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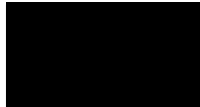
This thesis is written in the form of a journal article from Animal Behaviour.

Declaration

I wish to acknowledge the following assistance in the research detailed in this report:

All experimental design was done by Associate Prof. Culum Brown. Ms. Amanda Baxter provided animal husbandry assistance.

All other research described in this report is my own original work.



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08 October 2015

Energetic costs of sexual coercion—the price of persuasion

Females are often subjected to unwanted mating advances from males. Such advances can be costly to both parties. The costs of harassment to females have been widely explored in the literature; however, few studies have measured the direct fitness costs. Moreover, few have examined male costs. Conventional wisdom would lead us to hypothesise that when males and females are housed together, harassment would reduce foraging, growth and reproductive output. This study quantified harassment costs in both sexes by observing behavioural responses and long-term effects of unsolicited mating in a controlled setting. Sexually mature guppies were subjected to two housing treatments: equal sex ratios or single-sex groups. The effects of male coercion on males and females were assessed by measuring behaviour, growth rate and the number of offspring produced. Contrary to our expectations, our results indicated no significant differences in foraging and growth rates between mixed and single-sex shoals for either sex. Moreover, there was no significant difference in fry output between mixed and all-female shoals. Further, large males showed higher survival when housed with females. Thus, it appears that there were no direct costs of harassment for females in natural, mixed-sex shoals, but males appear to bear significant harassment costs. The study provides insights into reproductive behaviour and life history traits.

Keywords: sexual harassment; guppies; energetic costs; poeciliids; fitness costs

Animal mating strategies can be either cooperative or conflictive to various degrees (Pizzari & Gardner, 2012). There are two main theories that explain sexual selection and mating systems in nature—(1) conflict between the sexes over mating decisions (Chapman, Arnqvist, Bangham, & Rowe, 2003; Chapman, 2006; Davies, Hartley, Hatchwell, & Langmore, 1996; Gavrillets, 2000; Rowe, Arnqvist, Sih, & Krupa, 1994) and (2) adaptive mating strategies and the diversification of mating systems driven by variation in ecological conditions (e.g., Environmental potential for polygamy model, Emlen & Oring, 1977; Rowe, Arnqvist, Sih, & Krupa, 1994; polygyny threshold model, Verner, 1964; Verner & Willson, 1966; Orians, 1969; Weatherhead & Robertson, 1979). In most instances, males maximise reproductive fitness by mating as many times as possible with as many partners as possible (Arnqvist, 1989; Arnqvist & Nilsson, 2000; Bateman, 1998; Gowaty, Kim, Rawlings, & Anderson, 2010; Pilastro, Benetton, & Bisazza, 2003), because they have higher numbers of low cost gametes (Hayward & Gillooly, 2011; Yasui, 1997; Wedell, Gage, & Parker, 2002). In contrast, females have fewer high cost gametes (Hayward & Gillooly, 2011; Wedell, Gage, & Parker, 2002) and do not need to mate as frequently (Arnqvist & Rowe, 2013; Gasparini, Devigili, & Pilastro, 2011; Parker, 1979; Pilastro et al., 2003).

Harassment is a consequence of differing optimal mate numbers between males and females and the conflicting mating strategies that result (Chapman, Arnqvist, Bangham, & Rowe, 2003; Dadda, Pilastro, & Bisazza, 2005; Gavrillets, 2000) and is quite common in the animal kingdom (Clutton-Brock & Parker, 1995; Kohler et al., 2011), with females usually being the recipients (Pizzari & Gardner, 2012). Males maximise chances of paternity by mating at higher frequencies than females resulting in wasted time spent evading these mating attempts or ‘harassment’ for females (Davies, Hartley, Hatchwell, & Langmore, 1996).

This phenomenon has been observed across a wide range of taxonomic groups, including invertebrates (Okada et al., 2015); mammals (Clutton-Brock & Parker, 1995); bony and cartilaginous fishes (Magurran & Seghers, 1994); reptiles (Taylor, Price, & Wedell, 2014) and amphibians (Taylor et al., 2014). The costs for females may be many and varied, but range from physical damage (e.g., bean beetles *Callosobruchus maculatus*, harassment results in damage to the female’s reproductive tract, Pizzari & Gardner, 2012), enhanced risk of predation (e.g., eastern Mosquitofish *Gambusia holbrooki*, Dadda et al., 2005) and lost foraging opportunities (female bees

Anthophora plumipes, Stone, 1995) to high mortality costs (e.g., dung fly *Sepsis cynipsea*, Blanckenhorn et al., 2002).

Most research has concentrated on the costs of male harassment on females, but few have considered the male costs. One such study (Jordan & Brooks, 2010) explored the lifetime growth costs of increased reproductive efforts in male guppies and found that significant costs were incurred when males pursued unfamiliar mates. In addition, Clutton-Brock & Langley (1997) found that persistent coercive mating can affect longevity in both sexes of tsetse flies (*Glossina morsitans morsitans*), with males showing greater costs when the sex ratio was biased towards females. Similar results were found in Soay sheep, *Ovis aries*, when a bias towards females was present during winter months, resulting in higher male mortality (Bancroft 1993). Green and Madjidian (2011) attributed this deficiency in quantifying male costs to anthropogenic influences, wherein traditional male–female sex terms are used to describe animal mating systems and behaviour.

Fishes have been widely used as model organisms to study sexual harassment, particularly those in the Poeciliidae family (Plath, Makowicz, Schlupp, & Tobler, 2007). Harassment was found to cause declines in foraging rates and fecundity in female Trinidadian guppies (*Poecilia reticulata*) (Magurran & Seghers, 1994). Guppies are livebearing fish, i.e. they exhibit internal fertilisation and have a monthly brood cycle (Evans & Magurran, 2000; Kodric-Brown & Nicoletto, 2001). Males achieve insemination by either performing courtship displays by bending their bodies into an ‘S’ or sigmoid shape in front of or near females (Liley, 1966) or by ‘sneaky’ coercive mating or gonopodial thrusts (the 3rd, 4th and 5th anal fin rays are modified to form an intromittent organ called the ‘gonopodium’) (Howell, Black and Bortone, 1980) (Guevara-Fiore, Skinner, & Watt, 2009; Mathews, Evans, & Magurran, 1997; Pilastro & Bisazza, 1999).

Coercive mating resulted in a significant decrease in foraging rates and therefore body mass in focal (harassed) females (Magurran & Seghers, 1994). These findings are supported by work on coercive mating in Atlantic mollies by Kohler et al. (2011), who found significant reductions in foraging times in female fish and potentially increased energy expenditure in the presence of males. Several other studies (Pilastro et al., 2003; Dadda et al., 2005; Schlupp, McKnab, & Ryan, 2001; Plath, Parzefall, & Schlupp, 2003) also further substantiate the conclusion that coercive mating results in reduced foraging rates in focal females. While it has been proposed that sexual coercion is independent of male size (Plath et al., 2003), Schlupp et al. (2001) found that smaller-

bodied males impose a greater fitness cost on females because, unlike their larger counterparts, they rely on sneaky mating as opposed to display-based courtship. Tobler, Schlupp, & Plath (2011) suggest that reduced feeding success in female fish may be a result of a combination of factors such as intersexual and intrasexual competition for food along with coercive mating, as opposed to solely harassment alone. Findings from the study indicated that foraging success in females was dependent on two factors, namely avoidance of unwanted mating and, interestingly, active seeking of preferred males (Tobler et al., 2011).

