

Phase-shifting the light–dark cycle influences food-anticipatory activity in golden shiners

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Abstract

This study provides evidence that a circadian light-entrainable oscillator is at least partially involved in the timing of food-anticipatory activity (FAA) in a fish, the golden shiner, *Notemigonus crysoleucas*. Shoals of four golden shiners were fed for 11–20 days at a fixed daily time (either early night, midnight, late night, early day, midday, or late day). Most (78%) shoals developed peaks of FAA during that period of time. Food was then withheld for 7 days, and the light–dark (LD) cycle was either advanced or delayed by 6 h on the first of those days. The activity waveform of most (53–58%) shoals shifted along with the LD cycle, as indicated by significant correlation coefficients between pre- and postshift waveforms plotted relative to LD. Nonsignificant correlations were linked to low activity levels rather than to persistence of the activity peak at the old clock time. Activity shifts were gradual, taking 2–4 days, which indicates that the underlying mechanism is circadian rather than hourglass. © 2000 Elsevier Science Inc. All rights reserved.

Keywords: Food-anticipatory activity; Circadian; Phase-shift; Light-entrainable oscillator; Fish

1. Introduction

Food-anticipatory activity (FAA) is an increase in activity levels, preceding mealtime, that develops after the animal has experienced many days of food delivery at a fixed time. Various models exist to describe the internal mechanism that could lead to food anticipation [8]. Some of these models involve hourglass mechanisms: a periodic environmental factor, such as a meal or the onset of light, could set a timer in motion every day, and this hourglass timer would run a course that corresponds to the learned time span between the environmental factor and the next meal time. Activity levels would rise as the timer nears the end of its course. Other models resort to circadian mechanisms: for example, a circadian oscillator could be set in motion and entrained by the daily food arrival, and its phase would be determined by mealtime. Alternatively, a circadian oscillator could be entrained by light, and the animal could learn the phase of the oscillation at which food arrives, with activity levels increasing as this learned phase approaches.

Most of the studies that have sought empirical support for these various models have been conducted on mammals [8]. FAA has also been reported in fishes (mummichog, *Fundulus heteroclitus* [2]; bluegill sunfish, *Lepomis macro-*

chirus [2]; goldfish, *Carassius auratus* [4,17,19]; loach, *Misgurnus anguillicaudatus* [9]; mudskipper, *Periophthalmus cantonensis* [10]; sea bass, *Dicentrarchus labrax* [18]), but few studies have looked at fish with a view to distinguish between models of FAA timing [for exceptions, see [17,18]]. Here, we report on a study with a new species, the golden shiner *Notemigonus crysoleucas*, and a simple protocol, the shifting of the light–dark (LD) cycle, in an effort to distinguish between hourglass and circadian mechanisms and to show that light signals are important in the timing of FAA.

We let golden shiners learn the daily time of food delivery and develop FAA, but then we stopped giving them food while phase-shifting the LD cycle (advancing or delaying it by 6 h). On the premise that light signals may anchor FAA, we predicted that FAA would not remain at the old clock time, but would shift along with the new LD cycle. However, typical of circadian mechanisms, and contrary to hourglass mechanisms, the shift in FAA was predicted to be gradual rather than instantaneous. On the basis of a previous study with the same species where learned spatio-temporal patterns of activity shifted in 3 days after a 6-h shift in the LD cycle [13], we expected the gradual FAA shift to be of a similarly short duration.

2. Material and methods

Minnow traps were used to capture golden shiners in Folly Lake, 12 km south of Moncton, New Brunswick, Can-

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ada, between May and October. Only fish between 50 and 120 mm in total length were kept. These fish were placed in groups of 50–80 inside 180-l holding tanks, with water at a temperature of $16 \pm 2^\circ\text{C}$. The room was illuminated by windows, and the photoperiod was therefore natural. For a minimum of 2 weeks, the fish were fed Nutrafin food flakes at the surface, five times a day between dawn and dusk.

In nature, most golden shiner populations seem to be crepuscular [5,7,21], but others have been found to feed during both day and night [3]. We did not know the natural activity pattern of the population we used, but we knew from previous experience that their activity in the laboratory is correlated with food availability, irrespective of whether the food is given during the day or at night. From this prior experience we assumed that the five diurnal feeding times used in the holding tanks led to the fish being broadly diurnal at the beginning of the experiments.

