Original Article

Variation in the condition-dependence of individual sexual traits in male eastern mosquitofish, *Gambusia holbrooki*

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Most sexually selected traits are costly to produce and therefore tend to show condition-dependent expression. But individuals have a finite set of resources to invest across the multiple traits on which sexual selection acts. This necessarily leads to trade-offs among individual traits and between different reproductive stages. The effect of male condition on trait expression might therefore vary for different sexually selected traits depending on the marginal gains from investment into one trait rather than another. We manipulated the diet of eastern mosquitofish (Gambusia holbrooki) to test the condition-dependence of 4 components of male mating effort that are under precopulatory sexual selection (male–male aggressiveness, time spent with females, rate of copulation attempts, and male mate choice). We found positive condition-dependence of both the time spent with females and the rate of copulation attempts, but negative condition-dependence of male aggression towards rivals (all $P < 0.05$). By contrast, the level of male mating preference for larger, more fecund females did not vary significantly with male condition. Our results highlight the importance of incorporating variation in resource acquisition, hence condition, into allocation models that predict investment into multiple sexually selected traits.

Key words: mate acquisition, mate choice, mating effort, reproductive costs, sexual selection, sexual traits.

INTRODUCTION

Sexual selection acts upon male traits that affect reproductive success such as ornaments, courtship and weapons. These traits are often costly to produce and, consequently, tend to be condition-dependent (Johnstone et al. 2009). "Condition" is defined as the pool of resources that males can invest in fitness-enhancing traits (Rowe and Houle 1996). Many studies have demonstrated the condition-dependence of individual sexual traits, whereby males in better condition produce larger traits (Cotton et al. 2004; Warren et al. 2013; but see Bonduriansky and Rowe 2005). However, sexual selection does not act on individual traits in isolation, but rather on the net effect of multiple traits and the combined effects of investment into all stages of reproduction: from mate acquisition, to copulation, and then fertilization (Evans and Garcia-Gonzalez 2016). Since males possess finite resources, trade-offs exist among traits and between reproductive stages (Simmons et al. 2017). The effect of an improvement in condition might therefore vary for different sexually selected traits depending on the associated marginal

returns. This implies that expression of some traits will be more sensitive than that of others to male condition. For example, after a certain initial expenditure on a trait if the marginal returns are minimal there is no benefit to males in high condition of further investment. Such traits will appear to be weakly condition-dependence if the fitness function asymptotes at low levels of investment so that most males can afford to produce the trait, leading to little variation in trait expression.

The marginal returns of a trait are determined by its fitness function (see de Jong 1993), but this function is rarely independent of investment into other traits. For example, expenditure on traits that increase mate monopolization reduces the likelihood of females mating multiply, thereby lowering the returns from investment in sperm production (Parker et al. 2013; Lüpold et al. 2014). Understanding how and why the marginal fitness returns from different traits vary will enable us to better understand the optimal allocation strategy for a given absolute level of resource availability (i.e., condition) (Gross 1996). While the condition-dependence of individual traits has been investigated, far fewer studies have examined the effect of condition on allocation to multiple traits (although see Pike et al. 2010; Lewis et al. 2011, 2012; Devigili et al. 2013; Tigreros 2013; Rahman et al. 2013, 2014). It is possible that the optimal strategy will involve an increase, decrease

or no change in investment in different sexually selected traits as male condition improves (i.e., positive, negative, or no apparent condition-dependence).

To mate, males have to attract a female and potentially repel rivals, which selects for both the ability to win contests (e.g., fighting ability, weaponry), and being better at seducing or coercing females into mating (e.g., courtship, harassment, ornaments). Males in poor condition often perform worse on both counts. Allocating energy to courtship or aggressive behavior usually involves a trade-off with self-maintenance, which might further limit the ability of males in poor condition to invest in acquiring mates (Worden and Parker 2005). For example, male guppies reared with abundant food were more aggressive toward rivals, and courted more persistently, than those reared with less food available (Kolluru and Grether 2005). Furthermore, resource-limited male guppies spent less time in competitive interactions and more time foraging (Kolluru et al. 2009). This presumably facilitates future reproduction at the expense of their current reproductive success, highlighting how condition can also affect trade-offs between sexually and naturally selected traits.

