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Time-place learning in golden shiners (Pisces: Cyprinidae)

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Abstract

The goal of this study was to determine whether fish can learn to forage in different places at different times of the day, each place being associated with a specific time. Groups of eight golden shiners (*Notemigonus crysoleucas*) were kept in aquaria equipped with automatic feeders that dropped food on one side in the morning and on the other side in the afternoon, or on one side in the morning, the other side at midday, and back on the first side in the evening. After 3–4 weeks, food was withheld and the position of the fish within the aquaria was noted at 5-min intervals throughout the day. Consistent with time-place learning, most fish were on the correct side at the correct time. However, another experiment with three places instead of two provided only equivocal evidence of time-place learning; this could reflect the fact that, in the lakes they inhabit, golden shiners may need only distinguish between two places: open waters and littoral. Experiments with phase-shifts of the photoperiod showed that temporal discrimination is based on a circadian clock that can be gradually phase-advanced by 6 h in about 3 days.

Keywords: Circadian clock; Foraging; Golden shiner; *Notemigonus crysoleucas*; Time-place learning

1. Introduction

Most animals have endogenous circadian clocks that run with a periodicity of about 24 h. Many temporal aspects of an animal's behaviour are dictated, at least to some degree, by such a clock: daily wake and sleep onset, daily timing of feeding or reproductive activities, and timing of various behaviour in anticipation of night or day. These are daily single events, and therefore the clock needs to give a signal only once a day. Scarcer are examples of clocks being continuously consulted throughout the day. One such example is time-place learning. In time-place learning, an animal must learn to associate different times of day with different locations. In the absence of reliable cues related to sun height, temperature, or even light intensity, an animal must use a continuously consulted internal clock to distinguish between various times of day. (Conceivably, the animal could also use as many clocks as there are important times of day, each clock being consulted only once but also being precisely phased in relation to the others; functionally, this whole mechanism would be similar to a continuously consulted clock.)

Time-place learning is advantageous to an animal facing a food resource that varies spatio-temporally throughout the day but predictably from day to day. There are several examples of daily routines in which animals consistently forage in one place in the morning and in another place later on (Daan, 1981; Daan and Koene, 1981; Rijnsdorp et al., 1981). Movement from place to place could be a direct response to the presence of food, but it could also be the result of learning. Experimental evidence for time-place learning has been mounting recently (honey bees: Wahl, 1932; Gould, 1987; Moore et al., 1989; ants: Schatz et al., 1994; warblers: Biebach et al., 1989, 1991; Krebs and Biebach, 1989; starlings: Daan, 1981; Wenger et al., 1991; weavers: Falk et al., 1992; pigeons: Saksida and Wilkie, 1994; rats: Mistlberger, 1994).

Some fish species are highly mobile within their environment throughout the day, and such movements have sometimes been interpreted as reflecting spatial variation in food availability (e.g. McNaught and Hasler, 1961; Hall et al., 1979). Yet time-place learning has not been demonstrated in a fish species. Attempts with the minnow *Phoxinus phoxinus* (von Stein-Beling, 1935, cited in Daan, 1981) and the convict cichlid *Cichlasoma nigrofasciatum* (Reebs, 1993) failed to reveal the phenomenon, although such failure may have been caused by incomplete protocols rather than the fish's inability to learn. Here, I report evidence of time-place learning in a cyprinid fish, the golden shiner *Notemigonus crysoleucas*. Golden shiners are good candidates for studies of this kind because they are known to travel widely throughout the lakes they inhabit (Scott and Crossman, 1973) and to show regular daily migrations (Hall et al., 1979).

2. Materials and methods

All fish used in this study were minnow-trapped in Folly Lake, 12 km south of Moncton, New Brunswick, Canada. All measured 6.5–8.5 cm (total length). They were tested in the laboratory under a light-dark cycle of 12 h of light and 12 h of darkness, with abrupt transitions between light and dark. Water temperature was 17 ± 2°C. Feeding was done by automatic feeders that delivered 50 ± 19 mg (means ± standard deviation, based on 20 measurements) of small food flakes at the surface. Fish could not see the rotating container of the feeder in action unless they were directly below it. Moreover, fish probably could not detect vibrations produced by the feeder in action because feeders were supported by stands and were not in contact with the aquaria. This conclusion was bolstered by direct observations: fish that, because of their position, could not see the food falling onto the surface never showed evidence that they knew food had arrived; only after moving below the food floating on the surface did they react.

