Morphological and trophic divergence of lake and stream minnows (Phoxinus phoxinus)

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Abstract

Phenotypic divergence in response to divergent natural selection between environments is a common phenomenon in species of freshwater fishes. Intraspecific differentiation is often pronounced between individual inhabiting lakes versus stream habitats. The different hydrodynamic regimes in the contrasting habitats may promote a variation of body shape, but this could be intertwined with morphological adaptions to a specific foraging mode. Herein, I studied the divergence pattern of the European minnow (Phoxinus phoxinus), a common freshwater fish that has paid little attention despite its large distribution. In many Scandinavian mountain lakes, they are considered as being invasive and were found to pose threats to the native fish populations due to dietary overlap. Minnows were recently found to show phenotypic adaptions in lake versus stream habitats, but the question remained if this divergence pattern is related to trophic niche partitioning. I therefore studied the patterns of minnow divergence in morphology (i.e. using geometric morphometrics) and trophic niches (i.e. using stomach content analyses) in the lake Ånnsjön and its tributaries to link the changes in body morphology to the feeding on specific resources. Lake minnows showed a strong reliance on zooplankton and a more streamlined body shape with an upward facing snout, whereas stream minnows fed on macroinvertebrates (larvae and adults) to a higher degree and had a deeper body with a snout that was pointed down. Correlations showed a significant positive relationship of the proportion of zooplankton in the gut and morphological features present in the lake minnows. The results of this study highlight the habitat-specific divergence pattern in morphology and resource use in this ubiquitous freshwater fish, which may promote contrasting inter-specific interactions in the respective food webs.

Introduction

Natural selection can evoke adaptive phenotypic divergence of populations (1-3), which can lead to the formation of distinct populations or ecotypes (4, 5), and might even initiate speciation (6). In freshwater fish species, underlying ecological factors responsible for diversifying patterns in populations may include different predation regimes (7, 8), parasite occurrence (9), or different hydrodynamic conditions (10, 11). An environment that is characterized either by running or standing water may trigger divergence in fish populations with regard to morphological, physiological, developmental, or behavioral traits (12). To reduce the drag in the current, stream fishes often have a more streamlined body shape (13), which has been found, e.g. in pumpkinseeds (*Lepomis gibbosus*) or rock bass (*Ambloplites rupestris*) (14). Furthermore, divergence can be based on resource use, often referred to as trophic polymorphism (15, 16). Often, adaptations to different habitat types are intertwined with trophic niche partitioning (17). For example, in three-spined stickleback (*Gasterosteus aculeatus*) (e.g. 18) and juvenile sockeye salmon (*Oncorhynchus nerka*) (19), the lake ecotypes have a more streamlined body, as this morphology can be seen as beneficial to forage at relatively high speed and to cover larger areas in the lake habitat. In contrast, a deeper body generally supports a higher maneuverability in a structurally complex stream habitat when searching for the more cryptic benthic prey (20, 21).

In this study, I investigate the patterns of divergence in the European minnow (*Phoxinus phoxinus*), a

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common freshwater fish that has paid little attention despite its large distribution (22, 23). It is an understudied fish species, despite its profound ecological impact when introduced to new areas, where it can become invasive and has the potential to modify native ecosystems (24-26). Minnows were recently found to show phenotypic adaptions in lake versus stream habitats: Collin and Fumagalli (27) studied minnow populations in Switzerland, and Ramler, Palandacic (28) investigated minnows in Northern Italy and the Danube basin. The studies found opposing results: Collin and Fumagalli (27) found stream minnows being more streamlined, a body form that is beneficial to reduce the drag in the current. In contrast, Ramler, Palandacic (28) reported that a streamlined body form was more pronounced in lake minnows compared to stream minnows and lake minnows also had larger heads compared to stream minnows. This might be due to habitat-induced changes in head structures linked to different modes of foraging, as it is known, for example from European perch (*Perca fluviatilis*) (e.g. 29, 30). However, evidence on trophic niche divergence, incorporating morphological adaptations in minnows inhabiting lake versus stream habitats is missing.

