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Effect of stocking density on the growth and food use parameters of larvae of the Brazil strain of tilapia of the Nile *Oreochromis niloticus* (Linnaeus, 1758) during periods of sexual inversion by hapa installed in a pond in the ground

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Abstract
This study was conducted to evaluate the effects of stocking density on the survival, growth and feeding parameters of larvae of the Brazil strain of Nile tilapia, *Oreochromis niloticus* in breeding environments. Larvae of initial average weight 0.010 ± 0.002g and initial average total length 8.70 ± 0.83 mm were loaded at densities of 500, 1000, 1500, 2000, 2500 and 3000 larvae / m^2 called D1, D2, D3, D4, D5 and D6 respectively. The fish were all fed a commercial food (48% protein) distributed manually at a frequency of 6 meals per day. A rationing rate of 40, 30, 25 and 20% of the biomass was applied during the first, second, third and fourth weeks of feeding respectively. Growth controls on samples were used to determine the impact of stocking density on zootechnical parameters. The results show that the final average weight, daily growth and specific growth rate were inversely proportional to the increase in stocking density. However, the densities D1, D2, D3 and D4 do not differ (ANOVA; $p > 0.05$) from each other, but they are significantly higher (ANOVA; $p< 0.05$) than the other two densities (D5 and D6). Similarly, survival decreases with increasing density and the low values of this parameter were obtained at the highest loading densities, 83.11% at 2500 larvae / m^2 and 80.87% at 3000 larvae / m^2. In contrast to the first two parameters, the feed conversion rate is correlated with the increase in stocking density. These results show that for an improvement in fry production, a stocking density of 2000 larvae / m^2 can be applied in a happa installed in an earth pond.

Keywords: Stocking density, Brazil strain, tilapia, larvae

1. Introduction

In fish farming practices, stocking density is one of the factors that can affect survival (Luz *et al.*, 2012) [14]; growth and feed utilization parameters in fish (Wocher *et al.*, 2011) [26];. For maximum fish production in intensive farming, the proper use of farming structures (pond, enclosure, ponds, ponds, cages and catchers) can improve the financial profitability of the fish farm. Indeed, a high stocking density leads to stress and hinders the well-being of fish, which leads to an increase in energy requirements and therefore a decrease in growth performance and feed efficiency.

However, in recent years, particular attention has been paid to the breeding of Nile tilapia, the second most important fish species for aquaculture after carp (El-Sayed, 2002) [10]. Indeed, this species is reared in most tropical, subtropical and temperate regions due to its high growth rate, prolific reproductive performance, courtyard production cycle in captivity, tolerance to environmental stress and high market demand (Tahoun *et al.*, 2008; FAO, 2012) [25, 11]. In addition to the great potential of rearing this species, one of the main obstacles to the expansion of intensive rearing of this fish in most developing countries, particularly in Côte d'Ivoire, is the lack of production of good quality fry to meet the demand for male fry from the main actors in this activity. In other words, information on larval breeding, in particular, the effects of stocking density on zootechnical performance, is limited, sometimes controversial and above all unsuitable for breeding conditions. Indeed, according to García-Trejo *et al.* (2016) [12], the failures of small-scale fish farmers are due to insufficient knowledge of the stocking density of very small fish. However, most authors

(Sanches and Hayashi, 1999; El-Sayed, 2002; Tachibana *et al.*, 2018) [21, 10, 24] recommend relatively low densities in the range of 1 to 10 larvae / L. However, these densities are not appropriate for the different types of farming (extensive, semi-intensive and intensive) because experiments are generally carried out in limited spaces in a laboratory where most abiotic parameters are set. Thus, the present study aims to evaluate the effect of stocking density on the survival, growth and food use parameters of larvae of the Brazil strain of Nile tilapia raised in happas implanted with Chinese bamboo in an earth pond.

