
Divergence in trophic ecology characterizes colonization of extreme habitats

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Extreme habitats are characterized by the presence of physio-chemical stressors, but also differ in aspects of the biotic environment, such as resource availability or the presence of competitors. The present study quantifies variation in trophic ecology of a small livebearing fish (*Poecilia mexicana*, Poeciliidae) across four different habitats that included nonsulphidic and sulphidic surface waters, as well as a nonsulphidic and a sulphidic cave. Resource use in different habitat types was investigated using gut content analysis. Populations diverged in resource use from a diet dominated by algae and detritus in nonsulphidic surface habitats to a diet including invertebrate food items in the other habitats. *Poecilia mexicana* in cave habitats further exhibited a higher dietary niche width than conspecifics from surface habitats. The condition of *P. mexicana* was analysed using storage lipid extractions. Fish from sulphidic and cave habitats exhibited a very poor condition, suggesting resource limitation and/or high costs of coping with extreme conditions. Finally, divergence in resource use was correlated with variation in viscerocranial morphology. A common garden experiment indicated both a genetic and plastic basis to the morphological variation observed among field populations. It is suggested that the morphological diversification is an adaptation to the differential use of resources among populations. © 2008 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2008, **95**, 517–528.

ADDITIONAL KEYWORDS: cave fish – Cueva del Azufre – dietary niche width – eco-morphology – ecological diversification – fat content – gut content analysis – hydrogen sulphide – phenotypic plasticity – *Poecilia mexicana* (Poeciliidae).

INTRODUCTION

Extreme environments are characterized by the presence of physio-chemical stressors and any organism able to tolerate these conditions requires costly adaptations that are absent in most other species (Townsend, Begon & Harper, 2003). Usually, microbes are associated with extreme environments, and they have evolved a diversity of physiological and biochemical mechanisms to cope with adverse conditions (Rothschild & Mancinelli, 2001). Likewise, metazoans (even vertebrates) have colonized habitats characterized by extremes in temperature, pressure, salinity, oxygen, pH as well as the presence of toxicants (Howarth, 1993; Van Dover, 2000; Laybourn-Parry & Pearce, 2007; Weber *et al.*, 2007).

Studies on metazoans inhabiting extreme environments have primarily focused on mechanisms that allow organisms surviving and coping with the particular physio-chemical stressor(s) (Van Dover, 2000; Bergman, Laurent & Otiang, 2003; Ip, Kuah & Chew, 2004). Such adaptations may occur in biochemical and physiological pathways as well as in morphology, behaviour, life history strategies, and symbioses with other organisms. Extreme habitats, however, differ from adjacent non-extreme habitats not only in the presence or absence of physio-chemical stressors, but also in that the environmental gradient in abiotic stressors is correlated with a suite of ecological differences. For example, extreme habitats are usually less productive and have a lower species diversity (McMullin, Bergquist & Fisher, 2000; Tsurumi, 2003; Tobler *et al.*, 2008), which may lead to differences in resource availability and quality, changes in

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competitive interactions within and between species, as well as changes in the exposure to predators. Adaptations to extreme environments may thus not only include traits directly involved in coping with a particular physio-chemical stressor, but also traits that evolved in response to correlated differences in the biotic environment.

The present study investigated the trophic ecology of the small livebearing fish, *Poecilia mexicana* Steindachner (Poeciliidae), that inhabits four different habitat types in the southern Mexican state of Tabasco. Habitat types are characterized by the presence or absence of hydrogen sulphide and light (caves); besides nonsulphidic surface waters, this species has colonized sulphidic surface habitats, as well as a nonsulphidic and a sulphidic cave, which can be considered extreme conditions (Gordon & Rosen, 1962; Tobler *et al.*, 2006; Tobler *et al.*, 2008). Sulphidic habitats are characterized by high concentrations (up to 300 μM) of dissolved hydrogen sulphide (H_2S ; Tobler *et al.*, 2006). This respiratory toxicant is lethal to most metazoans even in micromolar concentrations (Bagarinao, 1992; Grieshaber & Völkel, 1998). Because the amount of oxygen available for respiration is generally limited in sulphidic habitats due to the correlated hypoxic conditions (Tobler *et al.*, 2006) and, at the same time, oxygen is required for coping with the toxic effects of H_2S (Bagarinao, 1992; Ip *et al.*, 2004; Plath *et al.*, 2007b), organisms in sulphidic environments are selected for an efficient oxygen uptake. Likewise, the cave environment is challenging (Poulson & White, 1969; Howarth, 1993; Langecker, 2000), and diurnally active species such as *P. mexicana* that predominantly rely on visual senses in normal surface habitats have to cope with conditions of complete darkness (Plath *et al.*, 2004).

Besides the described differences in abiotic conditions, the four habitat types differ in a number of biotic environmental factors. The energy basis is likely to differ both qualitatively and quantitatively among habitats. Compared with normal surface habitats, photosynthetic primary production is absent in the caves (Poulson & Lavoie, 2000) and probably reduced in sulphidic surface habitats because H_2S is also toxic to most algae (Bagarinao, 1992). H_2S in turn allows for chemoautotrophic primary production by sulphide oxidizing bacteria in sulphidic habitats (Nelson & Jannasch, 1983; Sarbu, Kane & Kinkle, 1996), and mats of sulphide-oxidizing bacteria are present in the sulphidic surface and cave habitats (Hose *et al.*, 2000). Finally, allochthonous input from terrestrial habitats is probably present in all habitat types, but is dominated by leaf litter and terrestrial insects in surface habitats and guano from bat colonies inside caves. Furthermore, habitat types differ in

the presence of potential competitors and predators of *P. mexicana*. Compared with normal surface habitats, sulphidic and cave habitats exhibit a reduction in fish species diversity, with *P. mexicana* being the predominant species (Tobler *et al.*, 2006; Tobler *et al.*, 2007). Hence, not only are competitors for similar food sources rare, but piscine predators that may affect foraging behaviour are also absent.

