

Sexual selection in sticklebacks in the field: correlates of reproductive, mating, and paternal success

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Male sticklebacks display multiple ornaments, and these ornaments have been shown to be preferred by females in laboratory experiments. However, few field data exist, and it is not known whether these preferences are simultaneously or sequentially operative in a single population. We report correlates of reproductive success in two stickleback populations that differ in their ecology, over several periods within their breeding season. In both populations larger males had higher reproductive success, but not in all periods of the breeding season. Reproductive success increased with redness of the throat only in the Wohlensee population, and only in one period that was characterized by low average success. In the Wohlensee population, the parasitic worm *Pomphorhynchus laevis* is abundant, and reproductive success decreased with the presence of the parasite. In the Roche population, males with nests concealed in a plant had higher mating success. These nests were less likely to fail, suggesting that females preferred to spawn in concealed nests because of higher offspring survivorship. The different sexual traits appear to reveal different aspects of male quality (multiple message hypothesis): females probably find large males attractive because of their higher paternal quality, but it seems more likely that red males are preferred for better genetic qualities. Females also discriminate on territory quality, and male traits may be important in competition for these territories. The correlates of reproductive success were not consistent during the season, probably due to changes in the availability of ripe females. Such fluctuating selection pressures will contribute to the maintenance of genetic variation in sexual traits. *Key words:* embryo survivorship, *Gasterosteus aculeatus*, mating success, multiple ornaments, nest concealment, optimum brood size, paternal effort, paternal success, *Pomphorhynchus laevis*, reproductive success, sexual selection, sticklebacks. [*Behav Ecol* 10:696–706 (1999)]

Female mate preference is a powerful selective force in the evolution of male secondary sexual traits. Female choosiness, in turn, may evolve for three reasons (Kirkpatrick and Ryan, 1991): (1) choosy females are rewarded by direct fitness benefits (e.g., paternal care), (2) the offspring of choosy females benefit by inheriting from their father either genes for attractiveness, or (3) the offspring benefit by inheriting genes for viability.

Related to the benefits for choosy females is the complexity of male sexual traits. Males of many species display multiple signals. There are three hypotheses to explain the evolution of complex displays (Johnstone, 1996; Møller and Pomiankowski, 1993). The “multiple message hypothesis” states that different signals reveal different aspects of the signaler’s condition. In the “redundant signal hypothesis” (also called the “backup signal hypothesis”), it is assumed that different signals allow a better assessment of a single aspect of the signaler’s condition. The “unreliable signal hypothesis” says that some signals are unreliable indicators of the signaler’s condition. A fourth hypothesis, the “multiple context hypothesis,” can be added, in which the different signals function in different contexts (e.g., in intra- and intersexual selection).

Obvious candidates for the study of the function of multiple ornaments are multiply ornamented species in which the male provides, in addition to genes to the offspring, direct benefits in the form of parental care. The three-spined stickleback, *Gasterosteus aculeatus*, is such a species. Male stickle-

backs develop conspicuous breeding coloration consisting of an orange-red throat and forebelly and blue-green eyes. They build a nest of plant materials in shallow water. Males court females with a so-called zigzag dance, and they may spawn sequentially with up to 30 females over several days of the nesting cycle. A typical female lays 150–200 eggs, and a typical nest, therefore, contains thousands of eggs. After spawning the male alone cares for the eggs by defending the nest against territory intruders that often include cannibalistic conspecifics, and by direct care for the nest and eggs. Direct care consists of changes in nest structure with increasing egg development, removal of dead and diseased eggs, and providing the eggs with oxygen and removing waste products by fanning movements of the pectoral fins (Wootton, 1976, 1984).

A number of male traits have been shown to be preferred by female sticklebacks in their choice of mates. Best documented is the preference of females for males with more intense red breeding coloration (Bakker and Milinski, 1991; Baube et al., 1995; McKinnon, 1995; McLennan and McPhail, 1990; Milinski and Bakker, 1990, 1992; Rowland et al., 1995b). There are reports of female preferences for large body size (Rowland, 1989b), blue eye color (Rowland, 1994), and courtship behavior (Jamieson and Colgan, 1989; but see Bakker and Milinski, 1991; Milinski and Bakker, 1990). Nest concealment has also been shown to attract females (Sargent, 1982).

The above traits were studied in isolation, by experiments in the laboratory or in enclosures. Therefore, the relative importance of the different male and nest-site traits in determining male reproductive success in nature remains unknown. Correlative field data of an unmanipulated nature are needed to understand how sexual selection actually operates. Only two studies exist that report correlations between male traits and reproductive success in the field (red coloration: Bakker and Mundwiler, 1994; ventral spine length symmetry:

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Bakker ThCM, Mazzi D, and Kraak SBM, unpublished manuscript). It is not known whether the various preferences are simultaneously or sequentially operative in a single population. No field studies have investigated whether males with the preferred traits are better fathers. Such knowledge is necessary to understand the evolution of multiple sexual traits and the preferences for these traits.

In this study we investigated two stickleback populations that differ in their ecology over several periods within their breeding season. Our aim was to test which male traits and nest-site traits are correlated with reproductive success. The reproductive success of a male is the combined result of mating success (i.e., the number of eggs he receives) and paternal success (i.e., the proportion of embryos surviving to hatching). For one of the two populations we measured mating success and paternal success separately so that we could investigate the question of whether correlates of mating success are associated with paternal success; that is, whether choosy females prefer good fathers.

MATERIALS AND METHODS

Study sites

For the Wohlensee population, we collected data during the breeding season of 1993 at the Hasli site, which is an approximately 2 m² area in the littoral zone of the Wohlensee, near Bern, Switzerland (46°57' N, 7°28' E). The shallow area (water level 10–25 cm) is a preferred breeding site of sticklebacks. This natural habitat is surrounded by a wooden platform and has free access to the river from three sides. The habitat is relatively homogeneous in spring and early summer when plant growth is limited. Current velocity is negligible.

The prevailing parasite in this stickleback population is an orange-yellow worm, *Pomphorhynchus laevis* (Acanthocephala) (Bakker and Mundwiler, 1999). It is transmitted by *Gammarus pulex* (Amphipoda) and resides in the posterior end of the intestine. The parasite reduces the stickleback's fitness, as suggested by correlative field data (Bakker and Mundwiler, 1999) and experimental infections (Mazzi D, Bakker ThCM, and Kuenzler R, unpublished data).

For the Roche population, we collected data during the breeding season of 1995 in a stretch (of about 140 m length) of a small channel (46°26' N, 6°55' E), draining eventually into Lake Geneva, Switzerland. The channel is plated with blocks of concrete (60 cm × 35 cm) along the sloping (about 45°) walls and has a wooden boarded floor. At the bottom the channel is 1.1 m wide; the water depth varied between 50 cm and 80 cm in the course of our study. The vegetation is sparse. Sticklebacks nest in cut-outs in one corner of each concrete plate, or under the boards that line the concrete slopes at the bottom.

The strength of the water current at the nest sites is high (velocities measured at nests ranged from 5 cm/s to 53 cm/s). Parasite loads are low, and there is no particular parasite prevailing in this population.

Measurement of male traits and nest contents in the Wohlensee population

We followed the reproductive success of a group of about 15 males that settled in the Wohlensee study area within a few days in spring (19–22 April 1993), and a second, similarly sized group that settled after removal of the early group (5–10 May 1993). After settlement of the first male in each group, we checked the two groups of males daily for changes in group composition, nest positions, and willingness of the males to show courtship to a dummy of a ripe female (details in Bakker and Mundwiler, 1994).

Male traits

After completion of the nest, the males were hand-netted and photographic slides were made from the anterior half of the ventral side and one randomly chosen lateral side of the fish in a standardized setup (details in Bakker and Mundwiler, 1994). We analyzed the slides with a densitometer (X-Rite 310 Photographic Densitometer). The optical densities of red (R, filter 700 nm), green (G, filter 546.1 nm), and blue (B, filter 435.8 nm) were measured at 10 defined points (0.5 mm diameter) in the red throat region. We calculated a red index for each point from the optical densities (corrected for differences in film development): $1 - R/(R + G + B)$. The red index is an appropriate measure of red intensity (details in Bakker and Mundwiler, 1994). We used the highest index for red on the throat in the analyses. In an analogous manner, the green and blue indices of the iris of the eye were calculated from the slides. For green as well as blue, we used the highest index of three defined points in the analyses. Before the males were released at their nest, their standard length and mass were directly measured to calculate their condition factor (mass/length³). To distinguish neighboring males, we marked nearest neighbors differently by clipping the tip of the first and/or second dorsal spine. All nests and owners were mapped. We have never noticed nest takeovers.

