

Sex recognition in surface- and cave-dwelling Atlantic molly females (*Poecilia mexicana*, Poeciliidae, Teleostei): influence of visual and non-visual cues

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Abstract Cave fishes need to rely on non-visual senses, such as the sense of smell or the lateral line to communicate in darkness. In the present study, we investigated sex identification by females of a cave-dwelling livebearing fish, *Poecilia mexicana* (cave molly), as well as its surface-dwelling relatives. Unlike many other cave fishes, cave mollies still possess functional eyes. Three different modes of presentation of the stimulus fish (a male and an equally sized female) were used: (i) the stimulus fish were presented behind wire-mesh in light, allowing the focal female to perceive multiple cues, (ii) the experiment was carried out under infrared conditions, such that only non-visual cues could be perceived and (iii) the stimulus fish were presented in light behind transparent Plexiglas, allowing for the use of visual cues only. Females of all populations examined preferred to associate with the stimulus female in at least one of the treatments, but only when visible light was provided, suggesting that far-range sex recognition is limited or even absent in the cave molly under naturally dark conditions.

Keywords Cave fish · Lateral line · Poeciliidae · Sexual harassment · Visual communication

Introduction

Cave animals typically evolved from surface-dwelling ancestors (Barr and Holsinger 1985; Culver et al. 1995; Weber et al. 1998; Weber 2000; Romero and Green 2005). One of the most significant changes in environmental conditions accompanying the colonization of a cave habitat with respect to the sensory ecology of an organism is the absence of light and consequently the impossibility to use the visual system (Langecker 2000). This is especially relevant if the ancestral form was diurnal and relied on vision during orientation, foraging and mating. As shown for a wide range of taxa, the constant absence of light often leads to the reduction of the visual system including the eyes and according areas of the brain (e.g., cave fishes: Culver 1982; Wilkens 1982, 1988; Weber et al. 1998; Jeffery 2001, 2005). To compensate for the lack of visual information, other, non-visual sensory organs are often more elaborated than in the ancestral surface-dwelling form (Weber et al. 1998; Langecker and Longley 1993; Langecker 2000).

To orient in darkness, cave animals may use their mechanosensory organs, i.e. the lateral line system in fishes (e.g., *Astyanax mexicanus* (also referred to as *A. fasciatus*): Schemmel 1967; Montgomery et al. 2001; Burt de Perera 2004a, b), or they may use an electric sense, e.g., ampullary organs in cave catfishes (Weber 1995) or cave amphibians (*Proteus anguinus*: Istenič and Bulog 1984). In a social context, chemical (i.e., pheromone-based) communication, which is a wide-spread form of communication also in many surface-dwelling animals (e.g., Crapon de Caprona and Ryan 1990; McLennan and Ryan 1997; Wyatt 2003;

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Fisher et al. 2006; Fisher and Rosenthal 2006), may be one of the most obvious mechanisms by which cave animals can communicate in subterranean habitats (Parzefall 1976, 1993a). For example, chemical cues enable cave salamanders to localize conspecifics (olm, *Proteus anguinus*: Parzefall 1976; Parzefall et al. 1980; Pyrenean mountain newt, *Euproctus asper*: Parzefall et al. 2000; Poschadel et al. 2005, 2007).

In the present study, we examined sex recognition (i.e. the discrimination between a male and a female) in females of surface- and a cave-dwelling populations of Atlantic mollies, *Poecilia mexicana* (Gordon and Rosen 1962). This small livebearing fish is widely distributed in Mexican surface freshwaters (Miller 1966, 2005). *Poecilia mexicana* has evolved a cave form inhabiting a limestone cave in southern Mexico, the Cueva del Azufre. The Cueva del Azufre is about 500–600 m deep and has several breaks in the ceiling in the front parts of the cave (Gordon and Rosen 1962). While the front cave chambers receive some (dim) light, the inner parts of the cave are lightless, and the molly population from the innermost cave chamber XIII constantly lives in the dark. Besides darkness, cave mollies have to cope with high concentrations of hydrogen sulfide (H₂S; Tobler et al. 2006). This respiratory toxicant enters the creek draining the cave through multiple sulfidic springs. A behavioral adaptation, aquatic surface respiration (Kramer and McClure 1982), seems to be mediating the short-term survival of cave mollies in the sulfidic water (Plath et al. 2007c), but what specific physiological adaptations help this population to cope with the long-term toxic effects of H₂S remains to be studied.