In the present study, several aspects of the trophic ecology of *P. mexicana* from different habitat types were investigated. First, was the colonization of extreme habitats accompanied by a divergence in resource use? In nonsulphidic surface habitats, *P. mexicana* feeds predominantly on detritus and algae (Darnell, 1962; Miller, 2005). Differential resource use among populations was predicted both due to differences in resource availability and fish community structure. Second, do *P. mexicana* from extreme habitats exhibit a wider dietary niche than conspecifics from nonsulphidic surface habitats? Ecological factors such as competition and predation are primary determinants of a population's niche width (Begon, Harper & Townsend, 1996). Competitive release due to a reduced species diversity in extreme habitats (MacArthur, Diamond & Karr, 1972; Schluter & McPhail, 1992) or a shift from inter- to intraspecific competition (Svanbäck & Bolnick, 2007), as well as resource limitation (Fenolio *et al.*, 2006; MacArthur & Pianka, 1966; Schoener, 1971), could lead to trophic niche expansion. Third, do *P. mexicana* from different habitat types differ in the body condition? Survival in stressful environments is considered costly (Townsend *et al.*, 2003). Short-term survival of *P. mexicana* in sulphidic water critically depends on energy availability and the potential to perform aquatic surface respiration, whereby fish skim the surface of the water where diffusion maintains a better-oxygenated layer (Plath *et al.*, 2007b). Likewise, energy availability may vary among habitat types. The condition of *P. mexicana* from different habitat types was examined by the extraction of storage lipids as a proxy of energy availability. Finally, did *P. mexicana* in different habitat types diverge in their trophic morphology? Because viscerocranial morphology frequently reflects trophic and dietary characteristics in fishes (Winemiller, Kelso-Winemiller & Brenkert, 1995; Horstkotte & Strecker, 2005), skull morphology and intestinal tract length in *Poecilia mexicana* from different habitat types were compared. Whether differences in jaw morphology have a heritable basis was also investigated by comparing fish from a common garden experiment with fish collected in the field. The trophic morphology in poeciliids has previously been shown to be phenotypically plastic (Robinson & Wilson, 1995; Ruehl & DeWitt, 2007).

MATERIAL AND METHODS

STUDY SYSTEM AND FISH COLLECTIONS

Poecilia mexicana is a common fish species inhabiting freshwater habitats on the Atlantic versant of Central America from northern Mexico to Costa Rica (Miller, 2005). All study sites were located near the village of Tapijulapa in the southern Mexican state of Tabasco. Four habitat types differing in their abiotic conditions were sampled (see Supporting Information, Fig. S1). Habitat types were characterized by the presence or absence of H₂S (sulphidic or nonsulphidic) and/or light (surface or cave), thus proving a natural 2 × 2 factorial design.

The Cueva del Azufre is a sulphidic cave. The cave is structured into different chambers, the nomenclature of which follows Gordon & Rosen (1962). The front chambers obtain some dim light, whereas the rearmost cave chambers are completely dark. The cave is drained by a creek fed by a number of springs occurring throughout the cave, most of which contain high levels of dissolved H₂S (Tobler *et al.*, 2006). The presence of H₂S allows for bacterial chemoautotrophic primary production (Hose *et al.*, 2000). Additional energy input into the cave comes from bats that reside in different cave chambers and deposit considerable amounts of bat guano. *Poecilia mexicana* occur throughout the cave and, for the present study, they were collected in chambers V and X.

The Cueva Luna Azufre is, despite its name, a nonsulphidic cave (Tobler *et al.*, 2008). The creek in the Cueva Luna Azufre is also fed by springs; however, these do not contain H₂S. The energy basis of this cave is thus thought to rely entirely on allochthonous input, especially in form of bat guano. *Poecilia mexicana* were collected south of the Entrada Marabunda (Tobler *et al.*, 2008).

The El Azufre is a sulphidic surface habitat originating in the hills southwest of the two caves and is fed by multiple independent sulphidic springs. Both caves drain into the El Azufre, which eventually joins the Río Oxolotan. H₂S concentrations are comparable to those in the Cueva del Azufre. The presence of H₂S allows for chemoautotrophic primary production, but photoautotrophic production and allochthonous input from terrestrial habitats is possible. *Poecilia mexicana* were collected near the uppermost sulphur springs as well as at the resurgence of the Cueva del Azufre.

Two nonsulphidic surface habitats were sampled. These habitats include a large river, the Río Oxolotan (most proximate to the other habitat types) and Arroyo Tacubaya, a nonsulphidic creek similar in size and structure to the El Azufre. The energy basis in these habitats is photoautotrophic primary production and allochthonous input from terrestrial habitats.

