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Effects of dietary phosphorus and zinc levels on growth and bone mineralization in fingerlings of rainbow trout, *Oncorhynchus mykiss*

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Abstract.
A laboratory based 2 × 2 factorial experiment was conducted to investigate the influences of dietary phosphorus and zinc levels on growth and bone mineralization in fingerlings of rainbow trout for 21 weeks. Two levels of phosphorus (19 and 30 mg/g) and two levels of zinc (55 and 103 μg/g) in the dry diets were tested. Duplicate tanks of 30 rainbow trout (average weight 1.56 ± 0.24 g) per 60L glass tank were fed experimental diets three times a day to apparent satiation level at 15 to 24°C water temperature. The results of the present study demonstrated that dietary phosphorus supplementation influenced the growth and bone mineralization whereas zinc levels significantly ($p<0.05$) influenced bone mineralization in rainbow trout. Further investigations in this area with different size and age groups of this fish are broadly needed.

Key words: Phosphorus, Zinc, Bone mineralization, Rainbow trout

Introduction

Rainbow trout, which belongs to the family Salmonidae, is originally taxonomically linked with the Atlantic or Eurasian trouts of the genus *Salmo*. However, on the basis of evolutionary evidence which showed that rainbow trout has a greater genetic affinity to the Pacific salmon than to the Eurasian trouts so it was renamed as *Oncorhynchus mykiss* (Groot 1996). Rainbow trout have been introduced widely in suitable habitat throughout North America and other parts of the world, such as South America, Europe, Southern Asia, Japan, Africa and Oceania (Groot 1996, Scott and Crossman 1975).

Rainbow trout has important commercial and sport value. Their simple requirements for incubation under artificial conditions have made it possible to introduce to many new areas in the world. New understanding of the biological requirements of this fish and refinements of incubation and rearing methods have opened important possibilities for trout farming (Groot 1996). In the natural

environment rainbow trout feeds on various invertebrates including plankton, larger crustaceans, fish, insects, snails, and leeches. Nutritional requirements of rainbow trout have been well studied (NCR 1993). Their growth is very rapid when fed on well-balanced dry pelleted diets. So in many places around the world, they are entirely raised in artificial ponds on trout farms. Rainbow trout is by far the most widely farmed trout in the world and one of the few species of fish that may be regarded as truly domesticated. Trout production is predicted to increase by 5% per year for the near future and will likely maintain its place in the top 15 finfish and crustacean aquaculture species produced in the world, as well as remain in the top 10 species, with respect to total value (Hardy *et al.* 2000).

Dietary phosphorus (P) is an essential nutrient for optimum growth and metabolism of fish. It is the most important mineral needed by fish, since its requirement and functions are superior to that of any other mineral element (NCR 1993, Satoh *et al.* 2002). Zinc is also essential in the diet since waterborne Zn is not efficiently absorbed by aquatic animals as they required (NRC 1993). It is also known to be an essential trace element for growth (Burch *et al.* 1975, Semard 1999). Zinc is largely deposited in muscle, kidney, liver, pancreas, and bones (Wapnir 1990). Its deficiency may result in growth retardation (Ninh *et al.* 1995, 1996, Dorup *et al.* 1991). Among the tissues, bone has the highest Zn level and it serves as a reservoir for this element. In addition, Zn like IGF-1 can increase the protein component of bone and may play a role in bone growth (Ma and Yamaguchi 2001) whereas Zn deficiency resulted in lower levels of growth hormone and growth hormone binding protein mRNA (McNall *et al.* 1995).

Therefore, the present study aimed to investigate the possible effects of dietary P and Zn supplementation on the growth and bone mineralization of fingerling of rainbow trout using practical type diets.

Materials and methods

Formulation and composition of the experimental diets are presented in Table 1. Practical diets were formulated to contain 19 and 30 mg/g P, using monocalcium phosphate and 54 and 103 μg/g Zn, employing zinc sulfate heptahydrate (Table 1). The diets were labeled as P0Z0, P0Z1, P1Z0 and P1Z1 according to factors (P and Z) and levels (0 and 1). The experimental diets were formulated to be isocaloric and isonitrogenous. The carbohydrate sources and binders were wheat flour and pregelatinized starch, and the lipid source was pollock liver oil. The experiment was conducted in a 2 × 2 factorial design with the factors 'dietary phosphorus level 'and 'supplemental Zn level'.

The mineral mixture used in this study was the modified form of Ogino salt mixture (Ogino *et al.* 1979). The experimental diets were formulated to be isocaloric and isonitrogenous. The diets were pelleted using the laboratory pelletizer (AEZ12M, Hiraga-Seikakusho, Kobe, Japan), dried in a vacuum freeze-drier (RLE-206, Kyowa Vacuum Tech., Saitama, Japan), and stored at 4°C until used. The proximate

composition and mineral contents of the experimental diets used in this study are shown in Table 2 and Table 3. The diet was prepared with 57% (FM) as the sole protein source. Ingredients used in the test diets were selected taking into consideration the amino acid balance of the whole protein sources (Watanabe *et al.* 1993).

Table 1. Formulation and composition of the experimental diets

Ingredients (%)	Diets			
	P0Z0	P0Z1	P1Z0	P1Z1
Jack mackerel meal	57	57	57	57
Wheat flour	20	20	20	20
Pregelatinized starch	5	5	5	5
Pollock liver oil	4	4	4	4
Soybean oil	5	5	5	5
Mineral premixture[a]	0	1	0	1
Zn free mineral mixture[b]	1	0	1	0
Ca $(H_2PO_4)_2$	0	0	4	4
Vitamin premixture[c]	1.5	1.5	1.5	1.5
Choline chloride	0.5	0.5	0.5	0.5
Vitamin E (50%)	0.1	0.1	0.1	0.1
Cellulose	5.9	5.9	5.9	5.9

[a] Mineral premixture (%): NaCl 5.0, Mg $SO_4 \cdot 7H_2O$ 74.5, $FeC_6H_5O_7 \cdot nH_2O$ 12.5, Trace element mix.[a*] 5.0, Cellulose 3.0 [a*] (%)—$ZnSO_4 \cdot 7H_2O$ 35.3, $MnSO_4 \cdot 5H_2O$ 16.2, $CuSO_4 \cdot 5H_2O$ 3.1, $AlCl_3 \cdot 6H_2O$ 1.0, $CoCl \cdot 6H_2O$ 1, KIO_3 3, cellulose 44.0.

[b] Zn free mineral mixture (%): NaCl 5.0, Mg $SO_4 \cdot 7H_2O$ 74.5, $FeC_6H_5O_7 \cdot nH_2O$ 12.5, Zn-free mineral mix.[b*] 5.0, Cellulose 3.0 [b*] (%)— $AlCl_3 \cdot 6H_2O$ 10, $CoCl \cdot 6H_2O$ 1, KIO_3 3, Cellulose 986.

[c] The vitamin mix (%):-Thiamine hydrochloride 6, Riboflavin 10, Pyridoxine hydrochloride 4, Cyanocobalamin 0.01, Ascorbic acid 500, Niacin 40, Ca-pantothenate, 10, Inositol 200, Biotin 0.6, Folic acid 1.5, *p*-aminobenzoic acid 5, Vitamin K_3 5, Vitamin A acetate 4000 IU, Vitamin D_3 4000 IU.

Table 2. Proximate composition of the experimental diets (dry matter basis)

Parameters	Diets			
	P0Z0	P0Z1	P1Z0	P1Z1
Moisture (%)	4.5	4.5	3.0	4.5
Crude ash (%)	11.1	10.8	13.7	13.6
Crude protein (%)	44.9	44.7	45.5	45.2
Crude lipid (%)	16.6	16.7	16.6	16.9
Gross energy (kcal/g)	5.2	5.2	5.0	5.0

Table 3. Mineral contents of the experimental diets (dry matter basis)

Macro elements (mg/g)	Diets			
	P0Z0	P0Z1	P1Z0	P1Z1
P	19.14	19.49	30.30	30.09
Ca	28.03	30.19	34.76	35.20
Mg	3.00	3.05	3.00	2.97
Na	3.86	3.41	3.58	3.82
K	4.71	3.75	4.32	4.50
Trace elements (μg/g)				
Zn	54.54	103.2	55.85	96.07
Mn	40.44	40.71	41.18	40.62
Fe	345.5	341.9	352.2	348.2
Cu	7.04	7.05	10.63	8.35

Eyed eggs of rainbow trout were obtained from Fuji Trout Farm of Shizuoka Prefecture Fisheries Experiment Station and hatched under laboratory conditions at the Tokyo University Marine Science and Technology. Fish with an average body weight of 1.56 ± 0.24 g were randomly selected from stock and distributed into 60 L tanks at a density of 30 fish per tank. Duplicate groups were assigned to each experimental diet and the feeding was conducted for 21 weeks. The fish were hand fed three times per day, 6 days a week to apparent satiation level. The tanks had a continuous water supply at a rate of 0.6-1.0 l/min and the temperature was 15 to 24°C.

The fish were starved for 24 h before being individually weighed at the initial day and every 21 days of the experimental period after being anesthetized with ethylene glycol monophenyl ether (300 ppm). At the same time 5 fish were randomly sampled from each tank and stored at –20°C for analyses.

Proximate composition and chemical analysis of the diets and fish whole body samples were made in three replicates as follows: moisture contents was measured gravimetrically, crude ash contents was determined by incinerating a known amount of sample in an electric muffle furnace (Yamato, FA-21) at 600°C for 8 hours, crude protein was analyzed using the Kjeltec Auto Analyser System 1035/38 (Netherland), and crude lipid was measured by following the method of Folch *et al.* (1957). Samples for minerals were digested in nitric acid using the MLS-1200 Mega Microwave Digestion System (Italy), cooled in flowing water for 30 minutes, and diluted with de-ionized water to the required volume. Concentration of each element was measured by a Polarized Zeeman Atomic Absorption Spectrophotometer (Hitachi Z-5010, Tokyo, Japan) except for phosphorus which was analyzed by a visible light spectrophotometry (Shimadzu, UV 265 FW, Kyoto, Japan) at 750nm.

Statistical analyses of the results were performed using one-way and two-way ANOVA with SYSTAT 8.0 software (SPSS Inc. Chicago, USA, 1998). Differences between treatments were evaluated by Tukey's test. The level of significance was set at $p<0.05$ for all tests.

