

Growth, Survival and Fecundity in Ornamental Fish, *Betta splendens* (Regan) as a Function of Stocking Density

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Effects of stocking density (10, 25, 50, 100 and 150 fish/50 l of water) on growth, survival and fecundity were studied in Siamese fighting fish, *Betta splendens*. An increase in stocking density reduced the growth, survival and fecundity in *B. splendens*. A significant ($P < 0.05$) negative correlation was obtained between density and feeding parameters or gonad weight or fecundity in *B. splendens*. Animals reared in higher densities elicited poor gonad development and it severely affected egg production and hatching. Accumulation of more ammonia and carbon dioxide and oxygen depletion with increase in stocking density could have reduced the feed consumption and growth which in turn affected the gonad development and fecundity in female *B. splendens*. However, fish reared in 10 and 25D groups could withstand ammonia stress of 1.01 mg l^{-1} without any ill effect on survival, growth, gonad development and fecundity. Fish reared in 10 D group displayed better rates of feeding and growth at early rearing period than those in 25D group. However, fish reared in 25D group showed significantly higher growth rate ($t=2.71$; $P<0.05$) and growth efficiency ($t=2.67$; $P<0.05$) than fish reared in 10D group and also in higher density groups when fish attained sexual maturity on day 42. Although fish reared in 10D laid more eggs per female *B. splendens* than 25D group, the total number of egg production was higher in 25D than 10D group. The percentage of egg hatchability between 10D and 25D groups did not show significant ($P<0.05$) difference between them. Fish reared in 25D group exhibited 100% survival like 10 D group. Based on the survival, growth rate, total number of egg production, and egg hatching rate, fish reared in 25D group is economically better than 10 D or higher than 25D groups and hence 25 fish 50 l^{-1} water is considered as optimum density.

Key words : Density, growth, survival, ammonia, fecundity, *Betta splendens*

Growth and production of cultured fish are affected by variables such as light intensity, temperature, dissolved oxygen, stocking density and quantity of food (Brown, 1957). In fish farming practice, intensive culture is resorted to maximize the utilization of available water resources and to enhance the yield. Two major constraints in high-density culture are

depletion of dissolved oxygen and accumulation of ammonia and other toxic products of metabolism (Armstrong & Boyd, 1982; Sampath, 1985). Both dissolved oxygen and ammonia concentrations are dependent on density, fish metabolism and water exchange in culture system (Soderberg *et. al.*, 1983; Sampath & Pandian, 1984). Previous workers have reported

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reduction of growth rate with increasing density (Trzebiatowski *et. al.*, 1981; Sampath & Pandian, 1984) on cultivable fishes; however, studies on the effect of density in ornamental fishes are scanty (Dahlgren, 1979).

This paper reports on the effects of stocking density on growth, survival and fecundity in the Siamese fighting fish, *Betta splendens*.

Materials and Methods

Healthy and active 30 days old *B. splendens* (14.86 ± 1.68 mm; 0.041 ± 0.07 g) juveniles (1005 numbers) were collected from the laboratory-bred brooders. They were randomly assigned to five different density groups (10, 25, 50, 100 and 150 individuals/cement tank and hereafter referred as 10, 25, 50, 100 and 150 D groups). Triplicates were maintained for each group. The experiment was conducted in circular cement tanks (size: 1.75 x 1.50'; capacity: 110 l) containing 50 l freshwater in a static condition.

Experimental animals were fed with minced pieces of beef liver *ad libitum* for 2 h a day at 0900 h ; uneaten feed was removed by pipette with minimum disturbance to fish and dried in hot air oven at 80°C. Feeding parameters (feeding and conversion rates and conversion efficiency) of experimental fish were measured at 14 days intervals by weighing all the individuals in the tanks. Feed consumption (mg) was estimated by subtracting the amount of unconsumed dry feed from the total dry weight of feed offered. The feeding rate (mg g^{-1} live fish day^{-1}) was computed as:

$$\text{Feeding rate} = \frac{\text{Amount of feed consumed (mg)}}{\text{Initial wet weight of fish (g) x Number of days}}$$

Mortality of experimental animals was recorded daily at 06.00 and 18.00 h and dead animals were immediately removed to prevent

the contamination of water. The tanks were drained twice in a week and 50 l of water was maintained in all the tanks during the experiment. The hydrological parameters, such as temperature ($28.5 \pm 0.7^\circ\text{C}$), pH, dissolved oxygen, carbon dioxide and ammonia (Solarzano, 1969) were estimated once in 14 days.

