

# Differences in resource assimilation between the unisexual Amazon molly, *Poecilia formosa* (Poeciliidae) and its sexual host (*Poecilia latipinna*)

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**Abstract** Unisexual sperm-dependent species depend on a sexual host for reproduction, and must live in sympatry with their sperm donor. If niche overlap between the species is substantial, the intrinsic faster population growth of the unisexual over sexual species can cause competitive exclusion of the host from resources, causing the demise of the unisexual species. However, theoretical models predict that coexistence is possible, even without niche differentiation, if the unisexual species is a poorer competitor than the sexual host and if the effect of the unisexual species on the exploitative abilities of the sexual species is smaller than the effect that the sexually reproducing individuals have among themselves. We tested these predictions in the unisexual-bisexual mating complex of *Poecilia formosa*, and one of its sexual hosts, *P. latipinna*. Fishes were housed from parturition for 76 days with both conspecific and heterospecific individuals under both limited and ad libitum food regimes. Only one of the predictions of the models was met: the effects of intraspecific competition for *P. latipinna* were stronger than the effects of interspecific competition. *Poecilia latipinna* raised with conspecifics with limited food stored fewer fats than both

heterospecific *P. formosa*, and conspecifics raised in any other treatment.

**Keywords** Amazon molly · Body condition · Competition · Gynogenesis

## Introduction

Unisexual-bisexual mating complexes are found whenever a unisexual species depends on a sexual host for reproduction. Gynogenetic females, for example, produce diploid eggs ameiotically, but must mate with males of closely related species to initiate embryogenesis (Dawley 1989; Schlupp 2005; but see Choleva et al. 2008 for exceptions). Nonetheless, the male's genetic information does not enter the egg (but see Schartl et al. 1995a) resulting in strictly clonal inheritance. Due to their mode of reproduction, gynogenetic species must live in sympatry with their sexual host species (Dawley and Bogart 1989 and references within). Asexual species are thought to have a short-term advantage over sexually reproducing species because they lack the cost of producing males (Maynard Smith 1968). However, this advantage can become a hindrance for gynogenetic species; by only producing females, gynogenetic species have a faster intrinsic population growth rate than their sexually reproducing host species (Maynard Smith 1971; Lively and Lloyd 1990). Consequently, in habitats inhabited by both gynogens and hosts, the relative frequency of gynogens should rise rapidly. Assuming all but the reproductive mode is equal between the two species, population growth in asexuals should lead to the

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displacement of sexual individuals and, subsequently, the extinction of gynogens, due to the lack of sperm donors (Schlupp 2005).

A stable maintenance of unisexual-bisexual mating complexes requires the mitigation of the two-fold population growth rate of asexual species. Ecologically, such a reduction of the asexuals' short-term advantage may be mediated through differential competitive abilities between reproductive forms, as competition has been widely documented to affect species coexistence and community structure in a wide variety of taxa (e.g., *Paramecium*: Gause 1934; flour beetles, *Tribolium* spp: Park 1948; barnacles: Connell 1961; and plants: Tilman 1977). Higher competitive ability in terms of resource exploitation in sexual species could effectively reduce the reproductive success of asexual sperm-parasites and could, therefore, contribute to mediating stable maintenance of unisexual-bisexual mating complexes. A model developed in Schley et al. (2004) predicts that coexistence between a gynogenetic species and its host can be reached and maintained whenever the effects of intraspecific competition are greater than the effects of interspecific competition in the sexual species. In other words, the effect of unisexuals on the ability of the sexual species to acquire resources must be smaller than the effect that the sexually reproducing individuals have among themselves (Doncaster et al. 2000; Schley et al. 2004).

