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Morphological segregation of Icelandic threespine stickleback (*Gasterosteus aculeatus* L).

BJARNI K. KRISTJÁNSSON[1,2]*, SKÚLI SKÚLASON[1] and DAVID L. G. NOAKES[2]

[1]*Hólar College, 551, Skagafjörður, Iceland*
[2]*Zoology Department and Axelrod Institute of Ichthyology, University of Guelph, Guelph, Ontario, N1G 2W1 Canada*

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Icelandic threespine sticklebacks show parallel sympatric morphological differences related to different substrate habitats in four Icelandic lakes. The level of morphological diversification varies among the lakes, ranging from a population with a wide morphological distribution to a population with clear resource morphs, where morphological diversification was reflected in diet differences. These differences in morphological divergence are closely related to the differences in the ecological surroundings of each population. This appears to be resource polymorphism, which may lead to population differentiation and speciation. Trophically related sexual dimorphism was also common in these sticklebacks, which is possibly the result of sexual selection or habitat segregation by the sexes.

ADDITIONAL KEYWORDS: diet – lava – morphology – mud– parallel patterns – resource polymorphism – sympatric

INTRODUCTION

The threespine stickleback (*Gasterosteus aculeatus*) occupies a wide range of habitats, both marine and freshwater throughout its northern circumpolar range (Wootton, 1984). It has very diverse morphology, including sympatric morphs in many locations (Blouw & Hagen, 1990; McPhail, 1994; Baumgartner, 1995; Cresko & Baker, 1996; Kristjánsson *et al*., in press). In five lakes in British Columbia, Canada, two forms of stickleback occur that represent biological species pairs. One is adapted to the pelagic habitat as a zooplankton eater; small and slender with a narrow gape and numerous long gill rakers. The other is adapted to the benthic habitat, eats larger invertebrates and has a more robust body form with smaller and fewer gill rakers (McPhail, 1994). The pairs are largely reproductively isolated and have become established recently and independently within each lake (McPhail, 1994; Taylor & McPhail, 1999), and represent a good example of parallel speciation (Schluter & Nagel, 1995; Nagel & Schluter, 1998; Rundle *et al*., 2000).

Icelandic freshwater habitats are numerous and diverse (GarQarsson, 1979; Skúlason *et al*., 1999), shaped by the volcanic nature of the island with lava substrate occurring in many lakes. All freshwater systems on the island are young (less than 14 000-years-old) having been formed after the latest glacial epoch. The island has been colonized by six species of fishes: arctic charr, *Salvelinus alpinus*, brown trout *Salmo trutta*, Atlantic salmon, *Salmo salar*, American eel, *Anquilla rostrata*, European eel, *A. anquilla* and threespine stickleback. This low species diversity is associated with diverse habitat use and subsequently evolution of resource polymorphism in the arctic charr (Skúlason *et al*., 1992, 1999; Gíslason *et al*., 1999). The greatest morphological diversity observed in arctic charr is in Thingvallavatn, where four morphs occur (Snorrason *et al*., 1994; Skúlason *et al*., 1999).

The benthic substrate of young volcanic lakes in Iceland is often complex. Lava flows have entered lakes resulting in complex benthic habitat, where in other parts of lakes the benthic habitat is character-

*Corresponding author. E-mail: bjakk@holar.is

ized by mud. These two substrate types offer very different habitats for fish, especially for a small fish like stickleback. Lava is a complex three-dimensional structure with many holes and crevices that can host a large number of invertebrates (Malmquist *et al.*, 1999). Mud is usually more two-dimensional with animals burying themselves into the substrate, although vegetation may add some complexity, and may host specific fauna (Kairesalo *et al.*, 1992; Á. Einarsson pers. comm.). Lava and mud will differ in foraging opportunities and exposure to predators. It can also be assumed that such muddy habitats are less temporally stable than lava habitats.

We hypothesize that Icelandic stickleback will show resource polymorphism. We predict that stickleback living in lava habitat will have morphological features suited to benthic feeding, and reduced features for predator defence. Stickleback in mud habitat are more exposed and will have morphological features related to more pelagic foraging and increased features for predator defence.

In similar ecological surroundings, natural selection will promote similar adaptations of fish resulting in parallel evolution across lakes (Schluter & Nage, 1995). We hypothesize that in the four lakes studied we will observe parallel patterns in the divergence of the sticklebacks.

We compared males and females from each substrate habitat within each lake, as some investigators have previous reported sexual dimorphism in some features in this species (Wootton, 1984).

To summarize, we hypothesize that we will observe parallel polymorphism in relation to mud and lava habitats in threespine stickleback in the four Icelandic lakes. We will also examine whether there are signs of sexual dimorphism of stickleback in these four lakes.

MATERIAL AND METHODS

STUDY SITES

We caught threespine stickleback in four lakes in Iceland (Fig. 1): HreQavatn in BorgarfjörQur in western Iceland, a small lake (area 1.1 km^2) with a maximum depth of 20 m; Mývatn, in North-Eastern Iceland, a large shallow lake (area 37.3 km^2, mean depth 2.33 m, max. depth 4 m); FrostastaQavatn in southern Iceland, a small highland lake (area 2.6 km^2); and Thingvallavatn in South-Western Iceland, a large, deep lake (area 83 km^2, mean depth 34 m, max. depth 114 m). Kristjánsson (2001) gives more detailed descriptions of these lakes. The lakes were chosen to be widely distributed within the neo-volcanic zone on the island. In each of these lakes we sampled stickleback from a lava habitat and a mud habitat that was often vegetated.

