Food-anticipatory activity of groups of golden shiners during both day and night

Martin Laguë and Stéphan G. Reebs

Abstract: For 12 days, captive groups each containing four golden shiners (*Notemigonus crysoleucas*) were fed by automatic feeders at two diametrically opposed daily times. These two times could be midday and midnight, late day and late night, or early day and early night. As measured by interruptions of an infrared beam underneath the feeder, golden shiners almost always expressed food-anticipatory activity. Beam interruptions started to increase a few hours before mealtime, reaching a peak within 1.5 h of food delivery. In at least half of the groups tested, food-anticipatory activity developed for both daily times simultaneously. This double anticipation was maintained for at least 5 days after food was withheld. These results show that golden shiner groups (though not necessarily individuals) can express two peaks of food anticipation at widely separated daily times, even if one occurs during the day and the other at night, providing further evidence for the great variability that fishes can display in their activity patterns.

Résumé : Pendant 12 jours, des groupes de quatre Chattes de l'Est (*Notemigonus crysoleucas*) ont été nourris au moyen de distributeurs automatiques deux fois par jour, soit au milieu de la nuit et du jour, ou au début de la nuit et du jour, ou à la fin de la nuit et du jour. L'intensité de leur activité, telle que mesurée par des rayons infrarouges sous les mangeoires, a commencé à augmenter quelques heures avant l'arrivée de la nourriture, atteignant un maximum à moins de 1.5 h du repas. Pour au moins la moitié des groupes, une telle anticipation de l'arrivée de nourriture s'est exprimée aussi bien la nuit que le jour. Cette double anticipation a continué à s'exprimer pendant les 5 jours qui ont suivi l'arrêt permanent de toute distribution de nourriture. Ces résultats indiquent que les groupes de Chattes de l'Est (mais pas nécessairement les individus eux-mêmes) peuvent anticiper l'arrivée de nourriture à des moments très distincts de la journée, par exemple une fois durant la nuit et à un autre moment durant le jour, une démonstration supplémentaire de la grande flexibilité des patrons journaliers d'activité chez les poissons.

Introduction

Daily activity rhythms in fishes are often flexible. Within the same species, fish can be nocturnal or diurnal depending on temperature (Fraser et al. 1993, 1995), season (Müller 1978; Linnér et al. 1990; Heggenes et al. 1993), light intensity during the day (Andreasson 1969; Ericksson 1978), habitat (Bowen and Allanson 1982), shoal membership (Kavaliers 1980), ontogeny, or simple individuality (Sánchez-Vásquez et al. 1995*a*, 1996; Brännäs and Alanärä 1997). Food availability can also determine the diurnal/nocturnal status of a fish. In the laboratory, for example, individuals or shoals that are fed only once a day reliably develop peaks of activity slightly before feeding time (Davis and Bardach 1965; Gee et al. 1994; Naruse and Oishi 1994; Sánchez-Vásquez et al. 1997), and some can do so irrespective of the time of day or night at which feeding time is programmed (Spieler and Noeske 1984; Sánchez-Vásquez et al. 1995*a*). This increase in activity up to a learned time of feeding is called food-anticipatory activity and its timing is regulated by circadian mechanisms (Mistlberger 1994).

In this study, we further tested the flexibility of activity patterns in fishes by asking whether food-anticipatory activity could develop within the same shoal (but not necessarily within the same individual) at two daily times simultaneously. We worked with the golden shiner (*Notemigonus crysoleucas*), a species that we knew could anticipate food at any single time of day or night (S.G. Reebs and M. Laguë, personal observation). Groups of golden shiners can also learn to be in one half of their tank in the morning, in the other half near midday, and back in the first half late in the day, in order to get food (Reebs 1996; for a similar example from another species see Reebs 1999). This ability, called time–place learning, suggests that golden shiners could anticipate food delivery at more than one daily time. Here, to provide a strong test we measured food anticipation at two daily times that were diametrically opposed: midday and midnight, late day and late night, or early day and early night.

