

Ecological character displacement among *Nothobranchius* annual killifishes in Tanzania

David O. Alila^{1,2,3, ID}, Hanna ten Brink^{2,4, ID}, Marcel Haesler^{1,2}, Ole Seehausen^{1,2, ID}

¹Department of Fish Ecology and Evolution, Centre of Ecology, Evolution, and Biogeochemistry, EAWAG Swiss Federal Institute of Aquatic Science and Technology, Kastanienbaum, Switzerland

²Division of Aquatic Ecology and Evolution, Institute of Ecology & Evolution, University of Bern, Bern, Switzerland

³Department of Biological Sciences, Mkwawa University College of Education, University of Dar es salaam, Iringa, Tanzania

⁴Department of Fish Ecology, Royal Netherlands Institute for Sea Research (NIOZ), Texel, The Netherlands

Corresponding author: Department of Fish Ecology and Evolution, EAWAG Swiss Federal Institute of Aquatic Science and Technology, Seestrasse 79, CH-6047 Kastanienbaum, Switzerland. Email: aliladavy@yahoo.co.uk

Abstract

Divergent ecological character displacement (ECD) is the competition-driven divergence in resource use-related phenotypic traits between coexisting species. It is considered one of the primary drivers of ecological diversification and adaptive radiation. We analyzed phenotypic and ecological variation in 2 African annual killifish species of the genus *Nothobranchius*: *N. eggersi* and *N. melanospilus* in sympatry and *N. melanospilus* in allopatry. Our aim was to test whether allopatric and sympatric populations of *N. melanospilus* differ morphologically from each other and from *N. eggersi* and examine whether these differences are consistent with the predictions of ECD. We find that sympatric *N. melanospilus* differ from allopatric *N. melanospilus* and differ from *N. eggersi* more strongly than the latter. Our data satisfy four criteria for demonstrating ECD: Differences in phenotypes between allopatric and sympatric *N. melanospilus* are greater than expected by chance; the divergence pattern between allopatric and sympatric *N. melanospilus* results from an evolutionary shift rather than from ecological sorting; morphological differences observed reflect differences in resource use; and, lastly, sites of allopatry and sympatry do not differ in food resource availability or other ecological conditions. Our results suggest that competition is the main driver of the observed divergence between two *N. melanospilus* populations.

Keywords: ecological character displacement, killifish, coexistence, interspecific competition, resource polymorphism, Tanzania

Introduction

Knowledge of the ecological forces causing population divergence and species diversification in resource use are central topics in evolutionary ecology and key to understanding speciation, adaptive radiation, and community assembly (Gray et al., 2005; Komine et al., 2019; Pfennig & Pfennig, 2010). Competition between species for resources is one such force with potentially large impacts on ecological diversification, community assembly, and community diversity. The “competitive exclusion principle” (CEP) states that two species with identical niches that compete for a single resource cannot coexist in the same space indefinitely (Gause, 1934; Hardin, 1960). However, one possible evolutionary outcome of resource competition is divergent ecological character displacement (ECD): Heritable divergence in resource-related phenotypic traits between coexisting species due to frequency-dependent outcomes of competition for a shared resource (Brown & Wilson, 1956; Dayan & Simberloff, 2005; Grant, 1972; Schluter & McPhail, 1992). This mechanism leads to exaggerated trait differences among species living in sympatry compared to where either species is found alone (i.e., in allopatry). This divergence in traits will reduce competition for shared resources and introduce negative frequency dependence into the trait-dependent interaction dynamic,

potentially stabilizing coexistence. Schluter and McPhail (1992) predicted that this process should lead to well-separated trophic niches of species when in sympatry.

Despite the importance of ECD, there are only a few convincing examples of ECD. Schluter and McPhail (1992) outlined six criteria that must be tested and met in order to qualify a case as likely ECD (Schluter, 2000; Schluter & McPhail, 1992). The few examples that meet all, or most, of these criteria include Darwin’s finches (Brown & Wilson, 1956; Grant & Grant, 2006), sticklebacks in lakes in coastal British Columbia (Gray & Robinson, 2002; Schluter & McPhail, 1992), American spadefoot toads (Pfennig & Pfennig, 2010), and *Anolis* lizards (Stuart & Losos, 2013).

Here, we assess whether competition between annual killifish species in the genus *Nothobranchius*, subgenus *Adinia*, may have led to ECD between sympatric species sharing small ephemeral habitats in Eastern Africa. *Nothobranchius* is a genus of small, annual freshwater fish in the family Nothobranchiidae, and part of the order Cyprinodontiformes (Blažek et al., 2013; Reichard et al., 2022; Wildekamp, 2004). Their distribution and occurrence are limited to the subtropical and tropical parts of central, Eastern, and South-eastern Africa that are subject to pronounced seasonality in rainfall. All *Nothobranchius* habitats receive seasonal rainfall (Blažek

Received May 7, 2023; revisions received December 22, 2023; accepted January 15, 2024

Associate Editor: Scott Taylor; Handling Editor: Miriam Zelditch

© The Author(s) 2024. Published by Oxford University Press on behalf of The Society for the Study of Evolution (SSE).

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

et al., 2013; De Wet van der Merwe et al., 2020; M. Reichard et al., 2022), but they vary greatly in terms of size, patterns of inundation, and connectivity (Bartáková et al., 2020; Nagy & Watters, 2021).

The coastal region of Tanzania harbors more than 20 endemic species of *Nothobranchius* and has the highest incidence of sympatry observed anywhere in the distribution range of the genus (Reichard et al., 2022; Terzibasi et al., 2009; Watters et al., 2019). This raises the question of how so many closely related species can coexist in the region. Interestingly, there is also a lot more ecomorphological variation among species in this region than anywhere else in the wide distribution of the genus. This includes a wide range of body sizes and multiple feeding specialists, such as surface and open water feeding fish, piscivores, as well as the widespread benthic generalists (Nagy & Watters, 2021). Given the extensive sympatry of closely related species, combined with larger than elsewhere ecomorphological variation, we hypothesize a role for ECD among sympatric species. *Nothobranchius* are confined to ephemeral water bodies where few if any other fish compete with them or prey on them. The resulting patchy distributions that all *Nothobranchius* species have, across numerous small waterbodies with different local species richness and community compositions, make this system especially suitable to explore the role of ECD among populations of closely related species in facilitating ecomorphological diversification.

The main objective of our study was to examine if allopatric and sympatric populations of *N. melanospilus* displayed morphological differences and then test if these differences were consistent with the predictions of the ECD hypothesis. We focused on the most widespread and most common species in the coastal lowlands, *Nothobranchius* (*Adinia*) *melanospilus* and its interaction with the next most widespread and common species in the region in the same subgenus, *N. (A.) eggersi*. Morphologically, *N. melanospilus* is overall larger (known max length: 60-mm SL male) than *N. eggersi* (known max length: 39-mm SL male) (Watters, 2009; Wildekamp, 2004).

Nothobranchius melanospilus has a wider and more continuous geographical distribution range than *N. eggersi*, the range of which is geographically more narrowly confined and the distribution more patchy within the range (Bartáková et al., 2020; Nagy & Kis, 2010; Nagy & Watters, 2021).

In the spring of 2019, we undertook a field study to determine resource use, prey availability, and morphological traits of *N. melanospilus* and *N. eggersi* living in either allopatry or sympatry. Of the six ECD criteria summarized in Schluter and McPhail (1992), our data allowed us to test four criteria (numbers refer to the numbering in Schluter and McPhail, 1992): (a) the observed pattern of exaggerated phenotypic difference in sympatry is not due to chance; (b) the divergence pattern between sympatric species results from an evolutionary shift (in situ displacement in trait values) rather than ecological sorting; (c) observed morphological differences must reflect differences in resource use; and (d) sites of allopatry and sympatry should not differ significantly in available food resources or other ecological conditions, which may affect the phenotype.

Materials and methods

Study area and species

Between June 19 and 23, 2019, we sampled 44 ponds in the Tanzanian lowlands and found 13 ponds with

N. melanospilus only, 11 ponds having both species but no other species, 20 ponds in which both coexisted with one or more additional *Nothobranchius* species and not a single pond in which *N. eggersi* occurred alone. For this study, we focus on 5 ponds with *N. melanospilus* alone and 5 ponds with both *N. melanospilus* and *N. eggersi* present, but no other killifish species. Due to constraints on time and resources, we could not analyze all the 24 ponds with *N. melanospilus* and/or *N. eggersi* and therefore focused on 10 of them. We chose these 10 ponds based on their proximity from each other, so as to minimize variation in abiotic factors that could otherwise confound our results.

