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Variation along the shy–bold continuum in extremophile fishes (*Poecilia mexicana*, *Poecilia sulphuraria*)

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Abstract One potential trade-off that bold individuals face is between increased predation risks and gains in resources. Individuals experiencing high predation and hungry individuals (or individuals with low body condition) are predicted to show increased boldness. We examined one behavioral trait previously reported to be associated with boldness (the time individual fish needed to emerge from shelter) in various populations of mollies (*Poecilia* spp.). Our study system included several southern Mexican surface streams with high piscine predation and high food availability, sulfidic surface streams with high avian predation, in which the inhabiting fish show reduced body condition, and a sulfidic cave, where predation and body condition are low. Our comparison revealed very short times to emerge from the start box in populations from non-sulfidic streams. In sulfidic habitats (whether surface or cave), it took individual *Poecilia mexicana* considerably longer to emerge from the start box, and the same difference was also found in an independent comparison between *P. mexicana* and the closely related, highly sulfide-adapted *Poecilia sulphuraria*. Fish reared under common garden conditions (in the absence of predators and hydrogen sulfide) showed intermediate boldness scores to the extremes observed in the field. Our data thus indicate that (a) boldness is shaped by environmental conditions/experiential effects, but is not heritable, (b) predation affects boldness in the predicted direction, but (c) low body condition leads to reduced boldness. Extremophile *Poecilia* spp. spend most of their time surfacing to survive under sulfidic and hypoxic conditions, which exposes them to increased levels of predations, but the fish forage on the bottom. Hence, in this system, increased boldness does not increase foraging success. We argue that energy limitation favors reducing energetically costly behaviors, and exploring novel environments may be just one of them.

Keywords Boldness · Extremophiles · Hydrogen sulfide · Poeciliidae · Predator regimes

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Introduction

Temperaments (sometimes also referred to as "personality traits") are now widely acknowledged to exist throughout the Animal Kingdom (Yerkes 1939; Wilson et al. 1993; Gosling and John 1999; Fraser et al. 2001; Gosling 2001; Réale and Festa-Bianchet 2003; Dingemanse et al. 2004; Sih et al. 2004; Dall et al. 2004; Svartberg et al. 2005;

Dingemanse and Réale 2005; Réale et al. 2007). One axis along which animal temperaments show pronounced variability across taxa and individuals is the shy–bold continuum (Wilson et al. 1994; Coleman and Wilson 1998; Réale et al. 2000; Rochette et al. 2001; Magnhagen 2007; Wilson and McLaughlin 2007; Webster et al. 2009). The terms shy and bold refer to the propensity to take risks, especially in novel situations, and shy individuals react to novelty by retreating, reducing activity levels, and becoming more vigilant, while bold individuals are more likely to approach novel objects and increase activity levels and exploratory behavior.

Boldness: effects of predation and resource gains

An individual's position along the shy–bold axis may be defined as the extent to which it is willing to trade off potentially increased predation risks for possible gains in resources. Consequently, predation plays a central role in shaping boldness (e.g., Brown et al. 2005, 2007a, b). For example, predation experience alters individuals' boldness in rock lizards (Lopez et al. 2006). In Panamanian bishops, *Brachyrhaphis episcopi* (Poeciliidae), populations from high-predation locations are generally bolder than those from low-predation sites, suggesting that habitat-specific selection pressures are likely to result in population variation of temperaments (Brown et al. 2005).

On the other hand, the relative benefits of being bold should depend on another ecological factor, namely, resource availability. Hungry individuals are predicted to be bolder because the relative payoff of being bold (increased food uptake despite or soon after a predator attack) will be higher. Hence, body condition should affect individuals' boldness. Contrary to this prediction, a previous study found individual wild-caught *B. episcopi* with low body condition to exhibit decreased boldness (Brown et al. 2007b). It was argued that in this case individual differences in boldness within natural *B. episcopi* populations are coupled with differences in competitiveness (see Ward et al. 2004), so boldness is thought to affect food uptake and thus, body condition, not vice versa (Brown et al. 2007b). It remains unknown so far how major differences in resource availability among different habitats affect boldness on a between-population level.

In the present study, we examined boldness in tropical live-bearing fishes of the genus *Poecilia* (mollies). We examined the effects of predation and body condition in a comparative approach. *Poecilia* spp. in our study system inhabit various habitat types, some of which are characterized by high and sustained levels of toxic hydrogen sulfide (H_2S). Ecological differences among sites allow testing for the simultaneous effects of different predator regimes and differences in resource availability/body condition. Differ-

ences in body condition among populations are immense (Tobler 2008; Tobler et al. 2006; Plath et al. 2007b; R. Riesch, unpublished data) and reflect reduced food uptake under sulfidic conditions as well as energetically costly adaptations to detoxify H_2S (see "Study system" for details). We considered three different selective regimes that should affect boldness: (1) in non-sulfidic surface streams, mollies experience predation by piscivorous fishes and birds (Table 1), but show high body condition (Tobler 2008; Tobler et al. 2006). (2) Fish inhabiting sulfidic surface streams experience reduced piscine predation but strongly increased avian predation (see "Study system"; Table 1) and show drastically reduced body condition. (3) Finally, fish inhabiting the front chambers of a sulfidic cave (where the fish still live under daylight conditions) experience no piscine or avian predation (Tobler et al. 2006; Table 1), and a giant water bug of the genus *Belostoma* (Plath et al. 2003; Tobler et al. 2007, 2008c) as well as some species of large-bodied spiders (J. Horstkotte, unpublished data) are the only known molly predators. Body condition of cave-dwelling mollies is even lower than that of mollies from sulfidic surface habitats (Tobler 2008; Tobler et al. 2006). Based on previous studies (Ward et al. 2004; Brown et al. 2005, 2007a, b; Lopez et al. 2006), we predicted that fish from sulfidic surface streams would be bolder than fish inhabiting non-sulfidic habitats because—in addition to the effect of predator experience—energy economy also favors high boldness due to an increased payoff in acquiring needed resources. Additionally, if predation alone played a role in shaping boldness, then fish from the Cueva del Azufre should be shyer than surface populations due to relaxed predatory regimes (however, it is unknown whether predation pressure differs from other habitats). If, on the other hand, body condition plays an additional (or even a more important) role, then these fish might show increased boldness just like fish from sulfidic surface streams.

