Effect of Stocking Density on the Resistance to Fasting, Growth and Survival of the African Catfish *Heterobranchus bidorsalis* (Geoffroy Saint-Hilaire, 1809) Larvae

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Abstract— *After artificial reproduction of African catfish Heterobranchus bidorsalis, larvae of two days old and 2.18 ± 0.35 mg of mean weight were used to perform two experiments in order to assess the effect of stocking density on their fasting resistance, growth and survival. During the first experiment which lasted 11 days with four batches of larvae at densities of 1, 2, 3 and 4 individuals/ml, results showed that density did not significantly affect (p<0,05) the resistance to fasting of larvae. However, first mortalities were observed at D5 for all the densities, the higher daily mortality was recorded at D10 and the last mortalities were obtained at D12.*

Results of the second experiment revealed that the weight and growth performance of larvae decreased with the increasing of the density after 28 days of rearing. In contrast, the larval survival rate increased with the density. The values of survival rate were respectively 30.53 ± 4.32 and 55.30 ± 21.70 % for the densities 1 ind./l and 20 ind./l.

Keywords— Fasting, Growth, Heterobranchus bidorsalis larvae, Stocking density, Survival

I. INTRODUCTION

The clariid catfishes constitute an excellent food fish of high commercial value in many countries of the world particularly in African countries like Nigeria (Adebayo and Popoola, 2008; Huda *et al*., 2006). In Côte d'Ivoire, studies on these fishes began at Oceanologic Research Center with *Heterobranchus longifilis* species (Legendre, 1986; 1991; 1992; Slembrouck and Legendre, 1988; Otémé and Gilles, 1995; Otémé *et al*., 1996). Since 1993, in order to diversify aquaculture species, the species *H. bidorsalis* was introduced and has been the subject of studies to optimize its breeding chain. However, the crucial phase of breeding these species remains undoubtedly larval rearing because the supply of fry is the major constraint to their commercial production (Imorou Toko *et al*., 2008; Alla *et al*., 2011). Many works showed that apart from feeding and water quality, weaning time and stocking density are cited as the main factors affecting larval rearing in a controlled nursery management system (Verreth and Van Tongeren, 1989; Hecht and Pienaar, 1993; Baras and Jobling, 2002; Kestemont *et al*., 2003). Studies revealed that during this stage, the density, which is an important factor of fish production especially in a commercial breeding (Agadjihouèdé *et al*., 2014), varies among species and the rearing system (Boyd and Tucker, 1998; Danaher *et al*., 2007) and influences the growth (Sahoo *et al*., 2004). Similarly, Celeda *et al*. (2007) estimate that in intensive aquaculture, it is important to pay special attention to stocking density, a decisive factor that directly affects the culture production. Moreover, according to Pedro *et al*. (2008), among production parameters to be optimized, stocking density has capital importance for its consequences on growth performance as well as for its likely effect on fish shape. Thus, the density is a very important factor in fish farming; that's why it was the subject of several works during the breeding of many species and particularly in Clariidae (Haylor, 1991; 1992; Kerdchuen and Legendre, 1992; Hossain *et al*., 1998; Bombeo *et al*., 2002; Sahoo *et al*., 2004; Baumgarner *et al*., 2005; Coulibaly *et al*., 2007; Imorou Toko *et al*., 2008; Atsé *et al*., 2009; Agadjihouèdé *et al*., 2014). However, most of these works were carried out on *Clarias gariepinus* and *Heterobranchus longifilis* and there is, in the literature, little information on the study of stocking density during the rearing of *H. bidorsalis*. That is why we are undertaking this work which aim is to evaluate the effect of this factor on the resistance to fasting, growth and survival of larvae of this species.

II. MATERIAL AND METHOD

To perform this work, larvae of two days old and 2.18 ± 0.35 mg of mean weight were produced in the nursery of Oceanologic Research Center after an artificial reproduction according to the method described by Legendre (1986), Slembrouck and Legendre (1988) and Gilles *et al*. (2001).

