Journal of Applied Ichthyology

J. Appl. Ichthyol. 29 (2013), 239–241

ISSN 0175–8659

Received: July 28, 2011
Accepted: April 9, 2012
doi: 10.1111/jai.12026

Short communication

Motivation and time of day affect decision-making for substratum granulometry in the Nile tilapia *Oreochromis niloticus*

By R. H. A. Freitas and G. L. Volpato

Laboratory of Animal Physiology and Behavior, Research Center on Animal Welfare – RECAW, Department of Physiology, IBB, CAUNESP, UNESP, Botucatu, SP, Brazil

Introduction

Determination of animal preferences has been stimulated to fulfill welfare requisites (Dawkins, 2006), assuming that they might have better welfare benefits when they receive preferential conditions (Volpato et al., 2007, 2009; Volpato, 2009). In this context, the preference for substratum granulometry was determined in the Cichlidae Nile tilapia, *Oreochromis niloticus*, an important aquaculture species in several countries.

Proper substratum has been shown to enhance growth, production, survival (Uddin et al., 2007) and reproduction (Takemon and Nakanishi, 1998; Galhardo et al., 2009) in Nile tilapia and other Cichlidae. Moreover, the Nile tilapia prepares nests for mating by digging in the substratum, a behavior affected by the type of bottom available (Mendonça and Gonçalves-de-Freitas, 2008). More importantly, because the Cichlidae use their mouths to dig, the particle size of the substratum might be relevant.

Therefore, the preference of Nile tilapia for substrata of various particle sizes was investigated. It was found that substratum size was chosen according to the behavioral context of nesting – or not nesting, a decision more consistently expressed in the morning.

Materials and methods

Oreochromis niloticus obtained from a monoculture fishery were held in tanks (1 fish/10 L) for 1 month prior to the begin of experiments. Fish were fed *ad libitum* once per day and maintained with continuous aeration and a 12-hour light/12-hour dark photoperiod.

Basically, fish were held in individual glass aquaria provided with five compartments: one with an acrylic base, and the others with different particle sizes of substratum. Fifteen fish (7.1 ± 0.7 cm standard length and 0.95 ± 0.26 cm mouth maximum gape) were allowed to visit each compartment freely so that the daily (morning and afternoon) registered fish position over 4 days could infer fish preference.

Five experimental aquaria (60 × 20 cm base; 25 cm high) had bottoms divided into five equal-sized compartments (12 × 20 cm base each) placed side-by-side and separated by opaque partitions from the adjacent compartments. This compartment distribution resulted in a 5-cm corridor for the fish to pass freely from once compartment to the next. The tested substrata (3-cm layer) were comprised of particles with mean (±SD) largest diameter of 0.12 ± 0.04 cm, 0.57 ± 0.07 cm, 1.04 ± 0.16 cm, and 1.64 ± 0.29 cm, as determined by random samplings of 15 particles each (significantly different from each other; ANOVA, $F = 213.23$, $P < 0.001$). Particle sizes larger than the maximum fish mouth gape were used because the omnivorous *O. niloticus* might inspect the particles for possible foraging. A non-substratum compartment was composed of a PVC plaque having the same height as the substrata throughout the compartments. Positioning of the substrate was controlled among the five aquaria. The relative position of the substrates in each aquarium was randomized and not repeated in the other aquaria, so that their relative positions in the laboratory were balanced (as suggested in Volpato et al., 2007).

The position of the eye of the fish relative to each substratum was recorded every 5 min for a period of 2 h twice daily (9:00–11:00 and 15:00–17:00 hour) over four consecutive days. Also registered was the substrata that was dug by the fish down to the glass bottom. For observations the researcher stayed about 2 m from the central aquarium (from the five aquaria positioned in line and observed at the same 30-s period) and noted the sequential fish positions (from left to right) in a computer program that converted the compartment numbers into substrate type (i.e. a kind of double blind control was assured).

