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ANIMAL SCIENCE

Behaviour of personality-screened juvenile *Oreochromis niloticus* in different challenges: Bold fish present more exploratory capacity and learning to face imposed challenges

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Abstract: The investigation of cultivation and management techniques that take into account behaviour and animal welfare becomes important due to their direct relationship with animal performance. This study aimed to classify juvenile *Oreochromis niloticus* by personality and compare their performance at exploration and competition tasks. The new object test was used to select bold and shy animals, which were then tested to see which first approached an object. The food competition test involved placing three feed pellets inside a cylinder and measuring the time taken until the pellet was eaten. The new object test found bold animals with a shorter mean time to approach the object than shy animals approaching 65% of the first object when compared to shy. The food competition test found that the bold animals ingested the first pellet more times and with a shorter average time of ingestion than shy animals, no difference in the second intake, whereas the results for the third were same as those for the first. Shy animals had higher intake of the second pellet. Bold animals explored their environment faster in both challenges, while shy individuals exhibited ability to learn from the challenges imposed in the study.

Key words: Stress, new object, feeding, personality.

INTRODUCTION

Nile tilapia, *Oreochromis niloticus*, is the most cultivated fish species in the world (Paredes-Trujillo et al. 2021). It has a fast growth rate, high resistance to diseases and stress, and the capacity to efficiently convert a wide variety of natural and artificial foods into high quality proteins (Bosu et al. 2016). The species presents competitive dominance and can negatively affect some native species (Champneys et al. 2020). Wing et al. (2021) found *O. niloticus* to have a competitive foraging advantage over another tilapia species (*O. amphimelas*). Mesquita et al. (2016) evaluated the behaviour of *O. niloticus*, classifying personalities as proactive and reactive using a T-maze. They showed that the two personalities learned to locate food efficiently during training, with similar decreases in search time on successive trials. In addition, proactive animals adapted more quickly to training and fed more efficiently in the T-maze than reactive individuals. However, there are no studies in the literature that have compared the competitive ability of Nile tilapia with different personality traits. Furthermore, it is necessary to investigate cultivation and management techniques that consider animal behaviour so that greater animal welfare can be guaranteed, which is directly related to animal performance (Torres et al. 2018).

The term "boldness" in behavioural studies is defined by placing an individual's level of risk acceptance during behaviours, such as foraging, exploration, and defense, along a boldness-shyness continuum (Budaev 1997, Coleman & Wilson 1998, Wilson et al. 1994). The terms used to refer to extreme behaviours in such situations are "bold" and "shy", to reflect bolder and shyer personalities, respectively. An environment which presents risks to a bold fish can lead it to perform general tasks, such as looking for food, whereas shy individuals tend to reduce swimming activity and, consequently, reduce their exploratory behaviour (Brydges et al. 2008). The classification of individuals according to personality is done through trials that measure their level of boldness, such as the exploration of a new environment or approaching a new object, for example (Mesquita et al. 2016).

Studies have shown advantages in doing personality screening because personality relates to learning, survival and resistance to diseases (Moiron et al. 2020, Ariyomo & Watt 2012). For instance, personality and level of boldness can affect an individual's choice of a sexual partner during couple formation, its level of aggressiveness, and its reproductive success (Moscicki & Hurd 2015), with bold individuals tending to have greater reproductive success than shy individuals (Wilson et al. 2010). Using a meta-analytic approach, Moiron et al. (2020) demonstrated that behavioural variation explains about 6% of the variation in survival for the different species of animals analysed. Furthermore, bold individuals tend to recover their appetite more quickly after stressful situations (Mas-Muñoz et al. 2011, Basic et al. 2012). However, personality classification methods can vary among species, thus studies with target species are required.

Thus, the aim of the present study was to screen *O. niloticus* by personality and compare their performance at exploration and competition tasks to identify differences between personalities in the face of challenges.