Two experiments were therefore carried out; the first was to study the effect of density on the resistance to fasting of *H. bidorsalis* larvae. To do this, four (4) batches of 30, 60, 90, 120 larvae in bowls each containing 1.5 liter of water

corresponding to respective densities of 20, 40, 60 and 80 larvae/l. Each batch was repeated three times. Larvae were not fed during the experiment. Every day, the water was renewed by half in each bowl and the mortalities were counted. The test ended when all the larvae in all bowls were dead.

The aim of the second experiment was to evaluate the effect of stocking density on larval growth and survival. It was performed with larvae (7 days old and 7.31 ± 2.84 mg of mean weight) of the same artificial fertilization. Thus, five batches of 50, 250, 500, 750 and 1000 larvae were constituted in aquaria of 50 liters corresponding to densities of 1, 5, 10, 15 and 20 larvae/l respectively. Each batch was repeated three times, making a total of 5100 larvae for this experiment. Larvae were fed to satiety three times a day (8h, 13h, 18h) successively with *Artemia salina*, the beef brains food and compound food CN+. Before starting the experiment at D7, larvae were weighed and measured to determine their mean weight and total mean length. The first sampling took place at D14 and every week, a sampling was conducted until D35. During the sampling, 30 larvae were collected by aquarium, weighted (mg) and measured (mm) individually using respectively a precision balance and a graduated ruler.

The experiment was conducted in a temperature-controlled water recirculation system with 50 liters glass aquaria. Temperature, dissolved oxygen and pH were measured daily in bowls and aquaria using respectively an YSI Model 58 oxygen meter (Yellow Springs Instruments) and a pH meter (model WTW). Every morning, aquaria were carefully cleaned by siphoning before feeding larvae. The number of dead fish was recorded daily. Survival (%) was determined at the end of the experiment by counting the remaining larvae in each aquarium. Coefficients of variation were determined as Standard deviation × 100/Mean weight or Total mean length; Condition factor (%) as Weight × 100/Length^3; Mean daily weight gain (g/d) as (Wf - Wi)/t where Wi and Wf are respectively the initial and final mean weights of the fish and t is the duration of the growth period in days; Total biomass (g) as Weight of remaining larvae in each aquarium for each density.

Statistical analyses were performed using STATISTICA 7.1 software. Data were expressed as means ± SE. The effects of density on the growth and survival were tested with one-way analysis of variance (ANOVA), followed by Turkey's test. The daily mortalities were compared by the chi-square test. Differences were considered significant when $p<0.05$.

III. RESULTS

3.1 Results

3.1.1 Water quality

During the two experiments, mean values of temperature, dissolved oxygen and pH obtained in bowls were respectively 27.9 ± 0.2°C, 6.5 ± 0.8 mg/l and 7.1 ± 0.3. In aquaria, they were 28.6 ± 0.2°C, 5.7 ± 0.8 mg/l and 7.0 ± 0.6 respectively.

3.1.2 Effect of stocking density on the larval resistance to the fasting

Fig. 1 shows variations of larval daily mortality rate according to their age and the stocking density. There was no mortality during the first three days of the experiment regardless of the density. First mortalities were observed at D5 with respectively 4.4%, 6.1%, 4.1% and 4.2% for densities 1, 2, 3 and 4. From there, these mortalities were increasing gradually until D10 where the highest daily mortality was recorded for all stocking densities. They represented 48.50% of the larval population for the density 1, 36.40% for the density 2, 33.00% for the density 3 and finally 38.30% for the density 4. From D11, the daily mortalities, which were respectively 6.60%, 9.10%, 7.40% and 6.50% for densities 1, 2, 3 and 4 decreased until the end of experiment at D12 where they reached their lowest value. That value was 1.10% for density 1, 2.8% for density 2, 2.6% for density 3 and 0.6% the last density. Moreover, there was no particular trend for mortalities recorded each day at all densities.

The comparison of the daily mortality average rate by the chi-square test revealed no significant difference ($p> 0.05$) between the mortality for all densities except at D10 where daily mortality for the density 1 was significantly different of the other three densities.

