



# Mutual mate choice in sticklebacks: attractive males choose big females, which lay big eggs

SARAH B. M. KRAAK & THEO C. M. BAKKER

Abt. Verhaltensökologie, Zoologisches Institut, University of Bern

(Received 17 November 1997; initial acceptance 26 January 1998;  
final acceptance 18 March 1998; MS. number: 5704)

## ABSTRACT

Brighter red three-spined stickleback, *Gasterosteus aculeatus*, males have been shown to be preferred by females in the laboratory but in the field, these males did not receive more eggs. Instead, they had heavier eggs in their brood. We investigated the hypothesis that sexual selection for red coloration in male sticklebacks acts through mate choice by preferred males, who can afford to be choosy, for high-quality females which lay heavier eggs. We assume here that heavier eggs provide a direct fitness advantage. In simultaneous choice tests males were presented with two females differing in size. The number of zigzags directed to and the time spent orienting to each female were measured. After the test the females laid eggs, which we counted and weighed. Bigger (i.e. longer and heavier) females laid significantly more and heavier eggs than smaller females. For all 23 males pooled together, the preferred female was the bigger of the two in 17 cases, laid more eggs in 18 cases, but laid heavier eggs in only 13 cases. When bright and dull males were analysed separately, we found that bright but not dull males spent more time oriented to the bigger female, and to the female that laid more eggs. Females preferred by bright males tended to lay heavier eggs than nonpreferred females, although this result was not quite significant. We conclude that in nature this preference for bigger females results in brighter males receiving on average heavier eggs. Assuming higher survival of bigger offspring, we propose that this can explain how brightness can be sexually selected in spite of brighter males not receiving more eggs.

© 1998 The Association for the Study of Animal Behaviour

Until recently, most studies of sexual selection by mate choice have made the assumption that only one sex engages in active choice. Classically, the sex with the highest parental investment was thought to be the choosy sex (Trivers 1972). Today this paradigm is replaced by one that states that the sex with the lowest potential reproductive rate should be choosy because the operational sex ratio is biased towards the other sex (Clutton-Brock & Vincent 1991; Clutton-Brock & Parker 1992). Parker (1983) was the first to consider mutual mate choice by both sexes. A more recent theoretical investigation of mutual mate choice is the study by Johnstone et al. (1996). Mutual mate choice may lead to assortative mating: high-quality females mate with high-quality males, and low-quality individuals mate among themselves. This may come about because only the preferred individuals can afford to be choosy in their choice of mate. Darwin (1871) proposed that male ornaments may

evolve by a process he called sexual selection, in which males that bear the ornament have a mating advantage over other males because females prefer them. We usually think of such a mating advantage in terms of ornamented males mating with more females. However, the advantage may lie in preferred males having access to females of better quality (Darwin 1871). In both cases, ornamented males leave more descendants. Thus sexual selection of an ornament may operate through mate choice by preferred individuals of the sexually selected sex for high-quality individuals of the other sex, that is, through mutual mate choice.

Mutual mate choice may be more common than we think. In the three-spined stickleback, *Gasterosteus aculeatus*, female mate choice has been thoroughly studied (e.g. Jamieson & Colgan 1989; Rowland 1989a, 1994; McLennan & McPhail 1990; Milinski & Bakker 1990, 1992; Bakker & Milinski 1991; Baube et al. 1995; McKinnon 1995; Rowland et al. 1995), but only a few studies have investigated male mate choice (Rowland 1982, 1989b; Sargent et al. 1986; Bakker & Rowland 1995). Why should we suppose that male mate choice takes place in the stickleback in nature? According to

Correspondence: S. B. M. Kraak, Zoological Laboratory, University of Groningen, PO Box 14, 9750 AA Haren, The Netherlands (email: s.b.m.kraak@biol.rug.nl). T. C. M. Bakker is at the Abt. Verhaltensökologie, Zoologisches Institut, University of Bern, Wohlenschtrasse 50a, CH 3032 Hinterkappelen, Switzerland.

Johnstone et al.'s (1996) model, the choosiness of a sex depends on the costs of choice in terms of the probability of finding alternative mates, and, less so, on the benefits of choice resulting from variation in quality in the other sex. The probability of finding alternative mates depends largely on the operational sex ratio, that is, the relative numbers of individuals of the opposite sex that are ready to mate. Limitations in the number of matings that males are willing to perform would effectively shift the operational sex ratio towards a female bias. Male three-spined sticklebacks might be limited by their sperm supply, since androgens inhibit spermatogenesis in all males in this species (Borg & Mayer 1995). Another limitation in the number of females with which a male will mate is probably set by the oxygenation of the eggs: for a given level of fanning, embryo survival decreases as the number of eggs in the nest increases (Th. C. M. Bakker, D. Mazzi & S. B. M. Kraak, unpublished data), so a male has to fan more for a larger brood to reach a certain egg survival rate. From this it follows that at a certain brood size it does not pay a male to acquire more eggs (Perrin 1995). Because the operational sex ratio is influenced by the respective potential reproductive rates of the sexes (Clutton-Brock & Vincent 1991; Clutton-Brock & Parker 1992), it may also be affected by environmental factors, such as food availability (as suggested by Wootton et al. 1995) or seasonal change in temperature (Kvarnemo 1994).