Heritability of boldness

Individual boldness has been described to be heritable in a variety of taxa (e.g., squid: Sinn et al. 2006; birds: van Oers et al. 2004; mammals: McCune 1995; Stein et al. 2001; Réale and Festa-Bianchet 2003; fish: Bakker 1986; Dingemanse et al. 2009; reviewed in Dingemanse and Réale 2005; Réale et al. 2007). Strong selective regimes are likely to favor heritable differences in boldness between populations. In poeciliid fishes, populations of *B. episcopi* that are exposed to different levels of predation pressure show heritable variation in boldness (Brown et al. 2007a). Also within the genus *Xiphophorus* (swordtails), *Xiphophorus birchmanni* tested within 7 days of capture from the wild are significantly bolder than the related *Xiphophorus*

Table 1 Piscivorous vertebrate species that we encounter on a regular basis during our annual field trips to the *Cueva del Azufre*, the *El Azufre*, the *Baños del Azufre*, and adjacent non-sulfidic habitats

	Species	Common name	Cueva del Azufre	El Azufre	Baños del Azufre	Non-sulfidic surface habitats
Mammals	*Lontra longicaudis*	Neotropical otter				x
Birds	*Phalacrocorax brasilianus*	Neotropic cormorant		x	x	x
	Egretta thula	Snowy egret		x	x	x
	Ardea alba	Great egret				x
	Egretta tricolor	Tricolored heron		x	x	x
	Butorides virescens	Green heron		x	x	x
	Ixobrychus exilis	Least bittern		x		
	Himantopus mexicanus	Black-necked stilt			x	
	Ceryle torquata	Ringed kingfisher				x
	Chloroceryle amazona	Amazon kingfisher		x	x	x
	Chloroceryle americana	Green kingfisher		x	x	x
	Chloroceryle aene	Pygmy kingfisher		x	x	x
	Pitangus sulphuratus	Great kiskadee		x	x	x
Reptiles	*Crocodylus moreleti*	Morelet's crocodile			x	x
	Trachemys scripta	Common slider				x
	Kinosternon leucostomum	White-lipped mud turtle		x[a]		x
	Drymarchon corais	Indigo snake		x		x
	Basiliscus vittatus	Striped basilisk		x		x
Fishes	*Astyanax aeneus*	Banded tetra		x[a]	x[a]	x
	Potamarius nelsoni	Lacandon sea catfish				x
	Rhamdia guatemalensis	Pale catfish			x[a]	x
	Rhamdia laticauda	Rock catfish				x
	Batrachoides goldmani	Mexican freshwater toadfish				x
	Strongylura hubbsi	Maya needlefish				x
	Heterandria bimaculata	Spottail killifish		x[a]	x[a]	x
	Bellonesox belizanus	Pike killifish			x[a]	x
	Ophisternon aenigmaticum	Obscure swamp eel	x[b]	x		x
	Centropomus undecimalis	Common snook				x
	'Cichlasoma' salvini	Yellowbelly cichlid		x[a]	x[a]	x
	Paraneetroplus gibbiceps	Teapa cichlid				x
	Archocentrus octofasciatus	Jack dempsey			x[a]	
	Parachromis friedrichsthalli	Yellowjacket				x
	Gobiomorus dormitor	Bigmouth sleeper				x

x refers to species present

[a] Only in little sulfidic up- or downstream parts of streams

[b] J. Parzefall, pers. comm.

malinche with regards to latency to emerge from shelter and time swimming actively in the open water, while natural hybrids show a range of intermediate phenotypes (J. B. Johnson, unpublished data). This suggests that differences may be genetic, even though laboratory studies in this direction still need to be conducted (G. Rosenthal, pers. comm.). In the present study, we tested for a heritable component in boldness by comparing the behavior of wild-caught *Poecilia mexicana* from different habitats to the behavior of fish from the same populations that had been

reared under standardized laboratory conditions (common garden rearing).

Specifically, we asked if populations of *P. mexicana* would differ in boldness (measured as the time to emerge from shelter when introduced into a novel environment), and if any variation detected would be heritable. By comparing the response of various populations with different ecologies with our a priori predictions about the direction in which the two environmental factors "predation" and "body condition" should affect boldness, we could estimate the relative importance of these selective agents.

Methods

Study system

Studied populations

P. mexicana is common in freshwater habitats on the Atlantic versant of Central America from northern Mexico to Costa Rica (Miller 2005). Near the village of Tapijulapa in the southern Mexican state of Tabasco, several locally adapted populations of that species inhabit ecologically different habitat types that are characterized by the presence or absence of H_2S and/or light (Tobler et al. 2006, 2008a; Plath et al. 2007a). All sites are within 10 river km distance of each other and the average distance between sites is about 3.5 river km. Sites sampled in this study include normal (non-sulfidic, surface) rivers and creeks, a sulfidic surface creek (El Azufre) and a sulfidic cave (Cueva del Azufre).

The Cueva del Azufre is structured into different chambers, the nomenclature of which follows Gordon and Rosen (1962). The cave is drained by a creek fed by a number of springs throughout the cave, most of which contain high levels of dissolved H_2S (Tobler et al. 2006). *P. mexicana* occur throughout the cave, even though their body condition is considerably lower than in non-sulfidic habitats (Plath et al. 2005; Tobler et al. 2006; Tobler 2008). Although all fish from the Cueva del Azufre have functional eyes (Peters et al. 1973; Körner et al. 2006) and readily respond to visual stimuli in visual preference tests (Plath et al. 2004, 2005), we chose only to test fish from chamber II for this experiment. This allowed us to minimize acclimation times to exposure of full daylight, since fish from chamber II obtain some daylight through holes and cracks in the ceiling.

Another sulfidic freshwater system occurs about 10 km west of Teapa, where a number of sulfidic springs enter a creek forming an area that is locally known as the Baños del Azufre. This habitat harbors two endemic species of poeciliid fishes: the sulfur molly, *Poecilia sulphuraria* (Alvarez del Villar 1948) and the largemouth mosquitofish, *Gambusia eurystoma* (Miller 1975). *P. sulphuraria* is a sister species of *P. mexicana* (Ptacek and Breden 1998) and may represent an even more sulfide-adapted form of *P. mexicana* than seen in the Cueva del Azufre system. While *P. sulphuraria* is clearly well adapted to high sulfide concentrations and is the only species found in the sulfurous areas directly below larger hydrogen sulfide springs, *G. eurystoma* has a lower sulfide resistance and occurs in the less sulfidic regions further away from the sulfur sources (Tobler et al. 2008b).

Ecological differences

In both systems, fish communities are strongly reduced in the sulfidic habitats (Tobler et al. 2006) and predatory fish species are absent (Table 1). By contrast, piscivorous birds are present in all habitats except the Cueva del Azufre (Table 1). Avian predation on *P. sulphuraria* in the crystal clear, highly sulfidic spring areas of the Baños del Azufre is so intense that this species forms dense schools (in contrast to the more common shoals seen in other poeciliids), which show remarkable coordinated escape maneuvers (R.R. and M.P., pers. observation). However, even fish from the Cueva del Azufre show pronounced refuge use, and hide, e.g., in crevices when disturbed or chased (authors, pers. observation).

H_2S affects body condition in that it inhibits the cytochrome *c* oxidase, blocking electron transport in aerobic respiration, thereby hampering the function of mitochondria and the production of ATP (Evans 1967; National Research Council 1979; Nicholls 1975). It can also modify oxygen transport proteins (Carrico et al. 1978; Park et al. 1986). There is some evidence that *P. mexicana* from sulfidic habitats have heritable physiological adaptations to detoxify H_2S that are absent in conspecifics from non-sulfidic habitats (Peters et al. 1973; reanalyzed in Plath and Tobler 2009). However, short-term survival of *P. mexicana* in sulfidic water critically depends on a compensatory behavior (aquatic surface respiration), where the fish exploit the more oxygen-rich air–water interface using their gills (Plath et al. 2007b; R. Riesch and M. Plath, unpublished data). *P. sulphuraria* further exhibit conspicuous lip appendages, which are thought to increase the efficiency of aquatic surface respiration (Winemiller 1989; Tobler et al. 2008b). Besides energetic costs for physiological detoxification mechanisms (Ip et al. 2004), aquatic surface respiration imposes costs as it detracts from the time available for feeding (Chapman and Chapman 1993; Kramer 1983; Weber and Kramer 1983; R. Riesch and M. Plath, unpublished data). Indeed, mollies from sulfidic

habitats are characterized by reduced gut fullness (M. Tobler, unpublished data).

