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Life on the edge: hydrogen sulfide and the fish communities of a Mexican cave and surrounding waters

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Abstract Most eucaryotic organisms classified as living in an extreme habitat are invertebrates. Here we report of a fish living in a Mexican cave (Cueva del Azufre) that is rich in highly toxic H_2S. We compared the water chemistry and fish communities of the cave and several nearby surface streams. Our study revealed high concentrations of H_2S in the cave and its outflow (El Azufre). The concentrations of H_2S reach more than 300 µM inside the cave, which are acutely toxic for most fishes. In both sulfidic habitats, the diversity of fishes was heavily reduced, and *Poecilia mexicana* was the dominant species indicating that the presence of H_2S has an all-or-none effect, permitting only few species to survive in sulfidic habitats. Compared to habitats without H_2S, *P. mexicana* from the cave and the outflow have a significantly lower body condition. Although there are microhabitats with varying concentrations of H_2S within the cave, we could not find a higher fish density in areas with lower concentrations of H_2S. We discuss that *P. mexicana* is one of the few extremophile vertebrates. Our study supports the idea that extreme habitats lead to an impoverished species diversity.

Keywords Extremophile teleosts · *Poecilia mexicana* · Cave fish · Hypoxia · Chemoautotrophy · Condition factor

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Introduction

Townsend et al. (2003) defined an extreme environmental condition as one that requires, of any organism tolerating it, costly adaptations absent in most related species. It is often claimed that habitats with extreme environmental parameters have reduced species richness (Begon et al. 1996; Townsend et al. 2003). For example, plant diversity is reduced on plots with low pH in the Alaskan tundra (Gough et al. 2000), and deep-sea hydrothermal vents possess a low species diversity due to extremes in temperature, hypoxia, sulfide, and heavy metals (McMullin et al. 2000; Price 2002; Tsurumi 2003).

However, not all habitats with reduced species richness are harsh. For example, low species richness in an apparently extreme habitat may also be explained by its limited size (MacArthur and Wilson 1967), low productivity, or low spatial heterogeneity (Begon et al. 1996). Hence, it remains an open question if harsh environments are in fact low in species diversity because of the abiotic stressors themselves (Townsend et al. 2003).

Physiochemical stressors like toxic chemicals are thought to directly influence the composition of ecological communities from zoogeographical to local scale

(Begon et al. 1996; Matthews 1998; Townsend et al. 2003). Hydrogen sulfide (H_2S) can clearly be considered an extreme environmental factor for all animal life, because it is acutely toxic (Bagarinao and Vetter 1989; Grieshaber and Völkel 1998; Lovatt Evans 1967; Smith et al. 1977; Theede 1973). Most known animals from sulfidic habitats are invertebrates, which cope with naturally occurring H_2S by (1) avoiding microhabitats with high sulfide concentrations, (2) switching to anaerobic metabolism, (3) excluding sulfide from sensitive tissues, or (4) oxidizing sulfide to more benign forms (Grieshaber and Völkel 1998; McMullin et al. 2000 for reviews). Previous research has focused on the impact of H_2S on species assemblages in deep-sea hydrothermal vents (Peek et al. 1998; Sarrazin and Juniper 1999; Van Dover 2000) or on H_2S as a chemical pollutant for invertebrates (Oseid and Smith Jr 1974) and fishes (Abel et al. 1987; Adelman and Smith Jr 1970; Bagarinao and Vetter 1989, 1990; Colby and Smith Jr 1967; Geiger et al. 2000; Smith Jr et al. 1976). Very little is known about the effects of naturally occurring H_2S and its influence on the composition of freshwater species communities (Dare et al. 2001).

Natural H_2S is present in a cave, Cueva del Azufre, and its outflow in tropical Mexico (Gordon and Rosen 1962). The dominant species in the cave is a cavernicolous form of a live-bearing fish, the Atlantic molly, *Poecilia mexicana* (the Cave molly; Parzefall 2001). Although *P. mexicana* from Cueva del Azufre and adjacent waters are used as a model system to study the evolution of cave adaptations (Parzefall 1969, 1993, 2001; Plath et al. 2003a, b, 2004, 2005, 2006), so far little is known about the environmental characteristics of their habitat. Here we used Cueva del Azufre and adjacent waters to study the effects of H_2S on the diversity of fish communities.

