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## Sequential male mate choice in a fish, the Pacific blue-eye *Pseudomugil signifer*

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**Abstract** Mate choice is not just a female preoccupation. Under some circumstances, males may also be choosy. However, studies of male mate choice have generally been confined to situations where males can make direct comparisons between potential partners. In contrast, sequential male mate choice has largely been overlooked despite its biological importance, especially if current investment in mate attraction diminishes a male's future mating opportunities. Using the Pacific blue-eye fish *Pseudomugil signifer*, we show that males are capable of exercising sequential mate choice. When presented sequentially with large and small females, males spent more effort courting the former. However, males did not appear to modify the time spent courting a given female based on the size of the female encountered previously. We suggest that greater attention to the sequential choice problem in males may help illuminate similarities and differences between the sexes when it comes to mating decisions.

**Keywords** Male mate choice · Pacific blue-eye · *Pseudomugil signifer* · Sequential mate choice · Sexual selection

### Introduction

Studies of mate choice have focussed almost exclusively on simultaneous choice where the choosing individual (usually a female) is able to directly compare two

potential suitors. This is true despite the fact that, in nature, the opportunity for simultaneous comparison of potential mates is often limited (Jennions and Petrie 1997). For example, it is recognised that females often need to sequentially visit potential mates and this has given rise to several theoretical models explaining how individuals might reach mating decisions when potential suitors are encountered in this manner (reviewed in Milinski 2001). Both theoretical models and empirical studies of sequential female choice suggest that females can discriminate among potential suitors encountered one at a time. Moreover, there is evidence that females may be able to rely on an adjustable internal ranking of what is attractive to guide their reproductive decisions as they accumulate information on variation in male quality (Bakker and Milinski 1991; Bateman et al. 2001; Pitcher et al. 2003). According to Milinski (2001), this is adaptive because a female that is insensitive to the local distribution of male qualities may end up mating with a low quality male in a population of high quality suitors.

What about males? In contrast to the small, but growing, number of empirical studies investigating sequential choice in females, sequential choice in males has been almost entirely overlooked, even though it is biologically relevant (Real 1990). For instance, in territorial species, males tend to rely on females visiting their territories (Saethers et al. 2001), and females rarely arrive simultaneously. In other taxa, males must actively search for females and can only assess potential mates one at a time, as in the amphipod *Corophium volutator* (Forbes et al. 1996). In both cases, males must strategically allocate their mating effort if females differ in reproductive value and/or if investment into current mating attempts reduces future mating opportunities (Bonduriansky 2001). At the same time, in many species the costs of a missed mating opportunity outweigh the benefits of male choosiness so males may be less discriminating than females, as in salamanders *Desmognathus santeelah* (Verrell 1995) and sticklebacks *Gasterosteus aculeatus* (Rowland 1982; but see Rowland 1989).

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Here we investigate whether male Pacific blue-eye fish *Pseudomugil signifer* exercise sequential mate choice. In other words, are males capable of expressing a preference in favour of particular females even when potential mates are presented one at a time? We ask whether this is (1) based on an absolute preference for females with an attractive phenotype (larger body size), and (2) whether males adjust courtship to a given female depending on the size of the female who was encountered last.

## Materials and methods

### Study species

The Pacific blue-eye is a small (<6 cm) freshwater fish from eastern Australia. Males do not build nests, but guard and maintain territories around submerged logs and rocks close to the riverbank. Females swim along the river's edge inspecting males along the way. Males try and entice females with conspicuous courtship displays (body tilted in a head-down position, raising and lowering his fins). If successful in his efforts, the female follows the male to his territory to spawn. Although females exercise mate choice and males are competitive (Wong 2004), under certain conditions, males may also be choosy. Specifically, female fecundity increases with body size and, in simultaneous choice tests, males preferred to court larger females (Wong and Jennions 2003). In the field, males do not venture far from their territories and rely on females to swim past. Females may swim along in small shoals thereby providing males with the opportunity to simultaneously assess females and direct their courtship efforts towards the more profitable female. However, females also swim past alone. Under these circumstances males have to make sequential mating decisions about how much to invest into courtship, especially as this may involve some energetic costs (Wong and Jennions 2003). Thus, Pacific blue-eyes are a promising candidate species for testing whether males are sensitive to the local distribution of female quality (Bakker and Milinski 1991), and adjust their courtship accordingly.

### Experimental procedure

We collected fish from the Johnstone River, Australia. The sexes were housed separately in 300-l aquaria on a 12L:12D cycle at 25°C and fed fish flakes and daphnia. Males and females were isolated for at least 4 months prior to the start of our experiments.

We conducted experiments in aquaria divided into three compartments using clear glass. The two smaller end compartments (length x width x height = 10x45x20 cm) each housed a gravid female during trials. The larger central compartment (40x45x20 cm) housed the test male. A large (standard length  $\pm$  SE. = 34.5 $\pm$ 0.5 mm) and a small (26.5 $\pm$ 0.4 mm) female were randomly assigned to one of the end compartments. A test male was then introduced into the main compartment. All fish were allowed to familiarize themselves with the test apparatus for 24 h prior to the start of each trial. To prevent males and females from seeing one another during this period, a black plastic sheet was inserted in front of each of the two dividers.

