



# Sex-specific responses to competitive environment in the mosquitofish *Gambusia holbrooki*

Samuel Brookes<sup>1</sup> · Maider Iglesias-Carrasco<sup>1</sup> · Loeske E. B. Kruuk<sup>1</sup> · Megan L. Head<sup>1</sup> 

Received: 13 March 2020 / Accepted: 17 September 2020 / Published online: 4 October 2020  
© Springer Nature Switzerland AG 2020

## Abstract

The competitive environment that animals experience during development constitutes an important source of selection that can influence the development, expression and evolution of traits. Here, we examine how the sex of focal and “competitor” individuals interact to affect development in the Eastern mosquitofish *Gambusia holbrooki*. We raised individuals of both sexes either alone or in the presence of a male or female conspecific (hereafter “competitor”), and measured their juvenile growth rate, time to maturity and size at maturity. For males, we also measured their gonopodium length, sperm quantity, and sperm velocity. We found that responses to the competitive environment were dependent on the sex of the focal individual, the sex of their “competitor” and sometimes an interaction between the two. When there was another fish present, regardless of its sex, males had slower growth rates and took longer to mature, but eventually matured at the same size. Females also showed slower growth rates in the presence of a competitor, but in contrast to males, reached maturity sooner and at a smaller size than when there was no competitor present. Presence of a competitor influenced male sexual traits, however there was little evidence that these effects were mediated by the sex of the other fish. Males reared with another fish had longer gonopodia for their body size, as well as fewer and faster sperm. Our results suggest that effects of the competitive environment are different for males and females, potentially due to sex differences in adult life history strategies. Further, for males, both life history traits and sexual traits were influenced by the competitive environment. For life history traits this effect appears to result from decreased resources and/or increased energy expenditure, but for sexual traits, effects appear to be mediated, in part, by the social environment.

**Keywords** *Gambusia holbrooki* · Intraspecific competition · Life history traits · Phenotypic plasticity · Poeciliid · Sex-specific · Sexual traits · Social environment

---

**Electronic supplementary material** The online version of this article (<https://doi.org/10.1007/s10682-020-10080-y>) contains supplementary material, which is available to authorized users.

---

✉ Megan L. Head  
megan.head@anu.edu.au

<sup>1</sup> Division of Ecology and Evolution, Research School of Biology, Australian National University, Canberra, ACT 2600, Australia

## Introduction

Interactions between conspecifics play an important role in determining the ecology of plants and animals. For example, the need to avoid competition over resources can lead to changes in the distribution of individuals as well as changes in the way individuals use resources (Bonin et al. 2015). Further, exposure to conspecifics can alter the developmental trajectories of juveniles and consequently have lasting effects on trait expression in adults (Fischer et al. 2003; Mayntz et al. 2003; Relyea 2004; Byrne et al. 2009) which may have important consequences for fitness. Such effects may be driven by a variety of processes including reduced access to resources, interference from other individuals or adaptive responses to variation in the social environment.

An important attribute of individuals that influences how they interact with other individuals is their biological sex. Males and females differ in a whole suite of traits that could dramatically influence how they respond to the presence of other individuals and likewise how they impact those around them. For instance, differences between the sexes in morphology, physiology and behaviour often lead to asymmetries in the competitive ability of males and females (e.g. plants, Varga and Kytöviita 2012; insects, Bedhomme et al. 2003; fish, Iguchi 1996; birds, Nicolaus et al. 2009). Such asymmetries could result in one sex securing a greater amount of a limited resource at the expense of the other. This may be particularly pronounced in species with extreme sexual dimorphism where large differences in adult size result from different growth trajectories during ontogeny (Badyaev 2002).

Just as the sex of a focal individual can influence its phenotypic response to interactions with conspecifics, so too can the sex of the individual with which a focal individual interacts, and as a result, the interaction between the two. While past research investigating competition over resources has often considered the effects of a focal individual's sex on competition (Bedhomme et al. 2003; Nicolaus et al. 2009; Oddie 2000), it is striking that few of these studies consider the sex of the competitor (but see e.g., Bonisoli-Alquati et al. 2011). This may be because it is expected that the effects of the competitor sex will simply mirror those of focal individuals. Past research focussing on how the social environment influences development and adult trait expression, on the other hand, usually deals more explicitly with the sex of interacting individuals. In this case, however, studies tend to focus on males as the focal sex. For instance, many studies show that males adjust investment in reproductive traits in response to differences in population parameters that provide information on the likely strength of reproductive competition and/or number of mating opportunities, like sex ratio and density (reviewed in Bretman et al. 2011). Presumably this focus on males arises because these studies are primarily interested in how the social environment influences investment in traits that function in mate competition. This, plus the tendency to manipulate population scale parameters, makes it difficult to look specifically at how interactions between the same or opposite sex individuals affect responses to rearing environments. Given the potential for effects of both competition over resources and strategic allocation of resources to different traits that depend on the social environment, the exact role that sex plays in determining responses to differences in competitive environments during development is likely to be complex and to depend on the biology and ecology of the species in question.

Here we test how the sex of interacting individuals influences the development of life-history traits of males and females, and sexual traits of males in the Eastern mosquitofish (*Gambusia holbrooki*). We do this by raising individuals of both sexes either alone or in

the presence of a conspecific of the same or opposite sex and then measuring the growth rate, size at, and time to maturity of male and female focal fish, as well as the gonopodia length, sperm quantity, and sperm velocity of male focal fish. Understanding whether and how the competitive environment affects these traits is important because they are likely to be closely associated with fitness and hence may influence evolutionary responses to variation in competitive environments. Although male and female mosquitofish are similar in size at birth, there is considerable variation in the time it takes males and females to mature (males: 18–56 days, females: 18–70 days, Pyke 2005), and by the time they reach sexual maturity females are on average larger than males. Once mature, males almost completely stop growing (Bisazza et al. 1996; Vega-Trejo et al. 2019), while females continue to grow throughout their adult life (Zulian et al. 1995), thus by late life females can be two times the size of males, and five times heavier (Bisazza and Marin 1995). Given that females are the larger sex we predicted that if responses to the presence of another fish are the result of competition over resources, then females would show greater plastic responses to the presence of another fish regardless of that other fish's sex. Also, being the larger sex we expected the greater resource requirements of females would mean that focal fish reared with females would fare worse than individuals reared with males. In addition, if the effects of the focal fish's sex and the sex of the fish with which they interact are compounding, this could lead to males faring worse than females when competing against females (i.e. a focal-sex  $\times$  other-sex interaction). Finally, we predicted that sexual traits (i.e. male gonopodium length, sperm number and sperm velocity) would be influenced by the sex of the other fish due to potential differences in future reproductive competition.

