Two endemic and endangered fishes, *Poecilia sulphuraria* (Alvarez, 1948) and *Gambusia eurystoma* Miller, 1975 (Poeciliidae, Teleostei) as only survivors in a small sulphidic habitat

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In the Baños del Azufre in Tabasco, Mexico, only two poeciliid species, *Poecilia sulphuraria* and *Gambusia eurystoma*, were found in sulphidic habitats, whereas adjacent non-sulphidic habitats were characterized by a vastly different fish community. Exposure of fishes to water from a sulphidic spring showed that all species except for *P. sulphuraria* quickly lost motion control, probably because of the toxic properties of H₂S. It is suggested that the fishes endemic to the Baños del Azufre are highly specialized, and competitive exclusion allows them to survive only in this particular habitat type.

Key words: adaptation; competitive exclusion; conservation biology; extremophiles; fish communities; hydrogen sulphide.

INTRODUCTION

The theory of island biogeography predicts allopatric speciation through isolation from mainland populations, and thus a high degree of endemism occurs on islands (MacArthur & Wilson, 1967; Begon *et al.*, 1996). In accordance with this theory, small isolated freshwater habitats like crater lakes in Cameroon (Schliewen *et al.*, 1994) and Nicaragua (Stauffer & McKaye, 2002), desert springs (Minckley, 1977) or caves (Romero & Paulson, 2001) often harbour endemic fish species.
Endemic species with very restricted, insular distributions can also be found in areas that are not physically isolated. For example, such species can be found in habitats that differ in environmental conditions from adjacent ones. The abiotic conditions in such isolated habitats can often be classified as extreme, preventing less adapted species of surrounding habitats from colonizing them (e.g. mountain tops that are connected by valleys; Begon et al., 1996).

By analogy, spring and stream habitats containing naturally occurring hydrogen sulphide (H₂S) can be considered islands. The H₂S competes with oxygen in the respiratory chain and is highly toxic for animals even in micromolar amounts (Lovatt Evans, 1967; Smith et al., 1977; Grieshaber & Völkel, 1998). Sulphidic freshwater habitats are usually part of a larger river system and are not physically isolated from adjacent non-sulphidic waters. Their toxicity, however, may restrict the migration of fishes that are not adapted to these conditions and allow only a few specialized species to survive, thereby creating an island-like situation.

Most known animals from sulphidic habitats are invertebrates (Grieshaber & Völkel, 1998; McMullin et al., 2000), and to date, very few freshwater fishes are known to tolerate the toxic effects of H₂S in the long term and to permanently inhabit sulphurous habitats. These include *Cyprinodon bobmilleri* Lozano-Vilano & Contreras-Balderas, 1999 (Cyprinodontidae), which is endemic to a sulphidic spring pool in northern Mexico (Lozano-Vilano & Contreras-Balderas, 1999), *Limia sulphurophilia* Rivas, 1980 (Poeciliidae) which was described from a sulphurous spring in the Dominican Republic (Rivas, 1980), and a distinct population of *Poecilia mexicana* Steindachner, 1863, from the Cueva del Azufre system in the Mexican state of Tabasco (Tobler et al., 2006). Furthermore, some fishes are known to live in temporary sulphidic habitats, where sulphidic conditions arise due to decay of organic matter, e.g. in salt marshes (Bagarinao & Vetter, 1989) and in floodplains of the Amazon (Val & Almeida-Val, 1995).

