J Ethol (2006) 24:195–200
DOI 10.1007/s10164-005-0175-1

SHORT COMMUNICATION

Helton C. Delicio · Ana C. Luchiari · Rodrigo E. Barreto
Ana L. Marcondes

Testing time–place learning in the cichlid fish Nile tilapia

Received: January 19, 2005 / Accepted: August 10, 2005 / Published online: October 22, 2005

Abstract Time–place learning based on food association was investigated in the fish Nile tilapia. During a 30-day period, food was placed at one side of the aquarium (containing three compartments) in the morning and at 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, 30th). During the test day, fish were not fed. The Nile tilapia did not learn to switch sides at the correct day period in order to get food, suggesting thus that this species does not have time–place learning ability.

Key words Time–place learning · Feeding · Fish · Nile tilapia · *Oreochromis niloticus*

Introduction

Food is the main requirement for all animals, which therefore spend considerable time and energy searching and acquiring food. Thus, to increase foraging efficiency is probably an important challenge in everyday life for animals. Investigations so far show that cognitive processes of learning may play an important role in the foraging behaviour in animals (Shettleworth 2001), including fish (Kieffer and Colgan 1992). Hence, the adaptiveness of particular learning abilities for complex foraging niches that increase animal foraging efficiency may result from the evolution of particular abilities (McLean 2001), among which we can highlight time–place learning (Reebs 1993, 2002). Time–place learning is the ability to associate different places at different times of a day. Generally, this phenomenon is associated with the synchronization of visiting a patch with available food in a specific time. This kind of learning has been reported for many animals, such as insects (Breed et al. 2002), birds (Wilkie et al. 1996), rats (Thorpe and Wilkie 2002), and fish (Reebs 1996, 1999). Indeed, food items may change daily, seasonally, and/or spatially; and it seems likely that some learning abilities play a role in foraging flexibility (Dill 1983). Accordingly, time–place learning specifically may have an important function in fish survival, as it might increase foraging efficiency due to an improvement of a fish's ability to locate and explore patches just when food is available. For instance, fish may save energy when seeking for food, because when fish have access to both abundant food and enough time for feeding, there is no immediate need to synchronize feeding time with food arrival, but when food availability and time for foraging is restricted, synchronization of feeding activity guarantees that the feeding window will not be missed (Volpato and Trajano 2005).

Time–place discrimination has been shown be reliable in several bird and insect species, but seldom in rat and fish (Widman et al. 2000). Several studies have been addressed to comprehend rat time–place learning, but very few studies have focused on fish; and more available information, therefore, is necessary for our understanding of this phenomenon in this taxon. To date, this learning ability has been reported in the cyprinid fish (*Notemigonus crysoleucas*; Reebs 1996) and in the galaxid (*Galaxias maculatus*; Reebs 1999). However, it was not observed when tested in the convict cichlid (*Cichlasoma nigrofasciatum*; Reebs 1993). Even so, cichlid is a fish family with several species inhabiting a wide variety of habitats, and consequently learning ability among them may vary largely, allowing us to suppose that time–place learning ability may be present in this fish family.

Reebs (1993) stated that failure of those convict cichlids to associate time and place may have been caused by some methodological points, such as a low cost of travel between corners, a limited number of rewards each day, and interference from learning the signal–food association. The feeding signal used might have impaired the association between food and patch, and fish could have learned the association food and signal independently of the patch. Moreover, the food patches provided allowed fish to easily inspect all corners when the food signal was displayed,

---

H.C. Delicio (✉) · A.C. Luchiari · R.E. Barreto · A.L. Marcondes
Departamento de Fisiologia, Instituto de Biociências, UNESP, Rubião Jr s/n, Botucatu, SP 18618-000, Brazil
Tel. +55-14-38116251; Fax +55-14-38113744
e-mail: hdelicio@ibb.unesp.br

decreasing the necessity of learning. Finally, a larger number of rewards could have improved the conditioning. These statements imply that the convicts failing to display this behaviour might have been either due to a real absence of this ability in this species or due to the experimental design.