## Materials and methods

### Origin and maintenance of fish

Focal and non-focal (hereafter referred to as “competitor”) fish used in the experiment came from two sources. “Lab fish” (Lab) were obtained from controlled matings between first generation lab-reared fish. “Wild-caught fish” (WC) were obtained from pregnant wild caught females. Both sources of fish originally came from the same population [Sullivan's Creek, Canberra, Australia (35° 16' 53" S, 149° 6' 45" E)]. Prior to giving birth both lab-reared and wild mothers were isolated in 1L plastic tanks with a mesh divider and plastic plants to provide refuge for new born fry. Tanks were checked for fry twice daily and when found, were separated from their mother and transferred to their experimental treatment.

### Experimental design

We used a  $2 \times 3$  factorial design where we manipulated sex of the focal fish (male, female) and experience with a conspecific ('no competitor', 'male competitor' and 'female competitor'), giving us a total of 6 'treatments' [focal male with no competitor (M), focal female with no competitor (F), focal male with a male competitor (MM), focal female with a male competitor (FM), focal male with a female competitor (MF) and focal female with a female competitor (FF)]. So that we could identify and follow focal fish in their treatments, we used, as non-focals, 21 day old fish that had been tagged with a visible elastomer implant (VIE). VIE is commonly used for fish identification and has been shown to have no

significant effect on growth or mortality in a variety of fish species (Frommen et al. 2015; Hohn and Petrie-Hanson 2013).

Focal fish were placed in their respective treatment on their day of birth and remained in these treatments until their last phenotypic measure was taken (i.e. until sexual maturity for females, and until sperm was stripped for males). Because mosquitofish cannot be visually sexed at birth we set up a third of tanks with no competitor and two-thirds with a competitor, on the assumption that (given equal sex ratios) this would result in an even distribution of sample sizes across the 6 treatments outlined above. This resulted in a total of 682 individuals (from 47 broods), of which 211 were assigned to no-competitor treatments and the remaining 471 were given a competitor. The exact sample sizes for the 6 different treatments were then determined when the fish had matured.

Throughout the experiment, fish were kept in a temperature-controlled room at 28 °C with a 14:10 h photoperiod. Experimental fish were kept in 3L plastic tanks and were fed twice daily with an approximately equal quantity of *Artemia* sp. nauplii delivered to each tank using a squeeze bottle. Thus, both space and food may have been limited in our competitor treatments. To control for variation in measured traits that may result from variation in micro-climate, tanks were randomly assigned to a shelf where they remained for the duration of the experiment. Shelf was included as a random effect in analyses.

## Measurement of phenotypic traits

During the juvenile stage, and when fish reached sexual maturity, we measured several resource-dependent phenotypic traits for which expression might be expected to vary in relation to the sex of the non-focal fish. We included both life-history traits of males and females as well as sexual traits of males, because we expected that the sex specific effects of competitive environment may differ between these two trait types.

## Juvenile growth rate

To estimate the growth rate of focal fish, we measured their length prior to being placed into treatment (i.e. on the day they were born;  $L_i$ ) and again after 21 days ( $L_f$ ). Growth rate (mm/day) was calculated from these measurements using the equation:  $\frac{(L_f - L_i)}{21}$ . To measure length, fish were first anaesthetised by submerging them in icy water, then placed in a container with 5 mm of water and photographed against a background of 1 mm graph paper for scale. Standard length was measured from these photographs using ImageJ (Abràmoff et al. 2004). Based on  $L_i$  there were no size differences between male and female focal fish ( $P=0.868$ ) nor between focal fish from different competitive environments ( $P=0.376$ ).

## Age, body length and gonopodium length at maturity

Fish were checked every second day for signs of maturation. Males were considered mature when their gonopodium was inflexible and clear (Meffe 1992) and its tip had developed an obvious spine. Females were considered mature when yolked eggs became visible through the abdomen wall in front of the anal fin (Meffe 1992). On the day fish were deemed mature, they were anaesthetised in icy water, photographed (to measure length: see above), and weighed to the nearest 0.01 mg (analyses of body weight (not presented) gave qualitatively similar results to body length). For males we also measured gonopodium length.

Males were first anaesthetised in icy water and then placed on their side, on a black background, with their gonopodium swung downward so it was perpendicular to their body. Males were photographed in this position and gonopodium length was later measured as the distance from the base to the tip using Image J. We then obtained values of relative gonopodium length. These were the residual values from a regression, in which ( $\log_{10}$ ) gonopodium length was the dependent variable and ( $\log_{10}$ ) body length at maturity was the independent variable. Relative gonopodium length can be interpreted as how much longer or shorter a males' gonopodium is compared to that which is expected for his body length. Once all measurements were made, fish were placed back into their treatment tanks.

## Sperm traits

We analysed sperm number and velocity of focal males, using CEROS Sperm Tracker and the HT CASA II program (Hamilton Thorne), following the methods of Vega-Trejo et al. 2016. Full details of sperm collection and processing protocols are provided in the online supplement. Briefly, males were anaesthetised in ice water and placed on a glass slide with their gonopodium swung forward. The male's abdomen was pressed gently to elicit the release of sperm. Two samples of 3 sperm bundles each were collected for sperm velocity measures and the remaining sperm bundles were collected and used for sperm counts.

Males were maintained in their respective treatments after maturity until the point of sperm sampling. All sperm samples were collected from males 4 to 7 weeks after they matured to reduce age related variation in our measures (Vega-Trejo et al. 2016). Within this window the exact day a males' sperm traits were measured was determined haphazardly allowing for logistic constraints (e.g. the maximum number of fish that could be measured in 1 day). However, we also recorded the number of days since maturation that a male's sample was taken, so that we could later control for any remaining age related variation in sperm traits in our analyses.

All experimental work was conducted with the approval of the Australian National University Animal Ethics Committee (Approval No. A2015/07). In accordance with animal ethics guidelines all tanks contained plants to allow fish to hide, all fish were provided enough food to meet energetic and growth demands and focal fish were added to experimental tanks after non-focal fish were fed to avoid potential cannibalism.