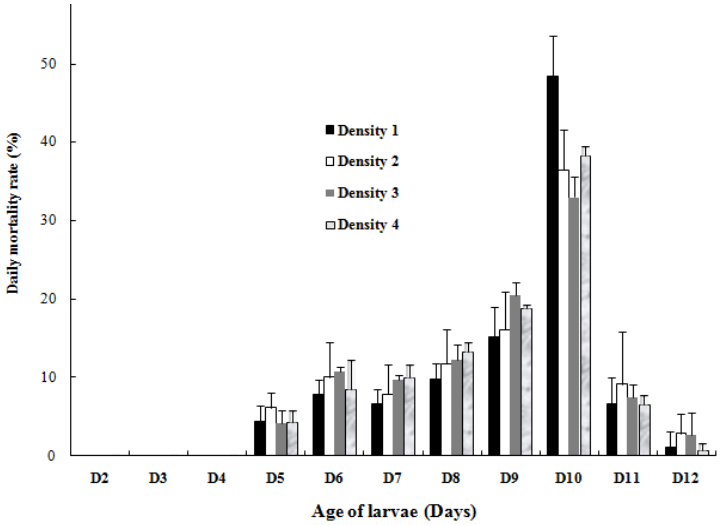

FIGURE 1: CHANGES OF DAILY MORTALITY OF *H. BIDORSALIS* LARVAE AT THE FOUR DENSITIES ACCORDING TO THEIR AGE

3.1.3 Effect of the density on larval growth and survival

The change of *H. bidorsalis* larval mean weight according to their age and the stocking density is represented by fig. 2.

Larval mean weight increased gradually from the start to the end of experiment for all densities. However, the increase was slow and virtually identical for all densities during the first two weeks of rearing (D7 to D21). The analysis of variance (ANOVA) revealed a significant difference in weight between the densities 1 and 20 ind/l at D14, between densities 1 ind/l, 5 ind/l and the other three densities (10, 15 and 20 ind/l) at D21 ($p < 0.05$). Mean weights recorded at D14 were 50.75 ± 20.73 mg for the density 1 ind/l, 48.71 ± 11.63 mg for the density 5 ind/l, 46.68 ± 20.54 mg for the density 10 ind/l, 46.13 ± 20.08 mg for the density 15 ind/l and finally 36.76 ± 11.91 mg for the density 20 ind/l. At D21, they were respectively 150.94 ± 61.20 mg, 149.96 ± 57.16 mg, 114.56 ± 61.76 mg, 85.13 ± 37.72 mg and 97.98 ± 44.32 mg for the five different densities.

After D21 and until the end of experiment, larval mean weight increased rapidly, passing at D28, from 702.95 ± 337.74 mg to 1557.75 ± 870.51 mg for the density 1 ind./l, from 362.30 ± 250.95 mg to 1538.48 ± 856.64 mg for the density 5 ind./l, from 339.01 ± 314.41 mg to 1338.08 ± 705.93 mg for the density 10 ind./l, from 258.79 ± 129.72 mg to 1022.87 ± 578.48 mg for the density 15 ind./l and from 281.04 ± 204.79 mg to 999.36 ± 297.76 mg for the density 20 ind./l. Larval mean weight at the density 1 ind./l was significantly different of that of the other four densities at D28 ($p < 0,05$). It is the same case of densities 1, 5 ind./l and the other three densities on the one hand and on the other hand, between that of the density 10 ind./l and the last two densities at the end of the experiment.

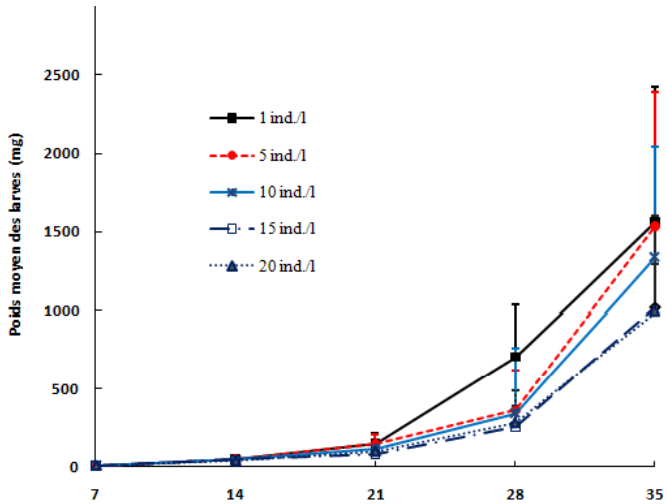

FIGURE 2: VARIATIONS OF *H. BIDORSALIS* LARVAL MEAN WEIGHT ACCORDING TO THEIR AGE AND DIFFERENT DENSITIES

The growth parameters of *H. bidorsalis* larvae reared at different densities during 28 days are summarized in table 1.

