Intraspecific phenotypic variation among alewife populations drives parallel phenotypic shifts in bluegill

Magnus Huss, Jennifer G. Howeth, Julia I. Osterman and David M. Post

Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT 06520, USA

Evolutionary diversification within consumer species may generate selection on local ecological communities, affecting prey community structure. However, the extent to which this niche construction can propagate across food webs and shape trait variation in competing species is unknown. Here, we tested whether niche construction by different life-history variants of the planktivorous fish alewife (*Alosa pseudoharengus*) can drive phenotypic divergence and resource use in the competing species bluegill (*Lepomis macrochirus*). Using a combination of common garden experiments and a comparative field study, we found that bluegill from landlocked alewife lakes grew relatively better when fed small than large zooplankton, had gill rakers better adapted for feeding on small-bodied prey and selected smaller zooplankton compared with bluegill from lakes with anadromous or no alewife. Observed shifts in bluegill foraging traits in lakes with landlocked alewife parallel those in alewife, suggesting interspecific competition leading to parallel phenotypic changes rather than to divergence (which is commonly predicted). Our findings suggest that species may be locally adapted to prey communities structured by different life-history variants of a competing dominant species.

1. Introduction

Phenotypic variation within species has largely been ignored in ecological studies; however, it is increasingly recognized that intraspecific variation may have a profound influence on ecological dynamics [1–3]. Furthermore, evidence that evolution can occur over ecological time scales and can have a measurable impact on concurrent ecological dynamics has inspired research on the feedbacks between ecology and evolution of life-history traits [1,4,5]. There are now several examples of the influence of evolutionary diversification within species on ecological processes, both in laboratory systems [6] and in natural systems among fishes [7–9], zooplankton [10] and plants [11,12]. These influences have effects ranging from changes in population growth rate [4] to ecosystem processes such as species richness and nutrient flux [9]. Phenotypic diversification caused by eco-evolutionary interactions may also propagate to affect other species. For example, there is a long-standing idea that species that compete for a set of shared limited resources should diverge in resource use and phenotype by means of natural selection (i.e. ecological character displacement [13,14]).

One striking example of the potential impact of predator trait divergence on the dynamics of ecological systems has been documented in coastal North American lakes inhabited by the planktivorous fish species alewife (*Alosa pseudoharengus*). Alewife populations occur in two genetically distinct life-history types: an anadromous and a landlocked form [15]. Anadromous alewifes display the ancestral life-history strategy of adults migrating into lakes in spring to spawn and young-of-the-year alewifes migrating to the ocean in autumn. By contrast, landlocked alewifes remain in lakes year-round owing to physical barriers isolating these populations from the ocean [15]. Alewifie predation has been shown to change zooplankton community structure by reducing the abundance of large-bodied species; however, the effects on zooplankton communities differ depending on prey selectivity of the two life-history forms and duration in freshwater [7]. In lakes with
anadromous alewife, large-bodied zooplankton can re-establish over winter, whereas landlocked alewives evert year-round predation pressure that eliminates large-bodied zooplankton [7]. As a result, lakes without alewife (‘no-alewife lakes’) contain large-bodied zooplankton, lakes with landlocked alewife (‘landlocked lakes’) contain small-bodied zooplankton and lakes with anadromous alewife (‘anadromous lakes’) contain large-bodied zooplankton in the spring, but small-bodied zooplankton in summer [7]. This divergence in prey community size structure has been shown to feed back to drive phenotypic divergence between anadromous and landlocked alewife populations, including foraging traits such as gape size and gill raker morphology [7,16,17]. As a result, anadromous alewives are better adapted to feeding on large prey items than are landlocked alewives [7], and there is direct evidence for divergence in feeding traits shaping zooplankton community structure [16]. Variation in alewife life-history and foraging traits has, in turn, caused evolutionary life-history diversification in their zooplankton prey [18], which has additional direct effects on lower trophic levels [10,19].

