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Feeding rates in the sailfin molly *Poecilia latipinna* and its coexisting sexual parasite, the gynogenetic Amazon molly *Poecilia formosa*

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Feeding rates of the gynogenetic Amazon molly *Poecilia formosa* and one of its sexual hosts, the sailfin molly *Poecilia latipinna*, were measured under winter and summer temperature conditions. Food consumption of the unisexual *P. formosa* in winter conditions was significantly higher than that of *P. latipinna*, and it is hypothesized that the resulting food stress might have an important influence on the population composition of these closely related fishes via higher winter mortality in *P. formosa*.

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The coexistence of a sexually reproducing species with a closely related asexually reproducing species is difficult to understand (Agrawal, 2006). Nevertheless, the all-female Amazon molly *Poecilia formosa* (Girard) coexists stably with either the sailfin molly *Poecilia latipinna* (Le Sueur) or the Atlantic molly *Poecilia mexicana* Steindachner, both of which are sexually reproducing. Indeed, this coexistence is essential for *P. formosa* because, as a gynogenetic species, it depends on the sperm of a bisexual species. Nevertheless, this coexistence is not easily explained because *P. formosa* should have a much higher population growth rate, given that no males are produced (the two-fold cost of males; Maynard Smith, 1978). Interestingly, the two sexual host species initially gave rise to the *P. formosa* in a single hybridization event *c.* 100×10^3 generations ago (Schlupp, 2005). One consequence of this is that *P. formosa* shares half of its genome with each of its hosts. The window for such a stable coexistence is quite narrow (Schley *et al.*, 2004; Kokko *et al.*, 2008). Theoretical work on this topic has underscored the importance of behavioural decisions,

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such as mate choice and sperm allocation by the host males (Moore & McKay, 1971; Moore, 1975; Stenseth *et al.*, 1985; Kawecki, 1988; Heubel *et al.*, 2009), as well as the importance of ecological differences (Case & Taper, 1986; Kirkendall & Stenseth, 1990; Schley *et al.*, 2004) and spatial dynamics (Kokko *et al.*, 2008) in maintaining the populations.

In coexisting populations of *P. formosa* and *P. latipinna*, the relative number of asexual females increases during the reproductive season, before the numbers of both species balance out again over the winter (Heubel, 2004). Thus, *P. formosa* appears to indeed realize a portion of its reproductive advantage during the reproductive season despite male discrimination against *P. formosa* females being present to some degree (Schlupp, 2005; Riesch *et al.*, 2008). Consequently, ecological differences between the species must exist, especially during winter. A different lower temperature tolerance is already identified as one ecological factor that could explain the higher overwinter survival rate of *P. latipinna* females compared with *P. formosa* (Fischer & Schlupp, 2009). A differential capability of the two species to cope with food stress could also be important, given that neonates of *P. formosa* are more sensitive to food stress compared with those of *P. latipinna* (Tobler & Schlupp, 2010). Clearly, differential susceptibility to reduced food availability during winter could also affect adult poeciliids, potentially resulting in the higher survival rate of *P. latipinna*. Moreover, different feeding ecologies during the summer could also lead to differences in the amount of resources each species can allocate to survival, somatic growth and reproduction.

All test fishes originated from collection sites in central Texas (Fig. 1). Multiple sites were used because of low numbers of individuals at single sites. Two sites were located near Martindale in the San Marcos River, with the one being a few kilometres downstream of the spring at County Road 101 (CO 101; 29° 51.43' N; 97° 53.80' W) and the other being a side branch near Martindale (SMA; 29° 51.48' N; 97° 51.85' W). The third site was the springhead of the Comal River in New Braunfels (COM; 29° 42.76' N; 98° 08.16' W).

