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Growth performance evaluation of genetically improved silver barb (*Barbonymus gonionotus* Bleeker) in different agro-ecological zones in Bangladesh

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Abstract
On farm trials of silver barb with other carps were carried out in 40 ponds during May to October 2005 in four agro-ecological zones viz., Trishal, Muktagacha, Parbotipur and Paikgacha in Bangladesh. In Trishal and Muktagacha zones, ponds were stocked with silver barb, silver carp and common carp at the stocking density of 11,500 fish/ha, whereas in Parbotipur and Paikgacha zones, ponds were stocked with silver barb, rohu, catla and mrigal at the stocking density of 10,000/ha. Among the ponds, 50% (20 ponds) were stocked with BFRI improved stock of silver barb (Treatment-1) and rest of the 20 ponds stocked with local silver barb stock (Treatment-2). The harvest weight of BFRI improved silver barb were 149±16.01, 168±18.06, 198±14 and 230±9.25g in Trishal, Muktagacha, Paikgacha and Parbotipur, whereas the data obtained at 113±15.52, 136±20.66, 170±17.0 and 205±12.10g for local stock of silver barb, respectively. In all trials, the harvest weight of BFRI improved stock showed significantly higher growth performance ($P<0.05$) over the local stocks.

Key words: Genetically improved silver barb, agro-ecological zones

Introduction

Silver barb (*B. gonionotus*) is a popular species among fish farmers of Bangladesh because it grows faster and well on low protein diets, whether feeding on certain aquatic plants or given supplementary feeds and can tolerate a wide range of environmental conditions. In Bangladesh, breeding is mainly carried out by hypophysation. For aquaculture, the farmers mainly depend on hatcheries for fingerlings of silver barb. There is a possibility of inbreeding in most of the small hatcheries where female and male are chosen from closed populations of very limited size (Hussain and Islam 1999). Genetic deterioration of existing stocks of silver barb has been reported (Hussain and Mazid 2001). Genetic stock improvement through genetic selection is one of the most useful ways of enhancing desirable traits in a founder stock with high genetic variability to reduce inbreeding in a hatchery population (Eknath *et al.* 1998).

The genetic stock improvement program of silver barb was initiated in 1996 at Bangladesh Fisheries Research Institute (BFRI) under the technical assistance of WorldFish Center (formerly ICLARM). It was initiated with three different stocks of fish from Indonesia, Thailand and Bangladesh and the follow up mass selection protocol was continued up to the seventh generation. The 7th generation of silver barb showed 32% higher growth in comparison to the base population of silver barb. The present paper deals with the results of the improved silver barb under different farming conditions in Bangladesh.

Materials and methods

Origin of improved silver barb and local silver barb

The improved fingerlings used in this experiment were developed from 7th generation of silver barb descended from mass selection experiment. The local silver barbs were produced in local hatchery in different locations of the country.

Study area

On farm trials of silver barb with other carps were carried out from 15 May to 15 October 2005 in four different agro-ecological zones at Trishal and Muktagacha in Mymensingh zone, Paikgacha in Khulna zone and Parbatipur in Dinajpur zone of Bangladesh. Each zone had 10 ponds with a range of area of 600 -1000 m^2 and a depth of 1.0-1.5m.

Experimental design and fish stocking

In Trishal and Muktagacha of Mymensingh zone, there were two treatments with five replications. Both the treatments were designed with silver barb (*B. gonionotus*), silver carp (*H. molitrix*) and common carp (*C. carpio*) at the stocking density of 7,500, 2,000 and 2,000/ha. In treatment-1, ponds were stocked with BFRI improved silver barb stock and in treatment-2 with locally available existing stock of silver barb. For the ponds in Paikgacha and Parbatipur, there were also two treatments with five replications. Both the treatments were designed with silver barb (*B. gonionotus*), rohu (*L. rohita*), catla (*C. catla*) and mrigal (*C. mrigala*) at the respective stocking density of 2500, 2500, 2500 and 2500 fish/ha. BFRI Improved stock of silver barb and locally available existing stock was stocked in treatment-1 and treatment-2, respectively.

Fish rearing and pond management

Stocked fingerlings were fed with commonly available agricultural by-products, rice bran (70%) and mustard oil cake (30%) at the rate of 3-4% of standing biomass of fish regularly. All the ponds were fertilized with organic fertilizer (cow dung) at the rate of 2,000 kg/ha/month.

Harvesting of fish and data analysis

After five months of rearing, all fish were harvested through seine netting and pond drying. During harvesting, fishes were counted and weighed from each pond to assess the survival rate and production. The growth and production data were analysed using a one-way analysis of variance (ANOVA). Results were tested to identify significant differences ($P<0.05$) between the means.

Results and discussions

Details of growth parameters and production of fish in different agro-ecological zones such as Trishal, Muktagacha, Paikgacha and Parbatipur are presented in Tables 1, 2, 3 and 4, respectively. In Trishal zone, at harvest the weight of improved silver barb (BFRI stock) was statistically significant and higher than that of local silver barb (149±16.01 vs. 113±15.52g in treatments-1 and 2, respectively). The weight of silver carp and common carp in treatments-1 and 2 were almost similar ($P> 0.05$). However, the total production of treatments-1 and 2 were significantly different (2,556 vs. 2,070 kg/ha/6 months, respectively).

Table 1. Harvesting weight and production performances of BFRI improved stock and local stock of silver barb along with other species in Trishal zone

Treatment	Species	Harvesting weight (g)	Survival (%)	Production (Kg/ha)	
				Species-wise production	Total production/ha
T-1	Silver barb (BFRI stock)	149±16.01*	92	1,035±66.6	2,556±108.33*
	Silver carp	523±49.46	84	888±97.16	
	Common carp	421±42.15	75	632±77.39	
T-2	Silver barb (Local stock)	113±15.52	88	755±125	2,070±177
	Silver carp	510±70.08	82	844±140	
	Common carp	383±93.39	61	471±53.04	

* Significant at 0.05% level

In Muktagacha zone, significant difference in harvest weight between the improved and local silver barb was also observed (Table 2). Although there were no significant difference in harvest weights of silver carp and common carp between the treatments. The total yield obtained in ponds stocked with BFRI improved silver barb was significantly higher than in ponds stocked with local silver barb strain (2,198 vs.1,790 kg).

Table 2. Growth and production performances of BFRI improved stock and local stock of silver barb along with other species in Muktagacha zone

Treatment	Species	Harvesting weight (g)	Survival (%)	Production (Kg/ha)	
				Species-wise production	Total production/ha
T-1	Silver barb (BFRI stock)	168±18.06*	93	1178	2,198±186*
	Silver carp	367±40.17	82	601	
	Common carp	282±41.11	74	418	
T-2	Silver barb (Local stock)	136±20.66	84	858	1,790±142
	Silver carp	322.55±34.81	88	516	
	Common carp	297.20±32.88	69	415	

* Significant at 0.05% level

In Paikgacha zone, polyculture of BFRI improved silver barb stock with Indian major carps (treatment 1) also obtained higher body weight at harvest than that of local silver barb stock in treatment-2 and they were significantly different ($P<0.05$)(Table 3). The final weight of catla, rohu and mrigal were not significantly different between the treatments. Total fish production after five months of culture was 2,353±102 and 2,305±114 kg/ha in treatments-1 and 2, respectively and slightly higher production in treatment-1 was obtained due to the presence of BFRI improved stock in it.

Table 3. Growth and production performances of BFRI improved stock and local stock of silver barb stock along with other species in Paikgacha zone

Treatment	Species	Harvesting weight (g)	Survival (%)	Production (Kg/ha)	
				Species-wise production	Total production/ha
T-1	Silver barb (BFRI stock)	198±14*	93	465±52	2,353±102
	Catla	370±28	81	758±64	
	Rohu	240±20	87	522±42	
	Mrigal	310±15	78	612±39	
T-2	Silver barb (Local stock)	170±17	83	353±40	2,305±114
	Catla	350±30	88	770±56	
	Rohu	246±24	85	523±35	
	Mrigal	326±12	80	660±24	

* Significant at 0.05% level

Silver barb reached to an average harvest weight of 230±9.25 and 205±12g in treatments-1 and 2, respectively in Parbatipur zone (Table 4). There were no significant differences in harvest weights of catla, rohu and mrigal between the treatments ($P>0.05$). Total production of fish in treatment-1 was 2,597±129g where BFRI stock of

silver barb was stocked, whereas in treatment-2 where local silver barb was stocked, it was 2,515±99g. The total production did not show any significant difference (P>0.05) showed between the treatments.

Table 4. Growth and production performances of BFRI improved stock and local stock of silver barb stock at Parbatipur zone

Treatment	Species	Harvesting weight (g)	Survival (%)	Production (Kg/ha)	
				Species-wise production	Total production/ha
T-1	Silver barb (BFRI stock)	230±9.25*	90	518±32	2,410±129^{NS}
	Catla	390±16.11	77	758±41	
	Rohu	260±22.20	80	522±28	
	Mrigal	340±11.25	72	612±44	
T-2	Silver barb (Local stock)	205±1210.29	80	430±28	2,296±99
	Catla	372±25.11	74	688±34	
	Rohu	275±17.28	81	563±48	
	Mrigal	328±23.29	75	615±36	

* Significant at 0.05% level

In all the regions, the survival rate of silver barb of BFRI stock showed higher survival than local stock. The survival rate of silver barb of BFRI stock in Trishal, Muktagacha, Paikgacha and Parbatipur were 92.38, 93.21, 93.92 and 90.16%, respectively. Whereas the survival rate were 88.92, 84.00, 83.04 and 80.84% incase of local stock of silver barb at Trishal, Muktagacha, Paikgacha and Parbatipur in respectively. From the results, it is clear that Local existing stock showed less survival rate over BFRI stock because the brood stock are being used by the hatchery operators might be of genetically deteriorated or inbred.

To compare the performance of present study, several previous studies could be illustrated. Kohinoor *et al.* (1995) reported a production of 1,718 kg/ha/6 months from the monoculture of rajpunti (*B. gonionotus*) with the stocking density of 15,000/ha. Kohinoor *et al.* (1999) conducted an experiment of silver barb (*B. gonionotus*) with silver carp (*H. molitrix*) and common carp (*C. carpio*) in on station management practice and obtained an average production of 2,056 kg/ha in six months period. In another study, Wahab *et al.* (2001) observed that polyculture of rohu, catla and common carp with rajpunti (*B. gonionotus*) yielded a gross production of 1,902 kg/ha/4 months culture period. The productions of present study were much higher than the above mentioned studies.

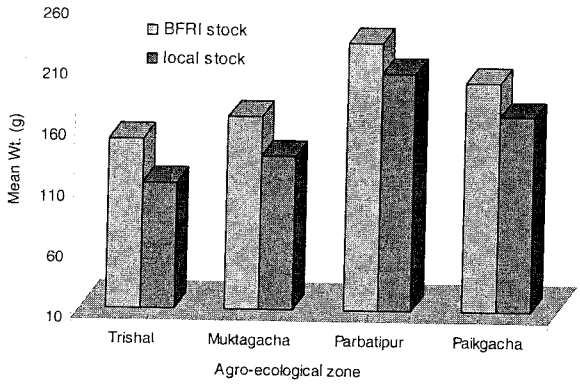

Fig. 2. Mean Wt. (g) of siiver barb of BFRI stock and local stock in different locations

It is evident that silver barb of BFRI improved stock had a higher growth and production over the existing local silver barb stock in all the treatments (Fig. 1). Local existing stock of silver barb showed less weight gain. The BFRI improved stock, on the other hand, showed higher growth performance due to its greater genetic gain, which developed over several generations of selection.

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Optimization of dose of methyltestosterone (MT) hormone for sex reversal in tilapia (*Oreochromis niloticus* L.)

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Abstract
This paper describes the optimization of dose of methyltestosterone (MT) hormone for masculinization of tilapia (*Oreochromis niloticus*). Five treatments (i.e. T_1, T_2, T_3, T_4 and T_5) with different doses such as 0, 40, 50, 60 and 65 mg of MT hormone were mixed with per kg of feed for each treatment and fed the fry four times a day up to satiation for a period of 30 days. The stocking density was maintained 10 spawn/liter of water. The growth of fry at different treatments was recorded weekly and mortality was recorded daily. At the end of hormone feeding the fry were reared in hapas fixed in ponds for another 70 days and at the 100th day the fish were sexed by the gonad squashing and aceto-carmine staining method. The analysis of growth data did not show any significant variation in length and weight of fish among the different treatments. High mortality of fry ranging 66% to 81.6% was observed in different treatments and highest mortality was observed during the first twelve days of the experiment. The sex ratio analysis showed that T_2 (40 mg/kg) and T_5 (65 mg/kg) produced 93.33% of sex reversed male and T_3 (50 mg kg) and T_4 (60 mg/kg) produced 96.66% sex reversed male, and these ratios were significantly ($p<0.05$) different from 1:1 male: female sex ratio. The control, T_1 (0 mg/kg) contained 43.33% male progeny. From these results it is suggested that either 50 mg/kg or 60 mg/kg of MT with a feeding period of 30 days could be considered as an optimum dose for masculinization of tilapia (*O. niloticus*).

Key words: Masculinization, Methyltestosterone, Tilapia

Introduction

Hormonal sex reversal is a technique of changing of sexes from one sex to another in fish by administering synthetic steroid hormones before and/or during the period of sexual differentiation. In this technique, the first feeding fry are treated with male hormones or androgens (i.e.17α-methyltestosterone), which develops testes and male sexual characteristics at maturity, while treatment with female hormones or estrogens (i.e.17β-estradiol) produces individuals with ovaries and female characteristics in fish (Hussain 2004). The sex reversal technique is very simple, economic, low inputs cost involving and ensures high production and high net profit which can be done by a

technician without sophisticated laboratory and equipment. A total of 35 tilapia seed production hatcheries have established that are producing 10-12 billions fry every year and a number of commercial farms have been established, which are producing roughly about 0.02 million tons of marketable size fish (Hussain 2008).

The production of tilapia for food has long been hindered by the precocious maturity and uncontrolled reproduction exhibited by these fish (Wohlfarth and Hulata 1981). These difficulties have been cited by Macintosh *et al.* (1985) as the reason for the commercial inviability of tilapia in Indian Major Carp culture systems. Female tilapias are capable of spawning every four to six weeks under ideal condition in their natural environment (Jalabert and Zohar 1982, Little *et al.* 1993). The excessive reproduction of tilapia species leads to overcrowding, competition for food and stunted the growth in aquaculture system, which resulted in low yields of harvestable size of fish. To overcome this problem and to increase the yield of tilapia several methods have been proposed and developed. These include the use of suitable predator species (Guerrero 1982), the generation of infertile fry through triploidy and the use of hybrid crosses to produce monosex broods (Pruginin *et al.* 1975, Hanson *et al.* 1983, Majumdar and McAndrew 1983). Monosex population offers several benefits in aquaculture, including faster growth and prevention of unwanted reproduction (Mair *et al.* 1991, Green *et al.* 1997). So, the culture of monosex population of tilapia is preferable.

The use of male sex steroids to induce sex inversions of genotype females into phenotypic males has proven to be one of the successful methods to produce monosex population (Hunter and Donaldson 1983). The androgen 17α-methyltestosterone (MT) (Ridha and Lone 1990) and the estrogen diethylstilbestrol (DES) are the most widely used hormones for sex inversion in tilapia. The direct masculinization of tilapias using hormone is the most common method for monosex male production (Shelton *et al.* 1978; Guerrero 1979, Guerrero and Guerrero 1988). In this process, male steroid is administered to first feeding tilapia fry so that the undifferentiated gonadal tissue of genetic females develops into testicular tissue, producing individuals that grow and function reproductively as males. However, in many cases the hatchery operators used very high or low dose of hormones and fed the fry with hormone mixed feed for shorter or longer period of time beyond the actual time needed for the sex conversion. As a result, 100% monosex population is not produced in their operation. So, optimization of hormonal dose and duration for feeding can minimize the production cost of the hatcheries and ensure to produce desired level of monosex population. For these reasons, the research was conducted to optimize the dose and duration of MT for the sex reversal of *O. niloticus*.

Materials and methods

Experiment site

The experiment was conducted in the hatchery of Fisheries Biology and Genetics Department, Faculty of Fisheries, Bangladesh Agricultural University (BAU),

Mymensingh. Three day-old spawns of tilapia (*O. niloticus*) were collected from a private tilapia hatchery named Reliance Aqua Farm Ltd., Bailore, Trishal, Mymensingh.

Design of the experiment

The experiment was comprised of five treatments (T_1, T_2, T_3, T_4 and T_5) of different doses (except control which was hormone free) of MT hormone. It was conducted in 5 glass aquaria of (45×25×24) cm^3 size each and contained 25 liter of water. 250 spawns were stocked in each aquarium. The average length and weight of spawns were 7.75±0.05 mm and 0.006 g respectively and the stocking density was maintained 10 spawns/liter of water. The spawns were reared for 30 days.

Four diets with different doses of MT hormone i.e., 40, 50, 60 and 65 mg/kg were prepared through ethanol evaporation method (Mair and Santiago 1994). To prepare 100 g feed for each treatment, required amount of MT hormone (i.e., 4, 5, 6 and 6.5 mg MT hormone for T_2, T_3, T_4 and T_5 respectively) was diluted with 60 ml of alcohol for homogenous mixing with the feed in each treatment. Ground and sieved fish meal was used for preparing the feed. In case of control, required amount of feed was prepared by mixing ethanol only. The prepared feeds were preserved in a refrigerator at 4^0C.

Feeding and sampling of fry

The spawns were fed with hormone mixed feeds 4 times (from 7:00 am to 07:00 pm with four hour interval) a day up to satiation. The water of each aquarium was exchanged by 75% of the volume with fresh water once a day in the morning to avoid water quality deterioration due to decomposition of left over feed and feces of the spawns. In addition, the faecal out-put and wastes of feed were removed from the aquarium by siphoning at 9:00 am and 5:00 pm daily. Additional oxygen was provided to aquarium through aeration for 22 hrs everyday from two aerators and was stopped for half an hour each time during feeding.

The fish were sampled at weekly interval to determine the increase in their size (length and weight). Sampling was done in the early morning when the fish stomach was about to be empty to avoid the biasness of weight due to the presence of excessive feed. Ten fry were randomly collected from each aquarium and the weight of all fry was taken together due to their small size in an analytical balance. The length (mm) was measured by placing the fish on a petri dish having a 1 mm graph paper underneath it. Mortality of the fry was recorded daily. The experiment was continued for 30 days and at the end of the experiment the fish were transferred to hapas fixed in a pond and reared them with the normal feed until being sexed. The hormone feeding was terminated at the 30th day of experiment but the fish were reared for another 70 days with normal feed for proper sexing. At the age of 100 days, the final growth and mortality (%) of fish were estimated. Water pH and temperature in each aquarium were estimated at seven days interval.

Fish sexing

The fish were sexed by gonad squashing and aceto-carmine staining method (Guerreo and Shelton 1974). The fish was killed and the viscera was removed to reveal the two thread like gonads lying along the upper surface of the body cavity on either side of the kidney. The gonads were removed and placed on a clean glass slide. A few drops of aceto-carmine stain were added and the gonads were squashed with a coverslip. The sex of the fish was identified by examining the slides under a microscope.

Statistical analysis

The length gain (mm), weight gain (g) and mortality (%) of fish of different treatments were tested using one way analysis of variance (ANOVA) followed by DMRT (Duncan's 1955) to identify differences among the means. This statistical analysis was performed with the aid of the computer software SPSS and MS excel programs.

Results

At the beginning of the experiment, the initial length and weight of 100 fry were taken. The average initial length and weight were 7.75 mm and 0.006 g respectively. Tables 1 and 2 showing the increment of length and weight of fry for the period of 30 days. During weekly sampling at 7th, 14th, 21st and 28th days of experiment, no significant variation in length and weight of fry was found although five different doses of hormone were administered. No significant growth variations were also found when the fish were sampled at the day of 100.

Table 1. Growth in length (mm) of tilapia fry (*O. niloticus*) during the hormonal (MT hormone) and non-hormonal (normal feed) feeding period

Treatments / Days	Av. length day 0	Av. length day 7	Av. length day 14	Av. length day 21	Av. length day 28	*Av. length day 100
T_1 (0 mg/kg, control)	7.75 ±0.05	9.10 ± 0.09	9.85 ± 0.21	10.50 ± 0.21	11.20 ± 0.13	66.00 ± 0.68
T_2 (40 mg/kg)		8.85 ± 0.08	9.35 ± 0.15	9.90 ± 0.17	10.30 ± 0.06	65.50 ± 1.03
T_3 (50 mg/kg)		9.25 ± 0.13	9.85 ± 0.18	10.30 ± 0.18	10.65 ± 0.05	65.00 ± 0.59
T_4 (60 mg/kg)		9.65 ± 0.15	9.80 ± 0.12	10.80 ± 0.16	11.35 ± 0.07	67.00 ± 0.93
T_5 (65 mg/kg)		9.85 ± 0.18	10.35 ± 0.19	11.10 ± 0.13	11.60 ± 0.08	70.50 ± 0.78

*The fry were reared up to 100 days with normal feeding after completion of hormonal feeding at different treatments.

Table 2. Growth in weight (g) of tilapia fry (*O. niloticus*) during the hormonal (MT) and non-hormonal (normal feed) feeding period

Treatments / Days	Av. wt. day 0	Av. wt. day 7*	Av. wt. day 14*	Av. wt. day 21*	Av. wt. day 28*	Av. wt. day 100**
T_1(0 mg/kg, control)	0.006	0.017	0.023	0.033	0.043	16.17±0.41
T_2 (40 mg/kg)		0.016	0.022	0.035	0.044	16.50±0.33
T_3 (50 mg/kg)		0.015	0.021	0.036	0.042	15.70±0.45
T_4 (60 mg/kg)		0.016	0.021	0.035	0.041	16.25±0.37
T_5 (65 mg/kg)		0.017	0.022	0.037	0.043	16.60±0.62

* During weekly sampling the weight of 10 fry was taken together due to its small size, therefore, it was not possible to calculate the standard error.

**The fry were reared up to 100 days with normal feeding after completion of hormonal feeding at different treatments.

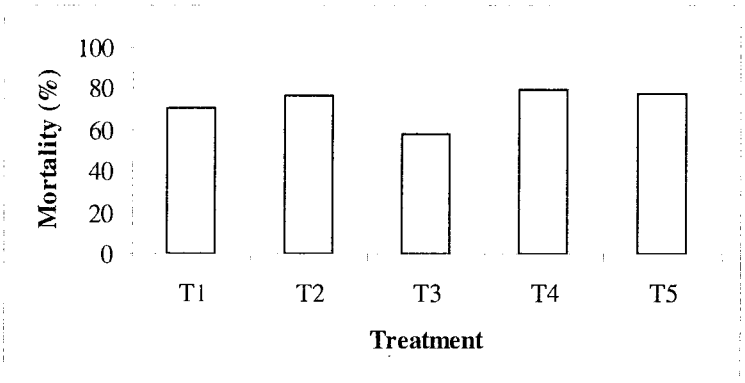

Fig 1. Mortality of *O. niloticus* fry fed with hormone (MT) – mixed feed at different treatments.

Fig. 1 shows the mortality of fry during the 30 days experiment. A high rate of cumulative mortality was observed in all treatments ranging from 58.00% to 79.60% and comparatively more mortality was observed in higher doses of hormone in first 12 days of experiment. The T_4 (60 mg/kg) showed the highest mortality (79.60%) while the T_5 (65 mg/kg) occupied the second highest position (77.20%). The mortality of T_1 (0 mg/kg, control) and T_2 (40 mg/kg) was 70.40% and 76.80% respectively which was not significantly different from T_4 and T_5. T_3 (50 mg/kg) demonstrated the lowest mortality (58.00%) and it was significantly ($p>0.01$) different from other treatments.

Fish from five treatments i.e. fish fed with 0, 40 50, 60 and 65 mg MT hormone/kg of feed were sexed at the age of 100 days and the result of sex ratios in different treatments were given in Table 3. T_3 and T_4 showed 96.66% male sex while T_2 and T_5 showed 93.33% male sex. All the treatments were significantly ($p<0.05$) different from 1:1 female: male sex ratio. The control group T_1 (0 mg/kg) contained 43.33% male sex

which was not significantly different from 1:1 sex ratio. The weekly measurement of pH and water temperature did not show any big fluctuation and both the parameters were in suitable range for tilapia fry. The ranges of pH and water temperature in different treatments were 6.75-7.50 and 26-27^{0}C respectively.

Table 3. Sex ratio of fish at different treatments. Fish were sexed at the age of 100 days

Treatments	No. of fish dissected	No. of female	No. of male	% of male
T_1(0 mg/kg, control)	30	17	13	43.33
T_2(40 mg/kg)	30	02	28	93.33*
T_3(50 mg/kg)	30	01	29	96.66*
T_4(60 mg/kg)	30	01	29	96.66*
T_5(65 mg/kg)	30	02	28	93.33*

*Significantly ($p<0.05$) different from 1:1 sex ratio.

Discussion

In the present study, treatments T_3 (50 mg/kg) and T_4 (60 mg/kg) produced 96.66%, and treatments T_2 (40 mg/kg) and T_5 (65 mg/kg) produced 93.33% sex reversed males from 30 days of masculinization experiment. The success of masculinization was quite high in all the treatments while the non-hormonal control group contained 43.33% male sex. Bombardelli *et al.* (2007) obtained 73.02% of masculinized Nile tilapia (*O. niloticus*) from 36 hours MT immersion bath. Gale *et al.* (1999) produced 83±3% of males when Nile tilapia fry were immersed in 100 μg/liter MT hormones for 13 days after fertilization. Mainardes-Pinto *et al.* (2000) compared the efficiency of 2 diets: 1(NUTRAVIT) and 2 (IP), both with 40% of crude protein, containing the synthetic androgen hormone MT and analyzed the most effective dose of this hormone on the sex reversal of Nile tilapia *O. niloticus*. A total of 9600 Nile tilapia fry at 7 days post hatching received the following treatments for 45 days: (A) 30 mg MT/kg diet 1: (B) 60 mg MT/kg diet 1: (C) 30 mg MT/kg diet 2: (D) 60 mg MT/kg diet 2 and two control groups E and F with diets 1 and 2 hormone free respectively. They found that the number of males in A, B, C and D treatments were higher than the controls groups and the dose of 60 mg MT/kg of diet as for the diets 1 and 2, was more efficient resulting in 98% of males during the experimental period.

High rate of masculinization in tilapia can be influenced by some important factors like hormone concentration, treatment duration, age and size of fry, availability of natural feed, stocking density and feeding frequency (Mair and Little 1991). In case of stocking density different studies used different stocking rates, for example, 3.6 fry/liter (Mair and Santiago 1994), 2.6 fry/liter (Shelton *et al.*1981), 1.5 to 7.75 fry/liter (Rosenstein and Hulata 1993) but the recommended stocking density for optimum masculinization of tilapia was 12 fry/liter (Mair and Little 1991). Low stocking densities can encourage the establishment of hierarchies among the treatment population where dominant fish preventing submissive fish from feeding, thus reducing the quality of

hormone ingested by the later (Mair and Santiago 1994). Considering the above important factors for sex reversal, the moderate stocking density (10 fry/liter) along with four times feeding regime, absence of natural feed and 30 days feeding durations had resulted high rate of masculinization in the present experiment and it could be a reflection of proper maintenance of the above factors.

In case of masculinization, fish size at the end of treatment period could be another factor. Dunhum (1990) cited that masculinization of *O. niloticus* fry could be unsuccessful if the fry failed to attain a standard length of 12 mm by the end of hormone treatment. All the fry in this experiment attained more or less 12 mm at the end of hormone feeding, might be one of the reasons for successful sex reversal.

The initial mortality was found high in all the treatments but mortality was decreased with the advancement of experimental time. At the end of the experiment (30 days), comparatively more fish was survived in the T_3 than those of all other treatments. In the T_1 and T_2 high mortality occurred due to electricity failure but the cause of high mortality in T_4 and T_5 could not be understood. T_3 had the lowest mortality (58.00%) and was significantly different ($p<0.01$) from other treatments. Mair and Santiago (1994) reported high mortality in both hormone and non hormone treatments. Therefore, it is difficult to predict any harmful effect of hormone on fish survival.

Although four hormonal doses as four treatments were applied to optimize the dose for masculinization, 50 mg/kg and 60 mg/kg doses of MT produced the highest percentage (96.66%) of male sex, therefore either 50 mg/kg or 60 mg/kg of MT could be recommended as optimal dose for masculinization of tilapia in hatcheries.

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Influences of dietary lipid and phosphorus levels on retention and excretion of phosphorus and nitrogen in fingerling red sea bream, *Pagrus major*

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Abstract
A laboratory based 2 × 3 factorial experiment was conducted for 12 weeks to investigate the influences of dietary lipid and phosphorus (P) levels on retention and excretion of phosphorus and nitrogen (N) in fingerling red sea bream. Two levels of lipid (210 and 260 g/kg) and three levels of phosphorus (17, 14 and 12 g kg^{-1}) in the dry diets were tested. Duplicate groups of 25 red sea bream (average weight 3.74±0.07 g) per 60L glass tank were fed experimental diets three times a day near to satiation level at 22 to 28°C water temperature. A reduction in dietary fish meal from 500 to 300 g/kg dry diet, corresponding to a supplementation in both dietary lipid and P resulted in significant increase in both P and N retention which resulted in the reduction of their excretion by red sea bream. The overall results of the present study demonstrated that both lipid and phosphorus supplementation are necessary for developing less-polluting feed which in turn, reduce fish meal level in the diet of fingerling red sea bream. Further studies in this regard with different size and age groups of red sea bream are warranted.

