

## The anticipation of night by fry-retrieving convict cichlids

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**Abstract.** Every day at dusk, many parental cichlids 'retrieve' their fry: they take wandering fry into their mouth and spit them into a previously dug pit. The present study investigated the relative importance of illumination changes and endogenous clock mechanisms in the daily timing of fry-retrieving behaviour in convict cichlids, *Cichlasoma nigrofasciatum*. Even when night onset was abrupt, most female cichlids were able to anticipate night: they started retrieving 0–12 min before night onset. Retrieving was more pronounced, however, when night onset was reliably preceded by a 15-min period of dim illumination. Dim illumination at other times of the day elicited no fry-retrieving response. When complete darkness was imposed in the middle of the day, retrieving took place but at lower levels than during the first 15 min of the preceding night. These results indicate a dual role of light levels and endogenous clock mechanisms in the anticipation of night by fry-retrieving convict cichlids. Light levels alone may not be sufficient because dusk is not the only time when light levels change, and endogenous clocks alone may not be sufficient because they are not accurate enough.

Circadian rhythms are widespread in animals. These rhythms are the expression of an endogenous clock whose periodicity approximates 24 h. The physiological mechanisms underlying this clock have been extensively studied (for reviews, see Kavaliers 1984; Rusak 1989) but the adaptive significance of circadian rhythms is less well understood. One common view is that circadian clocks allow animals to anticipate the arrival of day or night (e.g. Brady 1982). However, one could argue that because day and night are reliably preceded by changes in light intensity, animals could also anticipate these periods by simply responding to illumination changes rather than relying on an internal clock. To my knowledge, the relative importance of light and endogenous timing in day/night anticipation has never been studied experimentally.

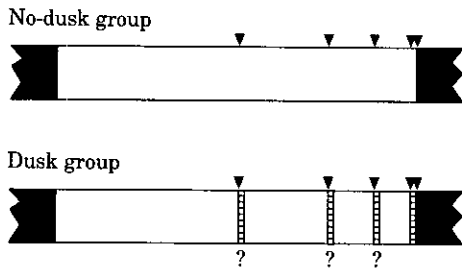
A behaviour that lends itself to studies of this kind is the fry-retrieving activity of parental cichlid fishes. Cichlids usually lay broods of several hundred eggs; these hatch into non-mobile wrigglers (embryos), which after a few days turn into free-swimming fry (larvae). The parents guard the fry, and when night approaches they start retrieving them, sucking several fry at a time into their mouth and spitting them into a previously dug pit (McKaye et al. 1979; but see also Baerends & Baerends-van

Roon 1950 for exceptions). This behaviour presumably allows parents to keep all of their fry in one place, which is more easily defended against nocturnal predators (the catfish *Rhamdia nicaraguense* is an example of nocturnal predator on cichlid fry; see McKaye et al. 1979). Retrieving is performed just before night to ensure that protection is given from the very beginning of darkness.

In the present study, I used convict cichlids, *Cichlasoma nigrofasciatum*, to investigate the relative importance of light levels and endogenous clock mechanisms in the timing of fry-retrieving behaviour. Convict cichlids are small biparental fish that are easily bred in the laboratory. In at least one aspect of their parental behaviour, fanning activity, they are known to exhibit endogenous rhythms and to be sensitive to light intensity (Reebs & Colgan 1991). Here I measured fry-retrieving and other related activities in the presence and absence of illumination changes, both during the afternoon and just before night onset.

### MATERIAL AND METHODS

All cichlids used in this study were first-time breeders (total length: 6.0–6.5 cm). I placed



**Figure 1.** Illumination regimes for the two experimental groups. The dark areas delimit a 12-h day. Hatched areas show 15-min periods of dim illumination. Arrowheads show observation times and question marks indicate periods of dim illumination that were not given on all days.

male–female pairs in separate 41-litre aquaria (51 × 26 × 31 cm) containing a 2-cm layer of gravel at the bottom. Water temperature was maintained at 26–28°C by water heaters whose pilot lights had been disabled to ensure complete darkness at night. In the middle of the tank was a clay flower pot (10-cm diameter at the opening) lying on its side. All fish spawned inside this pot and kept their wrigglers in it. Most fish also retrieved their young into the pot (some retrieved their young into a pit dug from under the side of the pot). To simplify data collection and interpretation, I removed the male from each aquarium at the end of the wriggler stage, before retrieving activity had started (preliminary observations revealed that males contribute to retrieving, but less so than females, and females can easily take care of the young by themselves when predators are absent). Females were fed once a day (in the morning) with food pellets, while young received *Artemia salina* nauplius larvae. Each female was in visual contact with a neighbouring female through one-third of one of the long sides of her tank.

