Two morphotypes of lacustrine threespine stickleback, *Gasterosteus aculeatus*, in Benka Lake, Alaska

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Synopsis

Within freshwater fishes, a common pattern of diversification of body form and trophic structure has recently been recognized. Two different suites of co-occurring characters appear to allow fish to efficiently forage on either benthic invertebrates or plankton. For threespine stickleback, *Gasterosteus aculeatus*, these suites of characters have been labeled 'benthic' and 'limnetic'. The forms differ in several morpholoical traits, with the limnetic having a more fusiform shape, larger eyes, longer and more numerous gill rakers, and a smaller more tubular mouth. Benthic and limnetic threespine stickleback are usually found in allopatry or parapatry, and less frequently in sympatry. Within the range of the threespine stickleback, which comprises perhaps tens of thousands of lacustrine populations, the sympatric occurrence of the benthic and limnetic forms has only been established for six lakes within a small region of the Strait of Georgia, British Columbia. We present the first evidence for the presence of sympatric morphotypes of threespine stickleback outside of British Columbia. We examine the nature and extent of this diversification, and present possible explanations for the sympatric occurrence of these morphotypes. We also explore possible reasons for the small number of documented sympatric benthic and limnetic forms of threespine stickleback despite the existence of thousands of apparently suitable lakes.

Introduction

Even within very limited geographic areas, populations of lake-dwelling fishes display a diversity of trait values that adapt them to the ecological conditions of their specific habitats (e.g., Echelle & Kornfield 1984, Bell et al. 1993). Despite this extensive adaptive variation in fishes, a consistent pattern has recently been recognized within this diversification (reviewed in Robinson & Wilson 1994). This pattern is manifested as two distinct suites of trophic and body shape characters which enable fish to for-age most efficiently either on plankton in the water column, or on benthic macroinvertebrates in the littoral zone. Each suite allows fish to utilize only one of these resources efficiently (Ehlinger & Wilson 1988, Schluter & McPhail 1992, Schluter 1995). Although the variation is continuous, in threespine stickleback the extreme forms have been termed 'benthic' and 'limnetic' to reflect the lake habitats in which they characteristically feed (McPhail 1984).

Benthic and limnetic forms are usually found in allopatry or parapatry, and less frequently in sympatry (Robinson & Wilson 1994). The presence of

two forms within a single lake has been interpreted in separate instances as evidence for two biological species (McPhail 1984), a genetic polymorphism within a single species (Robinson et al. 1993), and developmental plasticity (Meyer 1987). Study of the sympatric occurrence of these morphotypes is especially interesting because it can lend insight into character displacement and speciation (e.g., Wilson 1989, Wainwright et al. 1991, Snorrason et al. 1994, McPhail 1994, Robinson & Wilson 1994, Schliewen et al. 1994).

In the threespine stickleback, *Gasterosteus aculeatus*, this pattern of diversification has been studied in some detail (McPhail 1984, Schluter & McPhail 1992, McPhail 1994). The benthic morphotype forages primarily upon macroinvertebrates in the littoral zone of lakes, while the limnetic form feeds mostly on plankton in the pelagic zone. Limnetic stickleback are usually found in large, deep, relatively oligotrophic lakes, whereas benthic populations are typical of smaller, shallower, more eutrophic lakes. The forms differ in several morphological traits, with the limnetic having a more fusiform shape, larger eyes, longer and more numerous gill rakers, and a smaller more tubular mouth. Webb (1982, 1984) has shown that these forms make sense hydrodynamically, considering the demands of foraging in these two habitats.

Despite the holarctic distribution of the threespine stickleback, which comprises perhaps tens of thousands of lacustrine populations, the sympatric occurrence of the benthic and limnetic forms has been established for only six lakes within a small region of the Strait of Georgia, British Columbia. All six lakes were covered with ice during the last glacial advance. The initial invasion by stickleback is assumed to have occurred soon after the glaciers receded, and a local geological subsidence 2000 years later appears to have flooded the barriers between the ocean and the lakes, and allowed a secondary invasion of marine stickleback. It is hypothesized that the first stickleback evolved into the benthic species, and the second formed the limnetic. Because of this geological evidence, and also the lack of any other sympatric forms of stickleback throughout the entire range of *G. aculeatus*, an allopatric model of speciation has been presented for

the coexistence of these two species (McPhail 1994). This hypothesis has limited utility in other localities for explaining the sympatric occurrence of benthic and limnetic fish, even within stickleback. In other taxa of fish, such as cichlids in crater lakes, a sympatric model of speciation is more strongly supported (Schliewen et al. 1994).