2. Materials and methods

2.1 Experimental protocol

2.1.1 Larval production

The experiments were carried out in a private fish farm, located between latitude 5°40' N and longitude 4°6' W in the Azaguié sub-prefecture 25 km from Abidjan (Côte d'Ivoire). For breeding, 168 broodstock of the Brazil strain of Nile tilapia, *Oreochromis niloticus* were used. A sex ratio of 3:1 with 42 males (118.5 ± 5.97 g) to 126 females (80.5 ± 9 g) was used in a breeding happa (6 x 4.7 x 1 m) installed using Chinese bamboo in a 350 m^2 pond. The catchers are made of polyethylene nets with a mesh void of 1 mm. The larvae were collected on the 14th day after spawners were put into breeding. After narrowing the upper walls of the reproductive happa, in order to group them at the surface of the water, the fry menus are captured using a 1 mm vacuum mesh net. Before the reproductive happa was loaded again, each female's snout was examined to remove the eggs that had not been expelled.

2.1.2 Constitution of the experimental batches

The management of the larval monitoring hapa consisted of randomly collecting 3 samples of 200 larvae. These different samples were weighed to determine the initial average weight of each individual and the initial larval loading biomass for each density. Thus six (06) batches of larvae were constituted in triplicate, at the rate of three (03) per stocking density. The densities of 500, 1000, 1500, 2000, 2500 and 3000 larvae / m^2 were tested. Each lot was put in 1 happa of 1 m^2 or 18 hapas in total. All 18 hapas were installed in a 300 m^2 pond. A total of 18,000 larvae with an initial mean weight of 0.010 ± 0.002 g and an initial mean length of 8.70 ± 0.83 mm were used in this test.

2.1.3 Food preparation and larval feeding

The larvae were fed 6 meals a day with a commercial meal food containing 48% protein and a diameter of 0.3-0.5 mm. The food was mixed with the 17-α-methyltestosterone (DIMAC; Suisse) 60 mg hormone / kg food with 100 ml alcohol (Barry *et al.*, 2007) [6]. The larval rationing rate was 40, 30, 25 and 20% of the biomass of each density during the first, second, third and fourth weeks of larval monitoring respectively.

2.1.4 Monitoring of environmental parameters of the breeding environment

During the experiment, the temperature, dissolved oxygen and pH of the water were measured in situ three times a week and twice a day (between 6:30 am -7:00 am and between 3:30 pm - 4:00 pm). To do this, the OXYGUAR model oximeter was powered up and the probe was immersed in the water of each happa to record the dissolved oxygen and temperature values. As for the pH values, the WTW pH 330 model pH meter was powered up and the water was collected using a cap in which the pH meter probe was immersed.

2.1.5 Calculations and evaluation of zootechnical parameters

Weekly weight growth control fisheries were conducted on 25% of the population of each hapa. These controls made it possible to readjust the food ration for the week. At the end of the 28 days of breeding for each treatment, the fish biomass of each happa was determined and 30 randomly selected individuals were measured for total length and individual weight. From these data, different zootechnical parameters, namely (ITML) : initial total mean length (mm); FTML: final total mean length (mm); IMW: initial mean weight; FMW: final mean weight; mean weight gain (MWG) = (final weight – initial weight) / number of fish; specific growth rate (SGR) = 100 [(ln final weight – ln initial weight) / number of experimental days]; feed conversion ratio (FCR) = feed intake (g) / fish weight gain (g); condition factor (K) = 100 [final weight (g) / (final length (cm))3]; coefficient of variation of mean length (CVL) = 100 [standard deviation of final weight / mean length] and survival rate (SR) = 100 (final number / initial number).

2.1.6 Statistical analysis

The results are presented as mean ± standard deviation. The zootechnical parameters (final weight, daily average gain, specific growth rate, nutrient quotient and condition factor) were subjected to the analysis of variance using a criterion (ANOVA 1). This test was followed by the Tukey multiple comparison test for parameters with a significant difference ($p < 0.05$) to identify specific differences between batches taken in pairs. As for the survival rate, it was subjected to a contingency table analysis. These analyses were performed using STATISTICA 7.1 software.

3. Results

3.1 Physico-chemical parameters of the breeding environment

The minimum, maximum and mean values ± Standard deviation of the physico-chemical water parameters recorded during this study are shown in Table 1. Mean values for temperature, dissolved oxygen content, pH and transparency of breeding structures were similar for the different densities (ANOVA, $p > 0.05$). However, the mean values of dissolved oxygen and pH were inversely proportional to density.