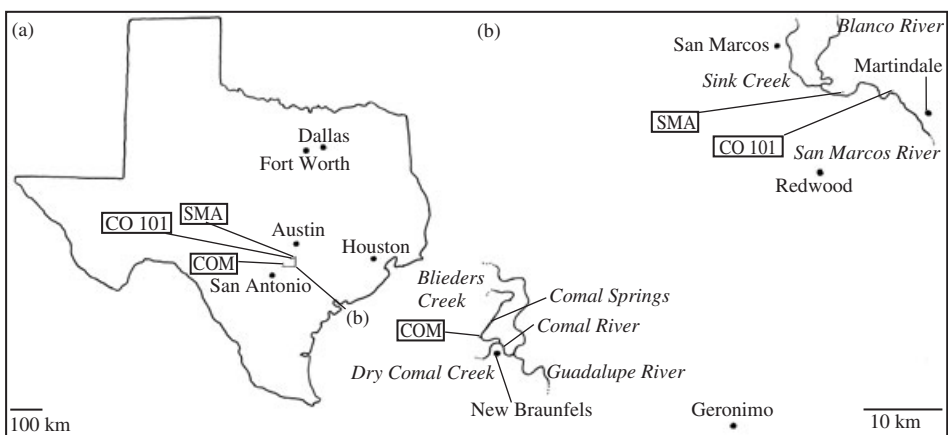


FIG. 1. Location of the three collection sites of the fishes used: SMA for *Poecilia formosa* (29° 51.48' N; 97° 51.85' W), CO 101 for *Poecilia latipinna* (29° 51.43' N; 97° 53.80' W) and COM for *P. latipinna* (29° 42.76' N; 98° 08.16' W) in (a) Texas and (b) in more detail in the area indicated in (a). Names of collection sites are in boxes. Only the rivers and streams in the vicinity of the collection sites are shown.

(COM; 29° 42.76' N; 98° 08.16' W). Eighteen *P. formosa* (originating from SMA) and 20 sympatric *P. latipinna* (11 originating from COM, nine from CO 101) were used. Before the experiment, the fishes were weighed and measured for standard length (L_S) and transferred individually from large stock tanks (c. 1000 l) located in a greenhouse into 5 l tanks in an air-conditioned room. The illumination cycle was 12L:12D. Sympatric *P. latipinna* and *P. formosa* tanks were placed in alternating order. Visual contact between the fishes was not blocked. All experiments took place at the University of Oklahoma.

Half of the fishes in the experiment were exposed to either winter or summer temperature conditions corresponding to the mean temperatures of each season (18.5 and 26° C, respectively; NOAA, 2003). Fishes were assigned randomly, but in even numbers to the two treatments. During the first 5 days, the fishes were allowed to acclimate to the new surroundings and conditions including temperature (Fischer & Schlupp, 2009) and were fed for only 15 min per day with thawed *Chironomus* sp. larvae (which are suitable for experiments with both species; unpubl. data). Feeding experiments began on day 6 by randomly selecting one neighbouring pair of fishes (i.e. one *P. formosa* and one *P. latipinna*) at a time to keep disturbances during the tests at a minimum. Eighty larvae were simultaneously offered to each paired set of test fishes, and the number of larvae remaining after 15 min was counted. All tests were conducted between 1300 and 1800 hours. Afterwards, the test fishes were weighed again before being returned to their stock tanks.

For statistical analyses, SPSS (17) (www.spss.com) was used. A GZLM with 'number of consumed chironomid larvae' as the dependent variable, 'species' [split according to collection site: *P. formosa* (SMA), *P. latipinna* (COM) and *P. latipinna* (CO 101)] and 'temperature treatment' (winter or summer) as fixed factors, and ' L_S ' and 'mass difference' (day 1 minus day 6) as covariates was conducted. The latter covariate corrects for the way in which each fish was able to sustain itself over the course of the experiment. Specifications were Poisson distribution, the hybrid (Fisher/Newton–Raphson) parameter estimation method, the deviance scale parameter method and computed means of the response to scale the estimated marginal means. Because the interaction of species and treatment was statistically significant, a *post hoc* pair-wise comparison of all-level combinations for the interaction was computed using sequential Bonferroni to correct for multiple comparisons.

Each of the species collection sites and temperature treatment in isolation as well as in combination and the covariates influenced the number of chironomid larvae consumed significantly (for details, compare Table I). The estimated marginal means of the number of chironomid larvae consumed as well as the origin, size and mass difference of the test fishes are presented in Table II. The consumption of larvae for the summer treatment did not differ significantly among species and collection sites. By contrast, during exposure to winter temperatures, *P. latipinna* originating from both sites consumed significantly fewer larvae than did *P. formosa* ($P < 0.05$ for COM *v.* SMA and $P = 0.01$ for CO 101 *v.* SMA), with the feeding rates among sites of *P. latipinna* not differing significantly. Due to the large number of comparisons, only the feeding rates of *P. latipinna* originating from CO 101 differed significantly between the summer and winter treatments after Bonferroni correction (CO 101: $P < 0.05$; COM: $P > 0.05$ and SMA: $P > 0.05$). The insignificant difference between summer and winter treatment for COM is probably due to both the higher thermal

