

Offspring number in a livebearing fish (*Poecilia mexicana*, Poeciliidae): reduced fecundity and reduced plasticity in a population of cave mollies

Rüdiger Riesch · Michael Tobler · Martin Plath · Ingo Schlupp

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Abstract Life history traits within species often vary among different habitats. We measured female fecundity in mollies (*Poecilia mexicana*) from a H₂S-rich cave and from a neighbouring surface habitat, as well as in laboratory-reared individuals of both populations raised in either light or continuous darkness. Compared to

conspecifics from surface habitats, cave-dwelling *P. mexicana* had reduced fecundity (adjusted for size) in the field. In the laboratory, the fecundity of surface mollies was higher in light than in darkness, whereas fecundity in the cave mollies was almost unaffected by the ambient light conditions. Our results suggest a heritable component to the reduction in fecundity in female cave mollies. Moreover, the reduced plasticity in fecundity of cave mollies in response to light conditions might be an example of genetic assimilation or channeling of a life history trait in a population invading a new environment.

R. Riesch (✉) · M. Tobler · I. Schlupp
Department of Zoology, University of Oklahoma,
730 Van Vleet Oval,
Norman, OK 73019, USA
e-mail: ruedigerriesch@ou.edu

R. Riesch · I. Schlupp
Biozentrum Grindel, Zoologisches Institut,
Universität Hamburg,
Martin-Luther-King Platz 3,
20146 Hamburg, Germany

M. Tobler
Zoologisches Institut, Universität Zürich,
Winterthurerstrasse 190,
CH-8057 Zürich, Switzerland

M. Plath
Institut für Biochemie/Biologie,
Abteilung für Tierökologie, Universität Potsdam,
Maulbeerallee 1,
14469 Potsdam, Germany

M. Plath
Institut für Biochemie/Biologie,
Abteilung für Evolutionsbiologie/Spezielle Zoologie,
Universität Potsdam,
Karl-Liebknecht-Str. 24–25,
14476 Potsdam, Germany

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Introduction

Life history strategies vary widely across species, but also between different populations of the same species (Stearns 1992; Roff 2002). Within species variation in life history traits, which may be genetically fixed or phenotypically plastic, can be influenced by several environmental factors, such as resource availability, predation and stressful environmental conditions (Sibly and Calow 1989; Stearns 1992; Roff 2002). Cave-living organisms provide a unique opportunity to investigate life history evolution, because they have colonized a radically different habitat and, in some cases, the

surface-dwelling ancestor is available for comparison. Life histories of cave animals are generally characterized by shifts towards longer life and generation times, older age at first reproduction and increased brood care behaviour, as well as a decreased fecundity combined with an increase in individual offspring size (Collembola: Christiansen 1965; *Gammarus minus* (Amphipoda): Culver et al. 1995; Teleostei: *Amblyopsis rosae* (Amblyopsidae): Winemiller 1992; *Astyanax mexicanus* (Characidae): Hüppop and Wilkens 1991; *Trichomycterus chaberti* (Trichomycteridae): Pouilly and Miranda 2003; for a review see Parzefall 2000). These traits were mostly interpreted as adaptations to food scarcity (Culver 1982; Hüppop 2000), because caves are usually nutrient-poor due to a lack of photoautotrophic primary production and reliance on nutrient-influx from epigeal habitats (Poulson and Lavoie 2000). Caves, however, also differ from surface habitats in other factors known to influence life history traits, including relaxed predation (Romero and Green 2005; Tobler et al. 2006, 2007a) or the presence of suboptimal gas concentrations and toxicants (Howarth 1993; Tobler et al. 2006); but the importance of different selective forces in the evolution of life history strategies of cave dwellers is as yet poorly understood.