A well-known unisexual-bisexual mating complex is found in the livebearing fish family, Poeciliidae. *Poecilia formosa* (Amazon molly, Girard 1859) is a gynogenetic fish that likely arose from a hybridization event between a female *P. mexicana* (Atlantic molly, Steindachner) and a male *P. latipinna* (sailfin molly, Lesueur 1821) (Awise et al. 1991; Schartl et al. 1995b; Schlupp and Riesch 2011). *Poecilia latipinna* and *P. formosa* are commonly found in mixed species shoals in the wild (Schlupp and Ryan 1996). *Poecilia formosa* sexually parasitizes both male *P. mexicana* and *P. latipinna* depending on the geographic region, and no significant trophic niche differentiation has been demonstrated between asexuals and their local sexual hosts (Scharnweber et al. 2011a). Consequently, niche differentiation likely does not facilitate coexistence between *P. formosa* and its sexual hosts, even though Balsano et al. (1981) suggested that there might be some spatial segregation. However, *P. formosa* has a reduced foraging rate in the presence of *P. latipinna*, whereas the foraging rate of the sexual species is not affected by the presence of gynogenetic individuals (Alberici da Barbiano et al. 2010). These results suggest

that the unisexual species may be a poorer competitor than its sexual host, which could contribute to mediating stability of the unisexual-sexual mating system (Schley et al. 2004). Alberici da Barbiano et al. (2010) only focused on the behavioural responses of adult subjects during 10 min foraging bouts in the presence of a heterospecific or a conspecific fish. The present study investigated whether differences in competitive abilities between *P. formosa* and *P. latipinna* from birth to a few months of age differentially affect the body condition of the two species. We tested this hypothesis using two treatments of food availability (ad libitum and limited) and two competitive treatments (intraspecific and interspecific). This approach allowed us to address two a priori predictions: (1) Under ad libitum food regimes, no difference in body condition or mortality should be found between the two species, whether they are reared with conspecifics or heterospecifics, because competitive effects should be low, and individuals should be able to equally invest in body condition. (2) Under limited food regimes, when competitive interactions are exacerbated (Schoener 1971), the body condition of individuals of either species should differ depending on whether they are reared with conspecifics or heterospecifics. In particular, following the predictions of the Doncaster et al. (2000) and Schley et al. (2004) models, we predicted that *P. formosa* would have a lower body condition than *P. latipinna*, when fish are reared with heterospecific individuals (interspecific competition). Additionally, *P. latipinna* should have a lower body condition when reared with conspecifics (intraspecific competition) than when reared with heterospecific individuals (interspecific competition).

## Materials and methods

### Experimental design

Gravid fishes were collected at the headwaters of the San Marcos River in San Marcos, TX (29° 89' N; 97° 82' W) in May 2011, and the experiment continued until September 2011. Each female was placed in a 19 L tank and isolated from other females to facilitate parturition of fry. A full set of experimental trials was initiated whenever a female *P. latipinna* and a *P. formosa* gave birth to offspring within 24 h of each other. Offspring of both species were assigned to 19 L aquaria that were fed either (a) ad libitum or (b) limited diet. Within each feeding regime, and for both species, the fish were housed in an

intraspecific competition treatment consisting of conspecifics ( $N=6$  conspecific individuals per aquarium) or in an interspecific competition treatment consisting of both species ( $N=3$  heterospecifics and  $N=3$  conspecifics per aquarium) where only one species served as the focal species. As a result there were eight experimental treatments across the feeding regime and competition treatments in a fully factorial design.

Each pair of field-caught *P. latipinna* ( $N=12$ ) and *P. formosa* ( $N=12$ ) provided enough offspring for all eight treatments, therefore each set of treatments was done testing juveniles from the same pair of mothers, and all treatments were tested using siblings. Dividing fish in this way was necessary because not enough females gave birth on the same day to be able to mix juveniles. Fishes were fed both one teaspoon/tank of live brine shrimp *Artemia* sp. (BioMarine; [www.aquafauna.com](http://www.aquafauna.com)) and 75 mg food pellets/tank (200 Purina AquaMax) daily in the ad libitum treatments, whereas fish in limited food treatments were only fed 25 mg of food pellets/tank, with one weekly supplement of one teaspoon of live brine shrimp. After performing several pilots, 25 mg of pellet food was found to be the lowest amount of food that did not cause the death of every fish in the tank within a couple of weeks. Standard length (SL) of all fish was recorded at 76 days, when they were euthanized using a high dose of ms-222 and fixed in a 5 % formaldehyde solution.