Condition factors (body mass per cubic standard length (SL^3)) drop from 0.032 ± 0.011 mg mm^{-3} (mean±SD) in sulfide-free surface habitats to only 0.023 ± 0.049 in the sulfidic El Azufre and 0.020 ± 0.010 in chamber V of the Cueva del Azufre (Tobler et al. 2006; Plath et al. 2007b). Likewise, the amount of storage lipids significantly differs among populations, and fish from non-sulfidic surface habitats around the Cueva del Azufre have approximately 9.46% (males) to 9.93% (females) storage lipids, while fish from the Cueva del Azufre have only 4.28% (males) to 6.21% (females) fat content, and the lowest values (2.74% in females and 2.92% in males) are found in the El Azufre population (R. Riesch, unpublished data; for similar results see also Tobler 2008). Probably in response to the lower condition factor of cave mollies relative to their surface-dwelling relatives, cave molly females prefer well-fed males over malnourished ones, while surface-dwelling females do not show such a preference (Plath et al. 2005).

Field experiments

Data were collected as part of the University course 'Tropical Ecology', co-taught by two of the authors (R.R. and M.P.) between September 7th and September 28th 2008 in Tabasco, Mexico [around Tapijulapa (Cueva del Azufre system) and around Teapa (Baños del Azufre system)]. We captured fish from sites with high and low H$_2$S concentrations in several interconnected habitats using standard seines (4 m long, 4 mm mesh width; Tobler et al. 2006). Average water temperatures were 28.15°C in Cueva del Azufre, 28.15°C in El Azufre, 25.49°C at Baños del Azufre, and 24.44°C (range 21.68–25.75°C) in the non-toxic surface habitats.

Upon capture, fish were transferred into closed and aerated (38 L, $43\times31\times32$ cm) black Sterilite® containers and were subsequently tested under field conditions. The only exception were fish from cave chamber II of the Cueva del Azufre, which were acclimated for 24 h before testing to ensure that fish were habituated to light conditions.

The experimental arena consisted of a 62-l ($51\times41\times31$ cm) black Sterilite® container, which was placed in the shadow next to the collection site. We had five identical experimental setups, so five fish could be measured simultaneously. All experiments were conducted during the daytime with water from the respective collection sites. Water depth was maintained at 10 cm and water was exchanged every few trials (approximately once every 60 min). Each arena was furnished with river gravel and stones. A start box constructed from a 710-ml Dannon® Activa Yogurt plastic container was positioned on one side

of the arena. A bright plastic sheet (marble-patterned contact paper, Con-Tact® brand; 2004 Kittrich Corp., La Mirada, CA, USA) was placed underneath the box to provide a strong background contrast to the color of the fish. The start box was equipped with a 4×4-cm trapdoor that could be opened via a remote pulley system (see Brown et al. 2005, 2007a for a similar experimental design). The observer was sitting quietly on the opposite side of the arena, approximately 1 m away, without disturbing the fish.

Within one species and population, all fish were tested in a random order with regard to sex or size. Each fish was placed into the start box and allowed to settle for 2 min before the remote pulley system was activated so as to open the trapdoor. The time taken for the fish to emerge from the box and explore the novel arena was recorded as an assay of boldness (Brown and Braithwaite 2004; Brown et al. 2005). We decided a priori that the criterion for this was the individual's head crossing the line of the door. Furthermore, any fish failing to emerge from the start box after 15 min was given a ceiling value of 900 s ($N=74$ fish failed to emerge: 45 females and 29 males). In total, we tested $N=424$ (272 females and 152 males) individual fish for their latency times to emerge from the starting box:

1. In the Cueva del Azufre system, we tested a total of $N=209$ individuals (150 females and 59 males) from six populations of *P. mexicana* [four from non-sulfidic surface sites (Arroyo Cristal, 36 females and ten males; Arroyo Tres, 31 females and nine males; Arroyo Bonita, 12 females and four males; Arroyo Tacubaya, 25 females and zero males), one from the sulfidic El Azufre (22 females and 12 males) and one from cave chamber II of the Cueva del Azufre (24 females and 24 males)]. Furthermore, we tested $N=64$ individuals of three additional species of poeciliids [*Heterandria bimaculata* (25 females and 15 males), *Heterophallus milleri* (15 females and 12 males), and the recently described *Priapella chamulae* (nine females and five males; Schartl et al. 2006)], which all stemmed from non-sulfurous surface habitats.

2. In the Baños del Azufre system, we tested $N=32$ *P. mexicana* (21 females and 11 males) from the non-sulfidic Río Teapao and $N=41$ *P. sulphuraria* (20 females and 21 males) from the Baños del Azufre. Additionally, we tested $N=61$ individuals from two further poeciliid species [*G. eurystoma* (18 females and 23 males) and *Xiphophorus hellerii* (14 females and six males)] occurring in the same region. *G. eurystoma* occurs only in the less sulfidic downstream regions of the Baños del Azufre system, while *X. hellerii* occurs only in non-sulfidic waters (Tobler et al. 2008b).

Laboratory studies

We asked whether the pattern observed in the Cueva del Azufre system would be due to heritable differences among *P. mexicana* populations (e.g., Brown et al. 2007a) or whether it represents a plastic response to different ecological conditions. To address this question, we repeated the experiment outlined above with three lab-reared stocks of *P. mexicana* (total *N*=73 individuals) from (a) the non-sulfidic Río Oxolotán (20 females and five males), (b) the El Azufre (15 females and nine males), and (c) the Cueva del Azufre, chamber II (11 females and 13 males). Test subjects were of the first to third lab generation originating from fish caught in the wild in 2007 and 2008; experiments were conducted in October 2008. Fish were maintained as large, randomly outbred stocks in tanks (1,000 l) in a greenhouse of the University of Oklahoma in Norman, reared under normal water conditions. The tanks contained naturally growing algae as well as a variety of naturally occurring invertebrates like chironomid larvae, copepods, and amphipods, on which the fish could feed. In addition, the fish were fed ad libitum amounts of flake food every 2 days.

Statistical analyses

In each study system, the time to emerge from the start box was used as a dependent variable for a general linear model (GLM), in which "species/population" and the sex of the focal fish were used as independent variables. Because boldness can be influenced by body size (with small individuals being bolder; Brown and Braithwaite 2004; Brown et al. 2005, 2007b), SL (measured to the closest mm directly after a test) was used as a covariate. Poeciliid species other than *P. mexicana* and *P. sulphuraria* were analyzed separately, whereby the sex of the focal fish (factor) and SL (covariate) were included in the GLMs.