Results and discussion

In rainbow trout, weight gain of the fish did not show any significant different among treatments of both P and Zn throughout the culture period. Likewise insignificant differences were obtained among dietary Zn Level treatments; SGR (Specific growth rate), FCR (Feed conversion ratio) and TGC were not significantly affected by the treatment (Table 4). The results of growth performance and feed utilization indicate that both P and Zn regardless of their supplementing levels had no influence on the feed intake and growth performance of fish. In addition, the results represent the stated parameters not to be potential and appropriate indices to assess P and Zn levels. This is in agreement with Apines (2000) who stated weight gain as an inappropriate index of Zn bioavailability in rainbow trout. Similar results were obtained by Hardy and Shearer (1985) and Li and Robinson (1996).

Table 4. Growth and feed performance of the experimental diets for 21 weeks

Diet group	Weight gain	SGR[1] (% day^{-1})	FCR[2]	TGC[3]	Condition factor
P0Z0	73.62^{a}	2.62^{a}	0.97^{a}	0.001125^{a}	1.161^{a}
P0Z1	74.61^{a}	2.62^{a}	0.96^{a}	0.001118^{a}	1.153^{a}
P1Z0	68.88^{b}	2.50^{b}	1.01^{b}	0.001047^{b}	1.162^{a}
P1Z1	61.54^{b}	2.51^{b}	1.02^{b}	0.001021^{b}	1.125^{a}
P	<0.05	<0.05	<0.05	<0.05	NS
Zn	NS	NS	NS	NS	NS
P × Zn	NS	NS	<0.05	NS	NS

[1] Specific growth rate; [2] Feed conversion ratio. [3] Thermal-unit growth coefficient. NS = Not significant
* Values in the same column not sharing a common superscript letter are significantly different ($p<0.05$).

Carcass proximate composition of rainbow trout at start and end of the experiment (Table 5) revealed significant ($p<0.05$) influence of P supplementation level on whole body crude ash contents, whereas that of Zn supplementation on crude ash was not remarkable.

Table 5. Proximate carcass composition of fish at start (n=30) and end (n=12) of the experiment

Diet group	Moisture (%)	Crude ash (%)	Crude protein (%)	Crude lipid (%)
P0Z0	67.37	1.97^{b}	16.46	14.35
P0Z1	67.33	2.03^{ab}	16.26	14.33
P1Z0	68.58	2.36^{a}	16.28	12.71
P1Z1	68.58	2.17^{ab}	16.31	13.22
P	NS	<0.05	NS	NS
Zn	NS	NS	NS	NS
P × Zn	NS	NS	NS	NS

Values in the same column not sharing a common superscript letter are significantly different ($p<0.05$).
NS = Not significant.

Insignificant variations were observed among different treatments of both P and Zn in the cases of whole body P, Ca, Mg, Na, K and Mn contents (Table 6). Conversely, whole body Zn content was found to increase significantly ($p<0.05$) in the treatments with higher Zn supplementation (Table 6). Supplementation of P also showed significant ($p<0.05$) increase in the whole body Fe content. Higher whole body Zn content with dietary Zn supplementation in this study is in agreement with the other study in Atlantic Salmon (Maage and Julshamn 1993). Zinc concentration of Abalone also increased linearly with dietary Zn (Tan and Mai 2001).

Table 6. Whole body mineral contents of fish at start (n=30) and end (n=12) of the experiment

Initial/ Diet group	P (mg/g)	Ca (mg/g)	Mg (mg/g)	Na (mg/g)	K (mg/g)	Zn (μg/g)	Fe (μg/g)	Cu (μg/g)	Mn (μg/ g)
P0Z0	4.43	3.94	0.33	0.7	3.56	15.2^{ab}	9.61	1.00	1.36
P0Z1	4.29	3.48	0.31	0.67	3.73	23.5^{a}	9.01	1.11	0.99
P1Z0	4.98	4.87	0.35	0.72	3.65	13.7^{b}	11.5	1.25	1.16
P1Z1	4.49	3.89	0.32	0.64	3.63	21.9^{a}	10.3	1.04	1.11
P level	NS	NS	NS	NS	NS	NS	<0.05	NS	NS
Zn level	NS	NS	NS	NS	NS	<0.05	NS	NS	NS
P level × Zn level	NS	NS	NS	NS	NS	NS	NS	NS	NS

Values in the same column not sharing a common superscript letter are significantly different ($P<0.05$). NS = Not significant.

Bone mineral contents of the fish are presented in the Table 7 and Figure 1. Dietary Zn and P supplementation significantly ($p<0.05$) influenced vertebral Zn and Fe contents in rainbow trout. Bone Zn content was significantly ($p<0.05$) affected by the dietary levels of P and Zn (Fig. 1). In addition, the interaction between P and Zn levels were also observed to have influence on Mg and Fe concentration of bone. Higher bone Zn accumulation obtained in treatments with higher supplementation for rainbow trout was similar to the findings of Satoh *et al.* (1987). In channel catfish, bone Zn increased linearly as $ZnSO_4$ and ZnMet increased (Li and Robinson 1996). Mohanna and Nys (1999) had similar results with chickens where tibia Zn concentration increased linearly with dietary Zn content. Zinc concentration of tissues of rats dependent on the dietary Zn levels (Roth and Kirchgessner 1983). The present results indicated that highest bone deposition was achieved with higher Zn supplemented diets. Zinc content in bones was higher than whole body probably because after absorption in the digestive tract, Zn was absorbed in the skeletal tissues (Knox *et al.* 1982) where it was deposited. Moreover, the skeleton acts as a reservoir for Zn (Yamaguchi 1998). The incorporation of Zn in the skeleton is relatively slow and it is firmly bound for long periods. When bones are already saturated, the excess amount of Zn is stored in the skin and muscle (Knox *et al.* 1982). Jeng and Sun (1981) similarly observed that Zn levels increased in the skeletal

tissues of common carp tissue then it deposits in the muscle tissues when fed high levels of $ZnSO_4$. Bone Zn has also been shown to be a more sensitive criterion of Zn status than weight gain in various animals (Gatlin and Wilson 1983, Forbes *et al.* 1984). Previous studies also indicated that whole body (Wekell *et al.* 1986) and bone levels (Huber and Gershoff 1970) have been used to quantify Zn status in animals. As in higher vertebrates, these results indicate that different tissues have varying rates of elemental deposition in fish.

Table 7. Vertebral mineral contents of fish at the end of the experiment (n=12) fed experimental diets for 21 weeks

Diet group	P (mg/g)	Ca (mg/g)	Mg (mg/g)	Na (mg/g)	K (mg/g)	Fe (μg/g)	Cu (μg/g)	Mn (μg/g)
P0Z0	112.57	206.14^{c}	4.22^{ab}	3.45	3.88	17.82^{a}	22.20	37.99
P0Z1	115.48	2.08.33^{bc}	4.12^{ab}	3.28	3.54	17.85^{a}	20.19	38.11
P1Z0	116.30	210.84^{ab}	4.78^{a}	3.48^{b}	4.01	16.44^{b}	32.24	35.51
P1Z1	116.86	212.76^{a}	3.76^{b}	3.73	4.21	10.24^{c}	11.44	40.69
P	NS	<0.05	NS	NS	NS	<0.05	NS	NS
Zn	NS	<0.05	<0.05	NS	NS	<0.05	NS	NS
P × Zn	NS	NS	<0.05	NS	NS	<0.05	NS	NS

Values in the same column not sharing a common superscript letter are significantly different ($P<0.05$). NS = Not significant.

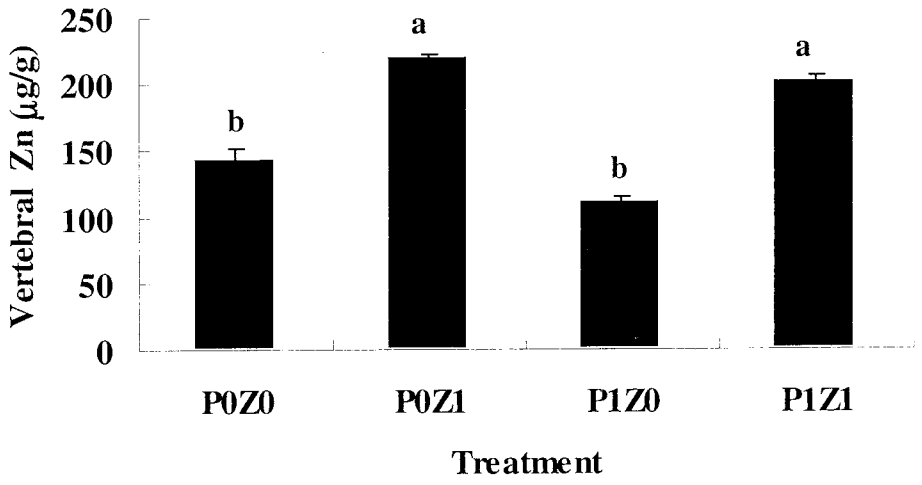

Fig. 1. Effect of dietary P and Zn levels on vertebral zinc contents of rainbow trout at the end of the experiment (n=12) fed experimental diets for 21 weeks.

The overall results of the present study demonstrated that P supplementation is not needed in the diet of fingerling rainbow trout for growth and bone mineralization. On the other hand Zn supplementation is needed. Yet, further studies in this area with different size and age groups of rainbow trout are warranted.

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Investigation of health status of juvenile exotic carps from various farming conditions of Bangladesh

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Abstract
Health status of juvenile silver carp, *Hypophthalmichthys molitrix* and silver barb, *Barbodes gonionotus* were investigated in three fish farms following different farming conditions through clinical and histopathological examinations for a period of nine months. Here the fishes and water quality parameters were sampled on monthly basis. Among the water quality parameters, water temperature has a distinct effect on fish health observed during the winter season. Different clinical signs like scale loss, dermal lesion, fin erosion were observed, while histopathologically necrosis, pyknosis, inflammation, haemorrhage, hypertrophy, vacuoles, missing of gill lamellae and clubbing were evidenced in the investigated fishes. The study showed that pathological symptoms were mainly increased during the winter season and *H. molitrix* exhibited severe pathological symptoms in compare to *B. gonionotus* during the investigation. It was also found that fishes of BAU farm was comparatively in the best condition, while, the fishes of other farms were severely affected during the experimental observations. In addition, disease like Epizootic Ulcerative Syndrome (EUS), protozoan disease and suspected bacterial colonies were clearly evidenced in the fishes of Government and NGO fish farms.

