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STUDIES ON THE LATERALIZATION AND AGGRESSION IN NILE TILAPIA (*Oreochromis niloticus*)USING THE MIRROR, DYADIC AND PREDATOR INSPECTION TESTS

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Abstract
Lateralization (eye preference, preference of one side over the other)was studied in Nile tilapia (Oreochromis niloticus), stocked in the laboratory in order to examine/determine how multiple tasks are performed simultaneously *for their survival, tenacity, responses, predator detection, etc. In this study, mirror and dyadic tests were used to determine, and compare the lateralization and aggression levels of Nile tilapia with its mirror image, an opponent and a potential natural predator. A total of 120 apparently healthy mixed-sex Nile tilapia (mean ± SE standard length 130.5 ± 0.3mm and weight 89 ± 0.1g)were used. Results showed that individuals showed more aggression towards (p = 0.001) mirror images than they did towards opponents in the dyadic test. Left and right eyes used in both tests showed individual differencesin the levels of lateralization. The individuals in the dyadic test used their left eye as much as (P = 0.83) they used their right eye in viewing their opponents. However, in the mirror test, their left eye was used more than their right eye when viewing opponents (P < 0.001), so they are left eye biased. Furthermore, test fish were found to also use their left eye more (p <0.001) than they used their right when viewing a predator.*

Keywords: *Lateralization, aggression, mirror test, dyadic tests, eye preference, Nile tilapia*

Introduction

Lateralization is the partitioning of cognitive functions into one hemisphere of the brain (Vallortigara and Rogers, 2005). Lateralization is the idea that the two halves of the brain are functionally different and that each hemisphere has functional specifications, e.g. the left is dominant for language and the right excels at visual-motor tasks. Cerebral lateralization or "eye-preference" can be measured in fishes using asymmetries in eye use (Facchin *et al., 2*009). Lateralization is the tendency by an individual to favor one side of the body/eye over the other. It reflects the fact that the right and left hemispheres of the brain may become specialized for certain tasks (Reebs, 2008). Lateralization is related to evolution and individuals with cerebral lateralization can respond to multiple stimuli simultaneously (Reddonand Balshine, 2010).

In most vertebrates, sight is an ideal measure for investigating brain lateralization, hence, with laterally placed eyes, images or information entering each eye are processed by the contralateral sides of the brain and this gives way and makes it easy to observe striking left-right asymmetries in the use of the eye while performing different tasks such as predator inspection, mirror image viewing, conspecific recognition, foraging and other fish behaviour (Workman and Andrew, 1986; Clotfelter and Kuperberg, 2007). Previous works have demonstrated functional specialization of the left and right side of the brain in various vertebrates (Frasnelli *et al*., 2013).

Many fishes have laterally compressed bodies with the eyes facing sideways. Therefore, it is not always easy for a fish to inspect an object with both eyes. A fish may instead turn its side towards the object and inspect it with one eye only (Reebs, 2008). This suggests that there may be a preference for one side (or eye) over the other (Reebs, 2008).

The display of aggression or aggressive behaviour is pronounced in fishes particularly during the breeding phase when males establish and defend territories for reproductive purposes (Hunting ford and Turner, 1987). Aggression plays an important role in survival, helps individuals monopolize resources, mates and protects offspring (Ariyomo and Watt, 2012). Individuals may show variation in aggressiveness which has been linked to differences in behavioural lateralization (Reddon and Hurd, 2008). Aggressive interactions in population-level are biased towards the right hemisphere (left eye use) of the brain in most vertebrates(Ariyomo and Watt, 2013) but left hemisphere bias (right eye preference) in some fish species (Miklosi and Andrew, 1999; Bisazza and De Santi, 2003).

This study was carried out using *Oreochromis niloticus,* (Nile tilapia) one of the first fish species cultured globally, and the most widely cultured tilapia species in Africa (FAO, 2005). Tilapias are laterally compressed and deep bodied and are cultured in various production systems in freshwater and saltwater (FAO, 2005).

