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Choosy males in a haplochromine cichlid: first experimental evidence for male mate choice in a lekking species

NOAM Y. WERNER & ARNON LOTEM
Department of Zoology, Tel-Aviv University

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Current theories of mate choice predict that the level of choosiness of males and females will depend on their relative investment in parental care. Males often invest less than females and are expected to be less choosy, especially in lekking species where males contribute only sperm. Our study of the haplochromine cichlid fish *Astatotilapia flaviijosephi*, a maternal mouthbrooder, provides the first experimental evidence for male mate choice in a lekking species. In this species the number of eggs spawned is positively correlated with female weight, thus making larger females potentially better mates. In the laboratory, we conducted a simultaneous choice experiment where males had the opportunity to associate with, and court, each of two females that differed in size. Males preferred to court the larger female and spent more time courting during experimental trials involving larger females. This selective allocation of courtship effort to more attractive (i.e. heavier) females suggests that there may be constraints on males in fertilizing multiple females, thus compelling them to be choosy.

Mate choice models predict that the level of choosiness of females or males during mate choice will depend on their relative parental investment (Trivers 1972; Owens & Thompson 1994; Johnstone et al. 1996). Females, which often invest heavily in parental care in both the pre- and postzygotic stages, are expected to mate with the best available male and, therefore, to be choosy. Males, on the other hand, typically show lower parental investment and hence are expected to be less choosy. None the less, some mating systems, such as those of sex role-reversed species, monogamous species and some promiscuous species, are characterized by considerable male parental investment (reviewed by Clutton-Brock 1991). In such systems male mate choice may be expected (Parker 1983; Johnstone et al. 1996) and, indeed, has been observed (e.g. reviews by Amundsen 2000; Bonduriansky 2001). However, in promiscuous species where only females care for the offspring and male parental investment is reduced to courtship and sperm, male mate choice is not predicted as its benefits are unlikely to outweigh the costs of lost mating opportunities (Parker 1983; Johnstone et al. 1996). None the less, several studies suggest that even in such systems male mating is not as cheap as previously thought: sperm may be a limiting factor to male reproductive rate through depletion (e.g. Dewsbury 1982; Birkhead 1991; Warner et al. 1995; Galvani & Johnstone 1998), and courtship behaviour may be energetically costly or risky (e.g. Vehrencamp et al. 1989; Magnhagen 1991; Höglund et al. 1992; Andersson 1994). If this is so, male mate choice may also be expected in systems with low paternal investment. None the less, in lekking species, renowned for their highly biased parental care (high maternal care and no paternal care), male mate choice has only recently been reported (Sæther et al. 2001). Sæther et al. (2001) showed male mate choice that was based on the sexual history of the individuals (i.e. whether the male and the female had already mated with each other). However, no study has reported that males in lekking species choose females based on their phenotypic features, as is frequently reported for female choice (reviewed by Fiske et al. 1998).

We explored whether male preference for larger and heavier females exists in a lekking species, the haplochromine cichlid *Astatotilapia flaviijosephi*. Haplochromine cichlids are promiscuous, maternal mouthbrooders with highly skewed parental investment (Fryer & Isles 1972). After spawning, females gather the fertilized eggs into their mouths, where they brood them, and subsequently the fry, for several weeks. Males, on the other hand, contribute only gametes to their progeny. As in many other fish species, female fecundity in *A. flaviijosephi* is related to female size (N. Werner, unpublished data). Hence, mating with larger females is expected to be more beneficial to males. If sperm production, or courtship, is costly, male preference for larger,

Correspondence: N. Y. Werner, Department of Zoology, Tel-Aviv University, Ramat-Aviv, Tel-Aviv 69978, Israel (email: wernerny@post.tau.ac.il).

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more fecund females may be expected, despite the lack of paternal investment. We carried out a laboratory male mate preference experiment, testing whether male haplochromine cichlids, which provide no paternal care, exhibit a preference for larger females.