It is widely assumed that the unwanted attention by males is stressful for female recipients. Previous studies have explored the negative ecological and metabolic effects of male harassment on female fish (Magurran & Seghers, 1994; Ojanguren & Magurran, 2007; Schlupp, McNab, & Ryan, 2001; Tobler, Schlupp, & Plath, 2011). In contrast, Kohler et al. (2011) observed no such physicochemical signs of stress (such as reduced foraging or reduced gill ventilation) in harassed females, possibly owing to stress habituation wherein temporal reduction in stress levels occurs on repeated exposure to a stressor. The authors conclude that females have become accustomed to the constant harassment by males and so do not show symptoms of distress (Kohler et al., 2011). Other studies also found no harmful effects on direct fitness in poeciliid females (Smith & Sargent, 2006; Smith, 2007). Indeed, one might expect evolution to impose mechanisms that counter-balance the potential impacts of male harassment in species in which it is commonly observed.

Females may show behavioural strategies to reduce the burden of harassment. Pilastro et al. (2003) observed that harassment encouraged shoaling with other females because proximity to other females diluted male sexual attention, providing more time for feeding (Pilastro et al., 2003). Similar observations have been made in other studies (Dadda et al., 2005; Agrillo, Dadda, & Bisazza, 2006; Darden & Watts, 2012). However, male harassment can also disrupt female social behaviour (Wearmouth et al., 2012) and social networks resulting in increased aggression among females, which likely has direct fitness costs (Darden & Watts, 2012). Harassment might also lead to niche segregation, where females seek out microhabitats that are not available to males (Wearmouth et al., 2012).

The costs of harassment in females (especially poeciliids) have been widely explored in the literature; however, few studies (Jordan & Brooks, 2010; Gasparini, Devigili, & Pilastro, 2012) have directly measured the direct fitness costs associated with harassment (i.e. reproductive output).

For instance, one such study (which evaluated the cross-generational effects of male sexual harassment on females) (Gasparini, Devigili, & Pilastro, 2012) found that sexual harassment did not affect lifetime fecundity in females, but caused a deterioration in offspring fitness. Another study (Jordan & Brooks, 2010) evaluated the lifetime costs of increased male mating efforts in males. Moreover, the focus of sexual harassment studies has most often been on female costs (Makowicz & Schlupp, 2013). The male costs of constantly chasing and displaying to females are seldom quantified. Recent evidence (Makowicz & Schlupp, 2013) suggests that harassment can cause a reduction in both female and male body condition. The present study quantified fitness costs to both males and females by observing behavioural responses to and long-term effects of unsolicited mating attempts in a controlled laboratory setting. We measured fitness directly by recording growth, mortality and reproductive output. We also recorded changes in both male and female behaviour in mixed-sex versus single-sex groups.

We hypothesised that males bear high costs from constantly displaying to and harassing females, which may be borne out by a reduction in foraging behaviour, reduced growth rate and higher mortality. Similarly, we expected females to show reduced foraging behaviour, growth rates and reproductive output in the presence of males.

Methodology

Sexually mature male and gravid female guppies originating from a feral population in Darwin, Australia were used in this study. They were first generation, captive-reared fish held at Macquarie University; thus, there is no expectation that captivity affected their behaviour. The population was kept in the laboratory in standardised environments. All experimental procedures adhered to institutional guidelines and were approved by the Animal Ethics Committee of Macquarie University (AEC Reference No.: 2014/005-5).

Fish were sorted into two groups based on body size (small-bodied and large-bodied, referred to as 'small' and 'large' individuals, respectively) (standard length range: small males, 7.89–22.35 mm; large males, 13.08–30.13 mm; small females, 6.67–23.23 mm; large females, 13.21–35.09 mm) and then housed in groups of 6. Note that in guppies there is a strong relationship between body length and age, thus larger fish are also older. We chose small and large guppies because small female guppies tend to grow fast but have low reproductive output, whereas large guppies tend to

grow little but have large reproductive output. In this fashion, we could monitor the effects of male harassment on two primary measures of female fitness. Similarly, small males mostly rely on sneaky mating (Houde, 1997; Price & Rodd, 2006) while large males rely on courtship displays (Houde, 1997); thus, we might expect to see different costs of harassment in different sized males. Group composition varied according to treatment: mixed-sex shoals (3 males and 3 females) and single-sex shoals (6 males or 6 females). After 3 months, stored sperm numbers were expected to be limited (multiply mated females would have higher sperm stores) (Evans & Magurran, 2000), so we briefly introduced males from the single-sex shoals into the all-female shoals once a month to ensure consistent sperm quality. Furthermore, frequent remating may reduce the costs of long-term storage (Constantz, 1984).

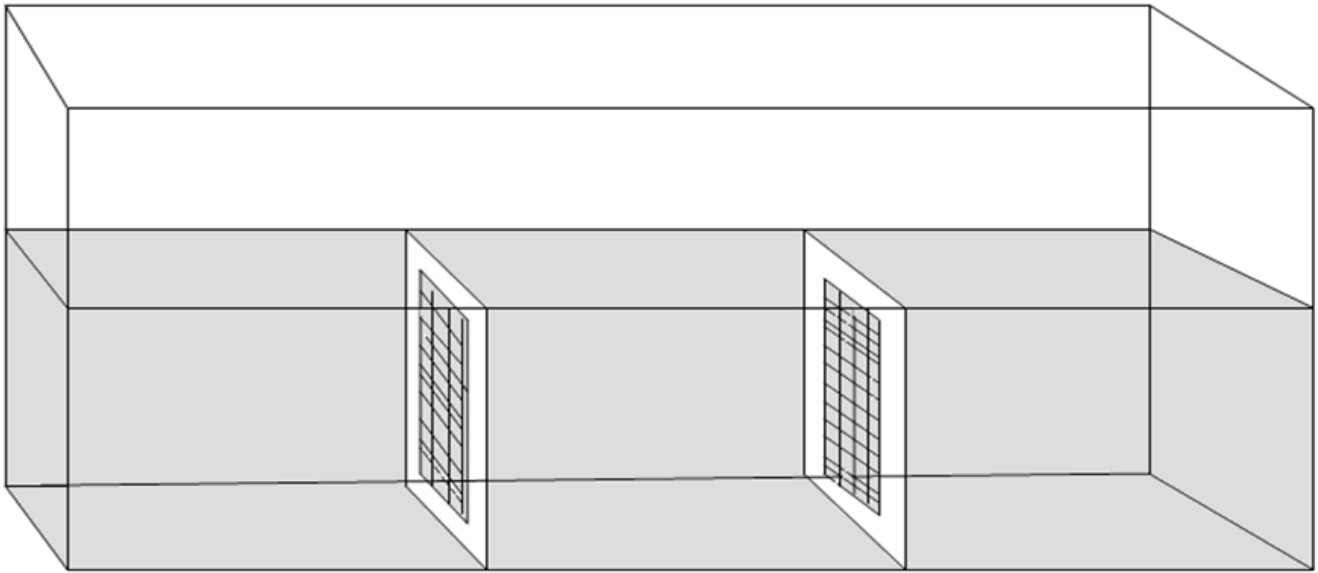


Fig. 1. Experimental tank design showing partitioning and fly mesh screens.

