



Correlates of the duration of the egg collecting phase in the three-spined stickleback

S. B. M. KRAAK*, T. C. M. BAKKER AND B. MUNDWILER

Abteilung Verhaltensökologie, Universität Bern, Wohlenstrasse 50a,
CH-3032 Hinterkappelen, Switzerland

(Received 16 September 1998, Accepted 25 January 1999)

Individual male three-spined sticklebacks in the field, often collected eggs for longer (up to 10 days) than had been reported for sticklebacks in captivity (3–6 days). The probability that a male stopped collecting eggs increased with the number of eggs already present, and possibly with the age of the eggs. Males with nests hidden under a plant were more likely to continue collecting than males with exposed nests. These results are discussed in the light of theoretical considerations that predict when males should stop collecting further eggs.

© 1999 The Fisheries Society of the British Isles

Key words: *Gasterosteus aculeatus* L.; three-spined stickleback; courtship phase; parental phase; paternal care; trade-off courting/caring; optimum brood size.

INTRODUCTION

Many fish species with exclusive male parental care breed in nesting cycles consisting of a courtship phase during which the male collects eggs from females, followed by a parental phase during which the male cares for the eggs until all have hatched [e.g. *Gasterosteus aculeatus* L. (van Iersel, 1953); *Lepomis cyanellus* Rafinesque (Hunter, 1963); *Oxylebius pictus* Gill (DeMartini, 1987); *Coraliozetus angelica* Böhlke & Mead (Hastings, 1988); *Symphodus tinca* (Forskål) and *S. ocellatus* (Forskål) (van den Berghe *et al.*, 1989); *Stegastes partitus* Poey (Knapp & Warner, 1991)]. In some species, however, males collect eggs continuously throughout the breeding season [e.g. *Blennius pholis* L. (Quasim, 1956); *Aidablennius sphynx* Valenciennes (Kraak, 1996); and see Ridley, 1978]. The question arises why, in the brood-cycling species, males do not continue to collect additional eggs as long as nest space is not limiting. Do males cease or decrease courtship at some point? Or do females discriminate against males whose nests contain many, or old, eggs? Reports of brood-cycling (references above) usually consist of descriptive remarks in the introduction, not of a systematic empirical study of the phenomenon. An exception is van Iersel's (1953) laboratory study on the causation of the transition from courting to caring in the three-spined stickleback, *Gasterosteus aculeatus*. Male sticklebacks stopped courting a stimulus female 2–6 days after they had received and fertilized eggs, and the more clutches received and fertilized, the sooner they stopped. In

*Author to whom correspondence should be addressed at: Department of Biology, University of Leicester, University Road, Leicester LE1 7RH, U.K. Tel.: 0116 252 3350; fax: 0116 252 3330; email: sk66@leicester.ac.uk

a study by Jamieson *et al.* (1992), typically captive male three-spined sticklebacks collected eggs daily over a period of 3–6 days and then stopped. Yet little is known about the patterns of the nesting cycles of wild sticklebacks. Two field studies suggest that females discriminate against nests with many (old) eggs (Belles-Isles *et al.*, 1990; Goldschmidt *et al.*, 1993).

Contrasting with the scarcity of empirical work on brood-cycling, several theoretical adaptive explanations have been offered. One of these assumes a trade-off between courting and caring for example, caused by increased risk of brood predation when the male is absent from the nest while courting. In this case it is adaptive that males stop courting some time after having acquired some eggs: although they could gain more eggs by continued courting, they risk losing the eggs they already have. Moreover, the more clutches the male has, the sooner he should stop courting, because the value of the brood increases with the number of clutches. This verbal adaptive argument has been confirmed by studying dynamic programming models (Sargent, 1990; Sargent *et al.*, 1995). Another adaptive explanation, which does not depend on the assumption of a trade-off between courting and caring, assumes a trade-off between the benefits and the costs of acquiring more eggs. More eggs need more oxygen, and hence, more fanning. This assumption is supported by the finding that when stickleback nests with eggs were deprived of male fanning, egg mortality was higher when the nest contained more eggs (Bakker *et al.*, unpubl.). And indeed males spend more time fanning when caring for larger broods (van Iersel, 1953; Coleman & Fischer, 1991). Perrin (1995) used this assumption in an analytical model addressing egg collection by stickleback males in relation to male quality. He found that at a certain brood size it does not pay a male to collect more eggs, and hence the male should stop courting at that point. Under this assumption females should also reject males at a certain brood size, because the amount of care per egg is decreased (Coleman & Fischer, 1991). In Perrin's (1995) model the critical number of eggs, both from the male's and the female's point of view, is higher for better quality males (quality is 'defined by its positive effect on [egg] survival under bad conditions', Perrin 1995, page 1044). A third adaptive explanation states that females should reject nests with older eggs present, if males tend to eat the remaining eggs after most have hatched (Rohwer, 1978).