In this study, I have analyzed stomach contents to understand the trophic niches of minnows in the lake Ånnsjön, Central Sweden and its tributaries. This method has the advantage to provide a direct insight into the foraging ecology, proving information on ingested prey with a high taxonomic resolution (31, 32). By combining the resource use assessment with morphological analyses by geometric morphometrics, I aimed to link the changes in body morphology to the individual resource use in the respective habitats. Streams are generally characterized by a lower abundance of zooplankton (e.g. 33). Therefore, I predict that in stream minnows, the dietary contribution of zooplankton would be lower compared to lake minnows. Furthermore, I predict that there is a relationship between morphological distance and dietary preference, indicating a specific morphology when consuming specific prey.

Material and Methods

Sampling and study area

Ånnsjön is located in Central Sweden (63.261212°N, 12.567719°E) at an elevation of 526m (Figure 1). Most of the approximately 57 km^2 lake is relatively shallow (below 2 m deep), but the deepest point is 39.5m (34). Minnows are the most common fish species and the fish community is further composed by brown trout*Salmo trutta* , Arctic charr *Salvelinus alpinus* , lake trout*Salvelinus namaycush* , and brook charr *Salvelinus fontinalis* (34). In Southern Europe, *P. phoxinus* belongs to a species complex (23, 35, 36). However, minnows inhabiting Norway and Sweden belong to one species only (37). In August 2018, minnows were caught from three lake locations (L1, L2, L3; Figure 1) using gill nets (1 m x 10 m with 6 mm mesh size) for up to 12 hours overnight. Furthermore, minnows were collected from three different slow flowing tributaries: downstream Stor Klockbäcken (location S1), downstream Sjöviksbäcken (location S2), and downstream Kvarnbäcken (location S3) (Figure 1). In the streams, minnows were caught using an electrofishing approach and killed with an overdose of benzocaine. Fish were frozen to -20°C and transported to the lab at Uppsala University.

In total, 279 minnows were analyzed, 158 from the lake locations (L1: 52, L2: 52, L3: 54) and 121 in the streams (S1: 50, S2: 50, S3:21). In the lab, fish were thawed and subsequently individual length (to the nearest mm) and weight (to the nearest 0.01 g) were measured. For geometric morphometric analyses, a photograph was taken on the left side of the fish with fins stretched out. The entire gut was collected and kept frozen at -20 °C for subsequent gut content analyses.

Geometric morphometrics

The body morphology of individual minnows caught in lake and stream locations was analyzed using a landmark-based geometric morphometric method (38). Digital lateral photographs were transferred to TPS-dig2 (https://life.bio.sunysb.edu/morph/) and 35 landmarks were determined, including 18 homologous and 17 semi-landmarks based on proportional distances between structures (Figure 2) (39).

Gut content analyses

Gut content was quantified from the entire gut of the minnows using a dissecting microscope. Gut fullness

was estimated (5 categories) and used to weight the volume proportion (equivalent to area proportion at uniform width) of each prey category observed in the sample which was estimated to the nearest 5 %. Food items were classified as a) zooplankton (Cladocera (*Eurycercus* sp., *Bosmina* sp.,*Daphnia* sp., *Alona* sp., *Ceriodaphnia* sp.,*Leptodora* sp., *Chydorus* sp.), and Ostracoda, b) benthic invertebrates (Amphipoda, Chironomidae, Ephemeroptera, Nematoda, Trichoptera, Bivalvia, Gastropoda, Coleoptera, Odonata, Arachnida, Oligochaeta, Polychaeta), c) terrestrial insects (adult Diptera), and d) unidentified items and mucus.

Statistical analyses

For minnow sampling, two different kind of gear was used (i.e. gillnets with one mesh size in the lake locations and electrofishing in the stream locations). To test if total length of minnows differed between habitats (i.e. lake and streams), thus to rule out a potential size-selective effect of sampling gear on my interpretations, I conducted an ANOVA with total length as dependent variable and location nested within habitat as independent variable.

Variation in morphology between the habitats (i.e. lake and stream), and locations was examined using MorphoJ v.1.06d (40). I checked for outliers using the "Find outliers" function. To correct the shape data for body size, I used a regression of the shape scores (Procrustes coordinates) on size (centroid size) for each location separately and the residuals of this regression was used for all further analyses (41). A Discriminant Function analysis (DFA) and a Canonical Variate analysis (CVA) were used to assess significance of shape differences between habitats. A second CVA was conducted for pairwise comparison between the six locations. The shape analysis was restricted to a maximum of 30 individuals of each location.