METHODS

Study Species

Astatotilapia flaviijosephi is endemic to Israel. It is locally abundant, mostly inhabiting rocky and densely vegetated areas in Lake Kinneret (the Sea of Galilee), the central part of the Jordan River and the numerous springs, pools, streams and canals that are connected with it (Goren & Ortal 1999). It is most closely related to central African haplochromine cichlids such as *Astatotilapia burtoni* (N. Werner & O. Mokady, mtDNA analysis, unpublished data) and shows similar behaviour (N. Werner, unpublished data). During the breeding season males aggregate in a certain area (i.e. a lek), defend small individual territories, 30–170 cm apart, and court passing females, which are often found in schools near the lek, at rates of 2–10 times/5 min (see also Fernald & Hirata 1977 and Kirchshofer 1953 for details of *A. burtoni* and *A. desfontainesi* behaviour in the field, respectively).

Courtship behaviour of *A. flaviijosephi* resembles that of other haplochromine cichlids (McElroy & Kornfield 1990). A courtship bout will often start with an interested male approaching the female. This may be accompanied by a lateral display in which the male spreads its dorsal fin. If the female remains or approaches the male, a more intense stage of courtship may follow, characterized, among other things, by a quiver, easily recognizable by the male's vigorous shaking in front of the female with its anal fin fully spread.

Housing Conditions

All experimental fish were caught in the area of Nahal HaKibbutzim, Beit-She'an Valley, Israel, with hand nets, cage traps or a small seine. In the laboratory males were kept in individual 45-litre aquaria, to prevent intrasexual aggression, while females were kept in several communal 145-litre aquaria. All aquaria were maintained at about 28°C, illuminated with 15-W daylight fluorescent lighting and exposed to a 13:11 h light:dark regime. The fish were fed once a day with commercial cichlid pellets and commercial flakes. The study was authorized by the Tel-Aviv University animal care and use committee, and fish were caught with permission from Mekoroth, the Israeli National Water Company. After the study the fish (20 females and 20 males) were kept in our laboratory for further experiments. The females were later released at the site where they were caught.

Experimental Set-Up

We divided a 145-litre aquarium (180 × 25 cm and 30 cm high) into three compartments by transparent Perspex barriers. All compartments were fitted with a heater, an air stone and halved flowerpots that served as shelters (two flowerpots for females, where they could hide completely from males, and one in the middle for males, for a symmetrical design). Each of the two side compartments was also fitted with an external water filter. For each trial we placed a male in the middle compartment (70 cm long) and a female in each of the two side compartments (each 55 cm long). All fish were measured (standard length to the nearest 0.5 mm), using callipers, and weighed (to the nearest 0.1 g), using an electrical balance, and we counted the egg spots on their anal fin. The experimental males weighed 10.5–62.1 g. In our laboratory, males as light as 5 g successfully fertilized eggs; thus, all our males were sexually mature. The two females in each trial differed in size (weight was used as the parameter for size, as it was more highly correlated with female fecundity than standard length was). The mean weight ± SE of the larger female in each pair was 9.8 ± 1.38 g (range 4.1–17.8 g) and of the smaller female 6.1 ± 1.07 g (range 2.8–12.8 g). Smaller females in each pair were on average 39% lighter than the larger ones (range 26–70%). This relative difference between the females was not correlated with the average pair weight that we later used for analysis (see Results; Spearman rank order correlation: $r_S = -0.28$, $N = 10$, NS). All our experimental females, like the experimental males, were sexually mature; in our laboratory we observed females as light as 1.9 g spawning and brooding eggs. We randomly allocated large and small females of the different pairs to the side compartments to control for effects of position on male choice. We introduced fish into the experimental aquarium for a minimum of 4 h before the experiment for habituation; during this time, opaque barriers prevented the fish seeing each other. To begin the experiment, we removed the opaque barriers, thus allowing visual contact between male and females. The interactions that followed were videotaped with a Sony 8-mm camcorder.