Another sulphidic freshwater system occurs c. 10 km west of Teapa (Tabasco, Mexico) where a number of sulphidic springs enter a creek forming an area that is locally known as the Baños del Azufre. This habitat harbours two endemic species of poeciliids: the sulphur molly *Poecilia sulphuraria* (Alvarez del Villar, 1948) and the largemouth mosquitofish *Gambusia eurystoma* Miller, 1975. The need for protection of these highly endemic fish species contrasts with the almost total lack of knowledge regarding their biology. Except for the taxonomic data published in the first descriptions and a few subsequent publications (Rodriguez, 1997; Ptacek & Breden, 1998), virtually no scientific work has been published about these fishes. Both species are listed as critically endangered by the IUCN (Groombridge, 1994) and are federally protected by the Mexican government (Norma Oficial Mexicana NOM-059-ECOL-2001: Appendix II). Nonetheless, the springs are used for recreational purposes as most of the springheads are situated within public pools that are used as therapeutic baths. Furthermore, intense agricultural practices of cattle grazing and sugar cane production occur within the immediate vicinity of the springheads. In this paper, data on the water chemistry of this unusual aquatic habitat are presented, and the fish communities in and around the sulphidic habitats are described. Moreover, measurements of sulphide tolerances of representative fish species that live in the Baños del Azufre and adjacent habitats are provided.
STUDY SITES

The Río El Azufre consists of two branches that merge west of the Baños del Azufre (17°33′ N; 93°0′ W) (Fig. 1). The southern branch (Río El Azufre S) contains clear, non-sulphidic water and a cobble streambed. The eastern branch (Río El Azufre E) is fed by multiple sulphidic springs and has a muddy bottom consisting of thick layers of anoxic sediments. Some of the larger sulphur springs are integrated in swimming pools before their water drains into the river. Beside the sulphidic sources, a small tributary draining into the Río El Azufre E has sulphide-free water.

WATER CHEMISTRY

Water variables were measured using a Hydrolab Multiprobe 4A (Hach Environmental, Loveland, CO, U.S.A.). Specific conductance was measured in mS cm⁻¹, dissolved oxygen in mg l⁻¹ and % saturation, and turbidity using a shuttered turbidity probe in nephelometric turbidity units (NTU). Data presented are means of several measurements (two to four) taken at the respective field sites. The H₂S samples were collected from various microhabitats by injecting 1 ml of water into a vial containing 1 ml of zinc acetate (0·12 M with 0·5 ml NaOH 1·5 M in a N₂ atmosphere) using a syringe. The vials were stored at room temperature and photometric measurements were conducted later in the laboratory according to Cline (1969).

FISH COMMUNITIES

To compare fish communities, fishes were collected in January 2006 at various sites (Fig. 1) using a seine (4 m long, 4 mm mesh width) and a cast-net (2·5 m in diameter, 6 mm mesh width). The catch effort was standardized by performing six seine hauls covering c. 20 m² each, as well as 10 hauls with the cast-net at each site. Fishes were counted and species identity was determined using keys provided by Miller (2005). After identification, the fishes were released at the collection site.

Fig. 1. Overview sketch of the Baños del Azufre with sampling sites (numbers). Major streets (—), and houses (■), water courses (— —) and sulphidic springs (●) are shown. The Río El Azufre S as well as the eastern-most part (site 1) of the Río El Azufre E are non-sulphidic. All other habitats contain H₂S.

