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Time spent close to a sexual partner as a measure of female mate preference in a sex-role-reversed population of the blenny *Salaria pavo* (Risso) (Pisces: Blenniidae)

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Abstract In most species females do not exhibit conspicuous sexual behaviours and female mate preferences are often measured by means of the time spent close to males. In spite of its widespread use, in only a few studies has this measure been validated as a reliable indicator of female mate preference. Sex-role-reversed species offer a better opportunity to test female preferences, as females usually court males. We tested in a sex-role-reversed population of the blenny *Salaria pavo* (Risso) if the time spent by females close to males related to the number of courtship displays directed towards males, and whether these measures of female preference reliably predicted matings. Females were simultaneously presented with two males behind a glass partition and the time spent close to each male was measured. We then allowed females to have access to the males' compartments and measured the courtship behaviours performed by females towards each male and recorded with which of the males females spawned. Females spent more time close to the male that subsequently received more courtship displays, and the preferred male had a more developed head crest. However, both measures of female preference failed to predict matings. Females were often attacked by males and probably had, in some occasions, to spawn with the less preferred male. Females that spawned with the previously preferred male had more swollen bellies than females that spawned with the less preferred male, suggesting a male preference towards more ripe females. These results validate the use of "time spent close to a sexual partner" as a measure of female preference in *S. pavo*. We argue that matings may not always reliably indicate mate preferences, particularly in the less choosy sex.

Keywords Female preference · Mate choice · Sexual selection · Sex-role reversed · *Salaria pavo*

Introduction

In most animal species sexual selection acts more strongly on males, and as a consequence, males usually have more elaborated secondary sex traits and display more complex courtship behaviours (Darwin 1859, 1871; Andersson 1994). Female preferences for specific male traits are, in part, responsible for the mating pattern occurring in a population (Jennions and Petrie 1997). Females usually do not exhibit conspicuous courtship displays and previous studies have relied on the time spent by females near the male as a measure of female preference (e.g. Basolo 1990; Milinski and Baker 1990; Kodric-Brown and Nicoletto 1997; Landmann et al. 1999).

Although in sex-role-reversed species females are the less choosy sex, the active courtship behaviours displayed towards males (e.g. the pipefish *Syngnathus typhle* L., Berglund et al. 1986; the Banggai cardinal fish *Pterapogon kauderni* Koumans, Vagelli 1999) offer a better opportunity to study female mate preferences. Courtship behaviours directed towards males are likely to be a good indicator of female propensity to mate with a particular male, although actual matings also depend on other factors such as costs associated with mate searching and male choosiness (Jennions and Petrie 1997). In these species it is possible to evaluate if the time spent close to a male correlates with more direct measures of female preference, such as courtship behaviours directed towards the male, and how these preferences influence the mating pattern. Time spent close to a sexual partner is an easy-to-measure and unambiguous variable and it constitutes a valuable tool if it can be demonstrated that it reliably indicates female preferences.

In spite of the widespread use of this variable as a measure of female preference, studies validating its application are scarce. Ideally, an association between the time spent close to the male with matings or with

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other measures of female preference must be shown. In fish it has been validated, for example, for the cichlid *Pseudotropheus (Metriaclima) zebra* (Boulenger) (Coultridge and Alexander 2001), the guppy *Poecilia reticulata* Peters (Kodric-Brown 1985), the swordtail *Xiphophorus nigrensis* Rosen (Ryan et al. 1990), the sailfin molly *Poecilia latipinna* (Lesueur) (Schlupp et al. 1994), the pipefish *Nerophis ophidion* (L.) (Rosenqvist 1990) and the sand goby *Pomatoschistus minutus* (Pallas) (Forsgren 1992).

We investigated in a Portuguese population of the peacock blenny, *Salaria pavo* (Risso), where sex-role reversal occurs, if the time previously spent by females close to males was correlated with another measure of female preference and whether these measures of preference were good predictors of matings.

S. pavo is an intertidal fish from the temperate Mediterranean and Atlantic coasts (Zander 1986). It exhibits a strong sexual dimorphism with males being larger than females, having a well-developed head crest and an anal gland in the first two rays of the anal fin (Papaconstantinou 1979; Patzner et al. 1986; Patzner and Seiwald 1987). Males defend nests in crevices or holes and take care of the eggs (Patzner et al. 1986). Females may lay eggs with several males throughout the breeding season and the same male may receive eggs from more than one female (Patzner et al. 1986).