Male investment in sexually selected traits that deliver advantages during male–male competition for females does not preclude male mate choice (Edward and Chapman 2011, Schlupp 2018). Male mate choice is sometimes analogous to conventional female choice: males choose whom to reject (e.g., Schlupp 2018). Alternatively, male choice can be more subtle, such as a male adjusting his mating effort depending on a female's quality (Bonduriansky 2001; Engqvist and Sauer 2001; Reinhold et al. 2002; Nandy et al. 2012; Godin and Auld 2013), and/or the size of the ejaculates he transfers (Simmons et al. 2007; Kelly and Jennions 2011). Here, male choice can be studied by investigating how males respond to females in a "no choice" context (i.e., a single female). How condition affects mate choice has predominantly been examined in the context of female choice (e.g., Hunt et al. 2005; Eraly et al. 2009; Dakin and Montgomerie 2014; Judge et al. 2014). Far fewer studies have investigated how condition affects male mate choice (Edward and Chapman 2011). There are reasons to expect condition to affect male choice since low-condition individuals often pay a higher cost. In studies that manipulate the costliness of male mate choice, males have been shown to be cost-sensitive. For example, in 2-choice trials when male Pacific blue-eye (*Pseudomugil signifer*) had to swim against a current to reach a large female, they were more likely to approach the smaller female (Wong and Jennions 2003). Similarly, Head et al. (2010) found that male guppies (*Poecilia reticulata)* performed fewer sexual displays and were less choosy with respect to female size when forced to swim against a current compared to those tested in still water. There is even some direct evidence for condition-dependent male choice. In the 2-spotted goby (*Gobiusculus flavescens*) males in good condition attract more mates, but are also more likely to reject prospective mates (Amundsen and Forsgren 2003).

By definition, access to food affects resource availability and thereby male condition. Manipulating food availability through diet is a simple way to alter condition to see how it affects investment into different sexually selected traits (e.g., Rahman et al. 2013). Here, we manipulated the diet (low or high food availability) of male eastern mosquitofish (*Gambusia holbrooki*) to quantify the condition-dependence of aggressiveness, mating effort, male mate choice and insemination success. In *G. holbrooki*, males inseminate females using a modified anal fin ("gonopodium") (Constantz 1989), and persistently attempt to sneak copulate rather than court (Wilson 2005). Males are also highly aggressive and nip and chase rivals. We first examined how male condition (manipulated through food availability) affects male aggressiveness, and the rate at which males approach and attempt to copulate with a female when 2 males (low and high food diet) compete. Second, we tested whether condition affected male mate choice by measuring the propensity to attempt to copulate with a female of specific quality (either a large or a small female), and whether or not there was successful transfer of sperm.

We predicted that low food diet males would: 1) be less aggressive, with reduced access to females when a rival was present, either because they conserve resources for less competitive, future mating opportunities, or because they simply have less energy to invest; 2) have a weaker preference for larger, more fecund, females, either because males in low condition have fewer mating opportunities, increasing the opportunity costs of rejecting a mate (Jordan and Brooks 2012), or because a weaker preference for large females decreases the risk of sperm competition (Edward and Chapman 2011), since larger females are generally preferred by males (e.g., Callander et al. 2012). It should be noted that we have limited confidence in our predictions due to the absence of a theoretical framework that considers optimal allocation by individuals in varying condition. Although there are general models that predict allocation into pre- and postcopulatory sexually selected traits (e.g., Parker et al. 2013), these assume that all males have the same resources to allocate to life-history traits. They predict the evolution of the optimal allocation strategy in a given selective environment. The total resources available to invest can, however, affect the allocation strategy by altering the relative strength of selection on different traits. This should lead to condition-dependent allocation strategies (see Hooper et al. 2018 for a related model of allocation into sexually or naturally selected traits).We return to the issue of the theoretical limitations of current allocation models for sexually selected traits in the Discussion.

METHODS

Origin and maintenance of fish

Experimental males were the offspring of wild-caught females from Canberra, Australia (35°18′027″ S 149°07′027″ E). Newborn fry were reared in sets of 5 in 20 × 12.5 × 13 cm tanks until they could be sexed, after which they were moved to single-sex tanks (30–50 males per 60 L tank). Fish were fed twice daily on *Artemia* nauplii and commercial fish flakes, and kept at 27 °C (± 1) on a 14:10 h photoperiod.

Diet and individual marking

We randomly assigned 120 males to competitive mating trials and a further 120 males to mate choice trials. Each male was anesthetized, photographed, and his standard length (SL) measured using ImageJ (US National Institute of Health, Bethesda, MD). For each trial type, males were ranked by SL and consecutively ranked males were paired (mean size difference = 0.07 mm). One male per pair was assigned to a low and the other to a high food diet. High food diet males were fed *Artemia* nauplii ad libitum twice daily, and low food diet males were fed once every second day (i.e., 25% of high diet) for 3 weeks. Males assigned to the competitive mating trials were housed individually in 1L aquaria for the full 21 days. These males were marked subcutaneously with fluorescent elastomer at the base of the dorsal fin (Northwest Marine Technology, Shaw Island, WA) for individual identification during competitive trials. The color was randomized so that subsequent observations were made blind to a male's diet treatment.