Key words: Lipid, Phosphorus, Nitrogen, Excretion, Red sea bream, *Pagrus major*

Introduction

The red sea bream is one of the most popular fin fish species in marine aquaculture through out the world due to its economic feasibility and traditional food habits. The aquaculture production of red sea bream (*Pagrus major*) is the second largest in Japan, followed by yellowtail (*Seriola quinqueradiata*) (Koshio 2000).

Aquaculture effluents contain P and N, which can contribute to excessive algae and macrophyte growth in receiving waters (Pillay 1992). Intensive fish production results in the release of organic wastes and soluble inorganic nutrients such as N and P, which can enrich as well as generate eutrophication in natural ecosystems (GESAMP 1996). As the original source of all aquaculture

waste is the feed fed to the fish, one effective way to reduce the waste load of fish farm effluent is to improve aquaculture diets with the aim of reducing excretion of P, N and total solids relative to fish growth (Lall 1989, Talbot and Hole 1994).

Nutritional strategy to reduce the waste load from aquaculture effluent is to produce high energy diet by reducing protein level and subsequently increasing fat level in the diet. The possibilities for reduced pollution load from fish feed are mostly related to improved feed conversion and reduced protein levels in the feed (Alsted 1991). In high energy diets the energy concentration is increased to improve feed conversion (Cowey and Cho 1991). However, the necessity of phosphorus supplementation in a fish meal-based diet has been reported at growing stage after juvenile (Masumoto 2002). Feed quality improvement involving ways to retain dietary P is one of the main strategies to reduce environmental impact of aquaculture (Lall 1991, Sugiura and Hardy 2000). Phosphorus is an essential dietary nutrient for fish and other animals, and is a major constituent of skeletal tissues, as well as an important component of the nucleic acids, DNA and RNA, energy transport compounds such as adenosine triphosphate (ATP), and phospholipids in cell membranes (Sugiura and Hardy 2000). Factors influencing the ingested P to the animal include the form of ingested P and its availability, the dietary P level, and physiological regulation of intestinal absorption and urinary excretion of P (Lall 1991, Vielma and Lall 1998, Sugiura *et al.*, 2000). Limited information is available on the metabolism, excretion and utilization of dietary P in fish (Lall 1991). Fish meal is the source of most dietary P in fish diets. In fish meal, P combines with Ca and forms the hydroapatite and/or tricalcium phosphate (TCP). Due to the structural complexity, P and Ca from TCP have been reported to be less viable to some fish species (Takamatsu *et al.* 1995, Shitanda *et al.* 1979, Watanabe *et al.* 1980, Hossain and Furuichi 1998). On the other hand, P from water soluble like monosodium/monocalcium phosphate is highly available to all fish. It has been reported that increasing dietary lipid relative to protein has been shown to increase protein retention in salmonids, and to reduce N excretion (Beamish and Medland 1986, Hillestad and Johnsen 1994, Helland and Grisdale-Helland 1998, Medale *et al.* 1995, Sugiura *et al.* 1998). No report is yet available on the effects of dietary lipid and P levels on retention and excretion of P and N by red sea bream. Hence, the present study aimed to investigate the possible effects of dietary lipid and phosphorus levels on retention and excretion of N and P in red sea bream fed fish meal based and alternative protein based diets and to observe whether or not red sea bream need lipid and phosphorus supplementation for optimum growth, feed utilization and utilization of P and N which in turn help in developing environmentally friendly diets.

Materials and methods

Six practical diets were formulated to contain two levels of lipid (210 and 260 g/kg) and three levels of phosphorus (17, 14 and 12 g/kg) in the dry diets

respectively. Increasing lipid level was achieved by addition of soybean oil in the diets. Changes in dietary P content from 17 to 12 g/kg was achieved by partial substitution of dietary fish meal with a combination of defatted soybean meal, corn gluten meal, feather meal and blood meal with the supplementation of monosodium phosphate. The diets were labeled according to factors (L and P) and levels (1-3) and they were designated as L1P1, L1P2, L1P3, L2P1, L2P2 and L2P3, respectively. The compositions of the experimental diets are presented in Table 1. Dietary protein of the experimental diets, in a range of from 471 to 454 g/kg dry diet and gross energy were from 23 to 25 MJ/kg. The carbohydrate sources and binders were wheat flour and pregelatinized starch while the lipid sources were pollock liver oil and soybean oil.

Table 1. Composition of the experimental diets (g kg^{-1} dry diet)

Ingredients	Diet code					
	L1P1	L1P2	L1P3	L2P1	L2P2	L2P3
Jack mackeral meal	500.0	300.0	300.0	500.0	300.0	300.0
Defatted soybean meal	50.0	150.0	150.0	50.0	150.0	150.0
Corn gluten meal	50.0	100.0	100.0	50.0	100.0	100.0
Feather meal	0.0	40.0	40.0	0.0	40.0	40.0
Meat flour	159.0	100.0	100.0	109.0	54.0	64.0
Blood meal	0.0	40.0	40.0	0.0	40.0	40.0
Pregelatinized starch	50.0	50.0	50.0	50.0	50.0	50.0
Pollok liver oil	135.0	150.0	150.0	135.0	150.0	150.0
Soybean oil	0.0	0.0	0.0	50.0	50.0	50.0
P-free mineral mixture[a]	10.0	10.0	10.0	10.0	10.0	10.0
NaH_2PO_4	10.0	20.0	10.0	10.0	20.0	10.0
Vitamin premixture[b]	30.0	30.0	30.0	30.0	30.0	30.0
Choline chloride	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin E (50%)	1.0	1.0	1.0	1.0	1.0	1.0
Cellulose	0.0	4.0	14.0	0.0	0.0	0.0

[a] P-free mineral mixture composition (%) : NaCl, 5 0; manganese sulfate, 74.5; iron (III) citrate n-hydrate, 12.5; trace element mix,a*5.0; cellulose, 3.0. a* The trace element mixture had the following components (%): zinc sulphate heptahydrate, 35.3; manganese sulphate, 162; copper (II) sulfate pentahydrate, 3.1; aluminium chloride hexahydrate, 1.0; cobalt chloride, 03; potassium iodate, 0.1; cellulose, 44.0.

[b] The vitamin mix had the following components (mg 100 g^{-1}}-Thiamine hydrochloride 6; riboflavin 10; pyridoxine hydrochloride 4; cyanocobalamin 0 .01; ascorbic acid 500; niacin 40; Ca-pantothenate. 10; inositol 200; biotin 0.6; folic acid 1.5; p-aminobenzoic acid 5; vitamin K_3 5; vitamin A acetate 4000 IU; vitamin D_3 4000 IU.

The diets were pelleted using the laboratory pelletizer, dried a vacuum freeze-drier (RI-E-206, Kyowa Vacuum Tech., Saitama, Japan) and stored at 4^0C until used. The minimum level of dietary P was estimated to be about 11.8 g kg^{-1} dry diets. The experiment was designed as a 2×3 factorial arrangement with the factors 'dietary lipid level' and 'phosphorus level'.

Fingerling red sea bream *Pagrus major* were obtained from Seiho Suisan Co. Ltd. (Mie, Japan) and fed commercial red sea bream feed prior to the start of the experiment. Twenty five fish (average weight 3.74 ± 0.07 g) were randomly distributed in each well-aerated 60-L glass tanks with two replications. The feeding trial was conducted in re-circulated artificial seawater (Sea Life®, Tokyo, Japan) at a flow rate of 700-800 ml/min. The water renewal rate in the system was 50% in every week. Important water quality parameters such as temperature, pH and salinity were monitored daily and dissolved oxygen was measured fortnightly. All the parameters were observed to be within the acceptable limits for fish culture. Water temperature ranged from 22 to 28°C. The fish were fed four times a day until near satiation for 12 weeks.

Initial weight data were obtained at the start of the experiment and growth of fish was measured every 3 weeks subsequently. Upon termination of the experiment, five fish from each tank were randomly selected for the chemical analyses of the whole body. Whole body samples were pooled from 5 fish and minced by a centrifugal mill (Retsch ZM 1. Germany) fitted with a 0.25 mm screen. The homogenate was collected and kept at -20°C until analysis. Proximate composition and chemical analyses of the diets and fish whole body samples were made in three replicates as follows: moisture contents was measured gravimetrically, crude ash contents was determined by incinerating a known amount of sample in an electric muffle furnace (Yamato, FA-21) at 600 °C for 8 hours, crude protein was analyzed using the Kjeltec Auto Sampler System 1035/38 (Netherland), and crude lipid was measured by following the method of Folch *et al.* (1957) (Table 2). Samples for P analysis were digested in nitric acid using the MLS-1200 Mega Microwave Digestion System (Italy). Phosphorus contents were analyzed by a visible light spectrophotometry (Shimadzu, UV 265 FW, Kyoto, Japan) at 750 nm.

Table 2. Nutrient contents of the experimental diets (g kg^{-1} dry diet)

Nutrients	Diet code					
	L1P1	L1P2	L1P3	L2P1	L2P2	L2P3
Crude ash	94.0	79.0	72.0	92.0	75.0	71.0
Crude protein	468.0	471.0	466.0	455.0	454.0	460.0
Crude lipid	216.0	217.0	200.0	264.0	266.0	262.0
GE* (MJ kg^{-1})	22.6	23.4	23.0	24.3	24.7	24.7
Protein/GE ratio (g MJ^{-1})	20.7	20.1	20.2	18.7	18.4	18.6
Total P	17.6	14.8	12.1	16.6	14.2	11.8

* GE, Gross energy

Results were analyzed using one-way and two-way ANOVA (Systat 8.0, SPSS, Chicago, USA). Differences between treatments were compared by Tukey's test. Values were considered significant at $P<0.05$.

Results and discussion

The results of overall growth performance and feed utilization in fish feeding on the experimental diets are presented in Table 3. Dietary lipid level did not show significant differences on weight gain, specific growth rate, thermal-unit growth coefficient, and feed conversion ratio (FCR) through out the rearing period. Reduction of FM with lipid and P supplementation (L1P2 and L2P2) had no significant effect on WG, SGR, and TGC compared to the control diet, L1P1 whereas, FCR was significantly improved. These results indicate that reduction of FM level (500-300 g/kg) has no remarkable influence on growth performance and feed utilization. Similar results were reported indicating no significant difference for growth in rainbow trout fed diets with different P levels (Green *et al.* 2002). Studies conducted with different species of fish have reported that fish fed monocalcium phosphate supplemented diets showed a significant improvement in weight gain and feed utilization, compared to fish fed diets without monocalcium phosphate (Kim *et al.* 1998).

Table 3. Growth and feed performance in red sea bream cultured for 12 weeks*

Diet code	Weight gain (g)	SGR[1] (% day^{-1})	TGC[2] × 1000	FCR[3]
L1P1	52.53 ± 1.31^{ab}	3.24 ± 0.00^{a}	0.939 ± 0.01^{ab}	1.14 ± 0.03^{ab}
L1P2	50.31 ± 2.06^{ab}	3.17 ± 0.06^{abc}	0.915 ± 0.02^{abc}	1.02 ± 0.01^{c}
L1P3	43.54 ± 1.18^{b}	3.01 ± 0.02^{c}	0.847 ± 0.01^{c}	1.16 ± 0.02^{a}
L2P1	53.78 ± 2.51^{a}	3.25 ± 0.06^{a}	0.948 ± 0.02^{a}	1.06 ± 0.01^{bc}
L2P2	52.66 ± 2..15^{ab}	3.21 ± 0.04^{ab}	0.936 ± 0.02^{ab}	1.01 ± 0.01^{c}
L2P3	44.19 ± 0.38^{ab}	3.05 ± 0.02^{bc}	0.857 ± 0.00^{bc}	1.09 ± 0.02^{abc}

*Values (means ± S.D.) in the same column not sharing the common superscript letters are significantly different ($P<0.05$). [1]Specific growth rate (SGR) = 100 × (ln final body weight - ln initial body weight)/days. [2]Thermal-unit growth coefficient (TGC) = (Final body weight$^{1/3}$ - Initial body weight$^{1/3}$)/(water temperature °C × days). [3]Feed conversion ratio (FCR) = Feed consumption/weight gain.

Proximate composition and P content of the whole body of red sea bream for the initial groups and at the end of the experiment fed different experimental diets are presented in Table 4. Increased crude ash contents and increased lipid contents were obtained for the final groups in contrast to the initial group. Whole body lipid content of the lipid supplemental group was higher than that of the group fed with the other diets. Highest body lipid content was achieved in the diet group L2P2 (127 g/kg). Whole body total P increased with reduction of FM and P supplementation, while low level P containing diets showed the lowest growth and feed performance among the treatments. Increasing dietary lipid level resulted in increased final whole body lipid content. These results indicate that the improved FCR is associated with increased dietary lipid content which might have increased the deposition of lipid in the body, because of a protein sparing effect of dietary lipid

in the red sea bream.

Table 4. Proximate composition of red sea bream at initial and end of the experiment*

Diet code	Moisture (g/kg)	Crude ash (g/kg)	Crude protein (g/kg)	Crude lipid (g/kg)	Phosphorus (g/kg)
L1P1	670 ± 4^{a}	39.50 ± 0.5^{b}	175 ± 1^{ab}	111 ± 1^{d}	7.04 ± 0.1^{b}
L1P2	676 ± 6^{a}	45.80 ± 0.2^{a}	173 ± 2^{bc}	116 ± 1^{cd}	8.59 ± 0.1^{a}
L1P3	682 ± 4^{a}	42.00 ± 0.2^{b}	!73 ± 0^{abc}	106 ± 1^{c}	7.05 ± 0.1^{b}
L2P1	667 ± 2^{a}	40.75 ± 0.1^{b}	178 ± 1^{a}	124 ± 1^{ab}	7.21 ± 0.0^{b}
L2P2	669 ± 1^{a}	42.95 ± 0.0^{b}	174 ± 0^{abc}	127 ± 0^{a}	8.17 ± 0.1^{a}
L2P3	675 ± 6^{a}	37.00 ± 0.7^{c}	171 ± 1^{c}	120 ± 2^{bc}	7.00 ± 0.1^{b}
Initial	754	45.5	155	47	7.42

*Values presented as means ± S.D. (n= pooled samples of 5 fish/tank) in a column not sharing the same superscript letters are significantly different ($P<0.05$).

Retention and excretion of P and N by red sea bream after 12 weeks feeding are presented in Table 5 and Table 6. Increasing lipid level significantly increased both P and N retention which in turn reduced their excretion. Reduction of FM with P supplementation in the diet significantly increased both P and N retention which in turn resulted in reduction of their excretion. Increasing dietary lipid slightly increased N retention (%), while significantly decreased N excretion (kg/ton) in this experiment. The results emphasized that retention efficiencies of dietary nutrients such as P and nitrogen are important for the evaluation of fish feed quality and might change according to fish weight, temperature, amount of feed consumed and feed composition (Lall 1991, Cho 1994).

Table 5. Retention and excretion of phosphorus in red sea bream fed diet supplemented with lipid and phosphorus for 12 weeks*

Diet code	P retention (%)[1]	P excretion (kg/ton)[2]
L1P1	35.46 ± 1.4^{d}	12.88 ± 0.7^{a}
L1P2	56.91 ± 0.4^{a}	6.46 ± 0.0^{c}
L1P3	49.52 ± 0.2^{b}	7.06 ± 0.0^{c}
L2P1	40.93 ± 0.3^{c}	10.40 ± 0.2^{b}
L2P2	56.74 ± 1.1^{a}	622 ± 02^{c}
L2P3	54.09 ± 0.1^{a}	5 85 ± 0.1^{c}
Lipid (L) & Phosphorus (P) level	<0.05	<0.05
L × P	<0.05	<0.05

*Values are presented as means ± S.D. (n = pooled samples of 5 fish/tank). Means in a column not sharing the same superscript letters are significantly different ($P<0.05$).

[1]Retention (%) = {(Final nutrient content - initial nutrient content)/nutrient intake} × 100.

[2]Excretion (kg t^{-1}) = [{FCR nutrient in diet (g) - nutrient retained in fish (g)/production (t)] × 1000.

Table 6. Retention and excretion of nitrogen in red sea bream fed diet supplemented with lipid and phosphorus for 12 weeks*

Diet code	N retention (%)[1]	N excretion (kg ton^{-1})[2]
L1P1	33.38 ± 1.3^{bc}	56.67 ± 2.8^{a}
L1P2	35.83 ± 0.3^{ab}	49.11 ± 0.0^{b}
L1P3	32.05 ± 0.3^{c}	58.80 ± 0.7^{a}
L2P1	37.26 ± 0.1^{a}	48.38 ± 0.5^{b}
L2P2	38.12 ± 0.4^{a}	45.38 ± 0.7^{b}
L2P3	33.94 ± 0.3^{bc}	52.76 ± 1.0^{ab}
Lipid (L) level	<0.05	<0.05
P level	<0.05	<0.05
L × P	NS	NS

*Values are presented as means ± S.D. (n = pooled samples of 5 fish/tank). Means in a column not sharing the same superscript letters are significantly different (P<0.05); NS: Not significant.

[1]Retention (%) = {(Final nutrient content - initial nutrient content) /nutrient intake} × 100.

[2]Excretion (kg t^{-1}) = [{FCR nutrient in diet (g) - nutrient retained in fish (g)}/production (t)] × 1000.

Therefore, the overall results of the present study demonstrated that both dietary lipid and P supplementation are needed in the diet of fingerling red sea bream for developing less-polluting feed. Hence, further study is necessary with different sized and aged red sea bream to determine the possible effect of dietary lipid and phosphorus supplementation for formulating environmentally clean diets.

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Preliminary success on hormone induced captive breeding of goldspot mullet, *Liza parsia* (Ham.)

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Abstract
An attempt was made to breed goldspot mullet, *Liza parsia* in captivity through hormone induction. The fish started spawning 35~36 hours after a single dose of 2ml ovaprim per kg body weight. Hatching of fertilized eggs completed within 42~48 hours after spawning. The mean hatching rate (%) was 71.33±12 corresponding to the fertilization rate (%) of 64±12. The larvae started its first external feeding on the third day and attained a length 2.5±0.25 mm. The salinity of both breeding and rearing cisterns was 20‰ and temperature was maintained at 22~23°C.

Key words: *Liza parsia*, hormone induced captive breeding.

Introduction

The fish *Liza parsia* (Ham.), under the family Mugillidae commonly known as goldspot mullet, is a catadromous fish and widely distributed in the coastal waters of tropical and sub-tropical regions extending from 42°N to 42°S (Talwar and Jhingran 2001, Nash and Shehadeh 1980). The fish *Liza parsia* is commonly available in shallow coastal waters, estuary and mangrove swamps of Bangladesh. The adults and juveniles are hardy, euryhaline and eurythermal. It is one of the most favorite, tasty and commercially important fish in Bangladesh as well as Southeast Asia, India and many parts of central and South America. The popularity of these species in aquaculture is due to high quality of its flesh, its extreme tolerance for a wide range of temperature and salinity, which is important for culture in intertidal ponds (Nlewadim and Deekae 1997).

A few works have been done on the biology of mullet, with brief accounts on fecundity, GSI, reproductive characteristics and spawning (Hsu *et al* 2007, Rheman *et al* 2002, Ergene 2000 and Cherif *et al* 2007) and only one study has been reported on artificial breeding of closely related species *L. subviridis* (Das, 1992). Ergene (2000) reported that the peak reproduction period of *L. ramada* lies in November through December. The mullet is a winter breeder and the suitable breeding temperature is 20~23°C (Hsu *et al* 2007, Huang and Su 1986, 1989, Kuo 1986, Shyu and Lee 1986).

As no attempt has so far been made in artificial breeding and fry production of *L. Parsia* and considering the fishery and aquaculture importance of the species, Bangladesh Fisheries Research Institute has been conducting research on its breeding and mass seed production in captive condition. This communication reports on the success of the hormone induced captive breeding and fry production of *L. parsia* for the first time in Bangladesh.

Materials and methods

The study was done in between October to December 2008 at the Brackishwater Station of the Bangladesh Fisheries Research Institute (BFRI), Paikgacha, Khulna.

Fish and experimental vessel

Live gravid females and ripe males, on the basis of their morphological criteria, of *L. parsia* were collected from a local shrimp farm. The gravid female has swollen belly with round and reddish genital papillae. The ripe male secrets milky white milt on gentle pressure in its anal region. The female broods were of 22~25 cm in total length and 142~174g in weight while the males were of 16~20 cm and 70~90g. The fishes were kept in a circular concrete cistern (2 m dia x 1 m depth), filled with filtered pond water of 7‰ salinity, which were gradually increased up to 20‰ by adding 150‰ brine in 72 hrs. Pelleted feed was given in a tray and cleaned up time to time. Fishes, with a female:male sex ratio of 1:2, were kept in a similar cistern containing 20‰ water after hormone injection. A continuous current flow was maintained (16 m/min) for 34 hrs and then stopped for creating calm situation for pairing of the fishes. There were no water shower but aeration was provided with portable electric aerator. Water temperature of the breeding cistern was maintained between 22~23^{0}C using electric thermostat.

Hormone dose and injection

A synthetic gonadotropin releasing hormone analogue (SGnRH) commercially known as "Ovaprim" (Syndel Lab. Ltd., Vancouver, Canada) was used in this study. After 72 hrs of acclimatization, fishes of both the sexes were injected hormone at a dose of 2ml kg^{-1} body weight. In case of both male and female, a single dose of hormone was injected in deep muscle at the base of the dorsal fin.

Spawning, fertilization and larval rearing

Spawning behaviour of injected fishes was closely observed visually. After 12 hours since the first spawning, all fishes were removed from the spawning cistern. The eggs were floating and drifting in nature. To determine the ovulation success, spent fishes were stripped and the females from which no egg to come out were considered fully ovulated. In case of any egg to come out, the spent females were dissected and eggs retaining in the abdomen were counted. The numbers of unreleased eggs were used to

calculate the number of egg released, taking into account the relative fecundity of 867, 949 and 644 eggs/g body weight of the species in the months of October, November and December, respectively (Rheman *et al.*, 2002). The information also demarcated that December is the peak breeding season of the goldspot mullet. A random 100-egg samples were studied under a trinocular microscope and classified as fertilized and unfertilized. The fertilization rate was calculated as the number of fertilized eggs divided by the total sampled number (n=100) of eggs. Hatching percentage was estimated by random volumetric sampling and counting of the newly hatched larvae. Meanwhile the newly hatched larvae were transferred to three circular fiber glass tanks having identical salinity and temperature. When about 80% of the hatchlings were observed with their absorbed yolk sac, feeding was started with boiled and screened hen's egg yolk for two days. Subsequently newly hatched *Artemia* was given for rest of rearing period (20 days).

Results and discussion

The spawning activity appeared to continue first after 36 hours post injection. This period is reported 34-35 hours for *M. parsia* (Radhakrishnan *et al*, 1976), 48-52 hours for *L. subviridis* (Das 1992), 40-50 hours for *M. cephalas* (Liao 1975). The fishes started pairing just before they spawned, males were observed more active during the time of mating. The first release of a small number of eggs stimulated the male to release spermatozoa. The female then responded with a jerk and release huge eggs, while spawning males stayed besides females close to the tail and fertilized releasing eggs as soon as those scattered. The spawning rates (%) were 65±8, 54±12 and 42±6 for October, November and December trials respectively. The fertilization rates (%) were 72±11, 64±9 and 56±16 in aforesaid three consecutive months (Table 1).

Table 1. Spawning, fertilization and hatching data in hormone induced breeding trials of *L. parsia*

Months	Size of brood (g)	Latency period (hr)	Spawning rate (%)	Fertilization rate (%)	Incubation period (hr)	Hatching rate* (%)
October 2008	♀ 96±13 ♂ 64±8	35	65±8	72±11	44±2	82±12
November 2008	♀ 134±11 ♂ 72±7	36	54±12	64±9	45±3	76±8
December 2008	♀ 165±8 ♂ 80±10	35	42±6	56±16	44±3	56±16

*Hatching rate was calculated on the basis of considering fertilized eggs as base unit (100%).

In the present trial, the eggs took 42~48 hours after spawning to hatch out. Das (1992) reported that the incubation period of *L. subviridis* is 32~36 hours at 22~25^{0}C and 38~42 hours at 20~21.5^{0}C of temperatures. The hatching duration of *L. parsia* was a bit wide, as every pair of brood did not laid eggs at a time but the time span was very close for all the three trials. The hatching rates (%) were 82±12, 76±8 and 56±16 for

October, November and December trials respectively. The hatching rates were calculated on the basis of considering fertilized eggs as base unit (100%). Similar observation was reported by Das (1992) having a hatching rate 62.5% for *L. subviridis* that were artificially bred on winter but the count was based on total laid eggs.

The time required to develop larvae from first cleavage to hatch out takes 44 hours, where myomers were differentiated after 40 hours of development. The day-old blackish larvae showed peculiar jerking/crippling movements. Development of mouth cavity was started at the 3rd day and the yolk sac and the oil globules were reduced and larvae started first feeding. A 12-day old fry looks like a complete fish with every fins, well developed gills, darker in color and observed to swim in school. The salient features of such day-wise development of different larval stages of *L. parsia* under captive breeding conditions are described in Table 2 and shown in Figs. 1-9.

Table 2. Salient features of larval development and behaviour of *L. parsia*

Age (day)	Total length (mm)	Description of development and behaviour
0	1.25	Newly hatched larvae had large yolk sac and oil globule; larvae were slightly curled; eye, mouth and anus were closed; notochord curved along yolk sac.
1	1.5	Yolk sac tended to reduce; jerking/crippling movement; mouth and anus still closed; digestive tube not well developed.
2	2.0	Formation of organ was in progress; pigmentation on eye and body; mouth was under development; crippling movement.
3 to 4	2.25 to 2.75	Development of mouth cavity was started; yolk sac and oil globule reduced; larvae started to take feed; dorsal and pectoral fins appeared; well developed mouth; gill development appeared.
5 to 7	2.75 to 2.8	Digestive tube was well developed; fin rays appeared; mouth opened; well developed eye; normal movement; formation of stomach, intestine, gall bladder; reduction of oil globule continued.
8 to 9	3.25 to 3.50	Complete disappearance of oil globule, Formation of gill filaments. It was the flexing point growth curved the growth started to be accelerated.
10 to 13	3.75 to 4.15	Fin fold moved backward; gill filaments well developed; body surface become dark in color; larvae swam in school.
14 to 15	4.25 to 4.75	Fry swimming in school; body surface getting darker.
22	4.80 to 5.5	Fry have every similarity of its parents, showed phototaxis during day time, swimming during night; eyes were very clear.

The physicochemical parameters of the water in the breeding cistern were measured periodically following standard methods (APHA 1995) and the mean values are given in Table 3. The salinity of water used in the present study was a bit lower (20%), but values

of other parameters were close to those have been reported by Das (1992) for *L. subviridis*.

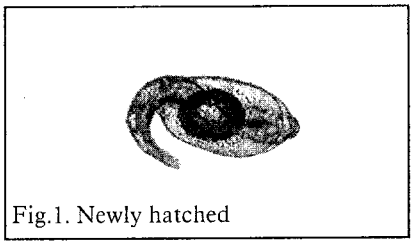

Fig.1. Newly hatched

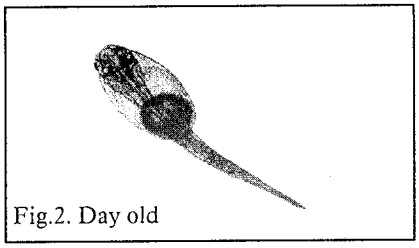

Fig.2. Day old

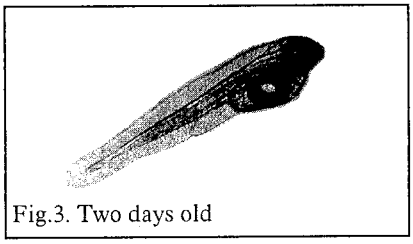

Fig.3. Two days old

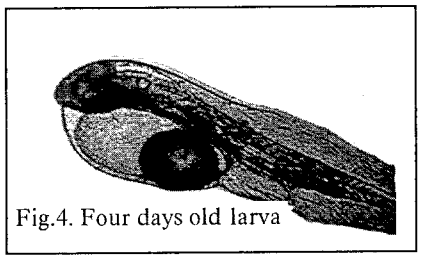

Fig.4. Four days old larva

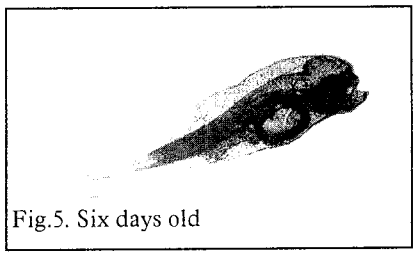

Fig.5. Six days old

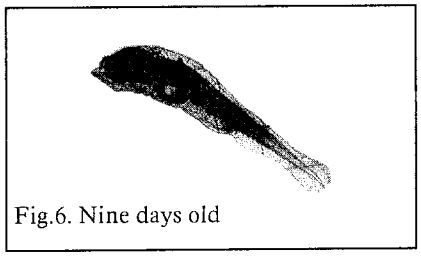

Fig.6. Nine days old

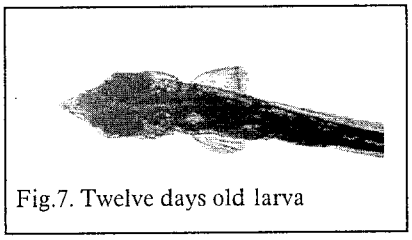

Fig.7. Twelve days old larva

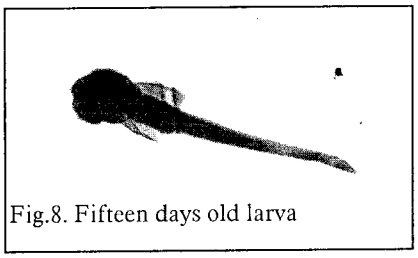

Fig.8. Fifteen days old larva

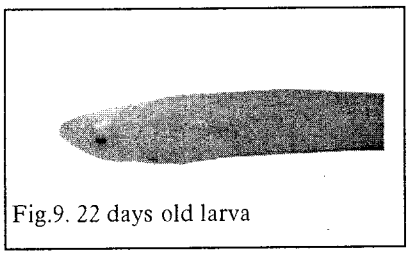

Fig.9. 22 days old larva

Figs. 1-9. Different development stages of *L. parsia* larvae at different days under captive breeding conditions.