TABLE I. Results of the GZLM with 'number of consumed chironomid larvae' as dependent variable, 'species' [including collection site (see Fig. 1): *Poecilia formosa* (SMA), *Poecilia latipinna* (COM) and *P. latipinna* (CO 101)] and 'temperature treatment' (winter or summer) as fixed factors and standard length (' L_S ') and 'mass difference' (day 1 minus day 6) as covariates

Factor and covariate	d.f.	Wald χ^2	P
Intercept	1, 38	20.502	<0.001
Treatment	1, 38	22.838	<0.001
Species	2, 38	10.33	<0.01
Species \times treatment	2, 38	8.542	<0.05
L_S	1, 38	7.086	<0.01
Mass difference	1, 38	3.953	<0.05

stability near the springhead in COM and the number of comparisons made, given that a mean \pm s.e. difference of 25 ± 10 consumed larvae is still quite high. In summary, all species had similar feeding rates at summer temperatures, but *P. latipinna* reduced food consumption at winter temperatures, whereas *P. formosa* did not. This main effect was slightly modified by habitat characteristics.

If feeding rates are indeed indicative of food demands in the examined species, then the lower feeding rates of *P. latipinna* under winter conditions could be an important factor explaining its higher survival rate during this time when food might be limited. Indeed, *P. formosa* did not gain mass during the winter treatment, despite displaying higher feeding rates. This suggests lower food conversion efficiency in this species at winter temperatures and thus a lower food stress tolerance in adult *P. formosa* at lower temperatures. Furthermore, the higher feeding rates would increase the time needed for feeding, which in turn increases the needed energy as well as potentially increasing exposure to predators or other dangers.

Generalization of these results needs to be done with caution, given that the sampling was restricted to central Texas despite the wider range in which *P. latipinna* and *P. formosa* occur in sympatry (Schlupp *et al.*, 2002). For example, the degree of character displacement in the mate choice of *P. latipinna* males was found to vary between different populations (Gabor & Ryan, 2001), and similar effects might be relevant here. Indeed, noticeable and sometimes significant variation in the feeding rates between populations was observed here. Another potentially important factor is that the sympatry of both species in central Texas is younger than that in the rest of their common range, with *P. latipinna* being introduced in the 1930s from Louisiana and *P. formosa* from Brownsville in the 1950s (Schlupp *et al.*, 2002). Given the observed differences between habitats (which cannot derive from their origin), however, it is clear that the fishes have already adapted at least partly to their new habitats.

Although the results reveal novel fundamental differences that may be important in understanding the maintenance of coexistence in this sexual–asexual species complex, further research is needed to confirm them. For instance, the fishes in this study could only interact visually but not directly, and the influence of males *via* harassment was not tested (Heubel & Plath, 2008). In addition, the finding that food conversion might be worse in *P. formosa* under winter temperatures but comparable

TABLE II. List of tested fishes with mean \pm s.d. standard length (L_S) and mass difference and estimated marginal means [parameter estimation method: hybrid (Fisher/Newton–Raphson); scale parameter method: deviance and scale of estimated marginal means; computed means of the response] of the number (N) \pm s.e. of chironomid larvae consumed in 15 min by the tested fishes either in summer (18.5° C) or winter (26.0° C) temperature treatment. Collection sites shown in Fig. 1

Species and collection site	Summer temperatures			Winter temperatures		
	L_S (mm) (n)	Mass difference (g)†	N^*	L_S (mm) (n)	Mass difference (g)†	N^*
<i>Poecilia formosa</i> (SMA)	38 \pm 8 (10)	-0.094 \pm 0.235	50 \pm 6	35 \pm 6 (8)	-0.075 \pm 0.099	40 \pm 6
<i>Poecilia latipinna</i> (COM)	34 \pm 3 (5)	-0.130 \pm 0.104	43 \pm 9	35 \pm 2 (6)	0.016 \pm 0.090	18 \pm 5
<i>P. latipinna</i> (CO 101)	46 \pm 4 (5)	-0.009 \pm 0.241	50 \pm 9	46 \pm 4 (4)	-0.052 \pm 0.110	13 \pm 4

*Covariates appearing in the model were evaluated at the following levels: L_S 38 mm and mass differences -0.06 g.

†None considered significantly different from 0 g mass difference (one-sample t -test with Bonferroni correction).

under summer ones needs to be tested rigorously. Finally, actual allocation into growth *v.* reproduction should be evaluated in the laboratory as well as in the field, and it should also be investigated whether food competition and food limitation are present in the field.

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