Table 1: Minimum, maximum and mean values ± Standard deviation of physico-chemical parameters of hapa water during larval breeding of the Brazil strain of Nile tilapia, *Oreochromis niloticus* according to loading densities.

		Oxygen (mg/l)			Temperature (°C)			pH	
	Mini	**Max**	**Mean**	**Mini**	**Max**	**Mean**	**Mini**	**Max**	**Mean**
D1	2.87	6.14	4.55±1.49	28.77	32.43	30.60±1.91	7.18	8.08	7.63±0.33
D2	2.57	6.1	4.32±1.54	28.33	32.03	30.18±1.92	7.53	7.92	7.72±0.33
D3	2.28	5.86	4.07±1.50	28.40	32.12	30.26±1.97	7.48	7.83	7.66±0.28
D4	2.12	5.56	3.84±1.28	28.65	32.11	30.38±1.83	7.27	7.85	7.56±0.32

D5	2.01	5.85	3.88±1.28	28.76	32.22	30.49±1.82	7.22	7.75	7.48±0.32
D6	2.18	5.48	3.83±1.41	28.88	31,97	30.43±1.61	7.27	7.95	7.61±0.40

3.2 Effect of stocking density on zootechnical parameters

3.2.1 Evolution of the average weekly weight

Figure 1 shows the profile of the average larval weight as a function of stocking densities over the 28 days of larval breeding. During the first week of breeding, all average weights show the same evolution in all stocking densities. During the control during the second week, a significant difference ($p < 0{,}05$) in the average weight was observed between the loading densities. The multiple comparisons of averages (Tukey's test) divides the loading densities into two groups. The highest densities 2500 and 3000 larvae per square met the lowest average weight. The low densities of 500, 1000, 1500 and 2000 larvae / m^2 recorded the highest average weights. No differences were observed between densities D1, D2, D3 and D4. During the third week, the average weights of densities D1 and D2 are distinct from those of densities D3 and D4 as well as those of densities D5 and D6. The analysis of variance for a given criterion shows a very significant difference ($p < 0.0001$) in the average weights as a function of densities. In the fourth weeks, the average weights obtained differ significantly ($p < 0.05$) from one density to another. The multiple comparisons of the means (Tukey's test) shows that the highest values of the mean weight are assigned to the lowest densities (D1 to D4).

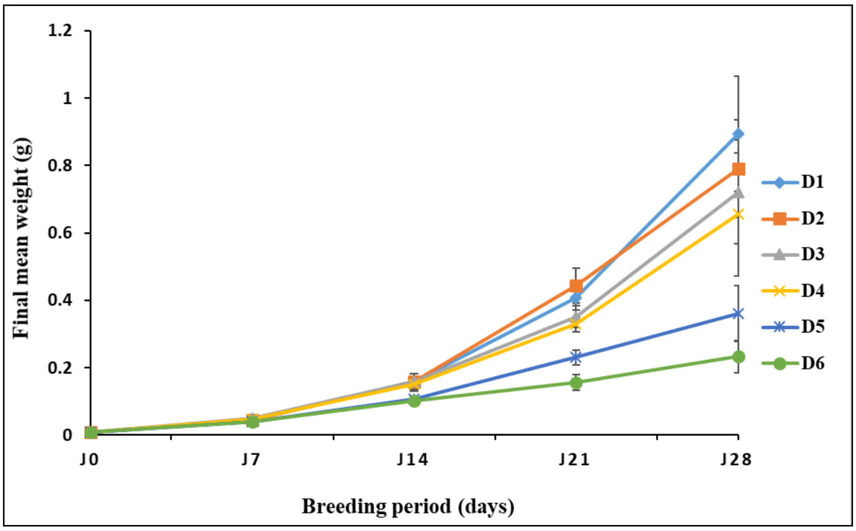

Fig 1: Evolution of the average weight of Nile tilapia larvae of the Brazil strain of *Oreochromis niloticus* according to loading densities (D1, D2, D3, D4, D5 and D6) during sexual inversion (vertical bar = standard deviation).