Table 3. Values of different water quality parameters of breeding and rearing cisterns of *L. parsia*

Water quality parameters	Mean values ± SE	
	Breeding cistern	Rearing cistern
Water salinity (‰)	20±1	20±1
Water temperature (^{0}C)	22.5±0.5	22.5±0.5
pH	8.0±0.4	7.8±0.3
Dissolved oxygen (mg/l)	9.0±1.2	8.5±0.8
Alkalinity (mg/l)	146±6.0	138±8.0

Conclusions

The results of this article reveal that hormone induced breeding of *L. parsia* in captive condition is possible and would open a new era in the country for aquaculture and conservation of this commercially important brackishwater species. Further research are required for brood management, improvement and/or perfection of captive breeding technique, larval food and rearing, and mass seed production.

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Necessity of dietary calcium supplement in file fish (*Monacanthus cirrhifer*)

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Abstract
File fish, *Monacanthus cirrhifer,* juveniles with initial mean body weight of 0.27 g were fed purified diets with or without calcium (Ca) supplement for 10 weeks at a water temperature of 27.0±1.4°C. Growth was significantly low in fish fed diet without Ca supplement than fish fed diet with Ca supplement. Feed efficiency and condition of fish were also significantly decreased in absence of a dietary Ca supplementation. Minerals contents of bone were similar in both the treatment groups and did not appear as a suitable indicator of Ca requirement. It appeared that Ca supplement to the purified diet is necessary for file fish for their proper growth and feed utilization.

Key words: File fish, Ca requirement, Bone mineralization

Introduction

The importance of Ca as an important nutrient for fish is well established. Until recent time, it was generally accepted that a dietary Ca supplementation may not be necessary for fish. Because, it has been reported that different species of fish can easily absorb calcium (Ca) from surrounding water through the gills, intestine and other organs (Love 1980, Ichii and Mugiya 1983, Ishihara and Mugiya, 1987, Takagi and Yamada 1992). However, our recent studies revealed that some marine species require a Ca supplement to the diet (Hossain and Furuichi 1998, 1999, 2000a,b). Therefore, further study is necessary to investigate whether Ca absorption from seawater is sufficient for marine fishes, i.e. whether they need a dietary Ca supplement. Accordingly, important aquaculture species need be tested for dietary Ca requirements. In the present study, the necessary of dietary Ca supplement for file fish *(Monacanthus cirrhifer)* has been investigated.

Materials and methods

Experimental diets

The composition of the experimental diets is shown in Table 1. Best quality ingredients were used for diet preparation. Protein sources were 50% vitamin-free casein. Lipid and sugar sources were pollack liver oil and starch and dextrin, respectively. Vitamin mixture, amino acid mixture and mineral mixture were prepared separately and added to the diets. Ca-lactate was supplied only to the control diet (with Ca) to obtain 0.3% Ca. Pollack liver oil was weighed upon the portion of casein weighed previously and was mixed with other ingredients. Ingredients were mixed 500 g at a time to facilitate a complete mixing. The mixed powder was then mixed with 20% distilled water and was pelleted with a laboratory type pelleter with sieve of appropriate mesh size. Pelleted diets were then cut into small spices appropriate to the mouth size of the fish to be fed. Then the feed was half dried with an air flow dryer at 60^{0}C and stocked at -20^{0}C until use. The proximate and mineral compositions of the experimental diets are shown in Table 2.

Table 1. Composition of the experimental diets for file fish

Diet	Control	No Ca supplement
Ingredient (%)		
Casein[1]	50	50
Amino acid premix[2]	4	4
Starch, pregelatinised	7	10
Dextrin	10	15
Pollack liver oil	10	10
Vitamin premix[3]	3	3
Mineral premix[4]	6	6
Carboxymethylene cellulose	4	5
Ca-lactate	2.308	-
Alpha-cellulose	3.692	6

[1] Vitamin free, from milk, 200 mg Ca/kg casein.

[2] Amino acid premix (g/kg diet): Arginine-HCl, 10; alanine, 10; glycine, 10; aspartate-Na, 10.

[3] Vitamin premix (mg/kg diet): Thiamine-HCl, 60; riboflavin, 200; pyridoxine-HCl, 40; vitamin B_{12}, 0.09; nicotinic acid, 800; Ca-pantothenate, 280; inositol, 4000; biotin, 6; folic acid, 15; PABA, 400; choline chloride, 8000; ascorbic acid, 2000; alpha-tocopherol, 400; menadione, 40; beta-carotene, 12; vitamin D_3, 0.05.

[4] Mineral mixture (mg/kg diet): KCl 3840; $MgSO_4 5H_2O$ 4080; $NaH_2PO_4 2H_2O$, 34,260; Fe-citrate, 1200; $AlCl_3.6H_20$, 45; CuCl, 7.9; KI, 1.9; $CoCl_2.6H_2O$, 0.7.

Table 2. Proximate and mineral compositions of the experimental diets for file fish

Ingredient (%)	Diet	
	Control	No Ca supplement
Proximate composition (% dm)[*1]		
Moisture	20.9	21.5
Crude protein	51.3	52.0
Crude lipid	9.1	9.3
Crude ash	5.1	5.0
Mineral composition (dm)		
Calcium (%)	0.34	0.03
Phosphorus (%)	1.00	1.05
Potassium (%)	0.19	0.18
Magnesium (μg/g)	385	390
Iron (μg/g)	260	255
Zinc (μg/g)	40.2	40.5
Manganese (μg/g)	22.4	23.0
Copper (μg/g)	10.2	12.4

[*1]dm = dry matter.

Fish and rearing procedure

Juvenile file fish attaching with the floating sea weeds/debris were collected using a scoop net from the open bay. The fish of similar size were shorted and adapted in rearing conditions for 2 weeks before starting of the experiment. Fish were fed the control diet during adaptation. The rearing experiment was carried out in 100-L round polycarbonate tanks with a continuous water flow of 1.5-2.0 L/min. Water temperature was 27.0±1.4°C. The rearing water contained approximately 400 mg Ca/L. A daily light:dark cycle of 12 h:12 h was maintained. At the beginning of the feeding trial, fish (average initial weight of 0.27 g) were weighed and distributed to 6 rearing tanks (three tanks for each treatment) as a group of 100 fish. The fish were fed the experimental diets to satiation twice a day for 10 weeks.

Sample collection

Final sampling was done after 16 h starving. Any abnormalities in external features were monitored and recorded. Fish of each tank were counted to record survivability. Then body length and weight were measured. After removing the internal organs, the whole body was washed with distilled water and preserved at -20°C for bone collection. For bone collection, preserved whole body carcasses were defrosted at room temperature, and then steamed on a boiling water bath for a few minutes. Vertebral column was separated from the body and cleaned in distilled water using a brush. Then each of the vertebrae was separated from the vertebral column and all together were rinsed vigorously to be cleaned properly. Finally, the vertebral bone samples were washed with distilled water for several times, and soaked on a cleaned filter paper. After drying in an

oven for 24 h at 105^{0}C, the samples were ground in fine grains using a mortar and pestle. The samples were then preserved in clean glass vials for further analysis.

Analytical methods

The proximate composition of experimental diets was analyzed according to the methods given in Association of Official Analytical Chemists (AOAC, 1980). For mineral determination, dried bone samples were digested with wet digestion method with a nitric acid-perchloric acid mixture. Minerals, except phosphorus, in the digested samples were determined with an Atomic Absorption Spectrophotometer (Perkin-Elmer 3300, Perkin Elmer, USA). Phosphorus in the digested samples was determined colorimetrically according to the molybdate method described by Taussky and Shorr (1953).

Statistical analysis

Data were analyzed for significant differences with student T-test using a statistical package (SPSS package programme).

Results and discussion

After the 10 weeks of rearing period, average final body weight and weight gain of file fish fed the diet without Ca supplement were significantly lower than that fed the diet with a Ca supplement (control diet) as shown in Table 3. Survival rate was 78.3 and 82.0 in fish fed control diet and Ca unsupplemented diet, respectively and was not statistically different from each other. Significantly lower condition factor was observed in fish fed Ca unsupplemented diet compared to the control diet. A deletion of Ca from diet decreased the feed efficiency of file fish. There were no differences in ash and mineral contents of vertebrae between two treatment groups except that an unsupplementation of Ca to the diet decreased the Fe content of vertebrae (Table 4).

Table 3. Growth and feed utilization of file fish after 10 months rearing period fed the experimental diets with or without Ca supplement

	Diet	
	Control	No Ca supplement
No. of fish at initial	100	100
Survival rate (%)	78.5	82.0
Av. body weight at initial (g)	0.27±0.02	0.27±0.02
Av. final body weight (g)[1]	2.47±0.47a	2.09±0.55b
Weight gain (%)[1]	815±13a	674±15b
Condition factor[1,2]	3.71±0.06a	3.57±0.05b
Feed efficiency (%)	91.8±1.8a	87.9±2.1b

[1]Significant difference ($p<0.05$), [2] Condition factor: Body weight (g) x 100/(total length in cm)^3.

Table 4. Ash and mineral composition of bone of file fish[1]

	Diet	
	Control	No Ca supplement
Crude ash (%)	55.8±0.7	55.5±0.7
Calcium (%)	22.4±0.3	21.9±0.5
Phosphorus (%)	10.2±0.7	10.5±0.3
Magnesium (%)	0.69±0.08	0.69±0.26
Potassium (μg/g)	92.3±13.9	99.8±18.2
Iron (μg/g)[2]	189±20a	165±12b
Zinc (μg/g)	120.0±1.8	116.9±6.8
Manganese (μg/g)	97.9±1.2	102.4±5.9
Copper (μg/g)	7.8±0.2	7.6±0.8

[1]Dry matter basis. Average values (mean±SD) of composite sample of bones from all the fish of each tank. [2]Significant difference.

It has been reported that fish can actively absorb Ca from surrounding water (Ogino and Takeda 1978, Love 1980, Takagi and Yamada 1992). Therefore, it is generally accepted that a dietary Ca supplement may not be necessary for marine fishes. However in the present study, the poor growth of fish feed Ca unsupplemented diet indicated that Ca absorption from seawater by file fish was not sufficient for their growth. Similar poor growth was observed in tiger puffer, Japanese flounder and scorpion fish in some previous studies (Hossain and Furuichi 1998, 2000a,b), which supports the findings of the present experiment. When experiencing Ca inadequacy, file fish maintained bone Ca content (Table 4), probably providing inadequate Ca for other physiological process leading to poor growth and food utilization. From the above discussion, it is clear that Ca uptake from seawater is not sufficient for proper growth and feed utilization of file fish and they need a dietary Ca supplement.

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Milt quality determination of a critically endangered fish, Olive barb (*Puntius sarana*, Ham.) in Bangladesh

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Abstract

The present study was aimed to evaluate the characteristics of the olive barb sperm. Milt was collected fortnightly from 49 male fish (mean weight 90.8 g and length 18.64 cm) from April to July in 2008. In the olive barb ejaculated milt, volume (μl/g), motility (%), duration of motility (s), concentration ($\times 10^{10}$/ml) and pH values were found to be 6.06±0.32, 88.27±0.71, 171.41± 7.41, 5.16±0.05 and 7.75±0.04, respectively. Milt volume was significantly ($P<0.05$) correlated with sperm concentration. Milt volume, sperm concentration, motility and duration of motility significantly varied ($P<0.05$) during spawning season.

Key words: *Puntius sarana*, Endangered, Milt quality

Introduction

Olive barb, *Puntius sarana* (Hamilton 1822), locally known as sarpunti is a very popular barb in Bangladesh and now critically endangered. It is the largest barb available in the Indian sub-continent. Though the fish was abundantly available in our open water system in the past, due to over exploitation and various ecological changes in its natural habitats, it is now critically endangered in Bangladesh (IUCN 2000). This important food fish will disappear from Bangladesh unless proper steps are urgently taken for development of appropriate aquaculture techniques along with proper conservation strategies.

Aquaculture farming requires good quality seed and for that proper management of male brood stock is a prerequisite .Management of broodstock is highly species-specific and its success depends upon many factors. (Billard *et al.* 1995). Determination of sperm density has been used to evaluate the sperm quantity (Rurangwa *et al.* 2004) that may influence the fertilization (Piros *et al.* 2002). For successful breeding programme it is essential to know the sperm characteristics. Various factors affect the sperm quality of fish including season, photoperiod, collection technique, temperature, time of collection, age and disturbances in spermatogenesis (Baynes and Scott 1989, Cabrita *et*

al. 2001, Asturiano *et al.* 2005). Sperm volume, spermatozoa concentration, percentages of motile sperm, sperm pH are usually considered for evaluating sperm quality (Bloom and Ottobre 2001; Tekin *et al.* 2003). No information is available pertinent to sperm quality of olive barb *Puntius sarana* in Bangladesh. Hence, the objective of the study was to evaluate the sperm quality of olive barb *Puntius sarana*.

Materials and methods

In the present study, *Puntius sarana* was collected from two natural depressions (Chalan *beel*, Natore district and Tola *haor*, Netrokona) during the month of January and February, 2008. The average size of male fish was 70g and 110g in Chalan *beel* and the Tola *haor*, respectively. The collected fish were reared in the ponds of Fisheries Field Complex, Bangladesh Agricultural University, Mymensingh. Fish were collected from the brood rearing ponds and kept in the tank of the hatchery of Faculty of Fisheries. Then collected broods were injected with PG at the rate of 2 mg PG/kg body weight for easy collection of milt samples. For sperm collection at first males were fished out from the tank using scoop net. Then fish was laid on the foam fixing in dorsal position and urogenital pore was wiped. Gentle pressure was applieed through the abdomen to remove urine, water, gut exudates and mucus and these were removed with tissue as much as possible for avoiding contamination. A 3 ml plastic syringe was used to collect the sperm. When the milt seemed concentrated, the mouth of the syringe was inserted into the urogenital pore to fill with sperm and then sperm was immediately transferred to the icebox. After collection, sperm samples were transported to the laboratory under cold conditions (7–10 °C). Ejaculated sperm volume was determined by the measuring pipette and expressed as μl. Milt pH were determined with a pH indicator strips (pH: 0–14; Merck, Germany). Sperm motility was evaluated visually for the percent motility (%) after activation in table salt (NaCl). The duration of motility (sec) was also recorded by stopwatch from the initial contact between the activation solution and milt until almost all of the spermatozoa (up to 20%) were immotile. One or two drop 0.9% NaCl was placed on a glass slide and then a drop of 1-2 µl fresh milt was poured to induce the initiation of motility. A light microscope (Novex K-range, Holland) was used at 400 magnifications to determine the percent motility. Sperm concentration was determined using haemacytometer (Germany) and expressed as number of cells$\times 10^{10}$/ml. Milt was diluted 4,000 times in a 0.9% NaCl solution. For preparing 4,000 times dilution, at first microtube containing 990 μl of the 0.9 % NaCl solution was taken and then 10 μl of milt was carefully added to the tube and the content was mixed carefully. From this microtube, 10 μl of milt suspension was taken out and transferred to another microtube containing 390 μl of 0.9 % NaCl solution and finally 4,000 times diluted milt was prepared. A droplet of the diluted milt was placed on a haemocytometer (depth 0.1 mm) with cover slip. The slide was left undisturbed for approximately 5 min to allow the milt cells to settle on focal plane. The number of milt in 5 large squares of the counting chamber was counted under the microscope at 40 times magnification.

Results

Evaluation of milt quality of olive barb

For evaluating the milt quality of olive barb, milt from forty nine fish of two stocks namely the Chalan *beel* and the Tola *haor* were evaluated. The size of *haor* olive barb ranged between 70 and 178 g (mean 111.69±5.73) in body weight and 18 and 24 cm (mean 20.23±0.35) in total length. On the other hand, size of Chalan *b*eel olive barb ranged from 35 to 98 g (mean 67.17±2. 90) in body weight and 15 to 20 cm (mean 16.85 ±0.25) in total length. The mean (±S.E.) of percentage of motility, sperm concentration ($x10^{10}$/ml), ejaculated milt volume (μL/g) and milt pH of *haor* olive barb were found 87.12±0.97, 5.18$x10^{10}$±0.06, 5.27±0.36, 7.79±0.05, respectively (Table 1). In Chalan *beel* olive barb the mean of percent motility of fresh milt, sperm concentration (×1010/ml), ejaculated milt volume (μL/g) and milt pH were found 89.57±0.99, 5.15×10^{10}±0.07, 6.97±0.49, 7.70±0.05, respectively.

Table 1. Milt characteristics of olive barb milt (mean ± standard error)

Items	Stock			
	Haor	Range	Chalan *beel*	Range
Number of fish	26		23	
Weight (g)	111.69±5.73	70-178	67.17±2. 90	35-98
Length (cm)	20.23±0.35	18-24	16.85±0.25	15-20
Milt volume (μL/g)	5.27±0.36	3.11-9.73	6.97±0.49	2.04-13.0
Milt pH	7.79±0.05	7.5-8.0	7.70±0.05	7.5-8.0
Sperm concentration	5.18 $x10^{10}$±0.06	4.3-5.6 $x10^{10}$	5.15 $x10^{10}$±0.07	4.3-5.7 $x10^{10}$
Fresh motility (%)	87.12±0.97	80-95	89.57±0.99	80-95
Duration of motility (s)	177.96±10.13	110-290	164.0±10.88	70-244

It was found that weight of fish was significantly correlated ($P<0.01$, $r = 0.68$) with milt volume. There was no significant correlation of pH with other parameters (Table 2). Length of fish was also correlated ($P<0.05$, $r = 0.62$) with milt volume. Fresh milt motility and milt volume were significantly correlated with duration of milt motility ($P<0.05$, $r = 0.3$) and sperm concentration ($P<0.05$, $r = 0.36$), respectively. There were no strong correlation found among length, weight, milt pH, fresh motility and duration of motility (Table 2).

Table 2. Correlations between spermatological parameters and body traits in olive barb

	Length (cm)	Milt volume (μL/g)	Milt pH	Sperm concentration	Fresh motility	Duration of motility
Weight (g)	0.92**	0.68**	0.19	0.05	- 0.04	0.14
Length (cm)		0.62**	0.16	0.05	- 0.11	0.1
Milt volume (μL/g)			- 0.11	0.36*	0.12	- 0.09
Milt pH				- 0.05	- 0.07	- 0.03
Sperm concentration					0.28	0.25
Fresh motility						0.3*

**P<0.01, *P<0.05

At the beginning of spawning season milt volume of olive barb was lower and it gradually increased with the time (Fig. 1). During the experimental period highest milt volume of olive barb was observed in 5th fortnight (7.42±1.61) and lowest value observed in 2nd fortnight (3.94±0.54). However, milt volume was significantly varied (P<0.05) among the sampling months during the spawning season.

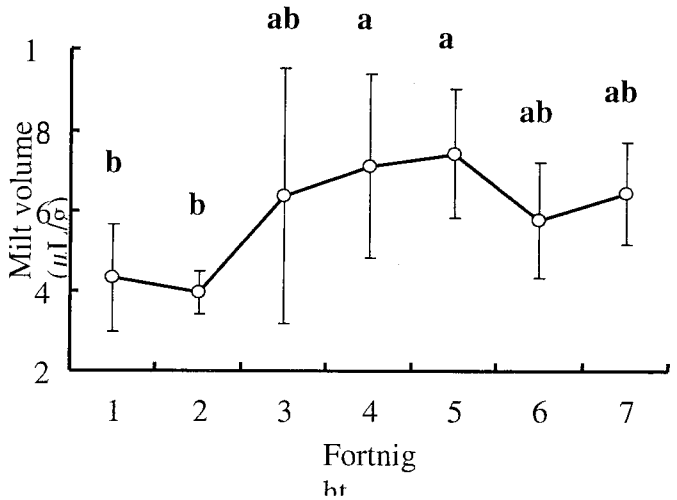

Fig. 1. Milt volume (μl/g) of olive barb at different fortnights during spawning season. Values superscripted by the same letter are not significantly different (P > 0.05). 1-2, 3-4, 5-6 and 7 fortnights represent April, May, June and July, respectively

Sperm concentration of fresh milt of olive barb was highest in 4th fortnight (5.40±0.16) of the sampling month. Like milt volume, sperm concentration was lower at the beginning of the spawning season and it gradually increased with time (Fig. 2). However, lowest concentration was found in the 7th fortnight (4.83±0.49) of the sampling months.

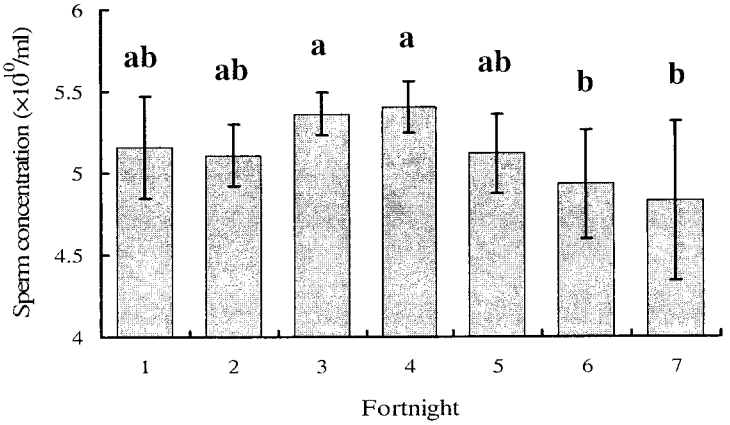

Fig. 2. Sperm concentration (x10^{10}/ml) of olive barb at different fortnights during spawning season.
Values superscripted by the same letter are not significant different (P>0.05).
Data represents mean (columns) and standard deviation (bars)

Almost similar milt pH was observed in spawning season and no significant different observed in different fortnight during the experimental period. Initial milt motility was rather low at the beginning of the season and a high variation (P<0.05) in milt motility of olive barb fresh milt was found among different fortnight of the sampling months. The milt motility was highest in the 4th fortnight (92.5±2.67) and lowest in the 1st fortnight (85±3.54) during the sampling month (Fig. 3).

Duration of motility (s) of olive barb was variable (P<0.05) among the fortnight during the experimental period. Duration of motility (up to 20% motility) gradually increased and observed highest in the 4th fortnight (222.38 ± 40.91) and then decreased (Fig. 4). However, lowest duration of motility was observed in the 1st fortnight (127± 34.02) of the sampling months.

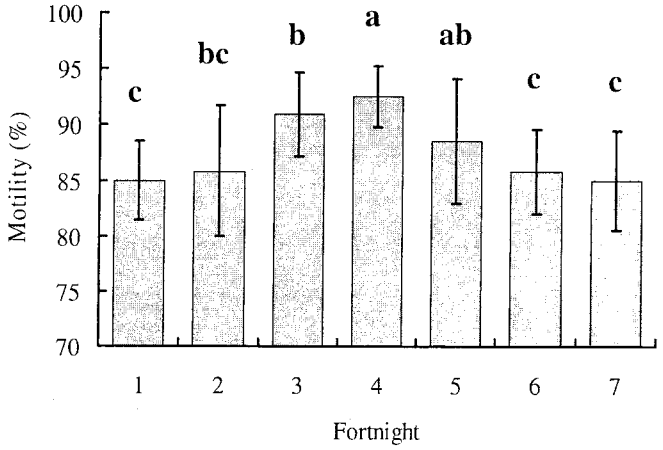

Fig. 3. Percentage of sperm motility of olive barb at different fortnights during spawning season.
Values superscripted by the same letter are not significantly different ($P > 0.05$).
Data represents mean (columns) and standard deviation (bars)

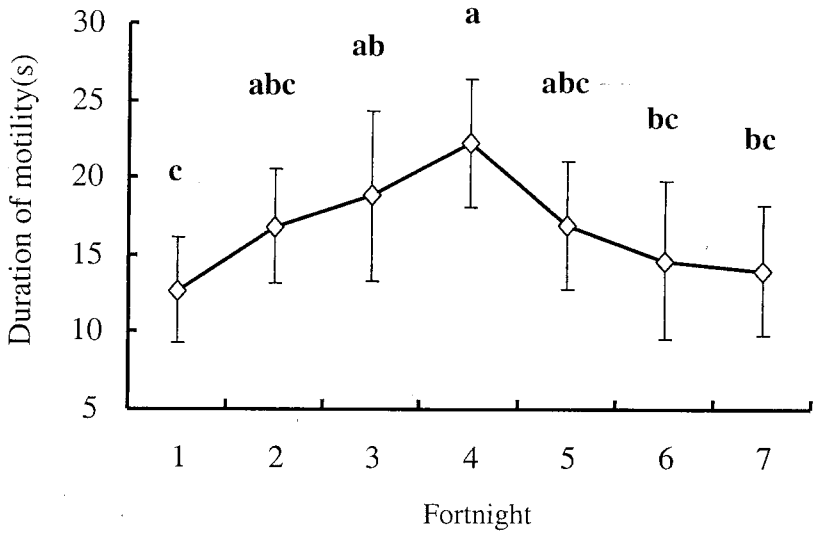

Fig. 4. Duration of sperm motility (s) of olive barb at different fortnights during spawning season.
Values superscripted by the same letter are not significant different ($P > 0.05$).
Data represent means (columns) and standard deviation (bars)

Discussion

Several factors contribute to variation in milt quality including biological characteristics of the brood stock (age, length and weight) (Trippel and Neilson 1992, Hoysak and Liley 2001), the rearing conditions for brood fish (Morisawa *et al.* 1979) and the methods of spawning induction (Caille *et al.* 2006). The milt quality of fish is changed with spawning, showing decreases in the duration of motility, percentage of motility and spermatozoa concentrations at the end of spawning (Lahnsteiner *et al.* 1998, 2005, Liley *et al.* 2002 and Aral *et al.* 2005). The present study confirms that the milt volume of olive barb was lower at the beginning of spawning season and it gradually increased with the time. During the experimental period highest ejaculated milt volume of olive barb was observed in 5th fortnight and lowest value observed in 2nd fortnight. These results suggest the milt volume changed during the spawning season. Sperm production increases from the beginning to middle of the spawning season and declines again at the end of the spawning season in many freshwater species (Lahnsteiner *et al.* 1998, Liley *et al.* 2002, Tekin *et al.* 2003 and Aral *et al.* 2005). Fish milt concentration has been assessed by three main techniques including haemocytometer counting, spermatocrit and spectrophotometry. Haemacytometer was used to determine sperm concentration in this experiment. Sperm concentration was linearly correlated with milt volume and sperm concentration was also varied between the sampling fortnights and highest in the middle of the spawning season. Fish sperm concentration is an important parameter in hatchery reproduction management and it is highly variable and depends on species, individuals, fish size, and season (Glogowski *et al.* 1999). In this study, the average milt motility of olive barb fluctuated in the sampling period. Lahnsteiner *et al.* (2005) reported that sperm motility pattern changed during spawning season. Milt motility in males could be due to either milt preparation procedures or the period of spawning season. The duration of milt motility was significantly varied in different fortnight. This result suggests that season also may impact on the duration of the motility. The milt pH in olive barb was found to be slightly alkaline in the present study. There was no significant difference observed in the milt pH among different fortnight in entire sampling months. The pH has been reported as one of the major sperm activating factors in fish species (Stoss 1983). The duration of sperm motility in *Petromyzon marinus* decreased with an increase in pH, but the percentage of motile cells did not change over the pH range 6.0–9.0 (Ciereszko *et al.* 2002). According to Ingermann *et al* (2002) on pH sensitivity of sperm motility in *Acipenser transmontanus* demonstrated that sperm maintained at high pH (more that 8.2) had appreciable motility when added to water but that the motility was inhibited when there was maintained at low pH (less than 7.5).

Conclusions

The olive barb, *Puntius sarana* farming should be expanded both for restocking in the natural habitat and aquaculture, there is an increasing need to improve the breeding process and for that largely standardized gamete management and handling. The study describes for the first time, milt characteristics of olive barb. Observation of milt characteristics represent valuable baseline information for establishing milt quality standard and provide background information that may be useful for breeding programs to save this species from extinction.

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Effects of irradiation on formaldehyde concentration and nutritional changes of formalin treated fish, *Pampus chinensis*

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Abstract
Formaldehyde is a very reactive compound capable of interacting with many functional groups of proteins including intermolecular and intramolecular cross-links of the molecules. The formation of cross-linking bonds may induce conformational change in proteins that favor further interaction of functional and hydrophobic groups. Formaldehyde which has been using illegally as a chemical preservative by some fish traders in our country. A study was carried out to determine the effects of irradiation (1.5 KGy) on formaldehyde concentration and nutritional (protein and lipid) changes of formalin (37% formaldehyde) treated fish (fresh) samples and found that the concentration of formaldehyde both in treated samples (0.37% formalin and 0.37% formalin with 1.5 KGy irradiation) were 37.0 μg/gm and 36.75 μg/gm. On the other hand, the amount of protein and lipid in treated samples before radiation (14.56% and 3.49%) and after radiation (14.15% and 3.25%). That means, radiation has no effect on the change of protein, lipid and formaldehyde.

