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Effect of feeding bioencapsulated *Lactobacillus* sp. in live *Tubifex* sp. on the growth performance of gold fish *Carassius auratus* Linnaeus, 1758)

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Abstract
An attempt was made to feed bioencapsulate *Lactobacillus* sp in live fish food organism *Tubifex* for use in the culture of gold fish *Carassius auratus*. The *C. auratus* fries when fed with bioencapsulated *Lactobacillus* sp. in *Tubifex* showed significant improvement in total wet weight gain ($p<0.007$) and FCR ($p<0.01$) compared to control. The specific growth rate and mean survival were slightly higher, although insignificantly ($p>0.05$) in bioencapsulated *Tubifex* fed group. None of the bacteriological parameters of the fish gut between the experimental and control groups differed significantly ($p>0.05$). *Lactobacillus* sp. was recorded at a level of log 5.11/g on the 90th day of experimentation. When the experimental *C. auratus* fries were infected with *Pseudomonas fluorescens*, the bioencapsulated *Tubifex* fed group resisted the infection. The survival was significantly highetr ($p<0.05$) in bioencapsulated *Tubifex* fed group (44%) than in control (22%). The *C. auratus* fed with bioencapsulated *Tubifex* showed less (55%) signs of tail/fin rot. Likewise, a significant improvement in total wet weight gain ($p<0.009$), FCR ($p<0.01$) and SGR ($p<0.04$) of *C. auratus* brooder fed with bioencapsulated *Tubifex* was seen compared to control group fed with depurated *Tubifex*.

Keywords: *Carassius auratus*, *Lactobacillus* sp, *Tubifex* sp, Bioencapsulation

Introduction

The use of probiotics in aquaculture is well studied with varying reports of their effect on growth, survival and disease resistance of different commercially important aquatic organisms (Douillet and Langdon 1993, Ringo *et al.* 1995, Gatesoupe 1999, Irianto and Austin 2002). In aquaculture sector, the ornamental fish breeding and trade provides excellent opportunities as a non-food fishery activity for employment and income generation. It is totally environmental friendly, socially acceptable, involves low investment with short gestation period for every cycle of breeding and growth, could be adapted as a small-scale backyard enterprise and ensures high profit. Application of

probiotics in ornamental fish rearing is also gaining importance. The beneficial effects of fish gut antagonistic bacterium as probiotic in ornamental fish culture have demonstrated in earlier reports (Mondal *et al.* 2003, Abraham and Banerjee 2007, Abraham *et al.* 2007a,b, Abraham 2008). The present study reports the bioencapsulation of probiotic in live fish food organism and its influence on the growth, survival and disease resistance of ornamental fish *Carassius auratus*.

Materials and methods

The live *Tubifex* sp. was procured from retail ornamental fish traders of Mohanpur, Nadia District as and when required. Before use, it was depurated for 2 days in running water with a flow rate of 6 L/h. An antagonistic bacterial strain, *Lactobacillus* sp. P21 isolated from *Cirrhinus mrigala* gut (Abraham and Banerjee 2007) was used as a probiont. For the purpose of standardization of optimum dose and time for the bioencapsulation of *Lactobacillus* sp. in *Tubifex*, a series of glass test tubes containing 10 ml each of de Man Rogosa Sharpe (MRS) broth were seeded with 24 h old probiont and incubated for 24 h. One gram each of depurated *Tubifex* was transferred in to the tubes containing 24h grown probiont for bioencapsulation. At regular intervals, the contents of the tubes were filtered through 60μ bolting silk cloth, sterilized by placing in boiling water for an hour, to screen out the broth from the *Tubifex*. The bioencapsulated *Tubifex* from each tube was then transferred aseptically into tubes containing 9 ml sterile saline separately. To determine the initial bacterial count of *Tubifex*, one gram of depurated *Tubifex* was transferred to a tube containing 9 ml sterile saline. Using a sterile glass rod, the *Tubifex* were macerated, vortexed and diluted by 10 fold serial dilution in sterile saline to appropriate levels. Aliquots (0.1 ml each) from appropriate dilutions were then spread plated on to MRS agar and incubated for 48 h. After incubation, the catalase negative colonies were counted along with total counts. For experimental purpose, bioencapsulation of *Lactobacillus* sp. in *Tubifex* was done for 45 min as above.

Carassius auratus fries of size 0.48–0.55 g weight and 29.10-33.40 mm length and brooders of size 7.71-7.89 g weight and 91.00–96.00 mm length were used. Forty *C. auratus* fries were introduced into each of the two 500L capacity fiberglass reinforced plastic (FRP) tanks containing 400L water. Nine brooders (5♀ and 4♂) were introduced into each of 500L capacity FRP tanks containing 400L water. The experiment was carried out for a period of 90 days. Both groups were fed with commercial pelleted feed at 5% of the body weight daily in two split doses. Bioencapsulated *Tubifex* were fed to the experimental fish at 5% of body weight on every 3rd day as against the 2nd dose of pellet feed. Simultaneously, the control fish were fed with depurated *Tubifex* at 5% of the body weight. The wastes and faecal matter were siphoned out and 50% of the water was exchanged on every 3rd day. The fishes were observed for mortality daily and the dead ones removed immediately and weighed. The length and weight of the fishes of all categories were noted at regular intervals. From these data, the survival percentage and

growth parameters such as wet weight gain, feed conversion ratio (FCR) and specific growth rate (SGR) were estimated.

Bacteriology was performed only for *C. auratus* fries. Two fish each were scooped out from experimental and control tanks and killed by placing them in separate glass beakers containing ice cubes. Gut was dissected out aseptically, macerated, serially diluted and enumerated the total plate count (TPC), total MRS count, *Lactobacillus* sp. count, motile aeromonads, presumptive pseudomonads, total coliforms, lactose fermenters and lactose non-fermenters as per APHA (1992).

A pathogenic strain *Pseudomonas fluorescens* 58C, from the collection of Kolkata University was used to test the disease resistance of the experimental *C. auratus* fries by immersion assay (Austin *et al.* 1995). From each of the bioencapsulated *Tubifex* fed and control *C. auratus* fries tanks, 10 each of fishes were scooped out and introduced into two glass aquaria namely B1 and B2. In a similar manner, 10 each of control *C. auratus* fries were introduced into two aquaria namely C1 and C2. To facilitate infection, two or three scales were removed from five fishes from each aquarium and reintroduced into the respective aquaria. All the aquaria contained 20L sand filtered water and the cell suspension of *P. fluorescens* 58C was added into B1 and C1 tanks in such a way to get a level of 2.0×10^6 cells/ml of rearing water. The aquaria B2 and C2 received no bacterial inoculum and served as control, respectively for bioencapsulated *Tubifex* fed and control groups. The test animals were maintained in their respective aquaria for 30 days and fed daily with pellet feed on demand. The accumulated faecal matter and other wastes were siphoned-out on every 5^{th} day. Mortality, external signs of infections and behavioural abnormalities were recorded daily. Statistical analyses (χ^2-test and student-t test) were as per Snedecor and Cochran (1974).

Results and discussion

The pre-weighed depurated *Tubifex* were kept in 10ml MRS broth culture of *Lactobacillus* sp. P21 for pre-determined time and the count of *Lactobacillus* sp. on MRS agar as confirmed by catalase test (i.e., catalase negative) were assessed at regular intervals. The results of four different trials made to standardize the bioencapsulation of *Lactobacillus* sp. in *Tubifex* are presented in Table 1. The counts were consistently the same (log 9.08-9.16/g) for up to 60 min, and thereafter, showed a decline to about log 8.88/g in 90 min. The depurated *Tubifex* had TPC in the range of log 7.08-8.18/g.

Table 1. Bioencapsulation of *Lactobacillus* sp. P21 in live fish food organism *Tubifex* sp

Time in min	Total MRS count/g	*Lactobacillus* sp. P21 count/g
0	7.67 ± 0.52	<5.00 ± 0.00
15	9.13 ± 0.06	9.12 ± 0.06
30	9.11 ± 0.04	9.08 ± 0.05

45	9.17 ± 0.06	9.16 ± 0.06
60	9.16 ± 0.06	9.15 ± 0.07
90	8.90 ± 0.22	8.88 ± 0.24

The *C. auratus* fries when fed with bioencapsulated *Tubifex* showed significant improvement in total wet weight gain ($p<0.007$) and FCR ($p<0.01$) compared to control. On the other hand, the SGR and mean survival were slightly higher, although insignificantly ($p>0.05$) in bioencapsulated *Tubifex* fed group than that of the control (Table 2). A significant improvement in total wet weight gain ($p\leq0.009$), FCR ($p<0.01$) and SGR ($p<0.04$) of *C. auratus* brooder fed with bioencapsulated *Tubifex* was seen compared to control fed with depurated *Tubifex*. The mean survival, however, showed no variation (Table 2). The results demonstrated the beneficial effect of feeding *Lactobacillus* sp to *C. auratus* brooder. In general, higher the weight gains, the higher the fecundity.

Table 2. Growth performance of *Carassius auratus* fries and brooder fed with bioencapsulated *Tubifex*

Growth parameters	*Carassius auratus* fries		*Carassius auratus* brooder	
Fries	Bioencapsulated *Tubifex* fed	Control	Bioencapsulated *Tubifex* fed	Control
Total wet weight gain (g)	41.23 ± 0.46^{a}	33.93 ± 0.38^{a}	84.27 ± 2.23^{c}	33.93 ± 0.38^{c}
Mean survival (%)	93.75 ± 3.75	86.25 ± 1.25	100.00 ± 0.00	86.25 ± 1.25
Food conversion ratio	4.38 ± 0.04^{b}	4.91 ± 0.04^{b}	5.28 ± 0.04^{d}	5.96 ± 0.06^{d}
Specific growth rate	1.30 ± 0.06	1.18 ± 0.02	0.91 ± 0.01^{e}	0.66 ± 0.05^{e}

Values sharing common superscripts within rows are significantly different. a: $p<0.0067$, $t= 12.19$, $df=4$; b: $p<0.011$, $t= -9.28$, $df=4$; c: $p<0.009$, $t= 10.20$, $df=4$; d: $p<0.01$, $t= -9.27$, $df=4$; e: $p<0.038$, $t=4.99$, $df=4$;

The results of all the above experiments were fairly uniform, probably as a result of supply of unknown growth factors or growth stimulators needed for the growth of *C. auratus*. The results of Gildberg and Mikkelsen (1998) also revealed that the specific growth rates of fish given different diets containing lactic acid bacteria at 10^{8}/g feed were fairly uniform. It can be inferred from the results of the present study that the bioencapsulation of *Lactobacillus* sp. play an important role in improving the dietary value of *Tubifex*, which favourably influenced the growth and survival of *C. auratus*. Likewise, feeding turbot (*Scophthamous maximus)* larvae with bioencapsulated lactic acid bacteria and *Bacillus toyoi* significantly improved the weight of turbot larvae (Gatesoupe 1999). The observed growth improvement in the present study could probably be attributed to the supply of essential nutrients and enzymes important in digestion process (Douillet and Langdon 1993) or due to alteration in host mechanism (Deeth

1984). de la Banda *et al.* (1995) reported increased enzyme activity in turbot larvae when fed with disabled lactic acid bacteria. According to them, supply of lactic acid bacteria seems to be an effective supplement to counter enzymatic deficiencies. Increased growth may also be attributed to production of vitamins by lactic acid bacteria (Goldin and Gorbach 1992).

As seen in Table 3, none of the bacteriological parameters of the fish gut between the experimental and control groups differed significantly ($p>0.05$). Feeding *C. auratus* fries with bioencapsulated *Tubifex* did not affect the dominant Gram-negative non-beneficial bacteria such as motile aeromonads, total coliforms, lactose fermenters and lactose non-fermenters. Although the presumptive pseudomonads were reduced in the gut of bioencapsulated *Tubifex* fed fish, the difference between this group and the control was observed only at $p<0.06$ level. The fact is that the probiotic strain *Lactobacillus* sp. was recorded at a level of log 5.11/g gut on the 90th day of experimentation, may be because of their inability to withstand peristaltic movement in the gut or lack of attachment site to effect colonization. This probably indicated that the population of *Lactobacillus* sp. was not sufficient enough in the gastrointestinal tract of *C. auratus* fries that could significantly affect the growth or exclude the non-beneficial bacteria. The results contradict with that of the *in-vitro* study of Abraham and Banerjee (2007), which demonstrated that *Lactobacillus* sp. was capable of inhibiting motile aeromonads, coliforms and pseudomonads. This, however, could not be transferred to the *in-vivo* situation when administered via live fish food organism *Tubifex*. Such inability could probably be attributed to the poor colonization of the *Lactobacillus* sp. in the gut of bioencapsulated *Tubifex* fed group. Bogut *et al.* (2000) observed reduction in the incidence of *Escherichia coli* in the gut of sheat fish, *Silurus glanis*, when fed with *Enterococcus faecium* for 58 days. It has been stated that by colonizing the intestinal mucous layer lactic acid bacteria may serve as first defense barrier against invading pathogenic bacteria (Ringo and Gatesoupe 1998). Once colonized, the probiont alter the microbial matabolism by the increase or decrease of relevant enzyme levels, competitively exclude the potential pathogen by the production of inhibitory compounds or competition for space or oxygen (Irianto and Austin 2002).

When the experimental fishes were challenged with *P. fluorescens* 58C, the bioencapsulated *Tubifex* fed group resisted bacterial infection. The survival was significantly high ($p<0.05$) in bioencapsulated *Tubifex* fed group (44%) than in control (22%). Further, the bioencapsulated *Tubifex* fed *C. auratus* showed less (56%) signs of tail/fin rot compared to control (78%, Table 4). Likewise, earlier studies on *Salmo salar* and *Onchorynchus mykiss* (Robertson *et al.* 2000), *C. auratus* (Mondal *et al.* 2003) also presented less evidence of minor health problem such as fin/tail rot in probiotic fed group. The results of the present study demonstrated that *Lactobacillus* sp. P21 was capable of improving the disease resistance and reducing the bacteria induced mortalities in *C. auratus*, besides improving the dietary value of *Tubifex*. Presumably, the

Table 3. Log counts of bacteria in the gut of *Carassius auratus* fries fed with bioencapsulated *Tubifex*

Bacteria	Bioencapsulated *Tubifex* fed				Control			
	Days of culture				Days of culture			
	0	30	60	90	0	30	60	90
Total plate count /g	9.93	9.25	9.45	9.30	9.93	9.38	9.36	8.66
MRS count / g	9.29	8.92	9.02	9.23	9.26	8.79	8.98	8.61
Lactobacillus P21 count / g	<5.16	<5.23	<5.26	5.11	<5.16	<5.32	<5.26	<5.39
Motile aeromonads / g	9.004	8.76	9.01	9.11	9.004	8.83	8.89	8.29
Presumptive pseudomonads / g[a]	3.16	3.09	3.19	3.13	3.16	3.24	3.46	3.34
MPN total coliforms / g	5.12	5.54	6.31	6.58	5.12	5.76	7.86	7.52
Lactose non-fermenters / g	8.94	8.79	8.65	8.91	8.94	8.56	8.41	7.55
Lactose fermenters / g	7.56	5.62	5.81	<5.39	7.56	5.69	5.97	<5.39

a: $P \leq 0.06$; t= -2.31; df= 6

Lactobacillus sp. P21 might have activated the cellular and humoral responses of the ornamental fish as has been suggested in earlier (Irianto and Austin 2002).

Table 4. Disease resistance in *Carassius auratus* fries fed with bioencapsulated *Tubifex*

Treatment	Survival (%)		Infectivity* (%)	
	Infected stock	Uninfected stock	Infected stock	Uninfected stock
Bioencapsulated *Tubifex* fed	44.44[a]	88.89	56.56[b]	11.11
Control	22.22[a]	77.78	77.78[b]	33.33

*: Percentage of fish exhibited tail / fin rot in 30 days of experimental infection. Infected with *Pseudomonas fluorescens* 58C at a level of 1.85×10^6 cells / ml. The pathogenic bacterial cells were inoculated into the rearing water on the 1st, 5th, and 20th days of experiment.

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Dietary protein and energy interactions- an approach to optimizing dietary protein to energy ratio in walking catfish, *Clarias batrachus*

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Abstract
An 8 weeks feeding trial was conducted in a static indoor rearing system to investigate protein to energy ratio (P/E ratio) in walking catfish *Clarias batrachus*. Six fishmeal based diets of two protein levels (25 and 35%), each with three lipid levels (5, 10 and 15%) resulted in P/E ratios ranging from 13.57 to 21.97 mg protein kJ^{-1} gross energy (GE) were fed to 50 fish in triplicate. Fish were fed 6% of their body weight three times per day adjusted fortnightly. Significantly higher ($p<0.05$) growth rates in terms of weight gain, % weight gain and specific growth rate (SGR) were evident in fish fed with higher protein diet. The highest growth rate was found by fish fed 35% protein, 17.06 kJ^{-} GE with a P/E ratio of 20.55 mg protein kJ^{-1} GE. Significantly better ($p<0.05$) feed conversion ratio (FCR) was also evident in fish fed with higher protein diet and best FCR was found by fish fed 35% protein, 10% lipid, 17.06 kJ^{-1} GE with a P/E ratio of 20.55 mg protein kJ^{-1} GE. Significantly indifferent ($p>0.05$) values of protein utilisation were found in between the both (higher and lower) protein diets. Higher lipid deposition ($p< 0.05$) in whole body was observed with increasing dietary lipid level at each protein diet and as higher ($p<0.05$) for the lower protein diets. The study reveals that *C. batrachus* performed best the diet containing 35%, 17.06 kJ g^{-1} and 20.55 mg protein kJ g^{-1} GE protein, gross energy and P/E ratio respectively.

Key wards: *Clarias batrachus*, Protein to energy ratio, Dietary protein and lipid levels

Introduction

Dietary protein to energy ratio (P/E ratio) in fish diets is of great importance and evidence of the use of dietary protein as an important energy source is widespread (Cowey 1980, Wilson 1989). If the dietary P/E ratio is unbalanced so that non-protein energy is inadequate, dietary protein may be catabolised and used as an energy source to satisfy maintenance before growth (Cowey and Sargent 1979, NRC 1993). Conversely, if excess dietary energy is provided, feed consumption and protein intake may be limited. The foregoing discussion does not consider other possible physiological effect like

different dietary protein levels and varying protein to energy ratios may impose on protein metabolism. In general, dietary protein requirements seem to be ≥ 35% for *Clarias batrachus* and somewhat higher for *Clarias gariepinus* and hybrid *Clarias* catfish (Machiels and Henken 1985, Degani *et al.* 1989, Singh and Singh 1992, Jantrarotai *et al.* 1996, Ali and Jauncey 2005). Unfortunately, dietary energy requirements of these works were not uniformly expressed (GE= gross energy, DE= digestible energy or ME= metabolizable energy) resulting ununiform comparisons between studies.

The walking catfish, *Clarias batrachus* is a promising species for aquaculture by virtue of its omnivorous feeding habits, air-breathing characteristics and higher market price. They are hardy, can survive in adverse aquatic environment and cultured with high stocking densities. Information on nutrient requirements and appropriate P/E ratios in walking catfish, *Clarias batrachus*, fed with fishmeal based practical diets are restricted. Therefore, it is important to estimate the optimum P/E ratio in a fishmeal based practical diets. Hence, the objective of the present study was to evaluate dietary protein and energy interactions and their influence on growth, feed and protein utilisation and body composition leading to optimisation of P/E ratio for *Clarias batrachus*.

Materials and methods

Experimental diets

Six experimental diets were formulated with two levels of protein (25 and 35%), each with three levels of lipid (5, 10 and 15%), to produce a range of P/E ratios. Gross energy contents (GE) of diets were ranging from 15.94 to 18.46 kJg^{-1} and P/E ratios ranged from 13.57 to 21.97 mg protein kJ^{-1} GE. Diets were referred to by two numbers separated by a '/', the first number being the dietary protein level and the second the lipid level. Ingredients and proximate composition of test diets, which are more practical for farming this species, are shown in Table 1.

Table 1. Formulation and proximate composition of the experimental diets (% dry weight basis)

	Diets					
Diet number	1	2	3	4	5	6
(Protein / Lipid), (%)	(25/5)	(25/10)	(25/15)	(35/5)	(35/10)	(35/15)
Ingredients						
Fish meal[1]	34.00	34.00	34.00	51.30	51.30	51.30
Mustard oil cake[2]	9.00	9.00	9.00	9.00	9.00	9.00
Rich bran (auto)[3]	9.00	9.00	9.00	6.60	6.60	6.60
Starch[4]	43.85	38.85	33.85	29.10	24.10	19.10
Alpha cellulose[5]	1.50	1.50	1.50	1.50	1.50	1.50
Soybean oil[6]	0.15	5.15	10.15	00	5.00	10.00
Binder (Carboxymethyl cellulose)[7,8]	2.30	2.30	2.30	2.30	2.30	2.30
Vitamin & minerals premix[9]	0.20	0.20	0.20	0.20	0.20	0.20

Proximate composition						
Crude protein	25.05	25.20	24.96	35.27	35.10	35.20
Crude fat	4.95	10.20	14.88	5.10	10.06	14.90
Ash	11.22	11.22	11.22	15.54	15.54	15.54
Fibre	3.46	3.46	3.46	3.31	3.31	3.31
NFE	48.38	43.38	38.38	33.12	28.12	23.12
GE (kJ g^{-1})	16.23	17.34	18.46	15.94	17.06	18.17
P/ GE ratio	15.43	14.45	13.57	21.97	20.55	19.27

NFE= Nitrogen free extractives, calculated as 100 – (% protein + % Lipid + % Ash + % Fibre);
GE= Gross energy content; P/ GE ratio= Protein to energy ratio in mg protein kJ^{-1} of GE.
[1] Crude protein: 60.05; crude fat: 4.54; fibre: 0.65; ash: 26.87.
[2] Crude protein: 34.78; crude fat: 9.03; fibre: 8.25; ash: 9.16.
[3] Crude protein: 16.39; crude fat: 28.42; fibre: 11.10; ash: 14.05.
[4] Marck Ltd., India. [5] Sigma, UK. [6] Teer, City group, Bangladesh. [7] Carboxymethyl cellulose – Sodium salt, high viscosity. [8] BDH, Poole, UK. [9] Novartis (BD) Ltd.

The all required amount of ingredients along with vitamin and mineral premix were weighed as per formulae of experimental diets (Table 1) and mixed homogenously. During mixing, oil was gradually poured into the mixture to assure homogeneity. Adequate amount of water was added to moisten the mixture to get a definite dough texture and then the mixture was extruded through 1 mm diameter die of a pellet machine (Hobart mixture machine, Model A200). The resultant feeds were then sun dried. The experimental diets were separately packed in air-tight polyethylene bag and stored in a deep freeze for further use.

Experimental system and animals

The experiment was conducted in a static indoor rearing system with 18 cylindrical fibre glass tanks (80 cm diameter, 75 cm deep, 70-L each). Artificial aeration was used to maintain an adequate level of dissolve oxygen in each test tank. About sixty percent of the water in the system was replaced biweekly to avoid accumulation of waste products. Water quality parameters such as temperature (27.00-34.50°C), pH (6.65–8.10), dissolved oxygen (5.50–7.50 mg/L) and ammonia (0.12–0.29 mg/L) remained within acceptable ranges for *Clarias batrachus* (Viveen *et al.* 1985, Hoffman *et al.* 1991). Eight hundred 10-week old (average weight 1.15±0.05 g) for *Clarias batrachus* fingerlings were collected locally. Before starting the experiment the fish were acclimatized to the experimental condition for two weeks.

Experimental procedure

Fish were randomly assigned into groups of 50 per 70-L cylindrical fibre glass tank. Each dietary treatment had three replications and the experiment was conducted for 8 weeks. The fish were individually weighed at the start and at the end of the experiment and bulk-weighed by tank fortnightly in between. Fortnightly bulk weights were used to adjust the daily feed ration for the following 2 weeks. The fish were offered the test diets three times daily at the rate of 6% of their body weight and sub-divided into three equal feeds at 9.30, 13.00 and 17.00 h. At the onset of the experiment, 15 fish were sacrificed

for analysis of initial carcass composition. At the termination of the experiment, 4 fish were taken from each replication for determination of whole body composition.

Analytical methods and analysis of data

Proximate composition of diet ingredients, diets and whole body fish were analysed following AOAC (1990) methods. Nitrogen-free extract (NFE) was calculated by difference. Gross energy was calculated according to Jauncey (1998). All samples were analysed in triplicate. Specific growth rate (SGR), %weights gain, food conversion efficiency (FCR), protein efficiency ratio (PER) and apparent net protein utilisation (ANPU) were calculated as follows:

SGR (%/day) = [(Ln. Final body weight – Ln. Initial body weight)/days xí 100]
% Weight gain = (Final body weight – Initial body weight/Initial body weight) x 100
FCR = Food fed (g dry weight)/Live weight gain (g)
PER = Live weight gain (g)/Crude protein fed (g dry weight)
ANPU(%) = (Final carcass protein – Initial carcass protein)/Total dry protein consumed x 100
ANEU(%) = (Final carcass energy – Initial carcass energy)/Total dietary energy consumed x 100

The growth performance, feed utilization, and whole body composition data were analyzed using one way ANOVA. Paired mean comparisons among the treatments were made using Duncan's Multiple Range Tests (Duncan 1955). A significance level of $p<0.05$ was used. Standard deviation (±SD) was calculated to identify the range of means. Percentage data were arc-sine transformed (Zar 1984) prior to ANOVA and reversed afterward. Third order polynomial regression analysis (Zeitoun *et al.* 1976) was employed to determine regression of specific growth rate (SGR, % day) on protein to energy ratio (P/E ratio).

Results

Growth performances in terms of final body weight, mean weight gain, specific growth rate (SGR, % day) and feed utilisation of fish fed the experimental diets were influenced by the levels of protein and energy as lipid (Table 2). The increase in average fortnightly fish weight is shown in Fig. 1. The relationship between SGR and P/E ratio at both protein levels depicted through a polynomial curve (Fig. 2) indicates that, with the form of energy provided, maximum growth rate could be achieved at P/E ratio of 20.55. Significantly higher ($p<0.05$) growth rates were attained fish fed at higher protein diets. However, the highest dietary energy level resulted in reduced performance. The highest growth performances and feed utilisation were found by fish fed 35% protein, 10% lipid and 17.06 kJ^{-1} GE with a P/E ratio of 20.55 mg protein kJ^{-1} GE. FCR values ranges between 1.25 to 1.66 with fish fed diet 5 (35% protein, 10% lipid and 17.06 kJ^{-1} GE with a P/E ratio of 20.55 mg protein kJ^{-1} GE) showing significantly ($p<0.05$) the lowest i.e. the best FCR. The fish fed the high protein with the highest lipid

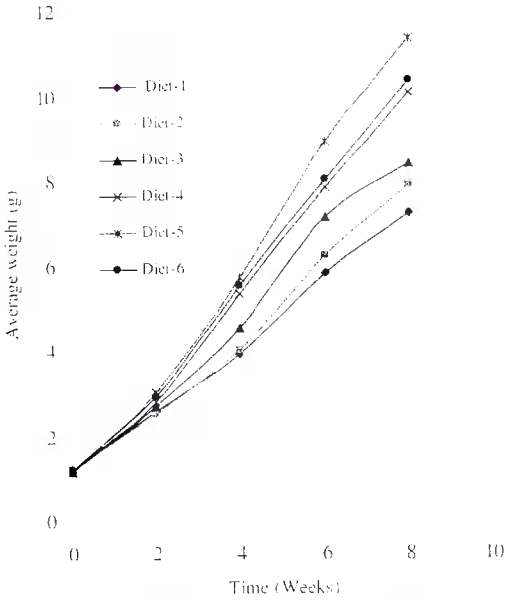
diet (6) exhibited a higher in FCR (Table 2). Protein utilisation efficiencies (PER and ANPU) did not significantly ($p>0.05$) different among the experimental diets. Fish fed diet 5 (protein 35%, lipid 10% and P/E ratio 20.55) had the highest ANPU value and diet 1 the lowest ($p>0.05$) (Table 2).

