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Size-dependent male mating tactics and their morphological correlates in *Poecilia gillii*

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Male alternative reproductive strategies are found in some species of most major animal taxa but are especially widespread in fishes. Mature males of the shortfin molly, *Poecilia gillii*, display extensive variation in size and morphology. We devised a field test of a priori hypotheses regarding the interrelationships between male size, coloration, morphology and mating tactics. Males did not occur in discrete size classes, but instead occurred in a size and morphological continuum. Large males exhibited darker and more orange-coloured dorsal and caudal fins, whereas small males exhibited lighter and more inconspicuous fin coloration. Furthermore, larger males had proportionately deeper bodies, larger dorsal and caudal fins and shorter gonopodia than smaller males. Our field study of male mating behaviour revealed a lack of courtship in this species, and similar levels of mating attempts (gonopodial thrusts) irrespective of male size. Instead, small males were significantly more likely to chase females than were large males. In contrast, large males exhibited higher rates of gonoporal nibbling (a likely means by which males determine, through chemical factors, whether a female is carrying fertilizable ova) and higher likelihood of chasing other males away. In total, we found evidence for the predicted associations between male size, coloration, morphology and mating behaviour. These associations appear likely to maximize mating success for males of a given body size and phenotype.

ADDITIONAL KEYWORDS: alternative reproductive strategy – female choice – male–male competition – mate guarding – *Mollienesia* – Poeciliidae – reproduction – secondary sexual traits – sexual selection.

INTRODUCTION

Sexual selection refers to differential reproductive success as a result of male–male competition and female mate choice (Darwin, 1871; Andersson, 1994). These dual processes can act as potent selective forces, leading to the evolution of exaggerated male traits involved in inter-male aggression (e.g. horns, antlers and large size) or that are favoured by females (e.g. sexually dichromatic coloration, ornamental display traits and elaborate courtship behaviours). In some species, sexual selection has given rise to males that exhibit divergent phenotypes and mating tactics, referred to as alternative reproductive strategies (Gross, 1996).

Alternative reproductive strategies are found in some species of most major animal taxa (Gross, 1996; Shuster, 2010) but are especially widespread in fishes (Neff *et al.*, 2003). For example, male bluegill sunfish exhibit one of three discrete life-history and mating strategies, termed parentals, sneakers and satellites. In the Canadian lake in which they were studied, parental males reach maturity after 7 years and attain large adult body size; they build and aggressively guard nests, attract females and provide parental care to the eggs spawned in their nest. In contrast, 'sneakers' and 'satellites' use alternative mating tactics to steal fertilizations from parentals. Specifically, sneakers mature in 2 years, at small size; they achieve fertilizations by darting into the nest while attempting to avoid the defensive parental male. Finally, satellites mature in 4–5 years and closely mimic females in both behaviour and appearance in order to obtain access to spawning females in the nests of parentals (Gross, 1982, 1991; Neff *et al.*, 2003). A similar phenomenon is found in salmon. For example, in Pacific coho salmon, large, late-maturing, 'hook-nose' males that are specialized for fighting also compete with small, early-maturing males, known as 'jacks', that are sneak-mating specialists

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(Gross, 1985). Finally, in some live-bearing poeciliid fish species, such as *Xiphophorus nigrensis*, males exhibit contrasting phenotypes and mating strategies: large, brightly coloured and ornamented males primarily court females to engender cooperative mating, whereas small males exhibit subdued coloration and engage entirely in sneak or coercive copulation attempts, and intermediate-sized males exhibit court or sneak tactics depending upon their size (Ryan & Causey, 1989; Ryan *et al.*, 1992).

Each of the above examples illustrates general principles derived from alternative mating strategy theory (Taborsky *et al.*, 2008). Firstly, there is an integration between male phenotype and the alternative mating tactics that are used. Specifically, small males use one set of mating tactics (i.e. parasitic) and have a phenotype (i.e. inconspicuous) that appears best suited to this overall strategy, and large males use different tactics (i.e. bourgeois) with corresponding phenotype (i.e. flashy) predicted to maximize success using this particular strategy (Taborsky *et al.*, 2008; Abbott *et al.*, 2019; Liotta *et al.*, 2019). Secondly, at least some measured phenotypes (body size, age at maturity and the presence or absence of a hook-nose or sword-like extension of the caudal fin) appear to be bimodally distributed or discrete characters. This pattern is predicted to be attributable to disruptive selection and reduced fitness for males expressing intermediate phenotypes (Emlen, 1996; Taborsky *et al.*, 2008). However, it has also been suggested that shared genetic architecture could constrain phenotypic divergence between alternative male morphotypes, an idea referred to as intralocus tactical conflict (Abbott *et al.*, 2019). Here, we test whether *Poecilia gillii*, a poeciliid species with large variation in male size but no courtship, exhibits a bimodal body size distribution, alternative male mating tactics as a function of body size, and predictable associations between size, coloration, morphology and mating behaviour.

The live-bearing fish family Poeciliidae has become a model system for the study of sexual selection owing to the remarkable variation amongst species in the development of secondary sexual characteristics and their tractability in the field and laboratory (Farr, 1989; Bisazza, 1993; Rios-Cardenas & Morris, 2011). All species in the family exhibit internal fertilization and all except one are viviparous, giving live birth to fully developed offspring (Rosen & Bailey, 1963). Internal fertilization is achieved by males inserting their intromittent organ, a modified anal fin referred to as the gonopodium, into the female gonopore and releasing spermatophores (Rosen & Bailey, 1963; Bisazza, 1993; Greven, 2005). Males of nearly all species reportedly engage in sneak or coercive copulations, in which they thrust the gonopodium forward and attempt insemination of unreceptive females (Bisazza, 1993). A subset of species have evolved courtship; courting males exhibit stereotyped swimming sequences, in which they display in front of the female with unpaired fins fully spread, in order to elicit cooperation during mating (Farr, 1989; Bisazza, 1993; Rios-Cardenas & Morris, 2011). Males in most courting species can exhibit either courtship or sneak copulation attempts, the frequency of which can depend on the social context, environmental conditions and male size or phenotype (Farr *et al.*, 1986; Ryan & Causey, 1989; Travis & Woodward, 1989; Reynolds *et al.*, 1993; Erbelding-Denk *et al.*, 1994; Ptacek *et al.*, 2005; Hankison & Ptacek, 2007; Hurtado-Gonzales & Uy, 2009; Becker *et al.*, 2012; Kolluru *et al.*, 2014).

In the majority of poeciliid species, males exhibit similar (drab) coloration to females (i.e. monochromatism), lack exaggerated ornaments, lack courtship and engage entirely in sneak copulations, have relatively long gonopodia as a proportion of body length, and have a high degree of reversed sexual size dimorphism (males smaller than females) (Pollux *et al.*, 2014). Each of these conditions is the probable ancestral state of the family (Furness *et al.*, 2019). However, there have been multiple origins of bright and sexually dichromatic coloration, exaggerated ornamental display traits, and courtship (Pollux *et al.*, 2014; Furness *et al.*, 2019; Goldberg *et al.*, 2019). All three traits are found together in some species in the genera *Poecilia* and *Xiphophorus*, most notably the sailfin mollies and swordtails. However, there are a number of other species that exhibit a subset of these traits (e.g. dichromatism, but not ornamentation or courtship). In these and other poeciliid species, there have been well-documented examples of substantial within-species variation in male reproductive tactics, coloration and morphology that are correlated with body size (Farr *et al.*, 1986; Ryan & Causey, 1989; Travis & Woodward, 1989; Erbelding-Denk *et al.*, 1994; Ptacek *et al.*, 2005; Hankison & Ptacek, 2007; Hurtado-Gonzales & Uy, 2009; Becker *et al.*, 2012; Kolluru *et al.*, 2014). These studies have primarily been conducted on species that exhibit a high degree of sexual selection (i.e. courtship, dichromatism and ornamentation). Comparative studies that synthesize data on sexual selection, mating system and male phenotype in a phylogenetic context highlight the importance of detailed studies in a whole range of species in order to provide a more general and comparative perspective on the importance of factors that have shaped the evolution of poeciliid mating systems. In this study, we derive a series of a priori predictions regarding the relationship between male body size, coloration, mating strategy and morphology and test them in the field using *P. gillii*, a species of shortfin molly.

