ORIGINAL RESEARCH ARTICLE

Effect of stocking density and age at stocking on growth and survival of *Heterobranchus bidorsalis larvae*

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ABSTRACT
Survival and growth performance of Heterobranchus bidorsalis larvae reared for 28 days at different stocking densities and ages were monitored to determine the optimum stocking density and age at stocking. Nine hundred fry were stocked at densities 100fry/m³ (SD1), 200fry/m³ (SD2), 300fry/m³ (SD3) and 400fry/m³ (SD4). Fry were stocked at day 3, day 6 and day 9 after hatching (A, B, and C respectively). They were fed with same commercial fry wean diet ad libitum. Growth indices (mean weight gain, mean total length, average daily growth rate, specific growth rate, performance index, condition factor) and survival rate were measured. The result showed that mean weight gain, mean total length, average daily growth and specific growth rate were stocking density dependent. Mean weight gain ranged from 0.09±0.03 in SD2A to 0.41±0.15g in SD1A. The least stocking density (100fry/m³) stocked at day three (SD1A) had the highest values of mean weight gain and mean length gain, while the highest survival was also recorded in the fish stocked at 100fry/m³ in day three (SD1A) and day nine (SD1C). The fish stocked in day three at 100fry/m³ had the highest performance index but the most consistent performance index was observed in fish stocked in day nine. It was concluded that <u>H.</u> bidorsalis fry should be stocked at 100fry/m³ at day three after hatching as this will enhance optimum survival and best yield.

Keywords: *Heterobranchus bidorsalis* fry, Performance index, Fish yield

INTRODUCTION
In many settings fish represent the principal animal source of food for the population, supplying both high quality protein and essential micro-nutrients for maintaining health and well-being (Kawarazuka and Bene, 2011). However, as at 2009, about 29.9% of fish stocks were overexploited, producing lower yields than their biological and ecological potential (FAO, 2012). Aquaculture therefore remains the most viable option to meeting this target. Species such as *Heterobranchus longilis* and *Clarias gariepinus* are widely cultured for their growth performance, resistance to intensive rearing conditions, and meat quality (Kerdchuen, 1992; Imorou Toko *et al*., 2008). However, in African catfish culture, supply of fingerlings for commercial production appears to be a major constraint. Many investigations have shown that this weakness could be related to difficulties encountered during the early stages of culture (*Alla et al. 2010*). Stocking density is one of the major factors determining growth (Engle and Valderrema, 2001) and the biomass harvested (Bonjard *et al.,* 2002). Closely related to this is the age at stocking of the fry in the nursery facilities. However, there is a dearth of information on these areas in African aquaculture, especially as relates to fish seed production. This research therefore investigated the effects of stocking density and age at stocking on growth and survival of *Heterobranchus bidorsalis* fry.

MATERIALS AND METHODS
The study was carried out at the Hatchery Unit, University of Ibadan Fish Farm. Hatchery raised 18 months old gravid bloodstocks were selected following Ayinla *et, al.,* (1994). Synthetic

hormone (Ovaprim) was administered at 0.5ml/kg body weight of female fish. After latency period; stripping, fertilization and incubation of eggs was carried out following the procedures described in Haylor, (1993). A 3x4 factorial arrangement in completely randomized design was adopted. Nine hundred fry were stocked and grouped into four treatments representing different stocking densities (100fry/m^3 (SD1), 200fry/m^3 (SD2), 300fry/m^3 (SD3) and 400fry/m^3 (SD4) in plastic aquarium tanks. Fry were stocked at day 3, day 6 and day 9 after hatching (A, B, and C, respectively). The fries were fed with encapsulated artemia 4 times daily *ad libitum*. They were raised for 28 days during which growth performance and survival rate were monitored. The initial weight and length of fry were taken per treatment. The initial length at day 3 was measured under ocular Microscope while subsequent measurements were taken with an electronic sensitive balance (OHAUS Model) and transparent graduated ruler and thread. Water temperature, pH, and nitrite were monitored with water test kits while dissolved oxygen was measured using Winkler's method (Boyd 1979). Growth performance was investigated by *determining the mean weight gain, mean length gain, average length gain, average daily growth, specific growth rate, performance index and condition factor using standard methods.

Data analysis
The data obtained were subjected to two-way analysis of variance. Differences between means were compared at p<0.05. Interactions between the parameters measured were established using correlation analysis.