Table 2. Growth performance, feed and protein utilization of *Clarias batrachus* fed diets containing various P/E ratios for 8 weeks

	Diets					
Diet number (Protein / Lipid), (%)	1 (25/5)	2 (25/10)	3 (25/15)	4 (35/5)	5 (35/10)	6 (35/15)
Initial body wt. (g)	1.15^{a} ±0.07	1.18^{a} ±0.04	1.15^{a} ±0.07	1.15^{a} ±0.07	1.13^{a} ±0.04	1.18^{a} ±0.04
Final body wt. (g)	7.20^{d} ±0.42	7.88^{cd} ±0.25	8.40^{c} ±0.28	10.05^{b} ±0.35	11.33^{a} ±0.60	10.35^{b} ±0.21
Weight gain (g)	6.05^{d} ±0.35	6.70^{cd} ±0.21	7.30^{c} ±0.35	8.90^{b} ±0.28	10.20^{a} ±0.57	9.18^{b} ±0.18
Percent weight gain (%)	526.14^{d} ±2.61	565.20^{cd} ±17.96	636.93^{c} ±69.90	774.62^{b} ±23.04	906.33^{a} ±21.80	780.98^{b} ±8.46
Specific growth rate (SGR) (% day)	3.06^{d} ±0.01	3.18^{cd} ±0.01	3.33^{c} ±0.16	3.62^{b} ±0.05	3.85^{a} ±0.04	3.63^{b} ±0.01
Food conversion ratio (FCR)	1.66^{a} ±0.02	1.59^{a} ±0.01	1.48^{b} ±0.05	1.40^{b} ±0.05	1.25^{c} ±0.03	1.35^{bc} ±0.04
Protein efficiency ratio (PER)	2.38^{a} ±0.05	2.52^{a} ±0.08	2.40^{a} ±0.05	2.45^{a} ±0.07	2.65^{a} ±0.08	2.50^{a} ±0.10
Apparent net protein utilization (ANPU, %)	37.25^{a} ±0.25	37.55^{a} ±0.38	38.10^{a} ±0.35	40.60^{a} ±0.50	43.25^{a} ±0.33	41.27^{a} ±0.28

Note: Values are ± SD of three replications. Figures in the same row having different superscript are significantly different ($p < 0.05$).

There was a trend towards higher whole body lipid content and lower body moisture content with increasing dietary energy level at each protein level, sometimes significantly Whole body protein content did not significantly ($p>0.05$) different among the experimental diets (Table 3). Body lipid content was positively correlated ($Y = -8.6 + 0.83X$; $r = 0.16$; $p<0.05$) with dietary energy content.



Diets 1, 2, 3, 4, 5 and 6 contained P/E ratio 15.43, 14.45, 13.57, 21.97, 20.55 and 19.27 mg protein per kJ^{-1} GE respectively.

Fig. 1. The mean fortnightly growth response of *Clarias batrachus* maintained on the six experimental diets over 8 weeks.

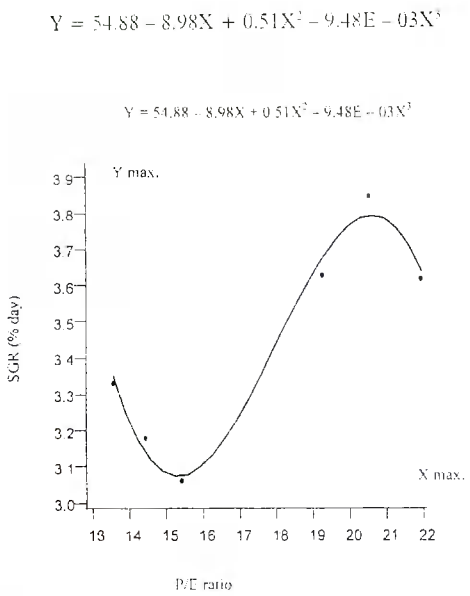


Fig. 2. The third order polynomial regression of specific growth rate (SGR, % day) with dietary protein to energy ratio (P/E ratio) in *Clarias batrachus*

Discussion

The results of the present study demonstrated that the level of dietary protein and energy influenced growth performance in *Clarias batrachus*. From the growth performance data and the third order polynomial regression analysis it observed that the best growth was achieved at 35%protein, 10% lipid, 17.06 kJ g^{-1} GE and P/E ration of around 20.55 (diet 5). A P/E ratio of 20.55 (20.55-mg protein kJ^{-1} GE) is suggested for optimum growth, in agreement with the related species *Clarias gariepinus* (Ali and Januncey 2005, Machiels and Henken 1985). However, increased dietary lipid above 10% at the high protein level did not further improve fish growth in terms of percent weight gain or SGR. Similar trends have been obtained with previous studies in *C. gariepinus* (Machiels and Henken 1985, Ali and Januncey 2005).

Table 3. Whole body composition (% wet weight basis) *Clarias batrachus* at the start and end of the experiment

		Diets					
Diet number (Protein/Lipid, %) Parameters	Initial	1 (25/5)	2 (25/10)	3 (35/5)	4 (35/10)	5 (45/5)	6 (45/10)
Moisture	73.15	72.20^{b} 1.22	69.62^{ab} ±0.82	68.65^{a} ±0.62	72.53^{b} ±1.24	70.59^{ab} ±0.92	69.92^{ab} ±0.34
Crude protein	14.53	15.26^{a} ±1.12	15.75^{a} ±0.71	15.08^{a} ±0.42	16.76^{a} ±0.74	16.29^{a} ±0.54	16.16^{a} ±0.64
Crude lipid	6.56	6.15^{b} ±0.05	8.87^{c} ±0.58	9.62^{d} ±0.19	5.34^{a} ±0.46	7.05^{b} ±0.48	8.87^{bc} ±0.36
Ash	2.38	2.55^{a} ±0.26	2.43^{a} ±0.15	2.58^{a} ±0.19	2.52^{a} ±0.31	2.32^{a} ±0.12	2.54^{a} ±0.28

Note: Values are ± SD of three replications. Figures in the same row having different superscript are significantly different ($p<0.05$).

At both dietary protein diets, poorest growth (% weight gain, SGR) was recorded for the lowest energy (as lipid) diet. At low dietary energy levels protein may be used for energy, as has been demonstrated in other *Clarias* species (Machiels and Henken 1985, Jantrarotai *et al.* 1998) and other fishes (Hassan *et al.* 1995, Yamamoto *et al.* 2000). The fixed ration level (6% of body weight) used might have prevented the fish from consuming more feed to compensate for energy supply from low energy diets. As a result, fish presumably catabolised dietary protein to meet some of their requirements for energy rather than using it as growth.

In the present study changes in feed conversion ratio (FCR) was improved with the higher protein diets. At the lower protein level FCR improved with increased lipid/energy level in the diet. At the high protein level FCR was best for the intermediate (10% lipid) diet (5). Improved FCR, up to a certain level of dietary energy inclusion (through lipid), has also been reported by earlier workers (Hassan *et al.* 1995, Jantrarotai *et al.* 1998, Yamamoto *et al.* 2000). *C. batrachus* fed the high protein with the highest lipid diet (6) exhibited poor FCR. This could be attributed to lower feed intake by fish. The high energy content of the diet, resulted in low protein intake (Grove *et al.* 1978), or to the hindrance of digestion and absorption of other nutrients by the high energy content in the diet (Dupree *et al.* 1979). Whole body composition analysis indicated that whole body lipid increased and moisture decreased with increasing dietary energy (as lipid) level at each protein level. Whole body lipid levels were higher in fish fed the lower protein diets. These observations seem in general agreement with results reported earlier for African catfish, *Clarias gariepinus* (Machiels and Henken 1985, Henken *et al.* 1986, Ali and Jauncey 2005).

On the basis of growth performance, feed utilisation and whole body composition, it may be stated that the diet 5, containing 35% and 17.06 kJ/g protein and gross energy

respectively, performed best. This diet presumably contained the most appropriate P/E ratio 20.55 (20.55 mg protein/ kJ of GE) in *Clarias btachus*. This study reveals that addition of dietary energy (lipid) at either protein level resulted in increases in growth and feed performance but that above 17.06 kJ g^{-1} GE at 35% protein performance was reduced. In conclusion, walking catfish *C. batrachus* performed best the diet containing 35%, 17.06 kJ g^{-1} and 20.55 mg protein kJ g^{-1} GE protein, gross energy and P/E ratio respectively.

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Optimum dietary carbohydrate to lipid ratio in stinging catfish, *Heteropneustes fossilis* (Bloch, 1792)

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Abstract
A feeding trial of 8-weeks was conducted in a static indoor rearing system to investigate the optimum carbohydrate to lipid ratio (CHO:L ratio) in stinging catfish, *Heteropneustes fossilis*. Five isonitrogenous (35% crude protein) and isoenergetic (17.06 kJ g^{-1} gross energy (GE)) fish meal based diets with varying carbohydrate to lipid (CHO:L g/g) ratios of 0.60, 0.98, 1.53, 2.29 and 3.44 for diets 1–5, were tested, respectively. The diets containing a fixed protein to energy ratio (P:E ratio) of 20.50-mg protein kJ^{-1} GE were fed to triplicate groups of 40 fish (per 70-L tank). Fish were fed 5% of their body weight per day adjusted fortnightly. Diet 1, containing 10% carbohydrate and 17% lipids with a CHO:L ratio of 0.60 produced the poorest ($p<0.05$) growth rates, feed and protein efficiency. Increasing carbohydrate content in the diets to 26% concomitant with a reduction in lipid content to 11% with a CHO:L ration of 2.29 of diet 5 significantly improved ($p<0.05$) growth rates, feed and protein efficiency. But did not differ with diet 4, containing CHO:L ratio 2.29. A further increase in dietary carbohydrate up to 31% and a decrease in lipids levels to 9% with a CHO:L ratio ranging from 2.29 to 3.44 (diet 4-5) did not significantly improve the fish performance. Apparent net protein utilisation (ANPU) of fish fed diet 5 was higher ($p<0.05$) than for diets 1& 2 but did not differ from diets 3 & 4. Higher lipid deposition ($p<0.05$) in whole body was observed with decreasing dietary CHO:L ratios as increasing lipid levels. Whole body protein of fish fed varying CHO:L diets did not show any discernible changes among the dietary treatments. This study revealed that *H. fossilis* can perform equally well on diets containing carbohydrate ranging from 26 to 31%, with 9 to 11% lipid or at CHO:L g/g ratio of 2.29–3.44.

Key words: *Heteropneustes fossilis*, Carbohydrate to lipid ratio, Protein utilization

Introduction

Carbohydrate and lipid are the major non-protein energy sources in fish diets and should be included at appropriate levels to maximise use of dietary protein for growth. Compared to dietary lipid, carbohydrates are relatively inexpensive and a readily available source of energy to many fish species. In warm water fish, dietary carbohydrate

utilisation is considerably high, and incorporation of this nutrient may add beneficial effects to the pelleting quality of the diet and to fish growth (NRC 1993, Wilson 1994). High levels of dietary lipid may create problems in the pelleting quality of the diet (Jauncey and Ross 1982) as well as, adversely affect the fish carcass/body composition (Hanley 1991). Any imbalance with respect to non-protein energy sources and/or their levels of inclusion may have a direct effect on growth, conversion efficiencies, nutrient retention, and body composition. In addition, the levels and forms of dietary non-protein energy that can be incorporated in fish feeds are not fully understood.

In isonitrogenous and isoenergetic diets (i.e. the same P/E ratio) testing various carbohydrate to lipid ratios, varying net protein utilisation and protein efficiency ratio reflects the ability of fish to use these nutrients to spare protein. In general, net protein utilisation and protein efficiency ratio peak at some point between extremes of lipid and carbohydrate concentration, sometimes nearer the lipid or carbohydrate extreme. Thus rainbow trout (Brauge *et al.* 1994), and *Tilapia zilli* (El-Sayed and Garling 1988) utilise lipids better than carbohydrate, while *Oreochromis niloticus* (Shimeno *et al.* 1993) and African catfish, *Clarias gariepinus* (Ali and Jauncey 2004) utilises carbohydrates better than lipids. Other investigators report peaks in protein utilisation and protein efficiency ratio at intermediate levels of carbohydrate such as in walking catfish, *Clarias batrachus* (Erfanullah and Jafri 1998), hybrid *Clarias* catfish (Jantrarotai *et al.* 1994) and channel catfish, *Ictalurus punctatus* (Garling and Wilson 1977). The variability of results could reflect not only the different capabilities of fishes to utilise carbohydrate, but also the various ranges of carbohydrate to lipid ratios tested and the varying sources of these nutrients.

The stinging catfish, *Heteropneustes fossilis* an air-breathing cat fish, is widely distributed throughout Indian sub-continent, inhabiting swampy and marshy water bodies. The fish has a high marketability due to its superior nutritive value, on is cultured extensively. Lack of information on its nutritional requirements is a major constraint in the development of intensive culture of this fish. Information on the nutrition of this species seems limited only to its protein requirements and lipid utilisation (Akand *et. al.* 1989, Anwar and Jafri 1992, Firdaus 1993). Information on carbohydrate and lipid utilisation and appropriate carbohydrate to lipid ratio in *H. fossilis* fed fish meal based practical diets is restricted. It is thus imperative to determine the optimum dietary carbohydrate to lipid ratio in this species that produces the best growth. The objective of this study was to investigate dietary carbohydrate to lipid interactions and their influence on growth, feed and protein utilisation and body composition leading to optimisation of CHO:L ratio for *H. fossilis*.

Materials and methods

Experimental diets

Five experimental isonitrogenous (35% CP) and isoenergatic (17.06 kJg^{-1} GE) diets were formulated with a P/E ratio 20.50 mg protein kJ^{-1} GE based on results from previous studies of optimised P/E ratio of *H. fossilis* . The non-protein energy was

adjusted by varying the ratios of lipid and carbohydrate in the diets so that the carbohydrate to lipid ratios (CHO:L, g/g) ranged from 0.60 to 3.44. Diets are referred to by two numbers separated by a "/", the first number being the percentage energy from dietary lipid and the second number the percentage energy from dietary carbohydrate. Composition of the experimental diets and their proximate composition are shown in Table 1.

Table 1. Formulation and proximate composition of the experimental diets (% dry weight) for cat fish, *Heteropneustes fossilis*

	Diets (Lipid/ Carbohydrate, % Energy)				
Diet number (Lipid / CHO), (%)	1 (80/20)	2 (70/30)	3 (60/40)	4 (50/50)	5 (40/60)
Ingredients					
Fish meal[1]	40.00	40.00	42.00	48.50	54.50
Mustard oil cake[2]	17.00	17.00	17.00	9.00	2.00
Rich bran (auto)[3]	26.00	26.00	17.50	9.75	1.70
Starch[4]	00	5.17	12.62	21.70	30.70
Alpha cellulose[5]	12.00	9.06	8.38	8.55	8.60
Soybean oil[6]	2.50	0.27	00	00	00
Binder (Carboxymethyl cellulose)[7,8]	2.30	2.30	2.30	2.30	2.30
Vitamin and minerals premix[9]	0.20	0.20	0.20	0.20	0.20
Proximate composition					
Crude Protein	35.02	35.02	35.00	35.02	35.04
Crude Fat	17.05	15.58	13.40	11.16	8.92
Ash	12.42	12.42	11.58	10.88	10.13
Fiber	15.98	13.04	11.68	10.46	9.25
NFE	10.18	15.35	20.47	25.56	30.72
GE (kJ g^{-1})	17.05	17.06	17.05	17.07	17.06
P: GE ratio	20.50	20.50	20.50	20.52	20.52
CHO: L ratio (g/g)	0.60	0.98	1.53	2.29	3.44

NFE= Nitrogen free extractives, calculated as 100 – (% protein + % Lipid + % Ash + % Fibre);
GE= Gross energy content; P: GE ratio= Protein to energy ratio in mg protein kJ^{-1} of GE.
CHO: L g/g ratio= % wt. in CHO/ % wt. in lipid.
[1] Crude protein: 60.05; crude fat: 4.54; fibre: 0.65; ash: 26.87.
[2] Crude protein: 34.78; crude fat: 9.03; fibre: 8.25; ash: 9.16.
[3] Crude protein: 16.39; crude fat: 28.42; fibre: 11.10; ash: 14.05.
[4] Marck Ltd., India. [5] Sigma, UK. [6] Teer, City group, Bangladesh.
[7] Carboxymethyl cellulose – Sodium salt, high viscosity.
[8] BDH, Poole, UK. [9] Novartis (BD) Ltd.

The required amount of ingredients along with vitamin and mineral premix were weighed (Table 1) and mixed homogenously. During mixing, oil was gradually poured into the mixture to assure homogeneity. Adequate amount of water was added to moisten the mixture to get a definite dough texture and then the mixture was extruded through 1 mm diameter die of a pellet machine (Hobart mixture machine, Model A200). The resultant diets were broken into smaller pieces and then sun dried. The experimental diets were separately packed in air-tight polyethylene bag and stored in a deep freeze for further use.

Experimental system and animals

The experiment was conducted in a static indoor rearing system with 15 cylindrical fibre glass tanks (80 cm diameter, 75 cm deep, 70-L each). Artificial aeration was used to maintain an adequate level of dissolved oxygen in each test tank. About sixty percent of the water in the system was replaced biweekly to avoid accumulation of waste products. Water quality parameters such as temperature (26.00-35.50^{0}C), pH (6.80-7.90), dissolved oxygen (5.70-7.80 mg/L) and ammonia (0.12-0.30 mg/L) remained within acceptable ranges for stinging catfish, *Heteropneustes fossilis* (Viveen *et al.* 1985; Hoffman *et al.*1991). Seven hundred 10-week old (average weight 1.72±0.02 g) shing fingerlings were collected from local fish vendors of Mymensingh. The collected fish were given a prophylactic treatment with salt (3% NaCl) solution for 10 minutes. During treatment sufficient oxygen supply was maintained through artificial aeration. Before starting the experiment the fish were acclimatized to the experimental condition for one week.

Experimental procedure

Fish were randomly assigned into groups of 40 per 70-L cylindrical fibre glass tank. Each dietary treatment had three replications and the experiment was conducted for 8 weeks. The fish were individually weighed at the start and at the end of the experiment and bulk-weighed by tank fortnightly in between. Fortnightly bulk weights were used to adjust the daily feed ration for the following 2 weeks. The fish were offered the test diets three times daily at the rate of 5% of their body weight and sub-divided into three equal feeds at 9.30, 13.00 and 17.00 h. Prior to weighing, fish were caught with a fine mesh scoop net and excess water was then removed from fish body by gently blotting on a soft tissue paper. Weight of fish in each sampling was measured by bulk weighing them using a digital electronic balanc. The sampled fish were handled very carefully. The experimental tanks were washed and cleaned during sampling time. At the onset of the experiment, 15 fish were sacrificed for analysis of initial carcass composition. At the termination of the experiment, 4 fish were taken from each replication for determination of whole body composition.

Analytical methods and analysis of data

Proximate composition of diet ingredients, diets and whole body fish (carcass) were analysed in triplicate following AOAC (1990) methods. Nitrogen-free extract (NFE) was calculated by difference. Gross energy was calculated according to Jauncey (1998).

Specific growth rate (SGR), %weights gain, food conversion efficiency (FCR), protein efficiency ratio (PER) and apparent net protein utilisation (ANPU) were calculated as follows:

SGR (%/day) = [(Ln. Final body weight – Ln. Initial body weight)/days x 100]
% Weight gain = (Final body weight – Initial body weight/Initial body weight) x 100
FCR= Food fed (g dry weight)/ Live weight gain (g)
PER = Live weight gain (g)/Crude protein fed (g dry weight)
ANPU(%) = (Final carcass protein – Initial carcass protein)/Total dry protein consumed x 100

The growth performance, feed utilization, and whole body composition data were analyzed using one way ANOVA. Paired mean comparisons among the treatments were made using Duncan's Multiple Range Tests (Duncan 1955). A significance level of ($p<0.05$) was used. Standard deviation (±SD) was calculated to identify the range of means. Percentage data were arc-sine transformed (Zar 1984) prior to ANOVA and reversed afterward.

Results

No mortality nor external clinical symptoms occurred in any treatment during the study. Growth responses, feed and protein utilisation of fish fed the experimental diets are shown in Table 2. The increased in average fortnightly fish weight is shown in Fig. 1. There was a trend of increasing growth with increasing inclusion of dietary carbohydrate and concomitant reduction dietary lipid level (increasing CHO:L ratio). Growth performances in terms of weight gain, %weight gain and SGR of fish fed diet 5 was significantly higher ($p<0.05$) than fish fed diets 1, 2 and 3, but did not differ ($p>0.05$) from diet 4 (Table 2). Food conversion ratio (FCR) values were better as the dietary carbohydrate level increased with concomitant reduction dietary in lipid level. Fish fed diet 5, containing CHO:L ratio of 3.44 g/g showed significantly ($p<0.05$) superior FCR but did not differ ($p>0.05$) from diet 4 (Table 2).

Protein utilisation efficiencies on the basis of PER and ANPU increased significantly ($p<0.05$) with increasing CHO:L ratio. Fish fed diet 5, significantly ($p<0.05$) highest PER value but did not differ ($p>0.05$) from diet 4. ANPU fish fed diet 5 was significantly higher ($p<0.05$) than diets 1 and 2 but did not differ ($p>0.05$) from diets 3 and 4 (Table 2). Except for body lipid content, whole body composition (body protein and ash) was not affected ($p>0.05$) by the dietary treatments. There was an overall trended of decreasing carcass lipid with decreasing inclusion level of dietary lipid (increasing CHO:L ratio, g/g). Body lipid content of fish fed diet 2 was significantly ($p<0.05$) higher than that of fish fed diets 4 and 5 (containing the lower dietary lipid) but did not differ from diets 1 and 3 (Table 3).

Table 2. Mean growth performance, feed and protein utilization of *Heteropneustes fossilis* fed diets containing various lipid to carbohydrate ratios for 8 weeks

	Diets				
Diet number (Lipid / CHO), (%)	1 (80/20)	2 (70/30)	3 (60/40)	4 (50/50)	5 (40/60)
Initial body wt. (g)	1.72^{a} ±0.02	1.72^{a} ±0.03	1.73^{a} ±0.01	1.72^{a} ±0.02	1.73^{a} ±0.01
Final body wt. (g)	5.78^{c} ±0.11	7.08^{b} ±0.04	7.55^{b} ±0.08	8.35^{a} ±0.07	8.75^{a} ±0.10
Weight gain (g)	4.06^{c} ±0.08	5.36^{b} ±0.06	5.82^{b} ±0.08	6.64^{a} ±0.06	7.03^{a} ±0.11
Weight gain (%)	236.72^{c} ±2.02	311.41^{b} ±8.81	336.42^{b} ±1.52	386.90^{a} ±2.90	405.82^{a} ±1.99
Specific growth rate (SGR) (% day)	2.43^{c} ±0.01	2.83^{b} ±0.04	2.95^{b} ±0.02	3.17^{a} ±0.01	3.24^{a} ±0.02
Food conversion ratio (FCR)	1.80^{a} ±0.02	1.66^{b} ±0.01	1.59^{b} ±0.10	1.35^{c} ±0.03	1.29^{c} ±0.05
Protein efficiency ratio (PER)	2.75^{c} ±0.09	2.79^{c} ±0.10	2.90^{ab} ±0.13	2.98^{ab} ±0.09	2.83^{a} ±0.10
Apparent net protein utilization (ANPU, %)	33.45^{c} ±0.44	35.70^{c} ±1.55	42.52^{ab} ±2.10	44.50^{ab} ±3.05	45.80^{a} ±3.20

Note: Values are ± SD of three replications. Figures in the same row having different superscript are significantly different ($p<0.05$).

Table 3. Whole body composition (% wet wt. basis) of *Heteropneustes fossilis* at the start and end of the experiment

		Diets				
Diet number (Lipid/CHO), (%)	Initial (all fish)	1 (80/20)	2 (70/30)	3 (60/40)	4 (50/50)	5 (40/60)
Moisture	78.16	73.85^{a} ±0.16	74.15^{a} ±0.55	73.30^{a} ±0.15	74.80^{a} ±0.65	74.98^{a} ±0.09
Crude Protein	14.24	16.60^{a} ±0.51	15.85^{a} ±0.43	16.75^{a} ±0.37	16.48^{a} ±0.74	16.58^{a} ±0.63
Crude Lipid	2.25	4.65^{ab} ±0.45	4.98^{b} ±0.85	4.79^{ab} ±0.47	3.64^{ab} ±0.40	3.48^{a} ±0.52
Ash	2.96	3.32^{a} ±0.70	3.24^{a} ±0.07	3.30^{a} ±0.23	3.15^{a} ±0.56	3.18^{a} ±0.28

Note: Values are ± SD of three replications. Figures in the same row having different superscript are significantly different ($p<0.05$).

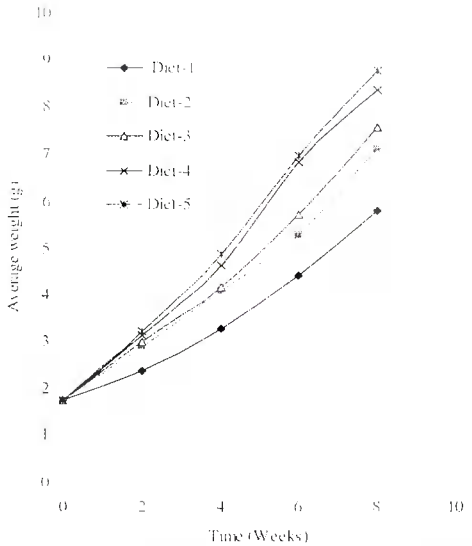

Fig. 1. The mean fortnightly growth response of *Heteropneustes fossilis* fed diets containing various lipid to carbohydrate ratios for 8 weeks
Diets 1, 2, 3, 4 and 5 contained CHO:L ratio (g/g) 0.60, 0.98, 1.53, 2.29 and 3.44

Discussion

The results of the present study demonstrated that growth performance and feed conversion efficiency are influenced by the dietary carbohydrate to lipid ratios in *H. fossilis*. From the growth performance data it observed that the the optimal dietary CHO:L ratio in *H. fossilis* which produced good growth rate and feed utilisation ranged from 2.29 to 3.44, which is smaller than the optimum ranges reported for African cat fish, hybrid *Clarias* catfish and walking catfish. In African catfish diets containing isonitrogenous (40% CP) and isocaloric (20 kJ g^{-1}GE), with CHO:L ratios ranging from 1.7 to 3.40 reported the production of fish with significantly improved growth performance and feed utilisation (Ali and Jauncey 2004). Jantrarotai *et al.* (1994) who fed hybrid *Clarias* catfish isonitrogenous (33% CP), isocaloric (12 kJ g^{-1} DG) diets containing CHO:L ratios ranging from 3.83 to 11.24, reported the production of fish with no significant difference in weight gain, feed conversion, or protein and energy deposition. Similarly, in channel catfish diets containing 24% CP and 12 kJ g^{-1} ME, with CHO:L ratios ranging from 0.45 to 4.5 there were no significant effects on fish performance (Garling and Wilson 1977). An investigation in walking catfish (*Clarias batrachus*) diets containing 40% CP and 14 kJ g^{-1} ME, with CHO:L ratios ranging from

0.02 to 3.38 reported the production of fish with significantly improved growth performance and feed utilisation (Erfanullah and Jafri 1998).

