Influences of Chosen Water Hardness on Gonad, Embryonic Development, Egg Hatchability in *Betta Splendens* (Regan)

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Abstract - The hardness is one of the important water quality parameters which determines the survival, development and growth of fishes and it varies from species to species. Rearing of *B. splendens* in four different hardness media (60, 260, 600 and 1200 mg CaCO3 l-3), reproduction, egg hatchability and development were studied for 56 days Mean body length and weight as a function of time. The fecundity and egg hatchability of *B.splendens* reared in the low water hardness of 260mg CaCO3 elicited maximum performance. Although fish reared in 600 and 1200 mg CaCO3 groups laid 308 and 265 eggs spawn1and its hatchability was drastically declined to 27 and 1.49%. An increase in water hardness, the developmental processes were advanced upto 600mg CaCO3*l*-1 and thereafter defective developments such as choriolysis, failure of yolk resorption, eye developed but no further development, tail curvature etc. Occurred in *B. splendens* eggs incubated at 1200mg CaCO3 *l*-1. The present study reveals that *B. splendens* prefer soft water (260 mg CaCO3 *l*-1) for maximum growth, reproduction, egg hatchability and development.

Key Words: Water hardness, egg hatchability, Fecundity, Choriolysis, Yolk resorption, Development, *B.splendens*

INTRODUCTION

Water hardness is most important to fish culture and it causes major effect on egg development and larval survival (Ketola *et al.,* 1988; James and Sampath, 2004; WurtsandStickney,1989). The quality of water depends on the dissolved minerals with which water becomes hard either temporarily or permanently based on the types of minerals present in water. Fishes usually obtain their mineral requirements from the dietary food intake or from the surrounding water (Hickman,1968). Water hardness is a measure of the quality of divalent ions (salts with two positive ions) such as calcium, magnesium and or iron in water. Calcium or magnesium is essential in the biological process such as bone and scale formation, blood clotting and other metabolic reactions in fish. Fish can absorb calcium and magnesium directly from the water or from the food. Otherwise fish reared in non suitable media cause stress which may alter the entire physiological condition and the normal functioning of the body organs. Each species of fish prefers a particular type of soft or hard water for the survival, and hatchability of eggs. Therefore, there is a need to identify the suitable hardness to attain the maximum growth of commercial important ornamental fishes. In fish, salt uptake is affected by external concentrations of calcium and magnesium (Fleming *et al.,* 1974) or the pH of ambient water (McWilliams,1980). Many published reports exist on the effect of water hardness on survival of fish egg (Lee and Hiu,1983; Gonzal *et al.,*1987; Ketola *et al.,* 1988). However, there is paucity of information on the impact of water hardness on egg hatchability, development and larval survival in oviparous aquarium fishes. Hence, the present study was undertaken to study the effect of water hardness on egg development, hatchability, and water hardness on egg development, hatchability, and reproductionin *Betta splendens, as* the success of a fish farming lies great lyon its water quality management program.

MATERIALSANDMETHODS

The sexual maturity, *B.splendens*threepairs of males and females were randomly selected and allowed to spawn in respective hardness separately. Once spawning is over, series of photographs were taken periodically still hatching of larvae and their subsequent developments. Fecundity, duration of incubation period etc. Were studied and the embryonic stages were identified. The chemistry and composition of chosen water samples were analysed in the laboratory at Palayagayal, Tamil Nadu, India (Table.1)

Table1. Composition and chemistry of tested four water samples in relation to hardness.