## Data analysis

Out of the total 682 replicates, 220 replicates were excluded from data analyses for various reasons (i.e. because the focal or competitor died before phenotypic measurements were made and/or because the focal or competitor exhibited spinal curvature or an 'atrophied' phenotype, which reduces the competitive ability of fish). Of the focal fish that died before measurements could be made 143 out of 471 were from the competitor treatments (30%) and 11 out of 211 were from the no competitor treatments (5%). Indicating that being reared with another fish, rather than being housed alone, is stressful. The final sample size used in the analysis of fish traits was therefore  $N=462$  fish ( $M=94$ ,  $F=100$ ,  $MM=64$ ,  $MF=55$ ,  $FM=78$ ,  $FF=71$ ), and for male sperm traits was  $N=193$  for sperm quantity ( $M=91$ ,  $MM=50$ ,  $MF=52$ ), and  $N=170$  for sperm velocity ( $M=79$ ,  $MM=46$ ,  $MF=45$ ).

To determine the effects of competitive environment (no competitor, male competitor, female competitor) on male and female traits, we modelled our data in ASReml-R (Butler et al. 2017). ASReml-R is an R package that allows the fitting of linear mixed models

using residual maximum likelihood. We fitted separate models for each of the six traits that we measured: growth rate, age at maturity, length at maturity, male relative gonopodium length, sperm number and sperm velocity. In models examining the effects on traits shared by both males and females (growth, age at maturity, length at maturity), we included the sex of the focal fish (male or female), whether another fish was present or not and, when another fish was present, the sex of this fish (male or female) as fixed effects in our model. We also included interactions between focal sex and presence/absence of a competitor, as well as focal sex and competitor sex when the latter was present. In addition to these primary factors of interest, we included as fixed effects the source of the focal fish (as a two-level factor), and (when a competitor was present) the source of the competitor fish and the competitor's length when initially placed with the focal fish (scaled to a mean of 0, so that main effects could be interpreted for average sized competitors). We included the source of focal and competitor fish as a fixed effects rather than a random effects because each had only two levels (Bolker et al. 2009). Random effects included in the models were the brood from which the focal fish came, the shelf within our controlled temperature room that the replicate was placed on (a 6-level factor, to control for potential effects of micro-climate) and (when a competitor was present) the brood from which the competitor fish came. Note that we were able to fit characteristics of the competitor (i.e. competitor sex, source, initial length, and brood) in the model because ASReml allows fitting of variables conditional on a given level of a factor using the code `at(treatment, "comp")`, i.e. fitting competitor variables only for focals with a competitor.

For the analysis of "male only" traits (relative gonopodium length, sperm number and sperm velocity), models were set up in the same way, except that we did not include sex of the focal fish nor interactions with it as fixed effects. When analysing sperm number and velocity we additionally included days since maturation (scaled to a mean of 0) as a fixed effect to control for potential effects of male age (Vega-Trejo et al. 2016). Days since maturation was nested within treatment, so that different parameters were fitted according to the presence of a competitor or not.

All model residuals were checked to see if they met assumptions of normality and when they did not (in the case of sperm number) variables were log transformed. For each analysis we ran two models—a "full" model containing the interactions between focal sex and presence/absence of another fish as well as between focal sex and competitor sex, and a "main effects" model containing only main effects, so we could interpret these in the absence of any potential interactions (Engqvist 2005).

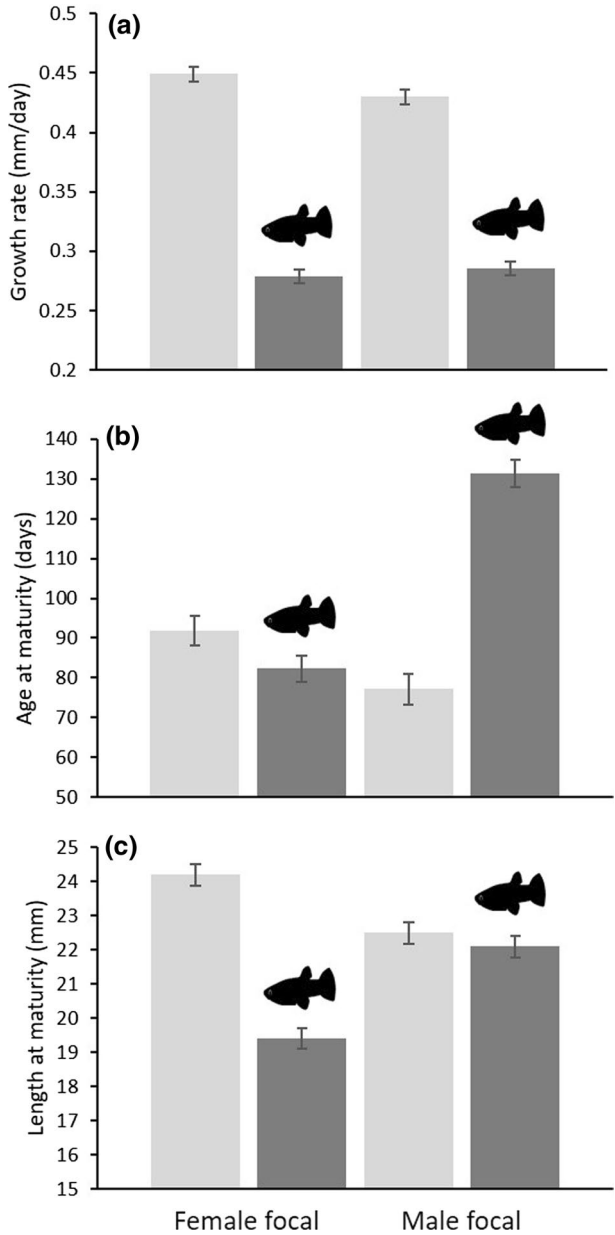
## Results

A summary of results are provided in Table 1. Full model outputs including parameter estimates and test statistics are provided in the online supplement (Table S1–S6). Correlation matrices for male and female life-history traits are also presented in the online supplement (Table S7).