Poecilia gillii, a live-bearing fish in the family Poeciliidae, is broadly distributed throughout Central

America (Lee & Johnson, 2009) and is the most common freshwater fish in Costa Rica (Bussing, 2002). It feeds on detritus and algae, reproduces throughout the year when conditions are favourable, and both males and females reach a maximal size of 11 cm total length (Chapman & Kramer, 1991; Winemiller, 1993; Bussing, 2002). Apart from studies of seasonal population dynamics (Chapman *et al.*, 1991), phylogeography (Lee & Johnson, 2009) and female associative behaviour (Jordan *et al.*, 2006), little is known about the mating system of *P. gillii*. What is known, from laboratory observations, is that *P. gillii* lacks courtship (Pollux *et al.*, 2014; Goldberg *et al.*, 2019) and, outside the three sailfin molly species, the male dorsal fin of *P. gillii* is among the largest of the non-courting shortfin molly species (Goldberg *et al.*, 2019). Our previous field and laboratory observations of *P. gillii* indicate that mature males exhibit a great deal of variation in body size, coloration and morphology, as has been observed in other species in the genus *Poecilia* (Snelson, 1985; Farr *et al.*, 1986).

We predicted that small males, which appear to have coloration similar to females, will engage in frequent sneak copulation attempts and will have relatively long gonopodia and reduced dorsal and caudal fin sizes (for their body size). Small body size, inconspicuous coloration and a long gonopodium are traits predicted to facilitate sneak copulations (Bisazza, 1993; Bisazza & Pilastro, 1997; Pilastro *et al.*, 1997; Greven, 2005; Pollux *et al.*, 2014). In contrast, we predict that large males will exhibit dominant phenotypic characteristics (i.e. dark coloration, aggression toward subordinate males, and female mate guarding) and will have proportionately larger dorsal and caudal fins and shorter gonopodia. Mate guarding and/or aggression towards subordinate males, and signalling phenotypes that enhance these traits, are characteristic of a large male strategy in some other poeciliid species (Baird, 1968; Constanz, 1975; Bildsøe, 1988; Erbelding-Denk *et al.*, 1994; Benson & Basolo, 2006; Prenter *et al.*, 2008). Furthermore, although known not to engage in courtship, we predict that large males might exhibit a fundamentally different mating strategy from small males. We also test whether male body size or any other phenotypic trait potentially involved in mating, male–male competition or female choice (i.e. gonopodium length, dorsal fin size and caudal fin size) exhibits a bimodal distribution. A bimodal body size distribution could be indicative of disruptive selection and a genetic polymorphism for age and size at maturation, as found in some other poeciliid species (Kallman, 1989; Ryan & Causey, 1989; Lampert *et al.*, 2010; Liotta *et al.*, 2019).

To test these predictions, we gathered data on male phenotype and mating tactics, in the field. Sexual selection studies in other species have sometimes included multiple populations but tend to measure a more limited set of phenotypes (i.e. only morphology or only behaviour). In this study, we adopt an integrated approach, by quantifying morphological traits (i.e. body size, fin coloration, body and fin dimensions, and length of the copulatory organ) and mating behaviour (i.e. mating and aggressive interactions) in the field in a single population.

MATERIAL AND METHODS

STUDY SITE

In late February 2019, during the dry season, we performed a field study of *P. gillii* mating strategy and phenotype. Our study site was a ~100-m-long stretch of the Pacuar river, where it crosses the 243 Hwy, in the Rio General drainage, Costa Rica (Fig. 1; GPS coordinates: 9.353150, −83.728517). *Poecilia gillii* was abundant and found at high density. Other fish species observed at this site included *Archocentrus nigrofasciatus*, *Astatheros altifrons*, *Astyanax aeneus*, *Brycon behreae*, *Bryconamericus terrabensis*, *Oreochromis* sp., *Parachromis dovii*, *Pimelodella chagresi*, *Poeciliopsis retropinna*, *Roeboides ilseae*, *Sicydium salvini* and *Tomocichla sieboldii*. At our study site, the river had a mean diameter of 13 m and was characterized by moderate to strong flow in the centre channel, bracketed by quiet waters. The river depth ranged from a few centimetres at one of the gently sloping banks to a single pool > 2 m deep (average depth 37.5 cm). The bottom substrate consisted of boulders, pebbles and sand. Much of the bottom substrate was covered in algae. *Poecilia gillii* mostly congregated in the quiet waters and lower-flow regions at the margins of the river. The canopy above the river was almost entirely open. One bank of the river had some tall grass hanging into the water; otherwise, with the exception of algae, the river was devoid of vegetation.

FIELD DATA COLLECTION AND ANALYSES OF MALE COLORATION AND MORPHOLOGY

Poecilia gillii were collected using a 4 ft × 20 ft seine net with 1/16 inch mesh (i.e. 1.2 m × 6 m seine net with 6.35 mm mesh). Females and immature individuals were immediately released, and males were placed into a bucket filled with water. In the field, at our mobile work station, males were individually anaesthetized with MS-222 (Sigma-Aldrich Co., St Louis, MO, USA) in preparation for having their photograph taken. The MS-222 solution was buffered with sodium bicarbonate, and for sedation the final concentration was estimated as 15–50 mg/L. Anaesthetized males were placed onto a white cutting board with a measurement scale and

