

## Photophilic behaviour in surface- and cave-dwelling Atlantic mollies *Poecilia mexicana* (Poeciliidae)

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The phototactic behaviour of surface- and cave-dwelling Atlantic mollies *Poecilia mexicana* was examined. Surface-dwelling Atlantic mollies, as well as light- and dark-reared cave fish showed photophilic behaviour under all light intensities used (620, 50 and 3 lx). © 2007 The Authors

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Cave fishes are emerging as a model system to study the evolutionary consequences of colonizing lightless habitats, such as the loss of eyes and pigmentation (Jeffery, 2001). Besides darkness, cave fishes often face reduced nutrient availability (with the exception of some tropical caves; Barr & Holsinger, 1985; Hüppop, 2000), because subterranean food webs typically rely on nutrient-influx from the surface (Poulson & White, 1969; Parzefall, 1993; Poulson & Lavoie, 2000). An exceptionally extreme subterranean habitat is the Cueva del Azufre near the village of Tapijulapa in the state of Tabasco, southern Mexico (17°26'30" N; 92°46'30" W). Its inhabitants need to cope with high levels of dissolved hydrogen sulphide (up to 300 µM H<sub>2</sub>S) and extreme hypoxia (Tobler *et al.*, 2006). H<sub>2</sub>S is a respiratory toxicant that causes high mortalities in metazoans even in micro-molar amounts (Smith *et al.*, 1977). Nevertheless, a large population of a cave-dwelling poeciliid fish can be found, the cave molly, a differentiated population of the widespread molly *Poecilia mexicana* Steindachner (previously described as a cave form of *Poecilia sphenops* Cuvier & Valenciennes; Gordon & Rosen, 1962).

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The cave mollies studied here originated from the innermost cave chamber (chamber XIII after Gordon & Rosen, 1962). The surface population originated from the El Azufre (the outflow of the cave) near the cave mouth, which contains less H<sub>2</sub>S (Tobler *et al.*, 2006). A comparison of mtDNA (D-loop) sequences suggested that the El Azufre population is the most recent ancestor of the cave molly (Möller, 2001). A microsatellite analysis has shown that the El Azufre population is genetically distinct from the cave population, and that gene flow between them is very limited and unidirectional from the cave to the surface habitat (Plath *et al.*, 2007). While many cave fishes have completely reduced eyes (Proudlove, 2006), the cave molly still possesses functional eyes that are slightly reduced in diameter (with the smallest eyes found in the innermost cave chambers; Peters *et al.*, 1973; Plath *et al.*, 2007a). Cave mollies can still respond to visual stimuli (Körner *et al.*, 2006; Plath *et al.*, 2006) and have modified several non-visual senses, *e.g.* cave mollies have an elaborated cephalic lateral line system and a higher density of taste buds on their heads (Parzefall, 2001).

Although the food web of this particular cave is considered energy rich due to chemo-autotrophic bacterial primary production and the input of bat guano (Langecker *et al.*, 1996), the *P. mexicana* caught in the cave exhibit a dramatically low body condition factor ( $K$ ;  $K$  is derived from  $K = ML_S^{-3}$ , where  $M$  is mass and  $L_S$  standard length; Tobler *et al.*, 2006), which may point towards costly adaptations to detoxify H<sub>2</sub>S. Furthermore, cave mollies spend 75% of their time breathing at the surface (aquatic surface respiration), which probably prevents them from feeding (M. Plath *et al.*, 2007b). The short-term survival in the natural habitat is critically dependent on energy availability (M. Plath *et al.*, 2007b). As an evolutionary response to energy limitation, several energy-demanding behaviours, such as aggression (Parzefall, 2001) and male mating behaviour (Plath *et al.*, 2003) are reduced in frequency or intensity.

