LATERALIZATION AND AGGRESSION IN NILE TILAPIA (*Oreochromis niloticus*)

BY

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DECLARATION

I, OLATOYE DOLAPO SALIM hereby declare that this project was written by me and it is a record of my own research work. All borrowed ideas were duly and properly acknowledged.

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21-03-2017

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CERTIFICATION

This is to certify that this project work was carried out by Olatoye Dolapo Salim with MATRIC NUMBER FAQ/13/0995 in the department of Fisheries and Aquaculture, Federal University Oye-Ekiti.

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DEDICATION

This project is dedicated to Almighty God, the alpha and omega, who preserved my life during the course of my five year programme. It is also dedicated to my ever supportive dear parents, Mr. and Mrs. Olatoye, my siblings (Wuraola and Olatunji), and to everyone out there contributing positively and immensely to science. You all are no doubt my greatest source of inspiration, without your support and encouragements I could not have completed this programme.

ACKNOWLEDGEMENTS

My deepest and utmost appreciation goes to Almighty God, the giver of life and breath, for His all-sufficient mercy and graceful grace, who has made it possible for me to attain another milestone. I tender my unalloyed praises to HIM for protecting me throughout the course of my five year degree programme.

I write with huge respect and appreciation also to my supervisor, Dr. T.O. Ariyomo, for her unending motherly care, support, assistance, guidance and knowledge she equipped me with during my project work and my entire stay in the university. I forever remain appreciative to the HOD, Dr. J.B. Olasunkanmi, my able Professors and all my lectures for the maximum support and precious time created to guide and put me through my project work, I pray God reward you all.

What more can I say about my family, Mr. and Mrs. Olatoye, my promising siblings (Wuraola and Olatunji) for their inspiration, love and counsel all the time? I am highly indebted to you and say a big thank you for contributing to my success story. May God in HIS infinite mercy shower you with resounding health and wealth.

Lastly, I will like to acknowledge a mentor, Engr. Akin Odumakinde, for impacting my world, to my supportive friend (Mr. C. A., Ibidapo), acquaintances, friends, departmental colleagues and fellowship members. I say a huge thank you to you all, God bless you forever more.

ABSTRACT

Lateralization (eye preference) or handedness has been studied over the years in different species of vertebrates in other to study how they perform multiple tasks simultaneously with respect to their survival, tenacity, responses, detection, etc. Mirror and dyadic test were used to test, determine, and compare the aggression and lateralization levels of Nile tilapia (*Oreochromis niloticus*) with its own mirror image, an opponent and potential natural predator. 120 apparently healthy Nile Tilapia about the same weight and length were used for the experimental trails. Results showed that individuals showed more aggression towards their mirror image than they did towards their opponents in the dyadic test. Left and right eye use in the both tests were different with individuals in both showing different levels of lateralization. The individuals in the dyadic test used there left eye as much as they used their right eye when viewing their opponents. However, tests fish in the mirror test used their left eye more than they used their right eye when viewing their opponents, so they are left eye biased.Furthermore, test fish were found to also use their left eye more than they used their right when viewing a predator. Findings were discussed on how the Nile Tilapia showed the various levels of aggression and interaction with their mirror images, opponents and the eye preferences during both aggression test and while viewing a predator.

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CHAPTER ONE

1.0 INTRODUCTION

Lateralization is the partitioning of cognitive functions into one hemisphere of the brain (Vallortigara & 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 by assessing asymmetries in eye use (Facchin *et al., 2*009). Lateralization is defined as 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 hemisphere of the brain can become specialized for certain tasks (Reebs, 2008). Lateralization is believed by some authors to be related to evolution and individuals with cerebral lateralization are able to respond to multiple stimuli simultaneously (Reddon & Balshine, 2010).

In most vertebrate, sense of sight is an ideal measure for investigating brain lateralization. Therefore, 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 task such as, predator inspection, mirror image viewing, conspecific recognition and other everyday behaviour of the fish (Workman & Andrew, 1986). Work done in the last decade has demonstrated functional specialization of left and right side of the brain in variety of species (Frasnelli *et al*., 2013). The importance of lateralization in fish can be seen in detection, categorization and response to ecological threats such as predator, etc. (Clotfelter & Kuperberg, 2007).

The body of many fishes is compressed laterally, which means that each eye tends to face 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 indicates that there may be preference for one side (or eye) over the other (Reebs, 2008).

Individuals display a variety of behaviours, one of which is aggression. Aggression or aggressive behaviour is pronounced in fish during the breeding phase when males of many species establish and defend territories for reproductive purposes (Huntingford & Turner, 1987). Furthermore, aggression plays important roles in survival, helps individuals monopolize resources, mates and protect offspring (Ariyomo & Watt, 2012). Individuals may show variation in aggressiveness and this has been linked to differences in behavioural lateralization (Reddon & Hurd, 2008). Aggressive interactions of most vertebrates in population-level is termed to be bias towards the right hemisphere of the brain (left eye use) (Ariyomo & Watt, 2013) but other studies in some fish species showed left hemisphere bias (right eye preference) (Miklosi & Andrew, 1999; Bisazza & de Santi, 2003).

