

# Daily food-anticipatory activity in golden shiners: A test of endogenous timing mechanisms

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## Abstract

Shoals of four golden shiners, *Notemigonus crysoleucas*, were fed daily at a fixed time, which could be the beginning, middle, or end of a 12-h artificial day, or beginning, middle, or end of a 12-h artificial night, depending on the shoal. Almost all shoals showed food-anticipatory activity (FAA), that is, a gradual increase in locomotor activity near the feeder, starting on average 4.5 h before mealtime. FAA started before the time of lights-on or lights-off even in shoals that were fed 1 h after these times. Increases in activity near the feeder generally did not appear at times unrelated to feeding, except for some shoals that were fed around the time of either lights-on or lights-off and that showed high activity at both of those times simultaneously. When food was withheld but the light–dark cycle was preserved, FAA persisted at the old clock time in 78% of the shoals. When the light–dark cycle was eliminated (fish placed in constant darkness), with or without a concurrent removal of the scheduled feeding, FAA persisted at the old clock time in only 22% of the shoals. These results can be explained by a model whereby the animals possess a circadian oscillator that is entrained by the light–dark cycle, with only weak potential for self-sustainability, and where a representation of the normal phase of feeding can be stored in memory. The possibility that FAA was generated by a food-entrainable oscillator can only be reconciled with the data by postulating that this oscillator is normally linked to a light-entrainable oscillator, and that the damping out of the light-entrained oscillator disrupts the action of the food-entrainable one. © 2000 Elsevier Science Inc. All rights reserved.

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## 1. Introduction

Food-anticipatory activity (FAA) is an increase in activity levels that occurs daily, several hours before meal time, when meals are delivered at the same time every day. This phenomenon is commonly studied with captive animals that receive only one meal a day while exposed to a light–dark cycle. FAA has been studied most extensively in rats, but it is also expressed in mice, hamsters, rabbits, mammalian carnivores, primates, birds, fishes, and bees [10].

Because the increase in activity precedes the actual delivery of food even in strictly constant laboratory conditions, we know that causal mechanisms are mostly internal. Several models have been put forward to account for the internal mechanisms that could underlie FAA [10]. These models make different predictions with regards to the maintenance of FAA after removal of the light–dark cycle, removal of daily food delivery, or removal of both of these factors simultaneously.

One model, which could be called “energetic hourglass,” is based on the notion that activity levels could rise with hunger, and that the animal could manage its energy reserves so that it would only start to become hungry 20–24 h after the last meal. FAA would, therefore, be caused by a long-term interval timer reset to zero every day by the scheduled meal. This model predicts that the daily peak of activity which corresponds to FAA should be maintained even in constant darkness (DD) as long as food is still delivered daily at the same clock time. However, if daily food delivery was permanently discontinued, irrespective of what happened to the light–dark cycle, then FAA should disappear because the animal would always be hungry and its activity levels would remain constantly high.

A second model could be called “photoperiodic hourglass.” This model also resorts to a long-term interval timer, but here the timer would be reset by a photoperiodic signal such as the time of lights-on or lights-off. The animal could learn the duration of the time period that separates the photoperiodic signal from the daily meal time, and activity would rise as this time span neared the end of its course every day. This model predicts the maintenance of a FAA peak even af-

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ter removal of the daily food delivery (provided that memory of the time span can be maintained without reinforcement for a few days), but that FAA should disappear immediately after the animal is put in DD. The model also predicts that the duration of FAA should not exceed the temporal distance between mealtime and the previous light signal. We might, therefore, expect different FAA durations if meals are scheduled 1, 6, or 11 h after the time of lights-on or lights-off.

The third and fourth models assume the existence of an endogenous circadian mechanism. The third model could be called “single LEO” (for single Light-Entrainable Oscillator). In this model, the animal would possess an endogenous circadian clock entrainable only by photoperiodic signals (most animals are in fact known to have such a clock). After a few days necessary for learning, the animal could store in memory a representation of the circadian phase at which meals are delivered, and could subsequently compare this marked phase with the current phase, with activity rising as the marked phase approaches [3,10]. The single LEO model predicts that FAA would respond to a change in conditions in the same way that a light-entrained oscillator would. If food delivery was removed, FAA would persist because food memory is already acquired and the oscillator on which the memorized phase is marked can still be entrained by the remaining light–dark cycle. If, on the other hand, the light–dark cycle was removed and DD was imposed, the persistence of FAA would depend on the characteristics of the oscillator. If the clock was undamped, the endogenous cycle would persist on its own for at least a few days and FAA would be expressed for that period of time. If the clock was damped, FAA could disappear in a day or 2.

