

## **Differential Sperm Priming by Male Sailfin Mollies (*Poecilia latipinna*): Effects of Female and Male Size**

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### **Abstract**

Recent interest in sperm competition has led to a re-evaluation of the ‘cheap sperm’ assumption inherent in many studies of sexual selection. In particular, mounting evidence suggests that male sperm availability can be increased by the presence of females. However, there is little information on how this interacts with male traits presumably affected by female mate choice, such as larger size. This study examines the effects on male sperm availability of female presence, male body size, and female body size in the sailfin molly, *Poecilia latipinna*. Individual males of variable body sizes were isolated in divided tanks for 3 d, after which time either a female or no female was added to the other side of the tank. Prior to the treatments, larger males had more stripped sperm than smaller males. Female presence significantly increased the amount of sperm males primed, but this effect was strongest in small males. Furthermore, males showed a greater priming response in the presence of larger females than in the presence of smaller females. These results demonstrate that the presence of sexually mature females increases the amount of sperm males have for insemination. Furthermore, traits that indicate female fecundity may be used by males as cues in male mate choice.

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### **Introduction**

Sexual selection has traditionally been described as the result of intersexual selection in the form of female mate choice, and intrasexual selection in the form of male–male competition (Andersson 1994). One reason for this generalization is that spermatogenesis has been viewed as an energetically cheap process compared with oogenesis, resulting in an abundance of cheap sperm (Trivers 1972). However, recent studies suggest that spermatogenesis can be energetically costly, and therefore limit male reproductive success (Dewsbury 1982; Nakatsuru &

Kramer 1982; Shapiro et al. 1994; review in Wedell et al. 2002), suggesting that males may exercise mate choice by differentially allocating sperm to maximize reproductive success.

Physiological changes associated with sperm production when males are provided with stimuli from females are referred to as the priming response (Olsén & Liley 1993; Bozynski & Liley 2003), which may be a mechanism by which males conserve energy associated with sperm production (Liley & Kroon 1995). In guppies (*Poecilia reticulata*), the availability of females significantly increases the amount of sperm that males have for transfer, suggesting that the priming response helps male guppies conserve energy resources (Bozynski & Liley 2003). This response, which may have evolved under selection pressures to conserve energy, may have been co-opted as an adaptation for males to exercise male mate choice.

Poeciliid fish have been used as a model system for studies of female mate choice (e.g. guppies: reviewed in Houde 1997; mollies: Ptacek & Travis 1997; Witte & Ryan 1998; platyfish: Hankison & Morris 2003). These fishes exhibit internal fertilization, and females give birth to live young. Males possess a gonopodium, which is modified from the anal fin, and is used to transfer spermatozuogmata (sperm bundles) to the female gonopore (Constantz 1989). Numerous studies have demonstrated a female association preference for larger poeciliid males (Morris et al. 1996; Ptacek & Travis 1997; Basolo 1998; Gabor 1999; Bisazza et al. 2001; Aspbury & Basolo 2002), yet few studies have demonstrated male mate preferences (measured as either an association preference or using actual mating attempts) for larger conspecific females in poeciliids (for exceptions see: Bisazza et al. 1989; Ptacek & Travis 1997; Gabor 1999; Herdman et al. in press). In many poeciliid fish females are larger than males, and larger females are often more fecund (Farr 1989), suggesting that large female size may be a trait males would prefer.

One poeciliid fish that has been the subject of many mate choice experiments is the sailfin molly, *P. latipinna*, which inhabits salt marshes and certain freshwater habitats along the southern Atlantic and Gulf coasts of the US and Mexico. As is the case of other poeciliids, female fecundity increases with female size (Travis et al. 1990), and male *P. latipinna* prefer to associate with larger females (Ptacek & Travis 1997; Gabor 1999). We therefore expect that male *P. latipinna* might manifest this preference for larger females as an increase in sperm production when with females. In addition, male *P. latipinna* vary in standard length (SL – tip of snout to caudal peduncle), and larger males exhibit higher rates of courtship displays to females, whereas smaller males attempt forced inseminations (an attempt to mate without female cooperation) at a higher rate (Farr et al. 1986; Travis & Woodward 1989; Travis et al. 1990; Travis 1994). Based on this observed linkage between courtship behavior and male size, we might expect that males of different body sizes will exhibit differences in the priming response.

