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Effects of dietary vitamin E on the growth and breeding performance of *Ompok pabda*

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Abstract
A 90 day feeding trail was conducted to investigate the effect of vitamin E on the growth and breeding performance of *Ompok pabda*. A total of 84 healthy female broodfish (41.10±0.44g) were divided into 4 treatments *i.e.* treatment T_1, T_2, T_3 and T_4 having three replications each. The fish were fed twice a day with a standard feed (40% protein) having 4 doses of vitamin E *viz.* 0 (served as control), 50, 100 and 150mg vitamin E/kg feed. At the end of the feeding trial, the broodfish were induced to breed with PG extract to observe the effect of vitamin E on feed. After rearing for 90 days with the experimental feeds, it was found that weight gain and specific growth rate of broodfish fed with 100mg vitamin E/kg feed (treatment T_3) was the highest (14.78±0.38g and 2.99±0.11) while 150mg vitamin E/kg feed (treatment T_4) fed fish gave the poorest result (2.97±0.89g and 1.21±0.32). There was no significant difference in terms of length gain of broodfish among the different treatments. The broodfish were induced to breed with equal dose of PG extract (18 and 12mg PG/kg body weight for female and male respectively) to observe the dietary effect of vitamin E on breeding performance. The highest ovulation, fertilization and hatching rate of eggs were found to be 81.48±6.41%, 84.04±3.53% and 68.59±5.03% respectively in the broodfish of treatment T_3 while the poorest (33.33±00%, 52.35±5.02% and 45.70±7.24% respectively) were found in the broodfish under treatment T_4. The results suggest that inclusion of 100mg vitamin E/kg feed is best for enhancing the breeding performance of *O. pabda* broodfish indicating that vitamin E content has a positive impact on reproduction of fish. The present results also imply that inclusion of higher level of vitamin E exerts an antagonistic effect in terms of growth and breeding performance of this species.

Key words: Growth rate, Breeding, Vitamin E, *Ompok pabda*

Introduction

Ompok pabda commonly known as pabda, is an indigenous, small freshwater catfish belonging to the family Siluridae of the order Siluriformes (Siddiqua *et al.* 2000). In Bangladesh, it inhabits in all types of freshwater habitats, especially in rivers, canals, beels, swamps and ponds. In spite of many advantages very little attempt has been made in Bangladesh to promote breeding and culture of *O. pabda*. The total production of *O. pabda* is only 144T from different waterbodies of Bangladesh (FRSS 2008). Its

production can be increased through culture practice but the availability of a large number of fry and fingerlings is a pre-requisite to flourish the culture of this species.

Nutrition in the diet of broodfish is known to have a profound effect on gonad development, fecundity, quality of eggs and larvae. Although precise information on the nutritional requirements of broodstock for gonad maturation is scanty, it has been found that quantity and quality of feed as well as the feeding regime is important for maintenance of egg quality and successful spawning. Vitamins are one of the most effective additives to nutritionally complete diets for fish production (Gaylord *et al.*, 1998). Vitamin E is an essential nutrient for all species of animals (McDowell 1989). As a fat-soluble vitamin, it is the most effective chain-breaking, lipid-soluble antioxidant in biological membranes, where it contributes to membrane stability. It protects critical cellular structures against damage from oxygen free radicals and reactive products of lipid peroxidation. Aquatic animals have high levels of unsaturated fatty acids to maintain cell membrane fluidity especially at low temperatures; it is assumed that vitamin E plays an important role in this context (Blazer 1992). The importance of vitamin E in fish reproduction has been reported by many scientists (Watanabe *et al.* 1970, Hamre and Lie 1995, Halver 2002 and Paul *et al.* 2004). For example, vitamin E caused higher gonadosomatic index, larger ova and more eggs than a control in a study of freshwater fish, *Cyprinus carpio* (Gupta *et al.* 1987).

Sufficient number of fry and fingerlings of this catfish is, however, quite difficult to obtain from natural waters for stocking in the ponds. Proper techniques of mass production of fry in commercial scale seem to be the most crucial factors in expanding culture practice for this species because market price of fish bears the special preference in aquaculture. Considering the above realities, the present research work was undertaken to observe the effect of vitamin E on growth and breeding performance of *O. pabda* broodfish.

Materials and methods

The research work was conducted in 12 cisterns ($120\times70\times40cm^3$) of the mini hatchery cum breeding complex, Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh. The breeding trials on the other hand, were carried out in the hapas of $150\times100\times100cm^3$ affixed in the pond beside the hatchery complex with the help of bamboo poles. Prior to stocking of broodfish, each of the cisterns was installed with all the facilities necessary to run the experiment efficiently. An inlet and an outlet were provided with each of the cisterns to facilitate renewal and removal of water concomitantly. Water hyacinths were kept floating at a corner of each cistern with the help of bamboo frame attached with float to provide shelter to the experimental fish. Water hyacinth was used to keep the water cool and clean.

Adequate number of mature and healthy *O. pabda* were collected from the wild source during the month of February and March 2009 and acclimatized in the cisterns. A total of 84 healthy, strong, and similar sized females were selected for rearing as broodfish for the research work. The brood rearing experiment was started on 1 April 2009 and

continued up to 30 June 2009. There were four treatments (*i.e.* T_1, T_2, T_3 and T_4) each with 3 replications stocked with 7 females. The broodfish of treatment T_1 served as control (i.e. fed vitamin E free diet) while that of treatment T_2, T_3 and T_4 were fed with a feed having 50, 100 and 150mg vitamin E/kg feed respectively.

The four different feeds containing 40% protein were prepared keeping all the ingredients (fish meal, soybean meal, mustard oil cake, rice bran, wheat bran, vitamin mineral premix) in equal amount except for vitamin E. The vitamin E used was in the form of α-tocopherol acitate, marketed as *E-vet* powder manufactured by the *ACME* Laboratories Ltd. The proposed amounts of vitamin E were weighed using a sensitive electric balance and were mixed thoroughly with the different experimental feeds. The feeds were prepared in the form of pellet (1.5mm) by using a pelleting machine and stored in refrigerator. The compositions of experimental feeds are shown in the Table 1.

Table 1. Formation and composition of experimental feed

Ingredient	Inclusion level (%) in treatments			
	T_1	T_2	T_3	T_4
Fish meal (%)	40.00	40.00	40.00	40.00
Soybean meal (%)	29.69	29.69	29.69	29.69
Mustard oil cake (%)	15.00	15.00	15.00	15.00
Rice bran (%)	5.15	5.15	5.15	5.15
Wheat bran (%)	5.15	5.15	5.15	5.15
Flour (%)	4	4	4	4
Vitamin mineral premix (%)	1	1	1	1
Vitamin E (mg/kg feed)	0	50	100	150

The experimental feeds were administered directly into the corresponding cisterns twice daily *ad libitum*. Each of the cisterns was siphoned every morning to remove faeces and uneaten feed particles. During sampling at 15 days interval, all the fish from each replication of a treatment were caught and weight (g) and length (cm) of each fish were measured by using a sensitive electric balance and a measuring scale respectively and recorded. During the experimental period temperature, dissolved oxygen and pH were recorded weekly.

For breeding trial, a total of 36 broodfish from four treatments were selected and kept in 4 separate cisterns for about 6 hours under gentle but continuous shower for conditioning prior to injection with PG extract at a dose of 12 and 18mg/kg body weight of male and female respectively. Mature and healthy males of *O. pabda* collected from the broodstock pond were kept in fiberglass tank.

After injecting PG extract, both females and males were kept together treatment wise in the hapa set in a pond for spawning. Most of the broodfish were found to oviposit within 9 to 10h post injection. The broods were removed from the hapas after 10.5h of injection when

the spawning was completed. Continuous water flow was maintained in the hapa with porous PVC pipes for aeration. When the breeding was completed the fertilized eggs were removed from the hapas and placed in separate trays ($101.6\times40.6\times12.7cm^3$) treatment wise for incubation. The trays were previously filled with filtered pond water to reduce the temperature difference and environmental shock. Gentle shower was maintained through porous PVC pipes for aeration of eggs.

For calculation of fertilization and hatching rates of eggs produced by the females of each treatment, a portion of the eggs were taken and incubated in 12 bowls of 40cm diameter corresponding to the treatments, *i.e.* three replications for each treatment. The remaining eggs were incubated in separate trays. Soon after fertilization, the embryonic development started and the fertilized eggs looked watery and slightly transparent. Within 6 hours of incubation, the numbers of fertilized and unfertilized eggs from each bowl for respective treatment were counted based on the appearance of the eggs. The unfertilized eggs turned opaque and whitish in colour few hours post fertilization. After completion of hatching, the number of larvae of each bowl was counted by siphoning them out. The hatching completed within 22h at 24-25°C.

In order to study the effect of different dietary levels of vitamin E on the growth and breeding performance of the broodfish, the following parameters were studied:

Weight gain (g) = Mean final weight – mean initial weight

$$\text{Weight gain (\%)} = \frac{\text{Mean final weight - mean initial weight}}{\text{mean initial wieght}} \times 100$$

$$\text{Specific growth rate (SGR, \% day)} = \frac{\text{Log}_e W_2 - \text{Log}_e W_1}{T_2 - T_1} \times 100$$

Where, W_1 = the initial live body weight (g) at time T_1 (day)

W_2 = the final live body weight (g) at time T_2 (day)

$$\text{\% ovulation} = \frac{\text{No. of fish ovulated}}{\text{Total no. of fish injected}} \times 100$$

$$\text{\% fertilization} = \frac{\text{No. of fertilized eggs} \times 100}{\text{Total no. of eggs(fertilized + unfertilized)}}$$

$$\text{\% hatching} = \frac{\text{No. of eggshatched}}{\text{Total no. of eggs}} \times 100$$

The gain in weight and length, specific growth rate of broods, ovulation rate, fertilization rate and hatching rate of eggs etc. were tested using one-way analysis of variance (ANOVA). Significant results ($p<0.05$) were further tested using Duncan's Multiples Range Test (DMRT) to identify significant difference between means. The statistical analysis was performed with the aid of the computer software SPSS programme.

Results

The growth performance in terms of weight gain, % weight gain, specific growth rate (SGR) during the experimental period of broodfish of *O. pabda* fed with different dietary levels of vitamin E under four feeding treatments is presented in Fig. 1. The average initial weights of the broodfish in four treatments were 41.17±0.78g, 41.08±0.49g, 41.62±0.76g and 40.55±1.14g in T_1, T_2, T_3 and T_4 respectively. At the end of the experimental period (90 days), the final weight of the broodfish of four treatments were found to be 45.09±0.62g, 48.88±0.78g, 56.39±0.59g and 43.51±0.54g in treatment T_1, T_2, T_3 and T_4 respectively. The highest weight gain was observed to be 14.78±0.38g in the broodfish of treatment T_3 (fed with feed having 100mg vitamin E/kg of feed) followed by 7.80±0.96g in treatment T_2 (50mg vitamin E/kg of feed), 3.93±1.10g in T_1 (0mg vitamin E/kg of feed) and 2.97±0.89g in T_4 (150mg vitamin E/kg of feed) (Table 2). Statistical analysis showed that both weight gain and percent weight gain of broodfish were significantly higher ($P<0.05$) in treatment T_3 compared to the other treatments. The highest specific growth rate (% SGR) of the broodfish of treatment T_3 (fed with 100mg vitamin E/kg of feed) was found to be 2.99±0.11% which was significantly higher ($p<0.05$) than those of other three treatments. Treatment T_4 yielded poorest result in terms of weight gain (2.97±0.89g), percent weight gain (7.32±1.41%) and specific growth rate (1.21±0.32) after the completion of the experimental period (Table 2).

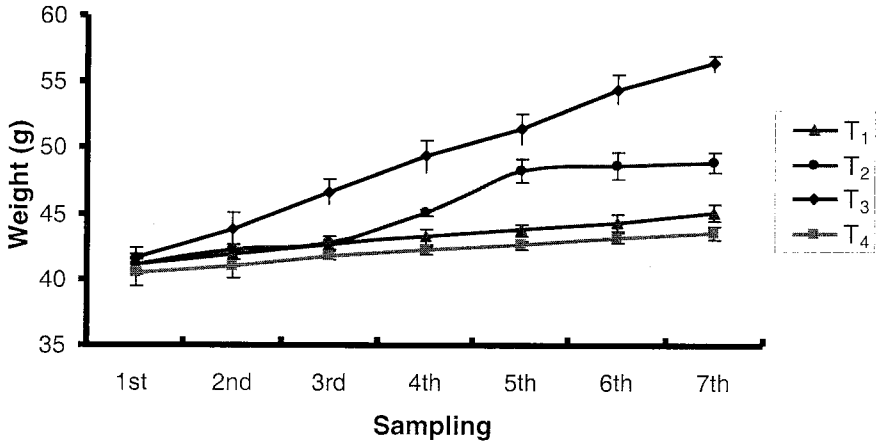

Fig. 1. The fortnightly growth response (weight) of *O. pabda* broodfish reared under different dietary levels of dietary vitamin E for a period of 90 days

Table 2. Weight gain, percent weight gain and specific growth rate of *O. pabda* broodfish under different doses of vitamin E (± SD)

Treatments	Initial weight (g)	Final weight (g)	weight gain (g)	weight gain%	SGR%
T_1	41.17±0.78	45.09±0.62	3.93±1.10^c	9.54±1.85^c	1.45±0.31^c
T_2	41.08±0.49	48.88±0.78	7.80±0.96^b	18.98±1.06^b	2.05±0.25^b
T_3	41.62±0.76	56.39±0.59	14.78±0.38^a	35.50±1.05^a	2.99±0.11^a
T_4	40.55±1.14	43.51±0.54	2.97±0.89^c	7.32±1.41^c	1.21±0.32^c

Values in the column with different superscripts are significantly different. SGR= Specific Growth rate

The average initial lengths of the broodfish of four treatments were 21.03±0.13cm, 20.88±0.57cm, 21.14±0.11cm and 20.63±0.28cm in T_1, T_2, T_3 and T_4 respectively (Table 3). The final lengths of the broodfish of four treatments were found to be 21.63±0.64cm, 21.69±0.83cm, 22.39±1.83cm and 21.16±0.28cm in treatment T_1, T_2, T_3 and T_4 respectively. The highest length gain was observed to be 1.25±0.11cm in the broodfish of treatment T_3 followed by 0.81±0.21cm in treatment T_2, 0.60±0.18cm in T_1 and 0.53±0.16cm in T_4. The highest percent length gain was observed to be 5.92±1.20% in treatment T_1 while the lowest was found to be 2.58±.78% in treatment T_4. Statistical analysis showed that there were no significant differences of means in terms of length gain and percent length gain among the treatments (Table 3).

Table 3. Length and percent length gain of *O. pabda* under different feeding treatments (± SD)

Treatments	Initial length (cm)	Final length (cm)	Length gain (cm)	% Length gain
T_1	21.03±0.13	21.63±0.64	0.60±0.18	2.83±0.76
T_2	20.88±0.57	21.69±0.83	0.81±0.21	3.87±0.55
T_3	21.14±0.11	22.39±1.83	1.25±0.11	5.92±1.20
T_4	20.63±0.28	21.16±0.28	0.53±0.16	2.58±0.78

The highest ovulation, fertilization and hatching rate of eggs were observed in the broodfish of treatment T_3 while the lowest was observed in treatment T_4 (Table 4). Statistical analysis showed that there was a significant difference ($p<0.01$) among the treatments. Duncan's Multiple Range Test (DMRT) showed that breeding performance of the broodfish of treatment T_3 was significantly higher ($p<0.01$) compared to the treatment T_1, T_2 and T_4. Treatment T_1 and T_2 were also significantly different ($p<0.01$) compared to treatment T_4. There were no significant difference between treatment T_1 and T_2.

Water temperature, dissolved oxygen and pH during the brood rearing period in the cistern were found to be in the desirable range according to Boyd (1979), Jhingran and Pullin (1985) and Rahman *et al.* (1982). There was no indication of the adverse effect of water quality parameter on the existence and growth of *O. pabda* broodfish. Temperature, pH and dissolved oxygen of water in bowls under different treatments ranged between 27.3 to 28.3°C, 6.8 to 7.5 and 5.3 to 6mg/l respectively.

Table 4. Breeding performance of *O. pabda* female broods reared under different doses of vitamin E when treated with equal dose (18mg PG/kg fish) of PG extract (±SD)

Treatment	Ovulation (%)	Fertilization (%)	Hatching (%)
T_1	59.26±6.4^b	71.56±3.41^b	57.95±2.34^b
T_2	62.97±6.41^b	68.16±3.12^b	58.53±4.72^b
T_3	81.48±6.41^a	84.04±3.53^a	68.59±5.03^a
T_4	33.33±0.00^c	52.35±5.02^c	45.70±7.24^c

Values in the column with different superscripts are significantly different

Discussion

The need of dietary vitamin E to maximize the breeding performance of *O. pabda* is clearly demonstrated in the present study. Weight of fish of different treatment increased with increase in dietary incorporation of vitamin E up to requirement level. The present result in terms of growth of the broodfish shows that the diet containing 100mg vitamin E/kg of feed (treatment T_3) is sufficient to support optimal growth of *O. pabda* broodfish. The result of the present study is in agreement with the reports of the earlier workers on *Cirrhinus cirrhosus* requiring 99mg vitamin E/kg feed (Paul *et al.* 2004), *Cyprinus carpio* requiring 100mg vitamin E/kg feed (Watanabe *et al.* 1970) and 80-100mg/kg (Halver 2002) and *Salmo salar* requiring 120mg vitamin E/kg feed (Hamre and Lie 1995).

On the other hand, higher amount of vitamin E in the diet of the broodfish (fed with 150mg vitamin E/kg of feed) resulted in poor growth. This finding is also in line with that of vitamin E requirement of broodfish of shing (*Heteropneustes fossilis*) and magur (*Clarias batrachus*) where higher doses (200mg vitamin E/kg of feed) showed an antagonistic effect on growth (Mollah *et al.* 2003, Roy and Mollah 2009). Studies with rainbow trout (*Salmo gairdneri*) (Cowey *et al.*, 1981, 1983) and channel catfish (*Ictalurus punctatus*) (Wilson *et al.* 1984) showed that weight gain did not respond to dietary vitamin E supplementation. Kiron *et al.* (2004) reported poor growth and feed utilization by incorporating 1000mg vitamin E/kg of feed in the diet of rainbow trout (*Oncorhynchus mykiss*). However, no significant difference was observed in terms of length gain of the broodfish of *O. pabda* in different feeding treatments. This seems to coincide with the result of that of Roy and Mollah (2009) and Jarboe and Robintte (1989). It is reported that vitamin E deficiency can lead to immunological malfunctions and reduce disease resistance in Salmonid fish (Lygren *et al.* 2000, Lygren *et al.* 2001) but there have been some discrepancies in literature regarding effects of higher dietary levels of vitamin E than normally used (Waagbø 1994, Wahli *et al.* 1998). Excess α-tocopherol inhibits the action of protein kinase C (PKC) in vascular smooth muscle cells leading to growth arrest (Boscoboinik *et al.* 1991).

Nevertheless, the beneficial effect of dietary vitamin E supplementation on fish reproduction was not found in many studies (Mollah, *et al.* 2003, Roy and Mollah 2009).

The result of the present study showed a positive impact of inclusion of vitamin E in the diet on the breeding performance of female *O. pabda*. The best ovulation rate of broodfish, fertilization rate and hatching percentage of the fertilized eggs produced, growth rate and survival rate of the larvae were obtained with the fish fed 100mg vitamin E/kg of feed i.e. treatment T_3. Other doses also showed positive result except treatment T_4 where the broodfish were fed with 150mg vitamin E/kg of feed. Takeuchi *et al.* (1981) conducted an experiment on the broodfish of 'ayu' *Plecoglossus altivelis* and observed better hatching percentage and survival of larvae with 3.4mg vitamin E/100g diet. Mollah *et al.* (2003) found better fertilization rate, hatching rate and survival rate of the larvae of *Heteropneustes fossilis* fed 200mg vitamin E/kg feed. Better fertilization and hatching rate and survival of larvae of *Clarias batrachus* was also observed when the broodfish were fed with feed having 50mg and 100mg vitamin E/kg of feed, however, Roy and Mollah (2009) recommended 50mg vitamin E/kg of feed for *Clarias batrachus* based on economics of brood rearing and larvae production.

Gupta *et al.* (1987) observed higher gonadosomatic index, bigger ova and complete spawning in three major carps (*Labeo rohita*, *Catla catla* and *Cyprinus carpio*) by adding vitamin E in their diet. Similarly Sutjaritvongsanon (1987) reported that a mixture of 35% fish meal, 30% soybean meal, 20% corn meal, 15% rice bran and 10mg/kg BHT together with 100mg vitamin E/kg of feed was suitable for stimulating gonad development and spawning in goldfish (*Carassius auratus*). 100mg vitamin E/kg of feed is also known to increase the number of pleopodal eggs significantly in freshwater crayfish, *Astacus leptodactylus* (Harlıoğlu and Barım 2004). Therefore, it seems that vitamin E requirement of fish is species specific so for as its requirement is concerned in gonad development and breeding performance of fish.

Inclusion of higher levels of vitamin E (150mg vitamin E/kg of feed) in the diet of broodfish drastically reduced the ovulation rate of broods, fertilization and hatching rate of the fertilized eggs. The result coincides with that of Roy and Mollah (2009), where higher doses of vitamin E in the diet of broodfish of *Clarias batrachus* also affected the fertilization and hatching percentage. Eskelinen (1989) found that a high dietary α-týocopherol level failed to increase the survival of eggs and fry in a study on the different diets on eggs production and egg quality of Atlantic salmon (*Salmo salar*). Generally higher levels of vitamin E can cause a condition of hypervitaminosis that is evidenced by retardation in growth (Harlıoğlu and Barım 2004). Excess vitamin might have deterred the usual maturation of the gonad leading to poor breeding performance.

Vitamin E has been found to have positive impact on breeding performances of some other species as well. It seems important to conduct experiments of similar nature to investigate the quantitative retention of vitamin E in the gonad and eggs and its (vitamin E) mode of action on gonad to understand the function and characterization. The success obtained through this work can serve as an important base for future research on this topic.

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Culture potentials of climbing perch, *Anabas testudineus* (Bloch) under different stocking densities at semi-intensive management

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Abstract
Anabas testudineus was cultured at different stocking density for the period of five months from May to September. Three stocking densities such as 50,000 (Treatment-1, T_1), 56,250 (Treatment-2, T_2) and 62,250/ha (Treatment-3, T_3) were tested with three replications. After five months rearing, the mean weights of koi were 46.74±2.59, 40.44±2.98 and 37.27±3.01 in T-1, 2 and 3, respectively. The calculated production of native koi in T_1, T_2 and T_3 were 1,916±314, 1,774.31±260 and 1,431±297 kg/ha, respectively which were significantly different ($p<0.05$) from each other.

Key words: *Anabas testudineus*, Stocking densities

Introduction

The climbing perch (*Anabas testudineus*), locally known as koi, is an important favourite small indigenous fish of Bangladesh. It can withstand harsh environmental conditions such as low oxygen, wide range of temperature and other poor water conditions (Habib and Hasan 1994). The species is considered as a valuable item of diet for sick and convalescents. The fish contains high amount of bionutritionally available iron and copper, which are essentially needed for hemoglobin synthesis. In addition, the fish also contains high amount of protein and easily digestible fat which is very low melting point and many essential amino acids (Saha 1971, Habib and Hasan 1994).

In late 1980s, the catches of the fish have drastically declined from open waters due to various ecological changes in inland water bodies. Keeping this aspect in mind, seed production technology through artificial propagation was developed in captive condition by the Bangladesh Fisheries Research Institute (Kohinoor *et al.* 1991). But proper culture technology has yet been optimized and evaluated with protein enriched feed. Therefore, research needs to evaluate its culture potentials in pond ecology. The present study attempted to evaluate the production potentials of native koi at on station management.

Materials and methods

Experimental design and pond management

Three ponds were selected having an area of 300 m^2 each. Each pond was equally partitioned in to three chambers by bamboo split (100 m^2 each with an average depth of 100 cm). Three stocking density of native koi (*A. testudineus*) such as 50,000, 56,250 and 62,250/ha were tested, which considered as T-1, T-2 and T-3, respectively. Prior to initiate the experiment, ponds were dried and pond bottoms were treated with lime (CaO) at the rate of 250 kg/ha and left for three days. After drying, ponds were filled with ground water and fertilized with cow manure at the rate of 2,000 kg/ha.

All the ponds clamberers were stocked according to the experimental design. After stocking, supplementary feed containing 35% crude protein (SABINCO Commercial feed) were applied at the rate of 20-4% of estimated fish biomass at twice daily at 10.00 h in the morning and at 15.00 h in the afternoon in all the treatments. The fingerlings were fed at the rate of 20% of their body weight for the first four weeks and it was reduced to 4% on the subsequent weeks. All the ponds were limed at the rate of 125 kg/ha at monthly interval during the culture period.