Sample collection

Samples of the two *Nothobranchius* species were collected from temporary pools in the Ruvu River Basin (5°S–7°S and 36°E–39°E) in Eastern Tanzania (Figure 1). The two species are listed as species of Least Concern by the IUCN and are endemic to Tanzania (Watters et al., 2020). To avoid the influence of seasonal variation, all fish and invertebrate samples were collected within a period of five consecutive days (June 19–23, 2019). All 10 ephemeral ponds that we chose have relatively similar sizes (66–120 m²). Population samples of *N. melanospilus* and *N. eggersi* were sampled using a seine net (depth 0.7 m, length 2.7 m, and its stretched mesh-size 4 mm) and a triangular dip-net (450 mm × 450 mm and its mesh-size 5 mm). The mesh-size was sufficiently fine to retain the smallest adult female *Nothobranchius*. (Frost et al., 1971; Watters, 2009). All collected fishes were identified to species level in the field. In total, we collected 384 adult individual fishes as sample specimens from the 10 sites, five ponds with *N. melanospilus* only (allopatric *melanospilus* from now onwards) and five with the two species in sympatry (sympatric *melanospilus* and sympatric *eggersi* from now onwards). These individuals were euthanized in clove oil and then fixed in 70% ethanol (Gray & Robinson, 2002). Sample specimens were shipped to the Institute of Ecology and Evolution; at the University of Bern. Field sampling, export, and import procedures followed the regulations of both the Tanzania and Swiss governments. Research permits, export, and import licenses were issued by the responsible authorities (research permit: DAARS/R/1/37; export license SL195/2019; Import license: 2407/19).

In the laboratory, fish were weighed by total mass to the nearest 0.01 g using an electronic balance. Standard length (SL) was measured with a digital caliper (to the nearest 0.01 mm) (Laufer et al., 2009; Önsöy et al., 2010). For stomach content analysis, we took a random sample of 10 individuals per species (5 of each sex), resulting in 10 (allopatric sites) or 20 (sympatric sites) individuals per sampling site, and a total of 150 individuals. The digestive tracts were taken out from the fishes. Prey items from the stomach were identified to order or, wherever possible, to the family level. A small number of prey items that were highly digested were excluded from the analysis due to identification difficulties. References for zooplankton and other aquatic invertebrates were consulted for identification (Marrero & Lopez-Rojas, 1995; Motta & Uieda, 2004; Ruppert & Barnes, 1994).

Three morphological traits considered to be relevant for foraging efficiency on different prey types (Bouton et al., 1997; Gray & Robinson, 2002; Olivier et al., 2019) were measured on the left side of each individual fish: In addition to SL, we measured lower jaw length (JL) and eye diameter

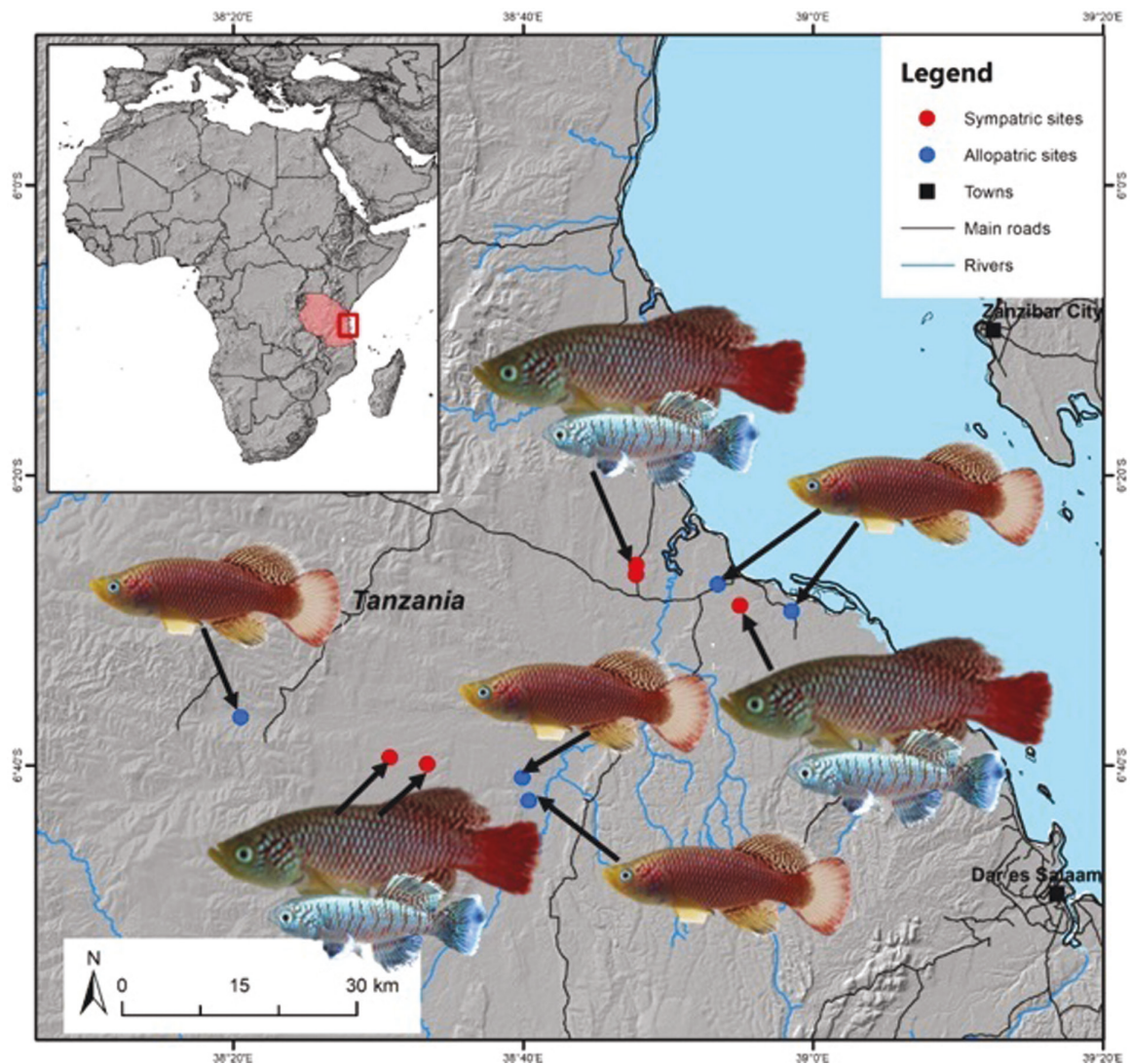


Figure 1. Map of the sampling area (Ruvo River Basin), located in Eastern Tanzania showing the allopatric sampling sites (*N. melanospilus*) and sympatric sampling sites (*N. melanospilus* and *N. eggersi*).

(ED) using a digital caliper (to the nearest 0.01 mm) (Costa, 2018, 2019; Schluter & McPhail, 1992).

A sample of aquatic invertebrates was collected at each sampling site at the time of fish sampling. We used a rectangular hand-net (20 cm × 15 cm, 500 µm mesh-size) for sampling both pelagic and benthic invertebrates (Meintjes, 1996; Žák et al., 2019). A total of 12 hauls (6 benthic and 6 pelagic) were completed at each site. The entire sampled contents were fixed in 70% ethanol. In the laboratory, all sampled invertebrates were carefully picked up from the organic debris, classified into their respective taxonomic groups, and counted (Meintjes, 1996). During the invertebrate identification process, observers were unaware of the sympatric/allopatric status of the ponds. Based on the total number of invertebrate individuals in each sample taken at a site, the proportional contribution of each taxonomic group to the invertebrate sample, and by inference, the assemblage was calculated for each site (Ruppert & Barnes, 1994).

Hypothesis testing

We assessed the evidence for ECD in our study system by testing four of the six criteria for ECD suggested by Arthur

(1982) and synthesized by Schluter and McPhail (1992). In this study, we were not able to test whether the phenotypic differences between the allopatric and sympatric populations have a genetic basis (criterion 2). Neither could we provide independent evidence for resource competition among individuals with similar phenotypes with our field data (criterion 6). These two criteria will need to be addressed experimentally in the future. Below, we describe how we tested the other four criteria for ECD. In the following, we summarize the predictions that each criterion makes and the tests we used to examine support for each. We prepared all graphs and statistical tests with R version 4.2.2 (R Core, 2020).

Criterion 1: The observed pattern of exaggerated character differences in sympatry is not due to chance

We visualized phenotypic character variation within and differences between the three different types of fish populations (*N. melanospilus* in allopatry, *N. melanospilus* in sympatry, and *N. eggersi*) using split histograms. We used nonparametric Kruskal–Wallis tests to assess whether the trait values of SL, JL, and ED differ between the three population groups.