Here we present the first evidence for the presence of sympatric benthic and limnetic forms of threespine stickleback outside of British Columbia. These forms were discovered in 1993 within Benka Lake, Alaska (62°11'15''N, 150°0'15''W). Benka Lake is 160 m above sea level, and 125 km north of Anchorage. It is a small lake (0.498 km^2) that is unusually deep (average 10 m, max. 23 m) as compared to most other Alaskan Lakes. Initial visual field observations suggested that two distinct groups of stickleback were present, one foraging on plankton in large schools towards the middle of the lake, and another feeding singly, or in small groups, on benthic prey in the littoral zone. If the two groups defined by our visual observations actually represent stickleback which are specializing on either plankton or benthic macroinvertebrates, then they should differ predictably in morphometric measurements and meristic counts. Specifically, open water feeders should be more fusiform in body shape, have larger eyes, a shorter snout, and have longer and more numerous gill rakers than those feeding in the littoral zone (Webb 1982, Webb 1984, Schluter & McPhail 1992).

Materials and methods

We collected threespine stickleback from Benka Lake on three separate occasions during July 1993. Individual stickleback were captured and preserved by foraging type after being observed by divers for a short period of time to determine the habitat in which they were foraging. Approximately 150 stickleback of each type were obtained. They were anesthetized using MS-222, fixed in 10% formalin for approximately 40 days, then transferred to 70% ethyl alcohol. Fish were stained using Alizarin red S to facilitate the measurements of bony structures.

Eight morphological attributes were assessed for

Table 1. Differentiation between two morphotypes of threespine stickleback in Benka Lake, Alaska, based on seven morphological traits. 'Raw data' are presented as means with one standard error in parentheses. 'Standardized data' are presented as percentages of standard length. 'Standardized canonical coefficients' were determined using a canonical correlation analysis on 79 benthic and 80 limnetic fish.

Morphological trait	Raw data		Standardized data		Univariate test results		Standardized canonical coefficients
	Benthic	Limnetic	Benthic	Limnetic	F-value	P-value	
Standard length (mm)	45.90 (0.69)	42.39 (0.59)					
Mass (g)	1.09 (0.05)	0.81 (0.03)					
Raker number	20.16 (0.14)	21.58 (0.16)			46.25	< 0.0001	0.7260
Raker length (mm)	0.90 (0.02)	0.96 (0.02)	1.97 (0.04)	2.26 (0.04)	26.24	< 0.0001	0.7641
Snout length (mm)	3.60 (0.08)	3.13 (0.07)	7.80 (0.09)	7.33 (0.09)	5.01	< 0.027	− 0.6750
Eye diameter (mm)	3.96 (0.05)	3.79 (0.05)	8.66 (0.07)	8.96 (0.05)	5.23	< 0.024	0.0754
Body depth (mm)	8.77 (0.14)	8.02 (0.12)	19.13 (0.13)	18.93 (0.13)	2.59	n.s.	− 0.0180

80 fish of each foraging type, randomly sampled from the overall collection (Hubbs & Lagler 1958, Baumgartner et al. 1988). Standard length and body depth were measured to 0.1 mm using dial calipers. The number of gill rakers on the first gill arch was counted using a dissecting microscope at 20–30X. An ocular micrometer was used to determine snout length, eye diameter, and the length of the fourth gill raker anterior to the angle of the arch. The mass of each fish was determined to 0.01 g using an electronic balance. The first gill arch of one benthic fish was destroyed while measuring the rakers, and therefore a total of 159 fish were included in the final analysis.

The relative contribution of each trait to discrimination was determined using canonical discriminant analysis (PROC CANDISC) in the SAS[1] System. A multivariate analysis of variance was used to determine whether the differentiation between the groups was significant. Univariate analyses of variance were also used to test the significance of individual morphological variables. A classification function was then constructed to predict the group to which each individual belonged, and the pattern of misclassifications was inspected to evaluate the accuracy of the function. Because some of the morphological variables were correlated with overall body size, relative measures of body depth, eye diameter, snout length, and gill raker length were constructed by dividing each measurement by standard length,

and applying an arcsine transformation (Sokal & Rohlf 1995). Gill raker number was not adjusted. Samples were tested for variance homogeneity and approximate normality prior to analysis.

Morphological traits found to be most important in separation of the foraging groups were investigated further. Some measurements were portrayed graphically, following adjustment to a common standard length, in order to examine the pattern of differentiation in more detail.

