

## Note

# Daily activity rhythms of the African catfish *Heterobranchus longifilis* (Clariidae) in an experimental enclosure

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**Abstract** – The swimming, air-gaping, and agonistic behaviours of *Heterobranchus longifilis* (318 ± 67 mm) were examined while fish were in a fasted state under 12L:12D and variable group size (2, 5, 10 and 15 fish) in a 1000-L aquarium. Fish exhibited a predominantly nocturnal activity pattern independent of group size. A diurnal peak of activity occurred, however, at the usual feeding time. A reduction in frequency of agonistic interactions was observed in larger groups. Five fish were then observed under 72L:0D and 0L:72D. The nocturnal activity pattern remained, contrary to the diurnal peak, and was independent of the duration of illumination or darkness. These results suggest the absence of biological clock in *H. longifilis*, although fish may somehow be influenced by past feeding experience. Behavioural plasticity in this species may provide potential for aquaculture in northern latitudes.

**Key words:** Catfish / Activity rhythm / Air gaping / Behaviour / Group size

## 1 Introduction

For several decades, there has been an increasing interest in some species of African catfishes of the Clariid family as candidates for aquaculture (e.g. Micha 1972; Teugels 1982; Legendre and Proteau 1996). In particular, *Heterobranchus longifilis* Valenciennes 1840 has one of the greatest growing capacities of cultured catfishes and inter-specific hybrids (Legendre et al. 1992). Catfishes have been shown to be essentially nocturnal foragers (e.g. Bruton 1979; Boujard et al. 1992; Avit and Luquet 1995; Boujard and Luquet 1996; Baras et al. 1998) growing faster when fed at night than at other times with equivalent amounts of food (Kerdchuen and Legendre 1991). They can cope with low levels of dissolved oxygen and exhibit air-gaping behaviours at the water surface, although this activity seems to require more energy from the fish than directly extracting oxygen from water (Bevan and Kramer 1987). Cannibalism is often reported to develop among catfishes (Hecht and Appelbaum 1988), and it is more frequent when animals are hungry (Baras et al. 1999).

These findings raise fundamental questions concerning the nature of biological rhythms in catfishes, especially when animals are subject to environmental synchronisers. Only a few studies have been devoted to the behaviour of *Heterobranchus* species (e.g. Baras et al. 1998; Poncin et al. 2002).

The paper presents data from a series of short experiments, which aim to examine the effects of light and group size on daily rhythms of activity of *H. longifilis* under controlled conditions.

## 2 Materials and methods

### 2.1 Rearing conditions

Fish were obtained from the Aquaculture Unit at Tihange, a station of the University of Liège rearing *Heterobranchus* from egg stage to maturity. They were transferred to experimental aquaria (300 to 1000 L) at the juvenile stage in the Laboratory of Fish Ethology and were observed while still immature (size: 318 ± 67 mm; weight: 321 ± 88 g). Fish were maintained at a constant temperature of 26 °C and under a 12L:12D photoperiod (light from 7:00 am to 7:00 pm). The size ranges (mm) of fish groups were: 344–381 ( $n = 2$ ), 347–404 ( $n = 5$ ), 351–392 ( $n = 10$ ), and 344–399 ( $n = 15$ ).

### 2.2 Experimental design

All observations were carried out in a 1000-L aquarium (2450 × 620 × 750 mm) with stagnant freshwater. Dissolved oxygen was recorded (WTW Oxy 330i) at the start

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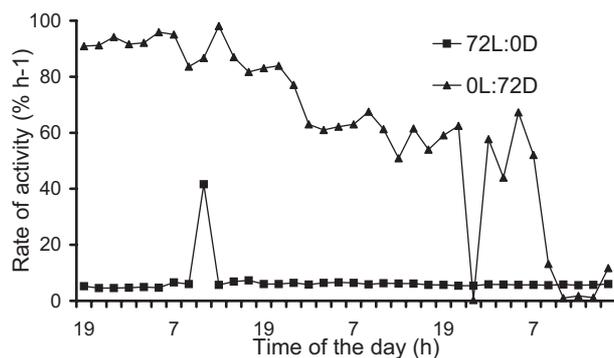
( $4.9 \pm 0.6 \text{ mg O}_2\text{L}^{-1}$ ) and the end ( $5.2 \pm 0.5 \text{ mg O}_2\text{L}^{-1}$ ) of each observation phase. The small variations observed in oxygen concentration could not be attributed to any photosynthetic activity since the aquarium was free from vegetation but was very likely due to fish-induced whirlpool and mixing of atmospheric oxygen through diffusion. No food was supplied during the observation phases, however fish were usually fed every day at about 11:00 am with dry pellets proportionally to their body weight (3% of body weight) before the observations began as well as between the different phases of observation, but not during the observations. The behaviour of fish was recorded after a period of acclimatization to the aquarium.