As for the benefits that male sticklebacks might have from mate choice, depending on variation in female quality, these are certainly present. Females vary in size, and female fecundity correlates well with body size (van den Assem 1967; Baker 1994; Fletcher & Wootton 1995). Indeed, in simultaneous choice tests male sticklebacks directed more courtship to the more fecund (larger or more distended) female dummy (Rowland 1982, 1989b) or female (Sargent et al. 1986). Also, the average mass per egg correlates with female body size (Fletcher & Wootton 1995). In addition, an increase in food ration increases egg mass, but does not change the chemical composition of the eggs (concentrations of protein, lipid, carbohydrate and nucleic acid; Fletcher & Wootton 1995), implying that bigger eggs contain more resources per egg. It is not known whether heavier eggs or the fry hatched from these eggs provide a fitness advantage in the stickleback, but for several other fish species it has been shown that larger eggs produce larger fry which have higher survival probabilities (e.g. Blaxter & Hempel 1963; Wallace & Aasjord 1984; Marsh 1986).

A third factor promoting male choosiness that was not considered by Johnstone et al. (1996) was mentioned by Bakker & Rowland (1995): females may pose a risk to the male (e.g. by transmitting parasites), his resources (e.g. by disturbing his nest), or his progeny (e.g. by cannibalism). Furthermore, conspicuous courtship may attract other egg cannibals (Foster 1994, 1995), sneakers and even predators. Therefore, a male should weigh the benefits of courting a female against these risks. In conclusion, we may expect mutual mate choice to take place in sticklebacks under certain conditions.

We have studied correlates of male mating success in a population of three-spined sticklebacks in the field at

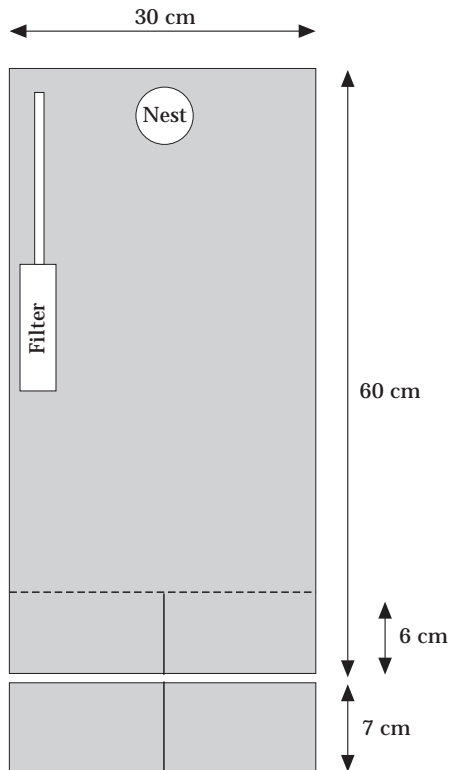
Roche, Switzerland. Contrary to the expectation for a putatively sexually selected ornament, males with redder throats did not have higher mating success in terms of numbers of eggs received (S. B. M. Kraak, Th. C. M. Bakker & B. Mundwiler, unpublished data). However, females of the same population did prefer redder males in the laboratory (Milinski & Bakker 1990, 1992; Bakker & Milinski 1991; Bakker 1993). This apparent contradiction between results from the field and from the laboratory requires further study. Nests of redder males in the field contained heavier eggs than nests of duller males (Th. C. M. Bakker, D. Mazzi & S. B. M. Kraak, unpublished data). Moreover, the following observations suggest that the operational sex ratio was relatively female biased in this population during our study. Females approached males' nests on average at a rate of 21/h per male and seemed very obtrusive (S. B. M. Kraak, Th. C. M. Bakker & B. Mundwiler, unpublished data). Males are thought to have a refractory period of about 1 h after fertilization of a clutch during which they are unwilling to court additional ripe females (Sevenster-Bol 1962). Certainly, males will not mate with 20 females/h. Indeed, males seldom zigzagged when ripe females approached; their behaviour to ripe females consisted more often of meandering and dorsal pricking (S. B. M. Kraak, Th. C. M. Bakker & B. Mundwiler, unpublished data), which has been interpreted as trying to keep the female away from the nest (Wilz 1970).

These observations led us to the following hypothesis. Because males are limited in the numbers of eggs they should care for because of problems of oxygenation, the attractive males with red throats in particular are approached by more ripe females than they should accept. Hence, these males should be choosy with respect to the quality, and not the number, of eggs that their mates lay. If a heavy egg has a higher fitness than a light one, choice by redder males for females laying heavy eggs, instead of more matings by redder males, may be the driving force selecting for the red throat of stickleback males in this population.

To investigate this hypothesis we conducted a simultaneous male mate choice experiment. Because it is plausible that egg mass is revealed to the male by female body size, we gave the males the choice between a bigger and a smaller ripe female. With this experiment we address the following questions. (1) Do bigger females of this population lay heavier eggs? (2) Do males prefer to court females that lay heavier eggs? (3) Are redder males choosier with respect to egg mass?

## METHODS

We used sticklebacks from the Roche population (near Montreux, Switzerland, 46°26'N, 6°55'E). Males were caught in March and April 1996. Ripe and semiripe females were caught weekly in May and June 1996 (when the experiment was run) such that females, when used in a test, had been freshly caught 1–3 days before. We thus minimized the positive influence of holding conditions and food ration on the traits we investigated, that is, fecundity and egg quality (Fletcher & Wootton 1995).



**Figure 1.** The experimental set-up. The big aquarium contained a male, his nest, some plant material and a water filter. Perpendicular to the front window was an opaque partition 6 cm long. In the first half of each test a transparent partition was placed parallel to the front window at a distance of 6 cm. In the small container were two females, one on each side of the opaque partition that divided the container in half.