Results

Cueva del Azufre system

Poecilia mexicana

Our comparison of latency times to emerge from the start box revealed a significant difference among populations, while we detected no significant effect of either standard length or sex (Table 2). Both populations from extreme (sulfidic) habitats (El Azufre and Cueva del Azufre) exhibited strikingly longer latency times than any other population examined (Fig. 1). Post hoc pair-wise comparisons revealed significant differences between the extrem-

Table 2 Results from general linear models on latency times to emerge from the start box in (a) six populations of *P. mexicana* from the Cueva del Azufre system, (b) *P. mexicana* and the "derived" (sulfur-adapted) *P. sulphuraria* from the Baños del Azufre system, and (c) in laboratory-reared individuals of three populations of *P. mexicana* from the Cueva del Azufre system

Source	df	Mean square	F	P
P. mexicana in the Cueva del Azufre system (R^2=0.553)				
SL [mm]	1	54,112.368	0.813	0.368
Sex	1	10,271.991	0.154	0.695
Population	**5**	**2,043,504.608**	**30.710**	**<0.001**
Error	200	66,541.384		
P. mexicana and *P. sulphuraria* (Baños del Azufre; R^2=0.229)				
SL [mm]	1	23,405.466	0.274	0.603
Sex	**1**	**357,899.573**	**4.182**	**0.045**
Species	**1**	**1,507,825.872**	**17.620**	**<0.001**
Error	69	85,575.645		
Lab-reared *P. mexicana* (Cueva del Azufre system; R^2=0.150)				
SL [mm]	1	163,144.501	2.267	0.137
Sex	1	23,335.657	0.324	0.571
Population	2	213,348.422	2.965	0.058
Error	66	71,960.963		

Interaction effects were non-significant in all cases and thus removed from the models. Significant effects are in boldface
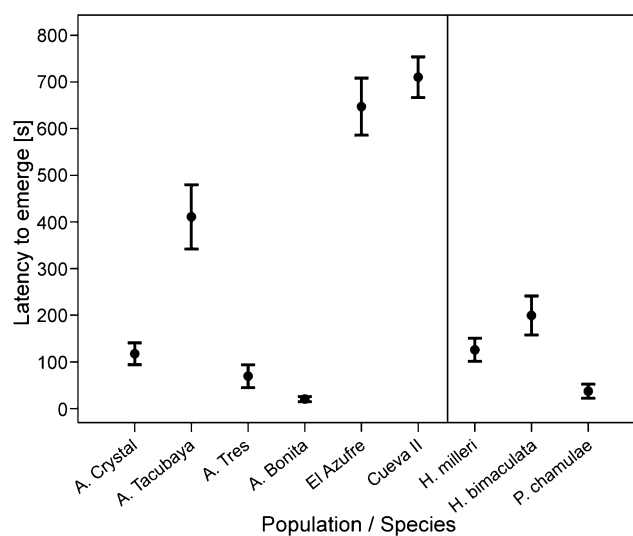

Fig. 1 The mean (±SE) time to emerge from the start box in four species of poeciliid fishes from the Cueva del Azufre area. From *left to right*: *Poecilia mexicana* from non-sulfidic surface streams (Arroyo Cristal: *N*=46, Arroyo Tacubaya: *N*=25, Arroyo Tres: *N*=40, Arroyo Bonita: *N*=16), *P. mexicana* from sulfidic habitats (El Azufre: *N*=34, Cueva del Azufre, chamber II: *N*=48), *Heterophallus milleri* from Río Amatan (*N*=27), *Heterandria bimaculata* from Clear Creek draining into El Azufre (*N*=40), and *Priapella chamulae* from Arroyo Tacubaya (*N*=13)

ophile populations and the four other *P. mexicana* populations ($P<0.001$ in all cases), while no significant difference was found between the two extremophile populations ($P=0.274$; Fig. 1). Unexpectedly, the Arroyo Tacubaya population also showed significantly higher latencies than the other three populations from non-sulfidic habitats ($P<0.001$ in all cases; Fig. 1).

Other poeciliids

When analyzing the latency to emerge from the start box in three other poeciliid species occurring in non-sulfidic habitats in the Cueva del Azufre area, equally low latency times were detected as in *P. mexicana* from the same habitat type (Fig. 1). Again, we found no influence of sex or standard length in two species (*H. bimaculata*, sex: $F_{1, 27}=0.011$, $P=0.916$; standard length: $F_{1, 27}=0.012$, $P=0.912$; *H. milleri*, sex: $F_{1, 40}=0.666$, $P=0.420$; standard length: $F_{1, 40}=2.406$, $P=0.129$), while a weak effect of standard length on latency to emerge was detected in *P. chamulae* (sex: $F_{1, 12}=0.500$, $P=0.496$; standard length: $F_{1, 12}=7.071$, $P=0.024$). As predicted, a positive correlation between SL and latency was detected (Pearson correlation: $r_p=+0.706$, $P=0.007$, $N=13$).

Baños del Azufre system

P. mexicana and P. sulphuraria

When we compared the latency times to emerge from the start box between *P. mexicana* from the non-sulfidic Río Teapao and *P. sulphuraria* from the highly sulfidic source region at the Baños del Azufre, a significant difference between species was detected (Table 2). Just like in the Cueva del Azufre system, the sulfur-adapted form showed longer latency times (Fig. 2). Contrary to the Cueva del Azufre system, males showed significantly shorter latency times than females (effect of the factor "sex"), while body size again had no significant effect (Table 2; Fig. 2).

Other poeciliids

The latency to emerge from the starting box in two other poeciliid species from the Baños del Azufre area was short in *G. eurystoma* from the less sulfidic downstream regions of the Baños del Azufre and was comparatively high in *X. hellerii* from the Río Teapao (Fig. 2). We found no influence of sex or standard length in the latter two species (*G. eurystoma*, sex: $F_{1, 38}=0.021$, $P=0.885$; standard length: $F_{1, 38}=0.010$, $P=0.920$; *X. hellerii*, sex: $F_{1, 17}=1.041$, $P=0.322$; standard length: $F_{1, 17}=0.032$, $P=0.861$).
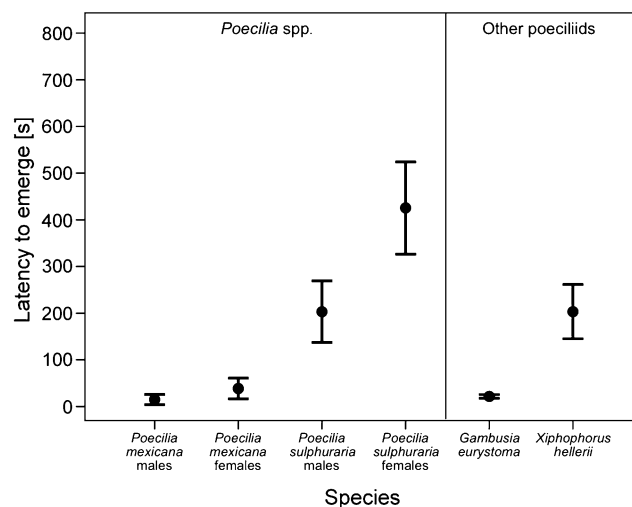