We first examined if male *P. latipinna* increased sperm availability (primed more) in the presence of a female for 7 d relative to males not presented with a female. We used males from three size classes in order to determine if males of

different sizes show differences in the priming response. We also examined how the size of females affects male sperm priming by presenting size-matched males with females varying in size for 7 d.

## Methods

The fish used in these experiments were collected from March to May 2003 from Spring Lake, in San Marcos, Texas. Females and males were housed separately in 38 l tanks for at least 30 d prior to the experiments. Because *P. latipinna* have a 30-d ovarian cycle, this separation allowed females to have dropped any broods they may have held prior to testing. Fish were fed O.S.I. Spirulina Flake mixed with O.S.I. Freshwater Flake food (Ocean Start International Marine Laboratory Inc. Hayward, CA, USA) twice a day and supplemented with live and freeze-dried brine shrimp and were maintained on a 14.00:10.00 hours light:dark cycle.

### Experiment 1: Priming Response and Male Size

On day 0 of the experiment, males were removed from their stock tank and anaesthetized in 400 ml of water containing several drops of clove oil. We modified sperm extraction and counting procedures from Matthews et al. (1997). After measuring SL, males were placed along the edge of a shallow Petri dish lined with wet cotton with their ventral side up. Gentle pressure was applied to the side of the male, going from the eye laterally, to the base of anal fin. This method resulted in no mortality of the test fish. Spermatozeugmata came out at the base of the gonopodium, and were collected using an aspirator [an aspirating mouthpiece attached to airline tubing, connected to a small (5 cm) glass tube, with a 1–200  $\mu$ l gel-loading pipette tip attached at the end]. This process was repeated on each male until no more spermatozeugmata were expelled from the male. The spermatozeugmata were placed into a micro-centrifuge tube with 100  $\mu$ l of 0.9% saline solution (0.9 g NaCl/100 ml water), and repeatedly drawn up and expelled from a pipette (to distribute sperm cells evenly). Sperm cells were counted five times on an improved Hy-Lite Neubauer chamber hemocytometer (Reichert, Buffalo, NY, USA) under 400 $\times$  magnification. The total number of sperm cells was determined by multiplying the mean cell count by the sample's initial volume (100  $\mu$ l) and dividing by the volume of the hemocytometer (0.1  $\mu$ l).

Following sperm extraction on day 0, individual males were placed in separate 18-l tanks, each divided into two sections by a clear Plexiglas divider. Males were divided into three size classes ( $n = 12$  males per size class) based on the distribution of male SL derived from the Spring Lake population [mean  $\pm$  1 SD SL (mm): small =  $23.98 \pm 2.50$ ; medium =  $31.21 \pm 3.13$ ; large =  $39.55 \pm 2.83$ ]. For each size class, half of the males were randomly assigned to the female present treatment and the other half were assigned to the no female treatment. On day 3 of the experiment, a single female [mean  $\pm$  1 SD SL (mm) =  $32.8 \pm 3.38$ ] was placed in the other section of the tank for the female present treatment males. On day 10, all fish were removed from the test tanks, sperm stripped, and the

number of sperm available from each male was counted again. We used Spearman rank correlation to determine the relationship between the total sperm cells stripped from each male on day 0 and male size (SL). We used a Mann–Whitney U-test to determine if female presence caused a priming response relative to no female present. To determine if males of different size classes differentially responded to the presence of a female, we used a Kruskal–Wallis test on the female present data. For the latter two analyses, we analyzed the increase in sperm stripped from males on day 10 relative to the amount stripped on day 0 (day 10 total cells – day 0 total cells), in order to control for variation in male sperm availability (independent of male size). Based on Bonferroni's inequality (Snedecor & Cochran 1980), we reduced alpha because each data set was analyzed twice ( $\alpha = 0.05/2$ ). All tests were two tailed.

