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Different stocking density levels on some growth parameters and survival of Heteroclarias fingerlings reared under Laboratory Conditions in Minna, Niger State.

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Abstract

An eight weeks study was carried out on the influence of different stocking density levels on some growth parameters and survival rates of Heteroclarias fingerlings reared under laboratory conditions. One hundred of four weeks old Heteroclarias fingerlings of initial mean weight (1.80±0.15g) were randomly separated into four experimental groups consisting of four treatments: 5 (control), 10, 15 and 20 with two replicates each reared in a plastic aquaria tank (19×29×29.5×30cm³) and fed with a commercial diet (coppens) to satiation twice daily. Growth and physicochemical parameters were determined weekly based on standard experimental procedures while survival rates were monitored daily. The results showed that fingerlings cultured at stocking density of five (5) had significantly (p<0.05) higher total length (16.02±1.30 cm), standard length(14.78±1.32cm), weight (24.26±7.99g) and survival rate (100±0.10%) respectively at the end of the study. However, there was significant reduction (p<0.05) in the total length of fingerlings cultured under 10, 15 and 20 stocking densities from week 1 to the end of the study. Physicochemical parameters measured were not significantly (p>0.05) affected by the different stocking densities and were found within the permissible levels for optimum growth of Heteroclarias fingerlings in the tropics. This study showed that the stocking density of five (5) fingerlings/aquaria had better growth parameters and survival rate.

Keywords: Heteroclarias, Growth parameters, Physicochemical parameters, Stocking densities, Survival rate.

INTRODUCTION

Fish is a food consumed by many species of animals including humans; it has a tremendous impact on the lives of many individuals and communities in almost all parts of the globe, primarily serving as a major source of relatively cheap and affordable animal protein (Mohammed and Adamu, 2019). Aquaculture is one of the fastest growing animal food

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producing sectors, and has overtaken capture fisheries as a fish food source (FAO, 2016). The Nigerian aquaculture industry has grown considerably, contributing to the production of about 20,475 metric tons of fish per year in the 1990s to about 85,087 metric tons per year in 2007 (Nwipie *et al.* 2015). Heteroclarias is an inter-specific hybrid of *Clarias gariepinus* and *Heterobranchus bidorsalis* which transfer or combine desirable traits of the two species (Bartley *et al.* 2000; David *et al.* 2018). Several studies have demonstrated that *C. gariepinus* and *H. bidorsalis* hybrid exhibit superior growth, improved survival and general hardiness than true breed of either *C. gariepinus* or *H. bidorsalis* (David *et al.* 2018).

In Nigeria, Heteroclarias has been known to be the most accepted fish species and there is increase in demand for Heteroclarias or Clarias hybrids than their pure breeds for aquaculture because of their resistance to disease, fast and high growth rate (Ayanwale *et al.* 2014). The effect of stocking density on growth, survival and yield on aquaculture are well known for different species, and seemed to impact production differently (Rahman *et al.* 2013). Consequently, identifying the optimum stocking density for specie is a critical factor not only to enable efficient management and to maximize production profitability, but also for optimum husbandry practices, (Rahman *et al.* 2013). Similarly, Mensah *et al.* (2013) also reported that stocking density is one of the most important factors in aquaculture because it directly influences growth, survival, behavior, health, feeding and production of fish under farmed conditions. Fish growth may differ according to method of culturing and their stocking density (Okeke, 2014). Stocking density in fishes is one of the most sensitive factor in determining the productivity of a culture system as it affects growth rate, size variation and mortality of the fishes in that particular environment and time (De-Oliveira *et al.* 2012; David *et al.* 2018). The utilization of maximum space for fish production through intensive culture also improves the profitability of the fish farm (Jha and Barat, 2005).

Increase in stocking density results in increasing stress, which leads to higher energy requirements, causing a reduction in growth rate and food utilization (Suleiman and Solomon, 2017; David, *et al.* 2018). Contrarily, in case of low stocking densities fish may not form shoals or group together and feel comfortable (Rahman *et al.* 2013). Consequently, identifying the optimum stocking density for a species is a critical factor not only for designing an efficient culture system, but for optimum husbandry practices (Suleiman and Solomon, 2017).

