

## Broods of attractive three-spined stickleback males require greater paternal care

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The relationship between egg number and survival in nests of three-spined sticklebacks *Gasterosteus aculeatus* was tested in the field. Nests were deprived of paternal care during a variable period by removal of the father while preventing egg predation by protection of the nest by a net. Upon male removal, a number of male traits were quantified. Nest-content variables and embryo survival were assessed at the end of the deprivation period. Proportional egg mortality was significantly positively correlated with the length of the deprivation period, the number of eggs present in the nest and egg size, thus suggesting that nests with more and larger eggs need more paternal care. Males with the most symmetrical ventral spines achieved the highest reproductive success as measured by the number of eggs in the nest. In addition, their nests contained relatively larger eggs. Spine length symmetry correlated with the blue intensity of the eye thus giving females several cues to assess male quality.

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Key words: egg number and size; embryo survival; fluctuating asymmetry; *Gasterosteus aculeatus*; male quality; paternal care.

### INTRODUCTION

In species where the male cares for the offspring, females could increase their fitness by mating with good fathers. Paternal quality comprises several aspects like male quality, territory quality and the number of offspring to be cared for. In teleosts with external fertilization, paternal care is widespread and often coincides with territory formation (Ridley, 1978; Blumer, 1979, 1982; Baylis, 1981; Gross & Shine, 1981; Gross & Sargent, 1985; Ah-King *et al.*, 2005). Paternal care often takes the form of egg cleaning, oxygenation of the developing embryos with fin movements (called fanning) and selection of a nest site that is defended against egg predators. In nest-building fishes, the eggs of

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multiple spawnings are often stuck together in one large egg mass. In such nests, competition for oxygen among embryos may lead to hypoxia when dissolved oxygen supply is interrupted for longer periods (Reebs *et al.*, 1984). Nest contents may thus be an important criterion of mate choice in nest-building fishes. Males with many eggs in their nest have to invest more in paternal care. When looking for mating partners, females of nest-building fishes may therefore trade-off between male quality and nest contents.

Perrin (1995) modelled the direct sexual selection situation outlined above for three-spined sticklebacks *Gasterosteus aculeatus* L. (and other fishes with male guarding behaviour) by an optimization model that links paternal investment, male mating success and signalling. Paternal investment is assumed to be a state-dependent decision, and signal a condition-dependent handicap by which males inform females of how much they are willing to invest. It is further assumed that embryo survival decreases with increasing egg number. Thus, males that have greater mating success must work harder (invest more time in fanning) than less attractive males to get a similar paternal success. Males are assumed to differ in quality or condition. Quality is defined here by its positive effect on survival under bad conditions. Low-quality males suffer more when increasing paternal investment. The optimal paternal investment (for a given egg number) correlates with quality. If females are able to assess male quality, they should prefer a male of high 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's (1995) model predicts that in a population at equilibrium paternal success (*i.e.* embryo survivorship until hatching) of males that differ in mating success (*i.e.* the number of mates acquired) is similar, because high-quality males receive more eggs. Thus, at equilibrium, female clutches are ideally freely distributed with respect to hatching success. Males of higher quality do provide better care, but better care is needed for their larger broods to reach similar embryo survivorship. Because attractive, high-quality males care for more eggs, similar embryo survivorship means that in absolute terms more embryos hatch.

Three-spined sticklebacks have exclusive male parental care. Eggs are collected during a limited number of days in a nest built of plant materials. Up to 2680 eggs (on average 28 females) may be spawned in a single nest (Kynard, 1978), or even more (Kraak *et al.*, 1999a, b; in exceptional cases up to 8000 eggs, representing 50 females or more, were found; T. C. M. Bakker, pers. obs.). Male care consists of nest defence, nest-maintenance behaviours, removal of dead and diseased eggs and oxygenation of embryos by fanning movements of the pectoral fins. Fanning may take up to two-third of a parental male's time (van Iersel, 1953). Experience in the laboratory with development of three-spined stickleback embryos outside the nest in containers with aerated water, and anecdotal evidence from care-deprivation experiments in the field at nest sites with hardly any water current (T. C. M. Bakker, pers. obs.) stress the importance of oxygenation for embryo survival in large egg masses (Blumer, 1986).