This study was carried out with *Oreochromis niloticus,* (Nile Tilapia) one of the first fish species cultured globally. The Nile tilapia is still the most widely cultured species of Tilapia in Africa. They are laterally compressed (making it a suitable species for the study) and deep bodied with long dorsal fins, characterized by an interrupted lateral line. A peculiar characteristic of the Cichlid family of fishes. Nile Tilapia are tropical fish endemic to freshwater in Africa, Jordan, and Israel and are being cultured in virtually all types of production systems in both fresh and saltwater in the tropical, subtropical and temperate climates (De Graaf & Janssen, 1996).

Traditionally, the mirror and dyadic testshave been used to measure aggression in species (Ariyomo & 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, a separate test termed 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.

There are different studies on the aggressive behaviour of several species of fish but this study was based on a left eye/right hemisphere or Right eye/left hemisphere dominance in relation to aggressiveness and predator inspection in the subject (*Oreochromis niloticus*).

1.1 JUSTIFICATION FOR THE STUDY

Oreochromis niloticus has limited information as regard its aggressiveness and "eye-preference", this research work will possibly provide more information as well as boost other researcher's interest towards conducting further research on the fish. However, fish may provide extremely valuable material for the study of the genetics of behavioural lateralization and for the investigation of the selective advantages associated with the specialization of function of the two halves of the vertebrate brain (de Santi *et al.*,2001).

However, most studies on Nile Tilapia are quite related to fish production or fish management due to importance of the species in tropics (Vijayen *et al.*, 1997).

1.2 OBJECTIVES OF THE STUDY

The aim of this research is to examine the extent to which the two hemispheres are specialized for aggressiveness through the succinct observation of the eyes. Moreover, there are some debates about whether interactions with a mirror image and with a real opponent measure the same aspects of aggression. (Larson *et al.,* 2006; Spence *et al.*, 2008; Ariyomo & Watt 2013).

Therefore, the objectives of this study were to:

1. Determine the level of aggression in Nile Tilapia while individuals were interacting with their mirror image in a mirror test and opponents or conspecific in a dyadic test.
2. Compare the level of aggression between a mirror image in the mirror test and real opponents or conspecific in a dyadic test.
3. Determine the level of lateralization (eye preference) while viewing a mirror image and a real opponent.
4. Determine the level of lateralization/eye-preference while viewing/inspecting a predator.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Taxonomy of Nile Tilapia (*Oreochromis niloticus*)

Species: *Oreochromis niloticus* (Linnaeus, 1758)

Family: Cichlidae

Order: Perciformes

Class: Actinopterygii

2.2 Description

The Nile Tilapia is characterized with a deep body covered with cycloid scales, usually appearing as silver colour with black/olive/Grey body bars with often flashes of red colour during breeding season (Picker & Griffith, 2017). They can grow to a maximum length of 62cm and weigh about 3.65kg at an estimated period of nine years of age. They possess an interrupted lateral line with truncated caudal fin. They have 27-33 gill rakers, 16-17 spines and 11-15 soft rays on the dorsal fin and 3 spines and 10-11 rays on the anal fin (Bwanika *et al.*, 2004).

2.3 Habitat and Biology

Nile Tilapia is known to be a tropical-based species that prefers to live in shallow water with preferred temperature range of 31 to 36 °C. Nile Tilapia is an omnivorous grazer that feeds on phytoplankton, periphyton, aquatic plants, small invertebrates, benthic fauna, detritus and bacterial films associated with detritus (FAO, 2006). It can also filter its feed by entrapping suspended particles, that include bacteria and phytoplankton, on mucous in the buccal cavity (FAO, 2006). Spawning begins when the water temperature reaches 24°C. The breeding process starts when the male establishes a territory, digs a craterlike spawning nest and guards his territory, ripe female spawns in the nest, and immediately the male fertilizes it. The female collects the eggs into her mouth and moves off. Female Tilapia incubates the eggs in her mouth and broods the fry after hatching until the yolk sac is absorbed. Incubating and brooding is accomplished in 1 to 2 weeks (depending on temperature) (FAO, 2006). After fry are released, they may swim back into her mouth if danger threatens. Egg number is proportional to the body weight of the female. A 100g female will produce about 100 eggs per spawn, while a female weighing 600-1000g can produce 1000 to 1500 eggs. The male remains and claims territory, guarding the nest, and is able to fertilize eggs from a succession of females (FAO, 2006). During cold period, spawning is suppressed. While the female is brooding, she eats little or nothing, therefore the males are normally used for aquaculture production in other to improve yield. Nile tilapia can live longer than 10 years and reach a weight exceeding 5 kg (FAO, 2006)

2.4 Role of aggression in fish

Aggression refers to negative attitude displayed towards another member by applying physical contact or force. Aggression is essential in the survival of a fish in its environment. According to Reebs (2008), the most food goes to the animal that eats fast, the fastest animal occupies the best shelter, and the largest share of eggs are fertilized by those males that produce the most sperm, hence aggressive display is necessary for survival. Aggression is noticed to be common in territorial fishes and many species of fish have a diversified repertoire of responses during agonistic encounter, (Turner, 1994).