	Waterparameters	Waterhardness(mg $CaCO3l^{-1}$)				Acceptablelimit (BIS)	Cause for rejection when exceeds (BIS)
		60	260	600	1200		
	Physicalexamination						
1	Appearance	Clear	Clear	Clear	Clear	-	-
2	Colour(ptco-scale)	Colourless	Colourless	Colourless	Colourless	5	25
3	Odour	None	None	None	None	Unobjectionable	
4	TurbidityNTunits	Nil	Nil	0.2	Nil	2.5	10
5	Totaldisssolidsmg/l	135	1915	2455	4380	500	2000
6	Electrical conductivitymicromho/cm	200	2860	3570	6540		
	II.Chemicalexamination						
7	pH	7.8	7.6	7.6	7.7	7.0-8.5	6.5-9.2
8	ph Alkalinityas$CaCO_3$	0	0	0	0		
9	TotalAlk.as$CaCO_3$	61	444	396	428		600
10	Totalhardnessas$CaCO_3$	61	256	607	1200	200	600
11	CalciumasCa	15	138	186	356	75	200
12	MagnesiumasMg	5	41	56	102	30	150
13	SodiumasNa	20	210	396	700		
14	PotassiumasK	3	30	40	72		
15	IronasFe	0.02	0.18	0.10	0.10	0.1	1.0
16	Manganese	0	0	0	0	0.5	0.5
17	Freeammoniaas NH_3	0	0.02	0.02	0.08	-	-
18	NitriteasNO_2	0.14	0.28	0.30	0.28		
19	Nitrateas NO_3	2	28	35	40	45	45
20	ChlorideasCl	25	556	765	1969	200	1000
21	FluorideasF	0.1	0.2	0.4	0.4	1.0	1.5
22	SulphateasSO_4	9	194	315	369	200	400
23	PhosphateasPO_4	0	0.06	0.06	0.06	-	-
24	Tidystest4hrs.asO_2	0.58	1.80	2.50	2.10	-	-
25	Residualchlorine	Nil	Nil	Nil	Nil	Nil	Nil
26	Dissolvedoxygen ($ml O_2 l^{-1}$)	4.74	4.51	3.61	3.38	-	-
	III. Bacteriological examination	B28969	B28970	B28971	B28972		
27	Faecalcoliformper100ml	Nil	Nil	Nil	Nil	Nil	Nil/100ml

Feeding and Water Change

Fry of *Bettas* were fed by *Infusoria* (raised from hay or banana peels) in the first week followed by brine shrimp nauplii during the 2nd and 3rd weeks. After 21 days, the fingerlings and adults were fed *adlibitum* with minced pieces of fresh beef live rtwice a day at 0600 and 1700h. The unconsumed food was removed by pipette after 2 h of feeding and dried in hot air oven at 80°C. The water used was clean, un chlorinated well water and its quality was monitored biweekly. The tanks were drained twice in a week and replenished with fresh water to remove the accumulated feces at the bottom. The fish were kept under a natural photoperiod of about 12hr everyday throughout the experiment.

Hydrobiological Analysis

Water used for the experiment was clear and unchlorinated well water. During the experimental period, biweekly samples of water were analysed for dissolved oxygen by Winkler's lodometric method, salinity by Harvey's titration method, pH was recorded using pH meter following the procedure given by Strickland and Pearson (1972). Ammonia was estimated following the Phenol-hypochlorite method (Solorzano,1969) using deionised water. EDTA method was adopted to estimate the hardness of water as described by Trivedy and Goel (1986)

Estimation of Parameters

Gonad estimation

Test animals were taken and weighed separately at sampling period and dissected to remove their ovaries, which were then weighed. From these weights, the gonadosomatic index (GSI) was computed according to the following formula (Dahlgren,1979).

$$\text{Gonodosomatic index}(\%) = \frac{\text{Wet weight Wetweight}}{\text{Of gonad of fish}} * 100$$

After attaining the sexual maturity on day 56, three pairs of males and females were randomly selected and allowed to spawn in respective hardness separately. Once spawning is over, series of photographs were taken periodically still hatching of larvae and their subsequent developments, Fecundity, duration of incubation period etc. Were studied and the embryonic stages were identified. The chemistry and composition of chosen water samples were analysed in the laboratory at Palayagayal, TamilNadu, India (Table2).

RESULT

Fecundity and Egg hatchability

The gonad weight (238 mg wet wt.), fecundity (371 eggs laid spawn^{-1}) andegg hatchability (97%) of *B. splendens* were maximum in 260 H group and they were statistically significantly($P<0.01$) as compared to fish reared in other groups (Table2.). ANOVA (one-way) test revealed that water hardness elicited the significant ($P < 0.01$) impact on gonad weight, fecundity and egg hatchability (Table 3). Duncan multiple range test showed that, the above parameters were differed significantly ($P < 0.05$) with better values in fish subjected to 260 H group than other groups (Table 2). Although fish reared in 600 and 1200 H groups laid 308 and 265 eggs spawn^{-1} and its hatchability was drastically declined to 27 and 1.49% (Table 2).

Table2. Effect of water hardness ongonad weight, gonadoso matic index and fecundity in *Bettasplendens.* Each value is the mean (X±SD) performance of six observations.