This field study documents systematically individual variation in the duration of egg collection in a population of three-spined sticklebacks. It explores whether the patterns of nesting cycles observed in wild sticklebacks agree with the theoretical predictions (Rohwer, 1978; Perrin, 1995; Sargent *et al.*, 1995; note that these models' adaptive hypotheses are not mutually exclusive). More specifically, it tests whether the probability of terminating egg collection correlates with the number of eggs already present (as predicted by Perrin, 1995, and Sargent *et al.*, 1995), and with the age of the eggs present (as predicted by Rohwer, 1978, and Sargent *et al.*, 1995). The model of Sargent *et al.* (1995) seems to suggest that the age of the youngest clutch, and not of the oldest, influences the transition from courting to caring (Table 3 in Sargent *et al.*, 1995). Furthermore, Sargent *et al.* (1995) found that the timing of this transition relative to clutch age and number should depend on some ecological factors. Particularly, increases in male survival, mating probability, and clutch survival appeared to lengthen the courtship phase. Perrin's model (1995) suggests that

the number of eggs at which the male should stop collecting more, depends on male (parental) quality. These predictions are investigated by analysing which characteristics of the males or their nest sites correlate with the timing of terminating egg collection.

Another unresolved issue is the following. *Van Iersel's study (1953)* was conducted at 18–20° C, at which temperature it took the eggs about 7 days to hatch; the males' courtship phases extended over more than half of this period. In natural populations the temperature may be lower and egg development may take up to 2–3 weeks (*Wootton, 1976*). Should the courtship phase become longer proportionally or stay fixed at 2–6 days? This question was not addressed by *Sargent et al. (1995)*, since in their model time is relative to the duration of egg development: the present data will provide insight.

Knowledge of the duration of egg collection would also be of practical importance in field studies in which one estimates male mating success by determining the number of eggs in a male's nest (e.g. *FitzGerald et al., 1993*; *Bakker & Mundwiler, 1994*). Some males might not have completed their courtship phase at the time the nest content is assessed, meaning that they possibly could have collected additional eggs, while others might have stopped courting several days before, meaning that the older brood might have suffered mortality for more days (*Kraak et al., 1999*). To estimate male mating success correctly, it is useful to know in which phase of the nesting cycle the male is at the time of capture and nest inspection (*Bakker & Mundwiler, 1994*; *Kraak et al., 1999*).

MATERIALS AND METHODS

STUDY POPULATION

A permanent freshwater population of three-spined sticklebacks was studied at Roche (near Montreux, Switzerland, 46°26' N, 6°55' E) in the drainage system of the Rhone near Lake Geneva. The study site was restricted to *c.* 140 m of a small channel plated with blocks of concrete along the walls and with a wooden boarded floor. The vegetation was sparse. Sticklebacks nested in cut-outs in one corner of each concrete plate, or under the boards that lined the concrete slopes at the bottom. Almost daily observations were made during May, June, and July 1995. Several traits as well as nest site characteristics were determined for 69 males and the broods investigated. This work was part of a larger study (*Kraak et al., 1999*), in which correlates of mating and parental success of these males were reported.

MALE CHARACTERISTICS

The males were caught from their nests with a dipnet, and put in a bucket of water from the channel. Within a minute after being caught the anterior half of the ventral side of the fish was photographed (one slide) and one randomly chosen lateral side (two slides, sometimes one slide) in a standardized way (described in *Bakker & Mundwiler, 1994*).

Pectoral fin size was measured as the average of the lengths of the 10 fin rays on projection of these slides by a photographic enlarger divided by the magnification factor ($18\times$).

The male breeding colours on the slides were analysed 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, and at six defined points in the iris of the eye (on each of the two slides, hence 12 points in total). From these measurements for each male a red

index was calculated for the throat and blue and green indices for the eyes (for details see Frischknecht, 1993; Bakker & Mundwiler, 1994; Kraak *et al.*, 1999).

