

Assessing the cues required for mate choice copying in the plainfin midshipman fish

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When choosing a mate, females can rely on their own judgements of male quality or use social information from other females' choices. The use of social information to inform mating decisions is called mate choice copying. Theory predicts that mate choice copying should be strongest in species where females have few mates over the course of their life span because each mating constitutes a greater proportion of the female's expected reproductive value; however, most research on mate choice copying has thus far focused on species with highly promiscuous females. In this study, we use the plainfin midshipman, *Porichthys notatus*, a toadfish in which females typically choose one mate per year, to investigate whether females mate-choice copy and, if they do, which cues influence their decision making. We show that in the wild, plainfin midshipman females co-occur in nests more often than expected under random female choice. Additionally, we found that females in the laboratory did not base their mating decisions on the mere presence of another female or previously laid eggs; however, females were more likely to choose a male they had observed spawning with another female. Taken together, our results indicate that female plainfin midshipman do mate-choice copy, but only when they observe a spawning event. Understanding how different mating systems affect the strength of mate choice copying and which cues are necessary to elicit mate choice copying will help elucidate more broadly how this behaviour evolved and is maintained.

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Few decisions affect a female's fitness as strongly as her choice of mates (Barbosa & Magurran, 2006). A female's mate choice can elicit direct benefits, such as access to good territories, nuptial gifts and paternal care, or indirect benefits, such as good genes passed on to her offspring (Kirkpatrick, 1996; Tazzyman et al., 2012; Trivers, 1972). In general, females use their own experience and evaluations of mate quality; however, if discerning quality is challenging, they can use the public information provided by other females' choices to increase the likelihood of choosing well (Dugatkin, 2005; Nordell & Valone, 1998). This phenomenon, called mate choice copying, occurs if an observer perceives a potential mate as being more attractive after detecting cues in the environment that suggest the potential mate has been chosen

previously by others, increasing the likelihood of the observer choosing them as well (Pruett-Jones, 1992). The cues necessary to elicit mate choice copying vary from species to species and can include females simply observing other females near a male (Moran et al., 2013), observing a male caring for offspring of a previous female (Alonzo, 2008), or observing a male and a female in the act of mating (Grant & Green, 1996).

Mate choice copying is thought to be favoured under two circumstances. First, when an individual is unable to accurately discriminate quality among possible mates (Gibson & Höglund, 1992; Nordell & Valone, 1998; Vakirtzis, 2011), perhaps due to the individual's youth or lack of mating experience (Jones & DuVal, 2019). Second, individuals are more likely to mate-choice copy when the costs associated with mate assessment are particularly high because searching and mate comparison are energetically expensive or time-consuming, or lead to a high risk of predation. By copying the choices of others who have already suffered assessment

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costs, copiers can avoid having to pay these costs themselves (Dugatkin, 2005; Gibson & Höglund, 1992; Vakirtzis, 2011).

The benefits of mate choice copying may also stretch beyond improving mate discrimination, particularly in species with paternal care. Laying eggs in the same nest as previous females increases the total brood size of the caring male, which may increase his parental investment (Coleman et al., 1985; Sargent, 1988) and reduce the likelihood of him abandoning (Balshine-Earn & Earn, 1998; Bose et al., 2014) or fully cannibalizing his brood (Bose, 2022). Similarly, mate choice copying could lead to mating and brood development synchrony, enhancing the survival of a female's offspring by diluting predation risk during particularly vulnerable stages of development (Ims, 1990). Thus, mate choice copying may improve a female's fitness regardless of whether it leads to her choosing the highest-quality male.

However, mate choice copying is not always necessarily beneficial. If the first female to mate chooses poorly, then a copier risks making an equally poor mate choice (Dugatkin, 2005). Copiers could also risk choosing a sperm-depleted mate (Gibson & Höglund, 1992; Witte & Massmann, 2003): if the chosen male has recently mated, he may not have sufficient sperm to fertilize all the female's eggs. Copying can potentially increase the time spent mating, particularly when males can only mate with one female at a time and thus the copier must wait to gain access to the male (Gibson & Höglund, 1992). We would expect to observe mate choice copying only in species and individuals where the benefits of copying outweigh the potential costs.

Theory predicts that when individuals have few mates, each mating is relatively more important, and so individuals should be choosier (Davies et al., 2020); therefore, we expect to observe stronger mate choice copying in species where females have few mates compared to species where females mate with many partners. Most studies on mate choice copying, however, have been conducted on species with highly promiscuous females and so there are little data on mate choice copying in species where females have few mates (Davies et al., 2020; Scauzillo & Ferkin, 2019). We aim to fill this gap in knowledge by studying mate choice copying in plainfin midshipman, *Porichthys notatus*, a species where most females mate once per year.

Porichthys notatus, also known as the plainfin midshipman, is a species of toadfish that reproduces in the intertidal zone of the North American Pacific Coast (Arora, 1948). There are two male reproductive tactics in this species: sneakers and guarders. Sneaker males are obligate cuckolders and steal fertilizations from guarder males (Brantley & Bass, 1994). Guarder males compete with one another to acquire and retain nests excavated beneath rocks, produce acoustic courtships hums to attract fecund females and provide parental care (Brantley & Bass, 1994; Cogliati et al., 2013). Females generally prefer larger guarder males (Bose et al., 2018; DeMartini, 1988). They are thought to spawn only once per season for several reasons: (1) females' ovaries never contain eggs in more than one developmental stage (DeMartini, 1990; A. Harrison-Weiss, personal observations); (2) when females are permitted sufficient time to finish spawning, they will empty their ovaries (A. Harrison-Weiss, personal observations); (3) when females are given the option of spawning with two males, they will almost exclusively deposit all their eggs in a single nest (A. Harrison-Weiss, personal observations); (4) females are unlikely to have the time or energy to produce more than one clutch per year since their eggs are large (4.5–6.4 mm in diameter; Brown et al., 2021) and energetically expensive to produce (DeMartini, 1990). Once a female has chosen a guarder male to spawn with, they individually adhere each of their ~90–300 eggs to the ceiling or side of his nest cavity (Brantley & Bass, 1994; Brown et al., 2023; DeMartini, 1990) before returning to sea and leaving the guarder

male to provide sole parental care (Arora, 1948; Brantley & Bass, 1994).

