mesocosm experiments) and environmental conditions (e.g., natural environments).

Figure 3. Effects of Adult Stickleback on the Environment of Juveniles
Biomass variation in relation to the treatments established during phase 1, only including data from the 20 mesocosms used during phase 2 (Supplemental Experimental Procedures). Treatments during phase 1 are mesocosms with either no fish (NF) or lake or stream fish. Lines connect treatments where fish were reared on either benthic chironomids (solid lines) or pelagic zooplankton (dotted lines). All error bars indicate ±1 SE.

(A) Zooplankton biomass measured at the end each phase.
(B) Total abundance of Dreissena on the walls of each mesocosm at the end of each phase.
Overall, these three results (Figure 3) support our third prediction: adults can alter the biomass of communities (e.g., zooplankton and macroinvertebrates) and the abundance of species (e.g., Dreissena) that are known to have profound and durable impacts on ecosystems [32]. Transmitting environmental conditions across generations has previously been shown to alter the relationship between trait variation and fitness among environments [33]. In our case, ecosystem differences persisted well beyond the presence of the adults, and this might increase the likelihood of eco-evolutionary feedbacks.

**Do the Ecosystem Modifications of Adults Affect Juvenile Growth and Survival?**

Survival did not differ among mesocosms with and without fish (fish effect: $p = 0.49$; Table S4); however, mesocosms previously modified by adult stream fish had lower overall juvenile survival (genetic effect: $p = 0.002$; Table S4). The prior presence of adult stream fish reduced the overall survival of both stream and lake juveniles, but this was only significant for stream juveniles (Figure 4A; Table S4). Growth accrual measured in a common environment is a useful proxy of variation in relative performance between juveniles of different fish ecotypes, because attaining larger body sizes over the growing season can improve overwintering survival [34], reduce predation risk [35], and increase adult female fecundity [36]. To compare variation in growth among the surviving juveniles, we calculated (1) an average growth accrual ($GA = Length_{Nov} − Length_{Sept}$) for each ecotype in each mesocosm and (2) an average growth accrual differential ($GAL-S = GA_{Lake} − GA_{Stream}$) between co-occurring ecotypes in each mesocosm. We found that the differential growth between ecotypes ($GAL-S$) depends on the interaction between the ecotype and rearing environment (i.e., GxE effect) of the adult that was present in phase 1 (Figure 4C; Table S5; $GAL-S = 1.71$ mm, SE = 0.72, $p = 0.04$). Lake juveniles entirely lost their growth advantage over stream juveniles in ecosystems previously modified by lake fish that were reared to adulthood on lake-like food (i.e., plankton) (Figure 4C). This result provides our strongest evidence of an eco-evolutionary feedback (prediction 4) because the interaction between the genotype and rearing environment of adults causes differential modification of ecosystems (evolutionary effect on ecosystems) and this subsequently affects the relative growth (proxy of fitness variation) of co-occurring ecotypes (feedback on differential growth of co-occurring ecotypes).

Existing theory suggests that phenotypic plasticity can either facilitate or constrain adaptive divergence, depending on how plasticity affects the relationship between phenotype and fitness in a given environmental context [37, 38]. In our lake-stream pair, the extent of plasticity varies among traits [19], but we are unsure which traits underlie the ecosystem effects of stickleback. Our results support one additional study [3] showing that plasticity can alter ecosystem conditions in mesocosms (Figure 1), but we additionally find that the interactive effects of rearing environment and genetic background can have a bigger impact on growth differentials between lake and stream juveniles than does the prior presence/absence of adult fish (Figure 4C). Future work should identify the plasticity of traits underlying ecosystem effects and quantify which traits are related to fitness variation in the modified environmental conditions.
Our experiment provides evidence for multiple components of an eco-evolutionary feedback, but at least two significant uncertainties remain. First, does growth accrual adequately reflect variation in relative fitness between ecotypes? Our primary evidence of an eco-evolutionary feedback (i.e., Figure 4C) relies on the assumption that growth rate is a useful proxy of fitness variation both within and between ecotypes [34, 39, 40]. However, survival differences between lake and stream juveniles did not depend on the presence/absence, ecotype, or prior rearing history of the adults in the previous generation (Figure 4A).

Second, what is the consequence of ecosystem modification for adaptive divergence in this population pair? Our observation that growth differentials were never less than zero (Figure 4C) suggests that lake juveniles would often outcompete stream juveniles, particularly in environments that are modified by adults that are reared in, or that originate from, streams. However, our experiment was performed in mesocosms, which neither emulate the full range of environmental conditions experienced by natural populations nor reflect any one environmental (e.g., lake or stream) condition more than another. As a result, very little is known about what types of similar modifications might be present in nature and how they might interact with other agents of selection (e.g., competition, predation, parasitism, etc.) in more natural environments.

More work is needed to determine which traits underlie the ecosystem effects of organisms in natural settings [7] and how such ecosystem modifications might influence evolutionary responses [5]. Our results highlight the ecosystem consequences of very recent phenotypic and genetic divergence that can occur over fewer than 100 generations [19, 30]. The lake-stream pair that we used is much less divergent in its shape and morphology than many other well studied lake-stream pairs [30, 41], and so we might expect a greater divergence in the ecosystem effects of different ecotypes as phenotypic divergence between them increases [1]. If our experimental results can be transferred to more natural settings, then considering a broader range of mechanisms underlying eco-evolutionary feedbacks may be useful for ecological theories of adaptive divergence and speciation.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, three figures, and five tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.11.070.