Traditionally, the mirror and dyadic tests are used to measure aggression in species (Ariyomo and Watt, 2012, 2013; Way *et al*., 2015). The mirror test is used to measure aggression of an individual towards its mirror image while the dyadic test is used to measure aggression of an individual towards an opponent or a conspecific (Larson *et al*, 2006). In this study, both tests were used to measure aggression and level of lateralization (eye preference) of the test fish. Furthermore, another test, predatory inspection test (where test fish were allowed to view a predator) was also conducted to establish eye preference in test fish while viewing or inspecting a predator.

While studies have been conducted on the aggressive behaviour of several fishes, this study focused on a left eye/right hemisphere or right eye/left hemisphere dominance in relation to aggressiveness and predator inspection in *Oreochromis niloticus* as limited information is available on its aggressiveness and "eye-preference", This study examines the extent to which the two hemispheres are specialized for aggressiveness through the succinct observation of the eyes, hence, the objectives of this study were to a) determine the level of aggression in Nile tilapia while individuals were interacting with their mirror images in a mirror test and opponents or conspecific in a dyadic test, b) compare the level of aggression between a mirror image in the mirror test and real opponents or conspecific in a dyadic test, c) determine the level of lateralization (eye preference) while viewing a mirror image and a real opponent and d) determine the level of lateralization/eye-preference while viewing/inspecting a predator.

MATERIALS AND METHODS

Collection and Acclimation of Test fish

A total of 120 apparently healthy mixed-sex Nile Tilapia adults of similar weight and length (mean ± SE standard length 130.5 ± 0.3mm and weight 89 ± 0.1g) were obtained from a private farmin Ikole-Ekiti and transported in open plastic containers to the fish biology laboratory of the Federal University OyeEkiti. Fish were fed with commercial feed twice daily. The water temperature, dissolved oxygen, and pH were measured using standard methods (APHA, 1995).Before the start of the experiments, water temperature, dissolved oxygen, and pH were 26.4^{0}C, 7.93mg/L and 8.51 respectively. The water quality parameters were monitored at intervals during the one month study period.

Aggression test (mirror Image and Opponent)

The fish were initially housed in groups of 40in50 litrecircular plastic tanks containing fresh water. Standard length and weight of each fish were measured using a Vernier caliper and Mettler top precision loading balance respectively and fish were then housed singly in rectangular plastic tanks (30 X 20 X 20 cm) prior to the start of the experiments. The test fish were separated into two groups of 60 individuals per group. The first group was tested for aggression by presenting them with a mirror image in the mirror test. In another experiment, the second group of 60 individuals was tested for aggression by presenting them with an opponent in a dyadic contest. The first group of 60 individuals used in the mirror test was then used as the opponents in the dyadic contests. The test fish and their opponents were size matched based on their length and weight in order to minimize the effect of size and also to ensure that smaller individuals do not react submissively to larger ones (Ariyomo and Watt, 2013). For the mirror test, a mirror was placed at the side of a rectangular glass tank (60cm X 30cm X 30cm) filled with freshwater from the borehole that supplies the laboratory. A fish was added to the tank and left to acclimatize for 60 s. A piece of opaque Perspex was used to cover the mirror during the acclimation. After acclimation, the Perspex was removed and the aggressive interactions (displays with erect fins, bites, nips and fast bouts of swimming towards the image) that a fish conducted towards its mirror image were recorded using a digital camera for 5 minutes. Fish were then returned to their individual tanks after the test.

After 24 hours, they were tested in the second experiment for aggression towards an opponent of the same species. Test fish were paired with opponent fish, which were the fish used in the mirror test. The test fish and the opponent were placed each in rectangular glass tanks side by side, but the tanks were separated using opaque Perspex during the acclimation period (60 seconds), after which the Perspex was removed and the number of aggressive interactions was recorded using a digital camera. To ensure uniformity in hunger level, fish were fed only after the behavioural experiment on the testing day. Fish were returned to their individual tanks once all the experiments were completed.