Before beginning the experiment, total wet weight of test fish in each tank was taken using an electrical monopan balance. Five fish from the stock were sacrificed to estimate the water content (Maynard and Loosli, 1962); the initial dry weight of the experimental fish was determined using this estimation. All animals in each tank were collected every 14 days and wet weight was taken. Wet weight was converted to dry weight using the percentage water content of fish sacrificed before beginning the experiment. Growth was calculated taking the difference in dry weight between the beginning of the experiment and that on the day of calculation. The growth or conversion rate (mg g^{-1} live fish day^{-1}) and gross conversion efficiency (%) were calculated as:

$$\text{Conversion rate} = \frac{\text{Growth (mg)}}{\text{Initial wet weight of fish (g) x Number of fish}}$$

$$\text{Gross conversion efficiency (\%)} = \frac{\text{Growth (mg)}}{\text{Feed consumed (mg)}} \times 100$$

Test animals attained sexual maturity on day 42 and the feeding experiment was terminated and spawning study started. Two females from each treatment were sacrificed to estimate the gonad weight. Their ovaries were removed, weighed and the gonadosomatic index (GSI) was computed according to the formula of Dahlgren (1979).

$$\text{Gonadosomatic index (\%)} = \frac{\text{Wet weight of gonad (mg)}}{\text{Wet weight of the fish (mg)}} \times 100$$

Fish, feed samples, unconsumed feed and

ovary were weighed in an electrical monopan balance to 1 mg accuracy. After attaining sexual maturity on day 42, two males and two females were randomly selected from each replicate tank and used for the spawning studies. The remaining animals in the experimental tanks were removed and sacrificed for growth estimation. One male *B. splendens* was paired with one female in plastic trough containing freshwater upto 15 cm level. The female found shelter against the highly aggressive male, which built a foam nest, made of bubbles covered with saliva, on the water surface or under the plant tufts. Both the partners were set for spawning within 4 – 7 h. The female brooders laid eggs within 24-30 hours. Both the male and female collected the falling eggs and squeezed them into the nest. After the completion of spawning, female brood was removed from the trough to avoid male's aggressive attack on female and male showed extreme sense of incubating the egg. The number of eggs laid by the female was counted by using a sterilized needle, without causing much disturbance. Fecundity is the number of eggs laid in a single spawn by a oviparous female fish (Mc Fadden *et. al.*, 1965). The male incubates the eggs intensively for 36 h and after that the fry hatches. The number of fry hatched and the number of un-hatched eggs were also counted. The hatching rate was calculated by dividing the number of eggs hatched by the number of eggs laid multiplied by 100.

Students 't' test was applied to determine the significance of mean values between the different experimental groups. Two-way ANOVA was applied to detect the significant effects of density and rearing duration on chosen feeding parameters in *B. splendens*. Correlation and regression was applied following least square method (Zar, 1974).

Results and Discussion

The present study reveals that an increase in stocking density decreased the feeding parameters, survival and fecundity in *B. splendens*. The feeding rate of 10 D group was 174 mg g⁻¹ live fish day⁻¹ and fish reared at 25, 50, 100 and 150 D groups showed a reduced feeding rate with 128, 87, 75 and 66 mg g⁻¹ live fish day⁻¹ respectively on day 14. The results obtained for rate and efficiency of conversion were also similar to those of feeding rate. (Table1).

Subjecting the data to two-way ANOVA showed that density did not significantly ($P>0.05$) affect the feeding parameters, while rearing period significantly ($P<0.01$) affected the rate and efficiency of conversion. Significant negative correlation was obtained between the rearing period (0-42 days) and feeding rate or conversion rate or conversion efficiency at different densities.