However, if individuals from a social but slightly aggressive species are all placed within a water tank for the first time, there is tendency for aggressive display such as nipping, chasing of each other until one individual subsidies the other and a pecking order or hierarchy is formed. The emergence of this pecking order invariably benefits every member of the tank because fighting, nibbling and chasing are energy consuming and potentially injurious (Reebs, 2008). Nonetheless the low ranking fish are prone to not necessarily living the happiest existence because their access to food, mate, etc. will definitely be limited and this predictably affects other activities of their lives (stunted growth, stress, etc.) (Reebs, 2008)

According to most authors, aggressiveness in fish is triggered by the effect of some growth hormones by controlling the use of nutrients in tissues synthesis which will boosts the metabolic demand of the fish thereby resulting in aggression to compete for their daily needs. A number of factors affect the need for aggressive display in fish which ranges from pressure of mating, producing younger ones, intrusion from conspecific or other species, light intensity, food, etc. Light intensity influences aggressive behaviour, because at lower light level, there is a high risk of losing resources like mate and food which triggers aggression.

2.5 Lateralization in fish

One of the most important and prominent characteristics of most vertebrates in relation to their nervous system is its laterality with each hemispheres of the brain having different and likewise complementary functions and advantages. (Santi *et al.*, 2000)

With reference to Santi *et al.*, (2000) in terms of lateralization, vision is an ideal sensory modality for studying and investigating brain lateralization. In animals with laterally placed eyes, most of the visual input entering the right or the left eye is processed by the contralateral side of the brain, making it possible to observe the striking left-right asymmetries in the use of the eyes in viewing conspecifics, predator etc.

It is common among fish for pairs of individuals to leave their shoals in order to approach and inspect a potential predator. Hence, the risk to be preyed upon is shared if both fish simultaneously inspect the predator, but not if one of the fish remains at a distance. (Santi *et al.*, 2000)

2.6Physico-chemical parameters of water

Aquatic habitats are remarkably diverse with respect to the chemical and physical properties of the water, for example; salinity varies between full-strength seawater and near distilled water while pH may differ by as much as 6.0 pH units (Perry & Laurent, 1993). Furthermore, aquatic environments especially the freshwater ecosystems, are typically unstable and characterized by marked natural fluctuations of temperature, pH, dissolved oxygen, dissolved carbon-oxide and dissolved ions (Perry & Laurent, 1993). These factors can affect the fish's natural activities, therefore it is of essence to determine and maintain a favorable condition for the fish species in other to have a good result.

2.6.1 Temperature

Temperature is the degree of hotness or coldness of a body. In the aquatic environment, temperature is the major controlling factor (Barnabe, 1994). Temperature affects the physical, chemical and biological processes in water bodies and also, the concentration of many variables (Enujiugha & Nwanna, 2004). Usually, body systems will show a 50% increase in activity for every 5^0C rise in temperature. Furthermore, studies have shown that increased temperature increases the rate of chemical reactions and decreases the solubility of gases (especially oxygen) in water (Enujiugha & Nwanna, 2004).

2.6.2 Dissolved oxygen

Barnabe (1994) reported that oxygen is essential for living things/organisms. It is used in the oxidation of food, liberating the energy necessary for all vital activities such as swimming, hunting, reproduction, growth, etc. Dissolution of oxygen in water is dependent on temperature, at 5^0C for instance, water requires 12.7mg/l of oxygen to become saturated, whereas at 20^0C, 9.1mg/l of oxygen is needed (Templeton, 1995).

According to Barnabe (1994), the aquatic environment contains relatively little oxygen (less than $10cm^3/l$, contrasting $200cm^3/l$of air), thus, concentration of oxygen is close to saturation (and sometimes super saturation) in the natural environment and varies from $8cm^3/l$ in cold water to $4.5cm^3/l$ in tropical waters, being less in marine waters and shallow fresh water because of the photosynthetic activities of plants. Enujiugha & Nwanna (2004) reported that high oxygen depletion can be so severe to fish life, oxygen content of natural waters varies with temperature, salinity, turbulence, the photosynthetic activity of algae and plants and atmospheric pressure.

Chapman &Kimstach (1992) noted that dissolved oxygen concentrations below 5mg/l adversely affects the functioning and survival of biological communities and below 2mg/l may lead to the death of most fish. Different fishes have different minimum requirements of dissolved oxygen below which they will die (Banarbe, 1994; Templeton, 1995). Ross

& Ross (2002) reported that oxygen is the first limiting component of the aquatic environment and the minimum tolerated oxygen level varies with species. Tilapia, are relatively hardy and will tolerate dissolved oxygen as low as 3mg/l.