The reduction of species diversity in caves is usually attributed to the lack of light and the associated lack of photoautotrophy (Barr and Holsinger 1985; Hüppop 2000). Many cave ecosystems completely rely on organic matter washed in from the surface (Parzefall 1993; Poulson and Lavoie 2000; Poulson and White 1969) and only specialized cave dwellers are thought to be able to cope with these conditions. Cueva del Azufre is thought to be different from most other caves in that the food web appears to be energy rich even compared to surface habitats and to rely mainly on in situ chemoautotrophic bacterial primary production and the input of guano by bats (Langecker et al. 1996). Within the cave, mollies were reported to feed on bacterial detritus and bat guano (Langecker et al. 1996), and mosquito larvae were found in their guts (M. Tobler, pers. obs.).

The major objective of our study was an analysis of the abiotic environmental conditions in different habitats in and around Cueva del Azufre to estimate the effects of these parameters on the composition of fish communities. To account for possible interactive effects of darkness and the presence of H_2S, we also examined a sulfidic creek outside the cave, where the absence of light cannot have an influence on the fish communities, and contrasted it with nearby non-sulfidic, but otherwise similar habitats. For a comparison at a between-species level, we examined the fish communities of the different habitat types. For the only species that occurs in all habitats examined, the Atlantic molly (*P. mexicana*), data on body conditions across habitat types as well as population densities in two different cave chambers were determined, which allowed us to estimate how different environmental conditions might act as limiting factors on a within-species level.

Methods

Study sites

All study sites are located near the village of Tapijulapa in the state of Tabasco, south Mexico. All creeks studied eventually drain into the Río Oxolotan, which is part of the Río Grijalva drainage system. We included several habitats in the immediate vicinity (within a perimeter of about 2 km) of Cueva del Azufre (17°26.5′N, 92°46.5′W), where H_2S and darkness occur in varying combinations and/or intensities (Fig. 1; Table 1). (1) The cave itself is sulfidic and the front chambers obtain some dim light, whereas the rearmost cave chambers are completely dark. Nomenclature of the cave chambers follows Gordon and Rosen (1962). Chambers III, IV, V, X, and XIII were sampled. (2) The creek flowing out of

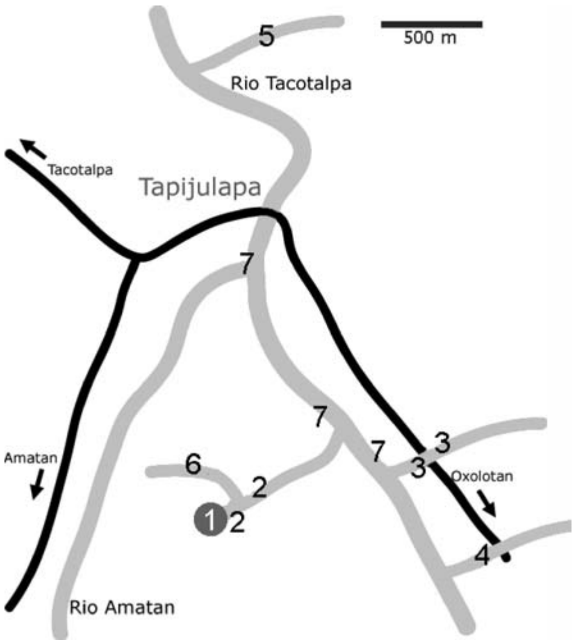

Fig. 1 Schematic view of the watercourses (*gray*) and collection sites (*numbers*) around the village of Tapijulapa (Tabasco, Mexico). Major streets are shown in *black*. Sulfidic habitats—*1* Cueva del Azufre (exit) and *2* El Azufre. Non-sulfidic habitats—*3* Arroyo Cristal, *4* Arroyo Bonita, *5* Arroyo Tres, *6* Clear Creek, and *7* Rio Oxolotan

Table 1 Brief comparison of the main sampling sites

	Cave	El Azufre	Arroyo Cristal, Bonita, Tres	Clear Creek	Ro Oxolotan
Light	Absent	Present	Present	Present	Present
H_2S	Present	Present	Absent	Absent	Absent
Width (m)	1–7	2–5	3—5	1–2	40
Mean depth (m)	0.5	1.2	1	0.3	?
Current	Low-moderate	Moderate	Moderate	Low	High
Predominant ground	Bedrock, silty sediments	Gravel	Cobble, gravel	Gravel	?
Surrounding	Secondary tropical rainforest, pastures, agriculture				
Sampling effort	High	High	Moderate	Low	Low

the cave (El Azufre) is sulfidic but is exposed to sunlight. The other surface waters sampled lack any sulfidic components and also have normal exposure to light. (3–5) Three creeks of similar size and structure to El Azufre were used for a direct comparison: Arroyo Cristal, Arroyo Bonita, and Arroyo Tres (the latter two were only sampled in January 2006). (6) A small freshwater tributary (Clear Creek) of El Azufre and (7) the Río Oxolotan were also included in our analysis.

