

Life-history differentiation of benthic and limnetic ecotypes in a polytypic population of threespine stickleback (*Gasterosteus aculeatus*)

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ABSTRACT

Resource-use polymorphisms are associations between heritable phenotypic variation and habitat use within populations. These polymorphisms can offer insights into the mechanisms by which adaptation can occur despite gene flow, and into the importance of these mechanisms for the formation of new species. Resource-use polymorphisms are relatively common in northern freshwater fishes, including the threespine stickleback (*Gasterosteus aculeatus*) complex. This complex exhibits parallel trophic variation at three nested levels: (1) species differences, (2) ecotypic variation across populations and (3) polytypy within populations. Of these levels, polytypic variation has been the least well characterized in the threespine stickleback, having been detected in only two populations (Benka Lake, Alaska; Cranby Lake, British Columbia). In each lake, individuals are specialized in body shape and trophic morphology along the benthic (bottom feeding) to limnetic (plankton feeding) axis. The morphotypes in each population differ in habitat use and diet. Here we demonstrate that in Benka Lake, Alaska, females of the two ecotypes also differ in reproductive allocation, with benthic females producing fewer, larger eggs. Our results offer the first evidence of reproductive life-history differences between morphotypes of stickleback within a single population. The difference in egg size between the morphotypes may reflect plasticity paralleling that in other aspects of the phenotype. We believe, however, it is more likely that the association between morphology and egg size is favoured by disruptive selection on fry size imposed by differences in water temperature between the habitats in which the morphotypes breed. Assortative mating in Benka Lake apparently has led to more pervasive phenotype divergence than previously suspected and possibly even to incipient sympatric speciation.

Keywords: benthic–limnetic, ecotype, egg size, gene flow, life history, polytypic variation, resource-use polymorphism, sympatric speciation, threespine stickleback.

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INTRODUCTION

Associations between phenotype and resource use can offer essential insights into the origins of biodiversity and the causes of speciation. Resource–phenotype associations have been documented in a number of taxa, most often among populations or species (e.g. Williams, 1972; Echelle and Kornfield, 1984; Grant, 1986; Schluter, 2000). Resource-use polymorphisms, associations between phenotype and resource use within populations, have been identified less frequently. These polymorphisms are of particular interest because they provide insight into the ways in which natural selection can forge correlations among traits without geographic isolation, potentially leading to sympatric speciation (Bush, 1994; Skulason and Smith, 1995; Smith and Skulason, 1996). Although controversial in the past, recent theoretical advances indicate that resource-based disruptive selection can lead to sympatric speciation under conditions likely to occur in nature (Kirkpatrick and Ravigne, 2002; Fry, 2003).

In freshwater fishes, resource-use polymorphisms that involve trophic differences are unusually common (Robinson and Wilson, 1994; Wimberger, 1994; Skulason and Smith, 1995; Smith and Skulason, 1996). Most often they involve associations between body form and trophic morphology that cause differential efficiency when foraging on benthic invertebrates in the littoral zone (benthic morphotype) or on plankton in the limnetic zone (limnetic morphotype). Differentiation along this benthic–limnetic resource axis (*sensu* McPhail, 1994) is continuous between the two extremes. Trophic divergence is not limited to variation within populations, but is also a common pattern of differentiation among populations of a single species and among closely related species (McPhail, 1994; Robinson and Wilson, 1994; Bell and Andrews, 1997). Because of the nested levels of differentiation that straddle species boundaries (Foster *et al.*, 1998), differentiation along this benthic–limnetic axis has been strongly implicated in the speciation of several taxa of post-glacial freshwater fishes (e.g. McPhail 1994; Hindar 1994; Bernatchez *et al.*, 1996; Schluter, 1996).

Research on benthic and limnetic ecotypes and species has focused primarily on the morphological features that promote efficient use of planktonic and benthic food resources and on behaviours associated with feeding (Ridgway and McPhail, 1984, 1988; Foster, 1995; Foster *et al.*, 1998; Boughman, 2001; Rogers *et al.*, 2002). In contrast, the reproductive life histories of ecotypes are poorly known (but see Skulason *et al.*, 1989; Baker *et al.*, 1998), even though life-history traits are predicted to be particularly tightly associated with adaptation to divergent environments (Roff, 1992).