Several studies have examined the effects of stocking density on the welfare of farmed fish, and have found it to be a source of chronic stress with commonly reported effects including reduced growth rates, alterations in the physical condition and health of fish, and the activation of stress responses. (Jha and Barat 2005; Ashley 2007; Louise, 2010; Suleiman and Solomon 2017 and David, *et al.* 2018). Stocking density is one of the factors that could potentially affect fish survival and production performance (Irwin *et al.* 1999), so it must be considered when determining the economic profitability of production systems (Baskerville-Bridges and Kling, 2000; Gomes *et al.* 2000). Furthermore, the use of the appropriate density is a commercially beneficial operation, focusing on maximizing the utilization of the rearing system and financial resources (Fairchild and Howell, 2001). Therefore this study is to investigate the influence of different stocking density level on some growth parameters and survival rates of Heteroclarias fingerlings under laboratory conditions in Minna, Niger state.

MATERIALS AND METHODS

Study Area

The study was conducted in Minna, Niger state. Minna is located in North Central Nigeria between the latitude of 9°31' and 9°45' North and Longitude 6°31' and 6°45' East of the

equator. The study area fall within the Southern Guinea savannah vegetation zone of Nigeria. Minna has two distinct seasons. Dry season from November to March which is completely devoid of rain and wet season from April to October.

Collection of Sample.

Four weeks old *Heteroclarus* fingerlings were bought from a private fish farm and transported to Animal Biology Laboratory of Federal University of Technology Bosso campus, Minna, in 50litres jerycan with opening on top well ventilated water to prevent mortality. The fishes were allowed to acclimatize to their new environment for a period of one week in the laboratory. During this period the fish were fed with a commercial diet (Coppens) to satiation in morning and evening in the month of March and April 2017 (Ayanwale *et al.* 2017).

Experimental design

The experimental design was a complete randomized design (CRD) consisted of four (4) treatments with two (2) replicates each. Treatment 1 (control), 2, 3 and 4 were stocked at 5, 10, 15, 20 fingerlings respectively with initial mean weight ($1.80 \pm 0.15g$) and length (6.0 cm) (Jamabo *et al.* 2009). The stocking density trials were conducted in an indoor aquaria plastic tank with dimension of $12 \times 29.5 \times 30cm^3$ supplied with 12litres of borehole water respectively. The fingerlings stocked in each of the tank were of same length and weight to avoid cannibalism (Jamabo *et al.* 2009). These experimental units consisted of a close system without water recirculation and the tanks were drained on Friday in a week and replaced with fresh borehole water between 08:00 and 10:00 hours. Left overfeed and faecal samples in the tanks were siphoned immediately after feeding (Ayanwale *et al.* 2018). The experiment was monitored for a period of eight weeks before termination.

Determination of Physicochemical parameters

Water Temperature

Water temperatures of the treatments were determined with mercury in bulb thermometer (10-110°C range). Temperature was determined by lowering the thermometer into the tanks in an inclined position for about 5 minutes to allow for equilibrium before taking the reading at 10.00am in the morning throughout the duration of the experiment.

Dissolved Oxygen

This was determined by using Winkler Azide method (American Public Health Association APHA, 2010). Water samples from the control and treatment tanks were collected by inserting 250 ml water sample bottles into the tanks and sampled water was fixed right in the laboratory with 1ml of reagent (I) (Manganous Sulphate) and 1ml of reagent (II) Alkaline iodide solution (KOH + KI). About 2 ml of Concentrated Sulphuric acid was added to each sample and 10ml of the sample was titrated with 0.025N Sodium Thiosulphate using starch as indicator until it turns colourless.