Water chemistry

Water parameters were measured in September 2002, August 2004, and January 2006 using a Hydrolab Multiprobe 4A, which measures several variables at the same time. Measurements and calibration of probes were conducted according to the manufacturer's recommendations. Specific conductance was measured in mS/cm, dissolved oxygen in mg/l and % saturation, temperature in °C, and turbidity using a shuttered turbidity probe in nephelometric turbidity units. Measurements of temperature and light over 24 h were conducted using Onset Stow Away loggers. Data presented are means of several measurements (2–4), which were collected at several sites within the mentioned habitats (Table 2).

To determine H_2S contents, represented by the total concentration of sulfide, samples were collected in August 2004 and January 2006 on site. One milliliter of water was injected into a vial containing 2 ml of zinc acetate (0.12 M with 0.5 ml NaOH 1.5 M) using a syringe. The vials were stored at room temperature, and photometric measurements were conducted according to Cline (1969). The data presented in Table 2 are means of one to four measurements.

Comparison of fish communities

In order to compare the fish communities, fish were collected in August 2004 and January 2006 at one to three sites within each habitat, and data from each site were pooled (Fig. 1; Table 3). Within the cave, chambers III, IV, V, X, and XIII, which include all major microhabitat types, were sampled. In El Azufre, data were collected right outside the cave exit, around the mouth of Clear Creek, and 250 m downstream. In Arroyo Cristal and Arroyo Bonita, fishes were collected in a 150-m long stretch, and in Arroyo Tres, in a 100-m long stretch. In Clear Creek, data were collected in a 200-m long stretch from the mouth upstream. In all surface creeks, sampling area included several riffles and pools. In the Río Oxolotan fishes were caught downstream of the mouth of Arroyo Cristal as well as at two boat ramps downstream of the mouth of El Azufre and in the village of Tapijulapa.

Because habitat structures differed strongly between sampling sites, various sampling methods were employed. In the cave, where the water is very shallow, fishes were caught with dip nets (13×14 cm, 1 mm mesh-width). In El Azufre, Arroyo Cristal, Arroyo Bonita, and Arroyo Tres, fishes were caught with a seine (4 m long, 4 mm mesh-width) and a cast net (2.5 m in diameter, 6 mm mesh-width). In Clear Creek, both dip nets and the seine were employed. In the Río Oxolotan, the seine, the cast net, and dip nets were used, and catches of local fishermen were qualitatively surveyed in January 2006.

Fishes were counted and photographed using a Nikon D70 digital camera. Species identity was determined ad hoc or using the photographs following Miller (2005). After identification, the fishes were released at the collection site. Abundance of fish species was classified in the following categories—rare, 1–5 individuals; common, 5–50 individuals; abundant, > 50 individuals. Nomenclature is in accordance with Miller (2005). For the comparison of the species diversity of each habitat type, the Shannon–Wiener diversity index (H) and the evenness index (J) were calculated with the combined data from 2004 and 2006 (Begon et al. 1996).

Population densities in the cave

We compared population densities in two sub-populations of the cave molly from two cave chambers (X and XIII). Both chambers are essentially dark. This

Table 2 Water chemistry of several chambers of Cueva del Azufre and adjacent surface habitats from 2002, 2004, and 2006. Nomenclature of cave chambers follows Gordon and Rosen (1962). For sites outside the cave refer to main text