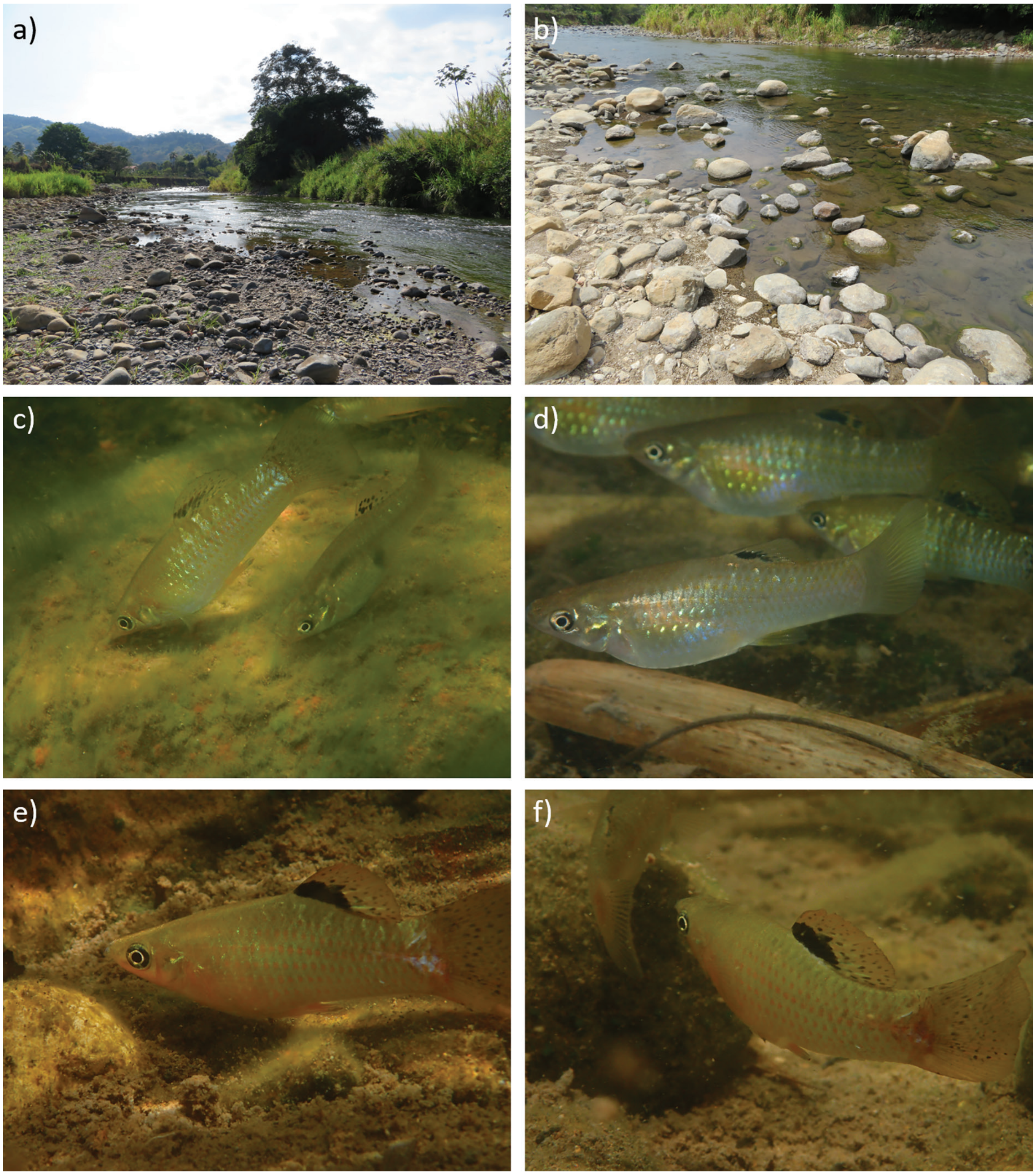

Figure 1. A, our field site was a ~100-m-long stretch of the Pacuar river, in the Rio General drainage, Costa Rica. B, *Poecilia gillii* congregated at high density in the slack waters near the river edge. C, a male (left) and small female (right) *P. gillii* pick at the algae-covered rocks. D, female *P. gillii* in a school. E, F, a large and colourful male *P. gillii* rests near the bottom (E) and makes a move towards a nearby female (F). Note that the large male, pictured in E and F, has sustained localized tissue damage around the caudal peduncle region. We surmise that this was probably attributable to an aggressive encounter with another large male.

had their gonopodium straightened and dorsal and caudal fins spread with the bristles of a fine-tipped, soft paint brush that had been wetted. The left side of each male was photographed, under natural lighting, with a Canon PowerShot SX720 HS camera attached to a tripod. Seventy-eight males were photographed. The first 20 males (ten small and ten large) were euthanized with an overdose of MS-222 and preserved in 95% ethanol for additional analyses. The remaining 58 males were released unharmed at the point of collection after having fully recovered from the light anaesthesia.

Data including male maturity, coloration and body and fin dimensions were obtained from the digital photographs. Sixty males were mature, as judged by the gonopodial hood extending beyond the distal tip of the gonopodium (Evans *et al.*, 2002), and 18 were immature. Only mature males (N = 60) were used in all further analyses.

Most studies of sexual dichromatism in Poeciliids have relied on photographs and published descriptions of the coloration of the fishes. However, such methods of colour quantification are based on human colour perception and are thus intrinsically subjective and anthropocentric. Here, using the photographs of each male and the image analysis software ImageJ (Schneider *et al.*, 2012; Rasband, 2014), we performed a quantitative analysis of male fin coloration as a function of body size. For each male we extracted: (1) the average red–green–blue (RGB) colour and perceived lightness of a small pixel subset of the dorsal and caudal fin; and (2) the proportion of black pixels in these fins. In males, the amount of black coloration in the fins varies. The inclusion of black coloration, and the darker coloration of the fin rays, can overshadow the non-black coloration of the rest of the fins when calculating average overall fin coloration. Therefore, a small (representative) subset of pixels was used to capture the non-black component of fin coloration. We selected a small circular region (mean ± SEM) of 177 ± 23 pixels for the dorsal fin and 113 ± 14 pixels for the caudal fin. This patch of pixels was taken from the central upper region of the dorsal fin and central lower region of the caudal fin. The exact size and positioning varied a bit between different individuals because it was chosen to avoid the inclusion of black pigmentation and the darker fin rays (for pixel sampling location on the dorsal and caudal fins of four representative males, see Supporting Information, Fig. S1). For this fin region, we extracted the average RGB colour (averaged over all pixels) using ImageJ. The perceived lightness (L^*) was subsequently calculated from the average RGB colour (Supporting Information, Calculating perceived lightness). The L^* is a measure of perceptual lightness that can take values between zero (black) and 100 (white) (Buckley & Giorgianni, 2015). To calculate the proportion of black pixels in the dorsal and caudal fins, we first converted the image of each male into an eight-bit greyscale image. Each pixel can then take a value between zero (black) and 255 (white). We extracted the greyscale value of each pixel for each fin of each male, and generated a histogram of all greyscale values of the dorsal and caudal fins (Supporting Information, Fig. S2). In the case of the dorsal fin, there is a clear separation between the black structures of the fin and the rest. This allowed us to define a threshold value (40 in this case; red vertical line in Supporting Information, Fig. S2) at which a pixel is considered to be black or white, respectively. We used the same threshold value for the caudal fin. We checked this threshold value in some of the images, and it nicely highlights the black structures of the fins, without capturing the slightly darker fin rays (Supporting Information, Fig. S1). Finally, we calculated the proportion of black pixels in each fin. We then fitted the perceived lightness and the proportion of black pixels (log-normal distribution) in the dorsal and caudal fins as a function of body size (i.e. standard length) using (generalized) linear models (LMs or GLMs) in R v.3.6.3 (R Core Team, 2020). The average RGB colour was subsequently used to graphically illustrate associated colour changes as a function of male body size.

The following male phenotypic traits were measured from the photographs of each male using ImageJ software (Schneider *et al.*, 2012; Rasband, 2014): (1) total length (TL); (2) standard length (SL); (3) caudal fin width (CFW); (4) body height (BH); (5) caudal fin height (CFH); (6) gonopodium length (GL); (7) dorsal fin width (DFW); and (8) dorsal fin height (DFH). The standard length (SL) was measured from the tip of the upper jaw to the outer margin of the hypural plate/base of the caudal peduncle. Gonopodium length (GL) was measured from the base to the distal tip of the male anal fin. Caudal fin width (CFW) was measured from the outer margin of the hypural plate to the outer margin of the caudal fin, and caudal fin height (CFH) from the highest point to the lowest point on the caudal fin. Dorsal fin height (DFH) was measured from the tip to the base of the longest fin ray, and dorsal fin width (DFW) from the anterior and posterior insertion points on the dorsal surface. Finally, body depth (BH) was measured from the highest point on the dorsal surface to the lowest point on the abdomen.

We generated a frequency histogram of each measured trait and used a Shapiro–Wilk normality test to determine whether the distribution deviated from normality. We next examined allometric relationships between the proportional size of body parts potentially involved in mating or sexual selection (i.e. BH, GL, CFH, CFW, DFH and DFW) and overall body size (i.e. SL). Specifically, the proportional size of each trait relative to overall body size (i.e. the trait divided by standard length) was fitted as a function of body size (i.e. standard length) in a linear model using ordinary least squares (OLS). A positive allometric relationship indicates that the given body part grows at a faster rate than does body size as a whole (i.e. large individuals have a proportionately larger body part than smaller individuals). In contrast, a negative allometric relationship indicates that the body part has a slower growth rate than does the body as a whole (i.e. small individuals have a proportionately larger body part than larger individuals).

FIELD DATA COLLECTION AND ANALYSES OF MALE MATING TACTICS

All field data on male mating tactics was gathered on 26 February 2019 between 08.54 and 13.44 h in the Rio Pacuar. In short, while snorkelling, individual males were observed for ≤ 5 min, and all mating behaviours were recorded in a standardized way. Male behaviours included gonopodial thrusting, gonoporal nibbling, chasing females, chasing males and being chased by a male. Gonopodial thrusting refers to a male approaching a female from behind, swinging his gonopodium forward and attempting to insert it in her gonopore (Bisazza, 1993). Gonoporal nibbling refers to a male making oral or nasal contact with the female's gonopore; this behaviour is thought to allow a male to determine, through chemical factors, whether a female is carrying fertilizable ova (Farr & Travis, 1986; Sumner *et al.*, 1994). Chasing refers to males rapidly swimming directly after another individual. Chasing could be directed at a fleeing female, toward another male or directed at the focal male. Below, we describe our data collection protocol in greater detail (for a description of a similar protocol, see Rios-Cardenas *et al.*, 2010).