In the course of cave colonization, *P. mexicana* altered several behavioral traits compared to adjacent surface populations, most of which are genetically fixed (Parzefall 2001 for a review). For example, cave mollies exhibit reduced shoaling (Parzefall 1993b), aggressive behavior (Parzefall 1974, 1979) and male sexual activity (Plath et al. 2003a, 2005a, 2007b). These reductions have traditionally been interpreted as consequences to life in darkness (Parzefall 2001), but the roles of energy limitation and the presence of H₂S have not been fully considered yet (for discussions see Tobler et al. 2006; Plath et al. 2007a, c). Beside the behavioral reductions, constructive trait evolution was also documented in this system. Contrary to females from surface populations, cave molly females have the ability to determine mate traits (such as size (Plath et al. 2004b) or nutritional state (Plath et al. 2005b)) in darkness. Cave mollies probably use their modified mechanosensory lateral line system — with widened pores in the head canal system (Walters and Walters 1965; Parzefall 1970) — to detect these traits (Plath et al. 2004b), whereas surface females appear to

rely on visual cues to discriminate between males. In previous studies investigating chemical communication, male *P. mexicana* from the milky surface creek outside the cave (El Azufre) did not discriminate between water from a tank containing conspecific females and control water (Wenzel 1997), and surface-dwelling males did not alter their behavior in water from tanks containing females (Zeiske 1968). This suggests that far-range chemical communication plays a minor or even no role at least in surface-dwelling Atlantic mollies. Communication using electrical or acoustical senses is unknown for poeciliids and no evidence indicates it evolved in the cave form.

Near-field sex recognition (i.e., the recognition of a female during direct body contact) in surface- and cave-dwelling *P. mexicana* males is probably mediated through nipping behavior, during which males touch the female genital region with their snout, thereby receiving “substrate borne” chemical information concerning sex and the female’s receptivity (Zeiske 1968, 1971; Parzefall 1969; Schlupp et al. 1991). In the cave molly, morphological changes of the female genital region (an enlarged “genital pad” with numerous secretory gland cells) and an enhanced gustatory system in males may facilitate such near-field communication (Parzefall 1970). In contrast to males, females do not nip and consequently would need to rely on far-field communication for sex recognition.

In poeciliids, females avoid associating with males when they are not sexually receptive (Liley 1983; Plath et al. 2001). Parental care as well as any kind of pair-bonding is lacking, and poeciliid mating systems are highly promiscuous. Poeciliid females have a roughly monthly sexual cycle (Parzefall 1973), and they can store sperm and thus require few copulations to ensure complete fertilization of several monthly broods (Constantz 1989). Males, by contrast, almost constantly exhibit sexual behavior (e.g., guppy, *Poecilia reticulata*: Magurran and Seghers 1994a, 1994b; Godin 1995; Houde 1997; Magurran 2001; mosquitofish, *Gambusia holbrooki*: Bisazza and Marin 1995; Atlantic molly, *P. mexicana*: Plath et al. 2003a, 2005a). Thus, females need to dedicate considerable time to avoiding unwanted copulations. Several studies have reported on costs for females arising from this male sexual harassment in terms of a reduction of female feeding efficiency in the presence of a harassing male (guppy, *P. reticulata*: Magurran and Seghers 1994a; Griffiths 1996; mosquitofish, *Gambusia holbrooki*: Pilastro et al. 2003; sailfin molly, *P. latipinna*: Schlupp et al. 2001; surface-dwelling *P. mexicana*: Plath et al. 2003a, 2007b). A notable exception is the cave molly: as a consequence of the decreased sexual activity of the males, sexual harassment is basically absent in this population (Plath et al. 2003a, 2004a, 2007b). Hence, another question of the current study was to

investigate if both surface- and cave-dwelling (non-receptive) *P. mexicana* females would avoid males and prefer to associate with another female.