SAMPLING AND HANDLING OF SAMPLES

We caught stickleback with minnow traps (Dynamic Aqua-Supply Ltd, mesh size 3.2 mm) except in the lava substrate in Thingvallavatn where we caught fish by electro-fishing. We anaesthetized fish using a CO_2

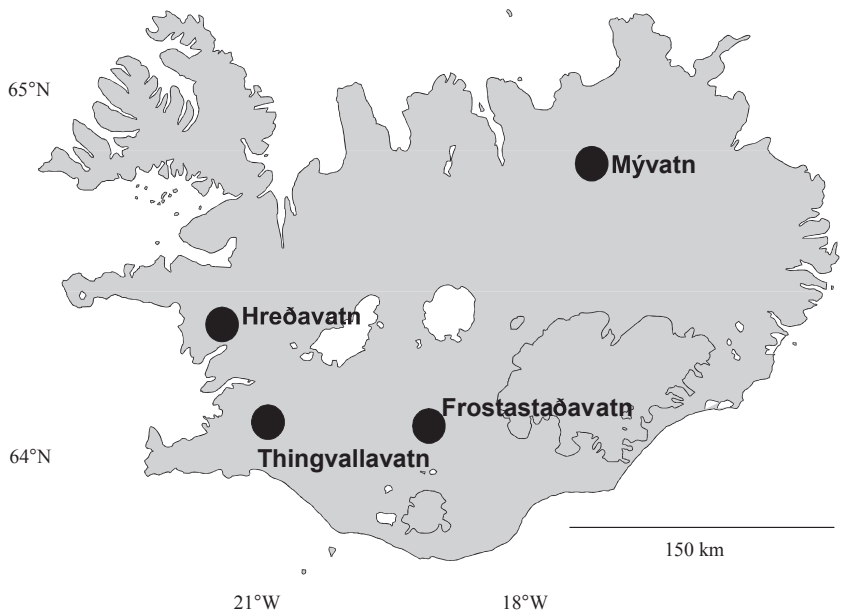

Figure 1. Sampling locations within Iceland.

solution, and then preserved them in buffered 10% formalin for at least 1 month before preserving them in 70% ethanol.

We chose 22–30 fish larger than 30 mm (see Bell, 1981) from each habitat for detailed analysis. We photographed each fish from lateral and dorsal angles (Minolta X700 camera, 55 mm macro lens, Kodacolor 100 ASA film), digitized 20 landmarks on each of the lateral pictures and 10 on each of the dorsal pictures (SigmaScan Pro 5, SPSS Inc.). We calculated 11 morphological distances from these landmarks (Fig. 2).

We measured fork length (FL) to the nearest 0.1 mm with a vernier caliper, and dissected the fish (Leica MZ12 dissecting microscope) to confirm sex. We removed the stomach (between the oesophagus and the pyloric sphincter) and counted and identified all prey organisms to the lowest taxonomic level possible. We opened the rest of the digestive tract and noted prey items to determine what fish had been eating because some had empty stomachs. The diet in the digestive tract may not necessarily give indications on the proportions of food groups eaten as different organisms are digested at different rates (Hall *et al.*, 1995).

We stained the fish after dissection with Alizarin Red in 1% KOH (e.g. Bell, 1982) to facilitate counting and measuring of meristic and morphometric characteristics. We used an ocular micrometer in a dissecting microscope (Leica MZ12, 8–101 times magnification) to measure eight additional morphological characteristics (Fig. 2). We counted gill rakers on the lower and upper branch of first gill arch and armor plates (designated as regular or keeled, e.g. Wootton, 1984) on the left side of each fish, the dorsal spines and rays on all fins. We counted all fin rays in the upper and lower parts of the caudal fin.

Statistical analysis

To standardize for body size, we regressed each of the 19 morphological measurements against fork length and calculated residual values for stickleback within each lake (Reist, 1985). For all further analysis we used the 'size free' residual values. We compared stickleback between habitats within each lake using both single variate as well as multivariate analysis. We used a jacknifed discriminant function analysis (DFA) to distinguish between sexes and habitats in overall body shape and meristic counts. This was carried out to obtain a relative measure of difference between habitats within lakes allowing for comparison across lakes. We used 2 ¥ 2 ANOVA with posthoc tests to evaluate differences in morphological measurements and meristic counts separately, as well as to compare discriminant scores among the four groups (lava males, lava females, mud males, mud females). Number of spines and number of pectoral fins rays differed only slightly. The proportion of those characteristics were compared using a non-parametric c^2-test (Sokal & Rohlf, 1981).