The main objective of the present study was to test whether there existed a negative relationship between egg number and embryo survival as assumed

by Perrin (1995) in his model. This was done by studying egg survival in the field under reduced oxygenation of the embryos in a population that breeds in a habitat with relatively strong water current (water velocities up to 50 cm s<sup>-1</sup>). To that aim embryos were deprived of paternal care during a variable period of time by male removal. Egg predation was prevented by applying a net over the nest. Because of high water velocities, survival chances of the embryos without care were expected to be higher than zero. A second aim of the study was to establish whether specific male traits correlated with reproductive success in the field. Laboratory experiments with fish from this population showed female mating preference based on the intensity of the male's red throat (Milinski & Bakker, 1990; Bakker 1993; Künzler & Bakker, 2001) and male body size (Künzler & Bakker, 2001), while field studies of this population found significant correlations of reproductive success with male body size and intensity of blue eye colouration (Kraak *et al.*, 1999a; Largiadèr *et al.*, 2001).

## MATERIAL AND METHODS

### BREEDING HABITAT CHARACTERISTICS

The study site was a stretch of *c.* 150 m length of a small channel in the neighbourhood of Roche (near Montreux, Switzerland, 46°26' N; 6°55' E) near its outflow in a wider channel that eventually ends in Lake Geneva. The study was done in July 1996. The water level in the channel was on average 47 cm. The channel has sloping walls (slope of 40°), is 1.1 m wide at the bottom and *c.* 2.1 m wide at the water surface. The walls of the channel consist of four layers of blocks of concrete (60 by 35 cm) until *c.* 65 cm above its boarded floor and then continue as grass walls until the level of the agricultural fields. There are no trees or bushes along the channel. The vegetation in the channel is sparse because macrophytes are removed every year before the start of the breeding season. Three-spined sticklebacks breed in small cavities (10 by 10 cm, sometimes 10 by 20 cm) that exist in the lower left or right corner of each concrete block. The concrete blocks are shifted half a block between stacked layers. Minimal nest distances are thus 60 cm in a horizontal plane and 46 cm between layers. The upper two levels were at or above the water surface during the study period, so that nests were located at distances of 5 or 25 cm from the bottom. Males also build nests under a log that separates the bottom from the concrete slopes. The water velocity at the nests ranged from 17 to 47 cm s<sup>-1</sup>, mean 29 cm s<sup>-1</sup> and temperature from 11.9 to 13.6° C (mean 12.9° C).

### MALE CHARACTERISTICS

Territorial males and their nest sites were located by observing breeding activities from the shore. Once the nest site of a particular male had been ascertained, the male was caught at the nest with a small hand-net. Males were selected randomly but it was impossible to catch some males or to locate some nests. Furthermore, males with hatched young in their nests were not taken. About half of the breeding males in this section of the channel were caught for the experiment. Immediately after catching, photographic slides were taken, of each male, from the anterior half of the ventral side and of one lateral side in a standardized set-up (Bakker & Mundwiler, 1994). The slides were analysed with a densitometer by measuring the optical density of red (R, 700.0 nm), green (G, 546.1 nm) and blue (B, 435.8 nm) at 10 defined points (diameter 0.5 mm) in the ventral red throat region. A red index ( $I_R$ ) was calculated for each point

from the optical densities (corrected for differences in film development):  $I_R = 1 - [R / (R + G + B)]^{-1}$ . The  $I_R$  is an appropriate measure of red intensity (Frischknecht, 1993; Bakker & Mundwiler, 1994). The highest index for red on the throat was used in the analyses. In an analogous manner, the green and blue indices of the iris of the eye (being the two major colour components of the blue-green eye) were calculated from the slides. Eye movements make the structural eye colour more difficult to photograph. Therefore, for each male two slides of one of his eyes were taken in quick succession. The highest indices of green and blue eye colour of 12 defined points (six points per slide that were evenly distributed round the iris) were used in the analyses. Recently discovered ultraviolet reflection of the eye (I. P. Rick, pers. comm.) could not be quantified with the photographic method.

