Short communication

Time–place learning in individually reared angelfish, but not in pearl cichlid

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Abstract

Time–place learning based on food association was investigated in the cichlids angelfish (Pterophyllum scalare) and pearl cichlid (Geophagus brasiliensis) reared in isolation, therefore eliminating social influence on foraging. During a 30-day period, food was placed in one side of the aquarium (containing three compartments) in the morning and in the opposite side in the afternoon. Learning was inferred by the number of correct side choices of all fish in each day of test (15th and 30th). During the test day fish were not fed. The angelfish learned to switch sides at the correct day period in order to get food, suggesting this species has time–place learning ability when individually reared. On the other hand, the same was not observed for pearl cichlid.

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1. Introduction

Time–place learning (TPL) is the ability to associate places (with biologically important events) at different times of day (Wilkie, 1995; Widman et al., 2000). Generally, this phenomenon concerns the synchronization of visiting patches with available food at specific feeding times. This kind of learning has been reported for many animals, such as rodents (Means et al., 2000; Thorpe and Wilkie, 2002), birds (Wilkie et al., 1996), insects (Breed et al., 2002), and fish (Reebs, 1996, 1999; Gómez-Laplaza and Morgan, 2005). In fish, this topic has received only limited attention, to our knowledge only five papers have addressed TPL in fish. Therefore, we feel that more information is needed to clarify some of the issues that have been raised in these few papers (Reebs, 1993, 1996, 1999; Gómez-Laplaza and Morgan, 2005; Delicio et al., 2006a).

Based on the fact that food items may change daily, seasonally and/or spatially, it seems likely that some learning abilities play a role in foraging flexibility (Dill, 1983). Thus, TPL may have an important function in fish survival, as it might increase foraging efficiency by improving its ability to locate and explore patches just when food is available (Reebs, 2002). For instance, fish may save energy when seeking for food because when they have access to both abundant food and enough time for feeding, there would be no immediate need to synchronize feeding time with food arrival, but when food availability and/or time for foraging is restricted, synchronization of feeding activity guarantees that the feeding window will not be missed (Volpato and Trajano, 2006).

To date all successful demonstrations of time–place learning in fish have been found only when the fish were tested in shoals. For instance, TPL was demonstrated in shoals of the cyprinid fish Notemigonus crysoleucas (Reebs, 1996), of the galaxid Galaxias maculatus (Reebs, 1999), and of the cichlid Pterophyllum scalare (Gómez-Laplaza and Morgan, 2005). While these studies provided important evidence of TPL in fish, group tests do not allow to exclude possible social influence on individual behaviour, and consequently on TPL. In a shoal of fish copying is a behaviour that has been observed: it is common for the leader of the shoal to “command” the group during foraging navigation (Reebs, 2000). Thus, most of the tested fish in shoals could have been in the correct side because they simply followed the leader instead of learning the time and place

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synchronization. To avoid this possible drawback, to test fish in isolation (1 fish/aquarium) would be a solution. On the other hand, if the leader of the shoal can learn TPL, it suggests that the other fish could also learn it. Particularly if they were under the right circumstances which, for instance, they were obtained the leadership of the shoal. Moreover, in the shoal condition there is a greater consequence for going to the incorrect side than in the individual condition, for instance, to lose a foraging opportunity (Reebs, 1996, 1999). Hence, an equally likely alternative is that the difference between the two conditions would be the response cost differences. Although the present study was not designed to distinguish between this two possibilities, it assessed if fish that demonstrated TPL in a shoal would also show it if tested individually, eliminating social influence on foraging, and decreasing response cost of learning tasks.

For this study, we chose two cichlid species: angelfish (P. scalare) and pearl cichlid (Geophagus brasiliensis). First, we tested cichlids in order to deepen our understanding of TPL in this fish family. To date three tests were conducted utilizing cichlid fish species, convict cichlid (Cichlasoma nigrofasciatum, Reebs, 1993), Nile tilapia (Oreochromis niloticus, Delicio et al., 2006a), and angelfish (P. scalare, Gómez-Laplaza and Morgan, 2005), but surprisingly just this latter one showed this learning ability. The other two cichlids species, although chosen as potential candidates for TPL because of their feeding circadian rhythmicity and food anticipatory behaviour, failed in their previous TPL tasks. Second, angelfish was chosen because it displayed TPL when tested in shoal (see Gómez-Laplaza and Morgan, 2005), and it was corroborated herein whether this species also displays this learning ability without influence of social factors on individual behaviour. Third, we investigated a new species, the pearl cichlid, increasing the range of fish tested for this phenomenon, besides this species has been poorly explored in terms of behavioural investigation. Thus, the aim of the present study was to evaluate whether both angelfish and pearl cichlid have time–place learning ability when tested individually housed in a daily TPL task.