We used 108 guppies in this study (males, $n = 54$ and females, $n = 54$), with 6 replicates (small individuals = 4 tanks and large individuals = 2 tanks). All experiments were conducted from January to July 2015 (6 months). Individuals were housed in 6 aquaria (95 cm x 53.2 cm x 35.9 cm) with about 23 cm of water and a layer (3–5 cm) of river gravel as substrate and clumps of Java moss for cover. Each aquarium was divided into three compartments (31 cm x 53.2 cm x 35.9 cm) using fly mesh screens (Fig. 1). Each compartment was assigned to different treatments: mixed-sex shoals (3 males and 3 females) and male and female single-sex shoals (6 males or 6 females each). Fish could see and smell but could not interact with individuals in the neighbouring

compartment. Fish were fed daily on a varied diet of commercial flakes (Tropical Colour™ fish food flakes) alternated with vegetable flakes, brine shrimp and spirulina (Fluval™). Water quality was maintained using internal filters and monitored through monthly water quality testing. Room temperature was maintained at 26 °C. A photoperiod of 12:12 light–dark hours was maintained. pH was maintained at 7.6.

Individual females were identified by injecting them with a small coloured polymer-elastomer tag (VIE; Northwest Marine Technology, Inc., Shaw Island, WA, USA) in one of 6 locations while under a mild anaesthetic (MS222; White & Brown, 2013). Males were identified by their unique colouration.

Mortality rate

The number of fish that died during the experiment was tabulated. Dead fish were immediately replaced with fish of the same sex and size from stock tanks. Mortality rate was calculated by dividing the total number of deaths per month by the number of females or males in the compartment to produce a per capita mortality rate (6 for female only groups and 3 for mixed-sex groups).

Growth rate

Standard length was measured from the tip of the snout to the end of the caudal peduncle (Fink & Weitzman, 1974) at the beginning of the experiment, after three months and at the end of the 6-month experiment. Monthly growth rate was calculated (mm/month) based on these measurements. Individuals were gently netted, placed on a grid and photographed. Images were analysed using ImageJ software (version 1.48) (Ferreira & Rasband, 2012). We chose to use standard length as opposed to weight, since the latter is heavily influenced by female reproductive status. Guppies are live-bearers and heavily pregnant females change mass across their cycle.

Reproductive output

The number of young produced in each group was monitored daily. Newly born guppies were removed from the experimental tanks using hand nets and housed in a rearing tank. Reproductive output was calculated by dividing the total young produced per month by the number of females in the compartment to produce a per capita birth rate (6 for all-female groups and 3 for mixed-sex groups).

Behaviour

Daily behavioural observation (5 min) of all shoals was conducted throughout the study period at three different times of day (lunch, 12–3 pm; afternoon, 3–6 pm and evening, 6–7.45 pm) resulting in 438 observation days. An observer sat motionless 1 m from the aquarium and recorded behaviour in real time using EthoLog (version 2.2; Ottoni, 2000). An Ethogram was created with predefined behavioural categories and associated key codes (Table 1). However, only behavioural data pertaining to sexual harassment were analysed and presented herein. An individual fish was selected at random and observed for the various types of behaviour, and observations for individuals from each shoal were entered into EthoLog (Ottoni, 2000). EthoLog generated output files of each session that summarise the amount of time spent (sec) on each of the behavioural categories which were then exported into MS Excel.

Table 1. Ethogram with user-defined key codes and behavioural categories

Category	Key Code	Description
Evasion	E	Females evading male mating attempts or males avoiding female aggression
Hunting	H	Foraging at the bottom of the tank
Pursuit	P	Individuals swimming towards/chasing those of the opposite sex
Shoaling	S	Individuals are 3–4 body lengths apart

Statistical Analysis

We used ANOVA to compare mortality rates and individual growth rates between treatments. The proportion of time individuals spent conducting various behaviour categories was compared between treatments using mixed models analysis, with treatment and body size as the main factors and observation day as the random effect. A mixed model approach was also used to compare the mean number of offspring produced, with month as the random effect. Analyses were performed using SPSS (version 21) and StatView software (version 5.0.1). In most cases, behavioural data were not normally distributed and were transformed prior to analysis.

Results

Mortality rate

There were no significant effects of treatment ($F_{1,8} = 1.123$, $P = 0.320$) and body size ($F_{1,8} = 0$, $P = 0.718$) on monthly mortality in females, with no significant interactions ($F_{1,8} = 1.123$, $P = 0.320$). On average, one female died every 5 months. Mortality in males was lower in all-male shoals compared to mixed-sex shoals ($F_{1,8} = 33.333$, $P = 0.0004$), particularly in larger males ($F_{1,8} = 8.333$, $P = 0.020$) (Fig. 2). There was significant interaction between shoal composition and body size ($F_{1,8} = 5.333$, $P = 0.049$). Male mortality for larger fish in mixed-sex shoals was more than 3-fold higher than in all other contexts. There was no significant difference in total mortality between females and males ($F_{1,142} = 0.031$, $P = 0.859$).

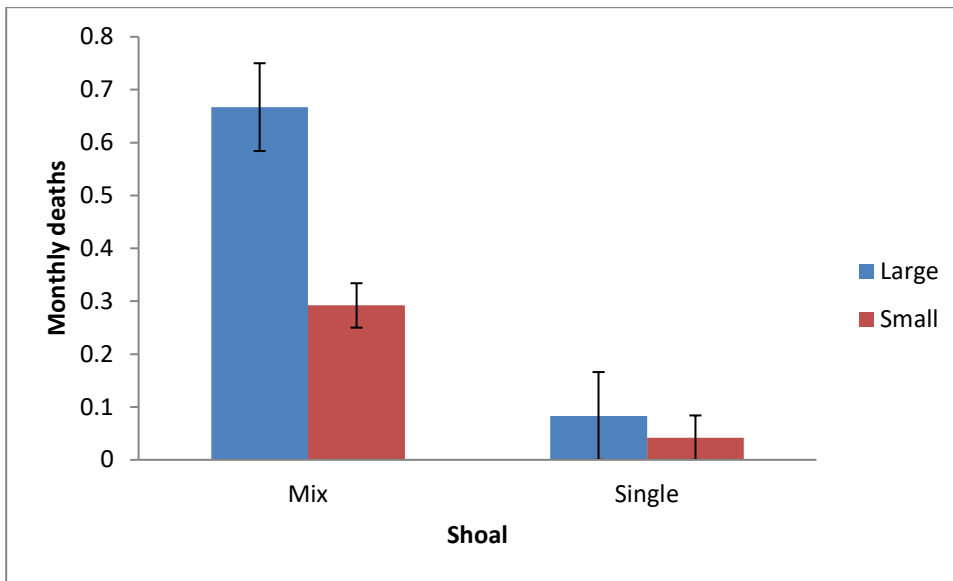


Fig. 2. Monthly mortality rates (\pm SE) in male guppies for mixed- and single-sex shoals.