AUTHOR CONTRIBUTIONS

B.M., B.L.-H., T.A., and O.S. conceived of the experiment. T.A., K.E.S., B.L.-H., and B.M. carried out the experiment. B.M. did the analysis and wrote the first draft of the manuscript. All authors edited and contributed to the writing.

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Supplemental Figures (S1-S3):

Figure S1.

(1)

(2)
Figure S2.
Figure S3.
Figure S1, related to Figure 1. (Panel 1) Photographs from the two sampling locations of the adult fish used in the mesocosm experiment. Lake fish (Blue border) were caught from the southeastern shores of Lake Constance (47°29'08.29"N 09°32'37.85"E), and stream fish were caught several kilometers upstream at Aubach, Oberriet (47°19'36.80"N 09°34'04.13"E). (Panel 2) Experimental design to complement results from Figure 1, showing (A) the time course of the experiment, (B) the design of the common garden phase where sticklebacks were reared in the lab prior to being used in Phase 1. (C) Shows the array of mesocosms.

Figure S2, related to Figure 2. Comparison of the absolute value of ecosystem effect contrasts of (on the x-axes) fish (LRR_F) with those of (on the y-axes) rearing environment (LRR_E), genotype (LRR_G), and genotype by environment interaction (LRR_GxE). All ecosystem metrics from Table S1 are included. Filled circles indicate a significant fish (F) effect, and large circles indicate a significant effect of the contrast on the y-axis. Significance testing was done as indicated in (Table S1).

Figure S3, related to Figure 3. Change in the density (#/L) of different zooplankton species over the three sampling weeks (Weeks 1, 3, and 12 correspond to May 9, May 25, and July 29, respectively) during phase 1. Significant effects of fish (F), environment (E), genetic (G), or genetic by environment interactions (GxE) are labeled accordingly (Table S1). For clarity, E and G effects are not labeled when GxE effects are significant. All error bars are +/- one standard error.

Table S1, related to Figure 1, 2, S2, and S3. Summary of effect sizes for every ecosystem metric measured in the experiment. Log response ratios (i.e. effects sizes) were calculated as described in the text, and significance was determined either by non-parametric tests (ORM: ordinal regression models) or by generalized linear mixed effects models (glme). Uncorrected p-values are reported, and those <0.05 are in bold. All p-values <0.007 are deemed significant when controlling for false discovery rates using the Benjamini & Hochberg correction [S1].

See accompanying excel table
Table S2, related to Table S1. Results of multivariate analysis for the last sampling date of phase 1. Contrasts are as defined in the main text: F is fish presence/absence, G is genetic background of fish, E is Rearing environment, and GxE is the interaction of GxE. Values with p<0.10 are highlighted in bold. The Multivariate Effect Size (MVES) was calculated as the Euclidean distance between centroids from a non-metric dimensional scaling analysis (NMDS).

<table>
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<th>Parameter</th>
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<th>p-value</th>
<th>MVES (NMDS)</th>
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</tr>
<tr>
<td></td>
<td>E</td>
<td>( F_{1,21} = 0.31 )</td>
<td>0.92</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>GxE</td>
<td>( F_{1,21} = 2.16 )</td>
<td><strong>0.06</strong></td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>( F_{1,37} = 3.43 )</td>
<td><strong>0.004</strong></td>
<td>1.15</td>
</tr>
<tr>
<td><strong>Abundance</strong></td>
<td>G</td>
<td>( F_{1,21} = 0.18 )</td>
<td>0.97</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>( F_{1,21} = 1.37 )</td>
<td>0.24</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>GxE</td>
<td>( F_{1,21} = 1.06 )</td>
<td>0.39</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>( F_{1,37} = 5.59 )</td>
<td>&lt;<strong>0.001</strong></td>
<td>1.52</td>
</tr>
</tbody>
</table>
Table S3, related to Figure 4. Statistical analysis of adult fish total biomass and growth over phase 1. Analysis was done using linear mixed effects models, with block as a random factor.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Total Biomass (Phase 1 start)</th>
<th>Total Biomass (Phase 1 end)</th>
<th>Growth (Length)</th>
<th>Growth (Weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic</td>
<td>$F_{1,21}= 1.61$ (0.22)</td>
<td>$F_{1,21}= 1.30$ (0.27)</td>
<td>$F_{1,21}= 0.02$ (0.87)</td>
<td>$F_{1,21}= 0.19$ (0.66)</td>
</tr>
<tr>
<td>Environment (Rearing)</td>
<td>$F_{1,21}= 0.48$ (0.49)</td>
<td>$F_{1,21}= 0.21$ (0.65)</td>
<td>$F_{1,21}= 1.62$ (0.22)</td>
<td>$F_{1,21}= 2.75$ (0.22)</td>
</tr>
<tr>
<td>Interaction (GxE)</td>
<td>$F_{1,21}= 0.36$ (0.56)</td>
<td>$F_{1,21}= 0.16$ (0.70)</td>
<td>$F_{1,21}= 1.51$ (0.23)</td>
<td>$F_{1,21}= 0.40$ (0.22)</td>
</tr>
</tbody>
</table>
Table S4, related to Figure 4. Statistical analysis of juvenile survival during phase 2 as shown in Figure 4A. Factors codes are as follows: Fish (F), Genetic (G), Rearing Environment (E), Juvenile Ecotype (JE). In all cases, we used the function `lmer` in R with Tank nested within Block (both as random factors), and did the analysis of variance with a Type II Wald Chi-square test.