Hardness(mg $CaCO_3$-1)	Gonadweight (mgwetwt.)	Gonado-somatic index (%)	No.ofeggslaid /spawn	o.ofeggshatched	Per-centhatching
60	133.33^{a}± 5.16	12.65^{a}± 0.72	285.67^{a}± 20.50	264.00^{c}± 23.00	92.35^{c}± 1.43
260	238.00^{c}± 8.37	20.29^{c}± 1.14	371.33^{c}± 26.50	368.00^{d}± 18.24	96.56^{c}± 0.79
600	198.00^{b}± 8.37	16.79^{b}± 1.28	307.67^{b}± 9.50	85.17^{b}± 6.91	27.19^{b}± 1.92
1200	190.00^{b}± 7.07	15.61^{b}± 1.03	265.00^{ab}± 22.01	4.00^{a}±1.00	1.49^{a}±0.26

Student's 't' test :(260Vs 600mg$CaCO_3l^{-1}$)

Gonad weight : t=4.78;P<0.01GSI

: t=2.87;P<0.05

Fecundity : t = 3.09; P < 0.05Per-

centhatching : t=68.91;P<0.01

Values (mean±SD) with different superscripts in the same column are significantly different (p<0.05).

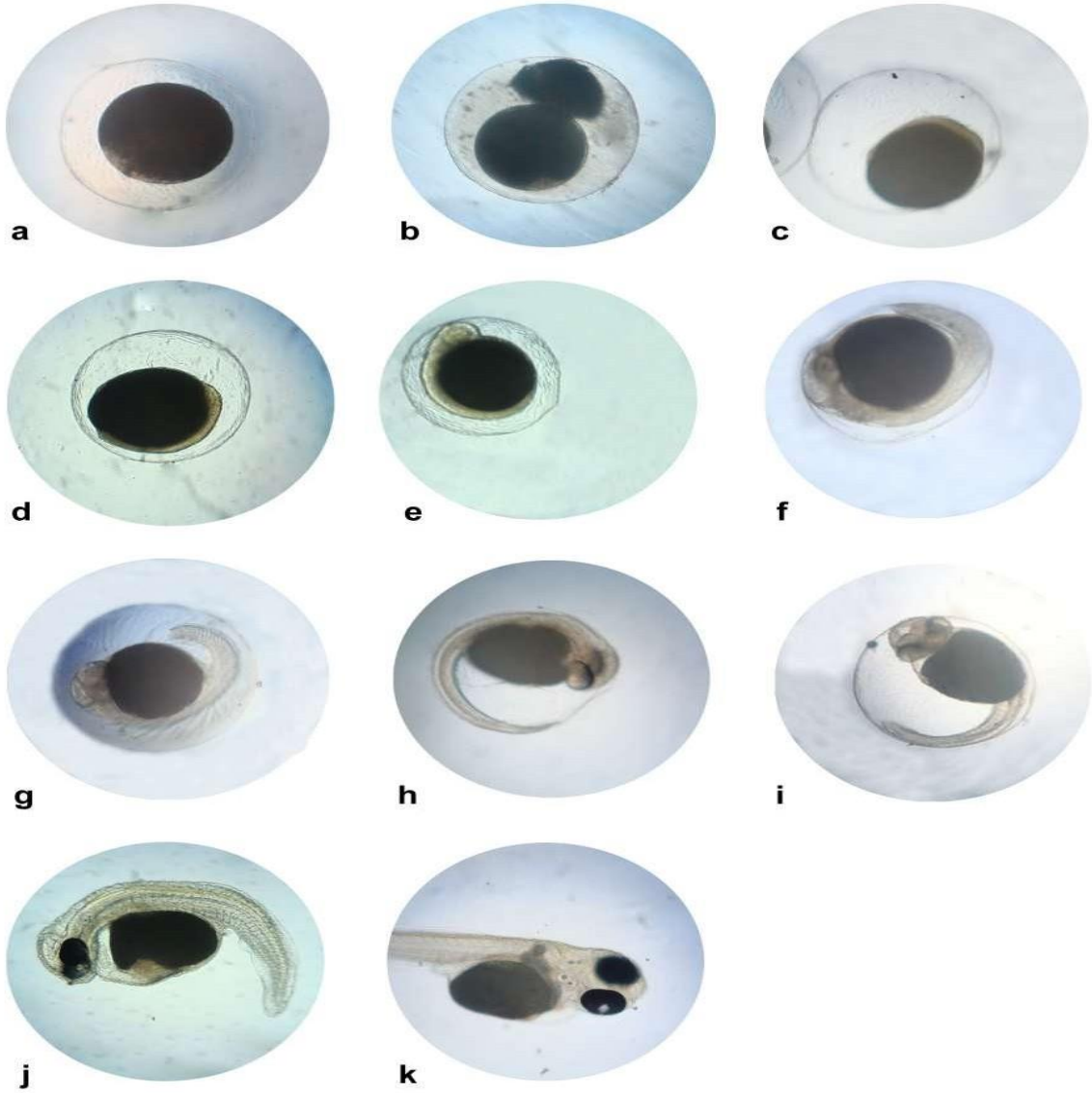
Table3.OnewayANOVA for gonad weight, gonadosomaticindex and fecundity in *Bettasplendens.*