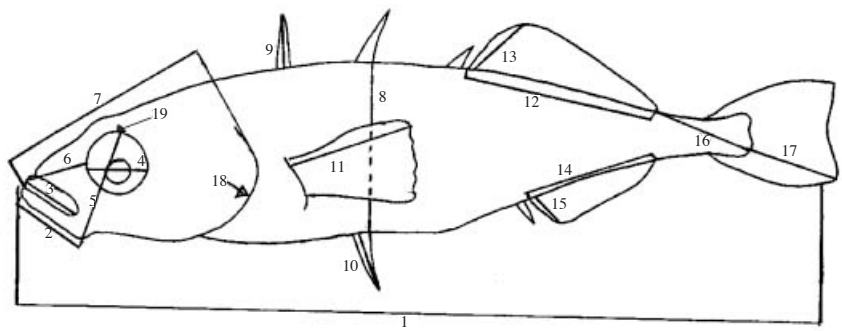

Figure 2. Morphological characteristics measured from the left side of each fish in the study: 1, Fork length. 2, Jaw length, the length of the lower jaw (P). 3, Maxilla length, The length of the maxilla from the snout to the end of the maxillary bone (F). 4, Eye diameter (P). 5, Height to top of eye, the distance from the snout to the dorsal part of the eye (P). 6, Snout length, the length from the snout to the anterior part of the eye (P). 7, Head length, distance from the angle of the lower jaw to the end of the operculum (P). 8, Body depth (P). 9, Dorsal spine length (F). 10, Ventral spine length (F). 11, Pectoral fin length, the length of the longest ray in the pectoral fin. 12, Dorsal fin length, The length of the dorsal fin, measured from the first fin ray to the end of obvious outgrowth (P). 13, Dorsal fin ray, length of first ray in the dorsal fin (F). 14, Anal fin length, The length of the dorsal fin from the first fin ray to the end of obvious outgrowth (P). 15, Anal fin ray, the length of the first ray in the anal fin (F). 16, Caudal peduncle length, The distance from posterior end of the dorsal fin to the end of flesh in the caudal peduncle (P). 17, Caudal fin length, the length of the longest ray in the caudal fin (F). 18, Gill raker length, the length of the longest gill raker on the longer arm of the first gill arch (F). 19, Distance between eyes, measured as seen from above (P). (P) indicates that the measurements were from a photograph and (F) indicates that the measurement came directly from the fish.

We conducted Principal Component Analysis (PCA) on the morphology of all fish from all the lakes combined to evaluate parallel evolutionary patterns. We excluded measurements on spines as they are believed to be affected by predation (Reimchen, 1994), and because the predation pressure differs among the lakes (Skúli Skúlason *et al.* unpub. results). Before conducting the PCA, we examined whether the morphological variables had a similar regression slope with standard length for the four populations (Reist, 1985). We did this by comparing slopes among lakes with analysis of covariance where fork length was the covariant. If the slopes did not differ between lakes, we concluded that the traits had similar allometry across the four lakes. We then used the residuals for each variable from each lake alone. If the regression slope of a variable differed between lakes, we removed size by calculating one regression slope for all the fish from the four lakes combined (Reist, 1985). To examine differences in PCA scores, we used ANOVA to compare lakes, habitat types and sexes on two PCA axes.

We excluded fish with empty stomachs from the analyses of diet. We grouped the diet in seven categories (Mollusca, Ostracoda, Chironomidae, pupae and flies (mainly diptera), Copepoda, Cladocera and other) and calculated the proportions of these groups within each stomach. We compared mean proportion of diet categories between habitats with non-parametric tests. The occurrence (number of fish each prey occurs in at each habitat) of all prey species and groups (taxonomic levels higher than species) in the stomach and digestive tract were compared between habitats using a c^2-test.

In all cases of multiple comparisons, we used sequential Bonferoni corrections to minimize the possibility of type I error (Rice, 1989). When calculating the correction factor the *P*-value was divided by the number of tests conducted on each set of data. In all statistical analysis we used alpha values of 0.05 to indicate significance.

RESULTS

HREÐAVATN

The sex ratio of the fish was similar in both habitats. Of 29 fish in the lava area, seven were male (24%) and of 30 fish in the mud area 11 were male (37%). The lava fish (mean FL 48.2 mm ± 4.15 SD) were larger than the mud fish (mean FL 45.5 mm ± 4.91 SD, ANOVA, $F_{1,58} = 5.2$, $P < 0.05$). Some variables differed between the sexes in the mud habitat. Males had longer maxilla ($t_{(27)} = 4.28$, $P < 0.05$) and gill rakers ($t_{(27)} = 3.88$, $P < 0.05$) than females. Mud females had longer anal fins ($t_{(27)} = -3.69$, $P < 0.05$) than the lava female. The four groups (lava males, lava females, mud males, mud females) differed in their overall morphology (DFA, Wilks $\Lambda = 0.115$, $c^2_{(54)} = 99.3$, $P < 0.05$, Fig. 3A. The model correctly classified all of the lava males, 91% of the lava females, 91% of the mud males and 89% of the mud females. The DF1 axis explained 58% of the variance and the model became non-significant when it was removed from the analysis. The variables that were significant between either sex or habitat had the highest loading on the discriminant axis as well as anal and dorsal fin height (Table 1). The discriminant scores on DF1 axis differed between fish from the two habitats (ANOVA, $F_{1,54} = 18.4$, $P < 0.01$) and between the sexes (ANOVA, $F_{1,54} = 11.5$, $P < 0.01$). The interaction of habitat and sex was significant (ANOVA, $F_{1,54} = 14.1$, $P < 0.01$). Post-hoc tests on the DF1 axis scores with Bonferoni correction showed that in both habitats there was sexual dimorphism. Females differed between the two habitats, but males did not (Fig. 3A). There were no differences in the meristic counts among the groups.

In the lava habitat, 50% of the fish had empty stomachs, compared to 33% in the mud habitat. The mean number of prey items in stomachs of the fish from the mud area (9.5 ± 11.4 SD 2.3 ± 2.15 SD) was higher than in the fish from the lava area (2.3 ± 2.15 SD) ($Z = -2.57$, $P < 0.05$). There was a similar trend in the mean number of diet groups (mud 3.3 ± 2.05 SD, lava 1.6 ± 1.12 SD, $Z = -2.57$, $P < 0.05$). The average proportion of each diet group did not differ between the two habitats (Fig. 4A). The main diets of both these groups were pupae and flies as well as cladocera, and the occurrence of diet in the stomachs and intestines of the fish did not differ between the habitats.