Growth rate

Mean growth rate in females was not significantly influenced by body size ($F_{1,50} = 0.482$, $P = 0.491$) or treatment ($F_{1,50} = 0.188$, $P = 0.666$). There was also no significant interaction between the two factors ($P > 0.05$).

There was no significant difference in growth rates between large and small males ($F_{1,50} = 2.526$, $P = 0.118$). Similarly, there was no significant effect of treatment on male growth ($F_{1,50} = 1.950$, $P = 0.168$). The interaction between shoal and growth stage was not significant ($P > 0.05$).

Females generally grew significantly faster than males ($F_{1,106} = 42.707$, $P < 0.0001$) (Fig. 3).

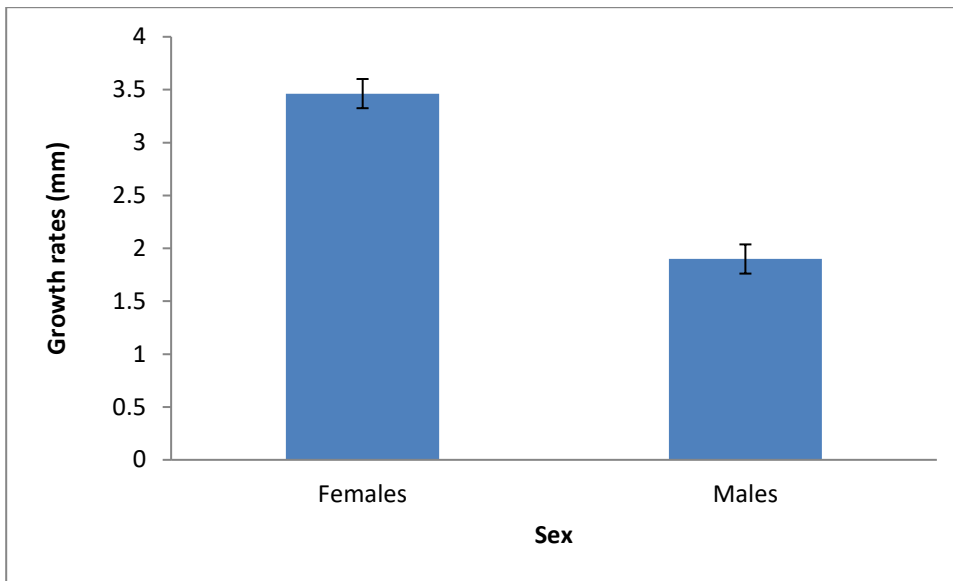


Fig. 3. Mean (\pm SE) monthly growth rates for female and male guppies.

Reproductive output

Notably, there was a tendency for mixed-sex shoals to produce more fry, but this was not statistically significant ($F_{1,74} = 1.253$, $P = 0.267$) (Fig. 4). Larger females tended to produce more fry than small females ($F_{1,74} = 3.735$, $P = 0.057$). There was no significant interaction between the two factors ($F_{1,74} = 0.50$, $P = 0.824$). Further, we found a significant variance across months ($F_{6,74} = 5.017$, $P < 0.001$). More fry tended to be produced towards the end of the experiment as the fish settled into the experimental tanks.

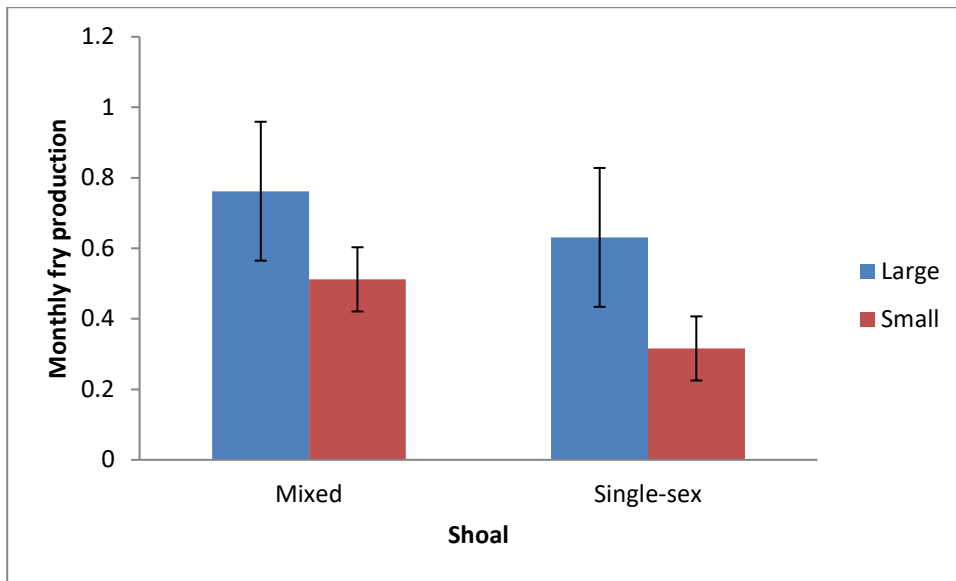


Fig. 4. Mean (\pm SE) monthly fry production between mixed-sex and all-female shoals.

Behaviour

Energy-time budgets

The time budgets for males and females respectively are summarised in Figures 5 and 6, showing the three key behaviours of interest. Visual observation suggests that there are big changes in behaviour between single- and mixed-sex groups.

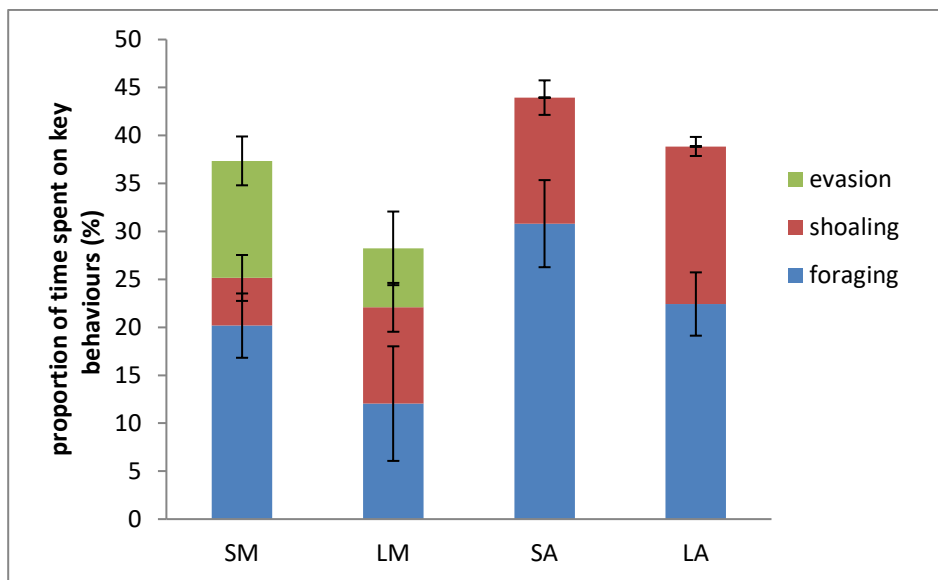


Fig. 5. Energy-time budget for females in all treatments. S = small-bodied females, L = large-bodied females, M = mixed-sex groups, A = all-female groups. Evasion is females evading sexual harassment.

