Giant water bug (*Belostoma* sp.) predation on a cave fish (*Poecilia mexicana*): effects of female body size and gestational state

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ABSTRACT

Background: Predation is an important driver of life-history trait evolution. Fish predators may prey selectively on certain prey size classes, or pregnant females, particularly those of livebearing prey species. This selectivity ought to affect the evolutionary trajectory of the prey population. In sulphidic and hypoxic habitats, Atlantic mollies (*Poecilia mexicana*) have to spend considerable time engaging in aquatic surface respiration, exposing themselves to predation by giant water bugs (*Belostoma* sp.). Compared with other females, pregnant *P. mexicana* experience greater oxygen demands leading to more aquatic surface respiration.

Questions: Are pregnant *P. mexicana* females more likely to be captured by *Belostoma* as a result of more aquatic surface respiration and decreased flight abilities (i.e. slower fast–start responses)?

Organisms and location: (1) Atlantic mollies (*P. mexicana*: Poeciliidae, Teleostei) inhabiting a sulphidic cave (Cueva del Azufre) in Tabasco, México. (2) A co-existing sit-and-wait predator, the giant water bug *Belostoma* sp. (Belostomatidae, Hemiptera), which catches surfacing fish at the water's edge.

Methods: Predation experiments inside the Cueva del Azufre. In Experiment 1, one randomly selected cave molly female was placed into a perforated bottle with one individual water bug. In Experiment 2, two size-matched females (one pregnant and one non-pregnant) were placed into a perforated bottle with one individual water bug.

Results: Capture rates after 24 h in Experiment 1 were correlated mainly with female body size. But larger females were also more likely to be pregnant, making it difficult to disentangle the effects of size and pregnancy. Experiment 2 isolated the effect of pregnancy, and water bugs clearly preferred pregnant over non-pregnant prey.

Keywords: aquatic surface respiration, cave fish, life-history evolution, Poeciliidae, size-selective predation.

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INTRODUCTION

Predators can profoundly affect the ecology and evolutionary trajectory of populations, as well as the behaviour and expression of defensive traits in individuals. Among the best-documented effects of predators are evolutionary changes on prey life-history strategies (e.g. Spitze, 1991; Martin, 1995). In guppies (*Poecilia reticulata*) and other livebearing fishes, for example, individuals are selected for earlier age of maturity, increased fecundity, and reduced offspring size under high predation (e.g. Reznick and Endler, 1982; Endler, 1995; Johnson and Belk, 2001; Reznick *et al.*, 2001), probably as a result of increased predator-induced mortality in adults. Furthermore, large newborn offspring may be able to escape gape-limited predators in low predator environments, where small predators and adult conspecifics are the main predation threat, providing fitness benefits for having increased offspring size (e.g. Hoyle and Keast, 1987, 1988; Persson *et al.*, 1996; but see Mattingly and Butler, 1994). In contrast, high predation environments select for many small offspring as a bet-hedging strategy (Einum and Fleming, 2004). Selection via differential predation pressures, however, may also directly act upon investments into reproduction. Especially in fish species with internal gestation, females carrying large clutches may be at a disadvantage when escaping from predators, because high reproductive investment can impair swimming ability and fast start responses (e.g. Olsson *et al.*, 2000; Ghalambor *et al.*, 2004). Here, we investigated differential predation by a giant water bug on female fish in various states of reproduction in an extreme environment.