Fish sampling

Fish sampling were done at fortnightly intervals through seine netting and weighing 50 fish to measure the growth, assess their health status and also feed adjustment.

Water sampling and analysis

Water quality parameters such as water temperature (°C), DO (mg/L), pH, alkalinity (mg/L) were monitored at weekly intervals from 0930 to 1000 hrs. Water temperature was recorded using a Celsius thermometer and transparency was measured by using a Seechi disc of 20 cm diameter. Dissolve oxygen and pH were measured directly using a digital portable oxygen meter (OAKTON) and portable pH meter (HANNA 8424). Alkalinity was determined following the titrimetric method according to the standard procedure and methods (Clasceri *et al.* 1992).

Fish harvesting

After a grow-out period of five months, ponds were drained by pump and all fish were harvested. Total bulk weight and number of fish from each pond were recorded. Specific growth rate (bw/day) was estimated as:

$$SGR = [\ln(\text{final weight}) - \ln(\text{initial weight}]/\text{culture period (days)} \times 100.$$

Data analysis

One-way ANOVA was carried out using STATGRAPHICS version 7 statistical package following Zar (1984). Significance was assigned at the 5% level of probability.

Results and discussion

The physico-chemical factors of the pond water under three treatments are presented in Table 1. The water temperature in T_1, T_2 and T_3 ranged from: 24.90- 32.2, 24.30-32.7 and 24.40-32.3°C, with the mean values of 25.20±0.51, 25.72±0.55 and 26.73±0.70°C, respectively. The variations in temperature among the treatment means were found similar ($p<0.05$) and were within the suitable range of growth of fish in tropical ponds (Rahman *et al.* 1982, Roy *et al.* 2002, Begum *et al.* 2003).

Table 1. Mean values of water quality parameters in different treatments

Water quality parameter	T_1	T_2	T_3
Temperature (°C)	25.20±0.51	25.72±0.55	26.73 ±0.70°C.
Transparency (cm)	30.20±1.35	26.20±1.40	24.62±1.72
pH	7.52 to 8.80	7.24 to 8.34	7.65 to 8.59
Dissolved oxygen (mg L^{-1})	5.22±0.12	5.09±0.15	4.92±0.20
Total alkalinity (mg L^{-1})	142↑1±11.20	132±7.54	139±5.37

The water transparency did not show any significant ($p<0.05$) difference among the treatment means. The mean values were 30.20±1.35, 26.20±1.40 and 24.62±1.72cm in T_1, T_2 and T_3, respectively. The values of transparency some times varied with sampling dates which could be due to differences in abundances in abundance of plankton. Boyd (1982) recommended a transparency between 15-40cm as appropriate for fish culture. Normally, the transparency value was low in this experiment because usually koi did not consume plankton. (Nargis and Hossain 1987, Singh and Samual 1981).

The level of pH varied from 7.52 to 8.80, 7.24 to 8.34 and 7.65 to 8.59 in T_1, T_2 and T_3, respectively. The pH in all pond water was alkaline throughout the experimental period which might be due to regular application of lime in all the ponds at monthly interval. Different authors have reported a wide variations in pH from 7.18 to 7.24 (Kohinoor *et al.* 1998), 7.03 to 9.03 (Roy *et al.* 2002), 6.8 to 8.20 (Begum *et al.* 2003) and 7.50 to 8.20 (Chakraborty *et al.* 2005) in fertilized fish pond and found the ranges productive.

The dissolved oxygen contents in the experimental ponds ranged from 4.6 to 6.9, 4.2 to 6.1 and 4.09 to 5.94 mg/L in T_1, T_2 and T_3, respectively, with the mean values of 5.22±0.12, 5.09±0.15 and 4.92±0.20 mg/L. Comparatively lower level of dissolved oxygen as observed in the experimental ponds appeared to be related to sampling time where the dissolved oxygen was monitored at about 9.00-10.00 am. Rahman *et al.* (1982) reported that dissolved oxygen content of a productive pond should be 5.0 mg/L or more. The values found in present experiment were around 5.0.

Total alkalinity ranged from 135 to 160, 118 to 162 and 134 to 169 mg/L with mean values of 1421±11.20, 132±7.54 and 139±5.37 mg/L in T_1, T_2 and T_3, respectively. These values did not show any significant difference among the treatments. The

variations in total alkalinity in all the treatments were found in productive range for aquaculture ponds (Wahab *et al.* 1995, Kohinoor *et al.* 1998).

Growth and production

The growth rates of koi under different stocking densities are shown in Table 2. The weight increments of koi by different treatments over the culture period are graphically shown in Fig. 1. During culture period it was observed that the growth rate was not varied among the treatments first three months but it was increased significantly in rest of two months. It can be also seen that the final weight was higher in T_1, which followed by T_2 and T_3. After five months of rearing, the mean weights of koi were 46.74±2.59, 40.44±2.98 and 37.27±3.0g in T_1, T_2 and T_3, respectively. The mean weight of T_1 showed significant ($p<0.05$) differences from T_2 and T_3, whereas significant ($p<0.05$) differences was also observed between T_2 and T_3. The results indicated that higher growth rate attained at lower stocking density.

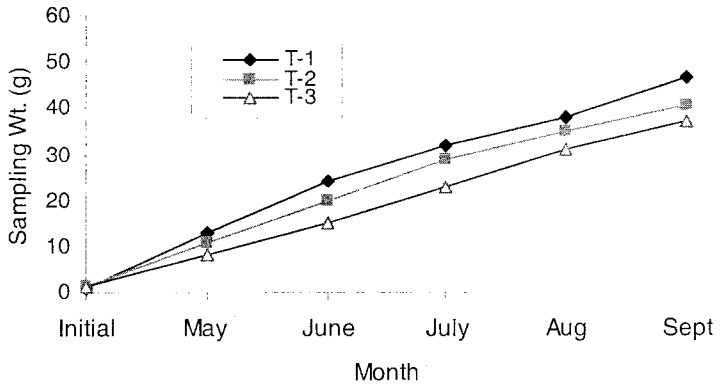

Fig.1: Monthly growth rate of Native Koi at different stocking densities

The specific growth rate (SGR) of koi at different stocking densities was observed to be 2.68, 2.59 and 2.55 for T_1, T_2 and T_3, respectively. The SGR of koi in T_1 was significantly ($p<0.05$) different from T_2 and T_3, whereas, T_1 and T_2 did not show any significant difference ($p>0.05$).

The mean survival rate of koi was found to vary with the stocking densities. The highest survival (82%) was observed in T_1, where stocking density was 50,000/ha and the lowest (76%) was obtained in T_3, where the density was 62,500/ha. The differences among the treatment means were found to be insignificant ($p>0.05$). The mean FCR value of T_1, T_2 and T_3 were 3.44, 3.76 and 3.81, respectively where T_1 resulted in the lowest FCR value, while the highest FCR value was found in T_3.

Table 2. Growth performance of native koi (*Anabas testudineus*) under mono culture management in different stocking densities

Treatment	Initial Wt. (g)	Harvesting weight (g)	Survival (%)	Production (kg/ha)	FCR	SGR (%)
T_1 (50,000/ha)	1.04± 0.22	46.74±2.59[a]	82	1,916±314[a]	3.44	2.54
T_2 (56,250/ha)	1.10± 0.22	40.44±2.98[b]	78	1,774±260[b]	3.76	2.44
T_3 (62,500/ha)	1.13± 0.20	37.27±3.01[c]	71	1,431±297[c]	3.94	2.39

* Dissimilar superscript indicates significant difference at 5% level of probability

The calculated production of koi T_1, T_2 and T_3 were 1,916±314, 1,774.31±260 and 1,431±297 kg/ha, respectively. The fish production was higher in T_1, where stocking was 50,000/ha and lowest production was observed in T_3, where the stocking density was 62,500/ha. Intermediate fish production results were obtained in T_2, where stocking density was 56,250/ha. The production level of T_1 was found to be significantly ($p<0.05$) higher than T_2 and T_3. But T_3, appeared to give the lowest production and differed significant ($p<0.05$) from T_2.

Correlation matrix among stocking density, harvesting weight, survival and production of koi is shown in Table 3. Stocking density showed a negative correlation with harvesting weight, survival and production. It means that if stocking density increased, then harvesting weight, survival and production decreased. While, harvesting weight showed positive correlation with survival, production and survival rate derived also significant positive correlation with production.

Table 3. Correlation matrix among stocking density, harvesting weight, survival and production of native koi under grow-out system

Parameter	Stocking density	Harvesting wt. (g)	Survival (%)	Production (Kg)
Stocking density	1.0000	-	-	-
Harvesting Wt. (g)	-0.9769	1.0000	-	-
Survival (%)	-0.9947	0.9937	1.0000	-
Production (Kg)	-0.9669	0.9991*	0.9881*	1.0000

* Significant difference at 5% level of probability

Thakur and Das (1986) reported that Koi (*Anabas testudineus*) production was 1,800 kg/ha in India by applying supplementary feed (rice bran, mustard oil cake and fish meal) with the stocking density of 60,000/ha in 170 days. They also stated that by applying the above feed, achieved 702 kg/ha over a period of 11 months, where the stocking density was 1,25,000/ha. Earlier study conducted by Akhteruzzaman (1988) evaluated the production potentials of koi in monoculture management at the density of 16,000/ha and obtained a production of 450 kg/ha in five months rearing with

supplementary feed consisted of rice bran (50%), mustard oil cake (30%) and fish meal (20%). The gross production of Koi in mono culture condition was 425 kg/ha at the stocking density of 20,000/ha, where rice bran (50%), mustard oil cake (30%) and fish meal (20%) was used as supplementary feed over a period of five months (BFRI Research Progress 1994-97). The production obtained in the present experiment was higher than the above mentioned results due to application of protein enriched (35%) supplementary feed which might gave the higher production and also regular water supply in the ponds might be another factor which enhanced the production.

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Effects of fish and prawn culture on physico-chemical parameters of water and rice yield in rice fields

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Abstract
An experiment was conducted with five treatments i.e rice combined with fish having regular urea fertilization (T_1), rice combined with prawn having regular urea fertilization (T_2), rice combined with fish with supplementary feeding (T_3), rice combined with prawn with supplementary feeding (T_4) and without fish and prawn (T_5) was kept as control. The dissolved oxygen values obtained in treatments with fish both in morning and afternoon were lower than the values of prawn containing treatments and control. The values of nitrate-N, ammonia-N, phosphate-P and chlorophyll-a were higher in fish containing treatments than the prawn containing treatments and control. Between the two fish containing treatments the higher gross (539.44 kg/ha) and net (440.14 kg/ha) yield were obtained in T_3 with supplementary feeding and the lower gross (424.88 kg/ha) and net (314.32 kg/ha) yield were recorded in T_1 without supplementary feeding. Again, between two prawn containing treatments the higher gross (108.69 kg/ha) and net (81.92 kg/ha) yield were obtained in T_4 with supplementary feeding and lower gross (64.32 kg/ha) and net (30.98 kg/ha) yield were recorded in T_2 without supplementary feeding. The highest yield of rice grain (3.45 mt/ha) and straw (6.37 mt/ha) were obtained in T_1 with fish having urea fertilization without feeding.

Key words: Rice-fish culture, Rice-prawn culture, Water quality parameters

Introduction

Rice and fish are the staple food for the people of Bangladesh. Fish is the main source of animal protein, providing 17.23 kg/year of the average per capita total intake of protein and 58% of the total animal protein intake in Bangladesh (DoF 2009). Crop land has already been declined in this country by 3.1% from mid 1980 to mid 1990 as reported by Alauddin (2004). In recent years, rice production has become less profitable for farmers due to stagnant yields and high input costs. Hence, there is a move towards diversification out of rice monoculture. One of these is the age-old practice of integrating fish culture with rice farming.

The practice of integrated farming of prawn with rice, fish, and vegetables is spreading, particularly among small-scale farmers, providing a year-round supply of crops for family subsistence, supplemented by a cash crop (USAID 2003). *Macrobrachium rosenbergii* species has been cultured in the integrated rice-prawn system since the 1980s in Mekong Delta of Vietnam (Hien *et al.* 1998). Rice-prawn culture can generate higher income than traditional rice-fish culture, because of the higher price of prawns (New 1995). The flooded rice field is a temporary aquatic environment subject to large variations in temperature, pH, dissolved oxygen concentration and nutrient status due to frequent disturbances through practices such as the use of agrochemicals (Watanabe and Roger 1985). From an aquacultural point of view, the rice field is not very suitable for fish production: dissolved oxygen and temperature values often exceed the fish tolerance limits and application of N-fertilizers can result in short term exposures of fish to unionized ammonia (Rothuis 1998).

Fish culture as an integrated and concurrent activity with rice culture in the same field is important for rational utilization of limited land resources, as well as a sustainable source of fish protein, additional income, and employment generation (Sollows and Thongpan 1986 and Ghosh 1992). Lightfoot *et al.* (1992) summarized rice yield data from 20 rice-fish systems from China, India, Indonesia, the Philippines and Thailand and found that rice yields ranged from 58 to 183% as compared to rice monoculture. Gupta (1998) conducted a survey on 256 farms in Bangladesh to assess the feasibility and economic viability of rice-fish culture. They found an average fish production of 233 kg/ha in the dry season and 212 kg/ha in the rainy season, and an average increase in the net benefit by 64.4% and 98.2% compared to rice monoculture, respectively. Frei *et al.* (2007) obtained the highest yield (586 kg/ha) in the carp/tilapia mixed culture followed by tilapia alone (540 kg/ha) and carp alone (257 kg/ha) in the rainy season and in the winter season, the highest yield (935 kg/ha) obtained in feed level II followed by feed level I (776 kg/ha) and the non-fed group (515 kg/ha).

Materials and methods

Experimental Site

The experiment was conducted at the Agronomy Field Laboratory, Bangladesh Agricultural University (BAU), Mymensingh during rainy season from July to November, 2007. The experimental site is under the old Brahmaputra Flood Plain Agro-ecological Zone having non-calcareous dark grey soils of silt loam texture and was situated in a relatively low land area near the deep tube-well of the field laboratory having 0.2 ha in size. The experimental area was divided into 15 plots, each comprising an area of 142 m^2 having rectangular in shape. Small water channels (70 cm width and 30 cm depth) were made between the plots to supply water in the experimental plots. Rainwater and irrigation water from the farm deep tube-well were the sources of water supply to the experimental plots. Each plot had common inlet and outlet in the dikes (height 60 cm, base width 50 cm and top width 40 cm) for regulation of water depth.

Nylon nets were fixed around each plot with the help of bamboo poles to prevent the entry of unwanted animals in the plot and escapement of stocked fish.

Experimental design

The experiment was conducted in a randomized complete block design (RCBD) with five treatments and three replications for each treatment. The treatments were: rice combined with fish having regular urea fertilization treatment I (T_1), rice combined with prawn having regular urea fertilization treatment II (T_2), rice combined with fish having supplementary feeding treatment III (T_3), rice combined with prawn having supplementary feeding treatment IV (T_4) and Treatment V (T_5) was kept as control i.e., without fish and prawn.

Field management

The experimental plots were ploughed two times using a power tiller. The weeds were removed and the land was then leveled by laddering. A small refuge pond was excavated in the middle of each plot, covering an area of 3 m^2 with 0.5m depth to provide shelter for fish during low water level and high temperature.

A basal dose of fertilizer was applied one day before transplanting according to the recommended dose BRRI (2004), i.e. 150 kg/ha triple super phosphate (TSP) and 75 kg/ha muriate of potash (MP). Urea was applied according to the BRRI (2004), i.e. 220 kg/ha in the T_1, T_2 and T_5 in three installments at 15, 30 and 55 days after transplanting (DAT) of rice seedlings with one-third of the total dose during each application.

The seedling of BR 11 were transplanted into the experimental plots at 48 days after seeding (DAS) in alternate row spacing of 35 cm and 15 cm as suggested by Hossain *et al.* (1990). The plant to plant distance in the rows was 20 cm. The alternate row spacing provides enough space for easy movement of fish and to penetrate sunlight in the water between the rows which improves the growth of plankton for fish feed.

Stocking and management of fish and prawn

The fingerlings of monosex tilapia (*Oreochromis niloticus*), common carp (*Cyprinus carpio*) and juvenile of prawn (*Macrobrachium rosenbergii*) were released in the experimental plots at 28 DAT and stocked at a density of 1 fish/m^2 and 2 prawns/m^2 respectively. Fish species were stocked at a ratio of 1:1. The fingerlings and juveniles were kept in a bucket in the experimental plots for about 15 minutes to adjust with the new environment. The healthy and strong fingerlings and juveniles were then gradually released into the central ditches. The average initial weight of fish and prawn were recorded at the time of stocking and they were 10.49 g and 1.5 g respectively.

Management of fish and prawn

Feeding was started five days after stocking. The feed ingredient were thoroughly mixed and made into 4 mm pellets. The feed composition was 50% fish meal, 44% wheat flour, 4% soybean oil and 2% mineral and vitamin premix. The proximate composition of feed on a dry matter (DM) basis was 34.9% crude protein, 12.7% crude lipid, crude ash

13.4% and gross energy19.5 kJ/g. Feed was provided to the fish @ 6.4 g of feed per kg metabolic body mass per day (g $kg^{-0.8}$/day) at 2 x maintenance feeding according to Becker *et al.* (1983). Feed was provided manually daily at 9 am. Feeding level was adjusted fortnightly based on the prospective fish biomass assuming a metabolic growth rate of 8 g $kg^{-0.8}$ /day (Frei and Becker 2005). The total amount of feed supplied was 6.5 kg (DM) in each plot. Water was supplied to the plots from the deep tube well and water level was raised gradually ranging from 15-25 cm with the growth of rice and fish.

For prawn feed was made into 3 mm pellets. The feed composition was 20% fish meal, 20% wheat flour, 10% meat and bone meal, 20% rice bran, 10% mustard oilcake, 15% soybean meal, 4% molasses and 1% mineral and vitamin premix *(Bright Fish Premix*, Anivet Agro Products Ltd.). The proximate composition of feed on a dry matter (DM) basis was 23.5 % crude protein, 9.4 % crude lipid, crude ash 12.3% and gross energy17.4 kJ/g. Feed was provided daily at 5 pm. Feeding level was adjusted fortnightly based on the sampling.

Water quality parameters

Water temperature, pH and dissolved oxygen levels were recorded weekly at 8 am and 3 pm using electronic probes and a portable multi-parameters instrument (Multi 340i, WTW, Weilheim; Germany). In addition, chlorophyll-a level was analyzed using the acetone extraction method (90% concentration) with cellulose nitrate filters (Whatman GF/C). Further water samples were taken fortnightly and analyzed for nitrate, ammonia and phosphate contents by using spectrophotometer (HACK-USA, DR 2010) and reagent of mineral stabilizer, polyvinyl alcohol, nessler for ammonia and pillow NitraVer 6, NitriVer 3 for nitrate and phosVer 3 for phosphate. These analyses were also done in duplicate.

Harvesting of rice, fish and prawn

Rice was harvested plot-wise at 125 DAT by cutting the plants at the water level with sickle. For determining rice yields, five samples were taken from each plot randomly placing a 1 m^2 frame and cutting the rice plants inside the frames at soil level. The rice sampled was threshed out manually. The grains were then cleaned and sun dried and weighed. Representative samples were taken for determination of the dry matter by drying overnight in a laboratory oven at 105 °C. The straw was also sun dried and the moisture content was determined. The yield data of grain and straw were then adjusted into mt/ha at 14% moisture level.

Fish and prawn including weed fish were harvested immediately after rice harvesting, i.e. 98 days after stocking fish fingerlings and prawn juveniles. The fish and prawn were collected from each experimental plot manually after draining out the water from the plots. They were then counted and weighed plot and species wise.

Data Analysis

Data are presented as mean values ± standard deviations. Mean values were compared by performing one way analysis of variance (ANOVA), followed by LSD test to detect

statistically significant differences between the treatments at $p<0.05$. The software used for statistical analyses was SPSS, Version 11.5 for MS Windows (Chicago USA).

Results

Water quality parameters in rice fields

The water temperature during the experimental period was more or less similar in all treatments (Table 1). Temperature showed weekly variations in all the treatments with more or less continuous decreasing trend towards the end of the experiment. There was no significant difference in morning and afternoon pH values among the treatments. The values of dissolved oxygen in morning were higher in prawn containing treatments and control than the fish containing treatments. The highest mean value of morning dissolved oxygen was recorded in T_2 and the lowest of the same was recorded in the treatment T_1. The highest afternoon of the same was recorded in the treatment T_2. The dissolved oxygen values obtained in treatments with fish both in morning and afternoon were lower than the values of prawn containing treatments and control. The highest mean value of nitrate- nitrogen (NO_3-N) was recorded in the treatment T_1 and the lowest of the same was recorded in the treatment T_4. In prawn containing treatments the NO_3-N value was lower than the fish containing treatments and control. The mean values of phosphate-phosphorus (PO_4-P) was significantly higher ($p<0.05$) in T_1 than the values of other treatments except in T_3 where difference was not significant. The mean value of ammonium-nitrogen (NH_4-N) was significantly higher ($p<0.05$) in T_1 than the values recorded in T_2 and T_4 and no such difference was observed with the rest of the treatments. However the lowest value was obtained in T_4. The highest concentration of chlorophyll-a was obtained in the treatment T_3 that was closely followed by the value of T_1. However the values of chlorophyll-a were higher in fish containing treatments than the prawn containing treatments and control.

Table 1. Mean values of water quality parameters recorded in different treatments during the experimental period

Parameters	Treatments				
	T_1	T_2	T_3	T_4	T_5
Temperature am (°C)	26.5 ± 1.9^a	27.1 ± 2.1^a	26.5 ± 2.2^a	26.5 ± 2.1^a	26.4 ± 1.9^a
Temperature pm (°C)	28.7 ± 2.5^a	28.9 ± 3.1^a	28.7 ± 2.9^a	28.9 ± 3.2^a	28.5 ± 2.7^a
pH am	7.0 ± 0.4^a	7.2 ± 0.5^a	7.1 ± 0.4^a	7.1 ± 0.4^a	7.0 ± 0.3^a
pH pm	7.3 ± 0.5^a	7.5 ± 0.6^a	7.4 ± 0.6^a	7.4 ± 0.6^a	7.6 ± 0.4^a
DO am (mg/l)	4.1 ± 2.1^b	5.3 ± 2.7^a	4.3 ± 2.6^b	4.7 ± 2.4^{ab}	5.1 ± 2.4^a
DO pm(mg/l)	6.1 ± 3.3^b	8.3 ± 3.2^a	6.1 ± 3.2^b	7.1 ± 2.7^{ab}	7.8 ± 3.1^a
NO_3-N (mg/l)	0.63 ± 0.30^a	0.36 ± 0.54^b	0.58 ± 0.48^a	0.31 ± 0.26^b	0.40 ± 0.40^{ab}
PO_4-P (mg/l)	0.26 ± 0.30^a	0.08 ± 0.06^b	0.20 ± 0.10^{ab}	0.09 ± 0.11^b	0.1 ± 0.1^b
NH_4-N (mg/l)	0.35 ± 0.21^a	0.22 ± 0.18^b	0.30 ± 0.23^{ab}	0.18 ± 0.07^b	0.28 ± 0.25^{ab}
Chlorophyll-a (μg/l)	36.4 ± 8.7^{ab}	23.3 ± 9.1^b	41.5 ± 10.6^a	22.3 ± 7.8^b	25.3 ± 8.9^b

Mean values with different superscripts in the same row were significantly different (*P<0.05*)

Survival rate of fish and prawn

The survival rate of fish and prawn were estimated separately and treatment-wise from the harvesting data and shown in Table 2. The average survival rate of common carp was higher than the average survival rate of tilapia. In fish containing treatments, the treatment-wise survival rate was higher in T_3 (87±6%) than the T_1 (52±7%). In prawn containing treatments, the survival rate were very low and these were 23±14% in T_2 and 39±30% in T_4.

Table 2. Survival rates of fish and prawn in different treatments in rice field

Treatments	Survival rate (%)			
	Tilapia	Carp	Tilapia/Carp	Prawn
T_1	41±17	70±9	52±7	-
T_2	-	-	-	23±14
T_3	83±11	91±2	87±6	-
T_4	-	-	-	39±30

Yield of fish and prawn

Yield of fish and prawn are shown in Table 3. The highest yield (539.44±84.9 kg/ha) was recorded in T_3 than the rest of the treatments. The yield was significantly higher ($p<0.05$) in treatments with fish than the treatment with prawn. Total gross yield was also significantly higher in the treatments with fish than the treatment with prawn. The yield of weed fish obtained in different treatments was more or less similar.