Experiment 1 assessed the propensity of *H. longifilis* to swim, air-gape and attack other fish (with or without biting) during light and dark periods depending on the number of conspecifics present in the aquarium. These daily rhythms of activity were described under 12L:12D in four group size conditions (2, 5, 10, and 15 fish) in the 1000-L aquarium. Fish were not reused. Behavioural data were recorded using a video camera system Sony<sup>®</sup>, Handycam 2006 (Poncin and Ruwet 1996). During the dark periods, two infrared spotlights Molyx<sup>®</sup> IR550 (830 nm filter, an undetectable wavelength for fish, Beach 1978) as well as a video camera Burle<sup>®</sup> TC300X sensitive to infrared light were used. Both cameras were alternatively placed in front of the aquarium in such a way that the whole aquarium can be filmed. No material (gravel, shelter, pump, etc.) was present in the aquarium where the behaviour of fish was recorded. Apart from the group size  $n = 2$  (see above), a 24-h observation phase consisted of reporting the activity of five fish, taken at random, for 10 min at the beginning of every hour. The observations were recorded on videocassettes and viewed on a TV screen. The data were collected manually and then analysed using a computer.

A second experiment was conducted on five fish (not reused) in order to examine their daily rhythms of activity under constant illumination or darkness over 72 h. A 3-d interval was introduced between the 72L:0D and 0L:72D conditions. To deprive the fish of food for 72 h should cause only minimal stress; these animals are able to withstand such conditions, sometimes encountered during the dry season (Poll 1959; Bruton 1979). Others studies even mention periods of food restriction as long as 3 weeks (e.g. Luquet et al. 1995). The same methodology as in Experiment 1 was used here. These experiments were carried out only once.

### 2.3 Statistical analyses

Air-gaping behaviours and agonistic interactions were counted (number  $\text{h}^{-1}$ ), and the total activity of fish (including swimming, air-gaping, and agonistic interactions) was quantified as the proportion ( $\% \text{h}^{-1}$ ) of different behavioural patterns expressed during each sample;  $t$ -tests were used to assess the effects of light/dark periods and of group size on the studied behaviours, as well as two-way analyses of variance (ANOVA) to determine potential interactions between light/dark conditions and group size on these behaviours. Variances were homogenous and distributed normally. Null hypotheses were rejected at  $p < 0.05$ .



**Fig. 1.** Distribution of activity (percent per hour) over 72 h (72L:0D and 0L:72D) for one group size ( $n = 5$ ).

## 3 Results

### 3.1 Total activity

Under 12L:12D, fish were significantly more active at night than during the light period ( $p < 0.001$ ) irrespective of the number of fish in the aquarium (2:  $t_{11} = 13.34$ ; 5:  $t_{11} = 12.62$ ; 10:  $t_{11} = 12.56$ ; 15:  $t_{11} = 11.48$ ). However, a peak of activity was observed about 11:00–12:00 am in all groups (the mean activity rate rises to  $57 \pm 3\% \text{h}^{-1}$ , whereas its value was  $6 \pm 4\%$  only 2 h before). This peak of activity happened at the usual feeding time of fish, although they were not fed during the experiment. When fish were maintained under 72L:0D, the peak of activity was only observed the first day; fish activity remained constant and at a low level for the next two days (Fig. 1). This indicates that the activity pattern of fish is not subject to any biological clock (Palmer 1976) but merely depends on the ambient light level, at least when no food is provided. As expected, fish proved to be much more active under 0L:72D, especially with respect to swimming. A significant decrease in activity gradually appeared between the first two days ( $t_{11} = 7.59$ ,  $p < 0.001$ ) and between the last two days ( $t_{11} = 4.65$ ,  $p < 0.001$ ), which was probably due to animal exhaustion.