We studied life history traits in a small livebearing fish, the cave molly, a divergent population of the Atlantic molly (*Poecilia mexicana*, Poeciliidae) from the Cueva del Azufre in Tabasco, Mexico (Gordon and Rosen 1962; Parzefall 2001). The water in this particular cave contains high concentrations of toxic hydrogen sulphide (H_2S , up to 320 μM ; Tobler et al. 2006). Migration into the cave from the outside is absent, and a source-sink relationship between surface and cave populations can be ruled out (Plath et al. 2007a). We compared the fecundity (size-corrected number of oocytes) of cave molly females with that of conspecifics from an adjacent non-sulphidic surface habitat using specimens collected in the field. Based on previous comparisons of epigeal and hypogean organisms, we expected a reduction of fecundity (Christiansen 1965; Hüppop and Wilkens 1991; Winemiller 1992; Culver et al. 1995; Parzefall 2000; Pouilly and Miranda 2003). In a laboratory experiment, we further examined whether differences in fecundity between surface and cave mollies are due to plasticity in response to environmental conditions (presence or absence of light) rather than fixed traits. Our null-hypothesis was that the fecundity of both cave and surface-dwelling molly

females would be fixed and that fecundity would not vary among the two treatment groups.

Methods

Study sites

All fish used in this study originated from the Cueva del Azufre and adjacent waters near Tapijulapa (Tabasco, Mexico). The waters studied eventually drain into the Río Oxolotan and are located within a radius of about 5 km. Within the cave, mollies were sampled from three chambers, V, X and XIII (Gordon and Rosen 1962), but data were pooled for analysis. For comparison, surface mollies were collected from a creek of similar size and structure lacking hydrogen sulphide (Arroyo Cristal). Details for the study sites can be found in Tobler et al. (2006).

Field study

Females of both the surface and the cave populations were collected in August 2004 and January 2006 with seines and dip nets (see Table 1 for sample sizes). Dip nets were used in the cave where low ceilings and rocky habitats preclude efficient seining. Fish were sacrificed with an overdose of MS 222 (Tricaine Methanesulfonate), and the standard length (SL) of each fish was measured. Then, the body cavity was opened and the number of ripe oocytes or developing embryos, if present, was counted.

Statistical analysis

The frequency of gravid females was compared between habitats and years using a binary logistic regression where “gravid (yes/no)” was the dependent variable and “population” and “year” were the independent variables. To test for differences in the fecundity of females between habitats and years, non-gravid females were excluded from the analysis, and data were square-root-transformed in order to achieve a normal distribution. Fecundity data were analyzed using a General Linear Model (GLM) with “number of oocytes (embryos)” as the dependent variable, “standard length” (SL) as a covariate, and “population” and “year” as independent variables. The interaction “population by year” was not significant ($F_1=0.002$, $P=0.964$), hence it was re-

Table 1 Descriptive statistics from the field study and the laboratory experiment

	Number	% gravid	Fecundity (mean±SD)	Fecundity (range)	SL of gravid females	Size-corrected fecundity (mean±SD)
Field data						
Surface						
2004	59	20.3	21.3±10.3	4–45	47.7±5.3	3.9±0.7
2006	29	27.6	24.5±11.0	11–47	52.5±10.9	3.9±0.8
Cave						
2004	48	75.0	3.0±1.4	1–6	35.6±3.7	2.0±0.7
2006	23	47.8	4.2±3.2	1–11	38.7±6.2	2.0±0.6
Laboratory data						
Surface						
Light	29	89.7	8.5±5.6	2–23	35.6±5.3	2.4±0.5
Darkness	32	65.6	5.1±3.5	1–14	32.3±2.9	2.1±0.5
Cave						
Light	67	71.6	4.3±2.6	1–13	32.6±4.1	2.0±0.5
Darkness	29	82.8	2.8±1.6	1–7	27.4±3.7	2.1±0.5

The size-corrected fecundity data refer to the estimated marginal means of the GLM

moved from the final model. Because of limitations in the field we did not attempt to stage or weigh the embryos. This will be reserved for future research. All statistical analyses were performed with SPSS 11 (SPSS Inc 2002).