Over the entire duration of the experiment, it was found that the mean daily weight gain of larvae gradually decreased with increasing density. Thus, it went from 55.37 ± 31.09 mg/j for the density 1 ind/l to 54.68 ± 30.59 mg/j for the density 5 ind/l, to 47.53 ± 10.63 mg/j for the density 10 ind/l, to 36.27 ± 20.66 mg/j for the density 15 ind/l and to 35.43 ± 20.33 mg/j for the density 20 ind/l at D35. For this parameter, mean values obtained for the three first densities were significantly different of those recorded for the two last ($p < 0.05$).

Larval total length also decreased as the density increased. From 55.27 ± 9.01 mm for the density 1 ind/l, it regressed at 54.03 ± 10.23 mm for density 5 ind./l, at 47.43 ± 13.89 mm for the densiy 10 ind./l, at 46.50 ± 9.52 mm for the density 15 ind./l before reach finally 45.67 ± 12.46 mm at the density 20 ind/l. Furthermore, there was a significant difference in larval size between the two first densities (1 and 5 ind./l) and the last ($p < 0.05$).

The coefficient of variation of final mean weight was between 29.80 ± 10.21 % at density 15 ind./l and 56.56 ± 18.19 % at density 20 ind/l. Those obtained at densities 1, 5 and 10 ind./l were respectively 55.89 ± 12.37 %, 55.68 ± 20.91 % and 52.76 ± 13.37 %. For the total length, the mean value of coefficient of variation increased from density 1 ind./l to density 5 ind./l and 10 ind./l. Their respective values were 16.31 ± l8.24 %, 18.94 ± 5.92 % and 29.29 ± 11.26 %. It decreased to 20.48 ± 10.04 % at density 15 ind./l before increasing again at the density 20 ind./l (27.29 ± 4.56 %).

At the end of the experiment, the condition factor varied in same direction as the coefficient of variation of the total length. The mean values recorded were 0.92 ± 0.13, 0.98 ± 0.08, 1.25 ± 0.18, 1.01 ± 0.20, 1.04 ± 0.78 respectively for densities 1, 2, 3, 4 and 5 ind./l. There was a significant difference between the values of condition factor at densities 1 and 10 ind./l ($p < 0.05$).

There was no particular change of larval total biomass. Its mean value was between 25.90 ± 4.75 and 69.28 ± 21.18 g respectively at densities 1 ind./l and 20 ind./l. The total biomass was 44.42 ± 21.14 g at the density 5 ind./l, 32.25 ± 14.26 g at the density 10 ind./l and 59.28 ± 33.24 g at the density 20 ind./l. The final total biomass was significantly different for all densities, except for densities 1 and 10 ind./l.

TABLE 1
GROWTH PARAMETERS OF *HETEROBRANCHUS BIDORSALIS* LARVAE REARED DURING 28 DAYS IN AQUARIA AT DIFFERENT STOCKING DENSITIES

Parameters	1 ind./l	5 ind./l	10 ind./l	15 ind./l	20 ind./l
FTMW (mg)	1557.75 ± 870.51^{a}	1538.48 ± 856.64^{a}	1338.08 ± 705.96^{b}	1022.86 ± 578.48^{c}	999.36 ± 297.76^{c}
MDWG (mg/j)	55.37 ± 31.09^{a}	54.68 ± 30.59^{a}	47.53 ± 10.63^{a}	36.27 ± 20.66^{b}	35.43 ± 20.33^{b}
CVFTMW (%)	55.89 ± 12.37^{a}	55.68 ± 20.91^{a}	52.76 ± 13.37^{a}	56.56 ± 18.19^{a}	29.80 ± 10.21^{b}
FTML (mm)	55.27 ± 9.01^{a}	54.03 ± 10.23^{a}	47.43 ± 13.89^{ab}	46.50 ± 9.52^{ab}	45.67 ± 12.46^{b}
CVFTML (%)	16.31 ± 8.24^{a}	18.94 ± 5.92^{a}	29.29 ± 11.26^{b}	20.48 ± 10.04^{ac}	27.29 ± 4.56^{b}
K	0.92 ± 0.13^{a}	0.98 ± 0.08^{ac}	1.25 ± 0.18^{b}	1.01 ± 0.20^{ac}	1.04 ± 0.78^{ac}
FTB (g)	25.90 ± 4.75^{a}	44.42 ± 21.14^{b}	32.25 ± 14.26^{a}	59.28 ± 33.24^{c}	69.28 ± 21.18^{d}