Sourceofvariation	SS	df	MS	F	Pvalue
Gonadweight					
Between hardness	16738.13	3	5579.38	102.98	P<0.05
Within groups	433.45	8	54.18		
Total	17171.58	11			
Gonadosomaticindex					
Betweenhardness	93.52	3	31.17	20.38	P<0.05
Withingroups	12.24	8	1.53		
Total	105.76	11			
No.ofeggslaid/spawn					
Betweenhardness	19072.92	3	6357.64	14.99	P<0.05
Withingroups	3394.00	8	424.25		
Total	22466.92	11			
No.ofeggshatched					
Betweenhardness	236329.00	3	78776.33	226.97	P<0.05
Withingroups	2776.67	8	347.0833		
Total	239105.70	11			
Per-centhatching					
Betweenhardness	20148.80	3	6716.27	4168.94	P<0.05
Withingroups	12.89	8	1.61		
Total	20161.69	11			

Water hardness on Development of egg

Female laid the eggs during spawning and male *B. splendens* collected all the eggs immediately and squeezed them in the already constructed bubble nest. Simultaneously, male (rarely female) started the incubation of eggs till hatching of the fry. On laying, the egg was buoyant in nature and 0.9–1.1mm in diameter. Embryogenesis occurred inside the chorion (Plate 1). First cleavage occurred at 30 min. after fertilization resulted the two cells. An extension of incubation period, the developmental stages were proceeded and advanced into fry on 36–40h. An increase in water hardness, the developmental processes were gradually advanced upto 600H group (Plate) and thereafter few defective development occurred in 1200 H group (Plate 3). At 20h, embryo developed at the cost of yolk inside the chorion and it became curved and attached over the yolk. It was more pronounced in eggs incubated at 60–600H groups while partial/ complete choriolysis was observed in eggs incubated at 1200 H group (Plate1-4). At 24 – 30 h, the caudal region became free from yolk and resorption of yolk increased with increase in water hardness was observed except egg incubated at 1200H group. The development of eye and mid-body region were observed in eggs incubated at 60–600H groups while complete choriolysis and tail curvature were observed in eggs incubated at 1200H group (Plate1-4). At 36 h, embryonic development was progressed with increase in water hardness and became active with little yolk content in embryos reared at 600H group as compared to embryos reared at 60 and 260H groups. However, yolk resorption, tail curvature and further development of embryo's were completely stopped in eggs incubated at 1200 H group from 24h onwards. The eggs incubated at 1200H elicited the varieties of defective developmental stages (Plate 4) in *B. splendens* and they are as follows:

1. Eggs were damaged and no development occurred,
2. Development began and arrested in earlystage,
3. Early embryo development started and then arrested,
4. Eye developed but no further development and
5. Yolk content was damaged prior to commencement of development.



a) Fertilized egg
b) 4 hours old embryo
c) 8 hours old embryo
d) 12 hours old embryo
e) 18 hours old embryo
f) 20 hours old embryo
g) 24 hours old embryo
h) 30 hours old embryo
i) 36 hours old embryo
j) 40 hours old embryo
k) 44 hours old embryo - head enlarged

Plate 1. Embryonic developmental stages of *Betta splendens*.