All tests involved groups of 8 fish. Groups were used instead of solitary fish because golden shiners are a shoaling species, and also to create a cost for not learning the time-place association. A fish that would not be in the correct place at the correct time would lose the food to its more adept shoalmates (as it would in nature).

Three experiments were conducted: the first experiment tested time-place learning involving two times of day and two places; the second experiment involved three times and two places; the third experiment featured three times and three places. These experiments are abbreviated as 2T2P, 3T2P, and 3T3P respectively.

2.1. 2T2P Experiment

2.1.1. Methods

Fish were captured on 17 August 1993. Three groups of 8 fish were placed in separate aquaria (140 × 30 × 45 cm). Food was delivered at one end (the 'morning side') of the aquaria every half hour during the third and fourth hour of the day, and at the other end (the 'afternoon side') every half-hour during the ninth and tenth hour. For a given group, the morning side could be left or right, originally determined at random but remaining the same thereafter. After seven days (acclimation period), an opaque plexiglas partition was installed in the middle of the aquarium, just below the outside-mounted filter which now straddled this partition. Fish could only go from one side to the other by crossing a rectangular (4 × 9 cm) opening at the bottom of the partition. This was day 0. On days 7 and 14 (observation days), the position of the fish was recorded for 1 s every 5 min throughout the day by means of a commercial video-recorder set on time-lapse. Food was still delivered on those days. On days 21 and 28 (test days), food was not delivered (the feeders remained in place but were turned off) and the fish's position was once again recorded throughout the day. (Food was still delivered between and after test days.) Finally, on days 35 and 36 (shift days), no food was given, and the lights went on and off 6 h earlier than usual. The position of the fish was recorded at 5-min intervals throughout these shifted days.

Shift days were used to see whether the clock mechanism underlying time-place learning had circadian properties, or resembled an hourglass set by light onset (see Biebach et al., 1991). If the clock is circadian, the preference of fish for a given side should shift ahead gradually (on a day-to-day basis), much like the wake-up time of a jet-lagged traveller. If the mechanism is an hourglass set by light onset, the preference should shift immediately ahead by 6 h on the first shift day.

During video playback, for each group, I counted the number of fish on each side at each 5-min intervals. I then added the number of times, during each 2-h feeding period, that more than half the fish were on a given side (a half-unit was assigned to times when exactly half the fish were present). The totals, out of 24, gave an estimate of the percentage of time spent by the majority of fish on each side. For each group and each test day, these totals were set up in a 2 × 2 contingency table with time of day as treatment and aquarium side as blocks. These tables were analysed with X^2 tests (here I am assuming that observations within the same group were independent, because the 5 min that separated subsequent observations was more than enough time for the fish to change sides if they wanted). Significance levels were set at $P = 0.05$. For representation on figures, the number of fish present on the morning side was arranged as a time series from lights-on (dawn) to lights-off (dusk) and submitted to a running average (window size: 5 recordings, or 20 min inclusive).

2.1.2. Results

Fish tended to be on the correct side at the correct time of day, both on observation days when food was delivered (Fig. 1, top row of panels) and on test days when it was not (Fig. 1, second row of panels). On test days, for each of the three groups, the majority of fish were on the morning side no less than 66% of the time in the 2-h morning period, but in the 2-h afternoon period this percentage dropped to no more than 12.5%. All six X^2 values (three groups × two test days) were significant (maximum $P = 0.0001$).

