

Factors influencing male mating behaviour in *Gambusia affinis* (Baird & Girard) with a coercive mating system

R. DEATON

*University of Oklahoma, Department of Zoology, 730 Van Vleet Oval,
Norman, OK 73071, U.S.A.*

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The effects of male and female body size, and correlated characteristics, on male mating behaviour were investigated in the western mosquitofish *Gambusia affinis*. Because larger females typically have larger broods in *Gambusia* sp., it was predicted that males would attempt more copulations with larger females. Two-way ANOVA showed that female body size was a significant predictor of male mating behaviour but male size was not. The effects of a suite of additional traits (both male and female) on male mating attempts were also tested. In a stepwise multiple regression, female standard length (L_S), size of the female gravid spot and male testes mass were significant predictors of male mating attempts, accounting for *c.* 27% of variation in male mating. Path analysis showed that differences between male and female L_S , male body condition and male testes mass were significant predictors of male mating attempts, and also accounted for 27% of the variation in male mating attempts. The two statistical models were very similar in their predictive power, but differed slightly in significant predictor variables. Results confirm that factors other than female size are important predictors of male mating behaviour in the western mosquitofish.

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Key words: coercive mating; female size; male mating behaviour; mosquitofish; multiple regression; path analysis.

INTRODUCTION

Since Darwin (1871), studies on sexual selection have focused primarily on two mechanisms: intersexual mate choice (female choice) and intrasexual competition (male–male competition; Andersson, 1994). It is clear, however, that other strategies, such as sexual coercion (forced copulations), play important roles in the evolution of mating systems (Clutton-Brock & Parker, 1995). Consequently, coercive mating has been proposed as a third mechanism of sexual selection (Andersson, 1994) and probably drives the evolution of male traits in many organisms (Clutton-Brock & Parker, 1995). It is the primary mating tactic in many animals (Clutton-Brock & Parker, 1995), including monarch

Present address: Department of Biological Sciences, Sam Houston State University, Box 2116 Huntsville, TX 77341, U.S.A. Tel.: +1 936 294 1550; fax: +1 936 294 3940; email: rdeaton@shsu.edu

butterflies (Solensky, 2004), water striders (Arnqvist & Rowe, 1995), garter snakes (Shine & Mason, 2005), bush crickets (Vahed, 2002), and macaques (Cooper & Bernstein, 2000). Coercive mating often is used as an alternative mating strategy (Gross, 1996) in organisms with pronounced male size polymorphism (Zimmerer & Kallman, 1989). In the guppy *Poecilia reticulata* Peters, for example, larger, more colourful males court females, while smaller males use sneaky or coercive behaviour to obtain mating success (Houde, 1997).

Poeciliids in the genus *Gambusia* (mosquitofishes) provide researchers with an excellent system for studies on mate choice when traditional mechanisms of sexual selection (*e.g.* female choice) are weak or lacking (Bisazza *et al.*, 2001). The *Gambusia* sp. mating system is largely male driven where males of all sizes force females to copulate (Pilastro *et al.*, 1997). Thus, male size (and other positively associated traits) is likely to be important in male reproductive success. In many livebearers, larger males are often more aggressive, and potentially better competitors for mates (Hughes, 1985; Riesch *et al.*, 2006). In the one-sided livebearer, *Jenynsia multidentata* (Jenyns), Bisazza *et al.* (2000) also demonstrated that larger males prefer to defend larger females, forcing small males to interact more with smaller, less fecund females. Bisazza & Pilastro (1997), however, showed a small male mating advantage in the eastern mosquitofish *Gambusia holbrooki* Girard, small males mated at higher rates than large males in a non-competitive situation, and suggested this as a potential mechanism for the coexistence of small and large males in natural populations. Hughes (1985) showed that small and large male western mosquitofish *Gambusia affinis* (Baird & Girard) differ in mating behaviours, where small males force-copulated (coerced) at higher rates than large males, suggesting size-correlated mating differences. This was true only when males were mated with sexually receptive females (Hughes, 1985). Further, small males of *J. multidentata* avoided mating with very large females, possibly to avoid predation by larger females (Bisazza *et al.*, 2000). Thus, there appears to be marked differences in mating behaviours between large and small males in many livebearers, possibly due to competition, body size, predator avoidance and female receptivity.

Female size can also be an important determinant of male mating success. In *J. multidentata*, males prefer to mate with larger females (Bisazza *et al.*, 2000), which is expected when larger females have larger broods (Marsh-Matthews *et al.*, 2005). Although it also has been suggested that overt female choice is relatively unimportant in mosquitofishes (Bisazza *et al.*, 1989, 2000; Bisazza & Marin, 1991, 1995), recent studies suggest that females may have more control over male mating than was previously thought (Bisazza *et al.*, 2001) and that female choice is important (Hughes, 1985, 1986; McPeck, 1992; Gould *et al.*, 1999; Bisazza *et al.*, 2001; Langerhans *et al.*, 2005).