Data Analysis

We used 10 female pairs. Each pair was presented to two males on separate days; hence we tested the responses of 20 males. For each of these 20 experimental trials, we analysed 15 min of videotape. Analysis started 1 min after we observed the male interacting with both females. We excluded two males, each tested with a different female pair, because they interacted continuously for over 10 min with only one female, without any indication that they had noticed the other female. Thus, 18 males were included in the final analysis. As the position of females within pairs was not changed between trials, we analysed data for female pairs rather than for individual males as independent data points, to avoid pseudoreplication. As a result, our sample size for the statistical analysis was reduced to 10 pairs. For analysis we used the average values of the behavioural parameters of the two males tested with each pair of females (this averaging was not necessary in the two pairs from which the two males mentioned earlier were excluded). We obtained similar results when we used males (N=18) as independent data points (N. Werner, unpublished data). Two parameters were quantified during the analysis: the total time that males spent near each of the two females (10 cm from the barrier or less), and the total time that males actively courted each female (identified by quivering behaviour, see above). Although courting is usually performed near the females, the parameter time spent near does not include the parameter time spent courting.

Within-trial comparisons indicated that males show a size preference between females presented to them simultaneously (see Results); we therefore determined whether courtship effort would also vary with female size in the absence of a simultaneous choice. This might be expected if males trade off present versus future investment in courtship, that is they might invest little in courting poor-quality females to conserve resources for courting possibly better females in the future. To test this possibility, we compared male courtship effort between trials. We predicted that total courtship effort would be higher for males tested with large than with small females. We used the average size of the two females presented in each trial as the measure for overall female attractiveness, and compared it with the total male courtship effort in this particular trial.

Because both male and female haplochromine cichlids have colourful egg spots on the anal fin (Goldschmidt & de Visser 1990; Goldschmidt 1991), which are thought to be involved in female choice (Wickler 1962; Hert 1989, 1991), we further analysed our results to assess whether female egg spots are involved in male choice. In general, there is a positive correlation between female size and the number of egg spots on their anal fin (N. Werner, unpublished data).

During our experimental trials, the two females presented to the males could see each other and, hence, could have established a dominance hierarchy. This might have affected male mate choice by causing one female to be less responsive than its pair mate. To investigate this possibility we quantified the time that each female spent near the male, and the number of times that she approached him, as a proportion of the time that the male spent near her or courted her.

For the statistical analysis we used Statistica, version 6. All data were treated as nonparametric. All P values are two tailed, based on the test statistic itself (Mundry & Fischer 1998) and corrected for ties (Zar 1999).

RESULTS

Within-trial Preference

Males allocated more courtship effort to the larger female in a pair. They spent significantly more time near the larger females (Wilcoxon signed-ranks test: T=0, N=10, P<0.01; Fig. 1a) and also invested significantly more time in courting them (T=3, N=10, P<0.02; Fig. 1b).

Between-trial Comparisons

Average female size in a trial was negatively correlated with the time that males spent near the females (Spearman rank order correlation: $r_S = -0.71$, N=10, P<0.05; Fig. 2a), but positively correlated with the time they actively courted them (r_S=0.77, N=10, P<0.02; Fig. 2b). The shorter times spent near larger females (Fig. 2a) were probably not the effect of males spending more time actively courting these females (Fig. 2b), because this result remained significant (although marginally) when courtship time and time spent near females were combined ($r_S = -0.65$, N=10, P=0.05).