### **Experiment 2: Effects of Female Size on Priming**

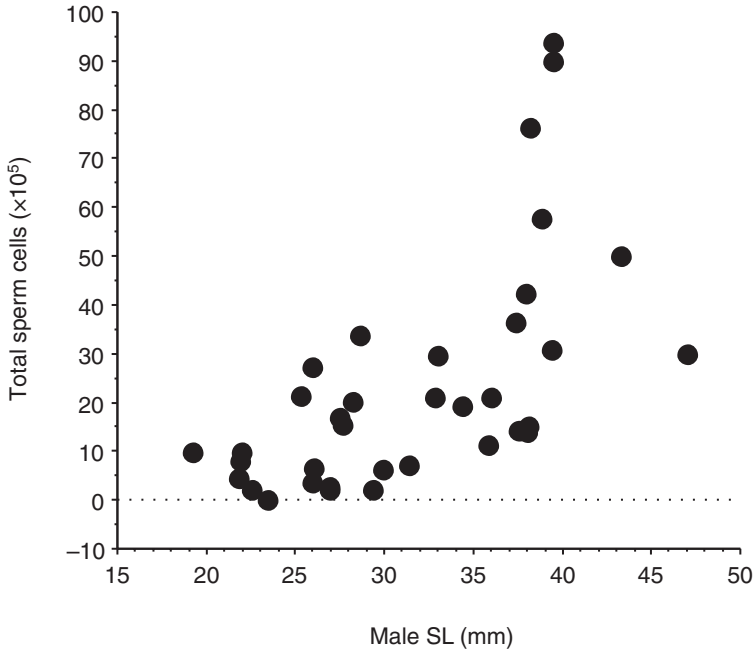
To determine how female size affects male sperm availability, we followed the same experimental design as experiment 1, with the exceptions that we used males of similar SL [ $n = 14$ ; mean  $\pm$  SD SL (mm):  $29.5 \pm 1.25$ ] and used females from the range of SL for the Spring Lake population [mean  $\pm$  SD SL (mm):  $34.1 \pm 3.48$ ]. All males had sperm stripped on day 0, and on day 3 were presented with a single female. On day 10, all fish were removed and sperm was stripped and counted as in experiment 1. All sperm counts were ln transformed, as this transformation normalized these data. We analyzed the increase in sperm stripped from males on day 10 relative to the amount stripped on day 0 (day 10 total cells – day 0 total cells) using simple linear regression with female SL as the independent variable.

## **Results**

### **Experiment 1: Priming Response and Male Size**

When measuring sperm availability on day 0 (after 30 d of physical but not visual separation), larger male *P. latipinna* have more sperm available than smaller males (Fig. 1; Spearman rank correlation between male SL and day 0 total cells:  $r = 0.696$ ,  $n = 36$ ,  $p < 0.0001$ ; mean values for size classes in Table 1). In the female present treatment, larger males had more sperm available than smaller males after 7 d (Spearman rank correlation between male SL and day 10 total cells:  $r = 0.615$ ,  $n = 18$ ,  $p = 0.011$  – mean values for size classes in Table 1). Also, in the female absent treatment, larger males had more sperm available than smaller males after 7 d (Spearman rank correlation between male SL and day 10 total cells:  $r = 0.543$ ,  $n = 18$ ,  $p = 0.025$  – mean values for size classes in Table 1).

There was a significant effect of female presence on male sperm priming. Males in the presence of a female for 7 d had a greater increase in the amount of sperm stripped than those males without a female (Mann–Whitney  $U = 74$ ,



*Fig. 1:* Relationship between male body size (SL; mm) and the total number of sperm cells stripped from males following 30 d of physical, but not visual separation from females

*Table 1:* Mean sperm counts for males in three size classes (L, large; M, medium; S, small), after different female exposure. On day 0 of the experiment, all males had been exposed to visual cues only of females for at least 30 d. Half of the males from each size class were then exposed to a female in a tank that prevented physical contact, but allowed both visual and chemical cues to be exchanged, for 7 d (female exposure = visual and chemical 7 d). The other half of the males were in a tank with no female cues for 7 d (female exposure = none 7 d)