Why do fishes enter such extreme sulphur caves at all? It has been suggested that nocturnal and photophobic animals are generally more likely to enter caves (Ludwig, 1942; Kosswig, 1960; Poulson, 1964; Barr, 1968). Indeed, among teleosts, the catfishes (Siluriformes), with numerous nocturnal species, constitute around one-third of all described cave fish species (Proudlove, 2006). Photophobic behaviour has been reported for several cave fishes, such as cave cyprinids, *Barbopsis devecchi* di Caporiacco (Ercolini & Berti, 1978), *Phreatichthys andruzzii* Vinciguerra (Ercolini & Berti, 1975), *Caecobarbus geertsii* Boulenger (Thinès, 1954, 1958) and *Garra barreimiae* Fowler & Steinitz (M. Timmermann & M. Plath, unpubl. data) as well as a cave loach, *Nemacheilus evezardi* Day (Cobitidae; Pati & Agrawal, 2002) and cave tetras, *Astyanax mexicanus* (De Filippi) [Characidae, previously described as *Astyanax fasciatus* (Cuvier) or *Anoptichthys jordani* (Hubbs & Innes); Langecker, 1989]. The aim of the present study was, therefore, to examine phototactic behaviour in the cave molly and its surface-dwelling ancestor. It was predicted that cave-dwelling *P. mexicana* would show photophobic behaviour, and that this response would also be found in the ancestral surface-dwelling form.

Test fish for the laboratory experiments were taken from large, randomly out-bred aquarium stocks. One lineage of the cave form as well as the surface population was maintained under a 12L:12D cycle. Another lineage was maintained in a lightless room (all test fish from this lineage were born in darkness).

Stocks were bred in aerated and filtered 150–200 l aquaria at 25° C. They were fed twice a day *ad libitum* with flake food, live *Artemia* sp. nauplii, *Daphnia* sp. and *Tubifex* sp.

The tests on the phototactic behaviour were conducted in a windowless room. The test tank (490 × 240 × 240 mm) was filled with aged tap water, and the temperature was adjusted at  $24 \pm 1^\circ$  C (mean  $\pm$  s.d.). A video camera was installed 1 m in front of the test tank, and videos were later analysed on a screen. The tank was divided in two halves by a black Plexiglas partition in a way that a small gap of 50 mm height above the tank bottom allowed the test fish to move between the two sides. Both compartments of the test tank contained a small filter and an aquarium heater. One half of the tank was covered on all sides by black plastic foil ('photonegative zone') and hence remained dark, the other half was not covered at the front and upper side ('photopositive zone'). A 60 W fluorescent bulb was installed 380 mm above the photopositive zone, and a transformer was used to adjust light intensities. Each fish was tested at three different light intensities in random order: (1) 620, (2) 50 and (3) 3 lx. Light intensities were measured at the bottom of the photonegative zone (under water) using a S-50 photoelement (R. Haase Scientific Supplies, Hanover, Germany) and read at (1) 1, (2) 0.5 and (3) 0.25 lx. Light intensities were probably even lower in the upper parts of the water column in the photonegative zone, but this could not be determined. To initiate a trial, a test fish ( $L_S = 31\text{--}51$  mm) was allowed to acclimate in the tank for 2 days. Only female mollies were used. The aquarium was illuminated using the respective light intensity for 12 h day<sup>-1</sup> (0700–1900 hours). On the third day, the phototactic behaviour of the focal animal was examined, once in the morning (0900 hours) and once in the afternoon (1400 hours). The time spent in either compartment and the number of changes between the two compartments were determined during both 30 min observation periods. Data from both parts of a trial were summed. Afterwards, another light intensity was adjusted, and the focal fish was allowed to acclimate to the changed illumination conditions for another 24 h. Then, the phototactic behaviour was measured under the changed illumination conditions as described above. Finally, the third light intensity was adjusted, the focal fish was given another 24 h for habituation, and measurements were repeated likewise. During this period of 7 days, the fish were fed in the late afternoon (after the second measurement) with *ad libitum* amounts of flake food, equally distributed between the photopositive and the photonegative zone.