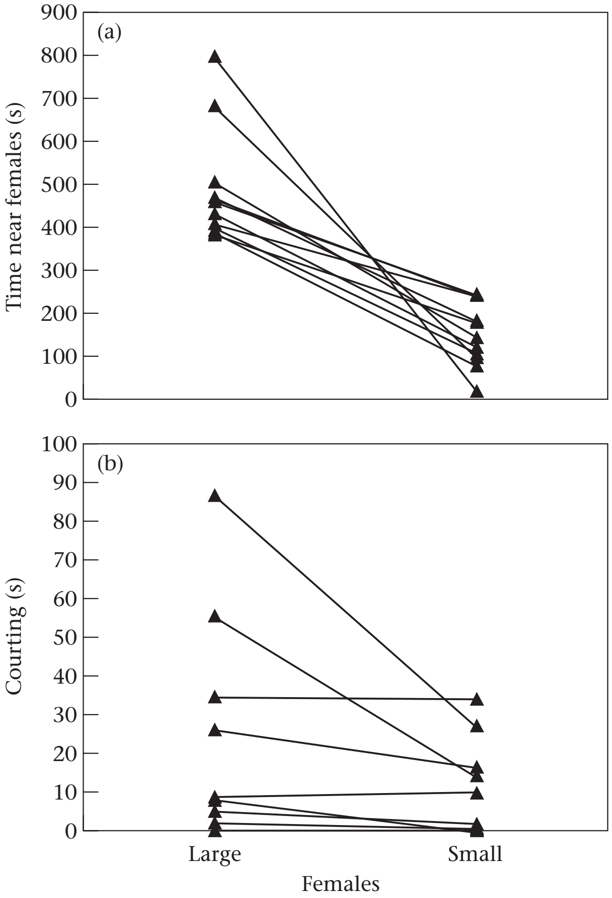

Figure 1. Within-trial preference: the average time that males (a) spent near and (b) courted the larger and smaller female of each female pair.

Female Egg Spots

Size differences between females within a pair were not significantly associated with number of egg spots (Wilcoxon signed-ranks test: T=18.5, N=10, NS). Male mate choice was also not significantly associated with the difference in the number of female egg spots within each trial (time near females: T=20, N=10, NS; courting time: T=8, N=10, NS) and we found no correlation between average number of egg spots on females in each pair and the time that males spent near them or courted them (Spearman rank order correlation: time near females: $r_S = -0.10$, N=10, NS; courting time: $r_S = -0.25$, N=10, NS).

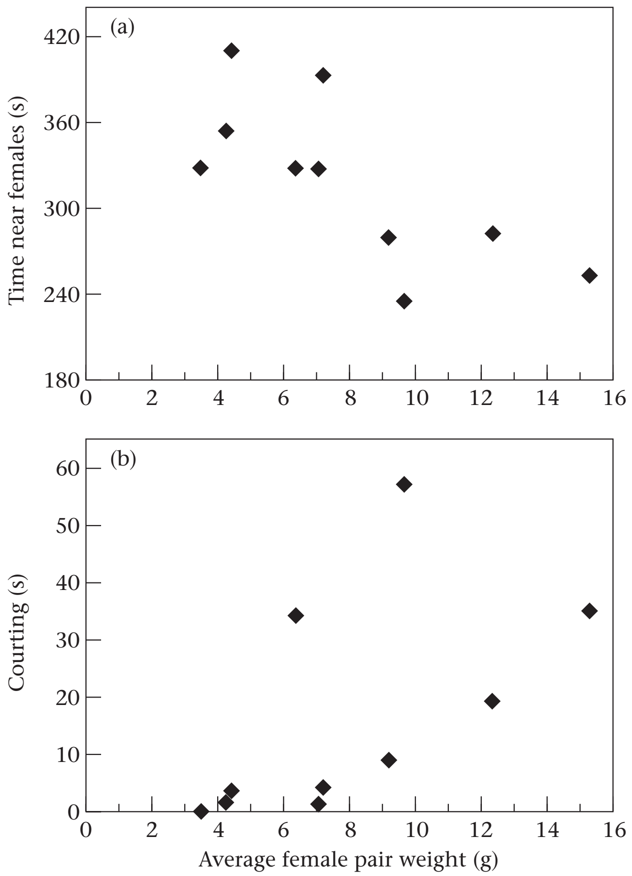

Figure 2. Between-trial comparisons: the effect of average female pair weight on the total time that males (a) spent near and (b) courted both females.

Female Behaviour

Within pairs there was no difference between large and small females in the time that they spent near the male (Wilcoxon signed-ranks test: T=14, N=10, NS) or in the number of times that they approached the male (T=19, N=10, NS). There was also no correlation between average female pair weight and either the proportion of time that the females spent near the males (Spearman rank order correlation: $r_S = -0.43$, N=10, NS) or the proportional number of approaches (r_S=0.12, N=10, NS).

DISCUSSION

In contrast with previous conceptions, the first report of male mate choice in a lekking species (Sæther et al. 2001) showed that males in a lekking bird species can recognize individual females, assess them simultaneously, and often reject copulations with certain females in favour of others. Our study provides the first evidence for male mate choice in a lekking fish.