Table 3. Production and efficiency parameters of fish and prawn in different treatments in rice field

Items	Treatments			
	T_1	T_2	T_3	T_4
Gross yield (kg/ha)	424.88±207.13^a	64.32±38.38^b	539.44±84.94^a	108.69±65.57^b
Net yield (kg/ha)	314.32±222.14^a	30.98±40.86^b	440.14±79.81^a	81.92±62.83^b
Weed fish (kg/ha)	29.58±12.81	29.58±20.86	25.59±12.68	27.23±12.15
Total gross yield including weed fish (kg/ha)	454.46±215.20^a	93.90±40.28^b	565.02±74.15^a	135.91±62.99^b

Mean values with different superscripts letters in the same row were significantly different ($P<0.05$)

Yield of Grain and Straw

All of the grain and straw yield are shown in Table 4. The highest yield of grain obtained 3.45±0.04 mt/ha in T_1 and the lowest of the same was recorded 2.94±0.49 mt/ha in T_2. No significant difference was observed among the treatments. The highest yield of straw obtained was 6.24±0.13 mt/ha in T_1 and the lowest (5.68±0.33 mt/ha) was recorded in T_5 i.e. control plot. No significant difference was observed among the treatments.

Table 4. Rice yield parameters in different treatments in rice fields

Items	Treatments				
	T_1	T_2	T_3	T_4	T_5
Grain yield (mt/ha)	3.45±0.04	2.94±0.49	3.16±0.10	3.32±0.21	3.01±0.22
Straw yield (mt/ha)	6.37±0.10	6.24±0.22	6.09±0.54	5.91±0.23	5.68±0.33

Values are mean ± standard deviation

Discussion

Temperature of water condition in a rice field is known to be one of the limiting factors for fish productivity. Water temperature in the rice fields fluctuated between 24.20-35.32°C among the different treatments of the present study. Almost similar ranges of water temperature were reported by various authors in rice-fish or prawn culture experiments in Bangladesh (Uddin 1998, Mondol 2001, Das 2002 and Kundu 2003). The pH levels in the rice fields tended to be lower in the presence of fish than in rice grown alone. In the present study, the pH values of water in rice fields ranged between 6.60-7.95, which are almost close to the neutral pH indicating suitable condition for fish and prawn culture. pH values were slightly higher in the afternoon than in the morning. Similar results were reported elsewhere (Rothuis *et al.* 1999, Vromant and Chau 2001, Frei and Becker 2005). Dissolved oxygen (DO) content is probably the single most important water quality parameter in aquaculture. Prolonged exposure to low DO concentration can be harmful for the aquatic life. In the present study, the DO levels of water ranged were between 2.2-8.8 mg/l which are almost similar to the values of 2.3-6.7 and 3.6-8.7 mg/l in rice fields reported by Rothuis *et al.* (1999) and Frei and Backer (2005). Higher values of dissolved oxygen in the afternoon might be associated with the high rate of photosynthesis in presence of sunlight. The high levels of DO in prawn containing treatments and rice only treatment may be attributed to the presence of filamentous algae which were comparatively low in rice-fish plots due to the grazing effect, very turbid water caused by fish specially *C. carpio* and consumption of oxygen by respiration of fish. The values of NO_3-N were also recorded higher in the treatments with fish than without fish which support the findings of Mondol (2001) and Sarker (2005). The phosphate concentration was also higher in the treatments with fish than in control which might be due to accumulation fish faeces and bioperturbation effect of fishes. Sarker (2005) also obtained relatively higher values of it in his study. The phosphate concentration were lower in prawn containing plots might be used phosphorus for their shell formation. Concentration of ammonia showed an increasing trend as the days of culture increased, probably due to higher metabolic deposition and organic load. The range of NH_4-N values recorded by Mohanty *et al.* (2004) in rice fields were 0.01-0.31 mg/l which are lower than the values obtained in the present study. Slight higher values of NH_4-N recorded in the treatments with fish than the control might be associated with the reasons stated above. High filtration rate of tilapia as depicted by Turker *et al.* (2003), which reduces phytoplankton abundance. This is substantiated by the chlorophyll-a values, which were significantly lower in the

presence of tilapia as compared to a situation with common carp only. During the study period, the values of chlorophyll-a were found to range between 10.3-61.5 μg/l among the treatments. Higher chlorophyll-a were obtained in treatment with fish might be the effect of organic accumulation due to fish faeces.

Haroon and Pittman (1997) reported survival rates of tilapia 66% in rice field which is more or less similar to the present study. The higher survival rate of tilapia and common carp in T_3 than the T_1 might be due to the use of supplementary feed in this treatment. The survival rates reported by Mondal (2001) for common carp (58%) were lower than the finding of the present study. The combined survival rates of common carp and tilapia obtained were 52% and 87% in T_1 and T_3 respectively. The survival rate recorded by Frei *et al.* (2007) for rice combined with common carp and tilapia (57 %) is lower than the survival rate of the same treatment in the present study. The lowest survival rate of prawn was in T_2 might be due to the absence of supplementary feeding.

The gross and net yield of combined culture of common carp and tilapia recorded by Frei *et al.* (2007) in rice field were 586 kg/ha and 460 kg/ha respectively which were higher than the yield obtained in the present study. Between the two prawn treatments the higher gross (108.69 kg/ha) and net (81.92 kg/ha) were obtained in T_3 with supplementary feeding and lower gross (64.32 kg/ha) and net (30.98 kg/ha) yield were recorded in T_2 without supplementary feeding. The cause of lower yield of prawn might be the lower survival rate of prawn. Sarkar (2006) recorded yield of prawn were 221.98 kg/ha to 388.38 kg/ha in his study that were explicitly higher than the present study.

The highest grain yield (3.45 mt/ha) was obtained in T_1 with fish having urea fertilization without feeding. Frei *et al.* (2007) also obtained the highest grain yield from the similar treatment in their experiment. The yield of rice grain obtained by Frei *et al.* (2007) in their study (3.4 mt/ha and 3.8 mt/ha) are almost similar to the yield of present study. Frei *at el.* (2007) obtained the yield of rice straw 6.2 mt/ha, 5.7 mt/ha, 5.8 mt/ha and 5.7 mt/ha in rice with common carp, rice with tilapia, rice with common carp/tilapia and rice only respectively that are more or less similar to the present study.

According to the findings of present study, it may be concluded that the introduction of fish culture in rice fields has more or less positive impacts on the availability of nitrate-N, ammonia-N, phosphate-P and chlorophyll-a of water. Therefore, this integrated rice-fish culture technology may be recommended for dissemination to the rural poor farmers through extension program which will benefit them economically and nutritionally. Between the treatments with fish and prawn, the treatments with fish may be recommended for dissemination to rural farmers considering higher yields and economic beneficial. Among the treatments the species composition of tilapia (*O. niloticus*) and common carp (*C. carpio*) was found to be most suitable for rice fish culture considering the survival and yield.

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Study on the growth and survival of *Channa striatus* (Bloch) postlarve using live feed

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Abstract
Feeding experiments were conducted on the postlarvae of *Channa striatus* with two different live feeds – a copepod (*Thermocyclops decipiens*) and cladocerans (*Moina micrura* and *Ceriodaphnia cornuta*) individually and in mixture. Food was provided at the rate of (500±50 Ind./L) and the experiments were carried out in 100 litre capacity tanks for 30 days. Results indicated better weight gain (951.85±28.77%) and survival (92.00%) of postlarvae fed with mixed live food than individual live feed organisms.

Key words: Postlarvae, Copepod, Cladocerans, *Channa striatus*.

Introduction

In larviculture artificial diets may perform poorly due to poor digestibility (Lauff and Hofer 1984), deficiency of growth factors (Higgs *et al.*, 1985), and insufficient stimulation of feeding behavior or pollution due to over feeding (Dave 1989). The cladoceran genera such as *Miona* and *Daphnia* have been used in freshwater fish larval rearing successfully, their biochemical profile with respect to organic and inorganic components are reported to be higher than the levels prescribed for freshwater fish larvae. Common carp and Atlantic salmon grew faster when fed on zooplankton (Kamler *et al.*, 1992) as compared to formulated diets. LeBrasseur (1969) observed higher growth rate and better food conversion in chum salmon (*Oncorhynchus nerka*) fed on live zooplankton. Watanabe *et al.*, (1983) reported an excellent protein efficiency ratio (PER) value of rainbow trout fed with *Daphnia* and *Moina*. Zooplankton are rich in essential amino and fatty acids (EPA and DHA) and should be sufficient as the first source of nutrients required by fish for growth (Kanazawa *et al.*, 1979). The exquisite value of copepods as live feed has been acquainted by the works of Kraul (1989) on the mass culture of these zooplankton. The

nauplii, copepodid stages and adults have wide spectrum of sizes ranging from 50μm to several millimeters rendering them highly suitable as live feed for many commercially important larvae of fishes, prawns, shrimps and mollusks. In order to get consistent results it is proposed to conduct experiments on the postlarvae of *Channa striatus* with different live zooplankton cultured in the lab comprising of a cladocerans (*Moina micrura* and *Ceriodaphnia cornuta*), and a copepod (*Thermocyclops decipiens*), to record feed acceptability, their survival and growth.

Material and methods

Experiments were conducted to study feed acceptability, growth rate and survival of postlarvae of *Channa striatus*. The postlarvae were collected from Centre for Aquaculture Research & Extension (CARE), St. Xaviers College, Tamilnadu. These postlarvae were transported to the laboratory and maintained in large tanks. For feeding experiments 4 batches of postlarvae having 25 numbers in each fibre tank with 50 litres of water were provided with different types of food. Cladocerans (*M. micrura* and *C. cornuta*) and Copepods (*T. decipiens*) cultured in the laboratory were fed to the larvae as individual and mixed feed.

Food was not offered to the larvae on the first day. The larvae were allowed to acclimatize in the laboratory condition for two days. During acclimatization feacal pellets and other debris were removed every day in the morning and 50% of the water was renewed. After third day, the entire water of the tank was renewed and food was given thrice a day at 8 hours interval at the rate of (500± 50 Ind./L) (Qin, *et al.*, 1997).

The postlarvae with similar length and weight (measured to the accuracy of 1 mm and 0.01 gm for length and weight respectively) were introduced in the circular fibre tanks @ of 25 larvae per tank. Experimentation was conducted in three replicates with these feeds for 30 days. Length and weight of the postlarvae were measured before and at the end of the experiment and the values were used for statistical analysis.

Results

Among the 4 types of live feed offered, highest growth was recorded in the fishes fed with mixture of copepod and cladoceran diet (SGR 3.97 ± 0.06, AWG, 951.85 ± 28.77) with significantly higher survival (92.00%) as shown in Table 1.

Among individual feeds *T. decipiens*, showed better results (SGR 3.97 ± 0.06, AWG, 442.66 ± 15.39) followed by *C. cornuta* (SGR, 3.49 ± 0.03 AWG, 404.66 ± 16.70) and *M. micrura* (SGR, 3.36 ± 0.09 AWG, 3.36 ± 0.09). Significantly better weight gain (2.57 gm) and increase in length (1.73 mm) was recorded in postlarvae fed on mixed zooplankton and lesser weight gain (1.00 gm) and increase in length (0.75 mm) in fishes fed on individual diet of *M. micrura.*

Table 1. Growth and survival in postlarvae of *Channa striatus* fed on different individual live feed and their mixed feed (Mean ± SE)

Feeds	Ceriodaphnia	Moina	T. decipiens	Mixed feed
Initial length (mm)	1.76 ± 0.01^{a}	1.75 ± 0.01^{a}	1.77 ± 0.01^{a}	1.77 ± 0.01^{a}
Final length (mm)	2.5 ± 0.01^{a}	2.40 ± 0.02^{a}	2.6 ± 0.02^{b}	3.5 ± 0.04^{c}
Increase in length (mm)	0.75^{e}	0.65^{f}	0.83^{g}	1.73^{h}
Initial weight (g)	0.26 ± 0.01^{a}	0.24 ± 0.01^{a}	0.27 ± 0.01^{a}	0.27 ± 0.01^{a}
Final weight (g)	1.31 ± 0.06^{a}	1.24 ± 0.05^{a}	1.46 ± 0.02^{b}	2.84 ± 0.01^{c}
Weight gain (g)	1.05^{e}	1.00^{e}	1.19^{f}	2.57^{g}
SGR (SGR %)	3.49 ± 0.03^{a}	3.36 ± 0.09^{a}	3.97 ± 0.06^{b}	8.56 ± 0.073^{c}
Weight gain (AWG %)	404.66 ± 16.70^{a}	3.36 ± 0.09^{a}	442.66 ± 15.39^{a}	951.85 ± 28.77^{b}
Survival (%)	80.00^{a}	80.00^{a}	84.00^{b}	92.00^{c}

Values in each row with different superscripts are significantly different at < 0.05 level of significance.

Discussion

The results suggested that postlarvae fed with mixed diet showed better growth and survival and were considered to be the best source of nutrients. The mixture of cladocerans (*M. micrura* and *C. cornuta*) and copepod (*T. decipiens*) might supply all required essential nutrients, thereby provides balanced diet. The movement of copepods and their nauplii triggers the feeding responses in fish larvae. The 'jerking' swimming action of most copepod nauplii and adults is believed to be an important stimulus for initiating feeding by fish larvae (Buskey 2005). The HUFA's are also likely to be present in the correct ratio to enhance survival and growth of fish larvae (McKinnon *et al.*, 2003). Due to the

smaller size and higher locomotive behaviour *C. cornuta* becomes most preferable species by the fish larvae (Suresh kumar 2000) which in turn results in frequent encounter thereby increasing the ingestion rate.

Snakehead can be successfully weaned by feeding them with live plankton from hatchling to larval stage and with combined diet (Live *Chironomous* larvae and formulated diet) during the fry stage (Haniffa and Arockia Raj 2000). Parameswaran (1975) reported that smaller postlarvae (5 – 15 mm) subsist on plankton of which zooplankton constituted the bulk (97.4%) consisting mainly of cladocerans (63.6 %), rotifers (27.9 %) and protozoan (5.4 %) the rest being other zooplankton. Large post larvae (16 - 30 mm) consumed in addition to above (Cladoceran 41.3 %, rotifer 6.2 %, copepods 27.9 % and phytoplankton 0.7 %), aquatic insects and other hemipterous and young shrimp. Hoff and Snell (1989) reported that snakehead larvae could be successfully reared using plankton, which can actively swim for 5 hours in freshwater, thereby extending their availability for larval consumption. Fregadolli (2003) has also reported predation of larvae of Brazilian fishes, *Piaractus mesopotamicus* and *Colossoma macropomus* on a cyclopoid copepod *Thermocyclops* sp. as first feed.

These findings suggest that snakehead can be successfully weaned by feeding the larvae with live plankton from hatchling to fry stage. Easy ingestion of live food organisms as well as their high content of essential factors, such as vitamins, enzymes are likely to be the plausible causes for better survival and growth of larvae and post larvae as observed by Kahan (1984). The ranking of protein quality on experimental diets based on EAAI (Essential Amino Acid Index) by Tacon (1990) is tuned with the present results, in which copepod fed diet stands first foreshadowing its utility.

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Studies on the growth and production of six major and exotic carps in Nasti baor, Jhenaidah, Bangladesh

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Abstract
An investigation on growth, production and fishery of three Indian major carps: rohu, *Labeo rohita*, catla, *Catla catla* and mrigal, *Cirrhinus mrigala* and three exotic carps: silver carp, *Hypophthalmichthys molitrix*, grass carp, *Ctenopharyngodon idella*, and common carp, *Cyprinus carpio* was carried out in Nasti baor during February to April months. In catch per unit effort (CPUE) study the highest catch/day/person (3.13 kg) and catch/day/gear (40.65 kg) was recorded in the month of March for *kochal* fishing. In *komar* fishing catch/day/person (15.08 kg) and catch/day/gear (1206 kg) was also found higher in March. *Komar* fishing was done only in March and April and its CPUE was greater in both the months than that of *kochal*. The average recovery rate (combination of all six species) was 37.80 considering the stocking from July month of the previous year. The recovery rate of common carp (54.1) was the highest and lowest (13.90) in case of silver carp. When the recovery was calculated on the basis of one year data and stocking, it was 55.6%. Analysis of production model revealed that the present production (54,806 kg/year) is less than both theoretical production (model I – 85,285 kg/year and model II –75,952 kg/year) estimated. Therefore, it may be concluded that the fish production from Nasti baor could still be increased from the present level of production.

Key words: Baor, *Kochal* fishing, *Komar* fishing

Introduction

In Bangladesh, inland water bodies are highly productive and contribute about 73% of total fish production (Hasan 1990). However, in recent years, the conditions of the inland capture fisheries of Bangladesh have deteriorated and production has either stagnated or even decreased for some major species (FRSS 2008). On the other hand, aquaculture in ponds and ox-bow lakes has emerged as an important option for increased fish production.

The oxbow lakes is locally called "Baor" in Bangladesh. Baors are closed waterbodies which occupy the dead channels of the rivers in the moribund delta of the Ganges. A baor normally is still part of the floodplain of the river, to which it is connected by inlets and outlets. Fish culture in baors is a practice by which an open water fisheries is converted by screening the inlets and outlets into a culture based

fisheries (PIU/DTA/BRAC 1994). This practice is akin to "Pen Culture", where fish are raised in an enclosure.

Fish culture in baors is being done on the basis of its natural productivity. It is therefore, essential to have a clear understanding of the biological basis of the systems and its productivity to utilize it fully. The growth and recovery rate of stocked fish play an important role in the culture system in the baor. To get a basic understanding of these factors, studies on production, growth and recovery of six Indian major and Chinese carps are undertaken for a period of three months (February – April) in Nasti baor.

Materials and methods

The study was conducted in Nasti Baor under Jhenaidah District. The total area of the baor is 66 ha. The average depth of baor is 2.64±0.15 m in winter and 4.32±0.17 m in monsoon. The sources of water of the baor are monsoon runoff and the underground seepage. Data collection was carried out for a period of three months from February to April 1995. The secondary data collected during the course of the study were also used.

Catch Per Unit (CPU) data

The Catch per Unit Effort (CPUE) data of six species were collected during the study period. The data recorded for CPUE study were:

a) Type of fishing gear
b) Number of gear used during each fishing
c) Number and weight of total fish harvested in each fishing
d) Number and weight of individual fish species harvested in each fishing
e) Number of fishermen attended in each fishing
f) Mesh size of the gear used in fishing, and
g) Number of hours of fishing.

Stocking and harvesting data

Stocking and harvesting data of the periods prior to start of the research were collected from Baor Record Book and analyzed stocking and harvesting for the period of one year. Species-wise stocking data except those of rohu and mrigal were available from July, 1994 to January, 1995 and the stocking data for total fish stocked were available from December, 1993 to January, 1995. There was no stocking after January, 1995. Species-wise harvesting data for all species were available from October, 1994 to April, 1995. The harvesting data for total fish harvested were available from December, 1993 to April, 1995. The harvesting data from February to April, 1995 were collected during the present study.

Gear used in baor fishing

Data were collected from the *kochal* (Purse seine net) and *komar* (Brush parks) fishing during the study period. These two gears are primarily used for fishing in the baor. The fishermen of the baor also use other nets for fishing. Those were chak jal (dip net), khepla jal (cast net) and koi jal (gill net).

Data analysis

Efficiency of gear and fishermen were estimated from the catch data for different months (February-April, 1995). Catch per unit effort (kg/day/person and kg/day/gear) were estimated from catch data for different months for different gear. Percentage of total carp harvested was calculated for each month from total catch data of three months.

Average harvested weight and production of different species for a seven month period were determined from the data of October, 1994 to April, 1995 since the species-wise data was not available prior to this period. Recovery rate for individual species (except that of rohu and mrigal) for the above period (stocking period = July, 1994 – January, 1995 and harvesting period = October, 1994 to April, 1995) was calculated. Recovery rate was estimated by the following formula:

$$\text{Recovery rate} = \frac{\text{Total no. of fish harvested}}{\text{Total no. of fish stocked}} \times 100$$

A difference of minimum 5 months period between stocking and harvesting is desirable, as after releasing the fingerling, a minimum of 5-6 months period is required for fish to grow to harvestable size. However, unavailability of species-wise data has limited the scope of analysis. The difference ratio between theoretical production and actual production was estimated by the production variable and production models by the using of stocking data from December, 1993 to November, 1994 and harvesting data from May, 1994 to April, 1995. Recovery rate of total fish was estimated for the same duration of production model. All calculation and analysis were done by using Excel 5.0 (Microsoft Corporation) Software.

Results

A total of 6,300 individual fishes were measured from the Nasti baor during study period. The total numbers of individual species measured were: rohu -1676, catla - 626, mrigal - 1434, silver carp - 687, grass carp - 952 and common carp - 925. The daily catch data were analyzed and total catch (kg), catch per unit effort (CPUE) in terms of catch per kg/day/person and catch per/day/gear were presented (Tables 1-4). During the period, total catch of *kochal* fishing was 9294.5kg and *komar* fishing was 3198 kg. In February, *komar* fishing was not done. In these periods catch per kg/day/person and catch per kg/day/gear were 2.27 kg and 29.32 kg in *kochal* fishing and these were 11.2 kg

and 935 kg for *komar* fishing. Percentage of total catch (kg) was estimated for the individual month by using three months catch data. Percentage of total catch in February was 16.8, and the values were 36.7% and 46.5% in March and April, respectively (Table 4).

Table 1. Gear wise catch data from *kochal* fishing for the month of February

Fishing days	Fm* no.	Gear no.	Catch (kg)	Catch/Day/Fm*	Catch/Day/Gear
1	75	6	228.00	3.04	38.00
2	75	6	157.00	2.09	26.17
3	75	6	123.00	1.64	20.50
4	75	6	122.00	1.63	20.33
7	75	6	141.00	1.88	23.50
8	75	6	79.00	1.05	13.17
9	75	6	25.00	0.33	4.17
10	75	6	50.00	0.67	8.33
11	75	6	68.00	0.91	11.33
13	75	6	43.00	0.57	7.17
14	71	6	85.00	1.20	14.17
15	71	6	72.00	1.01	12.00
16	71	6	110.00	1.55	18.33
17	71	6	84.00	1.18	14.00
18	71	6	17.00	0.24	2.83
19	71	6	125.00	1.76	20.83
20	66	5	30.00	0.45	6.00
Total	1242	101	1559.00	1.26	15.44

Fm* = Fisherman person

Table 2. Gear wise catch data for the month of March

Kochal fishing						*Komar* fishing				
Fishing Days	Fm* no.	Gear no.	Catch (kg)	Catch/ Day/Fm*	Catch/ Day/Gear	Fm* no.	Gear no.	Catch (kg)	Catch/ Day/Fm*	Catch/ Day/Gear
17	78	6	180.00	2.31	30.00	-	-	-	-	-
18	78	6	370.00	4.74	61.67	-	-	-	-	-
19	78	6	356.00	4.56	59.33	-	-	-	-	-
20	78	6	391.00	5.01	65.17	-	-	-	-	-
21	78	6	299.00	3.83	49.83	-	-	-	-	-
22	77	6	237.00	3.08	39.50	-	-	-	-	-
23	77	6	272.00	3.53	45.33	-	-	-	-	-
24	78	6	274.00	3.51	45.67	-	-	-	-	-
25	-	-	-	-	-	80	1	1206	15.08	1206
26	78	6	319.00	4.09	53.17	-	-	-	-	-
27	78	6	198.00	2.54	33.00	-	-	-	-	-
28	78	6	135.00	1.73	22.50	-	-	-	-	-
29	78	6	124.00	1.59	20.67	-	-	-	-	-
30	78	6	140.40	1.80	23.40	-	-	-	-	-
31	78	6	119.60	1.53	19.93	-	-	-	-	-
Total	1090	84	3415.00	3.13	40.65	80	1	1206	15.08	1206

Fm* = Fisherman person

Table 3. Gear wise catch data for the month of April

Kochal fishing						*Komar* fishing				
Fishing Days	Fm* no.	Gear no.	Catch (kg)	Catch/ Day/Fm*	Catch/ Day/Gear	Fm* no.	Gear no.	Catch (kg)	Catch/ Day/Fm*	Catch/ Day/Gear
1	80	6	228.00	2.85	38.00	-	-	-	-	-
2	80	6	266.00	3.33	44.33	-	-	-	-	-
3	80	6	232.00	2.90	38.67	-	-	-	-	-
4	80	6	97.00	1.21	16.17	-	-	-	-	-
5	80	6	29.00	0.36	4.83	-	-	-	-	-
6	80	6	333.50	4.17	55.58	-	-	-	-	-
7	80	6	20.00	0.25	3.33	-	-	-	-	-
8	80	6	243.00	3.04	40.50	-	-	-	-	-
9	80	6	153.00	1.91	25.50	-	-	-	-	-
10	80	6	320.00	4.00	53.33	-	-	-	-	-
11	-	-	-	-	-	80	1	861	10.76	861
16	80	6	58.00	0.73	9.67	-	-	-	-	-
17	80	6	320.00	4.00	53.33	-	-	-	-	-
18	80	6	12.50	0.16	2.08	-	-	-	-	-
19	80	6	295.50	3.69	49.25	-	-	-	-	-
20	80	6	215.00	2.69	35.83	-	-	-	-	-

21	80	6	279.00	3.49	46.50	-	-	-	-	-
22	-	-	-	-	-	80	1	662	8.28	662
24	80	6	266.00	3.33	44.33	-	-	-	-	-
25	80	6	283.00	3.54	47.17	-	-	-	-	-
26	80	6	147.00	1.84	24.50	-	-	-	-	-
27	80	6	202.00	2.53	33.67	-	-	-	-	-
28	80	6	150.00	1.88	25.00	-	-	-	-	-
29	-	-	-	-	-	80	1	469	5.86	469
30	80	6	171.00	2.14	28.50	-	-	-	-	-
Total	1760	132	4320.50	2.45	32.73	240	3	1992	8.30	664

Fm* = Fisherman person

Total no. of fish catch, total weight of fish (kg), average weight of fish (kg) at harvest, recovery rate of individual species and production of individual species (kg/ha) were estimated from the harvesting and stocking data of individual species (Table 5). The highest production was achieved for rohu (221.53 kg/ha) and the lowest was for mrigal (73.96 kg/ha). The highest individual weight was achieved by common carp (1.8 kg) followed by grass carp (1.6 kg) and silver carp (1.1 kg) and the lowest by mrigal (0.5 kg) (Table 5). The total production for the seven months periods (October 1994 to April, 1995) was 870.31 kg/ha. The total production was 1014.9 kg/ha when the harvesting data for one year (May, 1994 to April, 1995) was taken into consideration (Table 6). But the species-wise production for this period could not be estimated due to lack of species-wise harvesting data prior to October, 1994. The recovery rate of these species was 43.3% for catla, 13.9% for silver carp, 39.9% for grass carp and 54.1% for common carp (Table 6). Recovery rate of rohu and catla could not be calculated due to lack of stocking data prior to July, 1995. For the estimation of recovery rate the stocking data were undertaken from July, 1994 to January, 1995. The average recovery rate for all species was 37.8 (Table 5).