Site	Date	Water temperature (°C)	pH	Specific conductivity (mS/cm)	Turbidity (NTU)	DO (mg/l)[a]	DO Saturation (%)	Sulfide range (μM)
Cave (III hole)	August 2004	28	7.0	3.95	95.4	0.84	13.2	
Cave (III stream)	August 2004	28	7.0	3.95	81.4	0.85	13.6	
Cave (IV hole)	August 2004	28	7.0	3.99	67.9	0.72	11.4	
Cave (IV stream)	August 2004	28	7.0	3.95	76.8	0.91	14.2	
Cave (V)	August 2004	28	7.0	3.96	85.0	1.35	21.3	32
	January 2006	28	7.1	4.23	45.3	1.07	14.1	2–24[b]
Cave (IX below cascades)	August 2004	28	7.1	3.94	82.1	2.04	32.1	45
	January 2006	28	7.2	4.19	39.0	1.79	23.3	34–45[c]
Cave (X clear area)	September 2002	28	6.8	4.25	6.9	1.23	15.6	
	August 2004	28	6.8	3.95	43.9	1.44	23.1	199
	January 2006	28	7.0	4.20	9.0	2.45	32.0	
Cave (X spring I)	August 2004	28	6.7	4.18	10.1	0.32	4.8	234
	January 2006	28	6.7	4.20	9.8	0.38	6.0	129–190[c]
Cave (X spring II)	September 2002	28	6.7	4.20	18.4	0.29	3.8	
	August 2004	28	6.7	3.99	9.6	0.35	5.7	295–320[d]
	January 2006	28	6.7	4.32	8.0	0.90	3.3	124–148[c]
Cave (X turbid area)	September 2002	28	7.1	4.22	70.0	2.06	26.8	
	August 2004	28	7.1	3.93	74.6	1.95	30.6	137
	January 2006	28	6.9	4.25	39.0	1.65	21.6	2–27[c]
Cave (XIII)	September 2002	28	7.2	4.06	37.3	3.10	41.0	
	August 2004	28	7.2	3.94	33.0	4.01	64.1	0–1[c]
	January 2006	28	7.6	4.23	12.5	3.11	40.4	0–1[c]
El Azufre (cave exit)	September 2002	28	7.1	4.18	67.0	1.10	14.7	
	August 2004	28	7.1	3.94	68.4	1.18	18.9	37
	January 2006	28	7.1	4.22	104.3	1.05	13.8	23–41[c]
El Azufre (bridge 2)	August 2004	28	7.3	3.91	50.5	1.82	28.9	11
	January 2006	28	7.2	4.09	45.3	1.35	17.7	10–33[c]
Clear Creek	September 2002	27	7.3	2.17	55.0	2.7	34.1	
	August 2004	25	7.5	2.16	13.2	4.06	60.0	[e]
Arroyo Cristal	August 2004	28	7.8	0.38	28.6	5.75	82.8	
	January 2006	23	8.2	0.38	0.0	4.34	50.8	0[b]
Arroyo Bonita	January 2006	23	8.3	0.34	0.0	4.7	54.1	0[c]
Arroyo Tres	January 2006	22	7.6	0.29	80.5	3.1	36.3	0[c]
Río Oxolatan	September 2002	23	8.3	0.27	87.6	8.3	97.9	
	August 2004	26	8.3	0.39	155.6	6.25	95.0	[e]

NTU nephelometric turbidity units

[a] Oxygen-saturated water contains 6.4 ml/l O_2 at 20°C at sea-level

[b] Range of four measurements

[c] Range of two measurements

[d] Range of three measurements

[e] Samples were taken but produced no reliable readings

comparison was especially interesting because the two chambers differ in the presence of H_2S and oxygen concentrations (Table 2). A small cascade (1.5 m high) separates both chambers, so that migration is likely mostly unidirectional from chamber XIII to X.

Population sizes were estimated by using mark-recapture analyses and by calculating the Lincoln index (Mühlenberg 1989). Fish were caught with dip nets for 45 min by two persons. We marked the fish by clipping their dorsal fin. Observations from laboratory-reared mollies have shown that this procedure does not harm the fish, and the removed fin tissue usually regenerates within approximately 1 week. No dead fish were observed in the cave after releasing the handled fish. After 24 h, sampling was repeated. We counted the total number of mollies caught and the number of marked (recaptured) individuals.

Densities were calculated by dividing the mean values of the estimated population sizes by the area of the respective cave chamber. The area of chamber XIII was estimated as 10 m^2, that of chamber X as 85 m^2.

