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Extreme habitats as refuge from parasite infections? Evidence from an extremophile fish

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ABSTRACT

Living in extreme habitats typically requires costly adaptations of any organism tolerating these conditions, but very little is known about potential benefits that trade off these costs. We suggest that extreme habitats may function as refuge from parasite infections, since parasites can become locally extinct either directly, through selection by an extreme environmental parameter on free-living parasite stages, or indirectly, through selection on other host species involved in its life cycle. We tested this hypothesis in a small freshwater fish, the Atlantic molly (*Poecilia mexicana*) that inhabits normal freshwaters as well as extreme habitats containing high concentrations of toxic hydrogen sulfide. Populations from such extreme habitats are significantly less parasitized by the trematode *Uvulifer* sp. than a population from a non-sulfidic habitat. We suggest that reduced parasite prevalence may be a benefit of living in sulfidic habitats.

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1. Introduction

Living under extreme environmental conditions is usually associated with costs; however, very little is known about potential benefits. Townsend et al. (2003) point this out by defining an extreme environmental condition as one that requires, of any organism tolerating it, costly adaptations absent in most related species. These can include changes in morphology

and physiological pathways that allow coping with a physiochemical stressor, as well as behavioral adaptations and shifts in life history strategies. But why do organisms colonize extreme habitats if there are immediate costs ranging from the investment towards specific adaptations to an increased risk of death?

Given the associated costs, it could be argued that the organisms become trapped in an extreme habitat. However,

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many extreme habitats are not isolated but contiguous, and no permanent discontinuity prevents organisms from returning to their original habitat. Colonizing extreme habitats would be directly advantageous when individuals that have the ability to cope with the extreme environment confer an advantage compared to relatives living in non-extreme habitats. Selection favoring individuals with adaptations will lead to adaptive shifts within populations as colonizers of extreme habitats exploit new and unused niches (Romero and Green, 2005).

In either way, if organisms persist in extreme habitats over evolutionary time scales, resource investment into the costly adaptations that allow for survival must be traded off. Thus, organisms living in extreme habitats have to invest more into specific adaptations, but they also may maintain or even increase their fitness compared to adjacent populations in non-extreme habitats. Since extreme habitats often harbor impoverished biocoenoses (Begon et al., 1996), the advantages of living in extreme habitats may include the reduction of

competition and predation and the exploitation of new niches (Romero and Green, 2005); however, very few tests of these ideas have thus far been published.

Another potential advantage of living in an extreme habitat that has received no attention so far is that such habitats may act as “refuge” from parasites and diseases. Parasites are ubiquitous, and infections often have significant consequences for the host. Parasites can directly affect viability and fertility of the host with consequences for the host’s reproductive fitness (Bush et al., 2001). By avoiding or at least reducing the infection risk by parasites, hosts may increase their fitness and thereby trade off costly adaptations needed to survive in an extreme habitat.

There are basically two proximate mechanisms that can lead to a decreased risk of a parasite infection in extreme habitats (Fig. 1). Firstly, physiochemical stressors can have the same direct detrimental effect on free-living parasite stages as on every other organism. Thus, unless the parasite has the same ability to cope with the extreme environment as