The standard length and the weight was measured, and damage or obvious diseases recorded. Condition was calculated as $\text{weight} \times \text{length}^{-3}$. All fishes were marked by clipping the first or the second dorsal spine. Twelve males were marked individually by injecting a suspension of alcian blue under the skin in unique patterns of spots.

Each fish was kept in the bucket until his nest was replaced (see below), after which the male was returned to his site.

NEST SITE CHARACTERISTICS

Because all nests were located either in the cut-outs in the corner of the concrete plates or on the bottom, nests were present at only three discrete depths: c. 65, 45, and 20 cm under the watersurface. These levels were recorded for each nest. Whether or not the nest was hidden in a plant was recorded, and on how many sides it was protected by a wall of substratum (one to four sides and a roof). At each nest the temperature was measured in the afternoon.

BROOD CHARACTERISTICS

Immediately after the male was caught, his nest was placed upside down carefully in a Petri dish. Stickleback nests consist of plant filaments glued together with a secretion from the kidneys (Wootton, 1976). The eggs are contained in the nest as a lump, in the early stages stuck together, later more loosely. The nest was opened at its bottom and all the eggs were removed carefully into a small container with water from the channel. After the eggs had been dried on absorbing paper, the whole brood was weighed with a representative sample of about 40 eggs, to calculate the total number of eggs present (Kraak *et al.*, 1997). If fry were present in the nest they were counted individually.

Through a binocular microscope the whole brood was screened and all the developmental stages of the embryos recorded (25 stages, after Swarup, 1958). Throughout the season samples of about 40 (newly spawned) eggs were put in tea-balls in the channel, and the developmental stages of the embryos were monitored daily. Egg survival in the tea-balls was as good as, or better than, in nests with care, due to the strong current resulting in good water exchange. By this procedure the developmental stages could be mapped approximately onto age in days at different times of the season, i.e. for May, June and July separately. The age assigned to an egg in a brood was the earliest age (in days) at which its stage occurred in the tea-balls in that month. Because the durations of stage 21 and later stages were more than 1 day, usually it was not possible to determine the age of eggs older than stage 21. The ages were only approximations, since the rate of development of the eggs was probably influenced by the degree of oxygenation.

In 48 of the 69 cases the whole brood was dyed blue, by leaving it for at least 30 min in a suspension of alcian blue in channel water (about 10 g l^{-1}), in order to check at a later time whether new eggs had been added to the nest after the first collection. Dyeing did not influence embryo mortality significantly (Kraak *et al.*, 1997).

Finally the eggs were returned to the nest, which was repaired and put back in its place in the channel. Then the fish was released at his nest. The whole procedure of recording male and brood characteristics took between 30 min and several hours. The males accepted their nest in all but one case, including cases in which the nest contained all blue (dyed) eggs.

A sub-sample of 33 of the 69 males and their nests was collected again one or several times within the same nesting cycle, to check whether new eggs had been laid and to follow the fate of the eggs that had been present initially. In those cases where the brood had been dyed blue it was easy to distinguish between the initial eggs (blue) and eggs that had been laid in the meanwhile (eggs that are not dyed are yellow). In some cases in which the eggs had not been dyed, newly laid ones could be distinguished if their developmental stage was younger than the number of days that had passed since the first assessment. By measuring the weight of the remaining initial eggs and a representative counted sample parental success could be calculated, here defined as the proportion of

eggs surviving after 10 days. The manipulation of the nest and the eggs did not influence egg survival significantly (Kraak *et al.*, 1997).

DETERMINATION OF THE DURATION OF EGG COLLECTION

The duration of the egg collection phase was determined as the difference between the ages (in days) of the oldest and the youngest eggs present in the brood. If the youngest eggs were more than 1 day old, it was assumed that the male had stopped collecting eggs, because a male generally does not resume collecting additional eggs after an interruption of 1 or more days (unpubl. data). If the youngest eggs were aged less than 1 day, possibly the male would continue to collect additional eggs. Whether this had happened was verified in the sub-sample of nests that was collected again at a later date, by checking the developmental stages of the embryos again. Additional egg collection was obvious if uncoloured (yellow) eggs were present in a brood that had been dyed blue.

STATISTICAL ANALYSES

Whether the brood, male or nest site characteristics correlated with the probability of continuing egg collection was investigated by multiple logistic regression models (GLMStat 2.0.4). When the significance of a factor was investigated, it was first included into the model together with the interaction(s) between this factor and factor(s) already present in the model (if any). Subsequently, stepwise, first the interaction(s), and then the (least significant) factor were taken out again. At each step it was checked whether the model had changed significantly, assuming that under the null hypothesis the change in scaled deviance followed a χ^2 distribution. If the model had changed significantly (i.e. $P < 0.05$), then the factor (or the interaction) had a significant effect and must remain in the model.