RESULTS
Water quality
Results on water quality assessment (Table 1) showed that stocking density and age had significant effect on nitrate (p<0.05). Fry age and stocking density had significant effects on temperature, dissolved oxygen and hydrogen ion concentration (pH). Stocking density and fry age are jointly significant with Dissolved Oxygen. Interaction between the individual stocking density, stocking age and the joint effect of both showed that stocking density of 300fry/m^2 was significantly different from other stocking densities. Treatments with fry stocked on the ninth day had significantly different (p<0.05) nitrate, temperature and dissolved oxygen results from those stocked at day three and six.

Effect of stocking densities
The growth performance and survival of *H. bidorsalis* fry at different stocking densities and ages is shown in Table 2. The mean weight gain, specific growth rate and survival rate at different stocking density in *H. bidorsalis* are presented in table 3. The mean weight gain of *H. bidorsalis* stocked at different density as presented in the table shows that treatment SD1 (100fry/m^3) had the highest mean weight gain (value) while the SD3 (300fry/m^3) stocking density had the least value (Supply the value).

Table 1: Mean value of Temperature, Dissolved Oxygen, Nitrate and pH of culture water during the of treatments

Treatments	Temp.(^{0}C)	Dissolved Oxygen (mg/L)	Nitrate(mg/L)	pH
1A	24.35±0.66	5.383±0.50	0.108±0.01	6.960±0.33
1B	24.39±0.52	5.108±0.22	0.106±0.02	7.083±0.20
1C	24.85±0.93	5.241±0.62	0.103±0.01	7.292±0.26
2A	24.40±0.83	5.125±0.50	0.103±0.01	6.960±0.26
2B	24.42±0.55	5.008±0.21	0.109±0.02	7.210±0.26
2C	24.66±1.02	5.250±0.60	0.103±0.01	7.210±0.33
3A	24.28±0.55	4.908±0.20	0.154±0.04	7.000±0.00
3B	24.22±0.40	5.258±0.44	0.100±0.01	7.167±0.25
3C	24.70±0.96	5.300±0.20	0.130±0.01	7.250±0.30
4A	24.23±0.60	4.741±0.30	0.123±0.02	6.958±0.14
4B	24.21±0.60	5.016±0.10	0.108±0.01	7.083±0.20
4C	24.75±0.90	5.391±0.50	0.106±0.01	7.208±0.30

1= 100fry/m^3; 2= 200fry/m^3; 3= 300fry/m^3; 4= 400fry/m^{3}A = 3days old fry; B = 6days old fry; C = 9days old fry

Table 2: Growth Performance and Survival of *H. bidorsalis* larvae at different Stocking Densities and Ages

	SD1			SD2			SD3			SD4		
	A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
MIW(g)	0.009	0.012	0.014	0.009	0.012	0.014	0.009	0.012	0.014	0.009	0.012	0.01
MFW(g)	0.420	0.333	0.303	0.100	0.180	0.206	0.076	0.130	0.153	0.116	0.143	0.14
MWG(g)	0.411	0.321	0.389	0.091	0.168	0.192	0.067	0.118	0.139	0.107	0.131	0.12
MIL(cm)	0.85	0.90	1.00	0.85	0.90	1.00	0.85	0.90	1.00	0.85	0.90	1.00
MFL(cm)	1.92	1.68	1.43	0.47	0.78	0.66	0.27	0.42	0.22	0.67	0.75	0.56
MLG(cm)	2.77	2.58	2.43	1.32	1.68	1.66	1.12	1.32	1.22	1.52	1.65	1.56
ADG	0.015	0.011	0.010	0.003	0.006	0.007	0.002	0.004	0.005	0.004	0.005	0.00
SGR	5.93	5.14	4.75	3.75	4.21	4.14	3.32	3.68	3.68	3.96	3.81	3.57
SR	88.3	80.0	83.3	73.3	71.7	73.3	77.8	71.1	78.8	68.3	64.2	65.8
PI	1.25	0.88	0.83	0.22	0.43	0.51	0.16	0.28	0.39	0.27	0.32	0.33
K	1.30	1.50	1.41	1.12	1.42	1.60	1.10	1.40	1.54	1.01	1.22	1.10

K= Condition Factor, ADG=Average Daily Growth, PI= Performance Index, SGR=Specific Growth Rate, MIW=Mean Initial Weight, SR=Survival Rate, MFW= Mean Final Weight, MLG=Mean Length Gain, MWG=Mean Weight Gain, MWG=Mean Weight Gain, MFL=Mean Final Length