Previously, it has been suggested that organisms invading extreme habitats trade off increased costs of somatic maintenance due to the presence of physico-chemical stressors with reduced costs imposed by natural enemies, such as predators and parasites (e.g. Robb and Abrahams, 2002; Romero and Green, 2005; Rogowski and Stockwell, 2006; Springer *et al.*, 2007; Tobler *et al.*, 2007a; Springer, 2009). Many cave habitats, for example, are essentially predator-free habitats for fishes inhabiting them, so cave fishes typically rank in the uppermost portion in the trophic chain of subterranean ecosystems (Poulson and Lavoie, 2000). Recent studies on extremophile poeciliid fishes from southern México, however, indicate that predators may not always be absent, but that the kind of predation experienced in extreme environments may simply change. For example, predatory fish may be absent in toxic, sulphidic springs and caves (Tobler *et al.*, 2006, submitted; Riesch *et al.*, 2009a). However, poeciliids inhabiting sulphidic springs face disproportional predation risk by piscivorous birds (Riesch *et al.*, 2010a), while cave populations are preyed upon by giant water bugs of the genus *Belostoma* (Plath *et al.*, 2003; Tobler *et al.*, 2007b, 2008b; Tobler, 2009) as well as pisaurid and theraphosid spiders (Horstkotte *et al.*, 2010). *Belostoma* sp. occurs at high densities in the Cueva del Azufre; using mark–recapture analysis, the population size of this sit-and-wait predator was estimated as 336 ± 130 (mean ± S.E.) individuals in cave chamber V of the Cueva del Azufre, which results in a density of over one *Belostoma* per square metre (Tobler *et al.*, 2007b). Predation risk is higher in more sulphidic and thus hypoxic microhabitat patches, where the fish spend extended periods of time at the surface performing aquatic surface respiration, which maximizes oxygen acquisition in the hypoxic and sulphidic environment (Plath *et al.*, 2007b; Tobler *et al.*, 2009). Also, *Belostoma* predation is male-biased (Tobler *et al.*, 2008b), as cave molly males are known to have higher oxygen demands and thus spend more time showing aquatic surface respiration (Plath *et al.*, 2007b). Altogether, then, it seems that the presence of hydrogen sulphide (H_2S) forces cave mollies to the water surface, where they are exposed to elevated levels of predation. Hence, the presence of an abiotic stressor in this system leads to increased – not decreased – predation by *Belostoma* (Plath and Tobler, 2010).

In the present study, we wished to determine whether predation by *Belostoma* varies with gestational state of female cave mollies, potentially being one source of selection shaping the starkly divergent life-history strategies of extremophile livebearers (Riesch *et al.*, 2010b, 2010c, 2011). Pregnancies in poeciliids and other livebearing animals are physiologically costly and can lead to an increase in energy requirements, that is metabolic rates (e.g. Boehlert *et al.*, 1991; DeMarco and Guillette, 1992; Liu *et al.*, 2003; Speakman, 2008), and a decrease in swimming performance (e.g. Olsson *et al.*, 2000; Ghalambor *et al.*, 2004; but see Plaut, 2002). In cave mollies, this would force pregnant females to spend more time at the water surface engaging in aquatic surface respiration, while simultaneously reducing their fast-start response when being attacked by *Belostoma*. Thus, we hypothesized that pregnant females suffer from higher *Belostoma* predation rates than non-pregnant females.

METHODS AND MATERIALS

General procedure

Prey choice experiments were conducted in cave chamber V of the Cueva del Azufre (Gordon and Rosen, 1962) using animals collected on site. Generally, we use the terms 'choice (experiment)' or '(prey) preference' with the understanding that what appears to be a preference by *Belostoma* could in reality be a function of prey escape ability or target size and predator handling ability. Larger and more pregnant prey may be easier for *Belostoma* to detect and easier to make contact with given their specific feeding mode.

In Experiment 1, we tested for differential *Belostoma* predation in a 'no-choice' predation experiment; that is, water bugs could either prey on the one fish with which they were cohabitated in an experimental container or not feed at all. For this approach, a random sample of field-collected cave molly females was used as potential prey, so prey fish showed natural variability not only in gestational states, but also in body size. Standard length in Experiment (1) turned out to be a strong predictor not only of numbers of offspring per pregnant female (see also Riesch *et al.*, 2009b), but also of pregnancy itself (with large females being more likely to be pregnant) (Fig. 1). Furthermore, the hemipterans showed a strong predilection for larger prey fish (see Results). Consequently, we ran another experiment to investigate the potential role of gestational state of the prey females in more detail. In Experiment 2, a single water bug could choose to prey upon one of two simultaneously offered cave molly females that were size-matched, so that any confounding effect of body size was eliminated, and prey choice would be attributable to females' gestational state only. Together, these two experiments allowed us to disentangle the relative effects of prey body size (see Plath *et al.*, 2003; Tobler *et al.*, 2007b, 2008b) and gestation on water bug capture rates.