### Juvenile growth rate

Male and female focal fish both had a reduced growth rate on average when reared with another fish (Fig. 1a;  $P < 0.001$ , Table S1), however, females were more strongly

**Fig. 1** Effects of conspecific presence on male and female traits. **a** Growth rate, **b** Age at maturity, **c** Length at maturity. No competitor present in light grey. Competitor present in dark grey with fish animation above. Least square mean  $\pm$  S.E. obtained from linear mixed models testing the effects of focal sex and presence of another fish are plotted. These models controlled for the same factors as outlined for AsReml conditional models used to provide significance tests



affected by the presence of a competitor than were males (Fig. 1a; focal sex\*presence/absence,  $P=0.003$ , Table S1). When reared alone, females had higher growth rates than males, but in the presence of another fish males and females had similar growth rates (Fig. 1a). The sex of the competitor did not have a significant effect on the growth rate of either male or female focal fish (Fig. 2a,  $P=0.112$ , Table S1).

**Table 1** Summary of the effects of competitive environment on the traits of focal males and females as analysed in *ASReml-R* models

	Juvenile growth	Age at maturity	Length at maturity	Relative gonopodium length	Sperm number	Sperm velocity
Effect of focal sex	ns	♂ > ♀	♂ > ♀			
Effect of fish presence	Pres ↓	Pres ↑	Pres ↓	Pres ↑	Pres ↓	Pres ↑
Interaction between focal sex and fish presence	Pres: ns Abs: ♀ > ♂	Pres: ♀ < ♂ Abs: ♀ > ♂	Pres: ♀ < ♂ Abs: ♀ > ♂			
Effect of competitor sex	ns	♀-c > ♂-c	ns	ns	ns	ns
Interaction between focal sex and competitor sex	ns	ns	♀: ♀-c > ♂-c ♂: ♀-c < ♂-c			

Dark grey indicates  $P > 0.05$ , light grey indicates  $P < 0.05$ , with the direction of the effect given in the text; ‘ns’ is non-significant. ↑ indicates increase in trait; ↓ indicates decrease. ‘Pres’ refers to treatments where another fish was present; ‘Abs’ refers to treatments where focal fish were reared alone; ♀, ♂ denote the sex of the focal fish; ♀-c indicates the response in the focal with a female competitor and ♂-c the response in the focal with a male competitor (so, for example, ♀: ♀-c > ♂-c indicates that female focal fish housed with a female competitor had higher trait value than those housed with a male competitor)

### Age and size at maturity

Males reared in the presence of another fish took longer to mature than those that were reared alone, while females matured at the same age regardless of whether another fish was present or not (Fig. 1b; focal sex\*presence/absence,  $P < 0.001$ , Table S2). This meant that when there was no other fish present males matured earlier than females, but in the presence of a competitor males matured later than females (Fig. 1b). Further, males took longer to mature than females regardless of the sex of the competitor (Fig. 2b;  $P < 0.001$ , Table S2). Focal fish of both sexes took longer to mature in the presence of a female competitor (Fig. 2b;  $P < 0.001$ , Table S2), but there was no sex-specific response of age at maturity to competitor sex [i.e. no interaction between focal sex and competitor sex ( $P = 0.810$ , Table S2)].

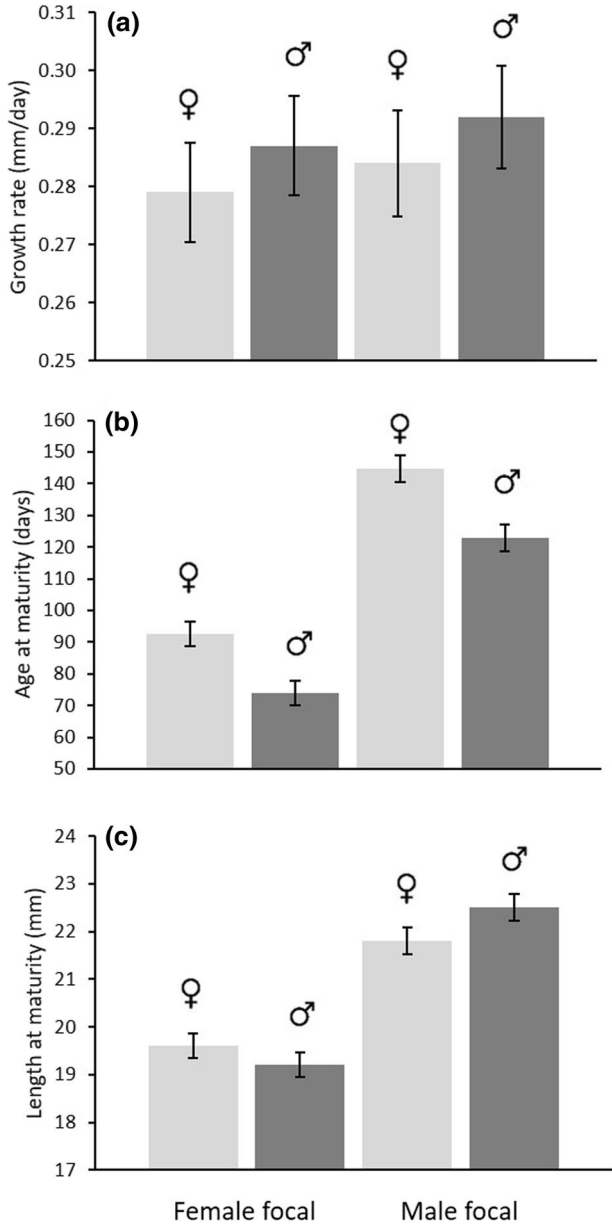
Females reared with another fish matured at a smaller size than those who were not, while there was no evidence that being reared with another fish affected male length at maturation (Fig. 1c; focal sex \* presence/absence:  $P < 0.001$ , Table S3). This meant that when females were reared alone they were larger at maturation than males, but when reared with another fish males matured larger than females (Fig. 1c). When considering the sex of the competitor, we found that while males matured at a larger size than females regardless of competitor sex (Fig. 2c;  $P < 0.001$ , Table S3B), both sexes matured larger when reared with a competitor of their own sex (focal sex \* competitor sex:  $P = 0.003$ , Fig. 2c).