The animals were released again in Roche after the experiment. Females were held in a 200-litre stock tank with water (at about 12°C) continuously flowing from a well, and some plants. Males were held individually in 'nesting tanks' with about 10 litres of water (15–16°C), an airstone, some plants, and a petri dish filled with sand. The males were visually isolated from each other by grey partitions between the nesting tanks. The majority of males built their nests in the petri dish. We stimulated them visually each day by placing a transparent 1-litre container with a ripe female in front of the tank, for undetermined lengths of time (minimally 10 min per day, up to several hours). We placed males that had built a nest in a 'test tank' together with their nest at least 5 days before the test took place. The test tanks measured 60 × 30 × 30 cm (length × width × height) and were filled with water (about 14°C) to a level of about 20 cm. The petri dish with the nest was placed at the back wall of the tank (Fig. 1). The water in the test tank was continuously filtered by a sponge through which the water was moved by an air-driven pump. Some plant material was also present. A grey partition 6 cm long divided the area at the front window in two (Fig. 1). This was done because the two females would be visible at the front window, and we wanted to be sure that a male approaching a female would interact with only one female at a time. The side

walls of the test tanks were covered with grey partitions, to isolate individuals from each other visually. Dark green curtains were hung in front of the test tanks. A day-night regime of 16:8 h was used. All fish were fed daily with frozen artemia and live tubifex.

Weekly, we tested six males that had either accepted their old nest after transfer to the test tank, or built a new one. Males were tested only if their nest was ready at least 1 day before, as indicated by the acts of creeping through or 'courtship glueing' (Sevenster & van Roosmalen 1985) when stimulated by a female. Of these six males each week we classified three as 'bright' and three as 'dull'. For this purpose, the intensity of red coloration was judged by S.K. (sometimes additionally by T.B.; the red intensity was later measured in a standardized way, see below). From 4 to 6 days prior to testing, each male was daily stimulated by two ripe females simultaneously, for three or four 5-min periods at intervals of 5 min to several hours. (The same intervals were used within a pair of one bright and one dull male.) The stimulus females were each held in 1-litre containers that were placed either at the front window of the test tank or at both side windows (after we had shifted the opaque grey partitions along the side walls), near the front. On the day of the test this was done for two 5-min periods, ending at least 1 h before the start of the test.

The test procedure was as follows. At least 15 min before the test, we placed a transparent partition in the test tank 6 cm from, and parallel to, the front window (Fig. 1). For each test male, we selected a pair of ripe females, differing at least 6 mm in standard length, from the stock tank (the bigger female was on average  $\pm$  SD 21.8  $\pm$  5.8% larger than the smaller female,  $N=23$  pairs). These females had not been used before, either in a test, or for stimulating males. To check a female's willingness to lay eggs, she was briefly put in a male's nesting tank (of a male that was not going to be used for testing). Only if the female started to enter the nest within 1 min (we prevented her from actually entering the nest by taking her away), was she selected for a test. Both females were put in a transparent container that measured 27 × 7 × 17 cm (length × width × height), separated by a black opaque partition in the middle. The females were acclimatized to the container for 15 min. Then we placed the container at the front window of the test tank (Fig. 1). We randomized the left or right position of the females according to size between tests, in such a way that the bigger female was on the left side first as many times as on the right side first. As soon as the females' container was in place, a video camera started to record the scene from the front, 'viewing' through the females' container into the test tank with the male. The camera stood between the green curtain and the test tank. After 5 min, we pulled up the transparent partition with a string (without a person having to go behind the curtain) and recorded another 5 min on video. Then we turned the females' container around, such that the females changed their left/right positions relative to the male, and the transparent partition was lowered again. This was immediately followed by another 5 min of recording on video, then the transparent partition was pulled up, followed by

5 min of video recording. We refer to the test period before we turned the females' container as the first subtest, and the period after we turned the females' container as the second subtest. Within each subtest, there was a period with the transparent partition down and one with the partition up. We intended the periods with the transparent partition down to be a pretest period during which the male could see the two females. Without such a partition we ran the risk that a male would immediately go to one of the females and stay there throughout the test period without ever seeing the other female; in the arrangement with the transparent partition, the male would see both females.

Immediately after the test, we photographed the male in a standardized way (see Bakker & Mundwiler 1994). From these slides we later measured the red intensity of the throat and calculated a 'red' index for each male (see Bakker & Mundwiler 1994). We also measured the male's standard length to the nearest 0.5 mm and his body mass to the nearest mg. Similarly, we measured the standard length and the body mass of each female and put her into the nesting tank of a male that was not going to be tested. If the female did not lay eggs within 1 h, we put her in a 1-litre container for a while, and then tried again, sometimes with a different male. Females that did not lay the first day were kept in a separate 10-litre tank overnight (without food), and put in a male's nesting tank again the following day, when they spawned within 1 h. We gave each female a 'willingness-to-spawn-score' ranging from 3 to 1, based on whether she had spawned within 1 h, after 1 h but on the day of testing, or the next day, respectively. After having laid eggs, the female was weighed again to the nearest mg, marked (by spine-clipping) and released in the stock tank. The relative mass was calculated, for females before and after spawning, and for males, as the quotient of body mass and standard length raised to a certain power (the exponent is the regression coefficient of the logarithm of mass regressed on the logarithm of standard length; this ratio is a common condition index, Bolger & Connolly 1989).

We carefully removed the eggs from the nest 1 h after they were laid (so that enough time had passed for them to be fertilized and to harden), and put them in a small container with a few dl of water and an airstone. An hour later, we separated and counted the eggs (at this time the eggs were in the two- or four-cell stage). We randomly took 25–50 eggs from the clutch, blotted them dry singly on tissue paper, and weighed them to the nearest 0.1 mg to calculate average egg mass.