Table 4. Gear wise catch data for the month of April

Name of Gear:	*Kochal*					*Komar*					% of total catch (kg)
Months	Fm* no.	Gear no.	Catch (kg)	Catch/Day/Fm* (kg)	Catch/Day/Gear (kg)	Fm* no.	Gear no.	Catch (kg)	Catch/Day/ Fm*	Catch/Day/ gear(kg)	
February	1242	101	1559	1.26	15.44	-	-	-	-	-	16.8
March	1090	84	3415	3.13	40.65	80	1	1206	15.08	1206	36.7
April	1760	132	4320.5	2.45	32.73	240	3	1992	8.3	664	46.5
Total	4092	317	9294.5	2.27	29.32	320	4	3198	11.2	935	100

Fm* = Fisherman person

Table 5. Average harvested weight, recovery rate and production (kg/ha) of different species during October, 1994 to April 1995

Species	Harvesting Month														Average weight of fish (kg)	Recovery rate of fish	Production (kg/ha)
	October		November		December		January		February		March		April				
	No. of Fish	Weight (kg)	No. of Fish	Weight (kg)	No. of Fish	Weight (kg)	No. of Fish	Weight (kg)	No. of Fish	Weight (kg)	No. of Fish	Weight (kg)	No. of Fish	Weight (kg)			
Rohu	2601	2234.9	3857	3144.4	3688	3523.9	1422	1312.5	531	374.0	816	729.0	861	644.0	0.87	-	221.53
Catla	2268	1657.2	2374	2303.0	3236	3813.4	1480	1469.8	358	286.0	396	453.0	1881	1033.0	0.92	43.30	203.99
Mrigal	1638	813.1	1138	611.9	1309	728.0	1761	932.0	306	171.0	240	173.0	1112	565.0	0.53	-	73.96
Silver carp	1856	2716.0	347	641.9	158	281.3	323	595.0	118	239.0	59	152.0	1965	787.0	1.12	13.90	100.23
Grass carp	804	1039.2	757	1304.4	634	1028.1	236	388.0	169	284.0	763	1257.0	686	1115.5	1.61	39.90	120.67
Common carp	521	835.4	569	982.2	643	1218.3	271	439.0	113	205.0	999	1413.0	1455	2168.0	1.77	54.10	149.93
Total	9688	9295.8	9042	8987.8	9668	10593.0	5493	5136.3	1595	1559.0	3273	4177.0	7960	6312.5	1.14	37.80	870.31

Note: Recovery rate of rohu and catla could not be calculated due to lack of stocking data for those species.
Production (kg/ha) were calculated by the use of standard area of baor which was 54 ha.

Two production models were prepared using production variables to estimate theoretical production of the baor (Table 7). The data were used for 1 year duration i.e., 1 year for stocking (December, 1993 – November, 1994) and 1 year for harvesting (May, 1994 – April, 1995). Analysis of production model revealed that the present production (54,806 kg/year) is less than both theoretical production (model I – 85,285 kg/year and model II – 75,952 kg/year) estimated (Table 7).

Table 6. Actual stocking and harvesting data of carps in Nasti baor during December, 1993 to April, 1995

Stocking status			Harvesting status		
Month	No. of fish	Weight of fish (kg)	Month	No. of fish	Weight of fish (kg)
December,1993	1110	92.5	December,1993	8799	4450.1
April, 1994	66946	3756.0	January, 1994	7368	5566.0
June, 1994	17873	1198.7	February,1994	4425	2809.7
July, 1994	10589	908.5	May, 1994	5187	5995.0
December,1994	62082	3857.0	June, 1994	1717	2750.0
January, 1995	8560	428.0	October, 1994	9688	9295.8
			November,1994	9042	8987.7
			December,1994	9668	10593.0
			January, 1995	5493	5136.0
			February, 1995	1595	1559.0
			March, 1995	3273	4177.0
			April, 1995	7960	6312.5
Total (Dec.93-Nov.94)	96518	5955.7	Total (May, 94 - April, 95)	53623	54806.0
Production (kg/ha) = 1015					

Table 7. Production variables and production models of Nasti baor for one year

1		2	3	4
Mean weight (kg)		Loge (w1)	Loge (w2)	Growth
Stocked (w1)	Harvested (w2)			Loge (w2) – Loge (w1)
0.06170559	1.02206143	-2.78538078	0.0218216	2.807202372

5	6	7	8	9		
Number Stocked	T/Stock weight	Number Harvested	T/Harvest weight	One year average weight		
				Mean stock	Mean harvest	Mean Bionass (B)
96518	5955.7	53623	54806	5955.7	54806	30380.85

Production models

A	B		C	
Average harvest weight/yr	Theoretical production model I	Difference ratio B : A	Theoretical production model II	Difference ratio C : A
54806	85285.1942	1.55612878	75952.125	1.385835949

Notes
Mean stock weight averaged from reported totals. Mean harvest weight averaged from reported totals
Annual production averaged from stocking wt. & harvesting wt. Time period assumed to be 1 year
Growth formula, G = {Loge (w2) – Loge (w1)} / time (yr.)
Production model I, P = G* time * B (mean) Production model II, P = B (mean) * T, where T is turnover ratio; This is assumed to be 2.5 for a warm water extensive system.

Discussion

In baor fishery, different types of gear are used for fishing but mainly *kochal* and *komar* fishing were being used in fishing during the study period. CPUE are expressed as kg of fish per 100m^2 of net area per day for gill net and seine net (BCAS 1989). For this study, fishing techniques in baors CPUE are expressed as kg/day/gear. *Komar* fishing was done in March and April indicating the higher CPUE than that of *kochal*. This is in close agreement with BBR (1994). The highest CPUE was found in March, though the highest percentage of catch was in April. It was due to low attendance of fishermen in fishing in March having low water depth of the baor. High fishing days was in April as the water level was going down as well as fishermen were badly in need of money to pay off their lease money.

Recovery rate of silver carp was comparatively less than those of other species. It can be explained that secchi depth was high and water colour was clear during 7 months after stocking (BCAS 1994) and the stocking density was very high (43% of total stock) resulting high mortality and adverse effect of growth. High recovery rate of common carp may be due to komar fishing and stocking low percentage of fingerlings. According to Baor Biological Studies (1994) recovery rate of common carp increase in *komar* fishing, seems to hold good with this view. Production model revealed that practical production was lower than those of production model I and II with an assumption that production of the baor can be increased by manipulating proper stocking ratio and density and improved management system. The turn over ratio (T) for warm water fish is <2.5 (Waters 1969). According to suggestion of Shawn Marriot (1994) T value was considered 2.5 in this study. However, it must be emphasized that the production achieved is in fact the production recorded. Loss of production due to poaching and unrecorded production could not be taken into consideration while analysis of production variables.

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Study on fish hatchery and nurseries in Mymensingh, Bangladesh

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Abstract
In generally, fish hatchery and nursery owners having both hatchery and nursery facilities were financially stronger, well-educated and well-trained than only nursery ponds owners in Mymensingh aquaculture region. On the other hand, only nursery pond owners were more experienced in fish seed business than only hatchery owners. Most of the owners were satisfied with existing communication facilities. Lack of technical knowledge was one of the major constraints which could be solved by ensuring proper training. This business can be made more profitable providing loan to poor farmers and improving law and order situation.

Key words: Fish hatchery, Nursery

Introduction

The fisheries sector of Bangladesh is a very important sector in respect to meet nutrition of the people and the export earnings of the country. Considering the importance of culture fisheries, the Government of Bangladesh has given a high priority on both freshwater and brackish water aquaculture development. The major input in culture fishery is quality fish seed, which mainly comes from Government and private hatcheries and nurseries. In the country, induced breeding of carps through hypophysation was initiated in 1967. Government of Bangladesh established a number of hatcheries in public sector in different parts of the country for supplying quality fish seeds to the farmers and also for transferring this seed production technology to the private entrepreneurs who were interested in establishing hatcheries on their own initiative to meet the increasing demand of quality fish seed. The induced breeding of carp has been so successful in Bangladesh that there is now an over capacity in the industry resulting a large drop in the price. So, many small or medium scale hatchery owners are not interested in maintaining quality brood stock to minimize this loss, resulting poor quality seed production.

The study was conducted to identify the existing physical facilities of the fish hatchery and nursery as well as experience and training status of manpower in fish seed farming and some other related matters. From this study the relevant officials of the Government, Semi-Government and private organizations will be aware of the various aspects of fish seed farms (fish hatchery and nursery).

Materials and methods

Study area and selection of fish farms

The study was conducted in 12 upazillas of Mymensingh district from February to April months. Primary information of fish seed farms were collected from District Fishery Office and Upazilla Fisheries Officers. On the basis of fish seed production practices, fish farms may by categorized into three groups: (i) only hatchery, (ii) only nursery, and (ii) farms with both hatchery and nursery. Finally a total of 81 farms were selected for the study of which 11 farms were only hatchery, 47 fish farms were only nursery ponds and the rest 23 farms were of the last category.

Preparation of survey schedule

An interview schedule was carefully designed so that the manager or owner of the fish hatchery and nursery can answer easily. The schedule includes questions on physical facilities, experiences, education and training status, price of seeds, source of money for operation etc. related to fish seed farms.

Data collection and analysis

Two methods were used to collect data- interview and direct observation. Both individual and group interviews were conducted on farm managers or owners using the prepared interview schedule. All the collected information were accumulated analyzed and then presented in textual, tabular and graphical forms to understand the present status and trends in fish seed farming. In this study tabular technique of analyses were carried out which includes classification of data in the form of tables. It is generally used to find out the crude association or differences between two sets of variable. This technique is based on arithmetic mean, percentage, ratio etc. Finally recommendation and conclusion was made on the total obtained results.

Results and discussion

Size of fish farms

On the basis of size, fish hatcheries and nurseries were divided into 3 categories: below 2 acres, 2 to 5 acres and more than 5 acres; and the selected farms were about 8.64%, 38.27% & 53.09% respectively (Table 1). It was observed that most of the farms were of large size (53.09%). The sizes were determined on the basis of total ponds and hatchery area used under the farms. Sarker (1995) found different sizes (average size 1.097 ha.) of fish hatchery in some selected areas of Bangladesh.

Table 1. Different size categories of fish hatcheries and nurseries in Mymensingh district

Size of fish hatcheries and nurseries (acre)	Farm's size category (%)			Total (%)
	Only hatchery	Only nursery	Hatchery + nursery	
<2	1.235	7.407	0	8.642
2-5	3.704	20.988	13.580	38.272
>5	8.642	29.630	14.815	53.087
Total (%)	13.581	58.025	28.395	100

Communication facilities

Well-developed communication system is helpful for the farm owners to ensure better economic returns through easy transportation of inputs and farm produced seeds. About 67.9% of fish hatchery and nursery owners mentioned the road communication facilities as excellent and only 2.48% as not good, i.e. unsatisfactory (Fig. 1). These results slightly differ from those of Alauddin (2001) who surveyed fish seed farms in three districts of Bangladesh, reported that about 45.24%, 38.09% and 16.67% of fish seed farm owners mentioned the facility as excellent, well and not good respectively.

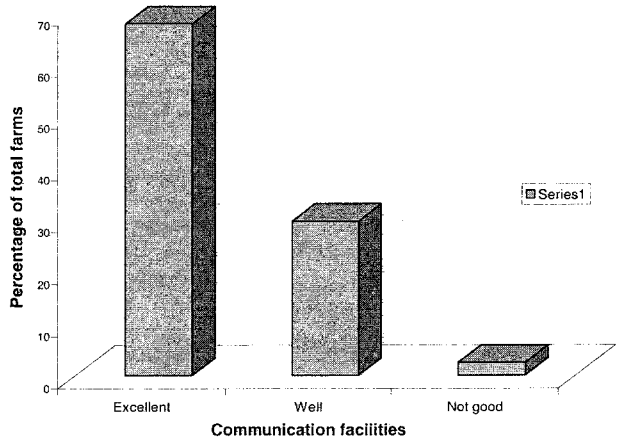

Fig. 1. Communication facilities of fish hatcheries and nurseries in Mymensingh district

Educational and training status

According to survey, only 6.18 % owners or managers had graduation or post graduation level education and 3.7% had professional degree i.e. graduation in Fisheries discipline (Fig. 2). About 13.59%, 11.12%, 33.34% and 16.05% of farm owners had primary and high school, SSC and HSC level education respectively and 16.05 % were illiterate. The result of present study was similar to that of Sarker (1995). But dissimilar results are reported by Malek (1997) and Zaman *et al.* (2006) that 46.67% and 23.3%

fish farmers were illiterate respectively. But Quddus *et al.* (1998) reported fully dissimilar result that there were no illiterate pond owners in Demra area in Dhaka. Alauddin (2001) also reported different result that no farm owner had professional degree.

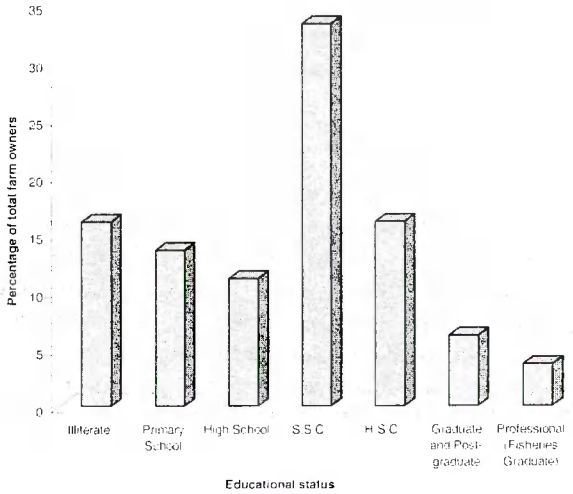

Fig. 2. Educational status of fish hatchery and nursery owners in Mymensingh District

Training on fish hatchery management and fingerling production is helpful for better management and economic returns. Training status of fish hatchery and nursery owners is shown in Table 2. About 43.21% fish hatchery and nursery owners had no training while about 49.38% fish hatchery and nursery owners received short-term training from Department of Fisheries (DoF) and/or from Bangladesh Fisheries Research Institute (BFRI). Similar results were also observed by Aladdin (2001).

Table 2. Training status of fish hatchery and nursery owners on fish seed production in Mymensingh district

Training status	Farm's size category (%)			Total (%)
	Only hatchery	Only hatchery	Hatchery + nursery	
No training	1.235	39.506	2.469	43.210
Short-term training	9.877	18.519	20.988	49.383
Consultation with UFO	2.469	0	1.235	3.704
Professional	0	0	3.704	3.704
Total (%)	13.581	58.025	28.396	100

Experience in fish seed production

Experience in fish seed production indicates the stability in fish seed production business. Experience in fish seed production was categorized into three types: (i) less than 6 years, (ii) 6-10 years and (iii) more than 10 years. About 29.63%, 43.21% and 27.16% owners had experiences for less than 6 years, 6-10 years and more than 10 years respectively (Table 3). So it is evident that fish hatchery and nursery owners were experienced enough in their business of fish seed production. Malek (1997) and Alauddin (2001) mentioned that 11.11% and 42.85% farm had experience in fish seed production of more than 10 years. This dissimilarity might be due to location wise variation in educational condition.

Table 3. Experience of fish hatchery and nursery owners on fish seed production in Mymensingh

Experience (years)	Farm's size category (%)			Total (%)
	Only hatchery	Only hatchery	Hatchery + Nursery	
<6	4.938	16.049	8.642	29.629
6-10	6.173	27.160	9.877	43.210
>10	2.469	14.815	9.877	27.161
Total (%)	13.580	58.024	28.396	100

Sources of fund for operation

Sources of fund for operation is shown in a pie chart in Fig. 3. It was observed that most of the farm owners (52.56%) use their own fund for farm installation and operation. Only 19.23%, 3.85%, 16.67% and 7.69% farm owners got their fund from banks, NGOs, Friends & relatives and moneylenders. CPP (1996) reported that 70% fish farmers took loan from money lender in Tangail district.

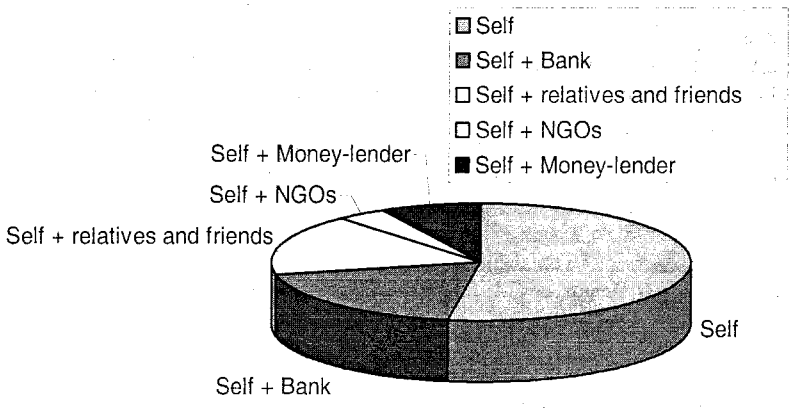

Fig. 3. Sources of fund of fish seed farms in Mymensingh

Price of outputs

Sale price of fish seeds determines the profitability of fish seed production. Maximum sale price of spawn of pangas and catla was highest (Tk. 5,000/kg) followed by carpio, grass carp, rui, mrigal, silver carp and sharpunti. Sarker (1995) reported dissimilar results that the price of spawn of catla (Tk. 7,000/kg). These results also differs from findings of Alauddin (2001) that maximum sale price of spawn of pangas was 6,000 Tk. per kg. Sale price of fingerlings of magur was highest (Tk. 4,500/1000 fish) followed by catla, carpio, Thai rupchanda, pangas, pabda, monosex tilapia, rui, grass carp, mrigal, sharpunti, silver carp and koi. These results are dissimilar to those of Siddique (1999) who reported market price of mirror carp (Tk. 300/1000 fish) was highest. Alauddin (2001) also reported different result that sale price of pangas fingerlings was highest (Tk. 1000/1000 fish) and lowest for silver carp, sharpunti and gonia (Tk. 200/1000 fish).

Considering the different observations during the study, Mymensingh was found to be potential area for fish seed production and Trishal and Bhaluka was considered to be very important upazillas in this regard. Lack of technical knowledge was an important constraint.

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Effects of gamma radiation and -20^{0}C temperatures on the shelf life of Hilsa, *Tenualosa ilisha* (Ham.-Buch. 1822)

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Abstract
The combined effect of radiation and refrigeration on the shelf life of hilsa, *Tanualosa ilisha* was studied by monitoring the microbiological, chemical and sensory changes of unirradiated and irradiated fish samples using low dose irradiation, doses of 300 krad, 600 krad and 900 krad. Irradiation (900 krad) dramatically reduced population of bacteria, namely total viable counts 48,850 cfu per gm for unirradiated, 31,850 cfu per gm and 19,600cfu per gm of 300 krad and 600 krad, respectively. The effect was more pronounced at the higher dose (900 krad), total viable count were 14,100cfu per gm. Another microbial indicator total mould counts (TMC) was 8,750cfu per gm, 6,350cfu per gm, and 19,600cfu per gm for 300 krad and 600 krad, respectively. The effect was more pronounced at the higher dose (900 krad) where total viable counts were 14,100cfu per gm. Total volatile nitrogen values increased slowly attaining a value of 101.02mgN per 100gm for unirradiated *T. ilisha* during refrigerated storage, whereas for irradiated fish, lower values of 71.13, 59.33 and 47.03mgN per 100 gm muscle were recorded. Sensory evaluation showed a good correlation with bacterial populations on the basis of overall acceptability scores.

Keys words: Gamma radiation, Shelf life, *Tenualosa ilisha*

Introduction

Fish is the main source of animal protein of the countrymen as 63% animal protein comes from fish alone (Chowdhury 2001). Bangladesh earns a lot of foreign exchange every year exporting fish and fishery products. Export of fish is mostly sold and preserved at the traders and consumers level with ice and freezing condition. All the prerequisites like proper handling and mode of preservation are essential since fish is an extremely perishable commodity. Rigor mortis started as soon as the fish dies and a large amount of fish is lost due to spoilage every year in Bangladesh. The cost of preservation should, however be low to ensure profitable return. The method of preservation should be simple, such as many are applicable on a commercial scale and suitable for the climate condition. There are various ways by which spoilage can be slowed down or stopped, processing and preservation. Bacteria and autolytic spoilage

are biological systems witch operate under certain optimum conditions. Therefore, altering these conditions can prevent or reduce spoilage. As bacteria require water and is sensitive to temperature, salt concentrations and pH, a number of approaches can be used for reduce autolysis by controlling enzyme activity. The most commonly used and practiced way of reducing autolytic action is to lower temperature, but enzymes can also be inactivated by using chemicals or by using radiation. Radiation in suitable doses can kill the microorganisms, insect and parasite which may present in food and inhibit enzyme activity (Clucas and Ward 1996). Ionizing radiation such as gamma rays emitted from the excited nucleus of ^{60}Co or electrons emitted from a hot cathode have been studied extensively with the objective of producing foods that are free of spoilage by microorganisms or pathogens or contain a greatly diminished number of spoilage organisms (Board 1983). In the above context, to extend the shelf life of hilsa fish using different doses of gamma radiation (300 krad, 600 krad and 900 krad) and at -20^{0}C the present study was carried out.

Materials and methods

Fresh hilsa used in this experiment were collected from the Kawran Bazar and then transferred to the laboratory of Food Processing and Preservation Division, AERE, Savar in presterilized polythene bag with ice. In the laboratory, the samples were processed in the following steps.

The samples were first of all randomly divided in two lots. The first lot was taken for chemical composition and second lot was used for preservation purpose under different techniques. The fish from the second lot were beheaded, gutted and washed in tap water. Then fishes were divided into the following sample categories. Sample A, stored at -20^{0}C temperature; sample B, control; sample C-E for 300 krad, 600 krad and 900 krad treatments. Except the sample A the other samples were subjected to irradiate in a Co-60 source. All samples were examined at the 0, 7, 14, 21, 28, 35, 42 and 49 days of storage periods. The stored samples were evaluated organoleptically for odour, appearance, color and texture by a panel of judge on a 9-point hedonic scale as described by Peryam and Pilgrim (1957). Trimethylamin (TMA) and total volatile nitrogen (TVN) were examined by using Conway micro diffusion technique method (Conway and Byrne 1933, modified by Pearson 1962) the total bacterial count (TBC) and total mould count (TMC) were estimated by the method of Sharf (1966). Determination of chemical composition, moisture, protein, ash, calcium, phosphorus, according to the method of AOAC (1975) and lipid by Floch (1957).

