



RESEARCH PAPER

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Optimizing the stocking density is crucial for growth and survival of catfish, *Clarias batrachus* larvae

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Abstract

The effect of four (12, 18, 24, and 30 larvae L⁻¹) stocking densities (SD) on growth and survival of catfish, *Clarias batrachus* larvae (1.47 cm and 0.03 g) was investigated in triplicates in glass aquaria for a period of 28 days. The larvae were fed with *Tubifex* sp. four times daily until satiation. The larvae reared at high SD (30 larvae L⁻¹) showed significantly slower growth performance than those held at low SD (12 larvae L⁻¹). Water quality parameters were found within the productive range (temperature: 28.7°C, DO: 6.1 mg L⁻¹, pH: 7.8, and alkalinity: 379 mg CaCO₃ L⁻¹) for all treatments. The specific growth rate varied significantly in fish reared at high SD than at low SD on day 14 only, but on day 7, 21, and 28 there were no significant differences among the four treatments. Heterospecific growth was observed in high SD resulting in increased coefficient of variance of length and weight on final day. On the other hand, there was no effect of stocking densities on the survival rate of the larvae during the experimental period. Based on these findings, a stocking density of 18 larvae L⁻¹ appears to be optimum for intensive culture of *C. batrachus* in indoor static water system.

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Introduction

Intensive fish farming has been based upon the premises that it utilizes a minimum of land and water resources while providing maximum profit to the farmer. The ability to raise fish at a relatively high density, thus maximizing usage of the fish production infrastructure, is of importance to get the maximum economic return from aquaculture. Density is one of the most deterministic factors in larviculture, affecting social interactions such as aggressiveness (Kaiser *et al.*, 1995; Sakakura & Tsukamoto, 1999), hierarchical phenomena (Huntingford *et al.*, 1990) and cannibalism (Moore *et al.*, 1994), resulting in variations in size, survival and growth performance in fish populations (Sheikh-Eldin *et al.*, 1997).

Both positive and negative relationships between stocking density and growth have been reported (Dambo & Rana, 1992; Esquivel *et al.*, 1997; Irwin *et al.*, 1999; Gomes *et al.*, 2000; Akter *et al.*, 2001; Rahman & Rahman, 2003 & Rahman *et al.*, 2005) and the pattern of this interaction appears to be species specific. The importance of stocking density on fish growth has been reported for several species, however, to our knowledge, the common practice of the very high densities and their effects on *Clarias batrachus* larvae culture has not been adequately studied, though preliminary attempts in lower densities have been performed by a number of authors (Mollah, 1991; Sahoo *et al.*, 2004ab & Samad *et al.*, 2005).

Until recently, the supply of *C. batrachus* fry comes from natural sources. This is one of the major limiting factors towards catfish farming. While it is now possible to obtain seeds through artificial means (Hossain *et al.*, 2006), larval rearing and subsequent development of fry remain to be investigated which withholds the widespread adaptation of these species commercially. Thus, the purpose of the present study was to test the effect of high stocking density on growth and survival of eight days old *C. batrachus* larvae in aquarium tanks.

Materials and methods

Brood collection and induced breeding

Mature healthy broods of *C. batrachus* were collected from the local market. Immediately after collection, broods were transferred to the fish rearing facilities at Khulna University and kept in a 500 L tank with aeration for 2 days. For this experiment, seven females and five males ranged from 130 to 190 g for male and 100 to 160 g for female were used. Spawning to obtain the larvae was induced following the procedure described by Hossain *et al.*, 2006.

Larval rearing

Eight days old larvae having an initial total length and weight of 1.47 ± 0.02 cm and 0.03 ± 0.001 g, respectively were reared in glass aquaria (50 × 30 cm; 20 L water) at a stocking density of 12 larvae L⁻¹ (SD 12), 18 larvae L⁻¹ (SD 18), 24 larvae L⁻¹ (SD 24), and 30 larvae L⁻¹ (SD 30) for 28 days. Each treatment was conducted with three replicates. Larvae were fed *Tubifex* sp. four times daily (8:00, 12:30, 18:00, and 22:00) until satiation. Aerator and PVC pipes were used to ensure oxygen supply and provide shelter, respectively for the larvae. Two PVC pipes of 7 inch long and 1 inch diameter each were used in each tank. About two-third of the water was replaced twice a day. During replacing water, feces, and dead larvae, if any, were removed. Adhered dirt inside the aquarium walls and PVC pipes were cleaned during water exchanges.