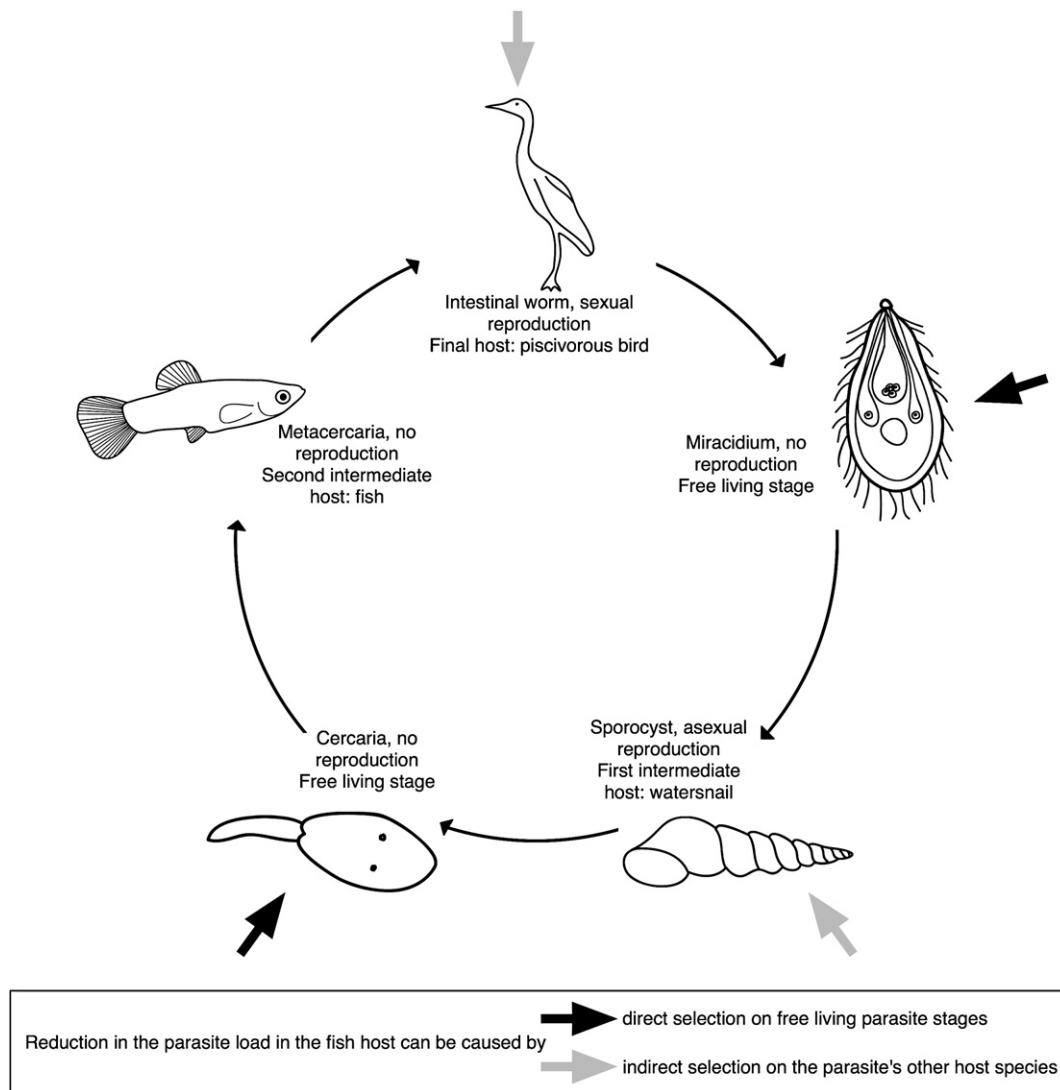


Fig. 1 – Life cycle of *Uvulifer* sp., a trematode parasite with an indirect life cycle. Hosts, e.g. the fish as second intermediate host, can escape infections by entering extreme habitats with environmental conditions that cannot be tolerated by either free-living parasite life stages or other hosts on which the parasite relies.

the host, it will be less successful and may even disappear from the habitat. Secondly, many parasites have indirect life cycles and rely on more than one host species as they go through different developmental stages. The lack of any necessary host species that does not survive in the extreme habitat interrupts the life cycle of the parasite. Thus, the absence of an obligate host species indirectly leads to the local extinction of the parasite.

Based on this hypothesis, two empirically testable hypotheses can be made. (1) Given that a parasite species has at least one of the above mentioned characters (free-living stages or multiple host species), its prevalence should be reduced in more extreme habitats. (2) On the level of parasite communities, it is predicted that hosts in more extreme habitats harbor fewer parasite species. Furthermore, parasite communities should generally shift towards species with a direct life cycle, species lacking long lasting free-living stages and species living inside rather than on their hosts.