Pearson r values were also calculated.

RESULTS

The duration of the egg collection phase [Fig. 1(a)] was determined for 40 of the 69 males (for the other males the data did not allow determination of this duration, e.g. because eggs were too old, or because at first assessment the male had not finished collecting and the nest was not revisited). Most males collected eggs during 2 or 3 days, but some males continued to collect eggs for up to 10 days. The minimum duration of a nesting cycle (time of egg development from spawning to hatching) was 19 days in May, 16 days in June, and 15 days in July. For each male the maximal proportion of the nesting cycle over which he had collected eggs [Fig. 1(b)] was usually much smaller than 50%.

The males collected eggs longer the fewer eggs they had received daily until the day of first capture (Fig. 2, $r = -0.688$ between the logarithms of both variables, $P < 0.001$), suggesting that males, on average, continued as long as needed to collect *c.* 3000 eggs. However, it is not known whether the daily number of eggs received before the first assessment of nest contents can be extrapolated to the period after first assessment. The daily rate of egg collection increased over the breeding season ($r = 0.495$, $P < 0.005$), while the duration of the collecting phase decreased ($r = -0.425$, $P < 0.01$). The latter relation became just non-significant when the duration of the collecting phase was taken as the proportion of the duration of the nesting cycle ($r = -0.288$, $P = 0.07$). Temperature at the nests (measured at only 28 of the 40 nests) ranged between 10.2 and 12.9°C and increased over the season ($r = 0.782$, $P < 0.0001$). The duration of the collecting phase decreased with increasing temperature ($r = -0.455$, $P < 0.05$), and again

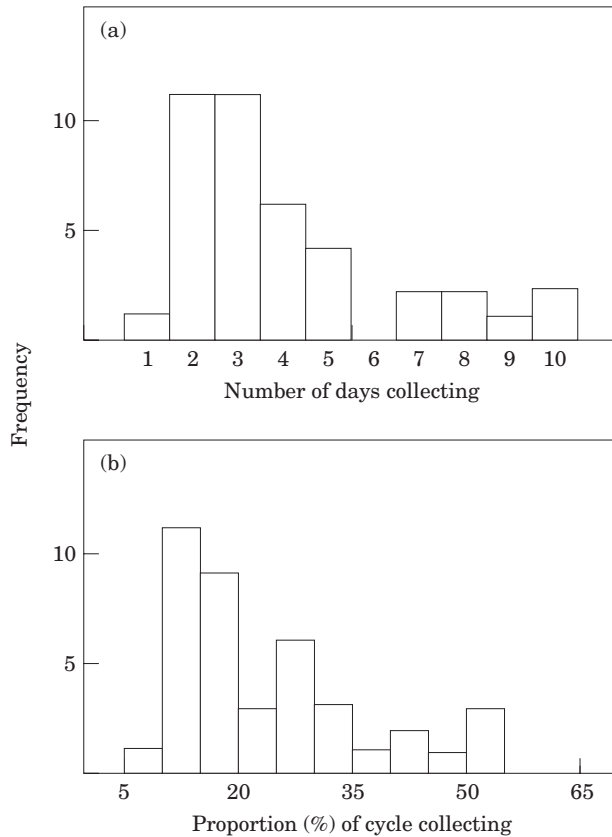


FIG. 1. The distribution of durations of the egg collection phase of 40 males: (a) in days, (b) as a percentage of the total duration of the nesting cycle (=average time needed for the development of the embryo to hatching in the respective month).

this relation became just non-significant with proportional duration ($r = -0.350$, $P = 0.068$). The daily rate of egg collection did not vary with temperature ($r = 0.027$, $P > 0.8$). This suggests that the relation (Fig. 2) is not mediated by temperature.