Key words: Fish disease, *Hypophthalmichthys molitrix*, *Barbodes gonionotus*, Histopathology

Introduction

Aquaculture development in the country has intensified recently through increased stocking densities, artificial feeding and fertilization (Mazid and Banu 2002). In this development, introduction of exotic fishes with the Indian major carps has brought a new dimension in augmenting the fish production ((Ullah and Khan 2003). Though intensification has magnified the fish production, it may also lead to water quality deterioration and susceptibility to infection (Mazid and Banu 2002). Thus, the carp fishes were affected by different range of diseases and parasites (Ahmed and Hoque 1999). Common diseases of freshwater fishes of Bangladesh are tail and fin rot, bacterial gill rot, dropsy, fungal disease, protozoan disease, parasitic disease, nutritional diseases and tumors (Chowdhury 1993). In composite carp culture system, fishes are often

stressed by various living and non-living factors resulting in the outbreak of diseases (Das *et al.* 2000). Basically, fish remains in such an environment, which is loaded with innumerable agents like chemical pollutants, bacteria, virus, parasites and fungus etc. that are either individually or in combination can inflict the body tissue or system producing disease of several kinds (Post 1987). Moreover, their internal and external biology is also altered by other physical, chemical and biological factors of the environment. These factors determine whether an etiological agent can or will cause disease among fishes or not (Post 1987). In addition, incidence of fish diseases was also depended on season, which tends to fluctuate with temperature. It was reported that up to 31% of extensive carp farms and 24% of semi-intensive carp farms were affected with diseases (Chowdhury 1997). In these view, it is obvious to investigate the occurrence of diseases by using suitable diagnostic techniques. However, clinical investigation provides information on the nature of diseases whereas; histopathological technique gives a way to unearth the fish health related condition. But in Bangladesh, these techniques have been using for diagnosing of fish diseases to a very limited extent due to poor technical know how and laboratory facilities (Moniruzzaman 2000). Thus, the present work was undertaken to identify the pathological changes and disease occurrence in *Barbodes gonionotus* and *Hypophthalmichthyes molitrix*, collected from different fish farming systems of Mymensingh district of Bangladesh for the assessment of their health conditions.

Materials and methods

The experiment was conducted for a period of nine months from April to December 2005 in three different fish farms i.e., Bangladesh Agricultural University (BAU) fish farm, Government and NGO fish farm. The BAU fish farm is located at the southern side of the Faculty of Fisheries, BAU, Mymensingh, whereas the other fish farms were at the Fulpur upazilla under the Mymensingh district. Silver barb (*Barbodes gonionotus*) and Silver carp (*Hypophthalmichthys molitrix*) were sampled from the three experimental ponds (one pond from each fish farm) considering as the experimental fish. Each pond was prepared following the standard procedure of semi-intensive carp polyculture system practiced in Bangladesh (Table 1). Stocking ratio were maintained at the rate of 35- 40 per decimal with *Labeo rohita* (rui), *Catla catla* (catla), *Cirrhinus cirrhosus* (mrigal), *Hypophthalmichthys molitrix* (silver carp), *Ctenopharyngodon idella* (grass carp), *Barbodes gonionotus* (silver barb), *Cyprinus carpio* (carpio) in BAU fish farm, whereas 60-65 numbers in NGO farm and 70-80 in Government fish farm maintaining the same species. Poultry droppings was applied in both the Government and NGO fish farms at a rate of 1.5 kg/ decimal and 1 kg/decimal respectively during October to December instead of cowdung for pond fertilization (Table 1).

Table 1. Data on Fish Farming Practices in different Farms included in the Experiment

Fish farm	Area (Acre)	Depth (m)	Stocking Density (no/dec.)	Fertilization (Kg/dec.)		Supplementary feeding		Fish spp.
BAU	0.02	1	35-40	Urea TSP Compost Cowdung	0.2 0.1 2 1	Rice bran: Oilcake Feeding rate (of body wt)	3:1 5%	Rui, Catla, Mrigal, Silver carp, Silver barb, Grass carp, Carpio
Govt.	0.023	2	70-80	Urea TSP Cowdung Poultry dropping	0.2 0.1 1 1	Maize bran: Oilcake Feeding rate (of body wt)	6:1 3%	
NGO	0.025	1.8	60-65	Urea TSP Cowdung Poultry dropping	0.1 0.1 1 2	Rice bran: Oilcake Feeding rate (of body wt)	3:1 4%	

Sampling was carried out at monthly intervals. During each sampling, 6 fishes from each farm were collected with the help of seine net. Plastic bags were used for transporting the fish samples in the 'Fish Disease Laboratory' of the 'Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. Water quality parameters i.e., pH, dissolve oxygen, temperature, total hardness, conductivity and total dissolve solids were estimated at monthly intervals between 0800 to 0900 hours by using a sterilized disposable bottle. Standard procedures and methods were followed by using HACH'S kit (Model FF-1A).

The sampled fishes were examined thoroughly by naked eye and then placed it under a low powered dissecting microscope to observe any kind of injury, infection or other abnormalities. Samples for histopathological study were collected from five organs i.e., skin, muscle, gills, liver and kidney through dissection. Then the samples were fixed in 10% neutral buffered formalin immediately after collection. After 12 hours of fixation, the samples were trimmed into a standard size (2 cm^3) and then processed in an automatic tissue processor for dehydration, clearing and infiltration (SHANDON, CITADEL 1000). The samples were sectioned at 5 μm by using microtome machine, stained with routine Haematoxylin and Eosin (H & E) and mounted with Canada balsam. The stained slides were then examined under a compound microscope (OLYMPUS) and photographs were taken by using a photo micrographic camera (OLYMPUS, Model CHS, Japan).

Results

Water quality parameters from different fish farms were studied for about nine months and the recorded values of the investigated fish farms were almost nearer during the experimental period (Table 2).

Table 2. Water quality parameters (Mean± SE) sampled at monthly intervals from fish farms

Water Quality Parameters	Mean± SE (From April to December)		
	BAU Fish Farm	Govt. Fish Farm	NGO Fish Farm
Temperature (°C)	27.44 ± 0.40	27.16 ± 0.62	27.65 ± 0.28
Dissolved oxygen (ppm)	5.36 ± 0.23	5.02 ± 0.27	5.17 ± 0.38
pH	7.21 ± 0.20	7.14 ± 0.22	7.05 ± 0.32
Hardness (mgl^{-1})	87.62 ± 0.49	91.93 ± 0.24	92.00 ± 0.19
Conductivity (μs)	210.36± 0.97	216.22 ± 0.19	220.33 ± 0.27
Total dissolved solids (ppm)	176.00 ±.024	180.66 ± 0.85	171.11 ± 0.32

Clinically both silver carp *(H. molitrix)* and silver barb *(B. gonionotus)* were observed bright, healthy and normal during the period of April to June whereas, scale loss with minor infectious symptoms in the skin were evidenced during July to October in all the investigated fish farms. These scenarios were found quite different during November and December. In the BAU fish farm, different symptoms like fin erosion, slight skin losses and haemorrhagic lesions were observed around the dorsoventral regions in *H. molitrix,* followed by *B. gonionotus.* Here, *H. molitrix* of Government fish farm had shown mild pathological symptoms during this period (November and December) having only rough skin and mild epidermal loss, while, serious scale losses was appeared in the lateral region of *B. gonionotus.* On the other hand, investigated fishes of NGO fish farm had several scale losses and rough skin symptoms observed in November. In addition, some other significant symptoms were appeared in the experimental species during December. *H. molitrix* had various scale losses along with bright reddish spot at dorsoventral region, while *B. gonionotus* had weak body and rough skin in caudal and ventral region.

Histopathologicallly skin and muscle of both the experimental species of BAU fish farm were severely affected during November and December other than April to June. Parts of epidermis along with dermis were lost and separated with sloughing off condition. In addition, necrosis, ruptured myotomes as well as missing were seen in many places, thus vacuolation was noticed. But mild pathology was observed during July to October. Similar symptoms were also observed in both the fishes of NGO and Government farms (Figs. 1& 6). However, several distinct and well developed fungal granuloma and its traces were noticed in the affected muscle of *H. molitrix* in the above two fish farms during the colder season.

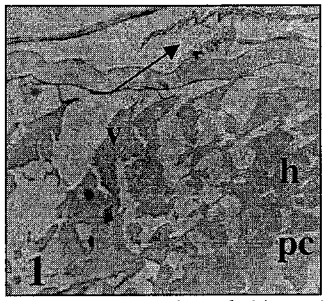

Fig. 1. Cross section of skin and muscle of sharpunti from NGO fish farm during July to October. Epidermal loss (↗), vacuoles (v), haemorrage (h) were noted. H & E x 125.

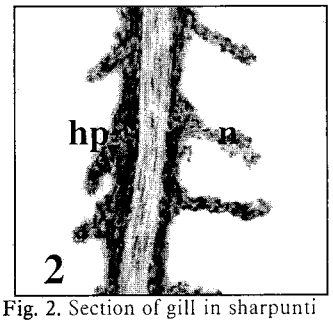

Fig. 2. Section of gill in sharpunti from Govt. fish farm during July to October. Secondary gill lamellae (SGL) were greatly hypertrophied (hp) and necrotized (n). H & E x 420.

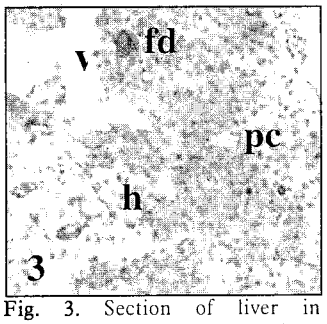

Fig. 3. Section of liver in sharpunti from NGO fish farm during November & December. Hepatocytes were highly haemorrhaged (h), had pyknotic cells (pc), fat droplets (fd) and vacuoles (v). H & E x 420.

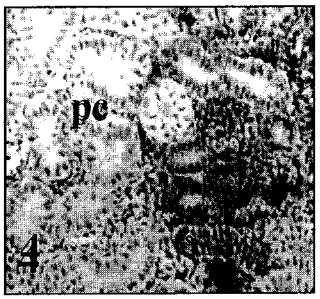

Fig. 4. Cross section of kidney in sharpunti from Govt. fish farm during July to October. Kidney tubules had pyknosis (pc). H & E x 420.