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SULPHIDE TOLERANCE

Based on the results of the species distribution in sulphidic and non-sulphidic habitat patches, it was tested whether or not the divergent water qualities may prevent migration of individuals towards more sulphidic habitats by transferring individuals from water of their collection site to water of a sulphidic spring was tested. Responses to water from a sulphidic spring were determined in \( P. sulphuraria \) [collected at sites number 2 (low H$_2$S concentration) and number 5 (high H$_2$S concentration); see Fig. 1], \( G. eurystoma \) (site number 2), \( Astyanax aeneus \) (Günther, 1860) (Characidae), \( Heterandria bimaculata \) (Heckel, 1848), \( P. mexicana \) and \( Xiphophorus hellerii \) Heckel (all Poeciliidae; collected at site number 10, which lacks H$_2$S). The last four species represent the most abundant species in the non-sulphidic waters of this area (Table I). Fishes were maintained in water from their respective collection site for a maximum of 4 h prior to the test. Individuals from the different species were tested in random order. For the experiment, individual fishes were introduced into a clear plastic bottle filled with 300 ml of water. Water was retrieved directly from one of the major sulphidic springs (site number 11; Fig. 1 and Table II) and was transported in a closed cooler to the hotel associated with the Baños del Azufre (Fig. 1), in which all experiments were performed. The time until a fish lost motion control (i.e. turned its abdomen upside down and stopped swimming) was measured and afterwards its standard length \( (L_S) \) was determined. Because of its toxicity, H$_2$S probably elicited the response of the fish in the experiment; however, it is important to note that other physio-chemical factors that correlated with the presence of H$_2$S (such as low oxygen, low pH, high-specific conductivity and high temperature; Table II) could have affected the test fish adversely and even aggravated the toxic effects of H$_2$S (Bagarinao & Lantin-Olagauer, 1999). The test water was not aerated because of the reactivity of H$_2$S with oxygen. Aeration leads to a rapid drop of H$_2$S concentration in the water because it is oxidized and ‘gassed out’ (unpubl. data). To control for changes in the water chemistry, the test water was replaced after each trial. After the experiment, fishes were immediately transferred to heavily aerated, sulphide-free water. None of the fishes died during this treatment, and all fishes were released at their respective collection sites after they had fully recovered. Data were analysed using a GLM with ‘time to loss of motion control’ as a dependent variable, ‘species’ and ‘sex’ as independent variables, and \( L_S \) as a covariate. Since the interaction terms were not significant \( (P > 0.05 \) in all cases), only the main effects were analysed.

RESULTS

WATER CHEMISTRY

The H$_2$S contents, represented by the total concentration of sulphide, were zero in the Río El Azufre S and the Río El Azufre E above the first sulphur spring. Sulphide was recorded in the Río El Azufre E below the first sulphur spring as well as downstream of the confluence of the two branches (Table II). The highest readings were obtained in the spring above site number 5, where 601 \( \mu \)M sulphide was measured. A pilot study in August 2004 revealed even higher concentrations at site number 5 (864–919 \( \mu \)M). The typical inverse correlation between H$_2$S and oxygen concentration was corroborated (Table II). Furthermore, sulphidic microhabitats around the Baños del Azufre generally had a higher temperature and specific conductivity as well as a lower pH than non-sulphidic microhabitats (Table II).

FISH COMMUNITIES

The fish communities in the non-sulphurous parts of the Río El Azufre consist of seven species, most of which are common in the Río Grijalva drainage
<table>
<thead>
<tr>
<th>Site</th>
<th>Poecilia sulphuraria</th>
<th>Gambusia eurystoma</th>
<th>Heterandria bimaculata</th>
<th>Poecilia mexicana</th>
<th>Priapella compressa</th>
<th>Xiphophorus hellerii</th>
<th>Astyanax aeneus</th>
<th>Rocio octofasciata</th>
<th>‘Cichlasoma’ salvini</th>
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<td>Site</td>
<td>Water temperature (°C)</td>
<td>pH</td>
<td>Specific conductivity (mS cm(^{-1}))</td>
<td>DO (mg l(^{-1}))</td>
<td>DO saturation (%)</td>
<td>Sulphide (µM)</td>
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<td>Río El Azufre E, above first sulphur spring</td>
<td>22.9</td>
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<td>Río El Azufre E, above large sulphur spring</td>
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<td>6.7</td>
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<td>Río El Azufre E, below large sulphur spring</td>
<td>28.7</td>
<td>6.5</td>
<td>2.850</td>
<td>0.81</td>
<td>10.8</td>
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<td>Río El Azufre E, 600 m above confluence with Río El Azufre S</td>
<td>28.6</td>
<td>6.6</td>
<td>2.864</td>
<td>0.68</td>
<td>9.0</td>
<td>124</td>
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<td>Río El Azufre E, 250 m above confluence with Río El Azufre S</td>
<td>26.6</td>
<td>6.7</td>
<td>2.179</td>
<td>1.06</td>
<td>13.0</td>
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<td>Río El Azufre, after confluence of E and S</td>
<td>25.2</td>
<td>6.8</td>
<td>1.663</td>
<td>1.56</td>
<td>19.2</td>
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<td>El Azufre S, above confluence with E</td>
<td>21.0</td>
<td>8.6</td>
<td>0.513</td>
<td>3.06</td>
<td>34.4</td>
<td>0</td>
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<td>11</td>
<td>Río El Azufre E, spring in swimming pool (water source for experiment)</td>
<td>31.7</td>
<td>6.3</td>
<td>4.169</td>
<td>0.48</td>
<td>7.4</td>
<td>530</td>
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</table>
system (Table I). In the sulphidic habitats, however, there is a remarkable shift in the species composition as only _P. sulphuraria_ and _G. eurystoma_ were found. The latter was absent directly downstream of the sulphurous springs where H$_2$S concentrations were highest. Only two individuals of _P. sulphuraria_ and no _G. eurystoma_ were caught at non-sulphidic sites.