We asked whether female cave mollies would discriminate between a male and a female in the absence of body contact. Do cues detected by the mechanosensory lateral line and/or water-soluble pheromones enable them to recognize females? In the cave molly eye size is slightly reduced (Peters et al. 1973; Plath et al. 2007a), but eyes are still capable of vision. Therefore, we were able to compare the response of surface- and cave-dwelling Atlantic mollies to visual and non-visual cues, using three different experimental treatments allowing for the use of (i) all sensory channels, (ii) non-visual sensory systems only, or (iii) visual channels only. More specifically, we tested (1) whether females of three *P. mexicana* populations discriminate between size-matched stimulus fish of different sex, (2) whether non-visual sensory pathways can be used for sex recognition in darkness, and (3) if populations differ in the use of sensory systems.

Material and methods

Study system

For a comparative approach, we used three populations of *P. mexicana* from adjacent, but vastly different habitats in Tabasco, southern Mexico near the village Tapijulapa (see Tobler et al. 2006 for details on the collection sites). One population inhabits a river, the Río Oxolotan. The second inhabits an affluent sulfidic creek, El Azufre, which is isolated from the Río Oxolotan by a waterfall approximately 15 m high. The water of this creek originates from inside a lightless limestone cave, the Cueva del Azufre. The third population used, the cave molly, stems from the innermost cave chamber (chamber XIII after Gordon and Rosen 1962). This population is isolated by a waterfall approximately 1.5 m high from other cave pools, which are also inhabited by mollies. A previous study using 10 unlinked microsatellite loci has demonstrated pronounced genetic differentiation among the populations studied despite their spatial proximity (Plath et al. 2007a). The three habitats differ in visibility conditions. The Río Oxolotan is a clear-water habitat, where females can use visual information for decision making, even though the water may be temporarily turbid after heavy rainfall. The water of the sulfur creek is milky due to a high amount of colloidal sulfur. Hence, vision may be restricted. Finally, inside the cave, visual communication is impossible and females need to rely on non-visual cues for decision making (see Zeiske 1968; Plath et al. 2004b).

Fish maintenance

All individuals used in this study were descendants of fish collected in the three aforementioned study sites. Fish were maintained as large, randomly outbred, mixed sex stocks in tanks of 50–200 l under artificial daylight with a 16 : 8 h light/dark cycle. Another lineage of the cave molly was maintained in a dark room, but under otherwise identical conditions. During animal care even the dark reared stock was exposed to dim light for a few minutes each morning. Thus, even these fish were familiar with light. This ensured that the dark-reared cave fish would behave calmly during the tests in light (Plath et al. 2004b). Temperature was kept between 25–30°C. Fish were fed *ad libitum* twice a day with commercially available flake food, *Artemia*-naupliae, water fleas and *Tubifex* worms. Test females were separated from males and maintained in 25 l-aquaria for four days before the experiment to standardize motivations. The stimulus males and females (see below) were taken from different tanks, i.e., they were taken directly from the stock tanks, such that an effect of familiarity on the observed association preferences (e.g., Griffiths 1997) could be ruled out.

All females used in this study were sexually mature and had interacted with the opposite sex before the test. Therefore, the females were most likely pregnant, and non-receptive.

Choice tests

We adopted the experimental set-up in which cave-dwelling *Astyanax mexicanus* females (Characidae) proved to be able to discriminate between a male and a female stimulus fish (Plath et al. 2006a): we used a standard choice aquarium (100×35×35 cm), filled to two thirds with aged tap water of 25–27°C. The bottom was covered with a thin layer of black gravel. We marked three equal compartments by vertical lines drawn on the front (two preference zones and a central neutral zone). During the tests in light, the tank was illuminated by a 40 W neon tube installed 28 cm above the tank. UV radiation was absent. During the tests in darkness, a 500 W infrared bulb (wavelength>800 nm) was used instead. A microspectrophotometry study has shown that the photo-receptors of Atlantic mollies are not sensitive in the infrared (Körner et al. 2006).