Factors other than body size also are known to affect the mating behaviour of males in many species, including age (Savalli & Fox, 1999), social dominance (Haley *et al.*, 1994) and condition (Kissner *et al.*, 2005). In many species, traits that affect reproductive success are strongly correlated with size, and therefore, may have indirect effects on male mating success (Wikelski, 2005). In fishes, several male and female characteristics have been shown to affect male mating behaviour and reproductive success, including territory size and quality (Kraak & Weissing, 1996), pheromones (Park & Propper, 2002), body pigmentation

(Amundsen & Forsgren, 2003), mating history (Dosen & Montgomerie, 2004), male dominance or aggression (Gozlan *et al.*, 2003), body condition (Kodrick-Brown, 1989) and female reproductive state (Bisazza *et al.*, 2000), many of which are correlated with body size.

In this study, the effects of female and male body size on male mating behaviour were examined in *G. affinis*. It was predicted that males would prefer larger females because they are more fecund (Pelabon *et al.*, 2003). A two-way ANOVA was used to test for size-correlated mating differences among males, which has been suggested for *G. affinis* (Hughes, 1985). It was also predicted that males of all sizes would prefer to mate with larger, more fecund females. Small males, however, may avoid mating with much larger females in order to avoid predation by those females, as has been suggested for other livebearers (Bisazza, 1993; Bisazza *et al.*, 2000). Finally, two different statistical approaches were used to test the effects of a suite of correlated characteristics (both male and female) on male mating to determine important predictors (in addition to body size) of male mating behaviour in *G. affinis*.

MATERIALS AND METHODS

Fish were collected from a small pond in Norman Oklahoma, U.S.A. (35°15' N; 97°29' W) in June 2005 and returned to a greenhouse laboratory. Fish were held in two 370 l community tanks and fed commercial flake food daily for 2 weeks. Due to the difficulty of collecting small males from the field, male offspring were reared from pregnant females collected from the same pond in May 2005. Thus, for this experiment, several of the small males used were obtained from laboratory-reared broods. Males reared in the laboratory were checked daily for maturation (formation of the gonopodium; Snelson, 1989) and held in isolation until behavioural observations began. Time to maturity in male *G. affinis* varies from *c.* 36 to 87 days (Campton & Gall, 1988a). There were no significant correlations between either age at sexual maturity (presence of gonopodium) on mating attempts (linear regression, $r^2 = 0.002$, $n = 24$, $P > 0.05$) or the number of days after sexual maturity on mating attempts (linear regression, $r^2 = 0.000$, $n = 24$, $P > 0.05$) for small males reared in the laboratory. In addition, to ensure that there was no effect of laboratory-rearing on male mating behaviour, ANCOVA was used to test for an interaction between male size and treatment (laboratory-reared *v.* non-laboratory-reared). There was no significant interaction in the model (ANCOVA, $F_{3,81} = 2.132$, $P > 0.05$) and, after removing the interaction from the model, there was no significant treatment effect on male mating behaviour (ANCOVA, $F_{2,81} = 3.342$, $P > 0.05$). Thus, laboratory-reared males, regardless of size, showed no differences in mating behaviours in this study.

Prior to behavioural trials, both males and females were separated into three distinct size classes (small, medium and large). The size classes included little to no overlap, with mean \pm s.d. standard length (L_S) for small, medium and large males 15.4 ± 1.0 , 18.4 ± 1.8 and 21.9 ± 2.5 mm, respectively, and for small, medium and large females 20.5 ± 3.9 , 27.1 ± 3.9 and 35.2 ± 3.3 mm, respectively. A medium male size class was included in the experiment to incorporate all natural size variation. No *a priori* predictions regarding mating behaviour of medium sized males, however, were made. Male mosquitofishes vary significantly in size, maturing from *c.* 11–24 mm L_S (Campton & Gall, 1988b). In this study population, males range in size from *c.* 11 to 27 mm L_S . Males in this study ranged from 13.5 to 26 mm, thus, much of the natural size variation was included in the experiment.

The experiment was conducted in July 2005, peak reproductive season for *G. affinis* in Oklahoma. A 'no choice' (Shackleton *et al.*, 2005), free-swimming (Houde, 1997) experimental design was used to test the effects of male and female body size on male mating

attempts (number of times males thrust the intromittant organ towards the female genital pore). Specifically, one male and one female were placed together for each behavioural trial. Although 'choice' experiments may be a more realistic setting for mate-choice studies, data are confounded by the presence of more than one female per replicate, causing non-independence of data (Houde, 1997). A no-choice design allows for the control of such confounding variables, and allows for full contact between the focal male and female (Shackleton *et al.*, 2005). Further, a free-swimming design was more appropriate for this study than the more traditional dichotomous mate-choice design. The reason being that, in order to test factors influencing male mating behaviours, the males need to investigate each female closely. Also the number of mating attempts by males towards females must be scored; therefore, the male and female dyads must be allowed to swim freely together during behavioural trials.