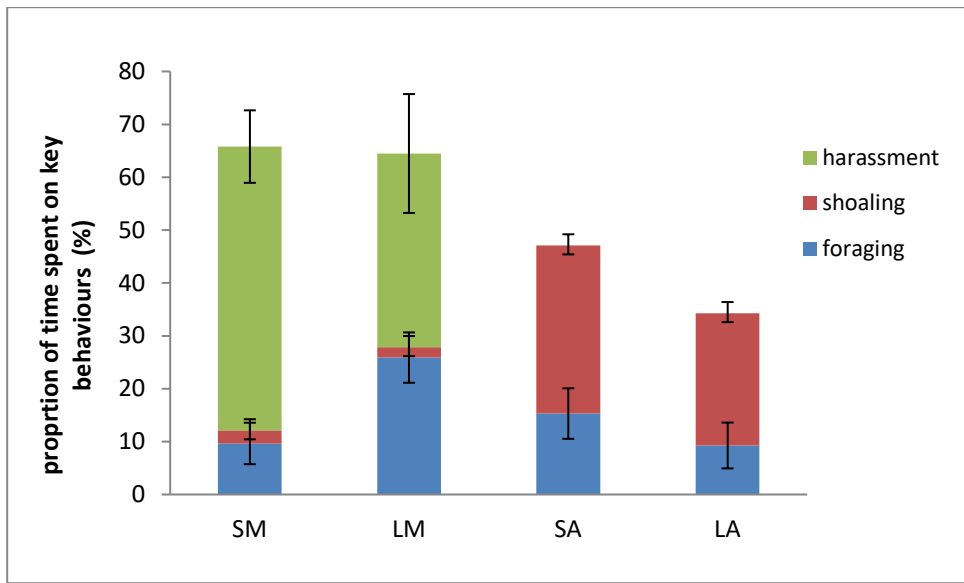


Fig. 6. Energy-time budget for males in all treatments. *S* = small-bodied males, *L* = large-bodied males, *M* = mixed-sex groups, *A* = all-male groups. Harassment refers to males harassing females.

Foraging

Females in mixed shoals spent less time foraging compared to those in single-sex shoals ($F_{1,188} = 3.897$, $P = 0.050$) (Fig. 7). Larger females tended to spend less time foraging than smaller ones (average time, 42.908 s vs. 74.774 s, respectively), but there was no significant effect of body size on foraging time ($F_{1,188} = 3.793$, $P = 0.053$) (Fig. 8). There were no significant interactions between the various factors and the time of day ($P > 0.05$). There was no significant variation across the repeated days of observations ($F_{20,188} = 1.519$, $P = 0.079$).

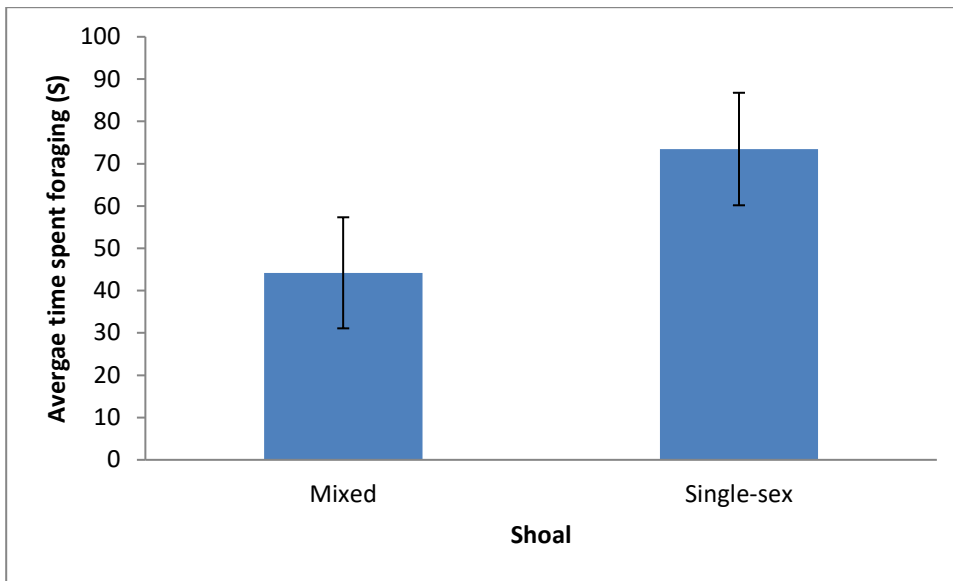


Fig. 7. Mean (\pm SE) time spent foraging in mixed- and single-sex female shoals

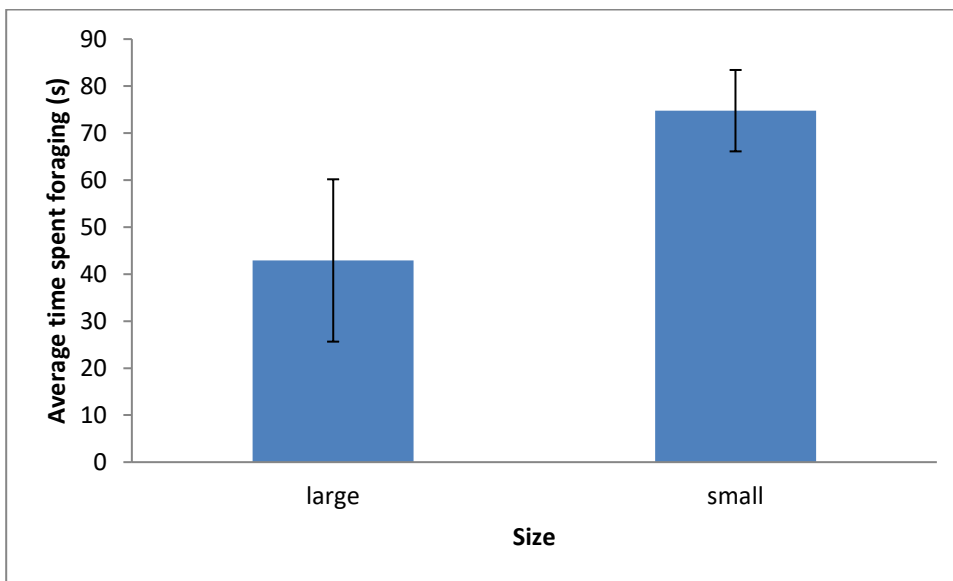


Fig. 8. Mean (\pm SE) time spent foraging for large and small female shoals

Males in mixed- and single-sex shoals spent similar times foraging (average time = 48.553 s vs. 34.848 s, respectively; $F_{1,190} = 0.957$, $P = 0.329$) (Fig. 9). Further, large males were observed feeding as often as their smaller counterparts (average time 46.1265 s vs. 37.2745 s, respectively; $F_{1,190} = 0.327$, $P = 0.568$). There was a significant interaction between shoal and body size ($F_{2,190} = 6.225$,

$P = 0.013$) (Fig. 9). Large males tended to spend more time foraging in the evenings when in the presence of females ($F_{20,190} = 1.833$, $P = 0.020$). There were no significant interactions between the other factors ($P > 0.05$). There was significant variation across the repeated days of observations ($F_{20,190} = 1.833$, $P = 0.020$).