Poecilia mexicana populations from the four habitat types are morphologically and genetically distinct, and there is striking reduction of gene flow between habitat types (but not different sites within habitat types) despite their spatial proximity and the lack of physical barriers (Plath *et al.*, 2007a; M. Tobler, T. Dewitt, I. Schlupp, F. J. Garcia de Leon, R. Hermann, P. G. D. Feulner, R. Tiedermann, M. Plath, in press). Fish were caught in January 2006 and May 2007. Because habitat structures differed between sampling sites, different methods were employed. In the caves, where the water is very shallow and low ceilings preclude seining, fishes were caught with dip nets (13 × 14 cm; 1 mm mesh-width). In the other habitats, fish were caught using a seine (length 4 m; 4 mm mesh-width). Fish were euthanized using MS222 immediately after capture and fixed in a 10% formaldehyde solution.

GUT CONTENT AND CONDITION ANALYSES

To test for differences in resource use and dietary niche width among habitat types, a gut content analysis was performed on *P. mexicana* collected in 2007. All individuals were measured for standard length (SL) to the nearest 0.1 mm and weighed to the closest 0.01 g (wet weight). Fish were dissected and entire digestive tracts were prepared. The content of the first quarter of the intestine was investigated under a stereomicroscope. All visible diet items were sorted and spread on a mm-square grid. The percent area for each food item was recorded. The area each diet item occupied was assumed to be proportional to its volume (Hellawell & Abel, 1971; Gido & Franssen, 2007). Items too small to identify or sort under the stereomicroscope were subsequently placed on a glass slide for examination under a light microscope. The relative proportion of each identifiable item on the slide was estimated and then multiplied by the area previously occupied on the mm-square grid.

Diet categories used in the gut content analysis were adapted as described previously (Winemiller, 1990) but, for the final analysis, the following groups were recognized: detritus (fine and coarse particulate organic matter); algae (filamentous algae, coccal algae, and diatoms); plant parts (parts of aquatic and terrestrial macrophytes, and seeds); aquatic arthropods (chironomid larvae, ostracods, isopods, annelids, and nematodes); terrestrial arthropods (collembola, adult chironomids, keroplatid larvae, and ants); gastropods; bat guano (lepidopteran scales and insect parts); and sand. Insect parts were classified as bat guano when they were present in small fragments (*P. mexicana* is not able to reduce insects into smaller pieces), whereas all other insects found were left intact.

Relative proportions of diet items were arcsine-squareroot-transformed and subjected to a principle

component analysis (PCA). Three principal components accounting for 60.7% of the variance were used as dependent variables in a multivariate analysis of covariance (MANCOVA) with habitat type and sex as independent variables, and SL as covariate. The assumptions of normal distribution and homogeneities of variances and covariances were met for this analysis. F -ratios were approximated using Wilks' lambda values. The interaction terms were not significant ($F_{9,491} \leq 1.79$; $P \geq 0.07$); thus, only main effects were analysed.

The dietary niche width of each individual was calculated using the inverse of Simpson's (1949) diversity measure (see Pianka, 1973; Pianka, 1986): $\beta = (\sum p_i^2)^{-1}$, where p is the proportional utilization of each dietary item i . Niche width values were \log_{10} -transformed to normalize distributions and then subjected to an analysis of covariance (ANCOVA) using habitat type and sex as independent variables, and SL as a covariate. The assumptions of normal distribution and homogeneities of variances were met for this analysis. The interaction terms were not significant ($F_{3,204} \leq 2.55$; $P \geq 0.06$); thus, only main effects were analysed.

To assess body condition, fish carcasses (without visceral organs) were dried at 55 °C for 7 days after the gut content analysis. Soluble nonstructural fats were then extracted during four 24-h extractions in petroleum ether (Meffe & Snelson, 1993; Heulett, Weeks & Meffe, 1995). The relative fat content [$(M_{\text{before extraction}} - M_{\text{after extraction}})/M_{\text{before extraction}}$] served as a proxy for individual body condition of the fish. Relative fat content values were arcsine-square-root-transformed to approximate normality and analysed using an ANCOVA with habitat type and sex as independent variables, and SL as a covariate. The interaction terms were not significant ($F_{3,227} \leq 1.83$; $P \geq 0.14$); thus, only main effects were analysed.

MORPHOLOGICAL DIFFERENTIATION

To test whether variation in resource use among habitats was correlated with changes in trophic morphology, skull morphology and intestinal tract length in individuals from different habitat types were examined and compared. The skull, eyes, and visceral organs of formalin-fixed fish collected in 2006 were removed and specimens were cleared and stained as described by Turner (1984). A digital picture of the dorsal side of the head was then taken using a Spot Insight digital camera mounted on an Olympus stereomicroscope. Skull traits were measured to the closest 0.01 mm using an image analysing program (Spot Advanced 3.5; Diagnostic Instruments). Six measurements of the skull were taken following Robinson & Wilson (1995), who investigated trophi-

cally induced morphological changes in a closely-related species, *Poecilia reticulata*: (1) the length of the dentary; (2) the length and (3) thickness of the premaxilla; (4) the length of the longest tooth on the premaxilla; (5) the width of the skull at the anterior margins of the orbitals; and (6) the length of the snout.

Variation in skull morphology among populations of *P. mexicana* was examined using a MANCOVA, in which the individual skull measurements were used as dependent variables, SL as a covariate (to control for allometry), and sex as well as habitat type as independent variables. The assumptions of normal distribution and homogeneities of variances were met for this analysis. Furthermore, a discriminant function analysis (DFA) was performed to test whether individuals were correctly assigned to the population of origin based on their skull morphology. To remove the effects of allometry and sex, a MANCOVA with the skull measurements as dependent variables, SL as covariate, and sex as independent variable was performed. The residuals of the MANCOVA were then used as independent variables in the DFA (consequently, the classification was only dependent on differences among habitats).