About 2 weeks after settlement, we removed all the males. Their wet mass was determined and they were transferred to the laboratory. The males were killed by decapitation and dissected to determine the severity of infection by *P. laevis*. We counted the number of live acanthocephalans in the intestine, and the number of dead worms or remnants of dead worms (whether encapsulated or not) outside the intestine. The number of acanthocephalans refers to the total number of live and dead worms. We determined the size of the pectoral fins on dead fish by measuring the length of the longest fin ray of the left fin with a digitized vernier calliper.

Nest contents

About 2 weeks after settlement, just before the eggs hatched, we determined the males' reproductive success. At the same time when the males were removed, their nests were collected and the eggs taken out. The eggs were usually counted directly. In case of large numbers of eggs, however, we weighed the entire egg mass, as well as a representative sample of a counted number of eggs (about 50), after having dabbed them on absorbing tissue. We then calculated the total egg number by extrapolation. The number of eggs determined by extrapolation agreed well with the actual number of eggs ($r = .99$, $n = 4$, $p = .011$). Eggs were transferred to the laboratory, where they were hatched at 15°C to verify the age of the eggs.

In the analysis of reproductive success, only males that had been settled for at least 10 days were taken into account. This eliminated five males in the early group and one male in the later group. These males all built their first nest ≤ 4 days before eggs were counted, and thus they had likely not finished egg collection (Kraak et al., 1999).

Measurement of male traits, nest-site traits, nest contents, and behavior in the Roche population

We sampled 69 males that had naturally settled in the study area in 1995, together with their egg-filled nests: 18 males were sampled between 27 April and 12 June, 36 males were sampled between 14 June and 26 June, and 15 males were sampled between 2 July and 23 July. These samples will be referred to as the May, June, and July samples, respectively. We did not remove any fish between collecting the data of these samples. Each individual is represented only once in the

data set (we were able to exclude from the later two samples a few males that had been sampled before because they were marked, see below). We have data on paternal success for 45 of these males: 15, 19, and 11 of the May, June, and July samples, respectively.

Male traits

We caught the males from their nests with a hand net and photographed them within a minute after catching, as we did with the Wohlfenchee fish. We measured fin size as the sum of the lengths of the 10 fin rays on a projection of the slide by a photographic enlarger, divided by the magnification factor (about 18 \times). As with the Wohlfenchee fish, we analyzed the slides with a densitometer and calculated a red index for the throat and green and blue indices for the eyes. As before, the highest index for red of 10 defined points on the throat was used in the analyses. However, for the green and blue intensities of the eyes, we decided to measure at 6 instead of 3 defined points on each of two different slides, taken a few seconds apart, of the same fish (12 points). This procedure gave more accurate estimates of the eye indices, because eye color is more variable between slides than throat color (Bakker ThCM, unpublished data). In the analyses we used the highest green and highest blue index of the 12 points.

After photographing the fish we measured the standard length and the mass of the fish, from which we calculated the condition (mass/length³). We marked each male by cutting his first or his second dorsal spine. A subsample of males was additionally marked with a unique combination of blue spots by injecting a suspension of alcian blue under the skin. The fish was kept in a bucket until we replaced his nest (see below), after which the male was returned to his site.

Nest site traits

We recorded several characteristics of the nest sites on the same day that we collected the male and his nest. Because all nests were situated either in the cut-outs in the corner of the concrete plates or on the bottom, nests were present at only three discrete depths: approximately 65 cm, 45 cm, and 20 cm under the water surface. These levels were recorded for each nest (the actual depths were also recorded). We calculated the velocity of the water current at each nest site (May and June only), from the number of revolutions per minute of a calibrated OTT propeller, type C20. Velocity was always higher at the deeper nests. At each nest site we measured the temperature and the concentration of dissolved oxygen using an oxygen sensor Cellox325 connected to a WTW Oxi325 Oximeter (May and June only). On each day we also measured the water level, the current, the temperature, and the dissolved oxygen concentration at a fixed reference point. For analyses we used standardized values of the nest sites (i.e., the values of the reference subtracted from the values of the nest sites). We recorded whether or not the nest was hidden in a plant and on how many sides it was protected by a wall of substrate (1–4 sides and a “roof”). We counted the number of snails and the number of caddisfly larvae present in a square of 50 cm \times 50 cm with the nest at its center only in May. Both snails and caddisfly larvae are potential egg predators.

Nest contents

Immediately after catching the male we carefully took his nest, opened it, and removed all the eggs, which we put in a small container with water from the channel. We dabbed the eggs on absorbing tissue and weighed the whole brood and a representative sample of a counted number of eggs (usually 40) to calculate the total number of eggs present. Immediately after weighing, the eggs were put back in the container with

water. If fry were present in the nest, we counted them individually. Through a binocular microscope we screened the whole brood and recorded all the developmental stages of the embryos present (25 stages; after Swarup, 1958). Throughout the season we put samples of about 40 eggs in tea balls in the channel and monitored the developmental stages of the embryos daily. By this procedure we could approximately map developmental stages onto age in days at different times of the season (i.e., for May, June, and July separately) (Kraak et al., 1999). From each brood of the May sample we put about 40 eggs in tea balls in the channel, and we monitored survival of these eggs daily.

In 48 of all cases we dyed the whole brood blue by leaving it for at least 30 min in a suspension of alcian blue in channel water (about 10 g/l). This allowed us to distinguish at a later time between eggs that had been newly laid in the meantime (if any) and the eggs that had initially been present. Dyeing did not significantly influence embryo mortality (Kraak et al., 1997). Finally, we put the eggs back into the nest, repaired the nest, and put it back in its place in the channel. Then we released the fish on his nest. The whole procedure of recording male and brood characteristics took between 30 min and several hours. The males accepted their nests in all but one case, which was excluded from analysis.

A subsample of 33 males and their nests (i.e., those that were still present) was collected again at a later date within the same nesting cycle, after an interval ranging from 2 to 19 days. For those cases where the eggs had been dyed blue, it was easy to distinguish between the initial eggs (blue) and eggs that had been laid in the meantime (undyed eggs are yellow). Also, in the cases in which the eggs had not been dyed, we could deduce which eggs had been newly laid when their developmental stage was younger than the number of days that had passed since the first assessment. By measuring the mass of the remaining initial eggs and a representative counted sample, we were able to calculate the parental success (i.e., the proportion of eggs surviving after a given number of days). The manipulations of the nest and the eggs did not significantly influence egg survival (Kraak et al., 1997).

Behavior

In May we recorded some behaviors while sitting on the shore near the nest, using The Observer (Noldus®) event recorder software. Throughout the first half of the nesting cycle of each male, we monitored behaviors associated with mating. The following events were recorded for 30 min once every 2 days: female approach into a circle with a radius of 30 cm around the nest; the male approaching a female; a female reaching the nest; a female entering the nest; a female ovipositing in the nest. Halfway through the nesting cycle (when eggs were at stage 20), we monitored behaviors associated with caring. On two occasions (on the same day or on 2 subsequent days) we recorded for 30 min whether the male was fanning or not (states), and the following events: gluing by the male on the nest, intrusions by conspecifics into a circle with a radius of 30 cm around the nest, and chases of the intruder by the male.

In July, we made video recordings of about 90 min each by placing a Hi8 Sony CCD-TR 808 camcorder in a waterproof housing in the water at a distance of about 25 cm from the nest (usually upstream of the nest). Later we analyzed these recordings. We noted how many individuals approached the nest and how many of them were ripe females, whether the male chased, courted, or ignored the individual, and whether females performed the head-up posture, reached the nest, entered the nest, or spawned in the nest.

Data analysis

All statistical tests applied in this study are two-tailed. The distributions of variables were checked for normality with the Kolmogorov-Smirnov test with p values after Lillifors correction. Some variables were not normally distributed ($p < .05$); in these cases we transformed the variable and checked for normality again. Paternal success (see below) was always transformed for statistical tests by arcsine square-root transformation.