Results

The canonical discriminant analysis clearly indicated that our two foraging types were distinct in multivariate morphological space (Wilks' Lamba = 0.573; $F_{5,153}$ = 22.77; p < 0.0001). The discriminant function scores show a distinct bimodal distribution, with most of the fish caught in the benthic zone having low scores, and most of the fish caught in the open water having high scores (Fig. 1). All traits except body depth contributed significantly to the discrimination (Table 1). The standardized canonical coefficients indicated that limnetic foragers have larger eyes, shorter snouts, and longer and more numerous gill rakers. Snout length, gill raker number, and gill raker length contributed most strongly to the differentiation. The classification function indicated that in 81.1% of the 159 stickleback examined, the initial visual assessment of foraging type was supported by their morphological attributes. Of the
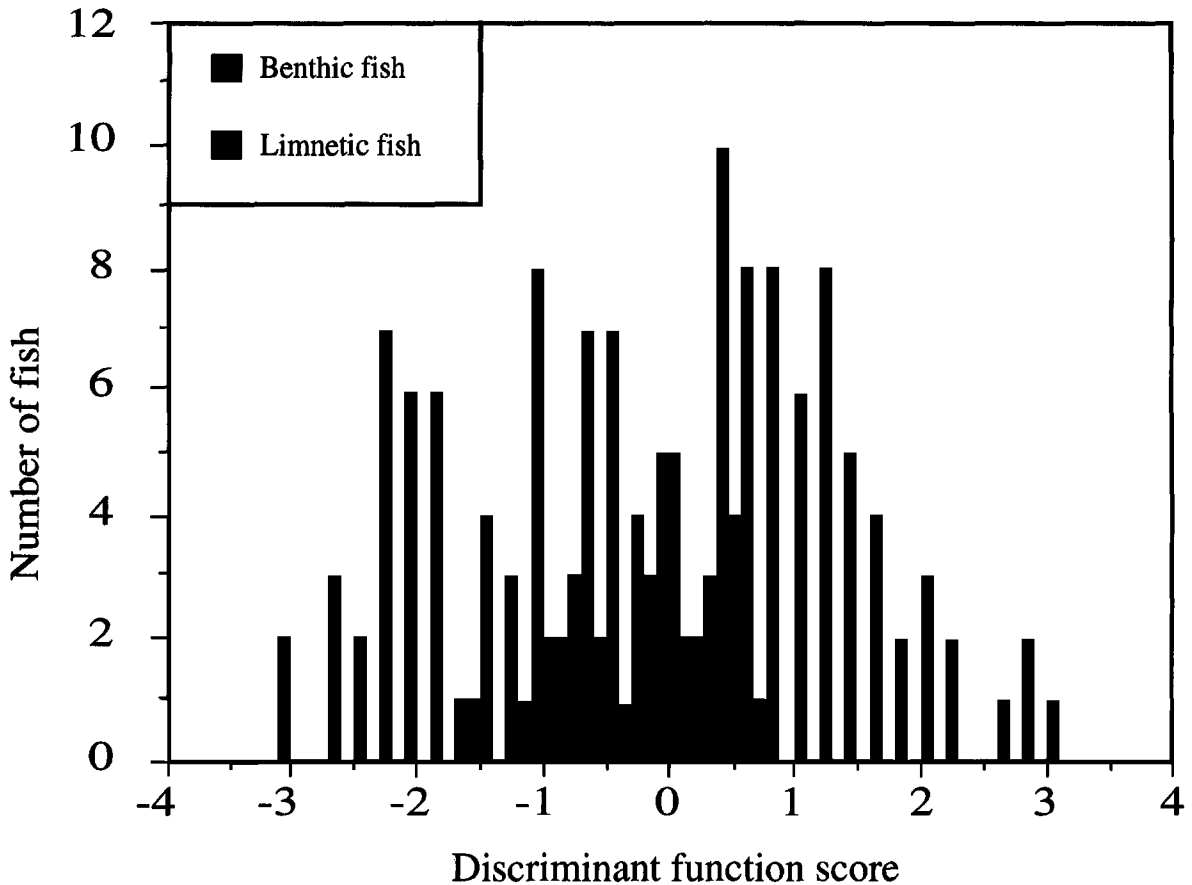
Fig. 1. Histogram of discriminant function scores of benthic and limnetic morphotypes of threespine stickleback in Benka Lake, Alaska.

two types, benthic foragers were classified correctly 78.5% of the time, whereas limnetic foragers were classified correctly 83.7% of the time.