Key words: Formaldehyde, Irradiation, *Pampus chinensis*

Introduction

Fish is the principle source of supply of protein food for the people of Bangladesh. Traditionally, the people consume fish of fresh water or near shore brackish water origins. Marine fish production has been started and increased considerably only in the recent part. Now, many of marine fish species become popular for consumption to our people than that of fresh water fishes. Marine fish is an excellent source of protein. About 80% of animal protein comes from fish. The protein content of fish on an average, 20%. Marine fish lipids are rich in fatty acid and also contain some glycogen. Fisheries sector plays an important role in the economic maturity of Bangladesh, contributing about 5.5% of the countries GDP during 2000-01 fiscal cycles (Chowdhury 2001). Formalin is a colorless strong-smelling chemical substance usually used in industry of textiles, plastics, papers, paint, constructions and well known to preserve

human corpse. It is water solution of formaldehyde (37% or 50%) which may contain up to 15% methanol as a stabilizer. The breakdown products of formaldehyde in air include formic acid and carbon monoxide that can cause irritation to the eyes, nose and respiratory tract, causing sneezing, sore throat, larynx constriction, bronchitis and pneumonia. Multiple exposures can lead to asthma. It can also affect the skin, causing dermatitis or allergic reaction. Small amount of formaldehyde develops in marine fish but its presence in freshwater fish is unexpected. Formaldehyde develops postmortem in marine fish and crustaceans, from the enzymatic reduction of trimethylamine oxide to formaldehyde and dimethylamine (Sotelo *et al.* 1995). While formaldehyde may be formed during the ageing and deterioration of fish flesh, high levels do not accumulation in the fish tissues, due to subsequent conversion of the formaldehyde formed to other chemical compounds (Tsuda *et al.* 1988).

In the aquatic environment data on the aquatic toxicity of formaldehyde are numerous. Various scientists such as Chou and Que Hee (1992) worked on the toxicity of formaldehyde for freshwater algae, microorganisms, invertebrates, fish as well as marine algae. They found the sensitivity of different organisms varies widely, however, the most sensitive aquatic effects identified were observed for marine algae. Sen (2002) reported the acceptable range of formaldehyde in fish muscle for human consumption is 1 mg/Kg for freshwater fish and 1-5 mg/Kg for marine fish. With a culture of malpractice seeping into every sector and level it is hardly surprising that it has reached the most important of our basic needs food. Many dishonest fish traders use formalin as a chemical that is mainly used with imported fish and it makes the fish stiff and keeps them looking fresh for a longer duration. So, the study was carried out to find out the effects of irradiation on formaldehyde concentration and nutritional changes of formalin treated fresh fish (*Pampus chinensis*) samples.

Materials and methods

All investigations were carried out in the laboratory of Food Processing & Preservation Division, Atomic Energy Research Establishment (AERE), Savar, Dhaka, Bangladesh. There the research was initiated through the collection of specimen fish. Specimen fish, Chinese pomfret, (*Pampus chinensis*) commonly known in Bangladesh as 'Rup Chanda' was selected in this study. Fresh pomfret used in this experiment were collected from the Malibag Bazar, Dhaka. Usually, collections were made early in the morning and after collection; samples were taken in a polythene bag with ice and immediately brought to the laboratory of Food Processing and Preservations Division, IFRB, AERE, Savar, Dhaka, Bangladesh. Fishes were divided into the following sampler- Sample A: Only formalin (0.37%) was treated for 20 minutes. Sample B: Both formalin (0.37%) and irradiation (1.5 KGy) were treated here. Panoramic Co-60 (1.5 KGy) source supplied by the Atomic Energy of Canada Ltd. Formaldehyde concentration of the fish tissue was measured by using perchloric acid ($HClO_4$) extraction and the Nash reagent, was developed by Castell and Smith (1973).

Perchloric acid (10%) was taken in a one liter volumetric flask and weighed 85.7 gm of 70% reagent grade perchloric. It was dissolved in a small amount of distilled water and diluted to volume. Potassium hydroxide (30%) was dissolved in 30 gm of KOH along with 65 ml distilled water, cooled and diluted to 100ml. Standard buffer solution was commercially available at p^H 6 and 8. Nash reagents were combined in 2 ml acetyl acetone with 75-80 ml distilled water in a 125 ml Erlenmeyer flask. It was caped and shaken vigorously. Ammonium acetate (150 gm) was dissolved with 300 ml water in a 500 ml beaker. The above solutions combined and diluted to 500 ml. It was made fresh daily and stored in refrigerator. Formaldehyde (1M aqueous) was taken in a 100 ml volumetric flask, weighed 8.12 gm of 37% w/w formaldehyde solution and diluted to volume with distilled water.

A dilution of 1 ml of 1 M HCHO (Stock-1) was prepared to 100 ml with distilled water. Stock-2 was prepared by taking two ml of stock-1 and diluted to 100 ml with distilled water. Working sample was prepared by taking 0, 1, 2, 3 and 4 ml aliquots to give final formaldehyde concentration of 0, 0.2, 0.4, 0.6 and 0.8 μ moles. Standard sample was prepared by diluted all taken standards to 5 ml with distilled water and added to 5 ml Nash reagent and mixed well on vortex. Tubes were heated for ten minutes in a 60 degree centregrade water bath and cooled in cold water for 5 minutes. Absorbance read at 415 nm.

Table 1. Concentration of formaldehyde and absorbance at 415 nm for standard curve

No of observation	Volume of formaldehyde solution (ml)	Formaldehyde concentration in standard solution (μ moles)	Absorbance at 415 nm
1	0	0	00
2	1	0.2	0.138
3	2	0.4	0.289
4	3	0.6	0.434
5	4	0.8	0.576

The samples were blended in food processor. Fifty to hundred gm minced samples were taken. Fifty to hundred gm pre-weighed portions along with 10% $HClO_4$ (two times of sample wt) were blended for 2 minutes. It was required few minutes for setting. Fifty ml of extracted aliquots were collected by filtering. p^H meter was standardized at 6 and 8. Fifty ml of extracted aliquots neutralized using 30% KOH. The volume of KOH was required for keep the samples neutralizing. Neutralized samples were taken of (1 to 5 ml) in 18*150 ml test tube. The samples were diluted to 5 ml with distilled water. Then Nash reagent (5 ml) was added and mixed well on vortex. The tubes were heated for ten minutes in a 60 degree centre grade water bath. Then it cooled in cold water for 5 min. Then the absorbance read at 415 nm.Concentration of formaldehyde in collected samples calculated by using the following formulas-

1. Prepared standard curve
2. (Formaldehyde) concentration expressed in μ moles/ gm fish

$FA = F*M*V_1/V_3*W*(50+V_2)/50$.....(i)

Where, F= μ moles FA read from standard curve, M= moisture content of fish (%), V_1= volume (ml) of perchloric acid added for 1:2 extraction, V_2= volume (ml) of KOH used to neutralized the sample, V_3= volume (ml) of extract added to tube, W= weight of fish used in 1:2 extraction

3. Expressed in μg/gm

$FA = F*M*V_1/V_3*W*(50+V_2)/50*G$.....(ii)

Where, G= 30 i.e. gram molecular weight of formaldehyde

Results and discussion

The protein and lipid content in treated fish samples before radiation (14.56% and 3.49%) and after radiation (14.15% and 3.25%) (Table 2). The concentration of formaldehyde both in treated samples (0.37% formalin and 0.37% formalin with 1.5 KGy irradiation) were 37.0 μg/gm and 36.75 μg/gm (Table 3, Fig. 1). That means, radiation has no effect on the change of protein and lipid. The concentration of formaldehyde at different treatments has remained nearly same. That means, radiation has no effect on change of formaldehyde. There have been no systematic investigations of levels of formaldehyde in a range of foodstuffs as a basis for estimation of population exposure. Although formaldehyde is a natural component of a variety of foodstuffs, monitoring has generally been sporadic and source directed.

Table 2. Determination of Protein and Lipid of treated fish samples

Nutrients	Treatments							
	Dip in formalin (0.37%) for 20 min.				Both formalin (0.37%) and irradiation (1.5 KGy)			
				Mean				Mean
Protein (%)	15.15	13.57	14.16	14.56 (±0.23)	13.57	15.10	13.78	14.15(±0.12)
Lipid (%)	3.26	3.45	3.76	3.49 (±0.03)	2.80	3.15	3.90	3.25 (±0.08)

Table 3. Concentration of formaldehyde (HCHO) of treated samples

	Treatments							
	Dip in formalin (0.37%) for 20 minute				Both formalin (0.37%) and irradiation (1.5 KGy)			
Formaldehyde (μg/gm)	F_1	F_2	F_3	Mean	F_{T1}	F_{T2}	F_{T3}	Mean
	38.45	36.20	36.35	37.00 (±0.07)	37.25	35.50	36.06	36.75 (±0.12)

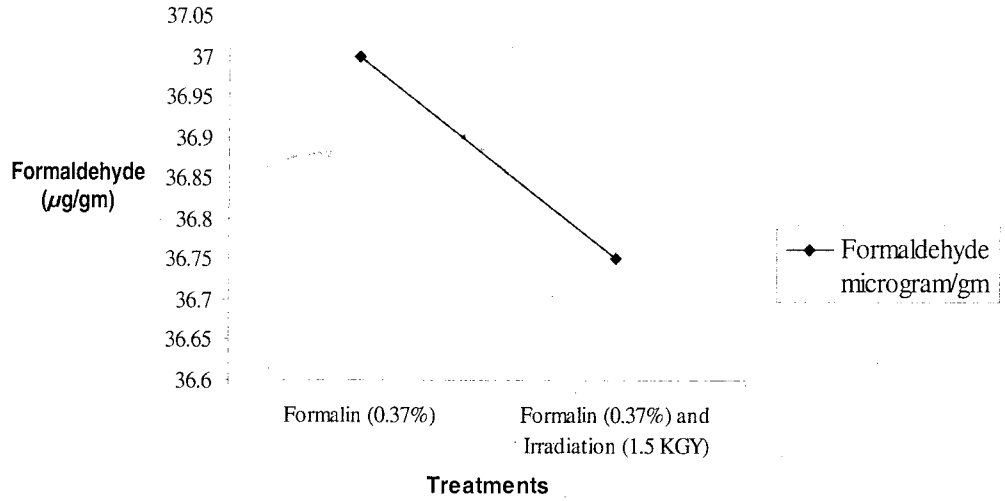

Fig. 2: Effect of treatments on the change of formaldehyde concentration in fish muscle

Lipids and DNA are particularly sensitive to ionizing radiation. Riebroy *et al.* (2007) were investigated the effects of irradiation at different doses (0, 2 and 6 KGy) on the microbiological, chemical and physical properties of Som-fug, a Thai fermented fish mince and they found that irradiation at high dose (6 KGy) might induce lipid and protein oxidation, though the growth of microorganisms was inhibited. Therefore, the irradiation at low dose (2 KGy) could be used to control the over fermentation of Som-fug up to 20 days at 4°C without adverse effects on quality and acceptability.

Crone *et al.* (1992) detected 2-alkyl-cyclobutone, a cyclic compound formed from fatty acids in irradiated but not cooked lipid containing foods. Yasuhara and Shibamoto (1995) suggested that fish containing the highest levels of formaldehyde (e.g., 10-20 mg/Kg) may not be considered palatable as a human food source. Again, in the few studies of the formaldehyde content of foods in Canada, the concentrations of formaldehyde were within the range <0.03-14 mg/Kg (Health Canada 2000). However, the proportion of formaldehyde in foods that is bioavailability is unknown. Available data suggest that the highest concentrations of formaldehyde naturally occurring in foods (i.e., up to 60 mg/Kg) and marine fish (Tsuda *et al.* 1988).

Study on the effects of radiation on the change of formaldehyde concentration in fresh fish was shown that there are no significant effects of radiation on the change of formaldehyde. But its presence in fishes is due to the use of formalin (as preservative) is evident without questions. For that it is recommended to avoid

the use of formalin in fish because it binds with protein of fish muscle and form a macromolecule, which is not digestible that means decline the nutritive value of fish.

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Effects of stocking density on growth and survival of *Mystus gulio* in nursery ponds

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Abstract
A study on the effect of stocking density on growth and survival of nona tengra (*Mystus gulio*) was carried out in brackishwater earthen nursery ponds (2 decimal each) for a period of 42 days. Five-day old captive bred tengra post-larvae (ABL: 4.53±0.83 mm and ABW: 3.33 mg) were stocked at four different densities of 200/m^2 (Treatment-1), 250/m^2 (Treatment-2), 350/m^2 (Treatment-3) and 450/m^2 (Treatment-4). Fries were fed twice a day with a mixture of fine rice bran, mustard oil cake and fishmeal at the ratio of 2:1:1. The specific growth rate (SGR) of larvae did not vary significantly ($p>0.05$) between T1 (5.096% mg/day) and T2 (5.08% mg/day), but it was found significantly ($p<0.05$) higher from T3 (4.98% mg/day) and T4 (4.91% mg/day), respectively. The final survival rates of 89.25±5.41% in T1 and 88.72±6.09% in T2 were found similar ($p>0.05$), but significantly higher ($p<0.05$) than those of, 76.20±4.77% in T3 and 70.34±5.71% in T4. The results indicate that 5-day old hatchery bred nona tengra post-larvae can be nursed in earthen pond at a stocking density of 200-250/m^2.

Key words: *Mystus. gulio*, stocking density, nursery ponds.

Introduction

Mystus gulio, locally known as nona tengra, is a euryhaline estuarine catfish commonly occurring in coastal waters of Bangladesh. This species is supporting the coastal fisheries to a great extent, both in point of commercial and local consumption views. This fish has also a demand in the export market. Up to now, this species is being caught from natural coastal waters throughout the year, with an abundance in monsoon period. *M. gulio* has also been trapped and grown as a significant additional harvest in most of the traditional coastal shrimp ghers. However, like many of fish species, abundance of *M. gulio* in natural coastal waters and its supply as well have been declining over the period, particularly due to overexploitation and habitat destruction. Concern over declining harvests and an obvious reduction of *M. gulio* in coastal fisheries biodiversity have led to develop its induced breeding technique (Alam *et al.* 2006), which has paved the way of establishing and expansion of aquaculture of the species.

Expansion of aquaculture of any fish species is greatly dependent on its ensured supply of seed for grow-out ponds. Successful controlled method of mass seed production includes the rearing of larvae at yolk sac stage and at stage after dissolving the yolk sac. The later part of nursing the larvae seems very sensitive, as they pass through a critical period of switching over from planktonic feed to other feeds and also need to adjust with new environment from hatchery to pond-nursery. After the yolk sac absorption, *M. gulio* larvae have been found to grow and survive well in hatching tank up to 2-3 days with boiled chicken egg yolk, but there has been a severe cannibalism afterwards (Alam *et al.* 2006). Shifting the post-larvae at this stage in pre-prepared earthen nursery ponds might have been a remedial measure of reducing cannibalism through providing them with larger space and natural food. Apart of that, short-term rearing of post-larvae in nursery ponds is a prerequisite to ensure the predictable supply of quality fry for stocking in grow-out ponds.

Under natural conditions the growth and survival of fish is in part dependent on the population density, however, there may be no relation between food abundance and growth when a space-limiting effect operates on the population (Backiel and Le Cren 1978). Such a situation tends to occur in intensive fish seed production practices, where the larvae are confined to restricted space. Though a few works have been done on biology (Kaliyamurthy 1981, Sarker *et al.* 2002) and induced spawning (Mijkherjee *et al.* 2002, Alam *et al.* 2006) of *M. gulio*, no information are so far available on mass-scale production of fry of this species at nursery phase in earthen pond conditions. Following the success of captive breeding of *M. gulio* for the first time in Bangladesh (Alam *et al.* 2006) and keeping the general relations among population density, space and food in aquaculture ((Backiel and Le Cren 1978), attempts have been made for development of mass-scale production practice of *M. gulio* fry prior to stocking in grow-out ponds. The present study presents the growth and production effects of stocking the hatchery reared *M. gulio* pos-larvae at different densities in earthen nursery ponds.

Materials and methods

The experiment was conducted in twelve earthen ponds at the Bangladesh Fisheries Research Institute (BFRI), Brackishwater Station, Paikgacha, Khulna from the period of July~August 2007. Twelve ponds having an area of 2 decimal each were prepared through sun drying and liming the bottom soil with agricultural lime @ 250 kg/ha and mustard oil cake @ 500 kg/ha. The ponds were filled in with brackishwater (≈5 ppt) up to a depth of 80 cm and inorganic fertilizers of TSP and urea were applied @ 35 kg/h with 3:1 ratio.

After 5 days of fertilization, nona tengra (*M. gulio*) post-larvae (ABW: 3.33 mg, ABL: 4.53±0.83 mm), were stocked in the ponds following different stocking densities of (i) 200 larvae/m^2 (Treatment-1), (ii) 250 larvae/m^2 (Treatment-2), (iii) 350 larvae /m^2 (Treatment-3) and (iv) 450 larvae /m^2 (Treatment-4). Each treatment had three replications and those were assigned into a completely randomized design. Prior to stocking, fish larvae were acclimatized for about 15 minutes.

The fish larvae were fed twice a day @ 100% of the biomass, with a mixture of finely powdered rice bran, mustard oil cake and fishmeal at the ratio of 2:1:1 during the first week of the stocking. Supplemental feed amount was decreased gradually @ 50%, 25% and 15% of the biomass in the consecutive 2nd, 3rd and 4th week onwards respectively. Growth performance of fish larvae in respect of total body length (BL), body weight (BW) was recorded weekly by dragging a hapa in the pond. Physico-chemical variables of pond water, *viz.*, temperature, transparency, salinity, pH, and dissolved oxygen were also monitored weekly following the standard measurement procedures (APHA 1992).

For the estimation of plankton abundance, twenty liters of water sample were collected from different areas and depths of each pond and passed through 25 μ mesh plankton net. The collected plankton samples were preserved in 5% buffered formalin in small plastic bottles. The quantitative abundance of plankton was estimated, using a Sedgewick-Rafter counting cell, following the method and formula:

$N = (Ax1000xC)/(VxFxL)$;

where, 'N' is the number of plankton cells or units per litre of original water; 'A' is the total number of plankton counted, 'C' is the volume of final concentrate of the samples in ml; 'V' is the volume of a field in cubic mm; 'F' is the number of fields counted; and 'L' is the volume of original water in litre (Stirling 1985). Plankton were identified following APHA (1992) and Bellinger (1992). The mean number of plankton was recorded and expressed numerically per litre of water. Triplicate sediment samples of bottom sediment were collected weekly from each pond with the help of an Ekman dredge (covering an area of 225 cm^2) to estimate the quantitative abundance of benthic organisms.

After 42 days of rearing, fingerling were harvested by repeated netting and finally by draining out the pond water. Growth (final length and weight), specific growth rate (SGR), survival rate and net length and weight gain were estimated at harvest. ANOVA was done to observe any difference in growth parameters of nona tengra and Duncan's New Multiple Range Test (DNMRT) was also employed for further analysis of the results.

Results and discussion

Water quality variables

Although the fish species are generally found in derelict or swampy waters and known to be very hardy, their culture in clean water results in higher yields due to the absence of a stress factor on their metabolism enabling a faster rate of growth (Tripathi 1996). Mean values of different water quality parameters are shown in Table 1. There was no significant difference ($P>0.05$) in any of the variables among the treatments. Water temperature was found to follow the trend of air temperature and almost similar in all pond waters throughout the experimental period. The variations in pH in the present experiment fall well within the suitable range of 7.0~8.5 for higher fish production (Banerjea 1967, Boyd 1982). Nursery ponds' water salinity varied from 2-7

ppt. The species feed and thrive well in low salinity and when salinity exceeds 10 ppt they migrates in to waters of low salinity (Pandian 1966). The observation of Grag and Batnagar (2000) on high alkalinity, as ranged from 134 ± 7.2~178 ± 2.94 mg/l supports the findings of the present experiment. The DO levels of 6.02~6.53 mg/l in pond waters under different stocking density treatments were above the minimal values or within the acceptable range, as reported by other authors (Kohinoor *et al.* 1998, Sarker *et al.* 2002, Grag and Bhatnagar 2000) in fish culture. The average concentrations of PO_4-P and NO_3-N of the present experiment are almost similar to the nutrient levels recorded by various authors (Milstein *et al.* 1995, Grag and Bhatnagar 2000) in their experimental fish ponds.

Mean variations in plankton and benthic organism production in pond waters under different stocking density treatments were found more or less similar *i.e.* not significantly difference at 5% level of significance, matching with the findings of Shah *et al.* (2008) in the brackishwater environment.

Table 1. Mean (±SD) values of different water quality variables in ponds under different treatments

Variables	Treatment-1 (200/m²)	Treatment-2 (250/m²)	Treatment-3 (350/m²)	Treatment-4 (450/m²)
Temperature (^{0}C)	25.45±2.81	25.64±2.98	25.28±2.99	25.30±2.75
Transparency (cm)	36.25 ±3.75	36.75 ±3.88	36.00 ±3.63	37.38 ±4.41
pH	8.32 ±0.19	8.35 ±0.17	8.29 ±0.15	8.30 ±0.24
Salinity (ppt)	4.32 ±2.82	4.38 ±2.92	4.25 ±2.96	4.23 ±2.13
DO (ppm)	6.43 ±1.13	6.53 ±1.02	6.02 ±1.41	6.33 ±1.81
Alkalinity (mg/l)	165.23 ±8.75	163.13 ±9.75	172.50 ±7.15	169.38 ±8.78
No_3-N (mg/l)	1.33 ±0.03	1.31 ±0.02	1.36 ±0.02	1.28 ±0.03
Po_4-p (mg/l)	1.13 ±0.03	1.18 ±0.03	1.15 ±0.04	1.08 ±0.05
Phytoplankton (x 10^4 cells/l)	13.86±1.21	13.71±1.25	13.14±1.21	12.86±1.57
Zooplankton (x 10^2 cells/l)	21.86±4.63	21.57±3.60	21.29±2.06	21.14±3.67
Benthic organism (cells/m²)	336.51±43.37	330.16±43.37	317.46±39.99	323.81±33.60

Values for all water quality variables among the treatments are insignificant ($p>0.05$)

Growth and production

Growth and production values (mean±sd) of nona tengra under four different treatments are furnished in Table 2. The average final body weight of 460.02±4.54 mg in T1 and 453.61±4.320 mg in T2 were found significantly higher ($p<0.05$), than that of 412.81±5.49 mg in T3 and 385.55±6.10 mg in T4. Similar to final body weight, the final body weight gain and the SGR were statistically similar ($p>0.05$) in T1 and T2, but significantly ($p<0.05$) higher than in T3 and T4. In case of final body length, T1 (31.15±1.62 mm) and T2 (29.25±0.35 mm) resulted in similar, but significantly higher

($P<0.05$) values compared to both T3 (25.44±0.65 mm) and T4 (23.6±0.66 mm). However, T3 and T4 had no significant difference ($P>0.05$). The lower final body weight in T4 and T3 might have been due to the higher total biomass compared to that of T1 and T2. It is interesting to note that, in spite of the variation in stocking density, the survival rate of fingerling was found statistically ($P>0.05$) similar among all the treatments. It clearly indicates that maximum growth in weight was exhibited by the fish when stocking density was low; while growth rate decreased with higher stocking density, showing a direct correlation between stocking density and growth of fish.

Hepher and Pruginin (1981) also stocked nursery ponds with spawn @ 0.5~1.5 million/ha. But Gupta (1981) recommended a stocking density of 4 million/ha, which is very much close to the present study. Tripathi (1975) reared spawns of rohu (*Labeo rohita*) and mrigal (*Cirrhinus cirrhosus*) for two months and obtained 32% survival with an average length of 70 mm and 65 mm respectively. Shigur (1974) recorded 71% survival of carp spawn when stocked at 6.0~7.5 million/ha. Tripathi *et al.* (1979) stocked rohu spawn @ 1 million/ha and obtained an average survival of 80.73%. Shahbuddin *et al.* (1988) found the survival rate of rohu fry to be 52~73% after 21 days of rearing in earthen ponds. Okamoto's (1969) experiment on red sea bream (*Pagrus major*) suggested that stocking density played a major role in the survival of cultured larvae. Survival of this species to the juvenile stage usually exceeded 5% when stocking density did not exceed 20/l, but decreased significantly at higher densities. African catfish (*Clarias gariepinus*, Burchell 1822) were cultured at four different densities of 50, 100, 150 and 200 fish per cage (1 m^3), respectively. Mean fish weights per cage were highest at the lowest density. Mean weights decreased with increasing density. Harvests and production estimates increased with increasing stocking density *i.e.*, total harvest and production were directly related to stocking density (Hengsawat *et al.* 1997). Coulibaly *et al.* (2007) tested a 90-day experiment of African catfish (*Heterobranchus longifilis*) with five different stocking densities (50, 100, 200, 500 and 1000 fish/m^3). The results showed that unlike final mean weight (Wf) and mean daily weight gain (Mdwg), weight variation coefficient (final Cv), cannibalism (Cr), mortality (Mr) and survival (Sr) were density dependent: best results of survival (68.0 ± 1.5%) were recorded at the lowest density (50 fish/m^3). Channel catfish were cultured for 177 days in circular tanks at combinations of five stocking densities (90–720 fish/m^3). Net yield increased as stocking density increased up to 540 fish/m^3 then declined at higher densities. Mean fish weight, feed conversion efficiency and survival all declined as stocking density increased (Allen, 1974). From the above finding of different authors in different ecological zone distinctly indicate that the survival and growth of fish larvae is highly correlated with stocking density. In the present study, the survival rate of nona tengra was found density dependent and higher than any other author that reviewed above, so this fish is hardy and can tolerate more stress compared to others. There is an exception findings in case of fry of Arctic charr, *Salvelinus alpinus* (L.), reared at seven different stocking densities during the initial feeding period (Wallace *et al.* 1988). The populations held at densities of 25 and 50 fry/l showed significantly slower growth and slightly higher mortality than the populations held at densities of 70–250 fry/l. It would

appear that high population density affects young Arctic charr such that agonistic behaviour was inhibited and schooling behaviour stimulated.

Table 2. Growth performance and survival (±SD) of *Mystus gulio* larvae for six weeks nursery rearing at different stocking densities

Parameters	Treatment-1 (200/m^2)	Treatment-2 (250/m^2)	Treatment-3 (350/m^2)	Treatment-4 (450/m^2)
Initial weight(mg)	3.33	3.33	3.33	3.33
Final weight (mg)	460.02±4.54^a	453.61±4.32^a	412.81±5.49^b	385.55±6.10^c
Net weight gain (mg)	456.69^a	450.27^a	409.48^b	382.21^c
Initial length (mm)	4.53±0.83	4.53±0.83	4.53±0.83	4.53±0.83
Final length (mm)	31.15±1.62^a	29.25±0.35^a	25.44± 0.65^b	23.6±0.66^b
Net length gain (mm)	26.62^a	24.72^a	20.91^b	19.07^b
Specific growth rate (% mg/day)	5.10^a	5.08^a	4.98^b	4.91^c
Survival rate (%)	89.25±5.41^a	88.72±6.09^a	76.20±4.77^b	70.34±5.71^b

Values in the same row with dissimilar superscripts are significantly different ($p<0.05$).

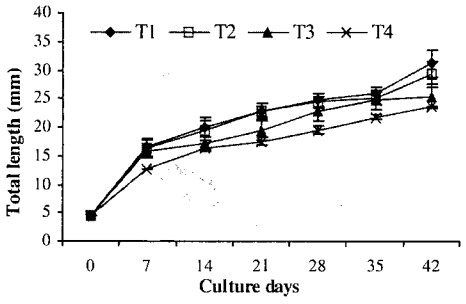

Fig. 1. The gain in length (mm±SE) of *Mystus gulio* fry in different treatments.

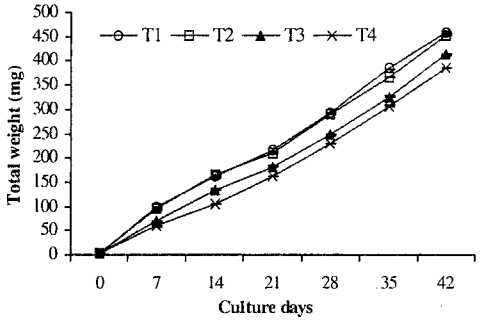

Fig .2. The gain in weight (mg±SE) of *Mystus gulio* fry in different treatments.

Growth increment of nona tengra in respect to body length (BL) and body weight (BW), throughout the culture period is shown in Fig.1 and Fig.2, respectively. The higher body length (BL) and body weight (BW) increment were found in T1 and T2, compared to that in T3 and T4 respectively, might be due to the lower stocking density. The specific growth rate (SGR % mg/day) was found statistically similar in case of T1 (5.10) and T2 (5.08) but significantly higher ($p<0.05$) than in T3 (4.98) and that obtained from T4 (4.91). From the above discussion it clearly indicates that the lower the stocking density the higher the growth performances in respect of both length and

weight. However, in between T1 and T2 it did not show any sharp differences as observed among the other three treatments. Though it was found slight better growth performance in T1 than in T2, but not statistically significant, it could be suggested to choose the stocking density up to 250/m^2 to get a maximum yield maintaining abiotic factors for enhancement of the growth performance and survival rate of nona tengra post-larvae during nursing stage in earthen ponds.

Stocking density is an important parameter which directly affects the growth of fish and hence its production (Backiel and Le Cren 1978). The question of stocking density becomes increasingly important in developing methods of intensive culture of fish larvae. The harmful effects that higher stocking density have on the culture of fish are the reduction of growth and survival rates and an increase of food conversion ratio (Powell 1972). Catfish fetch a very high price in India and Bangladesh and also have a good world market. Besides marine black tiger shrimp (*Penaeus monodon*) and different brackishwater finfishes, one can easily visualize the possibilities of exporting *M. gulio* for earning foreign exchange once their local production increases and a surplus is generated. Following the success in captive breeding of the species (Alam *et al.*, 2006), findings of the present investigation indicate that mass scale seed production is possible at a density of 200-250/m^2 for stocking grow-out ponds and increased supply in domestic and foreign market as well.