Fig. 2 The mean (±SE) time to emerge from the start box in four species of poeciliid fishes from the Baños del Azufre area. From *left to right*: *P. mexicana* from the non-sulfidic Río Teapao (males: $N=11$, females: $N=21$), *Poecilia sulphuraria* from the highly sulfidic upstream regions at the Baños del Azufre (males: $N=21$, females: $N=20$), *Gambusia eurystoma* from the slightly sulfidic downstream regions of the Baños del Azufre ($N=41$), and *Xiphophorus hellerii* from Río Teapao ($N=20$)

Laboratory studies on *P. mexicana* from the Cueva del Azufre system

The latency times to emerge from the start box did not differ significantly among lab-reared individuals from three different populations (Río Oxolotán, El Azufre, and Cueva del Azufre) of *P. mexicana* (Table 2, Fig. 3). There was also no effect of either sex or standard length (Table 2).
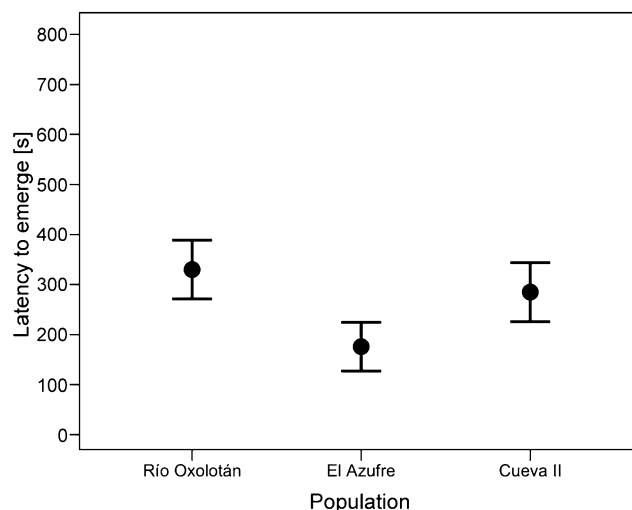

Fig. 3 The mean (±SE) time to emerge from the start box in laboratory-reared individuals of three populations of *P. mexicana* from the Cueva del Azufre system. From *left to right*: non-sulfidic surface river (Río Oxolotan: $N=24$), sulfidic surface creek (El Azufre: $N=25$), and sulfidic cave (Cueva del Azufre, cave chamber II: $N=24$)

Qualitatively, mean latency times converged to intermediate times relative to the extremes measured in wild-caught fish from the same and additional populations (Figs. 1 and 3).

Discussion

We predicted that fish inhabiting sulfidic surface habitats should be bolder than fish from non-sulfidic surface habitats because the relative payoff of being bold increases in the extreme environment. Contrary to our prediction, fish from all sulfidic sites (including the Cueva del Azufre) showed strongly decreased boldness. Based on the finding that fish reared in the laboratory (i.e., in the absence of predators and H_2S) showed intermediate boldness scores, we argue that two independent factors affect boldness in this system: high-predation risk in non-sulfidic habitats leads to increased boldness (i.e., decreased time to emerge), while presence of hydrogen sulfide appears to have an antagonistic effect and causes decreased boldness. We measured only one behavior associated with boldness (i.e., time to emerge from shelter) and did not measure consistency over time in this behavior; however, this measure of boldness is analogous to the widely utilized open-field test (e.g., Kilgour 1975; Yoshida et al. 2005). Furthermore, in another tropical poeciliid (B. episcopi), time to emerge from shelter has been shown to be a good predictor of other boldness behaviors such as the likelihood to approach novel objects (Brown et al. 2007b). Finally, measuring the same individuals repeatedly was logistically not feasible in the field and could have introduced some aspect of habituation to the setup, which we were trying to exclude.

Effects of predation

In the Panamanian bishop (B. episcopi), fish from high-predation sites are also bolder than fish from low-predation sites (Brown et al. 2007a, b). Increased boldness under high-predation risk seems counterintuitive at first because bolder individuals are also more exposed to predators. However, it was argued that fish from high-predation environments must be prepared to accept a higher risk for any given payoff, leading to bolder fish, for example, being the first to enter a habitat patch after a predator attack (see Brown et al. 2005 for a discussion).

In pupfish, Cyprinodon spp., Plath and Strecker (2008) compared several behavior patterns between seven sympatric species and found pronounced differences among species when analyzing the time until individual fish started to swim freely after being introduced into a novel test tank. The results from that study suggested that territorial, highly aggressive species like Cyprinodon beltrani or Cyprinodon

artifrons are bolder than the little territorial and less aggressive Cyprinodon simus. Again, the adaptive significance of higher boldness is thought to be linked to the fact that territorial species must re-enter their territories soon after disturbance, for example, after a predator attack, but this is not the case in the shoaling, less territorial C. simus (Plath and Strecker 2008).

Also on the level of individuals, correlated differences in suites of behavior (behavioral syndromes; Sih et al. 2004), e.g., between boldness and aggression, appear to be very common [e.g., in brown trout (Salmo trutta): Johnsson et al. 2001; three-spined sticklebacks (Gasterosteus aculeatus): Bell 2005; fiddler crabs (Uca mjoebergi): Reaney and Backwell 2007]. A recent study on three-spined stickleback and several reviews have emphasized that behavioral syndromes themselves appear to underlie natural selection (e.g., via predation regimes) and are probably not simply a by-product of genetic constraints, i.e., pleiotropic effects (Dingemanse and Réale 2005; Dingemanse et al. 2007; Réale et al 2007). Whether and how individual differences in boldness relate to other behavioral differences and whether behavioral syndromes differ among populations remain to be investigated in our study system.

Effects of hydrogen sulfide

Reduced boldness in extremophile populations cannot be driven by relaxed predation (as might be argued for the Cueva del Azufre population) because: (1) unlike fish from the sulfidic Cueva del Azufre, P. mexicana inhabiting the equally sulfidic El Azufre as well as P. sulphuraria from the sulfidic regions at the Baños del Azufre experience very strong predation by piscivorous birds (Table 1). During aquatic surface respiration, which mediates survival in the toxic and hypoxic water, fish are forced to stay immediately underneath the water surface, exposing them to increased levels of avian predation (see Kramer et al. 1983). (2) Individual fish from extremophile populations (including the Cueva del Azufre population) that were reared in the laboratory, i.e., without any predator experience and H_2S, were bolder than wild-caught fish from the same populations.

How can the effect of hydrogen sulfide be interpreted? We propose that, rather than predation alone, reduced body condition in mollies inhabiting sulfidic habitats (Plath et al. 2005, 2007b; Tobler et al. 2006) plays a crucial role in shaping boldness. Body condition has been shown to correlate with boldness behaviors in Panamanian bishop (B. episcopi), with lighter fish being shyer than heavier fish at any given body size (Brown et al. 2007b). The authors of the latter study argue that increased hunger of individuals with low body condition cannot explain reduced boldness—the opposite pattern would be predicted:

hungry fish should be more inclined to take great risks by emerging from shelter (Brown et al. 2007b). Brown et al. (2007b) therefore argue that differences in boldness may be responsible for differences in body mass, not vice versa. Similarly, Ward et al. (2004) found bolder sticklebacks to out-compete shy individuals and thus, grow faster. Such mechanisms, however, cannot explain the correlation between body condition and boldness among populations in our study system, where environmental conditions are responsible for major differences in body condition (see "Study system"). Therefore, we argue that body condition in our study system directly affects boldness.