Each trial consisted of four 15-min sessions with a 15-min interval between sessions. During each session, we either lifted or replaced the black plastic sheet in front of a female's compartment so that the male had visual access to only one female. The sequence of presentation involved alternating between the two females between sessions. After the four sessions, the male had seen each female twice, enabling us to compare male response to the first and second presentation of the same female (Bakker and Milinski 1991; see below). We conducted 30 focal samples for each session, noting, during each sample, whether or not the male was courting the female. We tested 30 males using a different pair of females for

each male. Half of the males saw the females in the following order: large, small, large, small ("LSLS treatment"). The others saw the females in the reverse order ("SLSL treatment"). We excluded males that failed to court in two or more consecutive sessions on the grounds that they were sexually unresponsive (Bakker and Milinski 1991).

### Statistical analysis

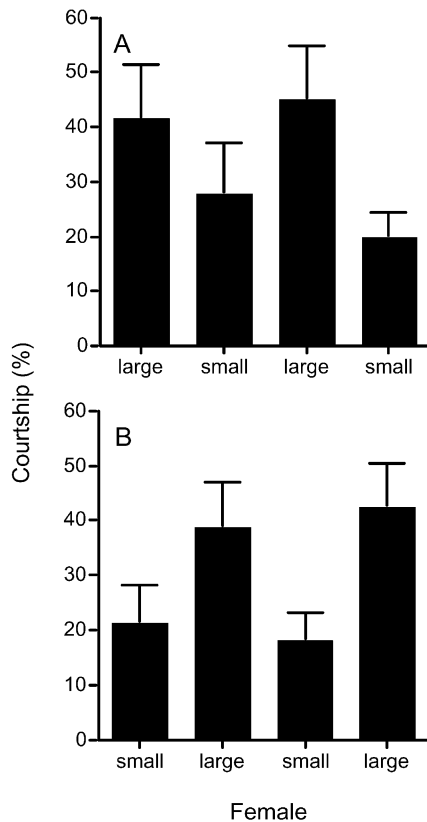
To test whether males have an a priori preference based on female size, we used a Mann-Whitney *U* test to test for a significant difference in percentage time spent courting between treatments when a male was *first* presented with a female (i.e. during session 1) (Bakker and Milinski 1991). Half of the males were presented first with a large female (LSLS treatment), the other half started with a small female (SLSL treatment). If males have an absolute preference for large females, LSLS males should court more than SLSL males during session 1.

Next, we examined whether courtship of a female in a given session was also influenced by the size of the previous female. Specifically, does courtship of a current female depend on who the male saw last? If there is a "previous female" effect, we expect male courtship to increase from the first to the second presentation of a large female because, in the interim, the male would have seen a small female (i.e. when the female presented in between is small). Conversely, we expect males to spend less time courting on the second presentation of the small female compared to the first because, in the interim, the male would have seen a large female (i.e. when the female presented in between is large) (Bakker and Milinski 1991). We tested these four inequalities (two/treatment) using Wilcoxon signed-ranks tests as described in Bakker and Milinski (1991).

## Results

Male Pacific blue-eye fish use a behavioural courtship rule based on the absolute size of the female. Specifically, males presented first with a large female courted more than those presented first with a small female (Mann-Whitney *U* test,  $P=0.037$ ; session 1, Fig. 1A,B), suggesting that males have an a priori mating preference for larger females.

If courtship of a given female was influenced by the size of the last female encountered we expect large 2 > large 1 and small 2 < small 1 for both treatments (see Materials and methods). This was not the case (Wilcoxon signed ranks test comparing difference in courtship between first and second presentation of large female: LSLS treatment:  $z=0.40$ ,  $P=0.69$ ,  $n=12$ , SLSL treatment:  $z=0.67$ ,  $P=0.51$ ,  $n=15$ ; small female: LSLS treatment:  $z=0.83$ ,  $P=0.47$ ,  $n=12$ , SLSL treatment:  $z=0.39$ ,  $P=0.70$ ,  $n=15$ ). We then tried an alternative analysis, combining data from all 27 males in a single Wilcoxon signed ranks test. Specifically we compared large 1 minus large 2 with small 1 minus small 2. If there is a 'previous female effect' we expect the two differences to take opposite signs (i.e. large 1 minus large 2 should give us a negative value whereas small 1 minus small 2 should give us a positive value). The results, however, were still not significant ( $z=-0.58$ ,  $P=0.563$ ,  $n=27$ ).



**Fig. 1A, B** Pacific blue-eye *Pseudomugil signifer*. Average ( $\pm$ SE) percentage time male courtship was directed towards a given female (large or small) during each of the four sessions. **A** LSL treatment. **B** SLSL treatment

## Discussion

In simultaneous choice tests, male Pacific blue-eye spent a greater proportion of time courting large, compared to small, females (Wong and Jennions 2003). This is probably because large females are more fecund (Wong and Jennions 2003) and prefer males that engage in more courtship activity (Wong 2004; Wong et al. 2004). Our results here suggest that males still preferentially invest in courting larger females even when they are presented with only one female at a time. Thus, we show, for the first time, that male blue-eyes are able to exert sequential mate choice.