MÝVATN

Sex ratios of the fish were similar in both habitats (31% males in mud, 40% males in lava). The mud fish (mean FL 64.4 mm ± 8.34 SD) were larger than lava fish (mean FL 43.4 mm ± 7.46 SD) ($F_{1,103} = 144.9$, $P < 0.01$). Sexual dimorphism was found in the mud area where males had larger heads than females ($t_{(55)} = 3.86$, $P < 0.05$). Mud males also had larger heads than lava males ($t_{(55)} = 4.40$, $P < 0.05$). The four groups differed in their overall morphology (Wilks $\Lambda = 0.276$, $c^2_{(54)} = 96.5$, $P < 0.05$, Fig. 3B. The model correctly classified all of the lava males, 83% of the lava female, 93% of the mud males and 40% of the mud females. The DF1 explained 73% of the variance and the model was only significant if that axis was included. The variables that were significant between either sex or habitat had the highest loading on the discriminant axis as well as anal fin length, snout length, gill raker length and ventral spine length (Table 1). All of these variables tended to be greater in the mud fish although these differences were not statistically

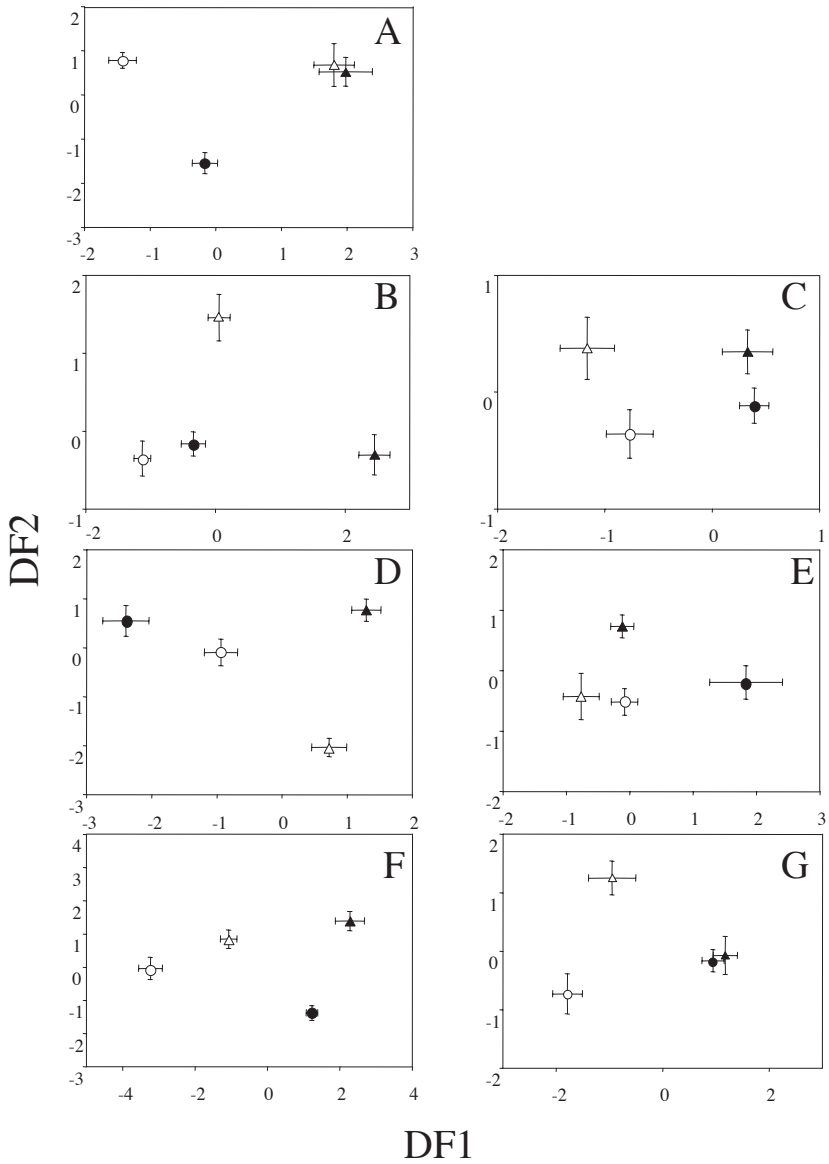

Figure 3. Discriminant scores of morphology and meristic counts of stickleback from two habitats within four lakes in Iceland; HreQavatn; (A) morphology, Mývatn; (B) morphology; (C) meristic; FrostastaQavatn; (D) morphology; (E) meristic; and Thingvallavatn; (F) morphology; (G) meristic. Each graph shows the mean of the four groups with one standard error. The groups are mud males ( ), mud females ( ), lava males and lava females ( ).

significant. The discriminant scores on that axis differentiated between fish from the two habitats (ANOVA, $F_{1,83} = 43.4$, $P < 0.01$) and between the sexes (ANOVA, $F_{1,83} = 67.7$, $P < 0.01$). The interaction of substrate and sex was significant (ANOVA, $F_{1,83} = 11.1$, $P < 0.01$). Post-hoc tests showed sexual dimorphism in both the habitats, and differences in the morphology of each sex between the two areas (Fig. 3B).