2. Materials and methods

Juvenile angelfish, P. scalare (Lichtenstein, 1823), and pearl cichlid, G. brasiliensis (Quoy and Gaimard, 1824), were held in glass aquaria (60 cm × 60 cm × 30 cm; ∼10 fish/tank) for about 2 months before experimental procedures. These juveniles composed the stock population (non-mixed species tank). The tanks were supplied with constant aeration with biological filter (recirculating system). During this time, temperature averaged 24 °C, and water was maintained in low levels of ammonia (<0.25 ppm) and nitrite (<0.50 ppm). The photoperiod was set from 7:00 to 19:00 h. Food was offered “ad libitum” once a day at the same time of day (noon) (38% protein; Purina Ltda, Campinas, SP, Brazil).

The fish from our stock were chosen by body size (standard length ∼5 cm; weight ∼3.5 g) and ten days were given to them for adjusting to test aquarium (100 cm × 30 cm × 30 cm) and lab conditions. Each fish was housed in social isolation in an individual glass aquarium (1 fish/aquarium). Each aquarium was divided into three identical compartments, approximately 33 cm in length each. These compartments were separated from each other by opaque acrylic partitions with a window (10 cm × 10 cm) in the centre so that fish could swim through. During this adjustment period, fish were daily fed ad libitum once a day, and food was released into the middle compartment of the aquarium in a random time of day (light phase). The food offered was extruding floating pellets (38% protein, Purina Ltda). After that, we started the experimental procedures as follows. We offered 50 mg of feed (~1.5% of fish body mass) of food twice a day, in one side of the aquarium on the morning (09:00 h) and in the opposite side on the afternoon (17:00 h). The leftover food was removed after 1 h. In addition, all aquaria were always cleaned up by siphoning to keep a good water quality. The sides, morning or afternoon, were chosen at random for each aquarium, being the same throughout the experiment. This procedure was repeated for 30 consecutive days, except for the behavioural quantification days (15th and 30th), in which fish were not fed. On these test days, the position of the fish in the aquarium (morning, middle or afternoon sides) was registered, each 30 s, during 1 h interval in the morning (from 9:00 to 10:00 h) and in the afternoon (from 17:00 to 18:00 h), totalling 120 observations for a single fish in each sampling. Eight fish of each species were tested. If fish were at the correct side, a right choice was scored. The association learning was inferred according to the number of correct choices of all fish in each period of the day. Fish distributed randomly among compartments before training suggesting no evidence of learning.

All sides of each aquarium were opaque, except the front. In front of the experimental aquaria an opaque curtain with small holes was installed for behavioural observations. Also, two pipes were fixed on each aquarium from behind the curtain reaching one compartment, left or right, of the aquarium (middle compartment had no pipe at all) in order to drop the food. This set-up allowed us to enter, move ourselves inside the lab, and feed the fish without being seen. Each aquarium was visually isolated from the others, and fish could see only the opaque sheeting and the two pipes of its own aquarium. All test aquaria were supplied with constant aeration (an air stone localized in the middle compartment). Thus, no external visual cues were provided for the fish, making it difficult for them to associate place with other extra environmental signals except time, an essential procedure to be adopted for time place learning tests (Reebs, 1996, 1999).

During the experiment, the water temperature averaged ∼25 °C, pH ranged from 6.6 to 6.9, water-dissolved oxygen ranged from 6 to 7 mg/l, and nitrite and ammonia were lower than 0.5 and 0.25 ppm, respectively. The test was conducted in a room supplied with artificial illumination (daylight fluorescent tube), under a light–dark cycle of 12 h light and 12 h dark controlled by a timer, with abrupt transition between light and dark. The light was on at 07:00 h and off at 19:00 h.

The data, in terms of total number of observations (960 total observations = 120 observations/period × 8 fish) in which fish were in each compartment, were compared by Goodman’s proportion test (Goodman, 1965). Goodman test refers to the comparison of two proportions at same time based on the observed
proportions (or frequencies) with a theoretical proportion (the maximum proportion). Goodman test calculates a $G$-value for two proportions that is compared to a expected $G$-value, in which observed $G$ is statistically different when it is higher than the expected $G$. Statistical difference were considered when $P < 0.05$.