Table 3: Mean Weight Gain (MWG), Specific Growth Rate (SGR) and Survival Rate (SR) of *H.bidorsalis fry* stocked at different densities

Treatment	MWG(g)	SGR	SR(%)
SD1	0.340±0.05 [a]	5.27[a]	82.2[a]
SD2	0.150±0.01 [b]	4.03[b]	72.8[b]
SD3	0.110±0.00 [c]	3.60[c]	75.9[b]
SD4	0.120±0.00 [c]	3.78[c]	66.1[c]

Means with the same superscript under each column were not significantly different at p>0.05

However, SD3 and SD4 were not significantly different. Result on specific growth rate followed the same trend with that of the total weight gain. Survival Rate was highest in treatment SD1 and decreased in the order of treatment SD3, SD2 and SD4. There was significant difference between all the stocking densities (p<0.05). Table 4 shows interaction between stocking density and survival rate while Table 5 presents values of Mean Length Gain, Performance Index (PI) and Condition Factor for the treatments. Mean Length Gain of the fry fish decreased in the order SD1 > SD2> SD4 >SD3. There was no significant difference between treatment SD2 and SD4. The lowest value of Performance Index was observed in treatment

SD3 while treatment SD1 had the highest. There was significant difference between treatments SD1 and SD2, which had the second highest PI. Condition factor decreased with increasing stocking density. The highest K value was observed in treatment SD1 while treatment SD4 had the lowest.

Effect of stocking age
The mean weight gain, specific growth rate and survival rate of *H.bidorsalis* stocked at different ages are presented in Table 6. Significant difference was observed in mean weight gain between stocking ages studied.

Table 4: Analysis of Interaction between stocking density and Survival rate

Stocking density	Mean values of survival rate
SD1 (100fry/m^3)	8.9722[a]
SD2 (200fry/m^3)	16.8056[b]
SD3 (300fry/m^3)	25.0833[c]
SD4 (400 fry/m^3)	32.416[d]

Means with the same superscript under each column were not significantly different (p>0.05)

Table 5: Mean Length Gain (MLG), Performance Index (PI) and Condition Factor (CF) of *H. bidorsalis* fry Stocked at different densities

Treatment	MLG(cm)	PI	CF
SD1	2.59±0.03[a]	0.99[a]	1.40[a]
SD2	1.55±0.04[a]	0.40[b]	1.38[b]
SD3	1.30±0.02[c]	0.30[c]	1.35[c]
SD4	1.50±0.01[b]	0.31[c]	1.11[d]

Means with the same superscript under each column were not significantly different (p>0.05)

Table 6: Mean Weight Gain, Specific Growth Rate and Survival Rate of *H. bidorsalis fry* stocked at different Ages

Treatment	MWG (g)	SGR	SR(%)
A (Day three)	0.169±0.05 [a]	4.24[a]	75.7[a]
B (Day six)	0.179±0.02[b]	4.21[b]	71.8[b]
C (Day nine)	0.187±0.02[c]	4.03[c]	75.3[a]

Mean with the same superscript under each column is not significantly different at p>0.05

The highest MWG was observed in stocking age of nine days (C) while age three (A) had the least. The highest specific growth rate value of 4.24 was recorded at stocking age of 3 days (A), followed by B, (six days), while nine days had the least with a value of 4.03. Survival rate was highest in stocking age of three (A) and decreased in the order to C (age nine). However, there was no significant difference between A and C in terms of survival rate. the mean length gain, performance index and condition factor of *H. bidorsalis* stocked at different ages is shown in Table 7. Mean length gain was highest in B which had a value of 1.81±0.20, followed by C and the least at A. There was no significant difference between stocking age three and nine. The PI was lowest at ages three and six days while age nine had the highest with values of 0.5, 0.5 and 0.51, respectively. Condition Factor increased with increasing stocking age, A, Band C had respective values of 1.13, 1.38 and 1.42. K values were significantly different among the ages.

Relationship between growth and water quality indices
Relationships between the growth, average weight, average length, survival rate, temperature,

dissolved oxygen, pH and nitrite are presented in Table 8. A strong positive correlation was found between average weight and growth. The longer the culture period the higher the increase in body weight. Average length had a very strong positive correlation with both growth week and average length. Survival rate had negative correlation with culture period, average length and average weight. Temperature had positive correlation with growth week, average weight, average length and a negative correlation with survival rate. The higher the temperature, the lower the survival rate and vice-versa. Dissolved Oxygen had significant positive correlation with culture period, average weight, average length and temperature but a negative correlation with survival rate. The higher the dissolved oxygen, the lower the survival rate. pH had a weak significant correlation with culture period, average weight, and dissolved oxygen but a strong correlation with temperature and no significant correlation with average length and survival rate.