At the field site, male standard length ( $L_S$ , cm) and wet body mass ( $M$ , g) were directly measured to calculate his condition factor ( $K$ ) from  $K = 100 M L_S^{-3}$  (Bolger & Connolly, 1989). Males were taken to the laboratory and their  $M$  was again determined after 2 days of starvation in order to obtain  $K$  that were not confounded by differences in stomach contents. They were then killed by decapitation and the asymmetry in ventral spine length determined by measuring the lengths of the left and right ventral spines with a digitized vernier calipers to 0.01 mm accuracy. The criteria for fluctuating asymmetry (FA) (Swaddle *et al.*, 1994) were tested using a random sample of 33 wild-caught fish from another Swiss freshwater population (Wohlensee; Bern 46°57' N; 7°28' E). The left and right ventral spines of each fish were measured twice using the same method while covering the displayed digits during measurement. Ventral spines showed FA: 1) the distribution of the lengths of left spines, right spines and signed difference between the lengths of left and right spines did not significantly deviate from normality (Kolgomorov-Smirnov test with  $P$  values after Lilliefors correction:  $P > 0.62$ ,  $P > 0.40$  and  $P > 0.47$ , respectively), 2) the signed difference between left and right spines was centred around zero (one sample  $t$ -test, d.f. = 32,  $P > 0.21$ ), 3) spine length was significantly repeatable: repeatabilities ( $r_I$ )  $\pm$  s.e. (Becker, 1992) for left and right spines were  $99.89 \pm 0.04$  and  $98.64 \pm 0.48\%$  respectively and 4) spine asymmetry was also significantly repeatable: mixed-model ANOVA (Swaddle *et al.*, 1994),  $F_{32,64}$ ,  $P < 0.001$ , that is, 2.7% of the variation in asymmetry was due to measurement error.

## DEPRIVATION OF PATERNAL CARE

After removal of nest owners, which were measured for a number of traits (outlined above), their nests were deprived of paternal care during a randomly assigned number of days: 7 days (two nests), 6 days (five nests), 5 days (one nest), 3 days (four nests) or 0 days (four nests). The entrance of the breeding cavity was closed by a double layer of a dark green, plastic net (mesh-size  $5 \times 5$  mm), which was spread and attached to the wall of the channel using hairpins. The mesh-size was large enough to let juveniles that hatched escape out of the nest cavity but prevented the main egg predators [adult and juvenile three-spined sticklebacks, and other piscivorous fishes like small perch *Perca fluviatilis* L. and rainbow trout *Oncorhynchus mykiss* (Walbaum)] from entering the nest cavity. After the deprivation period, the nest and eggs were collected, put in 500 ml vessels with water from the channel, transferred to the laboratory, and kept at 15° C under aeration, which guarantees good embryo survival.

The next day, eggs were separated from nesting materials and the number of eggs (including the number of empty egg cases in the nest) counted, the total egg mass determined after carefully dabbing the eggs on absorbent tissue and removing debris, the developmental stages assessed (Swarup, 1958) and, using a dissection microscope, whether or not the embryos were alive was determined. Embryos were considered dead when either the whole egg was opaque because it was 'mouldy' or it was milky white, or when only the embryo was opaque (milky white), or, in the case of late developmental stages, there was no heart beat or blood circulation. In some cases in which the status of the embryo was uncertain, the eggs were put in vessels with aeration at 15° C, and further development was checked.

## ASSUMPTIONS AND DATA ANALYSES

Developmental stages of the embryos were translated into age in days by using data from the developmental rate of separate eggs in July 1995 that were enclosed in tea-balls made of metal mesh in the channel (Kraak *et al.*, 1997). The tea-balls were fixed to the walls of the channel just under the water surface, and embryos checked daily for development. A complication of translating developmental stages into ages is that the developmental stages are of unequal duration: changes are much faster early in development, so that later stages last longer than early stages. Therefore the youngest age of each stage was used in the calculations. The number of days that the male had collected eggs before placement of the net was estimated from the difference between the youngest age of the most developed stage and that of the least developed stage of embryos in his nest. The number of days that males had cared for eggs before placement of the net was estimated from the difference between the youngest age of the most developed embryos and the number of days that the net had been in place.

Because of the probable greater water velocities and thus water exchange in the tea-balls than in the nest cavities (Kraak *et al.*, 1999b), the eggs in the tea-balls would have been oxygenated better and thus embryo development in the tea-balls would have been faster than that of embryos in nests that were deprived of paternal care. This became evident from the fact that in three cases the calculated number of days of care based on the developmental stage of the oldest embryos in the nest was negative. Also the exceptionally long, calculated egg-collection phases (five males had collected eggs for >10 days; Kraak *et al.*, 1999b) based on the developmental stages of the eggs pointed to a retardation of embryo development. The retarded development was further evidenced by the negative correlation between the calculated duration of the paternal care period and the length of the deprivation period; a quadratic regression was the best fit:  $r^2 = 0.70$ ,  $n = 16$ ,  $P < 0.001$  (Fig. 1). The calculated length of the paternal care period was corrected for the length of the deprivation period by taking the residuals of this quadratic regression. The residual values were used as the corrected ages.