<table>
<thead>
<tr>
<th>Response Variable</th>
<th>Factor</th>
<th>$\chi$ (df)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Overall Juvenile Survival</strong></td>
<td>F</td>
<td>0.47 (1)</td>
<td>0.49</td>
</tr>
<tr>
<td>(Fish effects)</td>
<td>JE</td>
<td>1.07 (1)</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>FxJE</td>
<td>0.17 (1)</td>
<td>0.68</td>
</tr>
<tr>
<td><strong>Overall Juvenile Survival</strong></td>
<td>G</td>
<td>5.83 (1)</td>
<td>0.016</td>
</tr>
<tr>
<td>(Treatment effects)</td>
<td>E</td>
<td>0.087 (1)</td>
<td>0.768</td>
</tr>
<tr>
<td></td>
<td>JE</td>
<td>0.44 (1)</td>
<td>0.508</td>
</tr>
<tr>
<td></td>
<td>GxE</td>
<td>0.17 (1)</td>
<td>0.680</td>
</tr>
<tr>
<td></td>
<td>GxJE</td>
<td>0.005 (1)</td>
<td>0.941</td>
</tr>
<tr>
<td></td>
<td>ExJE</td>
<td>0.05 (1)</td>
<td>0.825</td>
</tr>
<tr>
<td></td>
<td>GxExJE</td>
<td>0.005 (1)</td>
<td>0.942</td>
</tr>
<tr>
<td><strong>Juvenile Lake Survival</strong></td>
<td>G</td>
<td>3.44 (1)</td>
<td>0.063</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>0.138 (1)</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>GxE</td>
<td>0.138 (1)</td>
<td>0.71</td>
</tr>
<tr>
<td><strong>Juvenile Stream Survival</strong></td>
<td>G</td>
<td>4.46 (1)</td>
<td>0.035</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>0.01 (1)</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>GxE</td>
<td>0.09 (1)</td>
<td>0.76</td>
</tr>
</tbody>
</table>
Table S5, related to Figure 4. Statistical analysis of growth accrual, and growth accrual differential during phase 2. Factors codes are as follows: Fish (F), Genetic (G), Rearing Environment (E). An initial length (or length difference) was only included in the model if it lowered the overall model AIC value.

<table>
<thead>
<tr>
<th>Response Variable</th>
<th>Factor</th>
<th>$\chi$ (df)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth Accrual (Figure 4B)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entire population (diamonds)</td>
<td>F</td>
<td>9.24 (1)</td>
<td>0.002</td>
</tr>
<tr>
<td>Initial length</td>
<td></td>
<td>2.26 (1)</td>
<td>0.13</td>
</tr>
<tr>
<td>Stream juveniles (closed circles)</td>
<td>F</td>
<td>6.49 (1)</td>
<td>0.01</td>
</tr>
<tr>
<td>Lake juveniles (open circles)</td>
<td>F</td>
<td>9.43 (1)</td>
<td>0.002</td>
</tr>
<tr>
<td>Initial length</td>
<td></td>
<td>2.26 (1)</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Growth Accrual Differential</strong></td>
<td>G</td>
<td>0.86 (1)</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>2.68 (1)</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>GxE</td>
<td>4.20 (1)</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Supplemental Experimental Procedures:

*Design and setup of the mesocosm experiment*

Our experiment used a single lake-stream ecotype pair (from the Constance region) that has evolved in the past 150 years since the introduction of stickleback into the drainage system [S2]. Lake and stream ecotypes of stickleback have evolved independently in multiple lineages in coastal areas of the northern hemisphere [S3-5] and exhibit a broad range of adaptive phenotypic changes associated with lake and stream habitats [S6]. However, as our design did not include replicate lake-stream pairs, our experimental results cannot be safely extrapolated beyond the studied pair from Lake Constance [S7].

In the spring of 2010, we collected wild lake (L) and stream (S) stickleback from the Constance region (Figure S1), made within-population crosses in the lab (LxL and SxS), and reared the progeny to adulthood on either benthic or pelagic food sources. The following spring, these adult lab-reared fish were used in an outdoor experiment with 40 mesocosm ecosystems (1136 L, ~1.3m max depth). In each mesocosm, we added a mixture of gravel, sand, and soft sediment from a local lake (Lake Luzern), supplemented each mesocosm with nutrients, and added invertebrates and benthic substrate originating from three lakes and their tributary streams.