Calculation was based on the formula described by Boyd (1979) as follows

$$\text{Dissolved Oxygen (mg/L)} = \frac{\text{Volume}(\text{Na}_2\text{SO}_3) \times \text{Normality} \times 8 \times 1000}{\text{Sample volume (ml)}}$$

Biochemical Oxygen Demand (BOD₅)

Water samples from various experimental tanks were collected using a 250ml dissolved oxygen bottles without bubbles and were incubated in the dark for 5 days. The dissolved oxygen concentration was measured on the fifth day using the DO meter. BOD was calculated using the formula given by Boyd (1979).

$$\text{BOD}_5 \text{ (mg/l)} = \text{D1-D5}$$

Hydrogen Ion Concentration (pH)

The pH of the water samples were determined with Jenway 3305 pH meter model at room temperature. The pH meter probe was inserted into the sampled water for about 5 minutes until it stabilized before the reading was taken. The meter was standardized with buffer solutions of pH 4.0, 7.0 and 9.0 before the readings were taken.

Ammonia (NH₃)

100ml of the water sample from control and treatment tanks was pipetted into a Markham distillation apparatus (Kjeldal flask) and there after 5ml of 40% NaOH was added. The flask was connected to the condenser and the cooling water was turned on. About 10ml of 40% boric acid (H₃BO₃) solution was placed under the condenser ensuring that the tip of the condenser was immersed in the receiving solution and distilled slowly until 50ml of the distillate was collected in the receiving flask. The ammonia was determined from the distillate by titrating with 0.01M HCl until the colour at the end point changed from green to pink (APHA, 2010). Calculation was based on the formula below

$$\text{NH}_3 \text{ (mg/L)} = \frac{\text{Titre value} \times 14 \times 0.01 \times 1000}{V}$$

Where 0.01 = molarity of HCl used as titrant; 14 is the molecular mass of nitrogen; 1000 is the conversion to mg /litre and V is the volume of sample used.

Determination of Growth parameters

Total length and standard length

Five (5) *Heteroclaris* juveniles were randomly selected from each of the plastic aquaria tanks weekly by using a sieve. Each individual fish was placed with care on a plain paper so as to absorb the water on the fish specimen. Thereafter, the fish specimen was now placed on aluminum foil, and the lengths were measured using a transparent meter ruler graduated in centimeters (cm). The total length was determined by measuring the distance from the mouth of the fish to the caudal fin, while, the standard length was determined by measuring the distance from the mouth of the fish to the caudal penduncle (Ayanwale *et al.* 2017). This procedure was repeated for each of the fish samples from all the replicates.

Measurement of weight

Five (5) *Heteroclaris* juveniles were randomly selected from each of the plastic aquaria tanks weekly by using a sieve. The fishes were placed with care on a plain paper to absorb the water and the specimen fish was placed singly on a plastic petri dish cover whose weight was adjusted to zero and the weight of the fishes were determined using sensitive electronic scale (model EHA25). This procedure was repeated for each of the replicate. (Ayanwale *et al.* 2017)

Survival rate percentage (SR %)

This was calculated using the formula of Adewolu *et al.* (2008) that;

$$\text{SR (\%/)} = \frac{\text{Total number of fish harvested}}{\text{Total number of fish stocked}} \times 100$$

Data analysis

The data collected were analyzed for significant differences ($p < 0.05$) by the analysis of variance (ANOVA) using Microsoft Excel version 2003. Duncan Multiple Range Test (Duncan, 1955) method was used to separate the means where there were statistically significant differences ($p < 0.05$).

RESULTS

Total length

The results (mean \pm standard deviation) of Total Length (TL) of *Heteroclaris* fingerlings reared under different stocking density levels are presented in Table 1. The TL ranged from 6.64 ± 0.32 to 16.02 ± 1.30 cm of the fingerlings cultured at 5 stocking density level was significantly ($p < 0.05$) longer from week 2 to the end of the study. However there was a significant reduction in ($p < 0.05$) in the total length of fingerlings cultured under 10, 15 and 20 stocking density as the experiment progressed from week 1 to the end of the study.