Condition factor of *Poecilia mexicana*

Another factor we considered in this study was the general body condition of the only fish species present in all habitat types, *P. mexicana*. We determined the body condition factor for male and female *P. mexicana* living in the different habitats. The condition factor [1,000 × mass (g)/standard length (mm^3)] was determined in *P. mexicana* larger than 20 mm from cave chambers III, IV, V, XI ($N = 265$), and XIII ($N = 144$), from El Azufre ($N = 100$) and Arroyo

Table 3 Summary of the fish communities in the different habitats sampled in and around Cueva del Azufre in August 2004 and January 2006. The total number of species, the Shannon–Wiener diversity index (*H*) and the evenness index (*J*) is given for each habitat. Additionally, the abundance for each species within habitats is presented for 2004/2006

	Cave	El Azufre	Clear Creek	Arroyo Cristal	Arroyo Bonita[a]	Arroyo Tres[a]	Río Oxolotan
Number of species	1	2	3	11	9	6	14
H	0.00	0.05	0.82	1.79	1.70	1.59	1.92
J	–	0.06	0.77	0.81	0.76	0.80	0.78
Characidae							
Astyanax aeneus (Günther 1860)				a/a	a	a	a/a
Brycon guatemalensis Regan 1908							[b]
Ariidae							
Potamarius nelsoni (Evermann & Goldsborough 1902)							[b]
Pimelodidae							
Rhamdia guatemalensis (Günther 1864)						r	
Batrachoididae							
Batrachoides goldmani Evermann & Goldsborough 1902							r/–
Atherinopsidae							
Atherinella alvarezi (Díaz Pardo 1972)				–/r	c		c/c
Poeciliidae							
Heterophallus milleri Radda 1987					c		a/a
Heterandria bimaculata (Heckel 1848)			a/c	c/c		c	r/r
Poecilia mexicana Steindachner 1863	a/a	a/a	c/c	a/c	c	c	c/c
Priapella sp.[c]				c/a	c		
Xiphophorus hellerii Heckel 1848			r/c	r/c	r	c	
Centropomidae							
Centropomus undecimalis (Bloch 1792)							[b]
Cichlidae							
'Cichlasoma' salvini (Günther 1862)		r/–		–/r			r/–
Oreochromis cf. aureus (Steindachner 1864)							[b,d]
Paraneetroplus gibbiceps (Steindachner 1864)				r/r			
Thorichthys helleri (Steindachner 1864)				c/c	r		c/c
Vieja bifasciata (Steindachner 1864)				c/c	r	r	c/r
Vieja intermedia (Günther 1862)				–/r			
Eleotridae							
Gobiomorus dormitor Lacépède 1800					r		

a abundant, *c* common, *r* rare

[a] Only sampled in 2006

[b] Recorded in catches of local fishermen in 2006

[c] Undescribed species

[d] Introduced species

Cristal ($N = 100$). Standard lengths were measured to the closest millimeter using scale paper. Mass was measured to the closest 0.1 g using a Pesola scale. Based on the results of the water parameter analyses, *P. mexicana* from cave chamber XIII were treated as a separate population compared to the rest of the cave. This distinction is further justified due to the barrier between chamber XIII and the other parts of the cave (see above). Data were analyzed using 'population' as between factor and 'sex' as within factor in a two-way ANOVA. Since the interaction term was not significant ($F_{3,601} = 0.19$, $P = 0.90$), only the main effects were analyzed. For post hoc contrasts, Fisher's protected least significant difference was employed.

Results

Water chemistry

We found variation in H_2S and oxygen concentrations within the apparently homogenous cave, whereby H_2S and oxygen concentrations differed even within very short distances. The typical inverse relationship of oxygen and H_2S was found (Table 2). Furthermore, H_2S concentrations apparently vary over time. The values measured in 2006 were generally lower than those from 2004, possibly due to heavy rainfalls before and during sampling. The innermost cave chamber (XIII) exhibited the least extreme conditions within the cave. Water entering this chamber through cracks in the wall has relatively high amounts of oxygen and very low sulfide concentrations. By contrast, a small springhead only a few meters away in chamber X has almost no oxygen, but is very rich in H_2S with concentrations reaching 300 μM (Table 2). Downstream areas of the cave (chambers III, V) were richer in oxygen and had less H_2S. Turbidity within the cave appears to coincide with amounts of colloidal sulfur in the water, which is produced by the oxidation of H_2S. While the springs are clear and rich in H_2S, parts of the cave with mixing appear milky. Specific conductivity is uniform throughout the cave and pH is well buffered probably due to the limestone of the cave. Only in the immediate spring areas pH was lower than 7, which may reflect an interaction with the H_2S (Table 2). Typical for the cave habitat is a nearly constant water temperature of 28.3°C. Continuous temperature measurements over 24 h in February 1998 and August 2004 in chamber XIII revealed no variability in temperature. Continuous measurements over 24 h (1998) of the light intensities in chambers X and XIII read 0 Lux and confirmed complete darkness for these parts of the cave.