The threespine stickleback (*Gasterosteus aculeatus*) complex is considered to be a model system for the study of ecological speciation. Benthic and limnetic morphotypes have evolved repeatedly and in parallel: as allopatric ecotypes in thousands of lakes, as sympatric species pairs within six known lakes (e.g. Bell and Foster, 1994; McPhail, 1994; McKinnon and Rundle, 2002) and as partially differentiated ecotypes within two known lakes. These polytypic populations have recently been characterized with respect to morphology, foraging habitat, diet and foraging performance [Benka Lake, Alaska (Cresko and Baker, 1996; Cresko *et al.*, in prep.); Cranby Lake, British Columbia (Robinson, 2000)]. As expected, the limnetic form is more fusiform in shape, has larger eyes, more numerous gill rakers, and a smaller, more tubular mouth. Limnetic fish most often feed on plankton in the water column, whereas more benthic individuals forage on the bottom in the littoral zone (Robinson, 2000; Cresko *et al.*, in prep.). In Benka Lake, dietary information derived from gut content analysis and observation of foraging fish is confirmed by stable isotope analysis (Cresko *et al.*, in prep.). In both populations, the ecotypes perform best in the habitats for which they are trophically specialized, as measured by feeding efficiency

(Robinson, 2000) and growth rate (Cresko *et al.*, in prep.). Lastly, the phenotypic differences in trophic morphology persisted between the morphs from Cranby Lake under common laboratory rearing conditions, suggesting a heritable basis for the morphotypes (Robinson, 2000).

Here we provide the first description, from Benka Lake, Alaska, of life-history differences between benthic and limnetic stickleback in either the sympatric species pairs or the sympatric morphotypes. The primary difference involves the allocation of resources to egg size and number, with benthic females producing fewer, larger eggs. These life-history traits are unlikely to differ simply because they are genetically linked to trophic morphology or body shape – that is, they almost certainly have an adaptive basis. Phenotypic plasticity is one possible explanation. Females of each ecotype could assess conditions in their respective habitats and alter egg size appropriately, an ability demonstrated by guppies (Reznick and Yang, 1993), seed beetles (Fox *et al.*, 1997; Savalli and Fox, 2002) and plants (Callahan and Pigliucci, 2002). We suggest, however, that the allocation difference is more likely to reflect adaptation to predictably differing temperature regimes in the habitats in which the two morphotypes spawn and rear embryos. Like their morphology, egg size and number appear to have diverged despite strong potential for gene flow between the ecotypes (Cresko *et al.*, in prep.).

METHODS

Benka Lake (62°11'15"N, 150°0'15"W) is a small (0.5 km²), deep (average 10 m, maximum 23 m) lake located about 125 km north of Anchorage, Alaska. It is unusual among lakes in the region in that it has both large, shallow bays (benthic habitats) and steep shoreline regions that drop sharply to deep water (limnetic habitats). On 28 June 1998, stickleback were collected from sites that had previously been identified as either benthic or limnetic habitat (Cresko *et al.*, in prep.). The benthic breeding habitats were large, shallow (< 2 m deep) bays with a loose flocculent bottom supporting primarily benthic macroinvertebrate prey items. The limnetic breeding habitats were narrow rocky edges of the lake, and islands within the lake, where the bottom quickly dropped to depths of 10–20 m. The limnetic breeding habitats had little area to support macroinvertebrate communities, but were adjacent to large open-water areas that harboured planktonic prey. In benthic habitats, females foraged in loose groups on benthos in the same areas in which the males built their nests. In limnetic habitats, males built their nests along the narrow, steep shoreline and courted females foraging in large plankton-feeding groups just offshore. Within each habitat, individual fish were briefly observed to confirm foraging mode, then captured with a hand net. Fish were anaesthetized with MS222 and preserved in buffered 10% formalin.

A total of 50 gravid benthic and 60 gravid limnetic females were examined to determine standard length, blotted wet somatic mass, average egg dry mass, dry clutch mass and clutch size. Methods for assessing these traits are described in Heins *et al.* (1992), Heins and Baker (1993) and Baker *et al.* (1995, 1998). Slopes of the relationships of clutch mass, clutch size and egg dry mass to female mass were compared using reduced major axis regression if a significant correlation existed. This regression model may be a more accurate estimator of the allometric slopes when there is substantial error in the x-variable, as in our study (McArdle, 1988; Sokal and Rohlf, 1995). Female somatic mass was compared between the morphotypes via analysis of covariance (ANCOVA) using standard length as the covariate. Clutch mass, clutch size and egg dry mass were compared for the two morphotypes via ANCOVA using female somatic mass as the covariate. Variables were approximately normally distributed,

and variances were never statistically different (Levene's test, $\alpha = 0.05$). All variables were transformed (\log_{10}) before analysis, with the results presented as back-transformed values and 95% confidence intervals, as recommended by Sokal and Rohlf (1995).

