Nocturnal care of eggs and circadian rhythms of fanning activity in two normally diurnal cichlid fishes, *Cichlasoma nigrofasciatum* and *Herotilapia multispinosa*

STEPHAN G. REEBS & PATRICK W. COLGAN

Department of Biology, Queen’s University, Kingston, Ontario K7L 3N6, Canada

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Abstract. Breeding pairs of convict cichlids, *Cichlasoma nigrofasciatum*, and rainbow cichlids, *Herotilapia multispinosa*, were observed at night with an infrared visiophone to determine the extent of nocturnal egg care. In both species, females fanned eggs at night. The position of the female while fanning differed between day and night; the female’s snout was close to the eggs at night, and her lowered pelvic fins often brushed against the eggs. This suggests that she used olfactory and/or tactile cues to maintain proximity to her eggs in the dark. The percentage of time spent fanning and the duration of fanning bouts were always higher at night than during the day. The difference was caused partly by a direct effect of darkness and partly by an endogenous circadian rhythm, as demonstrated by experiments where dark pulses were given in the middle of the day, or where constant light and constant darkness were used. High activity levels throughout the night may represent a substantial energetic cost and may explain why females often fail to gain weight during the parental cycle.

Many fish exhibit parental care. At the egg stage, parental care involves guarding, cleaning and fanning of eggs (Keenleyside 1979). These activities are classical objects of study in ethology, and have been extensively described and quantified (e.g., van Iersel 1953; Mertz & Barlow 1966; Smith-Grayton & Keenleyside 1978). Such studies, however, have always been based on diurnal observations. The extent to which parental care is performed at night, when a fish cannot see its eggs (and the experimenter usually cannot see the fish), is poorly known.

There could be several benefits from knowing more about nocturnal parental care in fish. First, a more accurate assessment of time budgets would be obtained, and this could lead to a better determination of the energetic costs of reproduction (see FitzGerald et al. 1989). Second, the occurrence of egg care at night would force a modification of the view that visual stimuli are all-important in the performance of such a behaviour (this view is especially implied in the behavioural literature on cichlid fish; see Myrberg 1964; Weber 1970). Finally, field observations have indicated that normal dietary patterns of activity may break down during the reproductive season in fish (Helfman 1981), with the intriguing possibility that circadian rhythmicity may be temporarily lost in parental individuals. Comparing day and night behaviour would provide a direct test of this idea.

These potential benefits are offset by the difficulty in observing fish in the dark. The few nocturnal studies that have been carried out so far (Albrecht 1969; Moyer & Bell 1976; Ross 1978; Reeb et al. 1984) have taken place in uncontrolled field situations and have often relied on artificial illumination in the visible range, with uncertain consequences with regard to the alteration of behaviour. These difficulties, however, are not insurmountable: infrared technology can reveal the nocturnal behaviour of aquatic animals kept in the laboratory (e.g., Braski & Dumont 1987). Here, we used an infrared visiophone to observe the nocturnal behaviour of two parental fish species easily bred in the laboratory, the convict cichlid, *Cichlasoma nigrofasciatum*, and the rainbow cichlid, *Herotilapia multispinosa*. We compared nocturnal and diurnal behaviour, and attempted to explain the observed differences in terms of direct effects of darkness and in terms of circadian rhythmicity.

**GENERAL METHODS**

The cichlids used in this study were obtained from local pet suppliers and from the laboratory of M. H. A. Keenleyside, University of Western
Ontario. All fish were less than 8 cm in total length, and had not bred more than once previously. We used first- and second-time breeders indiscriminately and found no behavioural differences between them (see Colgan & Salmon 1986 for another study that found little effect of experience on parental behaviour).

Fish were originally housed together in a holding tank measuring 86 x 46 x 30 cm, and then moved in pairs to breeding tanks measuring 51 x 26 x 31 cm. Water temperature was 26–28°C for the convict cichlids and 28–30°C for the rainbow cichlids. Each breeding tank had a 100-W water heater, a 2-cm layer of medium-sized gravel at the bottom, a corner filter at the back and a nesting substrate at the front. This substrate was either a clay flower-pot 9-5 cm high with a diameter of 11 cm at the opening, lying on its side and facing the front glass, or half of a clay flower-pot (height = 12 cm, radius at opening = 7 cm) lying on its side and leaning against the front glass. Unless otherwise stated, each pair of fish was in visual contact with one other pair in a neighbouring tank. All fish were fed once a day with Nutrafin flakes, shrimp pellets and frozen brine shrimp, Artemia salina.