In the present study, the differences observed in optimal ranges of dietary CHO:L ratios from other species mentioned above due to different isonitrogenous, isocaloric diets containing varying levels of non-protein energy (varying CHO:L ratios). The differences also observed in growth and feed or protein efficiency indicate on ability of the stinging cat fish, *H. fossilis* to adapt to increasing levels of dietary carbohydrate (26-31%) which appears to be lower than the African cat fish (38%) (Ali and Jauncey 2004) but almost similar that reported for channel catfish (28%) (Garling and Wilson 1977) and walking catfish (27%) (Erfanullah and Jafri 1998).

Reduced growth rate and feeding or protein efficiency was found in stinging cat fish, *H. fossilis* fed the highest-lipid lowest-carbohydrate of diet 1, containing 10% carbohydrate and 17% lipid (CHO:L ratio 0.60). This could be the result of reduced feed consumption by fish due to the high dietary lipid level because of excessive food energy. This may prevent intake of the necessary amounts of protein and other nutrients required for maximum growth. Similar observations have been reported for African cat fish (Ali and Jauncey 2004), walking catfish (Erfanullah and Jafri 1998) and hybrid Clarias catfish (Jantrarotai *et al.* 1994). Crude fibre content (14%), as cellulose, in diet 1 is unlikely to be the cause of poor performance of *H. fossilis*. Since the diets were isonitrogenous and isoenergetic, the increase in PER and ANPU with increasing dietary carbohydrate (corresponding to increasing CHO:L ratios) could be attributed to the relative amounts of non-protein energy sources. This may indicate that *H. fossilis*, despite being omnivorous, can utilise dietary carbohydrate more efficiently than lipid although it is difficult to be certain. Because the diets were isoenergetic, higher lipid levels could be attained only by increasing the fiber content of the diet. There are several reports indicating that high dietary fiber levels reduce the utilisation of other nutrients (Hilton and Atkinson 1982; Anderson *et al.* 1984), but this view has been challenged (Jantrarotai *et al.* 1994). Thus, it remains unclear if reduced growth in fish fed the high lipid diets was due to inefficient lipid utilisation by fish, as compared with carbohydrate utilisation, or to the deleterious effects of the high dietary fiber level.

The different CHO:L ratios of the experimental diets had no effect on protein and ash content of the whole body of *H. fossilis*. However, increasing dietary lipid to 13% or higher (diets 1–4) resulted in increased total body lipid. Similar results have been reported in walking catfish, *C. batrachus* (Erfanullah and Jafri 1998), African cat fish (Ali and Jauncey 2004), hybrid Clarias catfish (Jantrarotai *et al.* 1994) and channel catfish (Garling and Wilson 1977). The inverse relationship between dietary CHO and whole body lipid content was interesting, since increased CHO did not produce undesirable fat accumulation in the body of the fish. This is in agreement with results reported for walking catfish, *C. batrachus* (Erfanullah and Jafri 1998), African cat fish (Ali and Jauncey 2004), hybrid Clarias catfish (Jantrarotai *et al.* 1994), channel catfish (Garling and Wilson 1977), tilapia (El-Sayed and Garling 1988) and Indian major carps (Erfanullah and Jafri 1998b).

On the basis of growth performance, feed and protein utilisation and whole body composition, it may be stated that the diet 5 of 40% lipid energy (8.92% lipid), 60% carbohydrate energy (30.72% carbohydrate) and carbohydrate to lipid ratio (CHO:L ratio g/g) of 3.44 performed best. In conclusion, this study reveals that stinging cat fish, *Heteropneustes fossilis* can perform equally well on isonitrogenous and isocaloric diets (35% protein, 17.05 kJ g^{-1} GE) containing carbohydrate ranging from 26 to 31% with 9 to 11% lipid or at CHO:L ratios of 2.29 to 3.44.

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Suitable stocking density of tilapia in an aquaponic system

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Abstract
An aquaponic system was studied through the integrated culture of mono-sex GIFT and two types of vegetables *viz.* morning glory, *Ipomoea reptans* and taro, *Colocasia esculenta* in a recirculating system for 15 weeks. Tilapia fry of uniform size of 0.76 g were released in three treatments (stocking densities): 106 fish/m^3 (T_1), 142 fish/m^3 (T_2) and 177 fish/m^3 (T_3) to assess the effect of stocking density on the growth performance of fish. Fish were fed with a commercial feed containing 25% protein. Weight gain (g) of tilapia ranged from 19.41 to 32.67 g and was inversely related with stocking density. Percent weight gain varied between 2553.99 and 4298.68 % and was significantly different among the treatments. SGR ranged from 3.09 to 3.59 % per day and varied significantly. FCR varied from 2.19 to 2.69 and had a positive correlation with stocking density. The highest survival rate (%) was achieved in T_1 (99%) followed by T_2 (98%) and T_3 (96%). Production of fish ranged from 3.43 to 3.52 kg/m^3 and was inversely related with stocking density. The present study demonstrated that 106 fish/m^3 was the best stocking density in terms of growth, food conversion ratio, survival and production for tilapia culture in the aquaponic system.

Key words: Aquaponics, Mono-sex tilapia, Stocking density

Introduction

Aquaponics is a bio-integrated system that links recirculating aquaculture with hydroponic (growing plants in a media without soil) vegetable, flower, and/or herb production. In aquaponics, nutrient-rich effluent from fish tanks is used to fertilize hydroponic production beds. This is good for the fish because plant roots and rhizobacteria remove nutrients from the water. These nutrients– generated from fish manure, algae, and decomposing fish feed– are contaminants that would otherwise build up to toxic levels in the fish tanks, but instead serve as liquid fertilizer to hydroponically grown plants. In turn, the hydroponic beds function as a biofilter– stripping off ammonia, nitrates, nitrites, and phosphorus– so the freshly cleansed water can then be recirculated back into the fish tanks. The nitrifying bacteria living in the gravel and in association with the plant roots play a critical role in nutrient cycling; without these microorganisms the whole system would stop functioning. The stocking

density of fish in the aquaponic system is very important for the proper functioning of the system. Stocking density of fish should be optimum to maintain the water quality suitable for fish and plant growth. Hence, the present study was conducted to observe the effects of stocking density on the growth and production parameters of tilapia in an aquaponic system and to determine a suitable stocking density.

Materials and methods

The experiment was carried out in an existing recirculating aquaculture system located at Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. The duration of the experiment was 15 weeks during April to August' 2009. Fry of mono-sex tilapia (*Oreochromis niloticus*) belonging to the same age group were collected from local hatchery of Mymensingh. The fry were acclimatized for seven days prior to the experiment. Two common vegetables (Morning glory, *Ipomoea reptans* and Taro, *Colocasia esculenta*) were selected for the experiment.

Experimental system

The system consisted of 12 equal sized cisterns and 2 smaller ones. Each cistern had inlet and outlet facilities connected to the bio-filter system which was located along the east and south sides of the system. The substrates of the bio-filter consisted of styrofoam, gravels, PVC nets and shells of mussels. The pump house was located at the south-eastern side of the system. The pumping unit consisted of two water pumps which ran alternatively for six hours to ensure continuous water flow and aeration.

Nine cisterns of uniform size were selected for conducting the experiment. The cisterns had a length, width and depth of 2.06 m, 1.8 m and 0.94 m respectively. So each cistern had an area of 3.708 m^2 and a volume of 3.486 m^3. Water level in the cisterns was adjusted at a depth of 0.76 m to maintain the water volume of 2.82 m^3. The desired level of water in the system was maintained by continuous recirculation of water at a rate of 15 l/min. In each cistern, water level was controlled by a PVC pipe placed at the opening of the outlet located at the middle of the cistern. The pipe was covered by a larger pipe (in terms of diameter and height) with four notches at the bottom which facilitated easy removal of waste materials from the cisterns. Water loss from the cisterns due to evaporation was adjusted every week by adding groundwater. The cemented cisterns were cleaned thoroughly with washing powder and washed with freshwater. After complete drying they were filled with groundwater up to a level of 0.76 m which was maintained throughout the experiment. Polyester net was placed over each cistern to prevent fish from predatory birds.

Preparation of media

Fifty four 5 l soybean oil containers were collected. The top of the containers were cut off. They were cut in the sides in rectangular shapes (2 cm×8 cm) and holes were made at the center of their bottoms to facilitate root growth and inflow of nutrients

(Plate 1). Three quarters of the containers were filled with decomposed water hyacinths and a thin layer of soil was placed on top of the water hyacinths to stabilize the plant seeds and stolons when planted. Six containers were then placed in each cistern. They were then half suspended into the water by hanging them from the cistern walls.

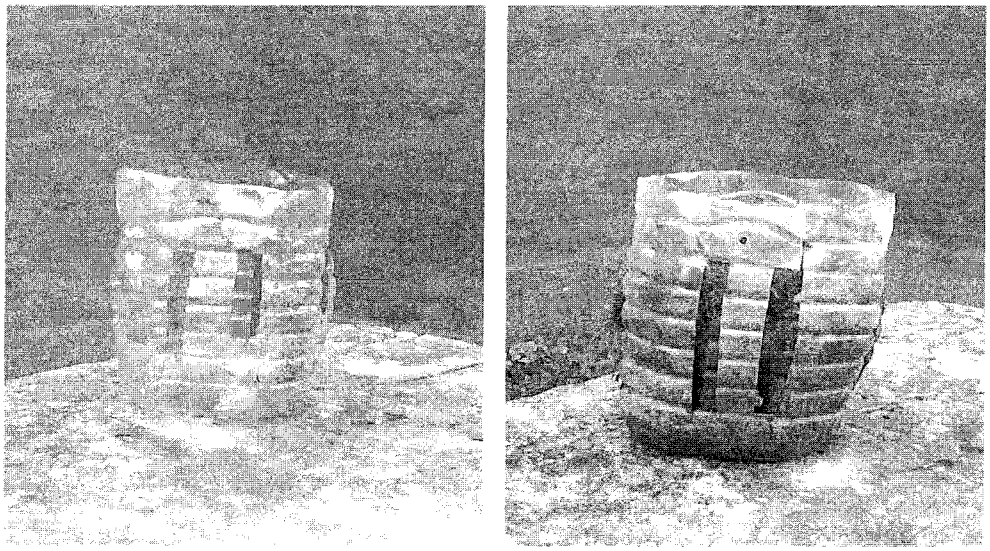

Plate 1. Container cut through the sides to facilitate nutrient flow and root growth and container filled with planting media.

Stocking and feeding of fish

Mono-sex tilapia fry of uniform size (0.76 g) were stocked in three treatments (stocking densities): 106 fish/m^3, 142 fish/m^3 and 177 fish/m^3 which were designated as T_1, T_2 and T_3 respectively. Fish were supplied with commercial feed made by Quality Feeds Limited having an average protein content of 25%. They were first supplied with starter feed for ten days, then nursery feed for twenty days and finally grower feed for rest of the days. At the beginning of the experiment feed was applied daily at a rate of 15% of body weight which was gradually reduced to 6% of body weight towards the end of the experiment. Feed was supplied three times a day, one-third at 9.00 AM, one-third at 1.00 PM and the rest at 5.00 PM.

Plantation

After one week of fry release, vegetables were introduced to the system. There were six containers in each cistern, three for morning glory and three for taro. Ten seeds of morning glory were sewed in each container, the seedlings were placed into the holes evenly spaced 3-4 cm apart and planted with two seeds per hole. Stolons of taro were planted, one in each container.

Water quality parameters

The water quality parameters observed were water temperature (°C), dissolved oxygen (mg/l), pH, alkalinity (mg/l) nitrite-nitrogen (mg/l) and nitrate-nitrogen (mg/l). Water temperature was measured everyday. Dissolved oxygen and pH were recorded at 15 days interval. Nitrite-nitrogen, nitrate-nitrogen and alkalinity were recorded at the beginning, middle and end of the experiment. The number of observations was limited due to economic deficiency. Water temperature was taken by an alcohol thermometer. Dissolved oxygen of water was measured by a dissolved oxygen meter (YSI MODEL-58, USA). Water pH was recorded with the help of a pH meter (Mettler Toledo MP 230). Total alkalinity of water samples was determined by acid titration method (APHA 1992). Nitrite-nitrogen and nitrate-nitrogen (mg/l) were measured in the Water Quality and Pond Dynamics Laboratory, Faculty of fisheries, BAU using a portable datalogging spectrophotometer HACH DR/2010, USA. Water samples were collected in small plastic bottles from the experimental cisterns on the sampling days.

Growth monitoring

Growth and production were monitored by randomly selecting ten fish from each cistern. Fish were caught by a big scoop net. The weight of fish was recorded in grams by a sensitive electronic balance. After 15 weeks of rearing the final weight of individual fish was recorded and the growth and production were calculated by the following formulae:

$$\text{Weight gain (g)} = \text{Mean final weight (g)} - \text{Mean initial weight (g)}$$

$$\%\ \text{Weight gain} = \frac{\text{Mean final fish weight} - \text{Mean initial fish weight}}{\text{Mean initial fish weight}} \times 100$$

$$\text{SGR (\% per day)} = \frac{\log_e W_2 - \log_e W_1}{T_2 - T_1}$$

Where

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

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

$$\text{FCR} = \frac{\text{Feed fed (dry weight)}}{\text{Live weight gain}}$$

$$\text{Survival rate (\%)} = \frac{\text{No. of fish harvested}}{\text{No. of fish stocked}} \times 100$$

$$\text{Production} = \text{No. of fish harvested} \times \text{Mean final weight of fish}$$

Data analysis

The data obtained on the growth of fish, FCR, survival rate and production were statistically analyzed to see whether the influence of different treatments (stocking densities) on these parameters were significant or not. One way analysis of variance (ANOVA) was done with the help of SPSS (Statistical Package for the Social Sciences).

Results and discussion

Water quality parameters

The ranges of water quality parameters such as temperature, dissolved oxygen, pH, alkalinity, nitrite-nitrogen, and nitrate-nitrogen during the experiment are shown in Table 1.

Table 1. Summary of water quality parameters during the experiment

Parameters	Value range
Water Temperature (°C)	28-33
Dissolved oxygen (mg/l)	6.2-8.5
pH	8.07-8.53
Alkalinity (mg/l)	40-78
Nitrite-Nitrogen (mg/l)	0.01-0.50
Nitrate-Nitrogen (mg/l)	0.03-1.16

The water quality parameters like temperature, dissolved oxygen, pH, alkalinity, nitrite-nitrogen and nitrate-nitrogen were within the suitable ranges for tilapia culture in recirculating system as described by Drennan and Malone (1992), Masser *et al.* (1999), Rashid (2008) and Swann (2009).

Growth and production of fish

The growth and production performances of tilapia in three treatments are summarized in Table 2. The best mean weight gain (32.67 g) and percent weight gain (4298%) was observed in case of the lowest stocking density T_1 and the lowest values were observed in case of highest stocking density T_3. This indicated an inverse relationship between weight gain and stocking density. This result was similar to the findings of Roy (2002), Carro-Anzallota and McGinty (2007), Gibtan *et al.* (2008) and Rashid (2008). In the present experiment SGR (% per day) of fish in T_1, T_2 and T_3 were 3.59, 3.32 and 3.09, respectively. The highest SGR was observed in the lowest stocking density T_1 and the lowest value in the highest stocking density T_3. This indicated a negative correlation between SGR and stocking density. Ridha (2005), Rashid (2008) and Alam (2009) found similar results.

Table 3. Growth and production of tilapia in three treatments

Treatment	Weight gain (g)	(%) Weight gain	SGR (% per day)	FCR	Survival rate (%)	Production (kg/m^3)
T_1	32.67±2.19	4298.68±30.86	3.59±0.012	2.19±0.026	99±1.0	3.52±0.03
T_2	24.54±1.83	3228.95±47.39	3.32±0.01	2.41±0.015	98±1.0	3.52±0.035
T_3	19.41±1.27	2553.99±63.04	3.09±0.001	2.69±0.023	96±1.73	3.43±0.01

The FCR values varied significantly in the three treatments. The lowest value (2.19) was found in T_1 followed by T_2 (2.41) and T_3 (2.69). This finding was similar to that of Ridha (2005), Hasan (2007) and Rahsid (2008) who also found a positive correlation between stocking density and FCR. The survival rates of the fish in the present study were 99%, 98% and 96% in T_1, T_2 and T_3, respectively and did not vary significantly ($p>0.05$). However, the highest survival rate was achieved in the lowest stocking density T_1 followed by T_2 and T_3. This indicated an inverse relationship which was also observed by Yi *et al.* (1996), Hasan (2007) and Rashid (2008).

In the present study the highest production of tilapia was observed in T_1 (3.52 kg/m^3) and T_2 (3.52 kg/m^3) followed by T_3 (3.43 kg/m^3). The finding was similar to that of Alam (2009). The lowest production in the highest stocking density might be attributed to the fact that the growth and survival rate of fish in T_3 was the lowest and the increase in biomass was limited by available space and greater competition. The growth parameters, FCR, survival rate and production reveal that of the three stocking densities tested in this experiment, 106 fish/m^3 might be the most suitable stocking density for all-male GIFT tilapia production in the aquaponic system.

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Effects of supplemental feed and fertilizer on growth and survival of *Macrobrachium rosenbergii* (de Man) post larvae in pond nursery system

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Abstract
The study was conducted to compare the performance of different nursing practices of giant freshwater prawn (*Macrobrachium rosenbergii*) post-larvae (PL). Three treatments such as only fertilizers (T_1), fertilizers with 5% supplementary feed (local feed) (T_2), and 10% commercial feed (T_3) were applied in the nursing system of prawn PLs in earthen pond. An earthen pond (315 m^2) was divided into nine equal small ponds by fine meshed nylon nets. Feeds were used once daily on a tray placed near the pond bottom. There was a significant difference ($p<0.05$) in some water quality parameters like pH and total alkalinity, but all measured water quality parameters *viz.* water temperature, transparency, dissolved oxygen and ammonia-nitrogen were within the acceptable range for nursing of prawn PL. The results showed that the mean final lengths of prawn post-larvae were 6.3±0.07 cm, 7.12±0.22 cm and 8.17±0.16 cm in T_1, T_2 and T_3, respectively. There were significant difference ($p<0.05$) in mean final length of prawn PL among the treatments. Significantly higher ($p<0.05$) average daily weight gain was observed in T_3 (0.071±0.007 g) than in T_2 (0.052±0.006 g) and T_1 (0.031±0.002 g). The specific growth rate (SGR) of T_3 (8.81±0.26) was found significantly higher ($p<0.05$) than T_2 (8.35± 0.22) and T_1 (7.42±0.11). Survival rate (%) was also significantly higher ($p<0.05$) in T_3 (66.24±1.58) than in T_2 (60.52±1.64) and T_1 (53.86±2.71). Therefore, it may be concluded that the growth and survival in prawn nursery was better in commercial feed than only fertilizers and fertilizers with local feeds.

Key words: *M. rosenbergii*, Post-larvae, Nursing, Commercial feed

Introduction

Among the wide array of prawn species available in Bangladesh, the long legged giant freshwater prawn, *Macrobrachium rosenbergii* with the popular Bangla name "Golda chingri" contributes to the major share of the exported prawns. This species remains by far the major subject of cultivation because of its global market evolved during the 1990s and is currently being further developed. Despite the immense potential,

expansion of prawn farming in this country is limited by several constraints. The most important ones are: (i) insufficient supply of quality prawn seeds in both coastal and inland areas, (ii) unpredictable initial mortality of prawn in grow-out ponds due not to stocking with nursed juveniles, (iii) lack of prawn grow-out management technologies appropriate for local conditions, and (iv) lack of appropriate research and extension works in technology development, synthesis and dissemination.

Though freshwater prawn grow, mature, fertilize, even hatch and about 90% of global prawn production is done in freshwater environment, their larvae neither can survive nor grow up to post-larval stage without brackish water. Therefore, prawn culture in this country is being developed in and around the coastal areas, depending on naturally collected seeds. Though only few prawn hatcheries are being operated, their production rate is not consistent and far below the country's requirement. This means the pressure on natural resources will be growing, resulting in shortages of natural seed supply. Therefore, proper management of freshwater prawn nursery has utmost importance, because availability of water and culture period restricts the grow-out management in Bangladesh.

From the above discussion, we can say that, the prawn seed supply is insufficient compared to the requirements of our country. So if the seeds are not nursed properly, mass mortality of prawn PL may occur and the farmers may loose their interests to prawn culture and if that happens, it will ultimately affect our national economy. At this initial stage of prawn nursery, the farmers must need adequate information on a proper nursing system to best serve their purpose. With this point of view, the present research has been designed primarily to understand some practical information on different feeding options including feeding on particular commercial feed.

Materials and methods

The study was carried out in a rectangular earthen pond of 315 m^2 at Freshwater Station of the Bangladesh Fisheries Research Institute, Mymensingh for 60 days from July to September. The large pond was divided into nine equal small pond of 35 m^2 each by fine meshed nylon net. There were three replications for each of the three treatments *viz.* only fertilizers (T_1), fertilizers with 5% supplementary feed (local feed) (T_2) and 10% commercial feed (T_3). Local feed was prepared by using the local ingredients such as rice bran 70% and mustard oil cake 30%. Saudi-Bangla shrimp feed was selected as commercial feed for this experiment.

Complete removal of all undesirable fish, insect and other aquatic organisms were done by drying the ponds. After one week of drying, lime was applied at a rate of 1kg 40/m^2. After 3 days of liming, the ponds were filled with deep tube-well water. All the ponds were equally fertilized with urea and triple super phosphate (TSP) at the rate of 100 g 40/m^2 each and cowdung at the rate of 10 kg 40/m^2. The water depth was maintained to a maximum of 1m using fine meshed PVC over flow pipe on the bank fixed at 1m above the pond bottom. Leaves of coconut tree were used in each pond to

provide shelter for prawn post-larvae. The PL of *M. rosenbergii* were collected from the hatchery of BFRI. The ponds were stocked with prawn PL having average weight 0.022±0.00 g, 0.021± 0.00 g and 0.022±0.002 g in treatment T_1, T_2 and T_3, respectively and average length 0.89±0.01 cm, 0.88±0.01 cm and 0.11±0.07 cm in treatment T_1, T_2 and T_3, respectively. The stocking density of prawn PL was 20/m^2. Cowdung, urea and triple super phosphate (TSP) were applied weekly throughout the experimental period at a rate of 3kg 40m^2, 75 g40 m^2 and 50 g/40 m^2, respectively. Feeds were supplied once daily in the ponds of T_2 and T_3 on tray placed near the bottom. All uneaten feeds were removed daily from the tray manually. The proximate compositions of local feed and commercial feed are shown in Tables 1 and 2, respectively.

Table 1. Proximate composition of local feed

Ingredients	Percentage (%)	Protein %
Rice bran	70	3.33
Mustard oil cake	30	13.65
Total	100	16.98

Source: BFRI

Table 2. Proximate composition of commercial feed (Saudi-Bangla Shrimp Nursery feed)

Food value	Percentage (%)
Moisture	11
Protein	30
Fat	4
Fiber	6
Ash	17
Carbohydrate	32

Source: Saudi-Bangla fish feed Ltd.

Throughout the experimental period, the water quality parameters were recorded weekly. Temperature (^{0}C), transparency (cm) and dissolved oxygen (mg/l) were measured between 7.00 and 8.00 am daily at the pond site. Alkalinity (m/l), pH and ammonia-nitrogen (mg/l) were measured weekly at Water Quality and Pond Dynamics Laboratory of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. Temperature and dissolved O_2 were measured by a digital DO meter (YSI model 58). Transparency was measured by using a secchi disc and pH with a pH electrode (Jenway, model 3020). Total alkalinity was determined titrimatrically. Ammonia-nitrogen (NH_3-N) was determined by HACH Kit (DR/2010 spectrophotometer). The chemical reagents used for this purpose were Nesslar reagent, mineral stabilizer and tri-methyl alcohol.

A good number of prawns PL (10-20 individuals) were sampled from each pond at every ten days interval and their length and weight of each individual was measured separately to assess the growth rate of prawns PL. At the end of the experiment, water was pumped out of the ponds and all prawn juveniles were collected, counted and weighed individually for each pond to assess the survival rate. Experimental data was collected and analyzed as follows:

a) Weight gain (g):

Weight gain = Mean final prawn weight − Mean initial prawn weight

b) Average daily gain (g):

$$ADG\ (g) = \frac{\text{Mean final prawn weight} - \text{Mean initial prawn weight}}{T_2 - T_1}$$

c) Specific growth rate (% day)

$$SGR\ (\%\ day) = \frac{Log_e W_2 - Log_e W_1}{T_2 - T_1} \times 100$$

Where,

W_1 = The initial live body weight (g) at time T_1 (day)

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

d) Survival rate = $\frac{\text{No. of prawn harvested}}{\text{No. of prawn stocked}} \times 100$

For the statistical analysis of the data, a one-way ANOVA and DMRT were done by using the SPSS (Statistical Package for Social Science) version-10.0. Significance was assigned at the 0.05% level. Duncan's test was used to tests the results of multiple ranges for comparisons of averages.

Results

The values of water quality parameters recorded from the experimental ponds during the study period are shown in Table 3. The average value of water temperature in T_1, T_2 and T_3 was 30.6±0.67 ^{0}C, 30.6±0.59 ^{0}C and 30.5±0.56 ^{0}C, respectively; transparency was 41.0±3.84 cm, 41.6±4.72 cm and 42.7±4.63 cm in T_1, T_2 and T_3, respectively; pH in T_1, T_2 and T_3 was 8.03±0.31, 7.89±0.25, and 7.81±0.23, respectively. Dissolved oxygen concentration recorded in T_1, T_2 and T_3, were 3.9±0.37 mg/l, 3.9±0.36 mg/l and 3.7±0.38 mg/l, respectively; total alkalinity in T_1, T_2 and T_3 were 118.7±5.58 mg/l, 126.3±5.46 m/l and 138.3±4.23 mg/l, respectively while the corresponding mean values of NH_3-N were 0.13±0.083 mg/l, 0.11±0.077 mg/l and 0.11±0.075 mg/l in T_1, T_2 and T_3, respectively. There was a significant difference ($p<0.05$) in some water quality parameters like pH and total alkalinity, but all other measured water quality parameters were within the acceptable range for nursing of prawn PL.