### Male relative gonopodium length and sperm traits

Males grew a significantly longer gonopodium than expected for their size when they developed in the presence of another fish (Fig. 3a,  $P < 0.001$ , Table S4). However, relative gonopodium length was not affected by the sex of the competitor (Fig. 4a;



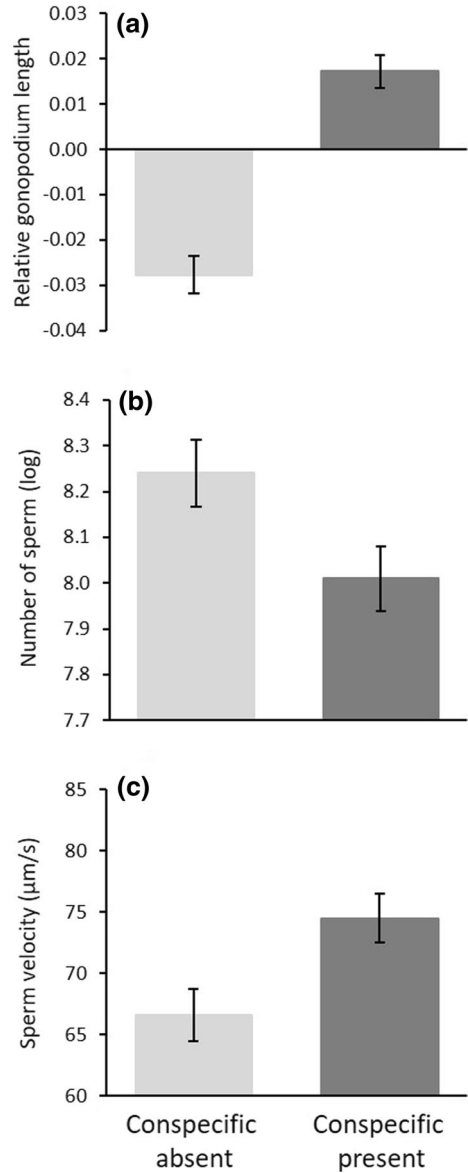
**Fig. 2** Effects of competitor sex on focal male and female traits. **a** Growth rate, **b** Age at maturity, **c** Length at maturity. Female competitor in light grey, male competitor in dark grey. Least square mean  $\pm$  S.E. obtained from linear mixed models testing the effects of focal sex and competitor sex are plotted. These models controlled for the same factors as outlined for AsReml conditional models used to provide significance tests



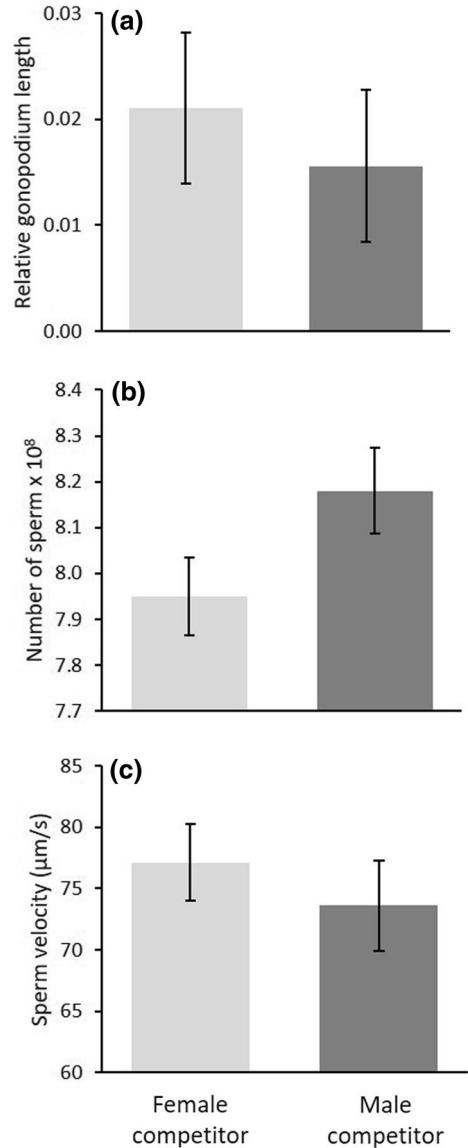
$P=0.190$ , Table S4). Males had shorter gonopodia than expected for their size when their competitor was initially larger ( $P=0.001$ , Table S4).

When reared in the presence of another fish, males produced fewer (Fig. 3b;  $P<0.001$ , Table S5) and faster-swimming (Fig. 3c;  $P=0.005$ , Table S6) sperm than when reared alone. However, neither sperm number nor sperm velocity were dependent on the sex of the competitor (sperm number—Fig. 4b,  $P=0.089$ , Table S5; sperm velocity—(Fig. 4c;  $P=0.629$ , Table S6). When considering the sex of the competitor,

**Fig. 3** Effect of conspecific presence on focal male sexual traits. **a** Relative gonopodium length, **b** number of sperm, **c** sperm velocity. Males reared alone in light grey. Males reared with another fish in dark grey. Least square mean  $\pm$  S.E. obtained from linear mixed models testing the effects of focal sex and presence of another fish are plotted. These models controlled for the same factors as outlined for AsReml conditional models used to provide significance tests



**Fig. 4** Effect of competitor sex on focal male sexual traits. **a** Relative gonopodium length, **b** number of sperm, **c** sperm velocity. Light grey bar represents focal male fish housed with a female competitor, dark grey bar represents focal male fish housed with a male competitor. Least square mean  $\pm$  S.E. obtained from linear mixed models testing the effects of focal sex and competitor sex are plotted. These models controlled for the same factors as outlined for AsReml conditional models used to provide significance tests



on average males produced the same number of sperm whether they had been reared with a male or a female.

## Discussion

We examined the sex-specific response of a range of resource-dependent phenotypic traits in Eastern mosquitofish that were reared alone, or with either a male or female conspecific. We found that responses to the competitive environment were often dependent on the sex

of the focal fish, their competitor, and sometimes an interaction between them. Our results are largely consistent with the idea that both male and female responses are driven by constraints of competition (i.e. competition over food resources) rather than strategic responses to competition (i.e. competition for mates). However, the latter may also play a role. Both males and females had slower growth rates when developing in the presence of a competitor, and although the reduction in growth rate when reared with another fish was stronger for focal females than for focal males, it was not dependent on the sex of the competitor. The effects of the competitive environment on age and size at maturity, on the other hand, were complex, and depended on the sex of both focal individuals and their competitors. In response to the presence of another fish, males took longer to mature but matured at the same size as when reared alone. This pattern was strongest when males were housed with a female competitor. In contrast, females responded to the presence of another fish by reducing their size at maturity regardless of competitor sex, and by maturing earlier if the competitor was a male. These sex-specific responses may reflect differences between males and females in how best to optimise the trade-off between age and size at maturity when resources are limited. Finally, when reared in the presence of another fish, males had longer gonopodia than expected for their size, and produced fewer, faster sperm.