Two snorkellers (A.I.F. and B.J.A.P.; hereafter, 'snorkellers') made observations on focal males, and A.H. recorded data (hereafter, 'recorder'). The snorkeller began by lying horizontally in the water, floating if the water was deep, gently lying on the bottom if the water was shallow, or with a hand or foot braced against the bottom if the water was shallow and flowing. The snorkeller then located a single focal male on which to make observations. Focal males were chosen at random. A trial began with the snorkeller glancing at his digital waterproof watch to note the time and then observing a focal male and making mental note of the number of times the male performed the following five behaviours: (1) gonopodial thrust; (2) chased female; (3) nibbled female gonopore; (4) chased another male away; and (5) was chased away by another male. The snorkeller slowly moved body position as required in order to keep the focal male in the visual field as he swam. Given that the snorkeller remained relatively still, fish appeared completely at ease and performed normal behaviours, including the mating behaviours listed above, in addition to feeding and swimming to within centimetres of the snorkeller's mask. Trials lasted a maximum of 5 min. If the focal male was lost before the 5 min mark, then the time at which the male could no longer be observed was noted and the trial ended. In practice, males tended to be lost when they swam rapidly out of the field of vision and could not be followed/located or when they swam rapidly into a school containing other *P. gillii* males of similar size and could no longer be identified individually.

When the trial ended, the snorkeller lifted his head from the water and reported the relevant data to the recorder, who was standing on the nearby river bank, with datasheet and clipboard in hand. The data included the size category of the male (small, intermediate or large), the number of times the focal male performed each of the five mating behaviours listed above, and the duration of the trial. Any other notable interactions or observations were also recorded. This process was then iteratively repeated (with intermittent breaks) by the two snorkellers working simultaneously on opposite sides of the river bank until a sample size of $N = 36$ males was reached (i.e. $N = 12$ for each of the three male size classes). Although possible, we consider it relatively unlikely that the same individual males were observed more than once by the snorkellers. This is because the density of *P. gillii* was very high in this site (in the thousands in this 100-m-long stretch of river) and males were selected at random for behavioural observation.

The use of a mask and snorkel to conduct behavioural observation studies has a long history in marine fish species (Ogden & Buckman, 1973; Pollux *et al.*, 2007; Muñoz *et al.*, 2010; Danylchuk *et al.*, 2019) and has been used successfully for the poeciliid species *Xiphophorus nezahualcoyotl*, using a very similar methodology to ours (Rios-Cardenas *et al.*, 2010). In *P. gillii*, the five mating behaviours were straightforward to observe and count (for examples of each behaviour, see Supporting Information, Videos S1 and S2). During each trial, we tracked only a single focal male, while ignoring all other fish (both males and females) unless they interacted with the focal male. Focusing our attention solely on the focal male allowed us to accurately count and remember the number of times this male performed each of the five behaviours during a maximal period of 5 min. Moreover, individual males never performed all five behaviours in the same trial; thus, in a given trial some behaviours were unobserved and scored as zero, which facilitated counts of the other observed behaviours (the mean and median number of different behaviours observed in a trial was two, with a range of zero to four). In other studies, a waterproof tablet and pen has sometimes been used to record data during trials (e.g. Pollux *et al.*, 2007). However, prior trials showed that it was difficult to follow a focal male and simultaneously mark data on the tablet. Looking away from the focal male to write down data, even if briefly, could mean losing the male. Since the behaviours were easy to score, we decided that counting and remembering the behaviours and conveying them to the recorder after the trial ended was the preferred method of data collection.

Estimating the relative size of focal males and placing them into one of three size classes (small, intermediate and large) was a necessity of our field data collection protocol. Although we do not have defined boundaries (on an absolute scale) between the three size classes, they approximate the lower, middle and upper third of the population size distribution. Both observers were familiar with the size distribution of *P. gillii* at this site, and the fish density was very high, meaning that males of all sizes were generally available for purposes of comparison (of relative size). For these reasons, we believe that our field scorings of size class are likely to be repeatable both within individual fish and across observers. Furthermore, any 'errors' in size classification or slight differences between observers in the way they scored size classes would be at the border between small and intermediate and between intermediate and large size classes. Given the degree of separation between the small and large size classes, it is very unlikely that any error could be made here. Importantly, our primary interpretations regarding male mating behaviour are between males in the small and large size classes; in a sense, the intermediate size class can be viewed as a buffer between the comparison of most interest: small males and large males.

The effects of male size on male mating strategies were analysed by fitting a zero-inflated negative binomial (ZINB) model to each male behaviour (i.e. gonopodial thrust, chased female, nibbled female gonopore, chased another male away and was chased away by another male) in R v 3.6.3 (R Core Team, 2020), using the package glmmTMB (Brooks *et al.*, 2017). The ZINB model accounts for overdispersion arising from both individual heterogeneity and excess zeros (Xie *et al.*, 2013). Overdispersion and zero-inflation were tested using functions implemented in the DHARMa package (Hartig, 2018). Fixed effects included a categorical variable for male size (small, intermediate or large), the duration of observation (in minutes), observer identity (A.I.F. or B.J.A.P.) and the interaction between male size and duration of observation. In all models, the interaction term was not significant, hence this covariate was excluded from any further analyses. Likewise, observer identity was not significant, except for 'chased another male away' and 'chased away by another male'. However, the inclusion of observer identity in these two cases resulted in model convergence problems, and thus, it was also excluded as a potential fixed effect in all models. All pairwise comparisons of male size classes for each male mating behaviour were performed with the package emmeans (Lenth, 2020). The *P*-values were adjusted for multiple testing using a Bonferroni correction.

RESULTS

Mature male *P. gillii* ranged in size from 22.6 to 51.8 mm standard length. There was significant covariation between male standard length and dorsal fin lightness (LM, $t_{58} = 74.519$, $P < 0.001$; Supporting Information, Table S1A) and caudal fin lightness (LM, $t_{58} = 80.085$, $P < 0.001$; Supporting Information, Table S2A); larger males tended to have darker, more orange-coloured dorsal and caudal fins, whereas the smallest males had brighter, uncoloured fins (Fig. 2). Furthermore, the amount of black pigmentation on the dorsal fin linearly increased with male size (LM, $t_{58} = 4.494$, $P < 0.001$; Supporting Information, Table S1B). In contrast, the amount of black pigmentation on the caudal fin increased exponentially with male size (GLM, $t_{58} = 11.774$, $P < 0.001$; Supporting Information, Table S2B). Thus, larger males had a significantly greater proportion of their dorsal and caudal fins covered in black pigmentation (Fig. 2).

The frequency distribution of male standard length did not differ from normality (Shapiro–Wilk normality test, $W = 0.980$, $P = 0.379$). Furthermore, the frequency distribution for each other trait (body height, caudal fin height, caudal fin width, dorsal fin height and gonopodium length), with the exception of dorsal fin height, did not differ significantly from normality (Supporting Information, Fig. S3; Table S3; Shapiro–Wilk normality test, $P > 0.05$). For dorsal fin height, the frequency distribution was right skewed (Shapiro–Wilk normality test, $W = 0.951$, $P = 0.014$).

We found a significant positive allometric relationship between body size and proportional body height (OLS regression, $t_{58} = 6.448$, $P < 0.001$), caudal fin height ($t_{58} = 5.391$, $P < 0.001$), dorsal fin height ($t_{58} = 6.151$, $P < 0.001$) and dorsal fin width ($t_{58} = 4.773$, $P < 0.001$). We found no significant relationship between body size and proportional caudal fin width (OLS regression, $t_{58} = -0.707$, $P = 0.482$). Lastly, we found a negative relationship between body size and proportional gonopodium length (OLS regression, $t_{58} = -14.740$, $P < 0.001$). Thus, large males had proportionately deeper bodies, larger dorsal and caudal fins and shorter gonopodia than smaller males (Fig. 3; Table S4; for allometric scaling coefficients see Supporting Information, Fig. S4; Table S5).