Nonetheless, the knowledge of time–place learning ability in a territorial cichlid remains unknown. In the present study, we investigated whether the territorial cichlid Nile tilapia, *Oreochromis niloticus*, displays time–place learning ability, considering some methodological aspects might have impaired Reebs (1993) from reporting it in convict cichlids: (1) no feeding signal was provided and (2) we used long aquaria divided in compartments with opaque partitions having one small window, to increase the difficulty of travelling between food patches, to ensure the fish could consider compartments as independent, and to prevent fish from seeing food when it arrived. Nile tilapia was chosen, first, because it shows evidence of a feeding circadian rhythmicity (Boujard 1999), which seems to be an essential part of the time–place learning mechanism. Second, previous studies reported that Nile tilapia can present food-anticipatory behaviour once conditioned (Marcondes 2002). Third, Nile tilapia has different food habits when compared with convicts. Both Nile tilapia and convict cichlids are omnivorous. However, the former is an omnivore with a herbivorous tendency showing much more food specialization, having a preference for specific aquatic macrophytes (Setlikova and Adamek 2004) and some fish and insect prey (Njiru et al. 2004), while the latter is an omnivore with a carnivorous tendency, showing no specific prey preference (Trujillo-Jimenez 1998). According with these statements, we assumed that the time–place learning ability is more important for a fish with a more specialized diet, like Nile tilapia, because it would save time and energy in order to get food, once the food availability tended to be somewhat limited. Finally, Nile tilapia is a territorial species that shows evident aggressive behaviour in groups when competing for food (Carrieri and Volpato 1991), and because of that, herein, we assessed whether it were possible to test them in groups, but, as expected, food patches were monopolized by dominant fish (further details in the Materials and methods). Thus, we conducted the main test using isolated fish (1 fish/aquarium).

Materials and methods

The stock population were juvenile fish (*O. niloticus*) held in an indoor 1200-l tank (~1 fish per 4 l; holding density ~0.75 g/l) for about 2 months. The tank was supplied with constant aeration and a dechlorinated water flow. During this time, the temperature averaged 23 ± 1°C and the water was maintained with low levels of ammonia (< 0.25 ppm) and nitrite (< 0.50 ppm). The photoperiod was set from 0600 hours to 1800 hours. Food was offered once a day (38% protein; Purina Ltd., Campinas, Brazil).

First, we decided to test the Nile tilapia in groups (5 fish/aquarium) to increase competition for food, providing an incentive for efficient foraging and creating a cost for not learning the time–place association. Reebs (1996, 1999) successfully used this procedure. However, Nile tilapia is a very aggressive species, with well evident territorial behaviour and establishment of a social hierarchy (Volpato et al. 2003; Corrêa et al. 2003). Thus, when Nile tilapia were tested in groups, the dominant fish defined their territories, having higher access to food or monopolizing a food patch and hindering fish in lower social levels from feeding. Moreover, some subordinate fish suffered cannibalism. As it was impossible to conduct the test in groups of fish, a second experiment was made using a single fish in each experimental aquarium. Two trials was performed, in each of which eight Nile tilapia were tested.

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 an abrupt transition between light and dark. The light came on at 0700 hours and off at 1900 hours. The test aquarium (100 × 30 × 30 cm) was divided into three identical compartments, each approximately 33 cm in length. These compartments were separated from each other by opaque acrylic partitions with a window (10 × 10 cm) in the center, so that fish could swim through. In front of the experimental aquaria, an opaque curtain with small holes was installed for behavioral observations. Also, two pipes were fixed to each aquarium from behind the curtain reaching one compartment, left or right, of the aquarium (the middle compartment having no pipe at all) in order to drop the food. This set-up allowed us to enter, move ourselves inside the laboratory, and feed the fish without had been being seen by them. Each aquarium was visually isolated from the others and then each fish could see only the opaque sheeting and the two pipes of its aquarium. Thus, no external visual cues were probably provided for the fish, making it impossible for them to associate place with any other signal that was not time, which is an essential procedure to be adopted for time–place learning tests (Reebs 1996, 1999).