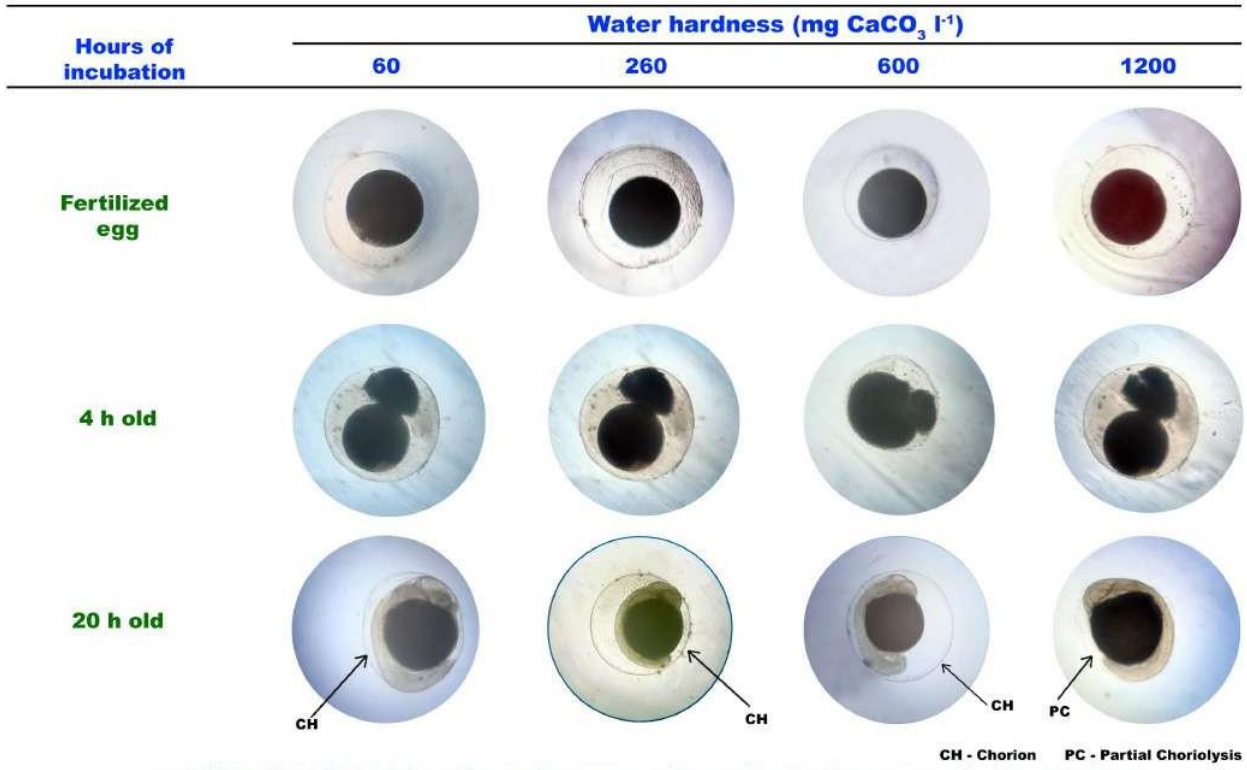


Plate 2 Effect of water hardness on embryonic development in *Betta splendens*.