Lateralization Test

The digital recordings of the test fish interactions were viewed using Microsoft Media Player, and the eye used by each fish was noted every two seconds for five minutes, based on the angle of approach of the test fish (Fig 1, Ariyomo and Watt, 2013). In the mirror test, eye use was recorded when each fish is at an angle to the mirror (Sovrano and Andrew, 2006). In the opponent test, eye use was recorded only when the test fish was positioned at an angle relative to the location of the opponent, such that the opponent was in the line of view of the test fish (Ariyomo and Watt 2013).

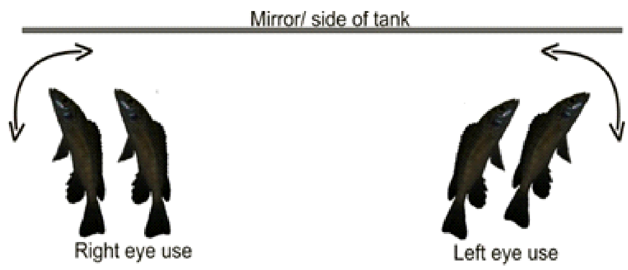

Fig. 1: Diagrammatic representation of eye use (adapted from Ariyomo and Watt, 2013). The arrows show the limits of the angle of eye use by test fish.

Predation Inspection Test

The adult catfish, *Clarias gariepinus*, (with standard length 354mm and weight 315g) used for the predation inspection test was obtained from a private farm in Ikole-Ekiti and transported in an open plastic container to the fish biology laboratory of the Federal University Oye Ekiti. Fish was kept in a circular 50 litre glass tank and fed with commercial feed twice daily.

The predator inspection test (Fig. 2) was recorded for five minutes while the fish used in the mirror test and those used in the dyadic/opponent test were inspecting the predator, *Clarias gariepinus,* following a 60 second acclimation period. Digital recordings of each fish viewing/inspecting a live predator were recorded and later viewed on a PC using Microsoft Media Player(© 2013 Microsoft, version 12.0.96600.18229), and the eye used by each fish was noted every two seconds for five minutes, based on the angle of approach, eye use was recorded only when the test fish was positioned at an angle relative to the location of the predator, such that the predator was in the line of view of the test fish.

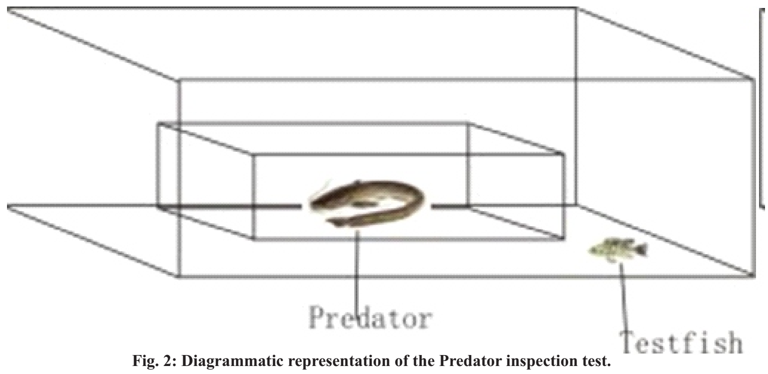

Fig. 2: Diagrammatic representation of the Predator inspection test.

Statistical Analysis

The aggressive interactions in both mirror and dyadic tests were analyzed using a two-tailed t-test. Eye preference when viewing a predator was analyzed using the paired t-test. Left and right use in the mirror and dyadic tests were compared using a generalized linear mixed model (GLMM) with binomial error distribution. Left and right eye use were treated as repeated measure observations for each fish. Individual fish was fitted as random effects in the model to avoid pseudo-replication. A model with the intercept only was fitted to determine differences between eye use in either test. All data were analyzed using *lmer* functions from the "lme4" package (Pinheiroand Bates, 2000; Bates *et al*., 2011) in R statistical software, version 3.5.1 (R Development Core team 2018).

Results

Aggression in the Mirror and Dyadic Tests

The number of aggressive interactions were significantly higher in the mirror test (mean = 33.23) than in the dyadic test (mean = 24.97; t = 3.3674, p = 0.001 (two-tailed), d.f = 115.45; Fig3). This indicates that test fish in the mirror test display more aggression towards their mirror images than the test fish in the dyadic test showed towards their opponents.