Data collection

Weekly measurements were carried out for weight (to the nearest g) with an electric balance (Mettler Toledo, B303-S; accuracy 0.0000 g) and total length (to the nearest cm) with a measuring scale for about 30 larvae from each aquarium. The dead larvae were counted for assessing the survival. Length and weight gain, specific growth rate (SGR), coefficient of variance (CV) of length and weight and survival rate were determined according to the following formula:

Length gain = Mean final length – mean initial length;
Weight gain = Mean final weight – mean

initial weight; $SGR = \{(\ln \text{ final weight} - \ln \text{ initial weight}) / \text{culture period}\} \times 100$; $CV \text{ of length} = (\text{Standard deviation of final length} / \text{mean of final length}) \times 100$; $CV \text{ of weight} = (\text{Standard deviation of final weight} / \text{mean of final weight}) \times 100$ and $\text{Survival rates} = (\text{Number of live fish} / \text{total number stocked fish}) \times 100$

Water quality parameters

Water quality parameters such as temperature, dissolved oxygen (DO), and pH were recorded twice daily during morning (08:00) and evening (20:00) in all tanks. Temperature and DO of each tank were recorded by a mercury thermometer and DO meter (Lutron DO-5510), respectively whereas the pH was recorded with the help of a pH meter (Hanna ISO 9001). Alkalinity of water was determined by titrametric method (APHA 1992).

Statistical analysis

All numerical results were expressed as mean \pm SD (Standard Deviation). Significance levels were analyzed by one-way ANOVA followed by the Tukey's multiple comparison tests. Differences were considered as statistically significant at a probability value of $P < 0.05$.

Results

Growth performance

Mean total length and body weight variations throughout the experimental period are presented in Table 1. There was a trend of decreased length of larvae with increasing SD, starting on day 14. Also starting on day 14 larvae reared at low SD (12 and 18 SD) were significantly higher in weight than those reared at high SD (24 and 30 SD). The average length of *C. batrachus* larvae gradually increased from 1.47 to 6.17, 1.47 to 6.12, 1.47 to 5.53, and 1.47 to 5.21 cm while the average weight of larvae of *C. batrachus* increased from 0.03 to 2, 0.03 to 1.98, 0.03 to 1.51, and 0.03 to 1.38 g for larvae stocked at 12, 18, 24, and 30 L^{-1} , respectively. SGR varied between weekly sampling but remained similar for all stocking densities except on day 14 at high SD (Table 1).

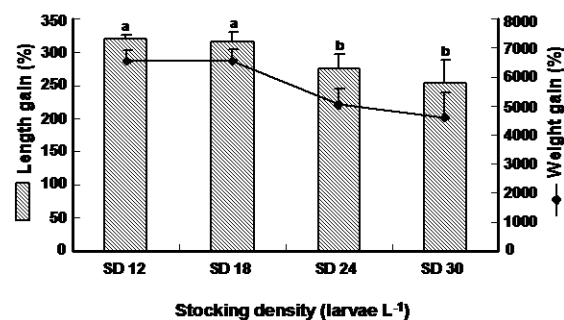


Fig. 1. Length and weight gain (%) of *C. batrachus* larvae reared at different stocking densities for a period of 28 days. Thick and thin bars represent mean and SD, respectively of three replicates with approximately 90 for each treatment. Bars with asterisks are significantly different between the treatments ($P > 0.05$).