In Portugal, a population of *S. pavo* occurs in an area where there is a scarcity of appropriate nest sites. Males compete strongly for the access to nests and do not defend any territory around the nest (Almada et al. 1994). At the peak of the breeding season most nests are fully covered with eggs and females have to compete for potential mates (Almada et al. 1994). This leads to a female-biased operational sex ratio and, unlike in other Mediterranean populations (Fishelson 1963), the sex roles are reversed, with females being the courting sex (Almada et al. 1995). Females approach males, adopting a typical nuptial colouration and beating the pectoral fins rapidly with synchronized opercula movements (Almada et al. 1995), while males usually adopt a more or less passive role.

Unlike the generality of sex-role-reversed species, in this population males have secondary sex traits more developed than females. Although it is still not clear why this happens, it should be noted that this is an atypical population of the species, with the sex roles reversed due to ecological constraints (Almada et al. 1995). Males still have the ability to court females when placed in an environment with an excess of nest sites, reflecting behavioural plasticity of courtship behaviour (Almada et al. 1995).

The conspicuous courtship display exhibited by females, together with the well-developed male secondary sex traits, makes this population of *S. pavo* ideal to study female sexual preferences. In this study it was tested if time previously spent by females close to males correlated with female courtship behaviour and whether these measures of preference were good predictors of matings.

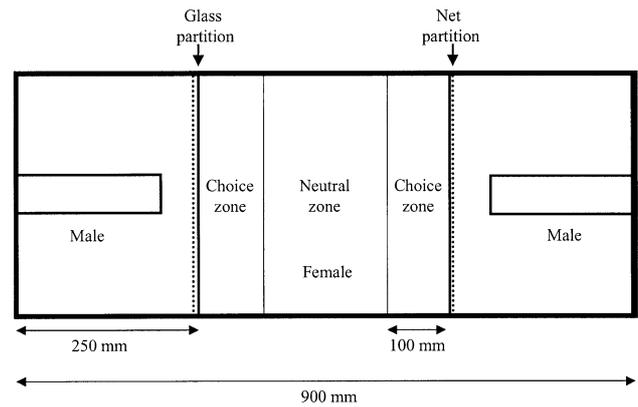


Fig. 1 Top view of the experimental set-up

Methods

Fish

All animals were captured in the Ria Formosa Nature Park (south Portugal, Algarve, 36°59'N, 7°51'W). Only males guarding nests with eggs in the field and ripe females (recognized on the basis of a distended belly) were used in the experiment, which was performed during the species breeding season (June/July). Males and females were kept in two separate stock tanks of 80×35×30 cm, bottoms covered with a layer of sand and provided with artificial nests. The tanks were illuminated by fluorescent lamps on a 16 h:8 h light:dark cycle. Salinity was kept at 35‰ and temperature varied between 20 and 25°C. The animals were fed daily with frozen common cockles (*Cerastoderma* sp.). Experiments were performed 2 days after fish capture. No mortality occurred during captivity and all animals were released in the same place where they were captured.

Choice tests

The experimental tank (90×35×40 cm) was divided into three different compartments by glass and a rigid net (Fig. 1). The bottom was covered with a layer of sand and the set-up was illuminated by two 30-W fluorescent lamps placed 20 cm above water level. During choice trials one male was placed into each of the outer compartments; an opaque glass box of 21×5×5.5 cm with an opening of 2.5×2.5 cm turned to the central compartment provided an artificial nest for males. The central compartment was divided by lines drawn on the front and back walls into two choice zones, adjacent to each of the male's compartments, and one neutral zone (Fig. 1). Two hours before trials males were placed in the end compartments and a female was released in the central compartment to acclimate to the experimental tank. During acclimation two opaque plates limited the female to the neutral zone and provided a visual barrier between the female and the males. All males adopted the provided nest and typically spent most of the time inside the nest with the head out, which is also the most common position observed in the field. Choice trials were divided into two steps: (1) after acclimation the opaque plates were removed and the female's behaviour and position were video recorded for 20 min. After this period the males' sides were switched and the female was again restricted to the neutral area by the opaque plates for 10 min; (2) the glass barriers separating the males' compartments from the central compartment were removed and only the rigid nets were left between these compartments. The opaque plates were removed and a second observational period of 20 min was video recorded.

The nets had square openings of 1×1 cm and, because in this species males are significantly larger than females, females were able to pass through the nets while males were not. This procedure

prevented physical contact between males, which could have influenced the female choice, and allowed access to both nests by the female. The fish were left in the tank for the next 2 days and both nests were checked for eggs every 3 h during the day.

The frequency of female courtship behaviours directed towards each male and the time spent in each of the choice zones were quantified from videotapes using Observer PC software V3.0 (Noldus Information Technology, Wageningen, The Netherlands). We had previously defined that if one of the males spent more than 50% of the time completely inside the nest (not visible to the female being tested) in any of the observational periods the trial would be aborted. However, this situation never occurred.