Males assigned to the mate choice trials were housed individually for the first 16 days of the diet treatment, after which they were experienced 5 days in "natural" conditions with full access to females. We set up 8 aquaria (60 L), each with 8 males (4 per diet treatment) and 12 stock females. This adult sex ratio is typical of that in the wild (Fryxell et al. 2015). During the 5 days, these males were still maintained on their respective diets. This was done by removing the males from their communal tank every evening and placing them into individual 1 L aquaria. We fed them their respective diet that evening, and again the following morning. We then returned the males to their communal tank 20 min after their morning feed. We tested male mate preferences at the end of the 21-day period (see Male mate choice below).

Competitive mating trials: male aggression and mating behavior

We allowed a pair of low and high food diet males to compete for a female in a 40 × 23 × 19 cm tank, which was set up with opaque screens at either end with one male behind each screen. We then placed a virgin stock female in a clear plastic cylinder in the center of the tank. After allowing 5 min for the fish to acclimate, we removed the opaque screens and recorded the behavior of both males for 10 min ("captive female" trials). We used the *iObserver* app to record aggression towards their rival (rapid approaches and nips), and the time spent oriented towards the female and within a body-length of her cylinder ("time near female"). We then removed the cylinder so that all 3 fish were free swimming, and recorded the males' behavior for another 10 min ("free swimming" trials). In addition to aggression towards their rival, we recorded the number of copulation attempts per male. We obtained data from 56 of 60 test pairs (4 males died).

Male mate choice and insemination success

To test whether diet affected the strength of male mate choice (i.e., reproductive effort directed towards either a large or a small female), we used a 2 × 2 factorial design in which males of each diet treatment were individually presented with either a large or small female (Total $\mathcal{N}$ = 120 males). Tests were conducted on the sixth day following 5 days of exposure to females to provide males with reasonable expectations of future mating opportunities (which is necessary to elicit mating preferences). We set up an experimental tank (40 × 23 × 19 cm) with gravel, 15 cm of water, and an opaque screen at either end behind which we placed the focal male and either a large or small female. Females were virgin, F_1 lab stock reared in single-sex tanks at different densities to yield large or small females (mean ± SE; 28.5 ± 0.2 mm vs. 35.3 ± 0.3 mm; t-test, t_{112} = 23.72, P < 0.0001). The test fish were given 5 min to acclimate after which we removed the opaque screens and observed them for 10 min. The frequency and duration of male sexual behaviors ("time with female" and copulation attempts) were recorded using the *iObserver* app. We successfully tested 114 males. Within 5 min of each trial ending, the female was anesthetized in an ice slurry, placed ventral side up in a polystyrene cradle and her gonoduct flushed to check for the presence of sperm (following Head et al. 2015). Briefly, we used a Drummond micropipette to inject 3 µL of saline solution (NaCl 0.9%) into her gonoduct and then collected the ejected solution. This process was repeated 3 times. The recovered solution was collected in a PCR tube, vortexed for 30 s to break up sperm bundles, and 10 µL transferred to an "improved Neubauer chamber" hematocytometer and viewed under 400× magnification for the presence of sperm.

Statistical analyses

To investigate male behavior during competitive mating trials the correlated aggressive behaviors of rapid approaches and nips ("captive female" trials: r = 0.513, P < 0.001; "free swimming" trials: r = 0.545, P < 0.001) were combined into an "aggression score" using a principal components analysis (PCA). The first principle component (PC1; hereafter "aggression") accounted for 75.6 and 77.2% of variation in "captive female" and "free swimming" trials, respectively. We then used paired t-tests to determine whether diet affected: 1) the level of aggression towards a rival, 2) time near the female (in "captive female" trials), and 3) number of copulation attempts (only measurable in "free swimming" trials). Effect sizes (d) were calculated following the formula in Table 1 of Nakagawa and Cuthill (2007) to compare 2 dependent groups.

To test for the effect of diet and female size on male mating effort, we ran 2 separate analyses using "time near the female" and "number of copulation attempts", respectively as the response variable in a generalized linear mixed model (GLMM) with Poisson error structure, with diet, and female size as fixed categorical factors, standardized male size as a fixed covariate and stock tank ID as a random factor. We included male ID as a random factor to correct for overdispersion. Four males were excluded from these analyses as they spent no time with the female (but their inclusion did not alter the significance of main effects or interactions). To test for an effect of diet on insemination success (yes/no), we ran a GLMM with binomial error structure with diet and female size as fixed factors, standardized male body size as a fixed covariate, and tank ID as a random factor.

All analyses were run in R (version 3.4.0). GLMMs were conducted using the *lme4* package, with *P*-values derived using the "anova" function of *lmerTest* package. Model assumptions were confirmed via inspection of residual plots. Unless otherwise stated all summary statistics are presented as Mean ± SE.