When facing two females, male *A. flaviijosephi* allocated more time to courting the larger female. This behaviour makes adaptive sense even if male reproductive rate is rarely limited (as long as the discrimination process itself is not too costly), as males can copulate with only one female at a time. However, our results also suggest that males adjust their courtship efforts on a larger time scale. The fact that male courtship effort and female size were correlated across experimental trials (Fig. 2b) may indicate that males trade current courtship effort against future mating opportunities. This behaviour makes adaptive sense only when mating is costly, and when males are likely to encounter more females over time (which is often the case in a lek). If, on the other hand, the cost of mating is negligible, the males' best strategy should be to court all females at maximal levels to avoid the risk of losing mating opportunities. Alternative explanations are that the correlation between female size and male courtship effort is due either to preference for more egg spots or to the lower receptivity of smaller females. However, in our experiment we found no association between male choice and the number of female egg spots, and no correlation between the number of female egg spots and male courtship effort. These results suggest that female size rather than number of egg spots was the main criterion in male choice. In addition, all the females in our experiment were sexually mature and we found no correlation between female size and their response to the courting males. Before the experiment females were kept for several weeks with no access to males. Therefore, no female had spawned for at least that period, which is long enough for sufficient recovery. Consequently, previous spawning was not expected to affect female condition or responsiveness during the trials. Furthermore, in a different study (N. Werner, unpublished data) we found no correlation, within a school of females, between female size and time until spawning after the introduction of a male, suggesting that egg maturation does not depend on a female's absolute size or its position within the school's size hierarchy. These results, again, suggest that at least in this experiment female size was the main criterion in male choice.

What are the possible costs of mating in *A. flaviijosephi*? Across experimental trials only active courtship was positively related to female size, while the time spent near females showed the opposite trend (Fig. 2a, b). This suggests that time per se may not be the major cost of mating in this system. Males tested with relatively smaller females might have needed more time to assess them, or might have taken longer to become sufficiently stimulated to initiate courting; however, the fact that they eventually spent more time near them suggests that time constraints were not their major concern. Clearly these results should be confirmed in the field, where time allocation for other activities is more meaningful than in the laboratory. However, it is possible that when a male is on the lek, its time is already mainly devoted to mating and, therefore, spending time assessing females may not be costly.

Courtship itself, on the other hand, may be costly in terms of energy or predation risk, while actual mating may be costly because of sperm limitation. In terms of predation risk, kingfishers, cormorants, herons and otters are all visually hunting piscivores that share the distribution area of *A. flaviijosephi*. A large territorial male venturing off its shelter to court a passing female may expose itself to much greater predation risk. Courtship may also be energetically costly. In cichlids, oxygen consumption during submissive behaviour that involves quivering is 3.3 times higher than routine metabolic rate (Grantner & Taborsky 1998). Courtship behaviour, which also involves quivering (see Methods), may be similarly costly or even more so. At the same time, sperm quantity can also limit male reproductive rate. Testes of monogamous cichlid species are empty after spawning (Polder 1971). Although males of promiscuous cichlid species have larger testes than males of monogamous species, this may be an adaptation to more intense sperm competition (Balshine et al. 2001). Therefore, even in promiscuous species such as *A. flaviijosephi*, sperm may still be in short supply. Consequently, males may choose not to court, or mate with, small (albeit sexually mature) females, to save sperm for future copulations with larger females. Our study gave no indication that males ignore small females. Males spent time near all the females, and sometimes courted small females, which suggests that they did not reject a priori the possibility of allocating sperm to such females. However, we cannot exclude the possibility that the cost of sperm limitation may still play a role in the overall tuning of courtship efforts.

Male mate choice may have implications for the study of promiscuous breeding systems. Sæther et al. (2001) noted that most great snipe, *Gallinego media*, females that mated multiple times shifted from a more popular to a less popular male and suggested that this trend originated from male mate choice. Similarly, our own study may suggest that male preference for larger, more fecund females may allow low-ranked males to mate more easily with small, low-quality females. Thus, male mate preference, in this case, may weaken the degree of reproductive skew among males in the lek, causing a higher genetic variance than would otherwise be expected; and, if the rejected smaller females then mate with subordinate males, male mate choice may result in some degree of assortative mating.

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