We do not report Bonferroni adjustments of our significance values because this would reduce our power too much (see Rothman, 1990, for justification). We chose $\alpha = 0.05$ as the significance level, but we also report cases with $.05 < p \leq .1$; when no p value is reported, $p > .1$.

We calculated the power of correlational tests following Welkowitz et al. (1982).

Reproductive success

Several field studies aiming to study correlates of reproductive success of paternal fishes (e.g., FitzGerald et al., 1993) estimated reproductive success simply as the number of eggs present in the nest, without regard to nest stage. However, as in our Roche samples, egg numbers were determined, as snapshots, at various stages of the nesting cycle. The eggs had suffered mortality in the nests for periods of various lengths of time. Moreover, some males had recently started their nesting cycle and had probably not finished collecting eggs yet. In Roche, the duration of a typical nesting cycle until hatching was minimally 19 days in May, minimally 16 days in June, and minimally 15 days in July. Accordingly, the sampled nests had ages ranging from 1 day to 20 days or even older. Obviously, one has to correct for this source of variation in egg number to compare reproductive success among nests.

We decided to standardize reproductive success by using the residuals of egg number to its fitted quadratic function of developmental stage of the oldest eggs present in the nest. We fitted this function separately for each of the 3 sample months (see Figure 1, as an example, for the June sample). This function has its maximum between developmental stage 15 and 16, corresponding to a nest age of about 3 days. We interpret the function as follows. The numbers of eggs in nests accumulate over the first 3 days because males typically collect eggs for 3 days (Kraak et al., 1999). Subsequently, egg numbers decline due to mortality in the nest (partial predation and cannibalism of viable and diseased or dead eggs). The residuals to this function indicate whether a male has relatively many or few eggs in his nest, taking into account how long ago he has started the present nesting cycle.

For the Wohlfensee data such a correction was not necessary; the males within each group were breeding to a large extent synchronously, and therefore no correlation existed between number of eggs present and the date of hatching in the laboratory [early group: $r = .09$, $n = 13$, $p = .8$; late group after square root ($x + 0.5$) transformation of egg number: $r = .17$, $n = 8$, $p = .7$; note that all nests were in the parental phase, meaning that no eggs were being collected any more and that only ongoing mortality had to be considered].

These estimates of reproductive success are the combined result of mating success (i.e., the number of eggs a male received) and parental success (i.e., the survival rate of eggs in the nest).

Mating success

Mating success (i.e., the number of mates acquired) is reflected by the number of eggs received (one female typically lays 150–200 eggs). We standardized mating success such that it is relatively unconfounded by the ongoing egg mortality in the

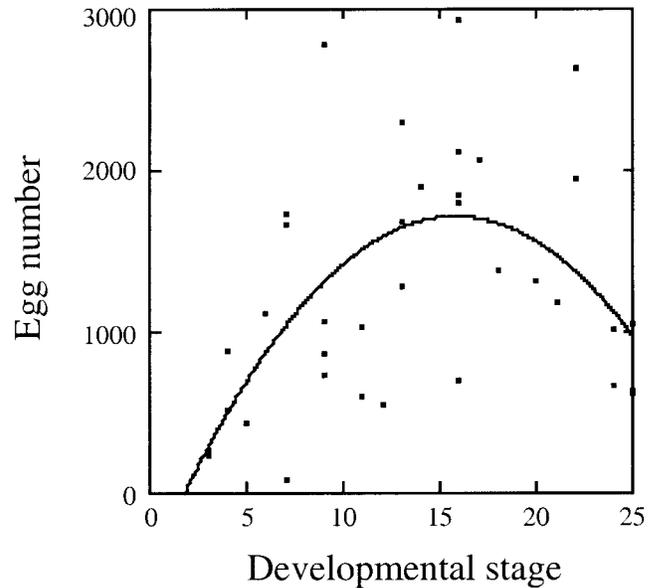


Figure 1

Relation between the number of eggs present in the nest and the developmental stage of the oldest eggs, for the 36 males of the June sample of Roche, 1995. The curve is the fitted quadratic function ($y = 8.8x^2 + 276.9x - 469.9$, $r^2 = .33$, $F = 8.21$, $p < .001$). We used the residuals of egg number as an estimate of reproductive success.

nest (which is an aspect of parental quality) for the Roche data as follows. We used a subsample of males that had not yet finished egg collection (as deduced if the youngest eggs were maximally 1 day old; see Kraak et al., 1999) and had collected eggs for no more than 3 days. We calculated the residuals of egg number to its fitted linear function of developmental stage of the oldest eggs. Here we assume that nests accumulate eggs monotonously with egg stage. The residuals indicate whether a male has received relatively many or few eggs, taking into account how long (in terms of egg development) he has been receiving eggs. We assume that the variance due to total egg mortality over 1, 2, or 3 days is negligible.

Paternal success

Paternal success is reflected by embryo survivorship until hatching: the better the male cleans, oxygenates, and defends his eggs and the fewer eggs he cannibalizes, the more that will survive to hatching. In our study in Roche some nests disappeared before their planned collection for a second time (that is, before hatching). The respective males have a paternal success of zero. For the remaining nests we calculated the average daily mortality percentage from the egg number present at first assessment and the number of those initial eggs that had survived until subsequent assessment, and the time interval in days. Based on these mortality rates, we calculated the proportion of eggs that would have survived for a standard interval of 10 days in each nest; this proportion we will call "paternal success." The choice of the standard interval of 10 days is arbitrary; we chose it to be shorter than the duration of egg development until hatching, which ranged from 15 days to more than 20 days.

We excluded from analyses those nests that we had partly destroyed, or nests from which >20 eggs floated away when putting the nest back after our first manipulation.

Any variation in egg survivorship between nests may be due to variation in egg quality in addition to variation in paternal quality. Because we are interested in the latter, we would like

Table 1
Correlates of reproductive and mating success (outcomes of multiple regression analyses)

Variable	β	t	p
Wohlensee, reproductive success			
Early group ($n = 13$, $r^2 = .36$, $F = 6.06$, $p = .032$)			
Body size	0.60	2.46	.032
Late group ($n = 10$, $r^2 = .79$, $F = 13.07$, $p = .004$)			
Red intensity of throat	0.49	2.56	.037
Presence of parasites	-0.56	-2.91	.022
Roche, May			
Mating success ($n = 10$, $r^2 = .72$, $F = 9.10$, $p = .011$)			
No. of snails	0.70	3.49	.010
Date of start of nest	-0.61	-3.02	.019
Roche, June			
Reproductive success ($n = 36$, $r^2 = .21$, $F = 4.29$, $p = .022$)			
Body size	0.31	1.97	.057
Presence of plant	0.31	2.01	.053
Mating success ($n = 23$, $r^2 = .51$, $F = 4.67$, $p = .009$)			
Body size	0.62	3.06	.007
Presence of plant	0.47	2.82	.011
Condition	0.36	1.67	.113
Standard O ₂ concentration	0.32	1.54	.140
Roche, July			
Reproductive success ($n = 11$, $r^2 = .74$, $F = 11.33$, $p = .005$)			
Body size	0.49	2.62	.031
Proportion of females headup	-0.85	-4.54	.002
Mating success ($n = 10$, $r^2 = .64$, $F = 6.28$, $p = .027$)			
Body size	0.83	2.99	.020
Red intensity of throat	-0.091	-3.29	.013
Mating success, largest male excluded ($n = 9$, $r^2 = .63$, $F = 5.15$, $p = .050$)			
Body size	0.41	1.66	.149
Red intensity of throat	-0.73	-2.94	.026
Roche, pooled			
Reproductive success ($n = 66$, $r^2 = .13$, $F = 4.59$, $p = .014$)			
Body size	0.28	2.37	.021
Red intensity of throat	-0.021	-1.77	.082
Mating success ($n = 44$, $r^2 = .20$, $F = 5.11$, $p = .010$)			
Body size	0.41	2.90	.006
Red intensity of throat	-0.20	-1.45	.154

to control for the former. In May, but not in June and July, we monitored survivorship of representative eggs from each brood in tea balls. Tea ball egg survivorship was always higher than egg survivorship in the nest. In the tea balls effects of paternal quality are excluded. Thus, only for the May sample, we controlled for variation in egg quality by subtracting the daily mortality of the tea ball eggs.