The growth rate of both male and female juveniles was slower in the presence of a competitor. This slower growth rate of juveniles of both sexes in the presence of another fish is likely associated with a reduction in food availability caused by competition. We found that the larger sex, in this case the female, was more detrimentally affected by the presence of a competitor. This is consistent with results from previous studies which suggest that individuals of the larger-bodied sex will be disadvantaged when resources are limiting, due to the costly production of large bodies (Bonneaud et al. 2016; Wikelski and Thom 2000). Alternatively (or additionally), the observed reduction in focal growth rates in the presence of another fish could be a consequence of harassment. Competitors in our experiment were older and larger than focal fish and thus were likely dominant. Harassment by large, dominant conspecifics can induce stress responses in fish, consequently reducing food intake, or leading fish to allocate resources away from growth toward other energy-demanding processes that are necessary for survival (Sadoul and Vijayan 2016). For example, in crowded populations of fish with high intraspecific competition, continuous aggressive interactions increase energy expenditure and metabolic costs, which reduces growth (Bonin et al. 2015). This is consistent with anecdotal observations during our experiment, in which fish were often seen hiding in the plastic plants provided as a refuge. Regardless of whether reduced growth rate of focal fish in the presence of another fish is the result of reduced food availability or increased energy expenditure, it is interesting to note that this effect was not dependent on the sex of the competitor.

Our results for age and size at maturation suggest that changes to developmental strategies in response to the presence of a conspecific are sex-specific. Males matured after achieving a particular length, regardless of whether or not they competed for food resources throughout their development. As male growth rate was reduced by the presence of another fish, however, it took significantly longer for them to achieve their target size, and as a consequence the age at which males matured in the presence of another fish was greater than for males reared alone. Conversely, females appeared to mature after a particular period of time, regardless of whether or not they were reared with a competitor fish throughout their development. Combined with our result that female growth rate was greatly reduced by the presence of another fish, this meant that females reared in the presence of a competitor matured at a significantly smaller length compared to those reared alone. One possible explanation for the observed sex-specific responses is differences in

life history between adult males and females and how these interact with size. In male mosquitofish, size is largely determinate and growth almost ceases at maturation (Bisazza and Marin 1995; Zulian et al. 1995). Despite this, however, large males are often at a selective advantage compared to smaller males. This is because they are dominant in aggressive interactions, out-compete them for access to females, and can be preferred by females as mates (Bisazza et al. 2001; Mcpeck 1992). Therefore, when resources are limited, delaying maturation to achieve a larger body size may be beneficial for males because it means that they will be more competitive upon reaching maturity. Females, on the other hand, continue to grow after maturation, and whether they mate or not is unlikely to be limited by their size. This means that despite the fact that fecundity increases with body size (Mautz and Jennions 2011), females that mature sooner (albeit smaller) could still, have greater lifetime reproductive success. An alternative explanation, however, is simply that sex-specific responses to the presence of other fish are due to the potential for greater plasticity in growth for the larger sex (Stillwell et al. 2010). Such plasticity could lead females to grow rapidly and achieve larger body sizes when conditions are suitable, but reduce growth rates and reach smaller body size in disadvantageous conditions.

Interestingly, our results for size and age at maturation also suggest that developmental plasticity was induced by a strategic response to the social environment, and not only constraints arising from competition for food resources. This idea comes from the fact that the interaction between the sex of focal fish and competitor fish was also important in determining the phenotype of focal fish, such that both males and females matured at a larger size when reared with another fish of the same sex than with a fish of the opposite sex. One possible explanation for this effect is that focal individuals increase their competitive efforts against conspecifics of the same sex in anticipation of future intrasexual competition for mating opportunities. Effects of juvenile social environment on maturation time and size are commonly found in poeciliid males (e.g. Borowsky 1973; Kolluru and Reznick 1996; Walling et al. 2007; Magellan and Magurran 2009) but have been less well documented in females. Despite this, investing in growth to out-compete individuals of their own sex is expected to be advantageous both for males and females. Large males and females could both experience a fitness advantage due to increased competitive ability in a mating context [i.e. since both males and females prefer to mate with large individuals (Bisazza et al. 2001; Mautz and Jennions 2011; Mcpeck 1992)]. Large individuals of both sexes could also experience an advantage when competing for resources if the sexes segregate due to sex driven niche partitioning, as has been found in other poeciliids (Croft et al. 2003; Magurran and Garcia 2002). Of course distinguishing between responses arising from constraints over competition for food resources and strategic responses to perceived future environments is difficult with our experimental design. Therefore, when interpreting this result we cannot disregard the alternative possibility that growth is retarded when developing with the opposite sex, rather than increased when developing with the same sex. In the case of females reared with males, reduced female growth could be related to continuous harassment by males [males start harassing females weeks before maturation (Bisazza et al. 1996)], which may reduce feeding rates (Pilastro et al. 2003). In the case of males competing with females, male growth might be affected by the sexual asymmetry in competitive ability that seems to confer a benefit to the larger females (Uller 2006).