DISCUSSION
Hepher (1988) observed that interpretation of the effects of stocking density on fish growth and body composition is complex as the results are

Table 7: Mean Length Gain, Performance Index and Condition Factor of *H. bidorsalis* Stocked at different Ages

Treatment	Mean Length gain (g)	Performance Index	Condition Factor
A (Day three)	1.68±0.15[a]	0.50[a]	1.13[a]
B (Day six)	1.81±0.20[b]	0.50[a]	1.38[b]
C (Day nine)	1.72±0.17[a]	0.51[a]	1.41[bc]

Mean with the same superscript under each column is not significantly different at p<0.05

Table 8: Correlations between growth, Average weight, Average length and Water parameters

Variables	GW	AW	AL	SR	TEM	DO	pH	NIT
GW	1.00							
AW	0.64*	1.00						
AL	0.78*	0.94*	1.00					
SR	-0.21*	-0.55*	-0.46*	1.00				
TEM	0.54*	0.49*	0.49*	0.17*	1.00			
DO	0.37*	0.41*	0.38*	-0.20*	0.56*	1.00		
pH	0.23*	0.17*	0.16	-0.06	0.51*	0.41*	1.00	
NIT	0.17*	-0.00	0.06	0.19*	-0.05	-0.17*	-0.08	1.00

GW = Growth period, AW = Average Weight, AL = Average length, SR = Survival rate
TEM = Temperature, DO = Dissolved Oxygen, pH = Hydrogen ion concentration   NIT = Nitrate
*indicates significant correlation at $P<0.05$

affected by many interrelated factors such as water quality, composition of feed and size of the ration. The results on water quality measured in the culture media during the experiment showed that the values obtained were adequate for fish growth and within the optimal level. Dissolved oxygen was with an average mean level of 5.14mg/L which was within the optimum range of 4mg/L to 6mg/L reported by Boyd (1979) and Omitoyin (2007). The lowest value observed for temperature was below the value 26-28°C recorded by Boyd and Lichtkopper (1979), but within the optimum range of 22-35^{0}C as reported by Omitoyin (2007). Hydrogen ion concentration (pH) obtained in this study was in accordance with the values observed for culturing fry by Brazil and Wolters (2002). Nitrite was within and similar to the range required for African catfish reported in Omitoyin (2007) and Pangni *et al.* (2008) on *Chrysichthys nigrodigitatus*. Growth is the manifestation of the net outcome of energy gains and losses within the framework of abiotic and biotic conditions (Pangni *et al*., 2008). Mean weight gain, performance index, condition factor and specific growth rate decreased with increasing stocking density. This means that these factors can be functions of stocking. The results might have been related to space and it showed that higher stocking densities resulted in lower MWG, PI, K and SGR. These agree with the findings of Pangni *et al*., (2008). The survival rate can also be attributed to stress as a result of aggressive feeding interaction. The results revealed that the stocking age affected the total weight gain and specific growth rate of *H. bidorsalis*. It also revealed that proper stocking age for optimum growth performance might be a function of stocking density. The variability within densities indicates that other factors, aside from

stocking density, affect fish welfare and that focusing solely on stocking density is insufficient to control welfare. Similar results were found by Dawkins *et al.,* (2004) who reported differences among the environment provided for chickens and the consequent impact on welfare that were not attributable to stocking density. Correlation analysis reflected that the higher the stocking density, the lower the growth (weight and length). This agrees with Mollah (1985) and Das *et al.,* (1992). The survival rate and growth of fish were negatively correlated at the stocking density indicating that space has limiting effects on the population. This agrees with Samad *et al.* (2005).

In conclusion, the effect of stocking density and age at stocking on growth performance and survival of *H. bidorsalis* fry was established in this study. It was clearly observed that stocking density of 100fry/m^3 is good for *H. bidorsalis* fry in terms of growth performance as the fry responded and performed better in growth. However, stocking density of 100fry/m^3 at day three had the best mean weight gain on the first week and the best overall survival. Due to complexity of the effect of stocking density on fish growth and body composition, it is recommended that further studies be carried out to redefine the effect of these factors so that fish can be stocked at higher densities since aquaculture has been accepted globally as a means of increasing fish production.

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