MATERIALS AND METHODS

The experiments were conducted in the larviculture laboratory Laqua (Laboratório de Aquacultura) of Universidade Federal de Minas Gerais, Brazil, under protocol number 373/2013 of Comitê de Ética em Experimentação Animal. A total of 324 *O. niloticus* (40.1 ± 5.3 g, 13.5 ± 1.1 cm) were used for personality classification. The animals were placed in 16 tanks (200 L), with a stocking density of 5.41 g/L, in a recirculating aquaculture system with controlled temperature (28ºC) and pH (7.2 - 7.8) (model HI9146 Hanna instruments) and ammonia (<0.004 ppm) (Labcon kit), constant aeration (dissolved oxygen > 5 mg/L) (YSI 6920VZ2 multiparameter probe), 12h/12h photoperiod (digital timer, group Key West DNI), and feed (36% crude protein, 2.6 mm diameter, Laguna/Socil, São Paulo, Brazil) offered until apparent satiety twice a day (8:00am and 4:00pm). The animals were kept in this system for two weeks, with fasting for the last two days prior to personality screening.

Personality screening

Personality screening was done according to the new environment methodology of Mesquita et al. (2016). A tank (200L) was filled to ¾ with water, in which a divider was placed creating two zones — a light zone corresponding to 60% of total volume and a dark zone corresponding to 40%. The dark zone was completely closed form the entrance of light. The division possessed a circular form (5-cm radius) where there was a door mechanism that could be opened and closed, as necessary, to allowed access to the light zone (Figure 1a).

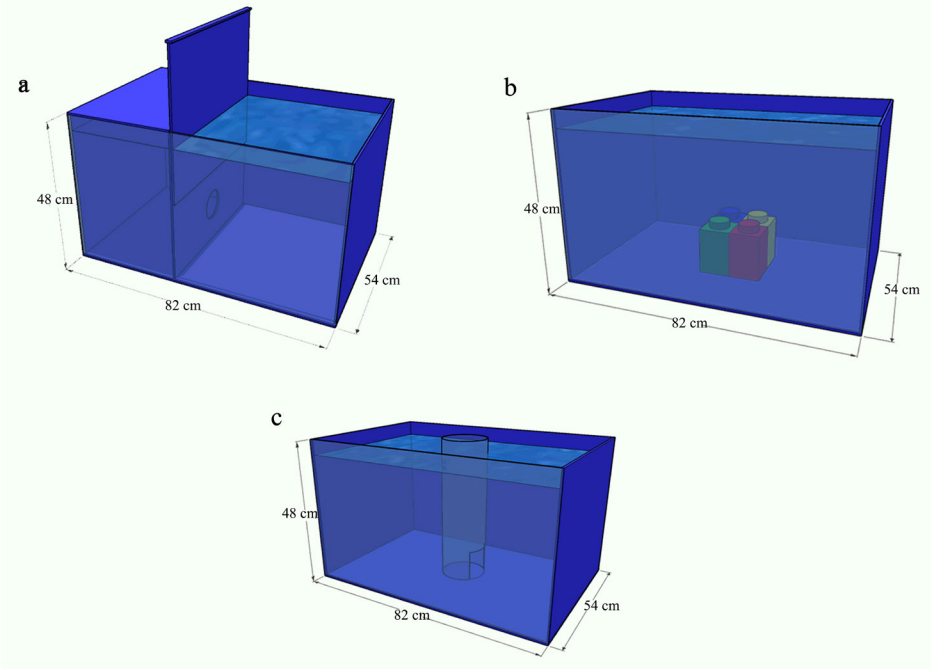

Figure 1. Tanks used in trials. a - Tank used for animal screening by personality; b - Tank used for new object test; c - Tank used for food competition test.

Six animals were randomly chosen and placed in the dark zone for 10 minutes to acclimatize to the new environment (Mesquita et al. 2016). The door mechanism was then opened allowing the animals to explore the new environment for 20 minutes, after which the door was closed. When the door was opened, 1 to 2 ml of a solution of water and dissolved feed was added in the light zone to stimulate animals in the dark zone to leave. Animals that remained in the dark zone were classified as shy animals while those that passed through the hole to the light zone were classified as bold animals (Mesquita et al. 2016). The water parameters of the trial tank were the same as those of the culture tanks. This trial was repeated 54 times until 120 animals were obtained with 60 bold and 60 shy. A total water change was performed before each trial to avoid problems from the stimulus solution used in the previous trial. Once classified, the animals were microchipped (microchip Partners) to monitor individual performance. Microchips were implanted intramuscularly in the dorsal region of the animals (close to the dorsal fin) using a syringe suitable for the application. The animals were anesthetized with a solution of 80 mg/L of eugenol for microchip implantation, as suggested by Ribeiro et al. (2015).