Correlations with male and nest-site traits

We used the following procedure to detect correlations between reproductive, mating, or paternal success as dependent variables, and various male traits, nest-site traits, and behaviors as independent variables. First we checked for correlations by single regressions. Then we performed stepwise backward regression analyses starting with all variables included, or, if that was not possible, at least all variables that singly correlated with $p < .1$, leaving out variables that singly correlated with the weakest. We report only the final outcomes of the stepwise backward multiple regressions. The total set of variables in-

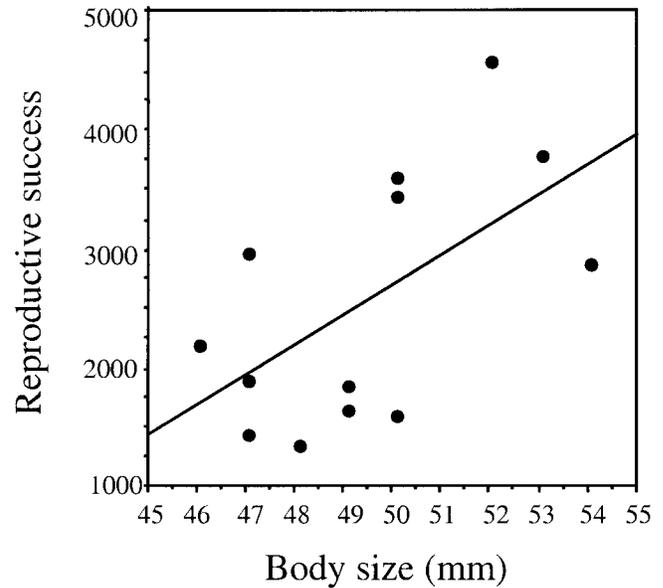


Figure 2

Correlation between reproductive success (number of eggs at the end of the breeding cycle) and male body size (standard length in millimeters) in 13 males of the early breeding group at the 1993 Hasli site. The line is the least square regression ($y = 2532.7x - 9959.8$, $r^2 = .36$, $F = 6.06$, $p = .032$).

vestigated in each case, and the transformations or corrections (see below) performed on them before analysis (if any), are reported in the appendix.

Some variables were corrected for correlations with the age of the nest (as inferred from the stage of the oldest eggs present) or date on which the nesting cycle had started. This was done by using the residuals of this variable to its fitted function of age (in days or developmental stage) or date, respectively. Usually a linear function was appropriate, but sometimes a quadratic function explained more of the variation of the variable. Within each sample we calculated standardized fin size as the residuals of fin size to its fitted linear function of standard length.

RESULTS

Correlates of reproductive success in the Wohlensee population

In the early group from the Wohlensee, the only male trait that correlated significantly with reproductive success was body size: larger males produced more progeny (Table 1 and Figure 2). Other potential criteria of female choice (condition, breeding coloration, standardized pectoral fin size, presence and number of parasites) were not significantly correlated with egg number. In the late group we found a significant positive correlation between egg number and the intensity of red throat coloration during egg collection (Figure 3) and a significant negative relationship between egg number and the presence of *P. laevis* (Mann-Whitney U test, $n = 9$, $U = 0$, $p = .05$). In a multiple regression analysis, both red intensity and presence of parasites emerged as the only significant variables (Table 1).

The number of eggs in the nests of males from the two successive breeding groups ranged from 0 to 4569. The availability of ripe females was higher in the early group (median egg number in the early group was 2181, range 1344–4569, in the later group 101, range 0–553; Mann-Whitney U test, n_1

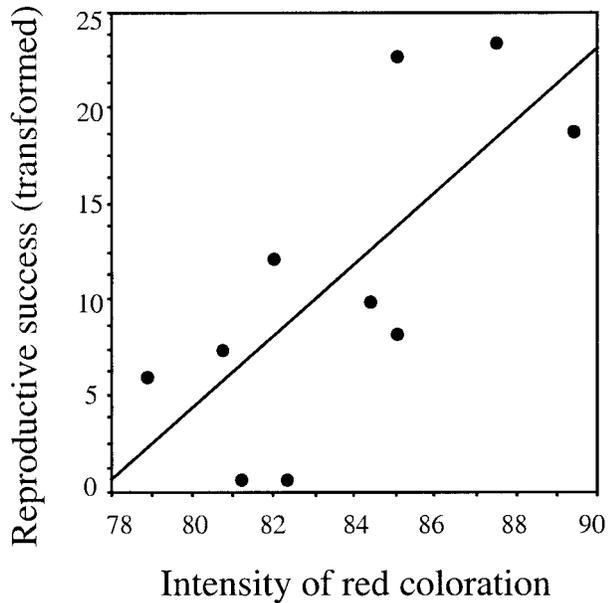


Figure 3
Correlation between reproductive success [number of eggs at the end of the breeding cycle, after square root ($x + 0.5$) transformation] and intensity of red throat coloration in 10 males of the late breeding group at the 1993 Hasli site. The line is the least square regression ($y = 185.9x - 144.3$, $r^2 = .53$, $F = 9.11$, $p = .017$).

$= 13$, $n_2 = 11$, $z = 4.14$, $p < .0001$). Males of the later group more often disappeared from the area (early group, 4 out of 22 males, 18.2%; later group, 12 out of 24 males, 50.0%; χ^2 with continuity correction = 3.82, $df = 1$, $p = .05$), and more of those that stayed shifted their nests than in the early group (early group, 1 out of 18 males, 5.6%; late group, 7 out of 12 males, 58.3%; $\chi^2 = 10.26$, $df = 1$, $p < .002$).

Males that left the area after a short, unsuccessful nesting period differed in a number of traits from males that stayed reproductively active for 10 days or longer. In the early group, the condition factor of deserters was lower (Mann-Whitney U test, $n = 13$, 3; $z = 1.95$, $p = .051$), and the blue intensity of their eyes was higher (Mann-Whitney U test, $n = 12$, 3; $z = 2.02$, $p = .043$) than those of stayers. There were no significant differences with respect to body size, red intensity, and green intensity of the eye (all $p > .31$). In the late group, deserters tended to be smaller (Mann-Whitney U test, $n = 11$ and 12, $z = 1.90$, $p = .057$) but were redder (Mann-Whitney U test, $n = 11$ and 7, $z = 2.31$, $p = .021$) than stayers. They did not significantly differ in the other traits (all $p > .39$). With the early and late groups pooled, deserters and stayers differed significantly only in size (deserters smaller: Mann-Whitney U test, $n = 16$, 25, $z = 2.02$, $p < .04$; all other traits $p > .29$).

Correlates of reproductive success, mating success, and paternal success in the Roche population

In the May sample (18 males), there was little variation in number of sides on which the nests were protected by substrate, presence of a “roof,” and presence of a plant. Therefore, these nest-site traits were not analyzed.

In May, reproductive success, linearly corrected for date, correlated with the following single variables: depth level of the nest ($r = .53$, $p = .025$), the nest’s distance upstream of the outflow of our channel into a bigger channel ($r = -0.65$, $p = .004$), the number of snails around the nest ($r = .53$, p

$= .027$), the number of intruders ($r = -0.57$, $p = .042$), and the number of female approaches ($r = -.75$, $p = .002$). However, because all these variables were correlated among themselves (e.g., significant correlations of depth level with, respectively: distance to big channel, positive; number of snails, negative; number of intruders, positive; number of approaches, positive), it is difficult to judge which of the factors might actually have a causal effect. None of the male traits was correlated with reproductive success, with any of the nest-site traits, nor with the numbers of intruders and approaching females.

We analyzed a subsample of 10 males of the May sample for mating success. None of the male traits correlated with mating success; only nests with a higher standardized temperature tended to have a higher mating success ($r = .63$, $p = .051$). However, a stepwise backward multiple regression indicated that male mating success decreased significantly in the course of the sampling period and that high mating success was associated with many snails around the nest (Table 1).

Two of the 15 nests of the May sample disappeared before hatching, and hence the males had zero paternal success. In the 13 remaining nests, on average, 43.6% of the eggs would have been left after 10 days (based on daily egg mortality without subtracting tea-ball mortality to make comparison with other months possible; median 50.8%, range 6.9–86.7%, SD 26.6%). Paternal success based on daily mortality after subtracting tea-ball mortality decreased with date ($r = -.71$, $n = 14$, $p = .004$), as did mating success. Among nine males for which we measured the condition a second time (11–20 days after the first time), there was a significant positive correlation between paternal success and daily loss of condition ($r = .80$, $p = .010$; actually, four males lost and five males gained in condition). This correlation suggests a cost of caring. However, this relation disappeared when we linearly corrected paternal success for date ($r = .14$, $p = .727$). This corrected measure of paternal success was positively correlated with the proportion of time that the male spent fanning ($r = .66$, $n = 11$, $p = .025$), but showed no relations with any male or nest-site trait. Paternal success, whether corrected for date or not, was not related to mating success.