The fourth model could be called “LEO-FEO” (for double, Light-Entrainable and Food- Entrainable Oscillators). This model assumes the existence of two separate circadian oscillators, one that is synchronized by the light–dark cycle and the other by daily food delivery, both being linked to one another so that the phase relationship of the two oscillators can remain fixed even when one of the two oscillators happens to be missing its synchronizer. FAA would be triggered by the FEO specifically, but a free-running FEO could be kept in phase by its link to the LEO. This model seems well supported by experimental tests with rodents [10,11] and to some extent [7] with birds [14,15]. Experiments with fishes have also been consistent with the LEO-FEO model, although not to the point of distinguishing it from the single-LEO model [21,22]. According to this LEO-FEO model, removing either the food cue or the photoperiodic cue should not obliterate FAA. If only food was removed, the LEO would still be sustained by the light–dark cycle and could keep the FEO running and generating FAA. If only light was removed, the FEO would still be sustained by the scheduled food delivery and could still generate FAA. However, if both light and food were removed, FAA might persist only more or less, depending on how damped or undamped the two free-running oscillators were.

We tested these various predictions in a fish, the golden shiner, *Notemigonus crysoleucas*. This is a freshwater cyprinid from Eastern North America. Time-place learning (learning to feed in one place at one time of day, and in another place at another time) has already been reported in golden shiners [16], suggesting an influence of food availability on daily activity patterns in this species. There is some indirect evidence for FAA in golden shiners [17], but FAA still has not been directly investigated in this species. Indeed, few fishes have been studied in that regard. To our knowledge, FAA has been convincingly reported in mummichogs, *Fundulus heteroclitus*, and bluegill sunfish, *Lepomis macrochirus* [2], goldfish, *Carassius auratus* [5,21,23], loach, *Misgurnus anguillicaudatus* [13], and sea bass, *Dicentrarchus labrax* [22].

Our goal was first to document FAA in golden shiners. For this we exposed shiners to scheduled food delivery at three different hours of the day and three different hours of the night (an improvement on previous studies, where usually only one or two daily times were tested). We established the daily activity patterns of the fish near the feeder, noted the timing of their activity peaks, and measured the duration of activity rises prior to food delivery. Then, to test the four models, we either removed the photoperiodic cues (we placed the animals in DD), or the food cue (we stopped feeding the animals for seven days, something these ectotherms can easily endure), or both. We knew from preliminary observations in this laboratory that endogenous oscillators underlying activity are mostly damped in golden shiners, with only few individual exceptions. We, therefore, predicted that removal of key time cues would result in the loss of daily activity peaks near the time of feeding, except in a few individuals that would happen to have less damped oscillators. Table 1 recapitulates our predictions for the four models we considered.

## 2. Materials and methods

Golden shiners were captured in Folly Lake, 12 km south of Moncton, New Brunswick, between May and October. All were between 50 and 120 mm in total length. Upon arrival in the laboratory, they were placed in groups of 50–80 inside 180-L holding tanks, and allowed at least 2 weeks of habituation before any experimentation began. Water temperature within the tanks was  $16 \pm 2^\circ\text{C}$ . Lighting came from windows and thus corresponded to the natural photoperiod. Fish were fed commercial food flakes at the surface, five times a day between dawn and dusk.

### 2.1. Documenting FAA

Shoals of four fish were placed in 37-L aquaria ( $50 \times 25 \times 30$  cm). Water temperature was  $18 \pm 2^\circ\text{C}$ . Photoperiod was light–dark 12–12 h, with abrupt transitions between light and dark. Light was provided by incandescent lights with an intensity of 700–900 lx at aquarium level. Gravel

Table 1

Predicted fate of food-anticipatory activity (FAA) after experimental removal of environmental time cues, according to four different models of the internal mechanism underlying FAA, in a fish species where endogenous oscillators are damped for most individuals

Model	Predicted fate of FAA after removal of		
	Scheduled food	Light–dark	Light–dark + food
Energetic hourglass	Disappears in all	Persists	Disappears in all
Photoperiodic hourglass	Persists	Disappears in all	Disappears in all
Light-entrained oscillator	Persists	Disappears in most	Disappears in most
Light-entrained + food-entrained oscillators	Persists	Persists	Disappears in most

The two-oscillator model assumes that both oscillators are equally entrainable by their respective zeitgeber.

covered the bottom of each aquarium, and a half flower pot provided refuge there. A small filter was mounted outside each aquarium at one end. At the other end, food was delivered by an automatic feeder that dropped Nutrafin® food flakes at the surface (about 40 mg, or approximately 0.1% of the combined weight of the four fish in each shoal; these commercial flakes are a balanced and complete food source for captive fish). A barrier prevented the floating flakes from spreading more than 20 cm away from that end.

For 12 days 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 (midday), or 11 h after lights-on (late day). Eighteen shoals were assigned to each of those six times, for a total of 108 shoals tested. (Shoals were used instead of individuals because golden shiners are a strongly shoaling species, and because we wanted to maximize the likelihood of detecting activity. In circadian studies on sea bass, shoals and single individuals yielded similar results [19,22]. In studies on European catfish, *Silurus glanis*, and white sucker, *Catostomus commersoni*, shoals yielded clearer and more robust activity cycles than single individuals [1,8]).