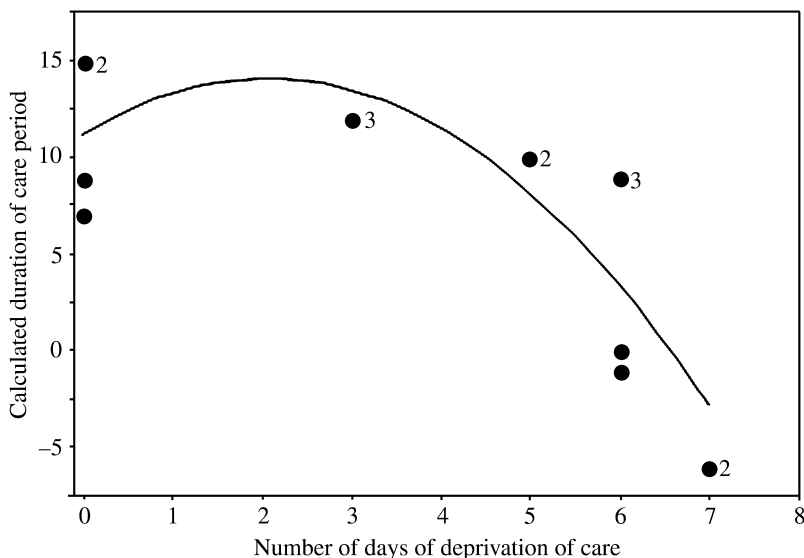


FIG. 1. Quadratic regression line ( $y = 11.09 + 2.90x - 0.70x^2$ ) used to correct the estimated number of days of the paternal care period before placement of the net for the length of the deprivation period in days. Numbers indicate the number of nests with equal data. The sample size was 16 nests.

Several variables change during the parental care period and have to be corrected when males and nests are sampled after a variable period of care (Kraak *et al.*, 1999a). The longer the paternal care period the greater the reduction in the number of eggs in the nest (due to mortality and cannibalism), red breeding colouration and, to a lesser extent, eye colouration (McLennan & McPhail, 1989), and physical condition (due to costs of care; Sargent, 1985). In some three-spined stickleback populations, the male's red colouration intensifies towards the end of the parental period (McLennan & McPhail, 1989). In the Roche population, males do not become redder at the end of the breeding cycle, so that there is a linear decrease of redness with the number of days that the male cared for eggs (data of Roche males and nests sampled throughout the breeding season of 1995:  $r = -0.44$ ,  $n = 63$ ,  $P < 0.001$ ; S. B. M. Kraak, T. C. M. Bakker & B. Mundwiler, unpubl. data). In the sample of the present study, none of the above-mentioned correlations, although negative, was significantly different from zero (all  $P > 0.5$ ). Correcting for the duration of the care period seems thus superfluous, which was confirmed by reanalyses of the data using the residuals of linear regression of relevant variables on corrected age. With a few exceptions, both analyses gave similar results. Therefore the results of the uncorrected data are presented.

More than half of the nests (*i.e.* 10 of 16 nests) contained some empty egg cases (median 37, range 0–284). Because almost all these nests also contained embryos of the latest stage before hatching, egg cases were counted as viable eggs thereby assuming that embryos had hatched and escaped through the net during the period of enclosure. In two nests, dead juveniles (28 and 42, respectively) were found. Dead juveniles were also counted as viable eggs, because they had developed until hatching and embryos can usually withstand lower dissolved oxygen concentrations than juveniles (Rombough, 1988). In one of these nests, there were very few eggs that had not hatched. The data on survival in this nest were discarded in the mortality analysis because it was unknown when the eggs had hatched. Because egg survival shows non-linear relationships with many variables (Rombough, 1988),  $\ln$ -transformed values of the variables were used in the analyses of egg mortality.

The distributions of variables were tested for normality using the Kolmogorov–Smirnov test with  $P$  values after Lilliefors correction. Variables that deviated ( $P < 0.05$ ) from normality were  $\ln x$  or  $\ln(x + 1)$  transformed in order to meet the normality assumptions for the multivariate analyses.

Due to technical difficulties, for some of the males data on egg mass (four males) and eye colouration (four males) were missing, thus making the data set unbalanced. Bonferroni adjustments of the significant values are not reported because this would reduce the power too much (Rothman, 1990). In order to reduce the number of comparisons, the following procedure was used to detect correlations between egg number or egg size as dependent variables and various male traits as independent variables. First, the data were checked for correlations by single linear regressions. Then multiple regression analyses with backward elimination of the least significant variables were performed starting with all variables that singly correlated with  $P < 0.1$ .