As exemplified in the alewife system, populations themselves may drive patterns of phenotypic diversification through their structuring effects on the environment (i.e. niche construction, here specifically referring to the process of populations structuring the communities and ecosystems in which they reside, sensu [11]; see [20] for an alternative definition that includes an evolutionary response). The implications of these altered selective landscapes on phenotypes and fitness of competing species are, however, not known. Here, we ask, using a combination of common garden experiments and a comparative field study, whether altered selective landscapes caused by landlocked alewife may drive phenotypic divergence and resource use in competing species. Specifically, we study whether growth rate, size selectivity and gill raker morphology of bluegill (Leperis macrochirus) are linked to divergence in prey community structure (i.e. dominance of small- or large-bodied zooplankton), caused by alewife presence and different alewife life-history types [1,7]. We measured gill raker morphology, because it is important to determine the size of prey consumed. In bluegill, as in other planktonivorous fishes, smaller inter-raker spacing typically favours the capture of smaller prey [21]. We hypothesize that bluegill will grow better in their native environment than in the alternative environments and thus predict that bluegill from landlocked lakes will be better adapted for foraging on small-bodied zooplankton compared with bluegill from other lake types. Specifically, we predict that bluegill from lakes with landlocked alewife populations will (i) grow better when fed small zooplankton and (ii) have a gill raker morphology better adapted for feeding on small-bodied prey, and accordingly (iii) select smaller prey items compared with bluegill from lakes with no alewife or anadromous alewife populations. Our results corroborate these predictions, suggesting that phenotypic shifts in bluegill foraging traits parallel those in the alewife populations, and show how niche construction by different life-history types can propagate across food webs.

2. Material and methods

(a) Study system

Bluegill used in experiments and the comparative field study were captured from June to September in 2007–2009 and 2012 by electrofishing (a common sampling technique that uses electricity to stun fish in bodies of freshwater) along lake shorelines in three no-alewife lakes (Black, Gardner and Hayward), three anadromous lakes (Bride, Dodge and Gorton) and three landlocked lakes (Pattagansett, Quonniapug and Rogers). All lakes are located in the state of Connecticut, USA (see map in [7]). Whereas the lakes without alewife have been isolated from the ocean over recent geological time, the lakes with landlocked alewife became isolated from the ocean by human-made dams approximately 300 years ago [15]. The lakes with anadromous alewife are connected to the ocean. There are no significant differences in lake characteristics among lakes, for example, in size and productivity [7,19], and all lakes contain similar fish communities (besides the lack of alewife in no-alewife lakes), with yellow perch (Perca flavescens), bluegill and pumpkinseed (Lepomis gibbosus) being the most common non-alewife zooplanktivorous fish [19]. Our study species, bluegill, is a dominant fish species in many North American lakes, often as the result of introductions (which is likely to be the case in our study lakes; Connecticut Department of Environmental Protection 2008, personal communication). As for alewife, large bluegills are positively size selective and availability of large zooplankton such as Daphnia may increase their growth rates [22], suggesting potentially strong resource competition between alewife and bluegill. Clearly, alewife is the most important species of the two shaping the zooplankton community as we observe high densities of large-bodied zooplankton in our no-alewife lakes that contain high densities of bluegill [7].

(b) Reciprocal transplant experiments

We performed reciprocal transplant common garden experiments during July–August 2012 to investigate (i) variation in bluegill growth rate in response to differences in prey community structure among three lake types (i.e. no-alewife lakes, anadromous and landlocked lakes) and (ii) variation in bluegill size selectivity among lake types. The common garden set-up allowed us to isolate the effects of phenotypic differences from the potential influence of community composition, density and seasonality. As the experimental fish were wild-caught, treatment responses are the sum of genetic and plastic effects. Black rubber stock tanks were used as experimental mesocosms. The tanks are 81 cm wide, 61 cm high and hold 265 l, but were only about 90% filled. A few days before the start of the experiment, the tanks were filled with water from a small no-alewife lake (Linsley) adjacent to the tanks. All tanks were covered with netting to prevent bird predation, colonization by insects and accumulation of leaves. The tanks were stocked with bluegill captured in late June/early July from three no-alewife lakes (Black, Gardner and Hayward), three anadromous lakes (Bride, Dodge and Gorton) and three landlocked lakes (Pattagansett, Quonniapug and Rogers). Until the start of the experiments, captured bluegill from each population were held separately in tanks and fed a mixture of small and large zooplankton (collected from Linsley and Quonniapug lakes).