Although the differentiation was in the predicted direction for all traits (Table 1), our use of standardized morphological characters in the canonical discriminant analysis obscured a trend in two traits of differing allometric growth between foraging types. Gill raker length of limnetics was always longer than in benthics of the same size, but the difference was greatest at larger sizes (Fig. 2). While eye diameter was also usually larger in limnetics than benthics, at very small sizes, this trend is reversed (Fig. 3).

Discussion

The results of our morphometric analyses confirm the presence of two morphotypes of threespine stickleback in Benka Lake. There are several hypotheses which may explain this divergence. The presence of two morphotypes could be evidence of phenotypic plasticity. Although we cannot presently dismiss this hypothesis, a genetic basis has previously been documented for differences in body shape and gill raker architecture in both sympatric and allopatric populations of threespine stickleback (Hagen 1973, McPhail 1984, Lavin & McPhail 1987). Alternatively, the morphotypes in Benka Lake might represent distinct species. However, the morphological differences between the two forms in Benka Lake are much less pronounced than those observed in the sympatric stickleback of the
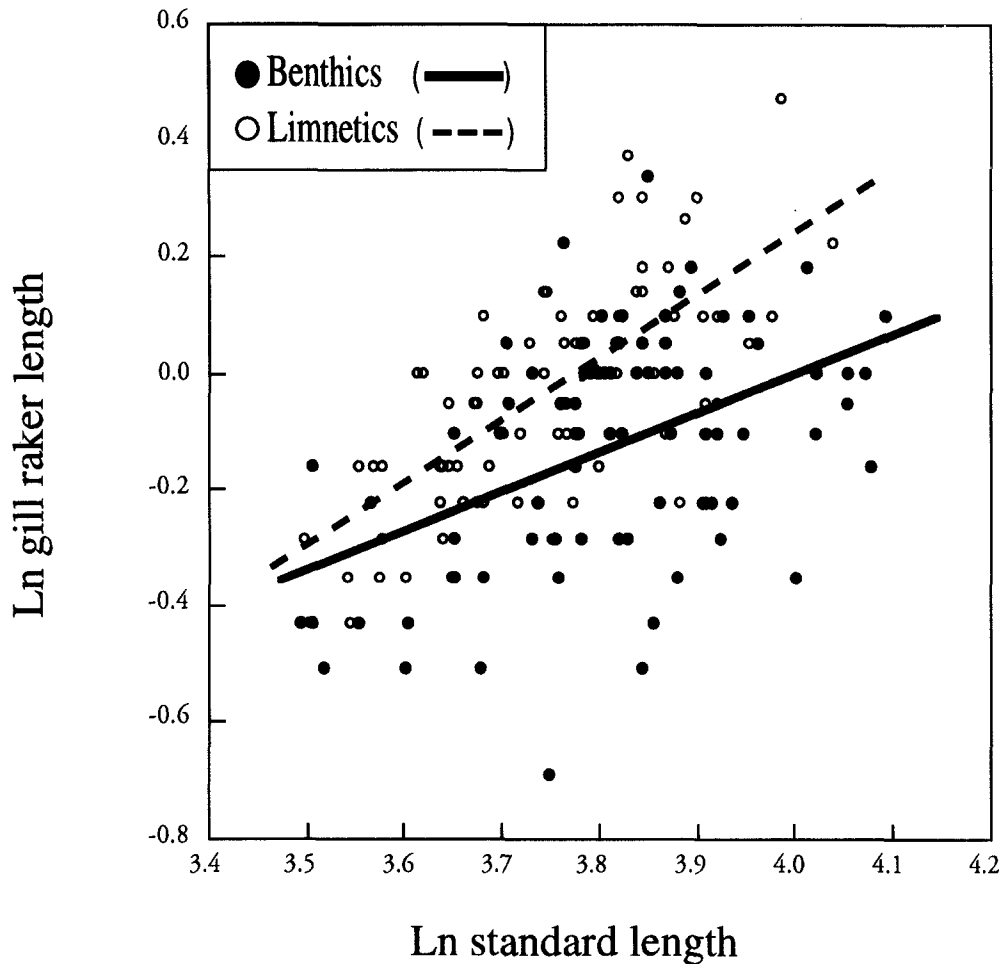
Fig. 2. Plot of gill raker length against standard length of benthic and limnetic morphotypes of threespine stickleback in Benka Lake, Alaska.