The animals were maintained in six tanks (200L each tank) in a recirculation system with each tank containing 20 animals of shared personality ("bold"-T1, "bold"-T2, "bold"-T3, "shy"-T1, "shy"-T2, "shy"-T3).

New object test

New object trials were performed immediately after classification in a 200L tank that was ¾ full of water. The water parameters were kept similar to those maintained in the culture system. Each trial used one randomly-chosen bold animal and one randomly-chosen shy animal, with each animal being used only once. The animals were transferred to the trial tank where they were allowed to acclimatize for 10 minutes. A coloured Lego block (the object) measuring 5x4x2 cm was then placed in the tank using a fishing line and observations were made for 20

minutes (Figure 1b). The time it took for the first animal to approach the object was recorded, and the animal was captured and had its microchip read, marking the end of the trial. Testing was repeated for 60 pairs of bold and shy animals.

Food competition test

Food competition trials were performed after the completion of the new object trials, in a 200L tank that was filled with water. A transparent cylinder (6-cm radius, 52 cm in height) with a square hole (4x4 cm) near mid-length was placed in the central region of the tank (Figure 1c).

For these trials, bold animals were marked with a green elastomer to visually differentiate them from shy animals in the tank, thus avoiding the need to remove animals from the tank for identification. Shy animals were submitted to the same marking procedure, but with a neutral substance, to avoid direct influences from marking only bold individuals. Two days after marking, the animals of both experimental groups were fasted. Each trial involved placing a randomly-chosen bold animal and a randomly-chosen shy animal in the tank, with each animal being used only once. The animals were allowed to acclimatize for 10 minutes, after which a feed pellet (36% crude protein, 2.6 mm diameter, Laguna/Socil, São Paulo, Brazil) was placed inside the cylinder. Thus, to acquire the pellet a fish had to enter the cylinder, pass through the lower part and swim to the surface of the water column where the pellet remained.

The time it took for a fish to consume the pellet, and whether the fish was marked by an elastomer (bold) or not (shy), were recorded. Next, a second feed pellet was placed inside the cylinder and the information described above was again recorded. Finally, a third feed pellet was placed inside the cylinder and the information again recorded. The timer was reset to zero at each inclusion of a pellet. The total time allowed to consume the three pellets was 20 minutes, and whether none, one or only two pellets were taken was also recorded.

Statistical analysis

All data were submitted to the Shapiro-Wilk normality test. Data of average time(s) of approach to the new object and average time(s) spent for the consumption of the different pellets that met normality were submitted to the Student's t-test at the 5% significance level. Non-normal data were transformed into Log 10 to meet normality and then submitted to the Student's t-test at the 5% significance level. Consumption time data for pellets 1, 2 and 3 of different personalities and consumption of each pellet by different personalities were submitted to ANOVA followed by the Tukey test (p <0.05).

RESULTS

New object test

Data for the new object trials are given in Table I and reveal that bold animals had a significantly shorter mean approach time to the new object than did shy animals (p <0.05). Of the 60 trials performed with bold and shy pairs, the bold individual approached the object first in 65% of the trials, the shy approached first in 21.6% of

Table I. Mean time (s) spent by Nile tilapia (*Oreochromis niloticus*) of different personalities to approach of the new object.

Personality	Average approach time of the new object
Bold	623.51±253.3[a]
Shy	1022.39±125.3[b]

Different letters indicate significant difference between bold and shy (t-test, p<0.05)

the trials, and neither bold nor shy approached the object in 13.4% of the trials.

Food competition test

The proportion of bold animals that ingested the first pellet (58.4%) was greater than that of shy animals (33.3%) and the pellet was not consumed in 8.3% of the trials (Figure 2). The number of second pellets ingested by the two personalities did not differ significantly, while bold animals had a higher mean ingestion (43.4%) of the third pellet than did shy animals (16.6%), while the third pellet was not ingested in 40% of the trials (p <0.05). There were trials in which all three pellets were ingested by bold or shy animals, but without significant differences (p >0.05). Bold animals had a higher consumption of the first pellet, shy animals had higher consumption of second, but this consumption was reduced again for the third pellet. There was a higher rate of non-consumption for the third pellet than for the others (Figure 2).