For each line. values with same letters as superscripts are not significantly different
FTMW : Final total mean weight ; MDWG : Mean daily weight gain; CVFTMW : Coefficient of variation of final total mean weight; FTML : Final total mean length ; CVFTML : Coefficient of variation of final total mean length ; K : Condition factor ; FTB : Final total Biomass.

Variations of larval survival rate at the end of the experiment are shown at the fig. 2. Survival rate increased with increasing density. The best survival rate was obtained at density 20 ind./l with 55.00 ± 21.70 % whereas the low value (30.00 ± 5.60 %) was recorded at density 1 ind./l. Mean survival rate observed at the densities 5, 10 and 15 ind./l were respectively 33.20 ± 15.80 %, 40.67 ± 17.93 % and 46.60 ± 24.97 %. There was a significant difference between the survival rate obtained at the density 20 ind./l and those of the other four densities (1, 5, 10 and 15 ind./l).

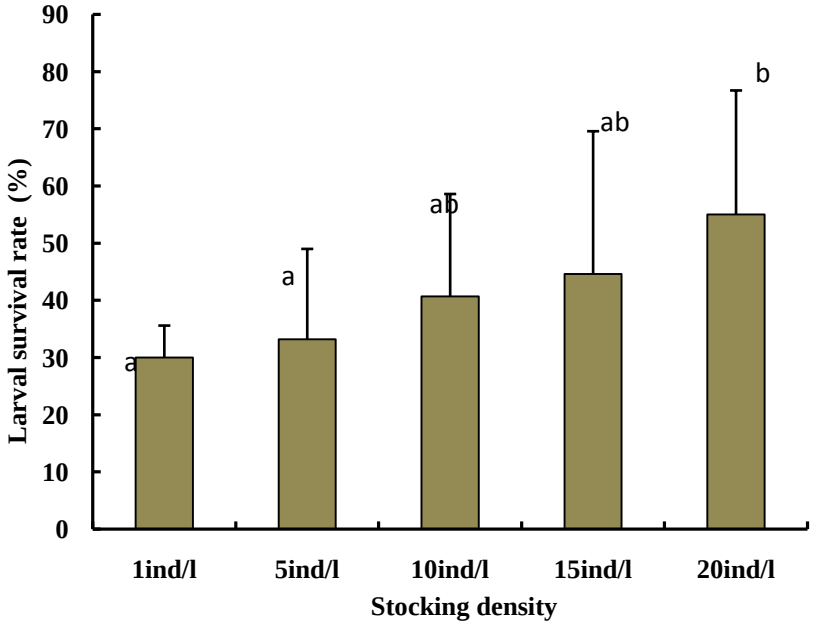

FIGURE 3: SURVIVAL RATE OF *HETEROBRANCHUS BIDORSALIS* LARVAE ACCORDING TO DIFFERENT STOCKING DENSITIES AFTER 28 DAYS OF REARING IN AQUARIA
**** Histograms with the same letters are not significantly different***

3.2 Discussion

Physico-chemical parameters (temperature, dissolved oxygen and pH) measured in bowls and aquaria varied very little during the experiments. So, they did not really affect not only the larval mortalities but also their growth and survival. Indeed, recorded values were in the range recommended by several authors for rearing juvenile catfish (Boyd, 1990; Lawson, 1995; Tarazona and Munoz, 1995). It is from 10 to 35 °C for the temperature, higher than 3 mg/l for dissolved oxygen and 6.5 to 8.0 concerning pH (Boyd and Tucker, 1998; Viveen *et al.*, 1985). The stability of these factors seems to be due, on the one hand to daily renewing water in bowls. On the other hand, the experiment was conducted in closed circuit with the same circulating water in aquaria.