The fish had been raised in the laboratory under a 12:12 h light:dark cycle with an abrupt light change between day and night. Lights came on at 0700 hours and went off at 1900 hours. Two experimental groups were formed (Fig. 1). In the ‘no-dusk group’ ( $N=8$  pairs), the original all-or-nothing light:dark cycle was maintained throughout the experiment. In the ‘dusk group’ ( $N=8$  pairs), fish were exposed during the day to 15-min ‘dim pulses’, starting 3–8 weeks before they spawned. During dim pulses some of the overhead

lights in the room were turned off; this reduced light intensity at tank level from the normal 100–200 lx to only 10–20 lx (measured with a Gossen Lunasix 3 photometer). A dim pulse was given every day at 1845 hours (15 min before night onset), thereby simulating dusk. Other 15-min dim pulses were given during the day, but the number of such pulses varied from day to day. A maximum of three such pulses could be given, one each at 1300 hours (mid-day), 1600 hours (mid-afternoon), or 1730 hours (late afternoon). At the beginning of each day, two coins were tossed to determine how many (0–3) and then which (1300, 1600 or 1730 hours) pulses would be given on that day. The only exceptions to this randomization were observation days, when invariably all dim pulses were given.

In each group the females’ behaviour was observed through a Sony CCD video-camera and remote monitor (Fuhrman Diversified Inc., LaPorte, Texas) on the third, sixth and 12th day of the fry stage. Observations lasted 15 min and started at 1300, 1600, 1730, 1845 and 1900 hours (coinciding with dim pulses in the dusk group, and with night onset in both groups). Observations at night were possible because I used infrared illumination (a bank of 36 light-emitting diodes) in conjunction with the infrared-sensitive camera. Cichlids are not sensitive to infrared illumination (Schwanzara 1967; Reeb & Colgan 1991).

The following acts were scored: (1) retrieving: the female took at least one fry into her mouth and spat it into the flower pot (or the pit dug under it), (2) nest entry: the female entered the pot (or stopped within one body length of the pit) with no fry in her mouth and (3) digging: the female took a pebble in her mouth and pushed it or carried it away. Note that, for the purpose of this study, my definition of retrieving corresponded to the kind of retrieving that usually takes place before night, i.e. ending in the nest or pit. Retrieving can also occur during the day, but usually it consists of a female retrieving a straggling fry into the school, not into the nest. I did observe such ‘diurnal retrieving’ but at very low rates compared to twilight retrieving. Note also that nest entries and digging were scored because they are contextually related to retrieving and could be viewed as an indicator of the parent’s motivation to retrieve. Conceivably, nest entries and digging could also represent an attempt by the parent to keep fry close to the nest where they could easily be

retrieved. Digging draws in fry because food is often lifted up from the gravel when the female moves pebbles. I predicted that rates of nest entry and digging would loosely parallel rates of retrieving.

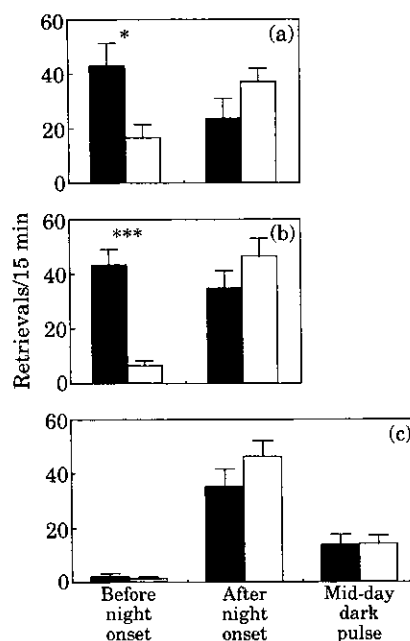
On the 13th day of the fry stage in both groups, a 15-min 'dark pulse' (all lights turned off) was given in the middle of the day (1300 hours). I carried out observations throughout this pulse and compared the results to those obtained during the first 15 min of the preceding night. I then removed the females from their tanks and observed the behaviour of fry at dusk and at night during the next few nights. This latter manipulation was done to determine whether fry-gathering at night could be the result of fry activity alone, or whether parental retrieving is necessary for it.