Results

Moisture content of hilsa comprised 76%, while protein accounted for 20%, lipid and ash about 2%. Calcium and phosphorus content accounted 250 and 110 mg/100g, respectively (Table 1).

Table 1. Biochemical composition of hilsa, *Tenualosa ilisha*

Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Calcium (mg/100g)	Phosphorus (mg/100g)
75.08	20.1	2.12	1.93	250	110

Organoleptic scores (OS)

Comparing the treatment, the highest organoleptic score was found in the 900 krad treatment group while the lowest score was measured in the control group (Table 2). The OS of 300 and 600 krad treatments were also different. Among durations, all treatments were significantly different.

Table 2. Organoleptic score in hilsa, *Tenualosa ilisha* sampled before and after treatment with irradiation of 300, 600 and 900 krad at 0, 7, 14, 21, 28, 35, 42 and 49 days (±SEM)

Duration (day)	Treatment (Krad)				Overall
	Control	300	600	900	
0	$8.65\pm.01_{a}$	$8.66\pm.06_{a}$	$8.65\pm.04_{a}$	$8.66\pm.04_{a}$	$8.65\pm.01_{a}$
7	$8.25\pm.01_{a}$	$8.48\pm.03_{a}$	$8.46\pm.05_{a}$	$8.57\pm.03_{a}$	$8.44\pm.05_{b}$
14	$7.55\pm.03_{b}$	$7.83\pm.01_{b}$	$7.86\pm.06_{b}$	$8.00\pm.05_{b}$	$7.81\pm.06_{b}$
21	$6.91\pm.04_{c}$	$6.94\pm.04_{c}$	$7.05\pm.03_{c}$	$7.17\pm.06_{c}$	$7.02\pm.05_{d}$
28	$6.05\pm.05_{d}$	$6.36\pm.06_{d}$	$6.58\pm.03_{d}$	$6.79\pm.03_{d}$	$6.44\pm.10_{c}$
35	$4.93\pm.23_{e}$	$5.15\pm.01_{e}$	$5.88\pm.22_{e}$	$6.23\pm.19_{e}$	$5.54\pm.25_{f}$
42	$4.08\pm.18_{f}$	$4.63\pm.03_{f}$	$5.23\pm.21_{f}$	$5.82\pm.05_{f}$	$4.94\pm.25_{g}$
49	$3.59\pm.04_{f}$	$4.28\pm.07_{g}$	$4.95\pm.08_{f}$	$5.28\pm.04_{g}$	$4.52\pm.25_{h}$
Total	$6.25\pm.46^{d}$	$6.54\pm.23^{c}$	$6.83\pm.33^{b}$	$7.06\pm.23^{a}$	6.67±.19

Rows with superscript and columns with subscript letters denote significant differences ($p<0.05$)

Total votalile nitrogen (TVN)

Comparing the treatment, the lowest TVN value was found in the 900 krad treatment group while the highest value was measured in the control group (Table 3). The TVN of 300 and 600 krad treatment was also different. Among durations, all treatment duration were significantly different.

Table 3. TVN values in hilsa, *Tenualosa ilisha* sampled before and after treatment with irradiation of 300, 600 and 900 krad at 0, 7, 14, 21, 28, 35, 42 and 49 days

Duration (day)	Treatment (Krad)				Overall
	Control	300	600	900	
0	$14.88\pm.37_{f}$	$12.20\pm.65_{f}$	$10.15\pm.65_{g}$	$8.98\pm.08_{g}$	$11.55\pm.87_{h}$
7	$26.55\pm.2.05_{f}$	$22.70\pm1.65_{e}$	$17.15\pm.65_{f}$	$13.33\pm.58_{f}$	$19.93\pm1.99_{g}$
14	$40.88\pm1.23_{e}$	$30.48\pm2.62_{de}$	$21.88\pm.63_{f}$	$17.60\pm.45_{e}$	$27.71\pm3.41_{f}$
21	$56.18\pm2.68_{de}$	$37.23\pm2.02_{d}$	$31.08\pm1.18_{e}$	$25.48\pm.72_{d}$	$37.49\pm4.42_{e}$
28	$63.25\pm2.75_{cd}$	$47.53\pm2.27_{c}$	$38.30\pm1.10_{d}$	$29.50\pm.50_{c}$	$44.64\pm4.77_{d}$
35	$73.72\pm2.52_{bc}$	$54.53\pm1.37_{bc}$	$43.93\pm1.57_{c}$	$36.33\pm.57_{b}$	$52.13\pm5.35_{c}$

42	83.38±2.22$_{b}$	61.68±1.43$_{de}$	51.17±1.12$_{b}$	39.00±.50$_{b}$	58.80±6.18$_{b}$
49	101.02±2.73$_{a}$	71.13±1.03$_{a}$	59.33±.57$_{a}$	47.03±.27$_{a}$	69.62±7.59$_{a}$
Total	57.48±7.04^{a}	42.18±4.88^{b}	34.12±4.15^{c}	27.15±.22^{d}	40.23±2.84

Rows with superscript and columns with subscript letters are significantly different ($p<0.05$).

Trimethyleamin (TMA)

Comparing the treatment, the lowest value of TMA was found in the 900 krad treatment group while the highest value was measured in the control group (Table 4). The OS of 300 and 600 krad treatment were also different. Among durations, all treatment duration were significantly different. The TMA value observed in the control was highest and similar to 1 week sampling. All the treatments were different each other and significantly different.

Table 4. TMA scores in hilsa, *Tenualosa ilisha* sampled before and after treatment with irradiation of 300, 600 and 900 krad at 0, 7, 14, 21, 28, 35, 42 and 49 days means

Duration (day)	Treatment (Krad)				Overall
	Control	300	600	900	
0	14.50±1.26$_{g}$	12.08±.13$_{i}$	9.98±.22$_{j}$	7.85±.05$_{k}$	11.10±.96$_{g}$
7	22.20±.55$_{fg}$	15.23±.33$_{ef}$	13.18±.58$_{ef}$	9.95±.10$_{ij}$	15.14±1.71$_{e}$
14	27.60±.70$_{f}$	19.60±.90$_{cd}$	17.45±.60$_{de}$	13.28±.22$_{ef}$	19.48±1.98$_{f}$
21	37.13±2.38$_{c}$	22.03±1.08$_{c}$	23.22±.64$_{d}$	17.45±.55$_{de}$	24.95±2.83$_{c}$
28	49.45±1.85$_{d}$	39.35±2.00$_{d}$	32.75±.25$_{c}$	21.67±.72$_{d}$	35.81±3.85$_{d}$
35	63.18±.53$_{b}$	1.85±1.25$_{b}$	37.35±.60$_{b}$	29.15±1.15$_{b}$	45.38±4.96$_{b}$
42	75.33±.67$_{b}$	69.65±2.85$_{b}$	48.58±1.72$_{b}$	39.92±2.18$_{b}$	58.37±5.57$_{b}$
49	90.10±2.40$_{a}$	81.03±2.07$_{a}$	60.08±2.07$_{a}$	54.70±1.80$_{a}$	71.47±5.56$_{a}$
Total	47.43±6.51^{a}	38.85±6.35^{b}	30.32±4.29^{c}	35.21±2.86^{d}	35.21±2.86

Rows with superscript and columns with subscript letters indicate significant differences ($p<0.05$)

Total bacterial count (TBC) (cfu/g^{-1})

Comparing the treatment, the lowest value of total bacterial count was found in the 900 krad treatment group while the highest value was measured in the control group (Table 5). The TBC of 300 and 600 krad treatments were also different among duration, all treatment duration were significantly different. To control, the TBC value observed in the control was highest and similar to 1 week sampling. The TBC values of first and second week are significantly different. All the other treatment were different and significantly different.

Total mould count (TMC)

Comparing the treatment, the lowest value of total mould count (TMC) was found in the 900 krad treatment group while the highest value was measured in the control group (Table 6). The TMC of 300 and 600 krad treatments were also different. Among durations, all treatment duration were significantly different.

Table 5. Bacterial density (cfu/g) in hilsa, *Tenualosa ilisha* sampled before and after treatment with irradiation of 300, 600 and 900 krad at 0, 7, 14, 21, 28, 35, 42 and 49 days

Duration (day)	Treatment (Krad)				Overall
	Control	300	600	900	
0	$1925\pm75_{h}$	$1250\pm150_{g}$	$750\pm20_{g}$	$550\pm50_{f}$	$1119\pm203_{h}$
7	$3350\pm100_{g}$	$2425\pm125_{f}$	$1900\pm200_{f}$	$1125\pm75_{e}$	$2250\pm337_{g}$
14	$5625\pm375_{f}$	$4250\pm150_{e}$	$3450\pm200_{e}$	$1875\pm125_{d}$	$3800\pm520_{f}$
21	$9075\pm325_{e}$	$6075\pm325_{d}$	$4450\pm150_{de}$	$2475\pm125_{d}$	$5519\pm918_{e}$
28	$15350\pm550_{d}$	$8700\pm600_{c}$	$5425\pm175_{d}$	$3875\pm125_{c}$	$8338\pm1673_{d}$
35	$23900\pm600_{c}$	$12750\pm250_{bc}$	$7950\pm200_{c}$	$5450\pm150_{b}$	$12513\pm2679_{c}$
42	$35300\pm600_{b}$	$10500\pm500_{b}$	$14450\pm250_{b}$	$7250\pm250_{b}$	$16875\pm4138_{b}$
49	$48850\pm850_{a}$	$31850\pm650_{a}$	$19600\pm400_{a}$	$14100\pm300_{a}$	$28600\pm5047_{a}$
Total	17947 ± 4077^{a}	9725 ± 2363^{b}	7247 ± 1586^{c}	4588 ± 1077^{d}	9877 ± 1392

Rows with superscript and columns with subscript letter indicate significant difference ($p<0.05$)

Table 6. Total mould count (TMC) (cfu/g) in hilsa, *Tenualosa ilisha* sampled before and after treatment with irradiation of 300, 600 and 900 krad at 0, 7, 14, 21, 28, 35, 42 and 49 days

Duration (day)	Treatment (Krad)				Overall
	Control	300	600	900	
0	$825\pm75_{h}$	$625\pm25_{h}$	$425\pm25_{h}$	$275\pm25_{d}$	$538\pm80_{g}$
7	$1300\pm50_{g}$	$1075\pm25_{g}$	$575\pm25_{g}$	$375\pm25_{d}$	$831\pm141_{f}$
14	$2175\pm75_{f}$	$1600\pm100_{f}$	$850\pm50_{f}$	$450\pm100_{d}$	$1269\pm254_{e}$
21	$3075\pm175_{e}$	$2425\pm75_{e}$	$145\pm5_{e}$	$825\pm75_{c}$	$1618\pm448_{e}$
28	$4050\pm150_{d}$	$3050\pm50_{d}$	$2175\pm75_{d}$	$1250\pm50_{bc}$	$2631\pm394_{d}$
35	$5625\pm125_{c}$	$3875\pm125_{c}$	$2825\pm75_{c}$	$1850\pm100_{ab}$	$3544\pm530_{c}$
42	$7125\pm125_{bc}$	$5175\pm75_{b}$	$3650\pm50_{b}$	$2525\pm75_{a}$	$4619\pm653_{b}$
49	$8750\pm250_{a}$	$6350\pm100_{a}$	$4675\pm75_{a}$	$3125\pm125_{a}$	$2597\pm791_{a}$
Total	4116 ± 687^{a}	3022 ± 487^{b}	1915 ± 407^{c}	1334 ± 259^{d}	2597 ± 272

Rows with superscript and columns with subscript letters indicate significant difference ($p<0.05$)

Discussion

The actual extension of shelf life is dependent on the level of dose and the condition of fish at the time of treatment (Liston *et al.* 1969). Several workers have demonstrated that the storage life of fish irradiated is an absolutely fresh condition is greatly in excess of that for fish irradiated after ice storage for even a very short period (Kazans *et al.* 1969). The use of irradiation in food preservation is based on the theory that at certain dose level of irradiation, spoilage is prevented and pathogenic bacteria destroyed without altering the characteristics of the food. Hussain *et al.* (2001) reported that low dose of ionizing radiation are known to reduce the spoilage causing factor in food and thereby extended the shelf life of irradiated products. Within storage period 49 days at

every 7 days the organoleptic test, chemical analyses, microbial analyses was carried out for assuring the degree of spoilage during the preservation.

Muscle of live fishes is more or less sterilized but after death autolytic, bacterial and other changes occur (Hussain 1986). The physical changes could be perceived with sense organs. Organoleptic score of hilsa, *Tenualosa ilisha* come down 8.65±.01 to 3.59±.04, 8.66±.06 to 4.28±0.07; 8.65±0.04a to 4.95±.08 and 8.66±.04 to 5.28±.04 in control, 300, 600 and 900 krad respectively after 49 days of storage. From the above discussion it was clear that with the increase of storage period the organoleptic score were rapidly decreased in the control fish than those irradiated fish of different doses. Hilsa irradiated with 900 krad has the longer shelf life other treatment and then 600 krad and 300 krad. Due to microbial spoilage with the increase of storage period, the appearance, odor, color and texture will also be deteriorated. So, the organoleptic scores are decreased.

Regarding the shelf life of control, irradiated hilsa, 300 krad, 600 krad, 900 krad stored at -20^{0}C, the TVN values were found to increase gradually from the moment of preservation. The initial TVN values were 14.88±.37 mgN/100g, 12.20±.65gmN/100g, 10.15±.65mgN/100g and 8.98±.08mgN/100g, respectively for control, 300 krad, 600 krad and 900 krad. And the TVN values were 101.02±2.73mgN/100g, 71.13±1.03mgN/100g, 59.33±57mgN/100g and 47.03±27mgN/100g respectively for control, 300krad, 600 krad and 900 krad at the end of 49 days storage periods. The total value of TVN was found 57.48±7.04mgN/100g, 42.18±4.88mgN/100g, 34.12±4.15mgN/100g and 27.15±3.22mgN/100g ($p<0.05$). It did not indicate and degree of spoilage but this value depends on non-protein nitrogen (NPN) of fresh fish (De and Nazrul 1964). It was observed that TVN value exceeds 30mgN/100g of muscle, the fish become unacceptable. Tanikawa (1935), Shewan (1975), Stansby *et al.* (1954) also found that the total volatile nitrogen (TVN) increased with the increase of time during spoilage and all of them suggested that 30mgN/100gm of fish muscle should be taken as the upper limit for acceptability.

The production of TMA was retarded considerably in the irradiated samples as compared to the control samples. The effects of irradiation were well effective in the rate of TMA accumulation. Suppression of TMA accumulation in the irradiated sample indicate that micro flora was capable of producing TMA were probably selected removed by irradiation (Change 1974). During the storage period of 49 days, the TMA values of all samples of -20^{0}C were comparatively lower due to retardation of enzymatic action at low temperature. Such pattern of spoilage was also obtained from the findings of Ahmed *et al.* (1981). Hussain *et al.* (2000, 2001). It was found from the present study that effect of treatments differ significantly ($p<0.05$).

It was found that in -20^{0}C storage temperature the population was increased with the increase of storage period. Bacterial density was shown at Table 5 at -20^{0}C storage temperature control, 300 krad, 600 krad, 900 krad irradiated fishes obtained 48,850±850cfu/g, 31,850±650acfu/g, 19,600±460acfu/g and 14,100±300acfu/g of fish muscle at the end of 49 days respectively. Hussain *et al.* (2001) has experimented the radiation effect of mackerel fish at low dose. He found that the irradiated degutted

sample remained acceptable up to 28 days at 4°C in the present investigation, TBC were lower than that of above mentioned findings but this result more or less supported to the present findings. Le *et al.* (2001) reported that irradiation significantly affected bacterial count. At the dose of 5KGY, shelf life was enhanced effectively by suppression of microbial growth and proliferation. Staving *et al.* (1966) reported that radiation dose 1 KGY to 3 KGY to be effective for reduction of bacterial load.

At -20°C storage temperature control, 300 krad, 600 krad, 900 krad irradiated fishes obtained 8,750cfu/g, 6,350cfu/g, 4,675cfu/g and 3,125cfu/g of fish muscles at the end of 49 days, respectively. From all of above mentioned discussion it can be concluded that irradiation (900 krad) in combination with -20°C temperature is the best method for long time preservation of fresh fish. Extension of shelf life at radiation doses 300 krad, 600 krad and 900 krad did not bring about any noticeable changes. And -20°C storage temperature is much more acceptable for long time preservation of fish.

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Effect of gamma radiation and freezing on the chemical and sensory changes in shrimp *Penaeus monodon* (Fabricius, 1798)

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Abstract
Gamma radiation (3, 6 and 9 kGy) in combination with low temperature (-20°C) were applied to retain the quality and shelf-life of shrimp, *Penaeus monodon* for a longer period. The quality was assessed by monitoring the chemical (TVN, TMA) and sensory changes in irradiated and non-irradiated (control) samples. Among chemical indicators of spoilage, total volatile nitrogen (TVN) values for irradiated shrimps were found to be 2.26, 2.18 and 1.57 mg N/100 g of sample at 3, 6 and 9 kGy respectively after 90 days whereas for nonirradiated samples it was found 2.45 mg N/100 g of sample. Trimethylamine (TMA) value for non-irradiated samples after 90 days were found 2.30 mg N/100 g sample whereas that for irradiated shrimps at 3, 6 and 9 kGy were found to be 2.10, 2.08 and 1.98 mg N/100 g sample respectively. The sensory scores of control sample were gradually decreased with the progress of storage period. From this study, it was clear that gamma radiation in combination with low temperature showed shelf-life extension (90 days) in each dose of radiation used but during the use of 9 kGy radiation, *P. monodon* showed best quality.

Key words: Gamma radiation, Freezing, Chemical properties, Shrimp

Introduction

Bangladesh has favorable conditions for shrimp farming. Fish is extremely perishable and requires quick preservation. In Bangladesh, considerable time required to reach the destination which varies according to location and often takes a considerable portion of normal shelf-life of lots of fish species (Coulter *et al.* 1987). To extend the shelf-life of fish and fishery products, ice storage and rapid chilling (Himelbloom et al., 1994) and rapid chilling, low temperature freezing, modified atmosphere packaging (Masniyom *et al.* 2002) organic acids, antimicrobials (Al-Dagal *et al.* 1999, Gelman *et al.* 2001) and ionizing radiation techniques are used (Venugopal *et al.* 1999). Shrimps are preserved by the application of low temperature but the shelf-life of preserved fish is not satisfactory. So the developed technique to extend shelf-life is essential to lessen the loss. Various experiments made it clear that no adverse health effects occur when irradiated foods are

consumed, using mean doses of irradiation of up to 10 kGy (Diehl *et al.* 1995, Rady *et al.* 1988). The validity of this technique is already recognized in many countries, including Canada, USA and the European Union (Tauxe *et al.* 2003) for many food products (Pszczola *et al.* 1990) including shrimps. Food irradiation inhibits sprouting, destruction of food borne insects and parasites, delay of physiological ripening and extension of shelf-life or improvement of food qualities (Kim *et al.* 2005).

Some chemical changes are produced by irradiation, which although lethal to living organisms like food borne bacteria (Alur *et al.* 1994), do not affect the nutritional quality of the food (Doyle *et al.* 1999) but results production of small amounts of radiolytic products (Merritt *et al.* 1988). These substances sometimes cause characteristic off-odors (Robins *et al.* 1991). Combination of food irradiation and freezing provide a means to increase shelf-life of fish products. In this study effect of gamma radiation (3, 6 and 9 kGy) in combination of refrigeration (-20°C) were applied on shrimp (*Penaeus monodon*) to observe the increase in shelf-life.

Materials and methods

Shrimp samples

Tiger shrimp used in this experiment were collected from Snow King Frozen Foods (Pvt) Ltd., Mirpur-1, Dhaka and the factory authority collected the tiger shrimp from Rupsa, under the District of Khulna. Then blocked (-20°C) tiger shrimp taken in a ice box and immediately brought in the laboratory of Food Processing & Preservation Division, IFRB, AERE, Savar, Dhaka. The entire samples were at first randomly divided into four lots: non-irradiated (control) and irradiated (3, 6 and 9 kGy). Control sample was kept at -20°C within polypropyl polythene bags for preservation purposes without radiation.

Irradiation

Samples were irradiated using a $Cobalt^{60}$ radiation source. Doses applied in this study were 3, 6 and 9 kGy. Before irradiation the samples were kept in polypropylene/polythene bags under atmospheric condition.

Storage conditions

The non-irradiated and irradiated samples were subsequently stored at -20°C. All samples were examined at the 0, 15, 30,45,60,75 and 90 days of storage period.

Chemical analysis

Conway microdiffusion technique was (Conway and Byrne 1933) employed for the estimation of TVN. Two gm sample was taken separately in conical flask from each batch. Then 10 ml of 10% Trichloacetic acid (TCA) solution was added with the sample and kept overnight. Then next day it was grounded in mortar and pastel and filtered through filter paper and volume made up to 50 ml in volumetric flask. Two ml of 2% boric acid solution was taken into the inner chamber of the Conway dish. Glass lid,

which was applied with grease surrounding the side way covering the Conway dish in such a way that outer chamber of the Conway dish was partially open. This was done to prevent the absorbed the N2 from atmosphere. Then 2 ml of sample extract was taken into outer chamber a finally 2 ml of saturated K_2CO_3 solution was added with sample. Then the lid was fixed immediately and left it over night at room temperature. On following day titration at the residual boric acid solution was done by standard N/70 H_2SO_4 solution through micropipette. Finally TVN values were calculated. In case of TMA, same procedure (procedure of TVN) was followed, except that 1 ml of 40% formaldehyde solution was added to outer chamber of Conway dish before adding K_2CO_3. The values of TVN and TMA were expressed in mg N/100 gm fish.

Sensory analysis

Sensory evaluation for the detection of freshness or shelf-life of the stored shrimp and consumer's acceptance was performed with high degree of reliability by sensory evaluation. Peryam and Pilgrim (1957) had developed a useful method for assessing the overall acceptability of food products. Nine points' hedonic scales were used for sensory evaluation by five judges (Miyauchi *et al.* 1964). Incase of sensory analysis, the shrimps were judged into some classes like appearance, colour, odour, texture, etc.

Statistical analysis

Analysis of variance (ANOVA) was employed to find out the level of significance between different treatments and days of storage. Effects of treatments were studied using ANOVA ($p<0.005$).

Results and discussion

Chemical analysis

TVN content of non-irradiated and 3, 6 and 9 kGy irradiated samples stored at -20°C is shown in Table 1. The initial TVN values were 1.05±0.01, 1.12±0.01, 1.17±0.01 and 0.88±0.01 mg N/100g respectively for control, 3, 6 and 9 kGy. The TVN values were 2.45±0.01, 2.26±0.01, 2.18±0.01 and 1.57±0.01 mg N/100g respectively for control, 3, 6 and 9 kGy at the end of 90 days storage periods. Comparing the treatment, the lowest TVN value was found in the 9 kGy treatment group while the highest value was measured in the control group. The TVN values were gradually increased with the progress of storage period. Under investigation best result of TVN value were found at the treatment dose of 9 kGy.

Tanikawa (1935), Yamamura (1938), Shewan (1942, 1975), (Stansby *et al.* 1954) and Ota (1985) also found that the total volatile nitrogen (TVN) increased with the increase of time during spoilage and all of them suggested that 30 mg N/100gm of fish muscle should be taken as the upper limit for acceptability. Wierzchowski (1956) estimated the acceptable limit of TVN in fresh water fish as 35mg N/100g of sample. According to Connel (1975) the acceptability of TVN was 30 to 35mg N/100g of sample.

Table 1. Total Volatile Nitrogen (TVN, mgN/100g) in control and irradiated tiger shrimp, *P. monodon* during 90 days storage period at -20°C

Duration (day)	Level of Radiation (KGy)			
	Control	3	6	9
Control	1.05 ± 0.01^{d}	1.12 ± 0.01^{e}	1.17 ± 0.01^{a}	0.88 ± 0.01^{d}
15	1.22 ± 0.01^{d}	1.28 ± 0.01^{de}	1.40 ± 0.01^{a}	1.06 ± 0.01^{cd}
30	1.58 ± 0.01^{c}	1.40 ± 0.01^{cd}	1.58 ± 0.01^{a}	1.16 ± 0.01^{c}
45	1.76 ± 0.01^{bc}	$1.64 \pm .001^{bc}$	1.75 ± 0.01^{a}	1.23 ± 0.01^{bc}
60	1.92 ± 0.01^{b}	1.86 ± 0.01^{b}	1.92 ± 0.01^{a}	1.28 ± 0.01^{bc}
75	2.28 ± 0.01^{a}	2.22 ± 0.01^{a}	2.11 ± 0.01^{a}	1.40 ± 0.01^{ab}
90	2.45 ± 0.01^{a}	2.26 ± 0.01^{a}	2.18 ± 0.01^{a}	1.57 ± 0.01^{a}

Rows within column and rows means (± SEM) with different letters denote significant differences ($p<0.05$); where different letter indicates significance between and within column

From the present investigation it was found that the TVN values were acceptable throughout the storage period. The TVN values were gradually increased with the progress of storage period. Under investigation best result of TVN value were found at the treatment dose of 9 kGy. The combination of irradiation and low temperature preservation used in this study results in very low level of TVN production after a storage period of 90 days. At the beginning of storage TMA value of control, 3, 6 and 9 kGy treated samples were 0.84±0.01, 0.87±0.01, 1.05±0.01 and 1.05±0.01 respectively. After 90 days storage period TMA value of that storage samples were 2.30±0.01, 2.10±0.01, 2.08±0.01 and 1.98±0.01 respectively. Comparing the TMA value, the lowest value of TMA was found in the 9 kGy treatment group while the highest value was measured in the control group (Table 2).