We measured two behaviours from the videotapes: the number of zigzags that a male directed to each of the females (scored by T.B., but compared with the counts scored by a student who was naive regarding this experiment), and the time (s) that the male oriented to each of the females (scored by the same student). The frequency of zigzagging is usually taken as a measure of the sexual tendency of a male (Bakker & Sevenster 1989). The zigzag counts correlated well between the two observers (Pearson  $r=0.97$ ,  $N=30$ ,  $P<0.0001$  for the females that were on the left side first; Pearson  $r=0.97$ ,  $N=30$ ,  $P<0.0001$  for the females that were on the right side first)

and for one observer (T.B. who scored the same 10 sequences twice: Pearson  $r=0.96$ ,  $N=10$ ,  $P<0.0001$  for the females that were on the left side; Pearson  $r=0.95$ ,  $N=10$ ,  $P<0.0001$  for the females that were on the right side). Because the scoring of time oriented to each female was straightforward, this was not repeated. Time scores were recorded only for the test periods during which the transparent partition was up, but zigzags were recorded for both test periods, with the partition down and up. Although we had originally not intended the period with the transparent partition down to be part of the test, we decided post hoc to add the numbers of zigzags of both periods (with partition down and up). This made the analyses more reliable because it increased the numbers of zigzags per male used for the analyses. Preference scores (proportions of total zigzags to the female on the left side) did not differ between the test periods with the partition down and up (paired  $t$  test first subtest:  $t_{22}=-0.371$ ,  $P=0.714$ ; second subtest:  $t_{22}=1.000$ ,  $P=0.328$ ), but correlated well (first subtest: Pearson  $r=0.51$ ,  $N=23$ ,  $P=0.012$ ; second subtest: Pearson  $r=0.60$ ,  $N=23$ ,  $P=0.003$ ). We used for analyses only cases in which the male zigzagged at least 10 times during each of the subtests of 10 min. This left us with 23 cases (seven cases were discarded).

## RESULTS

We collected data on 64 females and their clutches. Egg number as well as average egg mass increased significantly with female body size, regardless of whether expressed as standard length or body mass before or after spawning (Fig. 2), but did not correlate with the females' relative mass before or after spawning, nor with date of capture or willingness to spawn (all  $P>0.1$ ; power is 0.67 or 0.98 for a medium or a large effect size, respectively, Welkowitz et al. 1982). Body size was a better predictor of egg number than of average egg mass (Fig. 2).

The two females in the 23 pairs that were used in the tests differed between 6 and 17 mm in standard length (mean difference  $\pm$  SD =  $9.7 \pm 2.6$  mm; paired  $t$  test:  $t_{22}=18.4$ ,  $P<0.0001$ ); the big females varied between 50 and 68.5 mm standard length ( $\bar{X} \pm$  SD =  $54.7 \pm 4.5$  mm) and the small females between 41 and 58.5 mm ( $\bar{X} \pm$  SD =  $44.9 \pm 3.6$  mm). In all but one case the bigger female laid more eggs, but in only 15 out of 23 cases did the bigger female lay heavier eggs (egg weight;  $\bar{X} \pm$  SD; large females:  $3.048 \pm 0.284$  mg; small females:  $2.893 \pm 0.176$  mg; paired  $t$  test:  $t_{23}=2.26$ ,  $P<0.05$ ). Of the 23 males, we had classified 13 as bright and 10 as dull. The bright males had a significantly higher red index than the dull ones ( $t$  test:  $t_{21}=3.7$ ,  $P<0.005$ ) and a higher relative mass ( $t$  test:  $t_{21}=2.4$ ,  $P<0.05$ ), but they did not differ in standard length ( $t$  test:  $t_{21}=0.8$ ,  $P>0.4$ ; power is 0.22 or 0.48 for a medium or large effect size, respectively, Welkowitz et al. 1982). The absolute differences between the two females in a pair with respect to standard length, relative pre-spawning mass, willingness to spawn, egg number and average egg mass were not different for the bright and the dull males ( $t$  tests: all  $P>0.2$ ; power is 0.22