Table 3. Water quality parameters of different ponds (Mean ± SD)

Parameters	T_1	T_2	T_3	ANOVA
Temperature (^{0}C)	30.6 ± 0.67	30.6 ± 0.59	30.5 ± 0.56	NS
Secchi disc (cm)	41.0 ± 3.84	41.6 ± 4.72	42.7 ± 4.63	NS
pH	8.0^b	7.9^{ab}	7.8^a	*
DO (mg/l)	3.9 ± 0.37	3.9 ± 0.36	3.7 ± 0.38	NS
Alkalinity (mg/l)	118.7 ± 28.9^a	126.3 ± 28.4^{ab}	138.3 ± 21.9^b	*
Ammonia-N(mg/l)	0.13 ± 0.08	0.11 ± 0.08	0.11 ± 0.07	NS

* $p < 0.05$; NS, not significant

a, b and ab, superscript; Means with different superscripts are significantly *different* ($p < 0.05$)

The survival and growth of prawn PL as obtained from the different treatments are shown in Table 4. The initial mean lengths (cm) were 0.89± 0.008 cm, 0.88±0.01 cm and 0.87±0.03 cm in treatments T_1, T_2 and T_3, respectively; mean initial weights (g) were 0.022±0.00 g, 0.021±0.00 g and 0.022 ±0.002 g in treatment T_1, T_2 and T_3, respectively. At harvest, the mean final length gained by prawn post-larvae were 6.3±0.07 cm, 7.12±0.22 cm and 8.17± 0.16 cm in T_1, T_2 and T_3, respectively and the mean final weight gained by prawn post-larvae were 1.86±0.12, 3.14±0.33 and 4.26±0.44 g in T_1, T_2 and T_3, respectively. The variations in weight gain of prawn PL among the different treatments are shown in Fig.1. Average daily weight gains were 0.031±0.002 g, 0.052±0.006 g and 0.071±0.007 g in treatment T_1, T_2 and T_3, respectively. The specific growth rate (weight) obtained by prawn PLs were 7.42±0.11% per day, 8.35±0.022% per day and 8.81±0.26% per day in T_1, T_2 and T_3, respectively. Survival rates observed in T_1, T_2 and T_3 were 53.86±2.71% (only fertilizers), 60.52±1.64% (fertilizers with 5% local feed) and 66.24±1.58% (10% commercial feed), respectively. Significantly higher ($p<0.05$) growth performance and survival rate were observed in T_3 than T_2 and T_1.

Table 4. Growth performances of prawn larvae in different treatments (Mean ± SD)

Parameters	T_1	T_2	T_3	ANOVA
Initial length (cm)	0.89 ± 0.01	0.88 ± 0.01	0.87 ± 0.03	NS
Initial weight (g)	0.022 ± 0.00	0.021 ± 0.00	0.022 ± 0.002	NS
Final length (cm)	6.3 ± 0.07^a	7.12 ± 0.22^b	8.17 ± 0.16^c	*
Final weight (g)	1.86 ± 0.12^a	3.14 ± 0.33^b	4.26 ± 0.44^c	*
Av. daily gain (g)	0.031 ± 0.002^a	0.052 ± 0.006^b	0.071 ± 0.007^c	*
SGR (% per day)	7.42 ± 0.11^a	8.35 ± 0.22^b	8.81 ± 0.26^c	*
Survival rate (%)	53.86 ± 2.71^a	60.52 ± 1.64^b	66.24 ± 1.58^c	*

* $P < 0.05$; NS - not significant; SGR - Specific growth rate

Means with different superscripts are significantly different ($p < 0.05$)

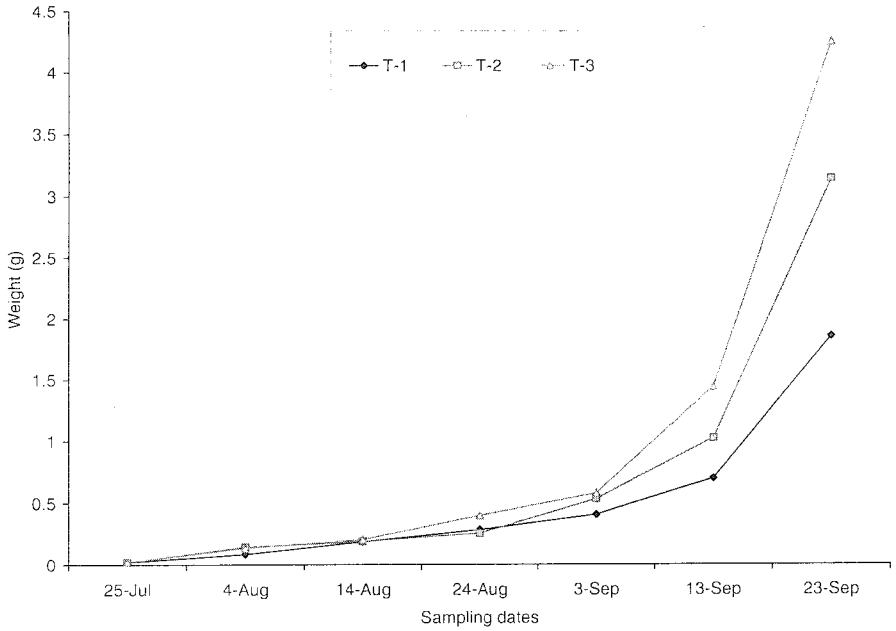

Fig. 1. Variations in weight gain of prawn PL among different treatments

Discussion

Water temperature in this experiment was found to vary from 28.9^{0}C to 31.6 ^{0}C over the entire period. The suitable temperature for nursing of prawn post-larvae was 27-31^{0}C (Fujimura 1974). However, the best growth of post-larvae at the temperature of 28^{0}C (Kneale and Wang 1979). Hoq *et al.* (1996) recorded that water temperature ranged from 27.5 to 30.5 ^{0}C was suitable for the growth of freshwater prawn. The water transparency was found from 32 to 51cm, which was more or less similar to the study of Latif *et al.* (1986), Azim *et al.* (1995). Wahab *et al.* (1995) reported that the transparency of productive water bodies should be 40 cm or less. and having the range from 2.2 to 6.5 mg/l, which was more or less similar to the study of the Hasan (1998), Paul (1998) and Mollah and Haque (1978) for the BAU campus ponds, Mymensingh. Wulff (1982) reported that juveniles of freshwater prawn could tolerate minimum oxygen levels of 1.0 to 1.5 mg/l and suggested not to allow the prawns at such levels for long time. Boyd and Zimmermann (2000) observed that the ideal environment for nursing of prawn post-larvae should have alkalinity of 20-60 mg/l.

pH values varied from 6.83 to 9.43 which were more or less similar to the findings of Jia-Mo *et al.* (1988), Hoq *et al.* (1996) and Hossain *et al.* (2000). According to Wickins (1976), the post-larvae of prawn could endure (without stress) total NH_3-N concentration of approximately 1.00 mg/l for some times. Straus *et al.* (1991) reported

that prawn juveniles should not be exposed to NH_3-N concentration higher than 1 mg/l or 2 mg/l at pH values of 9 and 8.5, respectively for a long period.

The authors *viz.* Sandifer and Smith (1975), Smith and Sandifer (1979), Kneale and Wang (1979), Shaha *et al.* (1989) have shown that prawn PL stocked at the rates between 100-700/m^2 for 45-60 days of nursery system resulted in final survival rates of 60-80%. Though Smith *et al.* (1983) reported that about 90% survival of prawn PL at stocking densities of 1000-1500/m^2 in an enclosed nursery system, only 28-37% survival has been reported by Angell (1994), for nearly same range of stocking density, in a case nursery system in Bangladesh condition. Chi and Oanh (1988) found that the survival rates of prawn post-larvae stocked at 10, 15 and 20 PL/m^2 were 56.1, 54.1 and 47.7% after 90 days, respectively. Nursery rearing of *M. rosenbergii* post-larvae in earthen ponds is limited (Williams and Berrigan 1977). Though Saha *et al.* (1989) reported 52% survival at the stocking density of 175 PL/m^2 in 30 days of rearing in earthen ponds and a mean final weight of 1-2 g after 40-90 days may be achieved in earthen pond nursery. The results in growth performances of *M. rosenbergi* in the present study more or less agreed with the above studies and it was clearly observed that the growth and survival of *M. rosenbergii* post-larvae were better in fed ponds where 30% protein rich commercial feed was supplied. Therefore, it may be recommended to the nursery that protein rich feed is a prerequisite for achieving success in nursery operations.

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Effects of stocking density on growth and production of GIFT (*Oreochromis niloticus*)

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Abstract
The study was carried out to assess the effects of stocking density on growth and production of GIFT for a period of 100 days. Three stocking densities were used 150, 200 and 250 fish/decimal; designated as treatment T_1, T_2 and T_3 respectively having two replicates for each. Commercial pellet feeds were fed at the rate of 30% body weight up to first 10 days and then gradually it was readjusted to 22%, 18%, 15%, 12% 10%, 8%, 6%, 5% and 4% respectively after every 10 days interval. The result showed that the fish in the treatment T_1 stocked with the lowest stocking density (150 fish/dec) resulted in best individual weight gain (148.65g) followed by those in treatment T_2 and T_3 respectively. The specific growth rates (SGR) at every 10 days were ranged from 6.59 to 1.11 in different treatments during the experimental period. The food conversion ratio (FCR) values ranged between 1.82 to 2.03 with treatment T_1 showing the lowest FCR. The survival rate ranged between 84 to 92 %. Treatment T_1 and treatment T_2 showed significantly higher survival than Treatment T_3. The fish production rate in treatment T_1, T2 and T_3 were 18.58, 23.87 and 26.78 kg/decimal respectively.

Key words: GIFT, Stocking density

Introduction

The GIFT strain was developed by the International Center for Living Aquatic Resources Management (ICLARM) through several generations of selection from a base population involving eight different strains of Nile tilapia, *Oreochromis niloticus* (Eknath *et. al.* 1993). GIFT tilapia is a hardy fish, which can survive in shallow and turbid water conditions and is a good converter of organic matter into high quality protein. Its rapid growth rate and tasty flavour (Balarin and Haller 1982, Pullin and Lowe-McConnel 1982) has been identified as one potential species. It also indicates that tilapia has made a significant contribution to food production, poverty alleviation and livelihoods support in the Asia and the Pacific. To optimize utilization of small water bodies for productive fish culture, researches have undertaken to identify suitable species for short

cycle aquaculture and developed low-cost management systems for optimizing production.

Stocking density is an important parameter in fish culture operations, since it has direct effects on the growth and survival and hence on production. It is an established fact that growth rate of fishes progressively increase as the stocking densities decreases and vice-versa. This was because of relatively less number of fish in a pond of similar size could get more space, food and dissolved oxygen at the same time. Stocking densities and management measures practiced by pond operators in Bangladesh are not based on scientific knowledge, thus resulting in poor growth and survival of fry. To obtain maximum economic returns it would be necessary to stock the ponds at optimum stocking densities for optimum growth in relation to inputs and productivity of the water body. The study was undertaken to study the effect of stocking density of GIFT tilapia culture under the same feed and management conditions.

Materials and methods

The experiment was carried out for a period of 100 days during February to May, 2007 in six selected experimental ponds of "*Nagla Fisheries Ltd.*", Haluaghat, Mymensingh. The size of each pond was 80 decimal and the ponds were indicated by the numbers 1, 4, 2, 5, 3, 6 respectively. The ponds were similar in respect of depth, basin configuration and pattern including water supply facilities. The water depth was maintained at a maximum of 1.2 m. There was well organized inlet and outlet system to maintain suitable water level. The ponds were surrounded by fine meshed nylon nets to prevent entering of frogs and fish eating animals. Water quality was maintained properly through exchange of water during the experimental period.

Experimental design

The ponds were selected randomly. The experimental layout is shown in Table 1.

Table 1. Experimental layout of GIFT tilapia culture

Treatment	Replication (Pond No.)	Pond Size (decimal)	Stocking density (nos./dec)	Total no. of stocking	Average wt. at stocking (g)
T-1	R-1 (1)	80	150	12000	3.0
	R-2 (4)	80	150	12000	3.0
T-2	R-1 (2)	80	200	16000	3.0
	R-2 (5)	80	200	16000	3.0
T-3	R-1 (3)	80	250	20000	3.0
	R-2 (6)	80	250	20000	3.0

After draining out of water, the ponds were fully dried and subsequently crushed limestone was spread on the pond bottom @250 kg ha^{-1}. After 3 days ponds were filled with water from deep tube-well and then cow-dung was applied @1000 kg/ha for phytoplankton production. The water depth was maintained at around 1.2 meter throughout the experiment. Fish were stocked after one week of fertilization.

The GIFT tilapia fries were collected from the Reliance Hatchery, Trishal, Mymensingh and transported to the farm in oxygenated polythene bags covered by jute bags. The fries were released to the culture ponds after sufficient acclimatization. Locally available commercial pellet feeds named "Quality feeds" was selected for the experiment. This pellet feed was examined and used due to having appreciable stability in water and nutritive value within the normal range. The proximate compositions of different types of "Quality feeds" are given in Table 2.

Table 2. Proximate composition of different types of tilapia feeds of "Quality Feeds Ltd" used for this experiment

Constituent	Amount (%)			
	Nursery-1	Nursery-2	Starter	Grower
Moisture	11	10	9	9
Protein	36	34	32	30
Lipid	8	7	7	7
Ash	6	5	5	5

At the beginning of the experiment feed was supplied at the rate of 30% of the body weight of reared GIFT and gradually it was readjusted to 22%, 18%, 15%, 12% 10%, 8%, 6%, 5% and 4% respectively after every 10 days interval. They were fed four times daily up to 30 days, then three times daily up to 70 days and then twice daily up to the end of the experiment. The feeding strategy is shown in Table 3.

Table 3. Feeding strategy for different types of feeds

Culture period	Types of supplied feed	Feeding frequency	Feeding rate (% of body weight)
1-10 days	Nursery-1	4 times	30%
11-20 days	Nursery-2	4 times	22%
21-30 days	Nursery-2	4 times	18%
31-40 days	Starter	3 times	15%
41-50 days	Starter	3 times	12%
51-60 days	Starter	3 times	10%
61-70 days	Starter	3 times	8%
71-80 days	Grower	2 times	6%
81-90 days	Grower	2 times	5%
91-100 days	Grower	2 times	4%

Analysis of water quality parameters

Water temperature (°C), pH, dissolved oxygen (DO), transparency (cm) and total alkalinity were recorded at 10 days interval between 9.00 A.M. and 11.30 A.M. using a digital meter (YSI model 58). Secchi disc was used to measure the transparency (cm) of the water. Total alkalinity was measured by Acid titration method.

GIFT tilapia sampling procedure

Sampling of tilapia was done at every 10 days interval using a cast net to observe the growth for adjustment of feeding rate was. Twenty fish were sampled from each pond. Weight of each fish was measured to assess the differential growth. The sampled fish were handled very carefully to avoid the handling stress.

Statistical analysis of data had been done to see whether the influence of different treatments (stocking densities) on the growth (weight) and production of fishes were significant or not. One way analysis of variance (ANOVA) was done to test the significance of difference among different treatment means. Significant differences among different treatment means were identified by Duncan's New Multiple Range Test (DMRT).

A simple economic analysis was done to estimate the net profit from different treatments. The cost of leasing of the ponds was not included in total cost. An additional 7.5% on total cost was included as operational cost according to ADCP (ADCP, 1983).

Results

Water quality parameters

The overall mean values of each water quality parameter of all treatments during the study period have been presented in Table 4.

Table 4. Water quality parameters (mean value ± S.E.) as recorded in different treatments during study period

Treatments	Parameters			Calculated
	T_1	T_2	T_3	F-Ratio
Temperature (°C)	28.66±0.33 (21.0-32.3)	28.90±0.06 (21.4-32.5)	29.21±0.04 (21.8-32.9)	4.17
Dissolved oxygen (mg l^{-1})	5.20±0.06 (4.88- 5.75)	5.08±0.04 (4.73-5.45)	4.96±0.04 (4.55-5.20)	13.19
pH	7.57±0.01 (6.4-8.5)	8.00±0.04 (6.9-8.9)	8.20±0.04 (6.9-9.0)	167.46
Total alkalinity (mg l^{-1})	101.18±0.00 (82-116)	101.23±0.06 (87-122)	96.82±1.29 (84-107)	23.13
Transparency (cm)	19.57±0.68 (17-23)	22.59±0.71 (18.5-28)	24.61±1.06 (19.5-29)	18.50

Growth in relation to stocking density

The growth rate of GIFT tilapia under different stocking densities were recorded 10 days interval and the results have been presented in the Table 5. This result indicates higher growth in weight (g) at lower stocking densities and the growth rate gradually decreased with increasing densities. For the evaluation of growth performance of fish in different treatments in terms of weight gain, average daily gain, specific growth rate (SGR % per day), food conversion ratio (FCR), survival (%) and production (kg/decimal/100 days) were calculated and are shown in Table 5.

Table 5. Growth parameters of GIFT tilapia observed in different treatments.

Growth parameters	Treatments		
	T_1	T_2	T_3
Mean initial weight (g)	3.0±0.25	3.0±0.25	3.0±0.025
Mean final weight (g)	151.65±4.74	141.2±3.96	130.85±2.19
Weight gain (g)	148.65±4.74	138.2±3.96	127.85±2.19
Average daily gain (g)	1.48±0.05	1.38±0.04	1.27±0.02
SGR (% per day)	3.92±0.03	3.85±0.03	3.78±0.02
FCR	1.82±0.02	1.92±0.03	2.03±0.01
Survival rate (%)	92.31±0.26	87.46± 0.08	84.52±0.38
Production (kg/decimal)	18.58±0.03	23.87±0.06	26.78±0.07

There was no significant ($p \leq 0.05$) difference in initial weight of fish under different treatments. In the study period the significantly higher mean weight gained by GIFT tilapia was 155.0 g in Treatment T_1. There were highly significant differences among treatment T_1, treatment T_2 and treatment T_3 in terms of weight gain when compared using ANOVA ($p \leq 0.05$). From the Table 5 the significantly highest average daily gain (1.48 g) found in treatment T_1. Significant differences were found among three treatments when compared using ANOVA ($p \leq 0.05$). The mean specific growth rate of GIFT tilapia in different treatments ranged between 1.11 and 6.59. The significantly ($p \leq 0.05$) highest SGR values (6.59) was recorded in treatment T_3 while the lowest (1.11) was obtained also in treatment T_3. Significant differences were found among three treatments when compared using ANOVA ($p \leq 0.05$).

The survival (%) in different treatments was fairly high. The survival ranged between 84 to 92 %. Treatment T_1 and treatment T_2 showed significantly higher survival than Treatment T_3. The mean values of survival (%) were 92.31±0.26, 87.46± 0.08 and 84.52±0.38 for treatments T_1, T_2 and T_3 respectively. There were significant differences among the three treatments when compared using ANOVA ($p \leq 0.05$).

The food conversion ratio (FCR) values among the treatments were ranged between 1.82 to 2.03. The significantly lowest i.e. the best FCR (1.82) was obtained with treatment T_1 while the highest (2.03) FCR i.e. the worst was obtained with treatment T_3.The mean values of FCR were 1.82±0.02, 1.92±0.03 and 2.03±0.01for treatments T_1,

T_2 and T_3 respectively. There were significant differences among the three treatments when compared using ANOVA ($p \leq 0.05$).

Fish production

The production of GIFT tilapia ranged between 18.58 to 26.78 kg/decimal/100 days in different treatments. The total production of fish in treatment T_1, T_2 and T_3 were 2,972 kg, 3,820 kg and 4,285 kg. In present study the highest production was 4285 kg and it was found in the treatment T_3 which was higher than treatment T_1 and T_2.

Economic analysis

A simple economic analysis was performed to estimate the net profit from this culture operation. The cost of production was based on the Mymensingh whole sale market price of the year 2007 in consideration of the inputs used. The prepared feed costs were calculated as Tk. 23.00/kg, 22.00/kg, 20.00/kg and 19.00/kg for nursery-1, nursery-2, starter and grower. The selling prices of GIFT tilapia were of Tk. 70.00/kg, 68.00/kg and 66.00/kg for treatment T_1, T_2 and T_3 fish. The cost of leasing ponds was not included in the total cost. An additional 7.5% on total cost was included in operational cost according to ADCP (1983). It was observed that the highest net profit (Tk. 246.88/decimal/100 days) was obtained with stocking density of 150 fish/decimal (T_1) while the lowest profit (Tk. 118.26/decimal/100 days) was obtained with stocking density of 250 fish/decimal (T_3).

Table 6. Economic analysis of GIFT tilapia production of the end at the study period

Investment (Tk.)	Treatments		
	T_1	T_2	T_3
Lime + Cowdung	1500	1500	1500
Cost of fry	24000	32000	40000
Feed cost	131280	168800	206600
Operational cost	11758.5	15172.5	18607.5
Total cost	168538.5	217472.5	266707.5
Cost of production/kg fish	56.71	56.93	62.24
Selling price/kg fish	70.00	68.00	66.00
Gross income (Tk.)from fish sale	208040	248304.2	278533.9
Net profit	39501.5	30831.73	11826.41
Net profit/dec./100 days	246.8844	192.6983	118.2641

Discussion

Water quality parameters

The results of the water quality parameters were found within the acceptable range of fish culture and all of them were more or less similar without any abrupt changes in

any parameters of the ponds. Murty *et al.* (1978) reported negligible monthly fluctuations in the values of physico-chemical properties of water in the fertilized and unfertilized ponds. During the present study, the highest temperature recorded in the month of May was 32.9°C in T_3 and the lowest 21.0°C in T_1 were found in February. The mean values of water temperature of the ponds under treatment T_1, treatment T_2 and treatment T_3 were 28.66±0.33, 28.90±0.06 and 29.21±0.04 respectively. Kabir (2004), Chowdhury (1998) found almost similar results. The observed value of pH (6.4 t0 9.0) recorded in present study indicate that pH in all treatments were within the range and suitable for fish culture. The concentration of dissolved oxygen (DO) in the present experiment was found 4.62 to 5.75 mg l^{-1}. The mean values of dissolved oxygen content obtained with treatments T_1, T_2 and T_3 were 5.20±0.06, 5.08±0.04 and 4.96±0.04 respectively. The lowest concentration of oxygen was observed in ponds (T_3); this might be due to over population that rapidly consumed the dissolved oxygen. The observed alkalinity levels (82 to 122 mg l^{-1}) of water of the experimental ponds indicate that the productivity of the ponds was medium to high. The observed secchi disc value ranged from 17.00 to 29.00 cm in different treatments. The mean values of transparency were 19.57±0.68, 22.59±0.71 and 24.61±1.06 for treatments T_1, T_2 and T_3 respectively.

Growth performances of GIFT

The effects of stocking density on growth and production of GIFT tilapia was investigated in this experiment. It was found that the growth rates varied in different stocking densities. The highest growth rate was found in treatment T_1 which was stocked with lower densities (150/dec) although same food was supplied in all treatments at an equal ratio. The lowest growth rate was obtained in the present experiment under the highest stocking rate. Leboute *et al.* (1994) obtained highest weight gain in lower stocking densities compared to high stocking densities, in case of tilapia culture in cages. Cruz and Ridha (1989) found no significant differences in mean individual final weight, daily growth rate and survival rate among three stocking densities, in case of (*O. spilurus*) in nursing phase for 68 days in cages. The average daily weight gain and specific growth rate in the treatment T_1 was significantly higher than that of T_2 and T_3 which might be due to less competition for feed in lower stocking density.

The percent survival as recorded in the present study varied between 84 to 92 %. The survival rate recorded in present study is higher than that the survival rate recorded by Hussain *et al.* (1987), which might be attributed to the relatively larger size of fingerlings (15g). The mean values of survival (%) were 92.31±0.26, 87.46± 0.08 and 84.52±0.38 for treatments T_1, T_2 and T_3 respectively. Treatment T_1 and treatment T_2 showed significantly higher survival than Treatment T_3. Survival rate was found to be negatively influenced by different stocking densities. It might be due to high competition of food and space among the fishes.

The total production of fish in treatment T_1, T_2 and T_3 were 1858 kg/acre/100 days, 2388 kg/acre/100 days and 2678 kg/acre/100 days. Although the mean weight gain in

treatment T_1 was highest but total production was highest in treatment T_3 which might be due to higher number of fishes. The present result supports the findings of Dimitrov (1976) who achieved the best production from higher stocking densities when compared to that achieved with the lower ones.

A simple economic analysis was performed to estimate the net profit from this culture operation. During the economic analysis it was found that gross profit was highest (Tk. 278,534) in treatment-T_3 where (Tk. 208,040) in treatment-T_1 and (Tk. 248304) in treatment-T_2 respectively, which might be due to higher stocking density but net profit was higher in case of lower stocking density. It was observed that the highest net profit (Tk. 246.88/decimal/100 days) was obtained with stocking density of 150 fish/decimal (T_1) while the lowest profit (Tk. 118.26/decimal/100 days) was obtained with stocking density of 250 fish/decimal (T_3). Thus, the results of the present study indicated that a stocking density of 150 fish/decimal is optimum for GIFT tilapia culture with formulated feed.

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Investigation on water quality in the Ashulia *beel*, Dhaka

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Abstract
The study was conducted to get an idea about the water quality of the Ashulia *beel*, and its temporal change over wet and dry seasons due to change of the physicochemical parameters. The water body has become a dumping ground of all kinds of solid, liquid and chemical wastes of bank side population and industries. Encroachment and illegal dredging has become a serious threat for the sound environment of the *beel*. The water parameters of pH 7.1-7.8 and alkalinity 30-63 mg/l in wet, and pH 7.1-8.4 and alkalinity 90-115 mg/l in dry season, respectively, which were within the standard range of DoE investigation. During wet season, EC 130-310 mg/l, TDS 80-132 mg/l, DO 1.1-2.1 mg/l and BOD -4.4-1.6 mg/l were measured. In dry season, EC 341-442 mg/l, TDS 207-276 mg/l, DO 0.5-2.0 mg/l and BOD 1.0-3.0 mg/l were measured. The comparative analysis showed that most of the water quality parameters of the Ashulia *beel* were suitable for aquatic organisms including fishes while the DO contents were much lower than the desirable level which may not be suitable for fishes.

Key words: Ashulia *beel*, DO, BOD, Alkalinity

Introduction

The *beels* are large surface water bodies that accumulate the surface runoff water through internal drainage channels. These depressions are mostly topographic lows produced by erosions and are seen all over the Bangladesh. From the ecological point of view, the *beel* has the potential for restoring and conserving habitats for rich biodiversity of flora and fauna, and conserving specific species with local and global significance and with vital roles to maintain ecological balance in the locality (Haque *et al.* 2005). The *beels* are freshwater wetlands which play a vital role in the improvement of water quality. The quality of aquatic environment generally depends on four kinds of factors, such as physical, chemical, biological and meteorological factors. Water quality is controlled and determined by the combinations of all kinds of factors in various ways and intensities (Rahman 1992).

Just by assessing the physical, chemical and biological characteristics of water, one can conclude about its quality (Barthwal 2002). According to Sabbir *et al.* (2010), water quality focuses on the various aspects of physicochemical parameters that detect the status of pollution and suitability of a particular water body for various aquatic

organisms. Seasonal or annual variations in the availability of freshwater may at times cause water quality degradation (EEA 1999, EGIS 2002). The Department of Environment (DoE), Institute of Water Management (IWM) and Water Resource Planning Organization (WARPO) have monitored surface water level and quality and found continuous deterioration of water quality of the surrounding rivers and lakes which are close to industrial districts or areas (Rahman and Alam 2005).