The mud fish had higher number of fin rays in the upper half of the caudal fin ($t_{(103)} = 3.27$, $P < 0.05$) than lava fish. This difference was only seen among males, when the sexes were examined independently ($t_{(33)} = 3.60$, $P < 0.05$). The four groups differed in their overall meristic counts (Wilks $\Lambda = 0.653$, $c^2_{(24)} = 41.8$, $P < 0.05$, Fig. 3C. The model correctly classified 75% of the lava males, 44% of the lava female, 39% of the mud males and 46% of the mud females. The DF1 explained 76% of the variance and the model was marginally non-significant when it had been removed (Wilks $\Lambda = 0.894$, $c^2_{(14)} = 11.0$, $P = 0.069$). The discriminant scores on the DF1 axis differed between fish from the two habitats (ANOVA, $F_{1,101} = 34.8$, $P < 0.01$, Fig. 3C). Differences

Table 1. Loading on Discriminant Function Axis 1 from analysis on sticklebacks in HreQavatn (Hre), Mývatn (Myv), FrostastaQarvatn (Fro) and Thingvallavatn (Thin) in Iceland

Variable residuals	Hre	Myv	Fro	Thin
Jaw length	0.304	0.254	0.433	0.061
Anal fin length	0.355	0.373	0.312	-0.277
Dorsal fin length	0.075	0.028	-0.001	-0.099
Snout length	0.267	0.391	0.228	0.243
Eye width	0.019	0.107	0.315	-0.143
Head length	0.244	0.477	0.308	0.046
Top of the eye height	0.095	0.147	0.185	0.087
Body depth	0.027	-0.093	-0.034	-0.147
Caudal peduncle	0.016	0.056	-0.238	0.053
Distance between eyes	0.063	0.149	0.201	-0.197
Dorsal fin ray	0.513	0.277	0.253	0.208
Anal fin ray	0.321	0.210	0.135	0.182
Caudal fin	0.172	0.194	0.060	0.211
Pectoral fin	0.222	0.133	0.186	0.120
Dorsal spine	0.177	0.023	0.057	0.368
Maxilla length	0.394	0.075	0.165	0.341
Gill raker length	0.372	0.346	0.290	-0.004
Ventral spine	0.006	0.412	0.303	0.385

between sexes or the interaction of those two variables were not significant ($P > 0.05$).

Diet was analysed only from fish in the lava area. The mud fish were kept in a refrigerator overnight before being fixed and therefore their diet would have been digested. The diet of the fish in the mud area has been studied previously (GuQmundsson, 1996). We used those data to compare the two habitats. The stomach contents of the mud fish came from 115 fish (20–81 mm) caught in the summer of 1990 (9 June–30 August). Only differences in proportions of the seven diet groups described here were analysed.

Fish from lava were more likely to have empty stomachs than fish from mud (lava, 30%, mud, 10%, $c^2_{(1)} = 7.3$, $P < 0.01$). There were considerable differences in the diet of the two groups (Fig. 4B). The proportions of cladocera ($Z = -5.2$, $P < 0.05$) and ostracoda ($Z = -3.0$, $P < 0.05$) was higher in the mud fish, while the proportion of chironomidae ($Z = -2.6$, $P < 0.05$) was higher in the lava fish.

FROSTASTAÐARVATN

There were differences in the sex ratio between the two habitats. In the lava habitat there were 11 males (34%), while in the mud habitat there were 23 (77%) ($c^2_{(1)} = 11.2$, $P < 0.01$). Sexual dimorphism was observed in the mud habitat, where males had longer maxillae ($t_{(27)} = 3.76$, $P < 0.05$) and larger eyes ($t_{(27)} = 3.55$, $P < 0.05$). Another notable difference was that mud fish had a greater distance between the eyes than did lava fish ($t_{(53)} = -3.49$, $P < 0.05$). The four groups differed in their morphology (DFA, Wilks $\lambda = 0.119$, $c^2_{(54)} = 91.4$, $P < 0.05$, Fig. 3D. The model correctly classified all of the lava males, 78% of the lava female, 81% of the mud males and all of the mud females. The DF1 explained 56% of the variance, when that axis was removed the model became marginally non-significant (DFA, Wilks $\lambda = 0.343$, $c^2_{(34)} = 45.9$, $P = 0.08$). The variables that were significant between either sex or habitat had the highest loading on the discriminant axis as well as jaw length, head length and gillraker length, which all were sexually dimorphic and the length of the anal fin where mud fish had larger fins (Table 1). We examined whether the discriminant scores on DF1 differed among the groups. The discriminant scores differed between the sexes (ANOVA, $F_{1,51} = 80.1$, $P < 0.01$), but not between habitats ($P > 0.05$). The interaction of habitate and sex was, however, significant (ANOVA, $F_{1,51} = 11.5$, $P < 0.01$, Fig. 3D). Post-hoc tests on discriminant scores showed that in both areas there was sexual dimorphism, and that females differed between the habitats. Because of the closeness of the model to significance when DF1 had been removed, we examined discriminant scores on DF2 (which explained 31% of the variance) among the groups. The discriminant scores differed between fish from the two habitats (ANOVA, $F_{1,51} = 33.3$, $P < 0.01$) and between the two sexes (ANOVA, $F_{1,51} = 8.3$, $P < 0.01$). The interaction of substrate and sex was also significant (ANOVA, $F_{1,51} = 13.1$, $P < 0.01$, Fig. 3D). Post-hoc tests on scores on DF2 revealed that the mud fish (male and female) differed only from the lava males. Significant sexual dimorphism was seen in the lava habitat (Fig. 3D).

No differences were seen in meristic counts when looking at individual variables. The groups differed in their overall meristic counts (DFA, Wilks $\lambda = 0.466$, $c^2_{(24)} = 42.0$, $P < 0.05$, Fig. 3E. The model correctly classified 54% of the lava males, 57% of the lava female, 48% of the mud males and 86% of the mud females. The DF1 explains about 57% of the variance and the model is only significant if that axis is included. Caudal fin length, where mud fish had longer fins, had the highest loading on the discriminant axis. The scores on this axis differed between fish from the two habitats (ANOVA, $F_{1,58} = 20.2$, $P < 0.01$) and between the sexes (ANOVA, $F_{1,58} = 21.4$, $P < 0.01$), as well as the interaction of habitat and sex was significant (ANOVA, $F_{1,58} = 4.9$, $P < 0.05$, Fig. 3E). Post-hoc tests on the scores on the DF1 show sexual dimorphism in the mud habitat and that mud females did differ from lava females (Fig. 3E).