RESULTS

For the subset of males measured before and after diet treatment ($\mathcal{N}$ = 114 of the 240), there was no significant difference in size prior to the diet treatment (High: 22.7 ± 0.2 mm; Low: 22.6 ± 0.2 mm; P = 0.621). However, there was a significant size difference afterwards (High: 23.1 ± 0.2 mm; Low: 22.4 ± 0.2 mm; P = 0.001), indicating that the diet treatment successfully altered the condition of males (using body size as a proxy for condition sensu Rowe and Houle 1996). On average, high food diet males grew significantly (Before: 22.7 ± 0.18 mm; After: 23.1 ± 0.2 mm; P = 0.033), whereas low food diet males showed a slight decline in size (Before: 22.6 ± 0.2 mm; After: 22.4 ± 0.2 mm; P = 0.248).

Competitive mating trials: male aggression and mating behavior

In "captive female" trials, when males were unable to mate, the occurrence of aggressive behavior (quantified as the combination of rapid approaches and nips, PC1) by males in a pair was positively correlated (r = 0.449, P = 0.001, $\mathcal{N}$ = 56 pairs). However, the low food diet males were significantly more aggressive than their high food diet rivals (difference in aggression: 0.41 ± 0.2, t_{55} = 2.12, P = 0.038, effect size d = 0.30). Low food diet males more often made rapid approaches to their rival (6.43 ± 1.7) than did high food diet males (4.02 ± 0.7), and more often nipped their rival (2.86 ± 0.7) than did high food diet males (mean 1.39 ± 0.4) (Figure

1a,b). In these "captive female" trials, there was no relationship between the time spent with the female by low and high food diet males within a pair ($r = 0.049$, $P = 0.486$, $N = 56$ pairs). The high food diet males spent significantly more time near the female (High: 155.3 s ± 18.6, Low: 61.5 s ± 10.3, t-test $t_{55} = 4.59$, $P < 0.001$, effect size $d = 0.85$, Figure 1c).

In "free swimming trials", when males had access to the female, there was no correlation between the occurrence of aggressive behaviors by males in a pair ($r = -0.022$, $P = 0.871$, $N = 56$ pairs). But, again, the low food diet male was significantly more aggressive (difference in aggression 0.64 ± 0.2, $t_{55} = 2.78$, $P = 0.007$, effect size $d = 0.52$). With access to females, low food diet males more often made rapid approaches to their rival (10.21 ± 2.4) than did high food diet males (4.79 ± 0.7) and also more often nipped their rival (1.27 ± 0.3) than did high food diet males (0.46 ± 0.1) (Figure 1d,e). There was a positive correlation between the number of copulation attempts by the low and high food diet males in a pair ($r = 0.405$, $P = 0.002$, $N = 56$ pairs); and high food diet males made significantly more copulation attempts than low food diet males (High: 27.11 ± 3.6, Low: 6.68 ± 1.7 S.E., t-test, $t_{55} = -6.17$, $P < 0.001$; effect size $d = 0.90$, Figure 1f).