Activity was recorded with an 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. The beam was modulated to make it insensitive to radiation from other sources such as room lights. Beam interruptions caused by moving fish 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 12-day training stage, to obtain daily activity waveforms. These waveforms were graphed (with TAU software, obtained from Mini-Mitter Co.) and examined to classify the shoals into one of the following categories: diurnal (above-average activity during the day only), nocturnal (above-average activity during the night only), crepuscular (above-average activity only within 3 h of either lights-on, lights-off, or both), indeterminate (no obvious peak of activity, or multiple peaks that did not cor-

respond to a crepuscular pattern). The waveforms were also examined for evidence of FAA (rising activity levels up to the time of food delivery). When such evidence was found, the duration of the rise was measured directly on the graphed waveform by measuring the distance from the time of initial activity increase to the time of food delivery, with the time of initial increase determined visually as a marked upward change in the slope of the waveform. Duration of FAA was compared between the six different times of food delivery with a one-way ANOVA (Systat 5).

## 2.2. Testing models

Of the 108 shoals above, 36 (six for each of the six different times of food delivery) stopped receiving food at the end of their training stage but were still exposed to the light–dark cycle (this was the still-light + no-food group), 36 were placed in DD but still received daily food (the still-food + no-light group), and 36 were placed in DD and stopped receiving food (the no-light + no-food group). Average daily activity waveforms were established over the first 5 days. To see if these “experimental stage” waveforms were similar to the previous “training stage” waveforms, the two types of waveforms were subjected to correlation analysis. For this, each 24-h waveform was reduced to 48 half-hour tallies of beam interruptions. A Pearson’s correlation coefficient was then calculated between the training and experimental waveforms for each shoal. Within each combination of daily feeding time and experimental group, significance level for the coefficients was originally set at  $p = 0.05/6 = 0.008$ , but it gradually increased to 0.05 according to a sequential Bonferroni correction [18].

We interpreted a significant correlation as a demonstration that FAA had been maintained, and maintained near the old clock time, indicating continued entrainment of the underlying oscillator or its free-run at a period close to 24 h. We interpreted a lack of significant correlation as the disappearance of FAA (implying the damping out of the underlying oscillator) or its free-run at a period very different than 24 h (a phenomenon which, over 5 days, could change the 24-h waveform sufficiently to prevent correlation).

The 108 coefficients were compared with a two-way ANOVA (Systat 5), with three experimental groups and six times of food delivery. The proportions of significant posi-

tive coefficients within each experimental group were also compared with a  $\chi^2$  test.

We worked with a battery of 24 aquaria. Each experimental group was made up of 24 shoals tested at one time of the year and 12 tested at another time. The times of the year at which we tested the groups were: September and December for the still-light + no-food group, February and May for the still-food + no-light group, and May and September for the no-food + no-light group.

### 3. Results

#### 3.1. FAA in golden shiners

Most (94 of 108) daily activity waveforms for the training phase showed peaks of activity that corresponded to the time of food delivery, with activity levels gradually rising

before meal time, and decreasing either precipitously or slowly after meal time (most top panels on Figs. 1, 2, and 3). Figure 4 shows that a great majority of the shoals that were fed in the middle of the night had nocturnal peaks of activity, while almost all of those fed in the middle of the day had diurnal peaks. Most shoals fed at the beginning or end of the night were nocturnal or crepuscular, while most shoals fed at the beginning or end of the day were diurnal or crepuscular. Interestingly, of the 29 shoals that were classified as crepuscular, at least 10 developed two peaks of activity—one near lights-on and one near lights-off—even though they were fed at only one of those times (e.g., see top panel for early day on the left side of Fig. 1).

On average, FAA duration was 4.5 h ( $\pm 1.5$  SD,  $n = 94$ ). There was no difference between feeding times in FAA duration ( $F(5, 88) = 1.81, p = 0.12$ , Fig. 5).