## RESULTS

### EMBRYO SURVIVAL AS A FUNCTION OF NEST CONTENTS

The number of eggs in nests that were subjected to deprivation of paternal care ranged from 63 to 2019 (mean  $\pm$  s.d. =  $795.8 \pm 638.2$ ,  $n = 16$ ), and was similar across treatments (Fig. 2; ANOVA,  $F_{3,8}$ ,  $P > 0.31$ ). The number of eggs that died during the period of deprivation ranged from zero to 1578, mean  $\pm$  s.d. =  $270.0 \pm 418.8$ . Some embryos could even withstand 7 days without care. When nests contain variable numbers of eggs, proportional egg mortality will give information as to whether egg survival is better in

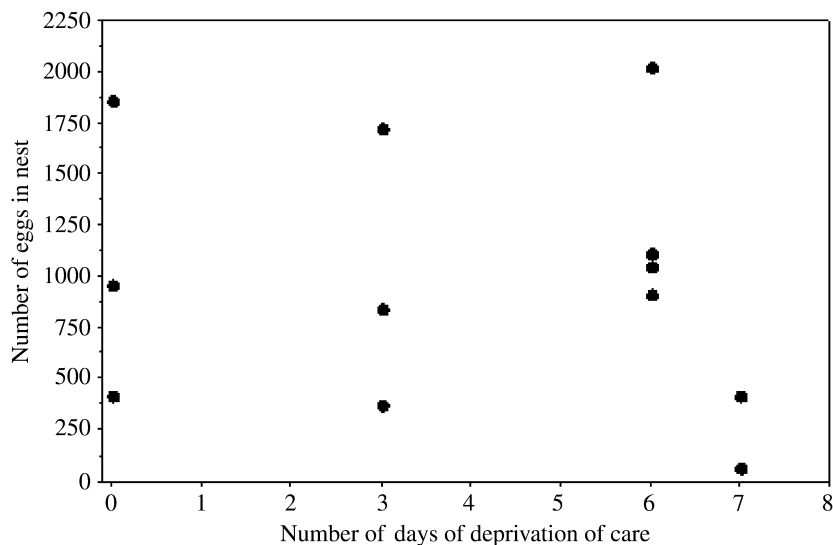


FIG. 2. The distribution of the number of eggs in nests across treatments.

some nests than in others. The proportion of eggs that died ranged between 0 and 78.2% (mean  $\pm$  s.d. =  $30.7 \pm 29.0\%$ ). In a multiple regression analysis, both the deprivation period and nest contents (egg number and egg size) were significantly and positively related to the proportion that died (Table I). The age of the eggs (age of oldest eggs at start of deprivation was deduced at the end of the deprivation period) did not enter the model ( $P > 0.33$ ). Even if age was included in the model, the effect of egg number on survival remained significant ( $P < 0.05$ ). The number and size of eggs in the nest did not significantly correlate with the age of the eggs corrected for the length of the deprivation period ( $r = 0.15$ ,  $n = 12$ ,  $P > 0.65$  and  $r = -0.005$ ,  $n = 12$ ,  $P > 0.98$ , respectively). How the egg number influenced percentage mortality is shown in Fig. 3. The effects of variation in the length of the deprivation period and in egg size on per cent mortality were controlled for by using residuals of the multiple regression of the proportion of eggs that died on deprivation period and egg size.

TABLE I. Multiple regression of the proportion of eggs that died on deprivation period (number of days of deprivation of care) and nest contents (number of eggs and egg size) ( $n = 12$ ,  $r^2 = 0.93$ ,  $F_{3,8}$ ,  $P < 0.0001$ ). The percentage of dead eggs were  $\ln(x + 1)$ , size and numbers of eggs  $\ln x$  transformed

Variable	Slope $b$	$t$	$P$
Deprivation period	1.01	10.38	<0.0001
Nest contents			
Number of eggs	0.28	2.85	0.021
Egg size	0.23	2.40	0.043