Table 1. Effect of density on the rates of feeding and conversion (mg g⁻¹ live fish day⁻¹) and gross conversion efficiency (%) in *Betta splendens* as a function of rearing period.*

Rearing period (days)	Density (No. of fish 50 l ⁻¹ water)				
	10	25	50	100	150
Feeding rate					
14	174.07±8.3	128.23±8.1	86.75±7.3	75.29±6.8	65.80±5.0
28	119.54±6.1	87.88±5.7	92.71±5.8	87.45±4.4	81.84±7.1
42	70.90±4.3	72.57±4.7	76.46±6.6	68.72±2.8	64.90±5.5
Conversion rate					
14	24.28±1.0	14.25±0.7	8.71±0.7	7.18±0.5	4.45±0.3
28	33.39±1.8	21.68±1.0	19.69±0.5	15.94±0.9	16.36±1.7
42	25.00±1.5	28.81±1.3	32.09±1.3	26.11±1.6	21.95±1.2
Gross conversion efficiency					
14	13.95±0.7	11.11±0.5	10.04±0.5	9.54±0.5	6.76±0.6
28	27.93±1.9	24.67±1.4	21.24±1.2	18.22±1.2	19.99±1.5
42	35.26±1.5	39.70±1.8	41.97±2.4	37.99±2.7	33.81±1.7

* Each value is the mean of three observations.

Student's 't' test on day 42

Feeding rate : 25 D Vs 10 D : t = 0.37; P>0.05

Conversion rate : 25 D Vs 10 D : t = 2.71; P<0.05

Conversion efficiency : 25 D Vs 10 D : t = 2.67; P<0.05

In the present study, the test animals were fed *ad libitum* and competition for feed may not be a factor responsible for reduction of feed intake. Under such conditions, however, the fish spent more time in avoiding each other than in feeding and it could have minimized the feed intake. In high-density groups (100 & 150 D) formation of dominant hierarchies followed by social constraints might also have caused the appetite and growth reduction.

Feeding rate of juvenile *B. splendens* was higher in early rearing period (14th day - except 50-150D groups) and it gradually declined when they grew; however, the rate and efficiency of conversion showed the opposite trend. An increase in metabolic rate was perhaps responsible for the higher rate of feed intake in juveniles during early period (Brett, 1971) and left less feed energy resulting in poor rate and efficiency of conversion. However, when juveniles grew, the rate and efficiency of conversion were more and it might be due to the diversion of more consumed feed energy for somatic growth and reproduction (Table 1 & 4). The reproductive cycle of *B. splendens* is very short and it requires more food energy for gonad development and egg production. Townshend & Wootton (1984) found that females channeled a higher proportion of the ingested feed energy for gonad development than the males.

An increase in density resulted in more accumulation of NH_3 and CO_2 and decline in dissolved oxygen. (Table 2). Accumulation of ammonia and carbondioxide and oxygen depletion with the increase in stocking density could have reduced the feed consumption and growth in *B. splendens*. Berg *et. al.*, (1996) found that low levels of oxygen reduced the growth rate in *Salmo salar* reared at different densities. Ammonia produces more serious ill effects than carbondioxide. Sampath (1985) reported that

Table 2. Levels of oxygen, carbon dioxide, total ammonia and pH at different densities of *Betta splendens* reared for 42 days. *

Density (No. of fish / 50 l)	Oxygen (ml l ⁻¹)	Carbon dioxide (mg l ⁻¹)	Ammonia (mg l ⁻¹)	pH
10	6.88 ± 0.18	19.00 ± 1.20	0.72 ± 0.05	8.55 ± 0.20
25	6.12 ± 0.08	28.00 ± 2.00	0.89 ± 0.10	8.45 ± 0.30
50	5.03 ± 0.14	36.00 ± 2.20	1.11 ± 0.07	8.35 ± 0.40
100	4.10 ± 0.26	43.00 ± 1.80	1.81 ± 0.11	8.40 ± 0.20
150	3.54 ± 0.19	51.00 ± 2.50	2.07 ± 0.21	8.10 ± 0.30

* Each value is the mean of three estimations.

Table 3. Effect of density on actual survival in *Betta splendens* as a function of rearing period.*.

Rearing period (days)	Density (No. of fish 50 l ⁻¹ water)				
	10	25	50	100	150
14	10 (100)**	25 (100)	45 (90)	82 (82)	110 (73.33)
28	10 (100)	25 (100)	45 (90)	79 (79)	100 (66.66)
42	10 (100)	25 (100)	45 (90)	78 (78)	96 (64.00)

* Each value is the mean of three estimations.