EXPERIMENTAL DESIGN AND STATISTICAL ANALYSES

A two-way ANOVA was used to test the effects of female and male body size on male mating behaviour. For each behavioural trial, male–female size dyads were chosen randomly (*e.g.* treatment = large male mated with a small female). Ten replicates for each of the nine treatments (male–female size dyad) were performed for a total of 90 observations ($n = 90$ individuals of each sex; see Table I). Each male–female pair was placed in a 20 l aquarium and allowed to acclimate together for at least 10 min prior to observations (Houde, 1997). The bottom of each tank was lined with a standardized amount of gravel (*c.* 20 mm). Male mating attempts (number of gonopodial thrusts) were measured during a 5 min focal observation period for each replicate pair. Because trials were conducted in early morning and evening (peak times for mosquito-fish sexual behaviour; C. Hubbs, pers. comm.), a time-of-day block was included as an effect of male mating attempts in the two-way ANOVA.

Female behaviours towards males were measured (to generate a dichotomous measure of female 'interest level'), assigning '0' to females that showed little to no interest (chasing and approaching males \leq two times, and mostly ignoring, moving away from males when approached, or moving to the bottom of the tank to avoid mating by males) and '1' to females that approached or chased the male at least three or more times during the 5 min observation. A one-way ANOVA was used to test for differences in female interest levels based on female and male L_S , and a two-sample *t*-test to determine whether male mating behaviour differed towards interested and uninterested

TABLE I. Randomly drawn male–female mating pairs based on size (small = 1, medium = 2 and large = 3) for two-way ANOVA. Each of nine pairs (treatments numbers 1–9) represents one mating observation per behavioural trial. Each treatment was replicated 10 times, for a total of 90 behavioural observations

Mating combination (size pair)		
Male size class	Female size class	Treatment
Small	Small	1
Small	Medium	2
Small	Large	3
Medium	Small	4
Medium	Medium	5
Medium	Large	6
Large	Small	7
Large	Medium	8
Large	Large	9

females. Logistic regression was used to test for correlations between female L_S and interest levels.

During each behavioural trial, the size of the female gravid spot was recorded on a scale from 0 to 4 (modified from Peden, 1973). The gravid (or pregnancy) spot is a black pigment spot on the abdominal region that forms as females develop ripe eggs (Farr & Travis, 1986) and is thought to be a fertility indicator to males (Snelson, 1989). Specifically, females with no spot were given a score of 0, those with a very small spot 1, medium sized spot 2, large spot 3 and very large spot 4. Females with no spot were typically females that were not carrying ripe eggs, while females that had very large spots (3 and 4) were very gravid and close to parturition. The size of the spot was scored based on relative size to the female body.

Following each behavioural trial, both fish (male and female) were stunned in ice water and immediately preserved in 5% formalin. The L_S and girth were measured to the nearest 0.5 mm, the ovary was removed, and eggs and embryos were counted and staged (following six-stage scale of Meffe, 1985). Viscera were placed back into the carcass and specimens were dried at 40° C to constant dry mass (10 days). To assess body condition, carcasses were weighed to the nearest 0.001 g, rinsed six times overnight in petroleum ether to extract soluble fat, dried again overnight at 40° C, and reweighed. Condition was quantified following Marsh-Matthews *et al.* (2005) and Marsh-Matthews & Deaton (2006), from residuals of linear regression of mass somatic fat (calculated as pre-extraction mass minus post-extraction mass) and initial mass (or pre-extraction mass).

Using those individuals for which all post-experimental measurements were available ($n = 80$), 10 male and 10 female characteristics were measured (Table II). Two statistical approaches were used to address the effects of L_S and other correlated variables on male mating behaviour: (1) stepwise multiple regression using residuals and (2) path analysis. Because several male and female characteristics were correlated with L_S ($r \geq 0.65$, $P < 0.01$), residuals of regressions of correlated traits were used as independent variables. The residual approach is used in structural linear modelling to remove correlations between independent variables (Brown & Prescott, 1999), which otherwise

TABLE II. Selective agents included in the path-analysis model defined by their hypothetical effects on male mating behaviour. Path numbers and abbreviations (denoted in parentheses) correspond to those given in Fig. 1

Hypothesis	Path
Direct effects	
Female characteristics	
Standard length (L_{SF} ; mm)*	1
Condition (K_F)*	2
Girth (G_F ; mm)*	3
Fecundity (F ; embryo number)	
Embryo stage size of gravid spot (S_{GS})	4
Male characteristics	
Standard length (L_{SM} ; mm)*	5
Condition (K_M)*	6
Testes mass (M_T ; mg)*	7
Gonopodial length (L_G ; mm)*	8
$L_{SM} - L_{SF}$ (mm)*	9
Unknown effect	
Variation in male mating that cannot be explained by the variables included in the model	10

*Independent variables included in stepwise multiple regression models.

would violate the assumptions of multiple regression (Sokal & Rohlf, 1997). Pilastro *et al.* (1995) found that size of the male relative to female was the best predictor of male mating success in the closely related *G. holbrooki*; therefore, the absolute difference in male and female size was also included in the model. Because this difference is a function of female L_S (L_{SF}) and male L_S (L_{SM}), two multiple regressions were conducted, the first including L_{SF} and L_{SM} and the second including only the absolute difference $L_{SM}-L_{SF}$. Number of mating attempts (gonopodial thrusts) was the response variable. Analyses were conducted in SPSS version 13.0 for Windows, and SAS, 2000.