I compared the results of the dusk and no-dusk groups using *t*-tests and MANOVAs for repeated measures (SPSS-PC). Groups were the between-subject factor, while days and daily times were within-subject factors. In some cases, data were log-transformed to render variances homogeneous.

If retrieving was under the control of an endogenous clock only, one could predict that retrieving would take place only before night onset, and equally in both groups (i.e. irrespective of the presence or absence of dim or dark pulses). However, if retrieving was under the control of light changes only, then the dusk group should retrieve more than the no-dusk group during all dim pulses, but equally to the no-dusk group during the dark pulse. A combination of these possible outcomes would indicate a dual role of endogenous clock mechanisms and direct light effects.

## RESULTS

In both groups, no retrieving into the nest took place during the mid-day, mid-afternoon and late-afternoon observations. Retrieving was performed only before and after night onset. On the third and sixth day, most females retrieved before night regardless of the presence or absence of a dusk-simulating dim pulse (the only exceptions were two females who did not retrieve before night in the absence of dusk). However, females in the dusk group retrieved significantly more than those in the no-dusk group (Fig. 2; third day:  $t=2.67$ ,



**Figure 2.** Fry-retrieving activity ( $\bar{X} \pm SE$ ,  $N=8$ ) of female convict cichlids before and after night onset on the third (a), sixth (b) and 12th (c) day of the fry stage, and during a mid-day period of complete darkness on the 13th day (c). ■: The dusk group; □: the no-dusk group. Asterisks show significant differences between the two groups (*t*-tests, \* $P<0.05$ , \*\*\* $P<0.001$ ).

$P=0.02$ ; sixth day:  $t=5.78$ ,  $P<0.001$ ). Whereas most (six of eight) females in the dusk group started to retrieve as soon as the dim pulse began, females in the no-dusk group started to retrieve only 0–12 min before night onset, and did so at a slower rate. I did not make a systematic attempt to count the number of fry carried during each retrieval, but my subjective impression was that the two groups did not differ in that respect.

After night onset, females in both groups continued to retrieve; activity in the dusk group was now lower than in the no-dusk group (possibly because most of the dusk-group fry had already been retrieved), but the difference was not significant (Fig. 2; third day:  $t=1.51$ ,  $P=0.15$ ; sixth day:  $t=1.24$ ,  $P=0.24$ ). Retrieving activity was still intense in the no-dusk group at the end of the 15-min period, and had I prolonged the observation the difference between the dusk and no-dusk groups would probably have become significant. In both groups, females retrieved at night by swimming with their snout close to the

bottom, stopping and backing up occasionally to suck in fry, and then returning to the nest, often in a straight line.

On the 12th day, retrieving activity became restricted to night only, and no significant differences were observed between the two groups (Fig. 2;  $t=1.19$ ,  $P=0.25$ ). On the following day, all fish retrieved during the mid-day dark pulse, at similar levels in both groups (Fig. 2;  $t=0.08$ ,  $P=0.94$ ), but at lower levels than during the first 15 min of the preceding night (Fig. 2; dusk group: paired  $t=5.68$ ,  $P<0.001$ ; no-dusk group: paired  $t=5.07$ ,  $P<0.002$ ).

After the females were removed from their tank, fry did not gather together in one place at night. There was no apparent change in their behaviour as night approached, even in the dusk group. When all lights went off, fry moved towards the bottom. There was little subsequent movement and, as a result, fry remained dispersed all night long.

In both groups, females entered nests mostly in late afternoon and just before night onset (Fig. 3). The dusk group tended to enter nests more often than the no-dusk group ( $F=4.85$ ,  $P=0.04$ ), and in both groups entries increased throughout the day ( $F=27.49$ ,  $P<0.001$ ) but not from day to day ( $F=0.45$ ,  $P=0.64$ ).

Digging activity tended to increase throughout each day ( $F=34.76$ ,  $P<0.001$ ) and from day to day ( $F=11.54$ ,  $P<0.001$ ) (Fig. 4). No significant differences were found between the two groups ( $F=0.15$ ,  $P=0.71$ ). Most digging took place near the nest.