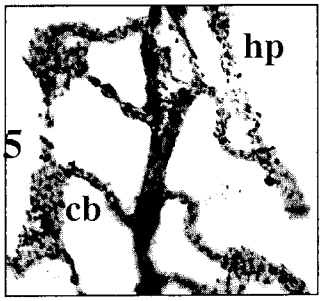

Fig. 5. Section of gill in silver carp from NGO fish farm during November & December. Secondary gill lamellae were greatly hypertrophied (hp) & clubbed (cb). H & E x 420.

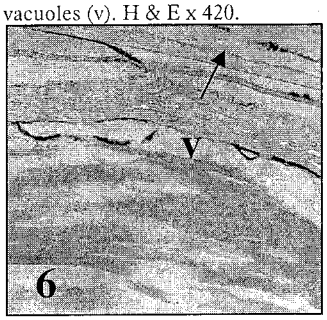

Fig. 6. Section of skin and muscle of silver carp from Govt. fish farm during November & December. Epidermal loss (↗), Vacuoles (v) was noticed. H & E x 125.

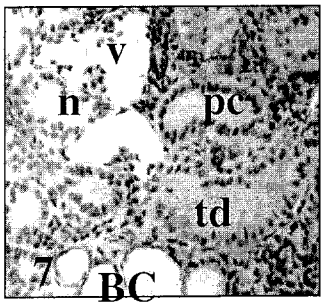

Fig. 7. Cross section of kidney in silver carp from Govt. fish farm during July to October. Necrosis (n), haemorrhage (h). vacuoles (v), pyknotic cell (pc), suspected bacterial colonies (BC) having tubular degeneration (td). H & E x 420.

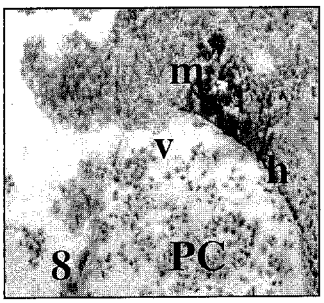

Fig. 8. Section of kidney in silver carp from Govt. fish farm during November & December. Necrosis (n), hemorrhage (h), vacuoles (v), melanin (m) pigments and protozoan cysts (PC). H & E x 420.

Among the organs, gills of the investigated fishes were also found affected in the BAU fish farm followed by NGO fish farm. It includes marked hypertrophy, clubbing, haemorrhage, missing of gill lamellae observed mainly in November and December (Fig. 5). But incase of Government fish farm, the intensity of gill pathology in *H. molitrix* was remarkably higher than the *B. gonionotus* causing severe hypertrophy, necrosis, clubbing, haemorrhage and pillar cells disruption in and between the primary and secondary gill lamellae. Both the gill lamellae were found to have swollen at its tips and many inflammatory cells were accumulated at the base (Fig. 2.).

Internal organs such as liver were severely affected in both the experimental fishes of NGO and Government fish farms having massive necrosis, pyknosis and inflammation (Fig. 3). But in the BAU fish farm, liver of the investigated fishes had shown less pathology compared to the other fish farms having some kinds of minor necrosis.

However, kidney of *B. gonionotus* of both the Government and NGO fish farms had shown different pathological changes like haemorrhage, pyknosis, and necrosis along with suspected bacterial colonies in the surroundings of renal cells during November and December (Fig. 4.). All the above mentioned symptoms were also observed in *H. molitrix* but more intensely (Figs. 7 & 8). Not only these but also, some protozoan cysts were evidenced in the kidney tubules of *H. molitrix,* which indicated it's susceptibly to disease (Fig. 8).

Discussion

Clinically both the investigated fishes were observed infected during the winter season. In *Hypophthalmichthys molitrix* rough skin, scales loss and weak body was observed in November whereas, haemorrhages with reddish bright spots were evidenced at dorsoventral region of skin during December. In contrast, scale loss and rough skin was observed in *Barbodes gonionotus* during November, but serious haemorrhages in dorsoventral and lateral region had found during December. However, both the fishes were recorded to have minor abrasions during July to October and had almost normal condition during April, May and June. Ahmed and Hoque (1999) mentioned that clinical symptoms like grey white necrotic areas were increased in December, January and February in various carps of Bangladesh. Similar symptoms have also been reported by Hoque (1998), Islam (1999), Moniruzzaman (2000) and Roy *et al.* (2006).

Nevertheless, histopathologically *H. molitrix* of both NGO and Government fish farms had several fungal granulomas and traces in its muscle. Noga and Dykstra (1986) were of the opinion that marked granuloma, inflammatory response were shown by fish infected with *Aphanomyces* sp. Hoque *et al.* (1999) also reported that large deep and whitish ulcers were recorded in the lateral region (near dorsal fin) and caudal region, where part of fins, scales and muscles were lost in most of the EUS affected fish. The result was also agreed with the observation of Moniruzzaman (2000), Roy *et al.* (2006) and Akter *et al.* (2006), Ahmed and Hoque (1999). Thus, it could be mentioned that *H.*

molitrix of NGO and Government fish farms were infected with Epizootic Ulcerative Syndrome (EUS).

Moreover, gills of investigated fishes were also observed infected in the investigated fish farms having hypertrophy, clubbing, haemorrhage, missing of gill lamellae occurred mainly in November and December. Islam *et al.* (1999) found that gills of local and exotic carp species were severely affected during colder months i.e., December and January when compared with other months. Almost similar gill pathology was also found by Ahmed and Haque (1998), Roy *et al.* (2006) and Akter *et al.* (2006). Thus, it could be reported that, infection of gills were also depended on seasonal variations.

On the other hand, histopathologically liver were severely affected observed in both the NGO and Government fish farms exhibiting necrosis, pyknosis and inflammation, while BAU fish farm had minor infections. Ram and Singh (1988) made similar opinion that liver exhibited varying degree of pathological changes. Afifi (1996) also observed that vascular changes in the liver due to the endothelial lining of the blood vessels and prevascular cuffing.

In kidney, investigated fishes of both the Government and NGO fish farms had different pathological changes and suspected bacterial colonies. Some protozoan cysts were also found in *H. molitrix* around its renal surroundings, which indicated it's susceptibly to disease. Similar opinion was also given by some authors, such as silver carp is susceptible to many diseases caused by parasitic protozoan (Ribelin and Migaki 1975). In addition, Bejerano *et al.* (1979) reported that bacterial infections can lead to death in silver carp. The scientist also observed that bacteria were introduced from poultry feces used to fertilize carp ponds. Mi *et al.* (1993) described septicemia, a common symptom of bacterial infection on silver carp, as a process of acute hemorrhagic inflammation accompanied with functional disorder in the heart, kidney and brain. He *et al.* (1992) isolated more than 10 strains of pathogenic bacteria from silver carp in Shashi District, China. From the result, it could be mentioned that *H. molitrix* of Government fish farm was severely affected and diseases like EUS, suspected bacterial colonies and protozoan infection occurred during colder months.

In contrast, when we consider the fish farms, BAU fish farm had comparatively less pathological symptoms in the investigated species among the other fish farms. Low stocking density of BAU fish farm could be one of the reasons having of less infection. On the other hand, clinically and histopathologically, internal organs like liver and kidney of the investigated fishes found affected in colder months. Ahmed and Hoque (1999) reported that histopathologically the internal organs like kidney and liver were more affected in compare with other organs. Similar pathological symptoms in kidney of major carp were observed by several authors like Islam (1999), Roy *et al.* (2006), and Akter *et al.* (2006). Thus, it could be mentioned that the health condition of BAU fish farm had in improved conditions, whereas Government fish farm was the most severely affected. Disease like Epizootic Ulcerative Syndrome (EUS) was clearly evidenced in the fishes of Government and NGO fish farms.

Clinically and histopathologically, different distinct effects of water temperature and season were also observed in the experimental fishes. Both the exotic carps had

almost normal condition during April to June whereas, minor pathological changes in organs were observed in July to October. But marked and distinguishing pathological changes in organs were evidenced in November and December. Similar results was also reported by different authors like Ahmed *et al.* (2004), Roy *et al.* (2006) and Akter *et al.* (2006), who examined through using clinical, parasitological and histopathological techniques on three small indigenous fishes and found that all fishes were severely affected during the month of November, December and January. It was also agreed by Hossain and Paul (1993), who observed that the outbreak of disease was highest when water temperature was at the lowest. So, it could be assumed that low water temperature might play a role in the health condition changes in exotic fishes.

Again when we consider individual fish species, *H. molitrix* was more susceptible to infectious diseases e.g., EUS, suspected bacterial infection and protozoan diseases than *B. gonionotus*. Furthermore, fishes of Government farm had shown different pathologies and infectious symptoms over the other fish farms. This is may be due to poor management practices i.e., high stocking density, poor quality of fingerling, low quality in feed management and less attention on the health condition of fishes. The correlation between disease and stocking density was observed by Duijn (1973) and stated that high stocking density often cause fish disease in cultural system. Thus, it could be suggested that more preventive and cautionary measures would necessary to be carried out in this fish farm to prevent diseases in order to have healthy and disease free fish. According to Srivastava (1975), success of the implementation of various fisheries development program depends, to the extend, on the intensification of fish disease research, as the improvement of fish yield can be achieved from healthy fish stock. In this situation, either facilities for disease diagnosis should be introduced or affected fish should be sent to the diagnostic laboratory immediately for diagnosis of diseases in order to have a healthy and disease free fish production.

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Histopathological studies of EUS affected shing, *Heteropneustes fossilis* (Bloch) from a fish farm of Mymensingh, Bangladesh

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Abstract
An investigation was carried out to observe histopathological changes in liver and kidney of suspected epizootic ulcerative syndrome (EUS)-affected shing fish, *Heteropneustes fossilis* (Bloch) collected from the "Agro-3 fish farm" situated at Boilor, Trishal, Mymensingh. Focal necrosis, haemorrhages and atrophy of the sinusoidal region were observed in the liver tissue. Fungal granulomas were found both in liver and kidney. In some cases fatty depositions were observed in all over the hepatic tissue. Degeneration and necrosis of renal tubular epithelial cells were also occurred. Missing of glomerulus and necrosis surrounding the Bowman's capsule in the kidney tissue were found.