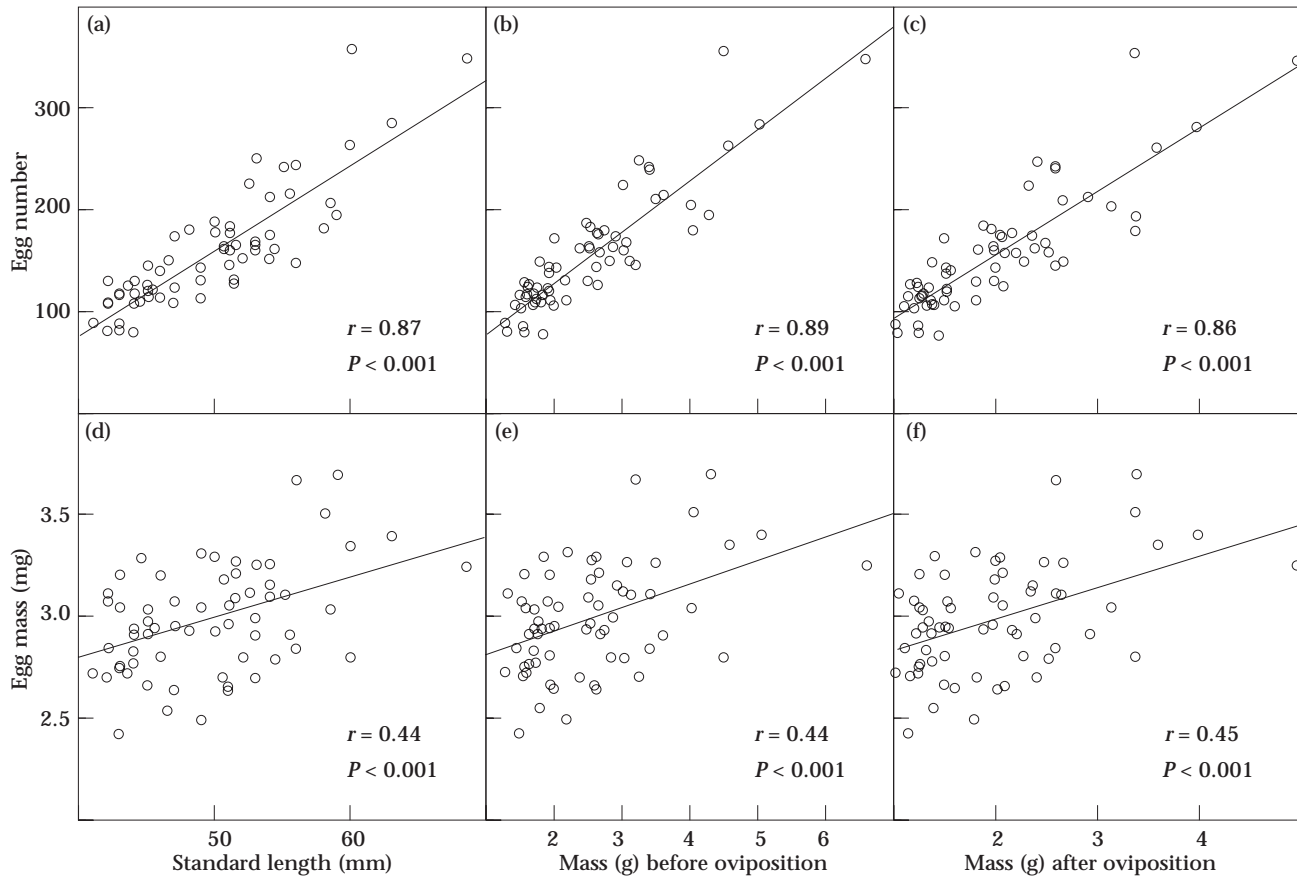


Figure 2. Number of eggs in a clutch plotted against (a) female standard length, (b) female mass before oviposition, (c) female mass after oviposition. Average egg mass of a clutch plotted against (d) female standard length, (e) female mass before oviposition, (f) female mass after oviposition;  $N=64$ . The lines represent the fitted linear regressions. Pearson's  $r$  and the respective  $P$  value are reported.

or 0.48 for a medium or large effect size, respectively, [Welkowitz et al. 1982](#)).

Preference indices using zigzags and time oriented, respectively, were significantly correlated (Pearson  $r=0.73$ ,  $N=23$ ,  $P<0.001$ ). Of the 23 cases, the bigger of the two females was preferred 17 times (sign test:  $P<0.05$ ), and the female laying more eggs was preferred 18 times (sign test:  $P<0.05$ ), but the female laying heavier eggs was preferred only 13 times (sign test:  $P>0.5$ ). Although there were four cases in which the male zigzagged more to the female to whom he spent less time oriented, the above results were the same, whether we defined as the preferred females those that received most zigzags or those to which the males spent most time oriented. The female with the higher relative pre spawning mass was preferred 13 times, considering zigzags, and only 11 times, considering time oriented (sign test: both  $P>0.5$ ).

Males spent significantly more time oriented to the big female than to the small female (Fig. 3a; paired  $t$  test:  $t_{22}=2.51$ ,  $P<0.05$ ). This difference was more significant among the bright males (Fig. 3b; paired  $t$  test:  $t_{12}=3.40$ ,  $P<0.01$ ), but was not significant among the dull males analysed separately (Fig. 3c; paired  $t$  test:  $t_9=1.02$ ,  $P>0.3$ ; power is 0.36 or 0.71 for a medium or large effect size, respectively, [Welkowitz et al. 1982](#)). Moreover, males spent significantly more time oriented to the female that

laid more eggs (paired  $t$  test:  $t_{22}=2.67$ ,  $P<0.05$ ), and again this difference was more significant among the bright males (paired  $t$  test:  $t_{12}=3.40$ ,  $P<0.01$ ), but was not significant among the dull males (paired  $t$  test:  $t_9=1.16$ ,  $P>0.2$ ; power is 0.36 or 0.71 for a medium or large effect size respectively, [Welkowitz et al. 1982](#)). There were no differences in time spent oriented to the female with the higher versus the lower relative pre spawning mass, or to the female that laid heavier versus lighter eggs (paired  $t$  tests: all  $P>0.1$ ). No differences were found in the numbers of zigzags directed to the bigger versus the smaller female, the female with higher versus lower relative pre spawning mass, the female that laid more versus fewer eggs, or heavier versus lighter eggs, whether analysed for all males pooled or for bright and dull males separately (paired  $t$  tests: all  $P>0.09$ ).

