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Short note/Kurze Mitteilung

Food selection in the sailfin molly and its coexisting sexual parasite, the gynogenetic Amazon molly

Futterwahl beim Breitflossenkärpfling und seinem Spermienparasiten, dem gynogenetischen Amazonenkärpfling

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Zusaamenfassung: In einem Caféteria-Versuch mit unterschiedlichem Frostfutter (pflanzliches und tierisches Futter) unterschied sich die Futterwahl von Breitflossenkärpflingen, *Poecilia latipinna,* und dem Amazonenkärpfling, *Poecilia formosa*. *Poecilia formosa* verbrachte mehr Zeit mit Roten Mückenlarven als *P. latipinna* und weniger Zeit mit Weißen Mückenlarven und Algentabletten als *P. latipinna*. Dabei verweilten beide Arten die meiste Zeit bei den Roten Mückenlarven. Trotz des künstlichen Versuchsaufbaus ist dies ein Hinweis auf einen Aspekt, der bei dem Versuch, die Koexistenz der sexuellen Art mit ihrem Spermienparasiten zu erklären, berücksichtigt werden sollte. Einerseits könnte Nahrungskonkurrenz zwischen beiden Arten bestehen und anderseits könnte *P. formosa* mehr Futter für den Grundstoffwechsel benötigen.

Stable coexistence of a sexually reproducing species with a closely related asexually reproducing species is difficult to understand (Agrawal 2006), as the later should have a higher reproductive output producing no males (two fold cost of males–if everything else is equal; Maynard Smith 1978). Nevertheless, the all-female Amazon molly *Poecilia formosa* (Girard) coexists with either males of the sailfin molly *Poecilia latipinna* (Le Sueur) and/or the Atlantic molly *Poecilia mexicana* Steindachner, both of which are sexually reproducing. This co-existence is essential for *P. formosa* because as a gynogenetic species it depends on the sperm of one of these species to initiate oogenesis. Consequently, if *P. formosa* would outcompete its host due to the higher reproductive output it would go extinct afterwards. Interestingly, *P. formosa* is closely related to its host species as they initially gave rise to the Amazon molly in a single hybridisation event more than 100.000 generations ago (reviewed in Schlupp 2005).

The window for stable coexistence of a gynogenetic species and its host(s) is quite narrow (Schley al 2004, Kokko al. 2008). Theoretical work on this topic has underscored the importance of behavioural decisions, such as mate choice and sperm allocation by the host males (Moore & Mckay 1971, Moore 1975, Stenseth al. 1985, Kawecki 1988; Heubel al. 2009) as well as the importance of ecological differences (Case & Taper 1986, Kirkendall & Stenseth 1990, Schley al. 2004) and spatial dynamics (Kokko al. 2008).

With regard to the ecological aspects, differences in feeding ecology are likely important, as feeding determines the resources species can allocate to their maintenance, growth and reproduction in the first place. But so far only the inferior ability of neonates of *P. formosa* to cope with food stress (Tobler & Schlupp 2010) and higher food demand of *P. formosa* females during winter temperatures (Fischer & Schlupp 2009) are known. This study explores which kind of food females of *P. formosa* and *P. latipinna* select in a cafeteria style experiment in order to test if there is the possibility of food competition in the field, e.g. whether both the beginning of the experiment each test fish was caught in plastic cylinders in order to be able to place a glass Petri dish cautiously at the small front site of the tank. The Petri dish had six different food items frozen onto the outer edge of its backside (fig. 1). These food items were frozen chironomid larvae, chaoborid larvae, brine shrimps, liver paste (chopped liver heated with baby food after DAVID REZNICK, pers. comm. with RÜDIGER RIESCH), algae tabs (algae thins) and chopped spinach (boiled prior to refrigeration). After releasing the fishes, they were filmed with a digital video camcorder for 15 minutes to avoid disturbance by an observer. The time spent (in seconds) with the different food items were determined using the video tapes, as the amount of food consumed can not be determined easily for thawed brine shrimps, spinach or the liver paste partly floating in the water at the end of the experiment.

Differences between both species in time spent with the offered food items were analysed with Wilcoxon tests using the Bonferroni method to correct for multiple comparisons and R (2008) to perform the tests. Additionally, the non-parametric Friedman Test with post hoc Dunn's multiple comparison tests was used to determine preferences for each species using Graph Pad InStat (3.0b).

Fishes were equally motivated to consume food, as there was no difference in overall time spent with the food items (*t*-test, $n = 20$, $P > 0.05$). Both species differed only significantly in the time spent with chironomid larvae ($P < 0.01$), chaeoborid larvae ($P < 0.01$), and algae tabs ($P < 0.05$). *Poecilia formosa* spent more time with the chironomid larvae than *P. latipinna* but less time with chaoborid larvae and algae tabs (fig. 2).