The 14 activity waveforms that did not show FAA were

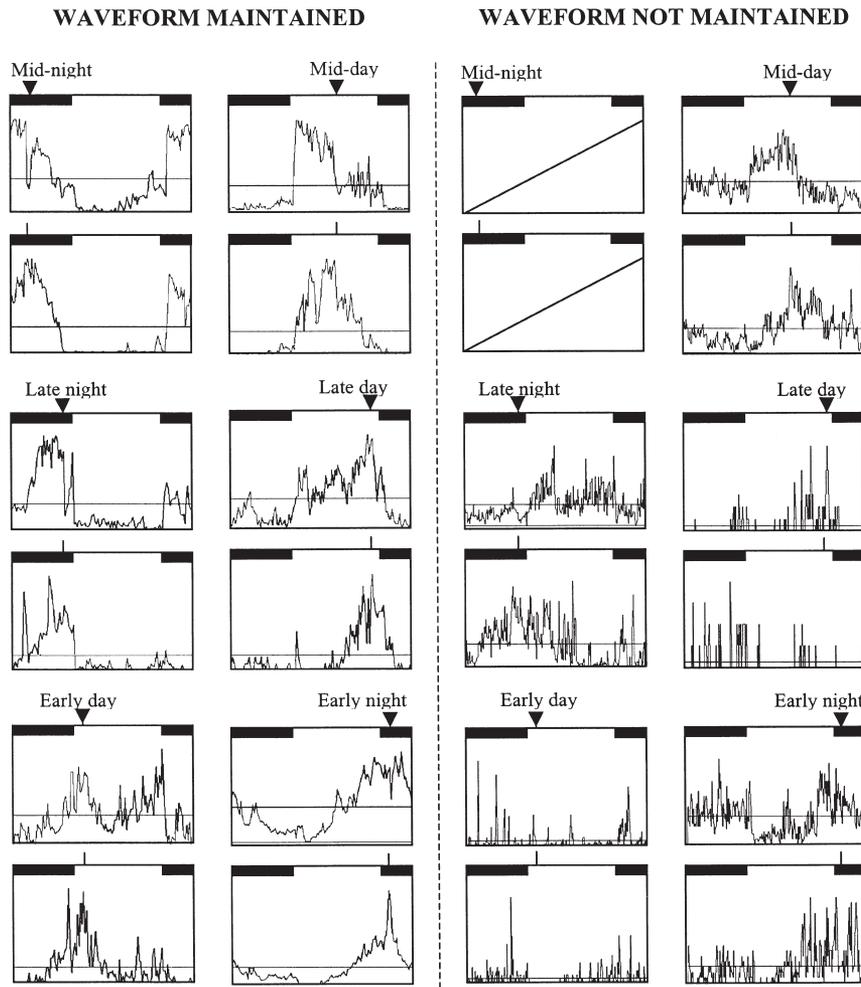


Fig. 1. Average ( $n = 5$  days) daily waveforms of activity in representative shoals of four golden shiners fed at different times (triangles above top panels) and then not fed (still-light + no-food, vertical bars above the bottom panels show the old feeding time). Dark horizontal boxes represent nighttime. On the left side, the members of each pair of waveforms were significantly correlated; on the right side, they were not. Diagonals across a panel indicate that no shoal fulfilled that condition.