Experiments took place throughout the year. Shoals of four fish were moved to another room which, as far as we can tell, was not exposed to daily factors (such as traffic noise, construction noise, regular entry of personnel) other than the photoperiod. The latter was light–dark 12–12 h, without twilight transitions between light and dark, and provided by incandescent lights with an intensity of 700–900 lux at aquarium level. Each shoal was placed in 37-l aquaria ($50 \times 25 \times 30$ cm). Water temperature was $18 \pm 2^\circ\text{C}$. Each aquarium was equipped with a half flower pot lying at the bottom to provide a refuge for the fish and with a small filter mounted at one end.

At the end of each aquaria, opposite the filter, food was delivered by an automatic feeder that dropped approximately 40 mg of Nutrafin food flakes at the surface. (From prior experience, we knew that 40 mg was a quantity that was always completely consumed within minutes by a shoal of four fish and that did not lead to notable loss of condition over the maximum 3-week period of our intended study. We also knew that these commercial flakes provide a balanced food source for shiners, ensuring long-term survival in captivity.) A plastic barrier prevented the floating flakes from spreading more than 20 cm away from the drop point. For 11–20 days (training phase), the fish were fed only once a day, at the same time every day. This time could be 1 h after lights-off (early night), 6 h after lights-off (midnight), 11 h after lights-off (late night), 1 h after lights-on (early day), 6 h after lights-on (mid-day), or 11 h after lights-on (late day). We chose these six times to see whether the response of FAA to shifted LD cycles could be generalized across many daily times of food delivery.

Twelve shoals were assigned to each of those six times, for a total of 72 shoals tested (288 different fish). Shoals were used instead of individuals because golden shiners, a very gregarious species, seldom display activity when kept single. In circadian studies on European catfish, *Silurus glanis*, and white sucker, *Catostomus commersoni*, shoals gave clearer and more robust activity patterns than single individuals [1,6]. In circadian studies on sea bass, no difference

was detected between the results of shoals and single individuals [16,18].

After the training phase, food was withheld for 7 days (healthy shiners can easily sustain such a level of food deprivation) and on the first of these days, the LD cycle was either advanced by 6 h (half the shoals, 6 for each daily time of food delivery) or delayed by 6 h (the remaining shoals). LD cycles were advanced by shortening the first dark period to 6 h instead of 12, and were delayed by lengthening the first dark period to 18 h instead of 12.

Throughout the experiment, activity was recorded with a modulated infrared beam that crossed the short length of the aquarium, directly below the feeder, approximately 4 cm away from the end and 8 cm below the surface. Beam interruptions caused by any of the four fish (or more than one fish simultaneously) moving in any direction were recorded by computer. The software (Dataquest III, Mini-Mitter Co., Sunriver, OR) tallied the number of interruptions for each aquarium in blocks of 6 min.

For each aquarium, and for each of the 6-min blocks of a 24-h period, the number of beam interruptions was averaged over the last 5 days of the training phase, to obtain daily activity waveforms. These waveforms were examined to determine whether FAA was present (FAA was defined as a gradual increase in activity levels above the daily average, culminating within 1 h of mealtime). The same was done for the last 5 days of the shift/no-food period (the first 2 days were omitted to allow for gradual phase-shifting of FAA). To see if the shift/no-food waveforms, graphed relative to the new LD cycle, were similar to the previous training stage waveforms (in other words, to see if FAA had followed the LD shift), we used a correlational approach. For this, each 24-h waveform was reduced to 48 half-hour tallies of beam interruptions. A Pearson correlation coefficient was then calculated between the training and shift/no-food waveforms for each shoal. Significant coefficients were considered indicative that the activity waveform had followed the shifted LD cycle. Within each experimental group of 6 shoals, level of significance started at $0.05/6 = 0.008$ but then gradually increased to a maximum of 0.05 according to a sequential Bonferroni correction [14]. A coefficient was also calculated for both waveforms plotted relative to the same clock time, rather than LD time. Significant correlations in this case would indicate that FAA did not shift along with the LD cycle and remained at the same old clock time.