Materials and methods

All manipulations were approved by the Comité de Protection des Animaux de l'Université de Moncton (protocol No. 96-16). 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. The water temperature in the tanks was 16 ± 2°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. As golden shiners that are fed exclusively during the day invariably show diurnal or crepuscular activity patterns (S.G. Reebs and M. Laguë, personal

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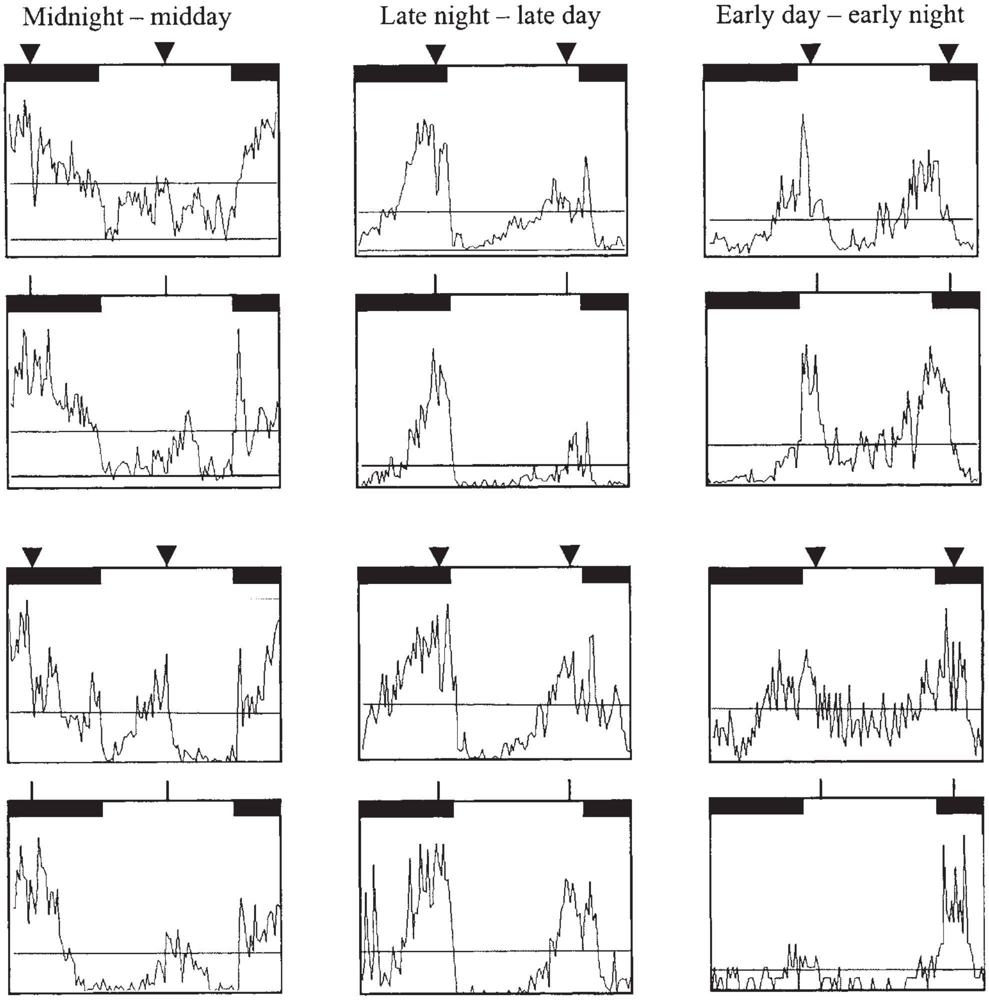
M. Laguë and S.G. Reebs.[1] Département de biologie, Université de Moncton, Moncton, NB E1A 3E9, Canada.

[1] Author to whom all correspondence should be addressed (e-mail: reebss@umoncton.ca).

Fig. 1. Six representative examples of pairs of daily activity waveforms (averaged over 5 days) for groups of four golden shiners (*Notemigonus crysoleucas*) that were fed at two daily times (top panel in each pair; ▼) and then not fed (bottom panel in each pair). The thick horizontal bar represents nighttime. The thin horizontal line indicates the average daily activity level for the group and, when different from 0, the minimum level.



observation), our pre-experimental feeding regime standardised fish activity to diurnal or crepuscular.