Previous studies have shown that trophic morphology in poeciliids can be phenotypically plastic (Robinson & Wilson, 1995; Ruehl & DeWitt, 2007). The present study therefore tested whether differences in skull morphology have a heritable component by comparing laboratory stocks with wild-caught specimens from the original collection site. If morphological differences were entirely caused by environmentally-induced phenotypic plasticity, differences among fish from different habitat types should disappear in the laboratory stocks housed under identical conditions. Likewise, if morphological differences were entirely determined by genetics, no differences between laboratory raised and wild-caught individuals would be expected. An intermediate results suggests the traits under investigation have heritable basis, but that phenotypic plasticity also plays a role. For this analysis, only three of the four habitat types were included because no laboratory stocks from sulphidic surface habitats were available. Laboratory stocks were founded in January 2006 and maintained as randomly out-bred populations in 1500-L tanks in a greenhouse at the Aquatic Research Facility of the University of Oklahoma. All stocks were exposed to identical environmental conditions. Algae, detritus, and invertebrates (amphipods) were present in the stock tanks, and fish were supplemented with commercially available flake food. A random sample of fish from stocks of each habitat type was collected in May 2007. At this point, the stocks were established in the laboratory for approximately three to four generations. Laboratory raised fish were subjected to

the same procedure as outlined above for wild-caught specimens.

Data were analysed with the same approach using a MANCOVA, in which the individual skull measurements were used as dependent variables, SL as a covariate (to control for allometry), and sex, habitat type, and treatment (i.e. wild-caught or laboratory-reared) as independent variables. The assumptions of normal distribution and homogeneities of variances were met for this analysis. The three-way interaction between sex, habitat, and treatment was not significant ($F_{12,224} < 1.77$; $P > 0.05$) and thus was omitted from the analysis. Also, a DFA was performed to test whether laboratory-reared individuals cluster with wild-caught specimens from their original habitat type. Again, a MANCOVA with the skull measurements as dependent variables, SL as covariate, and sex as independent variable was performed to remove the effects of allometry and sex. The residuals were then used as independent variables in the DFA.

Intestinal tract length was measured in all specimens that were subjected to a gut content analysis. Intestines were uncoiled and the length was measured from the esophagus to the anus to the closest 1 mm (Kramer & Bryant, 1995b). Variation in intestinal tract length was examined using an ANCOVA with intestinal tract length as the dependent variable and sex and habitat as independent variables. An individual's mass rather than its size has been suggested for use as the base in comparisons of intestinal tract lengths (Kramer & Bryant, 1995a); thus, a mass-based covariate ($10 \times \text{mass}^{1/3}$) adapted from the Zihler index (Zihler, 1982) was included.

RESULTS

GUT CONTENT AND CONDITION ANALYSES

A total of 251 individuals were examined for their gut contents (SL \pm SD; nonsulphidic surface: 35.6 ± 7.7 mm; sulphidic surface: 30.4 ± 4.5 mm; nonsulphidic cave: 24.8 ± 6.4 mm; sulphidic cave: 33.5 ± 6.2 mm). Thirty-eight individuals had empty intestines (0 of 58 from nonsulphidic surface habitats; nine of 74 from sulphidic surface habitats; two of 41 from the nonsulphidic cave; 27 of 78 from the sulphidic cave). The frequency of empty intestines differed significantly among habitat types ($\chi^2 = 37.24$, d.f. = 3,251, $P < 0.001$). Individuals with empty intestines were excluded from the PCA and the analysis of individual dietary niche width.

Poecilia mexicana from all habitat types ingested large amounts of detritus and, in the surface habitats, detritus was the primary dietary item (Table 1). Whereas individuals from nonsulphidic surface habitats consumed algae, those from sulphidic surface habitats consumed aquatic arthropods. Besides detritus, *P. mexicana* from cave habitats foraged primarily on bat guano. Based on gut contents, individuals from the two caves differed primarily in the amount of arthropods (abundant in the sulphidic cave) as well as the amount of algae, plant parts, and gastropods consumed (abundant in the nonsulphidic cave; Table 1). The MANCOVA using PCA scores as dependent variables (for the component matrix of the PCA, see Table 1) suggested that the resource use of *P. mexicana* in different habitat types was significantly different, but that neither sex nor size had a significant influence (Table 2).

Table 1. Proportions of dietary items averaged across individuals of each habitat type (including the sample size) and component matrix of the principal component analysis on the proportion of food items in the guts. Axis loadings for the first three principal components are shown (including the percent variation explained by each).

	Gut content				PC axis loadings		
	Surface no H ₂ S	Surface H ₂ S	Cave no H ₂ S	Cave H ₂ S	1	2	3
N/% variation explained	58	65	39	51	26.6	18.7	15.4
Detritus	0.71	0.70	0.29	0.29	-0.861	0.263	-0.020
Algae	0.12	0.00	0.09	0.00	0.036	0.746	-0.203
Plant parts	0.00	0.01	0.08	0.00	0.501	0.403	0.498
Aquatic arthropods	0.00	0.16	0.09	0.19	0.332	-0.578	0.450
Terrestrial arthropods	0.00	0.02	0.01	0.15	0.369	-0.315	-0.519
Gastropods	0.00	0.00	0.08	0.01	0.430	0.370	0.465
Bat guano	0.00	0.01	0.32	0.27	0.634	-0.081	-0.310
Sand	0.17	0.10	0.04	0.09	-0.549	-0.362	0.401

Table 2. Multivariate analysis of covariance results examining gut contents of *Poecilia mexicana* from different habitat types and analysis of covariance results examining the dietary niche width, relative fat content, and gut length of *P. mexicana* from different habitat types.