There are numerous reasons why female plainfin midshipman could benefit from mate choice copying. Females pay extremely high costs if they select a poor mate. Given that they entrust all, or at least the majority, of their eggs to a single guarder male per season, their reproductive success depends wholly on this male's nest defence and parental care (Bose et al., 2016; Brown et al., 2021). Yet, accurately discriminating quality among the many guarder males on the spawning grounds may be challenging. Guarder male courtship hums appear to be honest signals of quality, conveying information about male body size and condition to listening females (Balebail & Sisneros, 2022). Males that invest more heavily into sonic musculature also generally attract more females to their nests (Bose et al., 2018); however, females will often enter a male's nest and exit without spawning (Brantley & Bass, 1994; A. Harrison-Weiss, personal observations). This observation suggests that females may use additional information other than the male's humming to inform their mating decision. Additionally, guarder males cease humming shortly after a female enters their nests (Brantley & Bass, 1994). As a result, early arriving females may have more information, the male's song, from which to judge male quality compared to subsequent females. Thus, later arriving females may choose to mate-choice copy rather than rely on their own more limited information about the males' quality.

Female plainfin midshipman may also be unable to extensively sample mates because search costs are particularly high in the intertidal zone. Females face considerable risk of avian predation when moving between nests (Haupt, Bose, et al., 2020) and desiccation if they become trapped by the receding tide (Bose, Borowiec, et al., 2019). Since activity in the intertidal zone is restricted to the hours when nests are submerged, the tidal cycle imposes strict time limits for assessing the quality of multiple males (Craig et al., 2014; Gibson & Langen, 1996; Haupt, Borowiec, et al., 2020). Taken together, female plainfin midshipman would be expected to benefit greatly from mate choice copying as it can reduce the need for extensive and hazardous mate sampling.

In this study, we used multiple approaches to investigate whether female plainfin midshipman mate-choice copy. First, we used a large data set of field nest observations collected over multiple years and field sites to investigate the degree to which spawning females co-occur together in male nests. We controlled for male characteristics that females find attractive, to help clarify whether females in the wild may be making spawning decisions that are based on the actions of other females. Second, we conducted a series of laboratory experiments in which we systematically presented observer females with various cues of previous females' mate choices and then documented the observers' subsequent mating decisions. We tested whether females prefer to spawn with males whose nests contain other females over males with empty nests. We also tested whether females prefer to spawn with males whose nests contain eggs over males whose nests contain no eggs. Finally, we tested whether females prefer to spawn with males that they previously observed spawning with a demonstrator female. We predicted that females would prefer spawning with males in nests containing other females, with males whose nests contained previously laid eggs and with males they had previously observed mating with a demonstrator female.

METHODS

Field Study: Do Females Co-occur in Nests?

Between May and July (the *P. notatus* breeding season) of 2010, 2011, 2013 and 2015, we surveyed plainfin midshipman nests

found in the intertidal zones of nine field sites in British Columbia, Canada, Washington, U.S.A. and California, U.S.A. (for details of field sites, see Bose et al., 2018; Cogliati et al., 2014). At these beaches, we carefully overturned intertidal rocks to find nests with a guarder male and we recorded whether the male was guarding a brood of eggs or hatched embryos. If any other fish (females or sneaker males) were present in the nest, we recorded this information. We measured the nest-guarding male for standard length (to the nearest 0.1 cm) and body mass (to the nearest 0.1 g). These data have been previously published in Bose et al. (2018), but are reanalysed here to answer a new research question, namely how often do females co-occur together in male intertidal nests?

Laboratory Experiments: Do Females Mate-choice Copy and Which Cues Do They Use?

Fish collections, laboratory housing and experimental conditions

Our laboratory experiments were conducted over a 2-year period between May and July 2022 and 2023. In total, we collected 77 guarder male and 102 female plainfin midshipman from four intertidal field sites along the coasts of Vancouver Island and mainland British Columbia, Canada: Ladysmith Inlet (49°01'N, 123°83'W), Maple Bay (48°82'N, 123°62'W), Lantzville Beach (49°25'W, 124°07'W) and Crescent Beach (49°04'N, 122°88'W). The fish were transported in aerated, opaque plastic bins to the University of Victoria's Outdoor Aquatic Facility. Water temperature and oxygen were checked during transport. At the facility, guarder males and females were housed separately in outdoor 400-litre fibreglass tanks, each supplied with circulating filtered sea water (13 °C), a pea gravel substrate and one brick shelter per fish. Although reproductive plainfin midshipman cease feeding during

the breeding season (Bose et al., 2015; Cogliati et al., 2015), fish were offered a mixture of trout pellets and frozen shrimp once weekly to comply with university animal care recommendations.

The experiments were conducted in 16 175-litre indoor tanks (56 × 47 × 91 cm; width × height × length) supplied with free-flowing, filtered sea water (maintained at 13 °C) and lined with gravel. The lights were kept on a 12:12 h light:dark cycle. Because plainfin midshipman usually remain in dark nests under rocks, we covered all sides of the tank in black plastic sheeting to reduce ambient light. Each tank contained two identical nests, one on the right and one on the left, assembled out of eight 10 × 20 × 5 cm (width × length × height) bricks. Males were measured (body mass (g); standard and total length (cm)) and paired based on similar body size before being added to a tank. We used mass as an overall indicator of body size and calculated a percentage size difference for each pair (percentage size difference = difference in mass between the males/average mass of the two males × 100%). The males were given ~ 1 day to settle into the tank and select a nest before females were introduced.