On the first shift day, fish remained sluggish from the time of lights-on until midday (the previous clock time of lights-on). They tended to be on the morning side at the right time for the previous day,

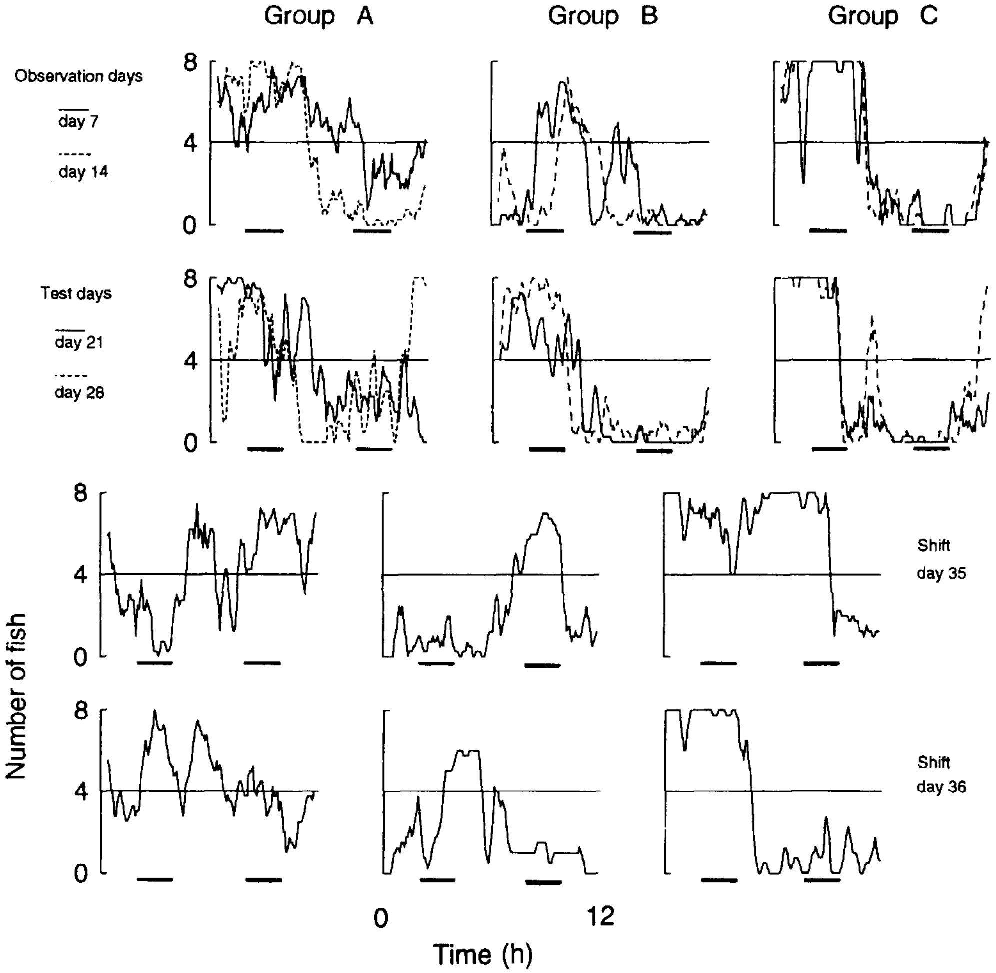

Fig. 1. Number of fish, out of a possible eight in each of three groups, present on the 'morning' side of an aquarium during a 12-h day. Data shown are the result of a running average applied to the original time series. The x axis (time of day) was drawn at y = 4 to show chance level. The two horizontal bars below each graph show the time when food was delivered on the 'morning' side (first bar) and on the opposite 'afternoon' side (second bar) of the aquaria. Food was delivered before, during, and after observation days but neither on test nor on shift days. Graphs of shift days are offset proportionally to their shift in photoperiod.

but the wrong time for the new day (Fig. 1, third row of panels). On the second shift day, their preference for the morning side had shifted ahead by about 4 h (Fig. 1, bottom row of panels).