Differences in the way the competitive environment affects male body size and male relative gonopodium length may indicate that these traits are influenced differently by resource limitation. While males reared with another fish extended the time to maturation to achieve similar body sizes, these males also matured with longer gonopodia for their body size. This result could arise if males favour investment in increasing

relative gonopodia length over growth when resources are limited. For instance, it might be expected that males would favour investment in gonopodia over growth in response to perceived high future mating competition, because males with relatively longer gonopodia have greater mating success (Head et al. 2017; Vega-Trejo et al. 2017). However, given that male gonopodium length was influenced by the presence of another fish and not the sex of that fish, this explanation seems unlikely. A more likely explanation might be that absolute gonopodium length is less resource dependent and more age dependent than male body length. If this is the case then increased maturation times which are needed for males to achieve a certain body size when in the presence of a competitor may also result in a longer gonopodia relative to body size simply because of increased time. Previous research investigating how diet restriction during development influences gonopodium size provides mixed support for this hypothesis. In the same population of *Gambusia*, Vega-Trejo et al. (2016) show that the allometric slope for gonopodium length is steeper for fish on an ad libitum diet than those on a restricted diet: small to average size males that have experienced restricted food had long gonopodia for their body size, while large fish that have experienced restricted diets had short gonopodia for their body size. In contrast, Livingston et al. (2014) show that the allometric slope for gonopodium length is shallower for fish that have experienced an ad libitum diet than for those that have experienced a restricted diet. Further, this result was generalizable across all males that had a restricted diet as juveniles so that in most of the size range of the males have short gonopodia for their body size. While it must be acknowledged that these previous studies differ considerably from ours (i.e. both studied effects of diet restriction rather than rearing with a conspecific, and both were examined compensatory growth and so did not carry out their manipulation all the way until maturity), their results combined with ours do suggest that resource limitation influences body size and gonopodia size may differ. As such we believe further investigation of how resource limitation influences the allometry of gonopodium length and how this impacts male mating success would be interesting.

Finally, we also found that males reared with another fish had fewer, faster sperm than males reared alone. While it is tempting to attribute this finding to a combination of variation in resource limitation and perceived sperm competition, it is important to note that males and females in our experiment were housed together until the male's sperm was stripped. Thus, the difference in sperm number could be driven partially by the fact that males were able to mate with females and thus had diminished sperm reserves. In line with this, there was a trend towards a difference between male versus female competitors in the reduction in sperm number ( $P=0.089$ ; Table S5). In fact inspecting Figs. 3b and 4b it is clear that there is a large degree of overlap in the standard errors for the number of sperm stripped from males reared alone and those reared with other males. This suggests that resource limitation is unlikely to drive this result. The way in which the presence of another fish affects sperm velocity is less easily explained by our methods. We found that males reared with another fish had faster-swimming sperm, which suggests that the social environment is more important than resource limitation in driving variation in this trait. This contrasts with a previous *Gambusia* study that manipulated food availability during development and found that males reared on restricted diets had slower swimming sperm and smaller sperm reserves (at least in recently matured males, like those measured here) than males reared on ad libitum diets (Vega-Trejo et al. 2016). Our results also contrast with studies in this system that manipulate adult environments. These previous studies found that male presence reduces sperm velocity but does not influence sperm number (Spagopoulou et al. 2020) and that restricted food decreases male sperm reserves as well as how quickly males can replenish these reserves (O'Dea et al. 2014). Clearly, more work is

needed to fully understand the contrasting effects of resource availability and social environment on male sperm traits.

## Conclusions

We have shown that there is a strong sex-specific response to presence of a conspecific during development and that this response is in part driven by the sex of the competitor. The sexes may differ in their resource requirements, and their ability to acquire those resources in the presence of another fish. In addition, the optimal development of resource-dependent traits might depend on the sex of the competitor if this alters the perception of future competition. While it is difficult to tease apart effects resulting from current constraints from strategic responses to perceived future competitive environments, our results suggest that rearing with the sex that has higher competitive ability (in this case the female) has strong effects in driving the direction of phenotypic trait expression. However for male sexual traits, these effects are likely mediated by strategic responses to perceived future reproductive competition.

**Acknowledgements** We thank the ANU animal services team for help with fish maintenance. Funding for this project was provided by the Australian Research Council (FT160100149 to MLH and FT110100453 to LEBK). We are grateful to Michael Jennions for discussions, and to Alastair Wilson, Kit Magellan and two anonymous referees for comments on the manuscript.

**Author contributions** SB, LEBK and MLH conceived the ideas and designed the methodology; SB collected the data; SB, LEBK and MLH analysed the data, SB and MI-C led the writing of the manuscript. All authors contributed critically to drafts and gave final approval for publication.

**Data availability** All data will be published on DRYAD and is available for reviewers at <https://datadryad.org/stash/share/INz54rPe9ZnLwzDCXDWYwb8s0cSWJfPsJse400gUOWU>.

## Compliance with ethical standards

**Conflict of interest** The authors have no conflict of interest to declare.

## References

- Abràmoff MD, Magalhães PJ, Ram SJ (2004) Image processing with imageJ. *Biophoton Int* 11(7):36–41
- Badyaev AV (2002) Growing apart: an ontogenetic perspective on the evolution of sexual size dimorphism. *Trends Ecol Evol* 17(8):369–378
- Bedhomme S, Agnew P, Sidobre C, Michalakakis Y (2003) Sex-specific reaction norms to intraspecific larval competition in the mosquito *Aedes aegypti*. *J Evol Biol* 16(4):721–730
- Bisazza A, Marin G (1995) Sexual selection and sexual size dimorphism in the eastern mosquitofish *Gambusia holbrooki* (Pisces Poeciliidae). *Ethol Ecol Evol* 7(2):169–183
- Bisazza A, Pilastro A, Palazzi R, Marin G (1996) Sexual behaviour of immature male eastern mosquitofish: A way to measure intensity of intra-sexual selection? *J Fish Biol* 48(4):726–737
- Bisazza A, Vaccari G, Pilastro A (2001) Female mate choice in a mating system dominated by male sexual coercion. *Behav Ecol* 12(1):59–64
- Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White J-SS (2009) Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol Evol* 24:127–135
- Bonin MC, Boström-Einarsson L, Munday PL, Jones GP (2015) The prevalence and importance of competition among coral reef fishes. *Annu Rev Ecol Evol Syst* 46(1):169–190