Of those 33 males in whose nest the youngest eggs had been laid on the day of first capture, indicating that these males had not yet terminated egg collection, 15 continued collecting eggs, whereas 18 did not. By logistic regressions, the factors were investigated which correlated with the probability of continuing egg collection. Starting with a model including the number of eggs and the age in days of the oldest eggs (and their interaction), only the number of eggs contributed significantly ($\chi^2 = 11.09$, d.f. = 1, $P < 0.001$). However, starting with a similar model, but with the age of the oldest eggs as developmental stage instead of in days, only the stage contributed significantly ($\chi^2 = 14.08$, d.f. = 1, $P < 0.0005$). Moreover, starting with the number of eggs and the stage of the youngest eggs, their interaction was significant ($\chi^2 = 10.44$, d.f. = 1, $P < 0.005$). The effects were in the expected direction: the more eggs present and the older the (oldest or youngest) eggs, the lower the probability of continuing egg collection. Of course age of oldest eggs and number of eggs were themselves correlated (for age in

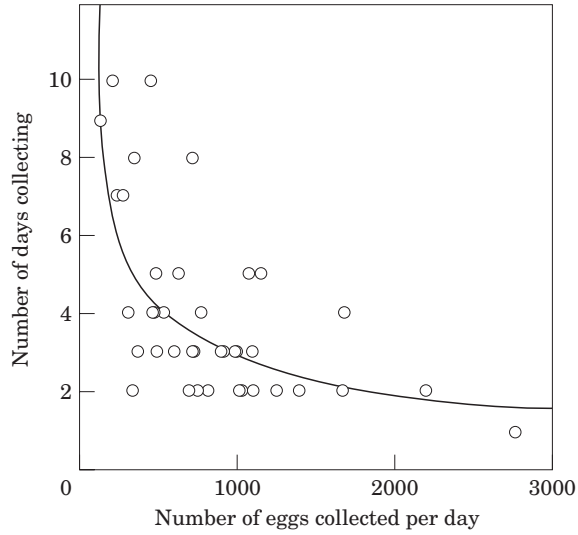


FIG. 2. The number of subsequent days that males collected eggs v. the daily number of eggs received until the first assessment of nest contents.

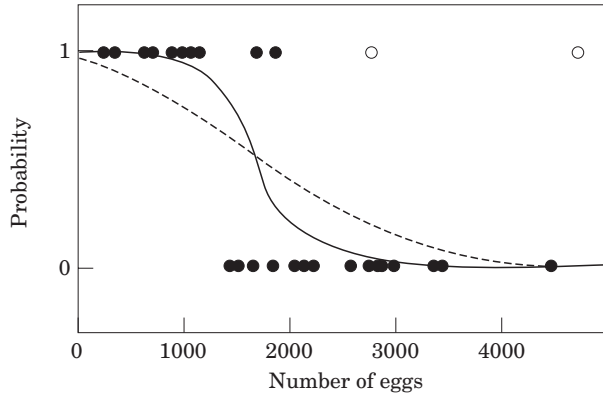


FIG. 3. The probability of continued egg collection after first assessment of nest contents as a function of the number of eggs present. ○, Two outliers. —, Fitted function based on $n=31$ (outliers excluded). ---, Fitted function based on $n=33$ (all data, including outliers).

days: $r=0.42$; for age in developmental stage: $r=0.68$), since age of the oldest eggs equals the time the male has been collecting eggs so far. Therefore, the analyses tested whether the one variable had an influence in addition to its effect through its correlation with the other. The robustness of these results was tested. Excluding the two outliers (Fig. 3), the analysis led to a model in which only egg number contributed significantly ($\chi^2=27.82$, d.f. = 1, $P<0.0005$). All males with <1400 eggs continued collecting; conversely, all but the two outlier males with >2000 eggs stopped collecting (Fig. 3). The outlier male that had collected almost 5000 eggs was quite large and had unusually large fins: the other outlier was not in any other respect atypical.

TABLE I. Variables whose effect on the probability of continuing egg collection is investigated, with the number of eggs included in the model

	χ^2 1	χ^2 2
Male traits		
Standard length	0.491	0.653
Pectoral fin size	0.109	0.014
Red intensity of throat	0.192	0.093
Blue intensity of eyes	2.941	1.923
Green intensity of eyes	0.220	0.335
Condition (=weight . length ⁻³)	1.656	0.244
Presence of wounds	0.006	0.001
Nest site traits		
Depth	0.919	0.189
Degree of protection	0.012	1.412
Presence of roof	2.060	1.660
Presence of plant	1.962	4.789*
Location on north or south shore	2.019	1.279
Date on which the male started the present nesting cycle	0.033	0.003
Mean egg weight	0.177	1.373
Egg survival after 10 days (calculated by inter- or extrapolation from egg mortality)	0.795	0.496

The χ^2 values are the changes in scaled deviance when the interaction of the variable and egg number is taken out of the model (1), and subsequently the variable is taken out of the model (2, both d.f.=1).