In El Azufre, H_2S concentrations are lower than in the cave and decrease with increasing distance from the cave exit. Although clear and most likely without H_2S, Clear Creek also has low oxygen and lowered specific conductance (Table 2). This contrasts with high values for oxygen in the other surface habitats (Table 2).

Comparison of fish communities

Extensive sampling in different chambers inside Cueva del Azufre revealed only one species of fish: the cavernicolous form of *P. mexicana* (Table 3). Juveniles and adults were caught. An estimation of the species richness revealed a pattern of low numbers of species in habitats containing H_2S and higher species richness in habitats without sulfur components (Table 3). A direct comparison of El Azufre to Arroyo Bonita, Arroyo Cristal, and Arroyo Tres, all streams of similar size and structure, revealed a considerably higher number of species in the non-sulfurous habitats (Table 3).

The low value of the evenness index in El Azufre compared to the benign surface habitats reflects the overdominance of one species: *P. mexicana*. Only one further fish species, the predatory cichlid '*Cichlasoma*' *salvini* occurs, but only in small numbers. The Río Oxolotan harbors a fish community comparable in species composition and diversity to that of the non-sulfurous creeks. In Clear Creek, a small stream that is directly connected to El Azufre, *P. mexicana* was not dominant, and *Heterandria bimaculata* occurred at high abundance. *Xiphophorus hellerii* was recorded in small numbers near the mouth of Clear Creek into El Azufre, but always in clear water.

Population densities in the cave

Population sizes were repeatedly analyzed in cave chamber XIII. The estimated population sizes were highly consistent between years (Table 4). Population densities were similar between the two cave chambers examined, with 12.47 ± 0.35 (mean ± SD) individuals per m^2 in chamber XIII and 19.58 ± 11.12 individuals per m^2 in cave chamber X.

Condition factors of *Poecilia mexicana*

A comparison of condition factors between *P. mexicana* from different habitats revealed pronounced differences between populations (ANOVA—$F_{3,604} = 64.54$, $P < 0.0001$; Fig. 2). Surface fish from Arroyo Cristal showed higher condition factors than cave fish and fish from El Azufre, which in turn had higher condition factors than fish from the cave. Furthermore, *P. mexicana* from chamber XIII showed a slightly, but significantly worse body condition compared to fish from the other cave chambers. Sex had no significant effect on the condition factors ($F_{1,604} = 1.58$, $P = 0.21$).

Discussion

Our study revealed high concentrations of H_2S in Cueva del Azufre and its outflow. In both habitats, the diversity of fishes was heavily reduced and *P. mexicana* was the dominating species. Compared to habitats without H_2S, *P. mexicana* from the cave and El Azufre have a significantly lower body condition. Although there are microhabitats with different concentrations of H_2S within the cave, we could not find a higher fish density in habitats with lower concentrations.

Environmental conditions

Except for the presence of H_2S in Cueva del Azufre and El Azufre, the water parameters reported here are in agreement with those reported for the wider area (Mayland 1984; Stawikowski and Werner 1998). Outside the cave, differences between habitats are best explained by the fundamental differences between rivers and small creeks. Within the cave, there was considerable variation in H_2S and oxygen concentrations within short distances. This patchiness was previously unrecognized. Sulfide concentrations seem to vary to some extent over time and are likely dependent on the discharge of the

Table 4 Cave molly population size estimates (mean ± SD) from two cave chambers (X and XIII). Juveniles (standard length < 30 mm) were not assessed in 1998 and 1999

Population	Year	Marked (day 1)	Caught (day 2)	Recaptured	Estimated population size	
Cave (XIII)	1998	53	54	28	102 ± 13	Adults
	1999	31	18	4	139 ± 62	Adults
	2002	39	34	16	309 ± 91	Adults + juveniles
	2004	83	55	12	380 ± 97	Adults + juveniles
Cave (X)	1998	52	96	3	1664 ± 954	Adults