Among the 36 males sampled in June, larger males ($r = .33$, $p = .049$) and males with their nests hidden in plants ($r = .34$, $p = .045$) had higher reproductive success. By stepwise backward multiple regression, we arrived at a model in which these effects became just nonsignificant (Table 1). None of the male traits was associated with the presence of a plant.

A subsample of 23 males of the June sample was analyzed for mating success, and we found that mating success, too, was higher for larger males ($r = .48$, $p = .022$) and for males with their nests in plants ($r = .50$ or $t = 2.67$, $p = .014$, $n = 8$ with plant, $n = 15$ without plant). After stepwise backward multiple regression, these factors remained significant in a model in which male condition and the standardized concentration of dissolved oxygen at the nest also had nonsignificant positive effects (Table 1).

The nests of 11 of the 19 males of the June sample disappeared, and therefore these males had zero paternal success. Of the other eight males paternal success was, on average, 49.3% (median 55.0%, range 9.8–82.8%, SD 21.0%). Comparing the 11 nests that disappeared with the 8 successful nests, we found that the former had a lower reproductive success before disappearance than the latter ($t = 3.07$, $df = 17$, $p = .007$). Males with successful nests had more intense blue eyes ($t = 3.77$, $df = 17$, $p = .002$), and their nests were subject to higher relative water currents ($t = 3.64$, $df = 12.6$, $p = .003$). Blue intensity of the eyes ($r = .47$, $n = 19$, $p = .044$) and relative current ($r = .86$, $n = 19$, $p < .001$) were correlated with the depth level. Nest success was correlated with

depth as well: all successful nests were at the deepest level, whereas of the unsuccessful nests, only two were at the deepest, two at the intermediate level, and seven at the shallowest level (Fisher's Exact test, $p = .001$). Six of the eight successful nests were hidden in a plant, whereas only one of the nests that disappeared was in a plant (Fisher's Exact test, $p = .006$). Since six of the seven nests in plants were at the deepest level, we do not know whether the relevant factor is concealment in a plant or depth level. Within the nests at the deepest level (eight successful nests and two unsuccessful nests), the effect of relative current was nonsignificant ($t = 0.95$, $df = 4.8$, $p = .388$), as was the effect of the presence of a plant (Fisher's Exact test, $p = .133$).

Within the eight successful nests, none of the male traits or nest-site traits was correlated with paternal success. Paternal success of these eight males was not related to mating success.

For the 15 males examined in July, standard length and fin size were not normally distributed. We did not transform the data. Regarding male standard length, this deviation from a normal distribution was due to one extraordinarily large male of 6.6 cm. The next largest in this sample was 5.5 cm. This 6.6-cm male and one other male had very large fins. The fins of the latter male were unusually large for his size. We calculated two standardized fin sizes: one based on the total sample of 15 males, and one based on a sample of 14 males excluding the male with relatively large fins. In the sample there was little variation in presence of a roof and presence of a plant; hence, these nest-site traits were not used in the analyses.

Our measure of reproductive success was positively correlated with the number of spawnings per hour ($r = .62$, $n = 12$, $p = .031$) and with the number of spawnings per approaching ripe female ($r = .60$, $n = 12$, $p = .039$) that we recorded on video. This gives us confidence that our measure of reproductive success makes sense. None of the male or nest-site traits were correlated singly with reproductive success. By stepwise backward multiple regression, we found that larger males produced more offspring, and that higher reproductive success was associated with a smaller proportion of approaching females performing the head-up display (Table 1). The effect of male size disappears, however, if we exclude the extremely large male from the analyses; only the effect of the proportion of females performing head-up displays remains significant ($r = -.65$, $n = 11$, $p = .032$).

We analyzed a subsample of 10 males of the July sample for mating success. This measure was also positively correlated with the behavioral measures for mating success [i.e., the number of spawnings per hour ($r = .69$, $n = 10$, $p = .027$) and the number of spawnings per approaching ripe female ($r = .63$, $n = 10$, $p = .051$)]. None of the male or nest-site traits were correlated singly with mating success. However, by stepwise backwards multiple regression, we found that larger males with less intense red throats have higher mating success (Table 1). Male standard length tends to be correlated with the red index among the males of this subsample ($r = .58$, $n = 10$, $p = .078$), but this is entirely due to the very large male who was also very red. If this male is excluded from the multiple regression model, red intensity is still significant, but the effect of male size becomes nonsignificant (Table 1). The number of individuals passing per hour, after logarithmic transformation, was positively correlated with mating success ($r = .65$, $n = 10$, $p = .043$). When the stepwise backward multiple regression is run with this variable added, only this variable remains included in the model. When the largest male is excluded from the analyses, we arrive at a model with only a significant negative effect of red index ($r = -.68$, $n = 9$, $p = .044$). Note that the number of passing individuals and the red index are negatively correlated ($r = -.74$, $n = 9$, p

$= .022$, or, excluding the large male, $r = -.91$, $n = 8$, $p = .002$); this makes interpretation difficult.

In July all 11 nests were successful. On average, 45.9% of embryos survived in these nests (median 52.0%, range 8.2–70.6%, SD 19.9%). Paternal success was only positively correlated with the green intensity of the eye ($r = .66$, $n = 11$, $p = .028$) and with the sum of the green and the blue index of the eye ($r = .73$, $n = 11$, $p = .011$). Paternal success was not related to mating success ($n = 9$).

To increase the power of our tests, we also analyzed the May, June, and July samples pooled (69 males). Presence of plant and presence of roof were not analyzed because they differed between the samples. Current, dissolved oxygen concentration, and temperature at the nest site were not analyzed because we did not measure them in July.

The only trait that correlated significantly with reproductive success was male size ($r = .30$, $n = 67$, $p = .014$). A stepwise backward multiple regression led to a model in which the red intensity of the throat had a nonsignificant negative effect besides the positive effect of male size (Table 1).

The mating success of 44 males was correlated with male size ($r = .40$, $n = 44$, $p = .008$) and the distance upstream of the big channel ($r = .29$, $p = .050$). Males that had settled farther upstream had a better condition ($r = .29$, $n = 67$, $p = .016$), and more intense green eyes ($r = .25$, $n = 67$, $p = .041$), but this was only true for the total pooled sample of 67 males, and not for the subsample of 44 males used for mating success (for both traits $p > .18$). In the July video recordings, the proportion of approaching individuals that were ripe females was higher for nests farther upstream ($r = .75$, $n = 12$, $p = .005$). By stepwise backward multiple regression, we found, again, besides the positive effect of male size, that the red intensity of the throat had a nonsignificant negative effect (Table 1).

The 14 nests that disappeared differed from the 32 successful nests in that the former had a lower reproductive success before disappearance ($t = 2.15$, $df = 44$, $p = .037$), their owners had less intense blue eyes ($t = 2.04$, $df = 44$, $p = .047$), and they were located at shallower depths (Pearson $\chi^2 = 10.5$, $df = 2$, $p = .005$), as was found in the June sample. Among the 32 successful nests, paternal success was not correlated with any male or nest-site trait. Paternal success of these males was not related to mating success ($n = 26$).

None of the relations with paternal success changed with respect to significance when the average mass of the eggs in the brood was included in the models. We tested this because average egg mass was shown to influence egg survival when nests were deprived of care (Bakker ThCM, Mazzi D and Kraak SBM, unpublished manuscript).

DISCUSSION

The male trait most generally associated with reproductive success is body size. Larger males had significantly higher reproductive success in the early group of the Wohlensee and in two of the three samples of Roche. Moreover, in these two Roche samples larger males also had higher mating success. We cannot exclude the existence of similar relations with body size in the late group of the Wohlensee and the May sample of Roche. For example, in the June sample of Roche, the effect of body size on reproductive success was of medium strength ($r = .33$), and the effect on mating success was large ($r = .47$). In the small May sample of Roche ($n = 18$ for reproductive success, $n = 10$ for mating success), the chances to detect effects of these strengths are not high (power = .22 and power = .32, respectively). It is not surprising that larger body size is associated with higher reproductive and mating success. In many fish species with paternal care for eggs in a

nest, larger males are of better paternal quality and therefore preferred by females (e.g., Bisazza and Marconato, 1988; Côte and Hunte, 1989; Downhower and Brown, 1980; Wiegman and Baylis, 1995; Wiegman et al., 1992). Large body size may also be advantageous in male–male competition (Rowland, 1989a).