Continuous aeration was provided in the passage area in front of the central compartment, interrupted 20 min before each observation period, and restored at the end of each observation period. Fish were fed at 17:00 hour each day with pellets of food spread randomly by aeration, introduced in front of the central compartment, avoiding fish conditioning. Water temperature was 22.9 ± 0.5°C. Water quality was measured in each aquarium at the beginning and end of the experiment whereby pH did not exceed 7.8, dissolved oxygen exceeded 3.00 mg O_2 L^{-1}), nitrite was <0.1 mg L^{-1} and ammonia was <0.5 mg L^{-1}, all of which were levels appropriate to this species (Mendonça et al., 2010).

Data were transformed by $\sqrt{(x + 0.5)}$ to reach parametric assumptions. Thus, frequencies of fish positions in each substratum (frequency of visits) were compared using ANOVA for repeated measures with three factors: day of observation, period of the day, and type of substratum. Frequencies of visits to each substrate irrespective of days and periods were compared by ANOVA for repeated measures. *Post-hoc* test LSD was applied to complement ANOVA.

Proportions of fish that were significantly more times in only one substrate (decided fish) were compared with proportions of fish that did not stay in any specific substrate (undecided fish) in each period of the day (morning and afternoon) by Goodman's test (1965). Proportions of fish

U.S. Copyright Clearance Centre Code Statement: 0175-8659/2013/2901–239$15.00/0

that dug in the substratum were compared by Goodman's (1964, 1965) test. All statistics considered $\alpha = 0.05$.

The protocol of this study was approved by the Ethics Committee for Animal Experimentation (CEEA) of the IBB-UNESP, Botucatu, SP, Brazil (Protocol No. 55/08).

Results

There was no effect of periods of the day on the number of visits (ANOVA, $F = 1.14$, $P = 0.31$), but there were effects of days of observation ($F = 15.52$, $P < 0.001$) and types of substratum ($F = 13.85$, $P < 0.001$). The 0.57-cm diameter substratum was the most visited of all eight observations (being significantly higher in five of them than in all other substrata – Fig. 1a). Additionally, this substrate was the only one that was always visited significantly more often than the PVC (Fig. 1a). Irrespective of days and period in the day, fish preferred the 0.57-size of substratum to the other particle sizes (Fig. 1b). Moreover, 14 of the 15 fish dug in one or more river substratum. This behavior, however, was predominantly in the finest particle substratum (Table 1).

Although substratum choice had not been affected by periods, a higher proportion of fish chose only one substratum in the morning [12 fish chose significantly only one substratum × three fish did not choose any specific substratum; Confidence Interval does not include zero for $A_{crit} = 3.84$; Goodman's (1965) test; $P < 0.05$]. That is, 12 fish stayed in one compartment at a significantly higher frequency, compared with three fish that did not stay predominantly in one compartment. In the afternoon these proportions were similar (9 × 6 fish).