Effect	<i>F</i>	d.f.	<i>P</i>
Gut content			
SL	0.31	3,499	0.817
Sex	2.19	3,205	0.091
Habitat	40.68	9,205	< 0.001*
Niche width			
SL	2.78	1	0.098
Sex	0.74	1	0.390
Habitat	16.03	3	< 0.001*
Fat content			
SL	1.62	1	0.205
Sex	9.65	1	0.002*
Habitat	36.11	3	< 0.001*
Gut length			
Mass	218.04	1	< 0.001*
Habitat	237.88	3	< 0.001*
Sex	24.49	1	< 0.001*
Habitat × Sex	47.98	3	< 0.001*

*Significant effect.

The individual trophic niche width also differed among habitat types (Fig. 1A, Table 2). Post-hoc tests indicated that individuals from surface habitats had a narrower spectrum of prey items in their intestines than those from cave habitats, and that fish from the nonsulphidic cave had a higher diversity than those from the sulphidic cave.

Poecilia mexicana from nonsulphidic habitats further had significantly more storage lipids than fish from the other habitat types (Fig. 1B, Table 2). Also, sex had a significant effect on the lipid content as males had less storage lipids than females.

MORPHOLOGICAL DIFFERENTIATION

Poecilia mexicana from nonsulphidic surface habitats were generally characterized by smaller heads (shorter head width and snout length), narrower mouths (shorter premaxilla and dentaries), and thinner premaxilla than fish from other habitat types (see Supporting Information, Tables S1, S2). Fish from sulphidic surface habitats were characterized by having longer teeth than individuals from the other habitat types. In the DFA, 67.7% of the individuals were correctly assigned to the habitat type of origin based on skull morphology (compared with a random expectation of 25%). The DFA also suggests that the skull morphologies of *P. mexicana* inhabiting sul-

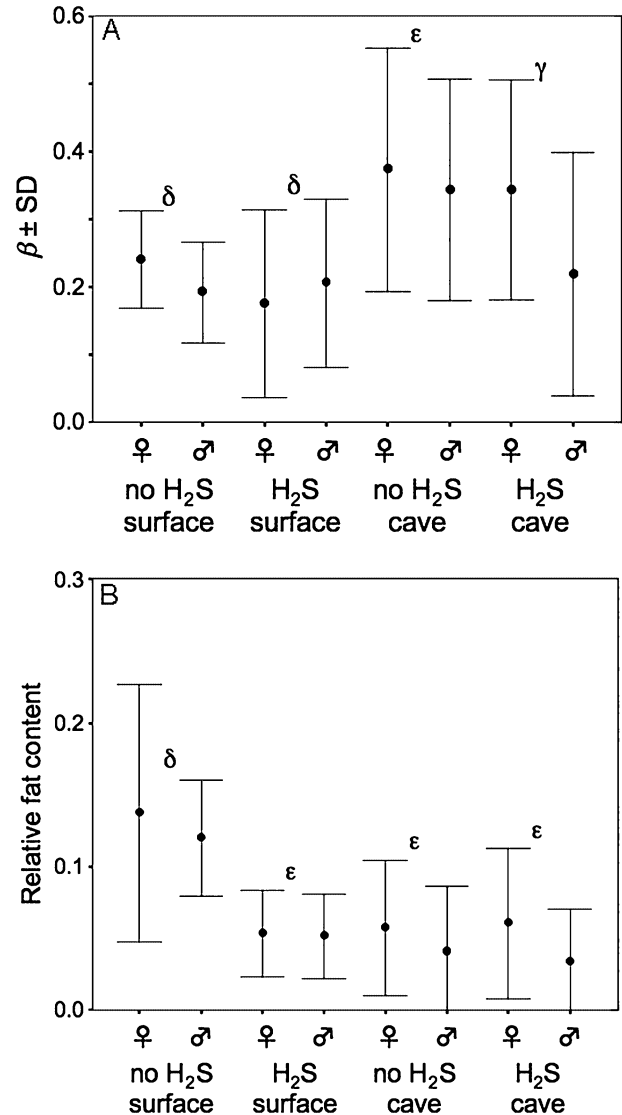


Figure 1. A, mean ± SD individual trophic niche width (β) of males and females from the different habitat types. B, relative fat content (\pm SD) of males and females from the different habitat types. Pairwise post-hoc tests (LSD, $\alpha < 0.05$) revealed which populations differed, as labelled by Greek letters.

phidic and/or cave habitats are more similar to one another than to the morphology of fish from the nonsulphidic surface habitat (separation along function 1: Fig. 2A, Table 3). Although skull morphology appears to be phenotypically plastic to some degree (see Supporting Information, Tables S1, S2), variation among populations appears to have a heritable basis. Laboratory-reared individuals clustered close to field-collected specimens of the respective habitat type (Fig. 2B, Table 3).