A potential model system to test this hypothesis is a small livebearing fish, the Atlantic molly (*Poecilia mexicana* Steindachner, Poeciliidae), which is widely distributed on the Atlantic versant from northern Mexico to northern Costa Rica (Miller, 2005). Besides normal stream and river habitats, this species also inhabits a limestone cave (the Cueva del Azufre) drained by a creek (Gordon and Rosen, 1962). The cave population of *P. mexicana* is also known as the Cave molly (Parzefall, 2001). The creek running through the Cueva del Azufre is fed by several springs with high concentrations of hydrogen sulfide (H_2S , Tobler et al., 2006). It eventually leaves the cave and forms a sulfurous surface creek, El Azufre.

Hydrogen sulfide is highly toxic for all animals, because it binds to the iron of the heme to replace O_2 . It also binds at the cytochrome c oxidase, where it prohibits electron transport in aerobic respiration (Lovatt Evans, 1967; Stallones et al., 1979; Grieshaber and Völkel, 1998). In the cave, H_2S ranges from 10 to 300 μM ; such concentrations usually are considered toxic (Arp et al., 1992; Völkel and Grieshaber, 1992). Consequently, the Cueva del Azufre and El Azufre can be viewed as extreme habitats (Tobler et al., 2006). How *P. mexicana* copes with H_2S is so far not well understood. A costly behavioral adaptation, aquatic surface respiration (ASR), where fish exploit the air–water interface, has been shown to be crucial for the survival in sulfidic water (Plath et al., submitted). Other fish are able to detoxify sulfide to some extent, e.g. by oxidizing sulfide to thio-sulfate (Bagarinao and Vetter, 1990), but physiological adaptations to H_2S remain to be studied in *P. mexicana*.

It has been suggested that in the Cueva del Azufre chemoautotrophic primary production provides *ad libitum* amounts of food for the mollies (Langecker et al., 1996). Mollies from sulfurous habitats, however, are in a worse nutritional state than mollies from non-extreme habitats and have a lower body condition, indicating that energy may in fact be limited (Plath et al., 2005; Tobler et al., 2006). Compared to adjacent surface habitats, the Cueva del Azufre and El Azufre harbor an impoverished fish fauna with *P. mexicana* as the predominant species (Tobler et al., 2006). Hence, interspecific competition for resources and predation by piscivorous fishes is reduced in the sulfidic habitats.

Other potential benefits of colonizing the Cueva del Azufre might also play a role in this system. Thus, we asked if living

in an extreme habitat confers an advantage to *P. mexicana* with regard to a reduced risk of parasite infection by testing the first of the aforementioned predictions. Mollies are known to harbor a diverse parasite fauna (Tobler and Schlupp, 2005; Tobler et al., 2005; Tobler, unpublished data). One of the most prevalent species is the digenean trematode *Uvulifer* sp., the metacercariae of which provoke the production of a fibrous capsule of host tissue around the parasite, which is followed by the migration of melanocytes into the cyst's wall, creating the characteristic appearance of a black spot (black spot disease, BSD; Spellman and Johnson, 1987; Bush et al., 2001). This reaction of the host is assumed to be costly, since the penetration of the skin causes mechanical damage. Until the parasite becomes encapsulated, the host's metabolic demand increases significantly so that energy reserves may decline. *Uvulifer* sp. has an indirect life cycle (Fig. 1). After encapsulation in the fish host, the parasite remains dormant until the intermediate host is consumed by a piscivorous bird, the final host in which the parasite reproduces sexually. Water snails are the first intermediate hosts in which the parasite multiplies asexually, and free-swimming cercariae are produced. These cercariae infect fishes as second intermediate hosts by penetrating the skin and transform into encysted metacercariae. This parasite thus has both characters to test the first prediction formulated above.

In the present study, we compared parasitization of *P. mexicana* by *Uvulifer* sp. among different extreme and non-extreme habitats. Furthermore, we attempted to investigate whether a potential reduction of parasitism in extreme habitats is caused by selection on free-living parasite stages or on other host species.

2. Materials and methods

2.1. Field sites

All study sites are located near the village Tapijulapa in Tabasco, Mexico. The creeks studied eventually drain into the Río Oxolotan. The Río Oxolotan itself joins the Río Amatán and forms the Río Tacotalpa, a tributary of the Río Grijalva-system.