2.2. 3T2P Experiment

2.2.1. Methods

Fish were captured on 5 October 1993 and moved to the experimental aquaria. The protocol was the same as in the previous experiment except that (1) four groups of fish were used instead of three, (2) feeding took place on one side during the second and third hour of the day, on the other side

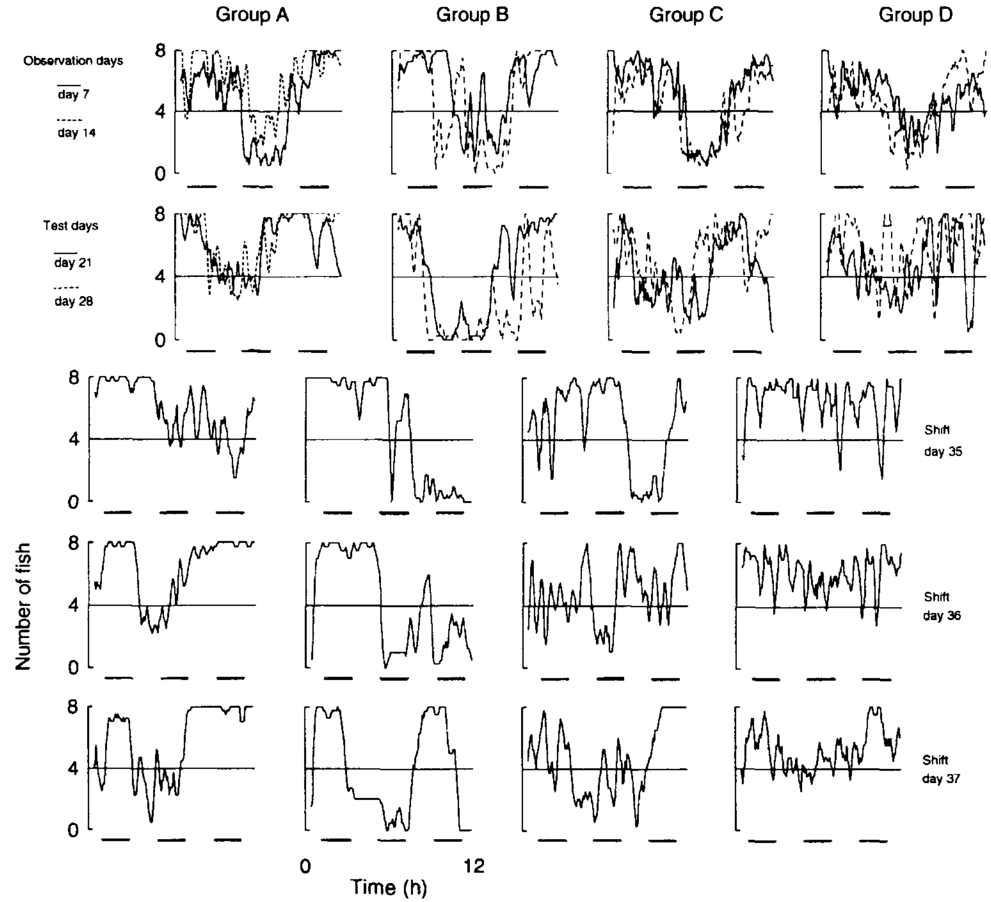

Fig. 2. Number of fish, out of a possible eight in each of four groups, present on the 'crepuscular' side of an aquarium during a 12-h day. The three horizontal bars below each graph show the time when food was delivered on the 'crepuscular' side (first and third bars) and on the opposite 'midday' side (second bar) of the aquaria. Other details as in Fig. 1.

during the sixth and seventh hour, and back to the first side during the tenth and eleventh hour, and (3) observations were made during three shift days instead of only two. The contingency tables involved three times and two places. Here I shall refer to the 'crepuscular' (morning and evening) side of the aquaria, and to the 'midday' side.