*Phase 1 –* The adult fish we used in this phase were individuals from the same lab populations that have been used in two previous studies [S7]. Briefly, in May of 2010, we collected the Lake fish ecotype from Lake Constance at Altenrhein 47°29'08.29"N 09°32'37.85"E and Stream fish ecotype from a small stream at Aubach, Oberriet 47°19'36.80"N 09°34'04.13"E. The two sampled populations are connected by streams and canals but separated by a minimal distance of
30 km from each other. Pairs of males and females were allowed to spawn in aquaria (60x30x40 cm), and then the adults were removed, and full-sib juveniles were reared on a common food sources for 5 weeks, namely brine shrimp (*Atermia sp.*) and small nematodes (*Panagrellus sp.*). After this time, each full-sib family was split into two groups and reared for 11 months on a “pelagic” diet, or a “benthic” diet, so as to establish contrasting rearing environments for both the lake and stream ecotypes. The pelagic diet consisted of live zooplankton (mainly *Daphnia* sp., *Diaptomus* and Cyclopoid copepods) collected multiple times per week from Lake Luzern, using a 170μm zooplankton net. The benthic diet consisted of frozen bloodworms (*Chironomidae* spp. larvae). Rearing lab bred sticklebacks on contrasting food sources from these populations has previously shown that lake fish grow faster than stream fish when reared on plankton [S2]. In addition, both linear morphometrics and body shape show some degree of plasticity, for both lake and stream ecotypes [S7]. Because we used individuals from the same rearing populations as two previous studies [S2,S7], we are confident that our plasticity manipulation created fish with different morphology, body shape, and potentially feeding behaviour. The previous morphological analysis on these fish [S7] is particularly relevant for understanding the phenotypic differentiation of the adults at the start of phase 1. In short, [S7] found that wild lake fish were larger, had shallower and more elongated bodies, deeper heads with longer jaws, and longer gill rakers (same number of rakers) than wild stream fish. Multivariate effect sizes for linear morphology (Mahalanobis distance (MD)=1.167) and shape (MD=1.66) were both significant. When fish were reared on a common food source (i.e. zooplankton or chironomids), lake fish had wider gapes (although there was an interaction between ecotype and rearing environment), more terminal mouths, dorso-caudally shifted maxilla, and shorter heads. In this case, multivariate effect sizes for linear morphology
(Mahalanobis distance (MD)=1.31) and shape (MD=2.201) were also significant. Finally, when comparing fish reared under different food conditions, those raised on chironomids had shorter heads with smaller eyes, deeper bodies, larger orbit, increased suspensorium, and a more downturned snout. Here as well, multivariate effect sizes for both linear morphology (Mahalanobis distance (MD)=1.63) and shape (MD=1.81) were significant. Overall, there was clear phenotypic differentiation between lake and stream fish, both in the wild and after rearing them on a common food source \[S7\]. This is broadly consistent with other previous studies that have found significant morphological and shape variation between this lake-stream pair \[S8-10\], two of which show that the amount of divergence in the Constance pair is small relative to other lake-stream pairs in Canada \[S10\], and other Swiss and Irish pairs \[S9\].

Phase 2 – The breeding was done in a similar way as in Phase 1, except that the juveniles were all kept on a constant diet (a mix of both plankton, and chironomids) throughout their entire rearing period. We used clutches from 10-12 independent lake and stream families, with several half-sib clutches (i.e. offspring had the same father). Families were raised separately (i.e. full and half sibs were in the same tank) until they were used in the mesocosm experiment. Each mesocosm received individuals from multiple families, and we did not track variation in survival and growth among individuals of different families. Hence, family was not used as a covariate in our analyses.

Design - The first phase of our mesocosm experiment (April 22-Aug 18, 2011) consisted of a factorial manipulation of stickleback genetic background (2 levels: stream [S] and lake ecotypes [L]) and rearing environment (2 levels: benthic [B] and pelagic [P]) and was set up in randomized complete block design, with 40 experimental mesocosms in 8 blocks of 5
mesocosms, where four were intended to hold either only males or only females. The blocks were set up spatially within the grid of mesocosms, and some sampling was done temporally by block. Sex determination of the adults was done following well established methods [S11]. We refer to the treatment combinations as LP, LB, SP, and SB (Figure S1), and a no fish control mesocosm (NF). This design allowed us to measure interactive effects of the genetic background and rearing diet of the lake and stream ecotypes on these aquatic ecosystems (Figure S1), and compare them to the ecosystem effects of fish presence. All of our analyses include block as a random factor.