So, why would fish in our study system be influenced by low body condition? (a) In the sulfidic systems described here, fish are only prone to predation (by birds or giant water bugs) while performing aquatic surface respiration, since piscivorous fishes are absent. To feed, mollies have to leave the surface and dive to the bottom to graze on bacterial mats, or eat detritus, aquatic arthropods, and snails (Tobler 2008; M. Tobler, unpublished data). This situation, however, is unique in that *Poecilia* spp. actually decrease predation risk during feeding, whereas fishes from other systems usually increase the risk of predation in order to obtain food (see Brown et al. 2007b for a discussion). Hence, in our system, fish do not have to be bolder to forage more efficiently. (b) Bodily energy reserves appear to be so low in extremophile *P. mexicana* that energy availability critically affects the survival of the fish in their natural habitat even over very short periods of time (Plath et al. 2007b). Over time, sulfidic spring residents in the Cueva del Azufre system have evolved a number of traits that appear to be adaptations to energy scarcity such as reduced male sexual activity (Plath 2008; Plath et al. 2003, 2007c), and shifts in life history strategies (Riesch et al. 2009; R. Riesch, unpublished data). Energy economy appears to dictate the reduction of energy-consuming behaviors here, and exploring novel environments may be just one of such behaviors. (c) Finally, fish in sulfidic habitats may have reduced responsiveness to predators, so reduced boldness would actually be beneficial. Even though empirical support for this idea is as yet missing, lower reaction times in fish from habitats with toxic H_2S may be due, for example, to sensory or neuromuscular impairment, and/or reduced cognitive or muscular function. Shyer individuals have longer response latencies to a predation threat also in other species [e.g., guppy (*Poecilia reticulata*): Godin and Dugatkin 1999; *Xiphophorus* spp.: G. Rosenthal, pers. comm.].

Experiential versus heritable effects

Lab-reared *P. mexicana* from different populations all showed very similar behavior, and times to emerge from the start box were intermediate to those observed in the field. We argue that the behavior seen in lab-reared individuals represents the reaction norm expressed under conditions when food is plentiful (high body condition) and predation is low like in our stock tanks. We conclude that differences in boldness are due to phenotypic plasticity and do not represent heritable differences. This finding contrasts with several other studies examining behavioral divergence in extremophile mollies. For example, *P. mexicana* in the sulfidic Cueva del Azufre show a heritable reduction of aggressive behavior (Parzefall 1974, 1979). Males from sulfidic habitats also show reduced sexual activity, and the same reduction is seen in offspring that were reared under normoxic laboratory condition (Plath et al. 2003, 2007c; Plath 2008). Likewise, reduced shoaling has a strong heritable component (Parzefall 1993; Plath and Schlupp 2008).

Boldness behaviors have a heritable component in some fishes (*Danio rerio*: Wright et al. 2003; *B. episcopi*: Brown et al. 2007a; *G. aculeatus*: Bakker 1986; Dingemanse et al. 2009), while other studies found differences in boldness detected in the wild to be lost when fish were maintained in the laboratory (*Lepomis gibbosus*: Wilson et al. 1993). Several other traits in *P. mexicana* inhabiting extreme environments, for example, morphology (Tobler et al. 2008a) or fecundity (Riesch et al. 2009), appear to have lost their plasticity (determined as the response to experimental exposure to different abiotic conditions) to different degrees. Therefore, it is possible that eventually the plasticity in behavioral traits such as shyness–boldness may be lost as well in extremophile mollies as they further adapt to their particular habitats (for a discussion on potential ecological speciation in this system please refer to Tobler et al. 2008a; R. Riesch, unpublished data).

Sex- and size-related differences

In *B. episcopi*, males are bolder than females (Brown et al. 2007a, b). This may be due to differences in life history priorities (Magurran and Macías Garcia 2000), especially mate competition among males, favoring males that "go back to normal" soon after a disturbance. In the present study, such an effect was found only in *P. mexicana* and *P. sulphuraria* from the Baños del Azufre system, but not in any other species or population. Moreover, an effect of body size on boldness was reported for *B. episcopi*, with smaller (i.e., younger, less experienced) individuals being bolder (Brown and Braithwaite 2004; Brown et al. 2005, 2007b). In our present study, such an effect of body size was detected only in *P. chamulae*, but not in any other species. It remains unclear so far what specific differences among species and/or populations cause these behavioral differences; however, based on our current results, it seems

that the findings of sex- and size-related differences among individuals cannot be generalized, but rather seem to apply to only some poeciliid species.

Experimental protocol

One might argue that the acclimation time of 2 min might be too short for a recently caught individual to consider the start box a safe haven, and that therefore the latency time to emerge from a start box could be a measure of flight response rather than increased willingness to explore a novel environment. However, in order to compare our results to those of another poeciliid (*B. episcopi*), we decided to follow well-established protocols (Brown and Braithwaite 2004; Brown et al. 2005, 2007a, b). Furthermore, prior experience with mate choice experiments on mollies (*Poecilia* spp.) in the field shows that most fish are already exhibiting mate choice behavior within minutes of being captured (R. Riesch and M. Plath, unpublished data). Finally, we would expect to find slight differences in the swimming behavior of fishes that try to escape the starting box with those that leave to explore. The vast majority of fishes left the start box slowly and started to slowly swim around in the outer container, while only few fishes dashed out of the starting box and immediately tried to hide again outside (all authors, pers. observation). Hence, although we cannot completely rule out that some of the tested fish may have shown flight response rather than exploratory behavior, we feel confident that overall the applied protocol was appropriate.

Additionally, some of the habitats we tested differed in water temperatures, which may have influenced the performance of the fish during our trials. However, it is important to note that the fish from the coldest habitats performed best (shortest latency times), while the fish from the warmest habitats (Cueva del Azufre and El Azufre) actually showed the longest latency times. Since we would expect fish from colder habitats to show longer latency times and vice versa, we feel confident that temperature did not confound our test results. In fact, one could argue that this lends even more support to our argumentation.