Lighting came from fluorescent tubes (F15 daylight, experiments 1 and 2) or from 40-W incandescent bulbs (experiment 3) placed directly above the tanks. Unless otherwise stated, the lighting schedule was 12:12 h light:dark, with 15 min of 'twilight' (provided by a single light in one corner of the room) at the beginning and end of the dark period. Pilot lights in the water heaters were disabled to ensure that no light was present at night.

Night observations were made with an AN/PAS 5 infrared visiometer (The Dutchman, Overland Park, Kansas). Illumination came from a 9-V flashlight equipped with an infrared gel filter (Kodak No. 81B). Transmittance values for this filter are 25% at 900 nm, 1% at 830 nm and less than 0.1% at 810 nm. We are confident that the fish could not perceive this light because first, the spectral sensitivity of the known visual pigments of many cichlids stops at 650 nm (Schwanzara 1967) and second, individuals of neither species interrupted their activities when the infrared flashlight was suddenly directed at them in the dark (when a normal light was used, the fish either sank to the bottom or swam to the front glass).

Day observations were made directly through a slot measuring 2 x 8 cm cut into a black plastic sheet that was suspended 20 cm from the front of the breeding tanks. Observations (day and night) lasted about 10 min (observations never started or ended during a fanning bout, which meant that some observations were slightly shorter or longer than 10 min). Throughout the observation period, the observer described into a tape recorder all activity within the nest. From the tapes, the following information was extracted for males and females separately: (1) percentage of time spent fanning, (2) mean fanning-bout duration, (3) fanning-bout frequency, (4) mean fin-beat frequency during fanning, (5) egg-mouthing frequency and (6) frequency of nest entry and departure. Fanning bouts were considered distinct if separated by 2 s or more (for a description of fanning, see results below). Egg mouthing was scored when the parent contacted an egg with its open mouth and then bucked away a short distance (after Smith-Grayton & Keenleyside 1978).

Statistical analyses followed Conover (1980) and Sokal & Rohlf (1981). Significance levels were set at 0.05. Neighbouring pairs that could see each other were never observed on the same day, and were therefore considered independent. With the exception of two pairs used in both experiments 1 and 2, all pairs were used only once.

**EXPERIMENT 1**

**Methods**

Eight pairs of convict cichlids and five pairs of rainbow cichlids were observed. From the time the eggs were laid until the time they hatched, observations were made during the fourth and tenth hour of the day, and during the fourth and eighth hour of the night.

**Results**

**Convict cichlids**

In seven of the eight pairs, only the female fanned the eggs (exclusive fanning by the female seems to be the norm in this species; see Weber 1970; Colgan & Salmon 1986; but also Townshend & Wootten 1985). All females fanned both during the day and the night, and for all eight females (P = 0.004, binomial test) the percentage of time spent fanning was higher on any given night than on any given day (Fig. 1). On any given night, the fanning bouts were longer (all eight fish, P = 0.004) and either less
numerous (six out of eight fish, \( P = 0.145 \)) or more numerous (the other two fish) than on any given day (Fig. 1). Fin-beat frequency during fanning was consistently lower at night (seven out of seven fish, \( P = 0.008 \), Fig. 1), but this decrease in fanning intensity was more than compensated by the increase in total time spent fanning. When the two variables were combined to calculate the total number of fanning fin beats per 10 min, the resulting values were twice as high at night (\( X \pm SE = 689 \pm 55 \)) as during the day (342 \pm 44). In the one pair where the male also fanned, fanning-bout characteristics were comparable for both sexes; however, the male did not fan at night.