Male preference for large females appears to be based on an absolute preference for such females. Males presented first with a large female spent a greater proportion of time courting that female compared to males that were first presented with a small female. Although there are few (if any) empirical studies of male mate choice that are directly comparable, this finding is consonant both with theoretical models (Milinski 2001) and empirical studies of sequential choice by females (e.g. Bateman et al. 2001; Pitcher et al. 2003). We cannot, however, exclude the possibility that the choosing male retained some memory of previously encountered females as they were wild-caught (see also Bakker and Milinski 1991).

It is also possible that males may simply be courting differently in response to possible behavioural cues from females. We did not score female behaviours in the present experiment but Wong (2004) found the amount of time spent by males in courtship was not reflected in changes to female behaviour. Specifically, males did not increase courtship rate even when females were expressing mating preferences in their favour. This finding is consistent with other species. In sticklebacks, for example, changes in female preferences in a sequential choice scenario, evidenced through changes in the duration of her head-up display, was not accompanied by any change in male courtship (Bakker and Milinski 1991).

We did not find any evidence that male courtship was attuned to the quality of the previously encountered female as males did not increase the percentage of time spent courting a large female if she was preceded by one that was small and vice versa. We are not aware of any studies that have attempted to test for a 'previous male effect'. Our result, however, contrast with those reported for sequential choice by females. Why? Our sample sizes are small, but comparable to those used in studies on female choice where strong effects for a "previous male" effect have been reported (e.g. Bakker and Milinski 1991). Nevertheless, our ability to detect an effect of a size comparable to those reported for sequential female choice in another fish (where  $r=0.51$ ) is only around 65% (guppies; Pitcher et al. 2003). Notwithstanding the possibility of low statistical power, there are also plausible biological reasons why our results for sequential male mate choice might differ from those reported for females.

One of these reasons could be due to differences in the way females and males maximise their reproductive success (Bateman 1948). The few studies on sequential female choice indicate that searching females are sensitive to the local distribution in attractiveness of potential mates and adapt their reproductive decisions accordingly (Bakker and Milinski 1991; Bateman et al. 2001; Pitcher et al. 2003). For females, there may be good reasons to fine-tune their internal expectation of what is attractive. Females increase their reproductive success by maximising the quality of their mating partners. A female that is insensitive to the local distribution of male qualities may therefore end up mating with a low quality male in a population of high quality suitors (Milinski 2001). In this regard, female choice for males in Pacific blue-eyes has a direct bearing on female fitness because a male takes care of the eggs by defending his spawning site and attractive males bring more eggs to the hatching stage (Wong 2004).

In contrast to females, males increase their reproductive success by maximising their number of mating opportunities (Bateman 1948). Hence, although large females may be more profitable, this alone does not mean that males should necessarily forego the opportunity to mate with smaller females (Rowland 1982). Male salamanders *Desmognathus santeelah*, for example, are choosy when presented with two females simultaneously but mated with small and large females at similar rates

when these were presented sequentially (Verrell 1995). The consequences of a missed breeding opportunity with any female might outweigh the benefits of fine-tuning courtship.

Mate choice, however, is context-dependent. Males, it seems, are more likely to be choosy if energetic and temporal constraints limit their mating opportunities (Judge and Brooks 2001, Wong and Jennions 2003), if mating is risky in terms of attracting predators (reviewed in Kotiaho 2001), and/or if resources necessary for mate attraction are in limited supply (Engqvist and Sauer 2001). It is plausible, therefore, that, under some circumstances, it may be adaptive for males to fine-tune courtship according to the local distribution of female quality (e.g. if females are very common or if cost of mating for males was very high). Certainly, there is evidence to suggest that males are capable of altering their courtship behaviours and even reversing their preferences for particular females (Bakker and Rowland 1995; Jenkins and Rowland 1997; Wong and Jennions 2003). Future studies might wish to manipulate female "density" and/or male costs under a sequential choice scenario by varying, for example, the time lag between successive females. In Milinski and Bakker's (1992) study on sequential female choice in sticklebacks, the strength of the previous male effect weakened when the length of the pause between sequential presentation of males was increased. This is because long pauses mimic a low mate density and thus a low probability of meeting a better partner, thereby negating any possible benefits of fine-tuning selectivity. Although we did not test for this in our study, such a possibility could also exist for males.

In conclusion, our understanding of female mate choice has benefited from considerable theoretical and empirical attention. Future studies may wish to improve our knowledge of male choice and, in particular, pay greater attention to the sequential choice problem that often confronts males. Broadly, more research directed towards this neglected topic may offer valuable insights into sexual selection. For instance, our study shows, under a sequential choice scenario, that males invest in greater signalling effort (i.e. more courtship) towards 'attractive' females. Phenotypic plasticity in the expression of male sexual displays has often been overlooked but is important because it can help inform our understanding of the costs associated with, and the evolutionary potential of, sexually-selected traits (Griffith and Sheldon 2001).

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