**SULPHIDE TOLERANCE**

The response to the exposure to sulphidic water differed significantly among the species examined (d.f. = 6, _P_ < 0.001; Fig. 2), whereby _P. sulphuraria_ exhibited a significantly higher tolerance than all other species (post hoc test, Fisher’s PLSD, _P_ < 0.001 in all cases). Furthermore, _P. sulphuraria_ caught at a site with high H$_2$S concentration exhibited a higher tolerance than conspecifics from a site with a lower concentration (_P_ < 0.001). The tolerance of all other species, including _G. eurystoma_, did not differ significantly (_P_ > 0.05 in all cases). The factors sex (d.f. = 1, _P_ > 0.05) and _L_ (d.f. = 1, _P_ > 0.05) did not have a significant influence on the sulphide tolerance.

**DISCUSSION**

The H$_2$S concentrations at the Ban˜os del Azufre were comparable to and in places even higher than those of the nearby Cueva del Azufre system (Tobler et al., 2006), and habitats of deep-sea hydrothermal vents inhabited by metazoans (McMullin et al., 2000; Nybakken, 2001; Price, 2002; Tsurumi, 2003). Even at lower concentrations, H$_2$S is known to play a role in natural fish kills (Bagarinao & Lantin-Olagauer, 1999; Luther et al., 2004). Sulphide resistance in

![Fig. 2](image-url). The mean ± s.d. time until loss of motion control in water from a H$_2$S-rich spring for six species of fishes (PSI, _Poecilia sulphuraria_ from spring area; PSII, _P. sulphuraria_ from the sulphidic creek; GE, _Gambusia eurystoma_; PM, _Poecilia mexicana_; XH, _Xiphophorus hellerii_; HB, _Heterandria bimaculata_; AA, _Astyanax aeneus_). PSI and PSII are significantly different from each other and from all other species (Fisher’s PLSD, _P_ < 0.001 in all cases).
fishes varies with habitat and lifestyle, and species that periodically encounter naturally occurring sulphide usually show increased resistance [e.g. the tarpon *Megalops atlanticus* Valencienes, 1847 tolerates concentrations up to 230 μM (Geiger *et al.*, 2000) and the catfish *Hoplosternum littorale* (Hancock, 1828) up to 87 μM (Affonso & Rantin, 2005)]. In fishes from shallow-water marine habitats, median lethal dose (LD50) concentrations of H2S range from 1 μM in open-coast inhabitants up to 700 μM in species inhabiting tidal-marshes with naturally occurring H2S (Bagarinao & Vetter, 1989).

In the Baños del Azufre, the occurrence of H2S was correlated with a shift in fish community composition. Only *P. sulphuraria* and *G. eurystoma* were recorded in microhabitats with sulphide, while other species were abundant in the non-sulphidic habitats. The presence of H2S thus has strong effects on the composition of animal communities leading to pronounced differences even within short spatial distances.