Strait of Georgia. There also appears to be a higher percentage of individuals which are intermediate between the two forms (about 19%) than in the Strait of Georgia Lakes (about 1%; McPhail 1984). Thus it seems likely that the morphotypes in Benka Lake are not reproductively isolated, but represent a polymorphism in a single population which is at least partially genetically determined.

Secondary contact between a differentiated population of stickleback and subsequent invaders from the ocean, the mechanism used to explain the species pairs of stickleback in British Columbia, most likely did not cause the phenotypic diversity observed in Benka Lake. Benka Lake is presently situated far away from the ocean (125 km), and is

160 m above sea level. There is no evidence for a local geological disturbance in Alaska, similar to the one in British Columbia, which would have closed the gap between Benka Lake and the ocean and allowed a secondary invasion of marine stickleback. Also, Benka Lake presently has no surface water connections to other bodies of water, and is surrounded by a ridge approximately 5–20 m high. Thus, it appears unlikely that the divergence in Benka Lake would be due to secondary contact between local freshwater populations of divergent stickleback, at least in the recent past. Although we have no way to completely eliminate alternative hypotheses, the morphological and ecological diversification of stickleback in Benka Lake appears to be
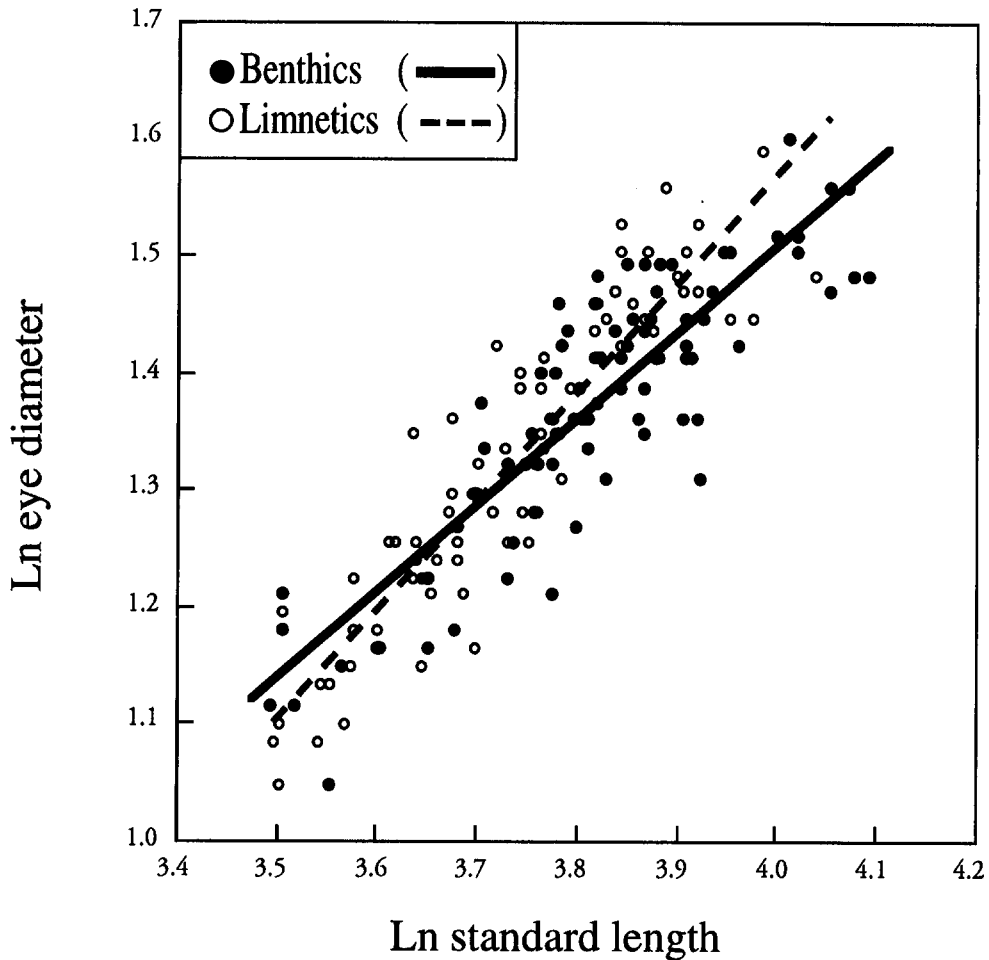
Fig. 3. Plot of eye diameter against standard length of benthic and limnetic morphotypes of threespine stickleback in Benka Lake, Alaska.

a process which is occurring in situ. Whether the pattern of diversification is due to secondary contact between previously allopatric forms, or due to in situ diversification, Benka Lake has the potential to provide unique insight into processes of evolution.