The fish from our stock were chosen by body size (standard length ~5 cm, weight ~3.5 g) and ten days were given them to adjust to lab conditions. During this adjustment period, fish were fed daily and food was released into the middle compartment of the aquaria. After that, we started the experimental procedures as follows. We offered 50 mg (~1.5% of the fish biomass) of commercial food twice a day, at one side of the aquarium in the morning (0900 hours) and at the opposite side in the afternoon (1700 hours). The leftover food was removed after 1 h. The two sides, morning or afternoon, were chosen at random for each aquarium and were kept the same throughout experiment. This procedure was repeated for 30 consecutive days, except on the behavioural quantification days (15th, 30th), during which the fish were not fed. In these test days, the position of the fish in the aquarium (morning, middle, or afternoon side) was registered every 30 s during a 1-h interval in the morning (from 0900 to 1000 hours) and in the afternoon (from 1700 to 1800 hours), totalling 120 observations for a single fish in each sampling. If the fish was at the correct side, a right choice was scored. Association learning was inferred according to the number of correct choices by all fish during each period of the day.

The data did not show normal distribution, requiring non-parametric statistics. The frequency of correct side choices was compared by Friedman test for morning and afternoon periods. For this data analysis, we regarded total frequency mean and mean frequency over time (considering the sum of frequencies of place choice in consecutive intervals of 5 min throughout the 1-h period of observation, totalling 12 blocks). Statistically significant differences were considered when $P < 0.05$. A power analysis indicated that the test run had 81% power to detect a difference of 3.5 between frequencies.

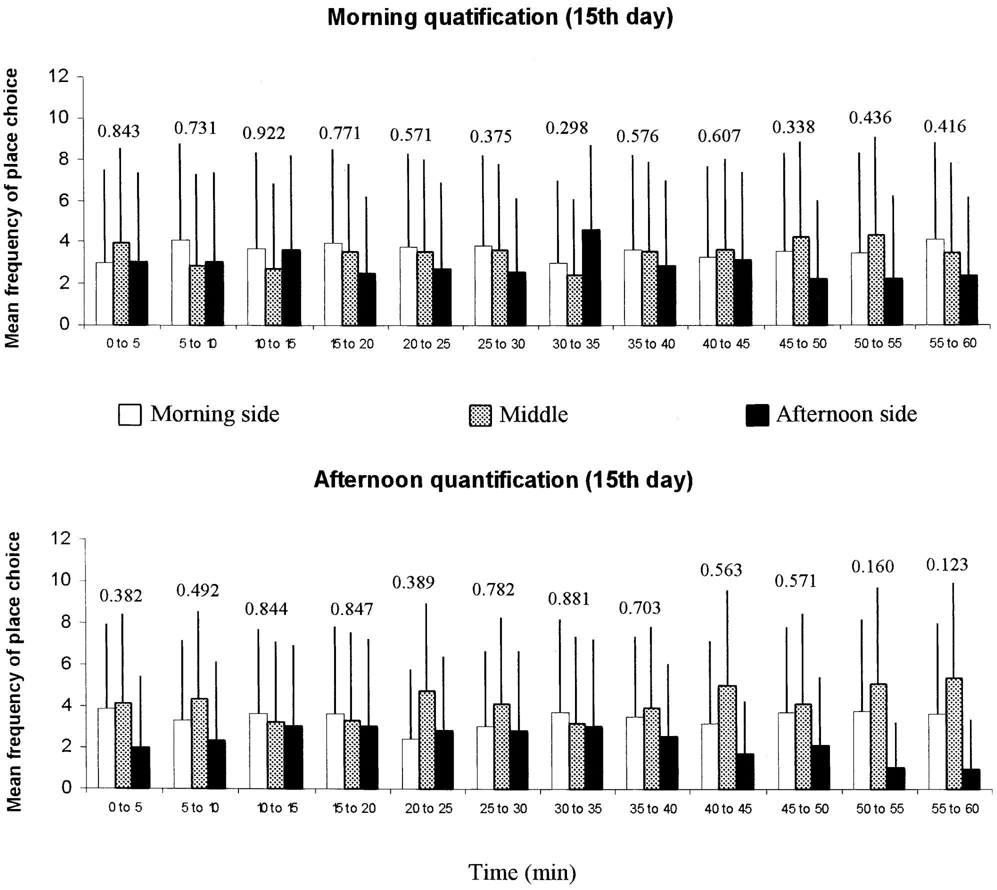
During the experiment, the water temperature averaged ~25°C, pH ranged from 6.6 to 6.9, water-dissolved oxygen ranged from 6 mg/l to 7 mg/l, and nitrite and ammonia were lower than 0.5 ppm and 0.25 ppm, respectively.

