Proximal cues for nocturnal egg care in convict cichlids, *Cichlasoma nigrofasciatum*

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Abstract. Visual cues are important for egg care in fish. Recent observations of nocturnal egg care suggest, however, that such cues are not essential. To determine what external stimuli allow fish to find and fan eggs in the absence of visual cues, the nocturnal behaviour of parental convict cichlids was measured after experimental brood substitutions. Parent fish neither fanned nor attended empty nests, showing that nest cues are not sufficient. Parents fanned eggs from other parents, indicating that a general property of convict cichlid eggs is involved. Parents did not fan or guard empty nests through which deoxygenated water was flowing, nor did they fan wax eggs, suggesting that respiratory features and tactile cues are not sufficient. Parents did, however, fan and attend tea bags containing their own eggs, showing that chemical cues are sufficient. Contrary to what has been implied previously for many cichlids, olfactory/gustatory cues can trigger egg care. The predominance of such cues, however, may depend on time of day or light intensity.

In the study of parental care in fish, ethologists have often tried to identify the external stimuli that trigger and orient egg-care behaviour. In species that lay eggs on a substrate (as opposed to species that carry their eggs with them), the main stimuli appear to be visual. For example, when a brood is replaced by eggs from another species (Noble & Curtis 1939; Greenberg 1961; Myrberg 1964) or even artificial eggs (Baerends & Baerends-van Roon 1950; Weber 1970), the parental fish continues guarding and fanning, but only if the new eggs look like the old ones. In contrast, removing eggs from a nest, bathing them in water, and then running this water through the nest does not trigger guarding and fanning, unless the eggs are close to hatching (Myrberg 1975, and references therein). The absence of any effect in this case has been taken as evidence that chemical cues are not sufficient for egg care behaviour.

All of the above studies were conducted during the day, when good illumination allowed efficient use of visual cues. In nature however, fish often have to care for eggs under low light intensity. For example, many species of cichlids spawn in dark places such as small caves (Barlow 1974; Lavery

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1991). Moreover, several of these species continue to fan their eggs at night, in the total absence of light (Reebs & Colgan 1991). Nocturnal egg care begs the question: in the absence of visual cues, what stimuli allow fish to find their eggs and properly guard and fan them? Obviously, tactile and chemical cues may assume a more important role at night than during the day. We investigated this question in the convict cichlid, a species that appears to rely predominantly on visual cues for egg fanning during the day (Weber 1970), but that also fans in complete darkness at night (Reebs & Colgan 1991).

METHODS

All fish came from the laboratory of Dr M. H. A. Keenleyside, University of Western Ontario, London, Ontario. They originated from wild fish caught in Costa Rica in 1984 and bred with other fish from aquarium dealers. All were less than 6 cm (total length) at the time of first breeding.

Individual pairs of convict cichlids were put in tanks measuring $51 \times 26 \times 31$ cm. They could spawn on the inside of a half of a clay flower-pot (height $= 12$ cm, radius at opening $= 7$ cm). This half-pot lay on its side edge and leaned against the front glass of the tank. An identical (alternative)

half-pot, to be used in future nest substitutions, lay on the bottom in a position inappropriate for spawning. Water temperature was 26–28°C. A 12:12 h light:dark cycle was provided by fluorescent tubes placed directly above the tanks. Pilot lights in the water heaters of each tank were disabled to ensure that no light was present at night. Fish were fed once a day with commercial pellets.

In a first series of experiments, 64 pairs were allowed to spawn. All were first-time breeders. Immediately after the lights went off on the first night following spawning, the male was removed (males usually do not participate in nocturnal egg care in this species, but can still disturb the female and cause unwanted variation in her behaviour) and one of eight nest substitutions (see below) was performed. Observations of the female's behaviour were made 1 and 6 h after the substitution. Observations were 10 min in duration and made with an AN/PAS 5 infrared visionscope (The Dutchman, Overland Park, Kansas). A 9-V flashlight equipped with an infrared gel filter (Kodak No. 81B) provided illumination. (For more information on the appropriateness of this equipment for unobtrusive observation of fish at night, see Reebs & Colgan 1991.) A vocal description of the behaviour was recorded on tape for later transcription. We later analysed (1) the percentage of time spent fanning (see Reebs & Colgan 1991) and (2) the percentage of time spent inside the nest.