2.2.2. *Results*

Fish tended to be on the correct side at each of the three times of day on observation days (Fig. 2, top row of panels). On the test days when food was not delivered, two of the four groups continued to be on the correct side at all correct times (see B and C on Fig. 2, second row of panels). On average for the two test days, the majority of fish in group B were on the crepuscular side 56%, 1%, and 76% of the time during each of the three respective 2-h periods ($X^2 = 51.72$ for the first test day and 16.58 for the second test day, $P < 0.003$); corresponding figures for group C were 59%, 3%, and 82% ($X^2 = 21.33$ and 7.28, $P < 0.03$). The other two groups tended to stay on the crepuscular side at all times, but they showed, at midday, a decrease in the time spent on the crepuscular side (see A and D in Fig. 2, second row of panels). On average for the two test days, the majority of fish in group A were on the crepuscular side 88%, 66%, and 97% of the time during each of the three respective 2-h periods ($X^2 = 6.78$ and 12.38, $P < 0.03$); corresponding figures for group D were 78%, 54%, and 72%, but when test days were considered separately, the three percentages were not significantly different from one another on either of the test days ($X^2 = 5.01$ and 3.33, $P = 0.08$ and 0.19 respectively).

The choice by fish on shift days can best be visualized by concentrating on the dip in the curves of the bottom three panels of Fig. 2. This dip shows the preference of the fish for the midday side (with the exception of group D, who once again did not go to this side). On the first shift day, this preference took place close to, or slightly ahead of, the correct time for the previous day. On the 2nd day, the preference had shifted ahead by about 3 h. On the third day, an additional but smaller shift had occurred, bringing the curves to an overall position and shape that resembled those before the shift (on Fig. 2, compare the second panels from the top with the bottom panels).

2.3. 3T3P Experiment

2.3.1. Methods

Golden shiners were captured on 8 August 1994. Four groups of eight fish were placed in separate, circular wading pools (1.15 m diameter, water depth: 20 cm) whose bottom was covered by a thin layer of lightly coloured gravel. Three opaque plexiglas partitions radiating from the center divided each pool into three equal areas. Passage from one area to another was possible through two small triangular holes (base × height: 7 × 5 cm) at the bottom of each partition. A wall of coloured cardboard surrounded each pool; a different cardboard colour (black, green, or blue) was assigned to each area within a given pool. This wall prevented fish from jumping outside of the pool, and supplied colour cues to distinguish each area. Areas could be further distinguished by the presence of different objects (pebbles, white plexiglas squares, black plexiglas rectangles) at the bottom of each area. Holes in the cardboard wall allowed outside stands to support feeders installed along the perimeter of the pools. A video-recorder was installed above each pool and left there at all times.

Other procedures were as in the 3T2P experiment, except that each time period was associated with a different pool area, originally at random for each pool, but consistently from day to day. I refer to these pool areas as morning, midday, and evening areas. No shift days were performed in this experiment. Finally, to build the 3 × 3 contingency tables, I counted all times when 4 or more fish were in a given area (the value 4 was given a full unit, not a half one as before).

2.3.2. Results

The behaviour of the fish in this experiment was characterized either by consistent preference for one of the 3 areas (e.g. group D on Fig. 3) or by much roving from area to area (e.g. group A on Fig. 3). To be consistent with time-place learning, the panels on Fig. 3 should show an increase of black in the first third of the day, of white in the second third, and of grey in the third third. This in general was the case on observation days, but not always on test days (Fig. 3). Group C showed good signs of time-place learning on both test days ($X^2 = 52.8$ and 41.4, $P < 0.0001$); preference for the morning area was maximum in the morning, preference for the midday area was maximum at midday, and preference for the evening area was maximum in the evening. Group B decreased its attendance in the