Experiment 1: presence of a female inside the male's nest (cue 1)

Once both males had taken up a nest, a clear Plexiglas tube (15 cm long, 8 cm diameter) with drilled holes was inserted into each nest (Fig. 1): one tube was empty (control) while the other tube contained a stimulus female fish (treatment). The tubes' open ends were covered in netting secured with zip ties, which allowed for visual, acoustic and olfactory cues to be transmitted, and the tubes were secured in the nest with bricks so that the males could not push them out of their nests. A random number generator determined which nests (left or right) the treatment and control tubes were inserted into. After the tubes had been inserted, one

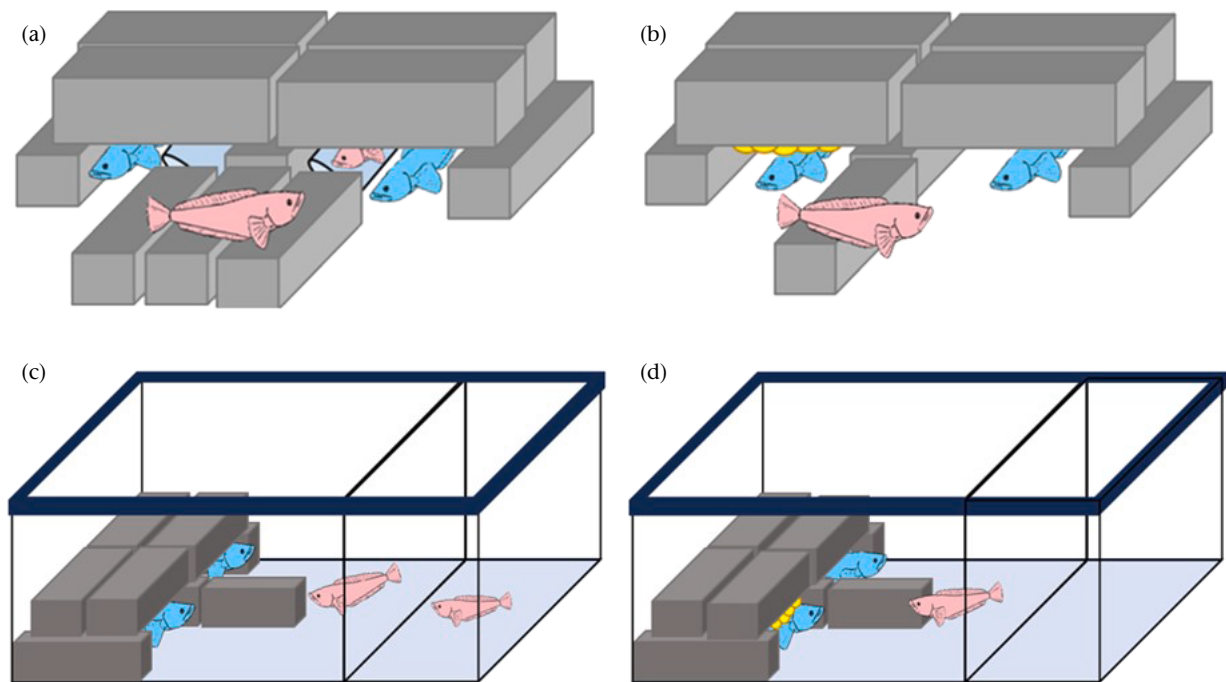


Figure 1. Experimental set-ups. (a) Experiment 1: presence of a stimulus female. For 3 days, focal females (in pink) were given the opportunity to spawn with one of two size-matched males (in blue). One of the males had an empty tube in his nest while the other male had a tube with a stimulus female in his nest. The tubes were secured in the nests by bricks that ensured the males could not forcibly eject them from their nests. These extra bricks were not used in experiment 2 or 3. (b) Experiment 2: presence of eggs. Females (in pink) were given the opportunity to spawn with one of two size-matched males (in blue). One of these males had a nest containing eggs (in yellow) and the other male had a nest containing no eggs. (c, d) Experiment 3: Female demonstrator. (c) A demonstrator female (in pink) was allowed to spawn with one of two size-matched males (in blue) while a second (and sometimes third) observer female could watch the spawning. (d) Once the demonstrator female had spawned, she was removed, and the observer female was then brought across the barrier. Observer females were given 3 days to spawn with either the male she had previously observed spawning or the male that had not spawned.

fecund focal female ($N = 24$) was added to each tank and given up to 3 days to spawn with either the male in the nest with an empty tube or the size-matched male in the nest with a tube containing a stimulus female. We recorded when the focal female laid eggs in either nest. If the focal female had not spawned after 3 days, she was removed from the tank. The stimulus female was also removed and replaced by a new stimulus female. A new fecund focal female was then added to the tank and a new trial was started. Note that not all trials resulted in a spawning, even after replacing the stimulus and focal females. As female plainfin midshipman spawning can last 20 h (Brantley & Bass, 1994), in all three experiments any focal female observed spawning (in an upside-down/inverted position) was given another full day to finish spawning and exit the nest before removing her, even if the spawning occurred on day 3. After spawning ended, the focal female and the two tubes (one empty and one with the stimulus female) were removed from the tank. The bricks with adhered eggs laid by the focal female were then removed, and the eggs were photographed with an Olympus Tough TG-6 camera.