Our field study of male mating behaviour revealed that male size had a significant effect on the number of times a focal male chased a female and nibbled a female's gonopore (zero-inflated negative binomial regression, $P < 0.001$; Fig. 4). Specifically, small males were significantly more likely to chase

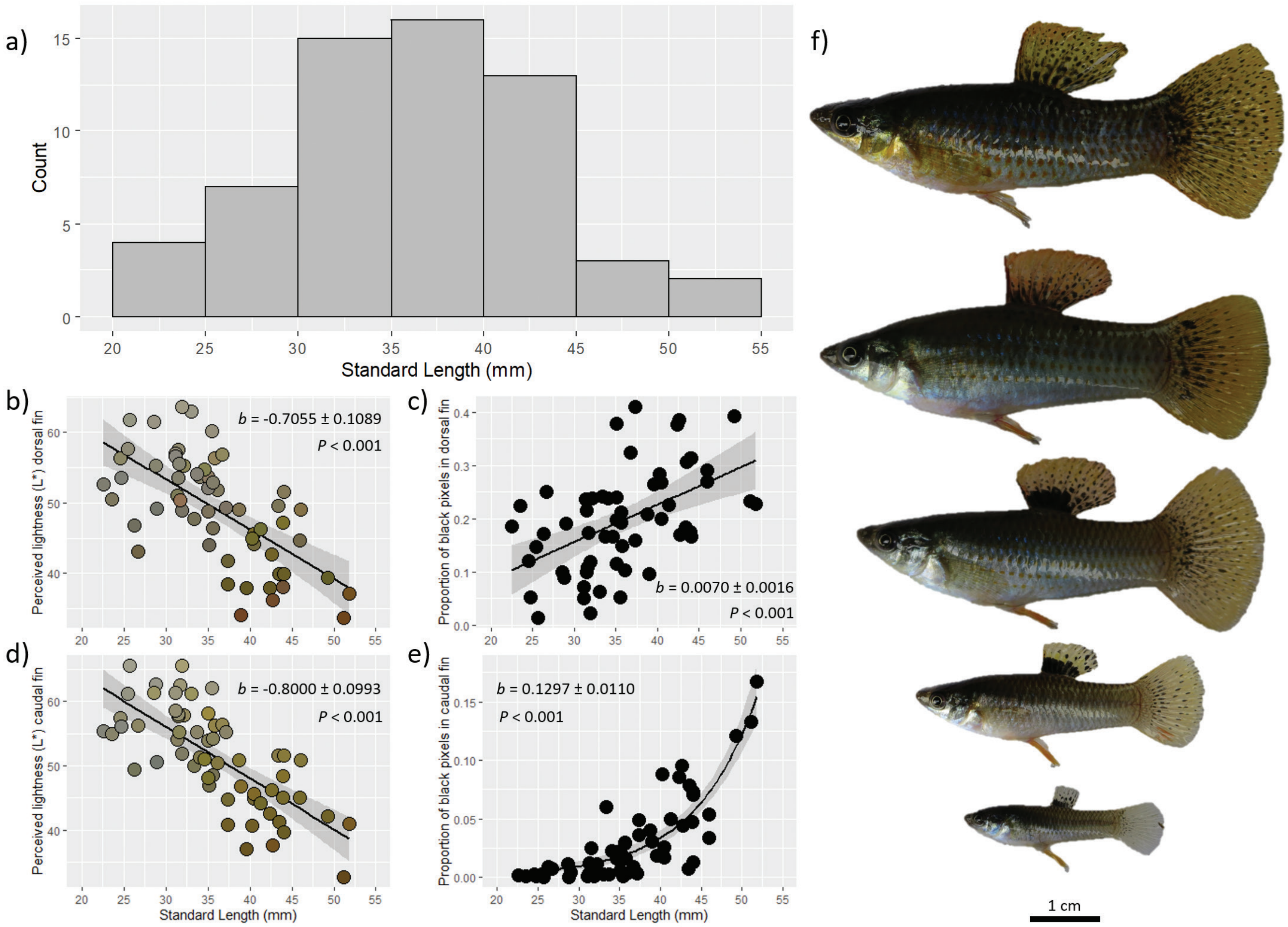

Figure 2. Variation in the size, lightness and coloration of mature male *Poecilia gillii* (N = 60). A, frequency histogram of male standard length. B–E, plots showing significant covariation between male standard length and dorsal fin lightness/coloration (B) and proportion of black pigmentation (C), in addition to caudal fin lightness/coloration (D) and proportion of black pigmentation (E). In B and D, points are colour coded according to dorsal and caudal fin coloration (i.e. average RGB). Grey shading around each line depicts 95% confidence intervals. The slope (b ± SE) and its significance (i.e. P-value) are given in each panel. F, males ranged in size from a maximum of 51.8 mm standard length (pictured top) to a minimum of 22.6 mm (pictured bottom).

females than were intermediate or large males (Fig. 4B), and large and intermediate-sized males nibbled a female's gonopore significantly more than small males (Fig. 4C). In contrast, male size had no significant effect on the number of gonopodial thrusts and the number of times males chased or were chased away by another male (zero-inflated negative binomial regression, $P > 0.05$; Fig. 4). However, in the case of 'chased away another male' the non-significance ($P = 1$) was attributable to a lack of variation within some size classes; specifically, only large males chased other males away, whereas intermediate and small-sized males were never observed to engage in this behaviour (Fig. 4D). Statistical output for all models of male mating behaviour can be found in Supporting Information, Tables S6 to S11.

DISCUSSION

Based on our prior informal observations of within-population body size variation and phenotypic differences amongst mature male *P. gillii*, in addition to empirical studies documenting alternative reproductive strategies in other species in the genus *Poecilia*, we anticipated that male *P. gillii* might exhibit alternative mating tactics that are correlated with body size and morphology. We therefore devised a field test of a priori hypotheses regarding the relationship between male size, coloration, morphology and mating tactics.

We found that male body size was continuously distributed and did not differ from a normal distribution (Fig. 2). This is consistent with patterns of body size found in the sailfin molly, *Poecilia latipinna*