** Values in parentheses are the per cent survival

Table 4. Effect of density on gonad weight (mg wet weight), gonadosomatic index and hatchability of eggs in *Betta splendens*. **

Parameters	Density (No. of individuals 50 l ⁻¹ water)				
	10	25	50	100	150
Wet weight of gonad (mg)	184.0±8.3	115.67±7.6	83.33±5.8	54.00±5.2	42.00±3.1
Gonadosomatic index (%)	32.29±1.8	19.73±1.4	17.58±1.8	15.15±0.8	10.41±1.1
Number of eggs laid female ⁻¹	477±22	373±30	230±25	139±14	112±11
*Total number of eggs laid	2385	4663	5175	5421	5376
Number of eggs hatched female ⁻¹	453±36	325±25	178±14	89±8	69±6
*Total number eggs hatched	2265	4063	4005	3471	3312
Egg hatching (%)	94.97±4.3	87.13±4.8	77.39±6.7	64.03±5.9	61.61±3.8

* Based on survival percentage in each density, 50% of population as female

** Each value is the mean of three estimations.

ammonia accumulation under high density culture might be responsible for reduction in feed intake and growth in *C. striatus*. The safe level of ammonia prescribed for a number of fish was 1.0 to 1.5 mg l⁻¹ (Liao & Mayo, 1974). In the present study, ammonia concentration in the fish reared in 10 and 25 fish 50 l⁻¹ water ranged between 0.72 and 1.01 mg l⁻¹ (Table 2) in the aquaria of *B. splendens* ($t = 0.24$; $P > 0.05$). This ammonia concentration did not significantly affect survival, feed intake and growth.

Moreover, ammonia accumulation more than 1.01 mg l^{-1} in *B. splendens* at higher densities (50 to $150 \text{ fish } 50 \text{ l}^{-1}$) evidently affected the survival (Table 3) and feed utilization parameters (Table 1). This suggests that *B. splendens* could withstand ammonia stress upto 1.01 mg l^{-1} without any ill effect on survival and feeding parameters.

B. splendens reared in 10 and 25D groups elicited 100% survival. However, the survival declined when the stocking density was increased beyond 50D. (Table 3). In salmonid species, mortality was positively related to stocking density (Refstie, 1977; Trzebiatowski *et al.*, 1981; Soderberg & Krise, 1986; Holm *et al.*, 1990).

Gonad weight, GSI and fecundity of *B. splendens* decreased with increase in density (Table 4) and exhibited significant ($P < 0.05$) negative correlation (density vs gonad weight: $Y = 200 - 34.57X$; density vs fecundity: $Y = 563 - 96.4X$). Reduction of gonad weight in higher densities evidently affected the egg production and hatching (Table 4). For instance, a female *B. splendens* spawned 477, 373 and 112 eggs and the hatching percentage was 95, 87 and 62 in animals reared at 10, 25 and 150 D-A groups respectively. In high stocking densities, reduction of feed intake and conversion could have reflected on ovary development and the fecundity. Besides, increased load of ammonia in high stocking densities (Table 2) considerably reduced the fecundity (Table 4) of *B. splendens*. Like fecundity, egg hatchability of *B. splendens* was also decreased with increase in stocking density (Table 4) and it might be due to the poor development of ovary, which was not able to produce good quality eggs.

The choice of optimum stocking density is based on a number of criteria, notably

economic considerations (Maguire & Leedow, 1983), survival and growth (Holliday *et al.*, 1993). The present study reveals that fish reared in 10 D group displayed better rates of feeding and growth at early rearing period than those in 25 D groups. However, fish reared, in 25D group showed significantly higher conversion rate ($t=2.71$; $P<0.05$) and conversion efficiency ($t=2.67$; $P<0.05$) than fish reared at 10D group when fish attained sexual maturity on day 42 (see Table 1). Regarding fecundity, the number of eggs laid per female reared in 10D or 25D groups were females, the total number of eggs laid was 2385 or 4663 (Fig. 1) during their spawning. This indicates that, female *B. splendens* reared in 25D group laid about 50% more number of eggs as compared to fish reared in 10D group. Even though, 10D group exhibited higher egg hatching than 25D group, the difference was not significant ($t=1.72$; $P>0.05$) (see Table 4). Moreover, 25D group exhibited 100% survival like 10D group. Based on the growth rate, survival, total number of egg production and egg hatching, fish reared in 25D group is economically better than 10D or higher than 25D groups and hence 25 fish 50 l^{-1} water is considered as optimum density.

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