Results

Statistical differences were not reported for the mean frequency overtime (Fig. 1) nor for the mean of total frequency (Fig. 2) during 15th-day observations. During 30th-day observations, no statistical difference was observed for morning quantifications, comprising both the mean frequency over time (Fig. 3) and the mean total frequency (Fig. 4). However, for afternoon quantifications, we noted a statistical difference only in the 25–30 min block, wherein the middle compartment had a higher frequency of visit than the afternoon one, while the frequency of visits to the morning compartment was statistically similar to both the middle and afternoon ones (Fig. 3). When we considered the mean total frequency, visits to the morning compartment were higher than visits to the afternoon one, whereas visits to the middle compartment were similar to the frequency of visits observed for the other two compartments (Fig. 4).

Discussion

In the present study, we observed that the cichlid fish Nile tilapia does not have a time–place learning ability based on food association. Even when some statistical difference was found, it corresponded to a higher level of wrong choices. Time–place learning related to food in fish has only been demonstrated in *N. crysoleucas* (Reebs 1996) and in *G. maculatus* (Reebs 1999). Similarly, no evidence of time–place learning ability has been observed in another cichlid, *C. nigrofasciatum* (Reebs 1993).


Morning quatification (15th day)

Afternoon quantification (15th day)

Fig. 1. A test of time–place learning in the cichlid fish Nile tilapia. Data are expressed as mean (+ SD; $n = 16$). No statistical difference was observed among the frequencies of visits into any compartment over time. The P value (Friedman test) is located above each column

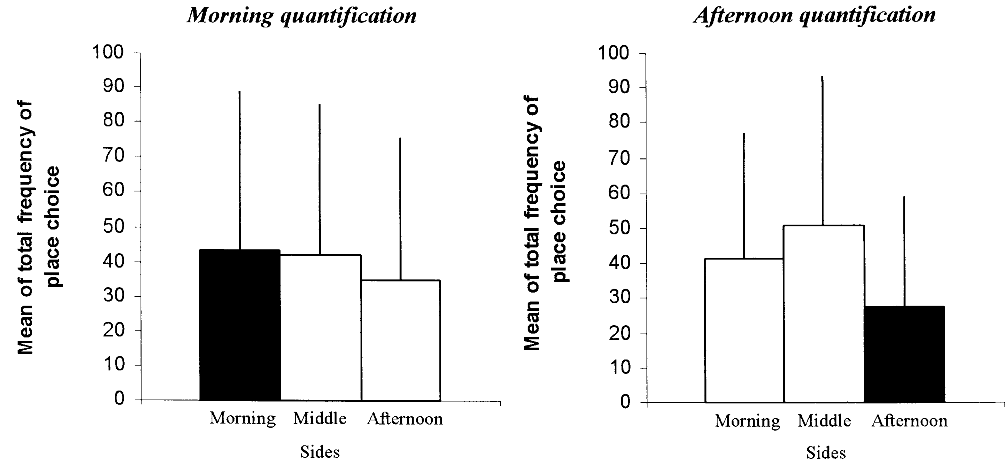


Fig. 2. A test of time–place learning in the cichlid fish Nile tilapia. Data are expressed as mean (+ SD; $n = 16$). No statistical differences were observed among the mean total frequency of visits into any compartment at both morning ($P = 0.890$; Friedman test) and afternoon quantifications ($P = 0.528$; Friedman test). Columns in *black* represent correct choices