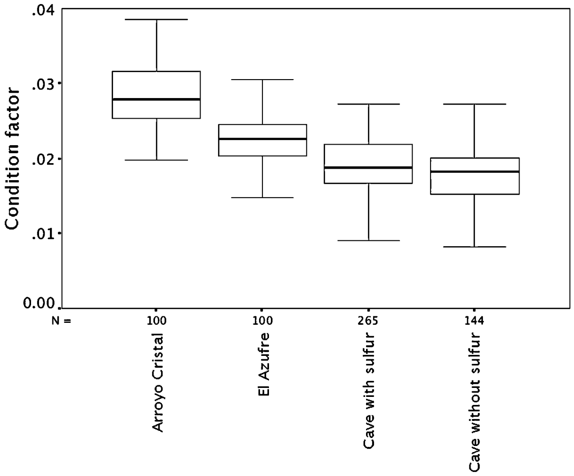

Fig. 2 Condition factors of *Poecilia mexicana* from different habitats. Clear water surface habitat (*Arroyo Cristal*), sulfidic surface creek (*El Azufre*), cave chambers with sulfidic components (*cave with sulfur*), and rearmost cave chamber XIII without sulfide (*cave without sulfur*). A post hoc analysis revealed that condition factors differed significantly between all populations (Fisher's protected least significant difference, $P < 0.01$ in all cases)

springs relative to the precipitation in the area. Thus, the toxicity of the water might peak during the dry season in February to April. Further studies are needed to estimate the degree and relevance of temporal variation in sulfide concentrations in the cave. The presence of H_2S indicates a chemically reduced environment. Potentially, other toxic substances, such as elevated concentrations of dissolved metals, coincide with H_2S, but Cave mollies have very low mercury concentrations compared to surface fish (Chumchal pers. comm.).

Two competing mechanisms apparently influence the oxygen content of the water. Oxygen concentrations clearly rise below areas of turbulence (e.g., chamber IX), but become low again downstream (chamber V). While mixing with air leads to increased oxygen, bacterial metabolism likely leads to decreased oxygen content. This can explain the relatively low oxygen values toward the exit and outside the cave. The close match of the readings from 2002, 2004, and 2006 indicates very high constancy of the abiotic conditions in the cave.

H_2S and fish communities

The fish communities documented here are typical for Central American fish communities in that cichlids and poeciliids were the dominant species (Miller 1976, 2005). A potential criticism of our study could be that the catch per unit effort was not identical across habitats and that we may have underestimated the fish diversity of some habitats, especially that of the Río Oxolotan, where water levels were high during our visits. However, catching efforts were lower in non-sulfidic surface habitats so that the reported high species diversity is a rather conservative estimate of the actual diversity. In contrast, catching efforts were very high within the cave and the El Azufre during several expeditions. Therefore, our data are suitable for a comparison of the fish communities between the different habitats studied.

Fish communities were most diverse in benign habitats, and were impoverished in both sulfidic habitats. In comparison to El Azufre, the non-sulfidic surface creeks of similar size and structure harbored a more diverse fish community. Our data therefore suggest that the presence of H_2S strongly influences the composition of the fish communities, which leads to pronounced differences even within short distances. We did not find any evidence for fine-scale changes of fish community compositions along the gradient of H_2S concentration in Cueva del Azufre and El Azufre, but the presence of H_2S rather seems to have an all-or-none effect, permitting only few species to survive in these habitats. The same pattern was found in other sulfidic freshwater habitats in southern Mexico, such as the Baños del Azufre (M. Tobler et al., unpublished data) and in metazoan communities in deep-sea habitats containing H_2S (McMullin et al. 2000; Price 2002; Tsurumi 2003). This supports the idea that extreme conditions directly translate into low species diversity (Townsend et al. 2003).

The small-scale distribution of oxygen-rich areas in the cave makes it likely that cave mollies can choose more favorable microhabitats by avoiding areas with extreme conditions. However, our estimations of fish densities in cave chamber X (high concentrations of H_2S) and XIII (low concentrations of H_2S) did not show pronounced differences. Possibly, food in chamber XIII is especially scarce, since primary production relies on H_2S, which is low in this chamber. Food shortage in chamber XIII is reflected by the eminently low condition factor of its inhabitants. Fish densities appear to be highest downstream toward the cave exit coinciding with intermediate H_2S and oxygen values, but a systematic survey there remains to be done.