Post hoc comparisons were performed using the Dunn's test to determine which population groups differed significantly. We predicted that trait values for SL, JL, and ED of *N. melanospilus* populations in sympatry would be significantly greater than those of allopatric populations. This expectation is based on the premise that an increase in these trait differences would enhance the contrast with the putative competitor, *N. eggersi*.

To test the null hypothesis that the trait overlap (in SL) between *N. eggersi* and sympatric *N. melanospilus* is the same as the trait overlap between *N. eggersi* and allopatric *N. melanospilus*, we used a randomization test. First, we calculated the difference between the mean values of the SL of *N. eggersi* and sympatric *N. melanospilus* for each of the five sympatric population sites. Next, we obtained a hypothetical difference in mean SL between *N. eggersi* and allopatric *N. melanospilus* by randomly sampling 30 individuals from all allopatric *N. melanospilus*, and calculating the mean SL of this sample. We then calculated the difference between the mean SL from each *N. eggersi* site and the random allopatric *N. melanospilus* sample, resulting in five values. We repeated this process 10,000 times to generate a null distribution of hypothetical mean SL differences. Finally, we compared the observed mean SL difference between *N. eggersi* and sympatric *N. melanospilus* with the null distribution of mean SL differences between *N. eggersi* and allopatric *N. melanospilus* to determine the probability of observing a similar or larger trait difference in the null distribution. If the result was less than 5%, we rejected the null hypothesis that the trait overlap between *N. eggersi* and sympatric *N. melanospilus* is the same as the trait overlap between *N. eggersi* and allopatric *N. melanospilus*.

To explore morphological differences other than size, we performed linear regression using log-transformed JL or ED with log-transformed SL, including the interaction between SL and fish group. Pairwise comparisons were then made on the slopes of the JL–SL and ED–SL relationships to assess variation in allometric scaling across the three groups. In addition, the estimated means of these traits were compared between groups at mean SL.

Criterion 3: The differences between allopatric and sympatric *N. melanospilus* are the outcome of an evolutionary shift (in situ displacement of mean trait values)

Ecological sorting through biased colonization or extinction of populations predicts that the trait means observed in sympatry are contained within the range of trait means that can also be observed in allopatry. An evolutionary shift, on the other hand, predicts that trait means observed in sympatry lie outside the range of means observed in allopatry (Grant, 1972; Gray & Robinson, 2002; Losos, 2000; Pfennig & Pfennig, 2010; Schluter & McPhail, 1992). This is because enhanced differences between sympatric species should not be the outcome simply of the inability of similar populations to coexist. We used frequency distribution plots to visualize trends of the trait mean values in allopatry and sympatry.

Criterion 4: Morphological differences reflect differences in resource use

Based on this criterion, we predicted that, relative to the sympatric *N. eggersi*, sympatric *N. melanospilus* would have greater differences in their diet compared with allopatric *N. melanospilus*. Because *N. eggersi* is the smaller species, we

can also predict the direction of the shift: sympatric *N. melanospilus* should feed on larger dietary items than allopatric populations do and the differences with *N. eggersi* should be larger still.

The stomach contents of a subsample of 150 fishes were taxonomically identified to the level of the order. Prey organisms were assigned to one of three major functional prey categories: Pelagic Soft-Bodied Crustaceans (SBC) (Copepods and Cladocerans); Epiphytic and benthic Hard-Bodied Crustaceans (HBC) (Ostracods and Conchostraca); and insect larvae, INL (Coleoptera, Odonata, Ephemeroptera, Hymenoptera, Hemiptera, Culicidae, and Chironomidae.) (Ruppert & Barnes, 1994). The total number of prey items in each category was counted. We then visually compared the diet between three different types of fish populations; *N. melanospilus* in allopatry, *N. melanospilus* in sympatry, and *N. eggersi* using box and whisker plots. We used PCA to visualize dietary niche separation among the three killifish types. The PCA was constructed using the absolute values of individual prey types found in the stomach of each fish. Furthermore, we used a nonparametric Kruskal–Wallis test followed by Dunn's post hoc test to assess the statistical significance of dietary variation among the three types of killifish. To account for the potential influence of size differences on total prey intake, we used proportions rather than absolute values in our statistical analysis.

Criterion 5: Sites of allopatry and sympatry do not differ significantly in food resource availability nor other abiotic ecological conditions affecting the phenotype

To test the assumption that allopatric and sympatric sites do not differ in the dietary resources available to killifish, we quantified and compared the invertebrate samples obtained by hand netting in the benthic and open water zones. We used nonparametric Mann–Whitney *U* test to test the null hypothesis of no differences in the three functional invertebrate categories between allopatric and sympatric ponds. Furthermore, we also used the nonparametric Mann–Whitney *U* test to assess the variation in the ecosystem size variables (pond size and depth) and physicochemical variables (pH, conductivity, and temperature). All samples were collected within a short period of time (5 days) to ensure that any temporal effects of sampling day were minimized.

Results

Criterion 1—Chance

Chance is unlikely to be the main cause of the observed pattern of exaggerated trait differences in sympatry. SL, uncorrected JL, and uncorrected eye size were all significantly larger in sympatric *N. melanospilus* compared to allopatric *N. melanospilus* and *N. eggersi* [Kruskal–Wallis test (SL: $H(2) = 220.4$, $p < .001$; JL: $H(2) = 163.6$, $p < .001$; ED: $H(2) = 131.6$, $p < .001$)], (Figures 2 and 3; Supplementary Figure 1). Dunn's post hoc test was then used to determine which of these groups differed significantly from each other. For all three traits, the post hoc test showed that sympatric *N. melanospilus* had significantly larger values compared to both *N. eggersi* and allopatric *N. melanospilus* (SL: $p < .001$; JL: $p < .001$; ED: $p < .001$, see Supplementary Table 1). Furthermore, randomization test showed that the mean body size (SL) overlap between *N. eggersi* and sympatric

N. melanospilus is significantly smaller ($p < .001$) than that between *N. eggersi* and allopatric *N. melanospilus* (see, e.g., [Supplementary Figure S1](#)).

To understand the interplay among JL, ED, and body size (SL) across our three killifish types, we performed size corrections for JL and ED. The pairwise comparisons between the slopes of regression lines for JL against body size (SL) did not reveal any significant differences between the three killifish types, including allopatric *N. melanospilus* vs. sympatric *N. melanospilus* ($p = .43$), allopatric *N. melanospilus* vs. sympatric *N. eggersi* ($p = .63$), and sympatric *N. melanospilus* vs. *N. eggersi* ($p = .87$). Additionally, the regression of ED against SL did not consistently show any differences between the slopes of regression lines for allopatric *N. melanospilus* vs. sympatric *N. melanospilus* ($p = .4$), allopatric *N. melanospilus* vs. *N. eggersi* ($p = .27$), and sympatric *N. melanospilus* vs. *N. eggersi* ($p = .96$) ([Supplementary Table 3: Supplementary Figure 2 & 3](#)).

With the aim of comprehensively examining the variation in each morphological trait (SL, JL, and ED) among the three killifish types, we conducted an analysis of differences in estimated marginal means (EMM). The pairwise comparisons of the EMM of JL at the mean SL value for each of the three types of populations showed that the JL of allopatric *N. melanospilus* was significantly different from that of sympatric *N. melanospilus* ($p = .001$), with allopatric *N. melanospilus* having a higher estimated marginal mean ([Supplementary Table 3](#)). There was marginally significant difference between the JL of *N. eggersi* and allopatric *N. melanospilus* ($p = .07$) and no significant difference between the JL of *N. eggersi* and sympatric *N. melanospilus* ($p = .64$).

The pairwise comparisons of EMM showed that the ED of allopatric *N. melanospilus* was significantly different from that of sympatric *N. melanospilus* ($p = .04$), with allopatric *N. melanospilus* having a higher estimated marginal mean at the mean SL. There was no significant difference between the ED of *N. eggersi* and either allopatric *N. melanospilus* ($p = .92$) or sympatric *N. melanospilus* ($p = .21$) at the mean SL.

Criterion 3—Evolutionary shift

The frequency distribution plots show clear trends in trait values between sympatric *N. eggersi*, allopatric *N. melanospilus*, and sympatric *N. melanospilus* ([Figure 3](#)). The result reveals that in all three traits *N. eggersi* are smaller followed by allopatric *N. melanospilus* and sympatric *N. melanospilus*. Only *N. melanospilus* from one sympatric site are exceptionally small, but even here *N. melanospilus* are significantly larger than the cooccurring population of *N. eggersi*.

SL and JL observed in populations of *N. melanospilus* that are sympatric with *N. eggersi* are not only shifted to significantly larger means, but they exceed the range observed in all allopatric populations combined by a large margin. While the largest fish in sympatric populations measured over 67 mm SL, and the fish with the longest jaws measured 5.7-mm JL, none of the fish in allopatric populations exceeded 55 mm SL and 4.6 mm JL ([Figures 2 and 3](#)). This implies that ecological sorting of trait variation that exists in allopatry and hence likely predates the establishment of sympatry is unlikely to explain the exaggerated divergence in trait mean values between *N. melanospilus* and *N. eggersi* in sympatry.