For the experiments, groups of four fish were placed in 37-L aquaria (50 × 25 × 30 cm) with the water temperature set at 18 ± 2°C. The photoperiod was 12 h light :12 h dark, with abrupt transitions between light and dark. Light was provided by incandescent bulbs with an intensity of 700–900 lx at aquarium level. Gravel covered the bottom of each aquarium, where a half flower pot provided refuge. A small filter was mounted outside each aquarium at one end. At the other end, food was delivered by an automatic feeder that dropped commercial food flakes (approximately 21 mg) at the surface. A barrier prevented the floating flakes from spreading more than 20 cm from that end.

For 12 days the fish were fed twice a day at the same times every day. These two times could be the middle of the day and the middle of the night (the midday–midnight condition), 1 h after lights-on and 1 h after lights-off (early day – early night condition), or 1 h before lights-off and 1 h before lights-on (late day – late night condition). Six aquaria were assigned to each of these three conditions, for a total of 18 groups tested. Groups were used instead of individuals because the golden shiner is a strongly shoaling species, and because we wanted to maximise the likelihood of detecting activity, as solitary golden shiners usually display little activity. In past circadian studies on sea bass (*Dicentrarchus labrax*), shoals and single individuals gave similar results (Sánchez-Vásquez et al. 1995*a*, 1995*b*). In studies on white sucker (*Catostomus commersoni*) and European catfish (*Silurus glanis*), shoals yielded clearer and more robust activity cycles than single individuals (Kavaliers 1980; Boujard 1995).

Activity was recorded with an infrared beam that crossed the short length of the aquarium directly below the feeder, 4 cm from the end and 8 cm below the surface. Beam interruptions caused by moving fish were recorded by computer. The software (Dataquest III, Mini-Mitter Co., Sunriver, Oreg.) tallied the number of interruptions for each aquarium in blocks of 6 min each. For each aquarium, and for each of the 6-min blocks during a 24-h period, the numbers of beam interruptions over the last 5 days of the 12-day feeding period were averaged to obtain daily activity waveforms. These waveforms were graphed (with TAU software, obtained from Mini-Mitter Co.) and examined for evidence of food-anticipatory activity. We considered any gradual rise in activity above the average daily level, starting before feeding time and culminating within 1.5 h of feeding time, to be evidence of food-anticipatory activity.

In rodents, food-anticipatory activity is known to persist for a few days after delivery of food ceases (Mistlberger 1994). To see whether this would happen, we withheld food for 5 days after the feeding period. For each aquarium, we established the daily activity waveform for this 5-day “no-food” period and compared it with the 5-day waveform for the feeding period. To the eye, it appeared that most patterns were indeed maintained, and to quantify this we used a correlation analysis. Each 24-h waveform was reduced to 48 half-hour tallies of beam interruptions. A Pearson’s correlation coefficient was then calculated between the food and no-food waveforms for each aquarium. In view of the large number of data points involved in each correlation ($n = 48$), we conservatively set the significance level at $P = 0.0005$. A significant positive coefficient was taken as an indication that the waveforms were similar, and therefore that the peak of activity had been maintained, and maintained near the original clock time.

Results

Midday–midnight condition

Of the six groups fed at midday and midnight, three showed peaks in food-anticipation activity around both midday and midnight. Of these, one had a higher peak at night (the pair of panels at the bottom left in Fig. 1), one had a higher peak during the day, and one had equal peaks. All of these three double-peak patterns were maintained during the no-food period: all three correlation coefficients between food and no-food activity waveforms were positive and significant ($r = 0.72$, 0.61, and 0.60; $P < 0.0005$).

Of the other three groups, one had low activity levels and did not show clear activity peaks at any time, while two showed only midnight peaks of food anticipation. These two nocturnal patterns of activity were maintained during the no-food period ($r = 0.80$ and 0.78, $P < 0.0005$). For both of those nocturnal groups, a diurnal peak was too small to be considered food anticipation according to one of our established criteria (above-average level) during the food period, but rose to above average during the no-food period (the pair of panels at the top left in Fig. 1).