*Significant at $\alpha=0.05$.

The outliers were excluded in further analyses. The effect of the variables listed in Table I on the probability of continuing egg collection was investigated, with the number of eggs included in the model. Only the presence of a plant raised the probability that the male continued to collect eggs (Table I).

DISCUSSION

The finding of factors that correlated with the probability that a male stops collecting eggs agree generally with the predictions from theory. Models (Perrin, 1995; Sargent *et al.*, 1995) predict that with an increasing number of eggs in the nest it is increasingly likely that a male stops collecting, which was found empirically to be the case. A positive effect of the age of the eggs is also predicted (Rohwer, 1978) and Sargent *et al.* (1995) seem to suggest that only the age of the youngest clutch, and not of the oldest, influences this transition. Rohwer (1978) reasoned that females should avoid nests with old eggs if males tend to eat the remaining eggs after most have hatched; according to this argument, too, the age of the youngest eggs is most important. In Perrin's model (1995) the effect of the age distribution of the eggs was not considered, although it might be important because older eggs require more care in terms of oxygenation (van Iersel, 1953). In the present study, the developmental stage of the oldest as well as the youngest eggs had a significant effect on the probability of continuing egg collection, but

these effects disappeared when two outliers were removed from the sample. Possibly a bigger sample would have given more robust results.

The finding that the presence of a plant in which the nest is hidden increased the probability that a male continued to collect eggs, is compatible with the theoretical predictions in two ways. *Sargent et al. (1995)* predicted that ecological factors related to increased mating probability and increased egg survival, lengthen the courtship phase. In a study of reproductive success of the same males (*Kraak et al., 1999*), the presence of a plant was indeed associated with higher male mating success and a higher probability that the brood survived until hatching. *Perrin (1995)* predicted that high male quality leads to prolonged courting. This may apply to high quality of the nest site as well. A nest in a plant may be of high quality since it has a higher probability that the brood survives until hatching. The higher mating success of males with nests in plants might reflect the females' appreciation of a plant as an aspect of quality (*Kraak et al., in press*). The advantage of nest concealment under a plant may be that it protects the male and his nest against predators and nest destruction (*Sargent & Gebler, 1980; Candolin & Voigt, 1998*).

None of the male traits and no nest site trait other than concealment under a plant had an effect on the probability of continuing egg collection. Some of the male traits might be expected to be indicators of paternal quality. *Kraak et al. (1999)* have suggested that in this population the red intensity of the throat was probably not an indicator of paternal quality, but only of genetic quality (good genes for viability, or sexy genes). Moreover, since redder males in this population collected bigger eggs (*Kraak & Bakker, 1998*), which required more oxygen (*Bakker et al., unpubl.*), their optimum brood size was expected to be lower rather than higher. Male size and green intensity of the eyes are suggested to affect paternal quality, and nest depth (*Kraak et al., 1999*) and average egg weight (*Bakker et al., unpubl.*) appeared to correlate with egg survival. It is not known why these traits did not affect the probability to continue egg collection.

Since neither male courtship display, nor female choice behaviour was monitored systematically, it is not known whether egg collection terminated because the males had stopped courting, or because they were rejected by females. Female rejection on the basis of egg age (*Rohwer, 1978*) cannot explain why some males continued to receive eggs for 10 days, while most stopped receiving eggs after 2 or 3 days. Female rejection on the basis of egg number (*Perrin, 1995*) cannot explain the two outliers, who continued to receive eggs when they already had huge numbers of eggs in their nest. Hence, it seems more likely that egg collection was terminated as a male decision.

In 17.5% of the cases the males collected eggs for more than 6 days, in contrast to *van Iersel's (1953)* laboratory experiment where males stopped courting, usually 3 and maximally 6 days, after having received their first clutch. *Van Iersel's (1953)* experimental design was artificial, in that the male was assigned matings only on day 0, on which day the male acquired his one to five clutches. On later days the male was presented with a stimulus female in a glass jar but was denied the opportunity to mate. So the male continued courting but could not collect additional eggs. Thereby all clutches had the same age. Since the male's courtship to a standard stimulus decreased with the age of the eggs, *van Iersel* concluded that the transition from courting to caring depended on the age of the

eggs. In nature, however, a male will have the opportunity to collect eggs throughout his courtship phase of several days, resulting in the daily addition of eggs of age zero to the brood. It is not known how the combination of the different ages of eggs in a brood triggers a male's transition to the parental phase.