The eggs of preferred females were not significantly heavier than the eggs of nonpreferred females when we tested for all males pooled together, whether we defined as the preferred females those that received most zigzags or those to whom the males spent most time oriented (paired  $t$  test: for zigzags:  $t_{22}=0.49$ ; for time:  $t_{22}=1.04$ , both  $P>0.1$ ; power is 0.67 or 0.97 for a medium or large effect size, respectively, [Welkowitz et al. 1982](#)). For the bright males analysed separately, however, there was an almost significant trend for the preferred females, in

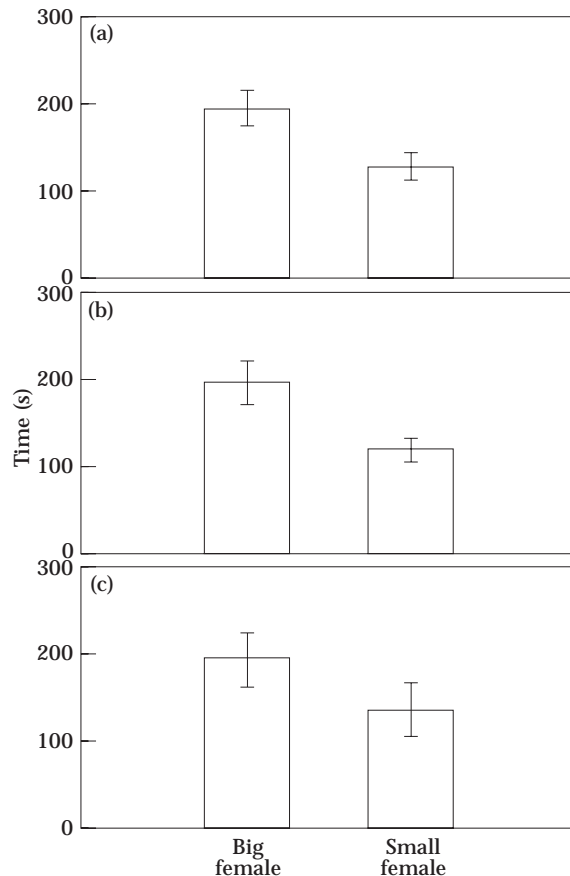


Figure 3. Time (s;  $\bar{X} \pm \text{SE}$ ) spent oriented to the bigger or the smaller female, respectively, by (a) all males,  $N=23$ , (b) bright males,  $N=13$ , (c) dull males,  $N=10$ .

terms of time spent oriented to, to have heavier eggs than the nonpreferred females (paired  $t$  test:  $t_{12}=2.01$ ,  $P=0.068$ ), but not if preference is defined in terms of zigzags ( $t_{12}=0.23$ ,  $P>0.5$ ; power is 0.44 and 0.83 for a medium or large effect size, respectively, Welkowitz et al. 1982), nor for dull males analysed separately (for time:  $t_9 = -0.40$ ; for zigzags:  $t_9=0.44$ , both  $P>0.5$ ; power is 0.36 and 0.71 for a medium or large effect size, respectively, Welkowitz et al. 1982).

## DISCUSSION

Bigger females laid heavier eggs, and males preferred bigger females. Furthermore, this preference was more pronounced in redder males, and not significant in dull males. However, we could not show that the preferred females laid significantly heavier eggs at  $\alpha=0.05$ . This appears to be a sampling problem, because female size and egg mass were not very strongly correlated (Fig. 2); only 15 of the 23 bigger test females laid heavier eggs. However, if redder males in nature consistently prefer bigger females, they will on average receive heavier eggs because of the positive correlation of egg mass with female size. It is not known whether male choice for big females is adaptive because the preferred females lay more eggs or because these females lay bigger eggs, or whether these effects are just side-effects of another adaptation. If

a male preference for big females had evolved because of benefits from bigger eggs, males, by implementing their preference, could not avoid choosing females laying more eggs as well. Female size revealed egg number more accurately than egg mass, but perhaps female size is the most accurate cue available to males for assessing egg mass. It is even advantageous, if males prefer females that lay bigger eggs, that these females lay more eggs as well. That way, a male that is willing to care for a limited number of eggs needs fewer matings to reach his optimal number of eggs, and consequently the male is less frequently exposed to the risks of courtship. Hence, the male preference may be an adaptation for reasons of egg mass as well as egg number.

It is not yet known whether heavier eggs have a fitness advantage in sticklebacks, but evidence exists for other fish that juveniles from bigger eggs survive better (Blaxter & Hempel 1963; Marsh 1986; Elliot 1989). Since in sticklebacks the chemical composition of large and small eggs is the same (Fletcher & Wootton 1995), it seems plausible that in bigger eggs the embryos are provided with more resources, whereby they can grow faster or reach a certain size sooner or safer (e.g. by not having to forage yet because their yolk is not yet depleted). This may be important if juveniles must outgrow the size range in which they are vulnerable to size-dependent predation (Foster et al. 1988). If differences in size or growth during the juvenile phase remain until the adult phase, it may be advantageous because large females enjoy higher fecundity (e.g. Fletcher & Wootton 1995; this study) and large males win more territorial fights (Rowland 1989a) and have higher mating success (S. B. M. Kraak, Th. C. M. Bakker & B. Mundwiler, unpublished data). Caring for bigger eggs may also be costly because they may require more oxygen. We found in the field that egg mortality in nests deprived of paternal care is higher when average egg mass is higher (Th. C. M. Bakker, D. Mazzi & S. B. M. Kraak, unpublished data). In that case the optimal number of eggs to care for (sensu Perrin 1995) is lower when eggs are bigger. Our argument still holds if the lower reproductive output in terms of number of fry is more than compensated for by these fry having higher survival chances because they hatched from larger eggs. This requires further study.