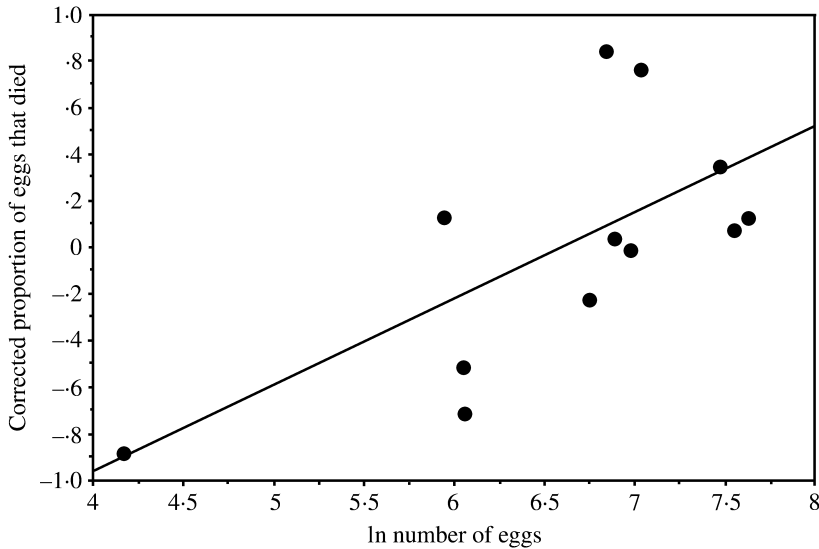


FIG. 3. Relationship between the number of eggs (ln transformed) in nests deprived of care and the proportion of eggs that died corrected for differences in the length of the deprivation period and egg size between nests by using residuals of a multiple regression analysis. The curve was fitted by:  $(y = -2.43 + 0.37x)$  ( $r = 0.67$ ,  $n = 12$ ,  $P < 0.05$ ).

### CORRELATES OF EGG NUMBER

One of the factors that impaired egg survival was egg number (Table I). The number of eggs in the nest of caring males can be regarded as a measure of reproductive success, *i.e.* the combined result of mating success and paternal success. Two males that were obviously still collecting eggs at the start of the experiment were excluded from the analysis.

As a first step in the analysis of associations, single linear regression analysis of the number of eggs in the nest was done on the following potential attractive male traits: blue and green intensities of the eye, red throat intensity,  $K$  after 2 days of starvation, ventral spine asymmetry, mean ventral spine length and  $L_S$ . The strongest associations ( $P < 0.1$ ), spine asymmetry and spine length, were then combined in a multiple regression analysis. The multiple regression revealed that only spine asymmetry (both absolute and relative) was significantly negatively associated with egg number (Table II). Males with longer ventral spines had more symmetrical spines: the relative asymmetry in spine

TABLE II. Multiple regression of egg number on male traits (asymmetry of ventral spines, *i.e.* unsigned difference in mm, and mean ventral spine length) ( $n = 14$ ,  $r^2 = 0.49$ ,  $F_{2,11}$ ,  $P = 0.025$ )

Variable	Slope $b$	$t$	$P$
Spine asymmetry	-0.52	2.32	0.041
Spine length	0.34	1.51	>0.15



length was smaller for males with longer mean spine length ( $r = -0.45$ ,  $n = 20$ ,  $P < 0.05$ ; the sample was supplemented with reproductively active males that were not used in the deprivation experiment), thus suggesting that ventral spines are subject to sexual selection (Møller & Pomiankowski, 1993).

Apart from the expected allometric relationship between mean spine length and  $L_S$  ( $r = 0.59$ ,  $n = 16$ ,  $P < 0.02$ ), significant associations among male traits were only evident between spine symmetry and blue intensity (Fig. 4). In agreement with former studies (Milinski & Bakker, 1990), redder males tended to have higher  $K$  values ( $r = 0.45$ ,  $n = 15$ ,  $P = 0.09$ ) but not after standardizing for corrected duration of paternal care ( $P > 0.15$ ). The other relationships between male traits were far from being significant (all  $P > 0.21$ ).

### CORRELATES OF EGG SIZE

In addition to egg number, egg size impaired egg survival (Table I). The average mass per egg in nests that were subjected to deprivation of paternal care ranged from 2.77 to 3.62 mg (mean  $\pm$  s.d. =  $3.12 \pm 0.25$  mg,  $n = 12$ ). The variation in mean egg size per nest was comparable to laboratory measurements of the variation in egg size between wild-caught females from the same population (Kraak & Bakker, 1998). Across nests, egg size was not significantly associated with egg number ( $r = 0.17$ ,  $n = 12$ ,  $P > 0.60$ ). A multiple regression analysis of the strongest single linear associations ( $P < 0.1$ ) between egg size and male traits revealed that spine asymmetry was significantly negatively associated with egg size and red intensity tended to do so positively (Table III).