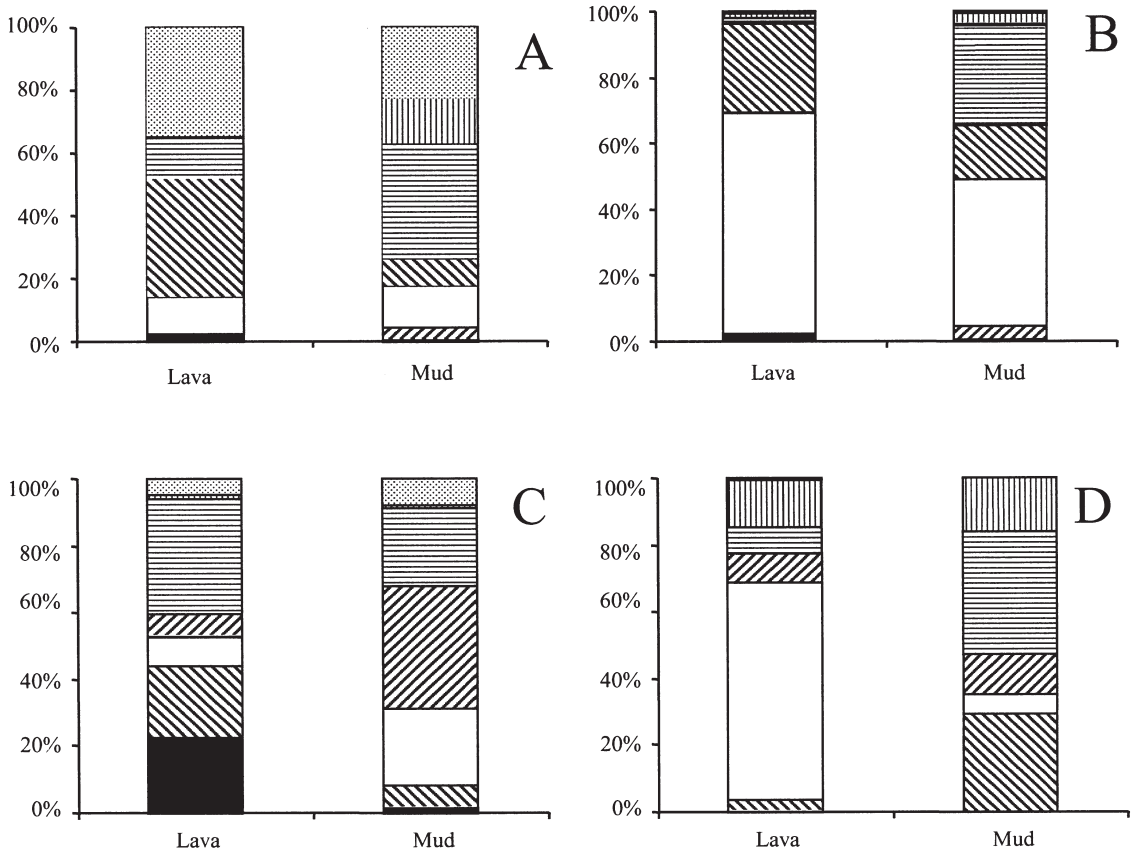

Figure 4. Mean stomach contents of stickleback from mud and lava habitat in four lakes in Iceland: (A) HreQavatn; (B) Mývatn; (C) FrostastaQavatn; and (D) Thingvallavatn. The bars show average proportion of seven diet groups: Other (dotted bars), Copepoda (vertical striped bars), Cladocera (horizontal striped bars), Pupae and flies (▨ diagonal striped bars), Chironomidae (white bars), Ostracoda (▨ diagonal striped bars), Mollusca (black bars).

The number of fish with food in their stomach was similar in both habitats (lava, 78%, mud 77%). Lava fish (25.7 ± 37.82 SD) had more food items in their stomachs than the mud fish (10.8 ± 12.15 SD, Z = -2.57, $P < 0.05$). Lava fish had a higher proportion of mollusca (Z = -3.5, $P < 0.05$) and ostracoda (Z = -3.7, $P < 0.05$) than mud fish (Fig. 4C). Ostracods ($c^2_{(30)}$ = 12.98, $P < 0.05$) and *Lymnea* sp. (Gastropoda) ($c^2_{(30)}$ = 9.64, $P < 0.05$) occurred more often in the intestines of fish from the lava area. Chironomidae (Chironomids) ($c^2_{(30)}$ = 12.92, $P < 0.05$) and orthocladinae (Chironomids) ($c^2_{(30)}$ = 10.46, $P < 0.05$) and sand grains ($c^2_{(30)}$ = 16.26, $P < 0.05$) were more often found in the mud fish.