Table 1: Mean total length of *Heteroclaris* fingerlings reared under different stocking density levels for the period of study

Trt	Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
5	4.91 ± 0.24^a	6.40 ± 0.32^b	6.64 ± 0.32^c	7.40 ± 0.49^c	9.48 ± 0.31^c	11.08 ± 0.71^c	12.88 ± 1.21^c	14.26 ± 1.47^b	16.02 ± 1.30^c
10	4.87 ± 0.21^a	6.14 ± 0.60^b	6.25 ± 0.18^b	6.66 ± 0.27^b	8.40 ± 0.27^b	9.99 ± 0.29^b	12.09 ± 0.56^b	13.04 ± 0.66^b	13.95 ± 0.68^b
15	4.73 ± 0.25^a	5.62 ± 0.20^a	6.09 ± 0.20^b	6.36 ± 0.23^{ab}	7.14 ± 0.61^a	8.39 ± 0.60^a	10.21 ± 0.62^b	11.04 ± 0.89^a	12.36 ± 0.88^a
20	4.92 ± 0.30^a	5.39 ± 0.16^a	5.71 ± 0.26^a	6.08 ± 0.20^a	6.87 ± 0.37^a	7.79 ± 0.23^a	8.81 ± 0.95^a	9.96 ± 1.19^a	11.40 ± 0.74^a

Note; values with same superscript in the same column are not significantly different ($p > 0.05$)

Standard length

The results (mean \pm standard deviation) of Standard Length (SL) of *Heteroclaris* fingerlings reared under different stocking density levels are presented in Table 2. The SL ranged from 6.55 ± 0.48 to 14.78 ± 1.32 cm of *Heteroclaris* fingerlings reared under 5 stocking density level was significantly ($p < 0.05$) higher from week 3 to 8. However, the standard lengths of fingerlings cultured under 15 and 20 stocking density levels were not ($p < 0.05$) affected from week 1 to the end of the study.

Table 2: Mean standard length of *Heteroclaris* fingerlings reared under different stocking density for the period of study

Trt	Initial	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
5	4.67 ± 0.25^a	5.59 ± 0.27^b	5.68 ± 0.29^b	6.55 ± 0.48^c	8.67 ± 0.40^c	10.21 ± 0.78^c	11.77 ± 1.29^c	13.22 ± 1.42^c	14.78 ± 1.32^c
10	4.27 ± 0.18^a	5.32 ± 0.59^b	5.36 ± 0.25^{ab}	5.60 ± 0.23^b	7.52 ± 0.24^b	9.16 ± 0.14^b	10.76 ± 0.55^c	11.83 ± 0.65^b	12.86 ± 0.69^b
15	4.08 ± 0.21^a	4.82 ± 0.22^a	5.16 ± 0.22^a	5.32 ± 0.29^a	6.30 ± 0.52^a	7.33 ± 0.52^a	8.97 ± 0.4^a	9.88 ± 0.63^a	11.26 ± 0.84^a
20	3.98 ± 0.32^a	4.52 ± 0.15^a	4.96 ± 0.41^a	5.24 ± 0.18^a	6.08 ± 0.38^a	6.88 ± 0.39^a	7.80 ± 0.91^a	8.77 ± 1.41^a	10.47 ± 0.82^a

Note; values with same superscript in the same column are not significantly different ($p > 0.05$)

Total weight

The results (mean \pm standard deviation) of body weight of *Heteroclaris* fingerlings reared under different stocking density levels are presented in Table 3. The weight of the fingerlings ranged from 2.15 ± 0.43 to 24.26 ± 7.99 g reared under stocking density of 5 was significantly ($p < 0.05$) affected from week 1 to 8. However, the weight of the fingerlings cultured under 15 and 20 stocking density levels was not significantly ($p > 0.05$) affected from week 4 to 7.