We placed a cylinder (12 cm diameter) in the center of each preference zone to hold one stimulus fish each (one male and one female). Stimulus fish matched the population of the focal female. Test females could swim around the cylinders. The cylinder material differed according to the treatments: We used wire mesh cylinders (mesh-width: 5 mm, wire diameter: 1 mm) for the treatments with availability of non-visual cues (all cues, treatment *i*; non-visual cues only, treatment *ii*; see Plath et al. 2006a, b), or

transparent Plexiglas cylinders for the treatment with availability of only visual cues (treatment *iii*). The mesh width of the wire-mesh material used during the tests in the absence of light was wide enough to allow non-visual cues (such as water-pressure waves) to pass through, as attested by the fact that cave molly females preferred larger over smaller males in exactly the same experimental set-up (Plath et al. 2004b).

Measurement of female association preferences

To initiate a trial, a male and a female were haphazardly taken from a stock tank and were introduced into one of the two cylinders each. Male and female stimulus fish were matched for size (males: 32.9 ± 0.4 mm, females: 32.9 ± 0.3 mm; mean size difference: 1.0 ± 0.1 mm). To avoid pseudo-replication, stimulus individuals were exchanged for each trial. Focal females were also tested only once; however, due to the limited number of mollies available from our stocks, some individuals have been used as a stimulus female in another trial, but never on the same day.

The stimulus pair was given five minutes of acclimatization. A trial was terminated if the two fish did not swim freely in their cylinders after five minutes. After this time, we introduced a focal female (mean (\pm SE) standard length, Río Oxolotan: 34.1 ± 0.5 mm; El Azufre: 33.4 ± 0.7 mm; cave, light reared: 35.6 ± 0.5 mm; cave, dark reared: 34.6 ± 0.5 mm) into the center of the neutral zone. Measurement was started when the focal female began to swim. We measured the time the female spent in both preference zones during an observation period of 10 minutes. Then, we reversed the position of the cylinders containing the stimulus fish from left to right and vice versa and measurement was repeated. This procedure was performed to detect side biases.

During the tests in light, the observer was sitting quietly about two meters in front of the tank. The tests in darkness were recorded by an infrared video camera and monitored in a neighboring room. The three fish involved in a trial were measured for size directly after a trial.

Statistical analysis

Association times near the male and the female stimulus fish during both parts of a trial were summed. We decided *a priori* to exclude the following trials: Trials in which the females spent more than 80% of their association time during both parts of a trial in only one preference zone, i.e. when the female did not follow the stimulus fish, were considered side biases (Río Oxolotan: 5; El Azufre: 7; cave, light reared: 2, dark reared: 7). Trials in which the females spent less than 50% of the observation time in either preference zones were discarded due to low response index

(Río Oxolotan: 2; El Azufre: 1; cave, dark reared: 1). To test for association preferences, we compared the times a female spent near the male and the female within each of the 12 test series using paired *t*-tests. Furthermore, we tested for differences between populations and treatments. For this, we calculated the strength of preference (SOP) as $[(\text{time}_{\text{female}} - \text{time}_{\text{male}}) / (\text{time}_{\text{female}} + \text{time}_{\text{male}})]$, whereby +1 would indicate maximum preference for the stimulus female, -1 would indicate maximum preference for the male, and 0 would indicate no preference at all. We checked that the data did not significantly deviate from the assumptions of normal distribution and equal variance. Data were compared using ‘population’ as between factor and ‘treatment’ as within factor for a two-way ANOVA. All *P*-values are two-tailed.

Results

We successfully tested 166 females. In the ANOVA, the factor ‘population’ had no significant effect on the strength of preference in the comparison between populations and treatments (two-way ANOVA: mean square=0.06, $F_{3,160}=0.80$, $P=0.50$), suggesting that females of all populations were equally motivated to prefer the female stimulus fish over the male stimulus. Indeed, females of all populations showed a preference for the female stimulus fish in at least one treatment (Fig. 1; Table 1).