Nocturnal and diurnal fanning differed qualitatively. During the day, fanning was similar to that already reported (e.g. Weber 1970): the female was broadside to the eggs or slightly facing them, and her head was at least 1 cm away from the egg batch. Her body was slightly curved in a ‘C’ opening towards the eggs. Ventilation was provided by her pectoral, dorsal and caudal fins. Her pelvic fins were up against the body. At night however, the female brought her snout close to the eggs (possibly touching them, although this could not be ascertained). The sagittal plane of her body was at an acute angle with the plane of the egg batch, and she moved slowly along the egg batch until her snout passed the edge of the batch, at which point she turned around (her snout still close to the eggs) and swam in the opposite direction. Her pelvic fins were down and often brushed against the eggs. Some females also swam along the egg batch with the snout and throat close to the eggs, and with the sagittal plane of the body perpendicular to the egg batch (this was similar to the ‘rocking’ motion of fanning rainbow cichlids, as reported by Smith-Grayton & Keenleyside 1978). This rocking motion was also observed during the day, but less frequently than at night.

There were other day/night differences. Egg mouthing was performed during the day, but never at night. Also, it was not uncommon for the females to leave the nest temporarily during the day (it occurred at least once in 96% of all day observations), and for the males to enter it (it occurred at least once in 81% of all day observations). However, females left the nest on only 33% of all night observations, whereas males entered it on only 28% of all night observations. At night the males swam about slowly or remained motionless just above the nest or the substrate.

![Figure 1](image.png)

Figure 1. Fanning behaviour (\( X \pm SE \)) of female convict cichlids from spawning to egg hatching. \( N = 8 \), except for fin-beat frequency where \( N = 7 \). Horizontal dark bars show night-time.

Rainbow cichlids

Both males and females are known to fan the eggs in this species (Baylis 1974; Smith-Grayton &
but only at night. Another male fanned about 10% of the time, day and night, while the last male fanned only once, 78% of the time on the last observation of the last night. Night fanning by males did not preclude simultaneous fanning by females; both fish often fanned side by side.

All females fanned day and night, and the 24-h patterns (Fig. 2) were similar to those of the convict cichlids. In all five females ($P=0.031$, binomial test), the percentage of time spent fanning was always higher at night, bout duration was always longer, and fin-beat frequency was always lower (although the total number of fanning fin beats per 10 min was higher). For three females, the fanning bouts were always less numerous at night, while the reverse was observed in the other two females.

Rocking was the only form of fanning witnessed at night, in both males and females. Rocking was also performed during the day, mostly by the females. The males tended to fan broadside to the eggs like the convict cichlids. Egg mouthing was also witnessed during the day, but not at night. Nest entry and departure were always less common at night.

Discussion

In all females, the results showed clear day/night differences. Changes in fanning-bout frequency varied individually, but nocturnal bouts were consistently considerably longer, resulting in a higher proportion of time spent fanning at night. These results can be compared with those from field studies on other species. Albrecht (1969) reported an increase in fanning activity at night in a damselfish, Abudelfal sauvin, including an increase in fin-beat frequency. His fish, however, were probably not in complete darkness, as he could apparently observe them without using artificial light. Reeds et al. (1984) used a veiled flashlight and a light magnifier to observe the nocturnal behaviour of threespined sticklebacks, Gasterosteus aculeatus, and reported longer and less numerous fanning bouts at night, and a higher (albeit not significantly so) percentage of time spent fanning. In contrast, Moyer & Bell (1976) and Ross (1978) observed a cessation of fanning at night in various species of anemonefish, Amphiprion spp., but they did not mention how their observations were conducted nor how intrusive they were. We have used the infrared scope for preliminary laboratory observations on two more cichlid species, C. severum and C. spirulum, and found

Keenleyside 1978). In our study, males varied greatly in their diel patterns of fanning. Two males spent 4–34% of the time fanning, but only during the day. One male spent 19–36% of its time fanning,
intense nocturnal fanning. Intuitively, nocturnal fanning makes adaptive sense: assuming that the eggs keep on consuming oxygen at night, it must be advantageous for the parent to continue ventilating them.

The question arises as to how the parent fish can detect and correctly orient towards its eggs in complete darkness. Visual stimuli, known to be important during the day (see Introduction), can safely be ruled out at night. The fanning posture may provide clues to the answer: proximity of the snout and pelvic fins to the egg suggests that chemical and/or tactile cues are involved. Experiments with wax eggs are in progress to provide a more definite answer.