For path analysis (Wright, 1921), a set of 34 *a priori* models that reflect hypothetical relationships between female and male characteristics and male mating behaviour were created (Johnson, 2002; Table III). All measured variables included in *a priori* models are depicted in a single global model (Johnson, 2002; Fig. 1). It was not intended to include all variables and all interactions between variables in each path model. Only models that were believed to be realistic, and thus, biologically significant predictors of male mating behaviour were generated (Johnson, 2002). Models focused on four major male characteristics, four major female characteristics, and $L_{SM}-L_{SF}$ (Table II). Path models were generated using the software Amos 4.0 (Arbuckle & Wothke, 1999) and were based on correlation matrices of measured variables on male mating behaviour. Akaike information criterion (AIC) values for each model were generated in Amos 4.0 to assess model fit (Johnson, 2002).

RESULTS

Two-way ANOVA showed no significant time-of-day block or male-female interaction effect on male mating attempts; therefore, those factors were removed from the model. When only male and female L_S were examined, the overall model was significant (two-way ANOVA, $F_{4,89} = 3.28$, $P < 0.05$, Table IV), with L_{SF} as the only significant factor ($P < 0.01$; Fig. 2). There was no significant effect of male size on male mating behaviour.

Female interest levels differed significantly across male size treatments [one-way ANOVA, $F_{2,89} = 8.256$, $P < 0.001$; Fig. 3(a)]. Tukey *post hoc* tests showed that female interest levels were greater towards large than small males ($P < 0.05$), but not towards medium sized males. Female interest differed significantly across all female size treatments [ANOVA, $F_{2,89} = 17.214$, $P < 0.001$, Fig. 3(b)] but did not affect male mating attempts. Also, using 87 females for which continuous size measurements were made, L_{SF} accounted for a significant amount of the variation in female interest levels, where smaller females showed greater interest than larger females (logistic regression, $X^2 = 19.8$, d.f. = 1, $n = 87$, $P < 0.001$).

For the 80 individuals for which all other measurements were available, L_{SF} [$P < 0.001$; Fig. 4(a)], testes mass [$P < 0.001$; Fig. 4(b)] and size of gravid spot [$P < 0.05$; Fig. 4(c)] significantly predicted male mating attempts (stepwise multiple regression, $r^2 = 0.272$, $n = 80$, $P < 0.001$). In a second stepwise multiple regression, $L_{SM}-L_{SF}$ ($P < 0.001$), testes mass ($P < 0.01$) and size of gravid spot ($P < 0.05$) were significant predictors ($r^2 = 0.234$, $n = 80$, $P < 0.001$). All other variables were removed from both models due to non-significance.

The path model explaining the most variation in male mating behaviour included the difference between male and female size ($P < 0.001$), male body condition ($P < 0.05$) and testes mass ($P < 0.001$) as significant predictors of male mating attempts ($r^2 = 0.27$, $n = 80$, $P < 0.001$; Fig. 5). This most explanatory model was chosen based on a combination of AIC and r^2 value (Table III).

TABLE III. An *a priori* set of 34 candidate models that denote biologically significant hypotheses to explain male mating behaviour in *Gambusia affinis* (measured as number gonopodial thrusts). Selective agents are as follows: female, female characteristics; male, male characteristics; female and male, both male and female characteristics. Models are defined as the path numbers shown in Fig. 1 and Table II. * The representative best path based on a combination of Akaike's information criterion (AIC) and r^2 values. Sample sizes (n) represent the number of replicates tested to generate AIC values and r^2 values in each path model