Male mate choice and insemination success

Parameter estimates, associated P-values, and effect sizes for factors affecting time spent with the female and the number of copulations when a male and female interacted are provided in Table 1. There was no effect of female size on the time the male spent with the female (Small female: 164.7 s ± 16.9; Large female: 185.9 s ± 17.1), or on the number of copulation attempts (Small female: 15.1 ± 1.8; Large female: 23.9 ± 2.9) (Table 1). There was also no significant interaction between diet and female size, suggesting that neither group of males exhibited choosiness based on female size (i.e., no difference in reproductive effort) (Table 1). The difference between the number of copulation attempts made with large versus small females was, however, much greater for high diet than for low diet males. High diet males made, on average, 14.6 ± 0.7 more copulation attempts when presented with a large than a small female, compared to only 3.5 ± 1.2 more copulation attempts with a large versus small female by low diet males (Figure 2). There was a significant interaction between diet and male body length (Table 1). Larger males on the low food diet spent more time near the female
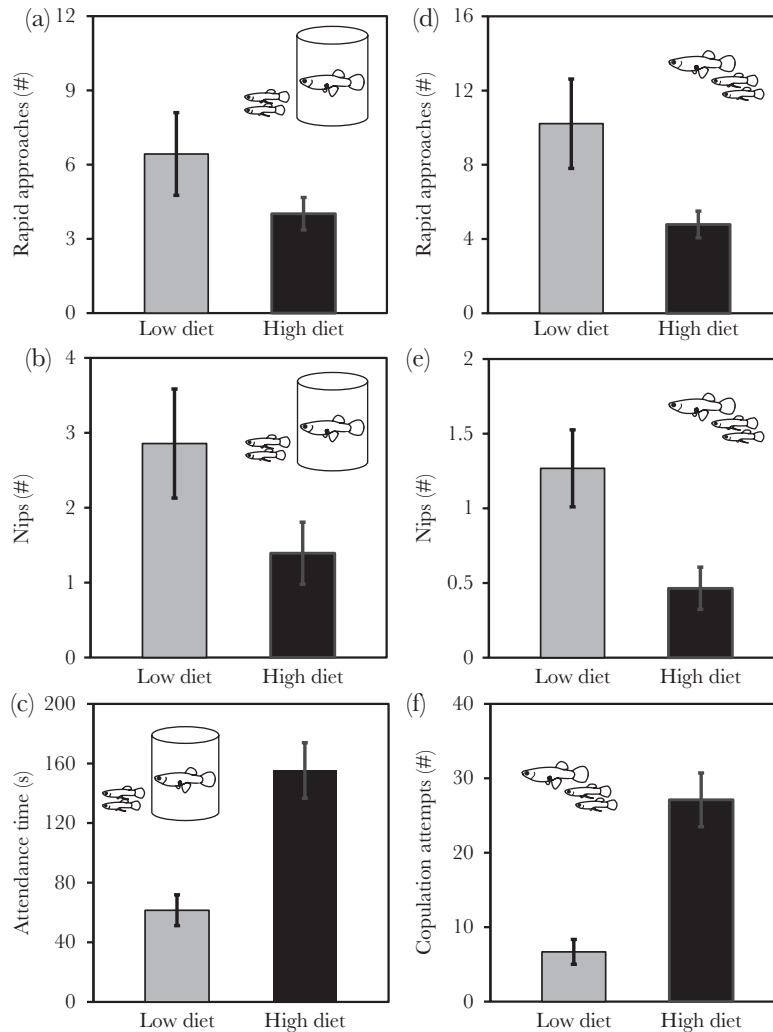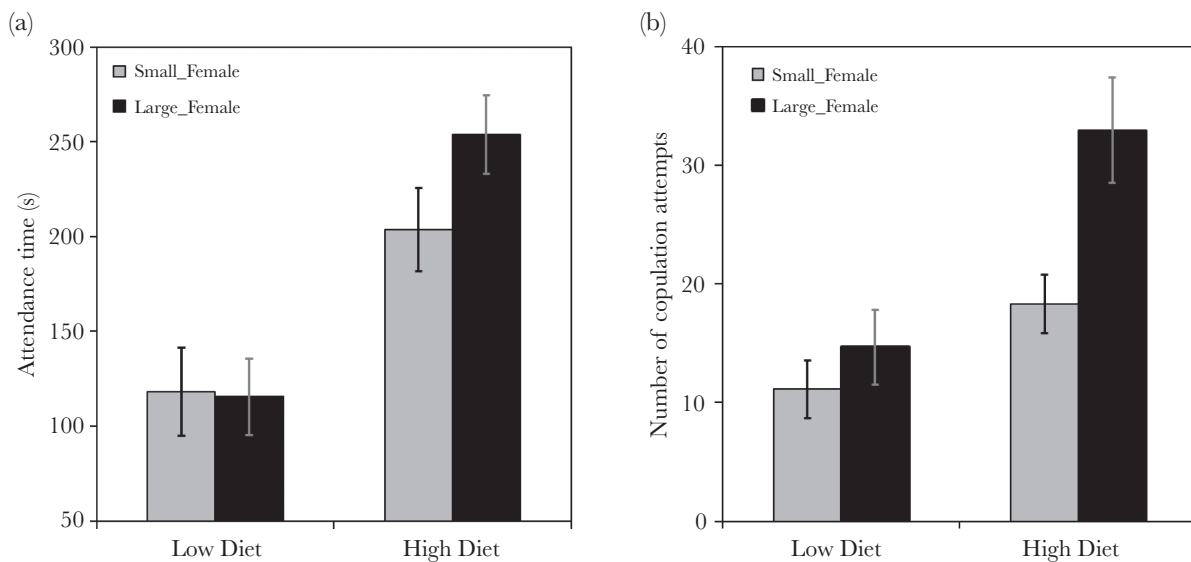
Figure 1
Effect of diet (low vs. high food) on the level of aggression (rapid approaches and nips) and mating behaviors (time with female and copulation attempts) shown by rival male *Gambusia holbrooki* in trials involving either a "captive female" (a–c) or a "free swimming female" (d–f) ($N = 56$ male pairs in each type of trial).