As minnows crush their food using pharyngeal teeth many individuals solely had unidentified items and mucus in their guts (37.5 % of all minnows caught) and these individuals were excluded from the analyses. Proportional data was arcsine-square root transformed prior to statistical analyses. Ordination of multivariate diet composition was based on Bray-Curtis similarities and analyzed using a PERMANOVA with location nested within habitat, setting location as a random factor and habitat as a fixed factor. The significance of the model was tested with unrestricted permutations (999 permutations) with type III sums of squares. To test whether the contribution of the three diet categories (i.e. zooplankton, benthic invertebrates, and terrestrial insects) differed between the individuals caught in the lake versus streams, I applied Mann-Whitney U-tests. I further conducted Kruskal-Wallis tests with Bonferroni –adjusted Dunn´s pairwise comparisons to analyze if the contribution of the three diet categories differed between the six different locations.

To test the relationship between resource use and minnow body shape, I used Spearman´s rank correlation on the individual proportions of zooplankton in gut content and the first axis of the CVA (CV 1).

PRIMER v 7.0.13 with the PERMANOVA add-on (Primer E Ltd. Plymouth, United Kingdom) was used to analyze the multivariate dataset, whereas univariate analyses were conducted using IBM SPSS v.25 (IBM Corp., Armonk, NY, USA).

Ethical statement

The study was approved by the Umeå Animal Ethic Committee with permit number: A21-2018. The permit for conducting electrofishing was received from Länsstyrelsen Jämtlands län.

Results

Total length of minnows caught in the different locations

Minnows caught in the six different locations varied in total length between 4.9 – 8.2 cm. ANOVA revealed no significant difference between minnow length of the different locations nested within habitats.

Geometric morphometrics

As revealed from DFA, differences in body morphology between minnow caught in the lake versus streams were significant (Mahalonobis distance$D = 5.3026$, $P < 0.001$). Further, DFA classified 90.0 % of all lake individuals and 92.3 % of all stream individuals correctly into the respective group. In general, lake minnows

were characterized by an upward facing snout and body shape was more streamlined, whereas stream minnows showed a snout that was pointed down-ward and the body was bulkier (Figure 3a). The first axis of CVA (CV 1) explained 59.8 % of the variation in the morphospace and along this axis, separation between minnows caught in lake and stream habitats occurred (Figure 3b). CV 2, which explained 18.3 % of the variation, indicated variability in body shape between the minnows caught in the different streams (Figure 3b). Pairwise comparisons of minnow body shape between the locations showed significant differences between lake versus streams, but further also between L1 and L3 in the lake habitat (Table 1). As seen from the ordination of CVA, minnow morphology of individuals caught in L3 were most similar to stream minnows (Figure 3b).

Gut content analyses

Gut content differed significantly between lake and streams (PERMANOVA: Pseudo-F $_{5,278}$: 3.7748, $P =$ 0.039), but further differed significantly between locations nested within habitats (PERMANOVA: Pseudo-F $_{5,278}$: 6.8904, $P = 0.001$). The contribution of zooplankton was significantly higher in lake locations (average 75.4 % $\pm$ 40.6 SD) compared to streams (29.8 % $\pm$ 42.4) (Mann-Whitney U: $Z_1 = $ -4.730, $P < 0.001$, Table 2, Figure 4). In contrast, the contribution of benthic invertebrates was significantly higher in streams (average 51.9 % $\pm$ 46.3 SD) compared to lake locations (average 21.5 % $\pm$ 38.8 SD) (Mann-Whitney U: $Z_1 = $ -5.601, $P < 0.001$, Table 2, Figure 4). Furthermore, the contribution of terrestrial insects was significantly higher in stream locations (average 18.2 % $\pm$ 35.8 SD) compared to lake locations (3.1 % $\pm$ 16.6 SD) (Mann-Whitney U: $Z_1 = $ -3.978, $P < 0.001$, Table 2, Figure 4). Pairwise comparisons of the three diet categories between the locations reflected the overall differences between lake and streams (Table 3). In addition, it showed variation in resource use between locations of the same habitat: minnows caught at location L3 had significantly lower proportions of zooplankton in their guts compared to L2, and L1, respectively (Table 3a, Figure 4). At this location, minnows were characterized by a higher contribution from benthic invertebrates, which explains the non-significant differences between the proportion of benthic invertebrates in L3 and S2, and S3, respectively (Table 3b, Figure 4). Furthermore, the proportion of terrestrial insects was significantly higher in location S3 compared to all other locations (Table 3c, Figure 4).

