

# Effect of predation on male mating behaviour in a unisexual-bisexual mating system

Andrea S. Aspbury<sup>1</sup>), Julia M. Coyle & Caitlin R. Gabor

(Texas State University-San Marcos, Department of Biology,  
San Marcos, TX 78666-4615, USA)

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## Summary

Mate choice for conspecifics is beneficial when closely related species live in sympatry, but mate choice can be costly in the presence of predators. Male sailfin mollies are sexually parasitized by gynogenetic Amazon mollies. Amazon mollies must mate with male sailfin mollies to initiate embryogenesis, but inheritance is maternal. We tested if male sailfin molly mate choice for conspecific females is affected by predation risk. Male mate choice was tested in one of four treatments: (1) predation/no refuge, (2) predation/refuge, (3) no predation/refuge and (4) no predation/no refuge. Predation consisted of dipping the beak of a great blue heron decoy in the aquarium prior to a mating trial. Refuge was provided by java-moss. For each trial the number of mating attempts toward each female was recorded. There was a significant interaction between predation and refuge on strength of preference (SOP) for conspecific females. The highest SOP was in the no predator/no refuge treatment, and the lowest SOP was in the predator/no refuge treatment. These results suggest that the cost of predation is higher than the cost of mating with heterospecifics, and that the presence of a refuge may reduce this cost. This could explain the continued maintenance of Amazon mollies.

*Keywords:* gynogenesis, mate choice, *Poecilia formosa*, *Poecilia latipinna*, predation risk.

## Introduction

Predation risk can affect time spent searching for mates, evaluating and courting potential mates, and copulating (Magnhagen, 1991; Sih, 1994). As such, there may be a conflict between maximizing the chance of mating

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<sup>1</sup>) Corresponding author's e-mail address: [aspbury@txstate.edu](mailto:aspbury@txstate.edu)

with a high-quality conspecific and minimizing the risk of being detected by a predator. Models on the evolution of female preferences that include the effects of predation indicate that a female's preference should decrease with greater costs of mate choice (Real, 1990; Crowley et al., 1991; Pomiankowski et al., 1991) and females are expected to be sensitive to predation risk and to become less selective with increasing levels of risk (Real, 1990). Both laboratory and field studies have demonstrated that female choice is affected by predation risk. For example, in some species the risk of associating with more conspicuous males in the presence of predators leads to a change in female preference, such that females favor less conspicuous males (Godin & Briggs, 1996; Gong & Gibson, 1996; Johnson & Basolo, 2003). In addition, predation risk also elevates the cost to females to search for and evaluate males with preferred traits (Forsgren, 1992; Hedrick & Dill, 1992). There is increasing evidence that males also exhibit mating preferences, and that males too can become less selective for high-quality females in the presence of predation risk (Berglund, 1993; Simcox et al., 2005; Dunn et al., 2008).

When closely related species occur in sympatry, mate choice involves identification of conspecific mates over heterospecific mates, as well as identification of high-quality mates. Therefore, species recognition is also likely to be affected by predation risk. One species complex that is a model system for examining species recognition occurs in the genus *Poecilia*. Poeciliid fish are livebearing and exhibit internal fertilization. Male sailfin mollies (*P. latipinna*) and male Atlantic mollies (*P. mexicana mexicana* and *P. m. limantouri*) are sexually parasitized by the Amazon molly (*P. formosa*), an all-female clonal species that reproduce by gynogenesis. Amazon mollies must co-exist and mate with males of the parental species to induce embryogenesis, but inheritance is strictly maternal. Amazon mollies putatively arose from a hybridization event between a female *P. m. limantouri* and a male *P. latipinna* or some other extinct sailfin species (Schartl et al., 1995). The original hybridization event that led to the formation of Amazon mollies is thought to have occurred about 100 000 years ago (or 200 000 generations; Avise et al., 1991; Schartl et al., 1995), but confidence intervals around this estimate are quite large (Dries, 2000, 2003). While males of the parasitized species show stronger preferences for conspecifics over Amazon mollies (Ryan et al., 1996; Gabor & Ryan, 2001), the continued persistence

of Amazon mollies illustrates that males of the parasitized species sometimes fail to discriminate against heterospecific mates. An unresolved issue is why male sailfin mollies mate with Amazon mollies. The maintenance of Amazon mollies should be evolutionarily unstable because natural selection is expected to work against males that mate with another species. Because the male's genes are not incorporated into the offspring of Amazon mollies, males obtain no direct genetic benefits from mating with gynogens (but see Schlupp et al., 1994 for indirect benefits of mate choice copying).