(i) Growth experiment

On day 1 of the experiment (13 July 2012), fish from each lake (except from Bride, from which we had only enough fish for the food selection experiment) were netted from holding tanks, weighed and stocked into the experimental tanks in groups of four. A full factorial design with lake type (anadromous, landlocked or no alewife) × prey community type (small- or large-bodied zooplankton) was replicated three times per treatment and randomly assigned to the tanks. Mean total length (64 mm) and weight (4.36 g) of bluegill at stocking did not differ significantly between treatments (ANOVA, p > 0.05). Two fish died within the first 24 h of the experiment. They were removed, weighed and replaced with new fish of similar size from the
same population of origin. During the experiment, bluegill were exposed to conditions of either small-bodied (representing land-locked lakes) or large-bodied zooplankton prey (representing no- alewife lakes). Note that anadromous lakes are characterized both by small- and large-bodied zooplankton, depending on time of year [7]. Zooplankton prey of the appropriate size were added to the tanks every second day throughout the experiment. Small-bodied zooplankton were collected from a landlocked lake (Quonnipaug) and large-bodied zooplankton from a no-alewife lake (Linsley). Zooplankton were collected by pulling a plankton net (150 µm mesh, 60 cm diameter) behind a boat at slow speed through the open water area (pelagic zone) of the lakes. The amount of zooplankton collected was adjusted to obtain similar zooplankton biomasses of small and large zooplankton (biomass estimates were done after every sampling, and sampling efforts adjusted accordingly). The small-bodied zooplankton samples had a biomass-weighted mean body length of 0.44 mm and were dominated by Ceriodaphnia lacustris (70% of total biomass). The large-bodied zooplankton samples had a mean body length of 1.47 mm and were dominated by Daphnia pulex (more than 99% of total biomass; see figure 1 for body size distributions of zooplankton added to the tanks). At the termination of the experiment (1 August 2012), all fish were removed, euthanized and frozen for preservation. The fish were measured to the nearest 0.1 mm total length, blotted dry and weighted to the nearest 0.1 mg wet weight. Growth rates are expressed as specific growth rate:

\[ G = 100 \times \ln W_{\text{end}} - \ln W_{\text{start}} \]

where \( G \) is the specific growth rate (%), and \( W_{\text{end}} \) and \( W_{\text{start}} \) are wet weights (grams) at the start and end of the experiment. The water temperature in the tanks was measured on three occasions and varied between 23°C and 24.3°C.

(ii) Prey selection experiment

We also carried out a short-term prey selection experiment using the same type of tanks used in the growth experiment to ask whether bluegill from different lake types differ in their selectivity for small and large prey items. We stocked the mesocosms for the prey selection experiment with identical zooplankton size distributions, so we could determine relative size–selection among bluegill from different lake types by comparing the final zooplankton size distributions among treatments. This prey selection experiment was set up on the second to last day of the growth experiment, using fish that had been held in the holding tanks and fed a mixture of small- and large-bodied zooplankton for approximately five weeks. Two fish (mean total length 67 mm) originating from each lake (except from Black lake, from which no fish less than 80 mm survived) were added to the tanks and fed a mixture of small- and large-bodied zooplankton added in similar biomasses (collected as above, with a size–distribution similar to that in figure 1). Treatments and population of origin were randomly assigned, and each population was replicated twice. After 20 h, fish were removed, euthanized and frozen for preservation. Treated were then gently stirred, and zooplankton were sampled using a depth-integrated sampler that collected 1.21 of water. The collected water was filtered through an 80 µm mesh net. Two zooplankton samples per tank were pooled and preserved in 70% ethanol. Zooplankton were identified and counted under a dissecting microscope. Fifteen (all if fewer) individuals from each taxon were length-measured and transformed to dry mass using length–weight regressions [23].