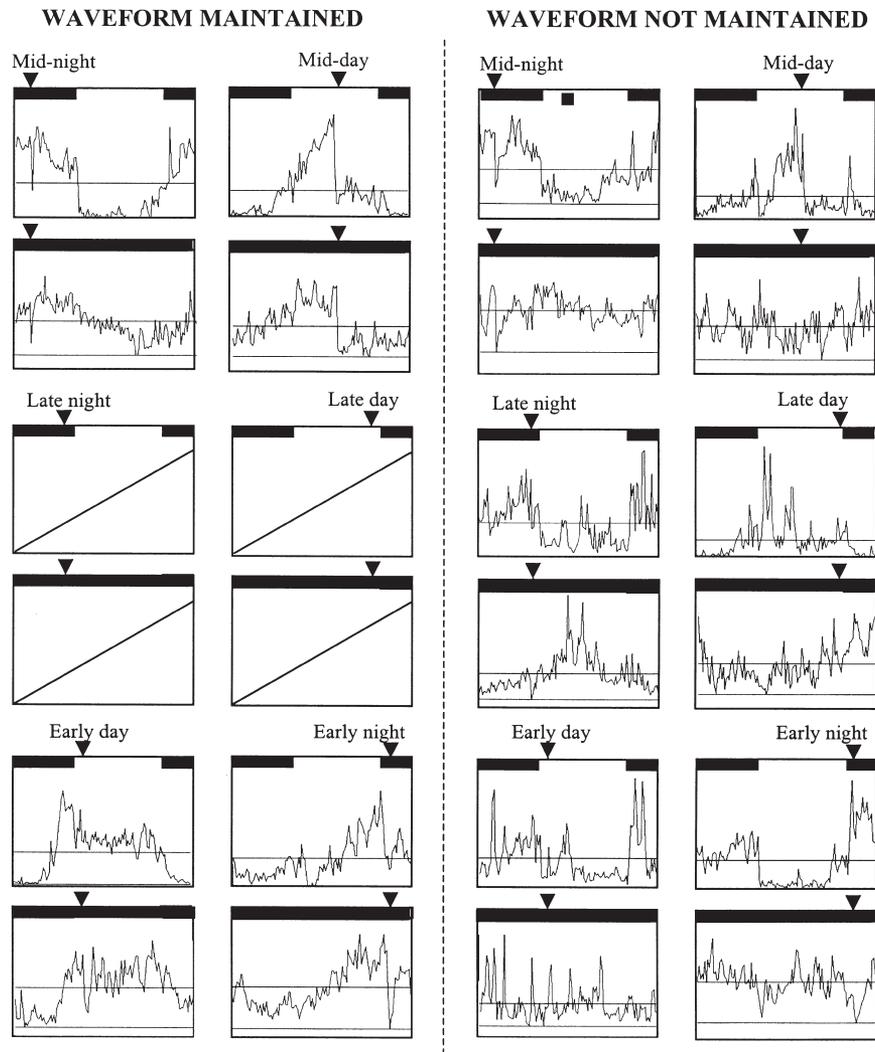


Fig. 2. Average ( $n = 5$  days) daily waveforms of activity in representative shoals of four golden shiners fed at different times (triangles above panels) and then still fed at the same clock time but placed in constant darkness (still-food + no-light group). Dark horizontal boxes represent nighttime. On the left side, the members of each pair of waveforms were significantly correlated; on the right side, they were not. Diagonals across a panel indicate that no shoal fulfilled that condition.

characterized either by uniformly low activity levels (e.g., top panel for early day on the right side of Fig. 1) or by activity peaks that did not start to rise before the time of food delivery (e.g., top panel for late night on right side of Fig. 1, top panel for midnight on the right side of Fig. 3).

### 3.2. Testing models

We could not identify major ways in which the three groups of 36 shoals differed before being submitted to the experimental removal of key time cues. For the still-light + no-food, still-food + no-light, and no-food + no-light groups, respectively, the number of indeterminate patterns of activity was 2, 3, and 3; the number of shoals for which no FAA was expressed was 4, 5 and 5; and the average length of FAA was  $4.5 \pm 1.3$ ,  $4.3 \pm 1.3$ , and  $4.9 \pm 1.6$  h. It

was important that the number of indeterminate and no-FAA cases be similar for all three groups, because these cases consistently led to nonsignificant correlations.

When the photoperiod remained but food was withheld (still-light + no-food group), 28 out of 36 shoals (78%) appeared unaffected. Their training and experimental daily waveforms were significantly and positively correlated (left side of Fig. 1, Table 2). The few shoals that did not yield significant correlations either had low levels of activity throughout, or had shifted peaks (bottom panels on right side of Fig. 1).

When the feeding schedule remained but DD was imposed (still-food + no-light group), only 8 of the 36 shoals (22%) had significantly correlated training and experimental waveforms (left side of Fig. 2, Table 2). Most of the other shoals did not show consistent peaks of activity during