Table 2. Trimethylamine (TMA, mg N/100 g) in control and irradiated tiger shrimp, *P. monodon* during 90 days storage period at -20°C.

Duration (day)	Level of Radiation (KGy)			
	Control	3	6	9
Control	0.84 ± 0.01^{f}	0.87 ± 0.01^{e}	1.05 ± 0.01^{d}	1.05 ± 0.01^{e}
15	1.05 ± 0.01^{ef}	1.03 ± 0.01^{e}	1.22 ± 0.01^{d}	1.28 ± 0.01^{d}
30	1.35 ± 0.01^{de}	1.28 ± 0.01^{d}	1.40 ± 0.01^{c}	1.40 ± 0.01^{cd}
45	1.57 ± 0.01^{cd}	1.40 ± 0.01^{cd}	1.57 ± 0.01^{c}	1.52 ± 0.01^{c}
60	1.75 ± 0.01^{bc}	1.63 ± 0.01^{bc}	1.84 ± 0.01^{b}	1.75 ± 0.01^{b}
75	1.92 ± 0.01^{b}	1.86 ± 0.01^{b}	1.95 ± 0.01^{ab}	1.86 ± 0.01^{ab}
90	2.30 ± 0.01^{a}	2.10 ± 0.01^{a}	2.08 ± 0.01^{a}	1.98 ± 0.01^{a}

Within column and rows means (± SEM) with different letters denote significant differences ($p<0.05$); where different letter indicates significance between and within column and row.

TMA is one of the volatile basis compounds, which is found in very low amount in freshwater fishes but this accumulates in spoiling marine fishes, as a result of bacterial reduction of TMAO (Trimethylamine oxide), reported by Ahmed *et al.* (1988). According to Yamamura (1938), in case of marine fish acceptable limit of TMA is 30 mgN/100gm of fish. But according to Connel (1975) and Huss (1986) suggested that the TMA value ranged from 10-15mgN/100g of fish muscle was the upper limit of acceptability. The production of TMA was retarded considerably in the irradiated samples as compared to the control samples. The effect of irradiation was well effective in the rate of TMA accumulation. Suppression of TMA accumulation in the irradiated samples indicated that micro flora was capable of producing TMA were probably selectively removed by irradiation (Chang 1974). During the storage period of 90 days, the lowest TMA value found in 9 kGy irradiated sample and storage of sample at -20°C after irradiation gives better result and is more acceptable. Such pattern of spoilage was also obtained from the findings of Ahmed et al. (1981), Hossain *et al.* (2000, 2001). It was found from the present study that effect of treatments differs significantly ($p < 0.05$).

Sensory analysis

Muscle of live fishes is more or less sterilized but after death autolytic, bacterial and other changes occur. The physical changes could be perceived with sense organs. These changes are the average of over all acceptability in respect of appearance, odor, color, texture. Organoleptic scores (OS) or sensory scores were shown in Table 3. Sensory scores of Tiger shrimp come down 9.00±0.01 to 6.00±0.15, 9.00±0.01 to 6.75±0.05, 9.00 ±0.01 to 7.07±0.04 and 9.00±0.01 to 6.58±0.07 in control, 3, 6 and 9 kGy respectively after 90 days of storage period. The sensory score were gradually decreased with the progress of storage period. Under investigation best result of sensory scores were found at the treatment dose of 6 kGy.

Table 3. Organolepticscores in control and irradiated tiger shrimp, *P. monodon* during 90 days storage period at -20°C.

Duration (day)	Level of Radiation (KGy)			
	Control	3	6	9
Control	9.00 ± 0.00^{a}	9.00 ± 0.00^{a}	9.00 ± 0.00^{a}	9.00 ± 0.00^{a}
15	8.25 ± 0.05^{b}	8.55 ± 0.10^{b}	8.60 ± 0.05^{b}	8.80 ± 0.05^{a}
30	8.00 ± 0.15^{bc}	8.10 ± 0.01^{c}	8.18 ± 0.02^{c}	8.37 ± 0.07^{b}
45	7.45 ± 0.05^{cd}	7.75 ± 0.05^{cd}	8.13 ± 0.03^{c}	8.03 ± 0.03^{c}
60	7.05 ± 0.10^{de}	7.35 ± 0.10^{de}	7.68 ± 0.03^{d}	7.18 ± 0.08^{d}
75	6.55 ± 0.20^{ef}	6.95 ± 0.10^{ef}	7.28 ± 0.07^{e}	6.90 ± 0.05^{d}
90	6.00 ± 0.15^{f}	6.75 ± 0.05^{f}	7.07 ± 0.04^{e}	6.58 ± 0.07^{e}

Within column and rows means (± SEM) with different letters denote significant differences ($p<0.05$); where different letter indicates significance between and within column and row.

Ingram and Rhodes (1962) claimed that the higher dose of radiation in the vicinity of 3 and 5 kGy had cause noticeable changes in odor, texture and appearance of fish. Desrosier (1963) observed that gamma irradiation prevented the microbial growth by sterilization the microorganisms and the rays also had effect on protein, fat, lipid and enzymes in fish. (Miyauchi *et al.* 1964) suggested that the average sensory score of 5 might be accepted in case of sensory test. Thus the control and treated sample of Tiger shrimp was acceptable up to 90 days of storage period.

In this study, it was found that with the increase of storage period the sensory score rapidly decreased in the control shrimp than those irradiated shrimp of different doses. Tiger shrimp irradiated with 6 kGy has the longer shelf-life than other treatments and then 9 kGy and 3 kGy. Due to microbial spoilage with the increase of storage period, the appearance, odor, color and texture deteriorate and the sensory scores decreases. Results of 6 kGy is better than 9 kGy which might be due to the fact that high dosing of irradiation damage the texture.

Due to lack of proper techniques of preservation, a significant amount of shrimps and prawns have been lost every year. So, inquest new and pertinent technology of preservation is essential to protect this loss worldwide. Various factors contribute to the spoilage and deterioration in the quality of the products. In the present study, attention was paid to investigation the effects gamma irradiation on frozen tiger shrimp. To extend the shel- life, Tiger shrimps were treated with gamma irradiation (3, 6 and 9 kGy) and stored at low temperature (-20°C) for 90 days for determining the shelf-life extension of these shrimp samples. Some parameters such as sensory score, total volatile nitrogen (TVN), Trimethylamine (TMA) were evaluated in every 15 days interval. The maximum shelf-life was found with radiation dose of 9 kGy. From the study, it can be concluded that irradiation (9 kGy) in combination with low temperature is an efficient method for long time preservation.

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Embryonic and larval development of *Mystus gulio* (Ham.)

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Abstract
Mystus gulio eggs are strongly adhesive and contain relatively small yolk (0.75~1.0 mm). The egg envelop is thick and transparent. First cleavage (two cells), four cells, eight cells, sixteen cells and multi cells stages were found 20, 25, 35~40, 60 and 70 minutes after fertilization, respectively. The morula stage was visualized within 1.5 h after fertilization. The heart beat visible and the circulatory system commenced after 16 h of fertilization. Embryos hatched 18~20 h after activation of egg. The newly hatched larva measured 2.82±0.03 mm in length and 0.32±0.06 mg in weight. The yolk sac was fully absorbed by the third day though larvae commenced exogenous feeding even before completion of yolk absorption. A 5-day old post larva began wandering in search of food. Ten-day old post larvae endowed with eight branched rays in dorsal fin and seven in caudal fin. Fifteen-day old post larvae had the pectoral spine become stout though the embryonic fin folds had to be disappeared. The length of fingerlings ranged from 25~30 mm after 30 days, and their external features were just like those of an adult except that they were not sexually matured.

Key words: Embryonic development, Larval development, Ontogenic events, *M. gulio*.

Introduction

Nuna Tengra, *Macrones gulio* renamed *Mystus gulio* (Ham.) is a native catfish of family Bagridae and distributed around India to Malay Archipelago especially in estuarine and tidal waters (Jhingran 1997). The decline in total catch of the species is in a state of vulnerable (IUCN, 2000 and Mijkherjee, 2002) in the nature. It is having high market demand and delicious in taste and it has an emerging trend as an aquaculture species in South-west Bangladesh. Despite a paucity of information available about its biology, except some observations on the fecundity, induced spawning, spawning behavior and larvae rearing of *M. gulio* by Sarker *et* al (2002), Alam *et al* (2006a and 2006b) and Islam *et al* (2007), hence quests have been made to study its embryonic and larval development. The present work was undertaken as a part of a study of the catfish, especially to observe early developmental stages.

Materials and methods

Mystus gulio brooders were collected from the reservoir ponds of the brackishwater station (BS) located in the south-west part of Bangladesh using cast nets. After a brief dip in potassium permanganate, the brooders were acclimatized in cisterns (4.5 X 1.5 X 1 m) without food. Spawning was introduced by an intramuscular injection of 1 ml/kg body weight of ovaprim (Alam *et al*, 2006a). Each breeding set consisting of two males and one female was released into a breeding hapa (1.2 x 0.9 x 0.9 m) after injection. The behavior of brooders was observed frequently after injection and the spawning activity appeared to continue first after some 5 hours post injection, though its latency period is 6-8 hours (Alam *et al*, 2006b). Fertilized eggs were collected every hour with the help of a dropper from the breeding hapa and observed its developmental stages. The eggs hatched out first after 18 hours of fertilization. When about 80% of hatchlings were observed with their absorbed yolk sac, feeding was given with boiled and screened hen's egg yolk. Newly hatched larvae and thereafter was also observed its developments. The lengths of the hatchlings were measured by ocular micrometer (1/100 fraction of a millimeter). Descriptions of the developing stages were made on the basis of examining eggs and larvae under microscope (Brand: CETI with JVC closed circuit digital camera and monitor) and digital still photographs of the developmental stages of eggs and larvae were also made.

Results

In the present study, spawning was observed within 6~8 hours after injection of the hormone. Fertilized eggs (Fig. 1) of *M. gulio* were adhesive, demersal and spherical in form. The yolk sphere contained no oil globule. Due to the adhesive nature of the eggs, considerable debris adhered to the capsule of the egg. The grayish-white egg capsule was translucent, where the yolk was brownish. The eggs became opaque as development progressed. The diameter of the egg capsule ranged 0.75~1.0 mm, while the yolk sphere ranged from 0.7~0.9 mm. The developmental stages of *M. gulio* were divided into six stages: embryo, hatchlings, larva, post larva, fry and fingerlings (Jhingran and Pullin, 1985), with each stage having typical anatomical and physiological features. A summary of the timing of the important ontogenic events and structures is presented in Table.1.

Embryo

The time required to develop from the first cleavage to formation of an embryo was about 1 h. The embryonic development of *M. gulio* was usually completed within 18~20 h after fertilization. The first cleavage commenced 20 min after fertilization when the blastodisc divided into two blastomeres (Fig. 2). Within another 5 min, the four cell stage was obvious. The eight cell stage was reached after 10~15 min. Sixteen blastomeres (Fig. 3) were noticeable within the next 20 min, and the number of cells doubled (64-cell stage) in the following 10 minutes. The morula stage (Fig. 4) was visualized within 1.5 h after fertilization. By about the seventh hour, the head and tail

ends of the embryo were distinguishable (Fig. 5). Myomers differentiated between 9 and 11 h of development (Fig. 6). In the 15-somite stage, the optic vesicles appeared, and in the 14th to 16th hours (Fig. 7).

Table 1. Ontogenic events in the early developmental stages of *M. gulio*.
(Each value is the average of five observations)

Age (h)	Ontogenic events
	Cleavage
0.2~1.0	2~16 cells stages
1.0~1.5	Morulla stage
	Formation of embryo
1.5~2.0	Blastulla stage
2.0~3.5	Germinal ring formed; embryonic shield formed; and more than half of yolk invaded
3.5~5.0	Yolk invasion two-thirds complete
5.0~7.5	Yolk invasion complete
	Differentiation of embryo
9.0	Embryonic rudiment distinct
11.0	2~3 myomers; eye vesicle demarcated
12.0	7~8 myomers; heart rudiment visible; demarcation of brain
14.0	12~17 myomers; heart and tail differentiated
16.0	Entire space inside egg occupied by embryo; heart beat visible; tail begins to separate from the yolk; blood circulation commenced; embryo making frequent movements
18.0	Hatching begins
20.0	Larva hatched (almost 80%)
	Larva (post hatching)
Newly hatched	2 mm long; unpigmented eyes; no fin buds; mouth not yet formed Head and body faintly yellow
3~8	3 mm long; displaying unpaired dorsoventral fin; heart and brain distinct; yolk sac elongated
12~20	Caudal fin begins to separate; pigmentation of eyes; alimentary canal distinct; pectoral fin buds appear
25~35	Mouth beginning to differentiate; pigmentation of body
40~48	Mouth opens; jaw movements begin; barbles formed
	Post larva
Third day	4 mm long; head prominent; yolk sac absorbed
Fifth day	5 mm long; body grayish black; pectoral fin clearly recognizable

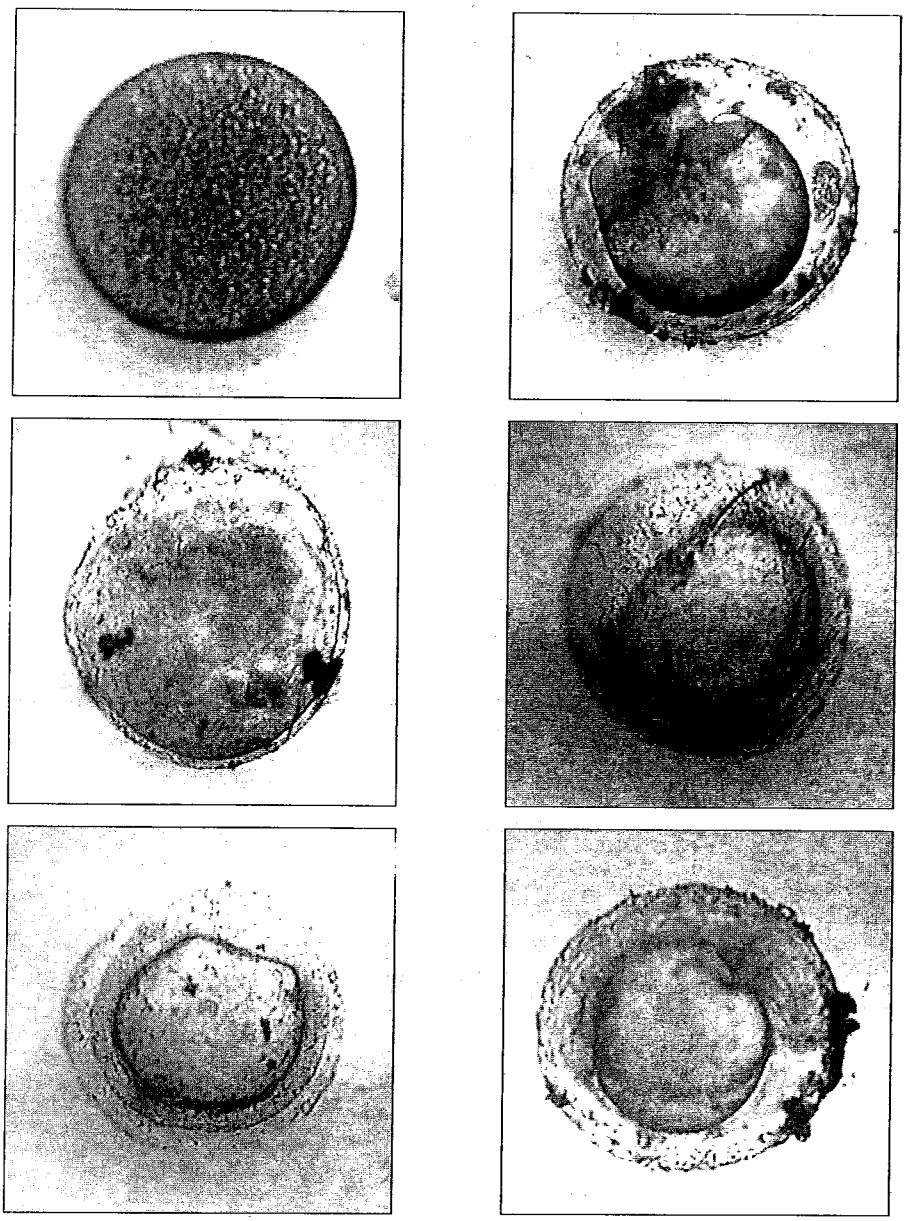

Plate 1. Developmental stages of *M. gulio*. Fig.1: Fertilized egg; Fig.2: Formation of two blastomeres; Fig.3: Sixteen cells stage; Fig.4: Morula stage; Fig.5: Seven-hour old embryo; Fig.6: Ten-hour old embryo.

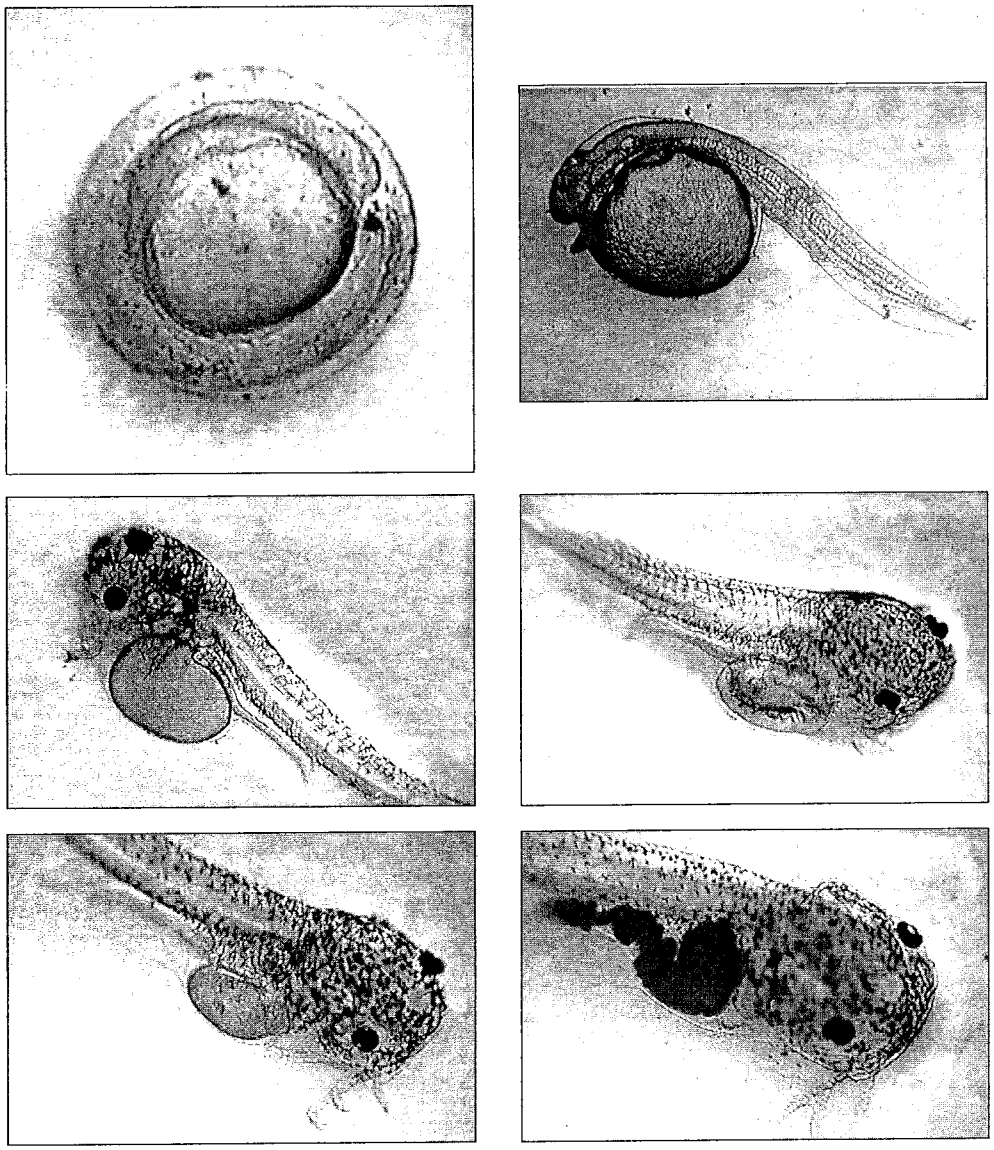

Plate 2. Developmental stages of *M. gulio*. Fig.7: Optic vesicle appeared (14^{th}~16^{th} h); Fig.8: Just hatched out larva; Fig.9: Six-hour old larva; Fig.10: One-day old larva with tiny protuberance of barbles; Fig.11: Two-day old larva with urinogenital opening distinct; Fig.12: Three-day old larva commenced exogenous feeding.

In the final stage of embryonic development, the growing embryo occupied the entire previtteline space and by about 1.5~2.0 h before hatching, it exhibits frequently twitching movement. After a pause of about 30 sec, this frequent movement suddenly culminated in a violent jerk breaking the previtelline membrane and the hatching emerged tail first (Fig. 8).

Hatchling

Length and weight measurements of newly hatched larva are given in Table 2. Hatchlings of *M. gulio* showed a laterally compressed transparent body, characterized by the presence of an almost round yolk sac, occupying about one-third of the total length. Chromatophores were completely absent. The mouth, alimentary canal and gills were not yet differentiated. The primitive streak of the notochord were quite prominent; about 25~27 myomers were distinct and another 7-8 were apparent in the tail region. The tip of the tail was rounded and the fin fold was differentiated, but not very distinct. Newly hatched larvae were not that active and generally they moves with water flow and few of them remain resting on the sides of the hapa where water current is much lower.

Larva

A relatively broad space appeared between the head and anterior margin of the yolk in 2~3 h old larvae (Fig. 9). This space facilitated the accommodation of the developing heart. Buccal invagination was appeared in 6~8 h old larvae and the alimentary canal formed as a straight tube emerging from the posterodorsal aspect of the yolk sac. The anal opening was closed and was situated between 10^{th} and 12^{th} myomers, which was about half of the length of the larva at this stage. The heart commenced to bit at a rate of 105~110 beats/min. Barbles appeared in the form of tiny protuberance in 1-day old larvae (Fig .10). The upper and lower jaws were formed, and the lower jaw showed occasional movements. The urinogenital opening was distinct and situated just posterior to the anal opening in 2-day old larvae (Fig.11). The heart beats at a rate of 120~130 beats/min. The pectoral fin buds appear as a moderate elevation. Intestinal coiling of the alimentary canal was noticeable. The yolk was exhausted by the end of the third day of development, and larvae commenced exogenous feeding even before completion of yolk absorption (Fig .12).

Post larva

In 5-day old post larvae, streaks denoted rudimentary rays, which appear in the caudal fin. The pectoral fin was differentiating and was in the form of a flap just behind the operculum; at this time, sidewise movement of the larvae commenced. The yolk was completely absorbed and began wandering in search of food. The phenomenon of aerial respiration began on the seventh day of development. Ten-day old post larvae endowed with eight branched rays in the dorsal fin and seven eight in the caudal fin, and at this stage, the outline of the brain in the cranial cavity could clearly be seen under a microscope. Fifteen-day old post larva showed seven-eight anal fin rays, and the pectoral spine had become stout. The embryonic fin folds had yet to disappear. Vertebral

segmentation of the notochord took place with distinct neural and hemal spines especially in the caudal region. Pigmentation was more pronounced throughout the head and body.

Table 2. Average measurement of hatchlings and post larva of *M. gulio*

Aging	Length (mm)	Weight (mg)
At hatching	2.82±0.03	0.32±0.06
10~12 h old hatchlings	3.16±0.06	0.47±0.04
1-day old hatchlings	3.36±0.09	0.65±0.08
2-day old hatchlings	4.05±0.25	0.87±0.08
3-day old hatchlings	4.63±0.08	0.98±0.08
4-day old hatchlings	5.65±0.07	1.15±0.07
5-day old hatchlings	5.87±0.08	1.25±0.07
10- day old hatchlings	14.87±1.45	55.68±3.21
15- day old hatchlings	17.64±2.11	89.63±4.52

Fry

Twenty-day-old fry ranged 19.0-21.0 mm in length. Fry swam actively and were observed to voraciously feed on plankton. Fry displayed a dorsal fin with branched rays. The body became dark due to the accumulation of pigments.

