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BRIEF COMMUNICATIONS

How to quantify embryo survival in nest-building fishes, exemplified with three-spined sticklebacks

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Egg survival in manipulated nests of three-spined sticklebacks *Gasterosteus aculeatus* in the field was not significantly different from that in unmanipulated nests.

Key words: embryo survival; sticklebacks; nest-building fish; paternal care.

For studies on the behavioural ecology of reproduction in nest-building fishes (Ridley, 1978), it is often desirable to quantify individual parental success in the field, reflected by the proportion of embryos that an individual male brings to hatching. Male three-spined sticklebacks *Gasterosteus aculeatus* L., build tunnel-shaped nests from plant material which is glued together with a secretion of their kidneys (Wootton, 1976). After building is completed, the males start to court females. Several females may deposit eggs in succession in a male's nest. After a few days (Iersel, 1953) the males stop courting and devote themselves to caring for the eggs until hatching and for the juveniles for some time after hatching. Care before hatching consists of aerating the eggs by fanning, removing dead or diseased eggs, maintaining the nest, and defending the brood against predators (often conspecifics). The males sometimes cannibalize their own eggs. The eggs are contained in the nest as a lump, in the early stages being stuck together, but becoming looser with time.

When a nest is taken from an individual male which has stopped courting in the field, the number of eggs present in the nest can be determined. However, the number of eggs reflects a mixture of mating success (i.e. the number of eggs received) and the rate of egg survival during the period of care. Here, a method is described for direct measurement of egg survival in nests of individual male sticklebacks, separated from their mating success.

The key to the method was that after having determined the number of eggs present in a nest, the nest was repaired, put back in place and left to the care of the male (Bakker & Mundwiler, 1994). At a later time, e.g. closely before the expected hatching time, the nest was examined again, and it was determined how many of the eggs that were initially present had survived. However, it was essential to be able to distinguish the eggs that were already present from eggs that could have been laid after the first inspection of the nest. Furthermore, it was assured that the manipulation of the nest and the eggs did not influence subsequent egg survival.

In the breeding season of 1995, 72 nests with eggs of individual male sticklebacks were collected from a Swiss population breeding in a channel system located near Roche/Montreux (46°26′N, 6°55′E). During the investigation of the nest contents, the male, which had been caught with a dipnet, was kept in a bucket with water from the channel. The nest was lifted carefully from the substratum and put upside down in a petridish.

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Using forceps the nest was opened at its bottom, all the eggs were removed carefully and put in water from the channel in a small container. All eggs together were weighed to the nearest mg after having been dried on absorbent paper. A counted subsample of about 40 eggs was weighed and the specific egg weight of the brood calculated, to determine the number of eggs from the total weight of the brood (mean egg number 1696, range 89–4713, $n=72$). The range of developmental stages of the eggs (according to Swarup, 1958) present in the brood was assessed using a binocular microscope (magnification 16 × or 25 ×). In 48 cases the eggs were put into a suspension of alcian blue in channel water (about 10 g l^{-1}) for minimally 30 min up to a few hours and then rinsed with clean channel water. The eggs were thus dyed deep blue. In all cases (including when the eggs had not been dyed) the eggs were put back in the nest, the nest was repaired (sometimes using some extra plant material collected from the channel to support the bottom of the nest), put back in place, and the male released near it. The males and their nests were out of the channel for 30–120 min while being manipulated. The degree of damage suffered by the nest was recorded (see below), and whether and how many eggs floated away when the nest was put back. In all but one case the male accepted his nest, i.e. continued to defend and care for it. However, in 13 out of 72 cases the nest was severely damaged and many eggs ($\geqslant 20$) were lost when putting it back. These cases were not considered further.

Fifteen samples of 10 to 43 eggs of known age (usually freshly laid), taken from some of the nests mentioned above, were put in tea balls (balls consisting of a fine metal grid) that were fixed to the substratum in the channel. These eggs were separated from each other. These eggs were monitored daily by viewing them through a binocular microscope and recording their developmental stages. In this way the developmental stage was mapped onto age in days, separately for May ($n=9$), June ($n=3$) and July ($n=3$), because water temperature influenced development rate. The time required for egg development until hatching was 19 days or more in May, 16–20 days in June, and 15–20 days in July.