Another question concerns the nature of the proximate factors responsible for elevated fanning levels at night. Three non-mutually exclusive hypotheses can be formulated. First, higher nocturnal fanning levels may be a direct consequence of darkness, unable to see (and to be distracted by) food, mate and neighbours, the parent fish can give long uninterrupted bouts of fanning. Second, an endogenous circadian clock (see Schwammann 1971) may cause the 24 h variation. Third, the parent may adjust its fanning activity to the rate of oxygen consumption by the eggs (see van Iersel 1953; Severson 1961) and this rate may be higher at night. Respiratory experiments are in progress to address this latter possibility. Hereafter we present the results of experiments testing the first two hypotheses. To look for a direct effect of darkness, we subjected parental fish to pulses of darkness in the middle of the day (experiment 2). To look for an endogenous rhythm, we put parental fish in constant light or constant darkness (experiment 3). In these experiments, only convict cichlids were used because of their longer egg-stage period at preferred breeding temperatures.

**EXPERIMENT 2**

**Methods**

Eight pairs of convict cichlids were allowed to spawn in the same conditions as before. In the middle of the first full day following spawning, all lights in the room were turned off for 1 h. An observation was made in the last 10 min of this 1-h pulse. It was compared with observations made at four control times: the morning (3 h after dawn) and night (3 h after dusk) immediately preceding the pulse, and the afternoon (3 h before dusk) and night (3 h after dusk) immediately following the pulse.

**Results and Discussion**

The nocturnal and diurnal values of all fanning characteristics were similar to those observed in experiment 1 (Figs 1 and 3). The values at the end of the dark pulse in the middle of the day tended to be intermediate between the day and night values (Fig. 3). This was significant (Friedman test followed by multiple comparison test) for percentage of time spent fanning and bout duration, but not for bout frequency, and not for fin-beat frequency where the pulse values were similar to the night values (Fig. 3).

Thus, darkness can have a direct effect on fanning behaviour. In the case of fin-beat frequency, this effect seems sufficient to account completely for day/night differences, as pulse values equaled night values. However, in the case of bout duration and percentage of time spent fanning, the pulse values were only intermediate between those of day and night. Therefore, it is unlikely that darkness alone could explain the elevated fanning levels observed at night. The possibility remains that pulse values would have been higher if the pulses had lasted longer. Values at the end of a 3-h pulse, for example, would be more comparable to our night values taken 3 h after dusk. Conversely, night values taken 1 h after dusk would be more comparable to those at the end of a 1-h pulse. We addressed this question by allowing eight more pairs to spawn in the same conditions as before: on the second night after spawning, we observed the pairs 1 and 3 h after dusk. The percentage of time spent fanning after 1 h ($X \pm st = 73.9 \pm 7.6$) was significantly lower than after 3 h ($81.4 \pm 6.0$; Wilcoxon test, $T = 2, 10, P < 0.02$) but also significantly higher than at the end of the 1-h pulses in experiment 2 ($47.5 \pm 8.8$; Mann–Whitney test, $T = 46, P = 0.01$). Mean bout duration after 1 h ($81.3 \pm 23.2$ s) was not significantly different than after 3 h ($97.6 \pm 26.4$ s; Wilcoxon test, $T = 10, P > 0.1$) but was significantly longer than at the end of the 1-h pulses in experiment 2 ($28.3 \pm 5.9$ s; Mann–Whitney test, $T = 47, P < 0.02$). Therefore, even when observation time is controlled, our conclusion remains that darkness contributes to, but does not explain all of the high levels of nocturnal fanning.
EXPERIMENT 3

Methods

For the constant-light experiment, eight pairs were allowed to spawn in the same conditions as before except that all tanks were visually isolated from each other. Once a pair spawned, the light of
its tank was left on continuously. Observations were then made every 6 h for 48 h, starting 3 h after what would have been the first dusk following spawning. The fish were fed after each observation (a single food presentation each day might have provided the fish with an artificial 24-h time cue).

Eight more pairs were used in the constant-dark experiment. Procedures were the same as in the constant-light experiment, except that we tried to minimize factors susceptible to distract the female. At the time of first dusk following spawning, the male was removed, the light was disconnected, and an opaque plastic hood was put over the whole tank to prevent any influence from small light leaks that showed through the partitions between tanks. At observation time, all lights that were still on in the room (other pairs that had not yet spawned) were temporarily turned off and the hood was raised to make the observation with the infrared scope. The females were not fed once they spawned.