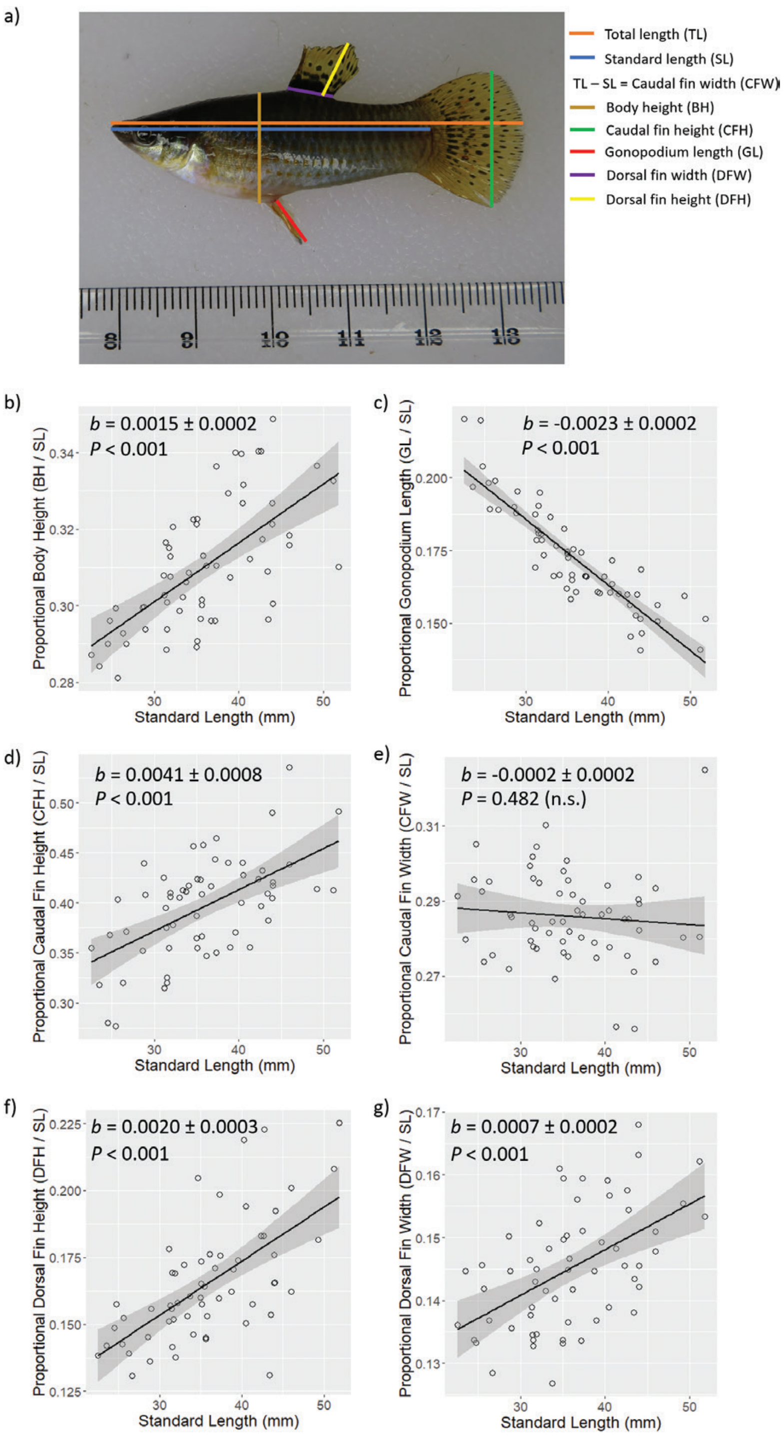

Figure 3. Allometric relationships between the proportional size of body parts involved in mating or sexual selection and overall body size. A, the male phenotypic traits that were measured included total length (TL), standard length (SL), caudal fin width (CFW), body height (BH), caudal fin height (CFH), gonopodium length (GL), dorsal fin width (DFW) and dorsal fin height (DFH). B–G, graphs depicting the relationship between standard length and proportional body height (B), gonopodium length (C), caudal fin height (D), caudal fin width (E), dorsal fin height (F) and dorsal fin width (G). Sample size was $N = 60$ males for all analyses. The fitted lines are from linear regression models using ordinary least squares (OLS). Grey shading around each line depicts 95% confidence intervals. The slope (b ± SE) and its significance (i.e. P-value) are given in each panel (for summaries of full model output, see Supporting Information, Table S4).

(Snelson, 1985; Farr *et al.*, 1986), but contrasts with patterns in some other poeciliid species, in which males exhibit bimodal or multimodal size distributions (Reznick *et al.*, 1993; Erbelding-Denk *et al.*, 1994; Kolluru & Reznick, 1996; Regus *et al.*, 2013; Cohen *et al.*, 2015). Furthermore, in addition to body size, all other measured male traits were normally distributed, except for dorsal fin height, which was skewed to the right (i.e. overabundance of males with high dorsal fin). Thus, in *P. gillii* males there was no evidence for discrete male size classes that exhibit alternative morphotypes. Nonetheless, we found it useful to divide mature males into three size classes (small, intermediate and large) for our field study of male mating tactics and to contrast the phenotype and mating tactics of males from opposite ends of the size distribution. When doing so, we found that small and large males differed in coloration, phenotype and mating tactics (Table 1).

Consistent with predictions, large males exhibited dark (orange) coloration on their dorsal and caudal fins, whereas small males were drab in coloration and had clear or very lightly coloured dorsal and caudal fins. The inconspicuous coloration of small males might facilitate cryptically approaching females from behind and avoiding aggression from large males (i.e. if they are not easily noticed or mistaken for small females). Furthermore, small male body size, relative to that of females, has been shown to facilitate sneak copulations in other poeciliid species (Bisazza & Pilastro, 1997; Pilastro *et al.*, 1997).

We also found a strong positive correlation between male body size and the amount of black pigment on both the dorsal and caudal fins. In some other shortfin molly species, dominant males have been described as assuming very dark coloration of the dorsal and caudal fins and the body (Ptacek *et al.*, 2005). In *P. gillii*, it is not yet known whether and how quickly black pigmentation can change conditionally, for example based on access to females or encounters with rival males. However, we did not observe rapid male colour change (e.g. from pale to dark black) based on transient social cues (i.e. interactions among males) (Kodric-Brown, 1998). Thus, the black pigment on the dorsal and caudal fins that was measured herein appears likely to be relatively stable over time and not something that can readily be made to appear and disappear based on short-term social effects.

The functional importance of fin coloration and the amount of black pigmentation on the fins has not been studied in *P. gillii*, but in other poeciliid species coloration is an important determinant of both female choice and male–male interactions (Constanz, 1975; Endler, 1984; Jirotkul *et al.*, 2000; Franck *et al.*, 2003; Kingston *et al.*, 2003; Ptacek *et al.*, 2005;Hurtado-Gonzales & Uy, 2009; Horth *et al.*, 2010; Kolluru *et al.*, 2014). Furthermore, natural selection might potentially contribute to the maintenance of differences in coloration among males of different sizes, for example if there is a trade-off between attractiveness to females and conspicuousness to predators (Endler, 1980; Hurtado-Gonzales *et al.*, 2010).

Large males exhibited body height, caudal fin height and dorsal fin height and width that increased at a faster rate than body size (i.e. positive allometry). In other words, these traits were underdeveloped in small males and overdeveloped in large males. In sharp contrast, and consistent with our a priori predictions, small males had a relatively long gonopodium relative to body size and large males a short gonopodium. A similar pattern has been found in *Limia perugiae* (Erbelding-Denk *et al.*, 1994), *Poeciliopsis occidentalis* (Constanz, 1975) and *Poecilia latipinna* (Snelson, 1985). A long gonopodium is thought to facilitate sneak copulations by increasing manoeuvrability during mating attempts (Bisazza, 1993; Greven, 2005). There is empirical evidence to back this: in guppies, a species that exhibits both courtship and gonopodial thrusts, there is a positive correlation between gonopodial thrusting and gonopodium length (Reynolds *et al.*, 1993). Furthermore, a similar relationship has been found across species; those with relatively long gonopodia engage in sneak copulations, whereas species with short gonopodia tend to exhibit courtship (Bisazza, 1993; Ptacek & Travis, 1998; Martin *et al.*, 2010; Pollux *et al.*, 2014). Thus, the relatively long gonopodium in small male *P. gillii* suggests a reliance on coercive mating, whereas the relatively short gonopodium in large *P. gillii* males suggests that mating cooperation might be elicited or, at the very least, females might be more receptive to the advances of large males.

Courtship in poeciliids involves the male displaying in front of the female with his dorsal and caudal fins fully spread, which can be accompanied by a sigmoid body posture (Rios-Cardenas & Morris, 2011; Goldberg *et al.*, 2019). Courtship behaviour was never observed in our field observations of *P. gillii*, nor has courtship behaviour been reported in laboratory observations of this species (Goldberg *et al.*, 2019). Given that *P. gillii* does not demonstrate courtship, why do large males exhibit exaggerated dorsal and caudal fins that are colourful? What is the advantage of growing and maintaining such, presumably costly, display traits? We envision two non-mutually exclusive possibilities. The first is that these traits are important in the context of male–male display and aggression (Bildsøe, 1988; Benson & Basolo, 2006; Prenter *et al.*, 2008; Goldberg *et al.*, 2019). While conducting field observations of *P. gillii* mating behaviour, B.J.A.P. observed two large males fighting with each other by biting each other's dorsal fin and caudal peduncle while circling each other head to tail. Furthermore, each male had its dorsal and