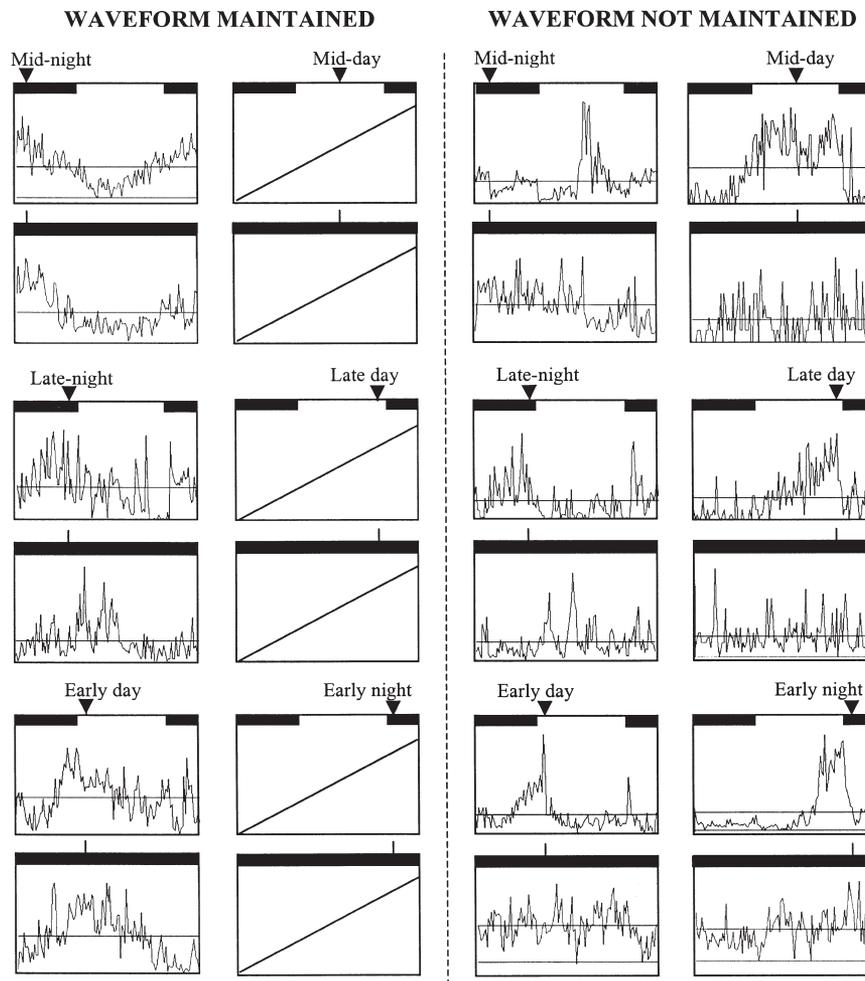


Fig. 3. Average ( $n = 5$  days) daily waveforms of activity in representative shoals of four golden shiners fed at different times (triangles above top panels) and then not fed (vertical bars above the bottom panels show the old feeding time) as well as placed in constant darkness (no-light + no-food group). Dark horizontal boxes represent nighttime. On the left side, the members of each pair of waveforms were significantly correlated; on the right side, they were not. Diagonals across a panel indicate that no shoal fulfilled that condition.

the experimental stage. Even though food was still delivered, and could thus affect activity patterns if only through a masking effect, peaks of activity ceased to appear around feeding times (bottom panels on right side of Fig. 2). There were a few cases of peaks still present (e.g., bottom panel for late-night on right side of Fig. 2), but they started to rise after mealtime, and, therefore, could not be considered FAA. Their position was so different compared to the training stage that the correlations turned out to be nonsignificant.

Results were similar when both food and photoperiod were removed (no-food + no-light group). Only 8 of the 36 shoals (22%) had significantly correlated training and experimental waveforms (left side of Fig. 3, Table 2). The other shoals did not show clear peaks of activity during the removal phase (bottom panels on right side of Fig. 3).

There was a significant effect of experimental groups on the value of correlation coefficients,  $F(2, 90) = 26.10$ ,  $p <$

0.001, but no effect of feeding times,  $F(5, 90) = 1.28$ ,  $p = 0.28$ , and no interaction between these factors,  $F(10, 90) = 0.82$ ,  $p = 0.61$ . The average coefficient was higher for the still-light + no-food group (Table 2). The proportion of significant coefficients varied significantly between the experimental groups ( $\chi^2 = 30.7$ ,  $p < 0.001$ ), being higher for the still-light + no-food group (Table 2).

## 4. Discussion

### 4.1. FAA in golden shiners

Golden shiners seem to represent a good subject species for the study of FAA. In a great majority of shoals, there was a clear rise in activity levels before meal time. Almost always, this rise was gradual and peaked at meal time, irrespective of when meal time was. The duration of this rise generally was 3–6 h, comparable to what has been observed

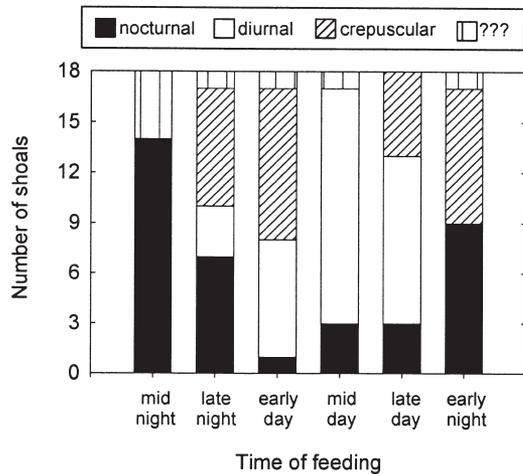


Fig. 4. Number of golden shiner shoals, fed at different daily times, whose food-anticipatory activity patterns were nocturnal, diurnal, crepuscular, or indeterminate (question marks).

in mummichogs [2], goldfish [2,21,23] and sea bass [22]. Rises in activity levels usually did not occur at other daily times beside meal time, except most notably for crepuscular individuals (to be discussed below).

Because our infrared beams were only located close to the feeders, the activity waveforms we obtained reflected FAA but not necessarily general activity. It is possible for a fish to show FAA peaks at a particular combination of time and place, and to show additional peaks of locomotor activity at other times and places that are not related to the search for food. In goldfish, FAA can be expressed near the surface before meal time, but other peaks of activity may also appear after a meal, this time near the bottom [21]. Some goldfish may also increase activity levels before a meal scheduled in the middle of the day while showing even higher levels throughout the night [21], a case of nocturnal individuals anticipating diurnal food, something our shiners never showed in our experimental setup. The point here is that the combination of golden shiners as a study species and the use of only one infrared beam near the feeder is a good choice for the study of FAA specifically, but no conclusion can be drawn about other types of activity, such as general locomotion, social interactions, or reproductive activities (see [24], for an example that, in medaka, *Oryzias latipes*, aggressive interactions, probably related to food site defense, can anticipate food arrival at different times of day while reproductive activities are only phased by the light–dark cycle; in our study, golden shiners were not aggressive, did not defend food patches, and did not exhibit reproductive activities).