*Phase 1 set up* - In April of 2011, we established experimental ecosystems in 1136 L mesocosms (dimensions: 1.5x1.5x1.3m) (Figure S1). We added approximately 40L of a mixture of sand, gravel, and mud to the bottom of each mesocosm to obtain a substrate depth of about 5 cm. We filled tanks with water from Lake Luzern and fertilized them with 2.46g NaNO$_3$ and 0.18 g NaH$_2$PO$_4$. After 1 week, we added to each mesocosm zooplankton collected from Lake Luzern (mesh size 65 µm), and lake sediment and benthic substrate (~5 L/tank) collected from multiple lakes (Lake Luzern, Lake Rot, and Lake Zug) and from multiple stream tributaries. We then waited for 2 weeks to allow the ecosystem to develop. This procedure ensured that each mesocosm received substrate, propagules, and a high diversity of living organisms from both benthic and pelagic habitats. Unfortunately the addition of sediment from these particular lakes unintentionally introduced two individual fish larvae into two of the tanks (see below). From April 22-May 4 2011 we added 7 adults sticklebacks to each of the 32 mesocosms, proceeding in 1-2 blocks per day. We chose fish from a narrow range of lengths and randomly assigned them to the tanks such that neither starting fish density (N=7 in all tanks), nor average length (mean
length=44.8 mm, SD=4.2), nor biomass (mean weight=0.71 g/fish, SD=0.22: mean biomass per tank: 4.95 g, SD=0.68) were significantly different among treatments (linear mixed effect models: all p-values >0.1 for length and biomass). Over the course of phase 1, mean survival rate of the adults was very high (Mean survival rate=90%, SD=14%: 17 tanks with 7/7 fish survived, 10 tanks with 6/7, 3 with 5/7, and 1 with 4/7, and 1 with 3/7) and did not differ among treatments (Friedman rank sum test: $\chi^2=3.16$, df=3, p=0.37). The adults increased in both length (average increase=2.8 mm, SD=2.5) and weight (average increase=0.39 g, SD=0.18), and this growth did not differ among treatments, and so the biomass of adults at the end of phase 1 (mean biomass per tank: 6.92 g, SD=1.87) was not different among treatments (Table S3).

Phase 2 set up - The second phase of our experiment (Sept 13 - Nov 23, 2011) aimed to test for an effect of ecosystem modification in the mesocosms on the survival and growth of juvenile stickleback. At the end of phase 1, we removed all the adults by a combination of trapping and dip netting. This procedure took approximately 3 weeks, because we had to ensure that all tanks had no remaining adults and we did not want to disturb the ecosystem too much by our fishing activity. To start phase 2 we decided to only use a subset of 20 of mesocosms, as opposed to all 40. This was because of the following three reasons. First, we found a loach (*Barbatula barbatula*: 6.5 cm, 2.1 g) in one mesocosm (Mesocosm 17, Block 4) and a burbot (*Lota lota*: 5.8 cm, 2.4 g) in another (Mesocosm 27, Block 6). These benthic fish presumably came into the experiment as larvae in the sediment (probably <1 cm) and avoided our detection throughout phase 1, probably by burrowing and foraging at the sediment interface. No additional individuals of either species were found in any of tanks at the end of phase 2 despite a thorough search of the sediment when the mesocosms were drained and emptied. Second, despite trying to keep the
sexes of the adult sticklebacks separate during phase 1 (4 blocks of females, and 4 blocks of males) we had two bouts of reproduction in two separate tanks (both with males). At the end of phase 1, we discovered 25 juveniles (< 1 week old) in each of tanks 25 (Block 6) and 35 (Block 8). Third, despite the high adult stickleback survival rate across the entire experiment the tanks with the lowest survival (5 tanks with a <6/7 survival rate) were spread across four blocks (Blocks =2, 4, 6, and 8). For these three reasons we decided to only continue with the four blocks (Blocks 1, 3, 5, and 7) for phase 2 of the experiment (all female tanks). Hence we compared ecosystem metrics between phases (Figure 3) as well as the survival and growth analyses (Figure 4) for only 20 tanks (4 blocks of 5 tanks).

Analysis of Ecosystem metrics  (phase 1 and 2)

Light extinction – Transmission of photosynthetic available radiation (PAR: 400-700nm mmols/s m²) was measured on cloudless sunny days using a 4π light sensor (Licor LI-193), and recorded on a LI-1000 Data logger (Licor Biosciences) at 6 different depths in each mesocosm: at 1, 10, 20, 30, 40, and 50 cm. A light extinction parameter (k) was calculated as the slope of the relationship between depth and ln(PAR).

Filtration- To sample water for analysis of algal biomass (chlorophyll-a), nutrients, and dissolved organic carbon, we filled a 1 L glass bottle with water from a central location in every mesocosm from a depth of about 30 cm. Samples were kept in the dark until filtration (within 3 hours of collection), whereupon we filtered between 500-1000 mL of water through 47mm ashed GF/Fs (6 hours at 450°C).
**Nutrients** - The filtrate was used to measure dissolved nutrients (NO$_3^-$, and PO$_4^{3-}$). Nitrate (NO$_3^-$) was measured with a Metrohm 761 Compact IC (Metrohm Schweiz AG Zofingen) with a detection limit of 0.25 mg/L (±0.1). Phosphate (PO$_4^{3-}$) was measured using a spectrophotometer Cary 50 (Varian) with a detection limit of 1.0 mg/L (±0.5). DOC was measured using total organic carbon analyzer Shimadzu TOC-V (Shimadzu Coporation 2011), with a detection limit of 0.5 mg/L (±0.5).

**Algal biomass** – Chlorophyll-a (µg/L) was extracted from the filters by immersing them in 8 mL of 90% ethanol, putting the vials in a 78°C water bath (10 min), and sonicating them (10 min). The extraction solution was then stored at 4°C until analysis on the subsequent day. Just prior to analysis, the solution was filtered with a FP 030/30 Rotband filter (to remove any filter particulates from the extraction procedure), and absorption was measured at 665nm using a Hitachi U 2000 spectrophotometer.