- Bonisoli-Alquati A, Boncoraglio G, Caprioli M, Saino N (2011) Birth order, individual sex and sex of competitors determine the outcome of conflict among siblings over parental care. *Proc R Soc B Biol Sci* 278(1709):1273–1279
- Bonneaud C, Marnocha E, Herrel A, Vanhooydonck B, Irschick DJ, Smith TB (2016) Developmental plasticity affects sexual size dimorphism in an anole lizard. *Funct Ecol* 30(2):235–243
- Borowsky RL (1973) Social control of adult size in males of *Xiphophorus variatus*. *Nature* 245:333–335
- Bretman A, Gage MJG, Chapman T (2011) Quick-change artists: male plastic behavioural responses to rivals. *Trends Ecol Evol* 26(9):467–473
- Butler DG, Cullis BR, Gilmour AR, Gogel BJ, Thompson R (2017) ASReml-R Reference manual, version 4. VSN International Ltd, Hemel Hempstead
- Byrne M, Ho M, Selvakumaraswamy P, Nguyen HD, Dworjanyn SA, Davis AR (2009) Temperature, but not pH, compromises sea urchin fertilization and early development under near-future climate change scenarios. *Proc R Soc B Biol Sci* 276:1883–1888
- Croft DP, Albanese B, Arrowsmith BJ, Botham M, Webster M, Krause J (2003) Sex-biased movement in the guppy (*Poecilia reticulata*). *Oecologia* 137(1):62–68
- Engqvist L (2005) The mistreatment of covariate interaction terms in linear model analyses of behavioural and evolutionary ecology studies. *Anim Behav* 70(4):967–971
- Fischer K, Bot ANM, Brakefield PM, Zwaan BJ (2003) Fitness consequences of temperature-mediated egg size plasticity in a butterfly. *Funct Ecol* 17:810
- Frommen JG, Hanak S, Schmidl CA, Thünken T (2015) Visible implant elastomer tagging influences social preferences of Zebrafish (*Danio Rerio*). *Behaviour* 152(12–13):1765–1777
- Head ML, Kahn AT, Henshaw JM, Keogh JS, Jennions MD (2017) Sexual selection on male body size, genital length and heterozygosity: consistency across habitats and social settings. *J Anim Ecol* 86:1458–1468
- Hohn C, Petrie-Hanson L (2013) Evaluation of visible implant elastomer tags in zebrafish (*Danio rerio*). *Biol Open* 2(12):1397–1401
- Iguchi K (1996) Sexual asymmetry in competitive ability in the immature ayu. *J Ethol* 14:53–58
- Kolluru GR, Reznick DN (1996) Genetic and social control of male maturation in *Phallichthys quadripunctatus* (pisces: poeciliidae). *J Evol Biol* 9:695–715
- Livingston JD, Kahn AT, Jennions MD (2014) Sex differences in compensatory and catch-up growth in the mosquitofish *Gambusia holbrooki*. *Evol Ecol* 28(4):687–706
- Magellan K, Magurran AE (2009) The effect of social environment during ontogeny on life history expression in the guppy *Poecilia reticulata*. *J Fish Biol* 74:2329–2337
- Magurran A, Garcia CM (2002) Sex differences in behaviour as an indirect consequence of mating system. *J Fish Biol* 57(4):839–857
- Mautz BS, Jennions MD (2011) The effect of competitor presence and relative competitive ability on male mate choice. *Behav Ecol* 22(4):769–775
- Mayntz D, Toft S, Vollrath F (2003) Effects of prey quality and availability on the life history of a trap-building predator. *Oikos* 101:631–638
- Mcpeek MA (1992) Mechanisms of sexual selection operating on body size in the mosquitofish (*Gambusia holbrooki*). *Behav Ecol* 3(1):1–12
- Meffe G (1992) Plasticity of life-history characters in Eastern Mosquitofish (*Gambusia holbrooki*: Poeciliidae) in response to thermal stress. *Copeia* 1992(1):94–102
- Nicolaus M, Michler SPM, Ubels R, van der Velde M, Komdeur J, Both C, Tinbergen JM (2009) Sex-specific effects of altered competition on nestling growth and survival: an experimental manipulation of brood size and sex ratio. *J Anim Ecol* 78(2):414–426
- O’Dea RE, Jennions MD, Head ML (2014) Male body size and condition affects sperm number and production rates in mosquitofish, *Gambusia holbrooki*. *J Evol Biol* 27(12):2739–2744
- Oddie KR (2000) Size matters: competition between male and female great tit offspring. *J Anim Ecol* 69:903–912
- Pilastro A, Benetton S, Bisazza A (2003) Female aggregation and male competition reduce costs of sexual harassment in the mosquitofish *Gambusia holbrooki*. *Anim Behav* 65(6):1161–1167
- Pyke GH (2005) A review of the biology of *Gambusia affinis* and *G. holbrooki*. *Rev Fish Biol Fish* 15(4):339–365
- Relyea RA (2004) Fine-tuned phenotypes: tadpole plasticity under 16 combinations of predators and competitors. *Ecology* 85:172–179
- Sadoul B, Vijayan MM (2016) Stress and Growth. In: Schreck CB, Tort L, Farrell AP, Brauner CJ (eds) *Fish Physiology*, Vol 35, Chapt 5. Academic Press, pp 167–205
- Spagopoulou F, Vega-Trejo R, Head ML, Jennions MD (2020) Shifts in reproductive investment in response to competitors lower male reproductive success. *Am Nat* 196(3):355–368



- Stillwell RC, Blanckenhorn WU, Teder T, Davidowitz G, Fox CW (2010) Sex differences in phenotypic plasticity affect variation in sexual size dimorphism in insects: from physiology to evolution. *Annu Rev Entomol* 55(1):227–245
- Uller T (2006) Sex-specific sibling interactions and offspring fitness in vertebrates: patterns and implications for maternal sex ratios. *Biol Rev Camb Philos Soc* 81(2):207–217
- Varga S, Kytöviita MM (2012) Differential competitive ability between sexes in the dioecious *Antennaria dioica* (Asteraceae). *Ann Bot* 110:1461–1470
- Vega-Trejo R, Jennions MD, Head ML (2016) Are sexually selected traits affected by a poor environment early in life? *BMC Evol Biol* 16(1):1–12
- Vega-Trejo R, Head ML, Keogh JS, Jennions MD (2017) Experimental evidence for sexual selection against inbred males. *J Anim Ecol* 86(2):394–404
- Vega-Trejo R, Fox RJ, Iglesias-Carrasco M, Head ML, Jennions MD (2019) The effects of male age, sperm age and mating history on ejaculate senescence. *Funct Ecol* 33:1–13
- Walling CA, Royle NJ, Metcalfe NB, Linström J (2007) Green swordtails alter their age at maturation in response to the population level of male ornamentation. *Biol Lett* 3:144–146
- Wikelski M, Thom C (2000) Marine iguanas shrink to survive El Niño. *Nature* 403(6765):37–38
- Zulian E, Bisazza A, Marin G (1995) Variations in male body size in natural populations of *Gambusia holbrooki*. *Ethol Ecol Evol* 7(1):1–10

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.