Regarding the effect of density on the larval resistance to fasting, it was observed in all, no particular trend in daily mortalities recorded for all densities. However, regardless of the density, the evolution of these mortalities depending on the duration of the fasting is similar to that observed when assessing the resistance to fasting of *H. bidorsalis* larvae by Alla *et al*. (2015) in the same experimental conditions. Indeed, these authors reported that first mortalities occurred at D5 while the highest were recorded at D10 and the last were observed at D12. These results point virtually in the same direction as those obtained in *H. longifilis* with three densities (Alla *et al*., 2014). For this species, the greatest number of larvae died at D10. As the experiment of the resistance to fasting, they explained these results by the fact that, after vitellin resorption, larvae used their body reserves to survive until D9 or D10. At that time, after exhausting, majority of larvae dead. These authors suggested that first mortalities observed in larvae occurred to the weakest populations and the latest concerned the most resistant which, after total depletion of their reserves, were able to withstand until two to three days before dying. These results seem to show that in population with the same age and environmental conditions, the weakest died and the strongest resist.

The results related to the larval growth depending to the stocking density showed that this growth was slow during the first two weeks of rearing and it was faster after this period until the end of the experiment for all densities. However, there was a difference in larval weight and length between densities 1 ind./l and 20 ind./l. The growth was changing inversely with the stocking density. Indeed, the growth parameters decreased with increasing density. These results are in agreement with those of Coulibaly *et al*. (2007), Imorou Toko *et al*. (2008), Atsé *et al.* (2009) and Agadjihouèdé *et al*. (2014) for the catfish *H. longifilis*. During their works, these authors reported effectively that larval growth in this species was better when they were reared at low densities. Generally, these densities which were less or equal to 5 individuals per liter according on the tests, were heavily dependent on rearing conditions (individual size, breeding structures and type of food distributed). Similar

results were also obtained in other farm fishes including tilapias (Mélard, 1986; Ruane *et al*., 2001; Ouattara *et al*., 2003) and other catfish (Hogendoorn and Koops, 1983; Hecht and Appelbaum, 1987; Haylor, 1991 ; 1992; Kaiser *et al*., 1995; Hengsawat *et al*., 1997; Bombeo *et al*., 2002; Barcellos *et al*., 2004).

At the end of the experiment, coefficient of variation of final mean weight were not significantly different from density 1 ind./l to density 15 ind./l and were superior than 30 % except at density 20 ind./l. Those of the length were less than 30 % for all densities. That means that their size was homogenous. Our results indicated that larval growth was heterogeneous at low densities whereas it was homogeneous at the high density (20 ind./l). This translates into the fact that the total biomass and the survival rate were high at this density indicating proportionality between larval survival and stocking density. The homogeneity of larval weight and length at high densities significantly reduces cannibalism which is one of the main causes of mortality of the larvae. Moreover, according to some authors (Kerdchuen and Legendre, 1992; Hecht *et al*., 1997; Baras and Jobling, 2002), the best survival achieved at high densities could also be explained by a proportional increase of the distributed food and environmental stress control at these densities.

These results confirm those previously obtained by several authors in *H. longifilis* (Kerdchuen, 1992; Kerdchuen and Legendre, 1992; Otémé and Gilles, 1995; Imorou Toko *et al*., 2008, Atsé *et al.*, 2009) and in *Clarias gariepinus* (Haylor, 1992; Siddiqui *et al*., 1993). Indeed, for these authors, the density is one of the factors that regulate aggressive behavior in Clariidae. This behavior would be responsible for cannibalism in these fishes. They claimed that at low densities, juveniles exhibit a sporadic aggressively which quickly leads to cannibalism while the aggression phenomena disappear at high densities.

IV. CONCLUSION

At the end of this experience, we accept that the density did not affect the resistance to fasting of *H. bidorsalis* larvae. Larval growth was better at high densities whereas this was not the case for survival.

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