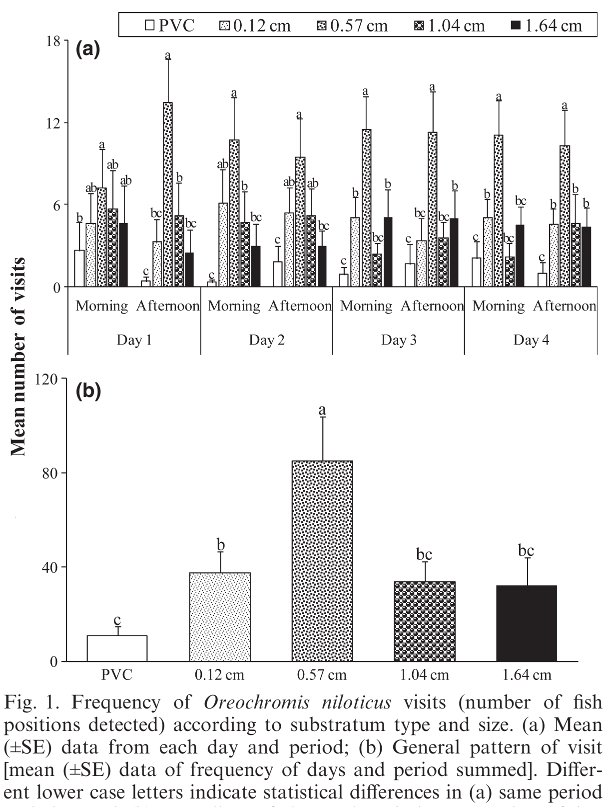

Fig. 1. Frequency of *Oreochromis niloticus* visits (number of fish positions detected) according to substratum type and size. (a) Mean (±SE) data from each day and period; (b) General pattern of visit [mean (±SE) data of frequency of days and period summed]. Different lower case letters indicate statistical differences in (a) same period and day and (b) regardless of day and period n = 12; three fishes presented outlier values and were excluded from analyses: (i) 175 times at 1.64 cm; (ii) 106 times at 1.04 cm; (iii) 52 times in PVC and 142 times at 0.57 cm

Table 1
Number of fish visiting different granulometry of substrate according to digging behavior

Substrate size (cm)	Digging	
	YES	NO
0.12	13a*	2
0.57	4b	11*
1.04	3b	12*
1.64	0b	15*

*Indicates higher proportions of fish. Confidence Interval does not include zero for $A_{crit} = 3.84$; Goodman's (1965) test; $P < 0.05$ that dug (YES) or did not dig (NO) in substrates. Lowercase letters = difference [$G_{calc} > G_{crit} = 2.64$; Goodman's (1964) test; $P < 0.05$] among proportion of fish that dug in each substrate.

Discussion

Nile tilapia were shown to prefer a particular size of natural substratum particle. Moreover, such decision-making is clearly more expressed in the morning than in the afternoon.

The Nile tilapia choice of a specific particle size of substrate appeared from the second to the subsequent periods of observation (Fig. 1a). This was expected because the fish were introduced into a novel environment, and an adjustment period was necessary (the first period of observation). In fact, exploration of the new environment has been attributed to explain the absence of a substratum preference before nesting (Galhardo et al., 2009; Mendonça et al., 2010). Nonetheless, the Nile tilapia consistently visited the 0.57-cm particle substratum more often in the last seven periods of observation, irrespective of the time of day (Fig. 1a). This consistent behavior indicates a Nile tilapia preference for the 0.57 cm-particle substratum, which is supported by the higher mean frequency of visits to this substratum over the entire period of study (Fig. 1b).

The preference of Nile tilapia for substratum size was altered when they dug their nests. A similar effect was described by Mendonça et al. (2010), who reported that male *O. niloticus* visited different substrata (sand, shell, stones and glass) at similar rates, but nested preferentially on sand. As this kind of excavation (nesting) is used by *O. niloticus* for attracting females and laying eggs (Mendonça and Gonçalves-de-Freitas, 2008), Mendonça et al. (2010) concluded that preferential nesting on sand indicates that the male Nile tilapia reproductive condition affects the choice of substratum type. In the present study the fish reproductive condition was not evaluated; however, 14 of 15 fish dug one type of river substratum, a reproductive behavior usually associated with reproductive status. Moreover, Nile tilapia performs non-seasonal (continuous) reproduction, at least suggesting that some reproductive stimulus is in charge. Furthermore, *O. niloticus* visited the 0.57-cm size particle more often but dug nests mostly in the 0.12-cm size particles (sand-like substratum). These data are in line with Mendonça et al. (2010), suggesting that the reproductive condition might also affect the choice of substratum granulometry.

The choice of particle size also depends on fish size and affects fish spatial distribution (Phelan et al., 2001; Stoner and Abookire, 2002). The 0.12- and 0.57-cm particles were preferred by the *O. niloticus*, perhaps because these particles were smaller than the largest mouth gape of 0.95 cm. Thus, suggested is that selection may involve the substratum handling capability of the fish. According to the optimum prey-size theory, the optimum prey width is close to 60% of the mouth gape of the predator fish (reviewed by Gill, 2003). Here, 60% of the mouth gape was 0.57 cm, exactly the same size of the most visited particle. Although a facility for 'mouth-management' should be involved, caution is need, as this percentage (60%) should be confirmed for other fish sizes.

