

Effect of Tank Structural Enrichment on Behavior and Growth of the Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT

Environmental structural enrichment is a method to increase environmental heterogeneity, which may reduce stress and improve animal welfare. Previous studies have shown that environmental enrichment can increase the growth rate, decrease aggressive and anxiety-like behaviors and reduce cortisol levels in animals. The purpose of this study was to investigate the effects of environmental enrichment on the behavior (boldness and aggressiveness) and growth (total length, standard length, weight) of the Nile tilapia (*Oreochromis niloticus*). Boldness, aggressiveness, total length, standard length, and weight were weekly measured over a 13-week period (during weeks 0, 1, 3, 5, 7, 9, and 11) for fish housed in three treatment tanks: no structural enrichment (NSE), moderate structural enrichment (MSE), and heavy structural enrichment (HSE). Significant differences were detected in boldness, aggressiveness, total length, standard length and weight among the three treatments as the weeks progressed ($P < 0.05$). Boldness was the highest in fish reared in tanks with heavy structural enrichment as the weeks progressed ($P < 0.05$). However, aggressiveness was lowest across weeks in fish reared in tanks with heavy structural enrichment ($P < 0.05$). The highest levels of growth, in terms of weight ($P < 0.05$), total length ($P < 0.05$), and standard length ($P < 0.05$), were recorded in the fish reared in tanks with heavy structural enrichments across weeks ($P < 0.05$). This study suggests that the growth of *Oreochromis niloticus* might be influenced by the structural enrichment of their rearing environment. Therefore, future related studies should consider environmental structural enrichment to enhance the growth of *Oreochromis niloticus*.

INTRODUCTION

In recent years, there has been a growing interest in finding strategies for improving the welfare of farmed fish, as evidenced by the increasing number of publications on fish welfare (Kristiansen *et al.*, 2004; Ashley, 2007; Näslund & Johnsson, 2016; Salena *et al.*, 2021). In land-based aquaculture systems, several husbandry parameters can compromise fish welfare if not controlled adequately, such as

water quality, high densities, sorting, transportation (**Huntingford *et al.*, 2012**) and also the lack of environmental stimulations (**Franks, 2018**). Structural enrichment plays a crucial role in enhancing the welfare of fish in captive environments. In the wild, fish live in complex ecosystems with diverse physical structures, such as plants, rocks, and crevices, which provide them with shelter, protection, and opportunities for exploration. However, in captivity, fish are often housed in bare, featureless tanks that lack these essential environmental stimuli. This can lead to boredom, stress, and compromised welfare. Recognizing the importance of replicating natural conditions, researchers and aquarists have increasingly focused on the concept of tank structural enrichment to improve the well-being of fish. Furthermore, structural enrichment of the rearing environment may considerably impact the behavior/personality traits of fish (**Näslund & Johnsson, 2016**).

Behavior/ personality traits are the qualities that make an animal distinct from another. Boldness and aggressiveness are two behavioral traits that have been of interest to behavioral ecologists (**Ariyomo & Watt, 2012**). Boldness is the propensity of an animal to engage in risky behavior. The shy–bold axis of behavior has received an increasing attention, especially from ecological researchers. Individual variation along the shy–bold axis may have important consequences in many contexts across an individual’s lifespan, given that boldness may influence success in mating competition, feeding, adjusting to environmental change and responding to predators, and subsequently influencing individual fitness (**Toms *et al.*, 2010**). Aggressive behavior refers to a negative attitude displayed toward another by applying physical contact or force.

Just like boldness, aggressive behavior has important consequences on the overall well-being and fitness of individuals. Studies have shown that the most aggressive, usually the fastest-eating animal occupies the best shelter and gets most of the food available in the environment, consequently influencing the growth rate of individuals within an ecosystem (**Reeb, 2008**). Therefore, the aggressive display may influence other facets of the life of individuals such as reproduction, foraging behaviors, ultimately impacting the survival and growth of some fish species in their environment (**Larson *et al.*, 2006; Spence *et al.*, 2008; Ariyomo & Watt, 2012**).