Also no surprise is the finding that the presence of parasites is associated with lower reproductive success in the Wohlensee. Males with parasites are likely of lower phenotypic quality and therefore not able to produce as many offspring as parasite-free males. It is impossible to tell from our data whether females preferred to mate with parasite-free males.

A puzzling result of our study is the fact that in the Roche samples we found either no association between throat redness and reproductive/mating success, or a negative one, and that only in the late group, but not in the early group, of the Wohlensee did we find that redder males have higher reproductive success. Laboratory experiments clearly showed a female mate preference for redder males in both populations (Bakker, 1993; Bakker and Milinski, 1991; Bakker and Mundwiler, 1994; Milinski and Bakker, 1990, 1992), which has led to a positive genetic correlation between preference and redness (Bakker, 1993). There may be several reasons for this discrepancy. (1) In the laboratory, variation in male traits other than red coloration were minimized and randomized. In the field, female preferences for traits other than red coloration may overrule red preferences. (2) Redder males may have reduced mating probabilities because aggression levels were too high in competitive situations (see Ward and Fitzgerald, 1987). (3) Costs of choice in the field (e.g., due to water current or predation risk) may reduce female choosiness with respect to red coloration (see Godin and Briggs, 1996; Milinski and Bakker, 1992). This may play a role particularly in Roche, where the water current is strong. (4) A female-biased operational sex ratio may reduce female choosiness (Clutton-Brock and Parker, 1992; Clutton-Brock and Vincent, 1991). (5) The mating advantage of redder males might not result in acquiring more eggs, but in acquiring eggs of better quality, via mutual mate choice and quality-assortative mating (Kraak and Bakker, 1998). (6) Our tests may have had insufficient power to detect the advantage of red intensity. For example, in the laboratory experiment of Milinski and Bakker (1990), the effect size of red intensity was only slightly more than medium ($r = .37$). Only with the three Roche samples pooled did we have a reasonable chance (power > 0.67) to detect a medium effect. However, because in the present study we even found (significant) negative relations of red intensity with mating and reproductive success, we think the lack of power is not a likely explanation. Large effects of red throat coloration as were found in the late group of the Wohlensee ($r = .73$; Figure 3) and in an earlier study of the Wohlensee population ($r = .69$; Bakker and Mundwiler, 1994) would have been detected even with the sample size of our June sample alone. Furthermore, in the breeding season of 1996 we also failed to find a positive relation between redness and reproductive success in Roche (Bakker ThCM, Mazzi D, and Kraak SBM, unpublished manuscript). Therefore, we think the lack of a positive relation is real.

Viewing our findings in more detail, we come up with the following explanation for the absence and presence of correlations between redness and egg number. The only sample in which we did find that redder males reproduce better was the late group from the Wohlensee. This group was characterized by very low reproductive success (median 101 eggs, versus 2181 in the early Wohlensee group, and 1820, 1266, and 2355, uncorrected for age, in the respective Roche samples). This may mean that only in the late Wohlensee group was the availability of ripe females limiting. The operational

sex ratio in Roche seemed female biased in July: females approached a male's nest at an average rate of 21 per hour (range 2–62, SD = 20). 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 21 females per hour. Indeed, males seldom zigzagged when ripe females approached (median 0.07 times per approaching ripe female; range 0–0.4). Their behavior to ripe females performing the head-up posture consisted more often of meandering and dorsal pricking (median 0.24 times per approaching ripe female; range 0–1). This behavior has been interpreted as trying to keep the female away from the nest (Wilz, 1970).

If ripe females are available in excess, males do not mate with an unlimited number of females. Survivorship of embryos deprived of care decreases with increasing brood size (Bakker ThCM, Mazzi D, and Kraak SBM, unpublished manuscript), probably as a result of oxygen limitation. As a consequence, males will experience a trade-off between the benefits of acquiring more eggs and the costs of oxygenating more eggs: they will refuse further matings when having acquired an optimum number of eggs (Perrin, 1995). This optimum depends on individual male paternal quality: better males can care for more eggs (Perrin, 1995). If red coloration is not associated with paternal quality, but body size is (see below), this may explain the absence of a positive correlation between egg number and red intensity on the one hand, and the presence of a positive correlation between egg number and body size on the other hand. If redder males are preferred by females for reasons other than paternal quality (e.g., genetic benefits), a positive relation between redness and egg number should only be found among males that have not reached their optimum brood size and that will not yet refuse matings. This may explain why only in the late Wohlensee group, in which males had collected low numbers of eggs, was red coloration positively correlated with reproductive success. If redder males are not better fathers, a negative correlation between mating success and red coloration, as in July in Roche, is even expected when it is taken into account that redder males care for bigger eggs and that bigger eggs have lower survival chances in the nest under oxygen limitation (Bakker ThCM, Mazzi D, and Kraak SBM, unpublished manuscript). Because it costs more to care for bigger eggs, males of equal paternal quality will have a lower optimum brood size if eggs are bigger.

Some of the sexual traits seem to play a role in male–male competition in the Wohlensee population. In the two breeding groups in the Wohlensee, several males disappeared after nest building during the observation period. They were probably outcompeted by other males. Male–male competition was probably more intense in the late group than in the early group, because mean reproductive success was considerably lower. Accordingly, in the late group many more males disappeared after building a nest than in the early group. Although we cannot exclude predation as a cause, competition for territories and females seems more likely. Deserters likely had lower competitive abilities: lower condition in the early group and smaller body size in the later group. On the other hand, deserters likely had traits that were attractive to females: more intense blue eyes in the early group and redder throat coloration in the later group. Especially in the late group where reproductive success per male was low, attractive males may achieve a higher reproductive success in a less competitive situation.

In the Roche population, we also have some indication that traits are involved in male–male competition. In July, the males with a better condition and greener eyes had settled farther upstream, where a higher proportion of passing indi-

viduals were ripe females, and the mating success was generally higher. Perhaps these males had been able to exclude other males from upstream territories, which might have been more attractive for females for unknown reasons. And in June, males with bluer eyes more often occupied nest sites at the deepest level, at which they were less likely to disappear (see below). However, competition for territories did not seem severe because there were always unoccupied nest sites left.

Territory traits seem to be important determinants of reproductive success. Nests that were hidden in a plant received more eggs. Nests concealed in plants enjoyed higher paternal success as well, in the sense that these nests were less likely to disappear before hatching. This suggests that females preferred safer places for their eggs. Particularly in June, many nests disappeared. We cannot distinguish between cases in which the male abandoned or was predated and the nest was lost subsequently and cases in which the nest was lost by destruction followed by the male leaving his territory. The nests that disappeared had relatively few eggs. This may mean that males were more likely to abandon because they had few eggs, or, alternatively, that males with few eggs were inferior males in more than one respect (i.e., mating and paternal success, and nest defense or self-defense). The probability of nest success was not only associated with concealment in a plant, but successful nests were also more often located at the deepest level, where the current velocity was highest (see also Whoriskey and FitzGerald, 1994). Although water current may positively influence embryo survivorship because embryos experience a better exchange of water, it is difficult to understand why nests at higher velocity sites should be less likely to disappear. Depth level and the presence of a plant, however, may influence the chances of total nest failure directly or, rather, the chances of disappearance of the father. The most common fish predator at Roche is the grey heron *Ardea cinerea*. Males at the deeper two levels (about 45 cm and 65 cm deep) are out of reach of this predator, and males fanning at a nest concealed in a plant are not as visible to this predator as males fanning at exposed nests. Concealment in a plant may also protect the nest against destructive raids by conspecifics (Mori, 1993; Sargent and Gebler, 1980). Males with nests in plants had a higher mating success, suggesting that females appreciate the protection of the nest and the father by the presence of the plant. This female preference for males with hidden nests may be indirect, however. It has been shown that males reduce courtship in the face of a predator, but less so when they court from a nest concealed by plants (Candolin and Voigt, 1998).