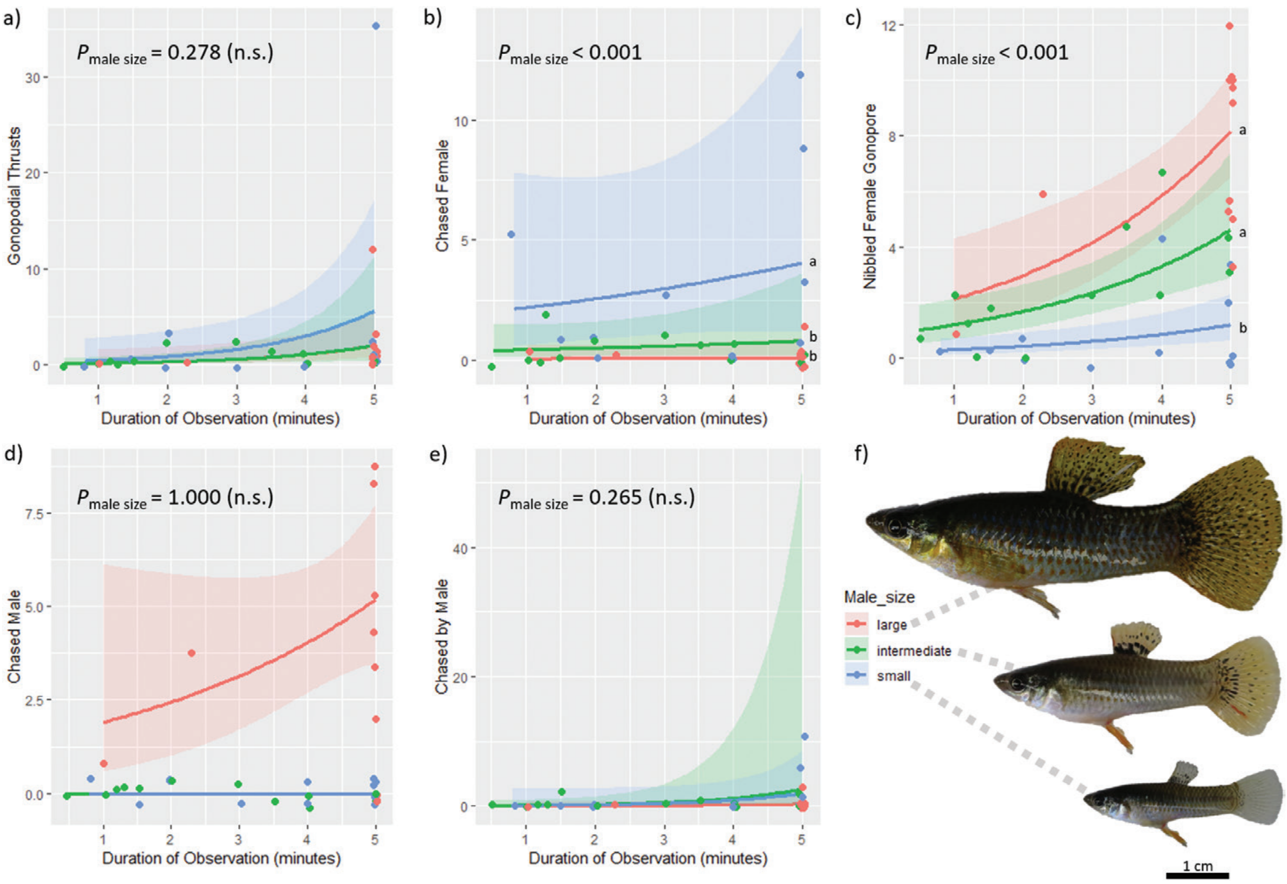

Figure 4. Male mating strategy as a function of body size. Each graph depicts the number of times a focal male performed the following five behaviours: gonopodial thrust (A), chased female (B), nibbled female gonopore (C), chased another male away (D) and was chased away by another male (E). The fitted lines are from zero-inflated negative binomial regression models that included male size as a categorical variable with three levels (large, intermediate and small) and the duration of observation as a continuous variable. The duration of observation refers to the length of time (range 0.5–5.0 min) that each focal male was tracked while snorkelling. Sample size was $N = 36$ males for all analyses. Points have been jittered for clarity. Coloured shading around each line depicts 95% confidence intervals. The significance (i.e. P-value) of the main effect of male size is given in each panel (for summaries of full model output, see Supporting Information, Tables S7–S11). For behaviours in which male size had a significant effect, differences among the three size classes are indicated as superscript letters along the right edge of the plot. Please note that in D, only large males chased other males away, hence the fitted lines for intermediate and small males both exhibit an intercept and slope of zero without a confidence interval. F, males to relative scale, depicting exemplars of large, intermediate and small size categories.

caudal fins fully spread during this fighting sequence. While snorkelling, we also observed other large males that had tissue damage to the caudal peduncle region in the exact area where the two large males were biting each other (Fig. 1). Only the largest males were observed with this injury. Given the way in which the dorsal and caudal fins were spread during this male–male fighting and the role that these traits play in male–male competition in other poeciliid species (Bildsøe, 1988; Benson & Basolo, 2006; Prenter *et al.*, 2008; Goldberg *et al.*, 2019), it is plausible to assume that these traits are important in the context of male–male display and aggression. Fighting was observed only a single time, between two equally sized and colourful males; however, large and colourful males were observed chasing away small, intermediate and large males on numerous occasions (Fig. 4D). Thus, having large and colourful dorsal and caudal fins that contain a high proportion of black pigmentation could serve as a badge with which male vigour or dominance is judged, thereby avoiding frequent direct fighting, except in the rare circumstance when two equally sized males meet and neither backs down based on display alone.

A second, non-mutually exclusive possibility is that although males do not engage in courtship, female

Table 1. Comparison of the coloration, phenotype and mating tactics of small and large male *Poecilia gillii*

Category	**Trait**	**Small ♂**	**Large ♂**
Coloration (Fig. 2)	Caudal fin colour	Clear or light yellow	Yellow/orange
	Caudal fin percentage black	Small	Large
	Dorsal fin colour	Clear or light yellow	Yellow/reddish orange
	Dorsal fin percentage black	Small	Large
Phenotype: proportional to body size (Fig. 3)	Body height	Small	Large
	Caudal fin height	Small	Large
	Caudal fin width	Proportional	Proportional
	Dorsal fin height	Small	Large
	Dorsal fin width	Small	Large
	Gonopodium length	Long	Short
Mating tactics (Fig. 4)	Courtship	No	No
	Gonopodial thrusts	Equal	Equal
	Chased female	Frequently	Rarely
	Gonoporal nibbling	Rarely	Frequently
	Chased male	Never	Frequently
	Chased by male	Equal	Equal

We emphasize that there is a continuous distribution of male body size, coloration, fin size and most mating tactics, and here we contrast these traits from males at opposite ends of the size distribution.

receptiveness to mating varies as a function of male size and phenotype. We observed that large and colourful males remain relatively stationary in a home territory and mate with nearby females. This is supported by our observations that large males frequently chase away other males (Fig. 4D) but are much less likely to chase females than are small males (Fig. 4B). Given that the number of mating attempts does not differ amongst male size classes (Fig. 4A), this suggests that females might be more willing to mate with large and colourful males (i.e. large males do not have to chase rapidly after females in order to mate with them). Furthermore, large males were much more likely to nibble the female gonopore than were small-sized males (Fig. 4C). This behaviour has been interpreted as males using chemical cues to judge whether a female has eggs that are at the appropriate stage to be fertilized (Farr & Travis, 1986; Sumner *et al.*, 1994). Females might be more likely to remain relatively stationary and allow large preferred males to nibble their gonopore as a prelude to mating, compared with small less attractive males. Alternatively, females might conceivably prefer large males because they frequently use gonopore nibbling to determine receptivity before attempting to mate and therefore harass them less (i.e. they do not attempt to mate if the female is not receptive). Thus, in summary, large males appear sometimes to engage in a strategy of territorial defence, chasing away other males of all sizes and occasionally fighting with other large males, while exhibiting a high rate of gonoporal nibbling and perhaps mating preferentially with fertile and receptive females. In contrast, mobile small males appear to engage in an indiscriminate mating strategy, in which they chase females that are often unreceptive and attempt coercive mating. Intermediate-sized males appear to engage in an intermediate or mixed strategy.

Our characterization of the *P. gillii* mating system makes for an interesting contrast with the closely related and well-studied sailfin molly, *Poecilia latipinna*. The biggest difference in the mating system of these two species is that *P. latipinna* exhibits courtship (and an enlarged, sail-like dorsal fin), whereas *P. gillii* does not. In *P. latipinna*, large males exhibit higher rates of courtship displays than small males (Farr *et al.*, 1986; Travis & Woodward, 1989; Ptacek & Travis, 1996). Furthermore, in *P. latipinna*, small males exhibit higher rates of gonoporal nibbling than large males (Farr *et al.*, 1986) or rates mostly independent of male size (Ptacek & Travis, 1996), whereas gonopodial thrusting rates (i.e. sneak copulation attempts) are more frequent in small males (Farr *et al.*, 1986; Travis & Woodward, 1989; Becker *et al.*, 2012) or independent of male size (Ptacek & Travis, 1996; Seda *et al.*, 2012). In *P. gillii*, we found that large males exhibit higher rates of gonoporal nibbling and that the gonopodial thrusting rate was independent of male size. In *P. latipinna*, females prefer large males with large dorsal fins (MacLaren *et al.*, 2004; MacLaren & Rowland, 2006). Males also prefer larger females, and larger males exhibit a stronger preference for larger females (Ptacek & Travis, 1997). Receptivity of female *P. latipinna* is determined through visual and direct contact cues (Farr & Travis, 1986; Sumner *et al.*, 1994). Large male *P. latipinna* are more interested in receptive females, whereas small males do not differentiate between non-receptive and receptive females (Sumner *et al.*, 1994). Furthermore, large *P. latipinna* males spend more time chasing away other males (Becker *et al.*, 2012) and are able to deny small males access to females, and thus might increase reproductive success, relative to the more common small males, by focusing their mating efforts on large receptive females (Sumner *et al.*, 1994). This is consistent with our observations in *P. gillii*; the significantly higher rate of gonoporal nibbling exhibited by large males might allow them to determine female receptivity and focus their mating efforts on such females.