RESULTS

Assuming that size at age is similar to that of other south central Alaska populations, both morphotypes of stickleback in Benka Lake consisted of a comparable mixture (Fig. 1a,b; top histograms) of age 1-, 2- and 3-year-old breeding females (*c.* 12, 24 and 36 months old, respectively). Presumed age 2 females formed the bulk of the breeders. The distribution of breeding female standard lengths was similar for the two morphotypes, but benthic females were heavier at all lengths (ANCOVA: $F_{1,117} = 66.5$, $P < 0.0001$; slope test, $P > 0.50$). At the overall mean standard length of 51.3 mm, limnetic females averaged 1.09 g (95% CI = 1.07 to 1.12 g), whereas benthic females averaged 1.26 g (95% CI = 1.23 to 1.30 g). This difference of 0.17 g represented an increase of 15.6% compared with limnetics.

The slope of the relationship between clutch mass and female somatic mass (Fig. 1; scatterplots) did not differ between the morphotypes (*t*-test, RMA slope estimates: $P > 0.40$), nor did clutch mass adjusted for body mass ($F_{1,95} = 0.02$; $P \gg 0.50$). At a common blotted wet somatic mass of 1.17 g, mean dry clutch mass was 0.048 g (95% CI = 0.046 to 0.050 g) for both morphotypes.

The allocation of clutch mass to size and number of eggs, however, did differ between the morphotypes. Slopes for the regressions relating clutch size to body size were similar (*t*-test, RMA slope estimates: $P > 0.50$), but clutch sizes adjusted to a common somatic mass (Fig. 2) were not ($F_{1,106} = 4.79$, $P < 0.031$). The back-transformed mean clutch size at a body mass of 1.17 g was 6.7% larger in limnetics (76.4 eggs; 95% CI = 73.5 to 79.3 eggs) than in benthics (71.6 eggs; 95% CI = 67.6 to 74.1 eggs). Individual eggs were correspondingly, and significantly ($F_{1,96} = 14.7$, $P < 0.0002$), smaller in limnetics by about 7.3% (Fig. 3). Neither morphotype displayed a significant relationship between egg dry mass and female somatic mass (benthics: $r = -0.20$, $n = 43$, $P = 0.20$; limnetics: $r = -0.07$, $n = 55$, $P > 0.50$), so the difference in egg size is not a simple consequence of the body size difference.

DISCUSSION

The reproductive traits of the morphotypes of stickleback in Benka Lake differ in two respects. One expected difference is that breeding benthic females are larger than breeding limnetic females. Although this difference in body size parallels that observed between the benthic–limnetic species pairs, it is much smaller (McPhail, 1994; Hatfield, 1997; Nagel and Schluter, 1998). The Cranby Lake morphotypes also differ little in body size. Although Robinson (2000) provides only standard length data for this population, extrapolation from our length–weight data suggests that the benthic ecotype is 14–16% heavier than the limnetic in Cranby Lake. This difference is nearly identical to that we observed in Benka Lake. Thus, females of both ecotypes in these lakes begin breeding at age 1 and at a similar size. In Benka Lake, the morphotypes also have similar growth trajectories, as indicated by our size-at-age plots and by indistinguishable growth rates measured *in situ* (Cresko *et al.*, in prep.).

In contrast, a more than two-fold difference (0.7 g vs 1.6 g) appears to exist between breeding females in the Paxton Lake species pair (Nagel and Schluter, 1998: estimation of mass from lengths in their Figure 1). Males in Paxton Lake show a similar degree of difference. Baker (1994: Table 6.1)