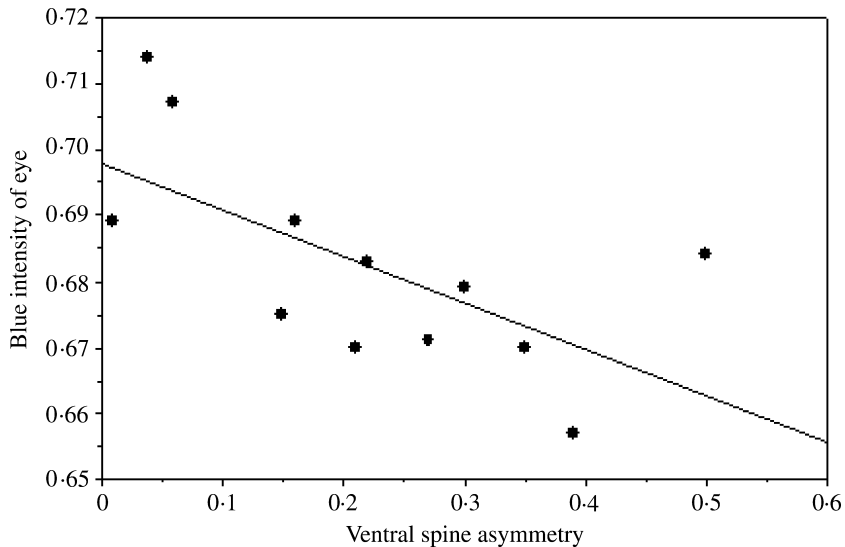


FIG. 4. Relationship between the ventral spine asymmetry (unsigned difference in mm) and the blue intensity of the iris of the eye. The curve was fitted by: ( $y = 0.70 - 0.07x$ ) ( $r = -0.65$ ,  $n = 12$ ,  $P < 0.05$ ).

TABLE III. Multiple regression of egg size (mean mass per egg in mg) on male traits (asymmetry of ventral spines, *i.e.* unsigned difference in mm, and red intensity of throat) ( $n = 11$ ,  $r^2 = 0.73$ ,  $F_{2,8}$ ,  $P = 0.01$ )

Variable	Slope $b$	$t$	$P$
Spine asymmetry	-0.68	3.69	0.006
Red intensity	0.40	2.17	0.062

## DISCUSSION

Depriving three-spined stickleback nests of paternal care while preventing egg predation caused disproportionately more eggs to die in nests with many eggs, thus justifying Perrin's (1995) assumed negative relationship between egg number and proportional survival. When females aim to maximize the direct benefits of mate choice, they therefore not only have to take into account the male's ability and willingness to care (Trivers, 1972) but also nest contents (Perrin, 1995). This may explain observations that female three-spined sticklebacks often refuse to spawn after having 'nosed' into the nest entrance (Wootton, 1976; Goldschmidt *et al.*, 1993). The present finding that broods of attractive three-spined stickleback males require greater paternal care could have been expected because males increase the amount of time spent fanning with increasing number of clutches spawned into the nest (van Iersel, 1953). Furthermore, female three-spined sticklebacks prefer to spawn in nests that already contain some but not too many eggs (Belles-Isles *et al.*, 1990; Goldschmidt *et al.*, 1993).

Deprivation of paternal care clearly led to retardation of embryo development and increased mortality confirming that oxygenation of eggs by male fanning promotes embryo development and survival (van Iersel, 1953; van den Assem, 1967). Even in a habitat where strong water flow replaces male fanning to some extent, male fanning behaviour seems not to be superfluous. In such habitats, fanning combined with nest-maintenance behaviours may additionally prevent the accumulation of silt on the nest (Potts *et al.*, 1988). Inside the nest cavities, water velocity was reduced, but there was obviously sufficient water exchange in the nest to let some embryos even survive to 7 days without care. These may have been embryos that suffered less hypoxia because they formed the outer layer of the egg mass. Alternatively, surviving embryos may have been at a more advanced stage of development when the male was removed because eggs are less tightly stuck together at later stages of development. Oxygen will become less quickly restrictive for single eggs than for eggs in an egg mass; in the laboratory single eggs develop well without additional aeration, and a recently discovered new form of three-spined stickleback in Nova Scotia has been emancipated from paternal care by distributing the eggs in fields of algae or in crevices in the intertidal zone (MacDonald *et al.*, 1995; Blouw, 1996).