Besides the presence of a plant, no other male or nest-site trait that was correlated with mating success was also correlated with paternal success. However, if we want to conclude that no relationship exists, we have to take into account the power of our statistics. In May, the correlation between mating success and relative temperature at the nest site was strong ($r = .63$). If an equally strong relation exists between temperature and embryo survivorship, the chance of detecting it in a sample of $n = 11$ would be about half (for $p = 0.63$ power = .52). Similarly, the relation between male body size and mating success in June and in the pooled sample was of medium strength ($r = .33, .30$, respectively). In our samples of $n = 8$ for June and $n = 32$ for the pooled sample, the chances of detecting a medium effect of body size on paternal success would be 0.13 and 0.40, respectively.

Moreover, if we want to conclude that preferred males were not better fathers, we have to consider first whether we should expect that embryo survivorship is higher in the nests of better fathers. One has to realize that the more eggs there are in a nest, the more oxygen is needed to ensure a given level of embryo survivorship (Bakker ThCM, Mazzi D, and Kraak

SBM, unpublished manuscript): paternal care is not shareable (sensu Wittenberger, 1983). Thus, males that enjoy a higher mating success and collect more eggs have to work harder in terms of fanning than less attractive males to achieve a similar paternal success (Perrin, 1995). If females can assess paternal quality, they should prefer a male of high paternal quality only until a certain egg number in his nest is reached; beyond that her eggs may have higher survival chances in the nest of a lower quality male with fewer eggs present (Perrin, 1995). Perrin's (1995) model predicts, counterintuitively, that at equilibrium males of high paternal quality have a higher mating success but have a similar embryo survivorship compared to low-quality males. Males of higher paternal quality do provide better care, but better care is needed for their larger broods to reach similar embryo survivorship. Thus, according to this model it is not expected that male traits that correlate with mating success correlate with embryo survivorship as well.

We can still use Perrin's (1995) model in combination with our data to answer the question of whether females prefer certain males because they are better fathers (direct benefits). If body size is an indicator of paternal quality, then large males should receive more eggs and have equal paternal success, measured as embryo survivorship, compared to small males. Conversely, if females prefer large males for indirect benefits only (i.e., for good genes or sexy genes), then the larger males with more eggs would have higher egg mortality. Our results correspond to the prediction that follows when body size indicates paternal quality. Redder males did not have higher mating success in our study (Roche), implying that they were not better fathers. At equilibrium, males of similar paternal quality should only have differential mating success, if the genetic benefit that a trait confers to the female's offspring compensates for the lower embryo survivorship. If not, then females should not favor genetically attractive males over males that have fewer eggs in their nests.

Our data tentatively suggest that red intensity of the throat does not signal paternal quality. However, some earlier studies have suggested that redder males are better fathers. For example, redder males may be better able to defend the nest because redness also plays a role in male-male interactions (Bakker, 1985, 1986, 1994; Bakker and Milinski, 1993; Bakker and Sevenster, 1983; Baube, 1997; Rowland et al., 1995a). Furthermore, the intensity of red coloration is sometimes correlated with physical condition (Bakker and Mundwiler, 1994; Frischknecht, 1993; Milinski and Bakker, 1990), but this is not always the case (Bakker and Mundwiler, 1994; Baube, 1997; Rowland, 1994). Further research is needed to clarify the relation between the red ornament and paternal quality.

It is unclear how we should interpret the positive correlation between green intensity of the eyes and paternal success in Roche in July. Males received similar egg numbers irrespective of eye color. It is unclear why females would not use green intensity of the males' eyes as a quality indicator or why males with greener eyes would not signal their superior quality. In the Wohlensee population, the green intensity of the eyes of parasitized males correlated positively with the number of acanthocephalan worms present (Bakker ThCM and Mundwiler B, unpublished data). Heavily parasitized males might invest more in paternal care of the present brood because of reduced survival probabilities. However, we did not investigate correlates of parasite load in the Roche population.

In conclusion, our findings agree with the multiple message hypothesis of sexual ornamentation (Møller and Pomiankowski, 1993). The various sexual traits were not correlated with each other (Wohlensee: Bakker ThCM and Mundwiler B, unpublished data), thus suggesting that they are not redundant signals. The multiple sexual traits reveal different aspects of male quality: females probably find large males at

tractive because of their higher paternal quality, but it seems more likely that red males are preferred for better genetic qualities (sexy or good genes). Females also appear to discriminate on territory quality. The strong indications that yet other male traits are important in male–male competition lends support to the multiple context hypothesis for sexual selection of multiple ornaments as well (see introduction). The correlates of reproductive success were not consistent during the season, probably due to changes in various ecological factors and changes in the availability of ripe females. Such temporally fluctuating selection pressures on male sexual traits will contribute to the maintenance of genetic variation in sexual traits.

APPENDIX

For the Wohlensee sample, reproductive success was transformed as square root ($x + 0.5$) for the late group, and the male traits analyzed were body size (e^x transformed for the late group), intensity of red throat coloration (Box-Cox transformed with a parameter value of 9.5 for the early group), intensity of blue (Box-Cox transformed with a parameter value of -14.5 for the early group) and green eye coloration during courtship, condition factor during courtship, presence/absence of acanthocephalans, and standardized pectoral fin size.

For the May sample of the Roche population, the following variables were analyzed: date on which the nest cycle started, body size, intensity of red throat coloration (corrected for developmental stage, linearly), intensity of blue and green eye coloration, condition (corrected for developmental stage, quadratically), standardized pectoral fin size, depth level, distance upstream of big channel, north/south shore, standardized current, dissolved oxygen concentration (2^x transformed) and temperature (4^x transformed) at the nest site, numbers of snails and caddisfly larvae around the nest (both after logarithmic transformation), number of intruders, female approaches, courtship bouts (after logarithmic transformation) per 30 min, and the proportion of intruders chased (after arcsine square-root transformation).

For the June sample of the Roche population, the following variables were analyzed: starting date of the nest cycle, body size, intensity of red throat coloration, intensity of blue and green eye coloration, condition (corrected for developmental stage, linearly), standardized pectoral fin size, depth level, distance upstream of big channel, north/south shore, standardized current, dissolved oxygen concentration and temperature at the nest site, number of nest sides with protection by hard substrate, presence of a roof, and presence of a plant.

For the July sample of the Roche population, the following variables were analyzed: body size, intensity of red throat coloration, intensity of blue and green eye coloration, condition, standardized pectoral fin size, depth level, distance upstream of big channel, north/south shore, number of sides with protection by hard substrate, numbers of approaching individuals (after logarithmic transformation), approaching ripe females, and chases per hour, proportion of approaches by ripe females, proportion of ripe females performing head-up (after logarithmic transformation), proportion of ripe females that elicited zigzag display (after arcsine square-root transformation), and proportion of ripe females that elicited meandering.

For the pooled sample of the Roche population, reproductive success is residuals of an ANCOVA of egg number with the sample month included as a factor and the developmental stage of the oldest eggs, quadratically, as covariate. Mating success is residuals of an ANCOVA of egg number with the sample month included as a factor and the developmental stage of the oldest eggs as covariate (these residuals were logarithmically transformed).

The following variables were analyzed: starting date of the nest cycle, body size, intensity of red throat coloration (corrected for sample month, by subtracting the sample mean, and age of the nest in days), intensity of blue and green eye coloration, condition (corrected for developmental stage), standardized pectoral fin size (corrected for sample month by subtracting the sample mean), depth level, distance upstream of big channel, north/south shore, number of sides with protection by hard substrate.