THINGVALLAVATN

There was no statistical difference in the sex ratio (lava, 45% males, mud, 40% males) of these fish. Considerable differences were found in the morphology of stickleback in Thingvallavatn. In the mud habitat there was a higher proportion (25%) of four spined individuals ($c^2_{(44)}$ = 4,97, $P < 0.05$) than in the lava habitat (0%). Sexual dimorphism was observed in the mud habitat where males had longer snouts than female ($t_{(28)} = 3.65$, $P < 0.05$). The lava fish had shorter dorsal ($t_{(50)} = -5.51$, $P < 0.05$) and pelvic spines ($t_{(50)}$ = -4.64, $P < 0.05$), gill rakers ($t_{(50)} = -4.12$, $P < 0.05$), fin rays in the dorsal fin ($t_{(50)} = -3.47$, $P < 0.05$) and longer anal fin ($t_{(50)} = 4.56$, $P < 0.05$). When the sexes were examined independently, males differed between habitats in the length of the gill rakers ($t_{(20)} = -3.90$, $P < 0.05$) and anal fin ($t_{(20)} = 4.00$, $P < 0.05$), while females differed in the length of the dorsal ($t_{(28)} = -4.66$, $P < 0.05$) and pelvic spines ($t_{(28)} = -3.92$, $P < 0.05$) and in the length of fin ray in the dorsal fin ($t_{(28)} = -3.61$, $P < 0.05$). The four groups differed in their overall morphology (DFA, Wilks 1 = 0.058, $c^2_{(54)} = 114.1$, $P < 0.05$, Fig. 3F). The model correctly classified 90% of the lava males, 92% of the lava female, 83% of the mud males and 94% of the mud females. The DF1 explained 74%

of the variance and the model was only significant when that axis was included. We examined whether the discriminant scores on DF1 differed among the groups. The scores differed between fish from the two habitats (ANOVA, $F_{1,48} = 189.0$, $P < 0.01$), and there was difference in the scores between the sexes (ANOVA, $F_{1,48} = 32.1$, $P < 0.01$), the interaction of habitat and sex was significant (ANOVA, $F_{1,48} = 3.8$, $P = 0.05$, Fig. 3F). Post-hoc tests on scores on DF1 revealed sexual dimorphism in both habitats and that the each sex differed between the habitats (Fig. 3F).

Considerable differences were seen in the meristic counts of the fish from these two habitats. The lava fish had fewer gill rakers on the upper half of the first gill arch ($t_{(50)} = -4.40$, $P < 0.05$) but higher number of fin rays in the ventral half of the caudal fin ($t_{(50)} = 4.17$, $P < 0.05$). When the sexes were examined independently, the lava males had lower numbers of gill rakers ($t_{(20)} = -3.80$, $P < 0.05$), but higher numbers of rays in the anal fin ($t_{(20)} = 3.70$, $P < 0.05$) than mud males, while lava females had higher number of rays in the ventral half of the caudal fin ($t_{(28)} = 3.34$, $P < 0.05$) than mud females. Sexual dimorphism was seen in one characteristic in the lava habitat, where males had more rays in the dorsal part of the caudal fin than females ($t_{(20)} = 3.57$, $P < 0.05$). The four groups differed in their overall meristic counts (DFA, Wilks $\Lambda = 0.058$, $c^2_{(54)} = 114.1$, $P < 0.05$, Fig. 3G). The model correctly classified 90% of the lava males, 92% of the lava females, 83% of the mud males and 94% of the mud females. The DF1 axis explained about 73% of the variance and the model did not remain significant when it was removed. The fish differed in the discriminant scores on DF1 axis between the two habitats (ANOVA, $F_{1,48} = 73.1$, $P < 0.01$, Fig. 3G). The sexes did not differ and the interaction of substrate and sex did not differ as well.

The number of fish with food in their stomachs was similar in both habitats (lava, 100%, mud, 90%). The number of food items or the number of diet species and groups in the stomachs of the fish did not differ (lava; number of items = 15.5, number of species = 3.7, mud; number of items = 12.1, number of species = 3.6). There were significant differences in the proportion of diet groups (Fig. 4D). The lava fish had a higher proportion of chironomids ($Z = -5.6$, $P < 0.05$), while the mud fish had higher proportions of cladocera ($Z = -3.9$, $P < 0.05$) and ostracoda ($Z = -3.2$, $P < 0.05$). The chironomid group orthocladinae occurred in all of the lava fish, but in only 8 of the 30 mud fish ($c^2_{26} = 27.96$, $P < 0.05$). The cladocerans; *Acroperus harpae* ($c^2_{26} = 21.96$, $P < 0.05$), *Bosmina longirostris* ($c^2_{26} = 12.71$, $P < 0.05$), *Graptoleberis testudinaria* ($c^2_{26} = 15.46$, $P < 0.05$) and the copepod group Calanoida sp. ($c^2_{26} = 10.98$, $P < 0.05$) occurred more often in the mud fish than in the lava fish.

Parallel patterns in morphology among the lakes

The allometric slopes of length of fin rays in the dorsal and ventral fin, and caudal fin lengths differed among the lakes. For those variables, fish from all the lakes combined were used to remove size by regression. For all other variables, regressions conducted on fish within individual lakes were used. The residuals from these regressions were used in all further analysis.

The overall morphology of sticklebacks from all four lakes was summarized using principal component analysis. The first principal axis (PC1) explained about 32% of the variance. Variables in the head region had the highest correlation with that axis. The second principal axis (PC2) explained about 13% of the total variance. Gill raker length, the lengths of fin rays in anal and dorsal fins as well as the lengths of the pectoral and caudal fins had high positive correlations with that axis, while height to the top of the eye, eye size, head length and dorsal fin length had a high negative correlation. We conducted 2 ¥ 2 ¥ 4 ANOVA on both PCA axes using habitat, sex and lake as factors. There was no difference between habitats on PC1, but the sexes differed (males PC1, mean = 0.51, SD = 0.90, female, mean = -0.32, SD = 0.93 $F_{3,237} = 50.5$, $P < 0.01$). No interaction was significant, except for the interaction of lake and sex ($F_{3,253} = 3.3$, $P < 0.05$). There were significant differences in the scores on PC2 between fish from the lava and mud habitats (lava PC2, mean = -0.28, SD = 1.14, mud, fish mean = 0.21, SD = 0.82, $F_{3,237} = 10.3$, $P < 0.01$). The sexes also differed ($F_{3,237} = 4.3$, $P < 0.05$), but did not show any interaction. In summary, PC1 separates males and females, with males having most morphometric characteristics larger than the females. PC2 separates lava and mud fish, with lava fish having shorter rays in the dorsal and anal fin, as well as shorter pectoral and caudal fins, but larger heads and eyes.