2.6.3 pH

pH is an expression of the hydrogen ion concentration of water (pH= $-\log_{10} [H^+]$. It is a measure of the acidity or alkalinity of water which is expressed on a scale between 0 and 14. Between 0 and 7, the water is acidic; it is neutral at 7 and basic above this value (Barnabe, 1994). Enujiugha & Nwanna (2004) stated that pH changes can drastically affect the structure and function of ecosystem, both directly and indirectly, pH of any water body is dependent on temperature and temperature affects physical, chemical and biological processes in water bodies.

pH has a very important influence on the chemical environments, for example, the equilibrium between NH_4^+ and NH_3 in water is shifted towards the formation of NH_3 which is extremely toxic to fish as pH rises (Barnabe, 1994), while lower pH can adversely affect fishes' gills and can be detrimental to the growth of denitrifying bacteria (Cooper, 2004). The pH equilibrium depends on other interactions, mainly the carbon dioxide carbonate system and pH governs the carbonate content of waters.

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Materials

Perspex, rectangular glass tanks (60 x 30 x 30), stopwatch, mirror (30x30), Microsoft Media Player, Video camera (Canon HD CMOS 32X optical 200M image stabilizer), plastic tanks to hold individual fish, R Statistical system software for data analysis.

3.2 Collection and Acclimation of Test fish (assessed via a dyadic contest)

A total of 120 apparently healthy Nile Tilapia adults of about the same weight and length were bought from Gbogo Farms, Ikole-Ekiti and transported to the Fish biology laboratory of the Federal University Oye Ekiti. Fish were fed with commercial feed twice daily.

3.3 Water quality parameter determination

During the exposure period of trial and experiment, the water temperature, dissolved oxygen, and pH were succinctly determined and scrutinize at intervals using standard methods.

3.3.1 Temperature

Temperature was determined using mercury-in-glass thermometer calibrated in degree centigrade (^{O}C). It were inserted into the water in each of the plastic containers, containing individual fish then the readings were taken.

3.3.2 pH

pH was determined by using a pH meter. The probe was inserted into the sample bottles containing the different containers holding the fish, and readings were taken.

3.3.3 Dissolved Oxygen (DO) Concentration

DO was measured using a dissolved oxygen meter. The probe was inserted into each container holding the fish.

3.4 Aggression test (mirror image and opponent)

Fish were initially housed in groups in a large round plastic tanks. Fish were then separated and standard length (standard length, measured from the tip of the snout to the caudal peduncle) was measured using measuring tape. Fish were then housed singly prior to the start of the trials. The test fish were divided into two groups of 60 individuals each. The first group of 60 individuals were tested for aggression by presenting them with a mirror image in the mirror test (Fig. 1). In a different set of experiments, the second group of 60 individuals were tested for aggression by presenting them with an opponent in a dyadic contest. The first group of 60 individuals used in the mirror test were used as the opponents in the dyadic contests. The test fish and their opponents were size matched in order to minimize the effect of size and also to ensure that smaller individuals do not react submissively to larger ones. Fresh water were used for each trial.

For the mirror test, a mirror was placed at the side of a rectangular tank (60cm X 30cm X 30cm) filled with water. 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 acclimatization (Fig. 1). Once the fish had acclimatized, 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 over a period of 5 minutes. Fish were then returned to their individual tanks once they had been tested.

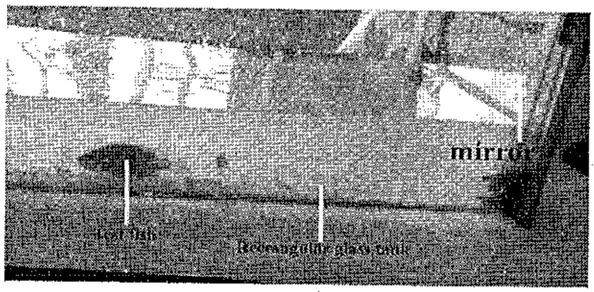

Fig 1: Test of aggression towards mirror image. Rectangular glass tank dimension 60cm X 30cm X 30cm, fish swimming away from it mirror image (adapted from Ariyomo& Watt, 2013)

Fish were left for 24 hours and then they were tested in a 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 tanks side by side, but the tanks were separated by an opaque Perspex during the acclimatization period (60 seconds), after which the Perspex was removed and the number of aggressive interactions were also recorded using a digital camera, as before (Fig 2). To ensure uniformity in hunger level, fish were fed only after the experimental trials on the day they were tested. Fish were returned to their individual tanks once all the trials had been completed.