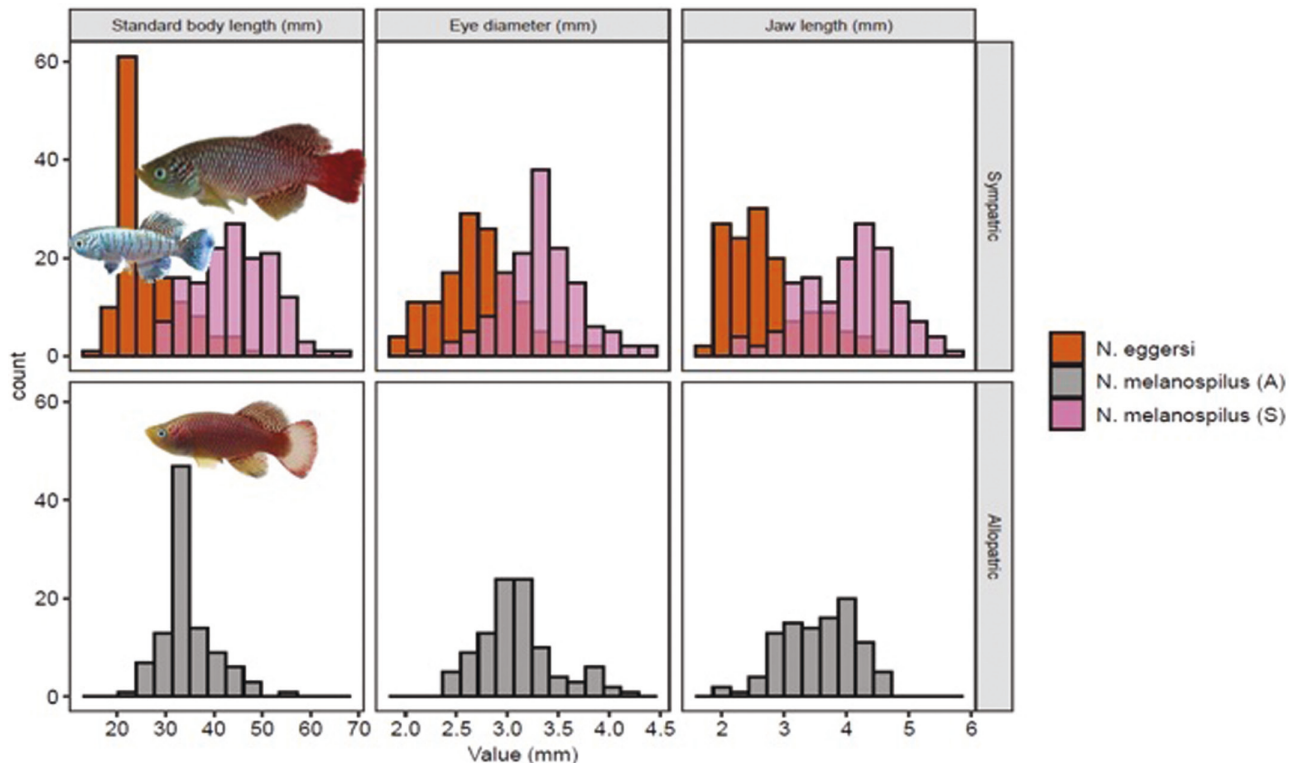


Figure 2. Frequency distributions illustrate the difference in standard length (SL) (left panels), eye diameter (ED) (middle panels), and jaw length (JL) (right panels) between sympatric (upper panel) and allopatric sites (lower panel).

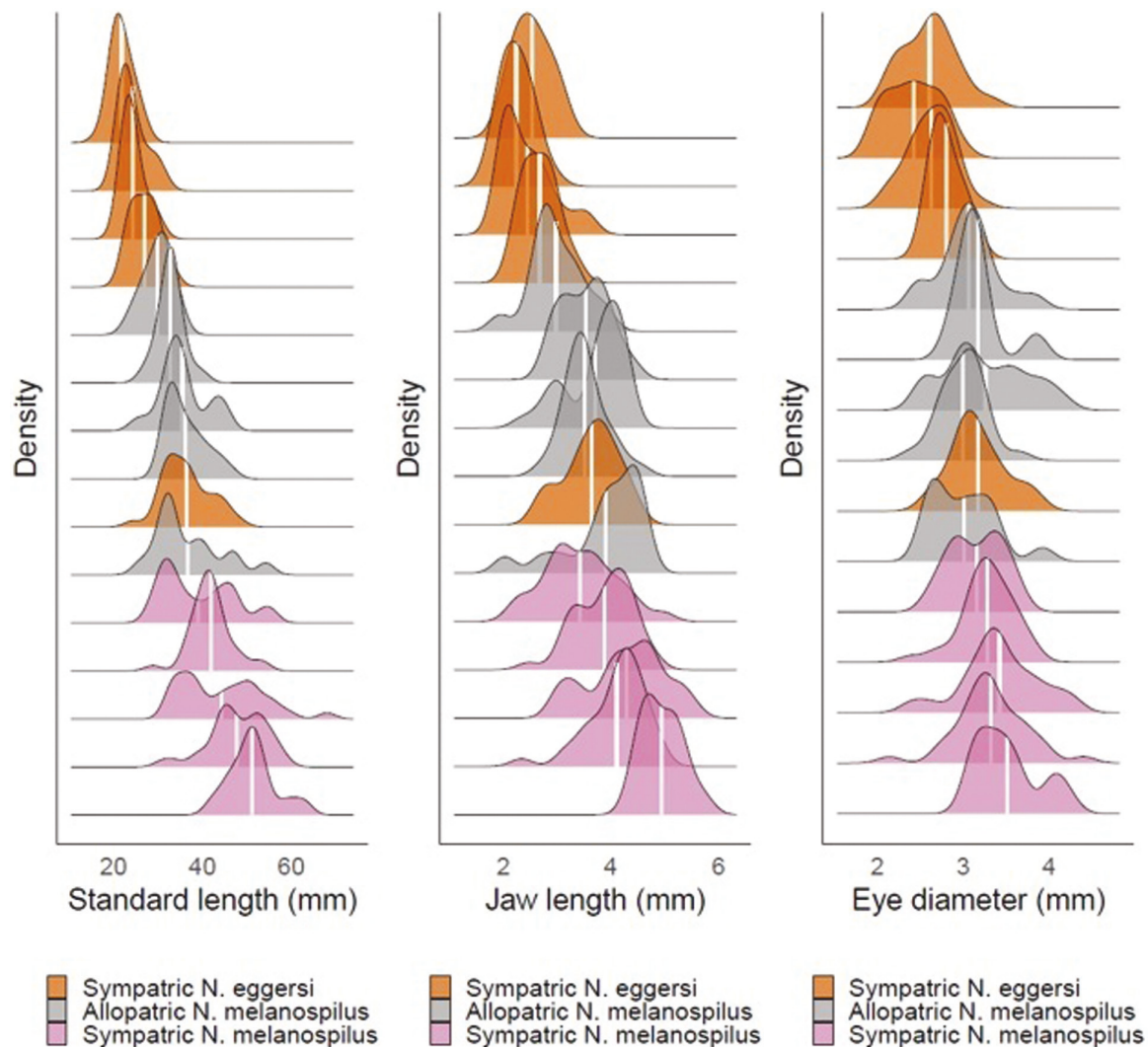


Figure 3. Frequency distribution of trait values in standard length (SL) (left panels), jaw length (JL) (middle panels), and eye diameter (ED) (right panels) for sympatric *N. eggersi*, allopatric *N. melanospilus* and sympatric *N. melanospilus* sampled in 10 sites. The white bars in each distribution indicate the mean value of the population. The sites are ordered based on ascending mean SL values.

Criterion 4—Resource use

Stomach content analysis showed that both species of *Nothobranchius* had diets based on aquatic crustaceans (Copepoda, Cladocera, Conchostraca, and Ostracoda) and insect larvae (INL) (Coleoptera, Odonata, Ephemeroptera, Hymenoptera, Hemiptera, Culicidae, and Chironomidae) (Figure 3). We found significant dietary niche separation between allopatric *N. melanospilus*, sympatric *N. melanospilus*, and *N. eggersi* (Figures 4 and 5).

The Kruskal–Wallis test results revealed the statistical significance of differences among the three types of populations for all the three diet types: SBC, HBC, and INL (SBC: [H2] = 67.96, $p < .001$; HBC: [H2] = 104.66, $p < .001$; INL: [H2] = 88.06, $p < .001$). The results of the post hoc Dunn's test showed significant differences between each all three pairwise contrasts of population types for all three diet types ($p < .001$ for all 9 comparisons).