Alternative reproductive strategies can be characterized by a genetic polymorphism, with equal fitness maintained by frequency-dependent selection (Gross, 1996; Shuster, 2010). In *P. latipinna*, a Y-linked locus mediates variation in body size and mating behaviour (Trexler & Travis, 1990; Trexler *et al.*, 1990; Travis, 1994a, b; Fraser *et al.*, 2014). If the mechanism is the same in *P. gillii*, then differences in male size are genetically based and not attributable to differences in age or growth rate. It is widely reported that male poeciliid fishes cease growth after becoming sexually mature. In a laboratory study, Snelson (1982) showed that male *P. latipinna* continue to grow after reaching maturity, but at a rate two to three times more slowly than females. Travis *et al.* (1989) also found that mature male *P. latipinna* can grow after reaching maturity, but growth rates in the laboratory were more than a magnitude higher than those observed in field enclosures. These authors concluded that post-maturational male growth is sufficiently low that it can be ignored as a source of body size variation within populations. In *P. gillii*, the largest immature males were as big as the largest mature males (Supporting Information, Fig. S5), suggesting that the variation in size of mature males is not attributable to post-maturation growth. Fitness as a function of body size, incorporating both pre- and post-mating sexual selection (Evans & Garcia-Gonzalez, 2016), has not been investigated in *P. gillii*, and it is possible that frequency-dependent selection by females and/or predators might maintain the size, morphological and behavioural variation that we have described.

Theory predicts that alternative reproductive tactics should become discrete or bimodally distributed owing to disruptive selection and reduced fitness for males with intermediate phenotypes (Taborsky *et al.*, 2008). Furthermore, within each alternative strategy there is predicted to be a matching between tactics and morphology (Taborsky *et al.*, 2008). In *P. gillii*, we found that male coloration, body size and all other measured morphological traits varied continuously. In other words, there were no clear 'alternatives', in which phenotypes were discrete or discontinuous. Things became a bit fuzzier for mating tactics. Specifically, some mating behaviours did not differ as a function of male size class (rate of gonopodial thrusting and being chased away by another male), some differed significantly among size classes, but the nature of the variation was continuous rather than discrete (i.e. rate of gonoporal nibbling and chasing females), and some were performed only by a given size class and therefore could reasonably be considered discrete (i.e. chasing other males and fighting between males was observed only for the large size class). Given the (mostly) continuous nature of morphological and behavioural variation, the weight of evidence suggests that *P. gillii* males do not fit the definition of having 'alternative reproductive strategies' because there are no clearly separable (i.e. dichotomous or discontinuous) alternatives. Nonetheless, within this continuous milieu of phenotypic variation, we found clear evidence for the predicted associations between male size, coloration, morphology and behaviour. These associations appear likely to maximize mating success for males of a given size. A similar pattern is found in *P. latipinna*, whereas in some other poeciliid species body size exhibits a bimodal distribution and males in each size class exhibit discrete phenotypic differences, i.e. small male *Xiphophorus multilineatus* lack the sword-like extension of the caudal fin, whereas large males have the sword (Liotta *et al.*, 2019). The cause(s) of these differences are unclear. Intralocus tactical conflict theory suggests that because males within the same population share the same genetic architecture there could be a constraint on phenotypic divergence, with behaviour being more labile than morphology and therefore diverging more readily (Abbott *et al.*, 2019). Additionally, perhaps the magnitude of divergence amongst mating tactics could differ as a function of the intensity of sexual selection and male–male competition in a given species.

CONCLUSION

This study documents differing reproductive tactics and morphologies across a broad size continuum in a poeciliid species that does not exhibit courtship. This makes for an interesting contrast with poeciliid species that display alternative reproductive strategies in the context of courtship and/or sneak mating. In spite of differences in some details, there is broad congruence between the mating systems of *P. gillii* and *P. latipinna*, including predictable associations between male size, phenotype and mating tactics.

The study of alternative reproductive strategies has necessarily focused on documenting patterns of variation in species in which they exist. However, many (perhaps most) poeciliid species apparently do not exhibit such strategies. It remains to be determined why males of some poeciliid species exhibit striking alternative reproductive strategies and other species apparently do not, or do so in much more subtle ways. What are the prerequisite conditions for the evolution of alternative reproductive strategies and mating tactics? In species that evolve such strategies, why do some exhibit continuous variation, whereas others exhibit discrete morphs (i.e. *Poecilia parae*)? The comparative variation and large number of detailed empirical studies of alternative mating tactics and phenotypic variation in the family Poeciliidae (see table 17.2 of Rios-Cardenas & Morris, 2011) makes these questions ripe for comparative study.

Lastly, our field snorkelling protocol used to characterize mating behaviour could be adopted in a variety of species, and we believe it can be an important complement to controlled laboratory studies of mating behaviour.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Supporting methods: Calculating perceived lightness.
Figure S1. Paired images of representative male *Poecilia gillii*, showing conversion to greyscale and application of threshold value used to define black and non-black fin coloration.
Figure S2. Frequency histograms of greyscale pixel coloration of the dorsal and caudal fins in male *Poecilia gillii*.
Figure S3. Frequency histograms of male *Poecilia gillii* phenotypic traits.
Figure S4. Allometric relationships between the size of body parts involved in mating or sexual selection and overall body size (i.e. standard length).
Figure S5. Frequency histograms of male *Poecilia gillii* standard length as a function of maturity.
Table S1. Linear models examining the relationship between male standard length and dorsal fin coloration.
Table S2. (Generalized) linear models examining the relationship between male standard length and caudal fin coloration.
Table S3. Results of Shapiro–Wilk normality test to determine whether the distribution of each variable differs from normality.
Table S4. Linear models (ordinary least squares regression) examining the relationship between male standard length and proportional size of body parts.
Table S5. Linear models (ordinary least squares regression) examining the relationship between $\log_{10}$-transformed male standard length and $\log_{10}$-transformed measurements of body parts.
Table S6. Changes in fit of the zero-inflated negative binomial (ZINB) regression that models each male mating strategy (i.e. gonopodial thrust, chased female, nibbled female gonopore, chased another male away and was chased away by another male) as a function of male size (small, intermediate or large) and the duration of observation (in minutes), when additionally including either: A, the interaction between male size (small, intermediate or large) and the duration of observation (in minutes); or B, observer identity (A.I.F. or B.J.A.P.).
Table S7. Zero-inflated negative binomial (ZINB) regression that models the number of gonopodial thrusts as a function of male size (small, intermediate or large) and the duration of observation (in minutes).
Table S8. Zero-inflated negative binomial (ZINB) regression that models the number of times a male chased a female as a function of male size (small, intermediate or large) and the duration of observation (in minutes).
Table S9. Zero-inflated negative binomial (ZINB) regression that models the number of times a male nibbled a female's gonopore as a function of male size (small, intermediate or large) and the duration of observation (in minutes).
Table S10. Zero-inflated negative binomial (ZINB) regression that models the number of times a male chased another male as a function of male size (small, intermediate or large) and the duration of observation (in minutes).
Table S11. Zero-inflated negative binomial (ZINB) regression that models the number of times a male was chased by another male as a function of male size (small, intermediate or large) and the duration of observation (in minutes).

Video S1. *Poecilia gillii* male mating behaviour. Examples of males of different size classes performing gonopodial thrusting, gonoporal nibbling, chasing females, chasing males and being chased by a male. Filmed in the Pacuar river, 25 and 26 February 2019.

Video S2. *Poecilia gillii* males performing gonopodial thrusts in slow motion (one-quarter speed). Filmed in the Pacuar river, 25 and 26 February 2019.

SHARED DATA

The data sets used in all analyses have been deposited in the Dryad Digital Repository (Furness *et al.*, 2020).