3.2.2. Survival, growth and use parameters of the feed

Data on survival, growth and feed utilization parameters in larvae of the Brazil strain of Nile tilapia, *Oreochromis niloticus* subjected to the effect of different loading densities during the sexual inversion period are summarized in Table 2.

After 28 days of treatment, the mean survival rate values recorded ranged from 80.87 ± 2.32 to 94.2 ± 1.63% for all densities. These values decrease with increasing density. The highest value (94.2 ± 1.63%) was obtained at density D1 and the lowest (80.87±2.32%) at density D6. The statistical analysis shows a significant difference (Contingency Table; $p < 0.05$) between the survival rate values obtained at densities D1, D2 and those of densities D5 and D6. However, no differences (Contingency Table; $p > 0.05$) were observed between densities D3; D4; D5 and D6. Regarding growth, the final average weights (Pf) reached by larvae at densities at the different densities tested oscillate from 0.895 ± 0.089 to 0.233 ± 0.047 g. The lowest (0.233 ± 0.047 g) and highest (0.895 ± 0.089 g) value was observed at density D6(3000) and D1(500) larvae / m^2 respectively. At the end of the experiment, the final average weight of the six loading densities was significantly different (ANOVA; $p < 0.05$). The specific growth rate (SGR) values recorded in this study ranged from 16.03 ± 0.366 to 11.193 ± 0.747% for all densities. The one-

Table 2: Zootechnical performance parameters of larvae of the Brazil strain of Nile tilapia, O. niloticus subjected to increasing stocking densities during larval breeding.

Parameters	**Densities**					
	D$_1$ (500)	**D$_2$ (1000)**	**D$_3$ (1500)**	**D$_4$ (2000)**	**D$_5$ (2500)**	**D$_6$ (3000)**
Initial total mean length (mm)	8.7±0.83^{a}	8.7±0.83^{a}	8.7±0,83^{a}	8.7±0.83^{a}	8.7±0.83^{a}	8.7±0.83^{a}
Final total mean length (mm))	37.26±1,26^{a}	36.53±0,46^{a}	35.51±1.36^{a}	33.88±1.61^{a}	27.88±1.23^{b}	24.04±1.56^{c}
Initial mean weight (g)	0.010±0.002^{a}	0.010±0.002^{a}	0.010±0.002^{a}	0.010±0.002^{a}	0.010±0.002^{a}	0.010±0.002^{a}
Final mean weight (g)	0.895±0.089^{a}	0.789±0.030^{b}	0.721±0.085^{c}	0.654±0.089^{d}	0.361±0.046^{e}	0.233±0.047^{f}
Mean weight gain (g)	0.031±0.003^{a}	0.027±0.001^{ab}	0.024±0.003^{ab}	0.022±0.003^{b}	0.011±0.001^{c}	0.008±0.001^{c}
Specific growth rate (%)	16.030±0.366^{a}	15.590±0.130^{a}	15.250±0.438^{a}	14.910±0.476^{a}	12.780±0.474^{b}	11.193±0.747^{c}
Condition factor	1.690±0.050^{a}	1.640±0.017^{ab}	1.60±0,02^{b}	1.670±0.026^{ab}	1.65±0,01^{ab}	1.660±0.019^{ab}
Feed conversion ratio	1.220±0.129^{a}	1.170±0.045^{a}	1.170±0.149^{a}	1.110±0.145^{a}	1.840±0.258^{ab}	2.601±0.572^{b}
Coefficient of variation of mean length (%)	6.18^{a}	6.12^{a}	6.64^{a}	6.72^{a}	6.86^{a}	8.16^{a}
Survival rate (%)	94.20±1.63^{a}	90.80±1.63^{a}	85.96±1.64^{a}	85.60±4.35^{a}	83.11±2.96^{b}	80.87±1.26^{b}