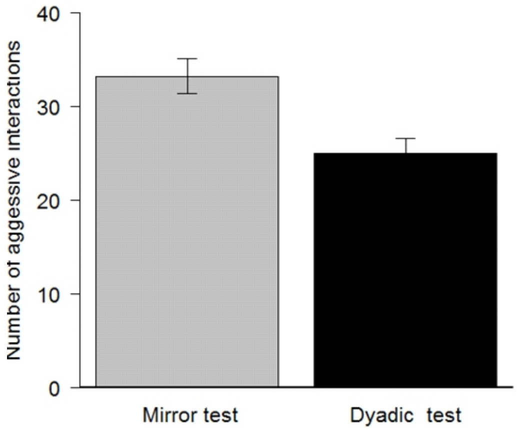

Fig 3: Mean (± SE) number of aggressive interactions in the mirror and dyadic tests.

4.2 Lateralization: Eye Use in the Mirror and Dyadic Tests

Based on the intercept, the mean left and right eye counts in the mirror test were not quantitatively different from the mean left and right eye counts in the dyadic test (Z = 0.399, P = 0.69). Although, there were significant differences in eye use in both tests (Z = 7.713, P < 0.001). There was no significant difference in the left and right use in the dyadic test, given that the left eye was used as much as the right (P = 0.83; Fig 4). However, eye use differed significantly in the mirror test (P < 0.001; Fig 4), with counts recorded for left eye use being significantly more than that recorded for the right eye use.

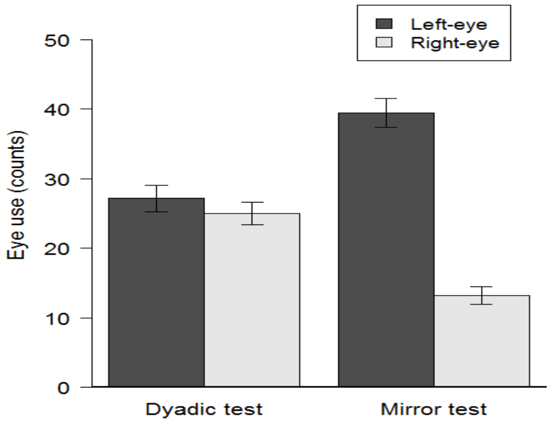

Fig 4: Mean (± SE) of left and right use in the dyadic and mirror tests.

Eye Use when Viewing a Predator

Left eye use was significantly higher than right eye use when viewing a predator (mean = 6.08; t = 5.91, p <0.001 (paired t-test), d.f = 59; Fig 5).

Fig

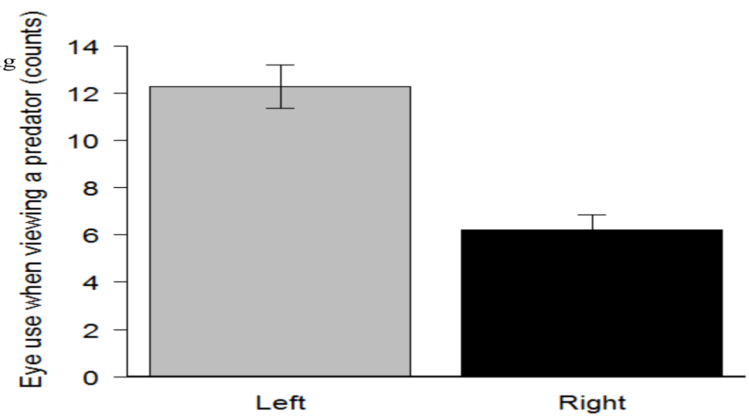

Fig 5: Mean (± SE) number of left and right use when viewing a predator.