Table 1

Parameter estimates for the effects of condition (high vs. low food diet), quality of potential mate (small or large female), and male body size (standardized covariate), on the strength of male mate preferences for either a small or a large female in one-choice tests

	Attendance time					Copulation attempts				
	Est.	Std. Error	χ^2	P	d	Est.	Std. Error	χ^2	P	d
Intercept	13.55	0.51				4.27	0.53			
Diet (Low)	−1.32	0.301	26.38	**<0.001**	0.85	−1.18	0.32	17.84	**<0.001**	0.71
Female size (Sm)	−0.78	0.69	0.048	0.827	0.23	−0.96	0.69	1.895	0.168	0.27
Male size (standardized)	−0.67	0.71	12.43	**<0.001**	0.19	−0.82	0.73	7.154	**0.007**	0.21
Diet × Female size	0.49	0.42	1.415	0.234	0.22	0.45	0.45	1.002	0.317	0.19
Diet × Male size	1.00	0.44	5.297	**0.021**	0.44	1.02	0.47	4.659	**0.031**	0.41

Mate choice preference is quantified as: 1) the time spent near the female and 2) the number of copulation attempts. Significance values of parameter estimates are from analysis of deviance (type II Wald chi-square tests) with significant effects ($P < 0.05$) in bold. Effect sizes (d) for the model parameters were calculated using the z value from the model following equation (10) in Nakagawa and Cuthill (2007).

Figure 2
Effect of diet (low vs. high food) on the mating effort of males presented singly with either a small or large female: (a) time spent near the female; (b) number of copulation attempts (mean ± SE) ($N = 27$ low diet, small female, $N = 29$ low-diet, large female, $N = 30$ high-diet, small female, $N = 28$ high-diet, large female).

and made more copulation attempts than did smaller males (Figure 3). For males on the high food diet (high condition), there was, however, no observable differences in the propensity of different sized males to attempt to mate (Figure 3).

In 32 of the 116 trials, the male successfully inseminated the female. Diet had no effect on the likelihood of insemination success ($\chi^2_{(1)} = 1.018$, $P = 0.313$; $N = 17$ high and 15 low food diet males), but larger males were significantly more successful ($\chi^2_{(1)} = 4.623$, $P = 0.032$), irrespective of diet treatment or female size (both interactions, $P > 0.34$). Full details of model outputs are given in Table 2.

DISCUSSION

We examined the consistency of the effect of a change in condition on different sexual traits for male mosquitofish (*G. holbrooki*). Given a diet-induced change in condition (sensu Rowe and Houle 1996), in what direction does the expression of sexually selected traits change? It is widely assumed that condition-dependence

means an increase in the expression of a trait with greater condition, but, as shown here, the opposite can also occur, or there may be no correlation between condition and trait expression (i.e., positive, negative, and no condition-dependence). We found evidence for positive condition-dependence of mating effort when 2 male *G. holbrooki* competed for a female (copulation rate), but negative condition-dependence of aggressiveness towards rivals. In contrast, the male preference for larger, more fecund females did not vary with male condition. Finally, in the absence of rivals, male size, rather than condition, was the best predictor of insemination success. Therefore, even across the small set of sexual traits examined in the current study (male mating effort, male aggression towards rivals, and male mate choice), we found evidence for all 3 possible relationships between condition and trait expression.

Condition-dependence of individual traits

When 2 male mosquitofish competed, low-condition males spent less time than high-condition males near a female. It was unclear whether low-condition males were prevented from approaching the

female by high-condition males, or if they were less motivated to mate. However, given the results of our mate choice experiment (Figure 3), it appears that low-condition males are less motivated to pursue mating opportunities as, even in the absence of a rival, they spent less time with the female. In another poecilid, the guppy (*P. reticulata*), low food diet males also have a lower mating effort (courtship rate), although they do not make fewer copulation attempts (e.g., Devigili et al. 2013; Rahman et al. 2013). If the benefits of mating attempts are the same for all males (but see Getty 2006), this suggests that the marginal costs of investing time in mating, are higher for males in low condition. This could be because they gain more by foraging, or could simply be because they have less energy and are therefore less active. Few empirical studies have manipulated food availability to explore how it affects the trade-off

between foraging and mating (Griffiths 1996). Future studies should examine the role of food availability, hence male condition, in modifying the trade-off between foraging and reproduction, to determine whether the lower motivation to mate of low-condition males is due to their absolute resource levels, higher marginal costs, or both (Scharf et al. 2013).