Gut length was measured in 58 individuals from nonsulphidic habitats (28 females), 74 from sulphidic

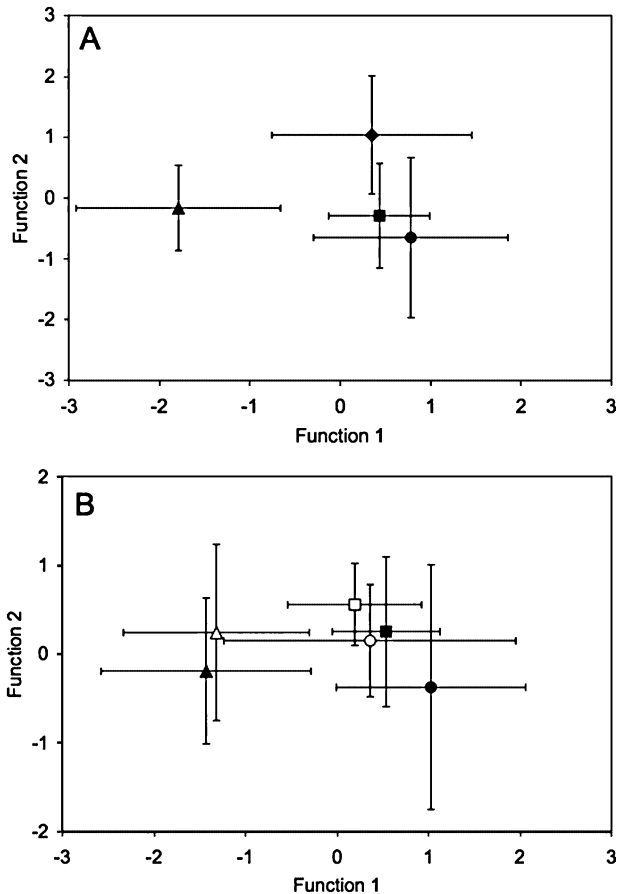


Figure 2. Discriminant function plots where functions 1 and 2 correspond to the discriminant functions from the analyses presented in Table 5. Depicted are the mean \pm SD discriminant function scores for each group. A, analysis of wild-caught individuals from all four habitat types. B, comparison between wild-caught and laboratory-reared individuals. \blacktriangle , nonsulphidic surface habitats; \blacklozenge , sulphidic surface habitats; \blacksquare , nonsulphidic cave; \bullet , sulphidic cave. Closed symbols represent wild-caught individuals, open symbols indicate laboratory-reared individuals.

surface habitats (46 females), 41 from the nonsulphidic cave (21 females), and 78 from the sulphidic cave (44 females). Both of the factors included in the analysis (sex and population), as well as their interaction and the mass-based covariate, had a highly significant effect on the gut lengths of *P. mexicana* (Fig. 3, Table 2). The significant interaction term between population and sex was driven by specimens collected in nonsulphidic surface habitats, where males had shorter intestines than females. Post-hoc tests revealed that average intestine lengths were significantly different between individuals from divergent habitat types (estimated marginal means of gut length in nonsulphidic surface > nonsulphidic

cave > sulphidic surface > sulphidic cave; LSD: $P < 0.001$ in all cases).

DISCUSSION

In southern Mexico, *P. mexicana* colonized a set of different habitat types, characterized by the presence or absence of light and H_2S . This provides an excellent system for studying the ecological and evolutionary consequences of life under stressful conditions. Previous studies have shown that *P. mexicana* in the different habitat types diverged phenotypically and genetically, thereby adapting to life in sulphidic and/or cave habitats (Plath *et al.*, 2004; Plath *et al.*, 2007a, M. Tobler, T. Dewitt, I. Schlupp, F. J. Garcia de Leon, R. Hermann, P. G. D. Feulner, R. Tiedermann, M. Plath, in press). The present study confirmed that the colonization of extreme habitats is accompanied by ecological differences that are indirectly related to the abiotic conditions of the different habitats. In particular, evidence is provided showing that the colonization of different habitat types in the Cueva del Azufre system was associated with a divergence in resource use and the trophic morphology of these fish.

DIFFERENCES IN RESOURCE USE AND NICHE EXPANSION

Poecilia mexicana in nonsulphidic surface habitats primarily fed on detritus and algae, which is consistent with previous studies investigating the food habits of closely-related species and *P. mexicana* in other parts of its range (Darnell, 1962; Winemiller, 1993; Kramer & Bryant, 1995a; Bussing, 1998; Miller, 2005). The most pronounced difference in diet was observed between the nonsulphidic surface habitats where diet was dominated by algae/detritus, and the divergent habitats where invertebrates were consumed by *P. mexicana*. Especially in the cave habitats, invertebrates made up the majority of the gut content. However, a substantial amount of this invertebrate diet (approximately 50%) stems from bat guano, not living invertebrates. This finding is also consistent with a previous study that investigated the gut content of *P. mexicana* from the sulphidic cave only (Langecker, Wilkens & Parzefall, 1996). Together, these studies suggest that colonization of extreme habitats in *P. mexicana* was accompanied by a differences in resource use. Similar differences towards the incorporation of invertebrate prey into the diet was found in a *Cyprinodon* species flock (Horstkotte & Strecker, 2005).

Several limitations preclude conclusions on the strength of resource use differences across habitats. First, the habitat effect in all analyses reflects multiple individuals from one or two sites per habitat type,

Table 3. Discriminant function analyses of the skull morphology in *Poecilia mexicana* from different habitat types, comprising an analysis of wild-caught individuals from all four habitat types and a comparison between wild-caught and laboratory-reared individuals.