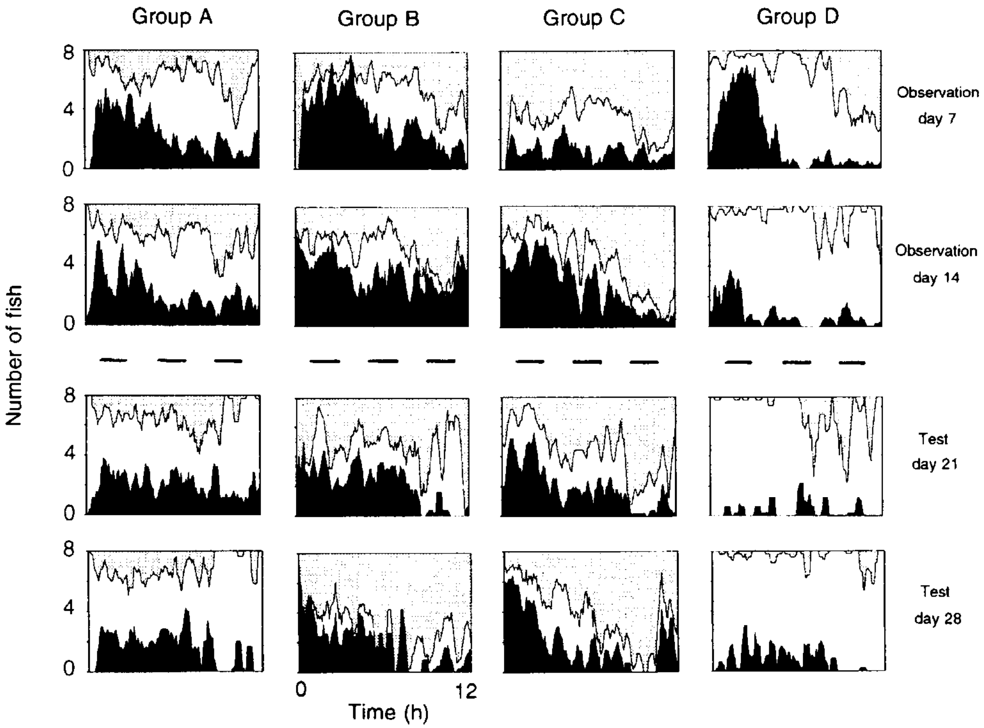

Fig. 3. Number of fish, out of a possible eight in each of four groups, found in the 'morning' (black), 'midday' (white), or 'evening' (grey) area of a pool during a 12-h day. The three horizontal bars below the second row of graphs show the time when food was delivered on the 'morning' (first bar), 'midday' (second bar), and 'evening' (third bar) area of each pool. Other details as in Fig. 1.

morning area and increased its attendance in the evening area throughout both test days ($X^2 = 15.3$ and 9.7, $P = 0.004$ and 0.05 respectively) but showed no increased preference for the midday area at the appropriate time. Group D on the first test day increased its attendance in the evening area at the correct time ($X^2 = 16.3$, $P < 0.003$) but showed no other evidence of time-place learning, including the second test day ($X^2 = 5.3$, $P = 0.25$). Group A either gave no evidence of time-place learning (first test day: $X^2 = 5.5$, $P = 0.24$) or showed an inappropriate increase in preference for the midday area in the evening (second test day: $X^2 = 11.86$, $P = 0.02$).

3. Discussion

Groups of golden shiners positioned themselves in their aquaria in ways that are consistent with time-place learning, at least in tasks involving up to 3 times and 2 places. One may argue that because groups were used, only a simple form of time-place learning could have been involved, requiring no more than one time-place association: it is conceivable that some of the fish could learn to be in one place at one time and move randomly otherwise, while other individuals would learn to be in the other place at the other time and move randomly otherwise. This would create a daily pattern that is suggestive of time-place learning at the group level, even though no individual would have learned more than one time-place association. This indeed could have been the case in the 3T3P experiment. However, it is unlikely to be so in the other two experiments: there, for many groups, almost all fish were found together consistently in the correct place at the correct time (for example, groups B and C in the 2T2P experiment, group B in the 3T2P experiment). This would not happen if many of the fish moved at random most of the time.

But one could also argue that fish may have learned only one of the time-place associations, and at other times copied the choice of other fish that had learned a different time-place association. Again this would create a daily pattern that is suggestive of time-place learning at the group level, even though no individual would have learned more than one time-place association. It is difficult to explain, however, what would cause some fish to learn only one of the time-place associations and some fish to learn another. A priori, one would expect all fish to learn at least the first time-place association of the day, given its early occurrence following a well-defined time signal (lights-on). It seems more reasonable to suggest that at least one fish has learned more than one time-place association.