Growth is the permanent increase in the size of an organism. The increase may be through body length, girth, and weight that occur when an animal is given adequate food, water and shelter. Additionally, the growth rate of fish populations is related to environmental conditions (**Khan & Khan 2014**), such as temperature, oxygen, the wavelength of light (color) and environmental enrichment. Environmental enrichment includes physical structures added to the captive environment to increase the structural complexity. Moreover, it involves the introduction of novel objects, social housing, and exercise opportunities to the home cage (**Green *et al.*, 2012**).

Environmental enrichment is one of the strategies investigated to improve the living conditions of animals and can improve the welfare of captive fish. For a change in an animal's environment to be considered, environmental enrichment must be biologically relevant, possess functional significance to the animal and result in an improvement and not merely a change in the animal's environment (Newberry, 1995). Its objective is to provide new sensorial and motor stimulation to help meet their behavioral, physiological, morphological, and psychological needs, whilst reducing stress and frequency of abnormal behaviors (Bockman *et al.*, 2018). In fish farms, rearing environments are usually designed from a human perspective and based on economic requirements, mainly for practical reasons for the farmer, with little consideration for the animal welfare. Throughout aquaculture production cycles, many farming operations can be stressful for fish, and enhancing the structural complexity of the environment may not only help them cope with these stressful events but also improve their overall welfare.

Enriching the environment of fish can have various positive effects on physiology, health, survival and therefore general welfare. Hence, this study investigated the effect of tank structural complexity on the behavior and growth of *Oreochromis niloticus* for an effective management, a sustainable exploitation, and as a prelude to making it an acceptable aquaculture system that can be practised and adapted across the world.

Little attention has been paid to the study of the structural complexity of rearing environments in aquaculture by researchers; the focus has been in recent times on aquaculture practices in barren environments (Näslund & Johnsson, 2016). Studies have shown that variation in habitat complexity would have various positive effects on physiology, health, survival and therefore general welfare. Hence, this research aimed to investigate the effect of tank complexity (environmental enrichment) on the boldness, aggression and growth of *Oreochromis niloticus*, an important food fish, for an effective management, a sustainable exploitation, and as a prelude to make it a suitable aquaculture system that can be adopted.

MATERIALS AND METHODS

1. Collection and conditioning of test organisms

All the fish used in these experiments were purchased from a reputable fish farm in Oyo State and transported in oxygenated plastic bags to the Fisheries and Aquaculture wet laboratory in Ikole campus, Federal University Oye-Ekiti. The collected fish were acclimated for two weeks in a plastic tank containing clean and unpolluted freshwater. After the acclimation period, the fish were assigned randomly in groups of 20 per tank into moderately enriched tanks, heavily enriched tanks, and tanks with no enrichment (control) for a culture period of 90 days. All fish were fed twice daily with commercial feed (35% crude protein), while the water quality parameters were strictly monitored

during the experimental period using a water test kit (Hanna-HI3817). To ensure uniformity in hunger level, feeding took place after the experimental trials. Behavioral testing for boldness and aggression is similar to that described in **Ariyomo and Watt (2012)**.

2. Tank complexity design and structural enrichment procedure

Nine transparent tanks measuring 41 x 28 x 23.4cm (Length x Breadth x Height) were used. Three tanks served as the control, with no structural enrichment (NSE) (Fig. 1a), another three had moderate structural enrichment (MSE) using stones (gravel) and artificial plants (Fig. 1b), while the remaining three tanks had heavy structural enrichment (HSE), with stones (gravel), artificial plants, PVC pipes, and a pan serving as a cover (Fig. 1c).