Experiment 1

In Experiment 1, we inserted one individual of *Belostoma* (body length, head to tip of abdomen: 18.0 ± 5.5 mm, mean $\pm$ S.D.) and one cave molly female (standard length: 33.0 ± 6.6 mm, size range: 22–54 mm) into a plastic bottle (1.5 L). Fish were checked for potential puncture wounds stemming from previous encounters with water bugs, and only fish without any wounds were used for our experiments. The bottles were perforated to
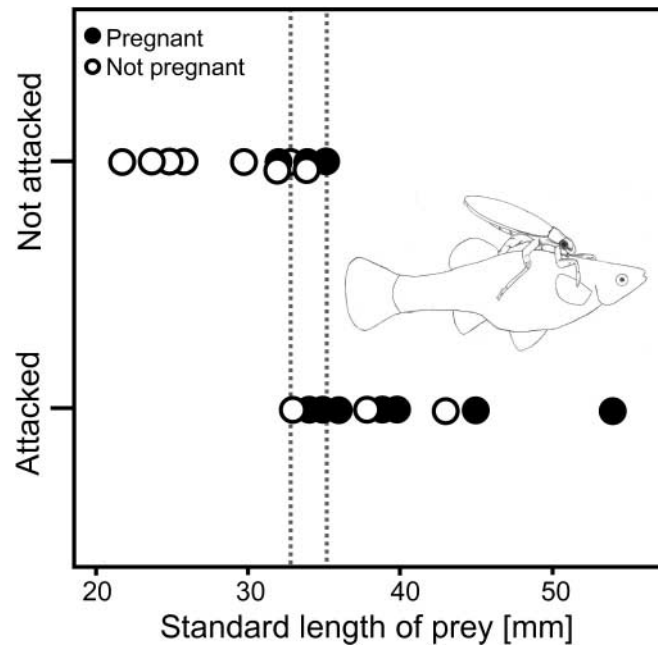
Fig. 1. Effect of body size (standard length, x-axis) and gestational state of the prey (●: pregnant cave molly females, ○: non-pregnant females) on the likelihood of capture by *Belostoma* sp. over 24 h in Experiment 1. Insert: water bug (*Belostoma* sp.) attacking a molly female.

supply the animals with fresh water and air. They were placed in shallow parts of the cave in a horizontal position such that a part of the bottle remained filled with air. Therefore, hemipterans could breathe and cave mollies could perform aquatic surface respiration (Plath *et al.*, 2007b; Tobler *et al.*, 2008b, 2009). *Belostoma* in the wild are typically found on rocky edges along the water (Tobler and Plath, submitted). However, we did not include rocks in the bottles, as this would have increased the risk of mechanical damage to the delicate fish skin during handling of the bottles. Still, the water bugs could sit on the walls of the bottles at the water's edge in a similar way as they ambush their prey in the natural environment. The bottles were covered on site with stones to prevent them from drifting away. We chose only places where hemipterans and fish naturally co-occurred to conduct the experiment. Twenty trials were conducted on 30 January 2010, and an additional 13 trials on the following day.

We checked the bottles after 24 h and noted whether a molly was wounded (i.e. had puncture wounds caused by *Belostoma* attacks) or was dead. Afterwards, all animals were measured for body size, and the water bugs were released at the collection site. The mollies were sacrificed using an overdose of MS222 and dissected on site. The ovary was inspected, and oocytes or developing embryos – if present – were counted. Due to limitations under field conditions, we could not determine the proportion of reproductive tissues per female body weight [i.e. gonadosomatic index (GSI)], as was done for Experiment 2 (see below).

Experiment 2

The experimental design for Experiment 2 was similar to that of Experiment 1, but with the following exceptions: we inserted one individual of *Belostoma* (body length: 18.3 ± 8.4 mm) and two size-matched cave molly females per 1.5-L bottle (standard length: 38.0 ± 2.4 mm, size range: 34–42 mm; mean size difference within pairs: 0.9 ± 0.9 mm, maximum size difference: 3 mm). Size-matched pairs were prepared so that females likely differed in their reproductive state/strategy (i.e. a female with large abdominal distension was always paired with a size-matched female with small distension). Gestational states were determined precisely after the termination of the experiment (see below). Twenty-two trials were conducted between 22 March and 24 March 2010.

Upon termination of the experiment, females were sacrificed using an overdose of MS222 and fixed in 10% formalin for subsequent analysis in the laboratory. There, females' carcasses were measured for standard length and weighed (blotted wet weight) on a Ohaus AV213 Adventurer Pro Digital Balance. Then females were dissected, reproductive tissues were removed and, if present, oocytes and developing embryos were counted to determine fecundity. We calculated the gonadosomatic index as the weight of all oocytes and developing embryos divided by the female's body mass.