A drawback to the use of groups is that one cannot ascertain exactly how many fish have learned correctly in each group. At least one fish has learned but all the others may just have been copying its choice. The sample size in the present experiments was 3 or 4, not 24 or 32.

Golden shiners represented a promising species for time-place learning (see Introduction), and I used groups because shiners are a strongly shoaling species. However, future research will benefit from testing individuals in addition to groups, relying perhaps on demand feeders and operant conditioning as in previous work with birds (see references in Introduction).

As compared to the experiments that involved two places, only equivocal evidence of time-place learning was obtained in the 3T3P task. This could reflect the fact that foraging shiners may not need to distinguish between more than two different places in their natural habitat. Hall et al. (1979) have described how golden shiners living in lakes feed in open waters at dawn, in the littoral zone during the day, and back in open waters at dusk. This situation resembles a 3T2P task, which shiners were capable of mastering in the present study. Beyond a dichotomy between open waters and littoral, natural habitats may not be heterogeneous enough, in terms of food availability at different times of day, to select for the capacity to distinguish between more than 2 places in time-place learning.

Other explanations may be sought to explain the relative lack of learning in the 3T3P experiment. These include (1) dominant individuals monopolizing particular areas and excluding most of the shoal from them (although, in many hours of direct observation, I never witnessed aggressive acts between individuals), (2) the adoption of a roving strategy from area to area, helped by a relatively low cost of movement, or (3) insufficient time to learn. These potential explanations all suffer in that they should also apply to the other two experiments, and yet fish performed well in those experiments. Explanation number 2 could be further studied by using very large basins, or operant conditioning routines that involve more effort. Explanation number 3 could be further studied by lengthening the duration of experiments, although already at this stage one can conclude that a long time (more than 5 weeks) would be needed before shiners learned time-place associations involving more than 2 places. The usefulness of time-place learning would then be limited to situations where day-to-day predictability lasts for more than one month.

It was not an objective of this study to measure the minimum time necessary to learn time-place associations. It appears to be less than 3 weeks in golden shiners. In comparison, birds seem to require about 3–10 days to reach 75% correct choices in 4T4P tasks, in set-ups that differ from the one I used

(Biebach et al., 1989, Biebach et al., 1994; Wenger et al., 1991). Bees need only 1–5 days in 2T2P tasks (Wahl, 1932; Gould, 1987).

Gradual shifting on shift days showed that time discrimination is based on a circadian, rather than hourglass, mechanism. Similar conclusions were reached for birds by Biebach et al. (1991) and Wenger et al. (1991). In addition, my results show that this circadian mechanism needs only 3 days to shift by 6 h in golden shiners. Shifts of circadian systems underlying time discrimination have been observed only once in another species, and only for one day: if lights go on 6 h earlier than normal, garden warblers immediately shift their spatial preferences ahead by 2.6 h on average (Biebach et al., 1991). In mammals, the closest comparison comes from the behaviour of food-anticipatory activity in rats subjected to shifts in mealtime. Unfortunately, most rats delay rather than advance in response to a 6-h advance of mealtime (Stephan, 1984, Stephan, 1992). Fish chronobiologists often feel that fish rhythms are more labile than those of birds or mammals (e.g. Ali et al., 1992); at least for birds, the above comparison does not support this contention.

Does the circadian clock need to be involved in time-place learning? In nature, could fish simply use cues such as sun position, light intensity, or temperature to estimate time of day? The problem with such cues is that they are not reliable enough. The sun is not always visible, and both light intensity and temperature can vary not only because of time of day but also because of weather or position of the fish (especially depth) within the lake. Light intensity can be used as a partial cue, but its reliability as a signal is improved if its action is gated by a circadian system (see Reebs, 1994). Golden shiners need an endogenous clock to know when changes in external cues truly reflect changes in time of day. They may then better outcompete other fish by anticipating, or at least not missing, the arrival of food in specific places at specific times.

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