Key words: *Heteropneustes fossilis*, EUS, Histopathology

Introduction

Among the commercially important fishes of Bangladesh *Heteropneustes fossilis*, locally known as "shing", is an important air breathing catfish in Bangladesh and generally grows in pond, lake, baor, beels and floodplains with natural care. Shing fish has been reported to be affected by some metazoan parasitic diseases (Sanaullah 1976) and bacterial diseases (Sahoo and Mukherjee 1997). In India, *Gyrodactylus neonephrotus* was detected from the skin of *H. fossilis* by Singh and Agarwal (1994). Srivastava *et al.* (1998) stated the toxicity of malachite green in liver of a shing during the pre-spawning phase. Detrimental effects on the liver cells, including hypertrophy and vacuolization, followed by necrosis and cirrohosis were observed at acute, sub-acute and sublethal concentrations in both the short and long term treatments. Sahoo and Mukherjee (1997) detected three Gram negative bacterial pathogens as *Aeromoas hydrophila*, *Edwardsiella tarda* and *Haemophilus piscicum* from shing fish *Heteropneustes fossilis*. The epizootic ulcerative syndrome (EUS) have been detected in many fishes of Asia Pacific region (Lilley *et al.* 1992). In Bangladesh the EUS have also been found in many natural and culture fishes by different authors (Barua 1989, Ahmed and Rab 1995, Chowdhury *et al.* 1996). Sahoo *et al.* (1998) detected the ulcer disease in the shing fish for the first time in India. Barua (1989) mentioned shing fish in his list of susceptible fishes to EUS. But

until 2006, this fish was not found to develop ulcer type disease in Bangladesh. However, in September, 2006, the fish was found to be affected by EUS like ulcer disease in a fish farm named "Agro-3 fish farm" in Bailor, Mymensingh.

In all countries detection of fungal granuloma together with other histopathological findings have been proved to be the confirmatory tests of the etiological agent *Aphanomyces invedans* in EUS affected fishes, includng Bangladesh by different autrhors. Haque *et al.* (1999) observed the fungal granuloma together with necrosis, haemorrhage and pyknotic cells in EUS affected catla fish. So the above diseased fish were undergone histopathological investigations in order to confirm the etiology of the ulcer type lesions, in one side and in another side, bacteriological investigations were done in order to understand the involvement of any bacteria with those lesions. The present paper deals with the histopathological findings of the fish. Report of their bacteriological investigation will be published elsewhere.

Materials and methods

Study area and duration

A fish farm namely "Agro-3 Fish Farm" was selected for the present experiment that was situated at Boilor, Trishal, Mymensingh. Duration of the study was September, 2006 to January, 2007.

Collection of sample

A total of 20 *Heteropneustes fossilis* (Shing) were selected as the experimental fish. They were collected from one of the ponds of the above farm, in which the fish were suffering from suspected EUS like lesions and were having severe mortality. Such diseased fish were caught by using seine net during sampling. No dead fish were taken. After netting, the fish were taken in a plastic bucket with pond water and immediately brought to the fish disease laboratory, Bangladesh Agricultural University, for study.

Fixation and preservation

Samples of liver and kidney were collected from each fish by a sharp scalpel and forceps for histopathological study. An I cm^3 portions of each organ, from each fish were taken and fixed in 10% neutral buffered formalin. The amount of fixative was 10 times to bulk of tissue fixed (Humason 1979). After at least 10 days of fixation, the samples were trimmed for histopathological preparations.

Histopathological procedure

The fixed samples were taken out with forceps from the vial and placed separately in a perforated plastic holder which was covered by perforated still plates. Marking was done with dark pencil (2B) in perforated plastic holder. The samples (blocks) were then arranged in a still rack and placed in an automatic tissue processor for dehydration, clearing and infiltration (SHANDON, CITADEL 1000). Alcoholic series of higher concentrations, xylene and paraffin wax were used in the processor maintaining at

various time schedules. After tissue processing (21 hours), the samples were embedded with melted wax on perforated plastic holder and still mold. The solid blocks were placed in a refrigerator (deep freeze) for half an hour and paraffin blocks were separated from the still molds. Surface and side of paraffin blocks were trimmed with the help of scalpel and microtome (Leica, JUNG RM 2035). Blocks having hard tissue before sectioning were decalcified by dissolving the surface of blocks in water for one hour followed by washing rapidly. The blocks were placed again in deep freeze for 30 minutes. Sections were taken from the blocks at a thickness of 5 micrometers. The ribbon with section was placed on a water bath at a temperature of 40°C, which were finally picked up over glass slides. The sections were then stained with haematoxyline and eosin and were mounted with Canada balsam. The stained slides were then examined under a compound microscope (Olympus). Photomicrographs were done by using a photomicroscope (OLYMPUS, Model CHS, Japan).

Results and discussion

From the severe outbreak of ulcer type disease in shing fish of the Agro-3 farm, first time in Bangladesh, round ulcers were observed on body surface. No such ulcer type lesions in *Heteropneustes fossilis* were found in Bangladesh prior to this occassion. However, Sahoo *et al.* (1998) found an outbreak of ulcer disease of shing fish in India.

The results of the histopathological study revealed necrosis and atrophy of the sinusoidal region in the liver of the infected fish (Fig. 1). Focal necrosis and haemorrhages were occurred in the liver tissue (Fig. 2). Fungal granulomas were found in liver tissue of the shing which proved that the causative etiology of the ulcerative disease was *Aphanomyces* like fungal pathogen. Prominent vaculations were also observed (Fig. 3). Fatty deposition was found in the whole hepatic tissue (Fig. 4). Degeneration of renal tubules were detected in the haematopoietic tissue (Fig. 5). Necrosis and vacoulation of renal tubular epithelial cells with fungal granuloma were present in kidney tissue (Fig. 6). Missing of glomerulus and necrosis surrounding the Bowman's capsule were found (Fig. 7). Hemorrhages occurred in the whole kidney tissue with fungal ganuloma (Fig. 8) ensuring the disease as EUS. In another part of this investigation (Hasan 2007), *A. hydrophila* bacteria were isolated from the above ulcers. So, to compare the present natural histopathology with that of experimental one by *A. hydrophila*, an experimental histopathplogical investigation of the shing fish was done by Islam (2006). He found that *A. hydrophila* caused focal necrosis in haematopoietic tissue together with hemorrhage and atrophy. Infected liver showed pathology like massive atrophy, haemorrhages, necrotic hepatocytes, focal necrosis and atrophy of hepatic sinusoids represented by necrosis of the sinusoidal lining cells. *A. hydrophila* cells were distributed all over the hepatic tissue.

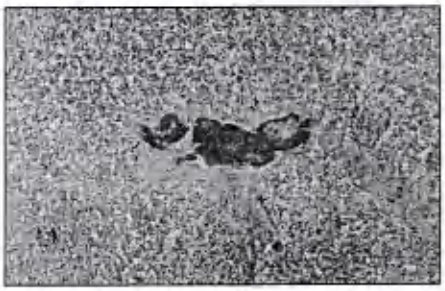

Fig. 1. Necrosis (n) and atrophy (↗) of the sinusoidal region in liver of naturally infected shing. H & E (×125).

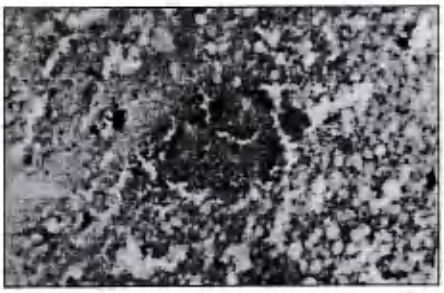

Fig. 2. Focal necrosis (↗) characterized by haemorrhagic lesion in the liver of a naturally infected shing fish. H & E (×440)

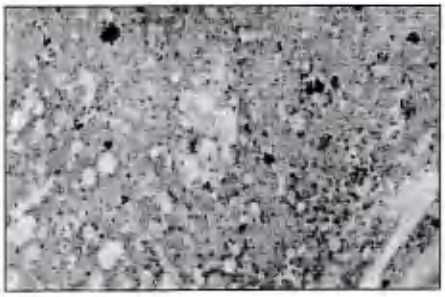

Fig. 3. Fungal granuloma (↗) were found in the liver with vacuolation (v) in a shing fish. H & E (×440).

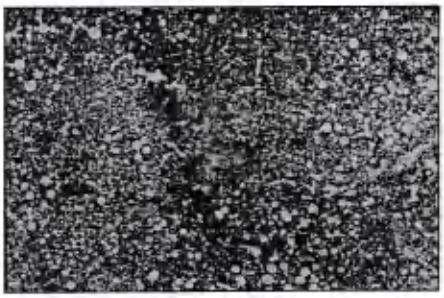

Fig. 4. Focal necrosis (↗), hemorrrhages (h) and fatty deposition occured in the liver tissue of a shing fish. H & E (×125).

Fig. 5. Degeneration of renal tubules (r) were detected in the haemato-poietic tissue. H & E (×440).

Fig 6. Necrosis (n) and vacoulation (v) of renal tubular epithelial cells with fungal granuloma (↗) in kidney tissue. H & E (×440).

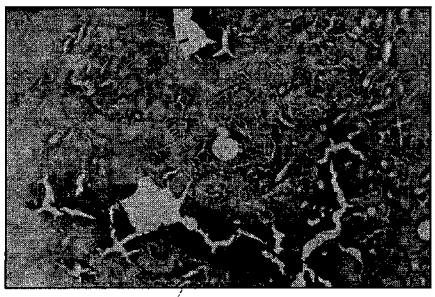

Fig. 7. Missing of glomerulas (↗) and also necrosis (n) surrounding the Bowman's capsule in the haematopoietic tissue. H & E (×125).

Fig. 8. Haemorrhages (h) occurred in whole kidney tissue with fungal granuloma (↗) of infected shing fish. H & E (×440).

The findings of Islam (2006) were considered to be similar with the present natural histopathological changes of the shing fish. So it may be concluded that the present histopathological changes might be the concurrent effect of both the fungal pathogen (*Aphanomyces*) and the bacteria *A. hydrophila*. EUS diseases of many fishes were found to be caused by both the above pathogens by many authors in different fishes. Faruk *et al.* (2002) studied some diseased carps and varying degree of pathology was noticed in the kidney of fishes, which included degeneration and necrosis of kidney tubules, vacuole, tubular granuloma, haemotopoietic necrosis and encystment of parasite in the kidney. Ram and Singh (1988) reported varying degrees of pathological changes from *Channa punctatus* including cytoplasmolysis, nuclear pyknosis, and necrosis leading to complete degeneration of hepatocytes. Ventura and Grizzle (1988) observed lesions associated with natural and experimental infections of *A. hydrophila* in channel catfish, *Ictalurus punctatus*.