Water resources of Dhaka city is the most important and is the burning issue in terms of extreme degradation of water quality of the surrounding water bodies, for example, rivers, lakes, ponds and canals. Huge quantities of industrial effluents; solid waste from river-side settlements; petroleum products from ships, launches, cargoes, boats; and untreated sewage regularly get dumped into the Buriganga, Balu, Turag and Shitalakshya rivers, which are already severely polluted (Rahman and Alam 2005). The Ashulia *beel* is located adjacent to Dhaka city which is connected with Turag river. Ashulia *beel* plays a vital role as catchments area in facilitating the drainage of water from Dhaka city in the wet season (Khan *et al.* 2007). In the present study, existing water quality parameters are emphasized for aquatic organisms in Ashulia *beel*. The water quality parameters are compared with the standard values of DoE as well as other relevant standards to know the present status of water quality of Ashulia *beel*. The study was made consciousness to the concerned authority in developing the present situation of the *beel* area and to make it environmentally sustainable.

Materials and methods

Study area

The Ashulia *beel* is located adjacent to Dhaka city which covers approximately 5,000 acres of low land connected with the Turag river. One branch of the Turag river extends over the north-eastern part of the Ashulia thana. The north and east of Ashulia mainly constituted of low lands which forms *beel*, locally known as Ashulia *beel*. The soil of the *beel* is of Madhupur tract on which sediments deposit each year during monsoon flood. Being low land, the lands remain submerged for 6 to 7 months due to monsoon in a year with a water depth of more than 180 to less than 275 cm (SRDI 1992). The water samples were collected for physiochemical analysis from Taltola (Site 1), Ashulia landing center (Site 2), Berulia (Site 3), Pam house (Site 4) and Sluice gate (Site 5) sites in wet (July-September, 2010) and dry (October-December, 2010) season, respectively. Each sampling sites were divided into four sampling stations or points, and from each sampling stations, 500 ml of water was collected by plastic bottles with double stoppers. Before sampling, the bottles were cleaned and washing with detergent solution and treated with 5% HNO_3 over night. The bottles were finally rinsed with deionized water and dried. After sampling, the bottles were screwed carefully and marked with the respective identification number.

Sample analysis

The water quality parameters such as temperature and pH were determined by the Thermometer and digital pH meter, respectively. Buffer solution containing pH 7.0 was used to calibrate the digital pH meter. Transparency was measured by Secchi Disc method. Electric conductivity (EC) and Total dissolved solids (TDS) were determined by digital EC meter and TDS meter, respectively. Dissolve oxygen (DO) was determined by digital DO meter where sodium thiosulphate (0.025N) was used as a reagent. Acidity was measured by titration with 0.05N NaOH after addition with phenolphthalein indicator which is known as Titration method. Alkalinity was measured by titration with 0.1N HCl after addition 2-3 drops of methyl-orange indicator. The EDTA method was used to determine the hardness of water where eriochrome black T was used as indicator and titration with EDTA solution. BOD was measured by two steps where initial BOD (BOD_1) was measured immediately after collection and after 5 days BOD (BOD_5) was measured by incubation in the dark condition at 20^{0}C for 5 days. Then the total BOD (BOD_1 - BOD_5) was measured according to Trivedy and Goel (1984), and Huq and Alam (2005).

Results and discussions

The water temperature was found 28.7-31.7^{0}C during wet season and 22.4-25.6^{0}C during dry season, respectively, which was found within the EQS (1997) standard ranged from 20-30^{0}C used for all purposes (Table 1). In the Ashulia *beel*, the temperature of the water samples descended from 31.7-22.4^{0}C in the month of July to December due to seasonal variation. The range of water temperature (wet season) of the studied *beel* indicated that almost suitable for fishes or aquatic habitat and breeding ground as well. The ranges of pH were investigated 7.1-7.8 during wet and 7.1-8.4 during dry season that confirmed the slightly alkaline nature of water of the beel (Figs. 1 and 2). The transparency of productive water bodies should be 40 cm or less (Rahman 1992). In this study, the transparency was found 6.85-21.50 cm during wet and 5.25-13.75 cm during dry season. It was indicated that the water of the studied *beel* was suitable for the aquatic organisms including fishes both in wet and dry season, because of transparency within the desirable range (Table 1). Due to current of water, it didn't possible to measure transparency in some sampling stations. In wet season the ranges of Electric Conductivity were 130-140, 200-210, 200-310, 200-210 and 200 μs/cm in Taltola, Ashulia landing center, Berulia, Pam house and Sluice gate, respectively. In dry season the ranges of EC were 420-435, 354-442, 341-427, 426-437 and 428-430 μs/cm in Taltola, Ashulia landing center, Berulia, Pam house and Sluice gate, respectively (Table 1). Due to seasonal variations, all sites showed lower EC value than the standard value of DoE (700 μs/cm). Among the five sites, Berulia and Ashulia landing center showed higher EC value during wet and dry season, respectively. The Taltola site showed lower TDS value in wet season than dry season. Other four sites showed relatively similar TDS value in the wet season but they were also lower than the standard limit of DoE (165

ppm). In the dry season, the TDS value ranged from 207-276 mg/l in the studied area which was higher than wet season and exceeded the standard limit (Table 1).

Table 1. Water quality parameters of the Ashulia *beel* in wet and dry season

Parameters	Sampling sites	Wet season (July-September)		Dry season (October-December)		Standard
		average*	range	average*	range	
Temperatures (^{0}C)	1	30.85		24.55		
	2	29.30		25.18		20-30 (EQS 1997)
	3	28.93	28.7-31.7	23.2	22.4-25.6	
	4	29.33		23.1		
	5	29.18		23.18		
Transparency (cm)	1	18.7		6.77		
	2	8.9		9.03		40 or less (Rahman 1992)
	3	9.9	6.85-21.50	8.27	5.25-13.75	
	4	9.2		9.5		
	5	9.05		13.25		
EC (μs/cm)	1	132.5		427.5		
	2	205		417.75		700 (EQS 1997)
	3	230	130-310	390.75	341-442	
	4	207.5		431.5		
	5	200		429		
TDS (ppm)	1	81.25		271.25		
	2	128.25		258.75		165 (Huq and Alam 2005)
	3	127.75	80-132	245.75	207-276	
	4	129.25		270.25		
	5	127		267.25		

*=average of 4 stations

The DO indicate the degree of pollution by organic matter, the level of decomposition of organic substances and level of self purification of water. Adequate DO is necessary for good water quality. Dissolved oxygen at levels of 3 ppm or lower should be regarded as hazardous to lethal under average stream and lake conditions (Ellis *et al.* 1946). The range of investigated DO was 1.1-2.1 mg/l during the wet and 0.5-2.0 mg/l during the dry season (Table 2). From the investigation, it was observed that the DO content was much lower than the desired limit of 5.0 (EQS 1997, EGIS 2002, Rahman 1992). So, the *beel* water quality was degraded and it was not suitable for fisheries and aquatic organisms.

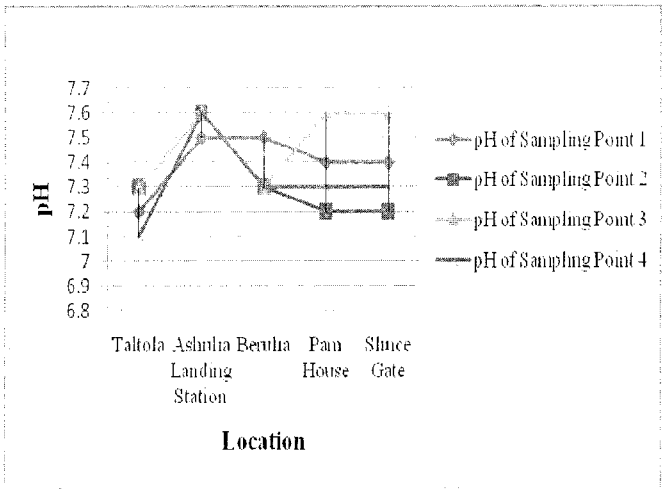

Fig. 1. The pH measured in Ashulia *beel* water during wet season.

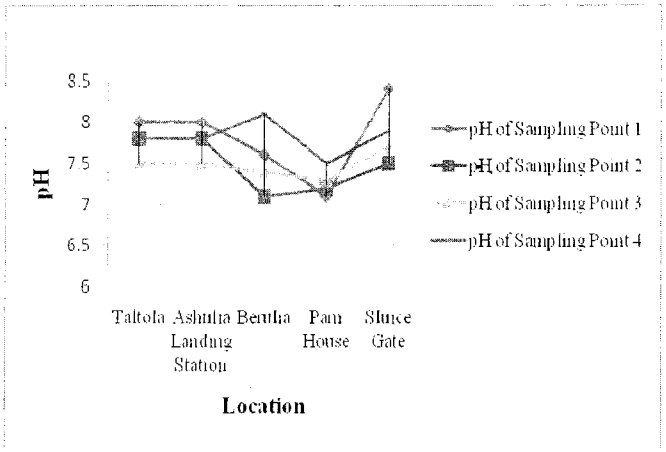

Fig. 2. The pH measured in Ashulia *beel* water during dry season.

The range of acidity along the Ashulia *beel* was 12.0-23.25 mg/l in the wet and 10.50-17.5 mg/l in the dry season (Table 2). According to Rahman (1992), total alkalinity more than 100 mg/l should be present in a highly productive waterbodies. Waterbodies having total alkalinity 40 mg/l or more are considered more productive than waterbodies of lower alkalinity (Mairs 1966). The concentration of alkalinity was found to vary from 30-63 mg/l in wet and from 90-115 mg/l in dry season. During the dry season, all the sites showed more alkaline water in comparison of the wet season (Table

2). A total hardness of 50 mg/l is considered as the dividing line between hardwater and softwater and 15 mg/l or more is suitable for fish culture (Swingle 1967). The value of hardness was found to vary from 30.0-91.3 mg/l in wet and from 115-127 mg/l in dry season (Table 2). During the dry season all the sites showed more hardness in comparison of the wet season. These variations were lower than the standard limit (123 mg/l) during wet season and around near the standard limit during dry season. Dry season showed about 3-folds higher hardness compared to wet season in Ashulia *beel*.

Table 2. Water quality parameters of the Ashulia *beel* in wet and dry season

Parameters	Sampling sites	Wet season (July-September)		Dry season (October-December)		Standard
		average*	range	average*	range	
Dissolved Oxygen (mg/l)	1	1.18	1.1-2.1	1.03	0.5-2.0	5.0 (EQS 1997)
	2	1.23		0.9		
	3	1.2		1.4		
	4	1.35		1.15		
	5	1.8		1.0		
Acidity (mg/l)	1	16.68	12.0-23.25	13.28	10.5-17.5	-
	2	20.19		13.35		
	3	12.25		11.8		
	4	12.88		11.81		
	5	13.77		11.54		
Alkalinity (mg/l)	1	61.25	30-63	112.35	90-115	>100 (Rahman 1992)
	2	50.88		110.85		
	3	30.75		111.38		
	4	38.0		91.75		
	5	57.98		111.29		
Hardness (mg/l)	1	30.9	30.0-91.3	116.0	115-127	123 (Huq and Alam 2005)
	2	51.13		117.25		
	3	35.95		119.88		
	4	36.5		118.65		
	5	32.7		124.0		

*= Average of 4 stations

The unpolluted waters typically have BOD values of 2 mg/l or less (Chapman 1996). BOD values were found to ranges from -4.42-1.6 mg/l in wet and 1.0-3.0 mg/l in dry season (Figs. 3 and 4). In dry season among the five sampling sites, Berulia (Site 3) showed the highest BOD concentration (3 mg/l). In the field study along the Ashulia *beel* revealed that the BOD concentrations higher than the desirable limit of drinking water (0.2 mg/l) in the dry season though fishing activities can be performed there. In wet season, BOD showed lower concentrations than the standard limit for fishes. So, this season revealed more or less positive condition of the water body.

Chatla *beel* is within the Hakaluki haor of the Sylhet division, in the northeast corner of Bangladesh. The Chatla *beel* represents the poor existing water quality that is being deteriorated day by day due to the different un-accepted human activities (Chowdhury *et al.* 2010, Jhingran and Pathak 1987). Peoples around Chatla *beel* use the water for their domestic and drinking purpose, and they also discharge their waste into that *beel* and consequently they are lacks of having safe drinking water (UNDP 2000).

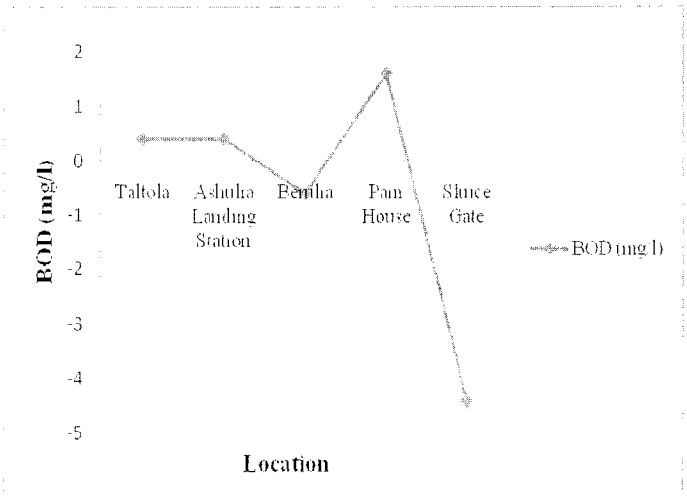

Fig. 3. Biological oxygen demand (BOD) of the Ashulia *beel* water during wet season.

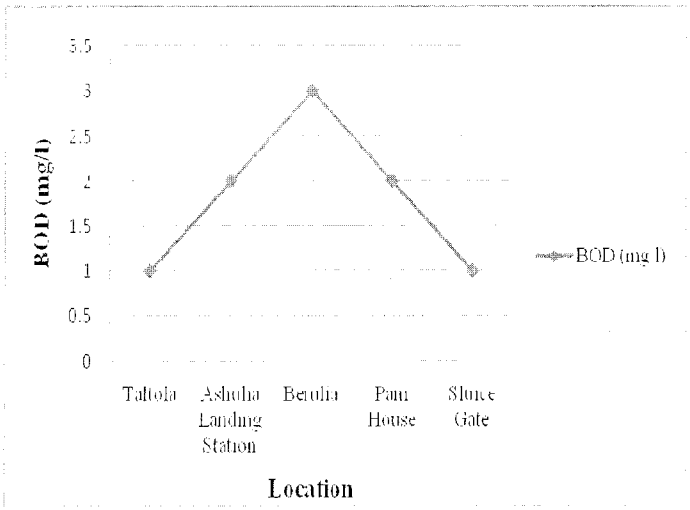

Fig. 4. Biological oxygen demand (BOD) of the Ashulia *beel* water during dry season.

The water qualities of the Ashulia *beel* and the Chatla *beel* were compared (Table 3). The water quality of the *beel* was evaluated based on the concentration of different parameters monitored above. The pH both in wet and dry season of Ashulia *beel* was suitable for fisheries where Chatla *beel* water was slightly acidic (Table 3). The DO value of Chatla *beel* was much better than Ashulia *beel* where the BOD value of Ashulia *beel* was lower than the Chatla *beel*. The hardness in Ashulia *beel* both in wet and dry season was within the standard limit where the hardness exceeded the limit in some points of Chatla *beel*. The alkalinity of Chatla *beel* was lower than the Ashulia *beel* water.

Table 3. Comparison of water quality parameters between Chatla *beel* and Ashulia *beel*

Parameters	Chatla *beel*	Ashulia *beel*	
		Wet season	Dry season
pH	6.5 - 6.9	7.1 - 7.6	7.1 - 8.4
DO (mg/l)	6.6 - 7.0	1.1 - 2.1	0.5 - 2.0
BOD(mg/l)	3.6 - 7.2	-4.42 - 1.6	1.0 - 3.0
Hardness (mg/l)	60 - 180	30 - 91	115 - 127
Alkalinity (mg/l)	25 - 35	30 - 63	90 - 115

The observed pH concentrations and transparency in Ashulia *beel* during both wet and dry seasons were within the standard limit which is sustainable for fisheries. Electrical conductivity (EC) was recorded higher in the Ashulia *beel* water during dry season. TDS content was much higher during dry season than the wet season. Total alkalinity of the sampled waters revealed the higher condition during dry season which was about 2-folds higher than the wet season and hardness was 3-folds higher when compared with wet season. The investigation revealed that higher BOD concentrations were recorded in dry season and Berulia site showed the highest BOD concentration. The waste effluents are disposed into the beel from the surrounding areas which are also responsible for higher BOD concentrations. The DO content was much lower than the desired limit. The average DO content was 1.35 mg/l in wet and 1.1 mg/l in dry season. The lower DO content is responsible for degradation of aquatic environment.

Surface water quality monitoring in Bangladesh is still very much limited and mostly on project based. Whatever the hydro morphological and water quality monitoring exists, mainly confined in the main river systems only. There is no regular monitoring or study on water quality of ponds, beels, etc so far and as a result no clear idea exists in this arena. In compliance to the study objective, investigation of water quality was done in this perennial water body (*beel*) to assess the quality of water, especially in the context of aquatic environment especially for fisheries. The investigation of COD, Nitrogen, Phosphorus, heavy metals and microbiological parameters such as total coliforms and fecal coliforms were not possible due to insufficient laboratory facilities. If the investigations of these parameters are possible then the water quality of *beel* water will be assessed properly. The *beel* is suffering from water quality problems which are a consequence of different water uses and the human

activities in the surrounding areas. If this condition is continuously going on then one day the *beel* will lose its existence and production capacities. For these reasons the study recommended to conserve the quality of the Ashulia *beel* water and its environment: i) regular monitoring of *beel* water quality with the standards of DoE, ii) industrial wastes and effluents must be treated before discharge into *beel* water, iii) halt encroachment, iv) restoration of aquatic habitat, v) keep records about fish species and their status, vi) illegal dredging must be stopped, vii) building awareness among the local people and conserve the *beel* with local participation, and viii) government should take initiatives to implement the recommended steps.

Acknowledgements

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Temperature effects on pathogenicity of selected *Edwardsiella tarda* strain to Japanese eel, *Anguilla japonica*

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Abstract
Temperature effect on the pathogenicity of selected *Edwardsiella tarda* V-1 strain to Japanese eel, *Anguilla japonica* was investigated. To evaluate the effects of both pathogen incubation temperature and fish cultivation temperature on pathogen pathogenicity a two-factor design was conducted. *E. tarda* was incubated at 15, 20, 25, 30 and 37±1°C, and the fish (mean weight: 100g) were reared at 15, 20, 25 and 28±1°C respectively. The fish reared at different temperatures were infected with the *E. tarda* incubated at different temperatures. The results of a 4-day LD50 test showed that temperature significantly affected the pathogenicity of *E. tarda* ($p<0.01$) and the interaction between the two factors was also significant ($p<0.01$). For fish reared at 20°C the pathogenicity of *E. tarda* was the highest at 30°C of pathogen incubation. When the fish rearing temperature was raised to 25 and 28°C, the pathogenicity of *E. tarda* incubated at all temperatures increased. Isolation testing demonstrated results similar to those of LD50. The selected isolate was virulent to eel, but pathogenicity varied with temperature.

Keywords: *Edwardsiella tarda*, Pathogenicity, Japanese eel (Anguilla Japonica)

Introduction

Edwardsiella tarda, the causative agent of edwardsiellosis in fish, is responsible for extensive losses in both freshwater and marine aquaculture. *E. tarda* infection of many commercially important cultured and wild fish has been reported, namely, channel catfish, eels, mullet, Chinook salmon, flounder, carp, tilapia, and striped bass (Thune *et al.* 1993). It has a wide host range, thus causing infection in higher vertebrates such as birds, reptiles (White *et al.* 1973), and mammals (Van Damme and Vandepitte 1984), including humans (Plumb 1993). In fish, it causes septicemia with extensive skin lesions, affecting internal organs such as the liver, kidney and spleen and muscle. These bacteria systemically avoid host defense mechanisms, thereby rapidly proliferating within the host and causing death. Pathogenesis of *E. tarda* is multifactorial, and many potential virulent factors have been suggested, namely dermatotoxins (Ullah and Arai 1983),

antiphagocyte killing (Ainsworth and Chen 1990), hemolysins (Hirono *et al.* 1997), serum resistance, and the ability to invade epithelial cells (Janda *et al.* 1991 and Ling *et al.* 2000). Although both virulent and avirulent strains were able to invade cultured cells in vitro, only the virulent strain could enter fish in large numbers via mucus, gills, and the gastrointestinal tract (Ling *et al.* 2001) and multiply inside various internal organs, causing death. It was also found that pathogenicity of *E. tarda* did not correlate with plasmid content, chemotactic motility, serum resistance, and expression of selected enzyme activities (Janda *et al.* 1991).

Serological studies have been carried out on strains isolated from Japanese eel, Japanese flounder, sea bream, and so on. The strains were classified into several serotypes. Pathogenicity of strains belonging to serotype A was the highest (Park *et al.* 1983 and Mamnur *et al.* 1994b). Some substances that may relate to the pathogenicity have been reported, such as siderophore (Kokubo *et al.* 1990 and Mathewl *et al.* 2001), hemolysin (Kusuda and Kitadai 1993), superoxide dismutase (SOD) (Yamada and Wakabayashi 1998), extracellular products (ECPs) and intracellular components (Icc) (Suprato *et al.* 1995). The invasion of *E. tarda* was also studied (Ling *et al.* 2000), however, very little is known about the pathogenesis of *E. tarda* in disease occurrence.

The present study was carried out to investigate the interaction of *E. tarda* with fish and environmental conditions that affect the occurrence of fish diseases, specially the effects of temperature on the pathogenicity of *E. tarda* to Japanese eel.

Materials and methods

Bacterial strain and growth conditions

E. tarda strain V-1 which is originally isolated from kidney of diseased eel (Anguilla japonica) in Japan. Sixty-one different serotypes of *E. tarda* have been differentiated according to the O-antigen (Tamura *et al.* 1998). *E. tarda* strain V-1 was temporally differentiated into serotypes by a cross absorption test of the O-antigen (Tamura *et al.* 1998). Different serotype strains of *E. tarda* V-1 strain used as the strain for antigen preparation and infection to test the vaccine efficacy against Edwardsiellosis (Liu *et al.* 2005). The bacterial strain *E. tarda* V-1 was used in this study to prepare a vaccine, was pre-cultured for 24 h at 30°C in brain heart infusion (BHI, Difco) broth and was inoculated into 1000 ml BHI broth, cultured with shaking at 30°C for 18 h. The cells were harvested by centrifugation at 4000×g for 15 min at 4°C and were stored at −80°C freezer until used. In the temperature experiment, *E. tarda* was incubated on plates covered with cellophane at 15±1°C for 15 days, 20±1°C for 10 days, 25±1°C for 5 days, 30±1°C for 3 days and 37±1°C for 1 day respectively. The medium contained yeast extract 1500, beef extract 1500, tryptone 5000, glucose 1000, NaCl 3500, K2HPO4 4800, KH2PO4 1320 and agar 15000 mg/L.

Experimental fish

Test fish, Japanese eels *Anguilla japonica* of an average weight 102.8±6.6g

(mean±SD, N = 25) were obtained from an eel farm in Yoshikawa at Kochi Prefecture, Japan and the fish had no previous occurrence of infection with *E. tarda* in this farm. Approximately 10 fish in each group and also for control were reared in 100 l tanks with well-aerated flowing water at 25°C. Fishes were fed with 0.5mm commercial dry pellets (Nissui) corresponding to 3% of the fish body weight per day for the entire experiment.

Infection and LD50 test

In order to evaluate the effects of temperature in both pathogen incubation and fish rearing, a two-factor experiment was designed. The temperature for the incubation of *E. tarda* was designed with five levels at 15, 20, 25, 30 and 37±1°C and the water temperature for the eel rearing at four levels, namely, 15, 20, 25 and 28±1°C. *E. tarda* was washed down from the cellophane with sterile saline solution. The bacteria of each temperature or salinity treatment were fivefold diluted into four dilutions. In the temperature experiment, the pathogen concentration was 106–109 CFUml/L when the fish rearing temperature was at 15±1°C, and 106–108CFU ml/L at 20, 25 and 28±1°C. Each dilution of 0.1ml was intraperitoneally injected into each fish in the three replicate tanks to ensure that each fish was infected with exactly same dose of the bacteria. The fish were fasted after injection, but other daily management practices were kept the same as before. After injection, the 4-day LD50 results were calculated. The results were expressed as Log CFU ml/L. A relatively short period (4 days) was used for LD50 calculation to more precisely evaluate the effect of pathogen incubation conditions (temperature) on its pathogenicity. A longer evaluation period probably abates the effects of the environmental factor treatments for pathogen incubation, because the pathogen and host will live in the same environment after challenge and then its pathogenicity probably changes with its environment in a longer duration.

Infection and isolation test

Based on the results of LD50 test, the concentration of *E. tarda* was varied in the different treatments of the isolation test. In temperature experiment, the pathogen concentration of the dilutions was 3×108 CFU ml/L when water temperature was at 15±1°C, and 3×107 CFU ml/L at 20±1, 25±1 and 28±1°C. Each dilution was injected intramuscularly into 20 fish with 0.1 ml per fish. Two fish in each group were randomly sampled to remove liver collection at 24, 48 and 72 h after injection. Fish were anaesthetization with 0.03 ml/L of 2-phenoxyethanol (ethylene glycol monophenyl ehter C6H5OCH2CH2OH, Nacalai Tesque. Inc, Japan). Liver was weighed and homogenized in a glass homogenizer containing 10 ml of sterile saline. The suspensions were diluted 10-fold to a series of dilutions. Each dilution of 0.1ml was incubated on plates of SS-agar at 25°C. For the 24 h sampling, the first to third dilutions of the suspensions were used for plate incubation, the second to fourth and third to fifth dilutions were used for the incubations of 48 and 72 h samplings respectively. After 48 h incubation the black clones of *E. tarda* were counted. The results were expressed as Log CFU g/L liver.

Statistical analysis

Data from each treatment were subject to one-way ANOVA, two-way ANOVA or t-test where appropriate. When overall differences were significant ($p<0.05$), Tukey's test was used to compare the mean values between individual treatments (Zar 1984). Statistical analysis was performed using the StatPlus 2007 Professional.

Results

LD50 test

In the temperature experiment, the LD50 test results are shown in Table 1. The results showed that both the pathogen incubation temperature and challenge temperature significantly affected the pathogenicity of *E. tarda* ($p<0.01$) and the interaction between them was also significant ($p<0.01$). At 20°C fish rearing temperature, the highest pathogenicity of *E. tarda* was observed at 30°C of pathogen incubation, followed by 15, 20, 25 and 37°C. When the fish rearing temperature was raised to 25 and 28°C, the pathogenicity of *E. tarda* incubated at all the temperatures increased. The mean value of LD50 decreased from 1010.3 to 107.9 CFU ml/L, and to 107.5 CFU ml/L respectively. The pathogen incubated at 37°C had a more obvious increase in its pathogenicity to Japanese eel. Its pathogenicity, however, was still lower than those incubated at 20 and 25°C. Incubation at 15°C always resulted in relatively lower pathogenicity.