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Introducing tilapia (GIFT) with shrimp (*Penaeus monodon*) in brackishwater rice-shrimp system: impact on water quality and production

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Abstract
Mixed rearing of tilapia (GIFT; Genetically Improved Farmed Tilapia) with shrimp (*Penaeus monodon*) in brackishwater rice-shrimp system was assessed for its impact on dry season's shrimp production. The experiment was conducted in pre-selected farmer's field located at Paikgacha Upazila of Khulna district and designed with three different densities (treatment) of GIFT, *viz*, 0.3, 0.4 and 0.5/m^2 with a constant stocking density of shrimp at 3/m^2. Each treatment had three replications. There had a set of control treatment where GIFT was not stocked. Results of the experiment revealed that tilapia did not exert any significant effect ($p>0.05$) on the water quality variables, even on survival rate of shrimp ($p>0.05$) under farm level condition in rice-shrimp rotational system, but a density dependent negative effect ($P<0.05$) on the growth of shrimp led apparently lower production rate of shrimp. Though tilapia provided the major augment of total production ($p<0.05$) in the respective treatments than in monoculture of shrimp, but not that of the economic return. However, economic loss due to sudden shrimp crop failure might be partially minimized by the tilapia crop.

Keywords: Tilapia, shrimp, concurrent culture, impacts

Introduction

Shrimp in Bangladesh is one of the largest foreign currency earning sectors. Due to continuous disease outbreak, poor management such as overstocking, and environmental degradation, not only the production per unit area seemed to be very low but total crop failure also occurs frequently. In this situation, farmers are looking for the alternative culture system of either polyculture, crop rotation and/or crop diversification, which may provide an opportunity to develop a sustainable aquaculture system leading to best use of coastal shrimp farms in Bangladesh reducing the risk of unexpected shrimp crop loss.

In a polyculture setting, shrimp and tilapia may utilize different niches. In extensive culture, tilapia can filter feed on phytoplankton and zooplankton in the upper

water column, while shrimp spend most of the time in the pond bottom grazing on bacterial films on the bottom substrate and on the detritus settling from above. Tilapia, as a filter feeder, can reduce excessive phytoplankton biomass in later stages of pond culture and recycle nutrients effectively (Stickney *et al.* 1979). A concomitant culture of Nile tilapia (*Oreochromis niloticus*) with shrimp has been reported (Perschbacher and Lorio 1993; Turker *et al.* 2003a, 2003b), but the red tilapia (*Oreochromis* spp.) has been found the best suite in shrimp ponds (Akiyama and Anggawati 1999).

Among the tilapias, GIFT (Genetically Improved Farmed Tilapia; improved strain of *Oreochromis niloticus*) is a most commonly cultured species in freshwater environment in Bangladesh. It could also be the best choice in brackishwater shrimp ponds as well due to its higher salinity tolerance ranging from 0 to 25 ppt (Hussain 2004). To date, most prawn and tilapia polyculture research has been conducted at fairly extensive stocking rates and under tropical or subtropical conditions in the world (Tidwell *et al.* 2000a, Yi and Fitzsimmons 2004). However, information regarding the concomitant culture of tilapia with shrimp at farm level coastal rice-shrimp system in Bangladesh is till scarce to nil. Therefore, the findings of the present study focused the rearing of tilapia and shrimp in a polyculture system and its effect on the culture environment, growth, survival and production at farm level conditions in the south-west Bangladesh.

Materials and methods

With the target of introducing GIFT (Genetically Improved Farmed Tilapia) and assessing its impact on growth, survival and production of shrimp (*Penaeus monodon*) as well as on the culture environment under field condition, an experiment was conducted during February to August, 2007 in 4 shrimp ghers, located at the Polder # 16/1 of Paikgacha, Khulna. Each gher was divided into three plots of almost identical size. The experiment was designed with three different stocking densities (T1=3000, T2= 4000 and T3= 5000 fingerling/ha) of GIFT along with a constant density of shrimp (30,000/ha). Each treatment had three replications. A set of control plots (T4) was also considered where GIFT was not stocked.

The plots were prepared followed by repairing dyke and liming the bottom soil with calcium oxide at a rate of 250 kg/ha. Tidal water was introduced up to a depth of 50 to 60 cm at the end of February. Phostoxin was applied @1tab/20 ton of water to kill the any unwanted animals introduced with tidal water. After three days, fertilizers were applied (Urea: 2.5 ppm, TSP: 3 ppm and MP: 0.6 ppm) and left over for the growth of primary producers. After seven days of fertilization (1st week of March), hatchery produced post-larvae (ABW, 0.008 g) of *Penaeus monodon* were stocked in the plots according to the design. GIFT (ABW, 3.37 g) stocking was done after 30 days of shrimp stocking (beginning of April) when shrimp reached in a size of juvenile. All the plots were fertilized with Urea (0.5-1.25 ppm) and TSP (1.0-1.5 ppm) at fortnight intervals for the first 2 months, but Liming was done at a rate of 5-8 ppm with dolomite (Ca Ma $(CO_3)_2$) for the entire culture period. Shrimp were fed with commercial pellet feed, once a day, with 100%, 60%, 30% and 10% of the estimated shrimp biomass in the 1st, 2nd, 3rd

and 4th week, respectively. However, the supplied feed varied from 2% to 3% of the standing shrimp biomass for the rest of the culture period.

During the entire culture period, gher water ecological parameters like, temperature, transparency, water depth, pH, dissolved oxygen, salinity and plankton population was monitored biweekly intervals following the standard methods of (APHA, 1985). After 90 days of rearing selective harvesting of shrimp was started using trap and continued up to 135 days. Harvesting of GIFT was done by dewatering of the plots at middle of August, 2007 just before rice plantation. Then growth, survival rate, FCR and production were estimated. Economic analysis was done considering all variable costs to the expenditure and respective shrimp and GIFT sales of the treatment to the gross return. ANOVA was done to observe the differences in growth, survival rate, production, FCR values and economic return among the different treatments.

Results and discussion

Water quality: Variations in water pH during the entire culture period have been presented in Fig 1. pH of water in all the treatments ranged within 7.2 to 8.6, indicating alkaline in condition. Alkaline water is more suitable, than neutral or acidic, for aquaculture . Acidic water restricts the growth of primary producer and also reduce feeding affinity of aquatic organisms (Boyd, 1990). However, water pH in the present trial was congenial for shrimp culture avoiding any unionized NH_3-N toxicity for prawn (New, 1995). Variation in pH during the entire culture period within the treatment was negligible and the difference among the treatments with increased stocking density of tilapia, even with the control one, was insignificant ($P>0.05$).

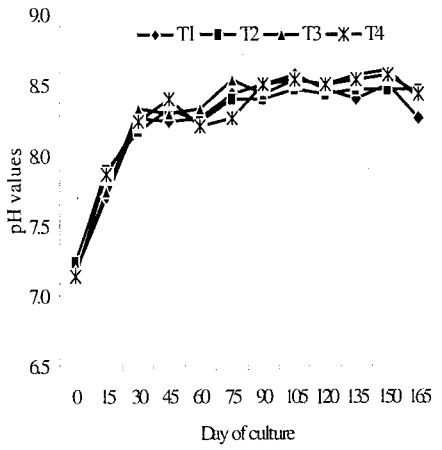


Fig. 1. Status in water pH under different treatments

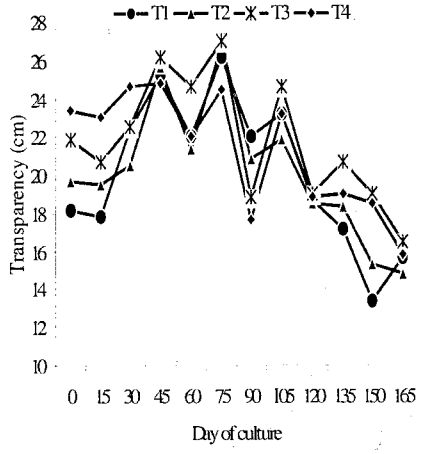
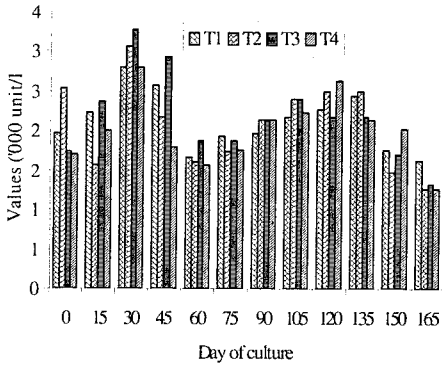


Fig. 2. Trend in transparency under different treatments

Water transparency in all the treatments was suitable from initial stage of culture to 60 days of culture (19.0 – 27.0 cm), afterwards it decreased in all the treatments with the increase in culture duration and reduced to minimum level (13-15 cm) at the end of culture (Fig. 2). Transparency in water might have been reduced due to heavy organic load (dense phytoplankton blooms) and/or due to excessive load in inorganic substances like, clay, silt, sand etc (Boyd and Tucker, 1989). At the same time, like that of transparency, the density of phytoplankton population also decreased with the progress in culture (Fig. 3). Values in dissolved oxygen showed minimum fluctuations among different sampling days (Fig. 4). Dissolved oxygen showed insignificant difference ($p>0.05$) among the treatments and always remained above sub-optimal levels (>4.0 mg/l), which was congenial for aquaculture, avoiding environmental stress for shrimp (Chanratchakool *et al.* 1995) and for GIFT (Hussain 2004).



ig. 3. Status in phytoplankton population in different treatments.

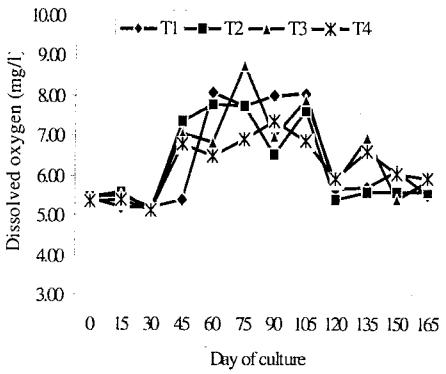


Fig. 4. Trend in dissolved oxygen under different treatments

Dense phytoplankton blooms with high photosynthetic rates can result in elevated pH levels (±10.0) in the afternoon, causing physical and physiological stress (Boyd and Tucker 1989) and even prawn mortality (Straus *et al.* 1991). Perschbacher and Lorio (1993), Turker *et al.* (2003a, 2003b) demonstrated that Nile tilapia, *Oreochromis niloticus*, has the ability of filter-feeding on phytoplankton and that subsequently reduces the pH level within the optimal ranges. Tian *et al.* (2001b) investigated water quality in a closed polyculture system containing Chinese penaeid shrimp with Taiwanese red tilapia and constricted tagelus. They found positive effect of polyculture on water pH control as well as controlling of planktonic blooms. Akiyama and Anggawati (1999) attributed positive effect to improving and stabilizing water quality, foraging and cleaning of the pond bottom, and having a probiotic type of effect in the pond environment by red

tilapia. But our observation on water quality differs with the above mentioned authors. In this experiment, there was no significant difference in water pH, transparency even in plankton population among the treatments including control ones. The reasons behind these might be due to the variation of experimental condition and difference in culture practice. The above authors conducted experiments under closed condition with semi-intensive culture practice of shrimp, but our experiment was based on improved traditional system under farm level condition. In this system, regular intake of tidal waters in each lunar cycle a general phenomenon to maintain a minimum water depth, which was naturally loaded with silt and clay particles, reducing the water transparency, photosynthesis rate and blooming of plankton population as well as restricted pH elevation. However, other water quality parameters were similar (Table 1) in all the treatments and were within the acceptable ranges for Brackishwater aquaculture under farm level conditions in South-west Bangladesh (Wahab *et al.* 2001; Wahab *et al.* 2003; Islam *et al.* 2002; Islam *et al.* 2005).

Table 1. Mean±SD of water quality variables under different treatments in shrimp-GIFT concomitant culture

Variables	T1 (G+S)	T2 (G+S)	T3 (G+S)	T4 (only S)
Temperature (^{0}C)	30.54±3.015	30.39±3.070	30.89±3.079	30.74±3.104
Transparency (cm)	20.18±3.935	20.26±3.541	21.79±3.278	21.53±3.384
Depth (cm)	36.11±3.408	37.76±4.196	34.53±3.238	41.69±3.995
Dissolved oxygen (mg/l)	6.34±1.221	6.27±1.044	6.47±1.122	6.22±0.716
Alkalinity (mg/l)	153.78±17.855	156.39±19.098	155.44±21.175	156.06±21.277
NO_3-N (mg/l)	2.87±1.725	2.64±1.689	2.54±1.548	2.55±1.650
PO_4-P (mg/l)	2.64±1.280	2.80±1.295	2.79±1.269	2.64±1.059

** G=GIFT, S=Shrimp.

Growth and production: Growth and production performance of shrimp and GIFT under different treatments have been shown in Table 2. Final body weight of shrimp was insignificant ($p>0.05$) among the treatments, where GIFT was introduced with different densities. But it seemed significantly higher ($p<0.05$) in T4 (25.87 g), where GIFT was not stocked (control). Survival rate of shrimp lied between 34.41 to 36.44% in all the treatments and the difference among treatment was insignificant ($p>0.05$). The production of shrimp was 246.33, 235.98, 222.64 and 283.30 kg/ha in T1, T2, T3 and T4, respectively (Table 2). Though the shrimp production was apparently higher in T4, where no GIFT was stocked, it did not differ significantly ($p>0.05$) amomg the treatments. Despite of insignificant survival rate and production among the treatments, significantly higher weight gain of shrimp was obtained in the control treatment (T4), indicating some negative impact of GIFT on growth of shrimp. Observation of the present study differs with the observation of Akiyama and Anggawati (1999) and Tian *et*

al. (2001a), who reported better growth, survival rate and production of shrimp in polyculture with tilapia than in monoculture of shrimp. This was probably due to the difference of culture practice. The authors conducted experiment under semi-intensive condition with higher stocking density of shrimp, where the deposition of organic matter was relatively higher than our condition, which supported available feed item for tilapia. But our experiment was based on improved traditional system with supplementation of commercial feed. GIFT as a fast feeder, required more feed with its body growth increment and would like to take the costly commercial feed faster than shrimp, and hence reduced shrimp growth. This observation was supported by Gonzales-Corre (1988), who observed negative effect of tilapia on shrimp growth and termed tilapia as a competitor of shrimp for food in polyculture settings.

Table 2. Production results of shrimp and GIFT under different treatments

Species	Particulars	T1 (G0.3/m^2+S)	T2 (G0.4/m^2+S)	T3 (G0.5/m^2+S)	T4 (only S)
Shrimp	Final weight (g)	23.03±0.51^{b}	21.68±1.08^{b}	21.55±0.96^{b}	25.87±0.45^{a}
	Survival rate (%)	35.57±6.19	36.07±7.20	34.41±4.98	36.44±5.96
	Production (kg/ha)	246.33±48.26	235.98±58.45	222.64±34.77	283.30±51.15
	FCR	1.93	2.15	2.27	1.84
GIFT	Final weight (g)	259.93±21.29	244.47±10.14	234.33±7.71	
	Survival rate (%)	53.02±6.28	49.42±6.42	42.20±4.16	
	Production (kg/ha)	413.79±59.71	484.40±77.48	495.32±63.84	
Total Production (kg/ha)		660.11±28.58^{abc}	720.38±48.47^{a}	717.95±30.66^{ab}	283.30±51.15^{d}

**Different letters in the superscript in same row indicate significant difference ($p<0.05$); G=GIFT, S=Shrimp.

Among the three tested stocking densities of GIFT, we observed relatively better growth of shrimp in the treatment where the stocking density of GIFT was lower (0.3/m^2). This indicated that lower stocking density of GIFT is more suitable in concomitant culture with shrimp under improved traditional culture in gher system. This observation was strongly supported by (Gonzales-Corre, 1988) who observed that the presence of Nile tilapia resulted in better growth and survival of shrimps at 0.4 tilapia/m^2, but poorer shrimp performance at a stocking density of 0.6 tilapia/m^2. Wang *et al.* (1998) also found that the optimum stocking density of Chinese shrimp and Taiwanese red tilapia was 6 shrimp/m^2 and 0.32 tilapia/m^2 (126.3 g in size), and shrimp growth and survival rate at all three stocking densities did not differ significantly among treatments. Tian *et al.* (2001a) reported that the best stocking rates were 7.2 shrimp/m^2,

0.08 tilapia/m^2 and 14 tagelus/m^2 in the polyculture of Chinese penaeid shrimp (*Penaeus chinensis*), Taiwanese red tilapia (*O. mossambicus* x *O. niloticus*) and constricted tagelus (*Sinonovacula constricta*).

Survival rate of GIFT was 53.02, 49.42 and 42.20%) in T1, T2 and T3, respectively, with insignificant difference ($p>0.05$) among the treatments (Table 2). Final body weight of GIFT was 259.93, 244.47 and 234.33 g in T1, T2 and T3, respectively and was also insignificant. Production of GIFT was 413.79, 484.40 and 495.32 kg/ha in T1, T2 and T3, respectively, which indicates a higher production rate from a higher stocking density. In this study, survival rate, weight gain and production of tilapia were density dependent. Akiyama and Anggawati (1999) reported density independent growth of Nile tilapia in polyculture with shrimp under semi-intensive culture condition.

Table 3. Economic return of shrimp and GIFT under different treatment

Particulars (Shrimp)	T1 (G0.3/m^2+S)	T2 (G0.4/m^2+S)	T3 (G0.5/m^2+S)	T4 (only S)
Production cost ('000 Tk./ha)	65.25±1.60	64.14±2.92	63.26±1.03	63.79±5.47
Gross return ('000 Tk./ha)	98.53±19.31	94.39±23.38	89.05±13.91	127.48±23.02
Net return ('000 Tk./ha)	33.28±17.88	30.25±20.47	25.79±12.90	63.69±21.93
BCR	1.51±0.26	1.46±0.29	1.41±0.19	2.00±0.34
Particulars (GIFT)				
Production cost ('000 Tk./ha)	5.93±0.44^{bc}	7.22±0.24^{ab}	8.32±0.44^{a}	
Gross return ('000 Tk./ha)	31.03±4.48	36.33±5.81	37.15±4.79	No GIFT
Net return ('000 Tk./ha)	25.10±4.23	29.11±5.79	28.83±4.38	
BCR	5.23±0.59	5.04±0.79	4.45±0.36	
Total for aquaculture				
Production cost ('000 Tk./ha)	71.18±13.68	71.35±30.80	71.58±0.78	63.79±5.47
Gross return ('000Tk./ha)	129.56±15.50	130.72±19.19	126.20±92.76	127.48±23.02
Net return ('000Tk./ha)	58.38±14.63	59.37±16.12	54.62±8.59	63.69±21.93
BCR	1.82±0.19	1.83±0.19	1.76±0.11	2.00±0.34

**Different letters in the superscript in same row indicated significant difference ($p<0.05$); G=GIFT, S=Shrimp.

The economic return of the present trial has been presented in Table 3. The net return from different treatments were similar ($p>0.05$), though it was apparently higher (63.69 '000Tk./ha) and BCR (2.00) from T4, where GIFT was not stocked. Similar to what has been reported by Akiyama and Anggawati (1999), GIFT concurrent culture of shrimp and GIFT augmented the total production through any undue reduction in shrimp and additional tilapia production. Despite of total lower production rate in T4 (Table 2), higher economic return (Table 3) was achieved, due to the quality of the product and higher market price, but at higher BCR. The lower size of shrimp in ponds with GIFT caused lower market price and economic return.

The results of the present trial focused that, though GIFT exerted a negative impact on the growth of shrimp, but not on survival and production. This might be due to feeding management and physico-biological conditions of gher that resulted in some degree of competition of GIFT with shrimp for food. Stocking of limited number of GIFT ($\approx 0.3/m^2$) might be helpful to increase the ecological condition of the farm by their omnivorous feeding nature. In concurrent culture, GIFT can reduce the production risk and economic losses to some extent, if the shrimp crop damaged due to any out-break of white spot viral disease. By changing the feeding schedule of GIFT (at day time) and shrimp (at night time) might be an option to reduce feed competition among GIFT and shrimp, because shrimp can eat well during the night time when Nile tilapia may not actively feed (Gonzales-Corre, 1988). Alternatively, tilapia could be confined in floating nets or cages to prevent them access to shrimp feed (Fitzsimmons, 2001). The present study has demonstrated that the tilapia-shrimp polyculture is technically feasible, and can be environmentally friendly and economically attractive with the appropriate feeding strategy. The use of cost effective diets and optimization of feeding inputs and schedules are therefore vital for sustainable shrimp-tilapia polyculture in coastal rice-shrimp system.

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Distribution of plankton population in shrimp *ghers* of Bagherhat, Bangladesh

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Abstract
Analysis of plankton sample recorded a total of 5 classes phytoplankton *viz* Bacillariophyceae, Chlorophyceae, Cyanophyceae, Dinophyceae, and Polyhymenophorea. Total 50 phytoplankton species were identified. Among the phytoplankton 18 species belonged to Bacillariophyceae, 12 to Chlorophyceae, 8 to Cyanophyceae, 7 to Dinophyceae, and 5 to Polyhymenophorea. Bacillariophyceae was the dominant group of phytoplankton throughout the study period. Toxin producing dinoflagelates were recorded from the shrimp ghers. A total of 11 kinds of different zooplankton genera were recorded, 4 of which were belonged to Copepoda, 3 to cladocera, 3 to Rotifera and 1 to Decapoda. Copepoda was the dominant group among the zooplankton which was followed by Rotifera and Decapoda. Temperature varied from 27°C to 32°C, transparency 24.5-29.6 cm and pH 6.7 to 9. Salinity fluctuated from 12 to 32.5‰ in both ghers. PO_4-P and NO_3-N ranged from 0.9 to 4.2 ppm.

Key word: Phytoplankton, Zooplankton, Water quality, Shrimp gher

Introduction

Coastal shrimp culture in Bangladesh has been expanded rapidly as elsewhere in this region over the last two decades. About 75% of these shrimp ponds are located in greater Khulna region such as Khulna, Bagherhat and Satkhira districts. Shrimp ponds in Bagherhat are primarily extensive . The extensive ponds here rely on tidal flushing for water exchange and post larval recruitment, so farmer have little control over the water quality in their ponds. A successful aquaculture largely depends on overall aquatic environment. Scientific management of a water body is closely related to the acquisition of knowledge of the environmental factors specially physico-chemical and biological factors that largely affect the aquatic productivity. Good water quality in shrimp pond is essential for survival and adequate growth (Boyd 1990, Burford 1997).Water quality determines the species optimal for culture under different environments (Jhingran 1991, Dhawan and Karu 2002). Moreover, suitable water quality enhance primary production, which in turn enhances secondary and tertiary production. The phytoplankton production represents a vital link in the food chain. The zooplankton forms the principal source of food for most of the fish.

Both the qualitative and quantitative abundance of plankton in water are of great importance in managing the successful aquaculture operation as they vary from location to location.

There is no information on plankton studies and water quality from the ghers of Bagerhat region. The present study aims to address the lack of basic information on water quality and plankton population in extensive shrimp gher in Bagherhat. By examining the samples of coastal water body, we will be also able to detect the beneficial and harmful plankton and thus will also be able to protect the coastal area from inimical affect of harmful planktons.

Materials and methods

Study area

The study was carried out in two large shrimp in Gher Bagerhat district, over a period of six months from April 2006 to September 2006. Gher I is situated in the village of Boroipara, and Gher II is situated in the village of Airpara in Fakirhat thana.

Analysis of water quality parameters

Water quality parameters (temperature, transparency, pH, PO_4-P, NO_3-N, and plankton analysis were determined fortnightly. Water samples were collected from Gher I and Gher II randomly from surface to a depth of 20 cm. between 9-12 A.M. Surface water temperature, transparency and pH were determined using a celsius thermometer, secchi disc and an electronic pH meter (Jenway 3020, Germany). Salinity was measured with a hand Refractometer. Nitrate-nitrogen (NO_3-N) and phosphate-phosphorus (PO_4-P) of collected water samples were measured in the using a data logging spectrophotometer (Odyssey 2500, HACH, USA) with high range chemicals (NitraVer 5 Nitrate Reagent Powder Pillows for NO_3-N, and PhosVer 3 Phosphate Reagent Power Pillows for PO_4-P analysis).

Plankton studies

For qualitative and quantitative analysis of plankton, water samples were collected a known volume (20 litres) of sub surface water from different location in each gher. Sample was passed through a plankton net (mesh aperture 25 μm). Each concentrated plankton was collected from the bucket (50 ml) and transferred to a plastic bottle and preserved in 5% buffered formalin with distilled water. The quantitative estimation of phytoplankton and zooplankton were done by Sedge-wick-Rafter counting chamber (S-R cell) method in Limnology Laboratory, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh using a microscope. A 1 ml of concentrated sample was put into the S-R cell and left for 10 min to allow the plankton settle. The plankton in 10 randomly selected fields in the cell was identified up to genus level and counted. This procedure was repeated three times for each sample and the mean number of plankton was recorded and expressed numerically per litre of water for each station. Plankton density was calculated according to Stirling (1985) using the formula:

$$N = \frac{A \times 1000 \times C}{V \times F \times L}$$

Where,
N= Number of plankton cells or units per litre of original water.
A= Total number of plankton counted
C= Volume of final concentration of the sample in ml
V=Volume of a field (mm^3)
F= Number of the field counted
L=Volume of original water in litre

Identification of plankton were according to Prescott (1962), Bellinger (1992), Needham and Needham (1962). Identification was made down to species level where possible.

Results

Water quality parameters

The monthly variation of surface water temperature at the collecting stations followed a clear maximum in April and a minimum in July for both Ghers. The highest recorded temperature (32°C in Gher I and 31.8°C in Gher II) was in April and May respectively and the lowest (27°C in Gher I and in Gher II) was in July, . During the study period salinity showed an irregular pattern for both sites, fluctuating from 12 to 28‰ in Gher I 12.6 to 32.5‰ in Gher II. In most of the sampling months pH of the water were above the neutral point. pH fluctuated from 6.9 to 9 in Gher I and 6.7 to 8.5 in Gher II respectively. Transparency ranged, 24.5 to 29.6 cm. Nitrate-nitrogen ranged from 0.9 to 2.1 ppm, the highest value was in September, and the lowest value in April. While in Gher II nitrate-nitrogen ranged from 1 to 1.8 ppm, the highest value was September and August and the lowest value in April and June. Phosphate-phosphorus ranged from 2.5 to 4.2 ppm in Gher I, the highest value was in September and the lowest value in June. In Gher II, phosphate-phosphorus ranged from 1.9 to 4.1 ppm, the highest value was in September and the lowest value in June 2006 (Table 1).

Phytoplankton

The phytoplankton population was identified up to genus level and re grouped in to the various classes or groups. The phytoplankton population was comprised of 50 genera belonging to Bacillariophyceae, Chlorophyceae, Cyanophyceae, Dinophyceae, and Polyhymenophorea groups. Among the different phytoplankton species recorded, 18 belong to Bacillariophyceae, 12 to Chlorophyceae, 8 to Cyanophyceae, 07 to Dinophyceae and 5 to Polyhymenophorea (Table 2). Bacillariophyceae appeared to be most abundant in Gher I (54.7×10^3 cells/L) and in Gher II (70.7×10^3 cells/L) in April, and least abundant in Gher I (9.7×10^3 cells/L) and Gher II (12.7×10^3 cells/L) in September respectively. Bacillariophytes showed another peak of abundance in June. Among the Bacillariophytes, the most abundant species were *Amphora ovalis, Nitzschia*

Table 1. Fortnightly variations of water quality parameters in the Bagerhat region (Fakirhat) of Bangladesh at both Ghers during the study period

Sampling date / Water quality parameters	Gher	1-Apr	15-Apr	1-May	15-May	1-June	15-June	1-July	15-July	1-Aug	15-Aug	1-Sep	15-Sep
Temperature (°C)	I	32	30.8	31	30.6	30.5	29.9	29	27	30	29	29.7	30
	II	31.5	30	31.8	31	30	29.7	29.5	27	30.1	30	29.2	29.7
Salinity	I	28	27.5	26	25.9	24	23.5	17	18	18	17.6	12	12.8
	II	32.5	31.9	27	26.1	23.4	22	16.8	17	15.9	16.1	12.7	12.6
pH	I	9	8.5	7.7	8	8.1	8	8	7.6	6.9	7.2	7.7	7.8
	II	8.5	8.4	7.2	7.6	7.7	8	7.9	7.5	6.7	7.1	7.9	7.5
Nitrate nitrogen (mg/L)	I	0.9	1	1.3	1.2	1.1	1.5	1.8	1.7	1.7	1.2	2.1	1.9
	II	1.1	1	1.4	1.2	1	1.2	1.7	1.4	1.8	1.1	2	1.8
Phosphate phosphorus	I	2.9	2.7	4.1	3.7	2.7	2.5	3.2	2.8	4.1	3.9	4.2	4.1
	II	2.7	2.8	3.7	3.5	1.9	2.2	2.9	2.7	3.7	3.7	4.1	3.9

acicularia, Coscinodicus lineatus and *Rhizosolenia alata.* Among the Chlorophytes, *Ulothrix aequalies, Cosmarium bioculatum* were most abundant species. Except in August and September, Bacillariophyceae was the dominant group of phytoplankton throughout the study period in both the sampling stations. Chlorophyceae, was the second most dominant group. The most dominant species of Cyanophyceae were *Trichodesmium erythraeum. Nostoc pruniforme,* and *Aphanizomenon flos-aquae.* Polyhomenophorea were most abundant in Gher I (2.7×10^3 cells/L) and in Gher II (1.9×10^3 cells/L) in April and found in least abundant during August and September in both ghers. During the study the period a number of toxin producing harmful algal species, namely, *Dinophysis caudata* were found. Bloom of *D. caudata* resulting fish kill were also observed for the first time in the area.