Fingerling

This stage began on the 30th day and lasted for the next 15 days. Thirty days after hatching, the pectoral, pelvic, dorsal, caudal and anal fins showed seven-eight, six-seven, eight, nineteen and seven-eight rays, respectively, representing the full complement of rays. On day 30, fingerlings were 25.0–30.0 mm in total length and externally resembled the adult suggesting the end of the fingerling stage.

Discussion

Changes in the pattern of the entire structure of an organ or of a specific organ in relation to the environment are decisive for evaluating the developmental pattern of a species (Balon 1999). Since the egg envelop is thick, translucent and sticky, observations on the development of *M. gulio* are difficult (Kovac 2000). Ontogenic events during the ovular phase (cleavage stage) did not markedly differ from those in *Heteropneustes fossilis* or *Channa marulius* (Khan 1926, Mookherjee 1945). Changes in structure emphasized the thresholds between embryonic, larval and post-larval development from the onset of cleavage, or at the time of organogenesis, respectively (Kovac 2000, Carlos *et al.*, 2002).

The first cleavage was found 30 min after fertilization in *Nandu nandus* and 20 min in *Ompok pabda* (Das *et al.* 2002 and Kohinoor *et al.* 1997). The yolk sac of *M. gulio* was fully absorbed by the third day, when the larvae measured 4.63±0.08 mm and weighing 0.98±0.08 mg (Alam *et al.* 2006). Whereas, yolk sac absorption was completed in 56 hrs and 48 hrs for *N. nandus* and *O. pabda,* repectively (Das *et al*, 2002 and Kohinoor *et al*,

1997). Disappearance of yolk sac for other similar catfishes like *Mystus montanus* (Raj *et* al, 2003) and *Mystus macropterus* (Wang *et al* 1992) was found also in the third day.

Fish farmers are much less familiar with the culture of catfish species because of the lack of breeding and feeding techniques and non availability of seeds from the wild (Meehan 2002). Despite this small scale operation have been attempted for *M. gulio* and the culture of other catfishes e.g. *Heteropneustes fossilis* and *Clarias batrachus* have been achieved in the past (Marguiles 1997). The high fecundity, short embryonic period, fast development of sense organs of *M. gulio* suggest that it may be a suitable species for commercial seed production.

Acknowledgment

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Species diversification in coastal aquaculture: Production potentials of shrimp (*Penaeus monodon*) with mono and mixed sex tilapia

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Abstract
An experiment of 120 days of culture was conducted in brackishwater earthen ponds having an area of 0.2ha each. The hatchery produced shrimp (*Penaeus monodon*) post larvae were stocked in the 40m^2 fine meshed nylon net nursery enclosures were fed with commercial pellet feed. After two weeks of nursing, juveniles were allowed to spread in cultural pond by opening the fence. Fingerlings of three different strain of tilapia were stocked as shrimp and Strain-1 all male (monosex) (T_1), shrimp and Strain-2 all male (T_2), shrimp and Strain-3 mixed sex population (T_3) @ 20,000/ha and 10,000/ha, respectively and shrimp only (monoculture) (T_4) @ 20,000/ha. The shrimp and fish were fed with farm made feed consisting of a mixture of fishmeal 29%, MOC 15%, rice bran 30%, soybean meal 16%, wheat flour 9% and vitamin premix 0.1%. The average final weight of shrimp was 24.9±1.13g, 23.41±3.26g and 26.67±1.89g that stocked with tilapia in treatments T_1, T_2, and T_3 respectively. The final average weight of shrimp in monoculture (T_4) was 27.41±0.76g, apparently higher but insignificant in treatments. The survival of shrimp was 42.17%, 32.38%, 39.45% and 61.98% in treatments T_1, T_2, T_3 and T_4 respectively. The production of shrimp in concurrent culture was 193.67, 154.26 and 210.41kg/ha in T_1, T_2 and T_3, respectively, while in monoculture (T_4) was 339.77 kg/ha. The growth and survival of tilapia among the treatments was insignificant. The growth of monosex tilapia ranged 225.29 and 291.31g and survival 62.77 and 72.20% in T_1 and T_2, respectively, in mixed sex was 193.0g and 83.20% (T_3). The production of tilapia monosex strains was 1676.69kg/ha (Strain-2 all male) and 1668.98 kg/ha (Strain-1 all male) while that of Strain-3 mixed sex population was 1622.92 kg/ha.

Key words: Species diversification, *Penaeus monodon,* Monosex tilapia

Introduction

Due to onset of viral disease and environmental degradation, the vertical productions of shrimp (*Penaeus monodon*) are interrupted. Diversification of culture with other than brackishwater but some hyopsaline suitable species in shrimp farming might be an important tool to safeguard crop loss of shrimp. Appropriate species culture practices,

that could suit to shrimp farming for better output. The growth and production of shrimp with some species (*Liza parsia*, *Rhinomugil corsula*, *Mugil cephalus*, *Pangasius hypophthalmus*, *Barbados gonionotus*) have been tried (Hossain *et al.* 1994, Ali 2000, Shofiquzzoha *et al.* 2001, 2003, Yang and Fitzsimmons 2007) while, the culture of tilapia (*Oreochromis* sp.) particularly GIFT with *P. monodon* in shrimp farms seems to be potentials (BFRI 2007).

Except GIFT, some other genetically improved strains of tilapia monosex all male *viz.*, Thai BD-1, GenoMar Supreme Tilapia™, BanglaFISHGEN etc. which are commercially marketed by some private firms and demanded successfully culture in freshwater. These strains were not yet been assessed on production and growth in brackishwater environment in coastal shrimp farms. The present experiment dealt with comparison of growth and production of mixed and mono sex tilapia strains *viz.*, BanglaFISHGEN all male (Strain-1), GenoMar all male (Strain-2) and BFRI-GIFT mixed sex population (Strain-3), in concurrent culture with shrimp.

Materials and methods

The experiment was conducted during March to June 2007 at the Brackishwater Station of the Bangladesh Fisheries Research Institute, Paikgacha, Khulna. Eight ponds of 2,000 m^2 each in the pond complex were selected and made ready. A 40m^2 nursery area encircled with nylon net fastened with bamboo fence was setup in each pond for nursing shrimp post larvae. The ponds were prepared by sun drying followed by liming with CaO @ 400 kg/ha and by fertilizing with mustard oil cake (MOC) @ 250 kg/ ha and TSP and urea (2:1) @ 35 kg/ha, respectively. After 7 days, the ponds were filled up to a depth of 80cm with tidal brackishwater from nearby the Sibsha river was entered into the ponds through a feeder canal and awaited for a week period for suitable water conditions.

Hatchery produced PCR tested shrimp (*P. monodon*) post larvae (PL) were stocked in the nursery enclosure and fed with commercial shrimp starter feed. After two weeks of nursing, at the 15th day, juveniles were allowed to spread in cultural pond by opening the fence. Fingerlings of different strains of tilapia were stocked at the 5th week of shrimp stocking. Under four different treatments, each two ponds were stocked with shrimp and Strain-1 (BanglaFISHGEN) all male (T_1), shrimp and Strain-2 (GenoMar) all male (T_2), shrimp and Strain-3 (BFRI GIFT) mixed sex population (T_3) @ 20,000 /ha and 10,000/ha, respectively while two ponds with shrimp only (T_4) @ 20,000/ha. The initial weight of shrimp was 0.005±0.001g and for Strain-1, Strain-2 and Strain-3 were 1.34±1.13g, 0.21±0.05g and 0.74±0.13g, respectively.

The shrimp were fed commercial pellet feed (Soudi-Bangla) and fish were fed with common prepared feed (approx. protein content, 30%) consisting of a mixture of fishmeal 29%, MOC 15%, rice bran 30%, soybean meal 16%, wheat flour 9% and vitamin premix 0.1%. Feed together was supplied twice daily @ 5-3% of shrimp and fish standing crop/day, twice at dawn and dusk.

The physicochemical parameter *viz.*, air and water temperature, salinity, pH and transparency (Secchi-disk reading) of the ponds were monitored weekly during the experimental period and growth of shrimp and fish were monitored fortnightly.

After 120 days of culture, both shrimp and fishes were harvested by de-watering the ponds and the growth, survival and production were estimated.

Data was compiled and analyzed using software MS Excel and following Zaman *et al.* (1982). The specific growth rate (SGR%) was estimated as per Dhawan and Kaur (2002) following the formula given below.

$$SGR\% = \frac{\ln Final\ \ weight\ (g) - \ln Initial\ \ weight\ (g)}{Number\ \ of\ culture\ \ days} X\ 100$$

Results and discussion

The physico-chemical parameters of the pond water are shown in Fig. 1. It shows that, water temperature varied from 26^0 to 32^0C (Fig.1a). Salinity was ranged from 8.0 to 17‰ (Fig.1b) and water pH was within 7.0-9.0 during the experimental period (Fig.1c). Water transparency was varying from 17.0 to 70.0 cm (Fig.1d). There was no significant difference in physico-chemical parameters of water among the treatments and congenial for shrimp culture is agreement with Grey (1990) and Jung and Co (1988). The body lesion of tilapia was observed when salinity of water increased more than 15 ppt agrees with scientists. Ridha (2008) reported similar observation when of tilapia strains were weighted over 200g and cultured in salinity above 20ppt for 34 days.

Variations in growth rate of shrimp and tilapia strains under different treatments are shown in Fig. 2. No significant growth variations of shrimp and tilapia strains among the treatment were observed. Shrimp attained its higher average weight in T_4 (27.41g) in monoculture, followed by T_3 (24.67g) shrimp with Strain-3 mixed population, T_1 (23.90g) with Strain-1 all male and T_2 (23.41g) with Strain-2 all male (Table 2).

The specific growth rate (SGR%) of shrimp was 7.06, 7.04, 7.09 and 7.17% in treatments T_1, T_2, T_3 and T_4, respectively. However, the SGR for the tilapia strains was 4.27, 6.03 and 4.59% was observed for Strain-1 all male, Strain-2 all male and Strain-3, respectively (Table 1). The final weight of tilapia strains was 225.29, 291.31 and 193.10g for Strain-1 all male, Strain-2 all male and Strain-3, respectively. The survival of shrimp was higher (63.33%) in treatment T_1, followed by T_4 (61.98%), T_3 (59.45%) and in T_2 (58.38%). Production of shrimp was higher of 339.77 kg/ha in monoculture (T_4), followed by 293.33, 290.02, 273.34 kg/ha in concurrent culture with Strain-1 all male (T_1), Strain-2 all male (T_2) and Strain-3 mixed sex pop^n (T_3), respectively. The survival, final weight and production of shrimp among treatments were insignificant. The estimated FCR value was 1.75, 1.73, 1.92 and 2.30 in treatments T_1, T_2, T_3, and T_4, respectively found comparative lower than Ridha (2008) but higher than Kamal and Mair (2005).

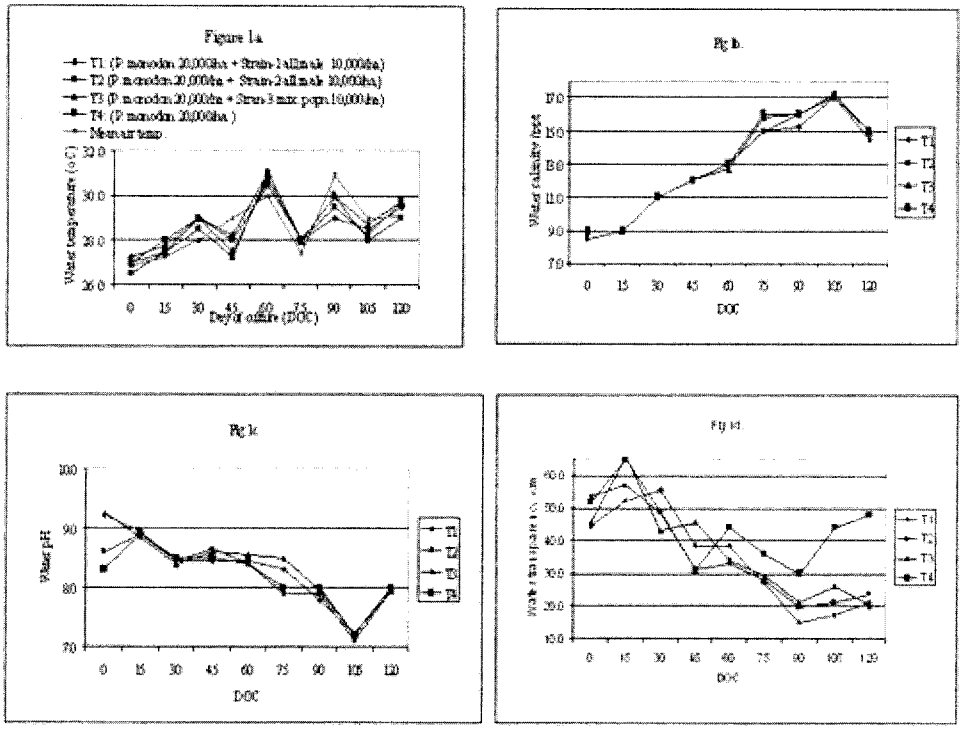

Fig 1: The variations in physico-chemical parameters of water of the experimental ponds under different treatments

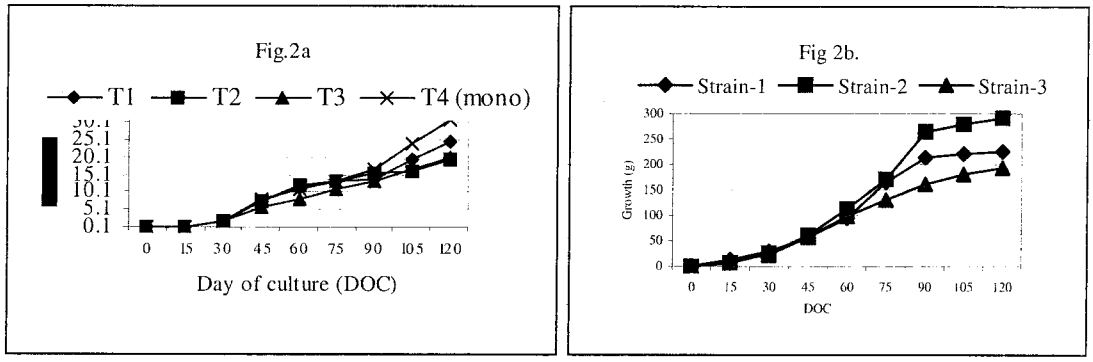

Fig. 2. Growth attainment of a. *P. monodon* and b. three different tilapia strains in concurrent culture.

Table 1. The growth, production characteristics and economics of shrimp and different tilapia strains under concurrent culture system

Treat-ments	Species	Growth attained (g)	SGR%	Survival (%)	Production (kg/ha)	FCR	Total cost (Tk./ha)	Gross return (Tk/ha)	Net return (Tk/ha)	BCR
T_1	*P. monodon*	23.90	7.06	63.33	290.02	1.75	1,43,230.00	S- 84,775.00 (38.84%)	74989.00	1.52
	Tilapia Strain-1 all male	225.29	4.27	72.22	1668.95			F- 1,33,516.00 (61.16%)		
								T- 2,18,291.00		
T_2	*P. monodon*	23.41	7.04	58.38	273.34	1.73	1,36,216.00	S- 73,259.00 (24.00%)	79203.00	1.58
	Tilapia Strain-2 all male	291.31	6.03	62.77	1676.69			F- 1,42,160.00 (66.00%)		
								T- 2,15,419.00		
T_3	*P. monodon*	24.67	7.09	59.45	293.33	1.92	1,34,914.00	S- 85,559.40 (39.72%)	80479.00	1.60
	Tilapia Strain-3 mixed pop^n	193.10	4.59	83.20	1622.92			F- 1,29,833.60 (60.28%)		
								T- 2,15,393.00		
T_4	*P. monodon*	27.41	7.17	61.98	339.77	2.30	85,615.00	T-1,35,720.00	50,105.00	1.59

Taka 70.0= 1.0US$

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Suitability of commercially available *Bacillus* probiotics on growth, survival and production of black tiger shrimp (*Penaeus monodon* Fab.)

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Abstract
Different forms of *Bacillus* probiotics was assessed in the earthen ponds on tiger shrimp (*Penaeus monodon*) culture. The experiment was designed with three different treatments depending on the mode of application (T1= oral probiotics; T2= spreading probiotics and T3= oral+ spreading probiotics). The shrimp was cultured for 120 days with the stocking density of 6-PL/m^2. Oral probiotics in the respective ponds were supplied with feeds. Whereas, spreading probiotics was applied to the pond water during pond preparation at 30, 60 and 90 days of culture period. Results of the experiment revealed that, all forms of *Bacillus* probiotic had effective role to keep the culture environment friendly in terms of mineralization of organic matter, nitrogen and phosphorus content in bottom sediment; holding of water transparency in a congenial state, increasing the density of planktonic biomass and boosting the THB-*Vibrio* ratio in water and sediment with insignificance ($p>0.05$) difference between different treatments. Whilst, spreading form of *Bacillus* probiotic showed higher weight gain (27.58±1.18g), survival rate (70.75±8.54%) and production (1167.66±109.62 kg/ha) and expected lower FCR (1.81±0.06) values with significant difference ($p<0.01$) with others methods of application, indicated its superiority in tiger shrimp culture.

Keywords: Probiotics, *Penaeus monodon*, shrimp culture

Introduction

Shrimp aquaculture particularly the culture of *Penaeus monodon*, has expanded rapidly throughout Asia in recent decades. Bangladesh has a trend of expanding culture areas as it has a high value of sea food products in the international market. One of the critical issues of shrimp culturists today involves the management of water and the volume of organic sludge being created within the pond during rearing periods and later being released into surrounding waterways. The use of probiotics in aquaculture is increasing with the demand for more environment friendly aquaculture practices (Gatesoupe 1999). A growing concern for the high consumption of antibiotics in aquaculture has initiated a search for alternative methods of disease control. In aquaculture, particularly

for shrimp, the use of beneficial bacteria such as probiotics is gaining in importance as an alternative to chemicals or antibiotics uses. A probiotics is generally defined as a live microbial support which improves the balance of the environment's micro flora (Fuller 1989). It has shown to have several modes of action in aquaculture: competitive exclusion of pathogenic bacteria through the production of inhibitory compounds; improvement of water quality; enhancement of immune response of host species and enhancement of nutrition of host species through the production of supplemental digestive enzymes (Thompson *et al.* 1999, Verschuere *et al.* 2000). In shrimp aquaculture, probiotics can be administrated either as a food supplement or as an additive to the bottom sediment via water (Moriarty 1998). *Bacillus* bacteria have the putative activities due to secretion of many exoenzymes (Moriarty 1996, 1998). The present study focused the suitability of different type (oral or spreading) of *Bacillus* probionts on micro flora, water and sediment quality management, growth, survival and production of tiger shrimp under zero water exchanged culture condition.

Materials and methods

Suitability of different forms (oral and spreading) of probiotics (beneficial microbs of *Bacillus spp*) on the culture environment, growth performance and production of tiger shrimp (*Penaeus monodon*) was assessed by a field level experiment. The experiment was designed with three different treatments (T1= oral probiotics; T2= spreading probiotics and T3= oral+spreading probiotics). Each treatment had three replications. The experiment was conducted in the earthen ponds having an average area of 0.1 ha each located in the pond complex at Brackishwater Station of Bangladesh Fisheries Research Institute. The experiment was designed for a culture period of 120 days with the stocking density of 6 PL/m^2, which was a bit higher than that proposed by Islam and Alam (2008) under modified improved culture system.

The ponds were prepared through drying, liming the bottom soil (@ 250 kg·ha of Ca_2O) and installment of in-pond nursery enclosure by fine nylon mosquito net and bamboo frame (about 10% of culture area in each pond). Ponds were filled up with tidal water to a depth of 90-110 cm and treated with Phostoxin (@1Tablet/20-25 ton of water) to kill all unwanted animals introduced with tidal water. After then fertilization was done with urea @1.0ppm, TSP @2.0ppm, MP @0.5ppm and molasses @3.0ppm to supplement the primary nutrient for primary producers. After 7 days of fertilization, stocking of shrimp post larvae (ABW 0.007g) was done within the prepared nursery enclosure in each pond according to the experimental design.

Shrimp larvae were initially fed with commercial feed of SAUDI-BANGLA shrimp nursery feed and reared for 14 days within the nursery enclosure. Then shrimp juveniles were released from the nursery enclosure by up folding the net to spread over the whole culture pond. After then SAUDI-BANGLA grow out feed was applied twice a day @ 3-4% of body weight for the entire culture period. Oral probiotics in the respective ponds were supplied (@5g/kg of feed) with adding the required feeds. Whereas, spreading probiotics was applied (@1.0ppm) at 30, 60 and 90 days of culture mixing with sand for

immediately reaching to the bottom sediment. Ponds were fortnightly treated with 6-10 ppm of dolomite for the entire culture period as a scheduled basis depending on the water pH. Additional liming was also done after every heavy rain fall and on demands. Ponds were fertilized with urea @0.5-1.0ppm, TSP @1.0-1.5ppm and MP @0.3-0.4ppm for the 1st two months of culture depending on the primary productivity.

Total heterotrophic bacteria (THB) and *Vibrio* sp. bacteria in water and sediment sample were monitored bi-weekly intervals following the methods of Barrow and Feltham (1993). During the entire culture period, pond ecological parameters like, temperature, transparency, water depth, pH, Dissolved oxygen and salinity were monitored bi-weekly intervals. Whereas, bottom sediment sample was collected at initial and end of the culture cycle, processed and analyzed. Analysis of water quality and soil sample was done following the methods of (APHA 1985).

After 120 days of rearing, complete harvesting of shrimp was done by dewatering the ponds. Then growth, survival rate, FCR and production were estimated. ANOVA was done with a significance level of $P<0.05$ to observe the differences in growth, survival rate, production and FCR values for different treatments.

Results and discussion

Soil pH of pond sediments reduced a little bit in all the treatments with the progress of culture period (Fig. 1). But organic matter, nitrogen and phosphorus content in sediment were found to a stable state in initial and end samples (Figs. 2-4) in all the treatments without any significant difference ($p>0.05$) among treatments. In case of improved shrimp farming (higher stocking rates and supplemented with feed, fertilizers etc) accumulation of nutrient enriched (N and P) organic matter in bottom sediment occurred (Briggs and Fungi-Smith 1994, 1998; Budford *et al.* 2003b; Islam and Alam 2008) due to uneaten feeds, molted shell and excreta, which increased metabolic toxicity (Briggs and Fungi-Smith 1998; Fast and Menasveta 2000). But in this experiment, we did not found so, might due to the positive effect of probiotics for immediate and subsequent mineralization of organic matters onto the bottom sediment. This observation was supported by Rengpipat *et al.* (1998a) and Rengpipat *et al.* (1998b), who stated that *Bacillus*, used as a probiotics, was able to colonize in both the culture water and sediment.

Water transparency in all the treatments was moderate at initial stage of culture but subsequently decreased with the progress of culture duration (Fig. 5), whereas, phytoplankton concentration increased with the increase in culture duration (Fig. 6), but there was no significance difference ($p>0.05$) among treatments. In intensive shrimp monoculture, wastes derived from feeding often stimulate phytoplankton growth and lead to dense blooms in ponds (Briggs and Fungi-Smith 1998; Fast and Menasveta 2000). Higher stocking density may lead higher in flask of organic matter onto the bottom sediment (Paez-Osuna *et al.* 1997). Bacterial activity on organic matter released available primary nutrient in water, which is preferable source of nutrient to the primary producer (Saha 2000) that stimulated the growth of primary producer in the

respective ponds might caused lowered transparency level in the respective treatments. In this study, observation on transparency and phytoplankton dynamics was supported by the above mentioned authors.

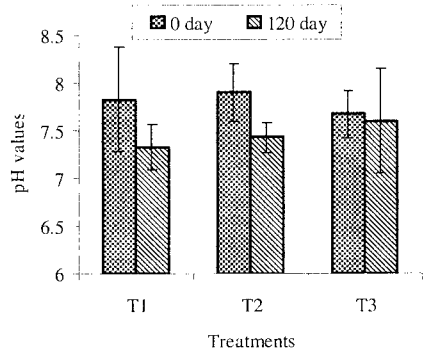

Fig. 1. Status in soil pH under different treatments.