Experimental Substitutions

The eight experimental nest substitutions ($N = 8$ in each group) were as follows.

Control (own eggs)

The half-pot bearing the eggs was taken out of the tank and then put back in the same position. The alternative half-pot was removed.

Other eggs

The half-pot with eggs was replaced with one bearing eggs laid by another pair on the same day. The new egg batch approximated the original one in size and position within the nest. The alternative half-pot was removed. With this group, we established whether any feature specific to the parent's own brood is necessary for egg care.

Empty nest

While laying during the day, the female might learn where her eggs are within the nest and then use conformational cues from the nest to fan in the eggs' direction. To test this hypothesis, the nest was replaced with the empty alternative half-pot already present in the tank.

Wax eggs

Weber (1970) showed that female convict cichlids can fan artificial eggs made of wax. We surmised that wax eggs could also be used to investigate the effects of tactile cues at night. After a female laid her eggs during the day, the alternative half-pot was removed, its inside surface was dried under a flame, and wax eggs were deposited at the spot corresponding to where the real brood had been. Following Weber (1970), eggs were made by dipping a probe into melted wax (Parowax) and touching the surface of the nest with the instrument, allowing the dripping wax to form a ball. As in Weber (1970), the wax eggs were rounder but similar in size to natural eggs. The shape index (width/length, $X \pm \text{SE}$) was 0.986 ± 0.003 for the wax eggs and 0.750 ± 0.013 for the real eggs ($t = 17.69$, $N = 20$, $P < 0.001$). The sectional area (length × width × $\pi/4$) for wax eggs was $2.05 \pm 0.06 \text{ mm}^2$, whereas that for real eggs was $2.08 \pm 0.05 \text{ mm}^2$ ($t = 0.38$, $N = 20$, NS). The female's nest was replaced with this new nest.

Used water

Because of egg respiration, oxygen in the boundary layer around the eggs becomes depleted while CO_2 becomes more abundant. Oxygen and CO_2 concentrations might therefore reveal the presence of eggs to the parent. This idea was first suggested by van Iersel (1953), who observed increased levels of fanning activity in three-spined sticklebacks, *Gasterosteus aculeatus*, after he ran used ('bad') water through their nest. Here, we adapted van Iersel's protocol to our procedure. The alternative half-pot was removed, two holes were drilled through its wall at the spot corresponding to the original brood's position, and the holes were connected to tubes (2·5 mm in internal diameter) that could deliver used water at a rate of 8–10 ml/min. Used water came from a 12-litre container in which eight males had resided for 4–10 h without aeration (after van Iersel 1953). The female's nest was replaced with this new nest. At observation time, the oxygen content of the water being delivered was 2–4 mg/litre (as measured with an oxygen meter, model 54, Yellow Springs Instrument).
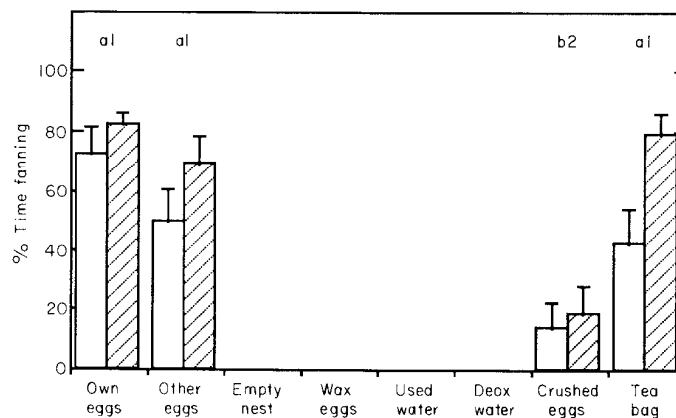
Figure 1. Percentage of time spent fanning by naive female convict cichlids 1 h (☐) and 6 h (▨) after their eggs were replaced by various stimuli at night. Open bars not sharing a letter are significantly different ($F_{3,28} = 5·64$, $P = 0·004$), as are hatched bars not sharing a digit ($F_{3,28} = 14·32$, $P < 0·001$).