Fig. 1. Pictorial representation of structural enrichment set up showing: **a)** No structural enrichment, **b)** Moderate structural enriched, **c)** Heavy structural enriched

3. Test of boldness

The open field test (**Burns, 2008; Ariyomo & Watt, 2012; Woodward *et al.*, 2019**) was used to determine boldness in the three modifications of tank structural complexity (Fig. 2). A tank measuring 41 x 28 x 23.4cm (Length x Breadth x Height) with a gridded base, marked into 36 rectangles (Fig. 3), filled with 15 litres of water was used as the open field. Each fish was placed in the middle of the tank and left to acclimatize for 60 seconds. After the acclimatization period, the number of lines crossed by each fish in the subsequent 180 seconds was recorded as a measure of boldness for each fish. A high rate of movement was also taken as an indicator of boldness since it reflected the fish's ability to explore its environment (**Ariyomo *et al.*, 2013**). Fish that crossed the highest number of lines while effectively utilizing the tank were deemed bold. Moreover, fish that crossed the least number of lines and did not utilize the tank were deemed shy. The time each fish spent freezing over a period of 180s was also recorded. The freezing time was excluded from the analysis since the majority of the fish tested did not freeze. Fish was tested for boldness during weeks 0, 1, 3, 5, 7, 9 and 11.

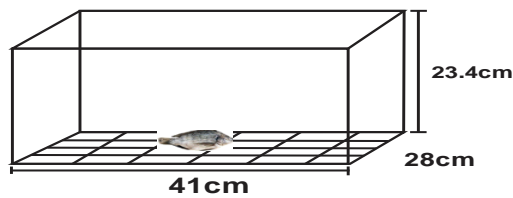


Fig. 2. Diagrammatic representation of the open-field test

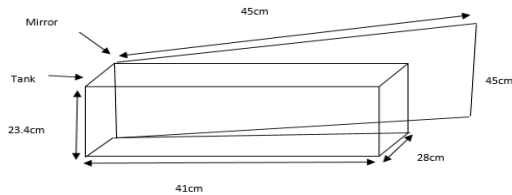


Fig. 3. Diagrammatic representation of the mirror test

4. Test of aggression

The mirror test was used to test for aggression (Ariyomo & Watt, 2015). All individuals were tested for thirteen weeks. A test tank measuring 41 x 28 x 23.4cm (Length x Breadth x Height) was filled with 15 litres of water, and a mirror (45 x 38cm) was placed at the side of the tank at an angle of 22.5°. Before the introduction of a fish into the test tank, an opaque material was used to cover the mirror, and then a fish was placed at the center of the tank and left to acclimatize for 60 seconds. The number of aggressive interactions the fish makes toward its mirror image in 300 seconds after the removal of the opaque material was counted and recorded (Ariyomo & Watt, 2015). They include the number of bites, nips, and fast bouts of movements toward the mirror image, and the number of displays were recorded immediately after counting. The total number of aggressive interactions was used as a measure of aggression. Fish were tested for aggression on weeks 0, 1, 3, 5, 7, 9 and 11.

5. Data analysis

Data collected during the experiment was subjected to statistical analysis using IBM SPSS Statistics version 25. The data were subjected to one-way statistical analysis of variance (ANOVA), and the significant differences in means was determined using Duncan's multiple range test. Statistical significance of the parameters measured was set at $P \leq 0.05$.

6. Ethical approval

All procedures were carried out in the Department of Fisheries and Aquaculture wet laboratory in accordance with the ethical standard of the animal ethics committee of Federal University, Oye Ekiti, Nigeria.

RESULTS

1. Boldness of *Oreochromis niloticus* during the open field test

The boldness of *Oreochromis niloticus* during the open field test across weeks is presented in Fig. (4). There were significant differences in the mean boldness in the rearing tanks with different levels of structural enrichment across weeks ($F_{7, 190} = 40$; $P < 0.05$). During the first test, boldness did not differ significantly among the rearing tanks with different levels of structural enrichment ($P > 0.05$). However, in the first week, boldness differed significantly among individuals in the rearing tanks with different levels of structural enrichment ($P < 0.05$), with the highest level of boldness recorded for fish housed in tanks with moderate structural enrichment; however, boldness did not differ significantly between fish in the tanks with no structural enrichment and those with heavy structural enrichment ($P > 0.05$). By the third week, there was a drastic decline in the boldness level of fish housed in the tanks that had moderate structural enrichment, followed by those in tanks with no structural enrichment, with the highest level recorded for fish housed in the tank with heavy structural enrichment.