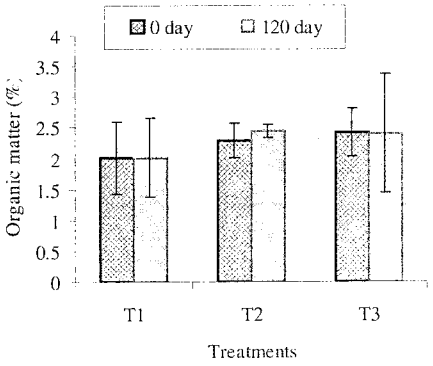

Fig. 2. Status of organic matter in bottom sediment.

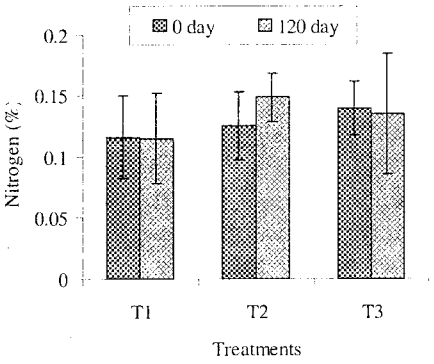

Fig. 3. Status of nitrogen in bottom sediment under different treatments

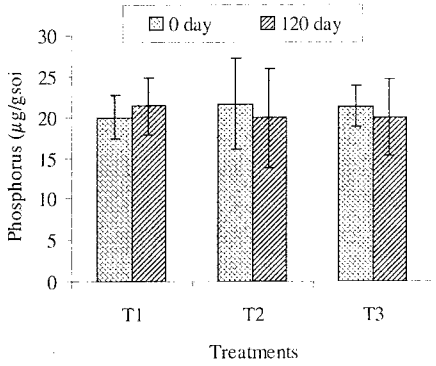

Fig. 4. Status of phosphorus in bottom sediment under different treatments

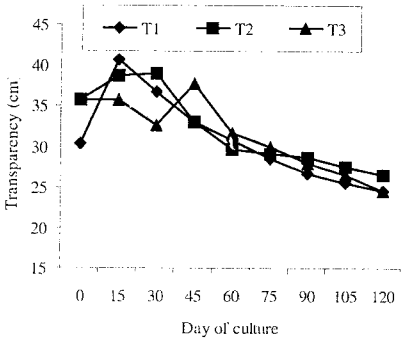

Fig. 5. Trend in transparency under different treatments

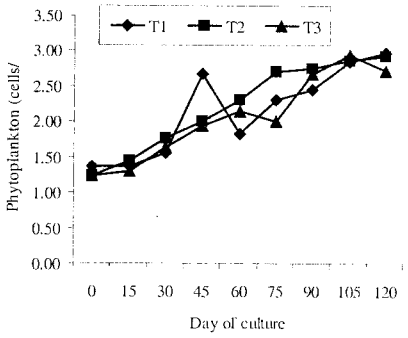

Fig. 6. Trend of phytoplankton concentration under different treatments

Other water quality variables (Table 1) were stable in condition during the culture period without any significant difference ($p>0.05$) among different treatments and were within the acceptable range of brackishwater aquaculture (Wahab *et al.* 2001; Wahab *et al.* 2003; Islam *et al.* 2004; Islam *et al.* 2005; Islam and Alam 2008).

Table 1. Water quality variables under different treatments

Parameters	T1	T2	T3
Temperature (°C)	32.04±1.42	31.94±1.36	31.87±1.39
Transparency (cm)	30.76±5.31	31.98±4.79	31.34±4.51
Depth (cm)	103.48±5.59	107.33±4.62	103.30±4.55
pH	9.02±0.16	8.99±0.26	9.02±0.19
Salinity (ppt)	10.91±1.21	11.13±1.14	10.69±1.29
DO (mg/l)	5.88±0.44	5.77±0.57	6.03±0.37

Status of bacterial load (THB-Vibrio ratio) in pond water and bottom sediment has been presented in Fig. 7 and 8, respectively. In the initial stages, the ratio of THB-Vibrio was very low and it increased subsequently with the progress in culture period in all treatments but the difference between treatments was insignificant ($p>0.05$). This might happened due to repeated application of probiotics and their continuous multiplication receiving the nutrients from the bottom sediment and that was the expectation for keeping the culture environment friendly.

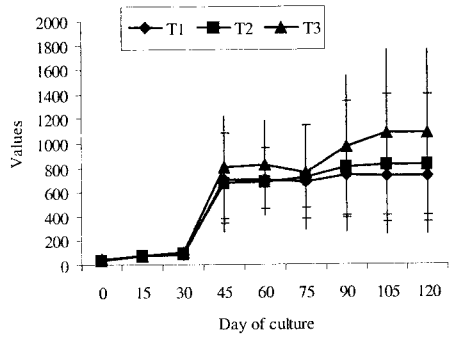

Fig. 7. Bacterial load (THB-Vibrio ration) in pond water under different treatments.

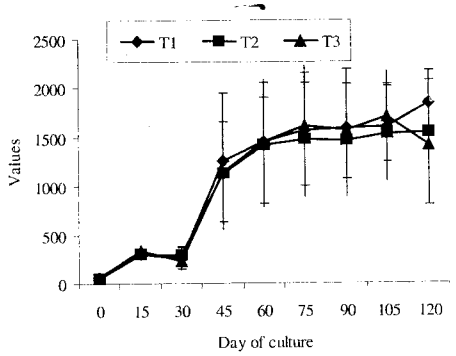

Fig. 8. Bacterial load (THB-Vibrio ration) in pond sediment under different treatments.

Bacillus bacteria in the environment, exclusion of other bacteria (especially harmful bacteria) by the probiont. The *Bacillus* also was able to replace *Vibrio* spp. in the gut of the shrimp (Rengpipat *et al.* 1998a). *Bacillus* bacteria are able to out-compete to other bacteria for nutrients and space and can exclude other bacteria through the production of antibiotics (Moriarty 1998; Verschuere *et al.* 2000). Many different antibiotic compounds are produced naturally by a range of *Bacillus* species, and it appears that other bacteria would be unlikely to have resistant genes to all of the antibiotics produced by the *Bacillus* probionts, especially if they had not been exposed to the *Bacillus* previously (Moriarty 1998).

Results on production performance of shrimp have been presented in Table 2. Final weight gain of shrimp (27.58±1.18g) was significantly higher ($p<0.01$) in T2 (spreading probiotics) followed by T3 (23.31±0.75g) and T1 (20.56±1.65g). Survival rate of shrimp was statistically insignificant ($p>0.01$) between T2 (70.75±8.54%) and T3 (52.97±4.06%), also between T3 and T1 (41.63±2.17%). But it was significantly different ($p<0.01$) between T2 and T1. Production of shrimp was significantly different ($p<0.01$) among the treatments. Highest level of production was obtained from T2 (1167.66±109.62 kg/ha) where probiotics was applied as spreading method followed by T3 (739.62±33.67 kg/ha) and T1 (512.79±38.47). FCR value was significantly lower ($p<0.01$) in T2 (1.81±0.06) followed by T1 (2.33±0.14) and T3 (2.34±0.11), but it was statistically insignificant ($p>0.01$) between T1 and T3. However, highest production of shrimp in T2 might due to higher body weight gain and also due to higher survival rate. Spreading probiotics might have effective activity to provide congenial environment for shrimp than that of oral ones by rapidly enhancing the proportion of *Bacillus* bacteria in the environment. Increased survival by shrimp might be due to exclusion of other bacteria (especially harmful bacteria) by the probiont, where the *Bacillus* bacteria were

dominant. The observed increase in survival in spreading probiotic treatments also might be due to enhanced digestion and increased absorption of food, which in turn contributed to the higher body weight gain and lower FCR values. Administering probiotics significantly improve survival but not growth (Ziaei-Nejad *et al.* 2005). *Bacillus* administration also has been shown to increase shrimp survival by enhancing resistance to pathogens by activating both cellular and humoral immune defenses in shrimp (Rengpipat *et al.* 2000). *Bacillus* surface antigens or their metabolites act as immunogens for shrimp by stimulating phagocytic activity of granulocytes (Itami *et al.* 1998). Whereas, Shariff *et al* (2001) and Mcintosh *et al.* (2000) stated that *P. monodon* and *Litopenaeus vannamei* treated with *Bacillus* probiotics did not significantly increase ($p>0.05$) either survival or growth.

Table 2. Details of production performance of shrimp under different treatments

Parameters	T1 (oral probiotics)	T2 (spreading probiotics)	T3 (oral+spreading probiotics)
Final body weight (g)	20.56 ± 1.65^{cb}	27.58 ± 1.18^{a}	23.31 ± 0.75^{b}
Survival rate (%)	41.63 ± 2.17^{cb}	70.75 ± 8.54^{a}	52.97 ± 4.06^{ba}
Production (kg/ha)	512.79 ± 38.47^{c}	1167.66 ± 109.62^{a}	739.62 ± 33.67^{b}
FCR	2.33 ± 0.14^{ba}	1.81 ± 0.06^{c}	2.34 ± 0.11^{a}

* Different superscript in the same row indicated significantly different ($p<0.01$)

It could be seen (Table 2) that, body weight gain, survival and production of shrimp under spreading probiotics was much more higher than that of Apud *et al.* (1984), who reported an average yield of 340 kg/ha/crop at stocking rate of 4–5/m^2 in monoculture with supplemental feed and improved water management. Body weight gain, survival rate and production of shrimp in this trial was also higher than the observation of Islam and Alam (2008), who reported highest body weight gain of 26.33g, survival rate of 57.76 % and total augmentation of shrimp production of 759.14 kg/ha with a stocking density 5/m^2 under modified improved culture system in a 120 days of rearing period.

However, findings of the present experiment focused that, among the tested forms of bacillus probiotics, spreading one (T2) showed the better performance of shrimp growth, survival rate and production. So, spreading type of probiotics seemed to be superior to the others and might be considered as a biotechnical tool for keeping the culture environment congenial under improved shrimp culture practices. Probiotics could be the best and alternative option as a preventive measure of shrimp disease instead of control by antibiotics for producing biological shrimp.

Acknowledgement

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Short Note

Effect of alum on the histological changes of silver barb (*Barbodes gonionotus*)

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Abstract
Studies were conducted to know the effects of alum on the histological changes of silver barb (*Barbodes gonionotus*) fry in the aquarium. The use of up to 0.5 g/L of alum for 120 hours as means of treatment of fish diseases is safe. At this level, no abnormal behavior and pathological alteration were observed in the organs of experimental fish. As the doses increased to 1.25 g/L and above (1.5 g/L), experimental fishes exhibited abnormal movement and with marked histopathological changes in the various organs. A dose of above 0.5 g/L should be strictly prohibited.

Key words: *Barbodes gonionotus*, Alum, Histopathology

The treatment of alum is mainly used in aquaculture to remove the turbidity of pond water and a concentration of 30 mg/L alum could reduce turbidity from an initial 340 NTU (Nephelometric Turbidity Units) to less than 30 NTU in four hours (Hart and McGregor 1982). But alum treatment rendered water acidic and not suitable for fish culture and a mixture of lime and alum removed turbidity and water was rendered suitable for fish (Biswal and Roy 1991). Alum treatment of ponds reduces soluble reactive phosphorus (SRP) and total phosphorus (TP) concentrations in ponds (Masuda and Boyd 1994). The alum was used as water purification material for drinking in remote areas of Bangladesh, where tube-well was not available. Recently, alum is using in aquaculture for fish health management. But the fish farmers do not know the suitable doses. Hence, the present work was under taken to know the tolerance level and histological changes on different organ of silver barb.

Six treatments were considered in the present experiment. First treatment (T_1) was control having no alum mixed, but treatment 2 (T_2), treatment 3 (T_3), treatment 4 (T_4), treatment 5 (T_5) and treatment 6 (T_6) were maintained at 0.5 g/L, 0.75 g/L, 1.0 g/L, 1.25 g/L and 1.5 g/L alum, respectively. The fishes were acclimatized in galvanized iron drum with tap water. Required amount of powdered alum were mixed in water and aerated for one hour. Then ten healthy fishes were released in each aquarium. Average size of the fishes was 11.42 $\pm$ 0.82 cm in length and 17.3 $\pm$ 2.21 g in weight. The death (due toxicity of alum) fishes were caught by hand net to collect samples for histological analysis. Number of fishes and time of mortality were recorded throughout the experimental period. Samples for histological

analysis were collected from skin, muscle, gill, liver and kidney and fixed in 10% neutral buffered formalin. Physico-chemicals parameters of aquaria water *viz.*, temperature, pH, total alkalinity (mg/L), total ammonia (mg/L) and dissolved oxygen (mg/L) were recorded. Confidence level (CL) were calculated using the formula (Gomez *et al.* 1986), C L = $100\{1\text{-}2(½)^N\}$. Where N was the number of exposed organism (fish).

No fish died at concentration 0.5/L during experimental period. However, fishes died at different time with the different alum concentration except treatments 1 and 2. Normal behaviors of fishes were changed especially at the time of death. Fish did not show any change of body color after treatment. In the treatment 3, 25% fishes died after 72 hours of the start of the experiment and no fishes died in the remaining period. All fishes died within 4 hours and 35 minutes at T_4, within 3 hours and 45 minutes at T_5 and within 1 hour and 35 minutes at T_6.

The range of dissolved oxygen (DO) of the aquarium water was from 4.9 to 5.9 mg/L. The water temperature ranged from 24.8 to 27.5°C during the experimental period. The range of pH was from 6.8 to 7.7, alkalinity from 140 to 160 mg/L and ammonia from 0.19 to 2.812 mg/L. The dissolved oxygen range and temperature of aquarium water was favorable for fishes. pH and total alkalinity of the aquarium water were slightly decreased with the increase of alum concentration at end of the experiment. But, the total ammonia was highly increased with increase of alum concentration of aquarium water. Mainly highly increased ammonia was found where fishes died. These remarkable changes in ammonia may be due to release of excess excreta for stress of fish before their death in the aquaria. These changes of water quality could affect the normal physiology of fishes (Subasinghe 1995).

Histological observation of skin and muscle of experimental fishes

In both the treatments 1 and 2, structure of skin and muscle was almost normal throughout the experimental period (Fig. A_1). Whereas, in treatments 3 and 4, myotomes of the muscle had slight disintegration showing minute vacuums (v) (Fig. A_2). However, in the treatments 5 and 6, epidermis of skin were lost partially, dermis splitted (↑) and muscles had necrosis (n) and vacuums (v) (Fig. A_3).

Histological observation of gill of experimental fishes

In the treatments 1 and 2, histological structure of gills was normal (Fig. B_1). But in the treatments 3 and 4, gill had cubbing (cb), necrosis (n), and pyknosis (p) (Fig. B_2). Whereas, in the treatments 5 and 6 gill exhibited hypertrophy (hy), hemorrhage (H), necrosis (n), loss of secondary gill lamellae (↑) (Fig. B_3).

Histological observation of liver of experimental fishes

In the low dose treatment (T_2) and control, histological structure of livers was almost normal (Fig. C_1). However, in the treatments 3 and 4 hepatocytes of liver had minute vacuums (v) and necrosis (n) (Fig. C_2). Whereas, marked necrosis (n) and wide vacuums (v) were noticed within the liver sections of treatments 5 and 6 (Fig. C_3).

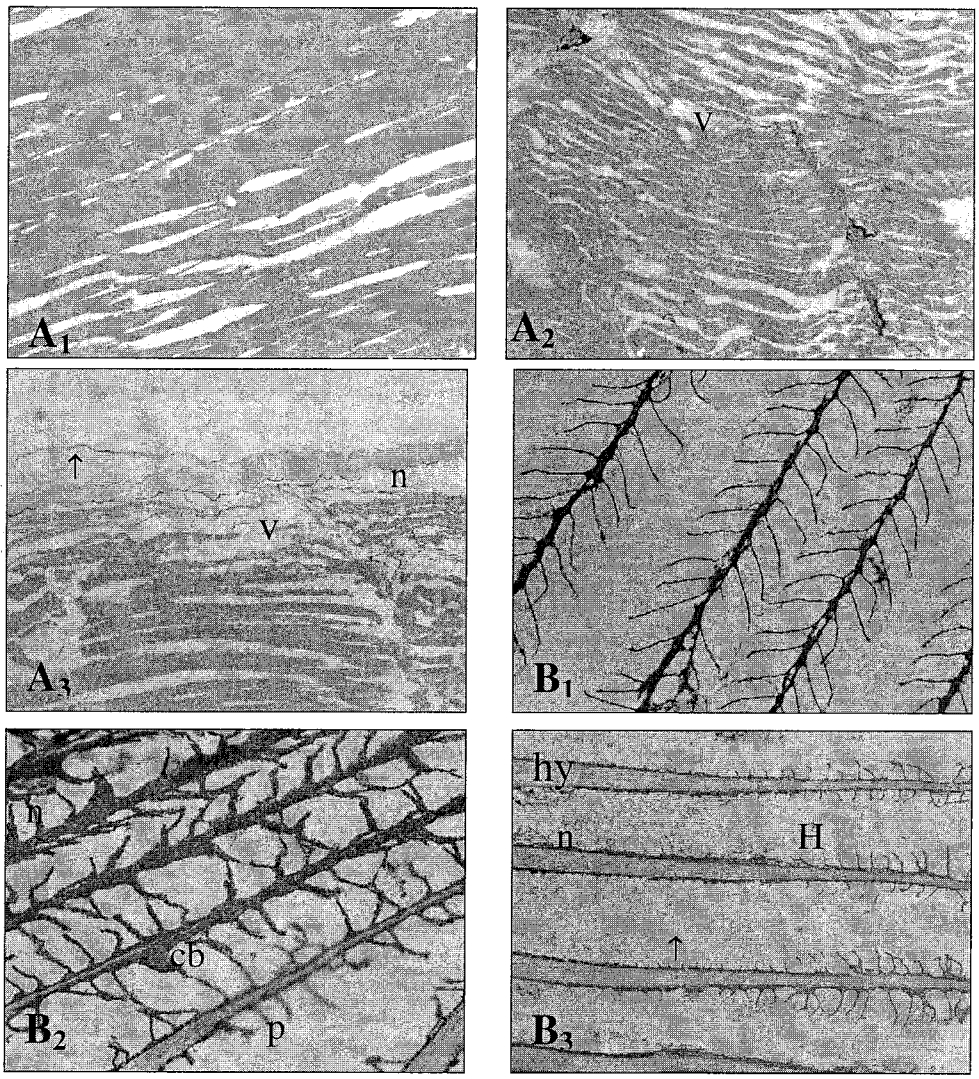

Fig. A_1 Section of the muscle from T_2 showing its normal structure. H and E × 175.
Fig. A_2 Section of muscle having minute vacuums (v) in T_4. H and E × 175.
Fig. A_3 Photomicrograph of the skin and muscle from T_6 showing loss of epidermis, splitting dermis (↑), necrosis (n) and vacuums (v). H and E × 175.
Fig. B_1 Section of gill from T_1 exhibiting normal structure of gill. H and E × 175.
Fig. B_2 Section of gill from T_4 with cubbing (cb), necrosis (n), and pyknosis (p). H and E × 175.
Fig. B_3 Photomicrograph of gill showing hypertrophy (hy), hemorrhage (H), necrosis (n), loss of secondary gill lamellae (↑) in T_6. H and E × 175.

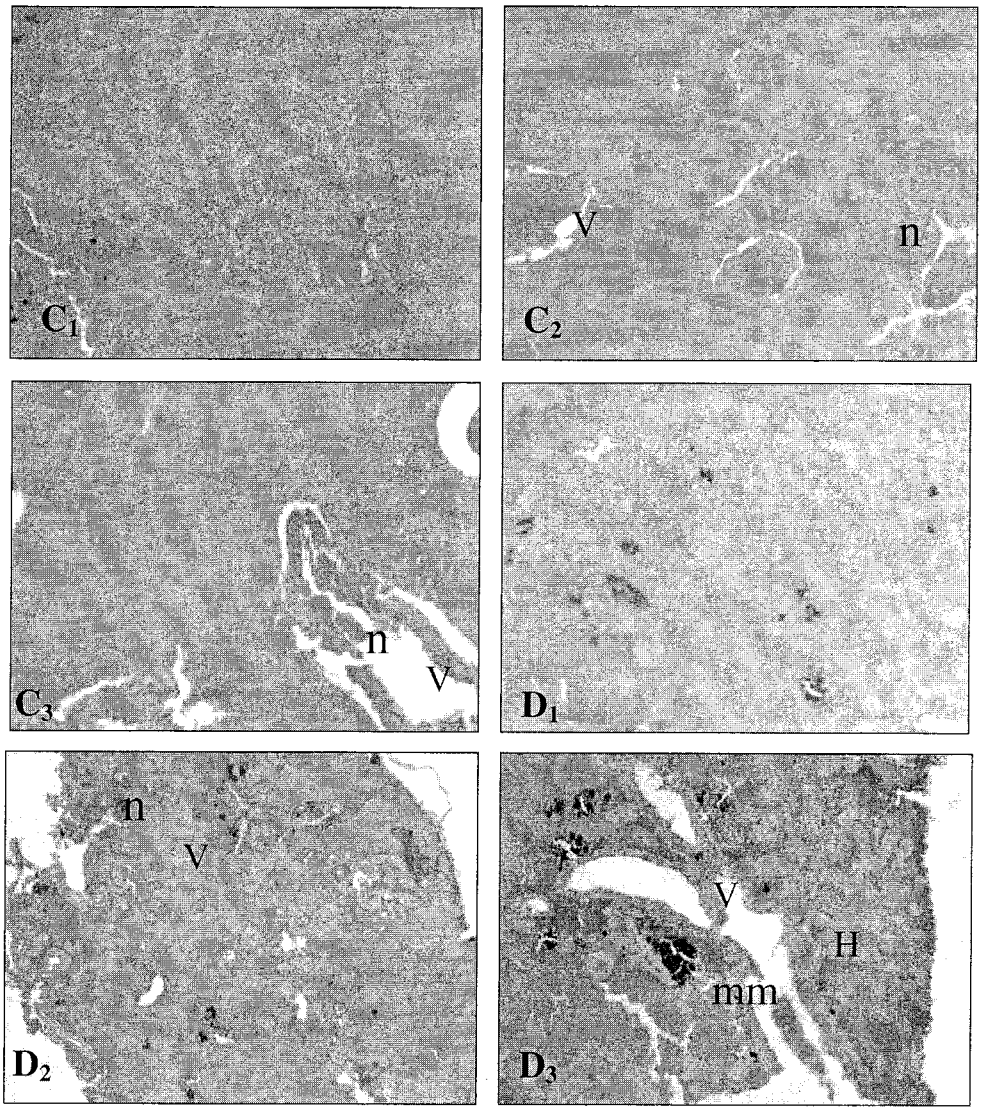

Fig. C_1 Section of liver from T_2 showing normal structure of liver. H and E × 175.
Fig. C_2 Photomicrograph of liver showing minute vacuums (v) necrosis (n) in T_3. H & E × 175.
Fig. C_3 Section of liver from T_6 with marked necrosis (n) and vacuums (v). H and E × 175.
Fig. D_1 Section of kidney from T_1 exhibiting its normal structure. H and E × 175.
Fig. D_2 Section of kidney from T_4 showing minute necrosis (n) and vacuums (v). H & E × 175.
Fig. D_3 Photomicrograph of kidney from T_5 with necrosis (n), melanomacrophages (mm), hemorrhage (H) and vacuums (v). H and E × 175.

Histological of structure of kidney of experimental fishes

Structure of the kidney exhibited normal in the treatments 1 and 2 (Fig. D_1). Again, the structure of kidney in the treatments 3 and 4 had minute necrosis (n) and vacuums (v) (Fig. D_2). However, in the treatments (T_5 and T_6) kidney structure had marked necrosis (n), wide vacuums (v), hemorrhages (H) and melanomacrophages (mm) (Fig. D_3).

Histological changes were observed in skin, muscle, gill, liver and kidney of the treated fishes except fishes treated with 0.5 g alum/L. In the treatments 3 and 4 myotomes had slight disintegration showing minute vacuums. Secondary gill lamellae were partially missing having clubbing and hemorrhage. The structure of kidney in T_3 and T_4, had tubular necrosis, vacuums and hemorrhage. In the high dose treatments (5 and 6), epidermis of skin was lost partially, dermis splitted and muscles had necrosis and vacuums. Again, primary gill lamellae were swollen, secondary gill lamellae were missing in many places having hemorrhage and necrosis.

Alum is a low cost and widely used chemotherapeutic in aquaculture of Bangladesh. From the finding of the present investigation, it was observed that use of up to 0.5 g/L of alum for 120 hours as means of treatment in fish diseases is safe. At this level, no abnormal behavior and pathological alteration were observed in the organs of experimental fish. It was also observed that as the doses increased to 1.25 g/L and above (1.5 g/L), experimental fishes exhibited abnormal movement and with marked histopathological changes in the various organs. Thus it needs proper attention in the application of alum as a drug in aquaculture. A dose of above 0.5 g/L should be strictly prohibited.

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