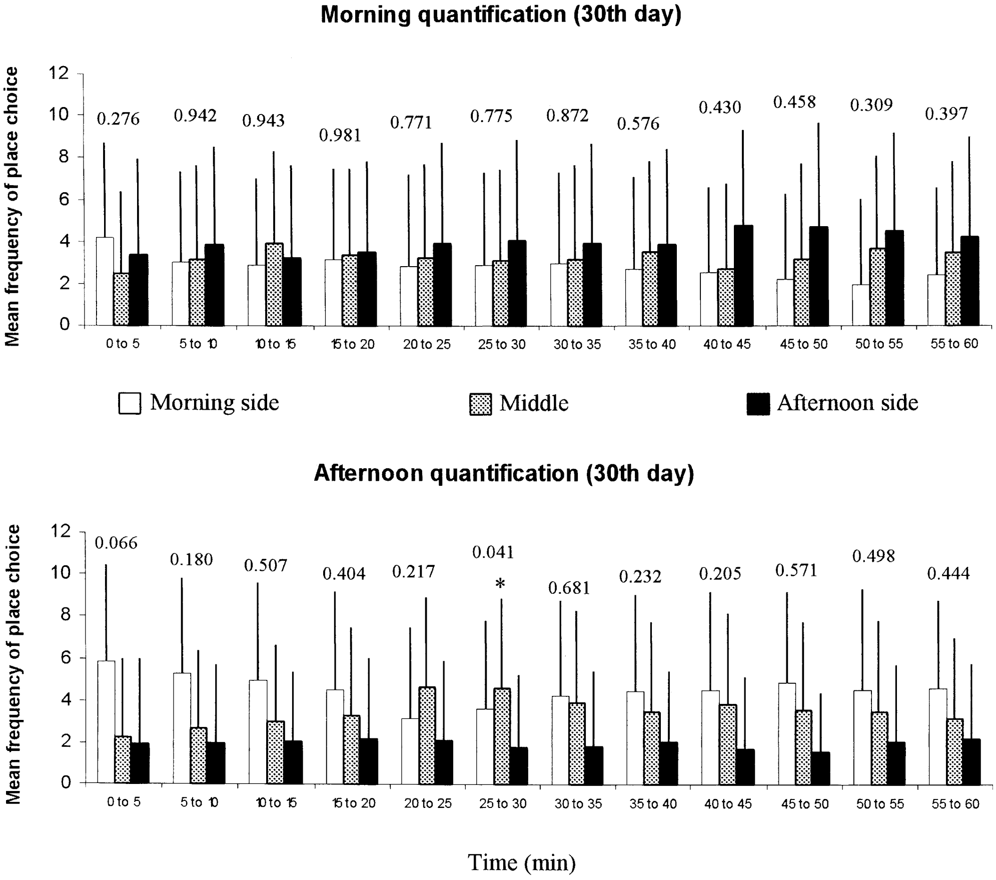


Fig. 3. A test of time–place learning in the cichlid fish Nile tilapia. Data are expressed as mean (+ SD; $n = 16$). No statistical differences were observed among the frequencies of visits into any compartment over time, exceptat the 25–30 min block, where the middle compartment had a higher frequency of visits than the afternoon one, showing a higher level of wrong choices. The *P* value (Friedman test) is located above each column