Late day – late night condition

Three groups clearly showed food-anticipation peaks around both the late day and the late night feeding times (middle pairs of panels in Fig. 1), with the nighttime peak always slightly higher than the daytime one. This double anticipation was maintained after food was withheld ($r = 0.75$, 0.85, and 0.86, $P < 0.0005$).

Of the other three groups, one anticipated the late-day feeding time only (and its pattern was maintained; $r = 0.73$, $P < 0.0005$), while two anticipated the late-night feeding time only (of these, only one was maintained, $r = 0.50$, $P < 0.0005$).

Early day – early night condition

Four groups showed food-anticipation peaks, of roughly the same intensity, before both the early-day and the early-night feeding times (the right-hand pairs of panels in Fig. 1). One more group was highly active during most of the day only, and another during most of the night only. Four of the six correlation coefficients were significant ($r = 0.58$, 0.60, 0.67, and 0.74, $P < 0.0005$). The two exceptions were the diurnal group and one of the doubly-anticipating groups, whose activity levels became low after food was withdrawn, resulting in correlation coefficients of 0.11 and 0.26, respectively.

Discussion

The results show that at the shoal level, restricted food availability can be anticipated during both day and night simultaneously. However, we cannot yet conclude that the same ability is possible at the individual level. It is conceivable that of the four fish within each group, some learned to anticipate the daytime food only, while others learned about the nighttime food only. At the group level, this combination of individual learning would result in double food anticipation. Casual but direct observation of shoal behaviour during the day revealed cohesive movements for the whole groups, but without tagged fish it was impossible to ascertain whether activity near the feeder was always initiated by the same individual(s). Only experiments with single fish could satisfactorily address the question of double anticipation by individuals. A loosely shoaling yet active species would probably be better suited to such a study. At this point, our study with golden shiners suggests only two possibilities without being able to distinguish between them: either double anticipation can develop within the same individual, or individuals within the same group, even a small one, where competition is presumably minimised, specialise on different time windows for feeding.

Whether double anticipation occurs at the individual or shoal level, the results add another facet to the great variability in activity phases that fish shoals can exhibit. Not only can some shoals develop peaks of food-anticipatory activity during the day or night (Spieler and Noeske 1984; Sánchez-Vásquez et al. 1995*a*), they can also develop them at both times simultaneously, based on food availability. Moreover, even within that phenomenon there is variability: only half of our groups convincingly showed such double anticipation, most of the others adopting a single peak in food-anticipatory activity that could be either diurnal or nocturnal. There was a tendency for nocturnal peaks to be higher or more common than diurnal peaks, but this may be related to the fact that during the night fish cannot rely on vision to determine from a distance whether food has arrived at the surface, and must therefore approach the feeder area more closely and more often.

We do not know what feeding-activity patterns are shown by golden shiners in the lake where we captured them. Most wild populations are known to be crepuscular with respect to feeding (Zaret and Suffern 1976; Hall et al. 1979; Keast and Fox 1992). This being so, the double anticipation displayed by the late night – late day and early night – early day groups may not be surprising (though one feeding still took place in complete darkness and the other in full light). However, the double anticipation displayed by the groups in the midnight–midday condition remains interesting. Moreover, double anticipation by the groups in this experimental condition may have been more prevalent than is suggested solely by the feeding period in the experiment. If one is willing to consider the no-food part of the experiment as a better condition for food anticipation to express itself (the fish were more hungry), then double anticipation was expressed in five out of six aquaria.

We must stress that we did not measure general activity. Because the infrared sensors were close to the feeders only, the daily patterns measured were truly of food-anticipatory activity rather than general activity. It is conceivable that fish might show one pattern at the surface, where food arrives, and another near the bottom (Sánchez-Vásquez et al. 1997).

Our correlation analysis showed that shoal anticipation in response to double feeding times can persist for a few days after food delivery is stopped. There is good evidence that food-anticipatory activity is based on a circadian mechanism (Mistlberger 1994). It seems that once it is established, even when two widely separated feeding times are used, the food-anticipatory circadian mechanism does not require the presence of a food synchroniser to maintain itself, at least for a few cycles.

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