Statistical analyses

We used logistic regressions to analyse survival of the fish ('1') or capture events ('0'; with the category 'captured' combining cases of dead and wounded mollies) in Experiment 1. Within the subset of pregnant cave molly females (16 of 33), a positive correlation between body size (standard length, SL) and numbers of offspring was uncovered (Spearman rank correlation: $r_S = 0.50$, $P = 0.047$, $N = 16$). Therefore, we did not include numbers of offspring as a covariate, but instead coded gestational state as a bivariate factor (pregnant vs. non-pregnant). However, the direction of the result did not change even when total numbers of oocytes and embryos were used as a covariate. Female body size (SL) was included as a covariate. We used an information theoretical approach based on the Akaike information criterion (AIC) to select the most likely model, ranging from the most complex model ('pregnancy' + 'SL' + 'pregnancy × SL'; AIC = 74.14), over a model including only the main effects ('pregnancy' + 'SL'; AIC = 76.69) to models involving only 'pregnancy' (AIC = 97.84) or only 'SL' (AIC = 68.76).

Since no-choice experiments, like Experiment 1, are basically akin to standard selection experiments [even though most studies use only certain components of fitness (Futuyma, 2005); in our case this was 24-h survival], we also included a selection analytical framework and calculated selection gradients (*b*-values) for female standard length and number of embryos [standardized partial regression coefficients, while forcing both main effects into a regression model (Lande and Arnold, 1983)].

In Experiment 2, we also ran binary logistic regressions on capture events (yes/no), in which block (mesocosm), prey fish SL, body mass, and gonadosomatic index (GSI) were explanatory variables. Again, we used a model selection approach based on AIC-values. The best model (AIC = 117.00) had the structure 'block' + 'body mass' + 'GSI'; the only difference in the second best model (AIC = 117.08) was that it included 'SL' instead of 'body mass'. All statistical analyses were performed in SPSS v.12.0 for Windows.

RESULTS

Experiment 1

When we terminated the experiment after one day, predation had occurred in 15 of 33 bottles (45.5%). In five cases, the molly was dead, while in another ten cases wounds resulting from *Belostoma* bites (1–5 per fish) were detected. A distinct threshold was discernible determining whether or not cave mollies were caught or attacked, with the largest non-attacked molly being 35 mm long, and the smallest attacked individual measuring 33 mm, and this pattern appeared to be unaffected by the gestational state of the prey (Fig. 1). Mean (± s.d.) standard length of attacked females was 38.5 ± 5.4 mm, and that of non-attacked females 29.4 ± 4.3 mm. However, pregnant females in this random sample of fish were distinctly larger (37.4 ± 5.4 mm, 32–54 mm) than non-pregnant females (29.9 ± 5.6 mm, 22–43 mm), which may have confounded an existing effect of pregnancy on capture success. This question, therefore, was also addressed in more detail in Experiment 2.

The most likely logistic regression model on survival rates (AIC = 68.76) included only SL (Wald = 4.69, d.f. = 1, P = 0.030). Indeed, when we calculated selection coefficients (standardized partial regression coefficients) we found a strong, negative value in the case of SL (b = −0.535) and a weaker value for pregnancy (b = −0.271).

Experiment 2

After 24 h, predation had occurred in 18 of 22 bottles (81.8%). In all cases, there was no immediate mortality, but wounds resulting from *Belostoma* bites (1–4 per fish) were detected.

In total, 19 of 44 (43.2%) cave molly females showed puncture wounds, and in most cases only one of the two fish per bottle was attacked. In 17 cases (89.5%) the captured female was the one with a higher GSI, in only one case did the water bug attack the female with the lower GSI (5.3%), and in one case (5.3%) both females were attacked. Mean (± s.d.) GSI values were twice as high in females that were attacked (0.15 ± 0.05) than in those that were not attacked (0.07 ± 0.05; Fig. 2). Similarly, attacked females had on average 4.8 ± 2.0 oocytes or embryos in their ovaries, compared with only 2.7 ± 2.2 in non-attacked females (Fig. 2).

The most likely logistic regression model (AIC = 117.00) had the model structure 'block' + 'body mass' + 'GSI'. However, only 'GSI' had a statistically significant effect ('block': Wald = 2.97, d.f. = 1, P = 0.085; 'body mass': Wald = 0.55, d.f. = 1, P = 0.46; 'GSI': Wald = 10.51, d.f. = 1, P = 0.001).