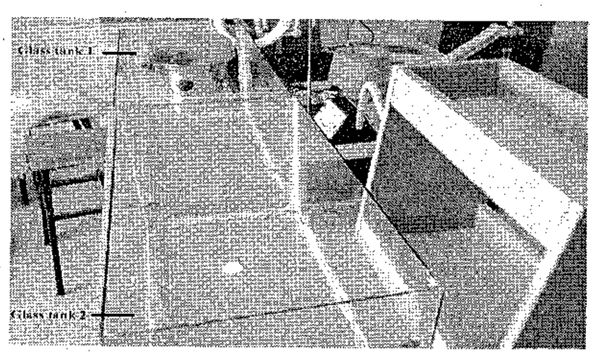

Fig 2: Experimental setup for the aggression towards an opponent. Two rectangular glass tankswith dimension 60cm X 30cm X 30cm placed side by sides (adapted from Ariyomo& Watt, 2013)

3.5 Lateralization Test

The digital recordings of the test fish interacting with their mirror images and opponents were viewed (Ariyomo & Watt, 2013) using Microsoft Media Player, and the eye used by each fish was noted every 2 seconds for 5 minutes, based on the angle of approach (Fig 3). In the mirror test, eye use was recorded when each fish is at an angle to the mirror (Sovrano & 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 it is looking at the opponent (Fig 4).

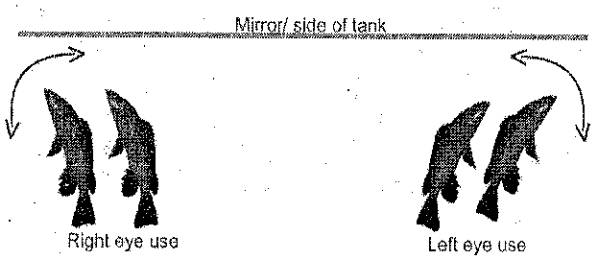

Fig 3: Diagrammatic representation of eye use (Ariyomo& Watt, 2013). The arrows show the limits of the angle of eye use by test fish.

3.6 Predation inspection test

The predator inspection test was recorded for 5 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 seconds acclimatization period. Digital recordings of each fish viewing/inspecting a live predator (Fig 4) was recorded and later viewed using Microsoft Media Player, and the eye used by each fish was noted every 2 seconds for 5 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 it is looking at the predator (Fig 4).

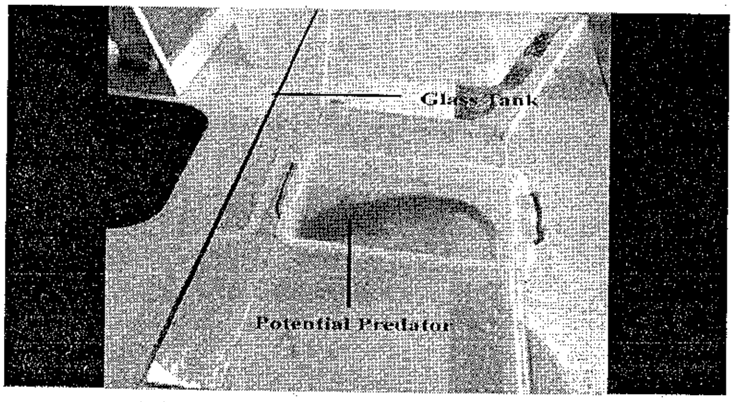

Fig 4: Experimental setup for the predator inspection test.

3.7 Statistical analysis

The rate of aggressive interactions (aggression) in the mirror and dyadic tests was analyzed using a two-tailed t-test. Eye preference when viewing a predator was analyzed using paired t-test. Left and right use in the mirror and dyadic tests was 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 individual. Individual fish was fitted as random effects in the model to avoid pseudo-replication. A model with the intercept only was fitted to determine the difference between eye use in either test. All data were analyzed using *lmer* functions from the "lme4" package (Pinheiro & Bates, 2000; Bates *et al.*, 2011) in R statistical software, version 3.5.1 (R Development Core team 2018).

CHAPTER FOUR

4.0 RESULTS

4.1 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).