Table 1. LD50 of *Edwardsiella tarda* incubated at different temperatures to *Anguilla japonica* reared at 15, 20, 25 and 28°C, respectively (mean Log CFU ml/L±SD)*

Fish rearing temperature	Pathogen incubation temperature				
	15°C	20°C	25°C	30°C	37°C
15°C	7.98±0.97ay	7.79±0.43by	7.65±0.21by	7.32±1.11by	8.89±0.29ay
20°C	9.91±1.38abx	9.92±0.23abx	8.63±0.21bx	11.40±1.84bx	11.92±0.56abx
25°C	8.27±0.11ay	7.27±0.30cz	7.18±0.14cz	7.18±0.11by	8.19±0.10ay
28°C	8.12±0.14ay	7.17±0.30cz	7.01±0.10cz	7.38±0.11by	8.57±0.12ay

Two-way ANOVA	F-value	P-value
Pathogen incubation temperature	112.83	9.27E−14
Fish rearing temperature	17.11	7E−05
Interaction	3.66	0.00311

* Means in the same row or column sharing a common superscript letter (a, b, c for rows, and x, y and z for columns) are not significantly different as determined by Tukey's test ($p>0.05$).

Isolation test

Table 2 showed that the pathogen incubation temperature significantly affected the proliferation rate of *E. tarda* in the liver of Japanese eel at 20°C of fish rearing temperature ($p<0.05$). The slopes of linear regression of *E. tarda* proliferation rates indicated that the bacteria incubated at 20°C had the highest proliferation rate during the 72 h infection test, followed by 25, 30 and 37°C. The slopes at 37 and 15°C were significantly lower than the slopes at 20, 30 and 25°C. At 20°C of fish rearing, the number of isolated *E. tarda* incubated at 15°C maintained at the lowest level, and the numbers of isolated *E. tarda* incubated at 20, 25, 30 and 37°C were not significantly different from each other ($p>0.05$) after 72 h infection. All the remaining fish died by the fifth day.

Table 2. Proliferation of *Edwardsiella tarda* incubated at different temperature in the liver of Japanese eel reared at 20°C (mean Log CFU g/L liver±SD)*

Incubation temperature	Time			Linear regression slope
	24 h	48 h	72 h	
15°C	4.33±0.58	4.54±0.12	4.87±0.11	0.0112b
20°C	4.25±0.35	5.54±0.36	6.22±0.61	0.0398a
25°C	5.43±0.18	6.43±0.43	6.76±0.39	0.0431a
30°C	5.49±0.32	5.65±0.43	6.61±0.63	0.0487ab
37°C	5.23±0.51	5.77±0.23	6.43±0.35	0.0190b
Analysis of covariance		F-value	P-value	
		6.321	$0.001<p<0.0025$	

* Slopes sharing a common superscript letter are not significantly different as determined by Tukey's test ($p>0.05$).

When water temperature of fish rearing was at 25°C (Table 3), the results showed that *E. tarda* incubated at 20°C could not be isolated during the 3 days, whereas the numbers of other treatments came up to about 106 CFU g/L liver. At the third day after infection the number of *E. tarda* incubated at 20°C was the highest, followed by 25, 30 and 37°C, but no significant difference between them was found ($p>0.05$). The remaining fish that were injected with *E. tarda* incubated at 20°C remained alive for 10 days till the experiment termination, but the others died by the fifth day. Again, the bacteria incubated at 20°C had the highest proliferation rate; the slopes of 20, 25, 30 and 37°C were 0.0743, 0.0554, 0.0734 and 0.0787 respectively. However, analysis of covariance showed that their proliferation rates were not significantly different ($p>0.05$).

Table 3. Proliferation of *Edwardsiella tarda* incubated at different temperature in the liver of Japanese eel reared at 25°C (mean Log CFU g/L liver±SD)*

Incubation temperature	Time			Linear regression slope
	24 h	48 h	72 h	
20°C	4.21±0.14	6.12±0.21	6.71±0.11	0.0743
25°C	3.88±0.32	5.61±0.21	6.28±0.25	0.0554
30°C	3.11±0.12	4.80±0.21	6.14±0.58	0.0734
37°C	2.97±0.76	3.95±0.56	5.92±0.86	0.0787

Analysis of covariance	F-value	P-value
	1.645	0.1<P<0.25

* *E. tarda* incubated at 15°C could not be isolated from the fish liver during the 72 h infection.

Discussion

It has been confirmed that *E. tarda* exists in the environment of eel farms even when the disease does not occur (Mamnur *et al.* 1994). Therefore, measures to establish or control the rearing environment to inhibit or reduce the pathogenicity of the pathogen are very important for aquaculture. In the present study, we looked that the pathogenicity of *E. tarda* is significantly influenced by incubation temperature and also the infection temperature. The pathogenicity of *E. tarda* was the highest at 25–37°C, and lowest at 15 and 20°C. This is accordance with the fact that edwardsiellosis has higher incidences when the water temperature is high. Statistical analysis revealed a significant interaction between the growth temperatures of pathogen and host. When the water temperature of fish culture increased, the LD50 values declined. Relatively higher temperature increased the proliferation rate of the pathogen. The pathogen incubated at 20°C could be isolated from fish infected at 20°C, but not be isolated from the fish infected at 25°C. This was likely due to the fact that the pathogen concentration used in the infection at 20°C was 3×108 CFU ml/L, but that used at 25°C was 3×107 CFU ml/L. This low concentration of the pathogen incubated at 20°C may not be able to proliferate fast enough to kill the fish. Additionally, higher temperature may also affect the immune response of the flounder. However, this requires further investigations to confirm. Considering the above conditions, it would be useful to maintain a relatively lower water temperature to alleviate edwardsiellosis as long as eel growth is not affected. Obviously, it is worth expanding study scope on the effects of environmental factors on pathogen pathogenicity to modify existing disease control strategies or to design new ones for aquaculture.

Lapaglia and Hartzell (1997) have been confirmed that environmental stresses can affect the physiological characters of microbes. *Archaeoglobus fulgidus*, an anaerobic marine hyperthermophile, can form a biofilm in response to environmental stress. *Deleya halophila* can produce several induced proteins in response to oxidative stress (Mylona and Katinakis 1992). Non-halophilic purple and green sulphur bacteria in response to osmotic stress can accumulate surrose and trehalose respectively (Welsh and Herbert 1993). Stephens *et al.* (1991) reported that growth temperature, dose size and route of infection affected the pathogenicity of *Listeria monocytogenes* strains to the mouse, and that some genes of this pathogen have been proved to be involved in stress response and pathogenicity (Cormac and Colin 1999).

Pathogen and host certainly interact with each other. The inner environment of fish is obviously different from that of the incubation medium. Its living environment also influences the immunity of fish. On the other hand, temperature may affect the production of some substances that contribute to the pathogenicity of *E. tarda*. This means that conditions that support fast proliferation of pathogens may not produce substances enhancing their pathogenicity. 10 strains of *E. tarda*, showing that neither 25 nor 35°C incubation temperature had any effect on the outer membrane protein (OMP) profiles of nine out of 10 *E. tarda* isolates (Darwish *et al.* 2001). In the present study, the conditions that produced lower LD50 values generally supported higher proliferation rates of this pathogen in the fish. It remains unknown, which contributes more to the pathogenicity of *E. tarda* for Japanese eel faster proliferation in fish tissue or some unknown secreted substances. From the study of Darwish *et al.* (2001) and the present one it can be seen that the OMP is possibly associated with the pathogenicity of *E. tarda*. OMP has been considered to be a factor related to the pathogenicity (Goullet *et al.* 1994).

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Microbiological quality study of *Macrobracium rogenbergii* (de man 1879) during storage at -20^{0}c temperature

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Abstract
The shelf life of fresh water prawn *Macrobrachium rosenbergii* by applying low temperature was investigated. *M. rosenbergii* preserved at -20^{0}C was subjected for quality assessment before storage and at 15, 30, 45, and 90 days of storage period. The quality assessments as done microbiological *viz.* total bacterial count (TBC), total mould count (TMC), total yeast count (TYC), total coliforn count (TCC) and salmonella count. All the samples were acceptable during 90 days because the upper limit of all spoilage indicator was not exceeding within the experimental time period.

Key words: *M. rogenbergii* Frozen storage, Shelf life extension

Introduction

Prawn and shrimp is the principal foreign exchange earring commodities among the frozen sea food of Bangladesh. Preservation methods keep the fish in afresh state so that the changes in texture, taste and appearance etc. are minimized. Bacteria and autolytic spoilage are biological systems which operate under certain optimum conditions. Therefore, altering these conditions can prevent or reduce spoilage. As bacteria require water and are sensitive to temperature, salt concentration and pH, a number of approaches can be used, control of autolysis is affected by controlling enzyme activity. The commonly used and practiced way of reducing autolytic action is to lower temperature. (Clucas and ward 1996). Generally, fish from tropical water have more bacteria than those from temperature zone and marine fish has higher bacteria than fresh water fish (Shewan 1976).

Difficulties with the international quality standard are being faced by some tropical prawn exporting countries including Bangladesh. The difficulties in meeting those standard are evident from the high level of rejection of prawn and shrimp exports by the importing countries with problem such as decomposition, high total bacterial counts, filth unexpected foreign matter and food born microbial organisms. The rejections

represent not only the monetary loss to the exporter but also more significantly, injury to the factory. In the year 1997 EU commission imposed a temporary ban an our export for hygienic quality of our product. Eventually this ban became a blessing to our processing pants because, each factory hurriedly developed to a considerable extent through the introduction of new facilities in the factory. The successful development of a prawn industry depends not only on improved supply of raw material but also on the quality of exports, poor quality of the raw material and poor sanitary performance of freezing plant affect in frozen prawn and shrimps by filth and also the freezing of decomposed prawn are major problems encountered by the seafood industry of Asian countries. (Dziezak 1997).

Preservation method are designed to inhibit the growth of organism and killing the organisms in fish in fresh minimizing the changes in texture, taste and appearance etc. There are several method of preservation such as freezing, drying by chemical preservatives, irradiation etc. The chief methods of preservation of fish and fleshy product are low temperature, drying and irradiation and combination of the above. The bacterial flora of fish and enzymes present in the tissue are adapted to the temperature at which fish lives i.e. around 5-10^{0}C for fish from cold water and 25-30^{0}C for tropical fish. By lowering or raising the temperature, bacteria and autolytic spoilage rate will be reduced (Frazier and Wasthoff 1988). Hence the present study was under taken to determine the microbiological changes in the freshwater prawn samples before and storage at -20^{0}C temperature.

Material and methods

Collection of samples

The fresh water prawn, *Macrobrachium rosenbergii* was collected from the Kawran Bazar fish market, Dhaka. Then samples were taken in pre-sterilized high density polypyrol polythene bag with ice and immediately brought in the laboratory of Food Technology Division. IFRB, AERE. Savar, Dhaka.

Microbiological tests

The microbial changes i.e. total bacterial count (TBC), total mould count (TMC), total yeast count (TYC), total coliform count (TCC) and salmonella count were estimated by total bacteriological count technique following Wittfogel (1962). Colonics that developed on the plates after incubation at 37^{0}C and 30^{0}C for 24 and 48 hours were counted with the half of colony counter. The number of bacterial, mould, yeast. coliform and salmonella colonies per gram of the samples were obtained by multiplying the number of colonies with dilution factor. The count were expressed as colony forming unit (cfu) per gram (cfu/gm).

Results

Total bacterial colony TBC (cfu/gm) levels raised over duration (Table 1). The levels (2.1×10^3cfu/g) of TBC found after storage was nine times the level (2.1×10^3 cfu/g) before storage. However, levels of TBC in other sampling duration treatments were also significantly different.

Table 1. Values of TBC in fresh water prawn *Macrobrachium rosenbergii*

Duration	Total bacterial colony
Before storage	2.3×10^2
After 15 Days	4.6×10^2
After 30 Days	3.0×10^2
After 45 Days	1.2×10^3
After 90 Days	2.1×10^3

Total moulds count (TMC) (cfu/g) levels elevated over duration (Table 2). The level (6.0×10^2cfu/g) of TMC found 90 days after storage was more than five times the level (1.1×10^2 cfu/g) before storage. However, levels of TMC found in other sampling duration treatments were also significantly different.

Table 2. Values total mould count (cfu/g) in fresh water prawn *Macrobrachium rosenbergii*

Duration	Total bacterial colony
Before storage	1.6×10^2
After 15 Days	2.1×10^2
After 30 Days	3.1×10^2
After 45 Days	4.1×10^2
After 90 Days	6.0×10^2

Total yeast count TYC (cfu/g) levels over duration (Table 3). The level (1.6×10^3) of TYC found 90 days after storage was more than five times the level (3.1×10^2cfu/g) before storage. However, level of TYC found is other sampling duration treatments were also significantly different.

Table 3. Values of total yeast count (cfu/g) in fresh water prawn, *Macrobrachium rosenbergii*

Duration	Total bacterial colony
Before storage	3.1×10^2
After 15 Days	4.6×10^2
After 30 Days	6.5×10^2
After 45 Days	1.0×10^3
After 90 Days	1.6×10^3

Coliform was absent during 90 days of preservation. Salmonella was also absent before and after storage.

Discussion

Total bacterial count (TBC) in increased with the increase of storage period and recommended that TBC 1.0×10^6 cfu/g of fish flesh is considered as maximum allowable limit (Kader *et al.* 1988). In the present investigation, TBC was found 2.3×10^2 cfu/g in the sample before storage. At -20°C storage temperature TBC was obtained 2.1×10^3 cfu/g of prawn muscle at the and of 90 days. According to international commission on the microbiological specification of foods guideline, acceptable total bacterial count for fish is 10^6cfu/g. According to the above mentioned suggestions, preserved sample remained acceptable up to 90 days of storage period. To detect the days when level of TBC exceed the acceptable limit research should be conducted long time.

In case of total mould count (TMC), it was found that the population increased with the increase of storage period. In the present study TMC was found 1.1×10^2cfu/gm in the sample before storage. At -20°C storage temperature TMC was obtained 6.0 $\times10^2$cfu/gm of prawn muscle at the end of 90 days.. From the present study it was found that the prawn, samples before storage contained less mould than the samples which stored at -20°C. And with the increase of storage time, the stored samples obtained more mould than that of the samples before storage.

Total yeast count (TYC) increased with the increase of storage period. TYC was found 3.1×10^2 cfu/gm in the sample before storage. At -20°C storage temperature TYC obtained 1.6×10^3cfu/g of prawn muscle at the end of 90 days. From the current investigation it was found that the prawn samples before storage contained less yeast than the samples which stored at -20°C. And with the increase of storage time, the stored samples obtained more yeast than the samples which stored at -20°C. And with the increase of storage time, the stored samples obtained more yeast than that of the samples before storage.

During 90 days of preservation period the TCC was absent in the samples before storage and the samples stored at -20°C. The acceptable total coliform count for fish is less than 5.0×10^2 cfu/g (Shewan 1975). So, the entire samples was acceptable during

whole investigation period. During 90 days of storage period salmonella was absent in the samples before storage and the samples stored at -20^{0}C. Acceptable salmonella for fish is 25cfu/gm. So, the entire sample was acceptable during whale investigation period. To detect the days of storage when salmonella developed in sample, research should be conducted for a long time.

In the present study, attention was paid to investigate the effects of low temperature on fresh water prawn. To extent the shelf life of the fresh water prawn were stored at low temperature (-20^{0}C) for 90 days for determining the shelf life extension of there prawn sample some parameters such as total bacterial count, total mould count (TMC), total yeast count (TYC), total coliform count (TCC) and salmonella count (TSC) were used before storage and at 15, 30, 45 and 90 days of interval during the storage period.

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Effect of gamma radiation in combination with freezing on the microbiological changes in frozen shrimp *Penaeus monodon*

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Abstract
In this study gamma radiation (3, 6 and 9 kGy) in combination with low temperature (-20°C) were applied to retain the quality and shelf-life of shrimp, *Penaeus monodon* for a longer period. The quality was assessed by monitoring microbiological changes (TBC, TMC, TYC, TCC and *Salmonella* count) in irradiated and non-irradiated (control) samples. Among microbiological indicators of spoilage, total bacterial count (TBC) values for irradiated shrimps were found to be 1875, 1625 and 1525 cfug^{-1} of sample at 3, 6 and 9 kGy respectively after 90 days whereas for non-irradiated samples it was found 2475 cfug^{-1} of sample. Total moulds count (TMC) value for non-irradiated samples after 90 days were found 425 cfug^{-1} sample whereas that for irradiated shrimps at 3, 6 and 9 kGy were found to be 275, 250 and 200 cfug^{-1} sample respectively. Total yeast count (TYC) value for non-irradiated samples after 90 days were found 4125 cfug^{-1} sample whereas that for irradiated shrimps at 3, 6 and 9 kGy were found to be 2850, 2150 and 1725 cfug^{-1} sample respectively. Total coliform count and *Salmonella* count showed that those were absent during 90 days storage period. From this study, it was clear that gamma radiation in combination with low temperature showed shelf-life extension (90 days) in each dose of radiation used but during the use of 9 kGy radiation, *Penaeus monodon* showed best quality.

Keywords: *P. monodon*, Gamma radiation, Freezing, Microbiological properties

Introduction

Bangladesh has favorable conditions for shrimp farming. The coastal belt of the country in the south and southeast regions are endowed with favorable tides, salinity and also soil conditions to grow shrimps. Fish is extremely perishable and requires quick preservation. It becomes spoiled by the attack of various pathogenic microorganisms. To extend the shelf life of fish and fishery products, ice storage and rapid chilling, low temperature freezing, modified atmosphere packaging, organic acids, antimicrobials and irradiation techniques are used (Himelbloom *et al.* 1994, Masniyom *et al.* 2002, Al-

Dagal and Bazarra 1999, Gelman *et al.* 2001, Venugopal *et al.* 1999). Shrimps are preserved only by low temperature blocking but the self life of preserved fish is not satisfactory. Various experiments made it clear that no adverse health effects occur when irradiated foods are consumed, using mean doses of irradiation of up to 10 KGy (Rady *et al.* 1988). The validity of this technique is already recognized in many countries, including Canada, USA and the European Union (Tauxe 2003) for many food products including shrimps (Pszczola 1990).

Combination of food irradiation and low temperature refrigeration provide a means to increase shelf life of fish products. In this study effect of gamma radiation (3, 6 and 9 KGy) in combination of refrigeration (-20°C) were applied on shrimp *Penaeus monodon* to observe the increase in shelf life.

Materials and methods

Fish samples

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

Irradiation and storage conditions

Samples were irradiated using a $Cobalt^{60}$ radiation source. Doses applied in this study were 3, 6 and 9 KGy. Before irradiation the samples were kept in polypropylene/polythene bags under atmospheric condition. The non-irradiated and irradiated samples were subsequently stored at -20°C. All samples were examined at the 0, 15, 30, 45, 60, 75 and 90 days of storage period.

Microbiological changes

The total bacterial count (TBC) was determined by decimal dilution technique followed by pour plate technique (Sharf 1966). The microbial changes were estimated by total bacteriological count technique following Withfogel (1962). TBC, TMC, TYC, TCC and *Salmonella* are the valuable measures to assess the degree of freshness of fish.

Results and discussion

Total Bacterial Count (TBC) analysis

TBC values of non-irradiated and 3, 6 and 9 kGy irradiated samples stored at -20°C is shown in Table 1. The initial TBC values were 250, 150, 100 and 100 $cfug^{-1}$ respectively for control sample, 3, 6 and 9 kGy irradiated sample. The TBC values were

2475, 1875, 1625 and 1525 $cfug^{-1}$ respectively for control sample, 3, 6 and 9 kGy irradiated sample at the end of 90 days storage periods. Comparing the treatment, the lowest TBC value was found in the 9 kGy treatment group while the highest value was measured in the control group. The TBC, values were gradually increased with the progress of storage period. Under investigation best result of TBC value were found at the treatment dose of 9 kGy.

Table 1. TBC ($cfug^{-1}$) sampled from control and different treatments of doses in irradiated frozen shrimp during 90 days storage period at -20°C. Within column and rows means (±SEM) with different letters denote significant differences (ANOVA HSD, $p<0.05$).

Duration (day)	Level of Radiation (KGy)			
	Control	3	6	9
Control	250 ± 50^{e}	150 ± 50^{e}	100 ± 50^{f}	100 ± 0.00^{e}
15	500 ± 100^{de}	300 ± 50^{de}	175 ± 25^{ed}	225 ± 25^{d}
30	775 ± 75^{cd}	450 ± 50^{cd}	400 ± 50^{bc}	375 ± 75^{cd}
45	1025 ± 125^{bc}	725 ± 25^{bcd}	675 ± 75^{ab}	525 ± 25^{bc}
60	1375 ± 25^{abc}	1025 ± 125^{abc}	925 ± 75^{ab}	925 ± 75^{ab}
75	1800 ± 50^{ab}	1575 ± 75^{ab}	1275 ± 25^{ab}	1275 ± 75^{a}
90	2475 ± 175^{a}	1875 ± 75^{a}	1625 ± 75^{a}	1525 ± 125^{a}

Total bacterial count (TBC) increased with the increase of storage period. Shewan (1975) recommended that TBC 1.0×10^{6} cfu/g of fish flesh is considered as maximum allowable limit. At -20°C storage temperature control, 3 KGy, 6 KGy and 9 KGy irradiated fishes obtained 2475±175 cfu/g, 1875±75 cfu/g, 1625±75 cfu/g and 1525±125 cfu/g of fish muscle at the end of 90 days respectively. According to international commission on the microbiological specification of foods (ICMSF 1982) guideline, acceptable total bacterial count for fish is 10^{6}cfu/g. According to the above mentioned suggestions, irradiated samples remained acceptable up to 90 days of storage period. To detect the days when level of TBC exceed the acceptable limit research should be conducted long time.

Total mould count (TMC) analysis

TBC values of non-irradiated and 3, 6 and 9 kGy irradiated samples stored at -20°C is shown in Table 2. In case of total mould count (TMC), it was found that the population increased with the increase of storage period. At -20°C storage temperature control, 3 KGy, 6 KGy, 9 KGy irradiated fishes obtained 425±25 cfu/g, 275±25 cfu/g, 250±50 cfu/g and 200±0.0 0cfu/g of fish muscle at the end of 90 days respectively. From the present study it was found that the shrimp samples treated by 9 KGy dose contained less mould than all other treated and controlled sample. And with the increase of storage time, the control samples obtained more mould than that of irradiated samples. So, from the total mould

count it was found that 9 KGy irradiated fish sample give the best result as preservation method.

Table 2. TMC (cfug^{-1}) sampled from control and different treatments of doses in irradiated frozen shrimp during 90 days storage period at -20°C. Within column and rows means (±SEM) with different letters denote significant differences (ANOVA HSD, $p<0.05$)

Duration (day)	Level of Radiation (KGy)			
	Control	3	6	9
Control	75 ± 25c	00^{c}	00^{c}	00^{b}
15	100 ± 0.00^{bc}	50 ± 00^{bc}	00^{c}	00^{b}
30	175 ± 25^{abc}	100 ± 50^{ab}	50 ± 00^{b}	00^{b}
45	225 ± 25^{ab}	175 ± 25^{ab}	125 ± 25^{a}	75 ± 25^{a}
60	250 ± 50^{ab}	200 ± 50^{ab}	175 ± 25^{a}	125 ± 25^{a}
75	325 ± 25^{a}	250 ± 50^{ab}	200 ± 50^{a}	175 ± 75^{a}
90	425 ± 25^{a}	275 ± 25^{a}	250 ± 50^{a}	200 ± 0.00^{a}

Total yeast count (TYC) analysis

Total yeast count (TYC) increased with the increase of storage period. Yeast density was shown at Table 3. At -20°C storage temperature control, 3 KGy, 6 KGy and 9 1725 ± 75 cfu/g of fish muscle at the end of 90 days respectively. KGy irradiated fishes obtained 4125±425 cfu/g, 2850±200 cfu/g, 2150±100 cfu/g and 1725±75 cfu/g. From the present study it was found that the frozen shrimp samples treated by 9 KGy dose contained less yeast than all other treated and controlled sample.

Table 3. TYC (cfug^{-1}) sampled from control and different treatments of doses in irradiated frozen shrimp during 90 days storage period at -20°C. Within column and rows means (± SEM) with different letters denote significant differences (ANOVA HSD, $p<0.05$)

Duration (day)	Level of Radiation (KGy)			
	Control	3	6	9
Control	500 ± 100c	300 ± 100d	150 ± 50d	75 ± 25d
15	625 ± 25bc	475 ± 25cd	350 ± 50cd	175 ± 25cd
30	925 ± 125bc	850 ± 150bc	725 ± 125bc	450 ± 100bc
45	1100 ± 150b	1225 ± 175ab	1125 ± 75ab	825 ± 75ab
60	2500 ± 300a	1650 ± 150ab	1450 ± 150ab	1050 ± 150ab
75	3025 ± 75a	2100 ± 150ab	1825 ± 125a	1475 ± 175a
90	4125 ± 425a	2850 ± 200a	2150 ± 100a	1725 ± 75a

Total coliform count (TCC)analysis

During 90 days of storage period the TCC was absent controlled and all treated sample which is shown in Table 4. According to ICMSF (1986) guideline, acceptable total coliform count for fish is less than 500 cfu/g. So the entire sample was acceptable during whole investigation period.

Table 4. TCC ($cfug^{-1}$) sampled from control and different treatments of doses in irradiated frozen shrimp during 90 days storage period at -20°C

Duration (day)	Level of Radiation (KGy)			
	Control	3	6	9
Control	Absent	Absent	Absent	Absent
15	Absent	Absent	Absent	Absent
30	Absent	Absent	Absent	Absent
45	Absent	Absent	Absent	Absent
60	Absent	Absent	Absent	Absent
75	Absent	Absent	Absent	Absent
90	Absent	Absent	Absent	Absent

Salmonella count analysis

During 90 days of storage period *Salmonella* was absent controlled and all treated sample which is shown in Table 5. According to ICMSF (1986) guideline, acceptable *Salmonella* for fish is absent cfu/25g. So the entire sample was acceptable during whole investigation period. To detect the days of storage when *Salmonella* developed in sample research should be conducted for long time.

Table 5. *Salmonella* ($cfug^{-1}$) sampled from control and different treatments of doses in irradiated frozen shrimp during 90 days storage period at -20°C

Duration (day)	Level of Radiation (KGy)			
	Control	3	6	9
Control	Absent	Absent	Absent	Absent
15	Absent	Absent	Absent	Absent
30	Absent	Absent	Absent	Absent
45	Absent	Absent	Absent	Absent
60	Absent	Absent	Absent	Absent
75	Absent	Absent	Absent	Absent
90	Absent	Absent	Absent	Absent

Shrimp industries in Bangladesh are playing a significant role in the national economy. The present study concludes that irradiation (9KGy) is the best method for long time preservation of fresh fish. To extend the shelf-life of the frozen shrimp, were treated with gamma radiation (3 KGy, 6 KGy and 9 KGy) and stored at low temperature (-20^{0}C) for 90 days for determining the shelf life extension of these fish sample some parameters such as total bacterial count (TBC), total mould count (TMC), total yeast count (TYC), total Coliform count (TCC) and total *Salmonella* count(TSC) were used in every15 days interval. The maximum shelf life was found with radiation dose of 9 KGy.