Fish in the tanks with no structural enrichment (NSE) and the tanks with moderate structural enrichment (MSE) had similar boldness levels ($P < 0.05$) and were the least bold in the fifth week, followed by the fish in the heavily enriched tanks whose boldness level was significantly the highest ($P < 0.05$).

In week 7, the boldness of fish in the tanks with no structural enrichment and those in tanks with moderate structural enrichment were not significantly different ($P < 0.05$) but the levels of boldness in the heavily enriched tanks were higher and differed significantly from the boldness of the individuals in the other tanks ($P < 0.05$). Fish in the tanks with moderate structural enrichment were significantly less bold than fish in the tanks with no structural enrichment ($P < 0.05$).

In the 9th week, boldness was significantly higher in fish raised in the tanks with heavy structural enrichment ($P > 0.05$) than those reared in the tanks with no structural enrichment and those with moderate structural enrichment. However, boldness did not differ significantly between the fish in the tanks with moderate structural enrichment or the tanks with no structural enrichment ($P < 0.05$), but the highest level of boldness was recorded in the fish housed in tanks with heavy structural enrichment ($P > 0.05$) in the 11th week.

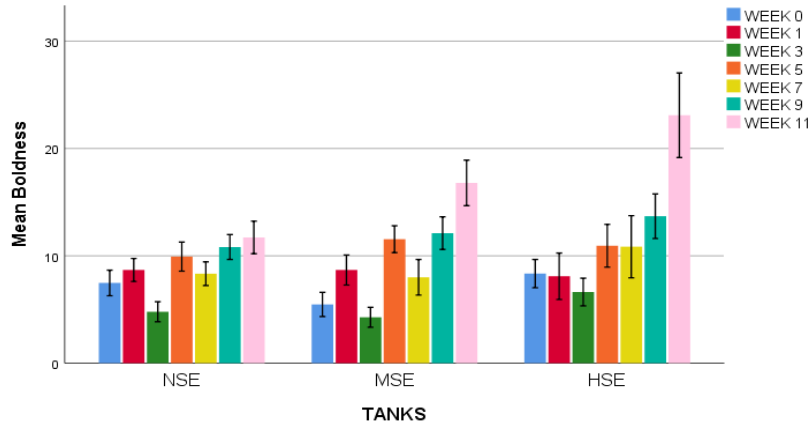


Fig. 4. Mean (\pm SE) boldness of *O. niloticus* in the different structurally enriched tanks across weeks at $P \leq 0.05$

2. Aggressive behavior in *Oreochromis niloticus* during the mirror test

The number of aggressive behavior in *Oreochromis niloticus* is presented in Fig. (5). The means of the aggressive interactions of fish in the different tanks with varying levels of enrichment across weeks were significantly different ($F_{7,190} = 10.13$; $P < 0.05$). Aggressive interactions of fish on the first day of the study were similar ($P > 0.05$) in all the tanks with different levels of enrichment.

Fish in the tanks with moderate structural enrichment and heavy structural enrichment were significantly more aggressive than those in tanks with no structural enrichment during the first week. Fish raised in the tanks with no structural enrichment and those in moderate structural enrichment showed an increase and decrease in the rate of aggressive interactions respectively in the third week. The rate of aggressive interactions in the 5th week was significantly higher in the tanks with no structural enrichment and those with moderate structural enrichment than in the tanks with heavy structural enrichment ($P < 0.05$). Furthermore, by week 7, mean aggressive interaction was significantly lower in fish raised in the tanks with heavy structural enrichment ($P < 0.05$) than those reared in the tanks with moderate structural enrichment and those in tanks with no structural enrichment. For week 9, there was a significant decrease in the rate of the aggressive interaction in fish raised in the tanks with heavy structural enrichment ($P < 0.05$) compared to those raised in moderately enriched tanks and tanks with no level of enrichment.