Golden shiners expressed FAA irrespective of the time of day or night at which food was delivered. Another study where multiple daily feeding times were tested in fishes is that of Spieler and Noeske [23] on goldfish. The daily times tested in that study were lights-on, midday, lights-off, and midnight. The goldfish showed FAA at all times. Mummichogs

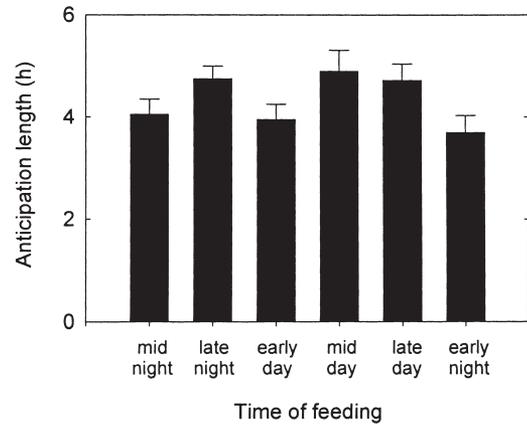


Fig. 5. Average ( $\pm$ SE,  $n = 14$ –17) duration of food-anticipatory activity in shoals of four golden shiners fed at different daily times.

also anticipate food arrival at lights-on and mid-day [2]. These studies and ours indicate that the timing mechanism that underlies FAA is flexible with regards to daily feeding time. This notion of flexibility in feeding patterns is further supported by studies of demand-feeding in sea bass [19] and goldfish [20]: even though all fish in those studies were kept in the same conditions and given the same access to a demand feeder, some individuals fed only at night while others from the same species fed only during the day. The reason for such within-species variability is unknown. As to golden shiners, although most populations in the wild are found to feed mainly at dawn and dusk [6,9,25], some are found to feed mostly during the day, or during both day and night [4]. This difference between populations may be linked to food availability, but it remains unproven.

Table 2

Average ( $\pm$ SD,  $n = 6$ , except overall where  $n = 36$ ) Pearson's correlation coefficients between the daily activity waveforms of the training stage and the experimental stage in shoals of golden shiners, for different feeding times during the training stage and for different experimental groups

Feeding time	Experimental group		
	Still light + No food	Still food + No light	No light + no food
Early night	0.53 $\pm$ 0.34 4/6	0.21 $\pm$ 0.33 2/6	0.04 $\pm$ 0.26 0/6
Midnight	0.71 $\pm$ 0.18 6/6	0.16 $\pm$ 0.35 2/6	0.28 $\pm$ 0.45 4/6
Late night	0.62 $\pm$ 0.35 5/6	−0.02 $\pm$ 0.23 0/6	0.03 $\pm$ 0.25 1/6
Early day	0.40 $\pm$ 0.19 4/6	0.23 $\pm$ 0.43 3/6	0.22 $\pm$ 0.28 3/6
Midday	0.52 $\pm$ 0.20 5/6	0.07 $\pm$ 0.34 1/6	−0.07 $\pm$ 0.07 0/6
Late day	0.45 $\pm$ 0.29 4/6	−0.03 $\pm$ 0.34 0/6	0.13 $\pm$ 0.12 0/6
Overall	0.53 $\pm$ 0.27 28/36	0.09 $\pm$ 0.33 8/36	0.10 $\pm$ 0.28 8/36

The proportion of significant positive coefficients is also shown.

One disadvantage of golden shiners for the study of FAA is that they are strongly shoaling, and tend to show little activity when not together. The necessity to test shoals means that individual behavior cannot easily be quantified. Moreover, to say that a certain percentage of shoals showed FAA does not necessarily mean that the same percentage of individuals would. It is conceivable that only one individual learned about the mealtime in each shoal, and that its shoalmates simply followed it.

#### 4.2. Testing models

The results from the experimental stage do not support the “energetic hourglass” model. This model predicted a correlation between activity and hunger, and therefore, constantly high activity levels when food was withheld, but this was not observed. In the still-light + no-food group, activity increased up to the normal time of feeding, but past that time activity decreased even though the fish remained hungry because no food had been delivered. This lack of evidence for an “energetic hourglass” model is consistent with the literature on other animals, inasmuch as we are not aware of any example where evidence for this model was substantiated with regard to FAA.