A storage fat content analysis was conducted to assess body condition in experimental fish. Specimens were air-dried at 65 °C for 5 days, at which time the dry weight (g) of each individual was recorded. Each fish was then placed in 3 consecutive 24 h petroleum ether baths, which extracts all soluble non-structural lipids from carcasses (Tobler 2008). The fish were then weighed again, and the relative fat content in each fish was calculated. This was done by subtracting the post-extraction mass from the pre-extraction mass and dividing by the pre-extraction mass (Tobler 2008). The data was log-transformed for the analysis. For the conspecific treatments, the average body condition of all fish alive at 76 days was used. For the heterospecific treatments, the average of the body condition of all fish alive of the focal species only was used.

Mortality per tank was calculated for each species after the 76 days by dividing the number of remaining fish over the number of fish at the beginning of the experiment (denominator = 6 for conspecific treatments and 3 for heterospecific treatments). Mortality was then

transformed into a nominal factor to be used in an ANCOVA, and treatments were categorized as having high, medium or low mortality (low mortality  $< 0.33$ ,  $0.33 < \text{medium mortality} < 0.66$ , high mortality  $\geq 0.66$ ). Mortality was included in the model because it was not independent of body condition. Additionally, it was not included in the model as a covariate because it was not a continuous variable.

### Statistical analyses

ANCOVA was performed to examine the effects of food regime (ad libitum or limited), species (*P. latipinna* or *P. formosa*), competitive treatment (reared with conspecifics or heterospecifics) and mortality (low, medium and high; note that replicates with mortality =1 were not used in the analysis because body condition could not be calculated) on body condition (log transformed). The model included SL as a covariate. Tukey's HSD comparisons were performed to further investigate any significant interactions between the main effects.

## Results

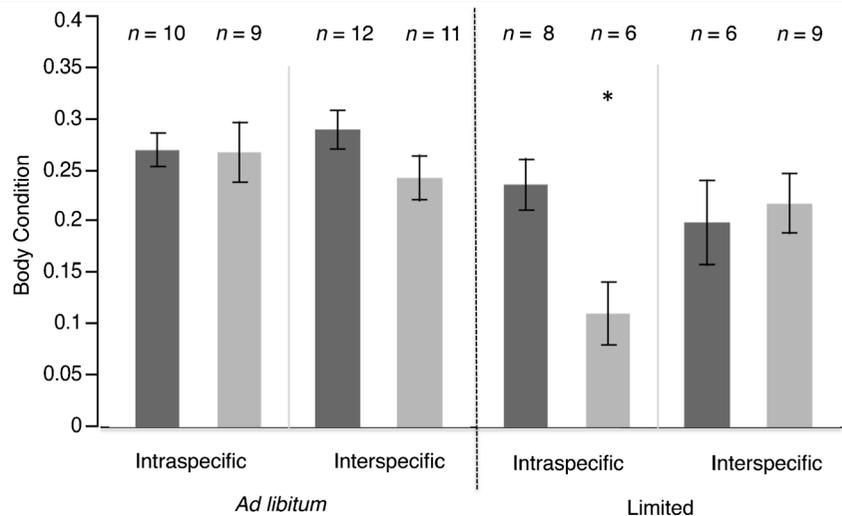
There was a significant three-way interaction between the food regime (ad libitum vs. limited), species (*P. latipinna*

**Table 1** Effect tests for GLM on body condition (pre-extraction mass – post-extraction mass/pre-extraction mass), with food regimes, species, competition treatments and mortality as main effects, and standard length (SL) as covariate. Only significant interactions shown in the table, full model available in supplemental information file