We caught *P. mexicana* in cave chambers III, IV, V, X and XIII of the Cueva del Azufre (Gordon and Rosen, 1962). Additionally, fish were caught in El Azufre, a surface habitat containing toxic H_2S . Currently, these are the only known sulfidic waters that harbor populations of *P. mexicana*. As a reference habitat, the closest comparable tributary to the Río Oxolotan on the opposite side of the river was chosen: Arroyo Cristal. This creek is comparable to the El Azufre in terms of size, structure and the adjacent surrounding. Details on the study sites can be found in Tobler et al. (2006). H_2S concentrations were highest within the cave (up to 300 μM); in El Azufre they ranged from 10 to 40 μM . H_2S was absent in Arroyo Cristal (Tobler et al., 2006).

2.2. Data collection

Fish were caught using small seines and dip nets. All trematode induced black spots on the body surface of the fish were counted. Furthermore, we estimated the occurrence of

Uvulifer's potential final and first intermediate hosts. Piscivorous bird species were qualitatively recorded in the morning and in the evening during 10 days in August 2004. Additionally, we identified the snail species inhabiting the different habitats, and we estimated their density by counting the numbers of snails in randomly selected patches (13 × 14 cm). A sub-sample of snails was checked for trematodes in the gonads by preparing the soft body parts of conserved specimens.

2.3. Data analysis

Quantitative descriptors of parasitism were calculated according to Bush et al. (1997) and analyzed as suggested by Rózsa et al. (2000). The prevalence, as the proportion of individuals infected, was calculated and compared between populations using a χ^2 test. Furthermore, the mean abundance, as the mean number of parasites per host examined, and the mean intensity, as the mean number of parasites per infected host, were calculated. Mean abundance and mean intensity were analyzed using a GLM where "number of cysts" was the dependent variable and "population" and "sex" were independent variables. Snail densities were compared between habitats using a Mann-Whitney U-test. Alpha levels were corrected according to the number of multiple comparisons using approximate Bonferroni adjustments ($\alpha' = 0.05/\text{number of multiple comparisons}$; Grafen and Hails, 2002). Statistical analyses were performed using SPSS 11 (SPSS Inc.).

3. Results

3.1. Parasite prevalence and intensity

The prevalence of BSD differed significantly between populations, whereby *Uvulifer* sp. was most prevalent in Arroyo Cristal, less prevalent in El Azufre and absent in the cave (Table 1, $\chi^2 = 218.69$, $P < 0.001$; $\alpha' = 0.0125$). The significant difference between populations was not only driven by eminently low prevalence of *Uvulifer* sp. within the cave, because when the prevalence was only compared between El Azufre and Arroyo Cristal, the difference was still significant ($\chi^2 = 21.017$, $P < 0.001$; $\alpha' = 0.0125$). Furthermore, the mean abundance of *Uvulifer* sp. differed significantly between populations, showing the same pattern as the prevalence (Tables 1 and 2). The factor "sex" had no significant influence (Table 2). Since the parasite was absent in the Cueva del Azufre, the mean intensity of BSD was only compared between the populations from Arroyo Cristal and El Azufre; however, no significant

Table 2 – Generalized linear models (GLM) on the mean abundance and mean intensity of BSD infections in the *Poecilia mexicana* populations studied. The interaction effect of "population" and "sex" was not significant in either case ($F = 0.012$, $P = 0.91$ and $F = 1.42$, $P = 0.243$, respectively) and thus only main effects were analyzed

Factor	df	Mean square	F	P	α'
Mean abundance					
Population	2	59.51	55.425	<0.001	0.0125
Sex	1	0.09	0.086	0.77	0.0125
Mean intensity					
Population	1	9.53	2.00	0.16	0.0125
Sex	1	3.28	0.69	0.41	0.0125

differences between populations and sexes were detected (Tables 1 and 2). The correction of the alpha levels did not influence the results.

3.2. Further hosts in the life cycle of *Uvulifer* sp.

Within the Cueva del Azufre, neither birds nor water snails could be observed. Around both surface habitats, however, several species of piscivorous birds (i.e., potential final hosts of *Uvulifer* sp.) were recorded. We observed Great egrets (*Ardea alba* Linnaeus), Green herons (*Butorides virescens* Linnaeus), Ringed kingfishers (*Ceryle torquata* Linnaeus), Snowy egrets (*Egretta thula* Molina), Least bittern (*Ixobrychus exilis* Gmelin) and Great kiskadees (*Pitangus sulfuratus* Linnaeus).