(c) Comparative field study: gill raker morphology

We collected bluegill in 2006–2009 and 2012 to compare foraging morphology. A subset of collected bluegill were euthanized and placed on ice for transport to the laboratory, preserved at −20°C, and processed for size and gill raker measurements. Gill raker measurements were made on bluegill ranging in size from 30 to 212 mm total body length. Population-level sample sizes were: Black, \( n = 57 \); Bride, \( n = 18 \); Dodge, \( n = 42 \); Gardner, \( n = 46 \); Gorton, \( n = 22 \); Hayward, \( n = 33 \); Pattagansett, \( n = 52 \); Quonnipaug, \( n = 47 \); Rogers, \( n = 47 \). The first branchial arch of each bluegill was removed, stained with Alizarin red [24] and preserved in 70% ethanol. The space between the first four gill rakers on the lower limb of the first branchial arch and the lengths and widths of these gill rakers (from tip to base) were measured under a dissecting microscope. For bluegill more than 50 mm total length, we also counted the number of gill rakers on the arch (small fish were excluded owing to difficulties removing gill rakers without damaging them). The means of the three measurements for space and four measurements for lengths and widths were calculated for each fish.

(d) Statistical analyses

Growth rates (log-transformed to normalize the data) were analysed with a linear-mixed model (using the lme function in the R package nlme) using restricted maximum-likelihood estimation, with lake type and zooplankton treatment entered as fixed effects, and replicate lake populations entered as a random effect nested within lake type. We used ANOVA to test whether the ratio of growth rate (arcsine-square-root-transformed to normalize the data) of bluegill fed large relative to small zooplankton (growth experiment) differed between lake types. Similarly, differences in zooplankton body size (prey selection experiment) were determined using ANOVA. Given a strong relationship between body length and gill raker morphology, differences in gill raker morphology (log-transformed) between lake types were determined using bluegill total body length (log-transformed) as a covariate, with lake populations entered as a random effect nested within lake type. The interaction term was used to test whether the slope of the relationship between gill raker morphology and body length differed depending on lake type. When the slopes differed significantly (\( p < 0.05 \)), we tested for mean differences within two specific size classes (33.3 ± 0.24 mm and 125 ± 0.95 mm, means ± s.e.), representing young-of-the-year and adult individuals, using ANOVA. Given a significant lake-type effect, differences among lake types were evaluated using post hoc Tukey’s tests. Statistical analyses were performed using R (R Foundation for Statistical Computing).
3. Results

(a) Growth experiment
We found a significant interaction between lake type of origin and zooplankton treatment on bluegill growth rates ($t = 4.07$, $p = 0.007$; figure 2a), but no significant main effects ($p > 0.05$). This was a result of differences in relative growth rate of bluegill fed large-bodied zooplankton relative to small-bodied zooplankton, depending on which alewife lake type the bluegill originated from (ANOVA, $F_{2,7} = 56.13$, $p = 0.045$, figure 2b). Bluegill from landlocked lakes grew equally well when fed small- and large-bodied zooplankton, but bluegill from anadromous and no-alewife lakes grew better when fed large- than small-bodied zooplankton (Tukey’s post hoc test, $p < 0.05$).

(b) Prey selectivity experiment
There was a clear difference in the effect of bluegill lake type of origin on zooplankton size structure (figure 3; $F_{2,15} = 9.06$, $p = 0.004$). Mean zooplankton body size was larger in communities with bluegill originating from landlocked lakes (similar to the added zooplankton body size; see the dashed line in figure 3) than in communities with bluegill from anadromous or no-alewife lakes (Tukey’s post hoc test, $p < 0.05$), indicating that bluegill from anadromous and no-alewife lakes were positively size selective, whereas bluegill from landlocked lakes were neutrally size selective.