	Wild-caught fish			Wild-caught and lab-reared fish	
	Function 1	Function 2	Function 3	Function 1	Function 2
Canonical loadings					
Dentary length	0.754	0.193	0.592	0.430	-0.278
Premaxilla length	0.701	0.253	0.362	0.417	0.126
Premaxilla thickness	0.587	-0.021	0.252	0.480	0.024
Tooth length	0.045	0.567	0.051	-0.004	0.120
Snouth length	0.636	0.339	-0.535	0.396	0.558
Head width	0.911	-0.288	0.046	0.930	-0.042
Canonical correlation	0.706	0.553	0.268	0.705	0.248
Eigenvalue	0.992	0.442	0.078	0.998	0.065
% variance	65.6	29.2	5.1	93.8	6.2
Chi-square	143.494	55.949	9.497	93.414	7.880
d.f.	18	10	4	12	5
<i>P</i>	< 0.001*	< 0.001*	0.050*	< 0.001*	0.163

*Significant effect.

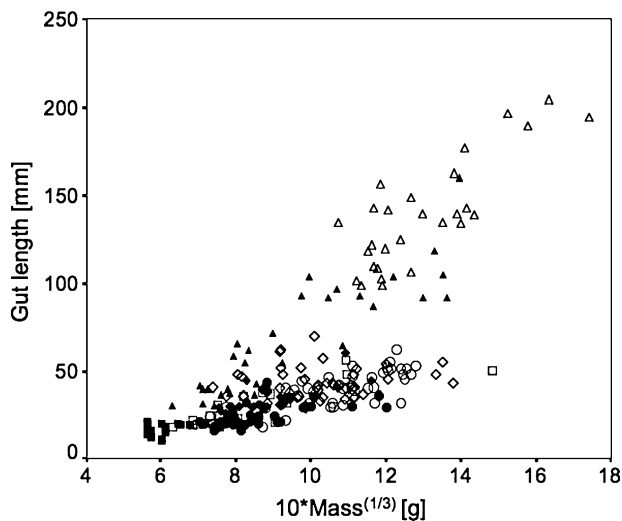


Figure 3. Gut lengths in males and females of *Poecilia mexicana* from different habitat types. ▲, nonsulphidic surface habitats; ◆, sulphidic surface habitats; ■, nonsulphidic cave; ●, sulphidic cave. Closed symbols represent males, open symbols indicate females.

thus replication of individual habitat types is low. Second, differences in resource use among habitats may be lower during different seasons. Seasonal differences in resource use have been documented in the closely related species *Poecilia gillii* Kner (in nonsulphidic surface habitats), but season affected only the frequency of different food items (detritus versus algae) ingested (i.e. there was no shift towards

an incorporation of invertebrates into the diet) (Winemiller, 1993). Third, the examination of gut contents may have underestimated the differences in resource use because small diet items are not readily quantifiable with the methodology used. Specifically, it was not possible to quantitatively differentiate between detritus and bacteria. Qualitatively, it was evident that specimens collected in sulphidic habitat had white filaments resembling the mats of sulphide oxidizing bacteria (Hose *et al.*, 2000) in their intestines, which were absent in specimens from nonsulphidic habitats. Consequently, the diet of *P. mexicana* from sulphidic and nonsulphidic habitats may differ more than this dataset suggests. Future investigations will use stable isotope analyses to elucidate the trophic structure of communities in the different habitat types (Peterson & Fry, 1987; Post, 2002).

It is likely that both differences in resources availability and the competitive regime contributed to differences in food resource use among habitats. Indeed, specimens collected in different habitat types not only differed in what they fed on, but also with respect to the diversity of food items present in their intestines; and fish from cave habitats exhibited a significantly higher diversity (i.e. a higher individual trophic niche width). It is unlikely that this increase in the trophic niche width was caused by a higher diversity in resources available because there is ample evidence that caves (and sulphidic habitats for that matter) exhibit a reduced species diversity compared with surface habitats (Poulson & White, 1969; Gibert & Deharveng, 2002). Niche expansion

as a response to competitive release (MacArthur *et al.*, 1972; Schluter & McPhail, 1992), increased intraspecific competition (Svanbäck & Bolnick, 2007), or as a response to resource scarcity (MacArthur & Pianka, 1966; Schoener, 1971; Fenolio *et al.*, 2006) may drive the increase in the diversity of resources used, but the exact mechanisms are unclear so far. All scenarios are not necessarily consistent with *P. mexicana* from sulphidic surface habitats having a low individual dietary niche width. At least in terms of fishes, the communities in the sulphidic surface habitats are as comparatively deprived as those in the cave habitats (Tobler *et al.*, 2006; Tobler *et al.*, 2008), and *P. mexicana* from sulphidic surface habitats are in an equally poor condition compared with conspecifics from the cave habitats (this study). High individual dietary niche widths in the two cave habitats may also be explained by nonselective foraging. *Poecilia mexicana* is a diurnal species relying on visual senses (Plath *et al.*, 2004) and, although fish from the sulphidic cave have evolved the ability for nonvisual communication in the context of sexual selection (Plath *et al.*, 2004, 2006), the derived cave-inhabiting *P. mexicana* may not be able to forage selectively on specific diet items. For example, the foraging efficiency of *P. mexicana* from the two caves in darkness is as low as the efficiency of surface populations in darkness (M. Tobler, unpublished data).

NUTRITIONAL CONDITION AND ENERGY LIMITATION

Previous studies examining body conditions in this system (by comparing length-weight regressions) found *P. mexicana* from nonsulphidic surface habitats to exhibit the highest body condition, whereas cave populations had the lowest, and specimens from sulphidic surface habitats were intermediate (Tobler *et al.*, 2006; Plath *et al.*, 2007b; Tobler *et al.*, 2008). Morphological differences among populations, however, affected these results because populations differ in body height (M. Tobler, T. Dewitt, I. Schlupp, F. J. Garcia de Leon, R. Hermann, P. G. D. Feulner, R. Tiedermann, M. Plath, in press.) and *P. mexicana* from sulphidic surface habitats have as low amounts of storage lipids as fish from the cave populations.

