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DETERMINATION OF OPTIMUM STOCKING DENSITY FOR *Heterobranchus longifilis* (BURCHELL, 1822) LARVAE IN AQUARIUM TANKS

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Abstract- *Heterobranchus* longifilis is highly esteemed fish in Africa, but high demand for the depleting natural stock and the deficiency of the fry production, poses major problem to fish farmers. To determine optimum stocking density that will yield highest growth performance in the aquaculture of *Heterobrachus longifilis* juveniles, 14 days-old fish juveniles were counted and stocked in triplicates, at five densities 2500, 3000, 3500, 4000, and 4500 larva per tank (5 larva/L (D5), 6larva/L(D6), 7larva/L(D7), 8larva/L (D8) and 9larva/l(D9) respectively). The results of the specific growth rate (SGR), mean daily weight gained (MDWG) and performance index (PI), condition factor (CF), survival rate (SR) and biomass showed lower values at higher stocking densities. However, the coefficient of variation (CV) and apparent food conversion ratio (AFCR) increased with stocking density, with highest values at D8 and D9 indicating that, high stocking density reduced feed efficiency and produce poor final biomass. The best values were therefore obtained in stocking density of 6 larvae /L (D5).

Keywords- Stocking density, *Heterobranchus longifilis,* Glass aquaria, specific growth rate, performance index, Condition factor

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Introduction

Members of the C*lariidae* are highly esteemed in Nigeria [1] and the species are endemic to tropical Africa and widely distributed in the Nile and most River Basins in West Africa [2]. Biologically they are most ideal aquaculture species; are hardy and adaptable principally as a consequence of its air breathing ability, feeds on a wide array of natural prey under diverse conditions.

However the deficiency of fry production poses a major problem to fish farmers moreover because of the high demand the natural stock is reduced [3]. They do not reproduce under captivity and when they breed in the wild, the survival of the offspring is extremely low due to climatic and biological hazards associated with the wild. That consequently, increases the cost of marketable fish production. Reliable and consistent source of fish seeds for commercially important species is important in maximizing production capability of marketable fish (400-450 g). Various management techniques such as environmental and hormone-induced breeding by hypophysation have therefore been developed for mass production of *Heterbranchus* and *Clarias* fry and fingerlings under controlled conditions [4-10]. Also there had been a general shift away from rearing larvae in nursery ponds to hatchery troughs and laboratory conditions due to environmental condition example water quality [11], which depress feeding activities [12]. Under laboratory conditions the problem of sibling cannibalism among clariid Juveniles [13-16] further reduce the chance of their survival. Sibling cannibalism among clariid juveniles has been attributed to high density fish culture conditions [17] and competition for food [18,19]. Stocking density had been identified as the main factor determining the growth [20,21] and the final biomass harvested [22]. This research will therefore determine optimum density that will yield highest growth performance in *Heterobrachus* juveniles under laboratory condition.

Materials and Methods

Study Site

The research was carried out in the fish hatchery complex of the University of Calabar. Water supply is from a perennial water reservoir 50m away. The water is pumped into overhead tank of about 2000 gallons located just beside the hatchery. By the use of filters, water is recycled at 2000m^3/day into aquarium tanks.

Breeding

Breeding of African catfish *H. longifilis* was carried out by hypophysation according to method described by Legendre [3] using purified carp pituitary extract at a dose of 4mg/kg body weight. Males (760.4 ± 102.8g and 42.4 ± 4.4 cm) and female (678.6 ± 111.3g and 40.5 ± 4.5cm) were selected. Fertilized eggs were incubated in water with flow through rat 1-31/min. mean hatching percentage was 80%. The fry were fed exclusively on live food (zooplankton) within the first 14days according to Hecht *et al* [23].

Experimental Design

Effect of Density

To determine the effect of density on the growth rate of catfish juveniles, 15 fibreglass tanks filled with 500L of water (20cm water depth) were used. Daily water exchange in the tanks was 1.5L/min. 14days-old fish juveniles were counted and stocked at five densities 2500, 3000, 3500, 4000, and 4500 larva per tank 5 larva/L (D5), 6larva/L(D6), 7larva/L(D7), 8larva/L (D8) and 9larva/l(D9) respectively). Triplicate tanks were constituted for each stocking density. Fish were feed ad libitum thrice daily at 10% biomass per day with dry food in form of crumbled non-pelletised feeds formulated according to Balagon and Ologbobom [24] with 40%, protein. Random samples of 100fish were measured for total length and weight before the start of experiment. Initial lengths were measured to the nearest half millimeter under the compound microscope using an ocular micrometer (10 fold magnification). Similarly, wet weight was recorded using an electronic digital balance (accuracy of ± 0.01). Initial mean total length and individual weight of 100 larvae were 1.8 ± 0.08 cm and 0.038 ± 0.004g respectively.