References

Alvarez del Villar J (1948) Descripción de una nueva especie de *Mollienisia* capturada en Baños del Azufre, Tabasco (Pisces, Poeciliidae). An Esc Nac Cienc Biol 5:275–281

Bakker TCM (1986) Aggressiveness in sticklebacks (*Gasterosteus aculeatus*)—a behavior-genetic study. Behaviour 98:1–144

Bell AM (2005) Behavioural differences between individuals and two populations of stickleback (*Gasterosteus aculeatus*). J Evol Biol 18:464–473

Brown C, Braithwaite VA (2004) Size matters: a test of boldness in eight populations of bishop, *Brachyraphis episcopi*. Anim Behav 68:1325–1329

Brown C, Jones F, Braithwaite VA (2005) In situ examination of boldness–shyness traits in the tropical poeciliid, *Brachyraphis episcopi*. Anim Behav 70:1003–1009

Brown C, Burgess F, Braithwaite VA (2007a) Heritable and experiential effects on boldness in a tropical poeciliid. Behav Ecol Sociobiol 62:237–243

Brown C, Jones F, Braithwaite VA (2007b) Correlation between boldness and body mass in natural populations of the poeciliid *Brachyrhaphis episcopi*. J Fish Biol 71:1590–1601

Carrico R, Blumberg W, Peisach J (1978) The reversible binding of oxygen to sulfhemoglobin. J Biol Chem 253:7212–7215

Chapman LJ, Chapman CA (1993) Desiccation, flooding, and the behavior of *Poecilia gillii* (Pisces: Poeciliidae). Ichthyol Explor Freshw 4:279–287

Coleman K, Wilson DS (1998) Shyness and boldness in pumpkinseed sunfish: individual differences are context-specific. Anim Behav 56:927–936

Dall SR, Houston AI, McNamara JM (2004) The behavioural ecology of personality: consistent individual differences from an adaptive perspective. Ecol Lett 7:734–739

Dingemanse NJ, Réale D (2005) Natural selection and animal personality. Behaviour 142:1159–1184

Dingemanse NJ, Both C, Drent PJ, Tinbergen JM (2004) Fitness consequences of avian personalities in a fluctuating environment. Proc R Soc Lond B 271:847–852

Dingemanse NJ, Wright J, Kazem AJN, Thomas DK, Hickling R, Dawnay N (2007) Behavioural syndromes differ predictably between 12 populations of three-spined stickleback. J Anim Ecol 76:1128–1138

Dingemanse NJ, Van der Plas F, Wright J, Réale D, Schrama M, Roff DA, Van der Zee E, Barber I (2009) Individual experience and evolutionary history of predation affect expression of heritable variation in fish personality and morphology. Proc R Soc Lond B 276:1285–1293

Evans C (1967) The toxicity of hydrogen sulphide and other sulphides. Quart J Exp Physiol 52:231–248

Fraser DF, Gilliam JF, Daley MJ, Le AN, Skalski GT (2001) Explaining leptokurtic movement distributions: intrapopulation variation in boldness and exploration. Am Nat 158:124–135

Godin J-GJ, Dugatkin LA (1999) Female mating preference for bold males in the guppy, *Poecilia reticulata*. Proc Natl Acad Sci U S A 93:10262–10267

Gordon MS, Rosen DE (1962) A cavernicolous form of the Poeciliid fish *Poecilia sphenops* from Tabasco, México. Copeia 1962:360–368

Gosling SD (2001) From mice to men: what can we learn about personality from animal research? Psychol Bull 127:45–86

Gosling SD, John OP (1999) Personality dimensions in non-human animals: a cross-species review. Curr Dir Psychol Sci 8:69–75

Ip YK, Kuah SSL, Chew SF (2004) Strategies adopted by the mudskipper *Boleophthalmus boddaerti* to survive sulfide exposure in normoxia or hypoxia. Physiol Biochem Zool 77:824–837

Johnsson JI, Sernland E, Blixt M (2001) Sex-specific aggression and antipredator behaviour in young brown trout. Ethology 107:587–599

Kilgour R (1975) Open-field test as an assessment of temperament of dairy cows. Anim Behav 23:615–624

Körner KE, Schlupp I, Plath M, Loew ER (2006) Spectral sensitivity of mollies: comparing surface- and cave-dwelling Atlantic mollies, *Poecilia mexicana*. J Fish Biol 69:54–65

Kramer DL (1983) The evolutionary ecology of respiratory modes in fishes: an analysis based on the costs of breathing. Environ Biol Fish 9:145–158

Kramer DL, Manley D, Bourgeois R (1983) The effect of respiratory mode and oxygen concentration on the risk of aerial predation in fishes. Can J Zool 61:653–665

Lopez P, Hawlena D, Polo V, Amo L, Martin J (2006) Sources of individual shy–bold variations in antipredator behaviour of male Iberian rock lizards. Anim Behav 69:1–9

Magnhagen C (2007) Social influence on the correlation between behaviours in young-of-the-year perch. Behav Ecol Sociobiol 61:525–531

Magurran AE, Macías Garcia C (2000) Sex differences in behaviour as an indirect consequence of mating system. J Fish Biol 57:839–857

McCune S (1995) The impact of paternity and early socialization on the development of cats' behaviour to people and novel objects. Appl Anim Behav Sci 45:109–124

Miller RR (1975) Five new species of Mexican poeciliid fishes of the genera *Poecilia*, *Gambusia*, and *Poeciliopsis*. Occ Pap Mus Zool Univ Mich 672:1–44

Miller RR (2005) Freshwater fishes of Mexico. University of Chicago Press, Chicago

National Research Council (1979) Hydrogen sulfide. University Park, Baltimore

Nicholls P (1975) The effect of sulphide on cytochrome *aa*3. Isosteric and allosteric shifts of the reduced alpha-peak. Biochem Biophys Acta 396:24–35

van Oers K, Drent PJ, de Goede P, van Nordwijk AJ (2004) Realized heritability and repeatability of risk-taking behaviour in relation to avian personalities. Proc R Soc Lond B 271:65–73

Park C, Nagel R, Blumberg W, Peisach J, Maliozzo R (1986) Sulfhemoglobin: properties of partially sulfurated tetramers. J Biol Chem 261:8805–8810

Parzefall J (1974) Rückbildung aggressiver Verhaltensweisen bei einer Höhlenform von *Poecilia sphenops* (Pisces, Poeciliidae). Z Tierpsychol 35:66–84

Parzefall J (1979) Zur Genetik und biologischen Bedeutung des Aggressionsverhaltens von *Poecilia sphenops* (Pisces, Poeciliidae). Z Tierpsychol 50:399–422

Parzefall J (1993) Schooling behaviour in population-hybrids of *Astyanax fasciatus* and *Poecilia mexicana* (Pisces, Characidae and Poeciliidae). In: Schröder H, Bauer J, Schartl M (eds) Trends in ichthyology: an international perspective. Blackwell Scientific, Oxford, pp 297–303

Peters N, Peters G, Parzefall J, Wilkens H (1973) Über degenerative und konstruktive Merkmale bei einer phylogenetisch jungen Höhlenform von *Poecilia sphenops* (Pisces, Poeciliidae). Int Rev Gesamten Hydrobiol 58:417–436

Plath M (2008) Male mating behavior and costs of sexual harassment for females in cavernicolous and extremophile populations of Atlantic mollies (*Poecilia mexicana*). Behaviour 145:73–98

Plath M, Schlupp I (2008) Parallel evolution leads to reduced shoaling behavior in two cave-dwelling populations of Atlantic mollies (*Poecilia mexicana*, Poeciliidae, Teleostei). Environ Biol Fish 82:289–297

Plath M, Strecker U (2008) Behavioral diversification in a young species flock of pupfish (*Cyprinodon* spp., Cyprinodontidae, Teleostei): shoaling and aggressive behavior. Behav Ecol Sociobiol 62:1727–1737