Female exposure	Male size class	Mean sperm cells ( $\times 10^5$ ) $\pm$ 1 SE	n
Visual (only 30 d)	L	45.87 $\pm$ 8.20	12
	M	17.05 $\pm$ 2.70	12
	S	8.14 $\pm$ 2.38	12
Visual and chemical (7 d)	L	45.93 $\pm$ 8.39	6
	M	19.76 $\pm$ 3.01	6
	S	20.82 $\pm$ 4.62	6
None (7 d)	L	49.83 $\pm$ 14.97	6
	M	13.91 $\pm$ 3.32	6
	S	12.33 $\pm$ 5.57	6

$n_1 = n_2 = 18$ ,  $p = 0.005$ ; Fig. 2). There was also significant variation between the three male size classes and the priming response; smaller males show the greatest priming response (Kruskal–Wallis test:  $H = 8.44$ ;  $df = 2$ ;  $p = 0.015$ ; Fig. 2).

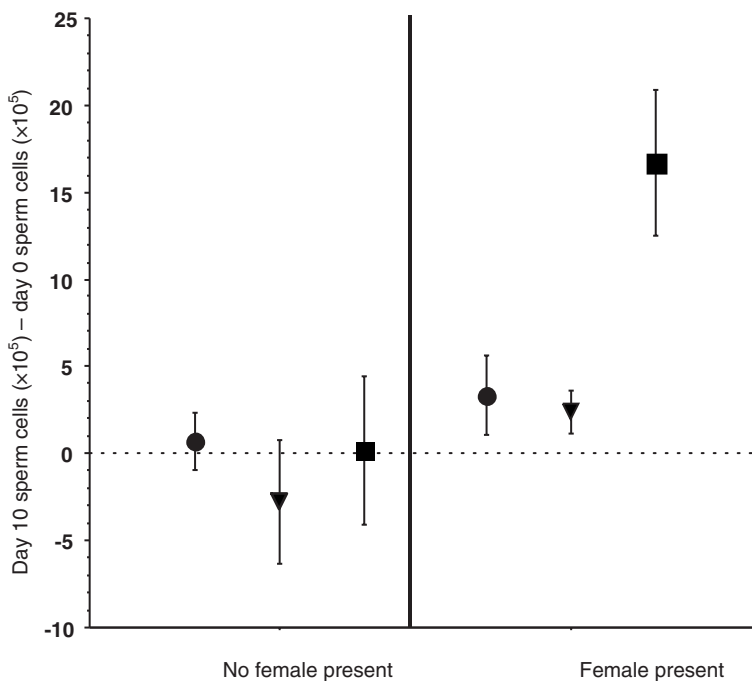


Fig. 2: Mean  $\pm$  1 SE sperm priming response (day 10 – day 0 total sperm cells) for males with no female present and with a female present. Circles are large males, triangles are medium males, and squares are small males

### Experiment 2: Effects of Female Size on Priming

There was a significant positive relationship between female size and the increase from day 0 to day 10 in the amount of sperm stripped from males (Fig. 3; simple linear regression:  $r = 0.640$ ,  $n = 14$ ,  $p = 0.014$ ). Males had more sperm available when presented with larger females for 7 d than when presented with smaller females.

### Discussion

Similar to studies in guppies (e.g. Matthews et al. 1997; Pilastro & Bisazza 1999; Pitcher & Evans 2001), we found that larger male *P. latipinna* have more sperm available after 30 d of separation (physical but not visual) from females than do smaller males. This result is not surprising, given that there are allometric relationships between body size and testis size in many species of fish (Stockley et al. 1997). We also found that males in the presence of females for seven days primed more sperm than those that were not presented with a female. This result is consistent with one other study in guppies, in which males with a female for seven days had more sperm available than those without a female (Bozynski &