**Periphyton biomass** - The growth of periphyton (mg/cm$^2$) was measured by quantifying the chlorophyll-a concentration from plastic strips that were placed in the center of every mesocom tank. We used a commercial 33m x 50mm PVC yellow flagging tape as substrate for the benthic algae to grow on. The tape was anchored to the mesocosm’s crossbeam and submerged (with weights) to about 50cm below the water surface. At sampling, we cut a strip of tape (Surface: 15 cm$^2$) and extracted chlorophyll from it as described above.
**Bacteria abundance and richness** - Sterile glass bottles were used to collect between ~150 mL of water from mesocosm tanks. To measure bacterial abundance, we used flow cytometry on raw water that was fixed in a final concentration of 0.01% paraformaldehyde and 0.1% glutaraldehyde. It was stored for 4-8 months at 4 °C until analysis. Bacterial cells were then stained with 10 μL mL⁻¹ SYBR Green (Invitrogen) and analyzed for flow cytometry [S12].

To quantify bacteria richness, bacteria was filtered through a 0.2 μm Supor® membrane filter (Pall Scientific). Filters were immediately placed in liquid nitrogen and kept in a -80 °C freezer until DNA extraction. Prior to DNA extraction, sterile forceps and scissors were used to cut up the filters into small pieces. Mechanical lysis was used to extract DNA from filters by a bead-beating step with 2 glass beads (2 mm) for 35 seconds at 5 ms⁻¹ on a FastPrep®-24 instrument in 1.2 ml STE buffer (10 mM Tris hydrochloride [pH 8], 1 mM EDTA, 100 mM NaCl). After lysis, we added 50 μL of 20 % sodium dodecyl sulfate (SDS), and then boiled the extract for 2 min. We transferred the liquid extract to new tubes with 400 μL phenol (pH 8) and 400 μL 24:1 chloroform isoamyl alcohol (CIA), followed by a vortexing step for 15 s. The extract was centrifuged for 5 min at 13200 rpm, followed by the repetition of the phenol/chloroform step. 100 μL volumes of 3M sodium acetate (pH 5.2) and 600 μL of isopropanol was added with the extract prior to precipitation on ice for approximately 2 hours. After precipitation, the samples were centrifuged at 13200 rpm for 30 min at 4°C. an aspirator was used to remove the supernatant, the pellet was washed with 70% ethanol and re-suspended in TE buffer. Quant-iT Picogreen (Invitrogen, Oregon, USA) was used to quantify DNA concentration following manufacturer’s protocols.
PCR reactions in volumes of 25 µL with 5ng of template DNA were made with the bacterial specific primers: 1406F fluorescently labeled with 6FAM on the 5’ end and 23Sr. These were used to target the intergenic spacer region between 16S and 23S rRNA for ARISA analysis. A mixture of 1 mL aliquot of PCR product, 9 mL highly deionized (HiDi) formamide, and 0.5 mL Liz1200 size standard (Applied Biosystems) was denatured on a PCR thermocycler for 3 min at 95 °C. Denaturing capillary electrophoresis of each fragment was run on a 3130XL Capillary Genetic Analyzer (Applied Biosystems) with a 50 cm capillary using POP-7 polymer. Southern size-calling method was used on ARISA fragments between 200 bp and 1250 bp, and a background cut-off level of 50 fluorescence units was employed. A binning window size of 2 bp was used to bin AIRSA peaks with the automatic and interactive binning R scripts [S13]. We exported the relative fluorescence intensity of binned peaks data for further analysis, and excluded any peaks present in blank extractions and amplifications. We operationally defined as the number of peaks detected in ARISA profiles as bacterial richness (R).

**Ecosystem metabolism** - Oxygen concentration (mg/L), temperature (°C), and light (PAR measured on a 2π sensor, mmols/s m²) were measured simultaneously for time periods of at least 24 hrs (up to 96 hours) in order to calculate gross primary productivity (GPP), net primary productivity (NPP) and respiration (R) for every mesocosm ecosystem. We used temperature sensors (Hobo Tidbit) to record temperature on 15-minute intervals, and an OXY-10 mini (PreSens) equipped with 10 m long fiber optic dipping probes (PreSens) to make oxygen measurements on compatible intervals. Because we only had 10 channels per on the OXY-10 mini, we spilt the 40 mesocosms into 4 sampling blocks of ten tanks, where each sampling block consisted of two of our 8 experimental blocks (each with 5 treatment combinations). From the
simultaneous measurements of water temperature, incident radiation (PAR), and dissolved oxygen (DO) over a minimum of 24 hours, we calculated gross primary production (GPP), net primary production (NPP), and ecosystem respiration (R). We calculated NPP as $[\text{DO}]_{t_1-t_0}$, R as $[\text{DO}]_{t_1-t_2}$, and GPP as NPP+R, where $t_0$=sunrise on first day, $t_1$=sunset on the first day, and $t_2$=sunrise on the following day [S14].