Finally, aggressive interactions on the last week of the test (week 11) across the different tanks were significantly different with the highest level of aggressive interaction recorded in the tanks with no level of structural enrichment followed by those raised in tanks that are moderate structural enrichment and the least value recorded in the tanks that had heavy structural enrichment ($P < 0.05$).

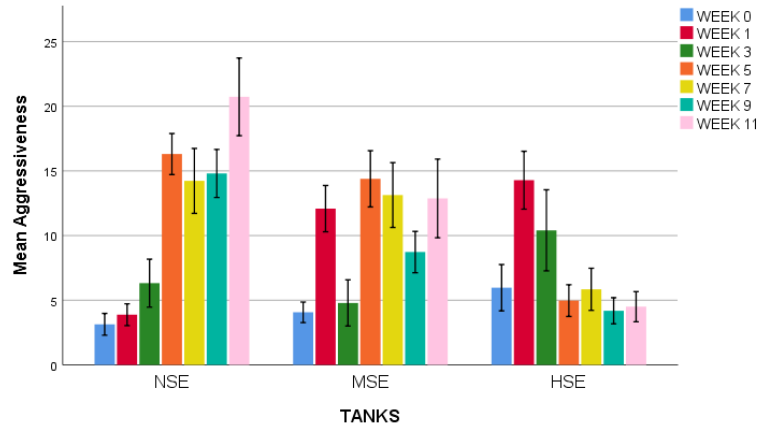


Fig. 5. Mean (\pm SE) aggressive interactions

Different superscripts are significantly different across the rows at $P \leq 0.05$

3. Weight of *Oreochromis niloticus* in the tanks with different levels of enrichment across weeks

Fig. (6) shows the weights of *O. niloticus* in the tanks with different levels of enrichment across weeks. There were significant differences in the weight of fish in the tanks with different levels of enrichment across weeks ($F_{7, 190} = 4.90$; $P > 0.05$). In the first test, fish in the tanks with moderate structural enrichment and heavy structural enrichment had significantly similar mean weights compared to the weight recorded for fish in the tanks with no structural enrichment, which was significantly higher ($P > 0.05$).

In the first week, fish in the tanks with no structural enrichment were significantly higher in weight than those in tanks with moderate structural enrichment and those with heavy structural enrichment. However, the weights of fish in the tanks with moderate structural enrichment and those with heavy structural enrichment did not differ significantly ($P > 0.05$). In the third week, the fish in the tanks with heavy structural enrichment achieved the best weights when compared with the weights of fish in the tanks with moderate structural enrichment and tanks with no structural enrichment. Fish in the tanks with heavy structural enrichment were higher in weight ($P < 0.05$) in the 5th week, with the least recorded in the tanks with no structural enrichment. In the 7th week, the highest weight was recorded in the tanks with heavy structural enrichment, while the least weight was recorded in the tanks with no structural enrichment ($P < 0.05$).

However, by the 9th week, there was a significant increase in the weight of fish in the tanks with no structural enrichment, followed by a decline in the weight of fish in the tanks with moderate structural enrichment. The highest record was achieved in the tanks with heavy structural enrichment. In the 11th week, the highest level of weight was recorded in the tanks with heavy structural enrichment, while the lowest weight was recorded in the tanks with no structural enrichment ($P < 0.05$).