Why then, in the entire holarctic range of stickleback, within which possibly tens of thousands of lacustrine populations exist, have sympatric benthic and limnetic forms been found in only six lakes within one small area in Western North America? Other species of fish exhibit this type of phenomenon much more frequently. In fact, the occurrence of this benthic-limnetic trend across various taxa of fishes has prompted some researchers to hypothesize the existence of two distinct niches within la-

custrine environments, regardless of the specific community structure in each lake (Robinson & Wilson 1994). One possibility is that threespine stickleback are usually found in environmental conditions which are not conductive to such diversification. However, Robinson & Wilson (1994) have found that trophic and morphological divergence is most often found in species-poor communities. When niches within a community are full, divergence into two forms may be less likely. In recently deglaciated areas, however, stickleback are often the only fish present in lacustrine communities, and should be excellent candidates for this type of diversification.

An examination of traditional sampling techniques for threespine stickleback may provide an alternative explanation. Seine nets and minnow

traps are most often used to collect stickleback. Seine nets are only useable in water depths of approximately 1.5 m or less, and minnow traps are most often deployed around the edge of a lake, and the traps themselves sit on the bottom. Even though limnetic fish are reproductively active in the littoral zone, they may be found much less frequently than benthic forms in shallow, near shore areas, within which traps and seines are most often used.

Even if unbiased samples are obtained, with equal numbers of stickleback and limnetic and benthic foraging individuals, it is possible that the presence of two morphotypes may still not be evident from a completely random sample of the overall population. For example, histograms of gill raker number of the two morphotypes in Benka Lake (Fig. 4) clearly support the presence of two morphotypes when the population is subdivided, but not when all fish are pooled. Using the pooled distribution alone, it would be very difficult for a researcher to uncover the existence of two morphs, even by examining a well defined character such as gill raker number. The morphological divergence in Benka Lake was only suspected because of our a priori knowledge of differences in foraging mode gained through visual observations. The possibility exists, therefore, that the lack of evidence of sympatric morphotypes of stickleback (and other fish) may not be due to the relative rarity of this phenomenon, but because of inadequate collection techniques. Once the divergence between forms of stickleback becomes large enough, a random sample would show a bimodal distribution, especially if several morphological characters are considered in conjunction in a multivariate analysis (e.g. Fig. 1). However, if one is interested in the process of diversification itself, the populations that are of the most interest are the ones undergoing incipient divergence (McPhail 1994), and are consequently the most likely to be missed.

Recently, complex feeding polymorphisms have been found in the well-studied species of bluegill sunfish (Ehlinger & Wilson 1988) and pumpkinseed sunfish (Robinson et al. 1993), prompting those researchers to suggest that 'adaptive variation may be more common than is currently perceived'. We believe this may also be the case for threespine stickle-
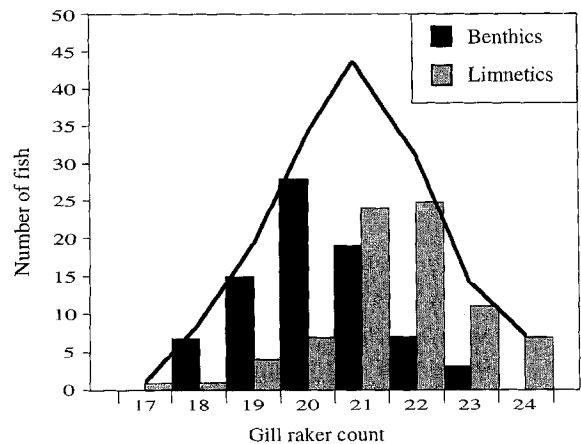

Fig. 4. Histogram of gill raker number of benthic and limnetic morphotypes of threespine stickleback in Benka Lake, Alaska. The solid line represents pooled gill raker numbers of benthic and limnetic stickleback.

back. Because our concept of the most likely history of evolution is determined by how common present-day patterns of diversification are in nature, it is important that these present-day patterns be accurately described. To uncover patterns and processes in nature, it is necessary for researchers to become intimately involved with their study organism. For studies on fish, careful underwater observation should be a part of any preliminary study to insure that collection techniques do not obscure patterns of diversification.

Acknowledgements

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