In the field, males with the most symmetrical ventral spines had the highest reproductive success measured in terms of eggs present in the nests of caring males. Other male traits were not significantly correlated with egg number.

Obtaining the data for the field experiment, however, was demanding and would not be possible with a very large number of nests. Limited sample sizes make the statistical power for detecting relationships low. The degree of FA is viewed as an indicator of the ability to cope with environmental and genetic stress (Parsons, 1990). There is some evidence that the degree of FA of male sexual traits plays a role in female mate choice, and indicates male quality (Møller, 1997; Møller & Thornhill, 1998; Vøllestad *et al.*, 1999). In the present population, the degree of FA of the ventral spines was negatively correlated with egg number due to either a greater mating success, greater paternal success, or both, of more symmetrical males. In laboratory experiments, female three-spined sticklebacks preferred males with symmetrical ventral spines over males with asymmetrical spines, but showed no preference with respect to the length of the pelvic spines (Mazzi *et al.*, 2003). Environmental stress increased pelvic spine asymmetry in juvenile three-spined sticklebacks (Mazzi & Bakker, 2001).

The fact that ventral spine symmetry was most strongly associated with reproductive success of all measured male traits may be due to the reduced genetic variability of this population as measured at 10 microsatellite loci (Heckel *et al.*, 2002). Female preference for symmetrical pelvic spines was significantly stronger in inbred than in outbred three-spined sticklebacks (Mazzi *et al.*, 2004).

Correlated with spine symmetry was the blue intensity of the iris of the male's eye thus providing females with several cues of male quality. Rowland (1994) showed that blue eyes are a criterion of female choice in an American three-spined stickleback population. The blue intensity of the eye is viewed as a long-term indicator of condition (Frischknecht, 1993) because it is correlated with growth and body size in several three-spined stickleback populations (Frischknecht, 1993; Bakker & Rowland, 1995). In a previous field study of the same Roche population (Kraak *et al.*, 1999a), blue eye colouration was more intense in males with successful nests than in males whose nests disappeared. Before disappearance the latter males had a lower reproductive success. Spine symmetry was not quantified in this population before the present study.

Better survival of embryos might be expected in nests of attractive males even when the male is removed either because attractive males contribute good viability genes to the progeny, or attractive males mate with females of higher genetic quality, or attractive males occupy better nest sites for embryo survival. Obviously these potential benefits of embryos in nests of successful males did not outweigh the higher oxygen demands of the larger number of embryos. The number of eggs would have to be standardized in order to be able to assess potential benefits of eggs in nests of attractive males. Egg numbers were not manipulated in this study because this would have changed nest structure and consequently water flow-through the nest.

Males with symmetrical ventral spines not only had the highest egg number but also had heavier eggs in their nest. The mean mass per egg was calculated by dividing the mass of the total number of (live and dead) eggs by the number of eggs. Because egg mortality was correlated with egg number, this may have led to a biased estimation of the mass per egg in nests with many eggs. In a single linear regression, the mean mass per egg was, however, not significantly related to the proportion of dead eggs ( $r = 0.09$ ,  $n = 12$ ,  $P > 0.78$ ).

Heavier eggs demanded more paternal care but these costs may be outweighed by advantages associated with egg size. Within and between fish species, larger eggs produce larger young (Chambers & Leggett, 1996). Larger body size at hatching may be advantageous in the present three-spined stickleback population because of the strong water flow. Larger young may be better able to escape predation (Foster *et al.*, 1988).

Males may obtain larger eggs by female mate choice, male mate choice, or both. In the present three-spined stickleback population, females that produce more eggs also produce larger eggs when not controlling for variation in body size (Kraak & Bakker, 1998). Males prefer to mate with larger females and will thereby obtain on average more and larger eggs when given a choice (Kraak & Bakker, 1998). This provides circumstantial evidence that the cost of such matings is smaller than the benefits.

In conclusion, in three-spined sticklebacks attractive males have to provide greater paternal care because depriving nests of care led to disproportionately high egg mortalities in nests with many eggs. Attractive males in this study were males with the most symmetrical ventral spines, which was correlated to the blue intensity of the iris of the eye. The expected covariance between male reproductive success and paternal effort must not necessarily hold for other forms of parental care. In the case of biparental care, which is the norm in birds, parental effort of attractive males may be reduced and compensated for by the female, as is the case in, for instance, barn swallows (de Lope & Møller, 1993). In systems with care by only the male, males do not have this option.

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