A total of 20 females (standard length, SL, mean \pm SE=6.1 \pm 0.16 cm) and 40 males (SL, mean \pm SE=9.8 \pm 0.18 cm) were used in the experiment, and no fish was used more than once. Before experiments the standard length of all animals was measured. In males the development of the anal gland was classified on an ordinal scale of 0 to 3 and the head height (including the crest) and body height were measured to calculate a relative index of crest development (head height/body height). In females the swelling of the belly, an indicator of the degree of ripeness, was also classified on an ordinal scale of 0 to 3.

As data did not conform to the assumptions of parametric tests, non-parametric procedures were applied. Unless noted otherwise Wilcoxon signed-rank tests were applied. All tests were two-tailed.

Results

Females were sequentially presented to the same pair of males with and without the glass partition. Sixteen of the 20 females consistently associated with the same male during the first and the second step of the experiment ($\chi^2=7.20$, $df=1$, $P=0.007$). The remaining four females did not court or spawn with any of the males and were excluded from the analysis.

During step 1 of the experiment, only one female did not spend more time close to the male that received more courtship displays during step 2 ($\chi^2=12.25$, $df=1$, $P<0.000$) and the average time previously spent by females close to this male was also significantly higher ($z=2.90$; $n=16$; $P=0.004$; Fig. 2). These results demonstrate a consistency between the two measures of

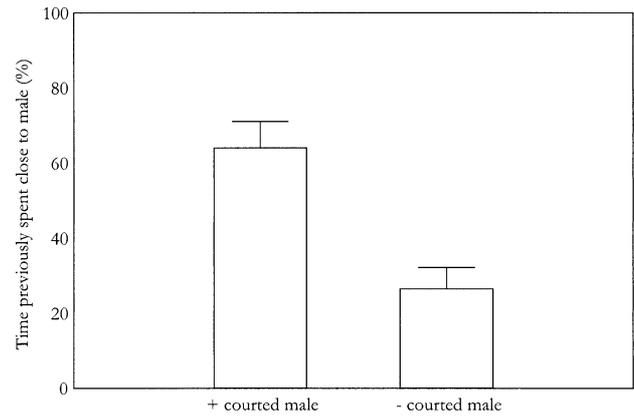


Fig. 2 Time previously spent by females close to the male that subsequently received more courtship displays and fewer courtship displays ($n=16$). Bars mean; whiskers SE

preference recorded: time spent close to the male and courtship displays directed towards the male.

Females preferred to associate with and to court the male that had the larger head crest (Table 1). No difference in body size or in the development of the anal gland was recorded between preferred and non-preferred males (Table 1).

Nine females spawned, always with only one of the males. Four females laid eggs during the 1st day and five during the 2nd day. Both measures of female preference failed to predict matings. During step 1 of the experiment females did not spend more time close to the male with whom they subsequently spawned ($z=0.47$; $n=9$; $P=0.636$), and during step 2, this male did not receive more courtship displays ($z=0.53$; $n=9$; $P=0.594$). No difference was recorded in any of the measured traits between the male that received eggs and the male that did not receive eggs (Table 1).

Females that spawned were larger than females that did not spawn and had more-distended bellies (Table 2). Four females spawned with the male that they had

Table 1 Comparison of the measured traits between the male that females preferred to associate with and the non-preferred male ($n=16$) and between the male that females spawned with and the

	Preferred male	Non-preferred male	Z	P	Male that received eggs	Male that did not receive eggs	z	P
Standard length (cm)	9.83 \pm 0.22	9.94 \pm 0.34	0.39	0.698	10.21 \pm 0.52	9.70 \pm 0.38	1.60	0.110
Anal gland development (0–3)	2.69 \pm 0.12	2.81 \pm 0.10	0.63	0.529	2.55 \pm 0.18	2.89 \pm 0.11	1.21	0.22
Relative crest size (head height/body height)	1.24 \pm 0.02	1.14 \pm 0.01	2.48	0.013	1.19 \pm 0.02	1.19 \pm 0.03	0.18	0.86

male that did not receive eggs ($n=9$). Values are mean \pm SE. Wilcoxon signed-rank tests were applied to analyse differences

Table 2 Comparison of the standard length and degree of belly swelling between females that spawned ($n=9$) and females that did not spawn ($n=7$). Values are mean \pm SE. Mann–Whitney tests were applied to analyse differences

	Females that spawned	Females that did not spawn	z	P
Standard length (cm)	6.37 \pm 0.24	5.60 \pm 0.25	-1.91	0.057
Belly swelling (0–3)	2.00 \pm 0.00	1.57 \pm 0.07	-3.40	0.000

previously spent more time close to. These females had more-swollen bellies in comparison with the five females that spawned with the previously less preferred male (Mann–Whitney test: $z=-2.11$; $P=0.034$). No difference in standard length was detected between these two groups of females (Mann–Whitney: $z=-0.98$; $P=0.324$).