Table 2 List of phytoplankton genus observed

Bacillariophyceae	Chlorophyceae	Cyanophyceae	Dinophyceae	Polyhymeno-phorea
Amphora	*Ankrisorodesmus*	*Anabaena*	*Balechina*	*Codonaria*
Asterionella	*Chlamydomonas*	*Aphanizomenon*	*Ceratium*	*Codoncella*
Bacteriostrum	*Clostridium*	*Aphanothece*	*Ceratocorys*	*Epiplocydoides*
Biddulphia	*Cosmarium*	*Microsystis*	*Dinophysis*	*Favella*
Coscinodiscus	*Golenkinia*	*Nostoc*	*Gonyaulax*	*Rhabdonella*
Cycloteua	*Gonatozygon*	*Oscillatoria*	*Noctiluca*	
Diatoms	*Pleurococcus*	*Spirulina*	*Pyrocystis*	
Fragillaria	*Scenedesmus*	*Trichodesmium*		
Gyrosigma	*Spirogyra*			
Hemiaulus	*Tetraedon*			
Navicola	*Ulothrix*			
Nitzschia	*Volvox*			
Rhizosolenia				
Surirella				
Synedra				
Tabellaria				
Thalassionema				
Triceratium				

Zooplankton

The zooplankton population were composed of different species of Rotifera, Decapoda, Copepoda and Cladocera. Out of 11 identified taxa, 3 belonged to Rotifera, 1 to Decapoda, 4 to Copepoda and 3 to Cladecora (Table 3). Some of them occurred during each sampling month and some did not. The zooplankton were most abundant in Gher I (107.5×10^3 cells/L) and in Gher II (132.5×10^3 cells/L). September and least abundant (36.75×10^3 cells/L) in Gher I and (35.5×10^3 cells/L) in Gher II in July. Copepods were the most dominant group among the zooplankton, 31-32% in Gher I and

Gher II of the total population. Each species of Copepoda showed noticeable fluctuations during the period of study. Four species of Copepoda such as *Cyclops*, Nauplius, *Diaptomus, Tigriopus*. Nauplius was observed during the study. They were abundant in April. Rotifera was the next dominant group represented by 3 genera, *Brachionus, Keratella,* and *Trichocerca* showed noticeable fluctuations in their abundance during the study period. Rotifers showed their maximum abundance in September in Gher I (32.5×10^3 cells/L) and in Gher II (39.5×10^3 cells/L) respectively. and Minimum abundance was observed in June.. Decapods were abundant in September in both the gher.

Table 3 List of zooplankton Genus observed

Rotifera	Decapoda	Copepoda	Cladocera
Brachionus *Keratella* *Trichocerca*	*Processa* sp.	*Cyclops* *Diaptomus* Nauplius *Tigriopus*	*Daphnia* *Diaphanosoma* *Moina*

Discussion

Temperature is the most important environmental factor with multisided effects on plants and animals. Temperature regulates the growth, reproduction, and metabolism as well as feeding intensity of fish. In the present study, water temperature was found to vary from 27-32°C and 27-31.8°C in Gher I and Gher II respectively. The highest temperature was found on April in Gher I and May in Gher II and the lowest was found in both Ghers in July . Santhanam and Srinivasan (1996) recorded temperature ranging from 28 to 30.5°C with the maximum in May 1994 in the Tuticorin Bay of India. During the study period the observed temperature are with in the optimal range.

The highest salinity 28‰ was found in April and the 32.5‰ was found April at Gher I and Gher II respectively. The lowest salinity of 12‰ and 12.6‰ was found in September in September at Gher I and Gher II respectively. Salinity showed variations during the study period due to circulation in the Bay, rivers discharge and dilution due to rainfall. Mahmood (1986) recorded the highest salinity 34‰ in the estuary of Matamuhuri river at Chakaria, Chittatong in the cost of Bay of Bengal in March.

The level of pH ranged from 6.9 to 9 at Gher I and Gher II. The highest pH was found in April. Islam and Hossain (1991) recorded pH values of 7.8, 7.7, 7.9 and 7.7 and 7.2 at six different sampling stations of the Bahgerhat water bodies of Bangladesh. Therefore the gher water was suitable for shrimp culture.

During the study period various types of phytoplankton and zooplankton were identified upto genera levels. The results seasonal variation environmental parameters and plankton suggest that the favourable period occurs from April to July when temperature rises and nutrients accumulate from fresh water run-off due to monsoon

rainfall and more coastal upwelling and eddy diffusion due to strong wind action during April to July. Maximum phytoplankton cell densities in the coastal water of during this study were (163.65×10^3 cells/L at Gher I and 154.3×10^3 cells/L at Gher II in June and April respectively. The phytoplankton community at the two sampling Ghers differed slightly from one to other. Although salinity, temperature, pH, nitrate-nitrogen and phosphate-phosphorus differences was observed between the stations were rather limited, these differences in environmental factors measured can explain the observed differences in species distribution and abundance. Indeed, phytoplankton species distribution was related to above factors. These differences in environmental condition can be explained by the position of the Ghers. Gher I positioned about 2 km upstream from Chitra river that are also connected more influenced by the discharge of the river waters, which explains the lower salinity. The rain cycle thus seem to be main factor controlling the seasonality of phytoplankton assemblages in the observed estuarine waters. There was no significant differences in phytoplankton species composition and abundance between the two Ghers.

The variations in monthy densities of total phytoplankton may be attributed to wide range of parameters including temperature, DO etc. The dominance in plankton population species during the various months of the study period was probably attributed to variations in the optimal conditions for particular species. In the present study different dinoflagellate species occurred almost round the year and among them *Dinophysis caudata* formed bloom during April and June when heavy freshwater flood was being discharge into the Bay through that rivers as run-off. Harmful algal blooms due to nutrient enrichment and the impacts of these algal blooms on the water quality and fishery resources have also been reported in different coastal locations of India (Santhanam *et al* 1996). The highest cell densities of phytoplankton were 163.65×10^3 cells/L at Gher I and 154.3×10^3 cells/L at Gher II were found during are June and April respectively. The zooplankton collected by the plankton net were mainly composed of herbivorous species. Normally phytophagous zooplankton grow more when phytoplankton are abundant. In the present study, zooplankton population was poor during the *Dinophysis caudata* bloom in April, 2006 and June. *Dinophysis caudata* is known to secrete toxins which possibly happened during the present reported bloom too. Possibly some zooplankton were killed by *Dinophysis* toxins as because fish mortality was also reported during the bloom period. High concentrations of phosphate phosphorus were recorded during the bloom months that might have a relationship with the bloom. Santhanam and Srinivasan (1996) reported that phosphate phosphorus accumulation through runoff waters in the coastal water of the Tuticorin Bay of India that triggered *D. caudata* bloom. Santhanam and Srinivaasan (1996) recorded the highest phytoplankton cell number (1.1×10^6 cell/L) during monsoon months in the Tuticorin Bay of India which was supposed to be caused by continuous discharge of sewage water during the rainy periods. They also observed a considerable variations in species composition during the study period and found diatom as a dominant group round the year. The dynamic characteristics of plankton distribution in the coastal habitats are also strongly influenced by tidal currents and salinity gradients, and thus

timing of sampling together with spatial location in relation to salinity and coastal environment mosaics will determine plankton type and concentration. Interrelationship among physico-chemical parameters such as light, temperature, pH and nutrients are important regulatory factor in the physiology and behavior of phytoplankton. Thus differential adaptation of phytoplankton to the physico-chemical parameters in their natural habitats can explain why in some genera become dominant in particular season or not. In this study, seasonal distributions of the large phytoplanktonic organisms inhabiting the gher water were determined together with their successions and environmental parameters.

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Effects of different types of feeds on growth and production of tiger shrimp, *Penaeus monodon* at Bagerhat region, Bangladesh

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Abstract
An experiment was carried out in farmers' *gher* (shrimp farm) at Bagerhat sadar upazilla, Bagerhat to ascertain the effects of three different types of feeds on the production and economics of brackishwater shrimp, *Penaeus monodon* for a period of 120 days. There were three treatments such as T_1 (BFRI dough feed containing of 30% fish meal, 10% protein conc., 10% soya meal, 15% mustard oil cake, 18% rice bran, 5% maize, 10% wheat flour, 1% oyster shell powder and 1% vitamin premix), T_2 (Commercial diet Saudi-Bangla grower) and T_3 (Saudi-Bangla special feed). Each treatment had two replicates and the stocking of shrimp in each *gher* was 3 nos/m^2. Water quality parameters did not differ significantly among the treatments except water depth. Average production and net return of shrimp in different treatments varied from 404.0 to 509.0 kg/ha and Tk. 56,493.99-Tk. 84,209.60, respectively. T_2 showed significantly ($p<0.05$) the highest production and economic return. The result of the study implied that T_2 is more suitable and economically viable than that of other treatments for shrimp farming.

Key words: Feed, *Penaeus monodon*, *Gher*

Introduction

Most of the shrimp farmers of Bangladesh do not use any type of feed for shrimp. Natural foodstuffs present in the shrimp pond are not sufficient to fulfill the demand of the growing biomass. Growth and production of farmed shrimp is largely dependent upon the supply and intake of dietary nutrient inputs and feed. Few farmers use single or mixed feed ingredients as farm-made feed. But single or two feed ingredients cannot fulfill the nutritional demand of the animal and not suitable for shrimp production and benefit. Formulated feed nutritionally sound and boost up shrimp production.

Shrimp farming requires more investment than other aquaculture activities. Maximum shrimp farmers of the country are not well to do and need to reduce investment in shrimp farming. Feed is the key component of modern shrimp farming but its cost is one of the most important items in shrimp production cost. Though

different types of commercial feed are available in the market but there is lack of suitable feed for shrimp farmers. Application of these costly feeds increases the production cost of the farmer. As a result, the farmers are deprived from getting more net income. To increase the shrimp production per unit area, low-cost balanced shrimp feed is prerequisite. Use of formulated low-cost feed with locally available feed ingredients instead of expensive commercial feed may be a means to reduce feed cost as well as production cost. In many times, all ingredients of balanced feed are not available al local level round the year. Easily available low-cost commercial feed may be used instead of locally made low quality feed for increasing shrimp production as well as for higher net return. Keeping these views in mind, the present research work had been undertaken to enhance the shrimp production applying different types of formulated feed.

Materials and methods

The study was conducted in six shrimp farms in Sadar upzilla of Bagerhat district, where shrimp are generally cultured in extensive traditional system. The area of the selected shrimp farms was 800 m^2 to 1,200 m^2.

Shrimp farm preparation: The dikes of the shrimp farms were constructed in such a way so that water can't overflow or pass through it. The entire farm was encircled up to 1.0 m height with fine meshed nylon net so that no animal from outside can enter and destroy the reared shrimp. The farms were dried and the surface soil was treated with agricultural lime ($CaCO_3$) at a rate of 250 kg/ha. After 7 days, farms were fertilized with mustard oil cake at a rate of 500 kg/ha. After that, the farms were filled up with tidal water up to a depth of 50-60 cm and treated with phostoxin tablet at a rate of 1 tablet/210 sqft of water to kill the unwanted animals of the farms. After remove all dead animals, water of the farms treated with lime at a rate of 250 kg/ha. The farms were then fertilized with triple super phosphate (TSP) and urea (2:1) at a rate of 35 kg/ha.

Stocking of shrimp post larvae (PL): After 7 days of fertilization, the post larvae of *P. monodon* having an average body weight of 0.006 g were stocked at a rate of 3 nos/m^2 in each farm. Before the stocking, the PL were acclimatized with the temperature and salinity of water of the farm.

Post stocking management: Shrimp PL were reared in nursery enclosure and fed with commercial nursery feed (Saudi Bangla nursery feed: starter 1, 2 and 3) @ 100% of the total biomass in 1st week, 60% in 2nd week and 30% in 3rd week. After 3rd week of nursery rearing, they were allowed to spread over the whole farm by opening the nursery enclosure. At this stage, different feeds (BFRI dough feed, Saudi-Bangla grower and Saudi-Bangla special feed) were applied @ 30%, 20% and 10% of the estimated shrimp biomass at 8 hrs intervals daily for the 1st, 2nd and 3rd week, respectively by spreading. Thereafter, feeding rate was gradually decreased from 5-3% for the rest of the culture

period. For maintaining productivity, farm's water was periodically treated with lime @ 50 kg/ha and inorganic fertilizer using TSP and urea (2:1) @ 30 kg/ha as needed. Additional lime was also applied @ 125-250 kg/ha after every heavy shower. Probiotics (@1 ppm) mixed with sands and made into balls was applied to the bottom of the farms to resist possible blackening of soil due to bacterial activity. The feeding behaviour and shrimp health were checked 1-2 days intervals through cast netting. To maintain undisturbed ecology of the farms and to control shrimp disease, water was not exchanged.

Water quality parameters *viz.*, water depth, temperature, salinity, pH, nitrate, ammonia and dissolved oxygen were determined at weekly intervals. Water temperature was recorded using a Celsius thermometer. Dissolved oxygen and pH were measured directly using a digital portable oxygen meter (Oakton) and portable pH meter (Hanna 8424), respectively. Salinity was recorded using portable refractometer. Nitrate and ammonia were determined following standard methods as mentioned by Strickland and Parsons (1968) and APHA (1992).

After grow out period of four months, shrimp farms were drained by pump and all the shrimp were harvested. Total weight and number of shrimp and economics in each farm were recorded. Farmers rally was arranged in the farm site to give hands-on experience to the interested farmers. Specific growth rate of shrimp was calculated as follows:

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

For statistical analysis of data, one way analysis of variance (ANOVA) was carried out to find the level of significance of difference among the different treatments. Significance was assigned at the 0.05% level.

Results and discussion

The physicochemical factors of the farm water under three treatments are presented in Table 1. Depth of water of the farms was 50.0—110.0, 66.0—130.0 and 59.0—135.0 cm in T_1, T_2 and T_3, respectively. The fluctuation in depth was due to evaporation and precipitation of water. Generally, depth of water of the traditional farms remains with 40-60 cm. This low depth of water provides poor space for the movement of the stocked shrimp. Besides, the stocked shrimp suffer from temperature shock during draught period. Inadequate water depth is one of the most important factors of shrimp mortality and low yield (Karim 2002). The water temperature in T_1, T_2 and T_3 ranged from 29.0—35.2°C, 29.0—35.0°C and 29.0—35.3°C, respectively with the mean values of 32.02±2.06, 31.87±2.07 and 32.04±2.06°C. The variation in temperature among the treatments were found similar ($p<0.05$) and was slightly higher than the suitable range for growth of shrimp (Boyd and Fast 1992, Apud 1989 and Latif and Islam 1995).

The salinity did not show any significant ($p<0.05$) difference among the treatments. The values of salinity ranged from 0.0—9.5, 0.0—9.0 and 0.0—9.0 ppt under T_1, T_2 and

T_3, respectively. The values varied with sampling dates, which might be associated with the differences in presence of salinity. Salinity ranging from 5.0—32.0 ppt is favourable for shrimp culture (Predalumpaburt and Chaiyakam 1994). The level of pH varied from 6.50 to 8.60, 6.50 to 8.40 and 6.5 to 9.15 in T_1, T_2 and T_3, respectively. The pH in all the farm water was more or less alkaline throughout the experimental period, which might be due to regular application of lime at fortnightly/monthly intervals. Several authors have reported a wide variation in pH 6.0—9.0 (Boyd and Green 2002), 7.5—9.2 (Hoq 2002), 7.68—8.35 (Shofiquzzoha *et al.* 2001) and 7.30—7.97 (Saha *et al.* 2001) in shrimp farms and found the ranges favourable for shrimp culture.

Table 1. Mean values (± SD with range) of water quality parameters as recorded from the shrimp farm under different treatments during the study period

Parameters	Treatments		
	T_1	T_2	T_3
Water depth (cm)	83.75±21.17[b] (50.0—110.0)	94.88±21.67[a] (66.0—130.0)	95.25±25.05[a] (59.0—135.0)
Temperature (°C)	32.02±2.06 (29.0—35.2)	31.87±2.07 (29.0—35.5)	32.04±2.06 (29.0—35.3)
Salinity (ppt)	3.05±2.96 (0.0—9.5)	3.0±2.83 (0.0—9.0)	3.0±2.28 (0.0—9.0)
Dissolved oxygen (mg/l)	4.0±0.58 (3.21—4.80)	4.0±0.51 (3.25—4.56)	3.94±0.53 (3.30—4.80)
pH	8.05 (6.5—8.60)	8.02 (6.5—8.40)	8.33 (6.5—9.15)
NO_3-N (mg/l)	0.013±0.002 (0.008—0.016)	0.012±0.002 (0.009—0.015)	0.012±0.002 (0.010—0.016)
NH_4-N (mg/l)	0.028±0.024 (0.002—0.063)	0.029±0.027 (0.002—0.070)	0.029±0.030 (0.002—0.080)

Mean ± SD, figures with different superscript differs significantly.

The dissolved oxygen content in the experimental farms ranged from 3.21—4.80, 3.25—4.56 and 3.30—4.80 mg/l in T_1, T_2 and T_3, respectively with the mean values of 4.0±0.58, 4.0±0.51 and 3.94±0.53 mg/l. Comparatively lower level of dissolved oxygen as observed in the farms appeared to be related to sampling time when the dissolved oxygen was monitored at about 10.00-11.00 am. At this time, dissolved oxygen remains lower in concentration. Apud (1989) and Boyd *et al.* (1994) reported that dissolved oxygen content of a shrimp farm should be >4.0 mg/l and >3.8-5 mg/l, respectively. In morning, dissolved oxygen of the farm water never decreased below 3.21 mg/l, which could be considered as congenial for shrimp culture.

Nitrate nitrogen ranged from 0.008—0.016, 0.009—0.015 and 0.010—0.016 mg/l with mean values of 0.013±0.002, 0.012±0.002 and 0.012±0.002 mg/l in T_1, T_2 and T_3, respectively. These values did not show any significant difference among the treatments.

Comparatively lower values of NO_3-N recorded at the farms might be attributed to wide uptake of this nutrient by the primary producers in the farms. The values found in the present study were within the suitable range for brackishwater aquaculture (Islam *et al.* 2004). The mean concentration of total ammonium-nitrogen as recorded in T_1, T_2 and T_3 ranged from 0.028±0.024, 0.029±0.027 and 0.029±0.030 mg/l, respectively with no significant difference among them. The values obtained in the experimental farms were far below the critical level of ammonia and the variations in ammonia-nitrogen in all the treatments were within the productive range for shrimp farming (Boyd 1998 and Chien 1992).

Growth and production

The stocking density, growth, survival and production of shrimp in the shrimp farms are shown in Table 2. The shrimps of all the farms were harvested after 120 days of culture. No disease symptom was observed in the experimental farms. Shrimps in T_1, T_2 and T_3 grew up to 20.89, 23.90 and 22.80 g after 120 days with a daily growth increment of 0.174, 0.199 and 0.190 g, respectively. The daily growth of shrimp applying highly valued CP feed (Tk. 85.00/kg) was 0.278 g after 120 days reported by Saha *et al.* (2006). The growth obtained from the present study applying low valued different feeds (Tk. 40.00-48.00/kg) was slightly lower than Saha *et al.* (2006). The survival of shrimp was 64.5, 71.0 and 69.5% in T_1, T_2 and T_3, respectively with no significant difference among them. These survival rates were higher than that of Alam *et al.* (2007), Alam and Islam (2007), Saha *et al.* (2006) and Hoq *et al.* (2001). There was no significant variation among the specific growth rate (SGR) of shrimp in different treatments. The SGR of shrimp was highest of 27.47-27.82 % in the 1st 15 days in all the treatments. In the 2nd 15 days, it dropped sharply to 7.5-8.80 % and then it was continued up to 1.46-1.70 % in the last 15 days of culture. The highest average production of shrimp of 509 kg/ha was obtained in T_2 and the lowest of 404 kg/ha found in T_1, which was significantly lower than that of T_2 and T_3. But there was no significant difference in production between T_2 and T_3. The food conversion ratio (FCR) was lower in T_2 compared to T_1 and T_3.

Table 2. Growth and production performance (mean±SD with range) of *Penaeus monodon* in different treatments during the study period of 120 days

Treatments	Shrimp farm size (ha)	PL stocked (/ha)	Final wt. (g)	SGR* (% per day)	Survival (%)	Production (kg/ha)	FCR **
T_1 (Dough feed)	0.089±0.01 (0.08-0.098)	2670±382 (2400-2940)	20.89±2.44 (18.3-22.8)	6.79	64.5±2.12 (63-66)	404.0±4.8[b] (400.6-407.4)	3.6
T_2 (Saudi-Bangla)	0.100±0.03 (0.08-0.12)	3000±849 (2400-3600)	23.90±2.60 (21.8-26.7)	6.91	71.0±4.24 (68-74)	509.0±33.5[a] (485.5-532.8)	2.3
T_3 (Saudi-Bangla special)	0.113±0.08 (0.106-0.113)	3390±297 (3180-3600)	22.8±2.13 (20.4-24.5)	6.86	69.5±4.95 (66-73)	476.0±57.4[a] (435.64-516.83)	3.1

* SGR=Specific growth rate, ** FCR=Food conversion ratio.

This production of shrimp is comparable to that of Alam and Islam (2007), who reported a production range of 425—660 kg/ha with the average body weight of 21.88—23.88 g after 110 days of rearing at Brackishwater pond complex, Brackishwater Station, BFRI, Paikgacha, Khulna and the stocking density of their research experiment was 5 PL/m^2. The higher production in some of the shrimp farms compared to the present study was due to high stocking density and high survival of about 90%. Low survival in the present study might be due to stress during long transportation. Stocked shrimp PL were packed about 30-32 hrs before stocking and carried from Cox's Bazar to Jessore by air and then by road from Jessore to Foila Shrimp Fry Marketing Centre, Foila, Rampal, Bagerhat. By this time, some of the stocked PL might become weak and died after stocking to the farm. However, Mazid *et al.* (2001) and Mazid (1994) reported a yield of shrimp (*P. monodon*) of 350.0-500.0 kg/ha at 1.0-2.5 PL/m^2 density. These findings of improved traditional culture system are closed to that of the present study. Rahman *et al.* (2002) stated that production of shrimp in Khulna was 158.47 kg/ha/yr. Production of shrimp in extensive traditional system in the Paikgacha area was 83.47-204.46 kg/ha as reported by Islam *et al.* (2005). Alam *et al.* (2007) reported that shrimp yield in improved traditional method at BFRI pond complex, Brackishwater Station, BFRI, Paikgacha, Khulna was 212.0 kg/ha at 2 PL/m^2. The national production of shrimp from culture sector in Bangladesh is 370 kg/ha (Anon, 2005). These productions are several times lower than the findings of the present study.

Cost of production and economic returns

The total gross return under T_1, T_2 and T_3 was Tk. 177,760.00, 225,557.88 and 209,541.20/ha with the benefit cost ratio of 1:1.47, 1:1.60 and 1:1.54, respectively. Higher net return (Tk. 84,209.60) was achieved in T_2 and lower (Tk. 56,493.99) in T_1. But there was slight variation in net return between T_2 and T_3. Benefit cost analysis implies that higher BCR (1.60) was also found in T_2 and lower (1.47) in T_1. The net return (Tk. 56,493.99—84,209.60/ha) obtained from the shrimp culture in the present study is much higher than that of Tk. 37,070.00/ha reported by Islam *et al.* (2005). Uddin (1998) and Ling *et al.* (2001), respectively also reported lower net reruns of Tk 35,600.00 /ha and Tk 35,500.00/ha from the traditional shrimp culture practice. Miah (2001) reported the net return of Tk. 57,056.00 of alternate shrimp-rice farming which is more or less similar with the present findings. Therefore, Saudi-Bangla grower is best feed than that of BFRI dough feed and Saudi-Bangla special feed. It implies that Saudi-Bangla grower is much more profitable, economically viable and socially acceptable than others feeds.

Most of the shrimp farmers of Bagerhat region are not familiar with the improved shrimp culture systems. Current shrimp production of Bangladesh is only 175-200 kg/ha, which is 2-3 times lower than that of the present findings. The on-farm research activities on modern shrimp culture techniques will grow awareness among the farmers. Farmers' training programs were organized at field level during culture and harvesting period. The whole culture practice was presented to them. The shrimp farmers showed keen interest to observe the production and economic returns from the culture practice.

It can be expected that national shrimp production would be boost up through the implementation of this practice in this region.

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Feeding habits of the sesarmid crab *Perisesarma bidens* (De Haan) in the mangroves of the Ryukyu Islands, Japan

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Abstract
Feeding habits of the sesarmid crab *Perisesarma bidens* (De Haan) was investigated in the mangroves of the Ryukyu Islands, Japan. Stomach content analysis showed that their diet consists mainly of mangrove leaves fragments, with small amounts of animal, algae and sediment matters, indicating that *P. bidens* is primarily detritivorous. The consumption rate of *P. bidens* was investigated under laboratory conditions by offering three different types of *Kandelia candel* mangrove leaves. Crab survived by eating green, yellow or brown leaves, preferring brown to either green or yellow leaves. Consumption rate of brown leaves was significantly higher when crabs were provided with green, yellow and brown leaves together, than when provided separately. It is considered that the brown leaves have a soft tissue, which is easily torn by the crab chelae and have apparently low C/N ratio. The C/N ratio of faeces, which indicated lower value than that of burrow leaves or sediments, derived from the symbiosis of bacteria in the stomach. The C/N ratio showed that sediments had C/N ratios 2/3 times lower than leaves sequestered in the burrow, indicating that mangrove sediments could have higher nutritional value than mangrove leaves. *Perisesarma bidens* showed significant consumption rates of mangrove detritus, therefore, it may have the important role as the grazer of mangrove detritus in view of the nutrient cycle in the mangroves.

Key words: Mangrove leaves, Sesarmid crab, C/N ratio

Introduction

The sesarmid crabs are diverse and abundant in mangroves: they affect the soil chemistry and primary productivity, mangrove zonation and colonization, food web dynamics, nutrient retention, litter decomposition, and offshore export of mangrove production (Smith *et al.* 1989, Skov and Hartnoll 2002, Camilleri 1992, Sheaves and Molony 2000). About fifty species of sesarmid crabs have been reported to be predominantly associated with mangrove ecosystems (Islam *et al.* 2002). Although most sesarmid crabs are opportunistic scavengers, many have essentially detritivorous as they feed on dead leaves that have fallen off the mangrove, in particular members of the

family Sesarmidae, which are important components of mangrove ecosystems in the Indo-West Pacific, Africa, the Caribbean and South America (Schoth *et al.* 1968).

The crab *Perisesarma bidens* (De Haan) is most common and typically associated with mangroves in tropical and subtropical estuaries and coastal lagoons in the Indo-West Pacific, being known from the Bay of Bengal to the Andaman Islands, Sri Lanka, India, Malay Archipelago, the Philippines, Hong Kong, Taiwan, Korea, and Tokyo Bay to Kyushu and the Ryukyu Islands of Japan (Sakai 1976, Dai and Yang 1991). This species live in burrow constructed in the edges or within the mangroves or in the reed marsh higher than ordinary high water mark, and among the roots, trunk, and lower branches of mangrove trees. This crab is commonly occurred in the mangroves of the Ryukyu Islands, Japan (Islam *et al.* 2000, 2002). The peak spawning season of this crab is early June to early August every year.

Stomach contents are the primary means of verifying the natural diets of many crustaceans that feed on macroscopic food, although the mouthparts and gastric mills of the crabs generally reduce the food to small fragments (Robertson 1991). In many cases, absolute or relative quantities of food ingested are difficult to measure, and the type of food is difficult to identify (Smith *et al.* 1989). To date methods for stomach contents analysis of crustaceans have been poorly documented in the literature. Williams (1981) established a method for analyzing the natural diet of portunid crabs. Stomach contents of varunid and sesarmid crabs were analyzed to get an idea about their natural diets by Mia *et al.* (2001) and Islam *et al.* (2002). Multiple field and laboratory studies leave no doubt that sesarmids ingest mangrove leaves. Leaves often comprise more than 85% of sesarmid stomach contents (Dahdouh-Guebas *et al.* 1999), and sesarmids may remove 79 to 95% of mangrove leaf fall from the forest floor (Sheaves and Molony 2000). The easiest method is the frequency of occurrence method which involves the examination of stomach contents visually, or with a microscope, with the different food items sorted and identified in each stomach. The percentage of stomachs an item is found in is used as a measure of its importance in the diet.

Freshly fallen (senescent) mangrove leaves have notably high C/N ratios several times greater than 17, a value suggested as a general maximum for sustainable animal nutrition (Russel-Hunter 1970, Twilley *et al.* 1997, Robertson 1988). Giddins *et al.* (1986) proposed that crabs might plaster leaves onto burrow walls to allow tannins to leach and increase the edibility of leaves. In addition, leaf nitrogen (N) content increases and C/N ratio decreases during breakdown, through microbial activity (Twilley *et al.* 1997, Cundell *et al.* 1979). Thus by not eating leaves immediately, but leaving them to age on the burrow wall, crabs might not only improve the digestibility of leaves but also decrease the C/N ratio of their diet (Giddins *et al.* 1986). Leaf material ingested by some sesarmid and xanthid crabs is incompletely digested, and much of it is returned to the environment in large faecal pellets, which contain more finely divided leaf materials (Misra *et al.* 1985). The aims of the present study were: to determine the diet of the crabs through an analysis of their stomach contents, the consumption rate of mangrove leaves, preference of different types of leaf, C/N ratios in leaves, sediments and faeces, and nutritional composition in the diet of *P. bidens*.

Materials and Methods

Study sites and experimental materials

Adult crabs of *Perisesarma bidens* were collected from two mangrove swamps: the Nuha River in the Okinawa Island (N=20) and the Shira River in the Iriomote Island (N=20), during April 2004 to March 2005 to analyze stomach contents.

Non-gravid, healthy adults of *P. bidens* and three types of *Kandelia candel* mangrove leaves (green, yellow, brown) were collected from the Nuha River mangroves at Okinawa Island, during July 2004, to examine the feeding habit and food choice of crabs under laboratory conditions.