Selective agents	Model (defined by paths)	AIC $n = 81$	r^2 $n = 81$
Female	1,10	6.0	0.11
Female	2,10	6.0	0.02
Female	3,10	6.0	0.04
Female	4,10	6.0	0.00
Female	1,2,10	12.0	0.13
Female	1,3,10	12.0	0.15
Female	1,4,10	12.0	0.13
Female	2,3,10	12.0	0.07
Female	2,4,10	12.0	0.02
Female	3,4,10	12.0	0.06
Female	1,2,3,10	20.0	0.16
Female	1,2,4,10	20.0	0.14
Female	1,3,4,10	20.0	0.16
Female	1,2,3,4,10	30.0	0.16
Male	5,10	6.0	0.02
Male	6,10	6.0	0.06
Male	7,10	6.0	0.10
Male	8,10	12.0	0.00
Male	5,6,10	12.0	0.09
Male	5,7,10	12.0	0.12
Male	5,8,10	12.0	0.03
Male	6,7,10	12.0	0.16
Male	6,8,10	12.0	0.06
Male	7,8,10	12.0	0.12
Male	5,6,7,10	20.0	0.17
Male	6,7,8,10	20.0	0.18
Male	5,7,8,10	20.0	0.12
Male	5,6,7,8,10	30.0	0.18
Female and male	1,6,7,10	17.5	0.23
Female and male	9,6,7,10*	20.0	0.27
Female and male	1,6,7,8,10	25.6	0.24
Female and male	9,6,7,8,10	30.0	0.27
Female and male	9,2,3,4,6,7,8,10	62.8	0.33
Female and male	1,2,3,4,6,7,8,10	65.5	0.28

DISCUSSION

Male preference for larger females is reported for many species (Werner & Lotem, 2003), including fishes (Sargent *et al.*, 1986), and expected when there is a strong female size and fecundity relationship (Pelabon *et al.*, 2003). This study confirms that female size is an important predictor of male mating

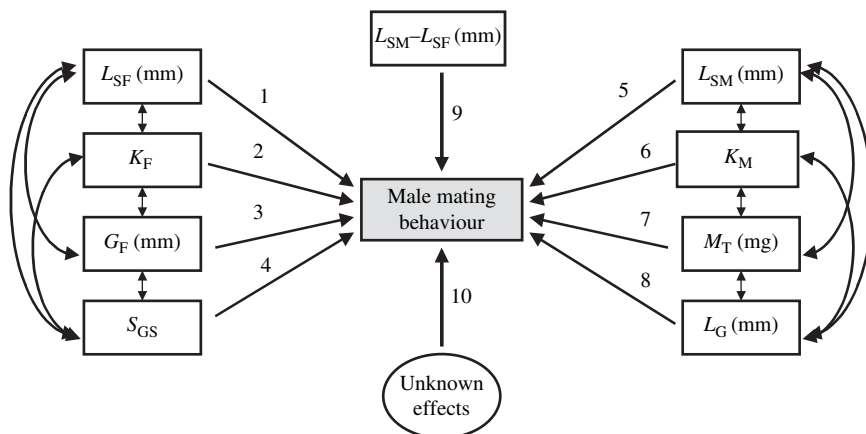


FIG. 1. A global model path diagram depicting putative selective agents on male mating attempts in the *Gambusia affinis* (represented by \square in the centre of diagram). Selective agents in boxes (see Table II) represent measured traits (both male and female) included as independent variables in both path analysis and multiple regressions. Variation in the model unexplained by the nine selective agents is represented by the unknown effects (shown in \circ). Arrows represent regressions of the selective agents on male mating behaviour. \longrightarrow represent direct effects of selective agents on male mating behaviour and \longleftrightarrow depict correlations among independent variables included in hypothesized *a priori* path models (shown in Table III).

behaviour in *G. affinis*. Similar results have been reported for other livebearers, including the one-sided livebearer, *J. multidentata* (Bisazza *et al.*, 2000), guppy, (Herdman *et al.*, 2004) and sailfin molly *Poecilia latipinna* (Lesueur) (Ptacek & Travis, 1997). Herdman *et al.* (2004), however, showed that female size is positively correlated with multiple paternity in the guppy and suggested that a male's preference for larger females might increase susceptibility to sperm competition.

Male size, on the other hand, was not an important predictor of male mating attempts, which is contrary to findings reported for other livebearers, including *J. multidentata* (Bisazza *et al.*, 2000), where male size and female size were important predictors of male mating behaviour (Bisazza *et al.*, 2000) and for *Brachyrhaphis rhabdophora* (Regan), where small males preferred smaller females (Basolo, 2004). Also, in the guppy and other livebearers, small males typically use sneaky tactics (forced copulations) and large males spend more time courting (Houde, 1997), showing marked differences in mating behaviours of males based on body size. These results, however, did not show size-correlated

TABLE IV. Two-way ANOVA showing *F*-statistic and associated level of significance for female standard length (L_{SF}) and male standard length (L_{SM}) effects on male mating attempts (number of gonopodial thrusts)

Source of variation	d.f.	<i>F</i> -value	<i>P</i> > <i>F</i>
Overall model	4,89	3.28	0.0148
L_{SM}	2,89	0.05	0.6548
L_{SF}	2,89	6.14	0.0032