Relationship between resource use and morphological distance

Along the first axis of CVA (CV 1) more positive CV-values were associated to the lake -bodyshape (Figure 3b). Spearman´s rank correlation showed a significant positive relationship between the dietary contribution of zooplankton and the morphological distances (i.e. values of CV 1) ($r_s = 0.460$, $P < 0.001$, Figure 5).

Discussion:

Minnows caught in Ånnsjön generally ingested more zooplankton, compared to minnows caught in the adjacent streams, which ingested benthic invertebrates and terrestrial insects to a higher degree. Thus, my results are in line with previous findings on minnow diet (22, 42). Furthermore, morphology differed between minnows caught in the lake and streams. This habitat-specific body shape was associated with the use of a specific set of resources, indicating that minnow morphology is an adaption to a specific foraging mode. While the streamlined morphology of lake minnows can be seen as an advantage to swim in a uniform environment, stream minnows were characterized by a downward pointed snout and a deep body that could help feeding on benthic prey while maneuvering in the structurally complex stream habitat (20, 21), where zooplankton is typically scarce (33).

Resource polymorphism will lead to intraspecific divergence within a single population (15, 16), and can be seen as an early stage of speciation (6). As predicted from niche evolution theory, morphologically divergence will reduce competition as less prey items are shared (43, 44), and this pattern could also be demonstrated empirically in the perch-roach system (45). Intraspecific differentiation may initially emerge from phenotypic plasticity (46), and depending on the stability of the selective regime, divergent phenotypes may become genetically fixed (47, 48). Unfortunately, genetic data for the minnows of this study is not available. Future studies to investigate the level of genetic differentiation are needed to characterize the position of the morpho-types of the European minnow in Sweden along the specification continuum that could vary from adaptive variation to complete and reproductive isolation (6, 49). However, results of morphological divergence, but

also resource use showed a strong variation within the habitats, between the different locations. Individuals caught at location S3 were feeding to a greater extent on terrestrial insects, compared to individuals caught in the other two stream locations. Furthermore, individuals caught at location L3 were feeding to a greater extent on benthic invertebrates and ingested fewer zooplankton than individuals caught at L1 and L2 and body shape were more similar to stream minnows. Such variation indicates a strong degree of plasticity in resource-morph formation, which would suggest that differences between minnows of the two adjacent habitats might not be genetically fixed. In contrast to the other lake habitats, water plants were abundant at L3, which could provide suitable microhabitats for benthic invertebrates. To understand the driving forces behind the variation in the degree of divergence, estimates of prey abundances at the different locations are needed.

In contrast to my results that are in accordance with the ones of Ramler, Palandacic (28), Collin and Fumagalli (27) found minnows inhabiting stream habitats in Switzerland to be more streamlined compared to conspecifics living in lakes. However, besides attributing these morphological adaptions to the hydrodynamic conditions occurring in stream habitats, they further reported a high predation pressure present in the lake habitats. A deeper body morphology can be seen as advantageous under such kind of ecological conditions, as muscle mass may enhance a rapid acceleration to escape predators (13, 50, 51). Only very few studies have tried to identify the specific factors that may shape the degree of variation in individual specialization (but see 52, 53, 54). Potentially, predation pressure could influence the strength and direction on the correlation of minnow morphology and diet, but further lab experiments are needed to resolve this relationship.

Scandinavian mountain lakes are characterized by a low species richness of fish. In many of these lakes, that are often remote, minnows were introduced as anglers used them as life bait (26). They are considered as being invasive, due to the fact that they can reach high densities, as for example, in Ånssjön (34). Næstad and Brittain (25) further showed that they have the ability to modify lake food webs, thus being responsible for a zoobenthos assemblage with a dominance of Chironomidae and Oligochaeta, and a low abundance of *Gammarus lacustris* . As lake minnows show a strong diet overlap with juvenile brown trout (24), they are also regarded as one of the factors contributing to the reduced recruitment and growth of the native brown trout in lake habitats (26). My results presented herein indicate that minnows inhabiting stream habitats may rely on different resources than the individuals inhabiting lakes. Therefore, interspecific competition target at different species in these contrasting habitats and patterns observed in lake habitats cannot be directly transferred to the interactions occurring in stream habitats. Nonetheless, the introduction of minnows into stream habitats may also pose a similar threat for the native fish populations of the stream, if a diet overlap would occur. Certainly, future studies need to determine the consequences of minnow invasions on the stream food webs.