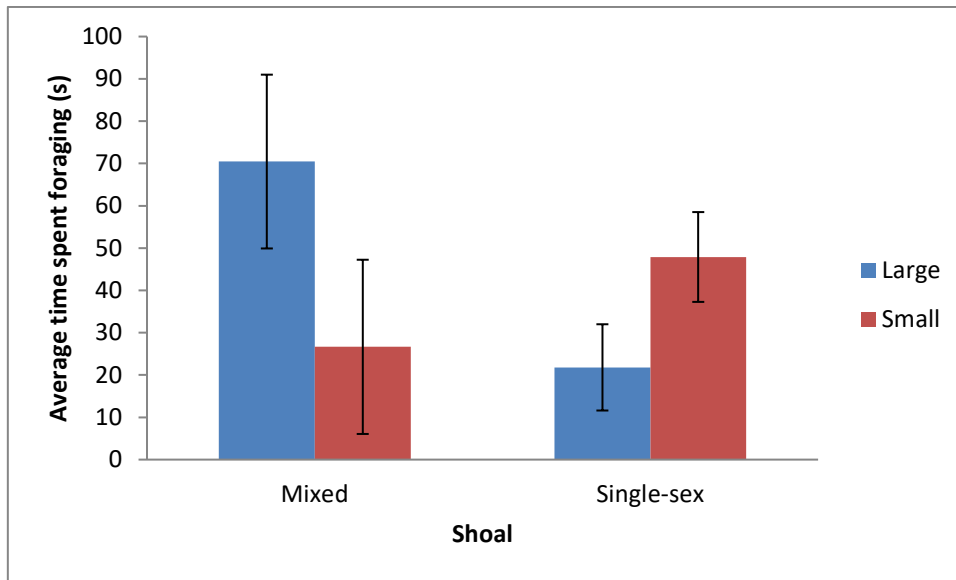


Fig. 9. Mean (\pm SE) time spent foraging for large and small males in mixed and single-sex shoals

Shoaling

Females generally spent less time shoaling when in mixed-sex groups compared with single-sex groups ($F_{1,188} = 11.888$, $P > 0.001$) (Fig. 10). Small fish tended to show lower shoaling tendencies than larger fish ($F_{1,188} = 7.790$, $P = 0.006$; 1.254 s vs. 1.558 s, respectively) (Fig. 11). Shoaling did not vary with the time of day ($F_{2,188} = 1.682$, $P = 0.189$). Smaller females also shoaled less when in the presence of males (1.236 s vs. 1.576 s), but this is true for both large and small females (1.558 s vs. 1.254 s, respectively) during lunch time observations. No other significant interactions were observed. There was no significant variation across the repeated days of observations ($F_{20,188} = 1.047$, $P = 0.409$).

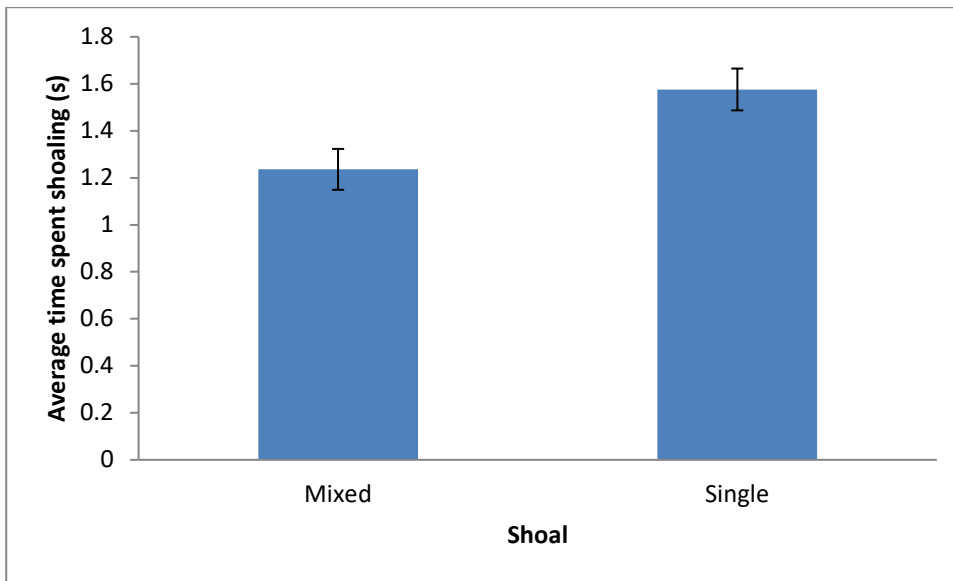


Fig. 10. Mean (\pm SE) time spent shoaling for mixed- and single-sex female shoals

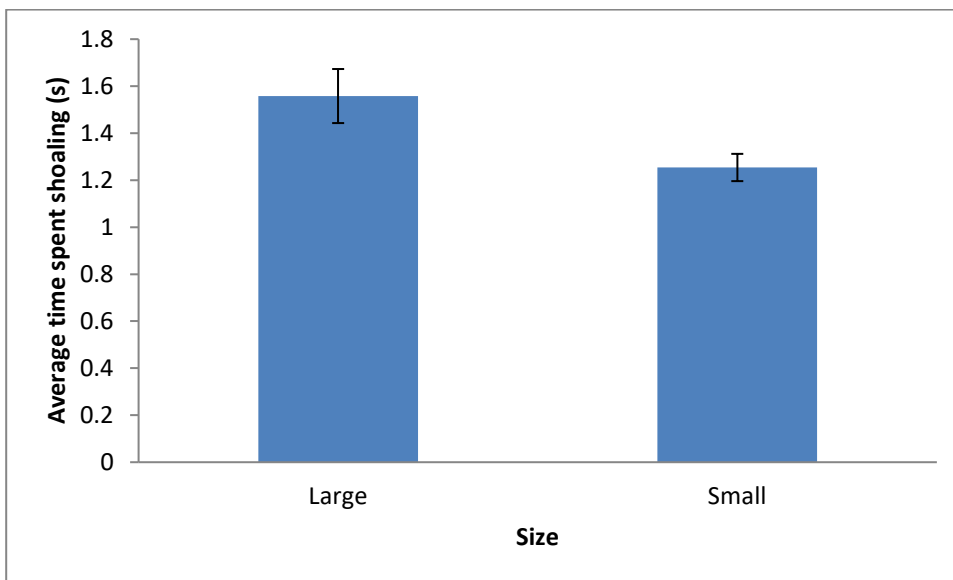


Fig. 11. Mean (\pm SE) time spent shoaling for large and small females

Male shoaling was significantly affected by shoal (treatment) ($F_{1,190} = 132.931$, $P < 0.001$) (Fig. 12). Shoaling did not vary with the time of day ($F_{2,190} = 1.530$, $P = 0.219$) and there was marginal interaction between treatment and time of day ($F_{2,190} = 2.873$, $P = 0.059$). In general, males spent more time shoaling when in all-male groups than in mixed-sex groups (1.8715 s vs. 0.9225 s, respectively), and this was most apparent during lunch time observations. No other significant interactions were observed ($P > 0.05$). There was significant variation across the repeated days of observations ($F_{20,190} = 1.989$, $P = 0.009$).