The interaction term ‘treatment’ x ‘population’ had a highly significant effect in the ANOVA (mean square=0.26, $F_{6,154}=3.48$, $P=0.0030$), indicating that females from

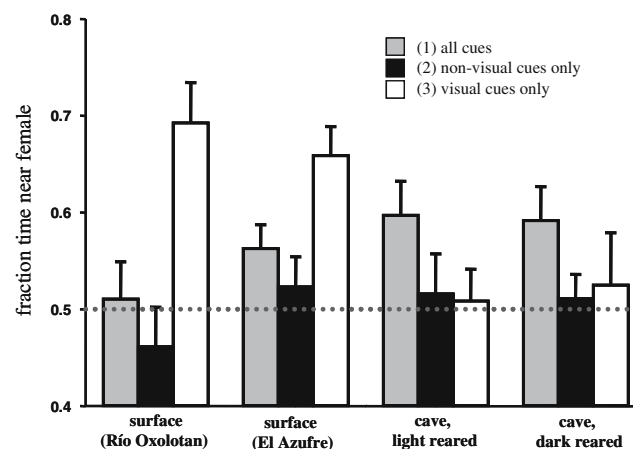


Fig. 1 The relative time (mean \pm SE) *P. mexicana* females from three different populations, originating from a clear-water surface stream (*Río Oxolotan*), a sulfidic, murky surface creek (*El Azufre*) and the cave, spent near the stimulus female when given a choice between a male and a female in simultaneous choice tests. Stimulus fish were presented behind a wire-mesh in light, providing multiple cues to the choosing female (treatment 1: all cues; gray bars), behind a wire-mesh in the absence of visible light (tr. 2: non-visual cues only; black) or behind clear Plexiglas in light (tr. 3: visual cues only; white). Values > 0.5 (dotted line) indicate a preference for the stimulus female

Table 1 Pair-wise comparisons of female association times near a conspecific female vs. male stimulus fish in three populations of *P. mexicana* (surface-dwelling from a clear water river; surface-dwelling from a sulfur creek and cave-dwelling). Three different modes of presentation (treatments) were used, which differed in the kind of cues available to the focal female (all cues-, non-visual cues- and visual cues treatment)

Population	Treatment	time near female (s)	time near male (s)	Paired <i>t</i> -test: t_{df} , <i>P</i>
Surface, clear water (Río Oxolotan)	(1) All cues	527.6±43.1	505.7±42.6	$t_{12}=0.26$, $P=0.80$
	(2) Non-visual cues	455.9±42.5	509.4±29.3	$t_{15}=-0.78$, $P=0.45$
	(3) Visual cues	743.3±49.6	326.6±43.4	$t_{11}=4.54$, $P=0.0009$
Surface, sulfur creek (El Azufre)	(1) All cues	519.3±29.4	400.8±23.6	$t_{15}=2.57$, $P=0.021$
	(2) Non-visual cues	451.9±25.4	414.6±26.2	$t_{14}=0.72$, $P=0.49$
	(3) Visual cues	641.8±35.5	329.1±28.1	$t_{11}=5.03$, $P=0.0004$
Cave, light reared	(1) All cues	601.8±43.9	396.5±28.7	$t_{11}=2.89$, $P=0.015$
	(2) Non-visual cues	484.1±38.7	455.6±38.8	$t_{13}=0.38$, $P=0.71$
	(3) Visual cues	512.1±35.5	494.8±35.3	$t_{12}=0.26$, $P=0.80$
Cave, dark reared	(1) All cues	632.1±39.9	435.8±38.5	$t_{14}=2.61$, $P=0.021$
	(2) Non-visual cues	465.1±24.6	444.4±24.8	$t_{13}=0.45$, $P=0.66$
	(3) Visual cues	556.4±55.5	507.0±58.1	$t_{13}=0.44$, $P=0.67$

the different populations showed differences in the strength of preference according to the type of treatment. Pair-wise comparisons of female association times revealed that surface-dwelling females from the Río Oxolotan spent significantly more time near the stimulus female only in the visual cues treatment (treatment *iii*; Table 1). Females from the sulfur creek (El Azufre) showed a preference both in the all cues treatment (*i*) and in the visual cues only treatment (*ii*). Both light- and dark-reared cave molly females showed a preference only in the all cues treatment (treatment *i*; Table 1).