During the experiment, left over feeds and waste were removed twice a day in the morning (600am) and in the evening (1600h). Dead fish in each tank were recorded and the tanks were uniformly aerated. Water temperature, dissolved oxygen and pH were measured daily at 6.00h. Water quality as phosphor, nitrate- nitrogen and nitrite-nitrogen were estimated weekly by the spectrometric method. At weekly intervals, 30 larvae were randomly sampled in each tank, and the total weight and length measured individually and daily food ration requirement was adjusted. After 49 days of rearing, all surviving larva from each tank were collected, counted measure for weight and length. The survival rate (SR), specific growth rate (SGR), body weight variation coefficient (CV), mean daily weight gained (MDWG), condition factor (CF) and apparent food conversion ratio (AFCR) was calculated as follows

$$SR\ (\%) = \frac{\text{Final number of larva}}{\text{Initial number of larva}} \times 100$$

$$SGR\ (\%) = \frac{\ln(\text{final body weight}) - \ln(\text{initial body weight})}{\text{Duration of rearing period (days)}} \times 100$$

$$CV\ (\%) = \frac{\text{Standard deviation of mean body weight}}{\text{Mean body weight}} \times 100$$

$$MDWG\ (\text{mg/day}) = \frac{\text{Final mean body weight (g)} - \text{Initial body weight (g)}}{\text{Duration of rearing period (days)}}$$

$$CF = \frac{\text{Final mean body weight (g)}}{\text{Mean total length}^3\ (\text{cm})}$$

$$AFCR = \frac{\text{Dry weight of total weight given (Kg)}}{\text{Harvested biomass (Kg)}}$$

To evaluate the effect of stocking density on production performance with more precision performance index (PI) was calculated [28,32]. The index was calculated by combining two responses such as growth and survival.

$$PI = \frac{\text{Survival rate (SR)} \times (\text{Final mean body weight(g)} - \text{Initial body weight (g)})}{\text{Duration of rearing period}}$$

Analysis

In all experiments the physio-chemical parameters of water were analysed weekly based on APHA (1980). The differences in the growth rate at different densities and feeding frequencies of *H. longifilis* juveniles were compared by one-way analysis of variance (ANOVA). Morphomeric relationship of fish were examined using correlation and regression models. Results of the water quality parameters are shown below

Results

Water quality monitored characteristics monitored throughout the study period showed water temperature range between 19.7 ± 0.4 and 20.6 ± 0.2˚C with no significant difference between treatment ($p>0.05$) [Table-1]. The pH mean values ranged from 6.7 ± 0.3 to 7.8 ± 0.2 and decreased with increasing stocking density. Also, values of dissolved oxygen are lowered significantly at higher stocking densities with the least value obtained in D9 and highest at D5. On the other hand, means of phosphor, nitrite-nitrogen and nitrate - nitrogen were significantly higher ($p<0.05$) with increasing stocking densities.

There was no much variation in the total length of larva stocked at different densities during the first part of the rearing period [Fig-1]. During the second quarter of the rearing period larva stoked at D5, D6, D7 significantly attained highest mean total length while the lowest values were obtained in higher stocking densities. However, there was significant variation in total weight throughout the rearing period, again with highest mean weight occurring at D5, D6 and D7. The larval growth was therefore influenced by the stocking density.

Table 1- mean ± SD of in-tank water temperature (T), pH, dissolved oxygen (DO), nitrite - nitrogen (NO_2-N), nitrate- nitrogen (NO_3- N) and Phosphorus (PO_4^{3-}- P) recorded during larval rearing in tanks. Each mean represents samples collected at daily (Temperature) and at weekly (other parameters) intervals during the 28 days rearing period.

Stocking Density	D5	D6	D7	D8	D9
Water quality					
Temperature (T˚C)	20.4 ± 0.1^{a}	19.7± 0.4^{a}	20.0 ± 0.1^{a}	19.8 ± 0.6^{a}	20.6 ± 0.2^{a}
pH	7.2 ± 0.3^{ab}	7.6 ± 0.1^{b}	7.8 ± 0.2^{b}	6.9 ± 0.2^{bd}	6.7 ± 0.3^{d}
Dissolved Oxygen (mg/l)	7.0 ± 0.1^{a}	6.4 ± 0.3^{c}	5.8 ±0.5^{d}	4.8 ±0.6^{e}	4.1 ± 0.4^{e}
NO_2 - N (mg/l)	0.01± 0.000^{a}	0.02±0.001^{b}	0.03±0.001^{c}	0.03±0.007^{c}	0.04±0.002^{d}
NO_3 - N (mg/l)	0.08±0.008^{a}	0.18±0.002^{b}	0.21±0.005^{c}	0.28±0.005^{d}	0.31±0.006^{e}
$(PO_4)^3$ - P (mg/l)	0.11±0.01^{a}	0.18±0.04^{b}	0.24±0.02^{c}	0.30±0.05^{d}	0.39±0.07^{e}