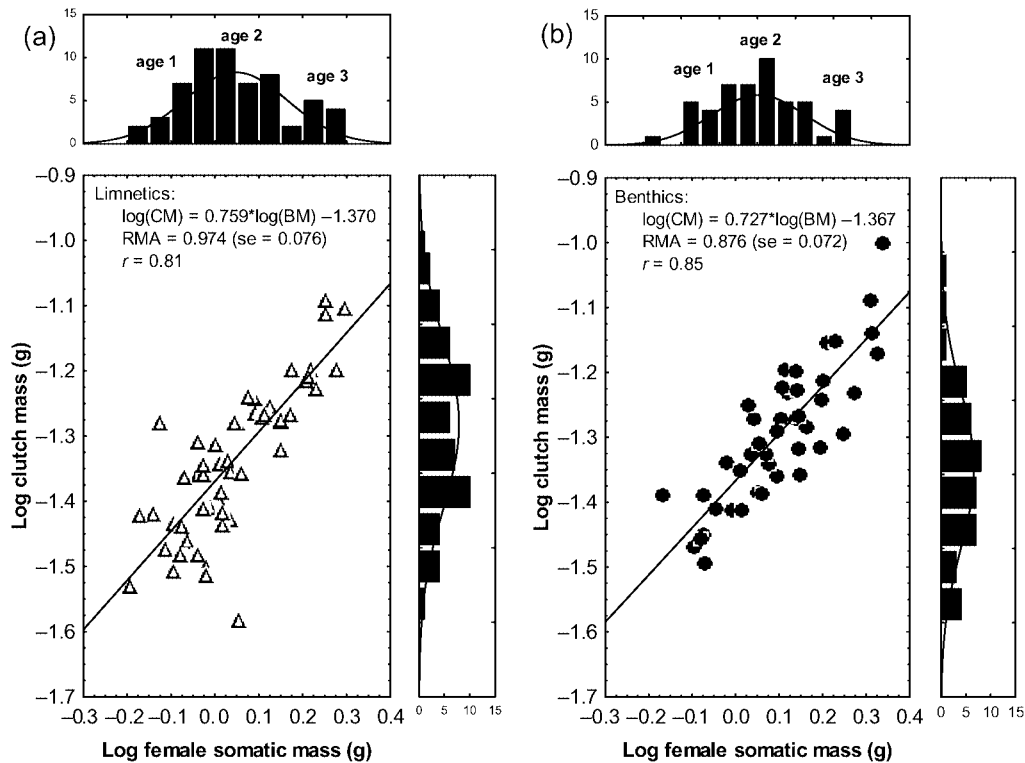


Fig. 1. The relationship between clutch mass and female somatic mass in breeding benthic and limnetic threespine stickleback from Benka Lake, Alaska. Top panels show the frequency distribution of female masses, while the side panels show the distribution of clutch masses. Probable ages are indicated based on size-frequency plots for larger samples of fish from Benka Lake, and from samples from more than 50 additional area lakes. In the ordinary least squares (OLS) regression equation provided, CM refers to dry clutch mass and BM to female blotted wet somatic mass, both in grams. The reduced major axis regression slope (RMA) is also given, with its standard error (se). The standard error for the OLS slope is the same as that for the RMA regression slope.

showed that stickleback from 34 allopatric freshwater populations were never as large as the benthics within Paxton Lake at age 1, and data for >50 Alaskan populations (J.A. Baker, unpublished data) indicate the same to be true. Canadian allopatric populations studied by McPhail (1977) were also more similar in size to the Paxton limnetics at first breeding (presumably at age 1). Although we cannot exclude the possibility that benthics in Paxton Lake may grow twice as large in one year as do limnetics, this seems to be an unlikely scenario. Rather, the magnitude of the difference suggests that limnetics in Paxton Lake are breeding at age 1, whereas benthics breed predominantly at age 2.

Lakes supporting the species pairs are shallow, and they likely offer limited limnetic feeding habitat. In such circumstances, limnetic females may profit most by reducing growth and beginning reproduction at a small size (and young age). In contrast, Benka Lake clearly provides abundant plankton. Stable isotope data suggest that each morphotype is feeding on its preferred food most of the time (Cresko *et al.*, in prep.). Thus, the planktonic diet of Benka Lake limnetics does not seem to limit their size at older ages or their maximum size and age.