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Production and quality assessment of fish pickles from mola (*Amblypharyngodon mola*) fish

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Abstract
Fish pickles (with olive and tamarind) were prepared from mola fish (*Amblypharyngodon mola*) and their nutritional and food quality were assessed. The quality of the pickle prepared with olive was excellent and the pickle prepared with tamarind was found good. Moisture content of the two pickle products were 43.85% (with tamarind) and 50.89% (with olive). The protein and lipid contents of tamarind added pickle were 19.13 and 35.64% respectively; pickle with olive contained less protein (13.16%) compared to tamarind added mola pickle. Lipid contents were almost same in both cases. Ash content of two pickles was also found similar (1.00%). The quality of mola pickles stored either in cool condition (4°C) with vinegar or at room temperature with Na-benzoate were found good for consumption up to 90 days of storage. All of the fish pickles preserved under different condition were found in acceptable condition up to 240 days storage and pickle with vinegar stored at 4°C was found good for consumption at the end of 240 days.

Key words: Fish pickle, Tamarind, Olive, Organoleptic quality.

Introduction

Among two hundred and sixty species of freshwater fishes available in Bangladesh, over one hundred and forty species are classified as small indigenous fish species (SIS). These fishes have been playing vital role providing the main source of animal protein for all rural and urban households as well. SIS's are also an important source of vitamin A, calcium and iron (Ahmed 1981, Wahab 2003). Among the SIS of Bangladesh, mola (*Amblypharyngodon, mola*n) bears prime importance in terms of availability and popularity. Unlike many other fish species mola is not seasonal fish and is available in ample quantity throughout the year (Kohinoor *et. al* 2001). Mola has high vitamin A content which is highest among 26 commonly consumed fish species of Bangladesh (Roos *et.al* 2002, Zafri and Ahmed 1981). Moreover bones of mola serve as a rich source of calcium and some other minerals as this species is normally cooked and eaten whole (Ghosh *et. al* 2004).

Pickling is one of the oldest methods used for preserving various food items including fish. It is largely remained as a household art in India. Pickling protects the food and also helps to retain its wholesomeness and nutritive value for a long time. A variety of methods have been reported for the preparation of fish pickle (Chandrasekhar *et al.* 1978, Vijayan *et al.* 1982, Muraleedharan *et al.* 1982, Bandyopadhyay *et al.* 1985). The method is essentially same for preparing all types of fish pickles and the manufacturing process is not very complicated and requires comparatively less capital investment. Pickles are widely consumed in many South and South-east Asian countries including Bangladesh. Like any other vegetable or fruit pickles, fish pickles have also gained popularity in the recent past. The demand for these types of ready to serve fishery products is increasing day by day among the non-vegetarian population in our country. Considering the importance of small fish in the Bangladeshi diet, mola was chosen in the study for preparing pickle and assessing its quality aspects. Information on the quality can give an idea about the nutritive value, food safety and acceptability of the pickled product from fish.

Materials and methods

Fresh mola fishes of average 2.53±0.3 gm body weight and 6.20 cm length were collected from a local market and transported to the Laboratory of Fisheries Technology, Bangladesh Agricultural University (BAU) in an insulated box with ice. Good quality mustard oil was collected from the market. Red chili, coriander and turmeric powder, garlic, cinnamon, cardamom, olive, tamarind and pachforon (mixed spice) etc. were used as ingredients. Vinegar and Sodium Benzoate were used as preservatives. After proper dressing 1 kg of processed mola was used for preparation of pickles with tamarind and another 1 kg of processed mola was used for preparation of pickles with olive. These samples were packed in sealed transparent glass bottles and stored separately both in ambient atmospheric temperature and cool condition at 4 C for 240 days. The quality of pickle samples were examined at 30 days time interval by examining the organoleptic aspects.

Processing and preservation of mola fish pickle with tamarind

After gutting, the samples were washed with salt water and then removed the water from fish. Then the fish were marinated with required amount of turmeric, red chili, coriander powder and salt for at least ½ an hour in a refrigerator at 4°C. Then lightly fried marinated fish in oil and then added tamarind sauce, required amount of chili powder, turmeric powder, coriander powder, garlic and salt. Required amount of spice such as cinnamon, pachforon (mixed spice), cardamom etc. are added toward the end of preservation. All the processes were done with adequate amount of the oil in the pan and total time required about 5-6 minute. The mixing continued until total mixture turned into dark. After cooking, the products were allowed to cool and then transferred it to an airtight bottle. Then required amount of Na-benzoate and vinegar was added to

bottles targeted for storage under ambient and cool temperature (4° C) respectively and were shaken thoroughly for proper mixing. Finally the products were stored at room temperature and cool condition at 4°C. A flow chart of mola fish pickle with tamarind has been presented in Fig.1.

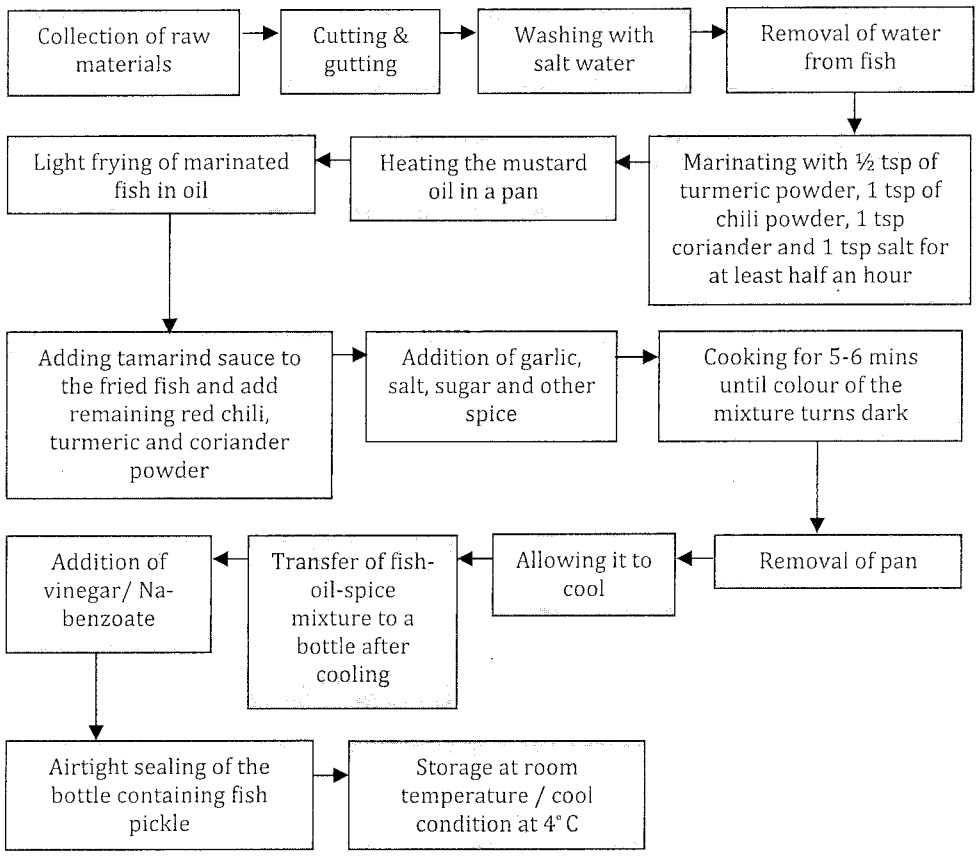

Fig. 1. A flow chart of mola fish pickle preparation with tamarind

Pickle composition

Ingredients		Mixture for marinating fish
1. Mola fish (Gutted): 1kg 2. Red chili powder: 4 tsp 3. Coriander powder: 3 tsp 4. Turmeric powder: 2 tsp 5. Garlic powder: 2 tsp 6. Pachforon: 2 tsp (Mixed spice)	7. Mustard oil: as required 8. Salt: to test 9. Sugar: as required 10. Tamarind (sauce): 6 tsp 11. Na-Benzoate: 5 g 12. Vinegar: 2 tsp	Turmeric powder: ½ tsp Red chili powder: 1 tsp Coriander powder: 1 tsp Salt: 1 tsp

Processing and preservation of Mola fish pickle with olive

After proper dressing, the samples were washed with salt water and then removed the water from fish. Then the fish samples were boiled with hot water. After de-boning the samples were mixed adding adequate olive paste then separately in the pan adequate amount of the mustered was heated to boil and then mixed with olive paste in the pan with subsequent addition of required amount of garlic, sugar and other spice. Then the total product in the pan was heated for 3-4 minute. After heating all the products were allowed to cool and then transferred it to an airtight bottle. By following the procedure mentioned above. Na-benzoate and vinegar was added to the pickle in two separate groups of bottles and stored under ambient and cool temperature (4° C) respectively. A flow chart of mola fish pickle preparation with olive has been presented in Fig 2.

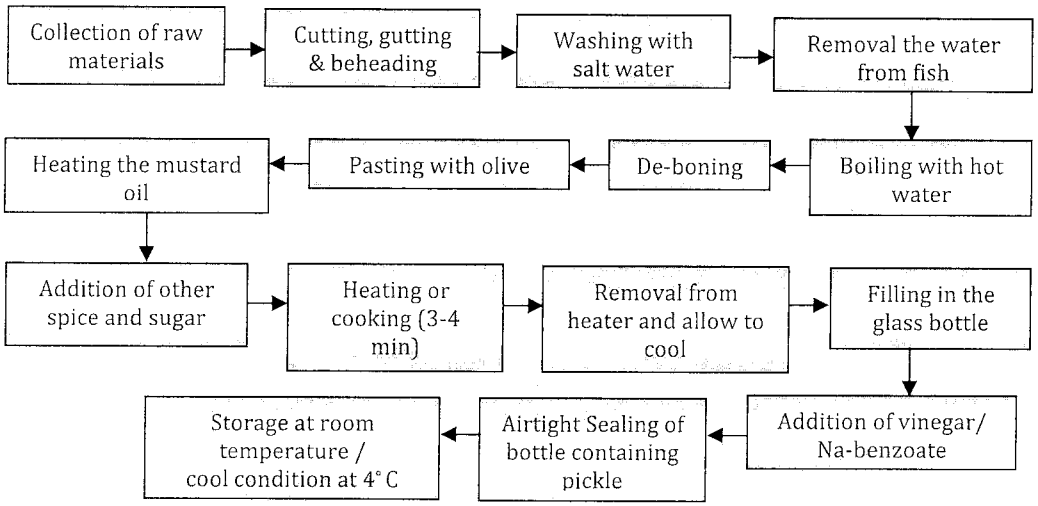

Fig. 2. A flow chart of mola fish pickle preparation with olive

Pickle composition

1. Mola fish: 1 kg	7. Mustard oil: as required
2. Red chili powder: 4 tsp	8. Salt: to taste
3. Coriander powder: 3 tsp	9. Sugar: as required
4. Turmeric powder: 2 tsp	10. Olive paste: 4 tsp
5. Garlic powder: 2 tsp	11. Na-Benzoate: 5 g
6. Pachforon: 2 tsp	12. Vinegar: 2 tsp

Organoleptic evaluation

Representative samples of pickle were taken on plate to assess the organoleptic characteristics such as general appearance, colour, flavour, taste, texture etc. and were evaluated by 5 member panel experts constituted in the Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh. The following set of guidelines (Table 1 & 2) has been prepared to get the maximum value from them by being able to compare the results.

Table 1. Determination of defect points of mola fish pickles

Characteristics of The product	Defect characteristics	Defect points	Quality
1. Colour	1. Pinkish red	1	Excellent
	2. Brownish red	3	Good
	3. Yellowish Brown	4	Average
	4. Blackish	5	Bad
2. Flavour	1. Natural flavour	1	Excellent
	2. Faint sour odour	2	Good
	3. Slight moderate odour	3	Average
	4. Moderate to strong odour	4	Bad
3. Taste	1. Feeling very good after chewing	1	Excellent
	2. Feeling good	3	Good
	3. Feeling average	4	Average
	4. Feeling bad	5	Bad
4. General appearance	1. Bright, shining	1	Excellent
	2. Loss of brightness	2	Good
	3. Slight dullness	3	Good
	4. Definite dullness	4	Average
	5. Dull	5	Bad
5. Consistency of flesh	1. Firm and elastic	1	Excellent
	2. Loss of elasticity	2	Good
	3. Moderately soft	4	Average
	4. Limp and floppy	5	Bad

able 2. Grading chart for mola fish pickles

Grade	Defect point	Degree of freshness
A	<2	Excellent
B	2 to 4	Good
C	4 to <5	Average
D	5	Reject

Biochemical analyses

Biochemical analyses such as estimation of moisture, crude protein, lipid and ash were carried out according to the methods given in AOAC (1980) with certain modifications. All determinations of fish pickles were done in triplicate and the mean value were reported.

Results and discussion

Organoleptic characteristics of mola fish pickle

The result of the organoleptic characteristics of the pickles immediately after processing was evaluated by four members of panel of experts. The organoleptic parameters such as general appearance, colour, flavour, texture, taste and overall quality of the products were examined. The organoleptic assessment of mola pickles with olive and tamarind immediately after are presented in the Table 3.

Table 3. Organoleptic characteristics of mola fish pickle

Product	General appearance	Colour	Flavour	Taste	Consistency of flesh	Grad e	Overall quality
Pickles with olive	Bright shining	Brown yellowish or Brownish red	Natural odour	Feeling very good after mouth chewing	Loss of elasticity	A	Excelle nt
Pickles with tamarind	Bright shining	Pinkish red or Brownish red	Natural odour	Feeling good after mouth chewing	Firm and elastic	B	Good

The general appearances of the both products were bright and shiny with yellowish-brown color or reddish-brown colour. The flavour of the product was natural. According to panel members, the taste of the products was very good after mouth chewing. In the pickles produced with tamarind, the consistency of the flesh of the product was firm and elastic whereas, the pickle produced with olive, there was a loss of elasticity in the flesh of the product which is in agreement with Vijayan *et. al* (1982). The overall quality of the pickles produced with olive was excellent. On the other hand the pickle produced with tamarind was good.

Table 4. Organoleptic quality assessment of prepared fish pickles under different storage conditions

Sample	Characteristics (Colour, flavour, texture, taste, consistency of flesh)	Storage time (days)						
		0	30	60	90	120	180	240
Mola pickle with olive vinegar added (cool condition at 4 C)	Colour	Excellent	Excellent	Excellent	Good	Good	Brown yellowish	Brown yellowish
	Flavour	Excellent	Excellent	Good	Good	Good	Good	Good
	Taste	Excellent	Excellent	Good	Good	Good	Average	Average
	Consistency of flesh	Good	Good	Good	Good	Moderately soft	soft	Soft
	Overall quality	Excellent	Excellent	Good	Good	Good	Acceptable	Acceptable
Mola pickle with olive Na-Benzoate added (Room temperature)	Colour	Excellent	Excellent	Excellent	Good	Brown yellowish	Brown yellowish	Brown yellowish
	Flavour	Excellent	Excellent	Good	Good	Faint sour odour	Slight moderate odour	Slight moderate odour
	Taste	Excellent	Excellent	Good	Good	Average	Average	Average
	Consistency of flesh	Good	Good	Good	Good	Moderately soft	Soft	Soft
	Overall quality	Excellent	Excellent	Good	Good	Acceptable	Acceptable	Acceptable
Mola pickle with tamarind vinegar added (cool condition at 4 C)	Colour	Excellent	Excellent	Excellent	Good	Good	Good	Good
	Flavour	Good	Good	Good	Good	Good	Good	Good
	Taste	Good	Good	Good	Good	Average	Average	Average
	Consistency of flesh	Good	Good	Good	Good	Slightly loss of elasticity	Slight loss of elasticity	Moderately soft
	Overall quality	Good	Good	Good	Good	Acceptable	Acceptable	Acceptable
Mola pickle with tamarind Na-Benzoate added (Room temperature)	Colour	Excellent	Excellent	Excellent	Good	Good	Brownish red	Brownish red
	Flavour	Good	Good	Good	Good	Slight off flavour	Slight moderate odour	Slight moderate odour
	Taste	Good	Good	Good	Good	Average	Average	Average
	Consistency of flesh	Good	Good	Good	Good	Good	Slight loss of elasticity	Slight loss of elasticity
	Overall quality	Good	Good	Good	Good	Acceptable	Acceptable	Acceptable

Changes in organoleptic characteristics of pickles during storage

Mola pickles prepared with olive and preserved in vinegar and Na-benzoate were stored at 4°C and at room temperature respectively for 240 days. In another batch, mola pickles were prepared with tamarind preserved similarly for 240 days. The quality of these products were evaluated by 4 members panel of expert time to time and the results of the quality assessment for fish pickles stored under different conditions are presented in Table 4. The quality of all the products stored either in cool condition at 4°C or at room temperature was considered good for consumption up to 90 days of storage. Then quality of all the products changed rapidly with further storage period and at the end of 240 days of storage colour in most of the products changed. However the taste of the olive and tamarind added mola pickles preserved with vinegar was found average when stored at 4°C after 240 days. Moreover colour and flavor of pickles (olive and tamarind added) with vinegar remained good for longer period than those treated with Na-benzoate. But no considerable difference of vinegar and Na-benzoate was observed with regard to taste and consistency of flesh. Present findings were in agreement with Erichsen and Molin (1964) who reported a prolonged shelf life of products like fish marinades and pickles (containing acetic, citric or lactic acids) as various micro organisms are easily destroyed in high acid (below pH 4.5) environment.

Biochemical composition

The biochemical compositions of two pickled products were analyzed and the results are presented in Fig. 3. The overall results of the proximate analysis of two types of fish pickles indicated some variation in their composition.

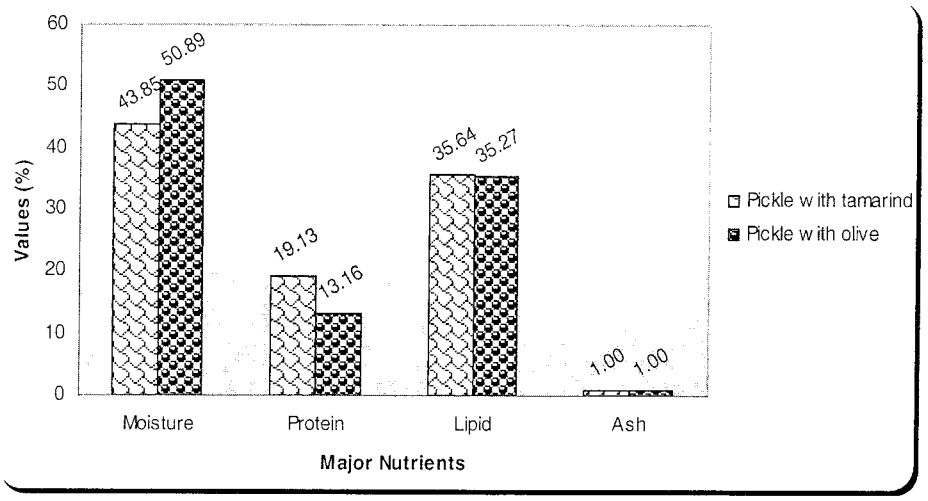

Fig. 3 Biochemical composition of mola fish pickle

Moisture content of the two products varied from 43.85 to 50.89% with the higher value recorded for mola pickles produced with olive. On wet weight basis, protein content varied from 13.16 to 19.13% with the higher value obtained from mola pickles produced with tamarind and lower value for mola pickles with olive. Lipid content varied from 35.64 to 35.27% on wet weight basis. There were little or no changes in ash content which were within 1.00% on wet weight basis. It was found that nutritional value of the pickled products with regard to major biochemical nutrient contents reported by Ghosh *et. al* (2004) and Bandyopadhyay *et. al* (1985) remain more or less unimpaired.

From the present study, it was observed that the nutritional quality of mola fish pickle prepared with tamarind and olive was quite satisfactory. Organoleptic assessment of product indicated that pickling can be a very efficient and practical way of preservation of mola fish even in ambient temperature for a considerable period of time. This can also augment the animal protein and vitamin-mineral availability for the general people of the country.

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Use of eye lens diameter and weight as an age indicator in the carangid fish, *Decapterus russelli* (Pisces: Carangidae) from Gulf of Oman: Preliminary observation

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Abstract
Specimens of *Decapterus russelli* have been collected from Lema, north of the Gulf of Oman. The ocular lens diameter and weight were tested as an additional age indicator to those already in use. The results showed that this technique could be adopted for determining the age of the species *Decapterus russelli* when the specimens are in the second year of age in case of eye lens diameter. On the other hand, eye lens weight failed to separate between the four age groups observed. The method is especially useful for age determination when otolith or scale ring are not visible or when false rings give erroneous reading.

Key words: Eye lens diameter, Eye lens weight, Ageing, *Decapterus russelli*

Introduction

Eye lenses as an age indicator have been applied to a wide variety of animals since proposed by Lord (1959) and used this technique for birds and animals other than fish Friend (1967). Teska and Pinder (1986) used eye lens weight to determine the effect of nutrition on age determination in vertebrates. The application of this technique is so limited in these animals, it being only possible to distinguish between juveniles and adults. Several authors concluded that both eye lens parameters (lens diameter and weight) can be used to estimate the age of fishes (Carlton and Jackson 1968, Crivilli 1980, Saleem *et al.* 1990, Douglas 1987, Al-Hassan *et al.* 1991, 1992, Al-Hassan and Al-Sayab 1994, Conides and Al-Hassan 2000, Jawad 2001, 2003, 2004, Jawad *et al.* 2001).

Age determination is an important step in the process of studying growth in fish species. The method involves counting of scale or otolith annuli and usually requires the measurements of a large number of specimens (Fletcher 1991). Otolith and scale readings require a variable and considerable effort to prepare each specimen and even then the readings are subject to both systematic and random errors in interpretation and

require independent validation (Beamish 1979). Thus, a considerable time is needed to acquire the skill necessary for consistent interpretation of the materials. Hence, the aim of this study is to determine the validity of the eye lens diameter and weight as age indicators in the Gulf of Oman fish, *Decapterus russelli* and to establish a faster method for ageing fishes beside the conventional methods of scale and otolith.

Materials and methods

Specimens of *Decapterus russelli* (395 nos.) were collected from the Lema north of the Sea of Oman (Gulf of Oman) during the period March to May 2010. Fishes were taken to the laboratory and the diameter and weight of the eye lens were taken to the nearest mm and g respectively following the procedure of Jawad *et al.* (2004). The lenses were extracted, dried at room temperature (25°C). The measurement of the lens in each side of the animal was kept separate. The large bone such as operculum and preoperculum were used to determine the age following Al-Hassan and Al-Sayab (1994). The bones on both left and right sides were twice read independently, using an ordinary dissecting microscope for verification. One way analysis of variance followed by Duncan's multiple range test (Harraway 1997) were applied to test the differences between the total length of the fish and its age.

Results and discussion

The age of *Decapterus russelli* samples ranging from less than one year to two years was determined. The total length observed in different age classes of the species in question showed that body size is variable within an age class and considerable overlap exists between these age classes ($p>0.05$). This is considered as one of the reasons for using eye lens diameter as an age indicator (Fig. 1).

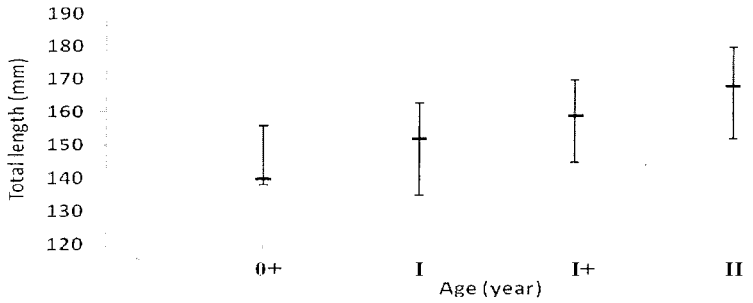

Fig. 1. Total length *vs* age (determined from opercular bone) of *Decapterus russelli* Vertical bars represent range of fish total length and horizontal lines represent mean fish length.

The average lens diameter showed a considerable increase with age for the species under consideration (Fig. 2). This increment is obvious in fishes belonging to age class I^+ and II ($p>0.05$). The overlap in lens diameter between young of the year class and Classes I & I^+ invalidates any accurate age determination for fish samples younger than two years of age. Carlton and Jackson (1968) and Jawad (2001) reached the same conclusion with carp and tilapia respectively when working on a small sample size and with fish not older than five years. Thus, only two years old can be effectively separated from the remaining age groups on the basis of lens diameter ($p<0.05$). On the other hand, it is not possible to differentiate fishes belonging to the four age groups on the basis of eye lens weight (Fig. 3).

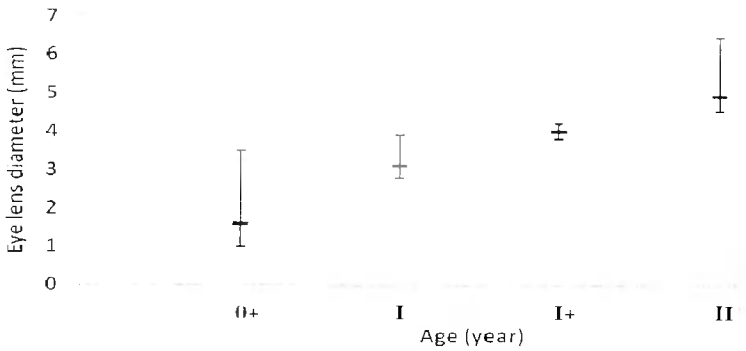

Fig. 2. Lens diameter *vs* age (determined from opecular bone) of *Decapterus russelli* Vertical bars represent total range of lens diameter and horizontal lines represent mean diameter.

Fig. 3. Lens weight *vs* age (determined from opercular bone) of *Decapterus russelli* Vertical bars represent range of lens weight and horizontal lines represent mean lens weight.

Gerking (1966) showed how different environmental factors could alter the growth rate in the bluegill *Lepomis macrochirus* and Swedberg (1965) summarized the various growth rates of drum, *Aplodinotus grunniens* from different areas in the United States. Environmental conditions must be considered in applying the lens technique (Burkett and Jackson 1971). Crivilli (1980), working on carp, stated that in the reproductive period energy is transformed from somatic to gonadal growth. Since the increment in lens diameter and weight is closely correlated with somatic growth, the variation in individual reproductive development could result in an increased variation in lens weight within an annual group. In other words, the growth rate during the reproductive period drops down due to the concentration of body on the reproductive metabolism. This drop in growth rate will affect the different parts of the fish body including the eye lens. This will end up giving variable results not in accordance with the general growth rate of the individual.

Acknowledgments

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Co-management approach on fisher group: A case study on *Ramsar* site, *Tanguar haor* in Bangladesh

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Abstract
In Bangladesh, wetlands are managed through leasing system traditionally from time immemorial. Recently the Government accepted co-management approach for wetland fisheries management and this approach is being practiced in few wetlands for maximize revenue income. A study was carried out to evaluate trend and impact of co-management in *Tanguar haor* (a *Ramsar* site wetland) on fisheries resources and livelihood of resident people in the immediate vicinity of the wetland. In *Tanguar haor*, conflict between leaseholders and the local community was a common phenomenon in the past. Since 2003 the district administration of Sunamganj has been managing the vast wetland resources, however, local people participation was ignored in *haor* management system. Average monthly fish catch of fishermen increased by 17% after introduction of co-management system and 7 fish species reappeared after introduction of co-management. Average monthly volume of fish catch has increased from 70 kg to 87 kg. A well-defined management structure has been developed for integration of all people of *Tanguar haor* which would enable them to raise voice jointly and influence policy in their favour.