Mean values with different superscript letters within a row are significantly different ($p<0.05$)

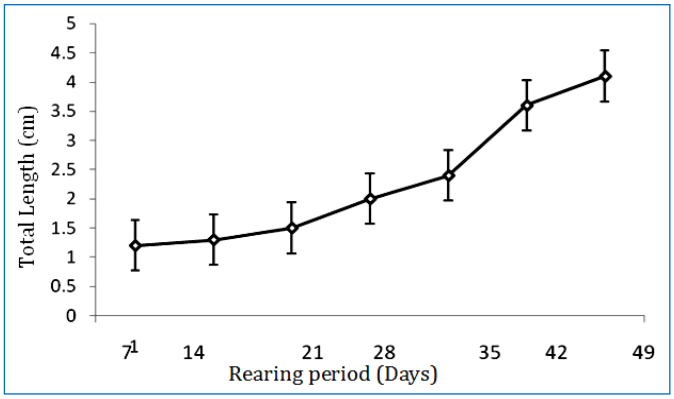

Fig. 1- Growth in total length of *H. longifilis* larva cultured at five densities in tanks for 49 days

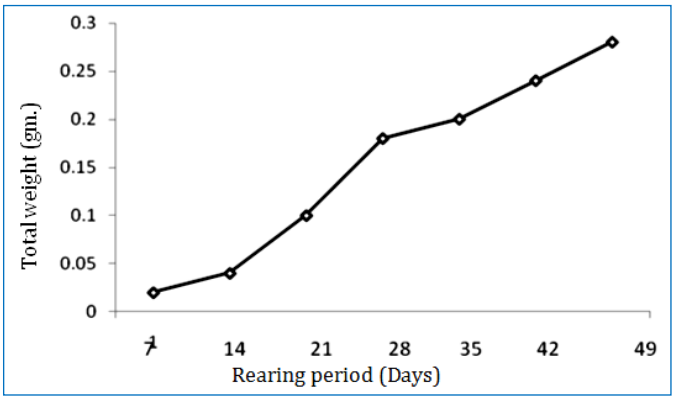

Fig. 2- Growth in body weight of *H. longifilis* larva cultured at five densities in tanks for 49 days

The performance of larvae at the end of the rearing period, are presented in [Table-2]. The results of the specific growth rate (SGR), mean daily weight gained (MDWG) and performance index (PI), condition factor (CF), survival rate (SR) and biomass showed lower values at higher stocking densities. The best values were therefore obtained in D5 and D6. On the other hand, the coefficient of variation (CV) and apparent food conversion ratio (AFCR) increased with stocking density with highest values at D8 and D9. Out of all treatments, cannibalism was only exhibited in D8 and D9 during this experiment. Mortality could therefore be due to natural death, manipulation and cannibalism.

Table 2- Mean ± SD of final total length, final body weight, final specific growth rate (SGR), daily weight gained (MDWG), final condition factor, final body coefficient of variation (CV), final apparent food conversion ratio, Survival rate, performance index and final biomass of Heterobranchus longifilis reared in tanks of five densities during the 28 days rearing experiments.

Stocking Density	D5	D6	D7	D8	D9
Growth Performance					
Total length, cm	4.2±0.23^{a}	3.9±0.17^{a}	2. 8±0.08^{b}	2.10±0.14^{c}	1.4±0.11^{d}
Body weight, g	0.28±0.045^{a}	0.22±0.034^{a}	0.15±0.016^{b}	0.08±0.001^{c}	0.02±0.011^{d}
SGR, % /day	7.23±0.12^{a}	6.44±0.16^{b}	5.66±0.11^{c}	5.33±0.18^{c}	4.32±0.22^{d}
MDWG, mg/day	5.21±0.10^{a}	4.33±0.17^{b}	3.45±0.15^{c}	2.88±0.16^{d}	2.08±0.21^{e}
Condition factor	0.86±0.07^{a}	0.80±0.01^{a}	0.76±0.04^{b}	0.67±0.02^{c}	0.61±0.05^{c}
CV, %	15.23±0.57^{a}	16.46±0.65^{b}	18.44±0.87^{c}	18.86±0.33^{c}	19.44±0.76^{d}
AFCR	1.65±0.01^{a}	1.82±0.03^{b}	1.86±0.07^{b}	2.16±0.01^{c}	2.74±0.22^{c}
Survival rate, %	99.56±0.17^{a}	90.43±1.23^{b}	88.36±1.37^{b}	80.45±1.65^{c}	70.86±2.67^{d}
PI	0.66±0.09^{a}	0.60±0.03^{b}	0.56±0.07c	0.50±0.02^{c}	0.46±0.04^{d}
Biomass, g/m^{3}	1034.7±8.57^{a}	994.8±7.77^{a}	890.6±9.47^{b}	789.8±8.57^{c}	711.6±8.76^{d}