In the present study, with temperatures ranging between 10 and 13° C, the length of the total nesting cycle (i.e. time of egg development to hatching) was more than twice as long as in [van Iersel's \(1953\)](#) aquarium with temperatures between 18 and 20° C. Yet, in the present study egg collection took 2–6 days in 80% of the cases, corresponding to van Iersel's courtship phase lengths in days, but representing a much smaller proportion of the nesting cycle. This suggests that the absolute length of the courtship phase, and not the length relative to developmental time, is important. On the other hand, the absolute, but not the relative (proportional) duration of the collecting phase decreased significantly over the breeding season and with temperature, suggesting the opposite. However, since the relations of proportional duration with date and temperature were nearly significant, the first conclusion is favoured, that the absolute number of days is important for the termination of egg collection. It would be interesting to see what theoretical models predict in this respect.

More empirical as well as theoretical studies should be undertaken to gain more insight into the question why males stop collecting eggs. The presented study has raised new questions. What should be the relative influence of the ages of the oldest *v.* the youngest (and all other) eggs in the nest? Should the termination of the courtship phase depend on the absolute age of the eggs in days or on the relative age, i.e. the developmental stage? Does the brood size at which males are expected to stop courting ([Perrin, 1995](#)) coincide with, exceed, or fall below the brood size above which females are expected to reject males? How would the models of [Perrin \(1995\)](#) and [Sargent *et al.* \(1995\)](#) be affected if it is taken into account that older eggs need more care in terms of oxygenation? Empirically, it should be established whether courting activity poses a cost in terms of reduced parental care resulting in lower egg survival (which is a major assumption of the model by [Sargent *et al.*, 1995](#)). [Jamieson *et al.* \(1992\)](#) reported evidence that three-spined sticklebacks experience a trade-off between courting and caring. This should be established separately for each individual fish species considered, since the extent to which such a trade-off is present probably varies among the different fish species, as argued by [Kraak \(1996\)](#). In any case, more fish species should be studied with respect to this topic to test the generality of theoretical considerations (Perrin's model was tailored specifically to the stickleback), and also because interspecies differences may be insightful. Furthermore, it should be investigated in sticklebacks whether males are likely to cannibalize the last laid eggs, as has been shown in two species of damselfish which appeared to result in females discriminating against old eggs ([Petersen & Marchetti, 1989](#); [Sikkel, 1989, 1994](#)). By detailed observations it should be distinguished whether egg collection is terminated because males stop courting or because females reject males. [Van Iersel's \(1953\)](#) experiment should be repeated with broods consisting of clutches of different ages. Finally, an experimental approach may be feasible for testing the more specific predictions, whether ecological factors ([Sargent *et al.*, 1995](#)) or aspects of male quality ([Perrin, 1995](#)) influence the termination of egg collection.

This study was financed partly by the Swiss National Science Foundation and the authors thank the Netherlands Organization for Scientific Research; W. Steenge for help in and outside the field; C. Fonseca for useful suggestions on the analyses; D. Nash for advice on software; V. Fries, R. Künzler, D. Mazzi, and S. Zala for discussion of an early version of the manuscript; and C. Magnhagen and anonymous referees for constructive comments.