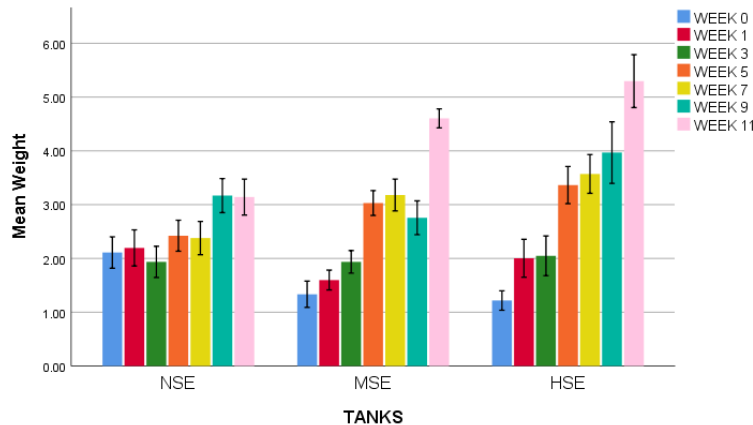


Fig. 6. Mean (\pm S.E) weight of *O. niloticus* in the different structurally enriched tanks across weeks at $P \leq 0.05$

4. Total length of *Oreochromis niloticus* in the different tanks across weeks

The total length of *Oreochromis niloticus* reared in the tanks with different levels of enrichment across weeks is presented in Fig. (7). In the first test, the mean total length of the fish in the tanks with no structural enrichments, moderate structural enrichment and heavy structural enrichment were significantly different ($f_{7,190} = 4.15$; $P \leq 0.05$). In the first week, fish in the tanks with heavy structural enrichment grew longer ($P < 0.05$) compared to the similar lengths recorded in the tanks with no structural enrichment and moderately enriched tanks, respectively. In the 3rd week, the fish in the tanks with no structural enrichment had a significantly lower total length ($P < 0.05$) than the similar total length of fish recorded in the fish raised in the tanks with moderate structural enrichment and heavy structural enrichment ($P > 0.05$). The mean total length of the fish in the tanks with no structural enrichments, moderate structural enrichment and heavy structural enrichment were not significantly different in the 5th week ($P < 0.05$). Fish in the tanks with no structural enrichment and moderate structural enrichment had similar mean total lengths ($P < 0.05$) that were significantly longer than the mean total length of fish in the tanks, with the heavy structural enrichment in the 7th week.

Fish reared in the tanks with different levels of enrichment increased in length in the 9th week. However, this increment was not significantly different between fish in the tanks with no structural enrichments and moderate structural enrichment ($P > 0.05$), while fish in the tanks with heavy structural enrichment had the highest increment in mean total length ($P < 0.05$). By the 11th week, the highest record of mean total length was taken from the fish reared in the tanks with heavy structural enrichment, followed by the moderately enriched tanks, with the lowest record obtained from the tanks with no structural enrichments ($P < 0.05$).

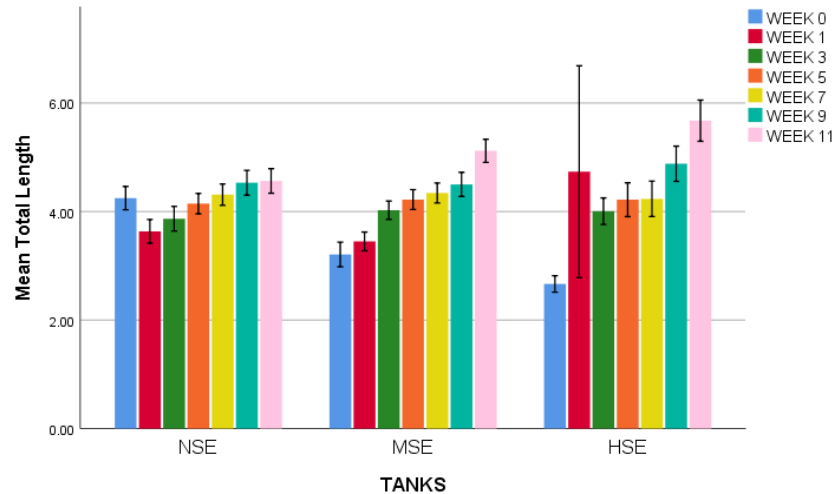


Fig. 7. Mean (\pm SE) total length of *O. niloticus* in the different structurally enriched tanks across weeks

Different superscripts are significantly different across the rows at $P \leq 0.05$