From the present study it was understood that the natural EUS lesions in concern of shing was caused by the fungus *A. invadans* together with *A. hydrophila* bacteria because the bacteria *A. hydrophila* was isolated from these lesions (Hasan 2007) and fungal granuloma were detected through histopathology. Akter *et al.* (2006) found well developed fungal granuloma from the kidney of EUS affected *Macrognathus aculeatus*. The fungal granuloma in the liver of *Cirrhinus mrigala* were confirmed by Hatai *et al.* (1994) and Roberts *et al.* (1994) to be proof of EUS by *A. invadans*. Hoque *et al.* (1999) observed necrosis, haemorhage, pyknotic cells and numerous fungal granuloma with some hyphae in kidney tissue of *Catla catla*. Islam (2006) did not find fungal granuloma in the shing fish because he infected shing fish artificially only by *A. hydrophila*. So pathology observed in naturally infected shing fish were understood to be caused by *A. hydrophila* together with *A. invadans* as supported by the findings of Islam (2006) in his artificial infection experiment. From the present study, it may be concluded that culturists may adopt proper management practices to avoid EUS and to get rid of the infection by such serious bacterial pathogen like *A. hydrophila*.

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Experimental pathogenesis of *Aeromonas hydrophila* bacteria in shing *Heteropneustes fossilis* (Bloch)

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Abstract
Pathogenicity of *Aeromonas hydrophila* bacteria was tested on the stinging catfish *Heteropneustes fossilis*. Before artificial infection the morphological, biochemical and physiological characters of *Aeromonas hydrophila* were studied. The infections were done by two different methods, *viz.*, intramuscular (IM) and intraperitoneal (IP) injection. In infection experiment, each group of 10 fish were injected either intramuscularly or intraperitoneally with one dose higher than the LD_{50} dose (9.6×10^7 CFU/fish). All the fish tested died within 1 to 9 days. Both in cases of intramuscular and intraperitoneal injection, external pathology were found. Haemorrhagic lesions were evident at the site of injection. The posterior end of the body surface was found to develop greyish-white lesion that was extended up to caudal fin. Hyperemic anal region and the fin bases were also observed. Total bacterial loads in liver, kidney and intestine were determined. *Aeromonas hydrophila* could be isolated from liver, kidney and intestine of the experimentally infected fish. In case of intramuscular injection the highest and the lowest bacterial load was found to be 2.4×10^7 CFU/g of liver and 2.1×10^2 CFU/g of kidney and in case of intraperitoneal injection they were found to be 3.6×10^6 CFU/g of kidney and 1.2×10^4 CFU/g of kidney respectively. It was concluded that *A. hydrophila* could cause serious disease condition to *Heteropneustes fossilis* and its pathogenesis in the fish was also very efficient.

Key words: Experimental pathogenesis, *Heteropneustes fossilis*, *Aeromonas hydrophila*

Introduction

Stinging catfish *Heteropneustes fossilis* is a hardy fish having much economic importance in Bangladesh. It can withstand wide environmental variation in relation to temperature and oxygen content. So it was supposed that the fish should be resistant to many fatal infections like epizootic ulcerative syndrome (EUS). However, only recently, the fish was found to be affected by EUS like lesion in an established farm in Mymensingh, Bangladesh. *Aeromonas hydrophila* could be isolated from those lesions (Hasan 2007). There are many evidences that *A. hydrophila* was associated with EUS in different fishes.

Laillier *et al.* (1980) stated that *A. hydrophila* might be the global bacteria associated with the fish diseases. *Aeromonas* spp. contributed to the pathogenesis of the

EUS disease (Lutwyche *et al.* 1995). Certain strains of *A. hydrophila* could induce EUS-like lesions were reported by Lio-Po *et al.* (1990). *Aeromonas* spp. contributes to the pathogenesis of MAS (Motile *Aeromonas* septicemia) disease (Roberts *et al.* 1990).

The present study was carried out to understand the pathogenesis of *A. hydrophila* in experimentally infected shing, *H. fossilis* and thereby to know the fate of the pathogen in the tissues of the fish. Thus the study might be helpful for the management and control of the aeromonads and other bacterial diseases in at least stinging catfish.

Materials and methods

Experimental site and duration of the study

The infection experiments were conducted at the wet laboratory of the Faculty of Fisheries. Duration of the experiment was from July to December 2006.

Experimental preparations

A recycle system was set with pipe fittings and electric motor in the wet laboratory. The recycle system consisted of 5 metallic drums each having 150 l capacity, twelve aquaria of fibre glass each having 40 l capacity and an over head tank. The water was supplied at first to the drums and then pumped up to the overhead tank. It was then drained to the aquaria by downward pipe ventilated to each aquarium. From the aquaria it was collected by a collecting tube which was passed through an ultra-violet tube light complex to sterilize the circulating water during the experimental period and then opened at the first drum. The circulation system was filled with both pond and supply water. Prior to the experiment the water was kept under circulation for 7 days. Glass wares (Petri dishes, test tubes, L-sticks, morter and pastle) were dry sterilized at 170°C for 1 hour by a dry sterilizer (Memmert). The tips were autoclaved at 121°C for 15 min and then dried at 70°C for overnight. Tryptic soya agar (TSA, Oxoid) plates were used for the culture of bacteria and TSA agar slants, for stocking of bacteria.

Fish stocking in the recycle system

The fish collected from the selected pond by seine net, were kept in the above mentioned 12 aquaria of the recycle system for 7 days for acclimatization. The aquaria were covered with synthetic fibre net to prevent fish escaping. Every day 50% of total water was changed. Aerators were set in the aquaria for continuous aeration during the whole experimental period.

Collection and characterization of Aeromonas hydrophila

An *Aeromonas hydrophila* isolate CK602 previously isolated, characterized and stocked by Sabur (2006) was collected, restocked in TSA slants and recharacterized before starting the experiment as follows.

Morphological characters such as shape, size, Gram character, flagellation and motility were observed using fresh 24-hour cultures. Biochemical characters such as, oxidase, catalase, oxidative-fermentative (OF), acid and gas production from sugars

(glucose, lactose, maltose, sucrose and manitol), methyl-red, Voges-Proskauer (VP), indole and H_2S production, decarboxylase and citrate utilization were studied. Physiological characters were checked by observing the growth of each isolate at temperatures of 4°C, 5°C, 37°C and 40°C and in different concentrations of NaCl such as 0%, 1%, 2%, 3% and 4%.

Experimental infections

For preparation of bacterial suspension *A. hydrophila* were freshly cultured onto TSA plate, incubated at 25°C for 48 hours and then 52 mg of the bacterial colonies were mixed with 4 ml of sterile physiological saline (0.85% NaCl in distilled water). For the experimental infection method 1 ml sterile plastic (disposable) syringe was used. Each of the 10 experimental fish was injected with 0.2 ml bacterial suspension either intramuscularly or intraperitoneally. One dose higher than the calculated LD_{50} (Mostafa 2007) was planned to be injected. Accordingly the suspension was made and the desired dilution was prepared and counted to be 9.6×10^7 CFU/ml.

An allocate of 0.2 ml of the above bacterial suspension was injected at the base of the dorsal fin of each fish after disinfection with 70% alcohol cotton. For intraperitoneal infection, bacterial suspension was injected in the peritoneal cavity at a dose of 0.2 ml of each selected fish. Injections were given with utmost care to avoid puncture of internal organs. At the injection period care also was taken so that the inoculum (bacterial suspension) would not come out after pushing back of the syringe.

All the injected fish were then transferred to the aquaria and observed for 15 days of the experimental period. No feed was supplied during this time. The injected fish were observed daily several times for any abnormal clinical appearances and were recorded properly. Water temperature was also recorded daily. Moribund fish were attended, observed and waited for their death. Freshly dead fish were collected immediately, transferred to the laboratory and used for bacterial isolation.

Isolation of bacteria from liver, intestine and kidney

Intestine, liver and kidney of each freshly dead fish were dissected out aseptically and placed in sterilized separate plastic petri dishes. After weighing, sample of each of the above organ was homogenized and suspended in sterile physiological saline (1 part of sample: 9 parts of PS) to obtain a stock solution. Two consecutive decimal dilutions, 10^{-1} and 10^{-2}, from the stock solution were made for each organ. The dilutions (stock, 10^{-1} and 10^{-2}) of each organ were spreaded onto duplicate TSA plates. All such plates were incubated at 25°C for 48 hours. Appeared colonies were counted and their numbers were used to interpret the pathogenesis of the pathogen in the organs of the experimentally infected fish, expressed by bacterial loads.

The bacterial load was calculated by using the following formula worked out by Mamnur Rashid *et al.* (1994).

$$\text{Bacterial CFU/g of fish organ} = \text{No. of colonies counted in the plate} \times 10^n \times 100$$

where, n is the dilution factor

Results and discussion

Morphological, biochemical and physiological characters of the *Aeromonas hydrophila* isolate (CK 602) have been shown in Table 1. They were found to possess same characteristics like those tested by Popoff (1984) and Sabur (2006).

Table 1. Characters of *Aeromonas hydrophila* isolates in comparison to other studies

Characters	Characterization by Popoff (1984)	Characterization by Sabur (2006)	Present results
Gram stain	-[1]	-	-
Shape	Rod	Rod	Rod
Motility	+[2]	+	+
Oxidase	+	+	+
Catalase	+	+	+
OF test	F[3]	F	F
Acid and gas production from Glucose	+	+	+
Acid production from			
Lactose	+	+	+
Sucrose	+	+	+
Maltose	+	+	+
Manitol	+	+	+
Insitol	-	-	-
Sorbitol	-	-	-
Rhamnose	-	-	-
Methyl-red test	-	-	-
Voges-Proskaur	+	+	+
Indole	+	+	+
H_2S production	+	+	+
Arginine decomposition	+	+	+
Lysine decarboxilation	-	-	-
Ornithine decarboxilation	-	-	-
Citrate utilization	+	+	+
Growth in : 4°C	-	-	-
: 5°C	-	-	-
: 37°C	+	+	+
: 40°C	+	+	+
: 0% NaCl	+	+	+
: 1% NaCl	+	+	+
: 2% NaCl	+	+	+
: 3% NaCl	+	+	+
: 4% NaCl	-	-	-

[1] Negative, [2] Positive , [3] Fermentative

The results of the pathogenesis of *A. hydrophila* in the liver, intestine and kidney *i.e.* their fate after experimental infection with different doses and by two different methods, *viz.*, intramuscular injection and intraperitoneal injection are shown in Table 2 and 3.