### 3.2 Air-gaping and agonistic behaviours

Under 12L:12D, air-gaping behaviours were more frequent in all groups during intense activity periods ( $p < 0.001$ ), that is, during darkness (2:  $t_{11} = 6.77$ ; 5:  $t_{11} = 7.91$ ; 10:  $t_{11} = 11.52$ ; 15:  $t_{11} = 12.22$ ) (Fig. 2a). A significant group size effect ( $p < 0.001$ ) was observed between all groups (2&15:  $F(1, 44) = 23.87$ ; 5&10:  $F(1, 44) = 4.07$ ,  $p = 0.049$ ; 5&15:  $F(1, 44) = 31.05$ ; 10&15:  $F(1, 44) = 20.71$ ), except 2&5 and 2&10. No significant interaction was found between light/dark conditions and group size with respect to air-gaping. The patterns of air-gaping behaviour under 72L:0D and 0L:72D did not highlight any rhythm in the activity and did not differ significantly ( $t_{70} = 1.22$ ,  $p = 0.226$ ). The number of times air was breathed at the water surface was similar under 12 and 72 h darkness ( $11.1 \pm 2.9$  and  $8.0 \pm 4.1$  respectively) but was smaller under 12 than under 72 h illumination ( $3.3 \pm 2.9$  and  $7.1 \pm 1.6$  respectively). A reduction in this activity was observed under 0L:72D

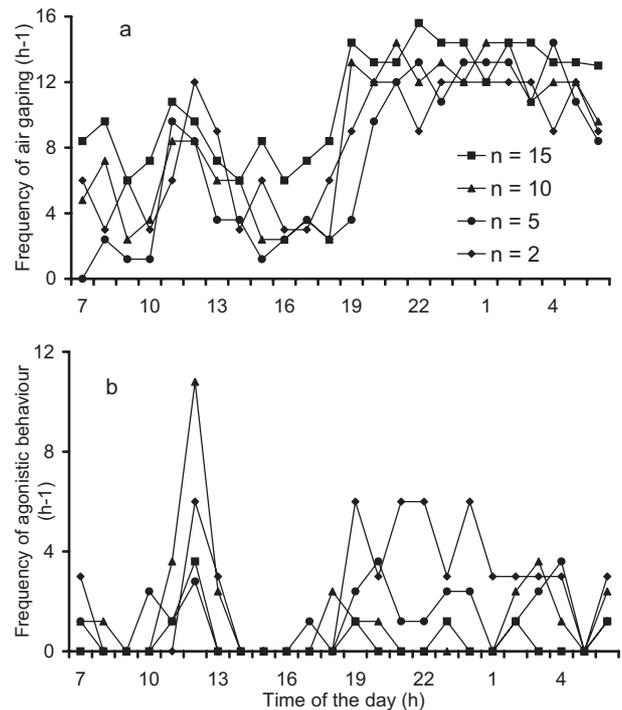
between the first and second days ( $t_{11} = 2.59$ ,  $p = 0.025$ ) and between the second and third days ( $t_{11} = 10.89$ ,  $p < 0.001$ ) and was probably a consequence of exhaustion in non-fed fish.

Agonistic behaviours were more frequent during darkness under 12L:12D (Fig. 2b), group sizes of 2 and 5 fish only (2:  $t_{11} = 4.75$ ,  $p < 0.001$ ; 5:  $t_{11} = 2.30$ ,  $p = 0.042$ ). Group size significantly affected agonistic behaviour between certain groups (2&5:  $F(1, 44) = 6.03$ ,  $p = 0.020$ ; 2&15:  $F(1, 44) = 21.33$ ,  $p < 0.001$ ; 5&15:  $F(1, 44) = 9.09$ ,  $p = 0.004$ ). An interaction between group size and light/dark was observed for the groups 2&10 ( $F(1, 44) = 8.23$ ,  $p = 0.006$ ) and 2&15 ( $F(1, 44) = 10.34$ ,  $p = 0.002$ ), suggesting that a difference in agonistic interactions is only observable when the smallest group is compared with the largest ones. As with air-gaping, an intense peak of activity was recorded about 11:00–12:00 am, the usual feeding time. No significant difference was observed in the number of attacks from one day to another under 72L:0D and 0L:72D, but there were significantly more attacks in the latter condition ( $t_{70} = 2.16$ ,  $p = 0.034$ ).