Plath M, Tobler M (2009) The evolutionary ecology of the cave molly (*Poecilia mexicana*) from the Cueva del Azufre system. In: Trajano E, Bichuette ME, Kapoor BG (eds) The biology of subterranean fishes (in press)

Plath M, Parzefall J, Schlupp I (2003) The role of sexual harassment in cave- and surface-dwelling populations of the Atlantic molly, *Poecilia mexicana* (Poeciliidae, Teleostei). Behav Ecol Sociobiol 54:303–309

Plath M, Parzefall J, Körner KE, Schlupp I (2004) Sexual selection in darkness? Female mating preferences in surface- and cave-dwelling Atlantic mollies, *Poecilia mexicana* (Poeciliidae, Teleostei). Behav Ecol Sociobiol 55:596–601

Plath M, Heubel KU, García de León FJ, Schlupp I (2005) Cave molly females (*Poecilia mexicana*, Poeciliidae, Teleostei) like well-fed males. Behav Ecol Sociobiol 58:144–151

Plath M, Hauswaldt JS, Moll K, Tobler M, García de León FJ, Schlupp I, Tiedemann R (2007a) Local adaptation and pronounced genetic differentiation in an extremophile fish, *Poecilia mexicana*, from a Mexican cave with toxic hydrogen sulfide. Mol Ecol 16:967–976

Plath M, Tobler M, Riesch RW, García de León FJ, Giere O, Schlupp I (2007b) Survival in an extreme habitat: the roles of behaviour and energy limitation. Naturwissenschaften 94:991–996

Plath M, Makowicz AM, Schlupp I, Tobler M (2007c) Sexual harassment in live-bearing fishes: comparing courting and non-courting species. Behav Ecol 18:680–688

Ptacek MB, Breden F (1998) Phylogenetic relationships among the mollies (Poeciliidae: Poecilia: *Mollienesia* group) based on mitochondrial DNA sequences. J Fish Biol 53:64–81

Réale D, Festa-Bianchet M (2003) Predator-induced natural selection on temperament in bighorn ewes. Anim Behav 65:463–470

Réale D, Gallant BY, Leblanc M, Festa-Bianchet M (2000) Consistency of temperament in bighorn ewes and correlates with behaviour and life history. Anim Behav 60:589–597

Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ (2007) Integrating animal temperament within ecology and evolution. Biol Rev 82:291–318

Reaney LT, Backwell PRY (2007) Risk-taking behavior predicts aggression and mating success in a fiddler crab. Behav Ecol 18:521–525

Riesch R, Tobler M, Schlupp I, Plath M (2009) Offspring number in a livebearing fish (*Poecilia mexicana*, Poeciliidae): reduced fecundity and reduced plasticity in a population of cave mollies. Environ Biol Fishes 84:89–94

Rochette R, Tetreault F, Himmelman JH (2001) Aggregation of whelks, *Buccinium undatum*, near feeding predators—the role of reproductive requirement. Anim Behav 61:31–41

Schartl M, Meyer MK, Wilde B (2006) Description of *Priapella chamulae* sp. n.—a new poeciliid fish from the upper Río Grijalva system, Tabasco, Mexico (Teleostei: Cyprinodontiformes: Poeciliidae). Zool Abh Dresden 55:59–67

Sih A, Bell AM, Johnson JC, Ziemba RE (2004) Behavioral syndromes: an integrative overview. Quart Rev Biol 79:241–277

Sinn DL, Apiolaza LA, Moltschaniwskyj NA (2006) Heritability and fitness-related consequences of squid personality traits. J Evol Biol 19:1437–1447

Stein MB, Chartier MJ, Lizak MV, Jang L (2001) Familial aggregation of anxiety-related quantitative traits in generalized social phobia—clues to understand "disorder" heritability? Am J Med Genet 105:79–83

Svartberg K, Tapper I, Temrin H, Radesater T, Thorman S (2005) Consistency of personality in dogs. Anim Behav 69:283–291

Tobler M (2008) Divergence in trophic ecology characterises colonisation of extreme habitats. Biol J Linn Soc 95:517–528

Tobler M, Schlupp I, Heubel KU, Riesch R, García de León FJ, Giere O, Plath M (2006) Life on the edge: hydrogen sulfide and the fish communities of a Mexican cave and surrounding waters. Extremophiles 10:577–585

Tobler M, Schlupp I, Plath M (2007) Predation of a cavefish (*Poecilia mexicana*, Poeciliidae) by a giant water bug (*Belostoma*, Belostomatidae) in a Mexican sulfur cave. Ecol Entomol 32:492–495

Tobler M, DeWitt TJ, Schlupp I, García de León FJ, Herrmann R, Feulner PGD, Tiedemann R, Plath M (2008a) Toxic hydrogen sulfide and dark caves: phenotypic and genetic divergence across two abiotic environmental gradients in *Poecilia mexicana*. Evolution 62:2643–2659

Tobler M, Riesch R, García de León FJ, Schlupp I, Plath M (2008b) Two endemic and endangered fishes, *Poecilia sulphuraria* (Alvarez, 1948) and *Gambusia eurystoma* Miller, 1975 (Poeciliidae, Teleostei) as only survivors in a small sulphidic habitat. J Fish Biol 72:523–533

Tobler M, Franssen C, Plath M (2008c) Male-biased predation of a cave fish by a giant water bug. Naturwissenschaften 95:775–779

Yoshida M, Nagamine M, Uematsu K (2005) Comparison of behavioral responses to a novel environment between three teleosts, bluegill *Lepomis macrochirus*, crucian carp *Carassius langsdorfii*, and goldfish *Carassius auratus*. Fish Sci 71:314–319

Ward A, Thomas P, Hart P, Krause J (2004) Correlates of boldness in three-spined sticklebacks (*Gasterosteus aculeatus*). Behav Ecol Sociobiol 55:561–568

Weber JM, Kramer DL (1983) Effects of hypoxia and surface access on growth, mortality, and behavior of juvenile guppies, *Poecilia reticulata*. Can J Fish Aquat Sci 40:1583–1588

Webster MM, Ward AJW, Hart PJB (2009) Individual boldness affects interspecific interactions in sticklebacks. Behav Ecol Sociobiol 63:511–520

Wilson ADM, McLaughlin RL (2007) Behavioural syndromes in brook charr, *Salvelinus fontinalis*: prey-search in the field corresponds with space use in novel laboratory situations. Anim Behav 74:689–698

Wilson DS, Coleman K, Clark AB, Biederman L (1993) The shy–bold continuum in pumkinseed sunfish (*Lepomis gibbosus*): an ecological study of a psychological trait. J Comp Psychol 107:250–260

Wilson DS, Clark AB, Coleman K, Dearstyne T (1994) Shyness and boldness in humans and other animals. Trends Ecol Evol 9:442–446

Winemiller KO (1989) Development of dermal lip protuberances for aquatic surface respiration in South American characid fishes. Copeia 1989:382–390

Wright D, Rimmer LB, Pritchard VL, Krause J, Butlin RK (2003) Inter- and intra-population variations in shoaling and boldness in the zebrafish (*Danio rerio*). Naturwissenschaften 90:374–377

Yerkes RM (1939) The life history and personality of the chimpanzee. Am Nat 73:97–112