Previous research indicates that there are significant costs to males that mate with Amazon mollies. In particular, male sailfin mollies show a greater mating preference for conspecific females than for Amazon mollies, and males from populations sympatric with Amazon mollies have a stronger preference for conspecific females over Amazon mollies than males from allopatric populations (Ryan et al., 1996; Gabor & Ryan, 2001). Additionally, male sailfin mollies produce more sperm in the presence of conspecifics than they do when in the presence of Amazon mollies (Aspbury & Gabor, 2004). These results suggest that mating with Amazon mollies is costly as observed by the general avoidance of them, the decreased sperm production in their presence, as well as the inter-population variation in male mating preferences. Selection against males that emphasize species recognition more than avoiding predation risk could be one potential mechanism contributing to the continued maintenance of Amazon mollies.

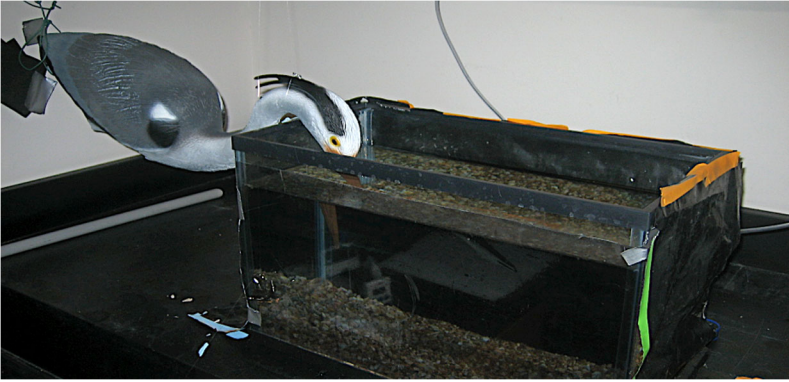
Most studies on the effects of predation risk in poeciliids consist of piscine fish predators. In guppies (*P. reticulata*) males have been shown to switch to alternative, sneaky strategies in the presence of predatory fish (Endler, 1987; Magurran & Seghers, 1990; Godin, 1995), and female guppy mating decisions are affected by predator abundance (Godin & Briggs, 1996). In sailfin mollies, however, Gabor & Page (2003) found that postpartum females preferred to associate with large males both in the presence and the absence of a piscine predator, the green sunfish (*Lepomis cyanellus*). These results suggest that at least for females, fish predators may not be an important factor in their mating decisions. Furthermore, in many of the sailfin molly populations sympatric with Amazon mollies, few, if any, piscine predators exist (Heubel, 2004; pers. obs.), and the piscine predators that do exist are not large enough to prey on most adult mollies. However, in many habitats occupied by sailfin mollies, there are wading bird predators that are known to prey on poeciliid fishes (e.g., wood storks, *Mycteria americana*, Ogden et al., 1976; white ibis,

*Eudocimus albus*, Kushlan, 1979; tri-colored herons, *Egretta tricolor* and little blue herons, *E. caerulea*, Smith, 1997; great egrets, *Ardea alba* and snowy egrets, *E. thula*, Trexler et al., 1994; Smith, 1997). Trexler et al. (1994) found that great egrets and snowy egrets preferred to prey on larger sailfin mollies, and for great egrets this effect was stronger in the absence of refugia (plant material). Additionally, Kushlan (1976) found that wading birds are more likely to forage in aquatic habitats when water levels fall during the dry season. In many sailfin and Amazon molly populations water levels fall dramatically during the dry seasons, and the dry season overlaps with the mating season. Thus, avian predation may be an important factor affecting male sailfin molly mate choice.

In this study we examined the effect of predation risk from a visually foraging, omnipresent bird predator, the great blue heron (*Ardea herodias*), on male sailfin mate preference for conspecific vs. heterospecific females. We also examined how the presence of a refuge might mitigate any negative effects of predators on male sailfin molly species recognition.