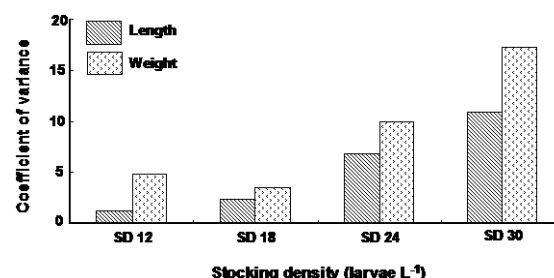


Fig. 2. Coefficient of variance of length and weight of *C. batrachus* larvae reared at different stocking densities.

Percent length and weight gain of larvae were higher at SD 12 and SD 18 than at SD 24 and SD 30. No significant differences ($P > 0.05$) were observed between SD 12 and SD 18 but varied ($P < 0.05$) from SD 24 and SD 30 (Fig. 1). The relationship between SD and CV of length and weight is presented in Fig. 2. The results indicated that values of CV for SD 12 and SD 18 were similar and increased for SD 24 and SD 30. In high SD, heterogeneous growth was observed while uniform growth was evident in low SD. CV values were obtained only from final length and weight and therefore, statistical analysis was not performed. On the other hand, survival rates between the treatments did not show any significant differences (data not shown).

Water quality parameters

The results of water quality data taken throughout the experiments are given in Table 2. All parameters

remained within the tolerance range for fish grown during the entire experimental period and did not

vary significantly except for alkalinity at the higher stocking density.

Table 1. Growth performances of *C. batrachus* larvae reared at different stocking densities. All values represent mean \pm SD of three replicates with approximately 90 for each treatment. Mean values in the same column having the same superscript are not significantly different ($P > 0.05$).

Stocking density (larvae L^{-1})	Culture period (days)					Overall (0 – 28 day)
	0	7	14	21	28	
Length (cm)						
12	1.47 \pm 0.02 ^a	2.41 \pm 0.01 ^a	3.48 \pm 0.08 ^a	4.98 \pm 0.02 ^a	6.17 \pm 0.07 ^a	4.70 \pm 0.05 ^a
18	1.47 \pm 0.02 ^a	2.41 \pm 0.06 ^a	3.48 \pm 0.05 ^a	4.93 \pm 0.03 ^a	6.12 \pm 0.14 ^a	4.65 \pm 0.12 ^a
24	1.47 \pm 0.06 ^a	2.39 \pm 0.04 ^a	3.27 \pm 0.12 ^a	4.50 \pm 0.12 ^b	5.53 \pm 0.37 ^b	4.06 \pm 0.31 ^b
30	1.47 \pm 0.03 ^a	2.36 \pm 0.12 ^a	3.06 \pm 0.45 ^b	4.22 \pm 0.44 ^b	5.21 \pm 0.60 ^b	3.74 \pm 0.54 ^b
Length gain (cm)						
12		0.93 \pm 0.02 ^a	1.08 \pm 0.08 ^a	1.50 \pm 0.07 ^a	1.19 \pm 0.05 ^a	
18		0.94 \pm 0.07 ^a	1.06 \pm 0.11 ^a	1.46 \pm 0.08 ^a	1.19 \pm 0.12 ^a	
24		0.91 \pm 0.10 ^a	0.88 \pm 0.08 ^a	1.23 \pm 0.12 ^b	1.03 \pm 0.50 ^b	
30		0.89 \pm 0.09 ^a	0.71 \pm 0.54 ^b	1.16 \pm 0.05 ^b	0.99 \pm 1.00 ^b	
Weight (g)						
12	0.03 \pm	0.12 \pm 0.00 ^a	0.28 \pm 0.04 ^a	1.08 \pm 0.04 ^a	2.00 \pm	1.97 \pm 0.10 ^a
18	0.00 ^a	0.12 \pm 0.00 ^a	0.28 \pm 0.03 ^a	1.07 \pm 0.04 ^a	0.09 ^a	1.95 \pm 0.07 ^a
24	0.03 \pm	0.12 \pm 0.01 ^a	0.23 \pm 0.05 ^a	0.84 \pm 0.06 ^b	1.98 \pm 0.07 ^a	1.48 \pm 0.15 ^b
30	0.00 ^a	0.12 \pm 0.00 ^a	0.21 \pm 0.05 ^b	0.75 \pm 0.14 ^b	1.51 \pm 0.15 ^b	1.35 \pm 0.24 ^b
	0.03 \pm				1.38 \pm 0.24 ^b	
	0.00 ^a					
	0.03 \pm					
	0.00 ^a					
Weight gain		0.09 \pm 0.00 ^a	0.16 \pm 0.04 ^a	0.79 \pm 0.06 ^a	0.92 \pm	
12		0.10 \pm 0.00 ^a	0.16 \pm 0.03 ^a	0.79 \pm 0.06 ^a	0.09 ^a	
18		0.09 \pm 0.01 ^a	0.12 \pm 0.05 ^a	0.61 \pm 0.02 ^b	0.91 \pm 0.06 ^a	
24		0.09 \pm 0.00 ^a	0.09 \pm 0.05 ^b	0.54 \pm 0.19 ^b	0.67 \pm 0.09 ^b	
30					0.63 \pm	
					0.30 ^b	
SGR (%)						
12		20.35 \pm 0.40 ^a	11.60 \pm 1.69 ^a	19.18 \pm 2.03 ^a	8.86 \pm 0.69 ^a	15.00 \pm 0.17 ^a
18		20.52 \pm 0.56 ^a	11.52 \pm 1.35 ^a	19.19 \pm 1.84 ^a	8.75 \pm 0.53 ^a	15.00 \pm 0.19 ^a
24		19.58 \pm 2.02 ^a	9.81 \pm 3.43 ^b	18.46 \pm 1.91 ^a	8.40 \pm	14.07 \pm 0.41 ^a
30		20.59 \pm 0.17 ^a	7.28 \pm 3.73 ^b	18.24 \pm 6.14 ^a	0.48 ^a	13.72 \pm 0.62 ^a
					8.77 \pm 3.82 ^a	