**Results and Discussion**

In both constant light and constant dark, a circadian rhythm in the percentage of time spent fanning and in fanning-bout duration persisted (Fig. 4). Values obtained at times that corresponded to the night (had the fish been left in a light-dark cycle) were significantly larger than values obtained during the ‘day’ (Friedman test followed by multiple comparison test). However, we found no clear evidence of a circadian rhythm in fanning-bout and fin-beat frequency (Fig. 4). Throughout the study, fanning-bout frequency was variable and did not yield clear results. In the case of fin-beat frequency, the absence of a rhythm in constant conditions is consistent with the results of experiment 2 in which we showed that darkness alone could explain the day/night differences observed in this variable.

There was evidence of a circadian rhythm in nest-departure frequency by the females in constant darkness: three of the ‘night’ values were significantly lower than any of the four ‘day’ values (Friedman’s $T = 3.66$, $P < 0.01$, followed by multiple comparison). A similar pattern was present in constant light but it was not significant (Friedman’s $T = 2.00$, $P < 0.1$).

The direct effect of darkness on fanning behaviour could be seen in this experiment. At all eight observation times ($P = 0.004$, binomial test), mean per cent time spent fanning and mean bout duration were higher in constant darkness than in constant light, whereas the reverse was true for fanning-bout frequency and fin-beat frequency (Fig. 4). These differences were the same as those observed between day and night in experiments 1 and 2 (Figs 1–3).

**GENERAL DISCUSSION**

Convict and rainbow cichlids are diurnal species. When not breeding, they spend the night resting on the bottom of tanks (personal observation). Reproductive activities and parental duties bring a change to this pattern, with females becoming active at night. Therefore, as asserted by Helfman (1981) for a number of other species, the normal patterns of activity of these parental fish can be said to break down during the reproductive season. This does not mean, however, that all rhythmicity is lost. Our study demonstrated that a circadian rhythm is still present.

It remains to be seen whether this rhythm is the product of a self-sustained internal clock. To demonstrate this, one needs to show that the rhythm persists in constant conditions for a long time (ideally with a periodicity different than 24 h). The limited duration of the egg stage in cichlids precludes such a demonstration. An alternative hypothesis is that the cycle is based on long-term, linear timing (interval clock) rather than on a self-sustained periodic clock (see Church 1984). To our knowledge, the capacity of fish to estimate long time intervals has never been studied.

The fanning cycle may also be the indirect results of other types of rhythmicity. For example, there may be a circadian rhythm of feeding activity (Randolph & Clemens 1976; Rusak 1981, and references therein). At times when feeding levels are low, fanning can express itself at a high intensity, but when the feeding levels are high, food-seeking activities may compete with fanning and curtail its expression. We found some evidence of a rhythm in
nest-departure frequency in constant conditions, and nest departure may reflect a need for foraging. The problem in this kind of analysis is to establish the right cause and effect relationship: does a fish forage more because its motivation to fan is low, or does it fan less because its motivation to feed is high?

Should a true rhythm of fanning activity be demonstrated, the question of its adaptive significance would arise. Why should a fish fan more at night? As mentioned before, the answer may lie in a possible circadian pattern of oxygen consumption by the eggs. The oxygen content of water may also be lower at night because of respiration and lack of photosynthesis by aquatic plants, hence a greater need for ventilation (see Reeb et al. 1984). Alternatively, high levels of fanning during the day might detrimentally compete with other essential and adaptive activities, such as foraging and chasing potential egg predators (however, egg predators may not be exclusively diurnal; see Moyer 1975; McKaye et al. 1979).

The observation that females spend a lot of time fanning at night sheds a new light on their time budget and on potential reproductive costs. In a study on male three-spined sticklebacks, Fitzgerald et al. (1989) showed that parental duties, which in this species consist mainly of fanning, entail a measurable cost (dry-weight loss). In convict cichlids, males often gain weight throughout the parental cycle, while females do not (e.g. Lavery & Keenleyside 1990). This may reflect not only reduced foraging opportunities for females because of the requirements of fanning, but also the energetic cost of fanning itself. Given the high levels of fanning throughout the night, this cost may be substantial. If we accept the idea that determining accurate time budgets may help to assess reproductive costs in fish, then future studies must consider more than just the diurnal half of a fish's life.

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