Laboratory study

Randomly out-bred laboratory populations of mollies, originally collected in the hindmost cave chamber XIII and the Río Oxolotan, were kept at the Biozentrum Grindel in Hamburg, Germany. These populations were established originally in the 1970s and repeatedly “refreshed” with wild-caught animals. Fish were reared and maintained in 100–200 L aquaria at 25–30°C. All populations were reared separately. Fish were fed *ad libitum* twice daily with commercially available flake food, *Artemia* nauplii, water fleas, and *Tubifex* worms.

Since these stocks were established, fish were subjected to two treatments. Three to five stock tanks of each population were kept either in continuous darkness or under a normal day–night light cycle (16:8 h using artificial fluorescent light). Hence, each population was kept both in their natural light regime as well as in an artificial light regime. Besides the differences in the illumination, maintenance conditions were identical in the two experimental treatments. As in the field study, females were sacrificed with an overdose of MS 222 for

the assessment of fecundity. The SL of each fish was measured, the body cavity was opened, and the number of ripe oocytes or developing embryos was counted if present.

Statistical analysis

The frequency of gravid females was compared between populations and light treatments using a binary logistic regression where “gravid (yes/no)” was the dependent variable, and “population” and “light condition” (light vs. darkness) were independent variables. For the analysis of female fecundity, we employed a similar analysis as described above, but the independent variables of the GLM were “population” and “light condition” (light vs. darkness).

It is important to note that there were size differences for both populations between the field and the laboratory treatments (Table 1). One possible explanation for this is that the growth patterns between natural populations and our laboratory-reared populations were different (e.g., due to differences in type and amount of food). However, this size difference does not have any influence on our interpretation, because body size was included as a covariate in the analyses, and the relationship between female size and offspring number in both populations is expected to be linear for the observed size range (as found in the field data: Table 3).

Table 2 Logistic regression on the frequency of gravid females

	-2 log likelihood	<i>B</i>	SE	Wald	<i>df</i>	<i>P</i>
Field data	179.585					
Population		1,591.396	751.842	4.480	1	<i>0.034</i>
Year		0.993	0.590	2.829	1	0.093
Population×year		-0.793	0.375	4.470	1	<i>0.034</i>
Laboratory data	167.041					
Population		-1.233	0.667	3.413	1	0.065
Light condition		-3.668	1.535	5.708	1	<i>0.017</i>
Population×light condition		2.155	0.909	5.624	1	<i>0.018</i>

Significant *P*-values are in italics

Results

Field study

Generally, a higher proportion of *P. mexicana* females from the cave were gravid as compared to fish from the surface habitat (Tables 1, Field data and 2, Field data). The frequency of gravid cave molly females was lower in January 2006 compared to August 2004. In surface-dwelling females, it was higher in 2006 compared to 2004.

Cave molly females had significantly lower fecundity compared to conspecifics from surface habitats (Tables 1, Field data and 3, Field data). The fecundity of both females from the cave and the surface habitat increased with female standard length.

Laboratory study

The laboratory experiment revealed significant differences in the frequency of gravid females among populations (Tables 1, Laboratory data and 2, Laboratory

data). The interaction “population by light condition” was significant: In surface mollies, a higher proportion of females were gravid in light than in darkness. In the cave molly, however, the difference in proportion gravid between light conditions was less pronounced (albeit not significantly), and was even slightly higher in darkness.

As in the field, fecundity increased with female standard length (Table 3, Laboratory data). In the GLM, cave mollies showed a trend of having a lower fecundity than surface mollies (Table 3, Laboratory data). There was a significant interaction between population and light condition. The fecundity of surface mollies was higher in light than in darkness, whereas fecundity in the cave mollies did not respond strongly to the light conditions (Table 1).