Under all testing conditions, the focal fish spent more time in the photopositive zone than in the dark zone (paired *t*-tests, d.f.<sub>surface</sub> = 6, d.f.<sub>cave, light-reared</sub> = 19, d.f.<sub>cave, dark-reared</sub> = 8,  $P < 0.05$  in all cases; Fig. 1). There was no significant difference in the time spent in the photopositive zone among the three different light intensities in any of the three groups of fish (repeated measures ANOVA: d.f.<sub>surface</sub> = 2 (repeated measurement) and 6 (between subjects), d.f.<sub>cave, light-reared</sub> = 2 and 19, d.f.<sub>cave, dark-reared</sub> = 2 and 8,  $P > 0.05$  in all cases). There was also no difference among the three groups in time spent in the photopositive zone under any light intensity (one way ANOVA: d.f. = 2 and 33,  $P > 0.05$ ). A statistically significant difference, however, was detected among the three groups in the number of changes between the two zones (one way

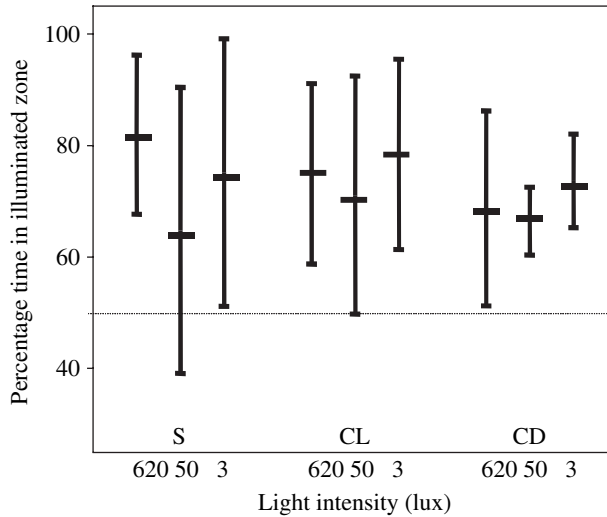


FIG. 1. The mean  $\pm$  s.d. percentage of time spent in the photopositive (illuminated) zone in surface-dwelling (S;  $n = 7$ ), light-reared cave-dwelling (CL;  $n = 20$ ) and dark-reared cave-dwelling (CD;  $n = 9$ ) *P. mexicana*. Each fish was tested at three light intensities (620, 50 and 3 lx). Values  $>50\%$  (.....) indicate photophilic behaviour.

ANOVA: d.f. = 2 and 33,  $P < 0.01$  for all light intensities). *Post hoc* pair-wise comparisons revealed that dark-reared cave mollies showed the largest number of changes and differed significantly from the two other groups (Tukey test: all  $P < 0.05$ ; Fig. 2). The number of changes did not significantly differ between the three different light intensities in any of the three groups of fish tested (repeated measures ANOVA: d.f.<sub>surface</sub> = 2 and 6, d.f.<sub>cave, light-reared</sub> = 2 and 19, d.f.<sub>cave, dark-reared</sub> = 2 and 8,  $P > 0.05$  in all cases).

Surface- and cave-dwelling *P. mexicana* showed photophilic behaviour. For surface fish, this is in accordance with the observation that this species is diurnal

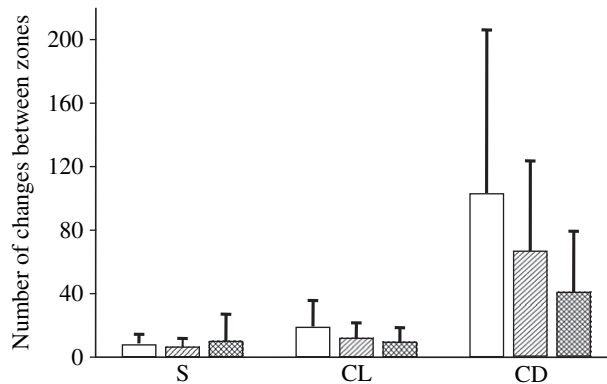


FIG. 2. The mean  $\pm$  s.d. number of times the focal fish changed between the photopositive and the photonegative zone during 1 h of observation time. Surface-dwelling (S), light-reared cave-dwelling (CL) and dark-reared cave-dwelling *P. mexicana* (CD) were tested at three light intensities [620 (□), 50 (▨) and 3 lx (▩)].