Why does the Nile tilapia choose the largest particle that is still smaller than their mouth gape for visiting (Fig. 1), but chooses a smaller particle for nesting (Table 1)? The answer should consider both fish foraging and energy conservation. Webster and Hart (2004) reported that the three-spined stickleback prefers a substratum more complex than sand (similar to 0.12-cm substratum of the present study) because this complex substratum makes food more available. In isolated fish, gravel offers additional opportunities for foraging and exploration for *O. mossambicus,* during which time nipping and dragging are observed (Galhardo et al., 2009). Food can be detected because the substratum particles are removed by the feeding motivation of the fish. In this sense, fish likely prefer the largest-sized substratum that can still be moved for foraging with the fish's mouth. As flat surfaces provide easier access to food (Falahatkar and Shakoorian, 2011), the PVC was less visited because the food was more easily detected on this surface and thus might be eaten more quickly (less food should remain for feeding later on – and thus less foraging in this area is expected). For nesting, however, the thinnest particles are preferable, because nesting requires moving large amounts of substrata, which might be energy-costly to the fish. Mendonça et al. (2010) also suggested that the Nile tilapia preferred to nest on sand because the fish can dig nests faster and be less energy-costly. Moreover, Volpato et al. (2004) showed that removing larger gravel mass was achieved by the more reproduction-motivated male Nile tilapia, a fact in line with the energy costs of excavation.

Another intriguing behavior was that fish chose one substratum size more consistently in the morning. As the same animals were observed in both periods of the day, this response may express changes in decision-making. Although the underlying factors involved in such a period-dependent response are unknown, some possibilities can be drawn. For instance, *O. niloticus* increased swimming activity (Fortes-Silva et al., 2010) and oxygen consumption (Biswas et al., 2002) in the afternoon, so fish could have visited more substrata, thus affecting their decision-making. As fish in the present study were fed at 17:00 hour, they also might have increased their activity by feeding anticipation, and consequently more fish visited more than one substrate. However, in mice the degree of attention is reported to vary within the circadian cycle (Hajós et al., 2008). In the *O. niloticus*, Delicio and Barreto (2008) showed that time-place learning occurred earlier in the afternoon, a result that reinforces effect of time of the day in processes that involve cognitive comparisons. This information lends support that the daily variation in decision-making by the Nile tilapia might be a biological phenomenon deserving further investigation.

Acknowledgements

The authors greatly acknowledge I. C. B. Fonseca (UEL, PR-Brazil) for the statistical analysis; A. L. Val (INPA, MA-Brazil), L. J. G. Barcellos (UPF, RS-Brazil), E. C. Urbinati, R. E. Barreto, F. P. C. da Silva and C. M. Maia (all from UNESP, SP-Brazil) for suggestions in a previous version; and A. K. C. Felício (FIRA, SP-Brazil) for technical assistance. This research was funded by CAPES (R. H. A. Freitas) and CNPq (proc. 307380/2009-2; G. L. Volpato).

References

Biswas, A. K.; Endo, M.; Takeuchi, T., 2002: Effect of different photoperiod cycles on metabolic rate and energy loss of both fed and unfed young tilapia *Oreochromis niloticus*: part I. Fish. Sci. **68**, 465–477.

Dawkins, M. S., 2006: Through animal eyes: what behaviour tells us. Appl. Anim. Behav. Sci. **100**, 4–10.

Delicio, H. C.; Barreto, R. E., 2008: Time-place learning in food-restricted Nile tilapia. Behav. Process. **77**, 126–130.

Falahatkar, B.; Shakoorian, M., 2011: Indications for substrate preferences in juvenile hatchery-reared great sturgeon, *Huso huso*. J. Appl. Ichthyol. **27**, 581–583.