**Sedimentation** – Sedimentation rate (mg/day) was measured using a 50 mL falcon tube that was weighted with a stainless steel nut, and suspended (from a wooden cross beam) in the middle of each mesocosm at a depth of 50 cm. The tubes were deployed for 3 weeks near the end of phase 1, and upon removal from the mesocosm we removed the sediment content of the tube and dried (at 50°C for 24 hrs) and weighed (analytical balance WBA-620) it on an aluminum weigh boat.

**Zooplankton sampling** - We sampled 9.5L of water using a tennis ball sampler (2 m long plexiglass tube, with a 5cm width) from 6 different locations in the mesocosm(all from the open water column), filtered it through a 100 µm Nitex mesh, suspended the zooplankton in approximately 35 ml tap water and preserved it in a 50ml Falcon tube at -20°C. The zooplankton community was identified to the lowest taxonomic level possible, counted and measured using a Leica DMI 6000B inverted microscope. The body length of either all or at a minimum 30 individuals per taxon was measured using the Leica microscope system at 1x to 100x magnification and the software LSA 3.7. All individuals were used for the calculation of abundance and species richness for each sampling date, but in order to compare similar taxonomic groupings among time periods, we aggregated organisms into the following taxonomic groupings: Calanoid copepods, *Bosmina*, *Daphnia*, Cyclopoids, Nauplii, Ostracods,
Small Cladocerans (*Alona, Eury cercus, Pleuroxus*), and Rotifers (*Asplanchna, Keratella, Trichocerca, Astramoeba*). Count data (i.e. number of individuals per sample of 9.5 L) was analyzed in order to compare with species from other communities in a common statistical analysis for the last sampling date of phase 1. In addition, zooplankton densities and biomass were calculated and subsequently analyzed using generalized linear mixed effect models (Figure 1B, Figure 2, 3). Macrozooplankton biomass was calculated from published length-weight regressions [S15].

*Macrozoobenthos sampling* – At the end of phase 1, we used a hand net (mesh size of 0.2 mm) to collect ~1520 cm³ of sediment from the top 0.5 cm of the benthic substrate in each tank, and stored the samples at -20°C. To quantify macrozoobenthos, each sample was thawed and individually washed through a stack of stainless steel mesh filters with a size range of 0.25-6.3 mm. All individuals retained on the sieve were then identified to the taxonomic level shown in Table S1. We measured the size of individuals in taxonomic grouping with >30 individuals across all the tanks, and used published length-weight regressions to estimate biomass [S16].

*Visual surveys* – At the end of each phase, visual surveys of *Dreissena* mussels were counted from the upper half of all 4 sides of the mesocosm walls (2 m²). We used the upper half because this was the region where variation in water clarity had no effect on our ability to see *Dreissena*, and because of how the mesocosms were constructed there was a clear demarcation at this level. A single observer counted all tanks within an 8-hour period (30-60 min/block).
Statistical analysis of ecosystem variables – We used generalized linear mixed effects (glme) models with a randomized block design to analyze all ecosystem metrics with a continuous data type, including abundance, density, richness, biomass, chemical, and physical parameters (Table S1). We made no mathematical corrections of p-values for multiple comparisons, however we indicate in Table S1 which p-values remain significant after controlling for false discovery rates using the Benjamini & Hochberg correction. All metrics based on count data were analyzed with ordered probit ordinal regression [S17], which assumes the response variable is ordinal. Such a semi-parametric analysis has several advances over other related approaches, for example, unlike many other rank related analyses of factorial experiments ordinal regression models allow for robust testing of interaction terms in a complete randomized block design [S17]. In addition, it provides a useful way to compare across multiple response variables that would otherwise need very different distributional assumptions for standard parametric analysis (e.g. glme models).

In order to compare the magnitude of effects across multiple metrics we quantified log response ratios (LRR) as:

\[ LRR_G = (\ln Y_{SP} + \ln Y_{SB}) - (\ln Y_{LP} + \ln Y_{LB}) \]
\[ LRR_E = (\ln Y_{SB} + \ln Y_{LB}) - (\ln Y_{SP} + \ln Y_{LP}) \]
\[ LRR_{GxE} = (\ln Y_{SB} + \ln Y_{LP}) - (\ln Y_{SP} + \ln Y_{LB}) \]
\[ LRR_F = \ln (Y_{NF}) - (\ln Y_{LB} + \ln Y_{LP} + \ln Y_{SB} + \ln Y_{SP})/4 \]

where \( Y \) is the mean ecosystem metric among mesocosms, after correcting for block means. The significance of F, G, E, and GxE effects were tested with either parametric (generalized linear mixed effect models) or non-parametric tests (ordinal regression).
For the last sampling date in phase 1, we performed a multivariate analysis (Redundancy analysis: RDA) of multiple groups of ecosystem metrics and communities, as listed in Table S1. For each community and grouping of ecosystem metrics, we performed an RDA on data that was either hellinger transformed (Macrozooplankton, Microzooplankton, Zoobenthos, Phytoplankton, and Macrophyte communities), log-transformed (Richness, Abundance), or standardized (Biomass, Ecosystem Function, Physical/Chemical). In all cases, significance testing of the fish effects and treatment effects were done via permutation (N=1000) within each block [S18]. To estimate multivariate effect sizes of each grouping, we performed non-metric multidimensional scaling (on transformed data) and calculated Euclidean distances between treatment centroids (using both NMDS axes) (Table S2).