Data for the food competition trials are given in Table II and reveal that bold animals had a significantly shorter mean first-consume time (first pellet) than the shy animals (p <0.001), while personalities did not differ significantly for mean second-consume time (second pellet; p = 0.1733) or mean third-consume time (third pellet; p = 0.3403). Figure 3 shows the mean time for consuming the first, second and third pellet, which did not differ significantly for bold animals. Shy animals, however, exhibited a significant reduction in consume time from the first to the third pellet (p <0.05).

DISCUSSION

In general, the comparison of the performance of bold and shy *O. niloticus* juveniles in the exploration and competition tasks, to identify differences between personalities in the face of challenges, presented interesting and important results for the development of this research.

The results of the new object test revealed that shy animals took longer to approach the object than bold animals. This result can be explained by the fact that shy animals are more afraid and slower at exploring environments than are bold animals, which explore environments quickly and superficially (Mesquita et al. 2016). In addition, bold animals can be considered a threat to shy animals, which in turn can be more sensitive to stressors (Hoglund et al. 2008). Thus, the presence of a bold individual as a social stressor may have discouraged shy animals from exploring the environment and, consequently, reduced their swimming activity

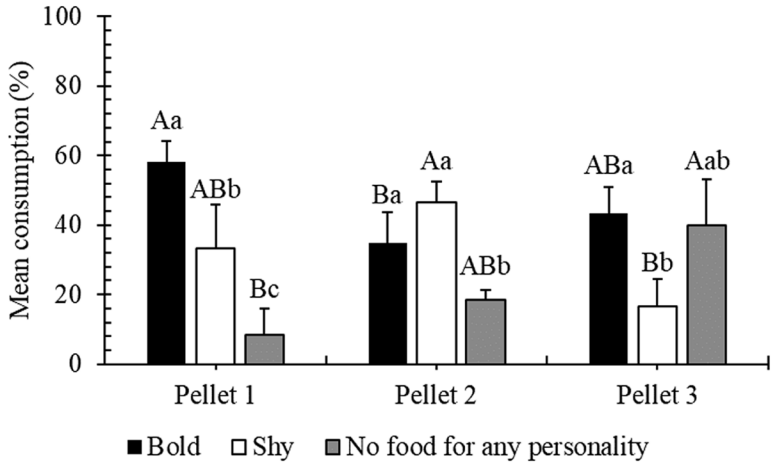

Figure 2. Mean values (± standard deviation) for consumption percentage of each pellet by Nile tilapia (*Oreochromis niloticus*) of different personalities. Different uppercase letters indicate significant difference in consumption among pellets for each personality between the different pellets. Lowercase letters indicate significant difference in consumption between personalities for each pellet. The results were submitted to ANOVA followed by the Tukey test (p <0.05).

(Brydges et al. 2008, Archard & Braithwaite 2011). When shy animals were the first to approach the object, they spent longer time than did bold animals arrived first. Shy animals tend to spend more time on tasks (Torres et al. 2018), since they explore environments more carefully (Mesquita et al. 2016).

In contrast, bold animals are more inattentive. Thus, bold individuals who did not approach an object may have been inattentive with the presence of the shy co-specific, leaving the initial task (Lego approach) in the background (Vaz-Serrano et al. 2011). The new object test is also considered a test for personality classification (Dahlbom et al. 2011). The results of the present new object test corroborate the initial personality classification carried out using the new environment method, since bold animals had a lower mean exploration time of the new object than did shy animals. The fact that shy individuals are more meticulous and slower can also explain the results of the food competition test, since it took more time for shy animals to feed than it did for bold animals. Therefore, shy animals may have spent more time exploring the environment looking for food. Furthermore, the fact that shy individuals exhibit greater signs of stress when exposed to a stress or challenge (Koolhaas 2008, Andersson et al. 2011), disfavours the activation of the hunger center (Andersen et al. 1991), potentially resulting in decreased interest in foraging. In contrast, bold individuals exhibit greater motivation to eat immediately after handling (Overli et al. 2007).