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Table 3: Mean weight of *Heteroclaris* fingerlings reared under different stocking density levels for the period of study

Tr	Initial	1	2	3	4	5	6	7	8
5	1.28±0.22 ^a	2.15±0.43 ^c	2.42±0.41 ^c	3.60±0.81 ^c	6.39±1.56 ^b	13.33±2.31 ^c	17.78±4.45 ^c	20.49±6.24 ^c	24.26±7.99 ^c
10	1.04±0.11 ^a	1.76±0.33 ^b	2.08±0.39 ^{bc}	2.32±0.37 ^b	3.72±0.49 ^a	9.42±0.71 ^b	13.61±1.93 ^b	16.26±2.57 ^b	18.57±3.05 ^{bc}
15	0.91±0.05 ^a	1.43±0.12 ^a _b	1.66±0.18 ^{ab}	1.83±0.26 ^{ab}	2.97±0.74 ^a	4.57±0.95 ^a	7.20±1.60 ^a	10.38±1.89 ^a	13.50±1.86 ^{ab}
20	0.93±0.13 ^a	1.18±0.13 ^a	1.47±0.20 ^a	1.66±0.15 ^a	2.85±0.63 ^a	3.74±0.51 ^a	6.61±1.75 ^a	7.64±1.96 ^a	9.97±1.68 ^a

Note; values with same superscript in the same column are not significantly different (p>0.05)

Survival rate

The results (mean± standard deviation) of survival rate of the *Heteroclaris* fingerlings reared under different stocking density levels are presented in Table 4. The survival rate (100.00±0.10%) of fingerlings cultured at stocking density of 5 was significantly (p<0.05) higher among other groups. However the survival rates of fingerlings reared at stocking densities of 10, 15 and 20 were not significantly (p>0.05) affected at the end of the study.

Table 4: Mean survival rate of *Heteroclaris* fingerlings reared under different stocking density levels for the period of study

Stocking density levels	Survival (%)
5 (Control)	100±0.10 ^a
10	93.2±0.51 ^b
15	92.3±0.50 ^b
20	93.2±0.49 ^b

Note; values with same superscript in the same column are not significantly different (p>0.05)

Physicochemical parameters

The results (mean±standard deviation) of water physicochemical parameters of *Heteroclaris* fingerlings reared under different stocking densities are presented in Table 5. The water temperature (26.83±0.50 to 27.11±0.44°C), pH (6.71±0.08 to 6.92±0.19), Dissolved Oxygen concentration (6.33±2.87 to 6.78±2.82 mg/l), Biochemical Oxygen demand concentration (0.67±0.50 to 1.22±0.67 mg/l) and Ammonia concentration (0.04±0.02 to 0.04±0.01 mg/l) from all the treatments were not significantly (p>0.05) affected during the experimental period.

Table 5: Mean water physicochemical parameters of *Heteroclaris* fingerlings reared under different stocking densities for the period of study

Parameters	5	10	15	20
Temperature(°C)	26.83±0.50 ^a	26.72±0.49 ^a	27.10±0.21 ^a	27.11±0.44 ^a
pH	6.92±0.19 ^a	6.83±0.12 ^a	6.71±0.08 ^a	6.83±0.24 ^a
Dissolved oxygen (mg/l)	6.78±2.82 ^a	6.33±2.87 ^a	6.78±2.77 ^a	7.56±3.13 ^a
Biochemical oxygen demand (mg/l)	0.67±0.50 ^a	1.22±0.67 ^a	0.89±0.78 ^a	1.00±1.00 ^a
Ammonia concentration (mg/l)	0.04±0.01 ^a	0.04±0.01 ^a	0.04±0.01 ^a	0.04±0.02 ^a

Note; values with same superscript in the same column are not significantly different (p>0.05)