The composition of fish communities in and around the Baños del Azufre was partially reflected in the tolerance to water from the sulphidic spring. Water from springs and non-sulphidic habitats differed in a number of physio-chemical factors. Due to its high toxicity even in micromolar amounts (Lovatt Evans, 1967; Smith *et al.*, 1977; Grieshaber & Völkel, 1998), H2S probably accounts for the rapid loss of motion control in the experiment, but based on the experimental design using water from the natural habitats, it is impossible to disentangle the effects of individual physio-chemical factors and their interactions.

Two surprising findings resulted from the sulphide tolerance tests. First, *P. sulphuraria* from a microhabitat with high H2S concentration had a higher H2S tolerance than conspecifics from a microhabitat with a lower concentration, suggesting that habituation or local adaptation to elevated levels of H2S may play a role in this species. Secondly, the sulphide tolerance of *G. eurystoma* did not differ from that of species recorded only from non-sulphidic water. This may, however, be an artefact of the experimental set-up, because water originating from one of the sulphurous springs was used, which contains high concentrations of sulphide and very low oxygen. A higher long-term sulphide tolerance in *G. eurystoma* compared to species from non-sulphidic habitats may only be evident at lower concentrations, but further experiments are needed to clarify this. It is important to note that the maximum tolerable sulphide concentration may differ in the species investigated if the sulphide concentration is increased slowly and individuals have time to acclimatize to the different conditions.

*Poecilia sulphuraria* is clearly well adapted to high sulphide concentrations and is the only species found in the sulphurous areas directly below larger hydrogen sulphide springs. How this species copes with the toxic sulphurous compounds is as yet unknown. In fishes, detoxification of sulphide is known through oxidation to thiosulphate in liver mitochondria (Bagarinao & Vetter, 1990) and binding to ferrous and ferric haemoglobin and other blood compounds (Torrans & Clemens, 1982; Bagarinao & Vetter, 1989). The short-term survival of fishes in water containing H2S has been shown to depend on the possibility to breath air or perform aquatic surface respiration (Brauner *et al.*, 1995; Plath *et al.*, 2007).
Especially in the spring areas with high concentrations of H₂S, *P. sulphuraria* can be observed performing aquatic surface respiration. The species possesses two conspicuous lip appendices, a character that is not known from any other poeciliid. It was previously hypothesized that these appendices function as taste organs (Wischnath, 1993), but a more plausible explanation is that they are an adaptation to the extremely hypoxic environment and maximize the efficiency of aquatic surface respiration as it has been shown in characids from seasonally hypoxic Amazonian waters (Saint-Paul & Soares, 1988; Val *et al*., 1998). A species-specific characteristic and eponymous of *G. eurystoma* is its extremely wide mouth, which may have a similar function as the lip appendices in *P. sulphuraria*.

*Poecilia sulphuraria* and *G. eurystoma* certainly belong to the few truly extremophile vertebrates and are a prime example of adaptive evolution in an extreme habitat. *Gambusia eurystoma* is closely related to *Gambusia sexradiata* Hubbs, 1936 (Miller, 1975), which is common in the region (Miller, 2005; unpubl. data). *Poecilia sulphuraria* is a sister species of *P. mexicana* (Ptacek & Breden, 1998), which was abundant in the non-sulphurous waters around the Baños del Azufre in the present collections. Although both species could be caught only a few metres apart, they were strictly separated in terms of water chemistry, suggesting niche partitioning between the congeners. Migration of *P. mexicana* into sulphidic habitats is probably limited by the presence of H₂S. Likewise, although *P. sulphuraria* can survive in non-sulphidic water, competitive exclusion possibly prevents this species from expanding its range.

The present survey clearly emphasizes the need for conservation measures in this system. The distribution of *P. sulphuraria* and *G. eurystoma* is restricted to a few river kilometres (Miller, 2005). In order to sustain this unique ecosystem, future development of the spring area should be accompanied by conservation measures, and an attempt to protect the area adjoining the sulphidic stream seems highly wanted.

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