(c) Gill raker morphology
There were no significant differences in the slope of number of gill rakers (interaction term, $t = 2.18$, $p = 0.091$) or the slope of gill raker width (interaction term, $t = 0.080$, $p = 0.94$) regressed against bluegill body length, nor in means of gill raker numbers ($t = 0.415$, $p = 0.52$) or width ($t = 0.168$, $p = 0.89$; figure 4). The slope of the relationship between gill raker spacing and body length (interaction term, $t = 3.31$, $p = 0.029$) and gill raker length and body length (interaction term, $t = 2.88$, $p = 0.048$) varied among lake types (figure 4). As the slopes differed significantly, we analysed mean differences for small and large bluegill separately. Mean gill raker spacing and length were significantly different between lake types for small bluegill (ANOVA, spacing: $F_{2,7} = 7.83$, $p = 0.029$; length: $F_{2,7} = 10.0$, $p = 0.018$), but not for large bluegill (ANOVA, $p > 0.05$). In the small size-group, bluegill had smaller gill raker spacing and longer gill rakers in landlocked than in no-alewife lakes (Tukey’s post hoc test, $p < 0.05$). Thus, small bluegill—the size class that most relies upon zooplankton as a resource—had significant differences in gill raker morphology that were consistent with the differences in size selectivity we observed in the prey selection experiment.

4. Discussion
Using common garden experiments and a comparative field study, we have shown that the strong effects of life-history differences among alewife populations on zooplankton...
Figure 4. Log-transformed (a) gill raker spacing, (b) gill raker length and (c) gill raker width as a function of log-transformed total body length for bluegill from landlocked (white circles, solid regression line), anadromous (crosses, dotted regression line) and no-alewife lakes (black circles, dashed regression line).

Communities have propagated across the food web to influence the phenotype and fitness of a competing species. We found that bluegill from different lake types differed in (i) growth rates when exposed to small- or large-bodied prey, (ii) prey size selectivity and (iii) gill raker morphology. Our results revealed clear divergence in foraging traits between bluegill from landlocked and no-alewife lakes, but only weak divergence in bluegill between anadromous and no-alewife lakes. Consistent predation by landlocked alewife shapes the zooplankton community [7] such that it produces strong selection for traits that increase foraging performance on small-bodied zooplankton [16]. Consequently, we found bluegill from replicated landlocked lakes to be better adapted to feeding on small-bodied prey; they were less selective for large prey than bluegill from anadromous and no-alewife lakes, and had smaller gill raker spacing than bluegill from no-alewife lakes. Taken together, these results suggest differences in bluegill foraging traits in response to variation in prey size structure caused by the presence or absence of alewife as well as life-history differences among alewife populations. This shift in foraging traits in response to prey size structure suggests there is a clear functional link between bluegill trait change and resource use, as would be expected based on theory of ecological character displacement [13,14] (for the role of plasticity in character displacement, see [25]); however, in contrast to most literature on character displacement, interspecific competition produced phenotypic shifts in bluegill foraging traits parallel to those in the alewife populations.

It has only recently been established that intraspecific phenotypic variation may generate diversifying selection on local ecological communities, affecting prey community structure and ecosystem function [8,9,16]. In the alewife system, variation in alewife life history drives divergence in Daphnia life history that in turn alters consumer–resource dynamics [10,18]. This study expands on these findings, suggesting that the effects of diversifying selection may propagate across natural food webs to affect competing species. Observed variation in bluegill growth rates when exposed to small- and large-bodied zooplankton suggests adaptation to prey communities similar to those in their lakes of origin. Landlocked lakes are characterized by dominance of small-bodied zooplankton year-round [7]. Thus, in landlocked lakes, selection should favour those individuals that can perform well feeding on small-bodied zooplankton. Accordingly, we found that bluegill from landlocked lakes select smaller prey and grow relatively better when fed small-bodied zooplankton than did bluegill from anadromous and no-alewife lakes. These findings are consistent with previous studies showing persistent ecological effects of landlocked alewife, leading to strong eco-evolutionary feedbacks shaping alewife foraging traits [16]. Our study shows that those ecological effects also shape bluegill foraging traits. By contrast, the lack of persistent environmental effects of anadromous alewife limits the potential for alewife with an anadromous life history to drive phenotypic variation in bluegill.