3. Results

The results obtained in the present study are presented in Fig. 1. Moreover, observed $G$ values referent to all comparisons pointed out below are presented in Table 1.

On day 15, during the morning quantification, angelfish had a proportion of choice for morning compartment significantly higher than for both afternoon and middle ones, besides it had a higher proportion of choice for afternoon compartment than middle one. During the afternoon quantification an opposite response profile was observed, wherein proportion of choice for afternoon compartment was significantly higher than for both morning and middle ones, while proportion of choice for morning compartment was higher than for middle one.

Table 1

<table>
<thead>
<tr>
<th>Sampling day</th>
<th>Fish species</th>
<th>Comparisons</th>
<th>Observation period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mor vs. Aft</td>
<td>Morning</td>
</tr>
<tr>
<td>Day 15th Angelfish</td>
<td>Mor vs. Mid</td>
<td>23.26</td>
<td>16.72</td>
</tr>
<tr>
<td>Day 30th Angelfish</td>
<td>Mor vs. Mid</td>
<td>9.03</td>
<td>6.63</td>
</tr>
<tr>
<td>Pearl cichlid Mor vs. Aft</td>
<td>0.83</td>
<td>6.40</td>
<td></td>
</tr>
<tr>
<td>Pearl cichlid Mor vs. Mid</td>
<td>14.79</td>
<td>9.66</td>
<td></td>
</tr>
<tr>
<td>Pearl cichlid Aft vs. Mid</td>
<td>13.89</td>
<td>2.06</td>
<td></td>
</tr>
<tr>
<td>Day 15th Angelfish Mor vs. Aft</td>
<td>3.81</td>
<td>4.38</td>
<td></td>
</tr>
<tr>
<td>Day 30th Angelfish Mor vs. Aft</td>
<td>0.77</td>
<td>8.74</td>
<td></td>
</tr>
<tr>
<td>Pearl cichlid Mor vs. Aft</td>
<td>1.39</td>
<td>6.06</td>
<td></td>
</tr>
<tr>
<td>Pearl cichlid Mor vs. Mid</td>
<td>1.77</td>
<td>1.55</td>
<td></td>
</tr>
<tr>
<td>Pearl cichlid Aft vs. Mid</td>
<td>3.16</td>
<td>4.49</td>
<td></td>
</tr>
</tbody>
</table>

If $G_{observed} > G_{expected}$, the null hypothesis is rejected ($P < 0.05$). In this case $G_{expected} = 2.39$. Mor, morning compartment; Aft, afternoon compartment; Mid, middle compartment.

a Fish species are angelfish, *Pterophyllum scalare*, and pearl cichlid, *Geophagus brasiliensis*.

Fig. 1. Total number of observations in which fish were in each compartment for both angelfish (*Pterophyllum scalare*) and pearl cichlid (*Geophagus brasiliensis*). Data were analysed by using the sum of proportions to each compartment of all tested fish (120 observations/feeding period $\times 8$ fish) at each period, morning or afternoon, on the test days. Proportions that do not share a same letter are statistically different to each other within a same period of each day of test (Goodman’s proportion test; $P < 0.05$). Compartments (aquarium position): (●) morning; (○) afternoon; (△) middle.
In the case of pearl cichlid, during morning sampling, it had a proportion of choice for middle compartment significantly higher than for both afternoon and morning ones. These two latter ones were similar. During afternoon quantification the proportion of choice for both middle and afternoon compartments was similar to each other and significantly higher than the proportion for morning side.

On day 30, during morning quantification angelfish had proportion of choice for both morning and afternoon compartments similar to each other and significantly higher than for middle one. During afternoon quantification proportion of choice for afternoon compartment was observed significantly higher than for both morning and middle ones, while proportion of choice for the morning compartment was higher than for the middle one.

Concerning pearl cichlid sampling, during morning, proportion of choice for morning and afternoon compartments was similar to each other. The same was observed when proportion of choice for morning and middle compartments was compared. However, proportion of choice for afternoon compartment was observed significantly higher than for middle one. During afternoon quantification the proportion of choice for the afternoon compartment was significantly higher than the proportion for both middle and morning sides, and these two latter ones were similar.