## DISCUSSION

The results reveal a dual role of endogenous timing and direct light effects in the anticipation of night by fry-retrieving parents. All fish retrieved fry during the mid-day dark pulse (a direct effect of light) but they did so at lower levels than during the first 15 min of the preceding night (endogenous timing). Also, six of eight fish in the no-dusk group retrieved before night despite the absence of dusk-like illumination changes (endogenous timing) but they did so at lower levels than in the dusk group (a direct effect of light). Finally, in the dusk group, no retrieval was observed during dim pulses other than at dusk (endogenous timing). A dual influence of light and

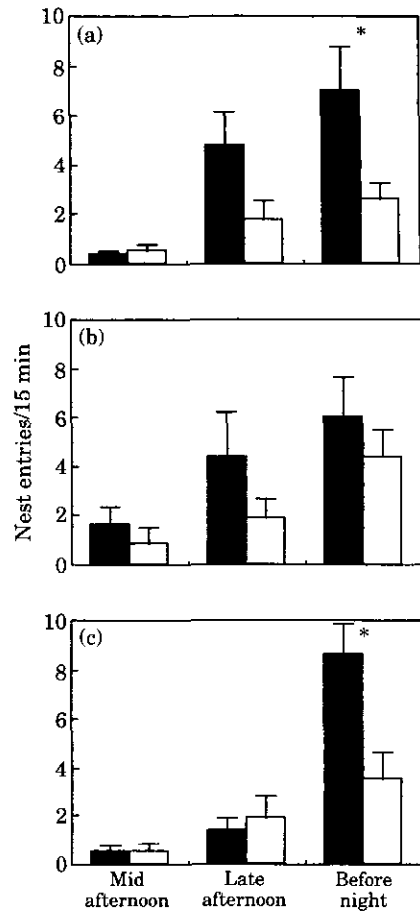
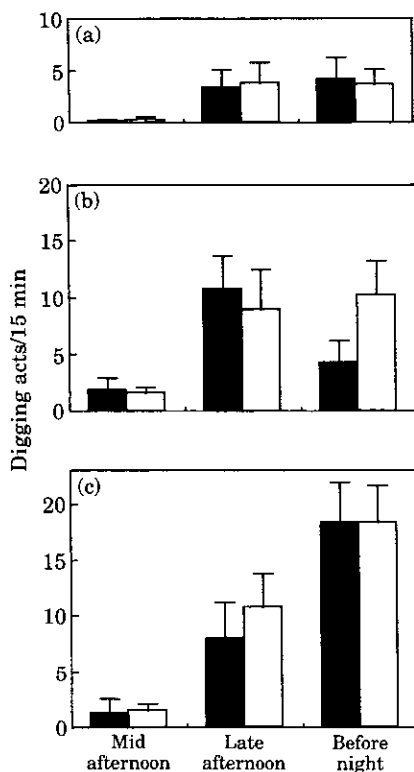


Figure 3. Nest entries without retrieval ( $\bar{X} \pm \text{SE}$ ,  $N=8$ ) by female convict cichlids at different times of the day on the third (a), sixth (b) and 12th (c) day of the fry stage. ■: The dusk group; □: the no-dusk group. Asterisks show significant differences between the two groups ( $t$ -tests,  $*P<0.05$ ).

endogenous timing in cichlids has previously been found in two other contexts not related to night anticipation: the fanning activity of convict cichlids (Reebs & Colgan 1991) and the elongation of retinal cones in Midas cichlids, *C. citrinellum* (Levinson & Burnside 1981).

Field observations in at least one other fish species also suggest a dual role of light and endogenous timing in night anticipation. In nocturnal eels, *Anguilla rostrata*, Helfman (1986a) found that light intensity determined onset and cessation of activity, with an inhibitory threshold of 10–100 lx, but that eels also made some anticipatory movements near the entrance of their



**Figure 4.** Digging activity ( $\bar{X} \pm SE$ ,  $N=8$ ) by female convict cichlids at different times of the day on the third (a), sixth (b) and 12th (c) day of the fry stage. ■: The dusk group; □: the no-dusk group.

cave before dusk, indicating an endogenous mechanism. Many other studies have found a robust correlation between light intensity and the onset of twilight activity in fish (e.g. McFarland et al. 1979; Helfman 1981; Hobson et al. 1981) but in these cases evidence of endogenous clock mechanisms was not or could not be found.

A dual role of light intensity and endogenous timing in night anticipation probably applies to other animals. For example, many studies have shown that birds tend to go to roost earlier on cloudy than on sunny days (e.g. Davis & Lussenhop 1970; Swingland 1976; Reebs 1986). That is an example of a direct light effect. Evidence of endogenous clocks also exist: all other things being equal, and even when well fed, birds tend to go to roost later on short days as compared to long days (e.g. Dunnett & Hinde 1953; Aschoff et al. 1970); moreover birds have not been seen going to roost during dark mid-

day storms (Swingland 1976), or even during total solar eclipses (Du Mont 1970; Elliott & Elliott 1974).