Reebs' (1993) experiment used 10 or 30 consecutive days of time–place training for convict cichlid and it was argued that they would have learned in a longer training period if the fish had not stabilized their time spent in each feeding area from the eighth day of conditioning. Moreover, similar periods of training were adequate to demonstrate time–place learning ability in *N. crysoleucas* (Reebs 1996) and in *G. maculatus* (Reebs 1999). Herein, a similar period of conditioning was used, and even when assessing the side preference at both the 15th and 30th days of training, this ability was not observed in Nile tilapia. In our experiment, fish also tended to stabilize the time they spent in the compartments; and fish even spent their time in the incorrect compartment. This provides us with evidence that the period of training had no influence on spatial–temporal task discrimination by tilapia.

In the present study, we used 100-cm long aquaria. To use long aquaria is one way to provide widely separated places, which is necessary for fish to consider the places as truly independent. Moreover, the experimental aquaria were divided in three well defined compartments by opaque partitions which prevented the fish from seeing all compart-

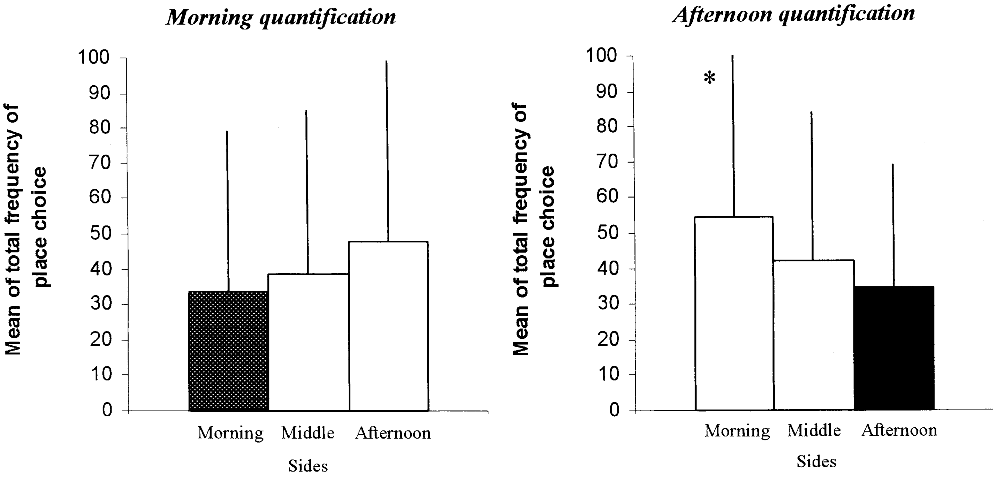


Fig. 4. A test of time–place learning in the cichlid fish Nile tilapia. Data are expressed as mean (+ SD; $n = 16$). No statistical differences were observed among the mean total frequency of visits into any compartment at the morning quantification ($P = 0.534$; Friedman test). *Asterisk* Mean total frequency of visits into the morning compartment was higher than those observed for the afternoon compartment during afternoon quantifications, indicating a higher level of wrong choices ($P = 0.0465$; Friedman test). Columns in *black* represent correct choices

ments; and each partition had just a window (10 × 10 cm) in the center whereby fish could swim through, decreasing the fish access between compartments. Also, the fish bodylength was about seven times shorter than each compartment length. Based on these facts, it is unlikely that tilapia considered the aquaria compartments as a single place and not associating two places with two feeding times. Reebs (1993) also could not observe time–place learning in the convicts when switching from smaller aquaria to longer. Thus, we assume that the length of the aquaria used was not a limiting factor to tilapia displaying time–place learning.