References

- Bakker, T. C. M. & Mundwiler, B. (1994). Female mate choice and male red coloration in a natural stickleback population. *Behavioral Ecology* **5**, 74–80.
- Belles-Isles, J.-C., Cloutier, D. & FitzGerald, G. J. (1990). Female cannibalism and male courtship tactics in threespine sticklebacks. *Behavioral Ecology Sociobiology* **26**, 363–368.
- van den Berghe, E. P., Wernerus, F. & Warner, R. R. (1989). Female choice and the mating cost of peripheral males. *Animal Behaviour* **38**, 875–884.
- Candolin, U. & Voigt, H.-R. (1998). Predator-induced nest site preference: safe nests allow courtship in sticklebacks. *Animal Behaviour* **56**, 1205–1211.
- Coleman, R. M. & Fischer, R. U. (1991). Brood size, male fanning effort and the energetics of a nonshareable parental investment in bluegill sunfish, *Lepomis macrochirus* (Teleostei: Centrarchidae). *Ethology* **87**, 177–188.
- DeMartini, E. E. (1987). Paternal defence, cannibalism and polygamy: factors influencing the reproductive success of painted greenling (Pisces, Hexagrammidae). *Animal Behaviour* **35**, 1145–1158.
- FitzGerald, G. J., Fournier, M. & Morrissette, J. (1993). Sexual selection in an anadromous population of threespine sticklebacks—no role for parasites. *Evolutionary Ecology* **8**, 348–356.
- Frischknecht, M. (1993). The breeding colouration of male three-spined sticklebacks (*Gasterosteus aculeatus*) as an indicator of energy investment in vigour. *Evolutionary Ecology* **7**, 439–450.
- Goldschmidt, T., Bakker, T. C. M. & Feuth-de Bruijn, E. (1993). Selective copying in mate choice of female sticklebacks. *Animal Behaviour* **45**, 541–547.
- Hastings, P. A. (1988). Female choice and male reproductive success in the angel blenny, *Coralliozetus angelica* (Teleostei: Chaenopsidae). *Animal Behaviour* **36**, 115–124.
- Hunter, J. R. (1963). The reproductive behaviour of the green sunfish, *Lepomis cyanellus*. *Zoologica* **48**, 13–24.
- van Iersel, J. J. A. (1953). An analysis of the parental behaviour of the male three-spined stickleback (*Gasterosteus aculeatus* L.). *Behaviour* (Suppl.) **3**, 1–159.
- Jamieson, I. G., Blouw, D. M. & Colgan, P. W. (1992). Parental care as a constraint on male mating success in fishes: a comparative study of threespine and white sticklebacks. *Canadian Journal of Zoology* **70**, 956–962.
- Knapp, R. A. & Warner, R. R. (1991). Male parental care and female choice in the bicolor damselfish, *Stegastes partitus*: bigger is not always better. *Animal Behaviour* **41**, 747–756.
- Kraak, S. B. M. (1996). A quantitative description of the reproductive biology of the Mediterranean blenny *Aidablennius sphyinx* (Teleostei, Blenniidae) in its natural habitat. *Environmental Biology of Fishes* **46**, 329–342.
- Kraak, S. B. M. & Bakker, T. C. M. (1998). Mutual mate choice in sticklebacks: attractive males choose big females, which lay big eggs. *Animal Behaviour* **56**, 859–866.
- Kraak, S. B. M., Bakker, T. C. M. & Mundwiler, B. (1997). How to quantify embryo survival in nest-building fishes, exemplified with three-spined sticklebacks. *Journal of Fish Biology* **51**, 1262–1264.
- Kraak, S. B. M., Bakker, T. C. M. & Mundwiler, B. (1999). Sexual selection in sticklebacks in the field: correlates of reproductive, mating, and paternal success. *Behavioral Ecology*, in press.

- Perrin, N. (1995). Signalling, mating success and paternal investment in sticklebacks (*Gasterosteus aculeatus*): a theoretical model. *Behaviour* **132**, 1037–1057.
- Petersen, C. W. & Marchetti, K. (1989). Filial cannibalism in the cortex damselfish *Stegastes rectifraenum*. *Evolution* **43**, 158–168.
- Quasim, S. Z. (1956). The spawning habits and embryonic development of the shanny (*Blennius pholis* L.). *Proceedings of the Zoological Society of London* **127**, 79–93.
- Ridley, M. (1978). Paternal care. *Animal Behaviour* **26**, 904–932.
- Rohwer, S. (1978). Parent cannibalism of offspring and egg raiding as a courtship strategy. *American Naturalist* **112**, 429–440.
- Sargent, R. C. (1990). Behavioural and evolutionary ecology of fishes—conflicting demands during the breeding season. *Annales Zoologici Fennici* **27**, 101–118.
- Sargent, R. C., Crowley, P. H., Huang, C., Lauer, M., Neergaard, D. & Schmoetzer, L. (1995). A dynamic program for male parental care in fishes: brood cycling and filial cannibalism. *Behaviour* **132**, 1059–1078.
- Sikkel, P. C. (1989). Egg presence and developmental stage influence spawning site choice by female garibaldi. *Animal Behaviour* **38**, 447–456.
- Sikkel, P. C. (1994). Why female garibaldi prefer males with young eggs: a test of the parental investment hypothesis. *Ethology, Ecology and Evolution* **6**, 191–211.
- Swarup, H. (1958). Stages in the development of the stickleback *Gasterosteus aculeatus* (L.). *Journal of Embryology and Experimental Morphology* **6**, 373–383.
- Wootton, R. J. (1976). *The Biology of the Sticklebacks*. London: Academic Press.