For the analysis of C/N ratios, mangrove leaves and sediments were collected from the burrow of *P. bidens* at the Nuha and the Shira River mangroves between April 2004 and March 2005. Leaves and sediment samples were collected from at least ten to fifteen burrows per station. The three types of *K. candel* leaves were also collected from this area for analyzing their C/N ratios and nutritional composition. Faeces of *P. bidens* were collected both from field and laboratory to determine its C/N ratios during April 2004 to March 2005.

Stomach content analysis

Crabs were fixed in 10% formalin solution immediately after collection. Stomach content analysis took place within one month of sampling. The stomach was carefully removed from each crab and the contents washed into a Petri dish with distilled water. A drop of stomach contents was placed on a glass slide and examined with a binocular stereomicroscope (Nikon SMZ-10, x25). The nature of the diet was determined using the percentage occurrence method described by Williams (1981). This method gives a measure of the regularity with which a particular food item is eaten in the population sample, and is recommended when the diet includes several different food items. The occurrence of each different food item is recorded for each stomach, and results expressed as the frequency of each item among the stomachs constituting the population sample. Stomach contents were scored for the following different food item categories: plant materials, animal materials, algae, silt/clay, and unidentified materials.

Feeding behavior

Crabs (N=20 for each leaf type) were housed separately for two days in ventilated plastic containers containing 100ml of brackish water (15‰ salinity) to acclimate them to laboratory conditions. Each container was tilted slightly for drainage. Crabs were starved for 12 hours prior to the experiments, so that faecal production ceased. Leaves were divided into green, yellow and brown, on the basis of their color. Green and yellow leaves were handpicked from the trees, while brown leaves were taken from the forest floor. Each crab received only a single type of leaf. Each leaf was weighed at the beginning of the experiment and daily to the nearest of 0.001g, using an electric balance. A new leaf of the same type was added to each container when less than 0.03g of the previous leaf remained. All leaves were photocopied at the beginning of the experiment,

so that the impact of feeding could be visualized. The carapace length (CL) and carapace width (CW), and body weight (BW) of all crabs was measured at the beginning of the experiment, and once a week to monitor growth. The experiment was conducted over a period of eight or more weeks. Water in all containers was changed on alternate days. The green, yellow and brown leaves of *K. candel* were provided together as food in order to investigate the leaf choice by the individuals of *P. bidens* separately. The experiment was conducted over a period of eight or more weeks. Growth changes of crabs by body weight and leaf consumption rates were monitored as described in the above experiment.

C/N ratio of burrow leaves and sediments

Collected leaves were rinsed with distilled water and dried in an oven at 60^0C with air circulation for 2 days. Dried samples were ground with a mortar and pestle. Ground samples were stored in desiccators until analysing in a Shimadzu (NC 80 model) high-sensitivity C: N Analyser. For each sample, 3 replicates of 0.1g dry weights were placed in ceramic sample boats and ignited at 830^0C for 1 minute. The connected Chromatopac recorder printed out the carbon (C) and nitrogen (N) amounts as detected by the Sumigraph Detector.

Sediment samples were washed with distilled water in plastic containers to remove salt, taking care not to lose any organic matter. After settling for 24 hours, the water was decanted and samples oven dried at 60^0C overnight. Dried samples were treated with diluted hydrochloric acid (HCl, 2N) overnight to remove carbonates and bicarbonates. Acid treated samples were oven dried again, ground using a mortar and pestle, and stored in a desiccators prior to analysis in a Shimadzu (NC 80 model) high-sensitivity C: N Analyser, as described for the leaf samples above.

C/N ratio of fresh leaves and faeces

Faeces were collected from crabs both in the field and laboratory (the latter supplied with green, yellow and brown leaves separately), oven dried at 60^0C overnight and analysed as described for the leaf samples.

Nutritional composition of leaves, sediments and waters

To assess the nutritional composition of mangrove leaves, samples of each type of leaf (green, yellow and brown) were analyzed by the Okinawa Environmental Technology Association, Japan. The amounts of energy and carbohydrate in 100g wets and dry leaf sample were assessed. The amount of carbohydrate and energy were estimated by the following equations: Carbohydrate = 100 - (Water + Ash + Fat + Protein), Energy = (Protein x 4) + (Fat x 9) + (Carbohydrate x 4). The concentrations of K^+, Na^+, NH_4^+, NO_2^-, NO_3^-, PO_4^{-2} and SO_4^{-2} were measured by spectro-photometer (DR/2000, HACH).

Statistical analysis

Comparative data on stomach fullness, food consumption and growth rate, and C/N

ratios of leaves, sediments and faeces were analyzed using multiple analyses of variance (MANOVA) on the statistical package Stat View 5. A two-factor ANOVA was used to evaluate differences between sites and treatments of the experiments. Fisher's PLSD, Wilk's Lambda, Roy's Greatest Root, Hotelling-Lawley Trace and Pillai Trace indicated where there were significant differences in the mean. Mean values are reported with 95% confidence intervals and 5% significance levels.

Results

Stomach contents

The diet of *Perisesarma bidens* consisted mainly of mangrove leaf fragments, complemented with some sediment materials (Fig. 1). Plant materials were the most common in all cases. The second most common category was clay or mud, which was also higher in winter than in summer. Relatively small amounts of animal matters were found in most of the individuals, during both the summer and winter seasons. Unidentified materials were also found in both seasons. No significant differences were detected between summer and winter diets ($P>0.05$, Fisher's PLSD).

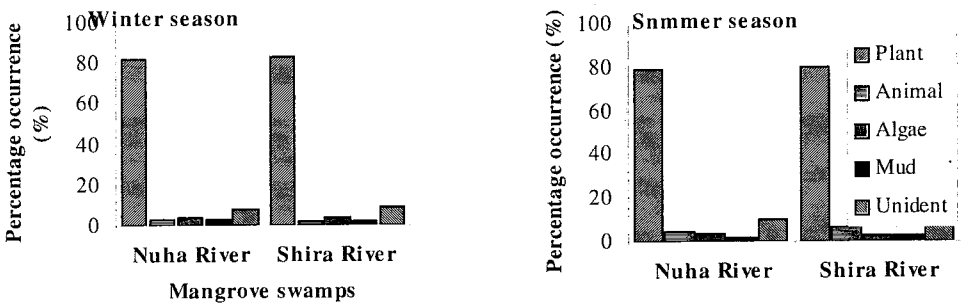


Fig. 1. Stomach contents of *Perisesarma bidens* (De Haan), collected from two different mangrove swamps. N=15.

Feeding behavior

Crabs were survived during experimental period, indicating that a diet of only green, yellow or brown leaves of *Kandelia candel* is sufficient for survival for an eight weeks period. Feeding commenced at varied parts of the leaves supplied (Fig. 2). Green leaves were consumed at the least and brown leaves to the most. Crabs preferred to feed on brown to yellow or green leaves, and also grew fastest on brown leaves than other two types of leaves ($P<0.0001$, Fisher's PLSD). When given alternatives, crabs preferred brown to yellow, and yellow to green leaves. The average weekly consumption rate of brown leaves was higher than that of green or yellow leaves during the course of experiment, as were weight changes ($P<0.0001$, Fisher's PLSD).

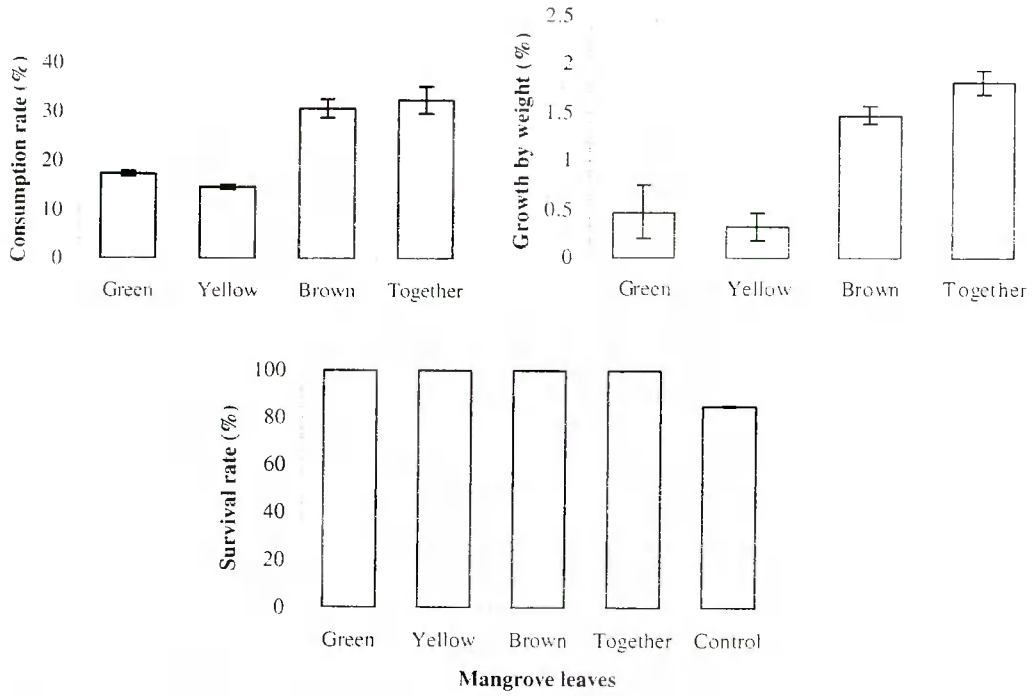


Fig. 2. Consumption, growth and survival rate of *Perisesarma bidens* (De Haan) when fed on green, yellow and brown leaves of *Kandelia candel*, separately and together. Data indicate mean (±SD) of eight weeks experiments, N=24. Significant differences ($P<0.0001$, Fisher's PLSD, 5% significance level) were found among three types of leaves supplied except survival rate.

C/N ratios

C/N ratios were always higher in leaves taken from burrows in the Shira River (60.01) than those taken from the Nuha River (43.89) sites (Fig. 3). The C/N ratio in burrow sediments of the Nuha River (20.76) was lower than in the Shira River (23.31) sites (Fig. 4). Significant differences ($P<0.0001$, Fisher's PLSD) were found among C, N and TOM contents and C/N ratios of burrow leaves and sediments between the Nuha and the Shira River sites. C/N ratios were always higher in green fresh leaves than in yellow or brown leaves ($P<0.0001$, Fisher's PLSD) (Fig. 5). The C/N ratio was lower in natural faeces than in faeces produced under laboratory conditions ($P<0.0001$, Fisher's PLSD) (Fig. 6).

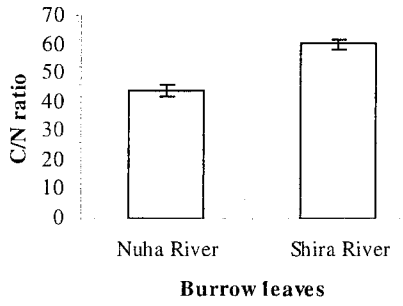
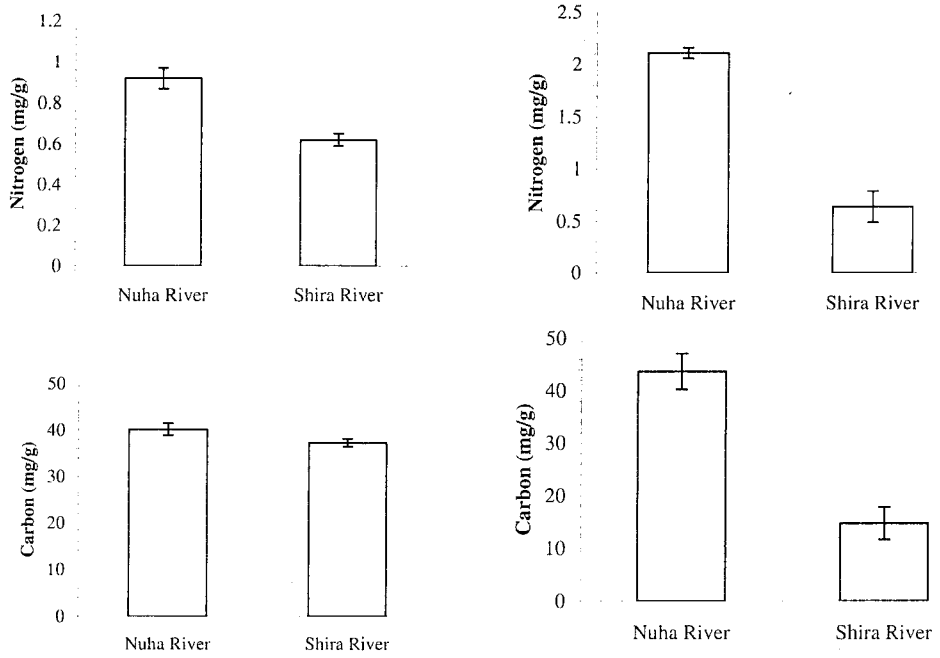


Fig. 3. Concentrations of C and N contents, and C/N ratios in burrow leaves of *Perisesarma bidens* (De Haan) from two different mangrove swamps. Data indicate mean (±SE), error bars = 95% confidence interval, N=15. Significant differences ($P<0.0001$, Fisher's PLSD, 5% significance level) were found between two mangroves.



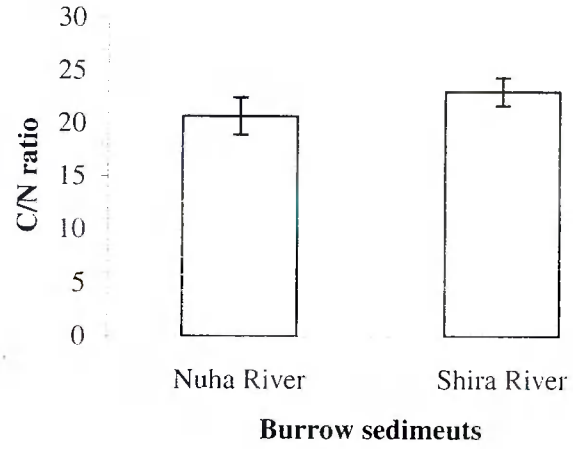


Fig. 4. Concentrations of C and N contents, and C/N ratios in burrow sediments of *Perisesarma bidens* (De Haan) from two different mangrove swamps. Data indicate mean (±SE), error bars = 95% confidence interval, N=15. Significant differences ($P<0.0001$, Fisher's PLSD, 5% significance level) were found between two mangroves.

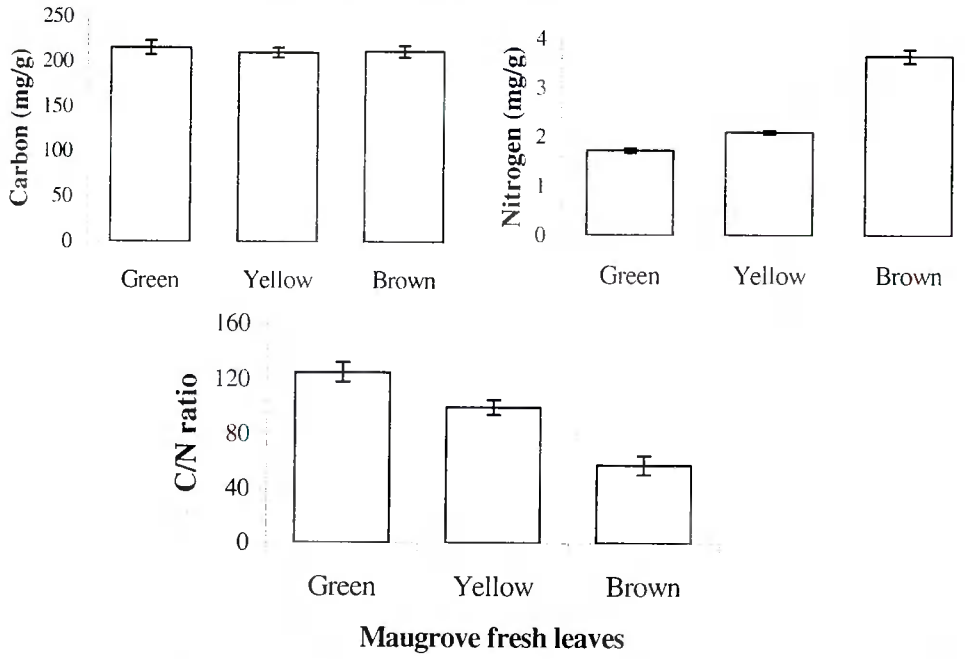


Fig. 5. Concentration of C and N contents, and C/N ratios in fresh leaves of *Kandelia candel* mangrove. Data indicate mean (±SE), error bars = 95% confidence interval, N=15. Significant differences ($P<0.0001$, Fisher's PLSD, 5% significance level) were found among three types of leaves.

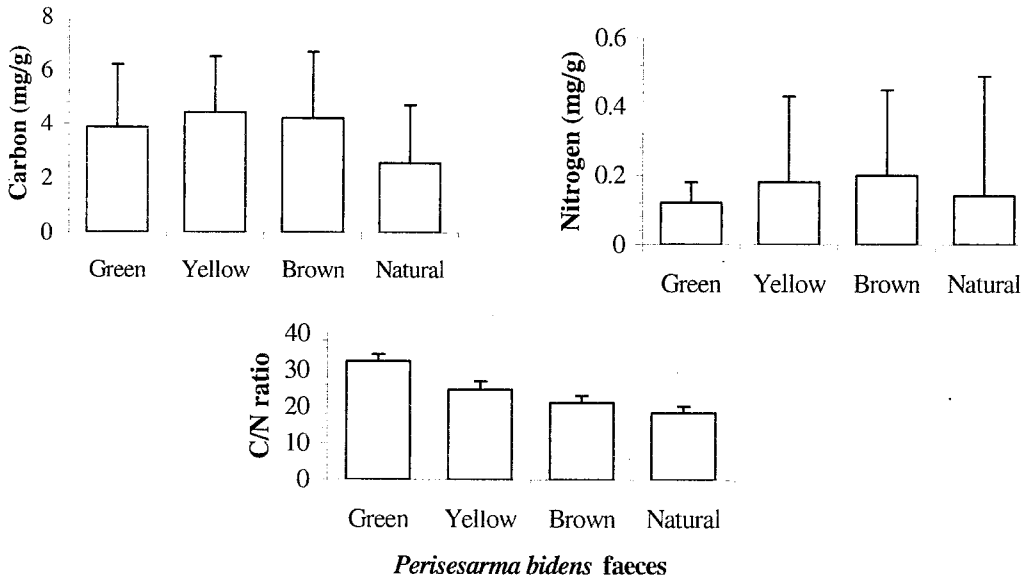


Fig. 6. Concentrations of C and N contents, and C/N ratios in faeces of *Perisesarma bidens* (De Haan) produced in the laboratory and the field. Data indicate mean (±SE), error bars = 95% confidence interval, N=15.

Nutritional compositions

Dry leaves were more nutritious than wet leaves in three different types (Fig. 7). The amount of carbohydrate and energy are always higher in brown leaves than that of yellow or green leaves (Fig. 7). The nutrients NH_4^+, NO_3^-, PO_4^{-2} and SO_4^{-2} are available in both mangrove habitats as well as in sediments and waters (Table 1).

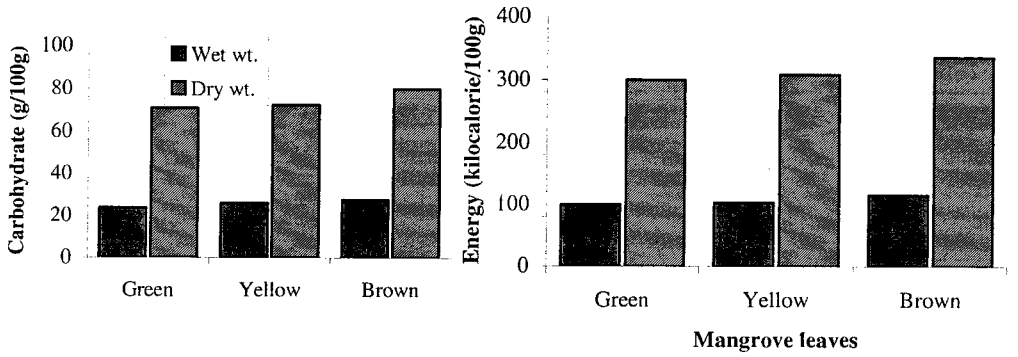


Fig. 7. Nutritional composition of *Kandelia candel* leaves in 100g wet and dry weights. Samples were originated from the habitat of *Perisesarma bidens* (De Haan) in the Nuha River mangroves on Okinawa Island. N = 5.

Table 1. Nutritional compositions of burrow sediments and waters

Sites	Nutrients						
	K^+	Na^+	NH_4^+	NO_2^-	NO_3^-	PO_4^{-2}	SO_4^{-2}
Sediments							
Nuha River	Nd	Nd	0.14	0.03	1.28	0.09	0.04
Shira River	Nd	Nd	0.12	0.03	1.30	0.08	0.04
Waters							
Nuha River	10.31	151.72	0.00	Nd	0.39	0.00	80.76
Shira River	2.11	57.19	0.03	Nd	0.34	0.08	14.95

Nd = not detected.

Discussion

The stomach contents of *Perisesarma bidens* shows that they are mainly detritivorous. This confirms the result of previous studies in related species of sesarmid crabs which are major players in leaf degradation and nutrient regeneration in mangroves (Islam *et al.* 2002, Dahdouh-Guebas *et al.* 1999, Smith *et al.* 1991). Silt and clay materials found in their stomach may have been incidentally eaten with leaf materials. More clay was found the stomachs during the winter than summer, where the crabs remaining in their burrows for extended periods, when they may consume clay to assuage their hunger. Only small amounts of animal material were found in stomach contents. This may be accidentally consumed together with the leaves, or when fallen leaves were insufficient in the crab surroundings. *Neosarmatium indicum* and *N. meinerti* descended from their burrows above the high tide mark to feed on mangrove leaves, which they took into their burrows (Dahdouh-Guebas *et al.* 1999, Islam *et al.* 2000). *Perisesarma bidens* showed the same activity in the Nuha River mangroves, where *Kandelia candel* is abundant, but little there is accumulation of leaf litter. This may be due to the presence of leaf-eating crabs like *P. bidens*. Based on the present study, it is concluded that the sesarmid crab *P. bidens* is detritivorous, and that there is no seasonal difference in its natural diet.

Consumption rate of brown leaves were higher than green or yellow leaves of *K. candel*. Therefore, *P. bidens* clearly preferred brown leaves to green or yellow. In a previous experiment of related species, *N. indicum* preferred brown leaves more than green or yellow ones (Islam et al. 2000). Giddins *et al.* (1986) reported that *N. smithi* consumed decayed leaves of *Ceriops tagal* much more than fresh and senescent leaves, which is consistent with the present findings. Micheli (1993) noted that *Perisesarma messa* and *N. smithi* did not exhibit a significant preference for newly fallen leaves of mangroves, and they consumed significantly more decayed leaves than senescent ones. The consumption rate of brown leaves by *Helice leachi* was significantly higher than that of green and yellow leaves (Lee 1998). It has been suggested that brown leaves have soft tissues that might be more easily torn by crabs.

Giddins *et al.* (1986) noted that *N. smithi* took leaves into their burrows several weeks before consuming. During this time, tannins were lost from the leaves through leaching, while nitrogen content increased through bacterial activity, resulting in a

higher quality food. Since *P. bidens* do not come outside their burrow during daytime, they must emerge during low tide at night and pull down leaf particles into burrows. The green leaves of *K. candel* ranked last in the preference hierarchy of *P. bidens*.

Skov and Hartnoll (2002) investigated that C and N contents and C/N ratios of leaves in crab burrows and found that they do not differ significantly from those of freshly fallen leaves. The leaf-ageing hypothesis, that sesarmid crabs may plaster leaves to their burrow walls in order to improve leaf palatability and nutritional quality, has been discussed in the literature for more than 15 years (Giddins *et al.* 1986, Skov and Hartnoll 2002). Several papers, for instance, have noted how sesarmids in the field and in the laboratory may prefer to ingest aged leaves rather senescent leaves (Giddins et al. 1986, Skov and Hartnoll 2002). The majority of studies have recorded C/N ratios in mangrove leaves that far exceed the Russel-Hunter ratio of 17. Leaves, in general, take a very long time to reach their lowest C/N values, and in most cases the lowest C/N ratios of decayed leaves are still at least double the Russel-Hunter ratio (Skov and Hartnoll 2002). The sediments in the present study had C/N ratios 2/3 times lower than leaves, indicating that sediments could have higher nutritional value than leaves. Bacteria may certainly reach high densities in mangrove mud and are highly digestible by crabs (Alongi 1988). The C/N ratio of fresh faeces of *N. meinerti* was 49, which is higher than the present study (Skov and Hartnoll 2002).

Green leaves of *K. candel* contain more protein than yellow or brown leaves in both wet and dry conditions (Islam et al. 2002, Lee 1998). Since carbohydrates are the primary energy source, brown leaves contain more energy (Islam *et al.* 2002). The leaf material is originally high in carbohydrates, lipids and protein content (Bhosle *et al.* 1976). Organic matter and nutrients are thus conserved in the forest rather than being washed out to the sea (Camilleri 1992). Thus, *P. bidens* plays an important role in the energy flow pathway in the mangrove forest by providing food for detritus feeders. However, *P. bidens* feeds on mangrove leaves and egests them as small particles; these particles are then utilized by bottom-dwelling detritivores inside and outside the mangrove forest. For this reason, *P. bidens* may be regarded as an important species in the nutrient cycles of the mangrove ecosystem.

Acknowledgements

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Effect of delayed icing on the quality of tiger shrimp (*Penaeus monodon* Fab.)

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Abstract
Effect of delayed icing on the quality of *Penaeus monodon* iced after three hours of harvest was studied in plastic and bamboo baskets. After harvest of three hours at ambient temperature (28^0-32^0C), ice was added to the shrimp at a ratio of 1:1 (shrimp: ice) and stored for 21 hours in both the baskets. Quality evaluation was carried out through visual assessment, biochemical analysis and microbial analysis for 24 hours. The organoleptic evaluation and scoring was done from the time of harvest treated as 0 hour and the average score was 10. At 9^{th} hour after iced condition quality of shrimp was found reduced to the next stage (acceptable) with a score ranged from 8.4-6.5 in both baskets. This acceptable stage was observed throughout the experiment for bamboo basket whereas in the plastic basket the quality was reduced to a small extent with a score of 6.4 (moderately acceptable). Till the end point of the experiment the quality of shrimp was acceptable in respect to biochemical analysis. The microbial load was found $\log_{10}$ 3.99±0.12 cfu/g to $\log_{10}$ 4.33±0.21 cfu/g and $\log_{10}$ 4.01±0.12 cfu/g to $\log_{10}$ 4.83±0.19 cfu/g in the bamboo and plastic basket respectively. The importers or buyers suggests for immediate icing to maintain good quality but results of the present experiment suggest that the quality does not vary drastically for first three hours.

Key words: Organoleptic assessment, Delayed icing, TMA-N, TVB-N

Introduction

Shrimp culture prospective is mostly recognized as the main exportable fisheries product after garments now and development of this sector is increasing day by day. Besides it plays a dominant role in foreign exchange earnings, employment, education, nutrition, poverty alleviation and other socio-economic development of the country. Though in Bangladesh, shrimp production is increasing, shrimp farmers do not get expectable amount of return from their harvest due to lack of proper handling and management of shrimp after catching, which results both qualitative and quantitative losses. Qualitative losses occur through spoilage or microbial attack and due to lack of proper preservation during the transportation of shrimp from Gher to processing center

whereas quantitative loss consists of losses in commercial value, but not in physical biomass through loss of quality. The spoilage process in shrimp results in the changes of organoleptic, biochemical and microbiological parameters (Ali *et al.* 2008, Coulter and Disney 1988). Bangladesh is facing many serious problems in fresh shrimp trade because of its perishable nature.

Preservation is particularly necessary during the period of abundance when all the shrimps are not possible to be consumed and if there is no efficient system for handling and transportation. It is generally agreed that at 0^0C fish and shrimp become unacceptable organoleptically, biochemically and microbiologically within two weeks (Cobb *et al.* 1976). TMA can also be used as an index of spoilage (Clucas and ward 1996). After harvesting the shrimp from the gher, the farmers usually use bamboo basket or plastic basket insulated with hogla mat or banana leaves for packing shrimp with or without ice during transportation and distribution. Quality of shrimp mainly depends on storage temperature (0^0C, 10^0C, 20^0c, and 30^0C). The higher the storage temperature, the greater the spoilage of shrimp. There are a great variation in the usage of ice (ratio of ice to shrimp), usually the ratio varies i.e., 1:0.5, 1:1, 1:2. However it is necessary to know the effect of plastic and bamboo basket on the quality of shrimp. Again information on the changes of ice-stored shrimp under various conditions is therefore needed in order to avoid qualitative and quantitative losses and also to ascertain or predict its quality standard (Ali *et al.* 2008). The present investigation was undertaken to assess the quality of shrimp during stored at delayed icing where ice was added after three hours of harvest with considering the effect of the medium (plastic and bamboo baskets) in which they are stored.

Materials and methods

Sample collection and preparation: In total twenty eight (28) shrimps were collected from the gher beside the river Shoalmari at Koiya under the Batiaghata thana in Khulna district of Bangladesh. Shrimps were kept in plastic and bamboo baskets immediately after harvesting using banana leaves. During sample collection water temperature of the gher was 28^0C. After three (3) hours of harvesting, shrimps were stored in ice at 1:1 ratio for 21 hours. After collecting and storing, they were immediately brought to the Quality Control Laboratory of Fisheries and Marine Resource Technology Discipline of Khulna University. From each basket, 5 shrimps were assessed for organoleptic evaluation intermittently for each hour until the end of study period. 3 shrimps from each basket were taken out and muscles were pooled for biochemical and microbiological analyses at different intervals during the 21 hour storage.