In the Introduction we argued that a male should become choosy when the operational sex ratio is female biased, or if more ripe females present themselves to him than he should mate with to acquire an optimal number of eggs. The males in our experiment might have perceived the sex ratio as female biased, as a result of having seen no other males for several months before the test, but several females per day. However, evidence against this argument is that the strength of the male preference for the bigger female decreased over time (correlation between the proportion of total number of zigzags to the bigger female and date: Pearson  $r = -0.44$ ,  $N=23$ ,  $P=0.036$ ). Conversely, perhaps the fact that on the days preceding the test the males saw only three or four pairs of females for 5 min each made them perceive females as a rather limited resource (especially since none of these females ever actually spawned with them). This could be

investigated experimentally, by manipulating the availability of ripe and willing females as perceived by the males. Such controlled perceived surplus of females might yield stronger results, for example, the difference in egg mass between preferred and nonpreferred females might become significant at  $\alpha=0.05$ . In this context, the fact that we found a difference between bright and dull males might not even have been expected. For this expectation, one needs to assume either a genetic correlation between male brightness and male choosiness, or that the bright males were aware of their own attractiveness, even though they did not experience more ripe and willing females than the dull males. The males might have perceived their own attractiveness because of differential behaviour by the females, during pretesting stimulation as well as during the test, for which we did not control in our experiment, and which we did not quantify.

We briefly exposed the test females to a courting male just prior to testing, to check whether the females were sexually responsive but did not record the brightness of these males. Therefore, we cannot control for the 'previous male effect' (Bakker & Milinski 1991), that is, the effect that a female's preference for a male of intermediate brightness is lower if she has been exposed to a bright male rather than a dull male just before. Through this effect, the responsiveness of our test females during the test might have varied, and this might have led to more 'noise' in our data. Another source of 'noise' in the data is that, if males based their choice on some aspect of female body shape, they had to judge some females when their eggs were not yet ready for oviposition (namely the females that delayed oviposition until a few hours after testing or even the next day).

We conclude that our evidence supports the hypothesis that redder males prefer to mate with females that lay bigger eggs. This may imply that (while Darwin 1871 had monogamous species in mind) even in a promiscuous species sexual selection does not necessarily act via a mating advantage in terms of numbers of mates, but that it may involve mutual mate choice, with high-quality mates providing direct benefits to the sexually selected sex. Hence, the three-spined stickleback may prove to constitute an empirical system for studies on mutual mate choice and its role in sexual selection.

### Acknowledgments

We are very grateful to Sarah Zala who analysed the videotapes, to Reto Künzler who constructed the female container, and to Willem Steenge who caught the fish. We thank Michael Taborsky for discussions, and Anders Berglund and two anonymous referees for critical evaluation of the manuscript. We thank the Swiss National Science Foundation for financial support and the Netherlands Organization for Scientific Research (NWO) for financially supporting S.K. with a stipend.

### References

van den Assem, J. 1967. Territory in the three-spined stickleback, *Gasterosteus aculeatus* L. An experimental study in intra-specific competition. *Behaviour (Supplement)*, **16**, 1–164.

- Baker, J. A. 1994. Life history variation in female threespine stickleback. In: *The Evolutionary Biology of the Threespine Stickleback* (Ed. by M. A. Bell & S. A. Foster), pp. 144–187. Oxford: Oxford University Press.
- Bakker, Th. C. M. 1993. Positive genetic correlation between female preference and preferred male ornament in sticklebacks. *Nature*, **363**, 255–257.
- Bakker, Th. C. M. & Milinski, M. 1991. Sequential female choice and the previous male effect in sticklebacks. *Behavioral Ecology and Sociobiology*, **29**, 205–210.
- Bakker, Th. C. M. & Mundwiler, B. 1994. Female mate choice and male red coloration in a natural stickleback population. *Behavioral Ecology*, **5**, 74–80.
- Bakker, Th. C. M. & Rowland, W. J. 1995. Male mating preference in sticklebacks: effects of repeated testing and own attractiveness. *Behaviour*, **132**, 935–949.
- Bakker, Th. C. M. & Sevenster, P. 1989. Changes in the sexual tendency accompanying selection for aggressiveness in the three-spined stickleback, *Gasterosteus aculeatus* L. *Journal of Fish Biology*, **34**, 233–243.
- Baube, C. L., Rowland, W. J. & Fowler, J. B. 1995. The mechanisms of colour-based mate choice in female threespine sticklebacks: hue, contrast and configurational cues. *Behaviour*, **132**, 979–996.
- Blaxter, J. H. S. & Hempel, G. 1963. The influence of egg size on herring larvae (*Clupea harengus* L.). *Journal du Conseil permanent international pour l'Exploration de la Mer*, **28**, 211–240.
- Bolger, T. & Connolly, P. L. 1989. The selection of suitable indices for the measurement and analysis of fish condition. *Journal of Fish Biology*, **34**, 171–182.
- Borg, B. & Mayer, I. 1995. Androgens and behaviour in the three-spined stickleback. *Behaviour*, **132**, 1025–1035.
- Clutton-Brock, T. H. & Parker, G. A. 1992. Potential reproductive rates and the operation of sexual selection. *Quarterly Review of Biology*, **67**, 437–456.
- Clutton-Brock, T. H. & Vincent, A. C. J. 1991. Sexual selection and the potential reproductive rates of males and females. *Nature*, **351**, 58–60.
- Darwin, C. 1871. *The Descent of Man, and Selection in Relation to Sex*. London: John Murray.
- Elliot, J. M. 1989. The critical-period concept for juvenile survival and its relevance for population regulation in young sea trout, *Salmo trutta*. *Journal of Fish Biology*, **35** (Supplement A), 91–98.
- Fletcher, D. A. & Wootton, R. J. 1995. A hierarchical response to differences in ration size in the reproductive performance of female three-spined sticklebacks. *Journal of Fish Biology*, **46**, 657–668.
- Foster, S. A. 1994. Evolution of the reproductive behaviour of threespine stickleback. In: *The Evolutionary Biology of the Threespine Stickleback* (Ed. by M. A. Bell & S. A. Foster), pp. 380–398. Oxford: Oxford University Press.
- Foster, S. A. 1995. Understanding the evolution of behaviour in threespine stickleback: the value of geographic variation. *Behaviour*, **132**, 1107–1129.
- Foster, S. A., Garcia, V. B. & Town, M. Y. 1988. Cannibalism as the cause of an ontogenetic shift in habitat use by fry of the three-spined stickleback. *Oecologia*, **74**, 577–585.
- Jamieson, I. G. & Colgan, P. W. 1989. Eggs in the nests of males and their effect on mate choice in the three-spined stickleback. *Animal Behaviour*, **38**, 859–865.
- Johnstone, R. A., Reynolds, J. D. & Deutsch, J. C. 1996. Mutual mate choice and sex differences in choosiness. *Evolution*, **50**, 1382–1391.
- Kvarnemo, C. 1994. Temperature affects male and female reproductive rates in the sand goby: consequences for operational sex ratio. *Proceedings of the Royal Society of London, Series B*, **256**, 151–156.