Values sharing at least one letter in common on each line in the table are not significantly different ($p > 0.05$)

factor analysis of variance shows that the loading density significantly affected ($p < 0.05$) these growth parameters. Indeed, the values of the final average weight and the specific growth rate decrease the increase in loading density. The multiple comparisons of means (Tukey's test) shows that the SGR recorded at densities D1, D2, D3 and D4 differ from those of densities D5 and D6. The recorded values of the condition factor (K) range from 1.60 ± 0.02 to 1.68 ± 0.026 for all treatments. These values vary slightly from one density to another. No significant differences were observed between the different treatments. Concerning the feed conversion rate (FCR), the highest value is noted at density D6 (2.6 ± 0.258) and the lowest at density D4 (1.11 ± 0.145). The value of FCR recorded at density (D6) is significantly higher (ANOVA, $p < 0.05$) than that of other loading densities. The lowest value of the coefficient of variation of the final length obtained in this study is noted at density D2 (6.12 ± 0.18%) and the highest at density D4 (9.22 ± 0.832%). These values do not differ significantly (Chi^2; $p > 0.05$) from one density to another.

4. Discussion

The importance of stocking density on fish growth performance has been reported in several species. Both positive and negative effects on growth performance have been reported and the pattern of this relationship appears to be species- and stage-specific (Azaza *et al.*, 2013) [4]. In this study, high loading densities were tested in contrast to most of the work done on low loading densities in aquariums in the laboratory.

The high larval survival rate values loaded at high densities in this study (80.87% for 3000 ind/m^2) reflect the ability of Nile tilapia larvae to intensive fry production (Alhassan *et al.*, 2012) [2]. Indeed, according to Bamba (2007) [5], a high survival rate can be linked to a good valorization of the feed distributed and a perfect adaptation to the breeding conditions.

The high survival of larvae in this study can be attributed to the continuous oxygenation of the water in the experimental pond throughout the test and the maintenance of the happa during each control fishery. Although high, the survival rates recorded in this study decrease with increasing loading density. These results are in agreement with those of El-Sayed (2002) [10] who tested densities in the range of 3 to 20 larvae / L. The latter found that average survival was negatively correlated with increased loading density. The same observations were made by Ouattara (2004) [18] in the lagoon strain of *sarotherodon melanoteron*. This author obtained mean survival rate values of 90.00; 89.00; 82.5 and 80.67% respectively for densities D20, D50; D100 and D150 ind/m^3. However, studies by Sanches and Hayashi (1999) [22] on Nile tilapia larvae with a low stocking density in the range of 2 to 10 larvae/L and by Tachibana *et al.* (2018) [25] with a density of 1 to 7 larvae / L, recorded survival was similar for the different densities tested. On the other hand, Breine *et al.* (1996) [7] observed an increase in survival with that of density in *Tilapia cameronensis*. These authors explain this phenomenon by a decrease in aggressiveness at high densities. In the current study, the decrease in survival with increasing density may be related to increasing aggressiveness with density (Ouattara, 2004) [18]. However, the survival rates noted in this work are excellent. Indeed, according to Morissens *et al.* (1987) [15], in a farm, when survival represents 75% of the initial population, it is considered good.

The final mean weights recorded in this study ranged from 0.895 ± 0.089 to 0.233 ± 0.047 g respectively for densities D1(500) and D6 (3000) larvae / m^2. This parameter is higher for low densities and decreases with high densities. In this work, the final average weights obtained decrease with increasing density. These results corroborate those of the work of Tachibana *et al.* (2008) [25]. Indeed, these authors observed a reduction in growth with the increase in density in a study conducted at low loading densities in the range of 1 to 7 larvae / L. The subjects were fed 5 times / day with a diet containing 48% protein for 30 days. Similarly, in a study or densities in the range of 2 to 10 larvae / L tested in a green water system and fed 6 times / day with a feed containing 43% protein for 28 days, Sanches and Hayashi (1999) [22] also reported a reduction in growth due to increased density. These same observations were made by Bamba (2007) [5], Diana *et al.* (2004) [9] and Suresh and Lin (1992) [24]. Indeed, these authors indicate that the final mean weights reached by the different populations are inversely related to the loading density applied. This decrease in density could be explained by an optimum density threshold above which the final average weights begin to decline. It could be linked to food competition and aggressive behaviour of fish that occur when interindividual contact increases as supported by the work of Ouattara *et al.* (2005) [19]. Contrary to the average final weight, production increases significantly with stocking density. The same observations were reported by Nobah (2007) [16] in male F1A hybrids. At low densities, individual growth is high and production low, while at high densities, individual growth is low and production high.