Main Effects	d.f.	F	P
Food regime	1	20.44	<0.01*
Species	1	11.59	<0.01*
Treatment	1	3.25	0.08
Mortality	2	2.39	0.11
Standard length (SL)	1	0.85	0.36
Food regime x species	1	6.08	0.01*
Food regime x treatment	1	5.36	<0.05*
Food regime x mortality	2	3.88	<0.05*
Food regime x species x treatment	1	6.07	<0.05*
Food regime x species x mortality	2	5.52	<0.01*
Treatment x mortality x SL	2	4.42	<0.05*
Residuals	28		

\* Indicates significant *P* values

**Fig. 1** Average body condition ((pre-extraction mass - post-extraction mass)/pre-extraction mass)  $\pm$  s.e. of *P. latipinna* (light grey) and *P. formosa* (dark grey) across food regimes and treatments. The figure depicts un-transformed fats, but for the analysis fats were log-transformed. Asterisk indicates treatment that is significantly different from others ( $P < 0.05$ ) after performing Tukey's HSD post-hoc comparisons

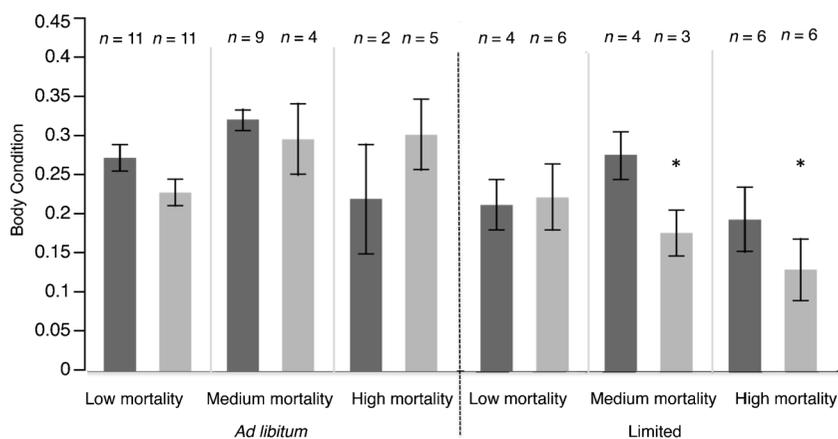


vs. *P. formosa*), and competition treatments (conspecific vs. heterospecific) on body condition (Table 1; Fig. 1). *Poecilia latipinna*, in the intraspecific competition treatments, had significantly lower body condition than fish tested in any other treatment and food regime (Tukey's HSD  $P < 0.05$ ; Fig. 1).

There was also a significant three-way interaction between food regime, species and mortality (Table 1; Fig. 2). *Poecilia latipinna* in limited food regimes experiencing high and medium mortality had a significantly lower body condition than *P. formosa* experiencing high and medium, and a lower body condition than conspecific or heterospecific fish tested in ad libitum treatments (Tukey's HSD  $P < 0.05$ ; Fig. 2).

## Discussion

The present study was designed to empirically test the predictions of the Doncaster et al. (2000) and Schley et al. (2004) models, which predict that for coexistence to be maintained between a unisexual and a sexually reproducing species, the unisexual species needs to be a poorer competitor than the sexually reproducing species, and negative effects of intraspecific competition in the sexually reproducing species must be higher than interspecific competition. The effects of intraspecific competition on body condition in *P. latipinna* were higher than the effects of interspecific competition on the body condition of *P. latipinna*. As a result, one of the requirements for coexistence between a sperm-dependent species and its



**Fig. 2** Average body condition ((pre-extraction mass - post-extraction mass)/pre-extraction mass)  $\pm$  s.e. of *P. formosa* (dark grey) and *P. latipinna* (light grey) across food regimes and mortality. The figure depicts un-transformed fats, but for the analysis fats were log-

transformed. Asterisks indicate treatments that are significantly different from others ( $P < 0.05$ ) after performing Tukey's HSD post-hoc comparisons

host was met. However, there was no evidence for *P. formosa* being a poorer competitor to *P. latipinna*. Neither food regime, nor intra or inter-specific competition, had a significant impact on the ability of *P. formosa* to allocate food resources to fat storage. In particular, the presence of *P. latipinna* in interspecific limited food treatments did not have an effect on the body condition of *P. formosa*.