## 4 Discussion

In agreement with empirical findings in several catfish species (*Clarias gariepinus*, Bruton 1979; *Silurus glanis*, Anthouard et al. 1987; *Hoplosternum littorale*, Boujard et al. 1990) as well as with previous studies on *H. longifilis* (Avit and Luquet 1995; Baras et al. 1998), fish placed under 12L:12D were essentially nocturnal. The present study provides further evidence that such an activity pattern was trained by light/dark alternation independently of the duration of each phase, and that starved fish show no activity rhythm under permanent illumination or darkness. However, it appeared that the activity of *H. longifilis* was lowered to minimum values within hours in the former condition and within days in the latter. Under 72L:0D, fish indeed maintained a constant, low activity rate ( $<10\%h^{-1}$ ) because they are used to remaining almost inactive under illumination. In contrast, under 0L:72D, animals were more active at the beginning of the experiment. The observed decrease in activity from day to day was probably due to the prolonged absence of food, which has indeed been found to depress activity in several species independently of the light/dark cycle (Boujard and Leatherland 1992). Fish seemed exhausted after a 48-h activity period (although exhaustion was not measured physiologically), which might indicate a biological limit of activity in non-fed *H. longifilis*.

Starved catfish were found to exhibit diurnal activity at the time they were usually fed but that peak appeared only once, that is, the day following normal feeding days. This suggests that fish could adapt their activity rate to a condition experienced before and that such training became ineffective over longer periods of time without food. Similar observations have been made in other tropical fish species, such as the blue tilapia *Oreochromis aureus* (Baras et al. 1996). In short, the peak of activity probably did not correspond to any biological rhythm of animals but rather to a mere conditioning process. An increase in fish aggression occurred during the peak of activity. With respect to aquaculture, this study confirms the extreme plasticity of activity as well as capacities of adaptation of *H. longifilis* to various meal timings and photoperiod regimes,



**Fig. 2.** (a) Air-gaping behaviours (number of times per hour) over 24 hours with dark periods from 19:00 to 07:00 with a group size of 2 to 15 individuals. Air was breathed  $3.3 \pm 2.9$  times under illumination and  $11.1 \pm 2.9$  times under darkness. (b) Frequency of agonistic interactions over the same 24 hours as in Fig. 2a.

which both reinforce the suitability of this species for tropical and northern latitudes. It also supports the idea that rearing *H. longifilis* under permanent darkness may be profitable, assuming that food remains available over the entire 24-h cycle (Baras et al. 1998).

The growth of Clariid catfishes has been shown to be density-dependent (Hogendoorn 1981; Kerdchuen and Legendre 1992; Baras et al. 1998), essentially because a higher population density is deemed to have a calming effect on fish (Kaiser et al. 1995) and lowers the amount of energy invested in agonistic behaviour. Fish are thereby less aggressive when stocked at high density. The mean number of attacks with two fish (2.4) was significantly higher than with 15 fish (0.4). In addition, fish had a propensity to gather on each other when they were stressed by human handling and during prolonged resting periods. Such behaviour might indicate the role of social interactions in stress regulation and their effect on fish aquaculture should be studied.

Gathering behaviour may seem strange in fish because these animals usually tend to maintain some distance between individuals (Bégout Anras and Lagardère 2004). In fact, rearing conditions inhibit territorial behaviours, especially when population density is high. This has been shown in several Teleostei, such as *Oreochromis niloticus* (Mélard 1986), *Salvelinus alpinus* (Jorgensen et al. 1993), and *Clarias gariepinus* (Hecht and Uys 1997). In particular, sensory performances are reduced (Howell 1994) and decreased vigilance is observed due to the absence of predators (Bégout Anras and Lagardère 2004).

The method used in these experiments did not allow us to collect data such as the speed or duration of swimming behaviour and the time fish passed at a given place in the aquarium. New video-tracking systems can be used in this aim (Ylieff and Poncin 2003; Delcourt et al. 2006) and should thereby improve the subsequent study of biological rhythms in fish.

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