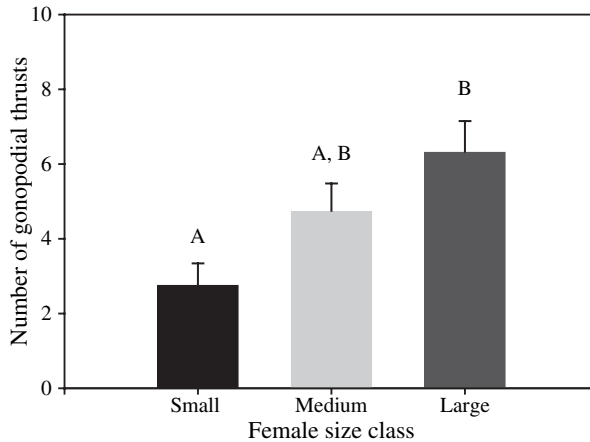


FIG. 2. Mean + s.e. ($n = 90$) number of male mating attempts (measured as number of gonopodial thrusts) by males towards females from each size class (small, medium and large). Upper case letters above bars represent significant differences between each size class (Tukey *post-hoc* comparisons, $P < 0.01$).

mating behaviours by males, as reported by Hughes (1985). Bisazza & Marin (1995) indicated a negative correlation between body size and successful mating (measured as gonopodial thrusts) in *G. holbrooki*, suggesting that small male body size is actually advantageous in that species. In this study, there was no difference in the number of mating attempts based on male size. Males of all size classes preferred larger females and small males did not avoid larger females as was expected. The path analysis, however, showed the size of the male relative to the female (measured as $L_{SM} - L_{SF}$) as the most important predictor of male mating behaviour. Similar findings have also been reported for *J. multidentata* (Bisazza *et al.*, 2000).

Female mosquitofishes rarely initiate mating; however, female interest levels may provide some insight into female choice. These results show that female size strongly influences female interest levels. Interestingly, small females showed the highest interest levels, suggesting that young (and possibly) naïve females are more receptive to males. Roberts & uetz (2005) showed that male wolf spiders preferred to mate with adult, un-mated (virgin) females, and suggested that males can assess potential receptivity of females. Bisazza *et al.* (2000) reported that females of the one-sided livebearer deprived of their sperm stores associated more with males, and also showed preferences for larger males. In this study, female interest levels increased with male size, showing that females were more receptive to larger males, but female interest levels did not influence male mating attempts. This may be because males prefer larger females, and larger females were not receptive to males. Hughes (1985), on the other hand, showed differences in small and large male mating behaviour in *G. affinis* when exposed to receptive females. It has also been suggested that guppy males increase their sexual activity towards receptive females (Houde, 1997). These results suggest that mating preferences of small females should be examined in more detail and female interest levels may be a good surrogate measure of female choice and receptivity in mosquitofishes.

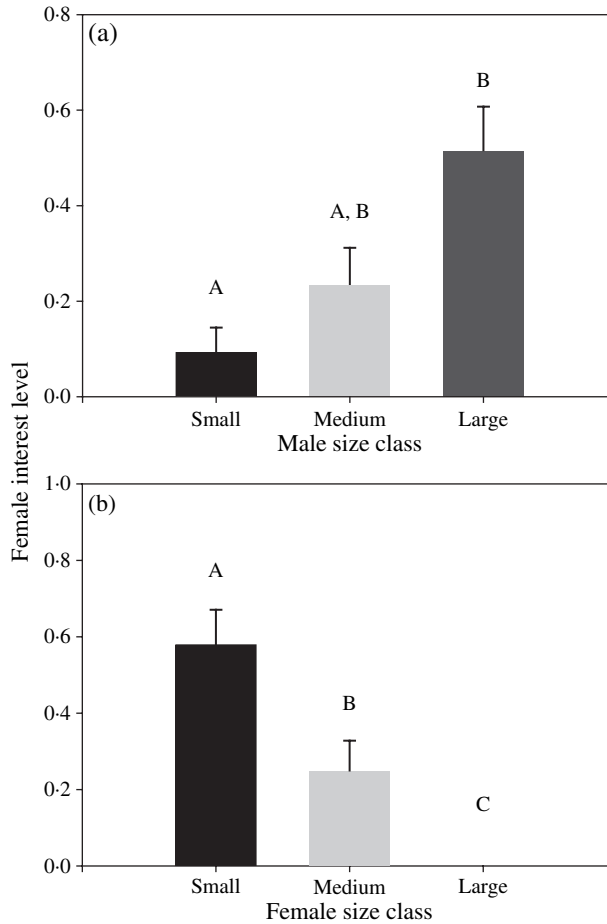


FIG. 3. Mean + s.e. ($n = 90$) female interest level (measured dichotomously as 0 or 1) (a) of females towards males in different size classes and (b) of females in different size classes. Upper case letters above bars represent significant differences between each (a) male and (b) female size class (Tukey *post hoc* comparisons, $P < 0.01$).

Most studies on the mosquitofishes have shown little evidence of overt female choice (although this has not always been the case, see Hughes, 1985, 1986; McPeck, 1992; Gould *et al.*, 1999); however, less obvious (*e.g.* female receptivity) or other cryptic mechanisms (*e.g.* sperm choice) of female choice may be important (Bisazza *et al.*, 2001). Females may have more control over male mating than is currently addressed, especially given that most behavioural observations are conducted in the confinements of aquaria. In this case, females may have significant control over male positioning, which is important for male mating success.