The diet of sympatric *N. melanospilus* was found to have shifted towards a higher proportion of HBC (mean values for SBC = 6, HBC = 12.3, INL = 10) compared to *N. eggersi* and allopatric *N. melanospilus* (Figures 4 and 5).

The major dietary component of the sympatric competitor *N. eggersi* consisted of SBC (mean values for SBC = 14.4, HBC = 2.54, INL = 1.76). In contrast, allopatric *N. melanospilus* in all five single-species ponds were found to be generalists, feeding on both HBC and SBC in relatively equal proportions (mean values for SBC = 10.4, HBC = 8.24, INL = 6.84). *Nothobranchius melanospilus* from sympatric populations consumed larger and harder-bodied prey compared to both *N. eggersi* and *N. melanospilus* from allopatric populations. Therefore, the observed morphological differences between allopatric and sympatric populations reflect clear differences in resource use.

Criterion 5—Ecological conditions

The Mann–Whitney *U* test results indicate that there were no significant differences in the densities of any of the three invertebrate groups (SBC, HBC, and INL) between the allopatric and sympatric sites (SBC: $W = 3.5$, $p = .07$; HBC: $W = 13.5$, $p = .92$; INL: $W = 7.5$, $p = .34$, see Supplementary Table 2).

All five single-species ponds and all five species pair ponds are located in the same river basin and have similar microclimatic

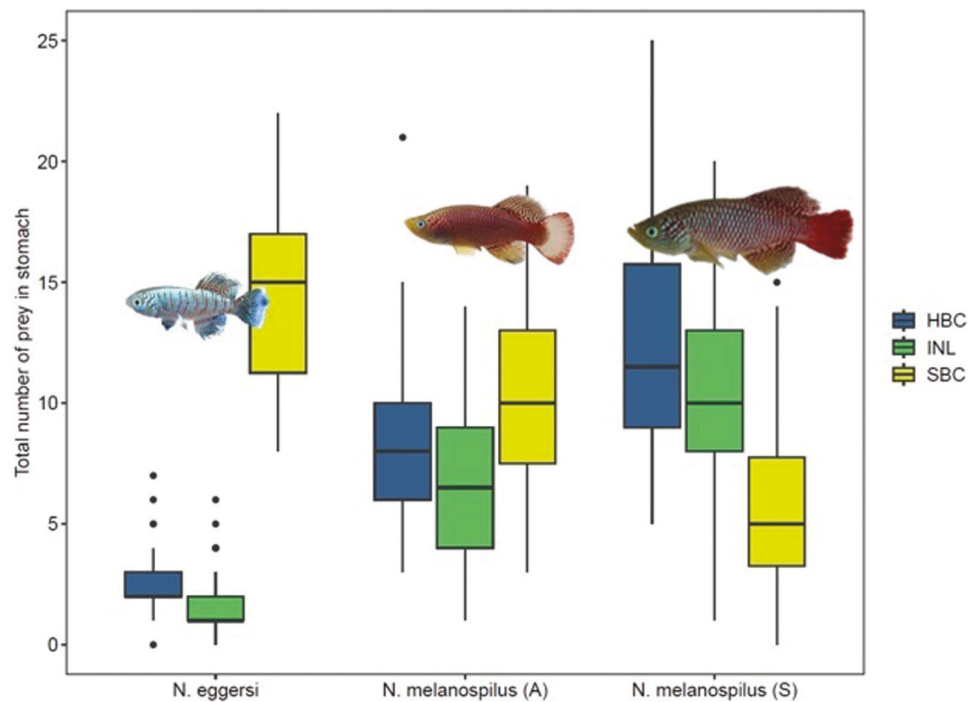


Figure 4. Stomach contents of *N. eggersi* in sympatry, *N. melanospilus* in allopatry, and *N. melanospilus* in sympatry. Box plots displays the median (horizontal line), interquartile range (box), and whiskers (1.5 times the interquartile range) to show the range of variation. Outliers are plotted individually as black points. The prey items are grouped into three major functionally different categories: hard-bodied crustaceans (HBC), insect larvae (INL), and soft-bodied crustaceans (SBC).

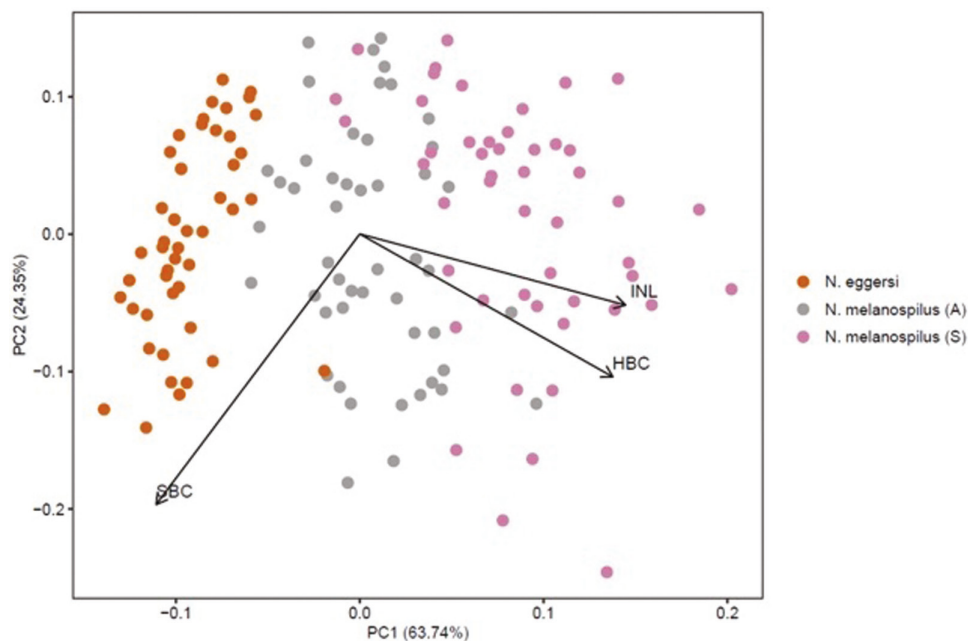


Figure 5. Principal component analysis of killifish prey (five allopatric and five sympatric sites pooled by species), consistent with ecological character displacement in sympatric *N. melanospilus*: *N. melanospilus* in allopatry has a diet intermediate between that of *N. melanospilus* in sympatry and *N. eggersi* in sympatry: soft-bodied crustaceans (SBC), hard-bodied crustaceans (HBC), and insect larvae (INL).

conditions. Mann–Whitney U test results reveal no significant differences between single-species and species pair ponds in terms of ecosystem size variables (pond size: $W = 15$, $p = .69$, and depth: $W = 11.5$, $p = .88$), nor in other physicochemical variables (pH: $W = 12$, $p = .94$, conductivity: $W = 15.5$,

$p = .59$, and temperature: $W = 9.5$, $p = .6$, see [Supplementary Table 4](#)). Therefore, environmental factors other than the presence or absence of a competitor seem unlikely to account for the observed differences between allopatric and sympatric *N. melanospilus*.

Discussion

ECD has been fully demonstrated in only a few examples. Most studies of ECD succeeded to demonstrate only some of the six ECD criteria (Arthur, 1982; Grant, 1972; Schluter & McPhail, 1992), with the first (chance ruled out) and fourth criteria (shift in trait matches the shift in ecology) having been demonstrated more often than the others (Stuart & Losos, 2013). The results of our study satisfy four out of the six widely accepted criteria for ECD, while the remaining two require additional investigation with common garden and quantitative genetics experiments. The support that we showed here for four of the criteria suggests a considerable possibility of the occurrence of ECD between closely related annual killifish species.

In support of the first ECD criterion, stating that an observed divergence pattern does not arise by chance, we show that all three morphological traits, (SL, JL, and ED) were significantly different among sympatric *N. melanospilus*, allopatric *N. melanospilus*, and *N. eggersi*. Subsequently, Dunn's post hoc analysis consistently revealed that sympatric *N. melanospilus* exhibited significantly larger values in all three traits when compared to both *N. eggersi* and allopatric *N. melanospilus*. This makes chance a very unlikely explanation for the shifts in body size between *N. melanospilus* that do cooccur with *N. eggersi* and those that do not.

The results for size correction of JL and ED suggest that body size (SL), rather than JL or ED, may have been the trait that experienced ECD, and it is the main trait distinguishing sympatric and allopatric *N. melanospilus*.