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REFERENCES

- Bakker ThCM, 1985. Two-way selection for aggression in juvenile, female and male sticklebacks (*Gasterosteus aculeatus* L.), with some notes on hormonal factors. *Behaviour* 93:69–81.
- Bakker ThCM, 1986. Aggressiveness in sticklebacks (*Gasterosteus aculeatus* L.): a behaviour-genetic study. *Behaviour* 98:1–144.
- Bakker ThCM, 1993. Positive genetic correlation between female preference and preferred male ornament in sticklebacks. *Nature* 363:255–257.
- Bakker ThCM, 1994. Evolution of aggressive behaviour in the threespine stickleback. In: *The evolutionary biology of the threespine stickleback* (Bell MA, Foster SA, eds). Oxford: Oxford University Press; 345–380.
- Bakker ThCM, Milinski M, 1991. Sequential female choice and the previous male effect in sticklebacks. *Behav Ecol Sociobiol* 29:205–210.
- Bakker ThCM, Milinski M, 1993. The advantages of being red: sexual selection in the stickleback. *Mar Behav Physiol* 23:287–300.
- Bakker ThCM, Mundwiler B, 1994. Female mate choice and male red coloration in a natural stickleback population. *Behav Ecol* 5:74–80.
- Bakker ThCM, Mundwiler B, 1999. Pectoral fin size in a fish species with paternal care: a condition-dependent, sexual trait revealing infection status. *Freshwat Biol* 41:543–551.
- Bakker ThCM, Sevenster P, 1983. Determinants of dominance in male sticklebacks (*Gasterosteus aculeatus* L.). *Behaviour* 86:55–71.
- Baube CL, 1997. Manipulations of signalling environment affect male competitive success in three-spined sticklebacks. *Anim Behav* 53:819–833.
- Baube CL, Rowland WJ, Fowler JB, 1995. The mechanisms of colour-based mate choice in female threespine sticklebacks: hue, contrast and configurational cues. *Behaviour* 132:979–996.
- Bisazza A, Marconato A, 1988. Female mate choice, male-male competition and parental care in the river bullhead, *Cottus gobio* L. (Pisces, Cottidae). *Anim Behav* 36:1352–1360.
- Candolin U, Voigt H-R, 1998. Predator-induced nest site preference: safe nests allow courtship in sticklebacks. *Anim Behav* 56:1205–1211.
- Clutton-Brock TH, Parker GA, 1992. Potential reproductive rates and the operation of sexual selection. *Q Rev Biol* 67:437–456.
- Clutton-Brock TH, Vincent ACJ, 1991. Sexual selection and the potential reproductive rates of males and females. *Nature* 351:58–60.
- Côte IM, Hunte W, 1989. Male and female mate choice in the redlip blenny: why bigger is better. *Anim Behav* 38:78–88.
- Downhower JF, Brown L, 1980. Mate preferences of female mottled sculpins, *Cottus bairdi*. *Anim Behav* 28:728–734.
- FitzGerald GJ, Fournier M, Morrisette J, 1993. Sexual selection in an anadromous population of threespine sticklebacks—no role for parasites. *Evol Ecol* 8:348–356.
- Frischknecht M, 1993. The breeding colouration of male three-spined sticklebacks (*Gasterosteus aculeatus*) as an indicator of energy investment in vigour. *Evol Ecol* 7:439–450.
- Godin J-CJ, Briggs SE, 1996. Female mate choice under predation risk in the guppy. *Anim Behav* 51:117–130.
- Jamieson IG, Colgan PW, 1989. Eggs in the nests of males and their effect on mate choice in the three-spined stickleback. *Anim Behav* 38:859–865.

- Johnstone RA, 1996. Multiple displays in animal communication: 'backup signals' and 'multiple messages'. *Phil Trans R Soc Lond B* 351:329–338.
- Kirkpatrick M, Ryan MJ, 1991. The evolution of mating preferences and the paradox of the lek. *Nature* 350:33–38.
- Kraak SBM, Bakker ThCM, 1998. Mutual mate choice in sticklebacks: attractive males choose big females, which lay big eggs. *Anim Behav* 56:859–866.
- Kraak SBM, Bakker ThCM, Mundwiler B, 1997. How to quantify embryo survival in nest-building fishes, exemplified with three-spined sticklebacks. *J Fish Biol* 51:1262–1264.
- Kraak SBM, Bakker ThCM, Mundwiler B, 1999. Correlates of the duration of the egg collecting phase in the three-spined stickleback. *J Fish Biol* 54:1038–1049.
- McKinnon JS, 1995. Video mate preferences of female three-spined sticklebacks from populations with divergent male coloration. *Anim Behav* 50:1645–1655.
- McLennan DA, McPhail JD, 1990. Experimental investigations of the evolutionary significance of sexually dimorphic nuptial coloration in *Gasterosteus aculeatus* (L.): the relationship between male colour and female behaviour. *Can J Zool* 68:482–492.
- Milinski M, Bakker ThCM, 1990. Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. *Nature* 344:330–333.
- Milinski M, Bakker ThCM, 1992. Costs influence sequential mate choice in sticklebacks, *Gasterosteus aculeatus*. *Proc R Soc Lond B* 250:229–233.
- Møller AP, Pomiankowski A, 1993. Why have birds got multiple sexual ornaments? *Behav Ecol Sociobiol* 32:167–176.
- Mori S, 1993. The breeding system of the three-spined stickleback, *Gasterosteus aculeatus* (form *Leiura*) with reference to spatial and temporal patterns of nesting activity. *Behaviour* 126:97–124.
- Perrin N, 1995. Signalling, mating success and paternal investment in sticklebacks (*Gasterosteus aculeatus*): a theoretical model. *Behaviour* 132:1037–1053.
- Rowland WJ, 1989a. The effects of body-size, aggression and nuptial coloration on competition for territories in male threespine sticklebacks, *Gasterosteus aculeatus*. *Anim Behav* 37:282–289.
- Rowland WJ 1989b. Mate choice and the supernormality effect in female sticklebacks (*Gasterosteus aculeatus*). *Behav Ecol Sociobiol* 24:433–438.
- Rowland WJ, 1994. Proximate determinants of stickleback behaviour: an evolutionary perspective. In: *The evolutionary biology of the threespine stickleback* (Bell MA, Foster SA, eds). Oxford: Oxford University Press; 297–344.
- Rowland WJ, Bolyard KJ, Halpern AD, 1995a. The dual effect of stickleback nuptial coloration on rivals: manipulation of a graded signal using video playback. *Anim Behav* 50:267–272.
- Rowland WJ, Bolyard KJ, Jenkins JJ, Fowler J, 1995b. Video playback experiments on stickleback mate choice: female motivation and attentiveness to male colour cues. *Anim Behav* 49:1559–1567.
- Rothman KJ, 1990. No adjustments are needed for multiple comparisons. *Epidemiology* 1:43–46.
- Sargent RC, 1982. Territory quality, male quality, courtship intrusions, and female nest-choice in the three-spine stickleback, *Gasterosteus aculeatus*. *Anim Behav* 30:364–374.
- Sargent RC, Gebler JB, 1980. Effects of nest site concealment on hatching success, reproductive success, and paternal behavior of the threespine stickleback, *Gasterosteus aculeatus*. *Behav Ecol Sociobiol* 7:137–142.
- Sevenster-Bol ACA, 1962. On the causation of drive reduction after a consummatory act (in *Gasterosteus aculeatus* L.). *Arch Neerl Zool* 15:175–236.
- Swarup H, 1958. Stages in the development of the stickleback *Gasterosteus aculeatus* (L.) *J Embryol Exp Morphol* 6:373–383.
- Ward G, FitzGerald GJ, 1987. Male aggression and female mate choice in the threespine stickleback, *Gasterosteus aculeatus* L. *J Fish Biol* 30:679–690.
- Welkowitz J, Ewen RB, Cohen J, 1982. *Introductory statistics for the behavioral sciences*. New York: Harcourt, Brace, Jovanovich, Inc.
- Whoriskey FG, FitzGerald GJ, 1994. Ecology of the threespine stickleback on the breeding grounds. In: *The evolutionary biology of the threespine stickleback* (Bell MA, Foster SA, eds). Oxford: Oxford University Press; 195–196.
- Wiegman DD, Baylis JR, 1995. Male body size and paternal behaviour in smallmouth bass, *Micropterus dolomieu* (Pisces: Centrarchidae). *Anim Behav* 50:1543–1555.
- Wiegman DD, Baylis JR, Hoff MH, 1992. Sexual selection and fitness variation in a population of smallmouth bass, *Micropterus dolomieu* (Pisces: Centrarchidae). *Evolution* 46:1740–1753.
- Wilz KJ, 1970. Causal and functional analysis of dorsal pricking and nest activity in the courtship of the three-spined stickleback *Gasterosteus aculeatus*. *Anim Behav* 18:115–124.
- Wittenberger JF, 1983. Tactics of mate choice. In: *Mate choice* (Bateson P, ed). Cambridge: Cambridge University Press; 435–448.
- Wootton RJ, 1976. *The biology of the sticklebacks*. London: Academic Press.
- Wootton RJ, 1984. *A functional biology of sticklebacks*. London: Croom Helm.