Mean values with different superscript letters within a row are significantly different (p<0.05)

Discussion

Environmental conditions in the cultured tanks influenced the optimum stocking density. High accumulation of organic matter and metabolic waste from the juvenile fish led to significantly higher concentration of nitrogen compounds which also lowered the dissolved oxygen in D8 and D9, compared to other densities. The gradual increase in the biomass in tanks with higher stocking densities, resulting in the higher consumption of dissolved oxygen explained the decreasing trend of dissolved oxygen in tanks D8 and D9. In this experiment, *Heterobranchus longifilis* larvae reared at high stocking densities responded with high levels of metabolites; such as urine and faeces. This stress response in fishes, changes water quality [24]. Water quality, mainly dissolved oxygen and pH levels are considered as limiting factor in intensive fish culture. High levels of dissolved oxygen increased growth in channel catfish *Ictalarus punctatus* larvae reared in tanks [11]. The result agree with findings of Sympath *et al* [26] who showed that air-breathing fish (*Channa striatus)* in higher densities consumed less and converted far less efficiently, spending greater energy surfacing. The high AFCR at density of D8 and D9 may be probably because at optimum density the fish consumed the highest ration and convert it with maximum deficiency, conserving energy by decreasing surfacing activity. In general, poor growth performance of cultured fish takes place at pH < 6.5 [27]. It is possible that decreasing dissolved oxygen and increasing of the other water quality parameters observed in the study, induced stress which results in the low growth at high densities.

The high survival rate in all tested densities shows that individuals reared in the maximum density produced maximum biomass. However the mean body weight of a single individual is less than that in optimum density. The optimum density which yields maximum production without sacrificing mean weight is profitable for aquaculture [28]. Individual growth rate can be increased by decreasing density because the effect of competition and cannibalism is removed.

In this experiment, high stocking density of *Heterobranchus longifilis* larva, reduced growth parameters (SGR, PI and biomass) indicating optimum production performance at stocking density of D5 where yield is significantly higher than D7 and almost equal to D6. Under crowded conditions at higher stocking densities, fish surfers stress as result of aggressive feeding interaction and eat less, resulting in growth retardation [29]. In a similar experiment using *Clarias batrachus* larvae reared in tanks, Sahoo et al [30] reported a similar effect of high stocking density on growth. The condition factor of *Heterobranchus longifilis* larva also decreased at high stocking densities D8 and D9 but was similar at lower densities D5 and D6. This result suggest that, at lower stocking densities all larva received adequate amount of food and are exposed to adequate environmental conditions, compared to these of higher densities.

On the other hand, the apparent food conversion ratio increased in high stocking densities. Food conversion ratio and feed efficiency were negatively correlated with stocking density in H. *longifilis* larva. Therefore, high stocking density reduced feed efficiency. This agrees with results from Jha and Barat [31] using *Cyprinus carpio* larva and Rahman et al [32] with *Tor putitora.*

Reduction in the mean body weight at higher stocking densities might be due to insufficient food as a result of competition leading to the poor growth of small sized fish. Differential growth and social dominance among the larva of clariid family had been observed [18, 33, 30]. At the beginning of this experiment there was variation in the body sizes of larva stocked.

Variation in the body sizes became pronounced amongst larva in higher stocking densities, D8 and D9, suggesting that these densities increased the dependence of growth on the initial size of the individual larva and thus the advantage gained by the largest in competing for food.

Survival rate was lower at high densities, and the dead larvae removed from the tanks were of small sizes with flattened abdomen indicating lack of food. High mortality among small sized larvae might be caused by the dominance of "shooters" (large size larvae) better equipped to out-compete small size larvae, for scarce food resources in high density tanks. It was even observed that in high

density tanks, larger individuals exhibited more active swimming behavior and always waiting in feeding areas of the tanks before and during feeding. While the larger sized larva spent more time feeding, the small sized larvae were reluctantly feeding at the bottom of tanks. The effect of stocking density on larval survival has also been observed to depend on the type of water receptacle. In the larvae of *Rachycentron canadum* [34], *Clarias batrachus* [30] and *Solea solea* [17] reared in tanks, survival rate decreases as stocking densities increase. However, stocking density did not affect survival of *Clarias gariepinus* larvae reared in cages [35]. Therefore, the rearing conditions of larva play a vital role in determining the optimum stocking density of fish larva.

Conclusion

Stocking density has significant effect on the growth of H. *longifilis* in tanks. Optimum stocking density for H. *longifilis* 6 larvae /L (D6). However, the same study must be conducted using different water holding structures such as cages, ponds and raceways.

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