Deoxygenated water

Procedures were as in the used water group, except that the tubes delivered water deoxygenated by blowing nitrogen through it. Oxygen concentration was 2–4 mg/litre.

Crushed eggs

The nest was taken out, all eggs were crushed, remaining pieces of egg membrane were removed, and the nest was put back. The alternative half-pot was removed. With this manipulation, we tested whether chemical cues (of the kind given by crushed eggs) are sufficient to trigger fanning.

Eggs in tea bag

The nest was removed, the eggs were scraped off and put into an empty tea bag that was then affixed, with strips of waterproof duct tape, to the dried side of the alternative half-pot. The tea bag prevented direct tactile contact with the eggs but still allowed chemical cues to pass through. With this substitution, we tested whether chemical cues (of the kind given by intact eggs) are sufficient to trigger fanning.

All of these substitutions were conducted in complete darkness with the aid of the infrared scope. The female never saw the experimental 'brood'. Her experience with her own brood at night was limited to the few seconds that preceded the substitution after lights went off.

In a second series of experiments, 21 pairs were allowed to spawn. All were second-time breeders. They had not been used in the first series of experiments. The control ($N = 8$), wax eggs ($N = 5$), and tea bag ($N = 8$) substitutions were performed as before, except that they took place on the second night following spawning. These experiments determined whether experience, with both a previous and the present brood, could affect the female's response.

Statistical Analyses

Statistical analyses were conducted on all groups for which there were non-zero data. Significance levels were set at $P = 0·05$. One-way ANOVAs followed by Scheffé's F-test for multiple comparison (Statview Statistical Package) were run separately on the data collected 1 and 6 h after the substitutions. Within groups, the 1-h and 6-h data sets were compared with two-tailed paired t-tests.

RESULTS

First-time Breeders

All control fish fanned their eggs. The control fish fanned, on average, 72·4% and 82·7% of the time after 1 h and 6 h, respectively (Fig. 1). The fish spent 88·9% and 94·3% of the time inside their nests, respectively (Fig. 2). The 1-h and 6-h data sets were not found to be significantly different. These results are similar to those observed in Reebs & Colgan (1991).
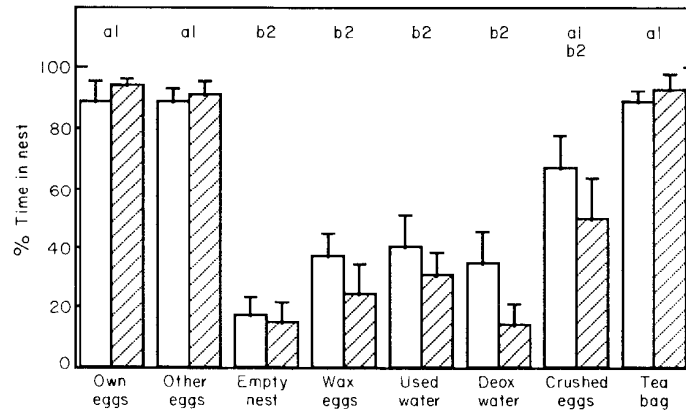
Figure 2. Percentage of time spent inside the nest by naive female convict cichlids 1 h (☐) and 6 h (▨) after their eggs were replaced by various stimuli at night. Open bars not sharing a letter are significantly different ($F_{7,56} = 12.75$, $P < 0.001$), as are hatched bars not sharing a digit ($F_{7,56} = 18.80$, $P < 0.001$).

At no time did fish fan empty nests, nests with wax eggs, or nests with used or deoxygenated water. Nest attendance in these groups was significantly lower than in the other groups (Fig. 2). No significant differences were found between 1 and 6 h, except for the group with deoxygenated water, where nest attendance was higher at 1 h ($t = 3.61$, $P = 0.009$). When in the nest, the fish were often near the artificial stimuli (wax eggs, holes) but did not appear to react to them.