In this study, two statistical approaches were used to determine which male and female characteristics are important predictors of male mating. The most predictive stepwise multiple regression showed that female body size (rather than the difference in female and male size), size of the female gravid spot,

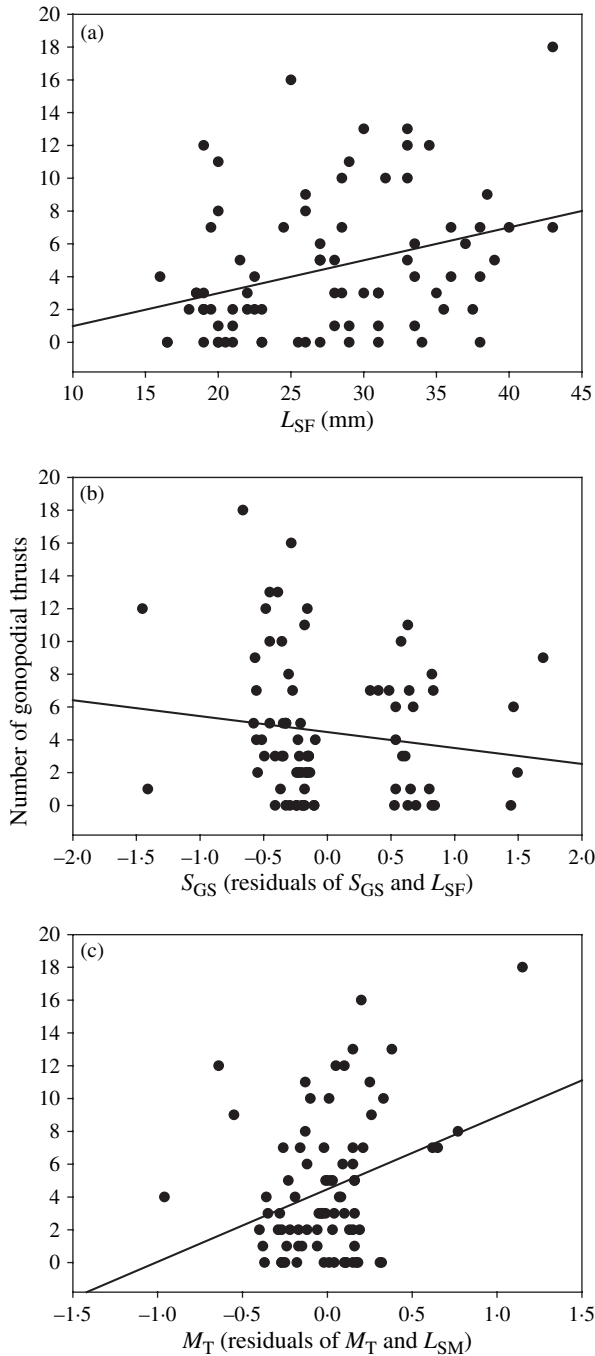


FIG. 4. Male mating attempts (measured as number of gonopodial thrusts) as a function of (a) female standard length (L_{SF}), (b) size of female gravid spot (S_{GS}) and (c) testes mass (M_T) for 80 males included in a stepwise multiple regression model ($y = 0.218 L_{SF} - 1.532 S_{GS} + 5.434 M_T - 1.493$; $r^2 = 0.272$, d.f. = 3,79, $F = 9.588$, $P < 0.001$).