In both the presence and absence of a rival, male *G. holbrooki* in better condition more often attempted to copulate, confirming the positive condition-dependence of male mating effort based on the proxy of the time spent near a female. When a rival was absent, this increase in the rate of copulation attempts did not, however, translate into greater insemination success. It should be noted, however, that the mean number of copulation attempts by low-condition males declined from 1.29 to 0.67 attempts/min when a rival
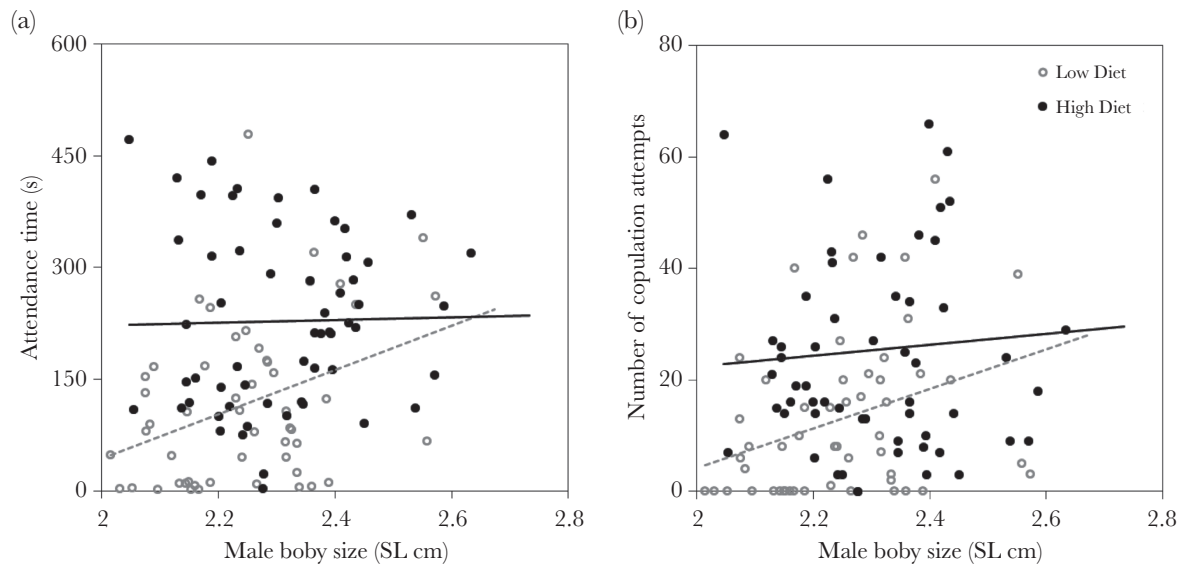

Figure 3
Effect of body size and diet on the mating behavior of a male presented with a single female: (a) time spent near the female. (b) Number of copulation attempts. The regression lines differ significantly for both behaviors (both diet × male size interactions, $P < 0.05$) (see text) ($N = 56$ low-diet, 58 high-diet).

Table 2
Parameter estimates for the effects of condition (high vs. low food diet), quality of potential mate (small or large female), and male body size (standardized covariate) on the insemination success (yes/no) of male *Gambusia holbrooki*

	Estimate	Std. Error	χ^2	P	d
Full model					
(Intercept)	−2.698	1.215			
Diet (Low)	1.282	0.743	2.973	0.085	
Female size (Sm)	2.222	1.524	2.125	0.145	
Male size (standardized)	1.949	1.680	1.346	0.246	
Diet × Female size	−1.436	0.994	2.088	0.148	
Diet × Male size	−0.543	1.090	0.248	0.619	
Reduced model					
(Intercept)	−11.304	4.920			
Diet (Low)	0.489	0.485	1.018	0.313	0.22
Female size (Sm)	0.156	0.483	0.104	0.747	0.07
Male size (standardized)	4.298	1.999	4.623	**0.032**	0.46
Comparison of fit					
	AIC (df)	logLik	χ^2	P	
Reduced	120.03 (5)	−55.014			
Full	121.90 (7)	−53.950	2.127 (2)	0.345	

Significance values of parameter estimates are from analysis of deviance (type III Wald chi-square tests) with significant effects ($P < 0.05$) in bold. Effect sizes (d) for the model parameters were calculated using the z value from the model following equation (10) in Nakagawa and Cuthill (2007).
No significant effect of removing interaction terms, so reduced model reported in Results.

was present, while that of high-condition males remained almost unchanged (2.6 vs. 2.7 attempts/min, Figures 1 and 2). The presence of a rival therefore markedly reduced the relative rate of copulation attempts by a male in poorer condition than his rival, which could potentially affect the number of sperm transferred, hence success under sperm competition (Simmons and Fitzpatrick 2012). As Head et al (2015) highlighted, one has to be cautious when inferring reproductive success from proxies such as copulation rate.

Male poeciliid fish establish dominance using aggression, and male–male competition plays a crucial role in determining male mating success in many poeciliid species (Kodric-Brown 1992). However, contrary to our initial prediction, male aggression was negatively condition dependent: low-diet males were more aggressive towards their high condition rival in the presence of either a "captive" or free swimming female. There are several possible explanations. First, diet restriction may increase general male aggression by favoring increased competitiveness for food. Second, if low-diet males perceive themselves to be less attractive, then a better alternative might be to invest relatively more into aggression to win contests for mating opportunities. Evidence from our study suggests, however, that this is an ineffective tactic since low-diet males were still not able to achieve as many copulation attempts as high-diet males in competitive trials. Third, high condition males may not need to exhibit higher levels of aggression if they had already monopolized access to the female (they spent more time close to the female and made more copulation attempts). There is also the possibility that the effectiveness of acts of aggression depends on male condition. For example, if competitive ability is greater for high condition males (e.g., Tomkins et al. 2004), then their lower rate of aggression could be because their aggressive behaviors are simply more effective.