Fortes-Silva, R.; Martínez, F. J.; Villarroel, M.; Sánchez-Vázquez, F. J., 2010: Daily rhythms of locomotor activity, feeding behavior and dietary selection in Nile tilapia (*Oreochromis niloticus*). Comp. Biochem. Physiol. A. **156**, 445–450.

Galhardo, L.; Almeida, O.; Oliveira, R. F., 2009: Preference for the presence of substrate in male cichlid fish: effects of social dominance and context. Appl. Anim. Behav. Sci. **120**, 224–230.

Gill, A. B., 2003: The dynamics of prey choice in fish: the importance of prey size and satiation. J. Fish Biol. **63**, 105–116.

Goodman, L. A., 1964: Simultaneous confidence intervals for contrasts among multinomial populations. Ann. Math. Stat. **35**, 716–725.

Goodman, L. A., 1965: On simultaneous confidence intervals for multinomial proportions. Technometrics **7**, 247–254.

Hajós, M.; Siok, C. J.; Hoffmann, W. E.; Li, S.; Kocsis, B., 2008: Modulation of hippocampal theta oscillation by histamine H3 receptors. J. Pharmacol. Exp. Ther. **324**, 391–398.

Mendonça, F. Z.; Gonçalves-de-Freitas, E., 2008: Nest deprivation and mating success in Nile tilapia (Teleostei, Cichlidae). Rev. Bras. Zool. **25**, 413–418.

Mendonça, F. Z.; Volpato, G. L.; Costa-Ferreira, R. S.; Gonçalves-de-Freitas, E., 2010: Substratum choice for nesting in male Nile tilapia *Oreochromis niloticus*. J. Fish Biol. **77**, 1439–1445.

Phelan, B. A.; Manderson, J. P.; Stoner, A. W.; Bejda, A. J., 2001: Size-related shifts in the habitat associations of young-of-the-year winter flounder (*Pseudopleuronectes americanus*): field observations and laboratory experiments with sediments and prey. J. Exp. Mar. Biol. Ecol. **257**, 297–315.

Stoner, A. W.; Abookire, A. A., 2002: Sediment preferences and size-specific distribution of young-of-the-year Pacific halibut in an Alaska nursery. J. Fish Biol. **61**, 540–559.

Takemon, Y.; Nakanishi, K., 1998: Reproductive success in female *Neolamprologus mondabu* (Cichlidae): influence of substrate types. Environ. Biol. Fish. **52**, 261–269.

Uddin, M. S.; Farzana, A.; Fatema, M. K.; Azim, M. E.; Wahab, M. A.; Verdegem, M. C. J., 2007: Technical evaluation of tilapia (*Oreochromis niloticus*) monoculture and tilapia–prawn (*Macrobrachium rosenbergii*) polyculture in earthen ponds with or without substrates for periphyton development. Aquaculture **269**, 232–240.

Volpato, G. L., 2009: Challenges in assessing fish welfare. ILAR J. **50**, 329–337.

Volpato, G. L.; Duarte, C. R. A.; Luchiari, A. C., 2004: Environmental color affects Nile tilapia reproduction. Braz. J. Med. Biol. Res. **37**, 479–483.

Volpato, G. L.; Gonçalves-de-Freitas, E.; Castilho, M. F., 2007: Insights into the concept of fish welfare. Dis. Aquat. Org. **75**, 165–171.

Volpato, G. L.; Giaquinto, P. C.; Fernandes-de-Castilho, M.; Barreto, R. E.; Gonçalves-de-Freitas, E., 2009: Animal welfare: from concepts to reality. Oecol. Bras. **13**, 5–15.

Webster, M. M.; Hart, P. J. B., 2004: Substrate discrimination and preference in foraging fish. Anim. Behav. **68**, 1071–1077.

Author's address: Gilson Luiz Volpato, Department of Physiology, IBB, UNESP, 18618-970 Botucatu, SP, Brazil.
E-mail: volpgil@gmail.com