Table 2. Fate of *Aeromonas hydrophila* in liver, kidney and intestine of stinging catfish, *Heteropneustes fossilis* artificially infected by intramuscular injection at a dose of 1.9×10^7 CFU/fish

Fish No.	Bacterial colony count		
	Liver	Intestine	Kidney
F_1	2.4×10^7	1.5×10^7	1.3×10^7
F_2	2.1×10^5	1.5×10^6	4.1×10^4
F_3	3.0×10^4	4.8×10^5	4.1×10^5
F_4	1.3×10^6	1.7×10^6	3.8×10^6
F_5	1.4×10^5	3.5×10^6	2.7×10^5
F_6	6.5×10^5	3.1×10^5	4.7×10^4
F_7	6.2×10^4	1.0×10^5	1.3×10^5
F_8	3.9×10^5	1.1×10^5	4.2×10^4
F_9	6.2×10^4	9.0×10^3	2.1×10^2
F_{10}	6.0×10^4	5.1×10^5	3.1×10^3

Table 3. Fate of *Aeromonas hydrophila* in liver, kidney and intestine of stinging catfish, *Heteropneustes fossilis* artificially infected by intraperitoneal injection at a dose of 1.9×10^7 CFU/fish

Fish No.	Bacterial colony count		
	Liver	Intestine	Kidney
F_1	2.1×10^5	2.2×10^5	1.4×10^5
F_2	3.9×10^5	5.0×10^4	3.6×10^6
F_3	2.0×10^4	1.3×10^4	1.2×10^4
F_4	1.9×10^6	2.9×10^6	3.7×10^5
F_5	2.0×10^5	3.7×10^5	3.6×10^6
F_6	$5.2 \times .10^5$	2.5×10^4	6.6×10^4
F_7	1.3×10^5	1.1×10^6	3.3×10^4
F_8	1.5×10^6	2.9×10^5	1.9×10^5
F_9	2.7×10^6	2.8×10^5	4.5×10^5
F_{10}	3.2×10^4	3.0×10^5	2.1×10^5

In the present study, *A. hydrophila* could be isolated from liver, kidney and intestine of experimentally infected shing by the homologous bacteria. Highest bacterial load was found to be 2.42×10^7 CFU/g of liver and the lowest, 2.1×10^2 CFU/g of kidney of *H. fossilis*. The range was 2.0×10^4 to 2.42×10^7 CFU/g of liver. In case of intestine, 3.5×10^6 CFU/g and 9.0×10^3 CFU/g was respectively the highest and lowest bacterial count. The bacterial load was found to be 1.3×10^7 to 2.1×10^2 CFU/g of kidney. Rahman and Chowdhury (1996) found total bacterial load in kidneys of carps to be 2.6×10^5 to

1.7×10^6 CFU/g. Iqbal *et al.* (1996) found total bacterial load to be 5.4×10^3 to 4.7×10^7CFU/g in slime and undetectable to 1.7×10^4 CFU/g in kidney of *Cirrhinus mrigala*. In the experimental infection of the selected *A. hydrophila* isolate (CK602) done intramuscularly and intraperitoneally to shing fish at a dose of $1.92x10^7$ CFU/fish, mortalities were 100% within 1-9 days. Alam *et al.* (1999) used 1.18×10^3 to 4.81×10^4 CFU/fish of *Edwardsiella tarda* bacteria for infecting Thai pangas (*Pangasius sutchi*) and 33% to 100% mortalities were achieved within 6 to 10 days. Pal *et al.* (1997) found 40-100% mortalities of silver barb (*Puntius gonionotus*) with a dose of $2\text{-}6\times10^6$ CFU/ml of *Pseudomonas fluorescens* to detect their pathogenicity. Miyashita (1984) found 40% death of tilapia by *Edwardsiella tarda* at doses of $10^{6\text{-}7}$ CFU/fish within 1-6 days. In the present study external clinical pathologies were observed in the moribund *H. fossilis* experimentally infected by *A. hydrophila*. Haemorrhagic lesions at the site of injection were observed. Wanna (2000) found petechial haemorrhages at the injected area and congestion around the anus of *Clarias batrachus* intraperitoneally with *A. hydrophila*. Hasan (2007) isolated *A. hydrophila* from naturally EUS affected shing and found the load to be 1.67×10^4 to 6.46×10^8 CFU/g in liver, 1.71×10^3 to 1.18×10^9 CFU/g in intestine and 1.47×10^4 to 3.70×10^8 CFU/g in kidney. Mamnur Rashid *et al.* (1996) found 100% mortality from an experimental infection of Japanese flounder *Paralichthys olivaceus* with *Edwardsiella tarda* by the dose of 10^5 CFU/fish in intra-peritoneal injection, 10^8 CFU/fish in oral intubation and 10^9 CFU/fish in immersion method respectively.

Through experimental infection it was proved that *A. hydrophila* was seriously pathogenic to shing fish which may cause heavy loss of its commercial production as also evidenced from the first observation by Hasan (2007). The bacteria *A. hydrophila* could be reisolated from the kidney, liver and intestine of the experimentally infected shing fish which proved that the pathogenesis of the pathogen in the organs of the shing fish is very efficient. Further studies are necessary to understand the histopathology of shing fish by *A. hydrophila* bacteria in natural condition as well as in experimental infection.

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Establishment of satellite nucleus of genetically improved farmed tilapia (GIFT strain) in Bangladesh

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Abstract
The aim of this study was to evaluate growth performance of the GIFT (Genetically Improved Farmed Tilapia) strain after one generation of selection for increased body weight at Bangladesh Fisheries Research Institute. Founder stock comprised of 30 families having 300 individuals of the GIFT strain were introduced from Malaysia through WorldFish Center in March 2005. The founder stock was reared in 100 m^2 hapa for three months and then individually tagged using Passive Integrated Transponder (PIT) at the weight between 30 and 40 g. After tagging, all the fish were communally grown out in pond until harvest. Breeding value for body weight was estimated using SAS and ASREML ranging from 4.17 to 9.70 g for males and 4.24 to 9.36 g for females. The best 40 females and 40 males from the founder stock were then selected to produce progeny of the first generation (F-1). From each family 25 female and 25 male fingerlings were sampled and tagged using PIT. A total of 2,000 tagged fish from 40 families were stocked in a pond (1000 m^2) for a continuation of the selection program. In addition, surplus fish after tagging were also reared together with progeny of the founder stock in cistern ecology for growth evaluation. The mean weight of the F-1 generation of GIFT fish was 7.2% greater than that of the founder population (non selected population).

Key words: Satellite nucleus, Genetically Improved Farmed Tilapia (GIFT)

Introduction

The last three decades have seen significant developments in farming of tilapias worldwide. In view of the increasing commercialization and continuing growth of tilapia industry, the commodity is not only the second most important farmed fish globally, next to carps but is also described as the most important aquaculture species of the 21st century (Shelton 2002). The fish is being farmed in about 85 countries worldwide, and about 98% of tilapia produced in these countries is grown outside their original habitats (FAO 2002). The main culture industries are in the Far East but they are increasingly being farmed in Caribbean, Latin America and recently, in temperate countries where

warm water through artificial means (thermal effluents or geothermal springs) are also available.

The development of Genetically Improved Tilapia (GIFT) technology that is based on traditional selective breeding as a means to improve commercially important traits of tropical farmed fish is a major milestone in the history of tilapia aquaculture (Azhar, *et al.* 2004). The GIFT was developed by WorldFish Center through several generations of selection from a base population involving eight different strains of Nile tilapia (Eknath *et al.* 1993 and 1998). Bangladesh Fisheries Research Institute (BFRI) received GIFT strain in 1994 and again 116 families in 1996 through WorldFish Center (Formerly ICLARM). In on-station and on-farm trials of BFRI, the GIFT strain was reported to show 35-57% superior growth than that of the existing strain of the country (Hussain *et al.* 2000). Further stock improvement of GIFT through mass selection was initiated in 1998. Through mass selection, six generations (F-1 to F-6) were produced. Through combined selection technology, the F-6 generation of GIFT strain achieved 33.7% growth over the existing GIFT strain. The rate of genetic gain in weight of fish was greater up to third generation but it decreased gradually after that and up to sixth generation. The reason behind such a decrease in genetic gain in particularly for body weight might have been the accumulation of inbreeding. Therefore, the genetic improvement strategy for GIFT was re-designed. Now the stock improvement program is being implemented through family selection protocol under the technical assistance of WorldFish Center. In this paper we report preliminary results of growth evaluation of F-1 generation of improved GIFT strain in Bangladesh.

Materials and methods

Stock improvement through family selection

Origin of stock

Founder stock comprised of 30 families having 300 individuals of GIFT Strain were introduced from Malaysia through WorldFish Center in March 2005. The founder stocks was reared in 100 m^2 hapa for three months. The fish were fed with SABINCO feed (28% crude protein) at the rate of 6% of estimated biomass. After three months rearing, the mean weight of female and male were 41.18±5.41 and 30.42±3.47g, respectively.

Tagging of founder stock

Then the female and male were tagged by using Passive Integrated Transponder (PIT). A PIT tag was injected into the peritoneal cavity of a fish and the number of tag was recorded. After tagging all the fish were transferred to a pond having 1000 m^2 area.

Rearing in pond

During rearing period, the fish were fed with supplementary feed six days in a week (28% crude protein) at the rate of 4-5% of estimated biomass. Fish were sampled at

fortnightly interval to assess the growth and feed adjustment. Water was supplied once in a week to maintain water depth at 1.0 meter. Pond was fertilized fortnightly with Urea and TSP at the rate of 12.5 and 25.0 kg/ha, respectively. After four months rearing, the fish were recaptured through seine netting and pond drying. The final body weight, sex and tag number of all harvested fish were recorded.

Estimation of breeding value

Breeding value was estimated for individual fish in a full pedigree, using SAS (SAS Inc, 1997) and ASREML (Gilmour *et al.* 1999).

Breeding in hapa

On the basis of breeding values of the founder stock, the best 40 males from 30 families were crossed with 40 best females (from 30 families) for the production of F-1 generation. For breeding, 40 breeding hapas ($1.0m^3$) were set up in a pond with bamboo poles. A pair of female and male breeders (1:1) was stocked in each breeding hapa. After 12 days of stocking, fertilized eggs were collected from brooding females. After that, collected eggs were transferred to the hatchery for incubation. Immediately after hatching, the larvae were shifted to a series of trays and were kept until their yolk sac resorption stage.