Fish from cave and sulphidic habitats may have a low condition for different reasons. *Poecilia mexicana* from the nonsulphidic cave exhibited low amounts of storage fats likely because resources are scarce. Caves relying on energy input from surface habitats are known to be energy limited (Streever, 1996; Hüppop, 2000; Poulson & Lavoie, 2000). Bat guano is thought to be the trophic base of cave food webs whenever bats are present and provide an energy-rich food base (Culver, 1982; Willis & Brown, 1985), but recent work

indicates that this is not necessarily the case (Graening & Brown, 2003).

Sulphidic habitats in turn have been suggested to be resource-rich (Langecker *et al.*, 1996). The paradox of fish with low body condition living in an apparently resource-rich environment may be explained in two (not mutually exclusive) ways. (1) Although resource-rich, sulphidic habitats may lack particular nutrients for fish or provide an imbalanced diet, which may negatively affect condition (Jeyasingh, 2007). (2) Coping with the toxic environment may be energetically costly. Although the physiological mechanisms of sulphide-tolerance are not well understood in *P. mexicana*, detoxifying H₂S has been shown to be energetically costly under hypoxic conditions in the mudskipper, *Boleophthalmus boddarti* Pallas (Ip *et al.*, 2004). Short-term survival of *P. mexicana* in sulphidic water is directly dependent on energy-availability and the possibility of performing aquatic surface respiration, where fish exploit the oxygen-rich air-water-interface (Plath *et al.*, 2007b). Aquatic surface respiration itself is physiologically costly and constrains an individual's energy budget, leaving less time for foraging (Kramer, 1983; Weber & Kramer, 1983; Chapman & Chapman, 1993). Reduced foraging activity in oxygen deprived environments also reduces body condition in an African cyprinid (Barrow & Chapman, 2006). *Poecilia mexicana* in sulphidic habitats thus appear to be living in a resource-rich habitat but paying the cost of coping with the toxic conditions. The high resource availability in these habitats may be one of the factors making life under such extreme environments possible at all. Future studies will need to examine the nutritional value of the food items ingested by *P. mexicana* as well as the costs of coping with the stressful conditions in the different habitat types.

DIFFERENTIATION IN TROPHIC MORPHOLOGY

The major difference in trophic morphology among populations living in different habitat types was found between *P. mexicana* from nonsulphidic surface habitats and those from extreme habitats, which parallels the major dietary differences with incorporation of invertebrates into the diet. Intestinal tract lengths in fishes are typically correlated with the amount of plant material ingested (Kramer & Bryant, 1995a), and *P. mexicana* from extreme habitats (which consume less plant material) had shorter intestinal tracts. They were also characterized by wider and thicker jaws, which may be advantageous in handling larger (Wainwright, 1996) and/or more evasive prey items (Hulsey & Garcia de Leon, 2005; Higham *et al.*, 2007).

Previous studies on *P. mexicana* body morphology using geometric morphometric analyses did not find

variation that was obviously related to trophic ecology (Tobler *et al.*, 2008; M. Tobler, T. Dewitt, I. Schlupp, F. J. Garcia de Leon, R. Hermann, P. G. D. Feulner, R. Tiedermann, M. Plath, in press.). Differentiation in skull morphology among populations inhabiting different habitat types is also less pronounced than the differentiation in general body shape, which appears to be driven predominantly by abiotic environmental factors [i.e. the lack of light (eye size reduction) as well as the presence of H₂S and hypoxia (increase in head and gill size, M. Tobler, T. Dewitt, I. Schlupp, F. J. Garcia de Leon, R. Hermann, P. G. D. Feulner, R. Tiedermann, M. Plath, in press.)]. Thus, differences in jaw morphology as reported in the present study are not simply explained by a correlated response to selection on other characteristics of the head (Chapman, Galis & Shinn, 2000), but may actually be adaptive to differential use of resources among populations. The finding that skull morphology is at least partly determined by genetics supports the idea that evolutionary divergence in skull morphology by natural selection is possible.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Measurements of skull traits (mean \pm SD) in males and females of *Poecilia mexicana* from different habitat types. Note that laboratory reared specimens from sulphidic surface habitats were not available during this study.

Table S2. Multivariate analysis of covariance results examining the skull morphology of *Poecilia mexicana* from different habitat types. For the tests of between subject effects, α -values were adjusted for multiple testing ($\alpha = 0.008$). Significant effects are bold. A, analysis of wild-caught individuals from all four habitat types. B, comparison between wild-caught and laboratory-reared individuals. Due to the lack of laboratory stocks from sulphidic surface habitats, no specimens of this habitat types were included in this analysis.

Figure S1. Map of the collection sites around the village of Tapijulapa (Tabasco, Mexico). Nonsulphidic surface habitats colour-coded are in blue (AT, Arroyo Tacubaya; RO, Rio Oxolotan), sulphidic surface habitats in yellow (EAI, emergence of the Cueva del Azufre; EAII, upstream springs), the entrance of the Cueva Luna Azufre (LA, nonsulphidic) in orange, and the entrance of the Cueva del Azufre (CA, sulphidic) in red. The star in inset indicates the location of the study area in Mexico.

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