Overall, the factor ‘treatment’ also had a significant effect (mean square=0.50, $F_{2,154}=6.75$, $P=0.0015$). A post hoc comparison revealed that the non-visual cues treatment (*ii*) differed from the all cues treatment (*i*; Fisher’s protected least significant difference: $P=0.013$) and from the visual cues only treatment (*iii*; $P=0.0007$), whereas the all cues treatment did not significantly differ from the visual cues only treatment ($P=0.33$).

In the ANOVA, light- and dark-reared cave mollies were treated as populations, although they represent two lineages of the same population. We controlled whether this has affected the results. However, excluding the dark-reared cave molly lineage from the analysis did not alter the results: the factor ‘population’ remained non-significant ($F=1.01$, $P=0.37$), whereas ‘treatment’ and the interaction term ‘treatment’ x ‘population’ remained statistically significant ($F>4.19$, $P<0.003$).

Cave molly females did not discriminate between a male and a female when only visual cues were presented (treatment *iii*). We hypothesized that this would be due to the pale coloration of cave mollies and the resulting less pronounced sexual dimorphism in coloration. In a control experiment, we therefore presented light reared cave molly females with a surface-dwelling female and a surface-dwelling male from the Río Oxolotan population in

treatment (*iii*), but no preference was detected (mean ± SE, near female: 587.5±60.8 s; near male: 416.8±48.4 s; paired *t*-test: $t_{11}=1.59$, $P=0.14$). There was no significant difference in SOP values between the experiment using stimulus fish from the cave form or from the surface form (two-sample *t*-test: $t_{23}=0.85$, $P=0.41$).

Discussion

Avoidance of males

In this study, females from all populations preferred to associate with females in at least one experimental treatment. All females used had mated before the tests, and although we did not check for pregnancy of the females (i.e. presence of developing embryos in their ovaries), it is very likely that most females were gestating developing broods. It is known from the closely related Trinidadian guppy (*P. reticulata*), that only virgin females, or females that had just littered a brood, are sexually responsive to male courtship (Liley 1966; Houde 1997). The formation of all-female shoals in surface-dwelling *P. mexicana* may thus represent a tactic to avoid male harassment, because females in shoals can avoid males in a fashion similar to predator avoidance via numerical dilution and/or increased vigilance (Pilastro et al. 2003; Dadda et al. 2005; Agrillo et al. 2006). Sexual harassment by males may thus explain the response of surface-dwelling *P. mexicana* females. In surface-dwelling *P. mexicana* the feeding times of individual females decreased from 100 % around another female to 35% in the presence of a harassing male (Plath et al. 2003a). This explanation, however, does not account for the observed association preference of cave molly females, because sexual harassment is lacking in the cave molly (Plath et al. 2003a, 2004a; 2007b, see below).

Differential use of sensory systems among populations

The three tested populations differed in their use of sensory systems for sex recognition. Because only laboratory-reared animals had been tested and all stocks were reared in clear water, we conclude that these differences between the populations are not learned, but rather have a genetic basis. Likewise, the different rearing conditions of the two cave molly lineages (light or darkness) did not affect the way cave molly females responded to visual and non-visual cues. Genetically fixed differences among the populations studied here in other behaviors have been described elsewhere (Parzefall 2001).

In the Río Oxolotan population, females discriminated between the sexes only when the stimulus fish were presented behind transparent Plexiglas, but not when visual plus non-visual cues were available to the focal female. This finding suggests that — although the all cues treatment provided additional (non-visual) information — the visibility of the stimulus fish may be obscured to some extent by the wire-mesh material used in that treatment. Possibly, females from the Río Oxolotan population rely more strongly on visual cues to differentiate between the sexes, which may be due to the fact that this population inhabits a clear-water habitat.