Data Accessibility Statement:

The data that support the findings of this study are openly available in DiVA at https://uu.diva-portal.org, reference number: urn:nbn:se:uu:diva-389472.

References:

1. Endler JA. Natural selection in the wild. Princeton, NJ: Princeton University Press; 1986.

2. Schluter D. The ecology of adaptive radiation. Oxford, U.K.: Oxford University Press; 2000.

3. Rundle HD, Nosil P. Ecological speciation. Ecology Letters. 2005;8(3):336-52.

4. Svanbäck R, Bolnick DI. Intraspecific competition affects the strength of individual specialization: an optimal diet theory method. Evolutionary Ecology Research. 2005;7(7):993-1012.

5. Svanbäck R, Bolnick DI. Intraspecific competition drives increased resource use diversity within a natural population. Proc R Soc B-Biol Sci. 2007;274(1611):839-44.

6. Hendry AP. Ecological speciation! Or the lack thereof? Canadian Journal of Fisheries and Aquatic Sciences. 2009;66(8):1383-98.

7. Scharnweber K, Watanabe K, Syväranta J, Wanke T, Monaghan MT, Mehner T. Effects of predation pressure and resource use on morphological divergence in omnivorous prey fish. Bmc Evolutionary Biology. 2013;13:doi: 10.1186/471-2148-13-132.

8. Walsh MR, Reznick DN. Phenotypic diversification across an environmental gradient: a role for predators and resource availability on the evolution of life histories. Evolution. 2009;63(12):3201-13.

9. Karvonen A, Wagner CE, Selz OM, Seehausen O. Divergent parasite infections in sympatric cichlid species in Lake Victoria. Journal of Evolutionary Biology. 2018;31(9):1313-29.

10. Ehlinger TJ, Wilson DS. Complex foraging polymorphism in Bluegill sunfish. Proceedings of the National Academy of Sciences of the United States of America. 1988;85(6):1878-82.

11. Webb PW. Body form, locomotion and foraging in aquatic vertebrates. Am Zool. 1984;24(1):107-20.

12. Walker JA. Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L (Gasterosteidae) body shape. Biological Journal of the Linnean Society. 1997;61(1):3-50.

13. Langerhans RB. Predictability of phenotypic differentiation across flow regimes in fishes. Integrative and Comparative Biology. 2008;48(6):750-68.

14. Brinsmead J, Fox MG. Morphological variation between lake- and stream-dwelling rock bass and pumpkinseed populations. Journal of Fish Biology. 2002;61(6):1619-38.

15. Skúlason S, Smith TB. Resource polymorphism in vertebrates. Trends in Ecology & Evolution. 1995;10(9):366-70.

16. Smith TB, Skúlason S. Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. Annual Review of Ecology and Systematics. 1996;27:111-33.

17. Robinson BW, Wilson DS. Character release and displacement in fishes - a neglected literature. American Naturalist. 1994;144(4):596-627.

18. Hendry AP, Taylor EB, McPhail JD. Adaptive divergence and the balance between selection and gene flow: Lake and stream stickleback in the misty system. Evolution. 2002;56(6):1199-216.

19. Pavey SA, Nielsen JL, Mackas RH, Hamon TR, Breden F. Contrasting ecology shapes juvenile lake-type and riverine sockeye salmon. Transactions of the American Fisheries Society. 2010;139(5):1584-94.

20. Anderson O. Optimal foraging by largemouth bass in structured environments. Ecology. 1984;65(3):851-61.

21. Ehlinger TJ. Learning and individual variation in bluegill foraging - habitat-specific techniques. Animal Behaviour. 1989;38:643-58.

22. Frost WE. The natural history of the minnow, *Phoxinus phoxinus* . Journal of Animal Ecology. 1943;12:139-62.

23. Kottelat M, Freyhof J. Handbook of European freshwater fishes. Cornol, Switzerland: Publications Kottelat; 2007. 646 p.

24. Museth J, Borgstrøm R, Brittain JE. Diet overlap between introduced European minnow (*Phoxinus phoxinus*) and young brown trout (*Salmo trutta*) in the lake, Øvre Heimdalsvatn: a result of abundant resources or forced niche overlap? Hydrobiologia. 2010;642(1):93-100.