Nothobranchius grow extremely fast (Dorn et al., 2014; Polačik & Reichard, 2010), and hence, size differences between populations could be due to different hatching or sampling dates. However, hatching is determined by the onset of the seasonal rainfall. All of our ponds are in close proximity to one another and experience synchronous start of the rainy season. Furthermore, it is expected that fish in sympatric and allopatric ponds hatch at the same time (Blažek et al., 2013). We avoided confounding time effects by samplings populations in random order within a timespan of five days. The observed trend of larger body size (SL) in sympatric *N. melanospilus*, in contrast to allopatric *N. melanospilus*, is consistently replicated across all five sampled ponds. Our results could also be caused due to phenotypic plasticity. Therefore, a common garden study is needed to further elucidate the underlying mechanisms and provide a more comprehensive understanding of the observed patterns.

In support of the third criterion of ECD, the divergence pattern between sympatric species likely results from an evolutionary shift (in situ displacement in trait values) rather than ecological sorting. We find no overlap in the ranges of population mean SL between allopatric and sympatric sites, that is, all five sympatric populations have mean SLs larger than the mean of the largest allopatric population. This implies that the pattern of exaggerated size differences between the species in sympatry cannot result from sorting of *N. melanospilus* populations by body size.

In support of the fourth criterion of ECD, observed morphological differences must reflect differences in resource use, stomach content analyses showed dietary niche separation between the three groups. Freshwater crustaceans form a large percentage of *Nothobranchius* diet, followed by insect larvae. In terms of volume, insect larvae are in general much

larger than crustaceans. Our data show that in allopatry, *N. melanospilus* feeds to roughly equal parts on both pelagic SBC and benthic HBC, while in sympatry with *N. eggersi*, *N. melanospilus* shifts its feeding niche to mainly Ostracods and Conchostraca (epiphytic and benthic hard-bodied crustaceans) and insect larvae. On the other hand, *N. eggersi* in sympatry mainly feeds on soft-bodied pelagic crustaceans (Copepods and Cladocerans). This is consistent with dietary character displacement permitting these phenotypically divergent species to coexist.

Importantly, we find that the differences in morphology between the populations in our *Nothobranchius* spp. are closely linked to resource use. The hard-bodied epiphytic and benthic prey were mainly consumed by the large-bodied population group (*N. melanospilus* in sympatry with *N. eggersi*). The larger *N. melanospilus* exploit a larger proportion of hard-bodied epiphytic and benthic Crustaceans (Ostracods and Conchostraca) and only a small proportion of pelagic and SBC (Copepods and Cladocerans) while the small *N. eggersi* predominantly feeds on SBC.

The *N. melanospilus* in populations living in sympatry with *N. eggersi* are bigger and thus have longer lower jaws. Even though, after size correction, their jaws are not longer than those of fish in allopatric populations, larger body and jaw size are likely responsible for an increased ability to consume and crush large hard-bodied prey. Thus, specialization on larger prey items may be enabled mainly by an increase in body size. Stomach content analysis of three other sympatric species of *Nothobranchius* in southern Africa has previously revealed that smaller species (*N. rachovii* and *N. furzeri*) feed on all types of crustaceans whereas the larger *N. orthonotus* is specialized in insect larvae and tadpoles (Blažek et al., 2013; Polačik & Reichard, 2010). This observation is also supported by other studies that have demonstrated a general correlation between fish-biting force and JL (Gray et al., 2005; Van Wassenbergh et al., 2007).

Finally, in support of ECD criterion 5, we demonstrate that sites of allopatry and sympatry do not differ significantly in available food resources or other ecological conditions which may otherwise affect killifish phenotypes. Different prey taxa sampled (crustaceans and insect larvae) were found in similar densities in all ponds. Because all sites are located in the same river basin with similar microclimatic conditions and same seasonality and they were all sampled within a short period of time (5 days) the similarity in abiotic facts is not surprising. This criterion is an important consideration when addressing the ECD concept (Gray & Robinson, 2002; Schluter & McPhail, 1992; Stuart & Losos, 2013).

Our results show that the key factor differentiating the allopatric and sympatric ponds is the respective absence and presence of *N. eggersi*. Hence, our findings suggest that competition for food resources with *N. eggersi* may be responsible for the pattern suggestive of ECD within *N. melanospilus*. As one important ecological process, competition can provide key explanations for the evolutionary origins of morphological diversity, through mediating dynamics within populations, evolutionary change, and change of community structure (De Lisle et al., 2018; Gray et al., 2005; Pfennig & Pfennig, 2010; Reifová et al., 2011).

Similarly, in another classical ECD study, Grant and Grant (2006) identified a compelling case of food niche separation. In sympatry, *Geospiza fortis* exhibited a specialization in

consuming smaller seeds, which was attributed to competition with *G. magnirostris*. This competition led to a reduction in beak size and shape within the population of the medium ground finch, *G. fortis*, on Daphne Major Island. Furthermore, the salamander species *Plethodon cinereus* and *Plethodon hoffmani* showed divergent snout-vent lengths when living in sympatry, while they were similar in size when living allopatry (Losos, 2000). We have identified a significant separation in the food niche between sympatric and allopatric *N. melanospilus* populations. This pattern of niche differentiation along the feeding axis is a recurring observation in studies investigating ECD.

In another ECD study by Gray and Robinson (2002) and Gray et al. (2005), a consistent pattern of replicated morphological character shift in sympatry was observed. This study focused on brook sticklebacks (*Culaea inconstans*) in sympatry with ninespine sticklebacks (*Pungitius pungitius*) across a series of lakes in Canada, revealing strong evidence for ECD (4 of 6 criteria met). The findings of this study indicated that brook sticklebacks in sympatry had undergone significant morphological changes compared to their allopatric counterparts. Specifically, the sympatric brook sticklebacks exhibited a more benthic body form characterized by a larger head, subterminal mouth, and deeper body. Additionally, they possessed fewer and shorter gill rakers in comparison to allopatric brook stickleback populations. Several other studies on ECD show food niche separation when two species compete for limited food resources (Anderson & Weir, 2021; Dufour et al., 2017; Dunham et al., 1979; Grant & Grant, 2018; Gray et al., 2005; Komine et al., 2019; Losos, 2000; Pfennig & Murphy, 2003; Reifová et al., 2011; Rice & Pfennig, 2010; Schluter & McPhail, 1992).

A total of 144 ECD case studies reported in Stuart and Losos (2013) satisfy at least one of the six ECD criteria summarized in Schluter and McPhail (1992). Among these, only a few studies were considered strong candidates for ECD (satisfying more than three criteria). These include Darwin's finches which fulfilled all six criteria, American spadefoot toads meeting five of six criteria, Anole lizards, satisfying five of six criteria and three-spined sticklebacks which met four out of the six criteria. (Dayan & Simberloff, 2005; Decru et al., 2017; Grant & Grant, 2006; Gray et al., 2005; Martin & Pfennig, 2011; Schluter & McPhail, 1992; Stuart & Losos, 2013; Wilson & Brown, 1956). We suggest that our study qualifies for adding African killifish to this short list of strong candidates for ECD, with 4 of 6 criteria fulfilled.

We can not yet investigate the remaining two of the six criteria for ECD (Schluter & McPhail, 1992). First, testing the evidence of interspecific competition between our two *Nothobranchius* species requires experimental manipulations in the field or in laboratory enclosures (Gray et al., 2005; Pfennig & Murphy, 2003; Pfennig & Pfennig, 2010; Schluter, 2000). We have recently conducted a large outdoor enclosure experiment that we will use to address this question in the near future. Moreover, to satisfy the final ECD criterion, a heritable basis for morphological differences between the allopatric and sympatric populations, growth rate differences between the lab-bred *N. melanospilus* from allopatric and sympatric populations should be evaluated in a common garden environment without a competitor (Gray et al., 2005; Grudemo & Johannesson, 1999; Losos, 2000). This aspect can be addressed in future studies. Testing to satisfy all six ECD criteria in a single study remains a challenging task, as it

demands the integration of fieldwork, large mesocosm experiments, and a variety of methods across the fields of ecology, evolutionary biology, and genetics. (Losos, 2000; Schluter, 2000; Stuart et al., 2017).

An alternative explanation for the observed differences in the body size and diet of *N. melanospilus* in the presence and absence of *N. eggersi* might be due to differences in the strength of competition experienced during ontogenetic development. Many species, including fish, use similar resources when small but diverge in niche when larger (Nunn et al., 2012; Werner & Gilliam, 1984). Since *Nothobranchius* spp hatch around the same time (Blažek et al., 2013), it is likely that sympatric species compete strongly for similar (small) food items early in life. This may result in high mortality rates (Terzibasi et al., 2009) and subsequently little recruitment to larger size classes.

To test this alternative explanation, one approach would be to design an experiment that manipulates the strength of both intra- and inter-specific competition, for example, by varying the density of the two species. By measuring survival and growth rates, as well as the diets of individuals, it might be possible to determine the direct effect of competition on the development and diet of *N. melanospilus*.