Discussion

In the field, cave mollies showed lower fecundity compared to conspecifics from surface habitats. In our experiment, however, where only the light conditions

Table 3 General linear model (GLM) of female fecundity (square root-transformed) in the field study and the laboratory experiment

Factor	<i>df</i>	Mean squares	<i>F</i>	<i>P</i>
Field data				
SL	1	11.401	31.370	< <i>0.001</i>
Population	1	22.505	61.923	< <i>0.001</i>
Year	1	0.000	0.000	0.983
Laboratory data				
SL	1	25.681	109.338	< <i>0.001</i>
Population	1	0.903	3.847	0.052
Light condition	1	0.035	0.149	0.700
Population×Light condition	1	1.404	5.978	<i>0.016</i>

Significant *P*-values are in italics

SL Standard length

differed among treatments, fecundity differences among cave and surface populations were less pronounced. Whereas fecundity of surface mollies was reduced in continuous darkness as compared to normal light conditions, cave molly fecundity did not vary across the two experimental treatments. Our design provided very benign conditions because we provided *ad libitum* amounts of high quality food, and toxic H₂S, which is highly prevalent under natural conditions (Tobler et al. 2006), was absent. Such conditions should allow cave mollies to express the maximum of their reaction norm. Hence, differences between cave and surface forms observed in the field are not caused by entirely plastic responses to light conditions.

While surface-dwelling *P. mexicana* show phenotypic plasticity in fecundity, size-corrected fecundity of cave mollies did not vary in response to the light treatment suggesting that the fecundity reaction norm became much smaller in this population. Phenotypic plasticity in fecundity may be advantageous in variable surface habitats (Via 1993; de Jong 1995), but plasticity was lost in the more stable cave habitat. Thus, phenotypic plasticity in fecundity might have been genetically assimilated or channelled in response to the persistent exposure of new environmental conditions (see Pigliucci 2005).

Incidentally, the reduction in fecundity is likely caused by selection on offspring size, which would constrain the maximum number of offspring a cave molly female could produce. Three different selective forces could be affecting fecundity directly and/or indirectly: (1) Caves are often considered predator-free environments. Different predatory regimes have been shown to affect life history traits in the related guppy (Reznick and Endler 1982; Reznick et al. 1990). Indeed, the predatory regimes differ between hypogean and epigeal habitats in that predatory fish and piscivorous birds are lacking in the cave (Tobler et al. 2006, 2007a), but the Cueva del Azufre is clearly not predator free. Giant water bugs (*Belostoma* sp.) are abundant in the cave system and are known to prey on mollies (Plath et al. 2003; Tobler et al. 2007b; Tobler et al. 2008). Therefore, lack of predation may not be responsible for the observed life history differences. (2) The Cueva del Azufre contains high amounts of toxic H₂S (Tobler et al. 2006). Costly adaptations to excrete or neutralize toxins have been shown to have a profound influence on life history traits possibly by draining resources that could have been invested into reproduction (Sibly and Calow 1989).

Indeed, short-term survival of cave mollies in sulphidic habitats critically depends on energy availability (Plath et al. 2007b), but physiological adaptations of cave mollies to H₂S remain to be studied. (3) Resources may be limited in the cave. In fact, food limitation has been documented in many cave systems, and cave animals often evolve morphological and/or behavioural traits to cope with food scarcity (Hüppop 2000). Resource limitation has been proven experimentally to influence fecundity in guppies (Reznick and Yang 1993; Reznick et al. 2001). The Cueva del Azufre was hypothesized to be rich in food resources because of bacterial chemoautotrophic primary production and the input of bat guano (Langecker et al. 1996), but a recent study has demonstrated reduced body condition in cave mollies compared to surface-dwelling fish from non-sulphidic waters (Tobler *in press*). This may indicate that the detoxification of hydrogen sulphide requires a substantial amount of energy and/or resource scarcity.

Current research will attempt to uncover differences in other life history traits in surface and cave mollies, the trade-offs between them and the extent to which they are plastic. The magnitude of fecundity differences among surface and cave mollies clearly highlights the value of this system in the understanding of life history evolution.

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