5. Standard length of *Oreochromis niloticus* in the different enriched tanks across weeks

The mean standard lengths (SL) of *Oreochromis niloticus* reared in the tanks with different levels of enrichment across weeks are presented in Fig. (8). The mean standard lengths recorded during the first test were almost similar ($F_{7, 190}=33.71$; $P < 0.05$) in the fish in tanks with moderate structural enrichment and heavy structural enrichment but differed significantly ($P \leq 0.05$) in the tanks with no structural enrichment. In the first week, similar mean standard lengths of fish were also recorded in tanks with moderate structural enrichment and heavy structural enrichment which were lower than the mean standard length recorded in fish in the tanks with no structural enrichment ($P > 0.05$).

The tank with moderate structural enrichment had fish with the longest standard length in the third week, while the shortest standard length was recorded for fish reared in tanks with heavy structural enrichment. In the 5th week, all the tanks had similar records of the standard length in all the fish raised. However, in the 7th week, the fish reared in the tanks with heavy structural enrichment recorded the highest standard length, while the lowest was recorded in the tanks with no structural enrichment ($P > 0.05$).

In the 9th week, the fish in the tanks with no structural enrichment and those with moderate structural enrichment recorded similar mean standard lengths throughout the tanks ($P > 0.05$) with the highest mean standard length achieved in the fish reared in tanks with heavy structural enrichment ($P < 0.05$). Finally, in the 11th week, the longest mean standard length standard was recorded in the fish reared in tanks with heavy structural

enrichment, while the lowest level was recorded in the tanks with no structural enrichments ($P < 0.05$).

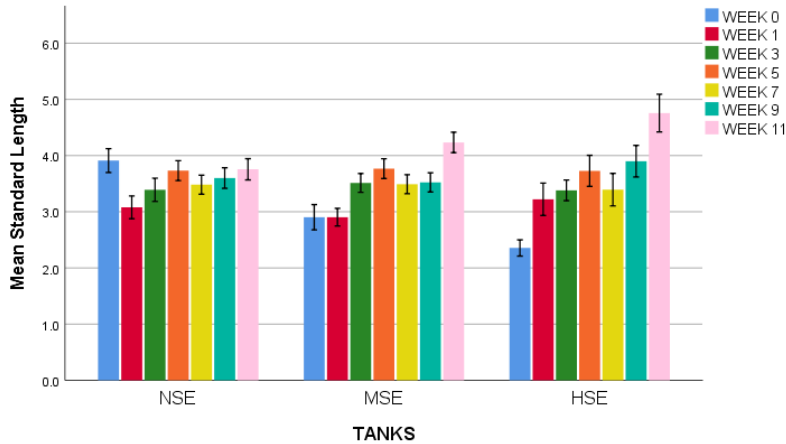


Fig. 8. Mean (\pm S.E) standard lengths of *O. niloticus* across weeks in different tanks at $P < 0.05$

DISCUSSION

This study examined important differences in the boldness, aggressiveness, and growth of *O. niloticus* reared in tanks with different levels of structural complexity; namely, tanks with no structural enrichment (NSE), moderate structural enrichment (MSE), and heavy structural enrichment (HSE).

Structural enrichment creates an intricate habitat that offers animals suitable hiding places, thus shielding them from environmental stress (Näslund & Johnsson, 2014). Thus, anxiety-like behaviors are reduced and consequently an improvement is observed in the overall well-being (Maximino *et al.*, 2010). In this study, fish in tanks with heavy structural enrichment were bolder in the open field test than fish reared in tanks with moderate structural enrichment and no structural enrichment. In contrast, von Krogh *et al.* (2010) found that zebrafish kept in barren environments had higher activity levels than those in enriched environments. While, studies of other species have found no structural enrichment effect on the boldness of the three-spine sticklebacks (Brydges & Braithwaite 2009), the guppies (Burns *et al.*, 2009), and the zebrafish (Woodward *et al.*, 2019) although there are variations in the methods used to test for boldness in the aforementioned studies. Boldness was tested in rearing tanks in some studies that were against testing in a separate tank as the case employed in the present study. Therefore, it

is unclear whether variations in the methods used to test for boldness affected the outcome of the tests.