Experiment 2: presence of eggs inside the male's nest (cue 2)

To determine whether females use eggs as a cue on which to base their mate choice copying, we conducted a second experiment. We used a similar tank set-up as the one employed in experiment 1. In this second experiment, eggs that had been laid in the previous experiment served as the stimuli. The top bricks were briefly removed, all eggs were photographed and the outline of the eggs on the brick was traced onto an acetate sheet with a permanent marker for later comparison. Bricks were then replaced so that one nest in each tank contained eggs and the other nest contained none. Whenever possible, we returned the eggs to the parental male; however, sometimes it was necessary to move eggs to a new male's nest. Previous research indicates that guarder males are unable to distinguish the paternity of eggs based on direct cues and that guarder males are willing to provide alloparental care to unrelated eggs (Bose et al., 2016). There were no indications throughout the trials that eggs returned to their parental male fared differently than those given to a new male. One fecund focal female was introduced to each tank ($N = 26$) and given up to 3 days to spawn with either the male whose nest contained eggs (treatment) or the male whose nest contained no eggs (control; Fig. 1b). The nests were inspected daily and any bricks with eggs were compared against the original acetate outline to determine whether there was a change in egg number. We recorded any reductions in egg number due to cannibalism or egg death and any additions in egg number due to spawning. If any new eggs were discovered, the eggs were immediately photographed and their outline was added to the original acetate sheet. We also inspected the bricks from the empty nest daily to ensure that equal disturbance occurred to both nests. Once a focal female had spawned, she was removed from the tank.

Experiment 3: observation of male spawning with a demonstrator female (cue 3)

In experiment 1, the stimulus females were kept in tubes and, thus, could not spawn with the guarder male. It is possible, however, that an actual spawning event is necessary to demonstrate mate choice for another female to copy (e.g. Grant & Green, 1996). To address this possibility, we ran a third experiment using a similar nest set-up to experiments 1 and 2. Again, each tank contained two side-by-side nests and a pair of size-matched males. In this third experiment, a clear Plexiglas divider with 30–40 2.5 cm diameter holes was added to each tank about 90 cm from the back (the side of the tank farthest from the nests; Fig. 1c). We then added up to three females to each tank: a single large

demonstrator female ($N = 24$) was placed in front of the transparent divider and one or two smaller focal observer females ($N = 26$) were placed behind the divider. Thus, the demonstrator females had access to the two males and their nests, while observer females could see, smell and hear the activity between the demonstrator female and the males in the tank. Demonstrator females were given up to 3 days to spawn with either of the two size-matched males while the focal females could observe the spawning (Fig. 1c). If the demonstrator female did not spawn after 3 days, all of the females were replaced with new fecund females. Once the demonstrator female had spawned in one of the two nests, she was removed. Any bricks with spawned eggs were also removed so they could be photographed and traced onto an acetate sheet for later comparison. Bricks from the opposite nest (the one without eggs) were also similarly handled, to control for handling disturbance. All bricks were then returned to their original nests. Next, we brought the largest of the two observer females across the barrier and gave her access to the two guarder males. This focal female was then given up to 3 days to either spawn with the same male as the demonstrator female (treatment) or the other male not chosen by the demonstrator female (control). After the first observer female had spawned, she was removed and the second observer female was moved across the barrier and given the opportunity to spawn. Nests were checked for new eggs daily by comparing the clutches against the acetate outline and any new eggs were photographed and traced. If the demonstrator female and first observer female spawned in different nests (meaning that the second observer female observed other females spawning in both nests and, therefore, could copy either decision), the second observer female trial was not run. This occurred four times over the course of the experiment.

Guarder male size matching for experiments 1–3

Matching was done visually at the start of experiment 1, but then the paired males were weighed at the end of their trials. For both experiment 2 and 3, males were weighed at the start and end of their trials. The size-matched males differed by a median $\pm$ SE 7.2 ± 5.7 g ($\sim 5\%$ of their body mass) at the start of experiment 2 and by 6.6 ± 4.0 g ($\sim 5\%$) at the start of experiment 3. At the conclusion of each experiment, males differed by a median of 6.8 ± 9.8 g ($\sim 8\%$) in experiment 1, 9.4 ± 9.0 g in experiment 2 ($\sim 8\%$) and 9.7 ± 9.4 g ($\sim 6\%$) in experiment 3. We examined what proportion of females chose to spawn with the larger of the two males in each experiment.

Statistical Analysis

All analyses were conducted in R (version 4.3.2; R Core Team, 2023). All model residuals were assessed for normality and heteroscedasticity (using the DHARMA package version 0.4.6; Hartig, 2022).

Field study

For the field nest occupancy data, we fitted a generalized linear mixed effects model (GLMM) assuming a negative binomial family with a 'log' link function to the number of females found in each nest on a given sampling day (using the 'glmmTMB' R package; Brooks et al., 2017). We included time in the breeding season (Julian date, continuous variable) and male standard length (in cm) as predictor variables. We also included random intercepts of study site and year to account for nonindependence arising from sampling multiple nests per site and year.

Next, we tested whether the observed distribution of females across male nests on any given day differed from random chance. That is, we asked whether females were observed to co-occur

together more often than if female choice had been completely random. To do this, we first extracted the model residuals from the above fitted GLMM. Positive residuals reflect cases where a greater than average number of females were found in a nest together (after accounting for the male body size and time in the season), while negative residuals reflect cases where fewer than the average number of females were found together in a nest. We calculated the variance of these residuals as a measure of how much spread there was in the female co-occurrence data. High variance can arise, for example, when all females that are mate searching on a given day choose the same male, thereby leaving the other nests empty. Alternatively, low variance arises when each female chooses a different male. We then repeated this procedure, but with permuted data. To permute the data, for each day sampled at a particular field site, we randomly redistributed the females among all the nests (however, we limited the number of co-occurring females to four per nest, which was the maximum in our observed data). We fitted a GLMM with the same structure as above to the randomized data, extracted its residuals, calculated their variance and repeated this 1000 times. We then calculated a P value based on the proportion of randomizations that produced variance estimates greater than our observed value.

Laboratory experiments

For all three laboratory experiments, we used an exact binomial test to compare the number of females that chose to spawn with the male in the treatment nest compared to the number that chose the male in the control nest. Although the two males were size-matched, there were still small differences in the paired males' sizes, and so we used additional exact binomial tests on the data collected from each experiment to investigate whether more females chose to spawn with the larger of the two males.