Our results are in agreement with the findings of Scharnweber et al. (2011b), where *P. formosa* was not found to be less efficient in feeding than *P. latipinna*. However, Alberici da Barbiano et al. (2010) found a decrease in the time allotted to foraging in adult *P. formosa* when foraging with heterospecifics compared to *P. formosa* foraging with conspecifics during brief trials (10 min). If this same behavioral difference in foraging occurred over the long-term period of the present study, then we would predict that *P. formosa* would have lower body condition when in interspecific competition treatments than when in intraspecific competition treatments. We found no support for this prediction. It is possible that gynogenetic individuals resume foraging efforts after being in the presence of heterospecifics for an extended period of time (as in the present study). In addition, in the current study we tested juvenile fishes, rather than adult fishes. It is possible that there are ontogenetic changes in foraging and nutrient sequestering in *P. formosa*, with younger individuals investing more in foraging and resource assimilation than older individuals (Jones et al. 2002). Additionally, behavioral differences in foraging ultimately might not be the only variables affecting growth and storage. For example, differential assimilation, allocation, and expenditure of resources could lead to similar body conditions between species.

We found support for one of the predictions of coexistence models in the unisexual-bisexual complex of *P. formosa* and *P. latipinna*; however, the second condition for coexistence was not met. Intraspecific competition in *P. latipinna* was higher than interspecific competition, but *P. formosa* was not a poorer competitor than *P. latipinna*. Additionally, even when food was limited and mortality was high, *P. formosa* still had a higher body condition than *P. latipinna*. These results could indicate that food competition alone is insufficient to explain the maintenance of this unisexual-bisexual mating complex, and several mechanisms might be working in concert to limit the population growth of the gynogenetic *P. formosa*. Tobler and Schlupp (2010) found significant differences in neonates survival between *P. formosa* and *P. latipinna*,

and *P. formosa* was susceptible to food stress. However, it is possible that *P. formosa* experienced higher mortality at the beginning of our experiment, but the stress caused by the limitation of food was tapered over the 76 days of testing. Our experimental design only focuses on one of many proxies that can be used to describe the competitive ability of a species. In fact, other ecological factors, such as a low tolerance to extreme temperatures have been shown to cause high mortality in *P. formosa* (Fisher and Schlupp 2009). Additionally, it must be noted here that the experimental design and the life history of poeciliid fishes have not allowed us to take into consideration the differences in food assimilation between males and females because we only tested juveniles. It is possible that the presence of juvenile males in the treatments consisting of *P. latipinna* might have partially influenced our results.

Together with other ecological factors, mating behavior is another proxy that might be playing a role in the maintenance of sperm-unisexual species. Male *P. latipinna* from many populations sympatric to *P. formosa* prefer mating with conspecifics and, therefore, limit the reproductive output of *P. formosa* (Gabor and Ryan 2001). Alberici da Barbiano et al. (2011) found that *P. formosa* does not have a two-fold reproductive advantage over host-species females based on the number of female embryos produced per female. However, Schlupp et al. (2010) found the opposite result. Additionally, Alberici da Barbiano et al. (2011) found no evidence of frequency-dependent male mate choice, yet, using a different population, Riesch et al. (2012) did find evidence of frequency-dependent male mate choice. These results suggest that, perhaps, different dynamics might be occurring concurrently in different populations of the same unisexual-bisexual mating complex.

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