Aggressiveness was found to reduce with increasing the structural enrichment. Fish in tanks with heavy structural enrichment had a lower rate of aggressiveness as the weeks progressed than fish in tanks with a moderate structural enrichment and no structural enrichment. When observed in their rearing tanks, the enriched fish displayed more shoal cohesion and fewer aggressive behaviors. The presence of complex structures decreased the aggression levels, as measured by significantly fewer flight behaviors and almost no fin erosion in the enriched groups. Similarly, habitat complexity was shown to decrease the aggressiveness in the zebrafish, *Danio rerio* (Carfagnini *et al.*, 2009), the redbreast tilapia, *Tilapia rendalli* (Torrenzani *et al.*, 2013), the Atlantic salmon, *Salmo salar* (Näslund *et al.*, 2014), and the catfish, *Clarias gariepinus* (Ojelade *et al.*, 2022). In contrast, Woodward *et al.* (2019) used a similar method for testing aggressiveness as that reported in this study and found that aggressive behaviors in the adult zebrafish housed in extremely enriched tanks were higher than those housed in moderately enriched and barren tanks.

Fish reared in tanks with heavy structural enrichment had a higher growth rate, and thus were larger in terms of weight and length (standard and total length) than fish in the tanks with moderate and no structural enrichments, given that the same amount of food was given to all fish in each treatment. Similarly, Ojelade *et al.* (2022) found that environmentally enriched housing tanks resulted in the best mean weight gain in the *Clarias gariepinus*. The *O. niloticus* fish is known for its aggressive and territorial behavior (Medeiros *et al.*, 2015). The heavy structural complexity of the tanks, providing adequate shelter (minimal in tanks with moderate structural enrichments and absent in tanks with no structural enrichments), allows subordinate fish to escape from the aggressive encounters. This structural complexity helps reduce stress levels and cortisol levels in fish. Stress may increase the cortisol levels in fish and increase fish's susceptibility to diseases and pathogens, and ultimately resulting in death. Therefore, cortisol elevation as a result of stress has been suggested as responsible for reduced growth rates of fish (Philip & Vijayan, 2015). Moreover, aggressive interactions may result in injuries, and the lower aggression levels observed in fish in the tanks with heavy structural enrichment led to a decrease in the damaged fins and other body parts, which can become entry points for pathogens that can affect the health and growth of fish (Nobel *et al.*, 2020). Remarkably, fish in the tanks with no structural enrichment had the least growth. The increased energy expenditure of fish by a restless state observed in the tanks with no structural enrichment might have negatively impacted the metabolic rate (Campos *et al.*, 2018), which in turn led to a decreased fish growth in this study. The increased aggression of the fish toward each other might lead to mortality. Although in

other situations, being bold and aggressive may be advantageous, for example in being able to secure a food source, territories, mate and evade predators (**Ariyomo *et al.*, 2017**).

Boldness increased in the heavily enriched tanks across weeks when compared to the other tanks, while the increased rate of aggressive behavior also explains the mortality rate recorded in the tanks with no enrichments. There were no structural enhancements unlike the tanks with moderate structural enrichment and heavy structural enrichment and as a result, fish in the tanks with no structural enrichment showed more aggression toward each other since they were able to sight other conspecifics better than in the moderately and heavily enriched tanks. Furthermore, the highest levels of growth rate in terms of weight and length increase were recorded in fish housed in the heavily enriched tanks.

CONCLUSION

Tank structural enrichment is an essential component of promoting the welfare of fish in captivity. By providing a stimulating and engaging environment that replicates their natural habitat, enrichment enhances the physical, behavioral, and psychological well-being of fish. Incorporating tank structural enrichment should be a fundamental consideration in the care and management of fish in captive settings to ensure the best possible welfare outcomes. It may be worthwhile to incorporate habitat enrichment into the culture of the *O. niloticus* to enhance the general welfare of this species.

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