Why is endogenous timing involved? Why do cichlids and perhaps other animals not simply use only illumination as a cue? The answer may lie in the fact that dusk is not the only event that decreases light intensity in an animal's immediate environment. For example, illumination decreases whenever a fish enters a shady area near the bank of a stream, forages under a log, or enters the nest (usually a dark cavity in the case of convict cichlids, see Lavery 1991). However, these events do not necessarily correlate with increased predator activity. Therefore, if fish were automatically prompted to retrieve fry every time such events took place, valuable time and energy, for both the parents and the fry, would be wasted. Endogenous clock mechanisms are necessary to 'tell' the fish that only the decrease in light intensity occurring at the end of the day truly announces the imminent risk of nocturnal predation.

But then, why are light effects involved at all? Could endogenous timing alone allow the fish to retrieve young at the appropriate moment? The answer here may be that circadian clocks in fish are not accurate enough. In diurnal fish under laboratory conditions, the free-running periodicity of circadian clocks usually deviates from 24 h by several hours (e.g. periods of 25–28 h in *Couesius plumbeus*, Kavaliers 1978; 24–26 h in *Fundulus heteroclitus*, Kavaliers 1980). The natural light:dark cycle synchronizes activity rhythms to 24 h, but in diurnal animals with endogenous periods greater than 24 h the synchronizing effect of light is thought to take place mostly at dawn (Daan 1982). Already by dusk the rhythms may be out of synchronization with the light:dark cycle by an hour or more. If only circadian mechanisms were involved in fry-retrieving, the fry could remain unretrieved and exposed to predators for a relatively long time early in the night. This could be costly as predator activity is often high at the beginning of the night (see Helfman 1986b). Note however that six of eight females in the present study anticipated night even in the absence of dusk; therefore their endogenous timing was still fairly accurate, although possibly not optimal (most of their total retrieving still took place at night). The two females who did not retrieve at all before night

onset may represent cases of endogenous clocks with periodicities much greater than 24 h.

The argument above relies on the presumed circadian nature of the endogenous timing mechanism. It is possible for other types of timing to be involved, such as an hourglass mechanism (defined as a direct evaluation of the time elapsed since day onset or any other reference point). However, hourglass mechanisms operating at the level of several hours or more have not yet been found in vertebrates. For example, a recent study on the time sense of garden warblers, *Sylvia borin*, in the context of time-place learning over a full day has revealed evidence of circadian rather than hourglass timing (Biebach et al. 1991). Moreover, it remains to be seen whether putative hourglass mechanisms could be any more precise than circadian clocks.

More specific aspects of the results are worth discussing here. First, dim pulses helped females anticipate night even though they were not perfect simulations of dusk. It is possible that the effect is not specific to light, and that any factor that reliably occurs at dusk could be learned by the fish as a cue to the imminent arrival of night. This view would emphasize the learning ability of the fish rather than a possible wired-in response to a photic signal. The relative efficiency of various factors as predictors of night awaits further study.

Second, because fry did not assemble at night in the absence of the female, it appears that the timing of fry-gathering is under the direct control of the female only. However, it remains possible that fry behaviour could influence the female's efficiency at retrieving. The observation that fry retrieval ceased to take place before night between the sixth and 12th day could be interpreted in that light: after 6 days, well fed fry become more mobile and tend to move out of the nest after being retrieved (personal observation; see also Hay 1976). Only in complete darkness are old fry sufficiently immobile and passive to allow effective retrieval.

The female may still try to increase the efficiency of her retrieval in special ways. By entering the nest and digging near it at dusk, the female may entice the young to stay near the nest where she can more easily find them after dark. Consistent with this view is the observation that nest entries and digging levels near the nest were significantly higher just before night than at the other times of day. Moreover, like retrievals, nest

entries were significantly higher in the dusk group. However, without further experiments, it is impossible to determine whether these results represent an attempt by the female to manipulate the young's location, or simply an expression of the female's preparation (in the case of digging) or motivation (in the case of nest entries) to retrieve.

A final aspect of the results worth pointing out is the ability of the parent fish to retrieve its young in complete darkness. The parent probably uses olfactory and/or tactile cues to find its fry over the gravel (see Myrberg 1975; Reeb & Colgan 1992). But how does it find the place where it gathers its young? Cognitive maps (Teyke 1989), information obtained through the lateral line, and orientation based on the location of a sound (the filter for example) may all be involved. This question is worthy of future study.

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