Herein, fish were tested in isolation. The absence of shoalmates obviously eliminated competition for food and the cost of not being in the right place at the right time was probably mitigated. Although it is not a clarified question, in fact *N. crysoleucas* (Reebs 1996) and *G. maculatus* (Reebs 1999) successfully displayed time–place learning when tested in school, but no test using isolated fish of these two species or others has been conducted. Regardless, this may have been the factor that prevented us from demonstrating this ability in Nile tilapia. However, as explained in the Materials and methods, it was impossible to conduct the test in groups of fish due to their aggressiveness. For instance, when in a group, dominant Nile tilapia monopolized a food patch, making it unavailable for the other fish (data not shown). Thus, we suggest that increased food competition by grouping fish would not have enhanced the experimental protocol in this species. However, if we had severely reduced the available feeding time (we used a 1-h feeding time) to only a few minutes, we might have increased the cost of wrong choices. However, we did not observe a clear anticipatory displacement for a food patch by any of the 16 fish tested during feed days—although anticipatory feeding activity has been reported for Nile tilapia in a learning task without time and place discrimination (Marcondes 2002). Overall, the fish only went to food areas when the food had already arrived. The amount of food offered was only ~1.5% of fish biomass, a lower amount than that suggested as suitable for tilapia (~5%; Stickney 1994) and this might have represented a cost in not learning. Even so, the fish did not pay more attention to the time–place association. Moreover, the fish did not need to socially interact or spend energy on other demanding activities or stressful situations, the energy requirement hence in the aquarium conditions may have been low and 1.5% may have represented an ideal amount of food for tilapia. Lukoyanov et al. (2002) reported that food restriction facilitates rat time–place learning and rats that were fed ad libitum failed to perform the task. Moreover, Widman et al. (2000, 2004) reported that rats augmented time–place discrimination when submitted to an increased response cost task, suggesting that rats are not able to promptly display time–place learning but that the response cost is an important trigger for this kind of discrimination. Accordingly, Nile tilapia might display this behavior in a task with increased cost or in a condition of food restriction, and these possibilities should be tested in future studies.

Herein we used Nile tilapia juveniles. It has been reported that learning capability in fish varies ontogenetically (Masuda and Ziemann 2000). Those authors reported that Pacific threadfin fish (*Polydactylus sexfilis*) of 50 mm and 90 mm had a better learning capability than did smaller (22 mm, 36 mm) or larger (130 mm) ones. This suggests that time–place learning might be affected by age and another result could have been reached, had we had used Nile tilapia adults. This possibility should be clarified in future investigations.

Finally, the two tests using a cichlid species, *O. niloticus* (present study) and *C. nigrofasciatum* (Reebs 1993) failed to demonstrate time–place learning. Although it is premature to assume that this learning ability is absent in this fish family, it would be one possibility. This might be a good hypothesis to be tested in further experiments using different species of several genera of cichlids. The cichlid family comprises several species dwelling in different sorts of habitats, which might have selected different learning ability among cichlid species. For instance, both Nile tilapia and convict cichlids are omnivorous with just some differences, the former being more feed-specialized than the latter, but even so neither presented time–place learning. The lack of this behavior might be involved with their omnivorous habit. It would be easier for them to switch food sources when one food becomes less abundant or available than another, thus decreasing the necessity to learn some time and place discrimination. Accordingly, a test of this learning ability in a carnivorous cichlid species would be more worthwhile, since synchronizing their activity with their prey might be profitable in terms of foraging and survival. Another possibility involves the territorial habit present in the cichlid family. A fish that has a defined territory could simply defend it and automatically protect its existing resources, like shelters and food, thus decreasing the necessity to display time–place learning for feeding. Thus, testing this learning ability in shoaling cichlids would also be valuable.

Acknowledgements The authors wish to acknowledge Dr. S.G. Reebs for valuable suggestions for this study and Mr. A.C.B. Tardivo for indispensable technical assistance. This research agrees with the Ethical Principles in Animal Research adopted by the Brazilian College of Animal Experimentation and was approved by the Ethical Committee for Animal Research (CEEA) of the Instituto de Biociências/UNESP, protocol 122/02.