To check whether the blue dye influenced egg survival, the fate of eight samples of eggs in tea balls was followed over periods of 9–20 days. In each sample, one half of the eggs was dyed with alcian blue. Samples usually consisted of 40 eggs but ranged from 20 to 42. On average 28·6% of the blue eggs died, whereas 27·1% of the unstained (yellow) eggs died (percentages of dead eggs ranged from 7·5 to 95%). According to Wilcoxon matched-pairs signed-ranks test, these percentages did not differ significantly ($n=8$, $T=19$, $P>0{\cdot}9$). Therefore, it is concluded that dyeing the eggs did not affect their survival significantly.

In 33 cases, the same nest was collected again, 2–19 days after the first time, and in five of these cases was collected a third time, 4–8 days after the second time. The nest was opened and the eggs taken out as on the first occasion. This time the eggs that had been present the first time were weighed separately, and a small counted sample of these eggs was used for the determination of specific egg weight. In the 21 cases where the eggs had been dyed blue, it was easy to see whether (10 cases) and which eggs had been laid after the first inspection: the eggs that had been dyed were still deep blue, whereas newly laid eggs were yellow without any trace of dye. In the cases in which the eggs had not been dyed, eggs that had been newly laid were identified when their developmental stage was younger than the number of days that had passed since the first inspection (six cases). From the number of initial eggs and the number that survived and the number of days in between, the daily egg mortality rate (or survival rate) could be calculated. This measure is not confounded by mating success. The mean daily mortality rate was 9% (range 1–23%, $n=33$). Assuming that 15 days are required for development to hatching (typical in July) these daily mortality rates of 1, 9, and 23% correspond to hatching success rates of 86, 24, and 2% respectively.

The final egg numbers present in the 33 nests that had already been collected once or twice before were subjected to ANCOVA's with the starting date of the nest and the developmental stage of the oldest eggs as covariates, and one of the following factors (mentioned below) at a time. In all cases only the developmental stage, but not the starting date ($P>0{\cdot}4$), were correlated significantly negatively with egg number ($P<0{\cdot}05$). None of the factors had a significant effect: whether the nest had been collected once or

twice before ($P>0{\cdot}5$), whether the eggs had been dyed blue or not ($P>0{\cdot}6$), whether new eggs had been added or not ($P>0{\cdot}2$), the degree of destruction of the nest [four classes: no destruction (eight cases), mild destruction but no eggs lost (two cases), mild destruction and up to 20 eggs lost (nine cases), wrong nest material (one case); $n=20$, $P>0{\cdot}2$]. The numbers of eggs present in these 33 nests was compared with those present in 15 nests that had not been collected before in which the oldest eggs had similar developmental stages (ranging from 19 to 25), in an ANCOVA with stage as covariate. Again, only stage had a significant effect ($P<0{\cdot}001$), but not whether the nest had been collected for the first time or not ($P>0{\cdot}7$), nor whether the nest had been collected for the first, second, or third time ($P>0{\cdot}6$). From these analyses it was concluded that manipulations of the nests and eggs did not significantly influence egg numbers, and, therefore, there was reason to believe that the estimates of egg survival were reliable.

These are the first descriptions of such methods for quantifying egg survival in stickleback broods, which is more difficult than estimating egg survival in fishes such as gobies (Forsgren *et al.*, 1996) or blennies (Kraak, 1996), where eggs are attached in one layer to a hard nest substratum. In these cases, areas occupied by eggs can be traced with pencil or pen (e.g. Kraak & Groothuis, 1994), and these areas can be used to calculate egg survival (Forsgren *et al.*, 1996; Kraak, 1996). It has been shown here that it is also possible to measure egg survival in broods that are lumps of eggs in nests of soft material.

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