Five of eight fish did not fan crushed eggs. Nest attendance for these five fish varied widely (8.9, 46.2, 56.5, 57.2 and 100% at 1 h; 0.0, 4.4, 21.5, 26.7 and 100% at 6 h). The other three fish fanned at levels somewhat lower than the controls (2.7, 45.6 and 62.8% at 1 h; 30.3, 44.8 and 73.0% at 6 h). Their nest attendance varied between 80.3 and 98.1%.

All fish fanned eggs from other parents, as well as tea bags containing their own eggs. No significant differences were found between these two groups and the control group in either time spent fanning (Fig. 1) or nest attendance (Fig. 2). There were also no significant differences between 1 and 6 h, except for the tea bag group, where fanning levels were significantly lower at 1 h ($t = 3.44$, $P = 0.011$). Females fanning tea bags had their snouts and pelvic fins close to the bag, just as they would with a normal brood.

Experienced Fish

Experience did not alter the females' general response to wax eggs or tea bags: no fish fanned wax
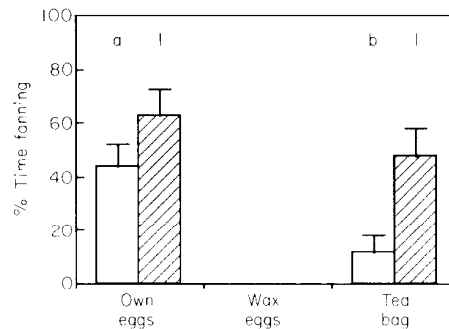

Figure 3. Percentage of time spent fanning by experienced female convict cichlids 1 h (☐) and 6 h (▨) after their eggs were replaced by various stimuli at night. Open bars not sharing a letter are significantly different ($F_{1,14} = 8.51$, $P = 0.011$). The two hatched bars are not significantly different ($F_{1,14} = 1.03$, $P = 0.327$).

eggs, but all fanned tea bags. At 6 h, the tea bag group did not differ significantly from the controls in either fanning activity (Fig. 3) or nest attendance (Fig. 4). However, fanning levels at 1 h for the tea bag group were lower in both within-group ($t = 5.08$, $P = 0.001$) and between groups (Fig. 3) comparisons.

DISCUSSION

In experiments such as those performed here, problems may arise because of dilution of cues over time (as in the treatment with crushed eggs), lack
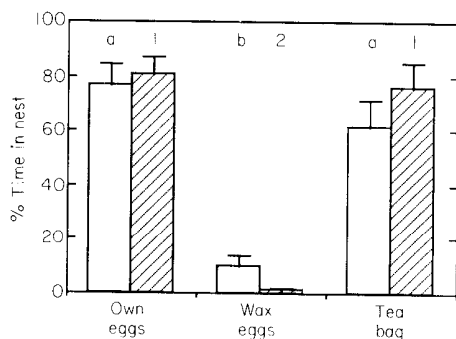
Figure 4. Percentage of time spent inside the nest by experienced female convict cichlids 1 h ( ) and 6 h (▨) after their eggs were replaced by various stimuli at night. Open bars not sharing a letter are significantly different ($F_{2,18} = 14\cdot31$, $P < 0\cdot001$), as are hatched bars not sharing a digit ($F_{2,18} = 29\cdot66$, $P < 0\cdot001$).

of habituation to potentially distressing cues (such as male scent in used water), or time required to adjust to new cues. Because of these potential problems, observations were made both early (1 h) and late (6 h) during the night. Almost no significant differences were found between these two times. Experiments were also carried out with both experienced and inexperienced breeders, and again no major differences appeared. There was evidence that fish with tea bags could detect the presence of these foreign objects within their nests, as fanning levels were significantly lower at 1 h in the tea bag groups (the same can be observed, incidentally, in all other groups including the controls, although the difference was never significant). At 6 h, however, fanning levels were high again and did not differ significantly from those of the controls, an indication that the fish had probably habituated to the tea bags. Because of this example, and the lack of difference between experienced and inexperienced fish, we are confident that the potential problems mentioned above were not important in our study, and that the following analysis is valid.