References

Boujard T (1999) The circadian rhythms of feeding activity in teleosts species. Cybium 23:89–112

Breed MD, Stocker EM, Baumgartner LK, Vargas SA (2002) Time–place learning and the ecology of recruitment in a stingless bee, *Trigona amalthea* (Hymenoptera, Apidae). Apidologie 33:251–258

Carrieri MP, Volpato GL (1991) Does snatching frequency really indicate food ingestion in the Nile tilapia. Physiol Behav 50:489–492

Corrêa SA, Fernandes MO, Iseki KK, Negrão JA (2003) Effect of the establishment of dominance relationships on cortisol and other metabolic parameters in Nile tilapia (*Oreochromis niloticus*). Braz J Med Biol Res 36:1725–1731

Dill LM (1983) Adaptative flexibility in the foraging behaviour of fishes. Can J Fish Aquat Sci 40:398–408

Kieffer JD, Colgan PW (1992) The role of learning in fish behaviour. Rev Fish Biol Fish 2:125–143

Lukoyanov NV, Pereira PA, Mesquita RM, Andrade JP (2002) Restricted feeding facilitates time–place learning in adult rats. Behav Brain Res 134:283–290

Marcondes AL (2002) Estresse e habilidade de aprendizado na tilápia-do-Nilo. MSc thesis, UNESP, Botucatu

Masuda R, Ziemann DA (2000) Ontogenetic changes of learning capability and stress recovery in Pacific threadfin juveniles. J Fish Biol 56:1239–1247

McLean AN (2001) Cognitive abilities – the result of selective pressures on food acquisition? Appl Anim Behav Sci 71:241–258

Njiru M, Okeyo-Owuor JB, Muchiri M, Cowx IG (2004) Shifts in the food of Nile tilapia, *Oreochromis niloticus* (L) in Lake Victoria, Kenya. Afr J Ecol 42:163–170

Reebs SG (1993) A test of time–place learning in a Cichlid fish. Behav Process 30:273–282

Reebs SG (1996) Time–place learning in golden shiners (Pisces: Cyprinidae). Behav Process 36:253–262

Reebs SG (1999) Time–place learning based on food but not on predation risk in a fish, the inanga (*Galaxia maculatus*). Ethology 105:361–371

Reebs SG (2002) Plasticity of diel and circadian activity rhythms in fishes. Rev Fish Biol Fish 12:349–371

Setlikova I, Adamek Z (2004) Feeding selectivity and growth of Nile tilapia (*Oreochromis niloticus* L) fed on temperate-zone aquatic macrophytes. Czech J Anim Sci 49:271–278

Shettleworth SJ (2001) Animal cognition and animal behaviour. Anim Behav 61:277–286

Stickney RR (1994) Principles of aquaculture. Wiley–Interscience, New York

Thorpe CM, Wilkie DM (2002) Unequal interval time–place learning. Behav Process 58:157–166

Trujillo-Jimenez P (1998) Trophic spectrum of the cichlids *Cichlasoma* (*Parapetenia*) *istlanum* and *Cichlasoma* (*Arconcentrus*) *nigrofasciatum* in the Amacuzac River, Morelos, Mexico. J Freshwater Ecol 13:465–473

Volpato GL, Trajano E (2005) Biological rhythms. In: Val LA, Val VMFA, Randall DJ (eds) Fish physiology, vol 23. Elsevier, San Diego

Volpato GL, Luchiari AC, Duarte CRA, Barreto RE, Ramanzini GC (2003) Eye color as an indicator of social rank in the fish Nile tilapia. Braz J Med Biol Res 36:1659–1663

Widman DR, Gordon D, Timberlake W (2000) Response cost and time–place discrimination by rats in maze tasks. Anim Learn Behav 28:298–309

Widman DR, Sermania CM, Genismore KE (2004) Evidence for time–place learning in the Morris water maze without food restriction but with increased response cost. Behav Process 67:183–193

Wilkie DM, Carr JAR, Siegenthaler A (1996) Field observations of time–place behaviour in scavenging birds. Behav Process 38:77–88