4. Discussion

The present study shows evidence that individually reared angelfish learns to switch tank sides at the correct period of the day in order to get food after a 15-day period of conditioning, although it was not consistent after 30 days of training. It suggests that this species may display TPL when isolated, as it had been previously reported for grouped angelfish (Gómez-Laplaza and Morgan, 2005). On the other hand, in the case of pearl cichlid, no clear pattern of side switching was found in this species for the TPL task imposed. As far as we know, there are just five studies regarding TPL in fish in the literature (Reebs, 1993, 1996, 1999; Gómez-Laplaza and Morgan, 2005; Delicio et al., 2006a) and the present study provides new data about this issue, it is the first investigation reporting evidence of TPL in social isolated fish (1 fish/aquarium).

Testing fish in isolation eliminated the possible drawbacks concerning social influence on feeding behaviour when studying grouped fish. In a shoal of fish it is not necessary that all individuals learn the time and place synchronisation, since the leader of the shoal can “command” the group during foraging navigation (Reebs, 2000). Most fish tested in shoals may be in the correct side because they simply followed their leader. However, this test also provided a task with decreased response cost, because of the lack of feeding competition among shoalmates. Hence, fish would have no further consequences if they had taken an incorrect place choice (they would not miss a foraging opportunity), what could decrease the chance of fish displaying TPL. Although angelfish presented this ability until some extent (because it occurred on the 15th test day, but not on the 30th one), the present study indicates that angelfish can display this ability, and in a favourable circumstance any angelfish that become shoal leader could conduct their shoalmates during foraging tasks.

The two tested species presented different patterns of navigation during experimental observations. While pearl cichlid seems to switch aquarium compartment at random, angelfish exhibited a more standardized pattern of compartment visiting. The pearl cichlid had no clear preference for any compartment, while angelfish clearly preferred the compartments where food arrived (afternoon and morning compartment instead of middle one). Even though angelfish visited all compartments during behavioural observations (the feeding window), they visited more frequently the correct side at 15th day observation, suggesting that angelfish considered the place with higher probability to find food dependent on the time of the day. Surprisingly, it was not consistent for 30th day observation. On this day, although angelfish chose the compartment correctly during afternoon sampling, it did not occur for morning one. For morning observation, frequency of choices between morning and afternoon compartment were similar to each other. A possible explanation would be that fish increased activity for searching food during morning sampling, exactly because food was not delivered on the test days (at morning compartment in this case). This fact would have induced angelfish visit or stay in the middle and afternoon compartment more frequent at morning interval. Consequently, it was enough to eliminate the statistical difference among morning and afternoon compartment observations. Conversely, for afternoon observation, they preferred the correct compartment, it might be due to afternoon test session is different from the morning test session because the fish are hungrier and they have had exposure to a test earlier in the day that might have affected their performance, facilitating their preference for afternoon side (the correct one in this case).

Pearl cichlid curiously spent a considerable time in the middle compartment during the test sessions, a place not associated with the feeding window and apparently any other resources. Angelfish even when they did not show TPL (30th sampling) they at least preferred the compartments associated with the feeding window. This would suggest that pearl cichlid did not know that it was feeding time or which places were associated with feeding. In the present experiment, the middle compartment was equipped with an air stone. Pearl fish tended to stay near the stone or even behind it, as they were considering this artefact a refuge (data not shown). It might be due to pearl cichlid behaviour, it is a very aggressive species, with well-evident territorial behaviour and establishment of social hierarchy, and on the contrary to angelfish, they do not shoal (Delicio et al., unpublished data). We observed that Nile tilapia, a cichlid with similar aggressive behaviour (Volpato et al., 2003) of pearl fish, prefers a place that has a refuge (shelter) instead of a non-enriched place with any resource and a non-enriched one where they were fed in a place preference test (Delicio et al., 2006b). Thus, it would be a plausible explanation for the considerable time that pearl cichlid spent in the middle compartment.

This study was performed in a laboratory equipped with an artificial fluorescent light to set-up dark and light cycle. This procedure does not allow a variation in light intensity during
the light-phase of the photoperiod. Hence, fish might not clearly distinguish time variation dependent on light intensity.

The aquaria had identical feeding areas, besides they had opaque background, bottom and small-side walls, and the front was covered with black sheeting with small holes for behavioural quantifications. Thus, fish were not able to visually detect the researcher and no aquarium extra cue was provided. Although fish could have perceived people movements in the lab, it was careful and random, thus undoubtedly minimising this potential cue. Hence, although exogenous extra cues might have taken place, they seemed rather unlikely.