DISCUSSION

There is great morphological variation in Icelandic stickleback, not only among different lakes, but also within lakes. In each of the four lakes studied, the stickleback show morphological divergence related to different benthic habitats, lava and mud. However, exactly which morphological structures differed and how much divergence was observed varied among the lakes. Sexual dimorphism occurred in all populations, which makes the interpretation of morphological divergence more complicated.

The morphological divergence of stickleback within lakes in relation to habitats supports our first hypothesis. Because of the apparent parallel morphological patterns in different lakes it is likely that this diver-

gence is promoted by natural selection. This supports our second hypothesis. The variability of sticklebacks among lakes may be related to differences in the ecological conditions in each case. It is not easy to generalize which exact ecological characteristics are likely to promote the divergence of the stickleback to the two habitats among lakes. For example, in Mývatn, where populations of sticklebacks are relatively dense (GuQmundsson, 1996), intraspecific competition may promote the divergence. On the other hand, in Thingvallavatn, where the stickleback populations experience high predation pressure from a specialized stickleback eater (piscivorous arctic charr, Sandlund *et al.*, 1992), predation may be the primary ecological factor causing their divergence of the stickleback populations (Doucette, 2001; Kristjánsson, 2001). Lava habitats are variable in complexity, size and groundwater flow among the lakes. This will produce differences in benthic habitats and levels of predation risk and competition among the lakes. In Hredarvatn, where the lava habitat is vegetated and limited in area and the stickleback are likely experiencing little predation pressure, they have not diverged much. In Thingvallavatn, where sticklebacks have likely been experiencing high predation pressure for a long time (Malmquist *et al.*, 1992), and the lava habitat is vast and complex, the stickleback have formed distinct resource morphs. The parallel evolution seen in Icelandic stickleback appears to be less advanced than the adaptations seen in the benthic/limnetic species pairs in British Columbia but this needs further validation (McPhail, 1994; Schluter & Nagel, 1995; Nagel & Schluter, 1998; Rundle *et al.*, 2000).

The fish in Thingvallavatn showed the greatest diversification between habitats and differed in morphology, meristic counts and diet. They represent two resource morphs. The morphological differences observed are well suited for the respective habitat of each morph. The lava fish had shorter spines than the mud fish, which allows them better access to holes and crevices in the lava. The mud fish experiencing higher predation pressure (Malmquist *et al.*, 1992) may also have longer spines because of predator defence. The stickleback also differed in fin structure, which likely reflects different methods of locomotion in the two habitats. The lava fish had shorter gill rakers than the mud fish. This is reflected in the different diet of the two morphs where the lava stickleback seems to be a specialized chironomid feeders while the mud fish are more generalist crustacean eaters.

We do not know whether the diversity of stickleback seen in this study is genetically determined, or based on phenotypic plasticity. Both of those factors can be important in the evolution of resource polymorphism and speciation (Robinson & Wilson, 1994, 1996; Skúlason & Smith, 1995; Schluter, 1996, 1998, 2000; Smith & Skúlason, 1996; Robinson & Schluter, 2000). We predict that in lakes where morphological divergence is small, e.g. Hredarvatn, phenotypic plasticity produces the differences. While in lakes where morphological divergence is advanced and the morphs differ as well in colouration, behaviour and diet, such as in Thingvallavatn, the morphological differences are more genetically based. This has been suggested to be a common trend in northern freshwater fishes (Skúlason *et al.*, 1999). In general, studies suggest this but the phenomenon requires to be examined more thoroughly.

Sexual dimorphism in trophic morphology was observed in all populations in this study. Males often had longer snouts, larger lower jaws and longer gill rakers. Gill rakers are believed to help fish eat small food, such as plankton, by acting as a sieve or to divert water flow within the buccal cavity (Sanderson *et al.*, 1991).

Sexual dimorphism is not uncommon in the threespine stickleback (Wootton, 1984), particularly where males are smaller than females. Studies on the behaviour of sticklebacks in Enos Lake suggested that males are more benthic than females (Bentzen & McPhail, 1984). However, the trophic morphology of the sexes suggests that males are more limnetic than females (Caldecutt & Adams, 1998; Kristjánsson, 2001). In the present study, males have longer head structures, such as jaws and gill rakers than females. These characteristics indicate that males are more limnetic in their morphology than females. In a stream population (Cook Inlet, Alaska, USA), females were more similar to a nearby benthic lake population than to their own males (Caldecutt & Adams, 1998). The cause of sexual dimorphism in trophic morphology of stickleback might be the result of different microhabitat use by the sexes and/or sexual selection. Sexual selection is well known in stickleback and in some cases it has caused reproductive isolation between morphs or species (Blouw & Hagen, 1990; Bakker & Mundwiler, 1994; McPhail, 1994). Usually, females select for male body colour or size (Bakker & Mundwiler, 1994; Nagel & Schluter, 1998; Scott & Foster, 2000). In the case of sexual selection on trophic morphology, it is possible that females select for one or more characteristics in the head morphology of males, and other characteristics, such as gill rakers, also respond as they are genetically or developmentally linked to the original characteristics. This is the subject of our ongoing study focusing on reproductive behaviour and diet.

In conclusion, this study demonstrates that Icelandic threespine sticklebacks are diverse and display important, and previously unknown, evolutionary adaptations to discrete resources. This phenomenon needs to be studied much further.

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