One snail species (*Pachychilus* cf. *indiorum* Morelet, Pachychilidae) was recorded in the two surface habitats. The snail density was significantly higher in the Arroyo Cristal (median = 55 snails/m² (interquartile range, IQR = 192), N = 31) than in the El Azufre (median = 0 snails/m² (IQR = 55), N = 26; U = 266.00, P = 0.015). Trematodes could not be recorded in any of the examined *P. cf. indiorum* from El Azufre and Arroyo Cristal (N = 29).

4. Discussion

Parasitism in *Poecilia mexicana* through *Uvulifer* sp. was high in the non-extreme surface habitat and reduced in habitats containing hydrogen sulfide. Within the cave, fish infected with BSD were absent from our samples. H₂S concentrations are especially high within the cave (Tobler et al., 2006) and potential final and intermediate hosts of *Uvulifer* sp. were absent. In

Table 1 – Prevalence, mean (± SD) abundance, and mean intensity of BSD infection within the three populations of *Poecilia mexicana* studied

	H ₂ S	Prevalence	Mean abundance (no. of cysts)	Mean intensity (no. of cysts)	Range (no. of cysts)	N
Arroyo Cristal	Absent	0.45	0.98 ± 1.72	2.17 ± 2.00	0–14	159
El Azufre	Low	0.20	0.55 ± 1.60	2.80 ± 2.65	0–11	128
Cueva del Azufre	High	0.00	0.00 ± 0.00	0.00 ± 0.00	0	456

contrast, potential final and intermediate hosts were present in both surface habitats, but snails were less abundant in the sulfur creek. The lower snail density in El Azufre could be due to toxic properties of the water or reduced food availability, as green algae cannot be found in sulfidic waters. We were not able to find any signs of trematode infections in the snails. This, however, may be due to the limited sample size.

Poecilia mexicana from the sulfur creek were significantly less infected with BSD compared to the surface creek without sulfide compounds. The mechanism leading to a reduced prevalence of BSD infection in the extreme habitats cannot be verified on the basis of the present field data. Since *Uvulifer* sp. has an indirect life cycle, the reduction in prevalence in its second intermediate host (*P. mexicana*) can be caused by lower snail densities that may reduce the infection risk for fish, or the toxic H₂S that may have a direct detrimental effect on free-living parasite stages (Fig. 1).

Potentially, the difference in BSD prevalence between the surface habitats may not only be explained by a lower abundance of parasites in the sulfidic habitat, but may rather be due to higher parasite-induced mortality in this harsh environment (compare McKeown and Irwin, 1997). An infection with *Uvulifer* sp. is costly (Spellman and Johnson, 1987; Bush et al., 2001), which might be especially relevant under extreme environmental conditions. BSD induced mortality might be higher in the sulfurous habitat, and increased parasite induced mortality would be an additional cost of living in an extreme habitat. If parasite-induced mortality was higher in extreme habitats, then differences in the mean intensity of BSD would be expected, as the habitat with toxic H₂S would lack heavily infected individuals. In our study, there was no significant difference in the mean intensity of BSD between the two surface habitats. Along with the low density of potential intermediate hosts, this suggests that lower BSD prevalence in the sulfidic habitat is not due to increased BSD induced mortality but rather due to a lower exposure to the parasite. Our study therefore suggests that reduced exposure to parasites may be a benefit of living in extreme habitats.

Parasite communities of hosts living in extreme environments remain largely unexplored. For example, a low diversity of parasites has been documented from hosts of deep-sea hydrothermal vents compared to other deep-sea habitats (De Buron and Morand, 2004). However, it is as yet unclear if the reduced parasite diversity in this system is caused by the presence of physiochemical stressors (Van Dover, 2000), the reduced diversity of potential hosts (Grassle and Maciolek, 1992; Biscoito et al., 2002), or simply the lack or research (De Buron and Morand, 2004).

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