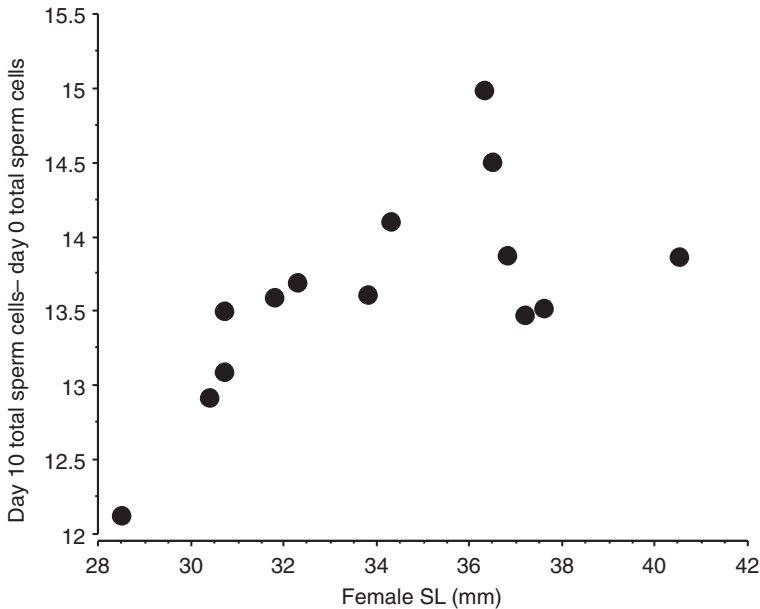


Fig. 3: Relationship between female body size (SL; mm) and the sperm priming response (ln day 10 - ln day 0 total sperm cells)

Liley 2003), and suggests that the availability of sexually mature females has a clear effect on how much sperm males have ready to transfer to females. It is likely that males without female stimuli do not prime sperm as a mechanism to conserve energy resources, as suggested by Bozynski & Liley (2003).

Interestingly, there was variation in the priming effect based on male size. Although small males had less total sperm after being in the presence of a female for seven days than large or medium males, small males showed the greatest priming response compared with medium and large males. In other words, although small males never have more sperm available than larger males (Table 1), they exhibit a large increase relative to their 'baseline' sperm counts when presented with a female, and large decreases relative to their 'baseline' sperm counts when not presented with females. This result suggests that sperm is costly and may be more costly for smaller males. Relative to larger males, smaller males have less energy available (Pitnick & Markow 1994), and therefore are more likely to show the strongest fluctuations in sperm availability dependent on female availability.

A general association preference for larger females by male *P. latipinna* (Ptacek & Travis 1997; Gabor 1999) is associated with a greater priming response when presented with larger females. There are at least two, non-mutually exclusive hypotheses to explain this result. First, males may have evolved a preference for female size because of the higher fecundity of these females, and therefore are prepared to invest more of their potentially limited reproductive resources in these matings. Secondly, males may be prepared to invest more sperm in matings with

larger females because these females are more likely to be the focus of greater male–male competition (Gage 1998). This may be likely in the case of *P. latipinna*, as suggested by the higher rates of multiply sired broods in larger females (Trexler et al. 1997).

We are currently examining whether any of the observed priming responses are related to the actual amount of sperm transferred by males. The relationship between sperm stripped and sperm transferred is unclear in guppies; Pilastro & Bisazza (1999) found a relationship between the amount of sperm stripped and the amount transferred to females, whereas Evans & Magurran (2001) found that sperm stripped was not a good predictor of fertilization success. One possibility is that larger males (who have higher sperm counts) transfer more sperm to females. In this case previously demonstrated female *P. latipinna* preferences for larger males (Marler & Ryan 1997; Ptacek & Travis 1997; Witte & Ryan 1998; Gabor 1999) could reflect a preference for direct fertility benefits (phenotype-linked fertility hypothesis – Sheldon 1994). In guppies males with traits preferred by females (e.g. larger size, higher proportion of orange coloration) transfer more sperm to females (Pilastro et al. 2002). Alternatively, larger, preferred males (which rely less on sneaky mating) may partition their sperm across several females, and therefore not show a relationship between primed sperm and sperm transferred. Warner et al. (1995) found that male bluehead wrasse (*Thalassoma bifasciatum*) that mated most often delivered less sperm per mating than those mated less frequently. This has yet to be resolved in *P. latipinna*.

In conclusion, we have shown that larger male *P. latipinna* have more sperm, males increase sperm availability when in the presence of females, and this response is strongest when presented with larger females. Our results suggest that males can exercise mate choice, which is a potentially important component of sexual selection in this species. Furthermore, our results suggest that smaller males may have higher constraints on sperm production and adjust their sperm availability when not in the presence of a female accordingly. Current studies will help elucidate if sperm priming responses relate to actual sperm transfer to females.

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