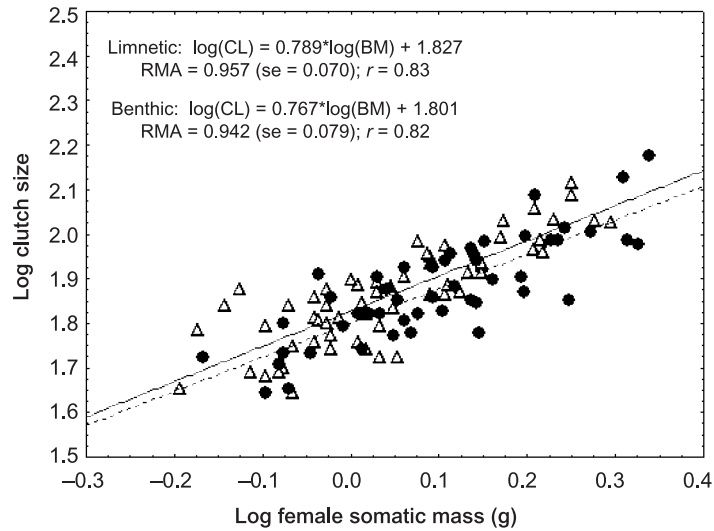


Fig. 2. The relationship between clutch size and female body size in two morphotypes of threespine stickleback from Benka Lake, Alaska. Open triangles and the solid line represent limnetic females, whereas solid circles and the dashed line represent benthic females. Regression information provided is as in Fig. 1.

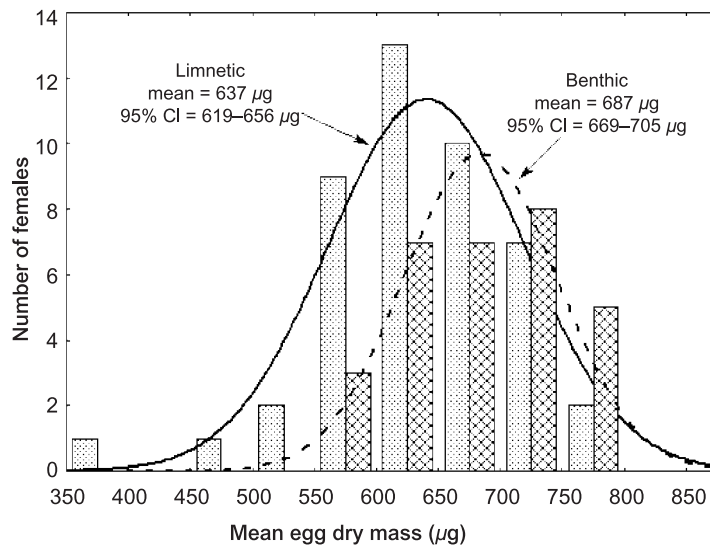


Fig. 3. The distribution of egg sizes in breeding female limnetic and benthic stickleback in Benka Lake, Alaska, during June 1998. Normal distributions are superimposed over the histogram for each morphotype.

Clearly, some limnetic females in Benka Lake are able to breed at age 1, at a size roughly similar to that of the Paxton Lake limnetics (Nagel and Schluter, 1998). The larger, older females may have deferred reproduction until age 2 (or even 3) to gain the advantage of increased clutch size (Roff, 1992), or they may have bred at age 1 and have survived to breed again. In

either case, the environment exploited by the limnetics in Benka Lake does not appear to place a significant constraint on their life history relative to benthics.

The second, more interesting difference is that, despite a common *total allocation* of resources to reproduction in the benthics and limnetics, significant differences existed in the allocation of these resources to *egg size* and *egg number*. Although we cannot rule out phenotypic plasticity (see below), we believe that the larger egg size of the benthic ecotype in Benka Lake is the result of selection. It is also likely that this selection is imposed on fry size, rather than on egg size itself. In fish, mortality rates are especially high in the first few weeks of life (Vetter, 1988), and the advantage of size for the youngest ages is well established (reviewed in Heath and Blouw, 1998). The fry of both benthic and limnetic stickleback initially feed on plankton in open-water habitats (reviewed in Hart and Gill, 1994), probably in mixed-type groups. In Benka Lake, benthic stickleback breed in shallow bays, a habitat in which the water temperatures are comparatively warm. Although limnetic fish also breed near shore, their breeding sites are on steep slopes in or immediately adjacent to deep, cool water. As a result, embryos of Benka Lake limnetics probably develop in cooler water than do those of the benthic form. The size of a larval fish produced from an egg of a given size is inversely related to the temperature at which the embryo develops (Heming, 1982; Beacham and Murray, 1985, 1990; Quattro and Weeks, 1991). Thus, benthic females must produce larger eggs if their fry are to be as large, and competitive, as those of limnetic stickleback in Benka Lake.