Actograms were also obtained for each shoal and examined to be subjectively classified into one of the four following categories describing the behavior of activity peaks following the LD shift: (a) indeterminate: impossible to tell whether activity peaks, if present at all, shifted or not; (b) no shift: activity peaks remained at the same clock time for both the training and shift/no-food phases; (c) immediate shift: the activity peak shifted completely after the first or second new light-to-dark or dark-to-light transitions that preceded the old clock time of food delivery; and (d) grad-

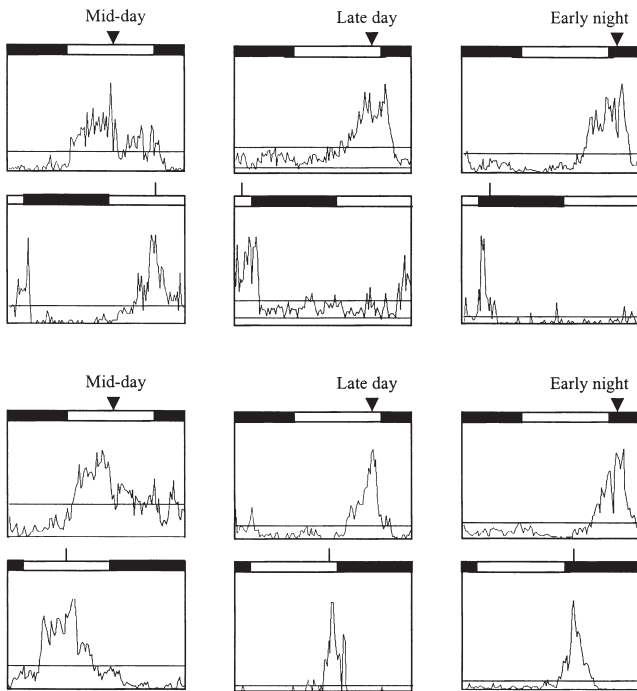


Fig. 1. Pairs of waveforms depicting the daily activity of golden shiner shoals fed at a fixed time every day (top panel of each pair). The top pairs show a 6-h light–dark delay, whereas the bottom pairs show a 6-h advance. Open and filled bars at the top of the panels represent light and dark times, respectively. Triangles above panels indicate the feeding time, whereas small vertical lines below panels show what would have been the new feeding time had food delivery been maintained and shifted along with the light–dark cycle. Horizontal lines across the waveforms represent the average daily level of activity. All waveforms are based on the average of 5 days.

ual shift: during at least 2 days, an activity peak started around the old clock time but eventually slid across to a new clock time that corresponded to the 6-h delay or advance of the LD cycle.

3. Results

Of the 72 shoals tested, 56 (78%) displayed FAA during the training phase (Fig. 1, top panels of each pair). Of the remaining 16 shoals, 5 showed consistently low activity levels at all times, and 11 had rising activity levels at an inappropriate time (of those 11, 8 were fed at midnight but had activity peaks during the day or at crepuscular times). A certain proportion (17%) of the shoals fed near “dawn” or “dusk” were bimodal, displaying peaks of activity not only before the normal time of food delivery but also before the other crepuscular time, even though no food was given at that second time.

Most of the shoals shifted their activity peaks along with the new LD cycle, be it for advances or for delays: 53–58% of the shoals had significant correlation coefficients between their training and shift/no-food waveforms (relative to LD time, Table 1, Fig. 1). Lack of significant correlation was not necessarily caused by persistence of activity peaks at the old clock time: only four shoals had significant coefficients when the shift/no-food waveforms were plotted relative to clock time (Table 1). Rather, on the basis of a visual examination of the waveforms, lack of significant correlation was caused mostly by very low activity levels after the LD shift.

On the basis of visual examination of actograms, more shoals were deemed to have shifted their activity patterns than revealed by the correlation analysis. Nearly two thirds of the shoals appeared to have shifted their activity peaks gradually (Table 2, Fig. 2). These gradual shifts took 2–4 days (Fig. 2). Less than one-sixth of the shoals had immediate shifts. Many of these shifts were not truly immediate, as a peak of activity often persisted for one day at the old feeding time (by the second day however, the peak had completely shifted). Most of the remaining shoals were classified as indeterminate. Only two shoals were deemed to have

Table 1

Average (\pm SD, $n = 6$, except overall where $n = 36$) Pearson correlation coefficients between the activity waveforms of golden shiner shoals before and after a 6-h shift in the light–dark (LD) cycle. The proportion of significant positive coefficients is also shown. The shoals were fed at various daily times before the shift, but not after.