## Material and methods

We used sailfin mollies collected from Spring Lake, Hays County, TX, USA (29°89'N, 97°82'W). This population is allopatric from Amazon mollies. We used Amazon mollies collected from Tamaulipas, Mexico (25°30'N, 97°86'W). All fish were maintained in the laboratory in standard conditions. Females used in this experiment were not visibly pregnant (30 day isolated, non-virgin females). Testing was performed from April–October 2008 from 1000–1500 h. Males were isolated for two days prior to the experiment. Individual males were tested in one of four treatment combinations ( $N = 15$  males per treatment): (1) no predation attempt with no refuge, (2) no predation attempt with a refuge, (3) predation attempt with no refuge, and (4) predation attempt with a refuge. Individual males were placed in an acclimatization container (a clear plastic cylinder measuring 12 cm diameter  $\times$  15 cm) in the center of a 38-l aquarium that also housed a female sailfin and an Amazon molly that were swimming freely (size matched within 2 mm standard length (SL)). After a 10-min acclimatization we released the male by gently removing the acclimation chamber. In the predator treatments, we used a pulley system to briefly dip and retract the beak of a great blue heron decoy



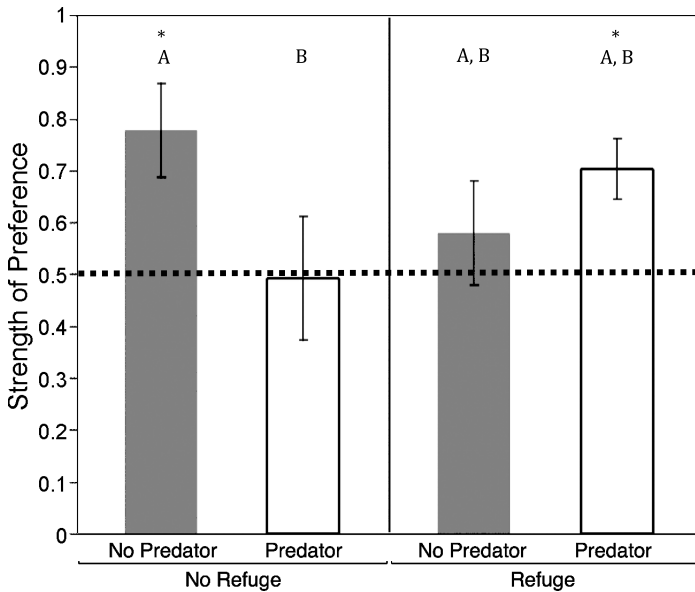
**Figure 1.** Image of testing aquarium with the great blue heron decoy beak dipped into the aquarium immediately preceding a mating trial that included a predation effect. This figure is published in colour in the online edition that can be accessed *via* <http://www.brill.nl/beh>

into the tank at this time (Figure 1). In the no predator treatment there was no stimulus introduced to the tank. We used a clump of java moss (*Vesicularia dubyana*) attached to the side of the middle of the tank as a refuge in the refuge treatments. We then recorded the number of gonopodial thrusts (mating attempts) directed at each female for 5 min after a male's first mating attempt. We used a 5-min mating trial so that we only measured the immediate response to the treatment.

In our analyses, we excluded males that failed to mate with a female during the 5-min trial. We calculated the strength of preference (SOP) as the number of gonopodial thrusts directed at conspecific females/total number of gonopodial thrusts. The SOP data were arcsine transformed to meet the assumptions of parametric analyses. We used ANOVA to examine the effects of the predator treatment, the refuge treatment, and their interaction on the SOP, and used post hoc paired *t*-tests to examine significant treatment effects. We also determined if the SOP was significantly greater than 50% (indicating a conspecific preference) in each treatment using Wilcoxon signed-rank tests. Finally, we examined if either of the treatments affected the total number of gonopodial thrusts, which could lead to differences in the SOP. We used an ANOVA with predator treatment, refuge treatment, and their interaction on the total number of gonopodial thrusts. All *p* values were two-tailed, the alpha level was set at 0.05, and analyses were performed using JMP v 7.01 (SAS Institute, Cary, NC, USA).

## Results

There were no main effects of the predator treatment, or refuge treatment on SOP (Figure 2, Table 1). There was, however, a significant interaction between the predator treatment and the refuge treatment (Figure 2, Table 1). The highest SOP was in the no predator and no refuge treatment, and the