In contrast to the Benka Lake situation, the limnetic member of the species pairs in British Columbia nests in shallower, and thus probably warmer, water than does the benthic species. If our hypothesis regarding fine-scale effects of temperature is correct, the relationship between ecotype and egg size (if any) should be reversed in the species pairs. This hypothesis cannot be evaluated at present, as data on female life-history attributes have not been published for any of the species pairs. This is especially unfortunate, as two of the four independently evolved species pairs no longer exist (Hatfield, 2001; Kraak *et al.*, 2001; Foster *et al.*, 2003).

Phenotypic plasticity cannot be ruled out as the causative factor in the egg size difference we found. In fact, thermal reaction norms are quite common in biology (Angilletta *et al.*, 2003). Reznick and Yang (1993) have demonstrated that guppies exposed to a dramatic reduction in diet respond by increasing embryo size appropriately. Similar adaptive plasticity in egg size is also shown by seed beetles (Fox *et al.*, 1997; Savalli and Fox, 2002). In these instances, however, the organisms are responding to a relatively long-term and probably an unpredictable change in their environment. Thus, they may subsequently experience a return to the original environmental conditions. In the seed beetles, for example, females encounter a host and then remain there for a sufficient time for egg size plasticity to be expressed. The female may then move to an alternative host and again respond adaptively. This is not the case with the Benka Lake stickleback. Although females are free to sample any environment within the lake, limnetics and benthics largely forage in their preferred environment after reaching the juvenile stage. We find no evidence that they alternate among environments, and thus there would seem to be no opportunity for plasticity to operate. All stickleback in Benka Lake remain offshore during the prolonged winter in Alaska, and benthic individuals only move inshore after ice-out at the start of the breeding season. Limnetic females remain offshore. The first clutch of the season is initiated before females move from shared offshore wintering areas to breeding grounds, and all females experience the same environment at that time. For at least the first clutch, then, plasticity appears unlikely. The size of eggs produced by benthics could, theoretically, move from larger to smaller as a plastic response to the increasing temperatures in shallow water as the breeding season progresses. However,

we think this is unlikely for two reasons. First, experimental evidence (Wootton, 1973, 1977) shows that female stickleback, in contrast to guppies, do not alter egg size substantially following onset of breeding, even when feeding regime is radically altered. Second, we (unpublished data) find no evidence of a seasonal change in egg size in any of nearly 50 natural Alaskan stickleback populations. If a plastic, adaptive response to temperature was at all common in threespine stickleback, it should occasionally be observed given the large seasonal temperature differences shown by Alaskan lakes (range of about 8–20°C during a typical breeding season). Instead, at least some level of genetic differentiation for egg size is indicated in shaping the present traits in Benka Lake stickleback (see Cresko and Baker, 1996, for a discussion of the possible role of plasticity in shaping morphometric traits).

We do not claim that phenotypic plasticity has played no role in shaping the present traits of the stickleback in Benka Lake. Adaptive phenotypic plasticity and genetic accommodation may have played important roles in the initial stages of divergence into benthic and limnetic ecotypes (reviewed in West-Eberhardt, 2003). Foraging choices made early in a fish's life may precipitate a cascade of developmentally plastic changes in habitat use, foraging morphology and allocation to reproduction. These plastic traits could then show microgeographic patterning within the lake because males and females reproduce in the areas closest to their foraging sites.

If the differences in egg size reflect genetic differentiation, it must have evolved in the face of high levels of gene flow. Two lines of evidence suggest high levels of recent gene flow (Cresko *et al.*, in prep.): (1) neutral mtDNA and microsatellite markers offer no evidence of genetic differentiation of the morphs, and (2) the allele frequencies at six microsatellite loci are in Hardy-Weinberg equilibrium. If, however, egg size is subject to strongly divergent patterns of selection in the two breeding habitats, and spatial separation of the breeding grounds has led to strong assortative mating, genetic differences could have evolved despite the potential for high levels of gene flow (Kirkpatrick and Ravigne, 2002; Fry, 2003). If mate recognition systems have also diverged such that mate choice decisions are based at least in part on ecotypic differences, all of the conditions for sympatric ecological speciation are fulfilled (Kirkpatrick and Ravigne, 2002; Fry, 2003). Studies of the genetic basis of the resource-use polymorphism and mate recognition system in this lake will provide critical data for understanding the role of natural selection, even in the face of gene flow, for the differentiation of populations into species.

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