25. Næstad F, Brittain JE. Long-term changes in the littoral benthos of a Norwegian subalpine lake following the introduction of the European minnow (*Phoxinus phoxinus*). Hydrobiologia. 2010;642(1):71-9.

26. Museth J, Hesthagen T, Sandlund OT, Thorstad EB, Ugedal O. The history of the minnow *Phoxinus phoxinus* (L.) in Norway: from harmless species to pest. Journal of Fish Biology. 2007;71:184-95.

27. Collin H, Fumagalli L. Evidence for morphological and adaptive genetic divergence between lake and stream habitats in European minnows (*Phoxinus phoxinus*, Cyprinidae). Molecular Ecology. 2011;20(21):4490-502.

28. Ramler D, Palandacic A, Delmastro GB, Wanzenbock J, Ahnelt H. Morphological divergence of lake and stream Phoxinus of Northern Italy and the Danube basin based on geometric morphometric analysis. Ecol Evol. 2017;7(2):572-84.

29. Svanbäck R, Eklöv P. Effects of habitat and food resources on morphology and ontogenetic growth trajectories in perch. Oecologia. 2002;131(1):61-70.

30. Scharnweber K, Strandberg U, Marklund MHK, Eklöv P. Combining resource use assessment techniques reveal trade-offs in trophic specialization of polymorphic perch. Ecosphere. 2016;7(8):e01387. 10.1002/ecs2.1387.

31. Hyslop EJ. Stomach content analysis - a review on methods and their applications. Journal of Fish Biology. 1980;17(4):411-29.

32. Manko P. Stomach content analysis in freshwater fish feeding ecology: University of Prešov; 2016.

33. Chandler DC. Fate of typical lake plankton in streams. Ecological Monographs. 1937;7(4):445-79.

34. Bergwall L, Berglund A. Fiskeundersökningar i Ånnsjön- Effekter av introducerad kanadaröding (*Salvelinus namaycush*) samt resultat av decimeringsfiske och fiskinventeringar 1992-2009. Länsstyrelsen Jämtlands län; 2010.

35. Kottelat M. Three new species of Phoxinus from Greece and southern France (Teleostei : Cyprinidae). Ichthyol Explor Freshw. 2007;18(2):145-62.

36. Geiger MF, Herder F, Monaghan MT, Almada V, Barbieri R, Bariche M, et al. Spatial heterogeneity in the Mediterranean Biodiversity Hotspot affects barcoding accuracy of its freshwater fishes. Molecular Ecology Resources. 2014;14(6):1210-21.

37. Holmen J. The Eurasian minnow: Post-glacial dispersal history and recent invasion patterns in Norway: University of Oslo; 2013.

38. Bookstein FL. Morphometric tools for landmark data. New York: Cambridge University Press; 1991.

39. Mitteroecker P, Gunz P. Advances in Geometric Morphometrics. Evol Biol. 2009;36(2):235-47.

40. Klingenberg CP. MorphoJ: an integrated software package for geometric morphometrics. Molecular Ecology Resources. 2011;11(2):353-7.

41. Klingenberg CP. Size, shape, and form: concepts of allometry in geometric morphometrics. Dev Genes Evol. 2016;226(3):113-37.

42. Michel P, Oberdorff T. Feeding habits of fourteen European freshwater fish species. Cybium. 1995;19:5-46.

43. Bolnick DI, Svanbäck R, Fordyce JA, Yang LH, Davis JM, Hulsey CD, et al. The ecology of individuals: Incidence and implications of individual specialization. American Naturalist. 2003;161(1):1-28.

44. Dieckmann U, Doebeli M. On the origin of species by sympatric speciation. Nature. 1999;400(6742):354-7.

45. Svanbäck R, Eklöv P, Fransson R, Holmgren K. Intraspecific competition drives multiple species resource polymorphism in fish communities. Oikos. 2008;117(1):114-24.

46. Pfennig DW, Wund MA, Snell-Rood EC, Cruickshank T, Schlichting CD, Moczek AP. Phenotypic plasticity's impacts on diversification and speciation. Trends in Ecology & Evolution. 2010;25(8):459-67.

47. Crispo E. Modifying effects of phenotypic plasticity on interactions among natural selection, adaptation and gene flow. Journal of Evolutionary Biology. 2008;21(6):1460-9.