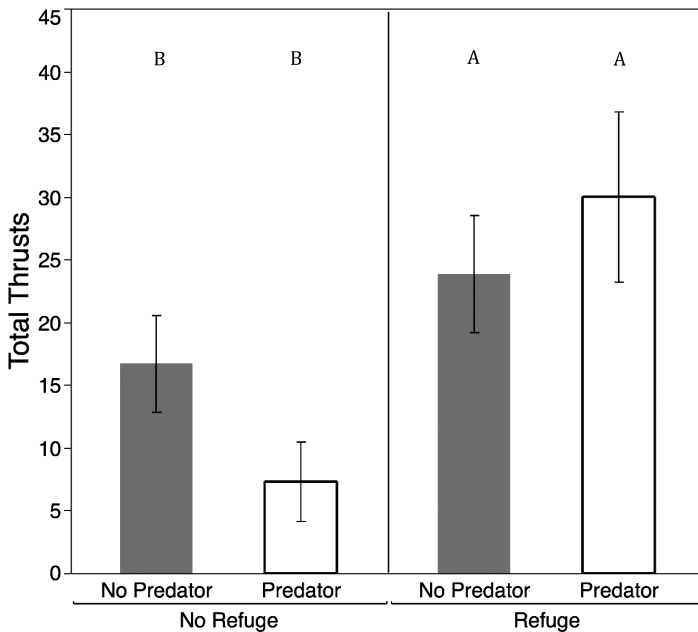
**Figure 2.** Mean strength of preference (SOP)  $\pm$  SE for conspecific females of male sailfin mollies when given the choice between a female conspecific and an Amazon molly. No refuge present treatments are on the left, and refuge present treatments are on the right. Grey bars are SOP for no predator treatments, and open bars are the SOP for the predator treatments. Different letters above the means indicate treatment differences (paired  $t$ -tests). A star above the bars indicates a SOP significantly greater than 0.5 (signed-rank tests).

**Table 1.** ANOVA on male strength of preference (SOP, arcsine transformed) with predation and refuge as main fixed effects, and the predation  $\times$  refuge interaction.

Source	df	$F$	$p$
Predation	1, 47	1.640	0.207
Refuge	1, 47	0.094	0.761
Predation $\times$ refuge	1, 47	4.123	0.048

lowest SOP was in the predator, no refuge treatment (Figure 2; mean SOP  $\pm$  SE: no predator and no refuge =  $0.778 \pm 0.091$ ; predator with a refuge =  $0.703 \pm 0.058$ ; no predator with a refuge =  $0.579 \pm 0.100$ ; predator and no refuge =  $0.492 \pm 0.120$ ). Males showed a significant preference for conspecific females (SOP greater than 0.5) in the no predator, no refuge treatment (Wilcoxon signed-rank test;  $W = 31.50$ ,  $N = 14$ ,  $p < 0.05$ ), and in the predator present, with a refuge treatment (Wilcoxon signed-rank test;  $W = 32$ ,  $N = 13$ ,  $p < 0.05$ ). Males did not show a preference for conspecific females (SOP not significantly different from 0.5) in the no predator, with a refuge treatment ( $W = 9.5$ ,  $N = 11$ ,  $p > 0.05$ ), nor did they show a preference in the predator, no refuge present treatment ( $W = 1.0$ ,  $N = 10$ ,  $p > 0.05$ ).

There was no effect of the predator treatment on total thrusts (Figure 3, Table 2), but the refuge treatment had a significant effect on the total thrusts (Figure 3, Table 2). The males had the highest amount of mating activity



**Figure 3.** Total number of thrusts (mating attempts)  $\pm$  SE by male sailfin mollies when given the choice between a female conspecific and an Amazon molly. No refuge present treatments are on the left, and refuge present treatments are on the right. Grey bars are thrusts for no predator treatments, and open bars are the thrusts for the predator treatments. Different letters above the means indicate treatment differences (paired  $t$ -tests).

**Table 2.** ANOVA on total number of male thrusts (mating attempts) with predation and refuge as main fixed effects, and the predation  $\times$  refuge interaction.

Source	df	<i>F</i>	<i>p</i>
Predation	1, 47	0.104	0.749
Refuge	1, 47	8.802	0.005
Predation $\times$ refuge	1, 47	2.410	0.128

in the presence of a refuge (Figure 3). There was no significant interaction between the predator treatment and the refuge treatment (Figure 3, Table 2).

## Discussion

The response of male sailfin mollies to the presence of a predator when given a choice to mate with a conspecific and a heterospecific female differs depending on the presence or absence of a refuge if a predator is present. Males had the lowest SOP for conspecific females when a simulated predation event preceded the mating trial, and when there was no refuge available. There was no difference in the SOP between the no predator, no refuge treatment and the predator, with the refuge treatments. Furthermore, males had a significant SOP for conspecific females in these two treatments, but lacked such a preference in the predator, no refuge treatment. Taken together, these results suggest that the presence of a refuge mitigates the risk of predation when male sailfin mollies are selecting between conspecific females and the heterospecific, sexually parasitic Amazon mollies.