Feeding time	6-h LD advance		6-h LD delay	
	Both waveforms relative to LD	Both waveforms relative to clock time	Both waveforms relative to LD	Both waveforms relative to clock time
Early night	0.61 \pm 0.37 5/6	−0.21 \pm 0.15 0/6	0.35 \pm 0.20 3/6	−0.14 \pm 0.30 1/6
Midnight	0.37 \pm 0.31 3/6	−0.10 \pm 0.22 0/6	0.17 \pm 0.26 3/6	0.12 \pm 0.27 2/6
Late night	0.52 \pm 0.15 6/6	−0.07 \pm 0.12 0/6	0.10 \pm 0.22 1/6	0.05 \pm 0.13 0/6
Early day	0.09 \pm 0.29 1/6	−0.06 \pm 0.12 0/6	0.46 \pm 0.40 4/6	−0.23 \pm 0.11 0/6
Midday	0.19 \pm 0.48 2/6	−0.09 \pm 0.22 0/6	0.29 \pm 0.35 3/6	0.03 \pm 0.32 1/6
Late day	0.33 \pm 0.41 4/6	−0.08 \pm 0.19 0/6	0.53 \pm 0.34 5/6	−0.25 \pm 0.10 0/6
Overall	0.35 \pm 0.37 21/36 (58%)	−0.11 \pm 0.17 0/36 (0%)	0.34 \pm 0.32 19/36 (53%)	−0.08 \pm 0.25 4/36 (11%)

Table 2

Number of golden shiner shoals classified into various categories based on the behavior of their daily activity peaks after a shift in the light–dark (LD) cycle

	Shift in daily activity peak			
	Gradual	Immediate	None	Indeterminate
6-h LD advance	22	6	0	8
6-h LD delay	24	3	2	7

not shifted at all, at least by this subjective evaluation (the correlation analysis on waveforms plotted relative to clock time in Table 1 indicated four no-shift shoals, two of which were classified as indeterminate by the visual method because they involved broad peaks of activity; the correlation coefficients of these latter two were significant irrespective of whether the waveforms were considered relative to LD or to clock time, but they were stronger in the case of LD).

4. Discussion

The results show that golden shiners can reliably develop FAA irrespective of the fixed daily time at which they were fed, with the exception of fish fed at midnight. The fact that

the fish were probably diurnal entering the study may account for their difficulty in developing nocturnal FAA, though it must be said that in other studies in this laboratory diurnal shiners did succeed in developing nocturnal FAA around midnight. We have no good explanation why most fish failed to show FAA at midnight in this study while they did in previous ones.

Our results also show that the timing of FAA can be anchored by the LD cycle in golden shiners, as a shift in LD resulted in a corresponding shift in FAA, at least in those shoals that maintained high activity levels. If the timing of FAA was solely dependent on food arrival, then after the LD shift and the removal of food FAA should have remained at the old clock time, or free-ran, or disappeared. In any of these cases we would not expect it to reappear at a new time 6-h distant. Yet this is what it did in most instances. In contrast, a maximum of only 4 shoals out of 72 yielded results that were consistent with FAA remaining at the old clock time.

Our results provide a falsification of hourglass models. Such models assume that the time of food delivery is measured by the running of a hourglass mechanism, which is triggered by an external signal whose phase is fixed relative to mealtime (the trigger could be the previous meal, or the time of lights-on or lights-off). Hourglass models are not compatible with the gradual shifts that were observed in the FAA of most shoals in this study. If the trigger was mealtime, FAA should have disappeared because food was not given after the LD shift. If the trigger was a light signal, the shift in FAA should have been immediate, a result that was observed but at a low frequency only. Those few immediate FAA shifts can be explained in other ways: they may be the result of a particularly labile circadian oscillator (that FAA was often maintained at the old clock time for 1 day before a complete shift took place on the second day is consistent with this idea) or they may reflect the influence of masking by light or darkness.