Key words: Co-management, Wetland, Fishers

Introduction

Wetlands of Bangladesh are rich in biodiversity and have great ecological, economic and social values in ensuring livelihood security of millions of poor people particularly fisher community in Bangladesh (Nishat 1993). Administrative arrangements for public inland fisheries in Bangladesh comprised only leasing of fishing rights from 1930 to 1986 (Ullah 1985, Naqi 1989). Since Bangladesh's independence in 1971, a range of initiatives have been taken attempting to find out an appropriate wetland fisheries management strategy to halt the further decline of fisheries resources (Craig *et al.* 2004). Recently, it has been argued that a community-based cooperative fisheries management, which is one of the property rights approaches in fisheries management, seems to be a viable option in many of the artisanal fisheries in developing countries (Wilson 2001).

Efforts are being exerted to introduce co-management system in Bangladesh and notable efforts have been made from Management of Aquatic Ecosystem through Community Husbandry (MACH) and Community Based Fisheries Management (CBFM) and Community Based resource Management (CBRM) projects.

Tanguar haor is the most important 'mother fishery' of Bangladesh. It provides subsistence and livelihoods to more than 40,000 people living in 88 villages situated in its periphery. The Government has declared *Tanguar haor* as an Ecologically Critical Area (ECA) in 1999 considering its critical condition due to overexploitation of natural resource. In 2000, the *Hoar* basin was also declared as the country's second *Ramsar* site-wetland of international importance (SDC). *Tanguar haor* is considered as a refuge for threatened fish and is also home to some of a bigger species (IUCN 2008). The management history of *Tangaur haor* has always been subject to "elite capture" and politically connected local elites. In leasing time, local people were almost ignored during resource extraction. The leasing system has been abolished from 2001 after the *haor* was designated as *Ramsar* site (Kabir and Amin 2007). In 2001, ownership of *Tanguar haor* was transferred to Ministry of Environment & Forest and subsequently the leasing system was banned effectively and its management was regulated under the direction of the district administration of Sunamganj District. Up to 2006, the *haor* was managed by the local administration. Though no leasing system was existed from 2002 to 2006 and local people deprived of resources extraction from the *haor*.

In 2002, IUCN Bangladesh on behalf of MoEF introduced a co-management approach in *Tanguar haor* as pilot basis. The Preparatory Stage (18 months) was started in December 2006 and ended in April 2009. The present study was carried out to evaluate the performance of the co-management system, especially co-management on fisheries resource in a *Ramsar* site where the government is committed to implement the *Ramsar* guidelines. However, this practice was recent in nature, by this time some indications have been obtained.

Materials and methods

This study was carried out during August 2009 to January 2010. For this study data collection methods was performed using of personal questionnaire surveys and Participatory Rural Appraisals (PRA) including direct field observations. Selection of study sites was done considering cluster of fisherman in and around the wetland areas within a convenient distance. The study was conducted in eight villages (*Indrapur, Mandiata, Lamagao, Jayantri, Rupnagar, Majharchara, Ranchi* and *Cowhani*) of the Dharmapasa and Tahirpur Upazilas (sub-districts) of the Sunamganj district (Fig.1). The villages were chosen on the basis of close proximity to *Tanguar haor* and were selected purposively assuming that the people of these villages are more dependent on the wetland resources in comparison to the villages that are further away from *Tanguar haor*. The semi-structured interviews and in-depth interviews were undertaken in all eight villages.

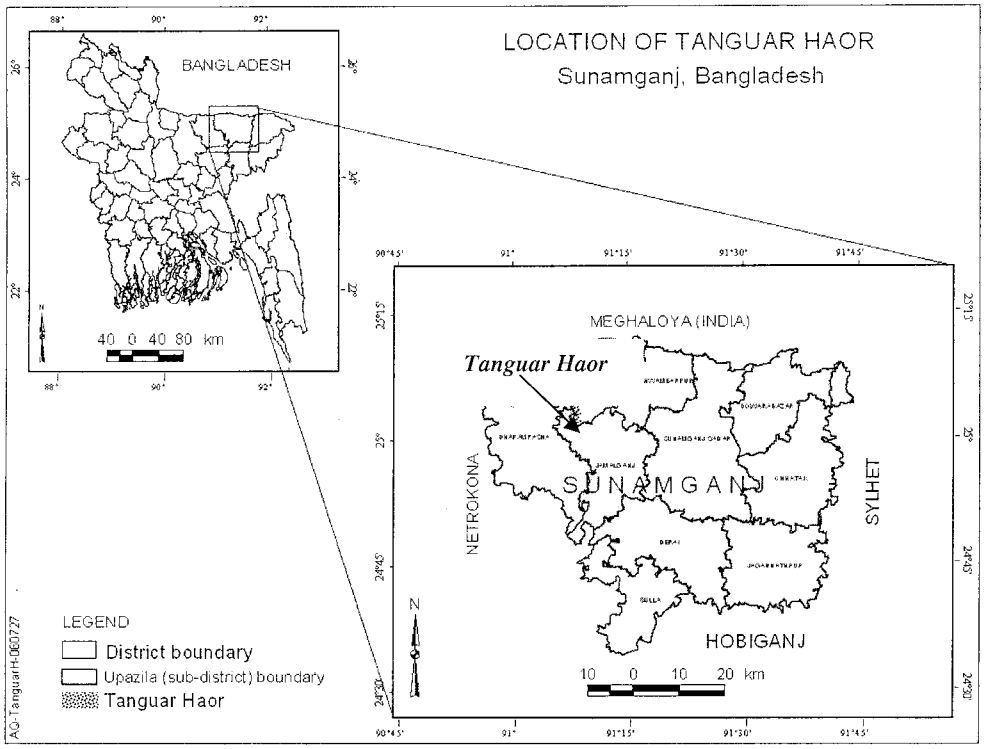

Fig 1. Location of Tanguar *haor*

Sampling: The sample design was prepared on the basis of base line census survey by IUCN. Based on the census information, a total of 80 fishermen and 40 fishmongers were initially selected for the survey. A total of 128 households were considered for the survey (Table 1).

Field data collection: Primary data was collected by field surveys using semi-structured questionnaire with the households and the key informants. Informal group discussions with people from different walks of life and direct field observations through field visits have been conducted also. PRA, such as Time Line and Trend Analysis, Seasonal Calendar and field observation were used to gather primary information from the local community. In this study, 4 FGDs were conducted at 4 different *Unions*. Member of the Union *Adhoc* committee, fisherman, fish monger and local people of other occupations were the participants of the FGD. The participants were invited in advance to a specific place on a specific date and time. Discussions were conducted in the *Union* council

office. Two officials from partner NGOs (CNRS and ERA) helped in organizing the groups.

Table 1. Distribution of samples according to union, village and occupation

Upazila and Police Stations	*Union*	Village	No. of interviews				
			Fish Monger	Fisherman	Focus Group Discussion	Security people (Police)	Total
Tahirpur	North *Sreepur*	i) *Indrapur*	5	10	1	2	33
		ii) *Mondiata*	5	10			
	South *Sreepur*	i) *Lamagaon*	5	10	1	2	33
		ii) *Joyatri*	5	10			
Dharampasha	North *Bangshikanda*	i) *Rupnagar*	5	0	1	2	33
		ii) *Majherchara*	5	10			
	South *Bangshikanda*	i) *Rangchi*	5	10	1	2	33
		ii) *Cowhani*	5	10			
Total sample	4 Unions	8 villages	40	80	4	8	132

Result and discussion

Fishery dependency of *Tanguar haor* people: People in the vicinity of *Tanguar haor* are dependent on fisheries resources to a great extent. Fishing is the most important economic activity of the *Tanguar haor* dependent people. More than 70% of households involved in fisheries activities in the floodplains either for income or food (Minkin *et al.* 1997). Table 2 illustrates the involvement of local people in fishing and related activities before and after co-management practices. The present study revealed that most of the local people around *Tanguar haor* are still engaged in fisheries related activities. Among the occupations of the respondents, more than half (67%) are full time involved in fish catching which was 48% before co-management practice. It is also evident that changes in the management practice (from leasing to government managed resources) resulted favorable environment for fish catching and aqua business. Before co-management, the local people were deprived fishing directly from *haor*. Interestingly observed that a number of seasonal fisher groups switched themselves in to fishing, fish business, and fish drying activities. Before co-management practice, these people were involved in

net/trap making/selling, boatman, fishing labour, ice selling and other indirect fisheries activities.

Table 2. Involvement of the local people in fishing and related activities

Types of involvement	Before co-management system		Present time	
	No. of respondent	%	No. of respondent	%
Fish catch	48	40.00	80	66.67
Wholesale fish business	13	10.83	15	12.50
Retail fish business	15	12.50	20	16.67
Fish drying activities	6	5.00	5	4.17
Trap making activities	13	10.83	0	0.00
Net/trap selling	4	3.33	0	0.00
Boatman	3	2.50	0	0.00
Fishing labor	12	10.00	0	0.00
Ice selling	4	3.33	0	0.00
Others	2	1.67	0	0.00
Total	120	100%	120	100%

The IUCN survey in 2008 found that 95 percent of the people reported some kind of dependency (through their occupation) with the *Tanguar haor*, and nearly 65 percent of the people were involved in fishing or related activities. The *Ramsar* Convention allows local inhabitants to use these resources to enhance their income. However, the new management is yet to develop a comprehensive management system.

Local people's participation in co-management practices: In the present study, an attempt was made to assess local people's willingness to participate in the management system. During FGD and personal interviews, a question was asked to the participants about the importance of the management and reasons for that. It is shown that that 75 percent of the interviewees expressed their opinion that present management system is "very important" to manage the resources properly and 20 percent of respondents shared that the present management system is "important" for managing the resources (Fig. 2). So all together 95 percent respondent had willingness to participate in the management activities and only 5 percent considered their participation as either 'not important' or 'less important'. However, all the respondents shared that their participation to the conservation practices is essential for both the betterment of the *haor* ecosystem as well as the local economy.

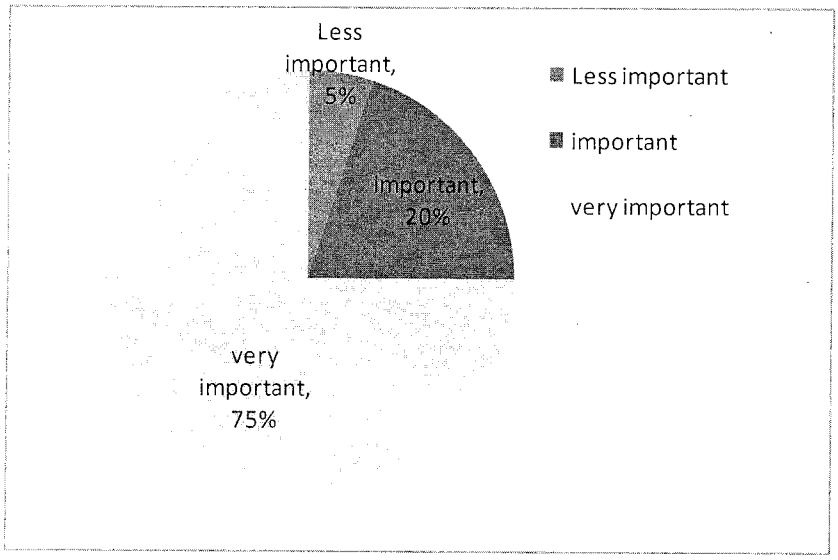

Fig. 2. Local people's opinion about co-management practices.

Tanguar haor management system has been running for nearly a decade. In the beginning, people of the *Tanguar haor* were against leasing of the fishing resources to individuals in the name of the fishing societies. Since local people living in the same fishing resources, they came in to conflicts with the leaseholders very quickly. The study revealed that over a long time the local people have realized that their participation in the co-management of *Tanguar haor* would be capable of changing their fate, and this is the only way they could establish their rights of access to and withdrawal of resources. As a result, the local community at *Tanguar haor* is very eager to be integrated into the management system, although they understand that they have to wait longer to have monetary benefits from the *haor*.

Causes of participation in the management practices: The question was asked about the reasons for participation in the current management system. Most respondents were aware that their access and ownership would be established if they are integrated into the current management system. Fig. 3 shows that nearly 43 percent of respondents were involved in the process in order to get benefit for the long run. Only 31 percent of the respondents wanted to participate in the management practices only to get benefit from the resources.

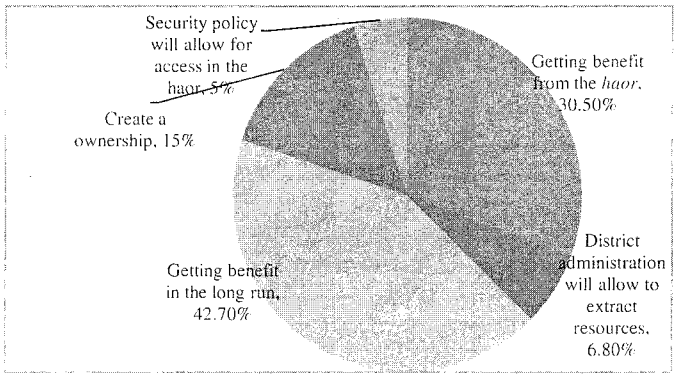

Fig. 3. Reasons of participation of local people in the management practices

About 15 percent of people were in favour of creating ownership in the wetland. The rest of the respondents opined that they want to participate in the management practices due to get permissions from district administration and security police. It is evident that there is close interrelationship and interdependency between *haor* and the local community. The major importance of the resources to the local community is to establish the rights of utilization that have been developed for a long period. Although the local people are willing to participate in the management system, however there are some constraints. Primarily, most of them have a low level of education. Secondly, they are not economically well off, and in most cases support their family by working as day-labourers. Therefore, it would be very difficult for the poor to find extra time to participate in these voluntary activities, unless the new management system has provision of providing substantial income (remuneration) in return for their services. However, the socio-economic status of the people will play an important role in integrating them into the management practices.

Benefits of co-management: Local people are willing to participate in the management system of *Tanguar haor* by their own accord, because they expect to receive some benefits. Fig. 4 shows that 46 percent respondents shared that their income will substantially increase by participating in the management practices. Thirty five percent respondents mentioned that *the* introduction of management system reduces illegal extraction of resources, 11 percent respondents think that the overall biodiversity status already increased. The rest of the respondents (28%) shared that the entire *haor* area would be a healthy ecosystem if the *Ramsar* guidelines were implemented with the active participation of the local community.

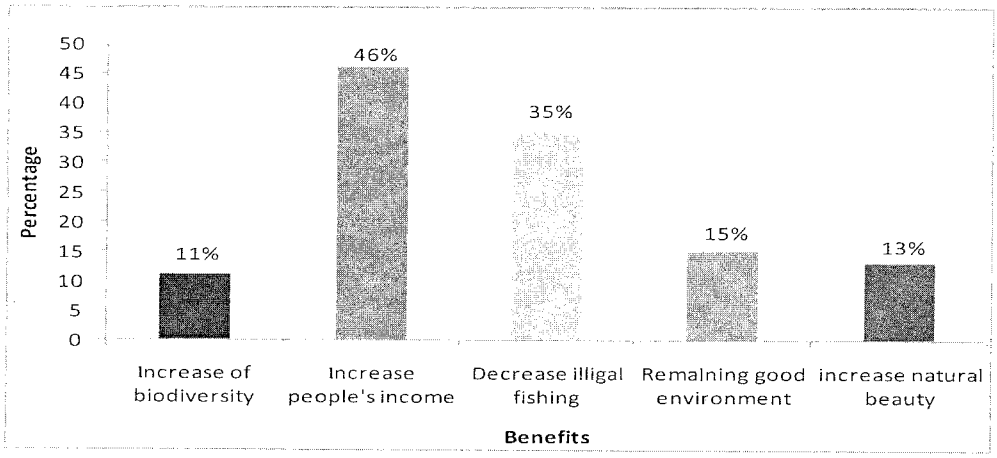

Fig. 4. Benefits of co-management in *Tanguar haor*

In the second half of the last century, especially during the past 50 years, the local community was systematically excluded from the use of the natural resources of the ecosystem. It is well known to the people at *Tanguar haor* that this wetland has recently been designated as a *Ramsar* site. In general, the local people perceive the gradual degradation of the resources, especially over-harvesting of fishery resources by the hired fisher folks. Most of the respondents believe that the leasing system is responsible for massive degradation and decline of fisheries, forest and water birds. At the same time, they also feel that the *haor* ecosystem has started getting return to its original state after the leasing system was abolished and the control of *Tanguar haor* was taken under co-management practices. Over a long period of time, the local people have realized that their participation in the management of *Tanguar haor* can enhance changing their rights of access to the *haor* and withdrawal of resources as well. The local community is eager to be integrated into the management system, although they understand that they have to wait for a longer period to have monetary benefits from the *haor* resources.

Institutionalization process of co-management: The concept of co-management has, however, been used to cover a large range of institutional arrangements, which have very little in common, and has been adapted very differently in various situations. From FGD it is found that a single organization has been formed integrating all people of *Tanguar haor* which enable them to raise voice jointly and influence on policy in their favour. A steering Committee is operating at the national level and regional platform is functional at district level, known as management committee. A well-defined organizational structure for co-management has been established (Fig. 5).

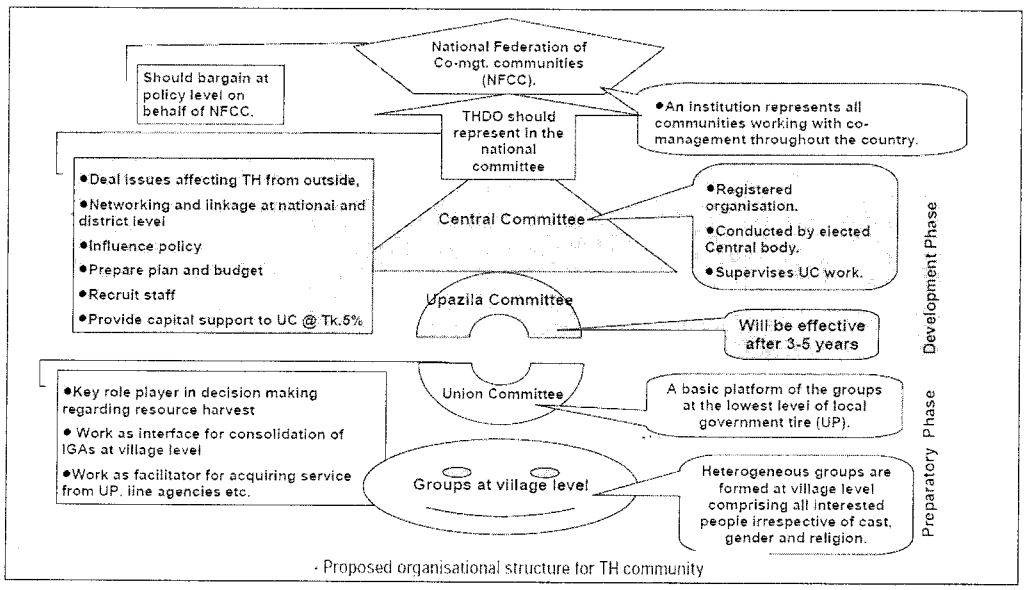

Fig. 5. Organizational structure for *Tanguar Haor* (IUCN 2009)

Four *Union Adhoc* Committees (UAC) are functional and it can be considered as the stepping stone for evolving resource governance structure. One of the milestones achieved, the benefit-sharing ratio from fish harvest. According to benefit-sharing ratio, the fish harvesters will get 40% (as traditional wage in *haor* area), 36% for the *Tanguar haor* community (4 *Union Adhoc* Committee as elected representatives of *Tanguar haor* community), and the rest 24% as government revenue which has to be invested in the *Tanguar haor* development through the concerned *Union Parisad*. This is a groundbreaking achievement and will usher future wetland management in Bangladesh.

This endorsement has given the legal basis to provide community people's access to the *Tanguar haor* resources. Establishment of terms and conditions for single and commercial fishing in the *haor* by the *Tanguar haor* management committee will stop indiscriminate fishing.

States of fish diversity due to co-management: The *Tanguar haor* is very rich in fish diversity along with other flora and fauna. Abundance of many nationally declared threatened species is interestingly abundant here. To assess the degree of increase or decrease in fish diversity, fishermen were asked about the total number of species they caught before and after co-management. Similarly, fishmongers were also asked to know the total number of fish species they buy or sell. The survey found that both fishermen and fishmongers given nearly the same information about the number of fish species exist. Form Fig. 6 it is clearly found that, during leasing time (before co-management)

fishermen and fishmonger found an average total of 23 and 22 fish species respectively. On the other hand, after introduction of co-management the number of fish species increased up to 30. It is very clear from this study 7 fish species re-appeared in the catch after the introduction of co-management (Fig. 6). According to fishermen and fish monger opinion the threatened species of fish in *Tanguar haor* are (mentioned in local name and scientific name): Nandina (*Labeo nandina*), Shorputi (*Puntius sarana*), Pangsh (*Pangasius pangasius*), Gozar (*Channa marulius*), Aor (*Mystus aor*), Gulsha (*Mystus cavasius*), Bacha (*Ailia coila*), Chitol (*Notopterus chitala*), Foli (*Notopterus notpterus*), Kali Baush (*Mylpharyngodon pisceus*), Gonia (*Labeo gonius*), Caski (*Corica soborna*), Pabda (*Ompok pabda*), Chela (*Salmostoma bacaila*), Kuccha (*Monopterus cuchia*), Tara Baim (*Mastacembelus armatus*), Chanda (*Chanda ranga*), Meni (*Nandus nandus*). Among the threatened species the following 7 species re-appeared: Nandina (*Labeo nandina*), Shorputi (*Puntius sarana*), Bacha (*Ailia coila*), Foli (*Notopterus notpterus*), Kali Baush (*Mylpharyngodon pisceus*), Meni (*Nandus nandus*), Gonia (*Labeo gonius*).

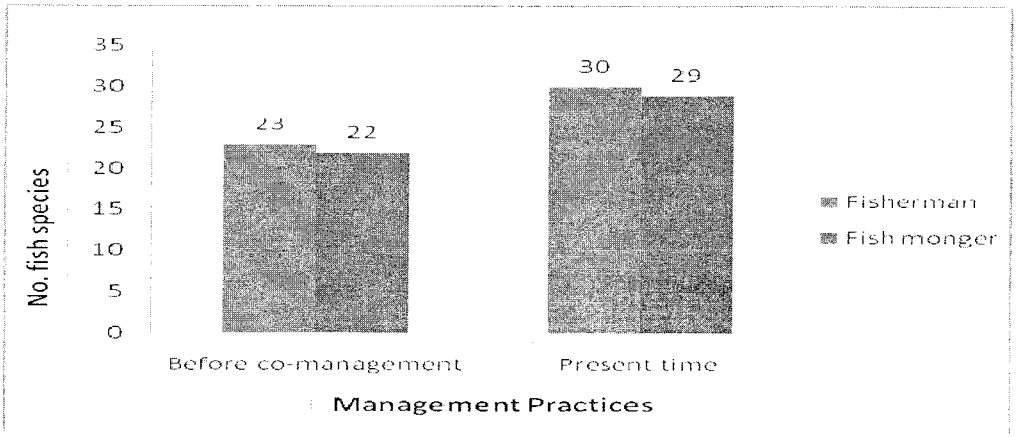

Fig. 6. Number of fish species observed before and after co-management

Tanguar haor has 52 *beels* which treated as single wetland. If it would divide as per number of groups/villages, the management of local community become destroy the natural system. Five *beels* viz. Rowa *beel,* Rupa boi *beel,* Alamer *duar,* Kawar *Khal and* Tegunne *beel* were established as sanctuaries to save the endangered fish species in *Tanguar haor.* During the dry season, dewatering of breeding places in the *haor* basin is an important reason for extinguishing fish species. After introduction of co-management system, the dewatering activity is nearly stopped.

Tanguar Haor is home to 141 varieties of fish (more than half of Bangladesh's 260 freshwater fish species). This includes 55 fish species that are threatened in Bangladesh, of which 28 are endangered. Of these 28 endangered fish species, 17 are found only in *Tanguar haor* (NCS 2007).

Status of a total fish catch after introduction of co-management: The issue of average fish caught before and after co-management was raised during FGD. Fig.. 7 shows that year round fish catch in *Tanguar haor* varied seasonally before and after co-management. April to October is the off-season to catch fish form *Tanguar haor.* Survey acknowledged the maximum catch for the months of December, January and February. On the other hand, the lowest fish catch is acknowledged for the months of June, July and August. It is found that the average monthly fish catch before co-management was 72 kg, which increased a bit 83 kg after co-management. It is clearly observed that average fish catch of *Tanguar haor* increased about 11% after introduction of co-management. The reasons behind the average fish catch increase was introduction of fishing licence among fishermen that stopped indiscriminate fishing. Not only that, creation of new sanctuaries and selective the fishing period other than whole year fishing.

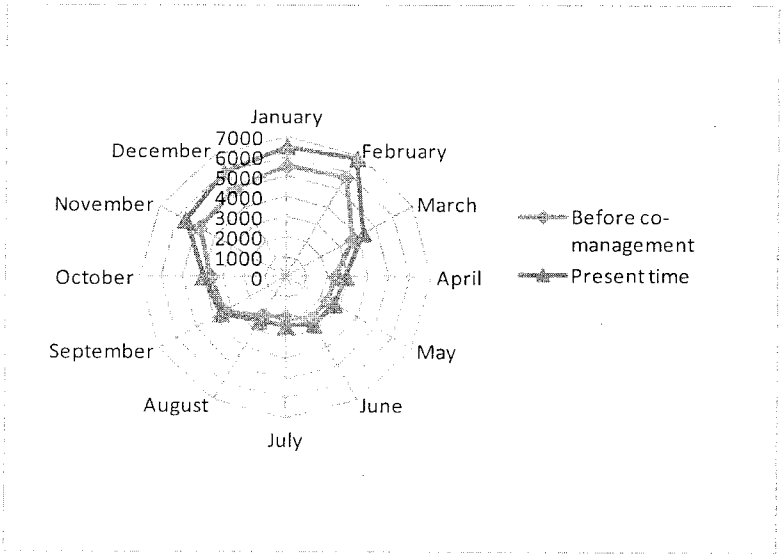

Fig 7. Average monthly fish catch (kg.) before and after co-management

Apart from that it is found from the personal fisher group interview that average monthly fish catch of fisher group before and after co-management w as70 kg and 87 kg respectively. However it is indicated that average monthly fish catch is increased 17% after introduction of co-management. Catch in *Tanguar haor* increased after the introduction of the co-management system due to the introduction of licensing system for commercial fishing among local fishermen. One of the important conditions of the licensing system is not to catch endangered and small species mentioned by the IUCN (IUCN 2008). Another reason is the close monitoring by the fish harvest committee formed under co-management approach.

Conclusions

To prevent further degradation and overexploitation of fisheries resources, better management is imperative. The present study finds that the *haor* resources are improving chronologically after the present management authority took over the management of the *haor*. Local people are satisfied with the existing management system because local community has been included in resources utilization. Majority of the respondents in the co-management practices considered their participation as "very important". The pilot scale efforts implemented in the *Tanguar haor* in regard to co-management and benefit sharing are landmark achievements. A close interrelationship and interdependence between the *haor* and the local community has been identified in this study. The local people intend to establish their rights of resource utilization, from which they have been deprived for a long period of time. Some of them have allegations against the existing management authority because the poor locals are often harassed and oppressed by the law enforcing forces.

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