Males also showed the highest amount of mating activity in the treatments with a refuge, and the lowest mating activity in the no refuge treatments. This result also supports the hypothesis that the presence of a refuge may mitigate risks of predation from wading bird predators. Further evidence to support this hypothesis is the observation that in the population that we studied, males that are courting females in shallow water are often found in close proximity to aquatic plants, such as hydrilla (*Hydrilla verticillata*) (pers. obs.). This behaviour, as well as the increased mating behaviour in the presence of a refuge could vary based on male size. Trexler et al. (1994) found that two species of herons preferentially prey on relatively large individual sailfin mollies. We would predict from this observation that large



males may be more sensitive to the risk of predation, but our data from the current study is not sufficient to test this hypothesis.

We also found that males did not have a significant SOP for conspecific females in the refuge present, no predator treatment. In addition the SOP for males from this treatment was not significantly different from the SOP for males from the predator present with no refuge treatment. Males in the refuge, no predator treatment had as high overall mating activity as they did in the refuge with a predator treatment, so a lack of mating responsiveness by males cannot explain this result. One potential explanation for this result is that female behaviour differed in the presence of a refuge. Although we did not quantify female behaviour in this study, we observed no general patterns during the mating trials that would support this hypothesis.

Our results demonstrate that male mating preferences are flexible, such that males become less choosy under predation. Heubel & Schlupp (2008) found that male sailfin molly association preferences for conspecific females were highest in the peak of the reproductive season. They suggested that seasonal differences in male preferences indicate plasticity in male mate choice, and that it may not pay males enough to always discriminate between conspecific females and Amazon mollies. Our study supports this hypothesis.

Our results could provide a mechanism for the continued paradoxical persistence of Amazon mollies. The persistence of such unisexual lineages is an evolutionary paradox, as they possess the disadvantages of both sexual reproduction (the costs of finding a mate and costs of mating) and unisexual reproduction (accumulation of deleterious mutations that cannot be purged by recombination) (reviewed in Schlupp, 2005). However, Amazon mollies have persisted for approximately 100 000 years, making them one of the oldest known vertebrate unisexual lineages (but see Dries, 2000, 2003). Prior studies have found that male sailfin mollies have conspecific mating preferences (Ryan et al., 1996; Gabor & Ryan, 2001), and that they produce more sperm when in the presence of conspecific females than when in the presence of Amazon mollies (Aspbury & Gabor, 2004). However, males also make mating mistakes, such as when Amazon mollies are larger than conspecific females (Gumm & Gabor, 2005), or when there are benefits due to mate choice copying (Schlupp et al., 1994). Our study suggests that in populations with little plant cover to provide a refuge, and with wading bird predators, the tradeoff between the risk of mating with an Amazon molly and the risk of predation may be biased towards making mating mistakes.

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### References

- Aspbury, A.S. & Gabor, C.R. (2004). Discriminating males alter sperm production between species. — *Proc. Natl. Acad. Sci.* 101: 15970-15973.
- Avise, J.C., Trexler, J.C., Travis, J. & Nelson, W. (1991). *Poecilia mexicana* is the recent female parent of the unisexual fish *P. formosa*. — *Evolution* 45: 1530-1533.
- Berglund, A. (1993). Risky sex: male pipefishes mate at random in the presence of a predator. — *Anim. Behav.* 46: 169-175.
- Crowley, P.H., Travers, S.E., Linton, M.C., Cohn, S.L., Sih, A. & Sargent, R.C. (1991). Mate density, predation risk, and the seasonal sequence of mate choices: a dynamic game. — *Am. Nat.* 137: 567-596.
- Dries, L.A. (2000). The evolutionary persistence of the gynogenetic Amazon molly, *Poecilia formosa*. — Ph.D. dissertation, University of Texas, Austin, TX.
- Dries, L.A. (2003). Peering through the looking glass at a sexual parasite: are Amazon mollies red queens? — *Evolution* 57: 1387-1396.
- Dunn, A.M., Dick, J.T.A. & Hatcher, M.J. (2008). The less amorous *Gammarus*: predation risk affects mating decisions in *Gammarus duebeni* (Amphipoda). — *Anim. Behav.* 76: 1289-1295.
- Endler, J.A. (1987). Predation, light intensity and courtship behaviour in *Poecilia reticulata* (Pisces: Poeciliidae). — *Anim. Behav.* 35: 1376-1385.
- Forsgren, E. (1992). Predation risk affects mate choice in a gobiid fish. — *Am. Nat.* 140: 1041-1049.
- Gabor, C.R. & Page, R. (2003). Female preference for large males in sailfin mollies, *Poecilia latipinna*: the importance of predation pressures and reproductive status. — *Acta Ethol.* 6: 7-12.
- Gabor, C.R. & Ryan, M.J. (2001). Geographical variation in reproductive character displacement in mate choice by male sailfin mollies. — *Proc. Roy. Soc. Lond. B: Biol.* 268: 1063-1070.
- Godin, J.-G.J. (1995). Predation risk and alternative mating tactics in male Trinidadian guppies (*Poecilia reticulata*). — *Oecologia* 103: 224-229.
- Godin, J.-G.J. & Briggs, S.E. (1996). Female mate choice under predation risk in the guppy. — *Anim. Behav.* 51: 117-130.
- Gong, A. & Gibson, R.M. (1996). Reversal of a female preference after visual exposure to a predator in the guppy, *Poecilia reticulata*. — *Anim. Behav.* 52: 1007-1015.
- Gumm, J.M. & Gabor, C.R. (2005). Asexuals looking for sex: conflict between species and mate-quality recognition in sailfin mollies. — *Behav. Ecol. Sociobiol.* 58: 558-565.