Table 2. Water quality parameters of *C. batrachus* larvae reared at different stocking densities. All values represent mean \pm SD. Mean values in the same row having the same superscript are not significantly different ($P > 0.05$)

Parameters	Stocking density (larvae L^{-1})			
	12	18	24	30
Temperature ($^{\circ}C$)	28.6 \pm 0.9 ^a	28.7 \pm 0.6 ^a	28.8 \pm 0.8 ^a	28.6 \pm 0.5 ^a
Dissolved oxygen (mg L^{-1})	6.0 \pm 0.35 ^a	6.2 \pm 0.54 ^a	6.1 \pm 0.45 ^a	6.4 \pm 0.07 ^a
pH	7.7 \pm 0.18 ^a	7.8 \pm 0.11 ^a	7.8 \pm 0.14 ^a	7.7 \pm 0.15 ^a
Total alkalinity (mg L^{-1} $CaCO_3$)	372.8 \pm 3.9 ^a	375.6 \pm 5.5 ^a	376.2 \pm 7.4 ^a	391.7 \pm 14.7 ^b

Discussion

Embryos reared at the high SD, in this study, showed significantly slower growth performances than those held at low SD. Growth performances in

terms of length and weight were comparable up to 14 days but significantly reduced after 14 days. Food was not a limiting factor during the experiments because larvae were fed four times daily until

satiation. The decrease in growth and survival of *C. batrachus* with increasing stocking density is consistent with the results of other studies on this species (Mollah, 1991, Samad *et al.*, 2005). This could be due to crowding, resulting in difficulties for fish to move and reach the food, thereby depressing the feeding rate (Dambo & Rana, 1992; Huang & Chiu, 1997 & Sahoo *et al.*, 2004a). Moreover, high density of fingerlings in combination with high concentration of food in the rearing system might produce a stressful situation, and therefore also reduce the growth of fish (Rahman & Rahman, 2003, Rahman *et al.*, 2005). Density dependent growth of *C. batrachus* fry was also observed by Sahoo *et al.*, 2004a when the fry were stocked (at SD 100 – 400 m⁻²) in concrete tanks (4 x 1 m). The authors observed that the fry attained about 0.22 to 0.96 g in weight and 3.19 to 5.07 cm in length at the end of 28 days when fed with prepared pellet and natural plankton developed by fertilizers. Nevertheless, *C. batrachus* larvae stocked in small aquaria (0.5 x 0.3 m) showed better growth than those reported by Sahoo *et al.*, 2004a due possibly to the live feed used in the present study.