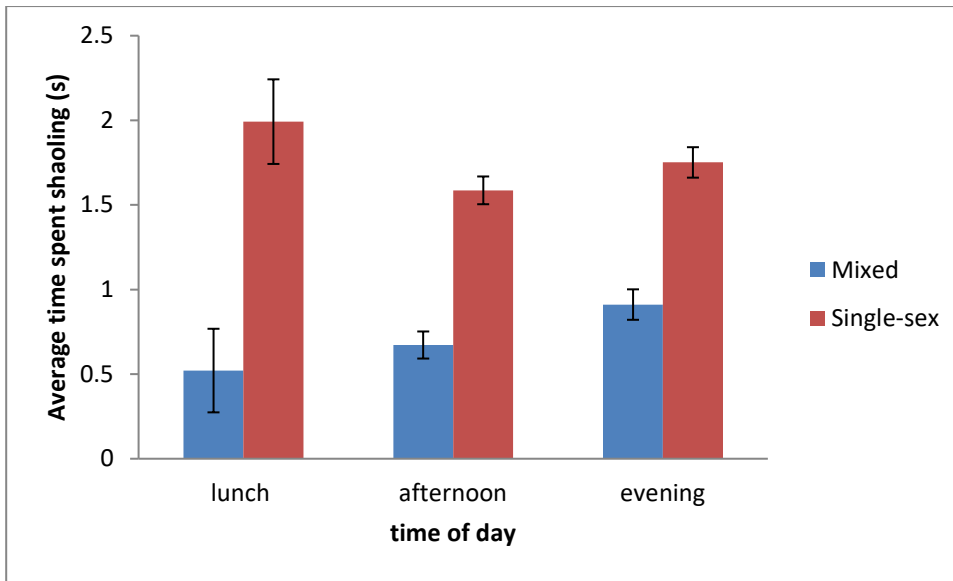


Fig. 12. Mean (\pm SE) time spent shoaling throughout the day for mixed- and single-sex male shoals

Harassment and evasion

Since harassment is generally a male behaviour and is orientated towards females, and evasion is typically the female response, only data from mixed-sex groups can be analysed. There were only 2 incidences of harassment initiated by females out of 111 observations, so we analysed data from males alone. There was only 1 recorded instance of a male evading violence from a large female out of all the observations, so we analysed evasion data for females alone.

Large and small males did not differ in the amount of time spent harassing females ($F_{1,86} = 1.857$, $P = 0.177$), although smaller individuals tended to spend more time harassing females than larger ones (proportion of time = 53.69% vs. proportion of time = 36.65%, respectively) (Fig. 13). There was a significant effect of time of day on harassment ($F_{2,86} = 8.162$, $P = 0.001$); males were most likely to harass females at lunch time. There was no significant interaction between size and time of day ($P > 0.05$). There was no significant variation across the repeated observations days ($F_{20,86} = 1.562$, $P = 0.082$).

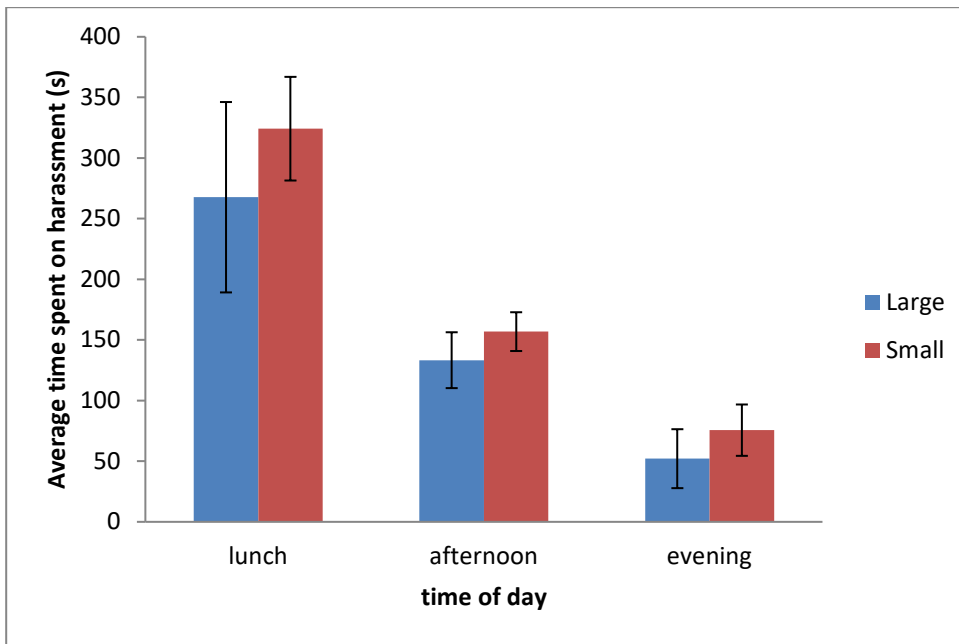


Fig. 13. Mean (\pm SE) Proportion of time spent on harassment throughout the days for large and small males

Equally, large and small females spent the same amount of time evading harassment ($F_{1,85} = 3.476$, $P = 0.066$), with smaller females tending to spend more time evading mating attempts than their larger counterparts (proportion of time, 12.20% vs. 6.15%, respectively) (Fig. 14). There was no significant effect of time of day and no significant interaction between size and time of day ($P > 0.05$). There was no significant variation across the repeated observations days ($F_{20,85} = 1.253$, $P = 0.234$).

When males and females were housed together, females spent 9.17% of their time evading mating attempts on average, whereas males spent 45.17% of their time budget harassing females.

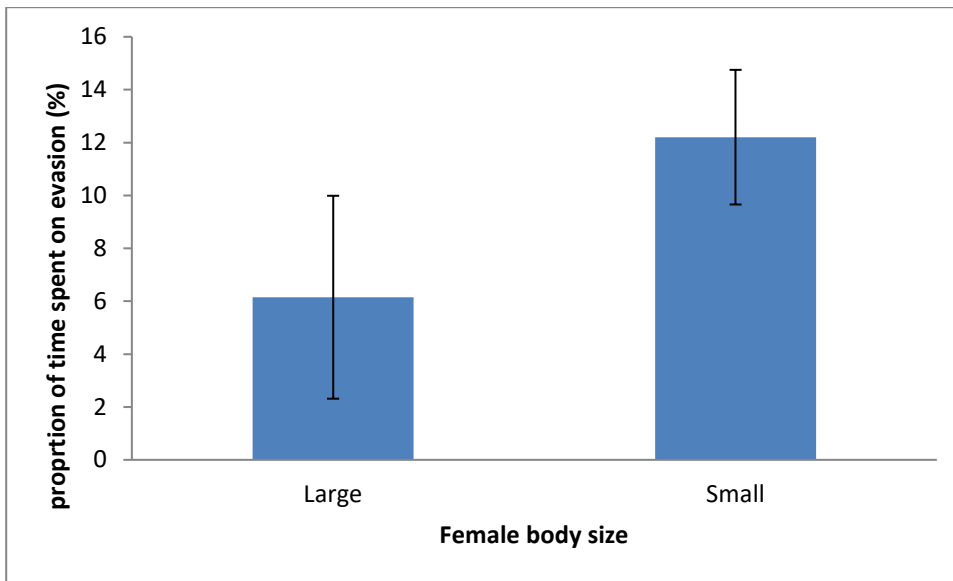


Fig. 14. Mean (\pm SE) Proportion of time spent on evasion for large and small females.