In general, we expect the marginal costs of mate choice to decline as an individual's condition increases allowing them to be more choosy (Jennions and Petrie 1997; Cotton et al. 2006). Previous studies of poecilids, including *G. holbrooki*, show that males tend to prefer larger females (review: Schlupp 2018). In our study, there was no significant difference in the extent to which males attempted to mate with small and large females. This can plausibly be attributed to the fact that most of the cited studies involve simultaneous choice between 2 females, while our study documented the mating effort expended on a single female. When 2 females are simultaneously available, there is no immediate cost to being choosy (Bonduriansky 2001; Barry and Kokko 2010; but see Servedio and Lande 2006 for a potential cost of greater sperm competition). In contrast, when females are sequentially encountered (i.e., singly), a male pays a fitness cost if he makes fewer copulation attempts, unless that short-term cost is offset by a greater probability of encountering, successfully mating with, and fertilizing, a more fecund female (Jennions and Kokko 2014). An alternative explanation for the our finding that males in higher condition did not show a greater propensity to mate with a large rather than small female is that males in high condition possess sufficient resources to mate with all available females (i.e., no effective resource constraint). Indeed, mate choice is expected to be greatest when the costs of reproduction are high, which is more likely for individuals in poor condition (Engqvist and Sauer 2001; Byrne and Rice 2006). There was a significant tendency for males in high condition to make a greater mating effort, but only when they were small (Figure 3). One possible explanation is that smaller males simply have lower energy reserves, so that the same dietary restriction has a larger relative impact resulting in greater sensitivity of sexual trait expression to condition.

Future directions: the condition-dependence of sexual selection

Many studies, partly motivated by existing theoretical models, have documented phenotypic correlations to examine trade-offs between pre- and postcopulatory sexually selected traits (reviews: Evans and Garcia Gonzalez 2016; Simmons et al. 2017). Less attention is given to the allocation of resources to sexual traits deployed at the same stage of reproduction (e.g., courtship vs. ornaments). In practice, phenotypic correlations do not necessarily identify trade-offs because variation in resource availability can generate positive correlations despite an allocation trade-off (van Noordwijk and de Jong 1986). Some researcher have therefore used experimental studies that manipulate food availability to test how condition affects the expression of different sexual traits (e.g., Devigili et al. 2013; Rahman et al. 2013, 2014). Unfortunately, the lack of theoretical framework to make predictions about the optimal condition-dependent resource allocation strategy remains an impediment. The problem, in a nutshell, is that the standard terminology of "condition-dependent traits" encourages thinking about the fitness maximization of traits in isolation, when our focus should be on how allocation changes with condition. The fitness functions of any given trait depend on the expression of other traits; so, it is misleading to think about individual trait fitness curves in isolation.

Current theoretical models that ask how males allocate resources to sexually selected traits are mainly about investment into 2 pools: traits under precopulatory or postcopulatory sexual selection (e.g., Parker et al. 2013). It is, however, difficult to make real-world predictions using these models. First, the optimal investment strategy depends on the extent of covariation in the strength of selection on each suite of traits (e.g., a and M in Parker et al. 2013), which is usually unknown. Second, these models assume independent functions that relate investment to components of fitness that are then multiplied to generate net fitness. But fitness often depends on interactions among traits. Third, these models assume that resource availability (R) does not differ among males. But variation in condition can change the marginal returns from different traits (see Kokko 2001, Hooper et al. 2018). It would be useful to have models that consider how R affects allocation decisions to sexual traits so that, for a given population distribution of male condition, we can predict the phenotypic correlation between traits. The only current models we are aware of that investigate condition-dependent allocation to sexual traits focus on the trade-off between sexually and naturally selected traits (that extend lifespan) (Kokko 2001; Hooper et al. 2018).

Finally, as in most studies, we have assumed that the plastic response to a high food diet by a male *G. holbrooki* equates to "private" information that he is in better condition than the average male. Another plausible view is that males treat greater food availability as "public" information that all males will have more resources to invest in reproduction. It is unclear, at least to us, how these 2 "interpretations" affect optimal allocation strategies. We suggest that further experiments could profitably explore: 1) the relationships between multiple sexual traits under a wider range of levels of resource acquisition, and 2) the information context in which resource allocation decisions are made. This should lead to a better understanding of resource allocation decisions into different sexual traits and better explain phenotypic correlations within and across populations or species.

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