The traditional focus of community ecology has been on the influence of species identity and abundance. It is also increasingly recognized that trait-mediated effects may be equally important [26], however, research on impacts of trait variation is commonly based on species means, ignoring variation within species. Our results suggest that niche construction by landlocked alewife, rather than by fish species composition and abundance, drives variation in foraging traits and performance in bluegill among lake types. This suggests adaptive divergence in bluegill in response to the ecological impact of a specific life-history type of a competing species. A key question is whether the observed divergence among bluegill populations is caused by genetic divergence or by phenotypic plasticity. Our common garden design showed that phenotypic differences remained when exposed to a shared environment and congruent patterns in experiments, and our comparative lake data suggest local adaptation is likely, but we have no information of heritability of measured traits in bluegill. Previous work indicates differences in gill raker morphology are likely to have a genetic basis [27,28], but a plastic component cannot be ruled out [29]. Whether or not trait variation caused by niche construction among populations of competing species is the result of genetic divergence or phenotypic plasticity would be an interesting topic for future studies on this and similar systems.

Although the occurrence of rapid evolution in nature is well established [4,30,31], we know little about its importance for natural ecosystems [5,7]. Generally, the importance of evolutionary change for ecological interactions is expected...
to depend on the strength of links between trait variation and ecological interactions, and the extent to which traits under selection can undergo rapid change [1,4]. The extent to which evolutionary change in one species can propagate through food webs, leading to cascades of evolutionary change, is particularly poorly studied. The cascading effects caused by landlocked alewife, as observed in this and previous studies [7,18,32], stress the importance of understanding how trait variation in dominant species drives selection, as this may be important for the evolution of whole communities. In this case, our results suggest that as alewife evolved to become a better competitor for small-bodied zooplankton it forced bluegill to increase foraging efficiency on that same resource, leading to shifts in bluegill foraging traits that parallel those in alewife. Although almost all literature on character displacement is about trait divergence, parallel trait changes are theoretically predicted given a narrow range of available resources and competition for essential resources [33,34]. Indeed, compared with most other study systems, alewife completely shift the prey composition leading to a very narrow size range of zooplankton observed in lakes with landlocked alewife. This may produce strong enough directional selection to produce the observed parallel phenotypic shifts in alewife and bluegill.

Given parallel phenotypic shifts in response to local ecological conditions, bluegill may even accelerate community divergence among lake types by reinforcing selective pressures (i.e. year-round dominance of small-bodied prey). Such a scenario suggests that eco-evolutionary feedbacks brought on by a dominant species that shapes its environment may not only drive the diversification of that dominant species and the species it directly interacts with (i.e. in predator–prey systems), but also indirectly affect whole guilds of competing species. Still, it is clear that alewife is the most important species shaping the zooplankton community, when present, as we observe high densities of large-bodied zooplankton in our no-alewife lakes that contain high densities of healthy bluegill [7].

In conclusion, we show how divergence of alewife may alter the selective landscape and cause variation in foraging traits and performance of bluegill, which suggests that competing species may be locally adapted to the niche constructed by different life-history variants of dominant species. Our findings support the hypothesis that life-history variation within species and niche construction by novel life-history types may have profound influence on natural food webs.

This study was conducted under our approved Yale University Institutional Animal Care and Use Committee Protocols no. 2006-10734, no. 2009-10734 and no. 2012-10734. Fish were collected under State of Connecticut Scientific Collector Permits no. 0508021, no. SC-07015 and no. SC-11016.

Acknowledgements. We thank Monica Ague, Jakob Brodersen, Paul Bucala, Andrew Jones, Matthew Walsh, Jerome Weis and Derek West for help in the field. Data accessibility. Data supporting the results in the article are deposited at Dryad (doi:10.5061/dryad.57n53).

Funding statement. This research was supported by Yale University, the USA National Science Foundation (DEB no. 0717265) and a postdoctoral fellowship from the Swedish Research Council to M.H.

References

22. Mittelbach GG. 1981 Foraging efficiency and body size: a study of optimal diet and habitat use by