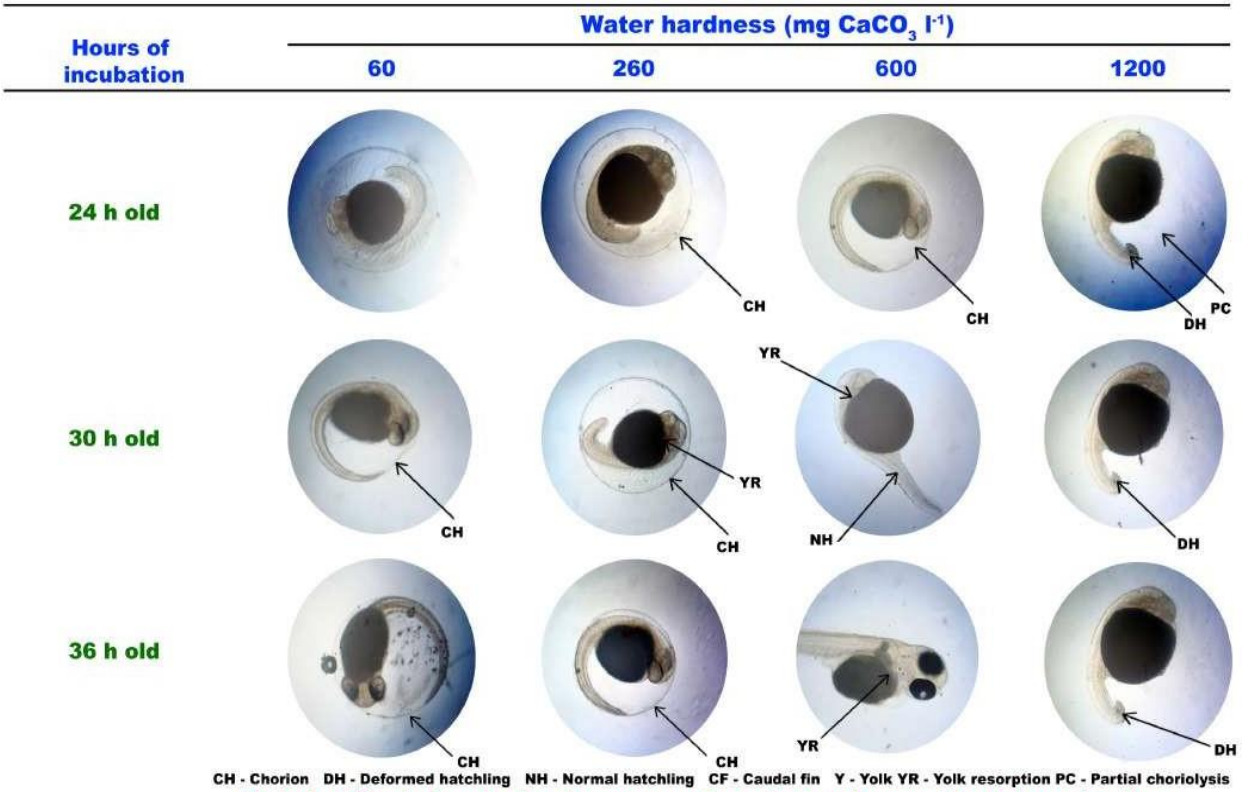
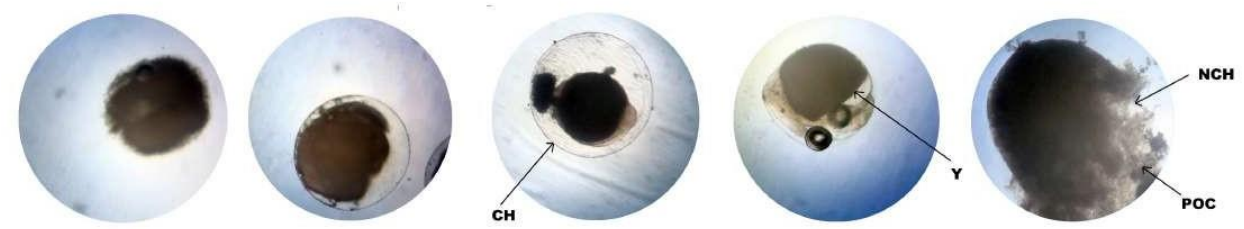


Plate 3. Effect of water hardness on embryonic development in *Betta splendens*.



CH - Chorion NCH - No chorion POC - Protrusion of cells Y - Yolk

Plate 4. A few defective developmental stages of *Betta splendens* eggs incubated at high water hardness.

DISCUSSION

B.splendens reared in 260H group exhibited better reproductive potential and egg development than those reared in higher water hardness (600 and 1200 mg CaCO3). It appears that *B. splendens* (oviparous) has preferred softwater for maturation, fecundity and egg development. James (1998) found thathigh growth rate, gonad weight and fecundity in *B. splendens* reared at 316 mg CaCO3 l^{-1}as compared to high water hardness (540 and 1018mg CaCO3l^{-1}) supports the present study.

The results obtained in *B. splendens* revealed that 260 H group elicited better performance in terms of growth, gonad weight and fecundity than those reared in60, 600 and 1200 H groups and this supports the findings of previous workers. Angelfish *P. scalare* grew well in soft water at 26^0C (Sweeney, 1992). Rathinam (1993) found that gonad development and maturity of *P. scalare* were suppressed due to the extreme hard water of the pond. The oviparous fish such as the rosy barb, *Barbus conchonius* and the tiger barb, *Barbus tetrazona* did not mature in hardness beyond 120ppm. *B. splendens* are found in freshwater systems like ponds, drainage channels, sluggish rivers and shallow warm water which have low mineral content(James,1998).