In our study, we investigated the ecological divergence between populations of *N. melanospilus* with and without sympatric *N. eggersi* because we did not find ponds with only *N. eggersi*. The cooccurrence of *N. eggersi* with *N. melanospilus*, rather than *N. eggersi* occurring alone, could be attributed to a complex interplay of ecological and evolutionary factors. One plausible explanation is the existence of a mutualistic or facilitative relationship between the two species, where *N. melanospilus* creates conditions favorable for *N. eggersi*, contributing to their coexistence (Krebs, 2014). Habitat preferences might also influence this dynamic, with *N. eggersi* showing preferences either for a subset of the conditions preferred by *N. melanospilus* or preferences aligned with the conditions created by *N. melanospilus*. Considering that *N. melanospilus* is more common compared to *N. eggersi*, it is plausible that *N. eggersi* occasionally exists in allopatry but that we did not find such ponds. Finally, historical factors shaped by shared evolutionary processes or historical events may also contribute to the observed patterns of cooccurrence between these species (Krebs, 2014; Molles, 2010; Reichard et al., 2022).

Conclusion

Morphological divergence and the shift in resource use in sympatric *N. melanospilus* may be explained by ECD between *N. melanospilus* and its congeneric species, *N. eggersi*, when they occur in the same pond. In our study, we focused on the ecological divergence between populations of *N. melanospilus* with and without sympatric *N. eggersi* since we did not find ponds with *N. eggersi* alone. Our results show a new strong case for ECD. Future studies should also examine if *N. eggersi* can be found in ponds without any other *Nothobranchius* species and if morphological divergence and resource use shifts between allopatric and sympatric populations occur in *N. eggersi* too.

Supplementary material

Supplementary material is available online at *Evolution*.

Data availability

We have archived the data on Dryad. Dryad identifier link (DOI): doi:10.5061/dryad.j9kd51ck7.

Author contributions

David O. Alila (Conceptualization—[Equal], Collection of field Data—[Lead], Data Curation—[Lead], Methodology—[Lead], Formal analysis—[Lead], Writing original manuscript draft—[Lead], Writing, review & editing—[Equal]), Hanna ten Brink (Conceptualization—[Equal], Data Curation—[Equal], Formal Analysis—[Equal], Methodology—[Equal], Writing, review & editing—[Equal]), Marcel Haesler (Project administration—[Equal], Conceptualization—[Equal], Data Curation—[Equal], Methodology—[Equal], Writing, review & editing—[Equal]), and Ole Seehausen (Funding acquisition—[Lead], Project administration—[Lead], Conceptualization—[Lead], Supervision—[Lead], Data Curation—[Equal], Methodology—[Lead], Formal analysis—[Equal], Writing—review & editing—[Equal]).

Funding

David Alila was supported by the EAWAG- Swiss Federal Institute of Aquatic Science and Technology through Ole Seehausen, the University of Bern through Ole Seehausen, and the Swiss Government Excellence Scholarship grant.

Conflict of interest: The authors declare no conflict of interest.

Acknowledgments

The authors express their gratitude to Martin Reichard for providing training in the field. Additionally, the authors thank Luiz Jardim De Queiroz and Edson Ishengoma for their valuable comments on the manuscript and the FishEc department at EAWAG for fruitful discussions regarding the article.

References

- Anderson, S. A. S., & Weir, J. T. (2021). Character displacement drives trait divergence in a continental fauna. *Proceedings of the National Academy of Sciences of the United States of America*, 118(20), e2021209118. <https://doi.org/10.1073/pnas.2021209118>
- Arthur, W. (1982). The evolutionary consequences of interspecific competition. *Advances in Ecological Research*, 12(C), 127–187.
- Bartáková, V., Nagy, B., Polačik, M., Blažek, R., Lamtane, H., & Reichard, M. (2020). Genetic diversity of a widespread annual killifish from coastal Tanzania. *BMC Evolutionary Biology*, 20(1), 1–13. <https://doi.org/10.1186/s12862-019-1549-2>
- Blažek, R., Polačik, M., & Reichard, M. (2013). Rapid growth, early maturation and short generation time in African annual fishes. *EvoDevo*, 4(1), 24. <https://doi.org/10.1186/2041-9139-4-24>
- Bouton, N., Seehausen, O., & Alphen, J. J. M. (1997). Resource partitioning among rock-dwelling haplochromines (Pisces: Cichlidae). *Ecology of Freshwater Fish*, 6, 225–240.
- Brown, W. L., & Wilson, E. O. (1956). Character displacement. *Current Biology*, 30(18), R1023–R1024.
- Costa, W. J. E. M. (2018). Comparative morphology, phylogeny and classification of African seasonal killifishes of the tribe Nothobranchiini (Cyprinodontiformes: Aplocheilidae). *Zoological Journal of the Linnean Society*, 184(1), 115–135. <https://doi.org/10.1093/zoolinnean/zlx102>
- Costa, W. J. E. M. (2019). Two new species of seasonal killifishes of the *Nothobranchius melanospilus* species complex from the East Africa biodiversity hotspot (Cyprinodontiformes: Aplocheilidae). *Vertebrate Zoology*, 69(1), 73–82.
- Dayan, T., & Simberloff, D. (2005). Ecological and community-wide character displacement: The next generation. *Ecology Letters*, 8(8), 875–894.
- De Lisle, S. P., Paiva, S., & Rowe, L. (2018). Habitat partitioning during character displacement between the sexes. *Biology Letters*, 14(6), 20180124. <https://doi.org/10.1098/rsbl.2018.0124>
- De Wet van der Merwe, P., Cotterill F. P. D., Kandziora, M., Watters, B. R., Nagy, B., Genade, T., Flügel, T. J., Svendsen, D. S., & Bellstedt, D. U. (2020). Genomic fingerprints of Palaeogeographic history: The tempo and mode of Rift tectonics across tropical Africa has shaped the diversification of the killifish genus *Nothobranchius* (Teleostei: Cyprinodontiformes). *Molecular Phylogenetics and Evolution*, 158, 106988.
- Decru, E., Vreven, E., Danadu, C., Walanga, A., Mambo, T., & Snoeks, J. (2017). Ichthyofauna of the itimbiri, aruwimi, and lindi/tshopo rivers (Congo basin): Diversity and distribution patterns. *Acta Ichthyol Piscat*, 47(3), 225–247. <https://doi.org/10.3750/aiep/02085>
- Dorn, A., Musilová, Z., Platzler, M., Reichwald, K., & Cellerino, A. (2014). The strange case of East African annual fishes: Aridification correlates with diversification for a savannah aquatic group? *BMC Evolutionary Biology*, 14(1), 1–13.
- Dufour, C. M. S., Herrel, A., & Losos, J. B. (2017). Ecological character displacement between a native and an introduced species: The invasion of *Anolis cristatellus* in Dominica. *Biological Journal of the Linnean Society*, 123(1), 43–54. <https://doi.org/10.1093/biolinnean/blx116>
- Dunham, A. E., Smith, G. R., & Taylor, J. N. (1979). Evidence for ecological character displacement in Western American Catostomid fishes. *Evolution*, 33(3), 877–896. <https://doi.org/10.1111/j.1558-5646.1979.tb04742.x>
- Frost, S., Huni, A., & Kershaw, W. E. (1971). Evaluation of a kicking technique for sampling stream bottom fauna. *Canadian Journal of Zoology*, 49(2), 167–173. <https://doi.org/10.1139/z71-026>
- Gause, G. F. (1934). *The struggle for existence*. The Struggle for Existence, Williams and Wilkins Company.
- Grant & Grant. (2018). Competition and character displacement. In *40 years of evolution*. Princeton Univ. Press (pp. 122–137).
- Grant, P. (1972). Convergent and divergent character displacement. *Biological Journal of the Linnean Society*, 4(1), 39–68.
- Grant, P., & Grant, B. R. (2006). Evolution of character displacement in Darwin's finches. *Science*, 313(5784), 224–226.
- Gray, S. M., & Robinson, B. W. (2002). Experimental evidence that competition between stickleback species favours adaptive character divergence. *Ecology Letters*, 5(2), 264–272. <https://doi.org/10.1046/j.1461-0248.2002.00313.x>
- Gray, S. M., Robinson, B. W., & Parsons, K. J. (2005). Testing alternative explanations of character shifts against ecological character displacement in brook sticklebacks (*Culaea inconstans*) that coexist with ninespine sticklebacks (*Pungitius pungitius*). *Oecologia*, 146(1), 25–35. <https://doi.org/10.1007/s00442-005-0184-3>
- Grudemo, J., & Johansson, K. (1999). Size of mudsnails, *Hydrobia ulvae* (Pennant) and *H. ventrosa* (Montagu), in allopatry and sympatry: Conclusions from field distributions and laboratory growth experiments. *Journal of Experimental Marine Biology and Ecology*, 239(2), 167–181. [https://doi.org/10.1016/s0022-0981\(98\)00198-1](https://doi.org/10.1016/s0022-0981(98)00198-1)
- Hardin, G. (1960). The competitive exclusion principle. *Science*, 131(3409), 1292–1297. <https://doi.org/10.1126/science.131.3409.1292>
- Komine, H., Watari, Y., & Kaji, K. (2019). Ecological character displacement in non-congeneric frogs. *Zoological Science*, 36(5), 410–416. <https://doi.org/10.2108/zs190037>
- Krebs, C. J. (2014). Ecology: Pearson new international edition: The experimental analysis of distribution and abundance. *Bios*, 56(4), 646.
- Laufer, G., Arim, M., Loureiro, M., Piñeiro-Guerra, J. M., Clavijo-Baquet, S., & Fagúndez, C. (2009). Diet of four annual killifishes: An intra and interspecific comparison.