In the case of the present study, fasting for two days, handling before trials and exposure to a new environment were potential stressors for animals with greater effects on shy individuals. For the number of pellets ingested, bold animals consumed a greater number of first pellets than did shy animals. These results, once again, corroborate the greater willingness of bold animals to eat after handling (Overli et al. 2007) than their shy co-specifics that tend to conserve energy, inhibit hunger by physiological factors related to stress and inhibit foraging behaviour. Greater feeding motivation for bold fish may be explained by a higher metabolic rate (Jolles et al. 2019), and thus greater energy requirements (Mesquita 2011). In contrast, the number of second pellets consumed by the two personalities (bold and shy fish) did not differ significantly, demonstrating an ability by shy animals to observe and make decisions in the second trial (Torres et al. 2018). Bold and shy animals differed with regard to the third pellet. During the first and second pellets, the bold animals learned that the food consume environment was safe, and thus explored the environment further (Brydges et al. 2008) until they felt the need to feed, at which point they returned to the feeding place. The feed pellets provide an olfactory stimulus, and so when placed in the water they are recognized by the animals as food, thus encouraging searching. The longer time taken by shy animals to consume the first pellet than the other pellets can be explained by it being the first time the animals experienced the trial. The decrease

Table II. Mean time (s) that Nile tilapia (*Oreochromis niloticus*) of different personalities spent to consume the different pellets in the food competition test.

Personality	Pellet 1	Pellet 2	Pellet 3
Bold	419.0 ± 210.1^{a}	720.0 ± 205.1^{ns}	992.3 ± 161.5^{ns}
Shy	780.3 ± 222.3^{b}	809.5 ± 234.6^{ns}	1046.0 ± 128.3^{ns}

Means with diferente letters in the same column indicate a significant difference by the T test (p <0.05). ns: not significant.

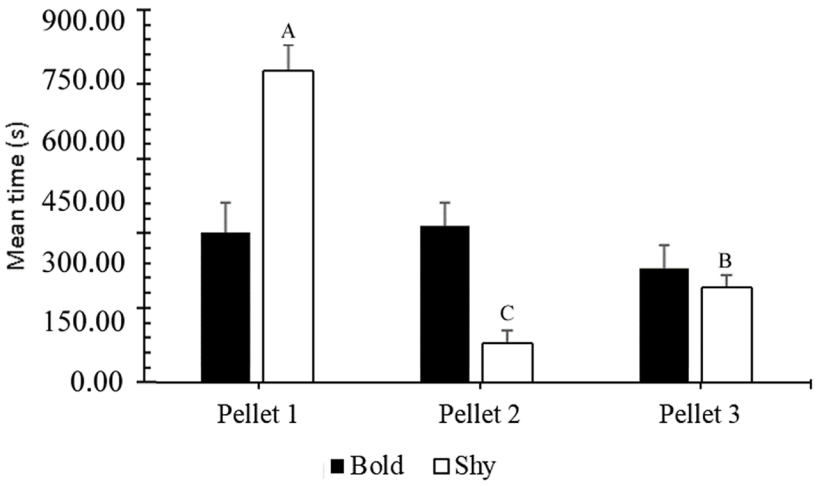

Figure 3. Mean values (± standard deviation) for consumption time among first, second and third pellets for Nile tilapia (*Oreochromis niloticus*) of different personalities. Different letters indicate significant differences between results by ANOVA followed by the Tukey test (p <0.05).

in the amount of time taken by shy animals to consume the second and third pellets can be explained by the fact that, as a species, *O. niloticus* is a fast learner of simple tasks, such as eating during the second repetition of a task (Torres et al. 2018). Therefore, after ingesting the first pellet, shy animals may have associated the feeding place with food, resulting in reduced ingestion time for the second and third pellets.

Bold animals, on the other hand, took less time to associate the task than did shy animals. However, the exploratory instinct of these animals lead them to explore new ways of eating and the environment (Brydges et al. 2008), which explains the fact that they maintained a pattern for capture time among all pellets offered. In addition, bold animals have the ability to store information longer during exploratory activities (Dammhahn & Almeling 2012, Jones & Godin 2010, Kareklas et al. 2016). Thus, bold animals recognize the place of food as a place without risks, leading them to a lessened fear in relation to food.

CONCLUSION

In conclusion, performance in exploration tasks and competition in the face of challenges imposed by different personalities demonstrated that bold *O. niloticus* exhibited better exploratory abilities than did shy individuals. In addition, shy *O. niloticus* exhibited a good ability to learn from the challenges proposed in the study. Overall, the study confirmed the advantages of doing personality screening that is related to learning.

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