In the present study, we used 100-cm long aquaria. Long aquaria provide widely separated places, necessary for fish to consider the tank compartments as truly independent. The experimental aquaria were divided in three well-defined compartments by using opaque partitions which hindering fish to sight all compartments and each partitions had just a window (10 cm × 10 cm) in the centre whereby fish could swim through, decreasing the fish access between compartments. Fish were also seven times shorter than each compartment length. Based on this, it is unlikely that fish considered the aquaria compartments as a single place, not associating two places with two feeding times.

Herein, fish were tested isolated. The absence of shoalmates obviously eliminated competition for food and the cost for not being in the right place at the right time was probably decreased. Accordingly, *N. crysoleucas* (Reebs, 1996), *G. maculatus* (Reebs, 1999), and *P. scalare* (Gómez-Laplaza and Morgan, 2005) successfully displayed time-place learning when tested in groups. Moreover, the only test using isolated fish failed to demonstrate TPL in Nile tilapia, *O. niloticus* (Delicio et al., 2006a). In the case of pearl cichlid, it is possible that isolation limited them to exhibit TPL. In a condition of higher competition for food, TPL could have been observed because grouping provides an incentive for efficient foraging and creates a cost for not learning the time-place association. However, pearl cichlid is a very aggressive species, with well-evident territorial behaviour and establishment of social hierarchy (Delicio et al., unpublished data). The territoriality inherent of some cichlid species is a limitation for conducting group studies, as reported in our previous paper for Nile tilapia (Delicio et al., 2006a). It was impossible to test Nile tilapia groups because dominant fish tended to monopolize food patches, which became unavailable to other lower-ranked fish. Another territorial cichlid, convict cichlid (*C. nigrofasciatum*, Reebs, 1993), when tested in groups also failed to display TPL. In the case of angelfish, which is a shoaling species (Gómez-Laplaza, 2005), although single housing affects its feeding and locomotion pattern (Gómez-Laplaza and Morgan, 1991, 1993), social isolation has not impeded fish to display TPL herein. Thus, we suggest that increased food competition testing grouped fish would not have improved the experimental protocol for pearl cichlid. Based on these preliminary evidences, we speculate that TPL in cichlid fish might be associated with their social characteristics, wherein the most territorial and aggressive species appear not to display this behaviour, while shoaling ones do. Nevertheless, angelfish can also be territorial (Chellappa et al., 1999; Gómez-Laplaza, 2002; Gómez-Laplaza and Morgan, 2003) and establish temporary feeding territories. In fact, there are practical reasons why we cannot test certain fish in groups. However, group testing is not the only way to increase response cost. We could give food for less time each day, make them swim further for it, put in more obstacles for going to the incorrect places, make them do an operant task in the correct location to get food. In any case, this raises new question about factors influencing TPL in fish. For instance, differences between species in habituation to housing in a new environment (experimental aquarium) could also play an important role. These aspects may be interesting research subjects to be addressed in future investigations.

Another possibility concerns fish feeding habits. While angelfish is a species that fed zooplankton (Nandini and Sarma, 2000), pearl cichlid is omnivorous (Vono and Barbosa, 2001). Thus, to synchronize feeding activity with prey locomotor activity or daily distribution is more relevant for a fish that fed zooplankton than for an omnivorous one. Zooplankton predated by angelfish varies vertically (Sarma et al., 2003). This restriction in the feeding habits of angelfish could be a factor facilitating they display TPL, once if they synchronized their foraging activity with zooplankton displacement through water column, they would have a more efficient foraging.

According with the above statements, angelfish presented TPL because it is a characteristic inherent to this species, although the mechanism underlying this remains unknown. However, pearl cichlid either is not able to present this ability or it is necessary some context for them to display TPL. For instance, it was previously reported for mammals that food restriction or increased response cost task induce these animals to display TPL (Widman et al., 2000; Lukoyanov et al., 2002; Widman et al., 2004). Thus, further investigations modulating cost of the task or motivation for foraging would be profitable for understanding this phenomenon in fish species that initially do not show TPL in single tasks of food and place synchronization, such as convict cichlid (Reebs, 1993), Nile tilapia (Delicio et al., 2006a), and pearl cichlid (present study).

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References