The overall growth performances in terms of average growth and length obtained in this study are almost double than those obtained by Mollah, 1991 & Samad *et al.*, 2005, and such variations could be due to feeding frequency. These authors also used *Tubifex* sp. but unlike four times daily ration as used in this study they fed *C. batrachus* larvae twice daily. The relationship between feeding frequency and growth rate varies between species (De Silva & Anderson, 1995). It was found that catfish fry commence feeding 5-6 times shortly after hatching (De Silva & Anderson 1995). Frequent feeding (frequency) reduces starvation and stunting of small fish; thus the group has better uniformity (Piper, 1982).

Provision of suitable diet during larval stage is vital and determines the rate of survival of the larvae. Our preliminary study reported that *C. batrachus* larvae preferred *Tubifex* sp. compared to *Moina* sp.

or prepared feeds (Islam *et al.*, 2004). The better growth performance in fish fed *Tubifex* sp. in the present study may be due to the supply of all the required essential nutrients and digestive enzymes for better digestibility and assimilation.

Another noticeable feature for higher growth could be the use of shelters in the rearing tanks. In this experiment, we used shelters as PVC pipes for *C. batrachus* larvae and during the whole experiential period larvae were aggregated in the PVC pipes except during feeding time. Use of different shelter materials and their placements could be some of the interesting variables to investigate in future experiments.

SGR varied significantly in fish reared at high SD than at low SD on day 14 only, but on day 7, 21, and 28 there were no significant difference among the four treatments. However, significantly reduced SGR on day 14 at high SD cannot explain the difference between treatments since larvae received food until satiety and water quality parameters remained similar in all treatments. Similar pattern of SGR values were also found by Bernardino *et al.*, 1993 & Gomes *et al.*, 2000 for *Brycon cephalus*. Nevertheless, SGR values at the end of experiment for all treatments were similar and no significant differences were observed between the treatments, showing the good growth potential of this species.

In this study, we observed heterogeneous growth of larvae with increasing stocking densities, which is usually related to social interactions, development of hierarchies, and establishment of territorial borders (Koebele, 1985; Lambert & Dutil, 2001). Furthermore, an increase in the coefficient of variance for fish within a population is considered to be indicative of the establishment of hierarchies (Huntingford *et al.*, 1990; Jobling, 1994; Irwin *et al.*, 1999 & Lambert & Dutil, 2001). Probably these factors are also important for the larvae of *C. batrachus* since coefficient of variance of length and weight increased with increased SD on final day.

Similar findings were also reported by Gomes *et al.*, 2000.

To evaluate the effect of SD on fish growth, physicochemical parameters must be within ideal ranges (Jobling, 1994). Water quality parameters remained within the range of tolerance for growth of *C. batrachus* during the entire period which could reflect the high survival (96-98%) in all treatments. The fact that SD did not alter survival of *C. batrachus* larvae suggests that intensive culture of this species at high SD is feasible.

In conclusion, this study clearly demonstrated that the growth of *C. batrachus* larvae in tanks is density dependent. Both growth in weight and length were negatively affected by increased stocking density. The maximum average growth rate was in SD 12 followed by SD 18, SD 24, and SD 30. Increasing densities result in heterogeneous growth rates and the suppression of growth of some individuals. In the present rearing trial, no effect of survivability was found fewer than four stocking densities. Considering overall growth performances, the 18 larvae L⁻¹ stocking level is proposed as the optimum density for intensive culture of *C. batrachus* larvae in tanks.

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