and shoals in shallow stream passages, where sunlight penetrates the water column. In contrast, the Mexican tetra *A. mexicanus* (another diurnal species) has been reported to be photophobic in most studies for both the surface- and the cave-dwelling form (Lüling, 1954; Thinès, 1954; Gertychowa, 1970; Romero, 1985). Breder & Gresser (1941) and Breder & Rasquin (1947) described indifferent or photophilic behaviour for this species. A potential problem of such studies on the phototactic behaviour in fishes is that many species seek shelter when introduced into a novel environment, and any disturbance by the observer will lead to a stress response. This is especially relevant for eyed fishes like surface-dwelling *A. mexicanus* and both surface- and cave-dwelling *P. mexicana*, which perceive visual cues from outside of the test aquarium. For example, when *P. mexicana* were tested in a similar set-up as used here albeit with a shorter acclimatization period (5 min) and were observed directly, they were 'photoindifferent' and showed signs of distress (freezing and enhanced gill ventilation rates; unpubl. data). In the present study, this problem was avoided: the test fish had sufficient time to acclimate to the test tank before the tests and no observer was present in the experimental room.

The present study suggests that photophobic behaviour is not a necessary prerequisite for cave colonization (Langecker, 1989; Romero & Green, 2005), and other factors promoting the colonization of the Cueva del Azufre by *P. mexicana* need to be considered. Accidental entrapment of cave mollies in the Cueva del Azufre is unlikely, because the stream draining the cave flows out of the cave, so that the early cave colonizers needed to swim against the water current, and there are no physical barriers that would prevent fish from leaving the subterranean habitat again (Tobler *et al.*, 2006). Despite their harsh environmental conditions, extreme habitats, such as the Cueva del Azufre, may actually provide several benefits to the colonizing organisms. Colonizers of extreme habitats may occupy free niches due to the absence of competing species (Romero & Green, 2005; Tobler *et al.*, 2006). The lack of piscine and avian predation may be another benefit of cave-dwelling (Tobler *et al.*, 2006). Although an aquatic heteropteran (*Belostoma* sp. Latreille) is known to prey on cave mollies (Plath *et al.*, 2003), a quantitative analysis of the predation pressures in and outside the cave has not yet been conducted. Finally, a reduced exposure to parasites may provide a benefit for cave colonizers, since the complex life cycles of parasites may be interrupted (Tobler *et al.*, 2007). For example, cysts of the digenean trematode *Uvulifer* sp. that are abundant in surface populations were never found in cave-dwelling mollies, because this parasite requires piscivorous birds as final host, which are absent in the cave (Tobler *et al.*, 2007).

In the front cave chambers of the Cueva del Azufre, several holes in the rooftop provide some light. Local densities of cave mollies are especially high in illuminated areas, but a quantitative analysis of local densities in different microhabitats remains to be carried out. If cave mollies show photophilic behaviour, why then are cave mollies also found in the inner, permanently dark parts of the cave? Interestingly, the number of changes between the photopositive and the photonegative zone was significantly higher in dark-reared cave mollies. Apparently, once cave mollies have been reared in darkness, they enter the dark zone more frequently. This may partly explain why cave mollies also enter dark pools in the Cueva del Azufre. It seems probable, however, that

other factors, such as food availability, need to be considered. For example, bat guano is not evenly distributed in the cave, but occurs in dark cave chambers with roosting bat colonies. Microhabitat patchiness within the cave, and especially the patchy distributions of food, light and hydrogen sulphide, clearly warrant future investigations.

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