DISCUSSION

Our results demonstrate that body size and female gestational state strongly predict the likelihood of capture with (a) larger fish being more likely to be preyed upon (see also Plath *et al.*, 2003; Tobler *et al.*, 2007b), and (b), at least when two potential prey fish are size-matched, pregnant females being more susceptible to predation than non-pregnant females.

Why were larger fish caught more often than smaller ones? The easiest explanation would be that larger prey may be easier for the water bugs to detect and make contact with given their specific feeding mode. A higher likelihood of large fish to be preyed upon, therefore,
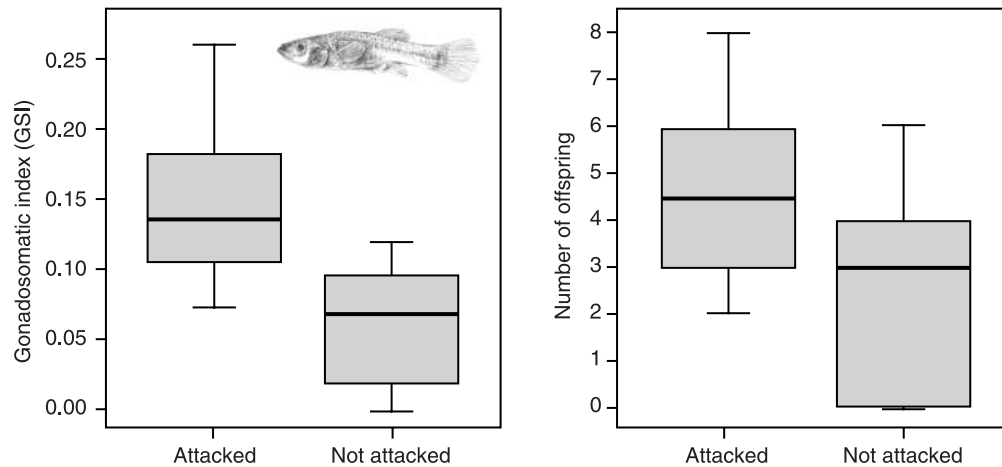
Fig. 2. Females that were attacked by *Belostoma* in Experiment 2 had a higher gonadosomatic index (GSI, left) and, congruently, had more offspring in their ovaries (right) than non-attacked ones. Boxplots showing the median (solid line), upper and lower quartiles (box), and the 5th and 95th percentiles (whiskers). Insert: a cave molly female.

does not necessarily involve an actual 'preference' on the side of the giant water bugs. On the other hand, if *Belostoma* had such a prey preference, this may be due to a cost–benefit trade-off: optimal foraging theory predicts that *Belostoma* should target fish that maximize the net energy intake per unit foraging time (Pyke, 1984; Perry and Pianka, 1997). Furthermore, if *Belostoma* were also susceptible to the toxic effects of hydrogen sulphide, which is likely (Bagarinao, 1992), then they should selectively target large cave mollies to maximize energy intake and obtain the largest benefit out of entering the toxic waters (e.g. Gilchrist *et al.*, 1998). On the basis of the present results, it is impossible to decide which of these hypotheses (or any combination thereof) plays a role, and future investigations will need to address this question in detail.

Why were pregnant cave molly females more likely to be caught by the hemipterans? Generally, the susceptibility of cave mollies to *Belostoma* predation is at least in part driven by the adverse environmental conditions in the sulphidic Cueva del Azufre (Tobler *et al.*, 2009). Hydrogen sulphide is highly reactive at room temperature and spontaneously oxidizes in water, which leads to and aggravates hypoxia in aquatic systems (Chen and Morris, 1972; Cline and Richards, 1969). During aquatic surface respiration, which mediates survival in hypoxic and toxic water, fish remain just underneath the water surface (Plath *et al.*, 2007b). Here, *P. mexicana* are vulnerable to attacks by *Belostoma*, which catch mollies with their raptorial forelegs while sitting on a rock and holding their abdomen into the air to enable gas exchange (Tobler *et al.*, 2007b). Male *P. mexicana* have a higher susceptibility to the adverse abiotic conditions than females and spend more time performing aquatic surface respiration (Plath *et al.*, 2007b). Consequently, male cave fish were found to be preyed upon more often than equally sized females (Tobler *et al.*, 2008b). Even though data on metabolic rates (oxygen consumption) of cave molly females in the course of gestation are lacking, a positive correlation seems straightforward to predict, and indeed there is evidence from other poeciliid species that pregnant females have higher oxygen demands (Timmerman and Chapman, 2003). In addition to potentially having increased exposure to water bugs, gestating females may also be at a disadvantage in

evading predators during an actual attack. Being pregnant, especially in high fecundity females, leads to considerable abdominal distension, which can affect swimming ability and fast-start responses (e.g. Olsson *et al.*, 2000; Ghalambor *et al.*, 2004; but see Plaut, 2002).