Organoleptic analysis: Organoleptic score sheet (Table 1) was used for the organoleptic quality assessment of shrimp developed by Ali *et al.* (2008). From the developed organoleptic score sheet, an overall acceptability ranking was done (Table 2). The organoleptic characteristics emphasized on odour, carapace color, carapace texture, eye

and shell color characteristics. While conducting the organoleptic analysis, the room temperature ranged between 28 ^{0}C to 31^{0}C.

Table 1. Organoleptic score sheet for shrimp developed by Ali *et al.* (2008).

Odor	Score	Carapace Color	Score
Fresh odour	10	Greenish(fresh)	10
Slightly fresh odour	9	Moderately greenish	9
Sweetly odour	8	Slightly greenish	7
Slightly spoilage odour	7	Slightly darken	5
Moderately spoilage odour	6	Moderately darken	3
Spoilage odour	5	Darken	0
Slightly off odour	4		
Moderately off odour	3		
Off odour	2		
Extremely off odour	0		
Carapace Texture		**Eye**	
Hard	10	Bright and transparent	10
Slightly hard	9	Moderately transparent	9
Moderately hard	8	Slightly transparent	8
Slightly soft	7	Slightly dull	7
Moderately soft	5	Moderately dull	5
Soft	3	Dull and opaque	3
Very soft	0	Fully dull and opaque	0
Shell Color	**Score**		
Bluish white	10		
Moderately bluish	9		
Slightly bluish	8		
Slight loss of brightness	7		
Loss of brightness and opaque	5		
Slightly reddish	3		
Radish (spotted)	0		

Table 2. Organoleptic score sheet of overall acceptability for shrimp.

Overall acceptability characteristics	Score range
Highly acceptable (HA)	8.5 – 10.0
Acceptable (A)	6.5 – 8.4
Moderately acceptable (MA)	4.5 – 6.4
Just acceptable (JA)	3.6 – 4.4
Just unacceptable (JU)	2.6 – 3.5
Unacceptable (U)	1.5 – 2.5
More unacceptable (MU)	0 – 1.4

Biochemical and microbiological analysis: TVB-N (Total Volatile Base Nitrogen) and TMA-N (Tri Methyl Amine Nitrogen) were determined according to the procedure of Siang and Kim (1992). Microbial analysis was done according to the procedure of ICMSF (1988). It involves the determination of standard plate count (SPC).

Results

Organoleptic changes of Penaeus monodon at delayed icing

Fig. 1 illustrates the organoleptic changes in *Penaeus monodon* during a period of 24 hours and 21 hours storage in ice. Shrimp were assessed 0 hour (immediately after arriving at the laboratory), 3rd hour (i.e. at that moment when ice was added to the sample after three hours of harvest), 4th hour, 5th hour etc up to 24th hour. The organoleptic evaluation and scoring had been started at the time of harvest of shrimp treated as 0 hour and the average score was 10. The initial average score on the 3rd hour (i.e. second observation of the experiment) was also 9.4 both in plastic and bamboo basket. However, the score gradually decreased over the range of time.

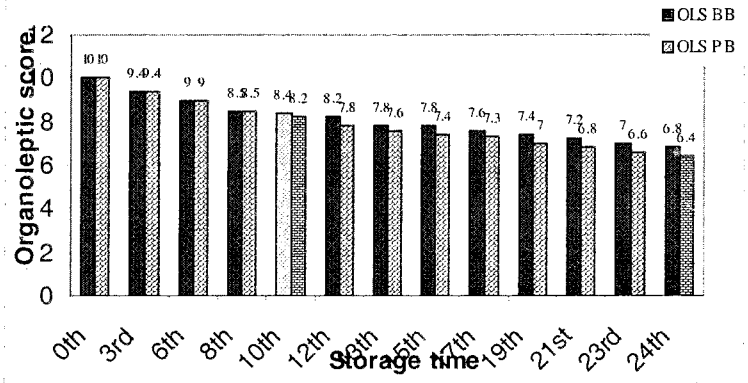

Fig. 1. Organoleptic score of shrimp stored at plastic and bamboo basket at delayed icing.

It was apparent that the quality of shrimp was under highly acceptable (HA) limit till 10th hour of observation and the quality was drastically changed soon after the 10th hour. It was 8.4 in the bamboo basket and 8.3 in the plastic basket. The difference of score was little but the quality attribute changed into another state.

Bio-chemical changes of Penaeus monodon at delayed icing

Total Volatile Base Nitrogen (TVB-N) in bamboo and plastic basket: The Fig. 2 shows the obtained TVB-N values for three different times and also shows the comparative values of the baskets in the same storage time period. In the present study the amount of

TVB – N was obtained over 24 hours storage including 21 hours in ice ranged between 2.56±0.32 mg-N/100g to 5.21±0.61 mg-N/100g whereas in the plastic basket it was 2.58±0.36 mgN/100g to 5.37±0.37 mgN/100g. In the 12th hour of the experiment the value was 3.64±0.66 mgN/100g in the bamboo basket and 3.73±0.58 mgN/100g in plastic basket.

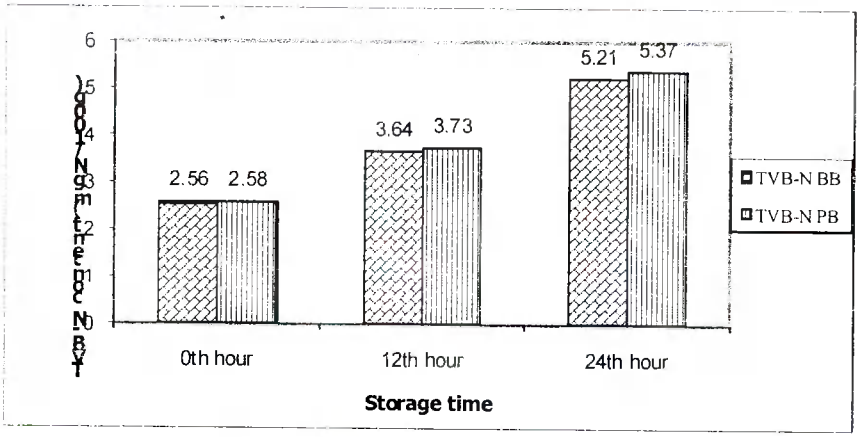

Fig. 2. Comparison of TVB-N in plastic and bamboo basket with storage time.

Trimethylamine Nitrogen (TMA - N) in bamboo and plastic basket: The Fig. 3 shows the obtained TVB-N values for three different times and also shows the comparative values of the baskets in the same storage time period. From the 0th hour to 24th hour of experiment the TMA-N content was 2.68±0.21 mgN/100g to 5.02±0.41 mgN/100g. This result was for the bamboo basket whereas in the plastic basket it was 2.72±0.23 mgN/100g to 5.16±0.47 mg-N/100g respectively.

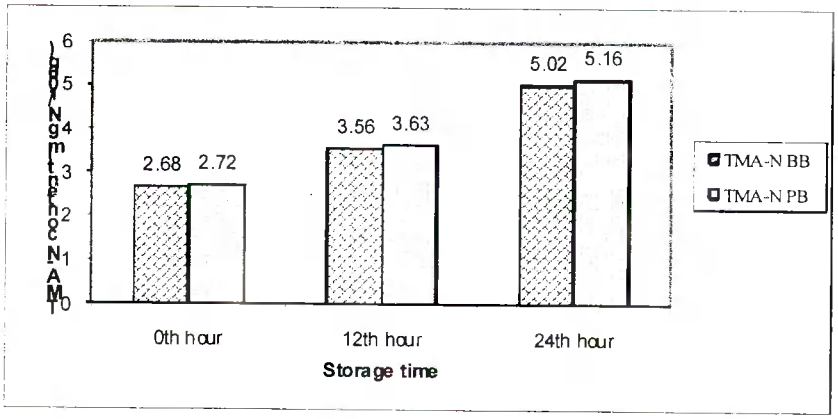

Fig. 3. Comparison of TMA-N in plastic and bamboo basket with storage time.

Microbiological (SPC) analysis of shrimp in plastic and bamboo baskets: Fig. 4 shows the microbiological quality changes in shrimp with storage time in both baskets. The average SPC counts obtained from these experiments varied between $\log_{10}3.93\pm0.12$ cfu/g to $\log_{10}4.33\pm0.21$ cfu/g respectively in the bamboo basket and in the plastic basket it was $\log_{10}4.01\pm0.12$ cfu/g to $\log_{10}4.83\pm0.19$ cfu/g respectively. SPC count was increasing in respect of storage time and relatively high in plastic basket.

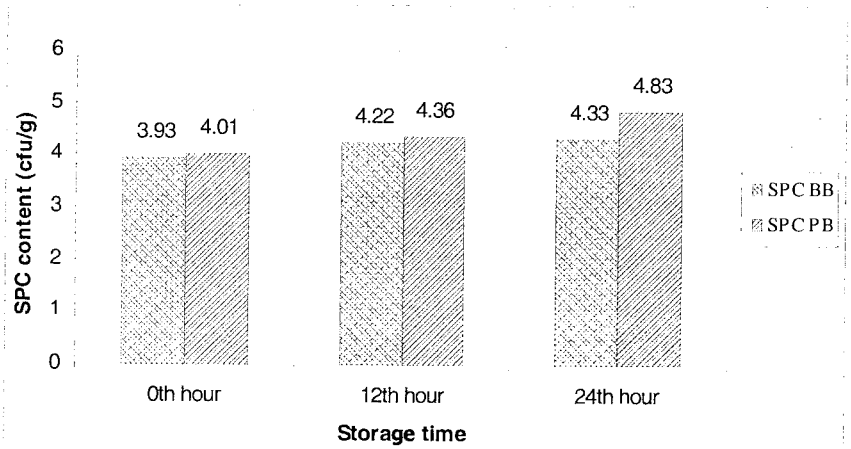

Fig. 4. Comparison of SPC in plastic and bamboo basket with storage time.

Discussion

Organoleptic changes in *Penaeus monodon*: The organoleptic evaluation and scoring had started after half hour of harvesting considered as 0^{th} (zero) hour and the average score on 0^{th} hour was 10 for both baskets. Then for each hour after 0^{th} hour was considered as 1^{st}, 2^{nd}, 3^{rd}, 4^{th} hour etc. From the Fig. 1, it was apparent that the quality of shrimp was under highly acceptable (HA) limit up to 10^{th} hour and values were 8.5 in both the baskets. Farooqui *et al.* (1978) reported that shrimp in ice maintained good quality for 0-2 days as judged by organoleptic quality was acceptable up to 7 days and rejected after 9 days. Reilly *et al.* (1985) has observed that shrimp lost their value as prime quality head-on produces after 2 days, during storage at 0^0C. They also found that shrimp iced immediately kept for 17 days, while 4h, 8h and 12h delayed iced shrimp kept for 16, 13 and 11 days respectively. Ali *et al.* (2008) conducted a similar experiment on quality changes in *P. monodon* at ambient temperature and reports that shrimp are rejected after 14^{th} hour in plastic basket but in bamboo basket after 15^{th} hour. However, the result of the present investigation assumed similarity with the above authors. After 10^{th} hour of storage time the result gradually changed in both the baskets and it was 8.4 and 8.2 in bamboo and plastic basket respectively. In the 13^{th} hour it was 8 and 7.6 respectively

whereas in the 15th hour storage time it was 7.6 and 7.4. Again in the 19th hour storage time it was 7.4 and 7. Here within four hour range the score in bamboo basket decreased a little in respect to plastic basket. Again in the 23rd hour storage time the score was 7 and 6.6 where the difference was same and it is in an acceptable range. But after within one hour there was a significant difference in the overall acceptability. In the last hour or 24th hour of experiment the score was 6.8 and 6.4 in which the score of the bamboo basket was in acceptable (A) region. On the other hand the score of plastic basket was in moderately acceptable (MA) region. Organoleptic characteristics of Shrimp stored in both plastic and bamboo basket indicated almost similar results for all shrimps. But the quality of shrimp stored at bamboo basket indicated a little bit better quality than that stored at plastic basket.

Total Volatile Base Nitrogen (TVB-N): Measurement of Total Volatile Base Nitrogen (TVB-N) is probably the first chemical method to be used as a potential and widely used index of freshness and it still the most popular indicator (Stansby *et al.* 1994). The level of total volatile nitrogenous bases increases after spoilage begins, both enzymically and bacterially and thus can be used as an index of spoilage. The low value of TVB-N initially is an indication of quality of fresh shrimp or fish while the high value may be due to autolysis and spoilage bacteria (Adebona 1982).

In the present study the amount of TVB – N was 2.56±0.32 mg-N/100g in the 0th hour of the experiment in the bamboo basket and 2.58±0.36 mgN/100g in the plastic basket. In the 12th hour of the experiment the value was 3.64 ± 0.66 mgN/100g in the bamboo basket and 3.73±0.58 mgN/100g. Again in the last observation (24th hour of the experiment) the result was 5.21±0.61 mgN/I00g in the bamboo basket and 5.37±0.37 mgN/100g in the plastic basket, which was also a little bit higher than that of the two. Here the obtained results between the baskets were almost similar but compared to storage time it was little bit high and also a little bit high in plastic basket. So, it can be suggested that preservation of shrimp in bamboo basket may give a better result.

Ali *et al.* (2008) found that the amount of TVB – N ranged between 2.68±0.19296 mg/100g to 12.46±0.3396 mg/100g for a storage period of 15 hours at ambient temperature and TVB-N values show a strong positive correlation with storage time. Putro *et al.* (1990) suggested that TVB-N of *gher* shrimp varied between 7 and 28mgN/100g. Connell (1975) stated that TVB-N content in shrimp has highly positive and highly negative correlation with storage time indicating that TVB-N is a good indicator of spoilage. The value of TVB-N in both condition indicated an acceptable limit as suggested by Connell (1975). Reilly *et al.* (1985) stated that TVB-N is not reliable as indices of quality. Boee *et al.* (1982) working on the storage of shrimp has observed on the storage of shrimp has observed that TVB-N increased evenly. Matches (1982) working on shrimp stored at 5 different temperature, found that TVB-N increased both with increase in time and temperature. Cann (1974) have found the increase in TVB-N to be low during the initial period of storage, with a rapid increase noted afterwards. This result supports clearly Cann (1974) as TVB-N content gradually increased over the range of storage time and shows a strong positive correlation.

Trimethylamine Nitrogen (TMA - N): Under the local conditions TMA was found to be a good indicator of freshness for white pomfret, Chinese pomfret and grouper (Siang and Kim 1992). TMA-N was also detected three times in the experiment at 0^{th} hour, 12^{th} hour and 24^{th} hour. The average results obtained in the present experiment were 2.68±0.21 mgN/100g, 3.56±0.53 mgN/100g and 5.02±0.41 mgN/100g respectively in the bamboo basket and in the plastic basket it was 2.72±0.23 mgN/100g, 3.63±0.53 mgN/100g and 5.16±0.47 mg-N/100g respectively. TMA-N values are slightly higher in plastic basket than in bamboo basket. The results clearly indicate that storage of shrimp in bamboo basket is better than plastic basket. Connell (1975) recommended 10 – 15 mg/100g of TMA-N in fishes or shrimp for human consumption. There is also wide variation in critical values suggested for individual species, like 5 – 7 mg/100g for herring and 1 –5 mg/100g for haddock (Castell and Triggs 1955).

The accepted limit of TMA-N is 12-15 mgN/100g (Monotgomery *et al.* 1970). Ali *et al.* (2008) observed the TMA-N level was 4.35±0.2089 mg/100g to11.78±0.141774 for a storage period of 15 hours at ambient temperature. The results found in the present investigation indicated much lower value during the storage period. Reilly *et al.* (1985) has observed that the level of 5mg/100g was never reached during ice storage of shrimps. However the result of the present investigation agreed with Reilly *et al.* (1985). Though the results were increasing gradually with respect to storage time, it was within in the front of acceptable range from the view of the writers above.

Standard Plate Count (SPC): The value of SPC ranged between $\log_{10}$ 3.82±0.29436 to $\log_{10}$ 5.11±0.16453 cfu/g in plastic basket for a storage period of 14 hours and between $\log_{10}$ 3.78±0.3629 to $\log_{10}$ 4.98±0.6226cfu/g in bamboo basket for a storage period of 15 hours, at ambient temperature (Ali *et al.* 2008). SPC was also detected three times in the experiment like as TVB-N and TMA-N at 0^{th} hour, 9^{th} hour and 24^{th} hour of the experiment. The average results obtained in these experiments were $\log_{10}$3.93±0.12 cfu/g, $\log_{10}$4.22±0.53 cfu/g and $\log_{10}$4.33±0.21 cfu/g respectively in the bamboo basket and in the plastic basket it was $\log_{10}$4.01±0.12 cfu/g, $\log_{10}$4.36±0.04 cfu/g and $\log_{10}$4.83 ±0.19 cfu/g respectively. Preservation of shrimp in bamboo basket showed lower microbial (SPC) count which will ensure better storage result in bamboo basket.

The accepted limit of SPC is 10^6 cfu/g (ICMSF 1988). SPC of freshly harvested shrimp ranged from 6.8×10^4 to 1.5×10^5 as observed by Lobrerra *et al.* (1990). These counts are within the range of reputed values (10^3-10^5) of shrimp from temperature environments (Lannelongue 1982, Matches 1982). Counts reported from tropical countries also ranged from 10^3 to 10^6 (Varma *et al.* 1982, Surendran *et al.* 1985). A report by De Silva (1985), however, indicated counts as high as 10^8/gm. Result of this present study showed that freshly harvested shrimps could meet existing standards for SPC, which is 10^6/g (ICMSF 1988).

Shrimp collected from all the three districts (Bagerhat, Khulna and Satkhira) and from all the points (Gher, Depot, Agent and Processing Plant) indicated value within 10^5cfu/g, is acceptable limit even when being practiced normally (Azam 2004). However, the result of our present investigation clearly supports the mentioned value. Besides

SPC contents gradually increased for both baskets over the range of storage time up to the completion of the spoilage of the sample.

Shrimp need to be produced to a level of quality, which will satisfy both the customer and statutory food legislation. The experiment here for the rural deprived shrimp farmer mainly because after harvesting, they cannot afford to preserve shrimp in immediate ice storage. Storage in ice into near market, it takes at least two or three hours. The experiment delayed icing after three hours has conducted to detect various quality measuring methods. Here the bamboo and plastic basket provided almost similar result. From the result of this experiment, it can be recommended that it is not very necessary for the rural farmers to apply ice on shrimp immediately after the harvest.

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Effect of water washing on biochemical and gel characteristics of minced meat from Croaker fish (*Johnius gangeticus*)

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Abstract
Quality of analog fishery products invariably depends on the gel characteristics and nutritional status of minced meat. With an objective to find out the effect of water washing on kamaboko gel, the minced meat from Croaker fish was washed for four times (5 minutes each) using chilled water at a temperature of 8-10^{0}c. Results reflected noticeable improvement in folding test and SSN % of kamaboko with essential decrease in fat content, water soluble proteins, expressible water and quality parameters like NPN,VBN, TMA, FFA and PV denoting superior gel quality than control sample after repeated washing. The results indicated that there was a definite improvement in functional properties such as gel forming ability, expressible water content of the croaker minced meat essential decrease in fat content, water soluble proteins, expressible water and quality parameters after each wash, but two washes of 5 minutes duration each was necessary to achieve satisfactory results.

Key words: Biochemical characteristics, Kamaboko, Water washing

Introduction

The rapid development of minced meat technology over the last ten years could make a major contribution to the increased utilization of underutilized fishes. Minced meat is the flesh separated in a comminuted form from the skin, bones, scales and fins of the source material. It represents a significant advance in an effort aimed at improving utilization of fish proteins in human food (Murray *et al.* 1980). In technical terms, any fish can be utilized to make minced meat, but it should have good gel forming ability which makes for an elastic texture, good taste and whiter appearance making it an important attribute for a particular kneaded product. One of the most critical steps in the preparation of minced meat is washing the meat of fish with water to remove blood, fat, soluble proteins and other nitrogenous compounds (Okada and Noguchi 1974). Such washing significantly improves the colour and odour as well as the texture of the final product (Miyauchi *et al.* 1973). An attempt has been made in the present study to find out the effect of water washing on biochemical and gel forming ability of minced meat from Croaker fish (*Johnius gangeticus*).

Materials and methods

Fresh Croaker (*Johnius gangeticus*) caught off Digha coast of West Bengal were used in the present experiment. During transportation and processing, the temperature of the raw material was maintained as low as possible by using sufficient crushed ice. The processing was done under hygienic condition. The fishes after dressing were deboned with a meat picking machine (Stadler make, Mumbai) and minced in a mincer (Stadler make, Mumbai). Minced meat was repeatedly washed four times with chilled water (8-10^{0}c) giving suspension time of 5 minutes for each wash following which water was removed by a screw press reducing the water content of washed meat to almost equal to the original moisture content of meat. Table salt at 0.3% was used for the last wash to render removal of water easier. Samples were taken after each wash (screw pressed) and analysed for bio-chemical, functional, microbiological and organoleptic parameters. Fish kamaboko gel was prepared from washed fish meat, stuffed into synthetic casing (Krehalon) and heat processed in water at 90±2^{0}c for 40 minutes (Suzuki 1981) and analysed for their quality. Proximate composition analysis were carried out according to the methods of AOAC (1995). Total lipid was estimated by solvent extraction method in soxhlet apparatus as described by Nambudiri (1985). Tri methyl amine (TMA) and Total volatile base nitrogen (TVB-N) was estimated by the method recommended by Beatty and Gibbons (1937). For estimation of salt soluble nitrogen (SSN) and water soluble nitrogen (WSP), by the method of Dyer *et al.* (1950) was used. Non-protein nitrogen (NPN) and free fatty acid (FFA) were estimated following the method of Nambudiri (1985). The peroxide value (PV) was determined iodometrically (Jacobs, 1958). Total plate count (TPC) was estimated by the method APHA (1984) . pH estimated by the method FAO (1981). α-amino nitrogen of the sample was estimated following the copper method of Pope and Stevens (1939) using TCA extract of sample. Different functional properties like Jelly strength, Expressible water and folding test measurement were estimated by the method as described by Okada, 1959 and Suzuki, 1981 respectively. Howard *et al.* (2006) have reported a 9-point hedonic scale for sensory assessment of any food, which was followed in the present study. Significant difference of various quality parameters between different washing treatments was studied by one way analysis of variance using Microsoft Excel programme. Pearsons correlation coefficient was calculated to assess significant relationship between different parameters analysed after each washing treatment.

Results and discussion

The dressing yield of croaker fish was 66%, which is in concurrence with results of Revankar et al (1981) who obtained dressing yield in the range of 50 - 58%. The fairly high yield obtained in the present study could be due to the comparatively bigger size of fish used. Subsequently the yield percentage decreased with the progress in washing process which could be because of the removal of blood, colour pigments, soluble proteins and other small bone particles etc (Table 1). The results of proximate

composition and other bio-chemical parameters of croaker fish were analysed and is presented in Table 2. Revankar *et al.* (1981) have reported the proximate composition of meat separated from croaker and the results obtained in the present study are in concurrence with the above reports within reasonable limits. The fishes were iced soon after landing, brought to the laboratory and analysed immediately and therefore the quality of the fish was fresh. The results of four chilled water washings of 5 minutes duration each on proximate composition is shown in Table 3 and that of the biochemical parameters in Table 4. The protein content decreased from 16.19 to 12.19% after the second wash accounting for a loss of 24%. The fat content decreased from 1.66 to 0.71% after the second wash, which accounts for a reduction of 57%. Adu *et al* (1983) working with rock fish reported 65% reduction of fat by water washing. The effect of water washing on the removal of fat depends on the species, the initial fat content, distribution of fat in the body and the nature of fat. With the increase in SSN, considerable amount of WSP, NPN, TMA, VBN, PV, FFA and α-amino nitrogen were removed due to water washing. Effect of water washing on the experimental values of NPN, TMA, VBN, FFA and were significant at 5% level. It is significant to note that, WSP to the extent of 16.06% was removed from the minced meat and its removal greatly benefited to improve the jelly strength of the meat, which is very much required for the preparation of products like kamaboko, sausage etc. (Suzuki 1981, Shamasunder *et al.* 1988). Joseph and Perigreen (1986) observed a loss of 40% TMA and 60% VBN from the meat of threadfin bream due to water washing. Removal of NPN in considerable amounts during water washing of picked meat has been reported by several workers (Agarwal *et al.* 1986, Joseph and Perigreen 1986, Shamasunder *et al.* 1988).

Table 1. Yield of meat at different stages of processing

Stages	Yield (%)
Dressing Yield	60.2
After meat separation	31.4
After mincing	29.2
After 1st washing	25.4
After 2nd washing	24.3
After 3rd washing	22.6
After 4th washing	22.1

Table 2. Proximate composition and other biochemical characteristics of raw material

Moisture (% of meat)	80.94	Non-protein nitrogen (mg/100g meat)	84.83
Protein (% of total nitrogen)	16.19	Volatile Base nitrogen (mg/100g meat)	2.2
Fat (% of meat)	1.66	Trimethyl amine (mg/100g meat)	0.27
Ash (% of meat)	0.9	Peroxide value (millimoles of O_2/kg fat	14.4
Salt soluble nitrogen (% of total N)	69.4	Free fatty acid (percent of oleic acid)	2.33
Water soluble proteins (g/100g meat)	3.05	α-amino nitrogen (mg/100g meat)	21.44

Table 3. Effect of number of water washings on proximate composition* on croaker meat

Proximate composition	Before washing	After 1st wash	After 2nd wash	After 3rd wash	After 4th wash
Moisture	80.94	86.19	86.73	87.26	88.15
Protein	16.19	12.41	12.19	11.68	11.09
Fat	1.66	0.74	0.71	0.60	0.37
Ash	1.0	0.34	0.19	0.21	0.19

* Percent of meat on wet weight basis

Kamabokos were prepared from croaker meat after each wash and their jelly strength, expressible water, folding test and pH were measured immediately after preparation, the results of which are presented in Table 5. The jelly strength of kamaboko after first wash which was 227 gm.cm increased to a value of 294 gm.cm after the second wash but decreased after 3rd and 4th washing respectively ($P \geq 0.05$). Similarly, the folding test grade improved from 'B' to 'A' and expressible water decreased from 38.7 to 38.5% between kamaboko prepared from meat of first and second wash respectively. The pH reduced from 6.5 to 6.45 after the second wash. The results indicate a highly significant improvement in jelly strength of kamaboko after 2nd wash ,but there was a decrease in subsequent washing. This could be explained by the fact that there was no substantial decrease in water soluble proteins after 3rd and 4th wash which effected the decrease in jelly strength in kamaboko product. Similarly the expressible water content reduced gradually in kamaboko prepared after each wash, which is also a significant quality factor in kamaboko, since it is connected with the cohesiveness of the meat. From the results of Pearson's correlation coefficient it was evident there was a negative relationship between SSN (% of TN) with total protein content (-0.62432) and expressible water (-0.66783), whereas direct positive relationship between SSN and jelly strength (0.348423). The corelationship coefficient were significant at 5% level. These increase in SSN % and jelly strength were presumably due to the considerable reduction in fat and water soluble proteins achieved during water washing. Suzuki 1981) opined that the removal of WSP fraction helps in increasing gel forming ability of the meat. Shamasunder *et al.* (1988) working with minced meat of pink perch and ribbon fish also found that the washing of the meat in chilled water helps in the concentration of SSN and increase of jelly strength of kamaboko. The organoleptic analysis of kamaboko product prepared after each wash also showed a gradual improvement in the mean panel scores for different sensory qualities, the values of which are presented in Table 6. The gradual improvement of different organoleptic attributes of kamaboko prepared after each wash could be directly related with the gradual change of the functional properties of Croaker minced meat during washing procedure. The results indicated that there was a definite improvement in functional properties such as gel forming ability, expressible water content of the croaker minced meat essential decrease in fat content, water soluble proteins, expressible water and quality parameters after each wash, but two washes of 5 minutes duration each was necessary to achieve satisfactory results.

Table 4. Effect of chilled water washing on different microbiological and biochemical parameters of Croaker minced meat

	TPC (Counts/gm)	SSN (% of TN)	WSP (g/100g meat)	NPN (mg %)	VBN (mg %)	TMA (mg %)	PV (millimoles of O_2/kg fat)	FFA (% oleic acid)	α-amino nitrogen (mg%)
Control (No washing)	2.5×10^6	69.4	3.05	84.83	4.4	0.51	1.44	0.51	21.44
After 1st washing	-	71.25	2.95	80.21	4.1	0.48	-	0.48	-
After 2nd washing	4.1×10^5	73.4	2.75	76.55	3.9	0.43	0.86	0.43	19.62
After 3rd washing	5.2×10^4	73.2	2.73	73.12	3.6	0.41	1.26	0.41	17.41
After 4th washing	5.8×10^4	72.6	2.66	68.55	3.3	0.39	0.95	0.39	15.36

- : No data

Table 5. Effect of chilled water washing on kamaboko gel characteristics of Croaker minced meat

Number of washings	Jelly strength (gm. Cm.)	Expressible water (percent of meat)	Folding test	pH
After 1st washing	227	38.7	B	6.5
After 2nd washing	294	38.5	A	6.45
After 3rd washing	243	37.3	A	6.42
After 4th washing	173	37.1	B	6.38

Table 6. Effect of chilled water washing on sensory quality of Croaker minced meat

Number of washings	Appea rance	Colour	Taste	Texture	Odour	Overall acceptability
Control (No washing)	6.0	7.0	6.8	6.3	6.6	6.3
After 1st washing	6.9	7.6	7.0	7.3	7.3	7.6
After 2nd washing	7.9	8.0	7.8	8.0	7.6	7.9
After 3rd washing	7.9	8.1	7.8	7.8	7.3	7.8
After 4th washing	7.8	8.2	7.9	7.5	7.3	7.6

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