The present study also showed that *B. splendens* reared in the highest water hardness (1200 H) even though laid more number of eggs spawn^{-1},the egg hatchability was poor (1.5%). It might be evidently due to the deposition of excessive Ca (356 mg l^{-1}), Mg (102 mg l^{-1}), CaCO3 (1200 mg l^{-1}), Na (700mg l^{-1}) and Cl2 (1969 mg l^{-1}) in highest water hardness (See Table 8.1) on egg surfaceresulting in hardening the eggs which possibly reduced the uptake of water and there by reducing the egg hatchability (1.5%). Ketola *et al.* (1988) observed that the eggs of Atlantic salmon (*Salmosalar*) and the rainbow trout (*Salmogairdueri*) hardened quickly in water with high concentration of calcium than at lower calcium concentration. Fisher(1963) stated that the previtelline fluid of the hardened eggs protects the embryo from mechanical injury and subsequent death and also excess calcium reduces the hatchability of eggs. Potts and Rudy (1969) found that water uptake by the *S. salar* eggs into the previtelline fluid was reduced by the presence of 10 mMCa^{2+}(400mgl^{-1}) in the ambient water. Hatchability of eggs of the silvercarp (*Hypophthalmichthysmolitrix*) was

maximum at water hardness of 300–500 mg $CaCO_3$ l^{-1} and it significantly reduced at water hardness of 600 mg $CaCO_3$ l^{-1} (Gonzal *etal.,* 1987); they also indicated that water uptake by the eggs of silver carp decreased as hardness increased but the direct involvement of calcium or magnesium was not demonstrated.

Normal embryonic development was observed in fish reared in 60 – 600 H groups. In the present study, first cleavage occurred at 30 min resulting into two celled stage. Previous reports showed different times for attaining two celled stage in fishes ranged from 30 – 75 min (Chaudhuri *et al.,* 1978; Abraham *et al.,* 1999; Haniffa *et al.,*2003; Sahoo *et al.,* 2007; Dhaneesh *et al.,* 2009), supports the present observation. Theeggs of *B. splendens,* incubated at 600 H group elicited 27% egg hatchability and it drastically declined to 1.5% in fish reared in 1200H group. The poor results might be due to the ionic and osmotic stress of water and also due to the less amount of dissolved oxygen (3.38 – 3.61 mlO_2l^{-1}) in 600 – 1200 H groups (Table 8.1). Holliday and Jones(1965) found that low quantity of oxygen reduced the egg hatchability in fish reared in higher salinity which supports the present study. Molokwu and Okpokwasili (2002) reported that, egg development, rates of hatching and survival and incubation time of *Clarias gariepinus* eggs were affected by salinity and the eggs were lethal beyond 200$CaCO_3$mg l^{-1}.

The eggs of *B. splendens* properly developed the chorion in 60 and 260 H groups (Plate1-4) which protected the larvae during its full development. However, partial or entire choriolysis was observed in *B. splendens* eggs incubated at1200 H (20 h) and it evidently due to the higher levels of Ca (356 mg l^{-1}), Mg (102 mg l^{-1}), Na (700 mg l^{-1}) and Cl_2 (1969 mg l^{-1}) contents deposition over the soft eggs which disturbed the chorion at early stage (20 h) as a result choriolysis and subsequently adverse development of embryo and death occurred (Plate 1-4). Mabee *et al.*(1998) identified unicellular hatching glands in the embryo of *Betta* facilitate normal hatching when they reared in suitable environmental condition. Therefore, choriolysis followed by premature hatching was due to an increase in Ca^{2+} ions and low oxygen tension (3.38 mlO_2l^{-1}) in 1200 H group and ultimately led to either mass mortality ofeggs or development of deformed hatchlings which thereby drastically decreased the per-cent egg hatchability (1.5%). Besides, *B.splendens* eggs incubated at 600H maintained their chorion upto 24 h safely and thereafter it began the choriolysis and thereby 27% egg hatchability and low larval survival were observed.

CONCLUSION

Based on the water hardness studies, it is inferred that, *B. splendens* highlypreferred the soft water for maximum egg hatchability, larval survival, and fecundity. The severity of choriolysis, premature hatching of embryo and development of deformed hatchings or mass mortality of eggs were high in *B. splendens* reared in1200mg $CaCO_3l^{-1}$ followed by fish reared in 600mg $CaCO_3l^{-1}$.

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