- Neotropical Ichthyology*, 7(1), 77–86. <https://doi.org/10.1590/s1679-62252009000100010>
- Losos, J. B. (2000). Ecological character displacement and the study of adaptation. *Proceedings of the National Academy of Sciences of the United States of America*, 97(11), 5693–5695. <https://doi.org/10.1073/pnas.97.11.5693>
- Marrero, C., & Lopez-Rojas, H. (1995). Quantitative evaluation of the point method for fish stomach contents analysis. *Journal of Fish Biology*, 47(5), 914–916.
- Martin, R. A., & Pfennig, D. W. (2011). Evaluating the targets of selection during character displacement. *Evolution*, 65(10), 2946–2958. <https://doi.org/10.1111/j.1558-5646.2011.01357.x>
- Meintjes, S. (1996). Seasonal changes in the invertebrate community of small shallow ephemeral pans at Bain's Vlei, South Africa. *Hydrobiologia*, 317(1), 51–64. <https://doi.org/10.1007/bf00013725>
- Molles, M. C. J. (2010). Ecology: Concepts and applications, fifth edition ecology: Concepts and applications. In *Oceanography faculty books and book chapters*. McGraw-Hill Companies.
- Motta, R. L., & Uieda, V. S. (2004). Diet and trophic groups of an aquatic insect community in a tropical stream. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, 64(4), 809–817. <https://doi.org/10.1590/s1519-69842004000500010>
- Nagy B., & Kis, A. H. (2010). Variation in habitat characteristics and the occurrence of *Nothobranchius* species in seasonal biotopes of Tanzania. *Journal of the American Killifish Association*, 43, 5.
- Nagy, B., & Watters, B.R. (2021). A review of the conservation status of seasonal *Nothobranchius* fishes (Teleostei: Cyprinodontiformes), a genus with a high level of threat, inhabiting ephemeral wetland habitats in Africa. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 32(1), 199–216.
- Nunn, A. D., Tewson, L. H., & Cowx, I. G. (2012). The foraging ecology of larval and juvenile fishes. *Reviews in Fish Biology and Fisheries*, 22(2), 377–408. <https://doi.org/10.1007/s11160-011-9240-8>
- Olivier, D., Lepoint, G., Aguilar-Medrano, R., Díaz, A. H. R., Sánchez-González, A., & Sturaro, N. (2019). Ecomorphology, trophic niche, and distribution divergences of two common damselfishes in the Gulf of California. *Comptes Rendus Biologies*, 342(9–10), 309–321.
- Önsoy, B., Tarkan, A. S., Filiz, H., & Bilge, G. (2010). Determination of the best length measurement of fish. *Journal of Zoology*, 7(1), 178–180.
- Pfennig, D. W., & Murphy, P. J. (2003). A test of alternative hypotheses for character divergence between coexisting species on JSTOR. *Ecology*, 84(5), 1288–1297. [https://doi.org/10.1890/0012-9658\(2003\)084\[1288:atoahfj2.0.co;2](https://doi.org/10.1890/0012-9658(2003)084[1288:atoahfj2.0.co;2)
- Pfennig, D. W., & Pfennig, K. S. (2010). Character displacement and the origins of diversity. *American Naturalist*, 176(Suppl 1), S26–S44. <https://doi.org/10.1086/657056>
- Polačik, M., & Reichard, M. (2010). Diet overlap among three sympatric African annual killifish species *Nothobranchius* spp. from Mozambique. *Journal of Fish Biology*, 77(3), 754–768. <https://doi.org/10.1111/j.1095-8649.2010.02717.x>
- R Core, T. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.
- Reichard, M., Janáč, M., Blažek, R., Žák, J., Alila, O. D., & Polačik, M. (2022). Patterns and drivers of *Nothobranchius* killifish diversity in lowland Tanzania. *Ecology and Evolution*, 12(6), e8990. <https://doi.org/10.1002/eece3.8990>
- Reifová, R., Reif, J., Antczak, M., & Nachman, M. W. (2011). Ecological character displacement in the face of gene flow: Evidence from two species of nightingales. *BMC Evolutionary Biology*, 11(1), 138. <https://doi.org/10.1186/1471-2148-11-138>
- Rice, A. M., & Pfennig, D. W. (2010). Does character displacement initiate speciation? Evidence of reduced gene flow between populations experiencing divergent selection. *Journal of Evolutionary Biology*, 23(4), 854–865. <https://doi.org/10.1111/j.1420-9101.2010.01955.x>
- Ruppert, E. E., & Barnes, R. D. (1994). *Invertebrate zoology*. Saunders College Publishing.
- Schluter, D. (2000). Ecological character displacement in adaptive radiation. *The American Naturalist*, 156(Suppl 4), S4–S16. <https://doi.org/10.1086/303412>
- Schluter, D. & McPhail, J. D. (1992). Ecological character displacement and speciation in sticklebacks. *American Naturalist*, 140(1), 85–108.
- Stuart, Y. E., Inkpen, S. A., Hopkins, R., & Bolnick, D. I. (2017). Character displacement is a pattern: So, what causes it? *Biological Journal of the Linnean Society*, 121(3), 711–715. <https://doi.org/10.1093/biolinnean/blx013>
- Stuart, Y. E., & Losos, J. B. (2013). Ecological character displacement: Glass half full or half empty? *Trends in Ecology and Evolution*, 28(7), 402–408. <https://doi.org/10.1016/j.tree.2013.02.014>
- Terzibasi, E., Lefrançois, C., Domenici, P., Hartmann, N., Graf, M., & Cellerino, A. (2009). Effects of dietary restriction on mortality and age-related phenotypes in the short-lived fish *Nothobranchius furzeri*. *Aging Cell*, 8(2), 88–99. <https://doi.org/10.1111/j.1474-9726.2009.00455.x>
- Van Wassenbergh, S., Herrel, A., Adriaens, D., & Aerts, P. (2007). No trade-off between biting and suction feeding performance in clariid catfishes. *The Journal of Experimental Biology*, 210(Pt 1), 27–36. <https://doi.org/10.1242/jeb.02619>
- Watters, B.R., Nagy, B., van der Merwe P. D. W., & Cotterill, F. P. D. (2019). Review of the *Nothobranchius taeniopygus* species group from central and western Tanzania with descriptions of five new species and redescription of *Nothobranchius taeniopygus* (Teleostei: Nothobranchiidae). *Ichthyological Exploration of Freshwaters*, IEF-1110, 29(3), 1–41.
- Watters, B. R. (2009). The ecology and distribution of *Nothobranchius* fishes. *Journal of the American Killifish Association*, 42, 37–76.
- Watters, B. R., Nagy, B., van der Merwe, P. D. W., Cotterill, F. P. D., & Bellstedt, D. U. (2020). Redescription of the seasonal killifish species *Nothobranchius ocellatus* and description of a related new species *Nothobranchius matanduensis*, from eastern Tanzania (Teleostei: Nothobranchiidae). *Pfeil Verlag*, 30(2), 151–178.
- Werner, E. E., & Gilliam, J. F. (1984). The ontogenetic niche and species interactions in size-structured populations. *Annual Review Inc*, 15, 393–425.
- Wildekamp, R. (2004). *A world of killies, volume 4: Atlas of the oviparous Cyprinodontiform fishes of the world*. American Killifish Association, NHBS Academic & Professional Books.
- Wilson, E. O., & Brown, W. L. (1956). Character displacement. *Systematic Zoology*, 5(2), 49.
- Žák, J., Vrtilek, M., & Reichard, M. (2019). Diel schedules of locomotor, reproductive and feeding activity in wild populations of African annual killifish. *Biological Journal of the Linnean Society*, 128(2), 435–450.