Discussion

The results revealed that individuals showed more aggression towards their mirror image in the mirror test than they did towards their opponents in the dyadic test, with the number of aggressive interactions in the mirror test significantly higher than the dyadic test. A similar result was observed by Earley *et al.* (2000) in killifish, *Rivulusmar moratus*, where authors found the rate of aggression differed between the individuals in the mirror test and the opponent test. However, It is possible that the mirror image was perceived more as a rival while the opponent fish was seen as just a peaceful conspecific (Cantalupo *et al*., 1996; Earley *et al*., 2000; Moretz *et al*., 2007a, b; Oliveira and Canário, 2011) hence the difference in the rates of aggression.

In the lateralization test, left and right eye use in both tests was different. This indicates that individuals in both tests showed different levels of lateralization. The right hemisphere/left eye bias observed in the mirror test is in accordance with previous results in similar tests/experiments (Bissaza *et al.,* 1988; Ariyomo and Watt, 2013). This indicates that the test organism's eye preference follows a general specialization of structures on the right side of the nervous system that deals with the social signal in most vertebrates (Bradshaw and Rogers, 1993). However, the individuals in the dyadic test used their left eye as much as they used their right eye when viewing their opponents. It is not clear, from this study, why this is so, given that all test fish came from the same population. A parsimonious explanation could be that individuals perceived and reacted to the mirror image differently than they did to a real opponent. Given that a mirror image did exactly what the test fish did or that the test fish tried to match the aggressive displays of the mirror image while this wasnot so in the opponent test. This may also explain why the rate of aggression was higher in the mirror test than in the opponent test. This could also mean that the mirror test and opponent test may not measure the same aspect of aggressiveness, at least in this species.

In the test for eye preference, while viewing a potential predator (*Clarias gariepinus*), individuals used their left eye more than they used their right eye when viewing a potential predator, an indication of a right hemisphere preference. This result is in line with similar research work on other vertebrates, for example, dog, *Canis, lupus familiaris,* where the experimental set-up consisted of the presentation of black silhouette drawings of different animal models (a dog, a cat, and a snake) to the dog's right and left visual hemisphere using two retro-illuminated panels. When stimuli were presented at the same time in the two visual hemispheres, the dogs preferentially turned the head with their left eye leading in response to alarming stimuli, the snake silhouette that is considered to be an alarming stimulus for most mammals (Siniscalchi*et al*., 2017). Therefore, the lateralized individual within a population may outperform and hence have higher fitness than those that are not lateralized because lateralization confers the ability to perform more than one takes simultaneously (Dadda and Bisazza, 2006).

Conclusion

There was evidence of functional lateralization in the Nile tilapia and how specialized the hemispheres are for aggressiveness. However, given the difference between the levels of aggression shown towards the mirror image by *O. niloticus*, compared to a conspecific, this study has provided evidence that does not agree with findings in other studies that reported that the interaction of some lower vertebrates (including fishes) with a mirror image and real opponent is similar and may measure the same aspect of aggression (Hirschenhauser *et al*., 2008; Desjardins and Fernald 2010; Ariyomo and Watt, 2013).

This experiment revealed a level of lateralization while test fish viewed a predator, which is in line with the results of similar experiments of the right-left eye preference, which is an evidence of the right hemisphere specialization.

The importance of lateralization cannot be overemphasized in the study of vertebrates' sense of attention, perpetual processing, motor response and control, emotions and inhibition of responses, survival, adaptation, feeding, territoriality, etc (Vallortigara *et al*., 2002). The tendency to focus more on one side/part of the body is evident in humans where some individuals are referred to as being left-handed and while others are right-handed. This claim has been studied in lower animals in order to study brain functionality in relation to being able to perform more than one task simultaneously like foraging, mate choice, predator inspection, etc. Lateralized individuals are superior (to individuals that are not lateralized) since they can multitask, for example, foraging while inspecting a predator (Dadda and Bisazza, 2006; Dadda *et al*., 2010). There may be dire consequences for individuals that are not lateralized as this may affect the outcome of important ecological interactions, such as predator-prey encounters (Domenici *et al*., 2014). Consequently, lateralized individuals may outlive those that are not lateralized while evading a predator in a predator-prey encounter context. The results obtained in this study will contribute to the database of findings in lateralization and hopefully stimulate the interest of other researchers into conducting further studies on Nile tilapia.

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