Time spent with different food objects differed significantly within the sympatric *P. latipinna* (non-parametric Friedman Test, $n = 10$, $P < 0.05$) as well as in *P. formosa* ($n = 10$, $P < 0.001$) from what was expected by chance indicating that food choice occurred. However, none of the *post hoc* Dunn's multiple

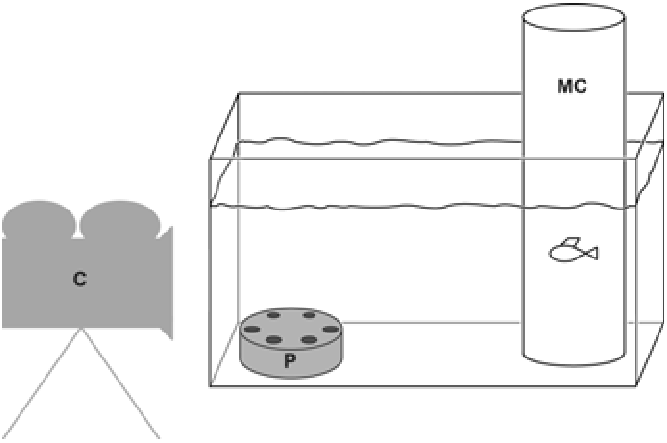

Fig. 1: Experimental setup: the tank (300 x 195 x 205 mm) was filled with 10 l water. To the outer edge of the backside of a glas Petri dish (P; diameter 75 mm, height 15 mm) six food items (chironomid larvae, chaoborid larvae, brine shrimp, liver paste, algae tabs and chopped spinach) were frozen. The dish was placed at the small side of the tank while the fishes were kept in the mesh cylinder (MC). After its removal the food choice of the fishes was filmed for 15 min with a camera (C).

Abb. 1: Aufbau des Experiments: Das Aquarium (300 x 195 x 205 mm) wurde mit 10 l Wasser gefüllt. Auf die Rückseite einer Petri-Glasschale (P; Durchmesser 75 mm, Höhe 15 mm) waren Rote Mückenlarven, Weiße Mückenlarven, *Artemia*-Nauplien, Leberpaste, Algentablette und gehackter Spinat angefroren. Die Petrischale wurde an der schmalen Seite des Aquariums platziert, während die Fische kurzzeitig in einem grobmaschigen Plastikzylinder (MC) untergebracht waren. Nach dessen Entfernung wurde die Futterwahl der Fische für 15 min mit einer Kamera aufgenommen (C).

select similar food and if one species prefers high energy food stronger than the other. Both would influence the amount of energy both fishes have and consequently could modify their reproductive output.

The test fishes were collected in 2006/07 in Brownsville, South Texas (Lincoln Park N 25°53.98, -97°28.77'). Prior to testing they were housed under 12h/12h artificial light conditions and a diet of commercial flake food in mixed groups (both for species and sex) in a 180 l tank at the University of Oklahoma. For the experiments ten *P. latipinna* females (L_S 30±4mm) and ten *P. formosa* females (L_S 31±4mm) were transferred into individual 10 l tanks and starved three days before the actual experiment started. The light regime was 12h/12h and the temperature 24 °C. At