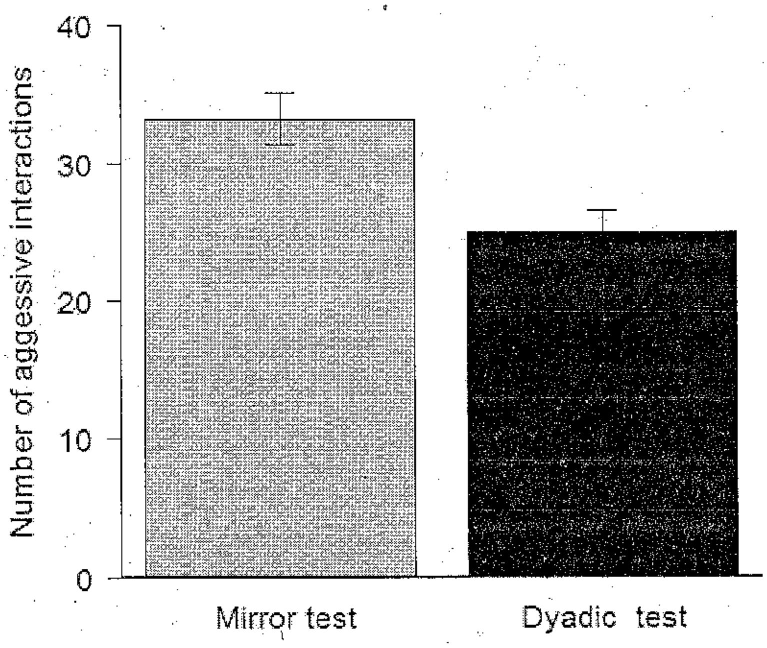

Fig 5: Mean (± SE) number of aggressive interactions in the mirror and dyadic tests.

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). However, there were significant differences in eye use in both test (Z = 7.713, P < 0.001). There was no significant difference in the left and right use in the dyadic test (P = 0.83). However, eye use differed significantly in the mirror test (P < 0.001).

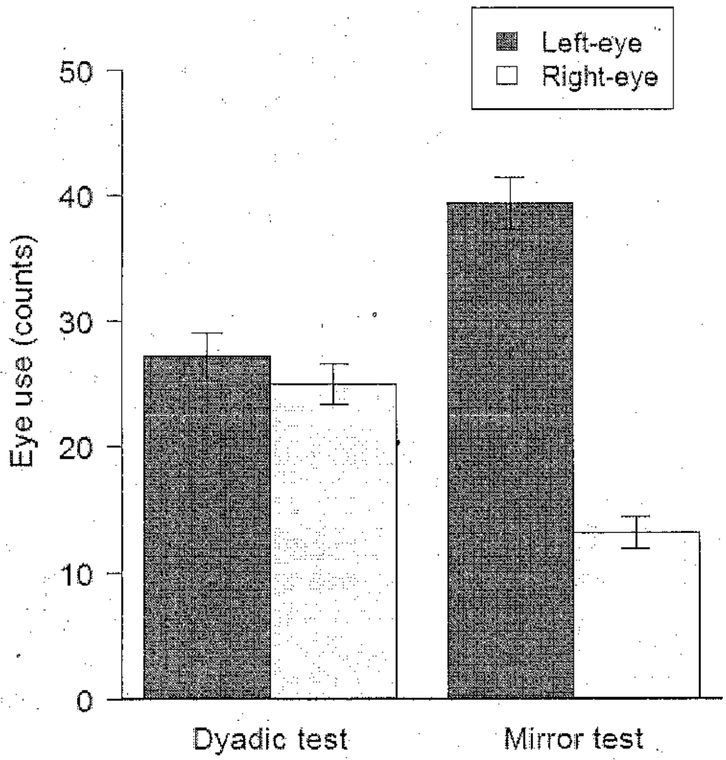

Fig 7: Mean (± SE) of left and right use in the dyadic and mirror tests.

4.3 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).

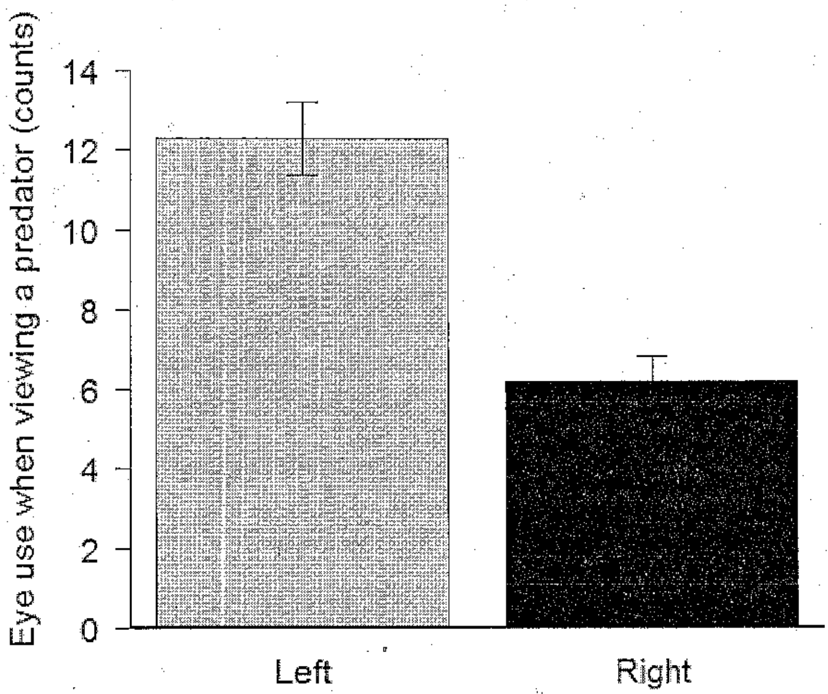

Fig 6: Mean (± SE) number of left and right use when viewing a predator.