In this study, the daily weight gain and specific growth rate obtained ranged from 0.028 to 0.006 g / day and from 16.03 to 11.19%/d at densities D1(500) and D6(3000) ind/m^2 respectively. A statistical difference ($p < 0.05$) was observed between the respective values of these parameters and the poisoning densities. The value of these parameters recorded at densities D1 D2 D3 and D4 differ ($p < 0.05$) from those of densities D5 and D6. These results disagree with those of Ronald *et al.* (2014) [21]. These authors observed no significant difference ($p > 0.05$) between the value of the parameters obtained by the densities tested, he suggested that the higher densities in *Oreochromis niloticus* had no apparent effect on fish growth. In addition, in this study, the value of these parameters decreases with increasing stocking density. This observation is consistent with that reported by Breine *et al.* (1996) [7]. Indeed, according to Aksungur *et al.* (2007) [1], an increase in loading densities leads to an increase in stress and therefore an increase in energy requirements, which leads to a reduction in the growth rate and use of the food. In contrast to this study, Osofero *et al.* (2009) [17] report an increase in fish growth related to an increase in stocking density. These authors attribute this increase in growth to the good quality of the diet and favourable physical and chemical conditions.

The condition factor recorded in this study varies slightly between loading densities. No link was observed between the values of this parameter obtained by the larvae of the Brazil strain of Nile tilapia and the loading densities applied during this work. Similar results were obtained by Dambo and Rana (1992) [8], who found no difference between the condition factors of the densities tested in *Oreochromis niloticus* fry raised in 2-litre to 2, 5, 10, 15 and 20 individuals / liter tanks. On the other hand, in juveniles of isolated and lagoon strains of *Sarotherodon melanotheron*, Ouattara *et al.* (2003) [20] have condition factor values that appear to vary with stocking density. In this study, no statistical differences ($p > 0.05$) were observed between the coefficient of variation in length recorded in larvae of the Brazil strain of Nile tilapia subjected to different loading densities. These results corroborate the work of Ouattara *et al.* (2003) [20]. These authors do not observe any variation in this parameter as the density increases. However, the values of this parameter seem to increase with the loading density.

The feed conversion rate recorded in this study is similar for the densities (D1; D2; D3 and D4) that recorded the best growth performance. The value of this parameter is low and ranges from 1.11 to 1.22 for densities D4 to D1. However, this parameter is high (1.84 and 2.6) for densities (D5 and D6) that have provided low growth performance values. This parameter is related to the increase in the loading density. The results of this study are similar to those obtained by Ronald *et al.* (2014) [21]. For these authors, fry uses food less well for somatic growth as stocking density increases. This situation can be attributed to the reduction in available oxygen levels and the development of a stress situation leading to an increase in energy requirements (Stickney *et al.*, 1972 and Allen, 1974) [23, 3]. According to Leatherland and Cho (1985) [13], this leads to a decrease in growth associated with high food consumption. In this case, it is obvious that the food distributed is used to meet energy needs instead of improving weight growth. However, unlike this study, no effect of stocking density on feed conversion rate was reported by Osofero *et al.* (2009) [17]. These authors justify this fact by using the same food in the same environment.

5. Conclusion

The results of this study show that the increase in stocking density to an extreme level in the larvae of the Brazil strain of Nile tilapia *Oreochromis niloticus* raised in hapa significantly affects the survival, growth and utilization parameters of the food. In this study, the negative effect of density was observed in larvae loaded at a density above 2000 larvae / m^2. This suggests that in larviculture of Nile tilapia *O. niloticus* in pond catchers a density of 1500 larvae / m^2 or at most 2000 larvae / m^2 is recommended for massive tilapia fingerling production in breeding environments.

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