48. Thibert-Plante X, Hendry AP. The consequences of phenotypic plasticity for ecological speciation. Journal of Evolutionary Biology. 2011;24(2):326-42.

49. Nosil P, Harmon LJ, Seehausen O. Ecological explanations for (incomplete) speciation. Trends in Ecology & Evolution. 2009;24(3):145-56.

50. Langerhans RB, Layman CA, Langerhans AK, Dewitt TJ. Habitat-associated morphological divergence in two Neotropical fish species. Biological Journal of the Linnean Society. 2003;80(4):689-98.

51. Walker JA, Ghalambor CK, Griset OL, McKenney D, Reznick DN. Do faster starts increase the probability of evading predators? Functional Ecology. 2005;19(5):808-15.

52. Bartels P, Hirsch PE, Svanbäck R, Eklöv P. Water transparency drives intra-population divergence in Eurasian perch (*Perca fluviatilis*). PLoS One. 2012;7(8).

53. Kristjánsson BK, Leblanc CA. Variation in the magnitude of morphological and dietary differences between individuals among populations of small benthic Arctic charr in relation to ecological factors. Ecol Evol. 2018;8(3):1573-81.

54. Scharnweber K, Strandberg U, Karlsson K, Eklöv P. Decrease of population divergence in Eurasian perch (*Perca fluviatilis*) in browning waters: role of fatty acids and foraging efficiency. PLoS One. 2016;11(9):20.

Tables

Table 1 : Results of Canonical Variate Analyses on pairwise comparison of body shape of minnows caught in the six different locations. Depicted are Procrustes distances (S) among groups and the P -value. Bold font depict significant differences.

		Lake locations	Lake locations	Lake locations	Lake locations	Lake locations	Lake locations	Stream locations	Stream locations	Stream locations	Stream locations	S
		L1	**L1**	**L2**	**L2**	**L3**	**L3**	**S1**	**S1**	**S2**	**S2**	**S**
		S	P	S	P	S	P	S	P	S	P	S
Lake locations	**L1**											
	L2	0.012	0.118									
	L3	**0.018**	**0.003**	0.011	0.220							
Stream locations	**S1**	**0.037**	**<0.001**	**0.031**	**<0.001**	**0.027**	**<0.001**					
	S2	**0.041**	**<0.001**	**0.034**	**<0.001**	**0.028**	**0.003**	0.017	0.073			
	S3	**0.035**	**<0.001**	**0.029**	**0.001**	**0.026**	**0.002**	0.013	0.253	0.019	0.127	

Table 2 : Diet composition of minnows caught in the lake and streams. Depicted are averages and standard deviation (SD) of the percentage of gut volume of each item, or sums of zooplankton, benthic invertebrates and terrestrial insects, including the sample size (N).

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	Lake locations	Lake locations	Lake locations	Lake locations	Lake locations	La
	L1 ($N = 32$) mean	**L1 ($N = 32$)** SD	**L2 ($N = 33$)** mean	**L2 ($N = 33$)** SD	**L3 ($N = 23$)** mean	**L3** SI
Eurycercus sp.	66.3	47.0	71.1	41.1	42.8	48
Bosmina sp.	0	0	0	0	3.3	15
Daphnia sp.	12.5	33.6	3.0	17.4	0	0
Alona sp.	3.2	17.7	0.3	1.7	4.3	20
Ceriodapnia sp.	3.1	17.5	6.1	24.2	0	0
Leptodora sp.	3.1	17.7	0	0	0	0
Chydorus sp.	0	0	0	0	0	0
Ostracoda	0		0	0	0	0
Gammarus	2.2	12.4	1.7	9.6	4.3	20
Chironomidae	3.1	17.7	3.8	12.3	8.3	21
Ephemeroptera	6.3	24.6	0	0	23.5	40
Nematoda	0.2	0.9	0	0	0	0
Trichoptera	0	0	3.2	17.4	0	0
Bivalvia	0	0	0	0	0	0
Gastropoda	0	0	0	0	0	0
Coleoptera	0	0	0	0	0	0
Odonata	0	0	0	0	0	0
Arachnida	0	0	0	0	0.4	2.1
Oligochaeta	0	0	0	0	8.7	28
Polychaeta	0.2	0.9	5.7	22.9	0	0
Diptera adult	0	0	5.2	20.9	4.3	20
Σ zooplankton	88.1	31.4	80.5	34.8	50.4	49
Σ benthic invertebrates	11.9	31.4	14.4	30.4	45.3	49
Σ terrestrial insects	0	0	5.2	20.9	4.3	20

Table 3 : Results of Kruskal-Wallis tests on pairwise comparison of volumetric proportion of a) zooplankton, b) benthic invertebrates, and c) terrestrial insects in the gut of minnows caught in the six different locations. Depicted are the results of the overall test, and adjusted P -value (Dunn´s correction) for pairwise comparisons of locations. Bold font depict significant differences.