CHAPTER FIVE

5.0 Discussion

The results carried out on *Oreochromis niloticus* (Nile Tilapia) 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 mirror test being significantly higher than the dyadic test. A similar result was observed in the test for cerebral lateralization and its relationship to phylogeny and aggression in *Anabantoid fishes* by Clotfelter & Kuperberg (2007) where they used fishes in genus *Betta*. 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 & Canário, 2011) hence the difference in the rate of aggressions.

In the lateralization test, left and right eye use in the both tests were different with individuals in both tests showing different levels of lateralization. The left eye bias observed in the mirror test is in accordance with previous results in similar tests/experiments (Bissaza *et al.*,1988). This indicates that the test organism' eye preference follows a general specialization of structures on the right side of the nervous system that deals with social signal in most vertebrate (Bradshaw & Rogers, 1993). However, the individuals in the dyadic test used there 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 while this may not be 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 a 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 hemisphere, 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. (Marcello *et al.*, 2017)

Similarly, *Bufo marinus* toads were tested in a group and were competing for prey (cricket) significantly more of their agonistic strikes at each other were directed to the left than to the right visual hemisphere. In addition, striking at the eye of a conspecific was avoided to a greater extent in the right hemisphere than in the left hemispheres. (Vallortigara *et al.*, 2002). This can only indicate that these vertebrates are evolved to visual inspection of potential predator/prey by their left eye/right hemisphere of the brain, a great feature that has contributed tit her success in survival.

5.1 Conclusion and Recommendation

Undoubtedly, there is quite a clear and explanatory connection and evidence of a functional lateralization in the Nile Tilapia and how specialized the hemispheres are for aggressiveness. However, this study has provided evidence that disputes findings in other studies that reported that the interaction of some lower vertebrates with a mirror image and real opponent measure the same aspect of aggression. Given the difference between the levels of aggression shown towards the mirror image by the *Oreochromis niloticus*, compared to a conspecific.

This experiment revealed the level of lateralization while test fish viewed a predator, which is in line with the results of similar experiment of the left eye preference, 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 of a man to focus more on one side/part of the body is evident in human where we refer tosome as being left handed and while some are right-handed. This this claim has been studied in other lower animals in order to study brain functionality in relation to their survival (a huge part of the lifestyle of any living organism). Therefore, 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 in Nile Tilapia.

REFERENCES

Ariyomo, T. O. & Watt, P. J. (2012). The effect of variation in boldness and aggressiveness on the reproductive success of zebrafish. *Animal Behaviour*, 83, 41-46.

Ariyomo, T. O. & Watt, P. J. (2013). Aggression and sex differences in Lateralization in zebraFish. *Animal Behavior*, 86, 617-622.

Barnabe, G. (ed.) (1994). Biology and Ecology of Cultured species.

Bates, D., Maechler, M. & Bolker, B. (2011). lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-39. http://CRAN.R-project.org/package=lme4

Bisazza, A. & de Santi, A. (2003). Lateralization of aggression in fish. *Behavioral Brain Research*, 141, 131-136.

Bisazza, A., Facchin, L., Pignatti, R. & Vallortigara, G. (1998). Lateralization of detour behavior in *Peocilid* Fish, the effect of species, gender and sexual motivation. *Behavior Brain Research, 91*, 157-164.

Bradshaw, J.L. & Rogers, L.J. (1993). The Evolution of Lateral Assymetries, Language, Tool use. *New York's Academic Press*.

Bwanika, G. N., Makanga, B., Kizito, Y. & Balirwa, J. (2004). Observations on the biology of Nile Tilapia, *Oreochromis niloticus L.,* in two Ugandan crater Lakes. *African Journal of Ecology* 42(s1): 93-101

Cantalupo, C., Bisazza, A. & Vallortigara, G. (1996). Lateralization of displays during aggressive and courtship behaviour in the Siamese fighting fish (*Betta splendens*), *Physiology and Behavior*, 60, 249-252.

Chapman, D. & Kimstach, V. (1992). The selection of water quality variables. In: Water quality assessments (Chapman, D. Ed). Chapman and Hall Ltd. London pp. 51-119.

Cooper, D. M. (2004). Basic fish care. Fish care.htm.

Clotfelter, E. D. & Kuperberg, E. S. (2007). Cerebral Lateralization and its relationship to phylogeny and aggression in *Anabantoid* fishes. *Brain. Behavior Evolution.* 69, 169-175

De Graaf, G. J. & Janseen, H. (1996). Artificial reproduction and pond rearing of the African Catfish Clarias gariepinus In Sub-Saharan Africa, A handbook. *FAO Fisheries Technical Paper.* 362, 73pp.

De Santi A, Bisazza A, Cappelletti M. & Vallortigara G. (2001). Prior exposure to a predator influences lateralization of cooperative predator inspection in the guppy, *Poecilia reticulata. Italian Journal Zoology*, 67:175–8.