Fish did not fan empty nests, showing that conformational properties of the nest are not sufficient to trigger egg care at night. In contrast, all fish fanned eggs that were not their own, indicating that a general property of convict cichlid eggs is involved. Among the properties of eggs that can be considered are physical consequences of respiration, tactile cues and chemical cues.

Fish did not react to a flow of used or deoxygenated water passing through their nest.

This suggests that respiratory features do not trigger egg care, but, as always with negative results, interpretation must be cautious. The water flowing through the nest may not have sufficiently mimicked the conditions found in the boundary layer around the eggs. Additional experiments with different flow rates, oxygen and CO_2 concentrations, and controls for the presence of fish scent in used water could yield different results. However, other considerations lead us to believe that respiratory features would be a poor cue for egg fanning. First, it would be difficult for a fish to approach eggs without disturbing the boundary layer it intends to sample. Second, egg metabolism is low in young eggs (Rombough 1988), presumably making respiratory features less detectable. Finally, respiratory characteristics alone would not allow distinction between eggs and other living things, such as algal clumps.

Two studies in three-spined sticklebacks have found that water with high CO_2 and low O_2 concentrations can elicit increases in fanning levels when passed through a nest (van Iersel 1953; Sevenster 1961). This is probably an adaptive response to low oxygen availability (see Reebs et al. 1984), but it does not mean that gas concentrations are the cue used to find the eggs and trigger fanning in the first place. In both studies, fish were already fanning before the stimulus water was presented. Sticklebacks may use nest cues for fanning as eggs are not very accessible in this species' intricate nest.

That fish did not fan wax eggs at night suggests that tactile cues are not sufficient for fanning. It remains possible that our wax eggs did not mimic some subtle tactile cues of importance to the fish. The ultimate test might be to present a normal brood, in the dark, to a fish that has been surgically or pharmacologically deprived of its senses of taste and olfaction. This, however, may be difficult to perform on a relatively small fish such as the convict cichlid.

Even if tactile cues did not trigger fanning, their importance for proper orientation should not be dismissed. At almost all times during the night, the pelvic fins are lowered and almost perpendicular to the body. During fanning, they brush against the eggs, perhaps helping the fish to stay close to them. On nocturnal forays outside the nest (which are infrequent but proceed at a steady pace, without any collision or apparent random movements), the pelvic fins could help the fish feel its way around.

Tactile cues coupled with an internal map of the nest surroundings (see Teyke 1989) could explain how a fish may find its nest in the dark.

Some fish fanned crushed eggs and all fish fanned eggs in tea bags. This indicates that (1) tactile cues from eggs are not necessary for nocturnal fanning, (2) chemical cues on the other hand are sufficient, and (3) the best cues come from intact eggs. As the parental fish approaches eggs, the first contact is almost always with the snout, and while the fish is fanning, its snout usually stays close to the eggs. This is consistent with the idea that the external cues responsible for the triggering and maintenance of fanning are chemical. Chemical cues have already been linked to recognition of young by cichlids (Myrberg 1975; McKaye & Barlow 1976; Lutnesky 1989). Olfaction is likely to be involved, although taste may play an additional role (see Hara 1986).

Weber (1970) and Myrberg (1975) have stated that olfactory cues are probably not involved in the care of cichlid eggs. This view should be amended to exclude nocturnal care. Perhaps it should also exclude situations where low light levels prevent the fish from seeing its eggs well. In nature, convict cichlids prefer to spawn in dark places (Lavery 1991). Conceivably, olfactory cues could supplement visual ones in the performance of diurnal fanning in some cichlids' natural environment. Whatever the answer, the results of our study, in conjunction with other studies such as Weber's (1970), indicate that parental behaviour can be triggered by more than one cue in the same individual, that these cues need not act on the same sensory modality, and that time of day and physical environment should be taken into account when determining the relative importance of such cues.

ACKNOWLEDGMENTS

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