		Lake locations	Lake locations	Lake locations	Stream locations	Stream locations	Stream locations
		L1	**L2**	**L3**	**S1**	**S2**	**S3**
zooplankton: **H5 = 42.679, P < 0.001**	**zooplankton:** **H5 = 42.679, P < 0.001**	**zooplankton:** **H5 = 42.679, P < 0.001**	**zooplankton:** **H5 = 42.679, P < 0.001**	**zooplankton:** **H5 = 42.679, P < 0.001**			
Lake locations	**L1**						
	L2	1.000					
	L3	**0.009**	**0.003**				
Stream locations	**S1**	**0.021**	**0.008**	1.000			
	S2	**0.005**	**0.002**	1.000	1.000		
	S3	**< 0.001**	**< 0.001**	1.000	0.613	1.000	

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		Lake locations	Lake locations	Lake locations	Stream locations	Stream locations	Stream locations
benthic invertebrates: $H_5 = 38.102$, $P < 0.001$	benthic invertebrates: $H_5 = 38.102$, $P < 0.001$	benthic invertebrates: $H_5 = 38.102$, $P < 0.001$	benthic invertebrates: $H_5 = 38.102$, $P < 0.001$	benthic invertebrates: $H_5 = 38.102$, $P < 0.001$			
Lake locations	L1						
	L2	1.000					
	L3	0.577	1.000				
Stream locations	S1	**0.004**	**0.042**	1.000			
	S2	**< 0.001**	**0.001**	0.480	1.000		
	S3	**< 0.001**	**0.003**	0.390	1.000	1.000	
terrestrial insects: $H_5 = 33.018$, $P < 0.001$	terrestrial insects: $H_5 = 33.018$, $P < 0.001$	terrestrial insects: $H_5 = 33.018$, $P < 0.001$	terrestrial insects: $H_5 = 33.018$, $P < 0.001$	terrestrial insects: $H_5 = 33.018$, $P < 0.001$			
Lake locations	L1						
	L2	1.000					
	L3	1.000	1.000				
Stream locations	S1	0.724	1.000	1.000			
	S2	0.884	1.000	1.000	1.000		
	S3	**< 0.001**	**< 0.001**	**< 0.001**	**0.002**	**0.004**	

Figure legends:

Figure 1 : Map of Ånnsjön and its position in Sweden including the locations where minnows where caught in the lake (L1, L2, L3) and the surrounding tributaries (S1, S2, S3). © Landmäteriet.

Figure 2: Position of the 35 digitized landmarks used in geometric morphometrics. Homologous landmarks (red dots): 1-4 : most posterior, dorsal, anterior, and ventral point of orbit; 5: insertion of pectoral fin; 6-8: most posterior, dorsal, and ventral margin of opercular (principal opercular bone); 9: tip of the snout; 12: dorsal transition head to body; 16: anterior insertion of dorsal fin; 17: posterior insertion of dorsal fin; 21: dorsal insertion of caudal fin; 22: ventral insertion of caudal fin; 26: posterior insertion of anal fin, 27: anterior insertion of anal fin; 30: anterior insertion of pelvic fin; 33: ventral transition head to body. Semi landmarks (blue dots) were based on proportional distances between homologous structures.

Figure 3: Results of geometric morphometrics. a) Shape differences between minnows caught in the lake (blue line) and the streams (green line). Shape-change outlines of Discriminant Function Analyses are magnified threefold. b) Ordination of shape based on Canonical Variate Analyses of minnows caught in the six different locations with confidence ellipses (probability 0.9) drawn for lake and stream habitats respectively.

Figure 4: Average proportions of gut content of minnows caught in the lake (L1, L2, L3) and the streams (S1, S2, S3).

Figure 5 : Relationship between proportion of zooplankton in the minnow guts and morphological distances (canonical variate 1), including results of Spearman's rank correlation.