Earley, R. L., Hsu, Y. & Wolf, L. L. (2000). The use of standard aggression testing methods to predict combat behaviour and contest outcome in *Rivulus marmoratus* dyads (Teleostei: Cyprinodontidae). *Ethology*, 106, 743-761.

Enujiugha, V.N. & Nwanna, L.C. (2004). Aquatic oil pollution impact indicators. *Journal Application Science Environmental Management*, 8, 1-75. www.bioline.org.br/ja

Facchin, L., Burgess, H. A., Siddiqi, M., Granato, M. & Halperu, M. E. (2009). Retaining the function of Zebra Fish epithalmic asymmetry. *Philosophical Transaction of the Royal Society (B)*,364, 1021-1032.

Frasnelli, E., Vallortigara, G., & Rogers, L. J. (2013). Left-right asymmetries of behavior and nervous system in vertebrates. *Neuroscience and behavioral Research*, 36, 1273-1291.

Food and Agricultural Organization of the United Nation (2006), Fisheries and Aquaculture Department, Cultured Aquatic Specification, Information Program, *Oreochromis niloticus.*

Huntingford, F. A & Turner, A. K. (1987). Animal Conflict. *Chapman and Hall Animal Behaviour Series.* Chapman and Hall: London. ISBN 0-412-28750-1. 448 pp

Larson, E. T., O'Malley, D. M. & Melloni, R.H. J (2006). Aggresion and vascotin are associated with dominant subordinate relationships in Zebra fish. *Behavioral Brain Research*, 167, 94-102.

Marcello S., Serenella d' I. & Augello, Q. (2017). Lateralized functions in the Dog brain. *Symmetry*,9,71. Doi: 103390/sym9050071.

Miklosi A., Andrew, R. J. (1999). Right eye use associated with decision to bite in zebrafish. *Behavior Brain Research*, 105, 199-205.

Moretz, J. A., Martins, E. P. & Robison, B. D. (2007a). Behavioral syndromes and the evolution of correlated behavior in zebrafish. *Behavioral Ecology*, 18, 556–562.

Moretz, J. A., Martins, E. P. & Robison, B. D. (2007b). The effects of early and adult social environment on zebrafish (*Daniorerio*) behavior. *Environmental Biology of Fishes*, 80, 91–101.

Oliveira, R. F. & Canário, A. V. M. (2011). Nemo through the looking-glass: a commentary on Desjardins & Fernald. *Biology Letters*, 7, 487-488.

Perry, S. F. & Laurent, P. (1993). *Environmental effects of fish gill structure and function In: Fish Ecophysiology*. Rankin, J.C. and Jensen, F. B. (edş.) Chapman and Hall, London. 230-264pp.

Picker, M. D. & Griffith, C. L. (2017). Alien animal in South Africa- composition, introduction history, origins and distribution patterns. *Bothalia-African Biodiversity & Conservation* 47 (2), 1-19

Pinheiro, J. C. & Bates, D. M. (2000). Mixed-Effects Models in S and S-PLUS. 1st edn. Berlin: Springer.

R Development Core Team 2018. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. http://www.rproject.org.

Reddon, A. R. & Balshine, S. (2010). Lateralization in response to social stimuli in cooperatively breeding Cichlid fish. *Behavioural Processes*, 85, 68-71.

Reddon, A. R. & Hurd, P. L. (2008). Aggression, sex and individual differences in cerebral lateralization in Cichlid fish. *Biology Letters*, 4, 338-340.

Ross, B. & Ross, L. G. (2002). Anaethetic and sedative techniques for aquatic animals *2nd Edition*. Blackwell Science Ltd.

Spence, R., Gerlach, G., Lawrence, C. & Smith, C. (2008). The behavior and ecology of the Zebra Fish, *Danio rerio. Biological Reviews*, 83, 13-34.

Sovrano, V.A., & Andrew, R.J. (2006). Eye use during viewing a reflection: behavioural lateralization in zebrafish larvae. *Behavioral Brain Research,* 167, 226-231.doi.org/10.1016/j.bbr.2005.09.021

Templeton, R.G. (1995). Freshwater Fisheries Management. Second edition.

Vallortigara, G., Angelo Bisazza, Lesley J. R. & Richard John. (2002). Comparative vertebrate lateralization. Cambridge University press, 2002. pp (94-98).

Vallortigara, G. and Roger, L. J. (2005). Survival with an assymetrical brain: an advantage and disadvantage of cerebral lateralization. *Behavior Brain Science.* 28, 575-589.

Walker S. F. (1980). Lateralization of functions in the vertebrates Brain: a review. *British Journal of Psychology*, 71, 329 - 367.

Way G. P., Ruhl, N., Snekser, J. L., Kiesel, A. L. & McRobert, S. P. (2015). A comparison of methodologies to test aggression in zebrafish. *Zebrafish*, 12, 144-51.

Workman, L. & Andrew, R.J. (1986). Asymmetries of eye use in birds. *Animal Behavior*, 34, 1582-1584.