DISCUSSION

Stocking density is one of the most important factors in aquaculture because it directly influences growth, survival behavior, health, feeding and production of fish or fingerlings under farmed conditions (Mensah *et al.* 2013). Ronald *et al.* (2012) also observed that the use of adequate densities, considering the number of animals and/or the biomass in the tank, can increase production and consequently the profitability of the activity. The significant increase in the Total Length, Standard Length and weight of the fingerlings cultured at 5 stocking density level during the greater period of the investigation could be attributed to favorable water quality factors and better feed quality (Osofero *et al.* 2009). Similarly, Russell *et al.* (2008) reported that suitable stocking density varies with species, water quality and feeding regimes, and is important for sustainable aquaculture. This finding agreed with that of Sorphea *et al.* (2010) who observed higher growth and survival rates at lower stocking densities for *Oreochromis* species in a pond. Significant reduction in the total length of fingerlings cultured under 10, 15 and 20 stocking densities from week 1 to the end of the study might be due to extra energy expenditure on competition for food and space (Levinus and Aviti, 2016). Furthermore, culturing fishes at densities less than optimal decreases the efficiency and profitability of culture system. It has been demonstrated that rearing fish at inappropriate stocking densities may impair growth and reduce immune competence due to factors such as social interaction and water quality deterioration which can affect both feed intake and conversion efficiency of the fish (Akinwole, *et al.* 2014; Tibile *et al.* 2016). Sahoo *et al.* (2004) and Nwipie *et al.* (2015) also made similar observations and reported that under crowded conditions, fish suffer stress as a result of aggressive feeding interaction and eat less, resulting in growth retardation. Increase in stocking density leads to increase in energy requirements due to stress, causing reduced growth and food utilization (Tibile *et al.* 2016).

The high survival rate recorded in the lowest stocking density (5) could be attributed partially to the physicochemical parameters of the water body and also due to the good health condition of the fish (Ofor and Afia, 2015). This observation also indicates an inverse relationship between survival rate and stocking density; it was also noticed that as the stocking density increases, the survival rate decreases and could probably be due to stress experienced as a result of aggressive feeding behaviour where energy meant for growth is used up in frenzy feeding activities (Okeke, 2014; Suleiman and Solomon, 2017). To support the above assertions, Rowland *et al.* (2004) and Schram *et al.* (2006) reported that increased stocking density has a negative effect on survival and growth of cultured fingerlings. Tibile *et al.* (2016) also noticed that lower stocking densities of fishes showed significantly higher growth and survival rate. Water quality or physicochemical parameters are critical for survival, health and growth of fish especially in control systems and for the production of quality fish seed in the hatchery. To maintain good water quality the physical and chemical properties of water should be kept within certain safe levels as well as biological properties (Mohamed *et al.* 2016). The physicochemical parameters measured were not affected by the different stocking densities and were within the acceptable range for fish culture in the tropics and fairly stable throughout the experimental period. The water temperature range recorded was within the ideal temperature required for catfish culture in fresh water. Water temperature of 26.72 ± 0.49 to 27.11 ± 0.44 °C were within the range of 25.00 to 32.00 °C acceptable for good fish growth (Ayanwale *et al.* 2014). Water pH range of 6.71 ± 0.08 to 6.92 ± 0.19 observed in this study fell within the recommended range of 6.5-9.0 as documented by Bryan (2004). The Dissolved Oxygen concentration of 6.33 ± 2.87 to 7.56 ± 3.13 mg/l recorded in this study was above the recommended range of 3.00 to 5.00

mg/l as reported by FAO(2016). This finding also confirmed that Dissolved oxygen was not limiting in the aquaria tanks as reported by Osofero *et al.* (2009) in a similar work but in bamboo net-cages. Similarly, the Biochemical oxygen demand concentration of 0.67 ± 0.50 to 1.22 ± 0.67 mg/l were also within the acceptable range of 1.0 to 5.00 mg/L recommended for fish growth in the tropics (CIESE, 2010). These results suggest no organic pollution from left over feed or faecal matter in the rearing media of Heteroclaris fingerlings throughout the experimental period which in turn increased the Dissolved oxygen concentration. The Ammonia concentration of 0.04 ± 0.01 to 0.04 ± 0.02 mg/l were within the range 0.01 to 1.55 mg/l for freshwater fingerlings as documented by Kohinoor *et al.* (2001).

CONCLUSION

The Heteroclaris fingerlings cultured at stocking density of five (5) had higher total and standard lengths, weight and survival rate. The physicochemical parameters measured were not affected by the different stocking densities and were all within the acceptable range for fish culture in the tropics. Similar study should be carried out in different receptacles such as concretes ponds, earthen ponds, and other aquatic environment so as to determine the appropriate stocking density for the fingerlings.

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