Next, for experiment 2 and 3, we counted the number of stimulus eggs from the photographs taken and tested whether the number of eggs in the nest influenced whether females copied by fitting a binomial generalized linear model and including the number of stimulus eggs as a fixed effect. We also determined the age of the stimulus eggs by calculating the number of days between when the eggs were laid and when the focal/observer female began spawning. We then tested whether the age of the stimulus eggs influenced whether females copied by fitting a binomial generalized linear model and including the age of the eggs as a fixed effect. Two observer females and one demonstrator female in experiment 3 did not make a clear choice and spawned in both nests; therefore, we excluded these three females from our analyses. Additionally, for one trial in experiment 2, which sides the males occupied were not recorded and so, this trial was excluded from the male size preference analysis.

Ethical Note

Plainfin midshipman are not considered a threatened or endangered species. All procedures in this study complied with the guidelines set by the Canadian Council on Animal Care (Olfert et al., 1993) and were approved by the University of Victoria Animal Care Committee (AUP 2021-12(2)) and the McMaster University Animal Care Committee (AUP 22-03-06). Animal collection (Licence Number: XR 104 2022, XR 137 2023) and transport (License Number: (2022) 128671, (2023) 134031) were approved by Fisheries and Oceans Canada. To minimize the number of fish removed from the wild, fish were reused for multiple trials: males were allowed to spawn with up to 10 females (well within their natural rate of mating; see Bose et al., 2018) and females were first used as a focal fish, then as a stimulus fish in experiment 1. In experiment 1, female stimulus fish were enclosed in tubes for no

more than 3 days and were monitored for signs of stress and ill health daily. As plainfin midshipman are benthic, fairly sedentary and shelter in small, confined rocky nests (Arora, 1948; Brantley & Bass, 1994), the tubes did not appear to interfere with their natural behaviours. Throughout the trials, no fish suffered injury or ill health from this confinement. At the end of the experiments, fish were humanely euthanized in a benzocaine bath (Neiffer & Stamper, 2009) followed by cervical severance, then reweighed (± 0.1 – 0.01 g) and remeasured (± 0.1 cm). All fish were dissected to confirm their reproductive status, their liver and gonads were removed and weighed, and their otoliths were removed and stored for other studies and analyses.

RESULTS

Field Study: Females Assort Nonrandomly Among Nests in the Wild

We sampled 652 plainfin midshipman nests on 73 sampling days across nine intertidal field sites and 4 years. In total, we found 183 females in the nests examined (Fig. 2). Four females was the maximum number of females found in a single nest, and this occurred in three different nests. We found three females in six nests, two females in 25 nests, one female in 103 nests and no females in 515 nests. Most females were found early in the season, and the average number of females per nest declined significantly across the breeding season (GLMM, Julian date: estimate $\pm$ SE = -0.054 ± 0.006 , $z = -8.50$, $P < 0.0001$). Surprisingly, the number of females found per nest was not significantly correlated with male body size (estimate $\pm$ SE = 0.019 ± 0.026 , $z = 0.75$, $P = 0.45$). Finally, our randomization test revealed that female co-occurrence in male nests happened more often than expected under random female choice, even after accounting for the effects of male body size and time in the breeding season ($P = 0.025$; Fig. 2b).

Laboratory Experiments: Females Copy the Choices of Other Females After Observing Spawning

In experiment 1, in the presence of a stimulus female, only eight out of 24 focal females (33%) spawned with males whose nests contained a stimulus female while 16 of 24 (67%) spawned with the other male. Hence, females did not demonstrate a clear preference for males whose nests contained another female (exact binomial test: $N = 24$, $P = 0.15$; Fig. 3). In experiment 2, in the presence of eggs, females did not show a clear preference for males in nests that contained eggs. Thirteen out of 26 focal females (50%) spawned with males who had eggs while the other 13 females (50%) chose to spawn with the males in empty nests (exact binomial test: $N = 26$, $P = 1$; Fig. 3). The average number of stimulus eggs on the bricks/nest ceilings was 190 (range 50–382 eggs), but the number of stimulus eggs did not affect female mate choice (GLMb: $\chi^2_1 = 0.64$, $P = 0.43$). The age of the stimulus eggs (average age = 5.3 days) also did not affect female mate choice (GLMb: $\chi^2_1 = 0.53$, $P = 0.47$). In experiment 3, during observation of a demonstrator female mating, 18 of the 24 focal females (75%) that made a clear choice chose to spawn with the same male as the demonstrator female while the other six females (25%) spawned with the male not chosen by the demonstrator female. Thus, females were more likely to spawn with the same male as the demonstrator females (exact binomial test: $N = 24$, $P = 0.02$; Fig. 3). There was no evidence that being the first or second choosing female influenced choice as this pattern also held when only first-choosing females were considered (exact binomial test: $N = 17$, $P = 0.049$). The average number of eggs on the bricks/nest ceilings encountered by observer females was 230 (range 106–519 eggs), but the number of eggs did not affect female copying (GLMb:

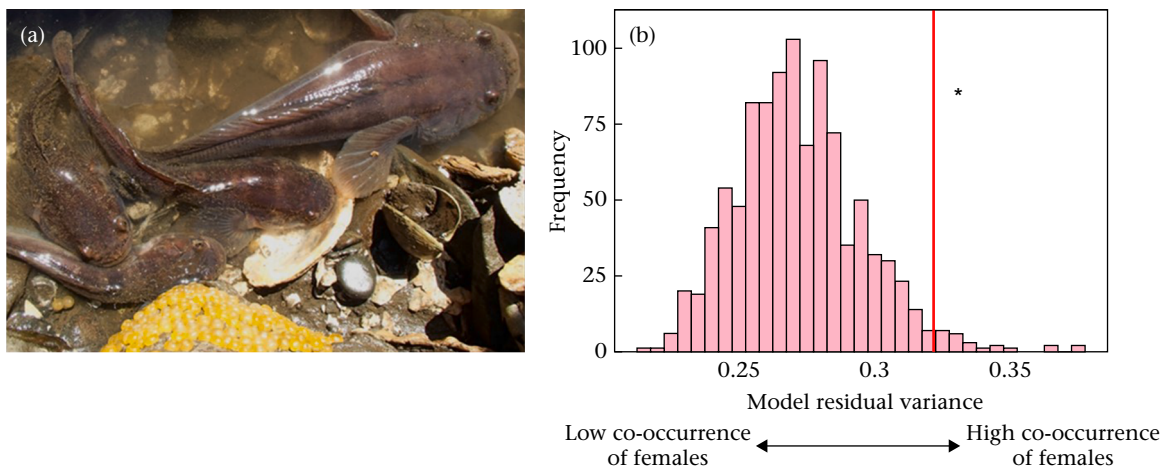


Figure 2. Co-occurrence of females in male nests in the wild. (a) Top-down view into the cavity of a plainfin midshipman nest uncovered in the intertidal zone by overturning a large rock. This nest contained a large nest-guarding male (top right), three females and a brood of eggs adhered to the rock surface. (b) Null distribution of model residual variances generated from the randomization test described in the Methods. Vertical red bar indicates our observed model residual variance. An asterisk indicates a significantly nonrandom observation of female co-occurrence in the wild.

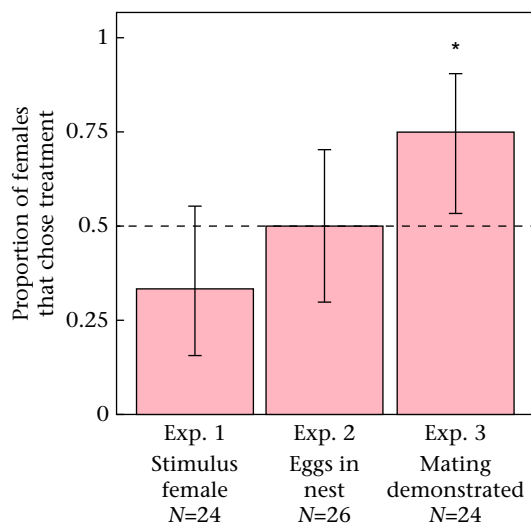


Figure 3. Proportion of females who chose to spawn with the males in the treatment nests. In experiment 1, the treatment nest contained a female in a tube; in experiment 2, the treatment nest contained a clutch of eggs; and in experiment 3, the treatment nest was where the demonstrator had previously spawned. The dashed line represents the null expectation of an equal preference between treatment and control males. P values were calculated using an exact binomial test and error bars represent the 95% confidence intervals. A significant effect of treatment is indicated by an asterisk.

$\chi^2_1 = 0.32$, $P = 0.57$). The age of the eggs (average age = 0.25 days) also had no effect on the likelihood of female copying (GLMB: $\chi^2_1 = 0.19$, $P = 0.66$).

Previous studies have shown that female plainfin midshipman prefer to spawn with larger males (Bose et al., 2018); therefore, to minimize any potential confounding effect of male size, we aimed to size-match the paired males in each trial (by matching their body mass and standard length). Because guarder male plainfin midshipman do not feed while breeding (Bose et al., 2015; Cogliati et al., 2015), the males lost mass over the course of the trials. The degree of weight loss was not identical between the two males in each tank and so, the paired males became less well-matched over time. Hence, we could examine whether females preferred to mate with the subtly larger of the two paired males in each trial (Fig. 4).

We found no significant preference for larger males in any of the three experiments. In experiment 1, 12 of 24 of the females (50%) chose to spawn with the larger of the two males (exact binomial test: $N = 24$, $P = 1$); in experiment 2, 13 of 25 of the females (52%) spawned with the larger of the two males (exact binomial test: $N = 25$, $P = 1$); and in experiment 3, 25 of 45 of all the females (56%; exact binomial test: $N = 45$, $P = 0.55$) and 12 of 22 of the demonstrator females (55%) chose to spawn with the larger male (exact binomial test: $N = 22$, $P = 0.83$).

DISCUSSION

In this study, we show that female plainfin midshipman engage in mate choice copying. To begin, our extensive field survey data revealed that females assort themselves nonrandomly among male nests in the intertidal zone. Spawning females were found to co-occur in certain nests more often than expected by random chance (even after accounting for the influences of time in the season and male body size, two major variables that can affect reproduction in the plainfin midshipman; Bose et al., 2018). This pattern implies that additional mechanisms, beyond a mutual preference for large males, affect female mate choice and promote concordant decisions. These mechanisms might include, among others, preferences for male traits that are uncorrelated with body size, preferences for certain nest architectures or neighbourhood characteristics, or as we argue from our laboratory experiments, female mate choice copying.

We discovered that females were more likely to mate with a male they had recently observed mating with another female, but they showed no preference for spawning near a nonspawning female or alongside previously laid eggs. We also found that these mating patterns could not be explained simply by shared preferences for larger males. Together, these results indicate that while female plainfin midshipman mate-choice copy, observation of a spawning event is necessary to elicit copying.