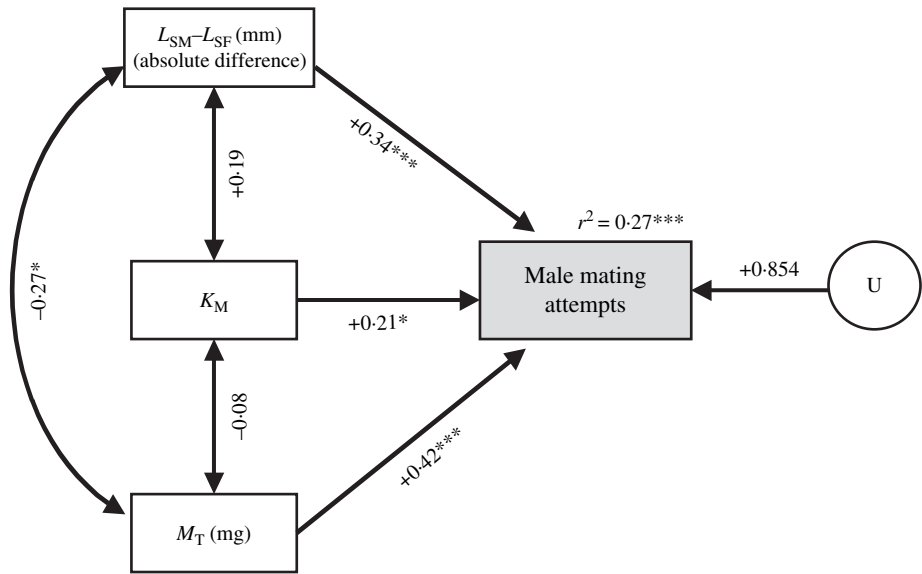


FIG. 5. The most predictive path model [based on a combination of r^2 and Akaike's information criterion (AIC) values] from all alternative *a priori* hypotheses (path models), showing significant predictors of male mating behaviour (number of mating attempts). \rightarrow represent direct effects on male mating, and numbers below lines represent path coefficients. \leftrightarrow represent correlations between independent variables (see Table II), and numbers to the right of the lines represent correlation coefficients. *, $P < 0.05$; **, $P < 0.01$; *** $P < 0.001$. r^2 value above dependent variable (shown in \square) represents total amount of variation explained in model. Unexplained variation is shown in the \circ (U).

and male testes mass were significant predictors of the number of mating attempts by males. But the most predictive path model (based on r^2 and AIC values) included only two significant predictor variables, the difference between male and female size and male testes mass. Both models were highly significant and accounted for 27% of the variation in male mating behaviour.

Other than body size, size of the gravid spot and testes mass also were important predictors of male mating. If the gravid spot is, in fact, a fertility indicator for males (Peden, 1973), expectations would be two-fold. First, females with ripe eggs would be expected to have the largest gravid spots and, second, males should increase the number of mating attempts towards females with the largest spots. These results, however, show a negative correlation between the size of gravid spot and male mating. In addition, previous studies (unpubl. data) have shown that the size of gravid spot is positively correlated with embryo stage (in contradiction to other reported results), and males avoid mating with females close to parturition (unpubl. data). Female mosquitofishes can store sperm for up to several months (Constantz, 1989), and sperm quality has been shown to decrease over time in 16 mosquitofishes (Hildebrand, 1917) and other species (*e.g.* birds; Wagner *et al.*, 2004). Thus, the negative correlation between male mating and the size of the gravid spot may be a result of males avoiding those females with late stage broods (no ripe eggs for immediate fertilization), which may be an evolutionary strategy used by males to avoid

reduction in fitness *via* reduced fertilization success. As far as is known, this has not been tested in livebearing fishes.

Testes mass also was a significant predictor of male mating attempts. Testes mass is usually highly correlated with male size, but this was corrected for in statistical analyses. In *G. affinis* larger males are more aggressive (Hughes, 1985), but larger males in this study population do not have larger testes per unit L_S than smaller males. Testes mass is probably correlated with male hormones driving sexual behaviour (*e.g.* testosterone, 11-ketotestosterone; Borg, 1994), which would explain why males with larger testes relative to body size attempt to mate at higher rates.

In this study, statistical models accounted for a significant amount of variation in male mating behaviour, but considerable variation was left unexplained. Non-significant variables included female girth, male body size (previously discussed), female and male condition and gonopodial length. It was surprising that female girth (as a surrogate for fecundity) did not influence male mating behaviour. In the one-sided livebearer, Bisazza *et al.* (2000) showed that males did not discriminate between gravid and non-gravid females. But, Park & Proper (2002) showed that male mosquitofishes change their sexual behaviour based on the reproductive state of females. Also, if males prefer to mate with larger females because they are more fecund, it might be expected that they would cue in on girth as a measure of size. Because body condition, as measured in this study (as soluble fat stores), varied little among individuals (all individuals were fed *ad lib*), it is not surprising that body condition did not influence male mating behaviour. Finally, Langerhans *et al.* (2005) showed considerable size variation in gonopodium length among species and populations of mosquitofishes, and that females associated more with males with longer gonopodia, both of which suggest that gonopodial length may be under sexual selection. In this study, there was little variation in gonopodial length (after correcting for male size), suggesting that gonopodial length may not be important for male mating success in all populations.

Importantly, this study illustrates that factors other than female size are important predictors of male mating behaviour in *G. affinis*. Results share similarities and differences with those reported for the one-sided livebearer (Bisazza *et al.*, 2000) but corroborate that female body size (or male size relative to female size) is perhaps the most important predictor of male mating behaviour. Livebearing fishes are an ideal system to make such comparisons because several mechanisms of sexual selection (*e.g.* female choice, male–male competition and sexual coercion) may be at play at any given time. In addition, abiotic factors, such as temperature (Wilson, 2005) have also been shown to be important predictors of male mating in *G. holbrooki*. This leaves a challenge to researchers studying factors influencing male mating behaviours in species with coercive mating systems, as a wide array of both abiotic and biotic factors appear to drive this mating system. Such systems are unquestionably complex and deserve considerably more attention to better understand mechanisms of sexual selection and other factors influencing male reproductive success.

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