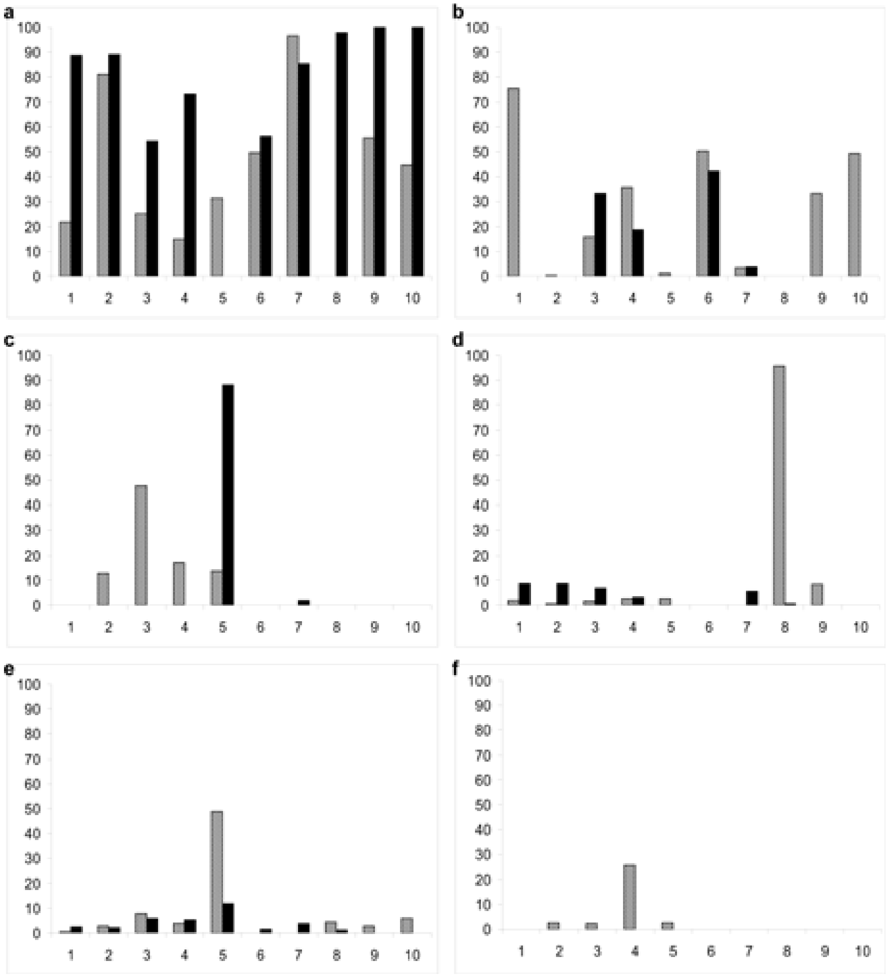

Fig. 2: Time spent with the different food items in percent of the time spent with any food item (y-axis, individual number on the x-axis). Black: *Poecilia formosa*; striped: *P. latipinna*. (**a**) Chironomid larvae, (**b**) chaoborid larvae, (**c**) brine shrimp, (**d**) liver paste, (**e**) spinach, (**f**) algae tabs.

Abb. 2: Zeit, die bei den verschiedenen Nahrungsangeboten verbracht wurde, in Prozent der Zeit, die insgesamt bei der Nahrung verweilt wurde (y-Achse, Nummer des Individuums auf der x-Achse). Schwarz: *Poecilia formosa*; gestreift: *Poecilia latipinna*. (**a**) Rote Mückenlarven, (**b**) Weiße Mückenlarven, (**c**) *Artemia*-Nauplien, (**d**) Leberpaste, (**e**) Spinat und (**f**) Algentabletten.

comparisons were significant for *P. latipinna* (n = 10, all $p > 0.05$), which reflects the conservative nature of this test to detect preferences. In contrast, for *P. formosa* the difference between the chironomid larvae and brine shrimp ($P < 0.01$) as well as the algae thins ($P < 0.001$) were significantly different underscoring the preference for the chironomid larvae; all other differences were not significant ($P > 0.05$).

Despite the artificial setup, not including direct competition and only the sight of the other species not feeding at the same time, the food selection between both species differed partly. There was, however, considerable overlap between the two species for the highly preferred chironomid and chaoborid larvae. Furthermore, while *P. latipinna* spent some time with the offered plant food, *P. formosa* did nearly not spend time with it at all. This hints at two aspects that need to be considered in explaining the coexistence of both species.

First, it is not clear whether there is competition for food resources between the two species. Given the different food preferences reported here, it might be that the two species are generally different, potentially because *P. formosa* only shares half of its genome with *P. latipinna* (being a hybrid between *P. latipinna* and *P. mexicana*, reviewed in SCHLUPP 2005). Consequently, food competition would not be very important in this system. But one could also imagine that especially *P. latipinna* displays a slightly different food choice due to the presence of *P. formosa* and thus needs to explore all different food items present to reduce potential conflict with *P. formosa*. If the latter should be the case food competition might play a role in this system. Furthermore, there is considerable overlap in food preferences, which might also cause some food competition, despite the differences reported here. If there is food competition it would be interesting to investigate which of the species is the superior competitor as this would influence the coexistence of both species to some degree.

Second, both species preferred food with high energy content (chironomid and chaoborid larvae), but *P. latipinna* spent more time with vegetable food than *P. formosa*. If this hints at a higher energy demand in *P. formosa* this would be a disadvantage for the fish with the potential higher reproductive output (HEUBEL 2004, SCHLUPP et al. 2010) and the overlap for high energy animal food should be an important factor promoting the occurrence of food competition in the field. However, it still needs to be carefully evaluated if *P. formosa* needs more resources for maintenance compared to *P. latipinna*, but this seems very likely (FISCHER & SCHLUPP 2010, TOBLER & SCHLUPP 2010). This would be a disadvantage for *P. formosa* which would reduce the amount of offspring it can produce if food is limited and it furthermore increases the time *P. formosa* must spend foraging. A potential explanation for the higher food demand would be the accumulation of deleterious mutations, which asexuals ex- perience over time due to the lack of recom-bination (CHARLESWORTH 1990).

Normally most of the diet of both fishes consists of plant food. For *P. latipinna* a previous study reported that 64 % of the aggregated food volume for 400 field caught individuals of this species consisted of plant material (vascular plants mainly, and small algae) and 31 % were *Aedes* mosquitoes (HARRINGTON & HARRINGTON 1961). Yet, this still might be a high proportion of animal food; normally the diet of both species mainly consists of detritus and algae, but sometimes parts of vascular plants and insects can be present, too (TOBLER pers. comm.). But herbivores usually do not obtain enough protein, as their primary food is especially low in protein content, which leads to omnivorous fishes occasionally feeding on invertebrates (BOWEN et al. 1995). In our study, the preference for animal food might be increased by the short starvation period, the absence of competitors and the fact, that handling time was reduced as the animal food was offered not alive but frozen. Especially the last point is important, because handling time is believed to be important for foraging decisions (CLEMENTS & RAUBENHEIMER 2006).

Altogether it indeed seems worthwhile to explore feeding ecology in this system further using direct competition and field surveys. In cafeteria style experiments living animal food should be used instead of frozen one in order to avoid biased results due to the reduction in handling time.

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