Our results highlight the need to find a species-relevant cue of mate choice for mate choice copying experiments. Many mate choice copying experiments use female proximity to a male to indicate mate choice (e.g., Moran et al., 2013; Nöbel et al., 2023; Schlupp & Ryan, 1997). Critically, this procedure often does not allow for the male and stimulus female to directly interact or mate,

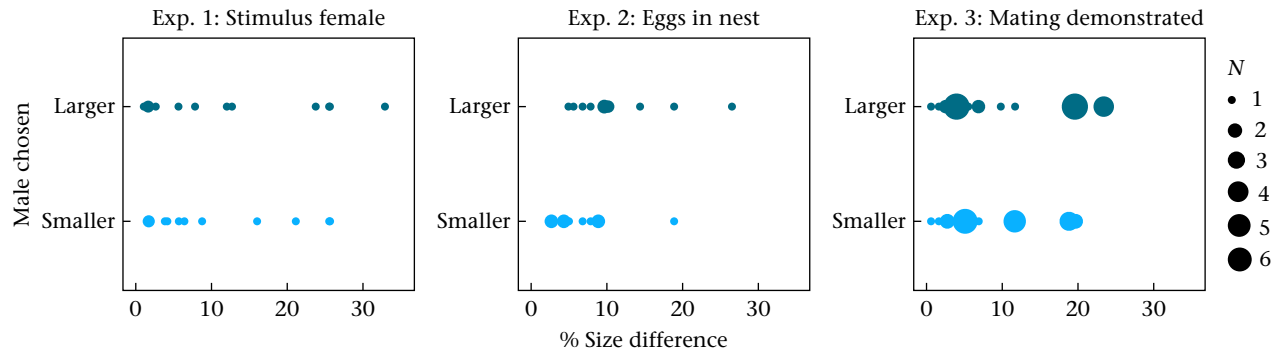


Figure 4. The relative size of the male chosen by each female given the percentage size difference in body mass between the two males. The size of each point indicates the number of males represented by that data point, ranging from one to six. See Methods for details on how we calculated the percentage size difference between males.

often because individuals are kept from physically interacting by experimental design (e.g. using transparent barriers to keep the male and stimulus female separate). While proximity seems to be a sufficient cue of choice in certain species, such as banded darters, *Etheostoma zonale* (Moran et al., 2013) and sailfin mollies, *Poecilia latipinna* (Schlupp & Ryan, 1997), mating is a necessary cue in others, such as Norway rats, *Rattus norvegicus* (Galef et al., 2008) and Japanese medaka, *Oryzias latipes* (Grant & Green, 1996). Similarly, our study indicates that female plainfin midshipman must observe an actual spawning event to mate-choice copy.

Why do female plainfin midshipman require a more explicit cue of mate choice to elicit copying compared to females of many other species? Perhaps because female plainfin midshipman spawn only once a year and are only reproductively active for a maximum of 8 years (Quindazzi et al., 2025), these females have undergone strong selective pressure to copy only the most reliable cue of mate choice, the observation of another female spawning, to protect against copying errors and mistakenly spawning with inferior males. The mere presence of a female in a male's nest may not reliably signal her mate choice because females will often enter a male's nest and exit without spawning (Brantley & Bass, 1994). Previously spawned eggs in a nest also may not reliably signal that a previous female has chosen the current nest owner as their mate. Males who take over nests from other males have been known to raise the previous nest owner's young alongside their own (Cogliati et al., 2013) and so eggs in a nest may not belong to the current nest owner. On the other hand, a female that mates with a male only after observing him spawning with another female generally ensures that her mate was, in fact, chosen by another female. Thus, by only using the most reliable signal of mate choice, the observation of a spawning event, female plainfin midshipman may be able to reduce copying errors. Theoretically, a female's willingness to copy more or less reliable cues of mate choice could be linked to their level of promiscuity: less promiscuous females may only copy reliable cues of other females' mate choices while highly promiscuous females may be more willing to copy less reliable cues. Whether this relationship exists across species is currently challenging to evaluate, however, because so few mate choice copying experiments have been conducted in species with nonpromiscuous females (Davies et al., 2020; Scauzillo & Ferkin, 2019). Future work should target mate choice copying in species with diverse mating systems.

Not only did females in experiment 1 show no preference for nests containing other nonspawning females, twice as many females (16 of 24) spawned in the nests with empty tubes. While this result suggests that the mere presence of a female in a nest is not a sufficient cue to elicit mate choice copying, it is also possible that incongruence among several cues reduce mate attractiveness.

That is, when a female observes another female in a nest, but she does not see eggs being laid, then this cue incongruence may reduce the likelihood of mate choice. Females seen not actively laying eggs in a nest may, in fact, be a signal of mate rejection. Rejection copying may then occur when females become less likely to choose a male after seeing another female reject him (Witte & Ueding, 2003). Similarly, the question remains whether female plainfin midshipman will mate-choice copy based exclusively on the cue of an observed spawning female, or whether they require an additional indirect cue of eggs in the nest following the spawning. In experiment 3, the eggs spawned by the demonstrator female were left in the tank when the observer female was given the opportunity to choose a male. To test whether observer females pay particular attention to the appearance of eggs after viewing a spawning event, a future fourth experiment will be needed. Here, the same experimental design as experiment 3 can be used, but with the demonstrator female's eggs removed before the observer is allowed to make a choice. This could help to tease apart more precisely which cues or cue combinations are necessary to elicit mate choice copying. Such an experiment would also help to clarify whether mate choice copying is more likely to benefit plainfin midshipman females by improving their ability to identify a higher-quality mate or through alternative mechanisms, such as improving paternal care or assuring mating synchrony to dilute danger for the developing embryos. If mate choice copying assists in choosing a high-quality mate, then egg presence may be inconsequential; however, if mate choice copying benefits females primarily through other mechanisms, then egg presence may be a necessary additional cue to elicit copying.

While our laboratory experiments suggested that egg presence in a nest does not influence female mate choice, it has previously been shown to affect male nest site selection (Bose et al., 2016). That is, guarder males prefer to take up nests that contain eggs even when those eggs are unrelated to them (Bose et al., 2016). Furthermore, guarder males will even provide alloparental care to some of the previous nest owner's brood (Cogliati et al., 2013; Bose, Lau, et al., 2019). Because take-over males appear to tolerate at least some unrelated eggs in their nests, we initially expected the presence of eggs in a nest to help to attract future females (Valencia-Aguilar et al., 2020); however, in experiment 2, we detected no clear female preference for nests containing eggs over empty nests. Why then do male and female plainfin midshipman respond differently to the presence of eggs in a nest? One explanation is that when guarder males select a nesting site, they are choosing a location to raise their offspring (Brown et al., 2021), and the presence of healthy eggs in a nest could indicate a suitable egg-rearing location. Females, on the other hand, are choosing not only where to lay their eggs, but also who will raise them (Arora, 1948).