- McKinnon, J. S. 1995. Video mate preferences of female three-spined sticklebacks from populations with divergent male coloration. *Animal Behaviour*, **50**, 1645–1655.
- McLennan, D. A. & McPhail, J. D. 1990. Experimental investigations of the evolutionary significance of sexually dimorphic nuptial colouration in *Gasterosteus aculeatus* (L.): the relationship between male colour and female behaviour. *Canadian Journal of Zoology*, **68**, 482–492.
- Marsh, E. 1986. Effects of egg size on offspring fitness and maternal fecundity in the orangethroat darter *Etheostoma spectabile* (Pisces: Percidae). *Copeia*, **1986**, 18–30.
- Milinski, M. & Bakker, Th. C. M. 1990. Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. *Nature*, **344**, 330–333.
- Milinski, M. & Bakker, Th. C. M. 1992. Costs influence sequential mate choice in sticklebacks, *Gasterosteus aculeatus*. *Proceedings of the Royal Society of London, Series B*, **250**, 229–233.
- Parker, G. A. 1983. Mate quality and mating decisions. In: *Mate Choice* (Ed. by P. Bateson), pp. 141–166. Cambridge: Cambridge University Press.
- Perrin, N. 1995. Signalling, mating success and paternal investment in sticklebacks (*Gasterosteus aculeatus*): a theoretical model. *Behaviour*, **132**, 1037–1053.
- Rowland, W. J. 1982. Mate choice by male sticklebacks *Gasterosteus aculeatus*. *Animal Behaviour*, **30**, 1093–1098.
- Rowland, W. J. 1989a. Mate choice and the supernormality effect in female sticklebacks (*Gasterosteus aculeatus*). *Behavioral Ecology and Sociobiology*, **24**, 433–438.
- Rowland, W. J. 1989b. The ethological basis of mate choice in male threespine sticklebacks, *Gasterosteus aculeatus*. *Animal Behaviour*, **38**, 112–120.
- Rowland, W. J. 1994. Proximate determinants of stickleback behaviour: an evolutionary perspective. In: *The Evolutionary Biology of the Threespine Stickleback* (Ed. by M. A. Bell & S. A. Foster), pp. 297–344. Oxford: Oxford University Press.
- Rowland, W. J., Bolyard, K. J., Jenkins, J. J. & Fowler, J. 1995. Video playback experiments on stickleback mate choice: female motivation and attentiveness to male colour cues. *Animal Behaviour*, **49**, 1559–1567.
- Sargent, R. C., Gross, M. R. & van den Berghe, E. P. 1986. Male mate choice in fishes. *Animal Behaviour*, **34**, 545–550.
- Sevenster, P. & van Roosmalen, M. E. 1985. Cognition in sticklebacks: some experiments on operant conditioning. *Behaviour*, **93**, 170–183.
- Sevenster-Bol, A. C. A. 1962. On the causation of drive reduction after a consummatory act (in *Gasterosteus aculeatus* L.). *Archives Néerlandaises de Zoologie*, **15**, 175–236.
- Trivers, L. R. 1972. Parental investment and sexual selection. In: *Sexual Selection and the Descent of Man* (Ed. by B. Campbell), pp. 136–179. Chicago: Aldine.
- Wallace, J. C. & Aasjord, D. 1984. An investigation of the consequences of egg size for the culture of Arctic charr, *Savelinus alpinus* (L.). *Journal of Fish Biology*, **24**, 427–435.
- Welkowitz, J., Ewen, R. B. & Cohen, J. 1982. *Introductory Statistics for the Behavioral Sciences*. San Diego: Harcourt Brace Jovanovich.
- Wilz, K. J. 1970. Causal and functional analysis of dorsal pricking and nest activity in the courtship of the three-spined stickleback *Gasterosteus aculeatus*. *Animal Behaviour*, **18**, 115–124.
- Wootton, R. J., Fletcher, D. J., Smith, C. & Whoriskey, F. G. 1995. A review of reproductive rates in sticklebacks in relation to parental expenditure and operational sex ratios. *Behaviour*, **132**, 915–933.