Implications for life-history divergence in *P. mexicana*

Our findings on size-selective predation suggest a more complex evolutionary scenario for life-history evolution in the Cueva del Azufre system than previously assumed (Riesch *et al.*, 2010c). According to life-history theory, organisms under strong predation should (1) mature at a younger age and smaller size, (2) invest more into offspring (i.e. large reproductive allocation), and (3) produce many small offspring compared with individuals of the same species from low predation environments (e.g. Gadgil and Bossert, 1970; Law, 1979; Michod, 1979).

In non-toxic surface habitats, *P. mexicana* clearly experience high levels of predation by a variety of different predators, including piscivorous fishes, reptiles, arthropods, and birds (Tobler *et al.*, 2006; Riesch *et al.*, 2009a, 2010a). Compared with surface mollies, cave mollies mature at approximately the same size, are characterized by slightly larger reproductive allocation, but also by an extremely low fecundity coupled with large offspring size (Riesch *et al.*, 2010c, 2010d; R. Riesch, unpublished data). If we follow the traditional cave literature (Romero, 1985; Tabuki and Hanai, 1999; Romero and Green, 2005) and assume caves to be a predator-free environment, two life-history traits (size at maturity and reproductive allocation) clearly do not meet life-history predictions (Gadgil and Bossert, 1970; Law, 1979; Michod, 1979). Hence, predation may actually act in a similar fashion on *P. mexicana* in the cave and surface habitats. In contrast, the third life-history trait (the offspring size–fecundity trade-off) meets the predictions for a predator-free environment. How are we to explain this confusing pattern?

It is important to keep in mind that several strong selective forces in the Cueva del Azufre are acting in concert: hydrogen sulphide toxicity, the cave environment (i.e. permanent darkness and limited resource availability), and predation. If we consider the influence of combinations of these selective forces on specific life-history characters, patterns of size at maturity and reproductive allocation are consistent with both *Belostoma* predation and H_2S toxicity, which select for small size at maturity and large reproductive allocation due to selection against large individuals (Gadgil and Bossert, 1970; Law, 1979; Michod, 1979; Tobler *et al.*, 2011). In contrast, patterns of different fecundities across populations are consistent with selection stemming from the cave environment and H_2S toxicity, which should select for large offspring and low fecundity (e.g. Culver, 2005; Riesch *et al.*, 2010b, 2010c), as would a low predation environment (Gadgil and Bossert, 1970; Law, 1979; Michod, 1979).

As we cannot disentangle the effects of different sources of selection on life-history traits, the relative importance of *Belostoma* predation on life-history evolution remains unclear. Nonetheless, several different scenarios are possible. First, *Belostoma* predation does play an important role in shaping cave molly life histories preventing divergence from surface populations in size at maturity and reproductive allocation. Strong selection from darkness and toxicity on large offspring size, however, simply overrides selection by predation on (smaller) offspring size. Second, if females are able to successfully reproduce several times prior to becoming targets of *Belostoma* predation, and males are predominantly targeted by *Belostoma* predation (Tobler *et al.*, 2008b), selection on female life-history traits from predation may be weak (compared with constant selection due to the cave environment and toxicity) regardless of the actual predation rates in an experimental setting. If this scenario were true, then we would still expect to find small size at maturity and large reproductive allocation

because of selection due to toxicity (Tobler *et al.*, 2011). Further studies are required to fully understand the intensity and evolutionary consequences of predation by *Belostoma* and other species on populations of extremophile fish. For example, it is possible that other predators [like piscivorous spiders (Horstkotte *et al.*, 2010) or crustaceans (Klaus and Plath, 2011)] do not share the same size-selective preference and also target smaller cave mollies at high rates. Nonetheless, our results clearly stress the importance of evaluating the effects of multiple selective factors when attempting to interpret the population-specific life-history evolution of a species (Endler, 1995).

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