Thus, females may attend to reliable signals of guarder male quality more than egg presence, particularly because there is no guarantee that spawned eggs belong to the current nesting male.

An alternative explanation for why we did not detect a female preference for nests with eggs is that our egg stimulus may not have been sufficiently attractive or salient. In experiment 2, our treatment nests had an average of 190 stimulus eggs, representing a clutch laid by one to two females (Brown et al., 2023; DeMartini, 1990; A. Harrison-Weiss, personal observations). While still ecologically relevant, these egg numbers constitute the lower end of brood sizes in the wild. Some males can accumulate up to 3000 eggs laid by many females; in fact, some guarder males' overall brood sizes may be limited by the space availability in their nests rather than by their female-attracting traits (Bose et al., 2018), i.e. by their extended phenotype rather than their phenotype. It, therefore, remains possible that females seek out much larger broods to contribute to, such that their offspring's survival probabilities can benefit by greater dilution effects (Rohwer, 1978). Choosing to spawn in nests with many eggs may also guarantee that the broods are sufficiently large to encourage heavy paternal investment (Sargent, 1988) and decrease the likelihood of guarder male abandonment (Balshine-Earn & Earn, 1998; Bose et al., 2014) or full brood cannibalization (Bose, 2022). It is possible that our experimental stimulus broods were too small to meaningfully differ in dilution benefits or paternal investment. Future studies could, therefore, test whether female mate choice is affected by larger broods.

The age of previously spawned eggs in a nest can also theoretically influence female decision making (Sikkel, 1989); in some species, females will avoid laying in nests with older eggs since males performing filial cannibalism might preferentially consume the youngest eggs in their nests (Petersen & Marchetti, 1989). By contrast, our results suggest that female plainfin midshipman were not influenced by egg age, perhaps because the eggs in our study were relatively young, given their prolonged developmental period (~60 days; Cogliati et al., 2013). Alternatively, plainfin midshipman females may not be deterred from laying in nests with much older eggs: in the wild, females regularly spawn in nests with young of highly varied ages (Brown et al., 2021).

Females copying the mating choices of demonstrator females cannot simply be explained by a shared preference for the same male. First, over the course of all three experiments, there were 22 cases when a second female was presented the same pair of males as another female, but without the second female observing the first female's choice. In 15 of 22 of these cases (68%), the two females chose to spawn with different males. Additionally, females in our three experiments did not consistently spawn with the larger male, the male characteristic most closely tied to male reproductive success (Bose et al., 2018; DeMartini, 1988), indicating that our males were fairly well-matched. Females only reliably chose the same male as a previous female when they were able to directly observe the previous female's selection. These results support the notion that our observed nonindependent mating was, in fact, a result of mate choice copying.

Female plainfin midshipman are unlikely to suffer serious costs from mate choice copying. Although copiers could risk choosing a lower-quality mate if the female they copy chooses poorly, mate choice copying would not increase this risk if the copier was unable to discriminate quality on their own and would otherwise be choosing randomly (Nordell & Valone, 1998). Even if copying does result in choosing a lower-quality mate, the benefits of laying eggs alongside other females' eggs may result in copying still being the best option. Guarder male plainfin midshipman also do not appear to suffer from sperm depletion: in the wild, it is common to find multiple females spawning within a nest (Bose et al., 2018;

Fitzpatrick et al., 2015). Guarder males appear to embed sperm in mucin trails that coat the nest surface (Miller, 2017), a fertilization tactic that could enable guarder males time to produce sufficient sperm for long-duration spawning (up to 20 h; Brantley & Bass, 1994) and with multiple females simultaneously. Thus, female plainfin midshipman may reap the benefits of mate choice copying while avoiding most potential costs associated with copying.

While male plainfin midshipman and their alternative reproductive tactics have been well studied (e.g. Balebail & Sisneros, 2022; Bass, 2024; Bose et al., 2014, 2015, 2019; Brantley & Bass, 1994; Brown et al., 2021; Cogliati et al., 2013, 2014; DeMartini, 1988; Fitzpatrick et al., 2015; Forbes et al., 2006; Houpt, Borowiec, et al., 2020; Lee & Bass, 2004, 2006; Miller et al., 2019; Pepler et al., 2021; Woods et al., 2022), females in this species have received far less attention. Research on female plainfin midshipman tends to be examined through a male lens, focusing on how females' decisions impact male reproductive success (e.g. Bose et al., 2018; Fitzpatrick et al., 2015); however, we will be unable to fully understand female decision making in plainfin midshipman unless we consider the female perspective. Previous work on female mate choice copying has focused largely on species that mate regularly and repeatedly, despite there being a theoretical expectation that mate choice copying could be more prevalent in species that mate only a few times in their lives. In line with this expectation, we show evidence for female mate choice copying in the plainfin midshipman fish, a species where females have relatively few mating opportunities during their lifetime. Further studies on mate choice copying in species where females have few mates are required to better clarify how mating systems affect the strength of mate choice copying and the mating cues necessary to elicit copying.

Author Contributions

Ainsley Harrison-Weiss: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Anna Grace Burgess:** Writing – review & editing, Methodology, Investigation, Data curation. **Madeleine G. Thomson:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Aneesh P.H. Bose:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Francis Juanes:** Writing – review & editing, Supervision, Funding acquisition. **Sigal Balshine:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Data Availability

The original data presented in this study are available at <https://doi.org/10.5281/zenodo.16333541>.

Declaration of Interest

The authors declare no conflicts of interest.

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