Discussion

Our findings suggest that, under laboratory conditions, sexual harassment has fatal direct costs to males unlike their partners, who are seemingly little affected by the constant unsolicited mating attempts. Unlike previous studies that tend to focus on harassment costs to females, we have also quantified the direct costs of harassment to males. While harassment clearly altered the time budget of both males and females, we found no significant differences in growth rates or birth rates between treatments. Both males and females in mixed-sex groups tended to spend less time shoaling than in single sex groups. Females also spend less time foraging in mixed-sex shoals. Thus, females seem to lose social cohesion and foraging opportunities as a result of male harassment. Overall, it appears that females incurred few direct costs of harassment in natural mixed-sex shoals, whereas male mortality was significantly higher under these conditions.

Sexual conflict in mating systems often results in an uneasy understanding, with both parties sharing a common goal of transmitting genetic material to the next generation (Arnqvist & Rowe, 2013). Male and female traits are believed to coevolve through either antagonistic coevolution or the Fisherian model of cryptic female choice (Evans, van Lieshout, & Gasparini, 2013). Females appear to have overcome the direct costs of harassment by maturing early and prioritising feeding to grow at a much faster pace than males, thus enabling them to withstand constant male advances,

particularly at the latter phase of their life cycle when females significantly outgrew males. This size advantage appeared to deter male harassment to a certain extent, although not significantly so. Conversely, at the early stages of their life cycle, when there is negligible difference in body size between the sexes, females experience comparatively higher levels of harassment. In addition to faster growth, females also appear to be habituated to the constant stressor of harassment (Kohler et al., 2011). Natural selection has acted to buffer the effects of harassment in multiple ways. For example, cerebral lateralization is much stronger in females compared to males. Dadda and Bisazza (2006), in their study on a poeciliid fish goldbelly topminnow *Girardinus falcatus*, showed that female fish could partition different tasks in different parts of their brains, enabling them to forage and avoid unwanted male attention simultaneously. Females were also found to be less bold than males (poeciliid *Brachyrhaphis episcopaei*, Brown, Burgess, & Braithwaite, 2007; *Poecilia reticulata*, Piyapong et al., 2010) and were harder to capture, which could be a result of lateralisation as well.

Males appear to bear comparatively higher costs—in terms of high mortality rates—of sexual coercion. These results support those of the previously mentioned study by Jordan & Brooks (2010), in which males were found to incur significant lifetime growth costs as a result of coercive mating. It is noteworthy that these costs are undoubtedly offset by increased mating success. However, the reverse maybe true if indirect costs are taken into consideration. According to Evans (2012), estimates of a female's lifetime reproductive success (LRS) are useful to identify any indirect costs (in addition to direct ones). Since the present study was designed to quantify direct, short-term fitness costs, its longevity did not allow for the manifestation of any such long-term, indirect costs (although 6 months is a substantial proportion of a guppy's life). So although females bear few direct costs, although unlikely, they may incur indirect costs in terms of long-term reproductive potential. Available literature pertaining to LRS provides mixed results with regard to female costs (Evans, 2012; Gasparini, Devigili, & Pilastro, 2012). It is noteworthy, however, that neither fry output nor growth rate was hindered in the presence of males over the 6-month study. Females in the single-sex treatments were expected to have higher reproductive outputs. One possible explanation for this discrepancy could be intrasexual competition. Borg et al. found similar results in their study on female guppies subjected to different treatments—exposure to larger or smaller female conspecifics (competitive treatments) and solitary treatment (Borg, Rosenqvist, Amundsen, & Forsgren, 2006). They found that solitary females had higher reproductive outputs when compared to those housed with other females, which was attributed

to higher energetic costs due to social interactions in the competitive treatments. Another study by Borg et al. found that female–female competition may affect resource budgets, and consequently, reproductive rates (Borg et al. 2012). Additionally, egg maturation (Borg, Rosenqvist, Amundsen, & Forsgren, 2006; mosquitofish, Lutnesky & Adkins, 2003) was found to be hampered by the presence of other females likely due to chemical inhibition.

Arnqvist & Rowe (2013) observed that parents do not have genetic interests in each other since they are unrelated, yet are equally genetically invested in their progeny. In terms of direct costs, males may appear to be at a disadvantage in that they incur comparatively higher costs of harassment than females; however, they gain significant benefits in terms of maximising paternity. Further, a study by Smith (2007) in Western mosquitofish *Gambusia affinis* indicated that females may have a greater propensity for cannibalising their offspring compared to males; the same may hold true for guppies and warrants further study. It is possible that males, although not the most cooperative mates (Smith, 2007), may inadvertently be more benevolent parents to fry owing to their less voracious appetites and smaller body size. This may, however, be a reflection of captive studies where fry have limited opportunity to disperse and escape from their mothers.

Notably, the experimental design resulted in changes in intrasexual competition, with males and females experiencing higher competition from members of their own sex when in single-sex shoals. Intrasexual aggression is energetically costly when resources are limited. Makowicz & Schlupp (2013) found that intrasexual aggression owing to competition resulted in a decline in male body condition. It is important to note, however, that the fish in our study were fed till satiated, so competition was likely minimal.

Conclusions

Our results suggest that sexual harassment appears to have significant direct costs to males in the form of higher mortality with limited direct costs to females. The loss of foraging opportunities by females did not seem to hamper growth or reproductive output over the 6 month study. It may be the case that females incur indirect costs that could be identified in a multigenerational study. We suggest that natural selection may shape female behaviour and life-history strategies in such a manner as to reduce the cost of constant male harassment. This topic could form the basis for future research.

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Appendices

Appendix I. Highlights

Highlights

- We measured direct fitness (growth/reproductive output changes) in both sexes.
- No effect of harassment on females in natural, mixed-sex shoals.
- Males showed higher survival when housed with females.
- Males bear significant costs of harassment.

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If the test statistic is conventionally quoted with degrees of freedom, these are presented as a subscript to the test statistic. For example:

ANOVA: $F_{1,11} = 7.89, P = 0.017$

Kruskal-Wallis test: $H_{11} = 287.8, P = 0.001$

Chi-square test: $\chi^2_2 = 0.19, P = 0.91$

Paired t test: $t_{12} = 1.99, P = 0.07$

If the test is conventionally quoted with the sample size, this should follow the test statistic value. For example:

Spearman rank correlation: $r_s = 0.80, N = 11, P < 0.01$

Wilcoxon signed-ranks test: $T = 6, N = 14, P < 0.01$

Mann-Whitney U test: $U = 74, N_1 = N_2 = 17, P < 0.02$

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