Given that the major differences of fish community composition are evident between Clear Creek and El Azufre, not between the cave and El Azufre, light seems to play a subordinate or no role on the between-species level. On a within-species level, the presence or absence of light appears to have a strong effect on the distribution of the two phenotypically distinct forms of *P. mexicana*: the cave form (Parzefall 2001) and the surface form living in El Azufre. It remains to be studied if and to what extent the phenotypic differences between the surface form and the cave form have a genetic basis, and how environmental effects, namely the presence of light and the availability of food, influence the ontogeny of this species.

Adaptations to H_2S

The most plausible explanation for how the presence of H_2S causes the observed reduction of species diversity is its toxic nature. High concentrations of H_2S are acutely toxic for most eucaryotic organisms, because H_2S binds at the iron of the heme to replace O_2 and also at respiratory chain proteins (e.g., the cytochrome c oxidase), where it prohibits electron transport in aerobic respiration (Bagarinao 1992; Carrico et al. 1978; Grieshaber and Völkel 1998; Lovatt Evans 1967; Nicholls 1975; Smith et al. 1977; Stallones et al. 1979; Theede 1973). Lower concentrations of H_2S are chronically toxic (Stallones et al. 1979). H_2S is known to potentially play a role in natural fish kills (Bagarinao and Lantin-Olaguer 1999; Luther et al. 2004) and sulfide toxicity is known to aggravate mortality due to hypoxia and low pH (Bagarinao and Lantin-Olaguer 1999). Sulfide resistance in fishes varies with habitat and lifestyle, and species that encounter naturally occurring sulfide usually show increased resistance [e.g., *Megalopus atlanticus* tolerate concentrations up to 230 μM (Geiger et al. 2000); *Hoplosternum littorale* up to 87 μM (Affonso and Rantin 2005)]. In fishes from shallow-water marine habitats, LD_{50} concentrations of H_2S range from 1 μM in open-coast inhabitants up to 700 μM in species inhabiting tidal-marshes with naturally occurring H_2S (Bagarinao and Vetter 1989).

Because of the typical inverse relationship between oxygen and H_2S concentrations, the reduction of species diversity may also be influenced by hypoxia. However, the Clear Creek also has very low oxygen concentrations, but harbors a completely different fish community compared to the H_2S-containing habitats. Although migration between Clear Creek and El Azufre is possible without having to overcome physical barriers or strong current, the composition of fish communities changes dramatically within about 3 m.

Previous experiments have shown that *P. mexicana* from non-sulfidic habitats lack any sulfide tolerance and that the short-term survival of cave mollies in water containing hydrogen sulfide depends on the possibility to perform aquatic surface respiration (ASR) and sufficient food availability (M. Plath et al., submitted for publication). During ASR, cave mollies exploit the air–water interface, which is relatively oxygen-rich compared to the rest of the water column (Kramer and Mehegan 1981; Kramer 1987). The fact that energy availability is crucial for survival (individuals supplemented with a high energy food source have a higher chance of surviving in toxic water; M. Plath et al., submitted for publication) highlights the biological significance of the low body condition of cave mollies and may point to costly physiological adaptations to detoxify H_2S. In fishes, detoxification of sulfide is known through its oxidation to thiosulfate in liver mitochondria (Bagarinao and Vetter 1990) and its binding to ferrous and ferric hemoglobin and other blood compounds (Torrans and Clemens 1982; Bagarinao and Vetter 1989). So far it is not clear how cave mollies cope with the long-term toxic effects of H_2S.

Our results suggest that the presence of H_2S reduces the diversity of fish communities. Furthermore, they contradict the hypothesis that food is abundant in this cave ecosystem (Langecker et al. 1996), or at least they suggest that the food base is of poor quality for the fish. Cave mollies have reduced a number of energetically costly behaviors like aggression, shoaling (Parzefall 1993), and male sexual activity (Plath et al. 2003b). Reduction of shoaling and aggression were previously interpreted as adaptations to the absence of light (Parzefall 1993, 2001). The results of this study, however, suggest that the presence of H_2S and restricted food availability are more likely explanations. Future research will have to answer which of the adaptations reported for this species are really adaptations to cave life per se (darkness), which are driven by the extreme water chemistry or low food availability, and how exactly these components interact.

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