Quantifying survival and growth rate of juvenile populations (phase 2)

Juvenile rearing and selection - Phase 2 began (Sept 13, 2011) with the addition to each mesocosm of 32 juvenile stickleback (16 stream, and 16 lake), which had been reared (June – September 2011) on a common food source, consisting of brine shrimp, nematodes for 6 weeks, and both plankton and chironomids for 10 weeks. These juveniles were reared from matings in the lab between adult Lake and Stream ecotypes that were collected in May 2011 from Lake Constance at Altenrhein (47°29'08.29"N 09°32'37.85"E) and Steinach (47°30'10.85"N 09°26'49.82"E), and in a small stream at Aubach, Oberriet (47°19'36.80"N 09°34'04.13"E). All the lake and stream juveniles were reared from about 25 successful matings in aquaria (60x30x40 cm), wherein we provided a petridish with sand, mud, and textile fibers. After mating, the eggs were removed from the nest, put in separate aquaria equipped with air pumps.
We reared the juveniles in the lab from June to September 2011. In order to minimize family effects we reared juveniles of different clutches in 25 different aquariums (11 Lake and 14 Stream families), and when selecting juveniles for phase 2, we randomly selected 16 juveniles from 8 different Lake families and 16 juveniles from 6 different Stream families, ensuring to get 1 to 4 individuals per family per mesocosm. Overall we used 1024 juveniles, 512 of each ecotype.

**Juvenile staining** - In order to distinguish lake and stream juveniles at the end of phase 2, we stained either the lake or stream population intended for each mesocosm with a Calcein dye (C_{30}H_{26}N_{2}O_{13}, CAS number 1461-15-0, Sigma # C0875), which fluoresces under UV light with excitation and emission wavelengths of 495/515 nm, respectively. We used a pH buffered solution of 125 mg/L of Calcein (Sigma, St. Louis, MO) and incubation time of 2 hours [S19]. We randomized whether the lake or stream fish were stained in each mesocosm.

After the staining of fish with calcein, every juvenile was photographed in a cuvette with a scale bar and a color reference. Standard length was measured using the photographs and the software ImageJ [S20]. Fish were transported to mesocosms in small plastic fish tanks, and acclimated to the new environment by slowly mixing water from the mesocosm with water from the lab tanks.

**Juvenile collection and identification** - At the end of phase 2, we collected juveniles by trapping and hand netting, and then froze them in individual tubes at -20°C. Each individual was observed under UV light using a Leica DMI6000 B inverted microscope. Every individual was screened for florescence at 6 different bony structures of the body. To minimize errors arising from weak
auto-florescence of some body parts, we inspected florescence of (i) bony scales on the head, (ii) pectoral spine, (iii) pectoral girdle, (iv) dorsal spine, (v) vertebrae, and (vi) teeth. A single observer did all the screening and the determination of whether an individual was stained or not. Standard length of each individual was measured with callipers prior to dissection.

Statistical analysis of juvenile growth –

At the start of phase 1, stream juveniles were slightly larger than lake juveniles (Lake juveniles: mean=19.2, SD=3.5, N=320; Stream juveniles: mean=20.6, SD=3.2, N=320), but were a similar length by the end of phase 2 (Lake juveniles: mean=25.8, SD=3.9, N=255; Stream juveniles: mean=26.0, SD=3.6, N=241). The entire juvenile population (lake and stream individuals) experienced positive growth (g) over the course of phase 2 in all mesocosms (mean growth accrual: g=5.14 mm, SD=1.5), suggesting that mesocosm conditions were amenable to test for variation in performance over the time period of the experiment. Consistent with previous laboratory experiments using the same population pair [S2], we found that lake fish grew consistently faster (higher GA) than stream fish (Figure 4B, Table S5). In addition, average growth accrual of the entire population was significantly higher in tanks that previously did not have adult fish (Figure 4B, Fish effect in Table S5). This provides additional support for a transgenerational effect of fish on juvenile growth, but provides no direct evidence of a feedback, because the effect is mediated by fish density rather than phenotype. In order to test for an eco-evolutionary feedback (prediction 4), we focused our analyses on the survival and growth accrual of co-occurring juveniles. We focused on growth accrual rather than final body length because there was a slight difference in mean body length of the two ecotypes at the start of phase 2 (Lake juveniles: mean=19.2, SD=3.5, N=320; Stream juveniles: mean=20.6, SD=3.2, N=320, t=5.37, p<0.001). We used linear mixed effects models (lmer package in R, Type II Wald
Chisquare test) with Tank nested within Block (both as random factors), to analyse the variation in juvenile length and growth accrual over the course of phase 2 (N=20 tanks). In all models, either an initial length (for growth accrual) or an initial length difference (for growth accrual differential) was included as a random covariate in the analysis, but it was only retained if the model had a lower AIC than the model without the covariate (Table S5).
**Supplemental References**