Discussion

Females consistently associated with the same male during the two sequential steps of the experiment and the preferred male had, on average, a more developed crest, demonstrating that female association with a particular male was not arbitrary.

The male with whom females preferred to associate during the first step of the experiment was also the male that subsequently received more courtship displays. This association between the two measures of female preference, time spent close to a male and courtship displays directed towards that male, validates the use of the former as an indicator of female mate preference in future experiments.

Previous laboratory studies have shown that the development of the male's crest correlates with the time spent by females courting a male (Gonçalves et al. 2002b) and with the number of eggs received by males (E. Barata, personal communication). Corroborating these results, in our experiment females preferred to associate and directed more courtship displays towards the male that had the larger crest. In the field, however, no relation between the development of the crest and the male's reproductive success has been demonstrated to date (Oliveira et al. 1999; Gonçalves et al. 2002a). These results suggest a female preference towards males with more developed crests, although in the field male choosiness and male–male competition for nest sites, with larger males having a higher reproductive success (Oliveira et al. 1999; Gonçalves et al. 2002a), probably dilutes the effect of this preference.

In our experiment successful males were not larger than unsuccessful males. This result may be explained by the small difference in size in paired males (mean difference: 0.7 cm; range: 0.1–1.8 cm), which could have made the assessment of males' size difference by females difficult. Alternatively, larger males can have a higher reproductive success in the field due to their advantage in male–male competition for the access to nests (as demonstrated for many species, e.g. *Pomatoschistus minutus*, Magnhagen and Kvarnemo 1989) and not to female preference for larger males.

Both measures of preference failed to predict with which of the males females would subsequently spawn. Female mate choice depends on female preferences but also on mate sampling costs and male choosiness (Jennions and Petrie 1997). In this population of *S. pavo*, females have the most active role in courtship and males are the choosier sex (Almada et al. 1994). Both in the field and in captivity males often reject courting females

(Almada et al. 1995; D. Gonçalves, personal observations). In our experiment males frequently attacked females during the second observational period, and it is likely that some of the females had to mate with the less preferred male. The fact that seven females did not spawn with any of the males, in spite of courting at least one of them, could indicate that these females were rejected by both males.

Females that spawned had more-distended bellies, which could indicate either a higher motivation to spawn, or that males preferred to mate with these females. Corroborating this last hypothesis, females that spawned with the previously preferred male had more-swollen bellies than females that spawned with the previously non-preferred male.

Females that spawned were larger than females that did not spawn, probably reflecting male preference for larger females. Larger females with more distended bellies are likely to have a higher fecundity and previous studies in other species have demonstrated male preference for larger females [e.g. threespine stickleback, *Gasterosteus aculeatus* L., Rowland 1982; Sargent et al. 1986; Jamaican beaugregory damselfish, *Stegastes leucostictus* (Müller and Troschel), Itzkowitz et al. 1998] and for females with more developed bellies (e.g. *G. aculeatus*, Rowland 1982, 1989).

In this population, small males cannot compete with large males for the access to nests and reproduce as sneakers (Gonçalves et al. 1996). Sneakers resemble females morphologically and approach nesting males imitating the female's courtship behaviour to achieve parasitic fertilizations of the eggs (Gonçalves et al. 1996). Thus, males can also reject small females with less-distended bellies because they can potentially be confounded with sneakers. During the breeding season females have bellies significantly more distended than sneakers and sneakers larger than 6–8 cm start to develop male secondary sexual ornaments (D. Gonçalves, T. Fagundes, and R. Oliveira, unpublished data). Thus, a male can benefit by choosing to mate with a large and round female not only because it will potentially receive more or larger eggs, but also because it may decrease the risk of parasitic fertilizations by sneakers.

In conclusion, we found a congruence between the two proximate measures of female preference used, validating “time spent close to a sexual partner” as a measure of female preference in *S. pavo*. Future sexual selection studies on this species may benefit from this result, using this easy-to-measure variable to evaluate female preference for male traits.

Both measures of female preference failed to predict matings, probably because in this population males are the choosy sex, often rejecting courting females. We argue that matings may not accurately indicate female preferences in sex-role-reversed species and male preferences in species with traditional sex roles. In the first case, males may reject females and force them to mate with a less preferred male and in the latter males may be rejected by females.

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