Gradual phase-shifts after LD changes are a characteristic of circadian clocks, so our results support the notion of the light-entrained oscillator being circadian. A previous study [13] had reached the same conclusion after advancing the LD cycle by 6 h and observing gradual 3-day shifts in the spatio-temporal activity patterns of golden shiners trained to a time-place learning task. This study confirms those previous results and add to them by showing that gradual shifts can occur in response to LD delays in addition to advances, and by testing a greater sample size distributed over a variety of feeding times.

Other studies have investigated the effect of LD shifts on fishes' general daily activity rather than on FAA. Both the catfish *Silurus asotus* [20] and the sea bass *Dicentrarchus labrax* [16] displayed immediate activity shifts in response to 6–12 h LD shifts. Published actograms strongly suggest an influence of masking by light in these studies. On the other hand, the hagfish *Eptatretus burgeri* took 7–13 days to resynchronize to a reverse LD cycle, displaying very clear

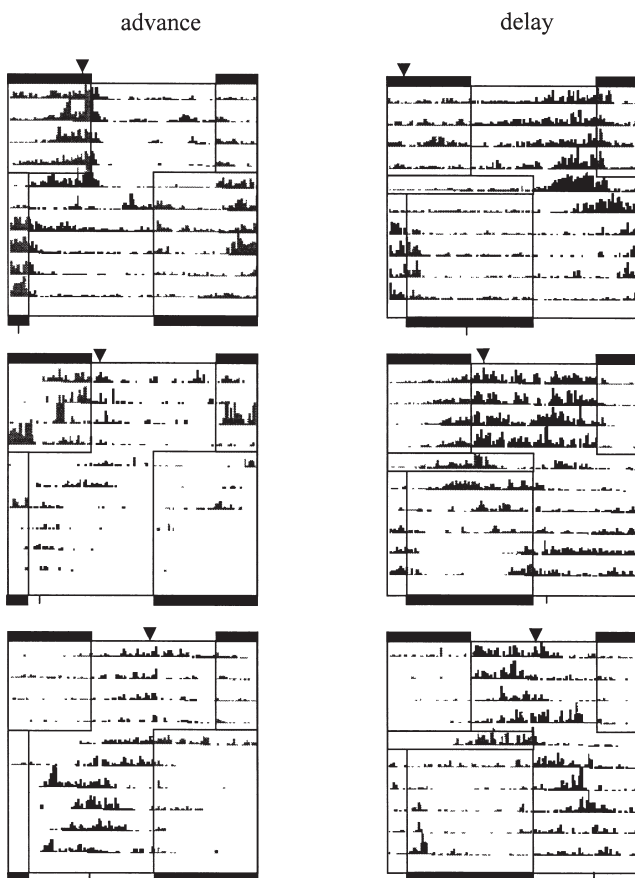


Fig. 2. Actograms from golden shiner shoals fed at a fixed daily time (triangles) and then deprived of food and exposed to a 6-h advance (left panels) or delay (right panels) in the 12:12-h light–dark cycle. Filled bars at top and bottom of panels represent dark time.

gradual shifts in activity [11]. Notwithstanding this latter and lone example, it is tempting to suggest that, in some fish species, FAA may be less susceptible to masking by light than general non-food related activity, and that it may represent a better marker of fishes' circadian clocks for experimental studies.

In conclusion, together with one previous study [13], the present work on golden shiners suggests the presence within these fish of a circadian light-entrained oscillator that can be used to anchor the anticipatory activity of daily food-related events. Our study, however, has nothing of substance to say about the possible involvement of food entrainment. It is possible that the circadian oscillator could also be entrained by food in addition to light. It is also possible that a second circadian oscillator, entrainable by food only, could be linked more or less strongly to the light-entrainable oscillator. Most reported studies on mammals, including two that used LD shifts [12,15], seem to favor models with food-entrainable circadian oscillators [8], though some implicitly recognize that a single light-entrainable oscillator could also explain their results [15]. Some studies in fishes are also consistent with the idea of food-entrainable oscillators, though they too cannot eliminate the possibility of a single light-entrainable oscillator [17,18]. Our results do not exclude the possibility of food entrainment. They only show that a circadian light-entrainable oscillator is at least partially involved in the timing of FAA in golden shiners.

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