- Hedrick, A.V. & Dill, L.M. (1992). Mate choice by female crickets is influenced by predation risk. — *Anim. Behav.* 46: 193-196.
- Heubel, K.U. (2004). Population ecology and sexual preferences in the mating complex of the unisexual Amazon molly *Poecilia formosa* (Girard, 1859). — University of Hamburg, Hamburg.
- Heubel, K.U. & Schlupp, I. (2008). Seasonal plasticity in male mating preferences in sailfin mollies. — *Behav. Ecol.* 19: 1080-1086.
- Johnson, J.B. & Basolo, A.L. (2003). Predator exposure alters female mate choice in the green swordtail. — *Behav. Ecol.* 14: 619-625.
- Kushlan, J.A. (1976). Wading bird predation in a seasonally fluctuating pond. — *Auk* 93: 464-476.
- Kushlan, J.A. (1979). Feeding ecology and prey selection in the white ibis. — *Condor* 81: 376-389.
- Magnhagen, C. (1991). Predation risk as cost of reproduction. — *Trends Ecol. Evol.* 6: 183-186.
- Magurran, A.E. & Seghers, B.H. (1990). Risk sensitive courtship in the guppy (*Poecilia reticulata*). — *Behaviour* 112: 194-201.
- Ogden, J.C., Kushlan, J.A. & Tilmant, J.T. (1976). Prey selectivity by the wood stork. — *Condor* 78: 324-330.
- Pomiankowski, A., Iwasa, Y. & Nee, S. (1991). The evolution of costly mate preferences. I. Fisher and biased mutation. — *Evolution* 45: 1422-1430.
- Real, L. (1990). Search theory and mate choice. I. Models of single-sex discrimination. — *Am. Nat.* 136: 376-404.
- Ryan, M.J., Dries, L.A., Batra, P. & Hillis, D.M. (1996). Male mate preferences in a gyno-genetic species complex of Amazon mollies. — *Anim. Behav.* 52: 1225-1236.
- Schartl, M., Wilde, B., Schlupp, I. & Parzefall, J. (1995). Evolutionary origin of a partheno-form, the Amazon molly, *Poecilia formosa*, on the basis of a molecular genealogy. — *Evolution* 49: 827-835.
- Schlupp, I. (2005). The evolutionary ecology of gynogenesis. — *Annu. Rev. Ecol. Evol. Syst.* 36: 399-417.
- Schlupp, I., Marler, C. & Ryan, M.J. (1994). Benefit to male sailfin mollies of mating with heterospecific females. — *Science* 263: 373-374.
- Sih, A. (1994). Predation risk and the evolutionary ecology of reproductive behaviour. — *J. Fish Biol.* 45A: 111-130.
- Simcox, H., Colegrave, N., Heenan, A., Howard, C. & Braithwaite, V.A. (2005). Context-dependent male mating preferences for unfamiliar females. — *Anim. Behav.* 70: 1429-1437.
- Smith, J.P. (1997). Nesting season food habits of 4 species of herons and egrets at Lake Okeechobee, Florida. — *Colonial Waterbirds* 20: 198-220.
- Trexler, J.C., Tempe, R.C. & Travis, J. (1994). Size-selective predation of sailfin mollies by two species of heron. — *Oikos* 69: 250-258.
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