The results are not easily explained by the “photoperiodic hourglass” model either. Admittedly, the better maintenance of FAA in the still-light + no-food group relative to both the still-food + no-light and the no-food + no-light group is consistent with the predictions of the model, which states that light signals are essential. However, the maintenance of FAA in 22% of the shoals in the still-food + no-light and the no-light + no-food groups cannot be explained by the model. Even more damning is the fact that in the experimental groups where food was scheduled 1 h after the time of lights-on or lights-off, anticipation began before the light signal. This is obvious from a visual examination of the waveforms, and it is also reflected by the fact that the duration of anticipation during the training stage did not differ between the experimental groups (4.5 h on average). It is possible, but hard to believe, that the animal would rely on the photoperiodic signal that occurred 13 h prior to mealtime rather than on the more proximate signal 1 h prior. It is also hard to believe that a biological hourglass mechanism could anticipate with equal precision events that are 6, 11, and 13 h in the future. At least when it comes to estimating time spans in the range of minutes, hourglass mechanisms (interval timers) are known to be less precise for the longer intervals.

This leaves the two circadian models. Our results best fit the single-LEO model (a circadian light-entrained oscillator with a representation of the phase of feeding time stored in memory). As predicted by the model, FAA was well maintained in the absence of food if the light–dark cycle remained present. (For similar results in goldfish, see [23]). Also as predicted, if the light–dark cycle was removed, only a minority of shoals still showed FAA, irrespective of

whether food was maintained or not. As opposed to the photoperiodic hourglass model above, the single-LEO model can explain away these few cases of FAA persistence by postulating that in some individuals, the LEO is sufficiently undamped to self-sustain its oscillation for a few cycles (in our experiment, “experimental stage” waveforms were based on the first 5 days) at a period close to 24 h.

Although we consider that the small number of nonsignificant correlations in the still-light + no-food group was linked mostly to a lack of FAA to start with, we contend that the much greater number of nonsignificant correlations in the other two groups was the direct result of the removal of light signals, which led to the damping out of the oscillator or possibly its free-run at a period very different than 24 h. This interpretation is consistent with the existence of a LEO.

In contrast, the “LEO-FEO” model, in its purest form, cannot easily explain why so many shoals ceased to anticipate food arrival when the light–dark cycle was removed but food delivery was maintained (the still-food + no-light group). According to this model, the FEO should have remained sustained by the scheduled food delivery and should have been able to maintain FAA on its own. This was not the case for 78% of the shoals tested in the still-food + no-light group. Even if nonsignificant correlations reflected free-running rather than damping out, the point would remain that the oscillator was not entrained by food delivery and so could not be called FEO.

The pure LEO-FEO model can be modified to accommodate our data. Perhaps there is a FEO anatomically separate from a LEO, but its proper functioning is dependent on that of the LEO, so that if the LEO damps out because of the lack of a light signal, the FEO is brought down with it, at least temporarily, and it may be many days before the FEO can generate FAA on its own. It would have been necessary for us to prolong our experiments for several days or weeks in constant conditions to assess the likelihood of this hypothesis.

An almost complete lack of anticipation to daily food in constant lighting conditions has been reported for sea bass [22]. On the other hand, goldfish [5], loach [13], and mummichogs [2] can develop FAA when fed daily in constant lighting conditions for many days. These observations on goldfish, loach, and mummichogs fit well with a FEO model, although they can also be reconciled with a LEO model if we accept that, in the absence of a light–dark cycle, the LEO can become synchronized by other zeitgebers, such as food, the activity associated with its search, or the competitive interactions associated with its defense. There are examples, from rodent and bird work, of LEOs being weakly entrained by novelty-induced exercise, social interactions, or other nonphotic synchronizers [12].

A good model should be able to account for unexpected data. Can the single-LEO model, which our experiments failed to falsify, account for the few shoals that were fed at only one crepuscular time (e.g., around lights-on) and that

showed increased activity at two crepuscular times (e.g., around both lights-on and lights-off)? Here is one possibility. In nature, golden shiners are usually crepuscular and feed during both dawn and dusk [6,9,25]. We do not know if this applies to our population in Folly Lake, but assuming that it does, the possibility arises that a strong memory already existed for two marked food phases—dawn and dusk. (The single-LEO model does not prevent more than one phase being marked [3]). This memory would not have been expressed when all-day feeding conditions prevailed (while the fish were in our holding tanks), but once part of the old conditions came back (food only available at one crepuscular time during the training stage), the old but resilient memory might have been reactivated (FAA expressed at both crepuscular times). This would be akin to known examples of rats developing FAA during a period of scheduled feeding, ceasing to express FAA during a subsequent period of ad lib feeding, and then spontaneously showing FAA again at the old circadian phase when completely deprived of food, even if this deprivation takes place 50 days after the last day of scheduled feeding [10].

The above speculation suggests one new way to differentiate single-LEO from LEO-FEO models experimentally. It would be interesting to feed animals at one single daily time for several months, then switch to another daily time just long enough for the old FAA phase to extinguish and a new FAA phase to develop, and then deprive the animals of food and see where FAA is expressed. Nothing in the LEO-FEO model could explain the reappearance of FAA at the old phase (should this be the observed result) but the single LEO model would fare better if one is willing to incorporate the notion of differential persistence of multiple memories.

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