Cave mollies failed to discriminate between the male and the female stimulus fish in the experimental set-up when visual cues were not presented and discriminated between the male and the female stimulus fish only when visual and non-visual cues were presented. When compared to the surface fish, visual cues alone appear to elicit a reduced response. Fish from the El Azufre are intermediate to the Río Oxolotan and the cave population and females responded when visual cues only and when all cues were offered. Differences in the use of sensory systems in sex recognition between the two surface populations may be due to differences in the sensory environments of the two habitat types: while the Río Oxolotan is only temporarily turbid (i.e. after rainfall), El Azufre is permanently turbid, because the dissolved hydrogen sulfide reacts with oxygen, and the resulting colloidal sulfur gives the water of that creek its milky appearance (Tobler et al. 2006). An alternative explanation to the intermediary position of the El Azufre population is its close relationship to the cave population (Plath et al. 2007a). Gene flow between all three populations studied is very limited, but it is higher between the cave and the El Azufre population than between the El Azufre population and proximate clear-water habitats (Plath et al. 2007a).

Lack of sex recognition in cave mollies in their natural environment?

Although cave mollies evolved the ability to use non-visual cues in communication (e.g., detection of the size of a

conspecific (females: Plath et al. 2004b; males: Plath et al. 2006b)), a previous study suggested that males — both from surface and cave populations — appear not to be able to discriminate between the sexes in darkness when body contact is impossible (Plath et al. 2003b). But whereas males from surface habitats could differentiate between males and females using visual cues only, cave mollies had to rely on a combination of visual plus non-visual cues (Plath et al. 2003a). This essentially means that far-field sex recognition is absent in male cave mollies in their natural lightless habitat, but also hints to an onset of a response to non-visual cues and a breakdown of a response to visual cues for sex identification.

A reduction of the response to visual cues in the cave molly is also known from another context. Surface-dwelling *P. mexicana* females prefer to associate with conspecific females over equally sized heterospecific females (swordtail, *Xiphophorus hellerii*), and this preference is based on visual cues (Riesch et al. 2006). Cave molly females, by contrast, showed no preference relative to the species identity of the stimulus fish, which may be attributed to the impossibility to use visual cues in the cave, as well as the absence of heterospecific fishes in that habitat (Riesch et al. 2006; Tobler et al. 2006).

Why cave mollies still used a combination of visual and non-visual cues for sex recognition and did not discriminate between males and females in darkness remains unclear. Other cave fishes evolved the ability to differentiate between the sexes in darkness. In cave populations of *Astyanax mexicanus* females most likely use chemical cues for far-field sex recognition (Plath et al. 2006a). Cave mollies have been assumed to be a phylogenetically young cave form compared to other cave fishes (Peters et al. 1973; Parzefall 2001). Therefore, the observed association preference in the all cues treatment may be a trait inherited from the surface-dwelling ancestor that is not currently under selection, and not enough time may have elapsed for the evolution of non-visual far-field sex recognition mechanisms. On the other hand, the lack of an association preference in the non-visual cues treatment may be explained by the reduction of male sexual activity and the simultaneous reduction of sexual harassment in the cave population (Plath et al. 2003a, 2004a, 2007b). Contrary to surface populations, females do not suffer from feeding time reductions when associating with males in the cave population of *P. mexicana* (Plath et al. 2003a, 2004a, 2007b). Thus, the selective pressure of actively avoiding male harassment by forming female shoals (Parzefall 1993b) might be lacking in the cave molly in their naturally dark habitat.

In summary, we found that (1) surface- and cave-dwelling Atlantic molly females discriminate between the sexes when in a dichotomous choice situation, (2) visual

cues are still being used by cave mollies for far-field sex recognition and thus (3) far-field sex recognition may be limited or even non-existent in their naturally dark habitat.

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