Nursing in hapa

After resorption stage, 300 fry from each family were transferred to 40 individual fine mesh nursery hapas ($2.0\ m^3$) in pond. The progeny were fed with nursery feed containing 30% protein at the rate of 30% of estimated body weight. After 45 days nursing, the mean weight of the fry was 2.80 ± 0.42g.

Rearing in hapa

Subsequently, 150 fry from each progeny group were transferred to 40 individual rearing hapa ($2.0\ m^3$ in size). Supplementary feed (Nursery feed) was applied in all the hapas at the rate of 15% of estimated biomass. After two months of rearing, the weight range of male and female were 36-43 and 28-32g, respectively.

Tagging

From each progeny group 25 male and 25 female fish were selected and tagged using Passive Integrated Transponder (PIT). Tagged fish from 40 families (2000 fishes) were stocked in a pond having $1000m^2$ area for communal rearing. Supplementary feed (25% crude protein) was supplied regularly at the rate of 6% of estimated biomass. After six months of grow-out in pond, the fish were harvested, and tag number, weight, sex, body depth were recorded. After harvesting, breeding values of F-1 generation were estimated from the complete data set, tracing back to the foundation population (F0).

Evaluation of Growth performances

This trial was conducted to compare growth performance between F-1 progeny of the selected fish (F-1 selected GIFT) and progeny of the non-selected population (founder stock) in cisterns (2.0 m^3) for a period of four months during April to July 2007. Progeny of the selected fish were produced from 40 single pair matings in separate hapas. Family rearing of the selection progeny was as described above. After tagging, surplus fish were sampled for this experiment. By contrast, the non-selected population (200 breeders) was stocked in a 300 m^2 pond for mass breeding. After 40 days of stocking, 6,000 fry were collected and reared in a 10m^3 hapa for a period of 3 weeks. From this population, fry samples were taken for growth evaluation. The initial mean weight of the selected fish and of the founder population (non selected population) were 2.95±0.65 and 2.65±0.82g, respectively. There were two treatments with three replicates. Before stocking the cisterns were cleaned and filled up with deep tube well water at the depth of 1.0 meter. Fry of GIFT strain were stocked at a density of 5 fish/m^3.

The fry of both treatments were fed twice a day in six days in a week with supplementary feed (28% crude protein) at 5-8% body weight. During grow out period, first and second months, feed was given at the rate of 8% and 7% of body weight, respectively, Then subsequently, 6% and 5% feed were given to the fish in the 3rd and 4th month, respectively. Fish sampling was done at monthly interval to assess the growth, and feeding ratio was adjusted on the basis of estimated weight of fish biomass. In every week cisterns were cleaned through siphoning and 80% water changed with deep tube well water. Average water depth was maintained in all the cisterns at 1.0 m during the experimental period. After four months rearing, all the fishes were harvested. After harvest, body weight was measured on individual fish. Statistical analysis was carried out to test significant differences in growth between the F1 generation fish and the founder stock.

Results and discussions

A total of 2000 fish (1000 males and 1000 females) of the first generation were harvested and measured of body weight in June 2007. General linear model analysis indicated that there was significant difference ($P<0.001$) in body weight between the two sexes, where the males were substantially heavier than the females (278 vs. 156 g) (Table 1). The effect of sex on size and growth is often found in aquaculture species (Ponzoni *et al.* 2005, Nguyen *et al.* 2007).

Table 1. Body weight of male and female

Sex	Number of records	Weight (g)
Male	1000	277.76±29.77
Female	1000	156.05±30.26

Therefore, the statistical model included sex as the fixed effect and the additive genetics of individual fish as the random term to estimate breeding values (EBV) of all animals in the pedigree. Based on EBV ranking, the best 70 females and 70 males from 40 families were selected to produce progeny for the second generation (F2). The EBV range for the selected males and females were 4.17- 9.70 g and 4.24-9.36 g, respectively (Table 2).

Table 2. Breeding values of selected male and female breeders

Sex	Number of animals	Breeding Values
Male	70	4.17 - 9.70
Female	70	4.24 - 9.36

The body weight data measured at different culture periods of the improved (selected) fish and founder stock (non selected population) are given in Figure 1. The initial mean weight was 30.23±0.41 and 31.70±0.60g for the improved (selected GIFT) and founder stock, respectively. Month wise sampling data showed that growth rate of the selected GIFT was always higher than the founder stock (Fig. 1). After four months rearing, the final cumulative mean weights were recorded at 168.67±3.51 and 157.33±2.52g for the selected and founder stock, respectively.

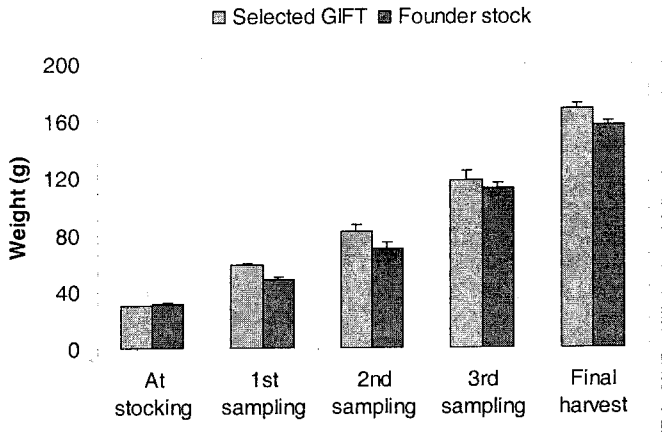

Fig. 1. Body weight of the progeny of the selected GIFT and founder stock over different culture periods.

Table 3 also presents net gain and daily gain for the F1 and founder stocks. The net gains for weight estimated for the selected GIFT was significantly ($P< 0.05$) higher than that of the founder stock (138.4 vs. 125.6 g). The final weight of the selected GIFT was 7.20% higher than that of the founder stock. In regard to survival rate, hundred percent survivals were obtained in both the stocks.

Table 3. Growth rate of the GIFT fish tested in cistern ecology at BFRI

Population	No of records	Net gain (g)	Daily gain (g)
Selected GIFT	30	138.43±3.40	1.15±0.03
Founder stock	30	125.50±3.30	1.04±0.02

To evaluate growth performance of the selected GIFT and founder population, the same ecological environment and culture condition were maintained where the selected GIFT and founder population representing more or less similar size were stocked at a density of 5 fish/m^2 and fed a formulated feed containing protein level 28% for 120 days. The results showed that the selected GIFT had a significant higher growth than the foundation stock, after one generation of selection. BFRI has initiated stock improvement program for GIFT through mass selection in 1998. Through mass selection, F-1 generation of GIFT showed 5% higher growth over average GIFT strain, which was introduced from ICLARM in 1994. Subsequent generations (F-2 to F-6) were produced in the same manner. Through combined mass selection technology, the F-6 generation of GIFT strain achieved 32.7% growth over existing GIFT strain (Annual Progress Report, 2007). In the present study, we applied family selection protocol, and an approximately 7.2% genetic gain was achieved after one generation.

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Performance of *Amblypharyngodon mola* with *Barbodes gonionotus, Cyprinus carpio* and *Macrobrachium rosenbergii* in rice-fish culture system

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Abstract
An experiment was conducted to assess the performance of mola (*Amblypharyngodon mola*) in rice fish culture system with freshwater prawn *Macrobrachium rosenbergii*), Thai silver barb (*Barbodes gonionotus*) and common carp (*Cyprinus carpio*) for a period of 4 months at the Agronomy Field Laboratory, Bangladesh Agricultural University, Mymensingh, Bangladesh. Four treatments viz., treatment-I (T_1) with *A. mola* and *M. rosenbergii*; treatment-II (T_2) with *A. mola*, *M. rosenbergii* and *B. gonionotus* ; treatment-III (T_3) with *A. mola*, *M. rosenbergii* and *C. carpio*, and treatment- IV (T_4) as control (without fish) were used in triplicate. All treatments were equally fertilized with urea (200 kg/ha), TSP (150 kg/ha) and MP (75 kg/ha). The mean values of water quality parameters viz., temperature, dissolved oxygen, pH, nitrate-nitrogen showed a very small variations among different treatments, but phosphate-phosphorus and chlorophyll-a were relatively higher in T_4 without fish (i.e., control). The fish production of 480.5 kg/ha in T_3 was significantly higher than that of 355.6 kg/ha T_2 and 223.8 kg/ha in T_1. The values of soil organic matter, total-nitrogen, phosphorus and potassium at harvest were significantly ($P<0.05$) higher in the treatments with fish than without fish, but pH did not show any significant differences. The yield of rice grain and straw was also obtained significantly ($P<0.05$) higher in the treatments with fish. The increase in grain was higher over the control by 11.81%, 9.41% and 14.76% and that in straw was by 9.83%, 4.77% and 13.29% in T_1, T_2, and T_3 respectively.

Key wards: Rice-fish culture, *Amblypharyngodon mola*, *Macrobrachium rosenbergii*, *Barbodes gonionotus, Cyprinus carpio*

Introduction

Bangladesh is a fishery resourceful country. The country offers a large potential for the development of inland fishery and aquaculture. Fisheries sector plays an important role in the economy of Bangladesh in terms of nutrition, income, employment generation and foreign exchange earning. This sector contributes about 4.07% of total GDP, 22% of agricultural production, 4.90% of export earning and about 63% of animal protein to

our daily diet (DoF 2008). The country has a vast potential of integrating fish and prawn culture with rice farming. Integrated rich-fish farming probably the best example of integration where rice and fish are directly benefited by each other. Activities of fishes in the rice fields enhance the fertility and environment of rice fields and as a result rice yield is increased by 10-15% with very few exceptions (Khoo and Tan 1980, Zhang 1986, Li 1988, Cruz *et al.* 1992, Kamp and Gragory 1993). Lightfoot (1990) stated that the integrated rice-fish farming offers possibilities of increasing rice yields by as much as 15% and at the same time harvesting up to 500 kg/ha of fish in every rice crop. Bangladesh has more than 2.83 million ha of seasonal paddy fields where water remains 4-6 months (DoF 2008). The carrying capacities of these lands are not utilized to the fullest extent, but there exists tremendous scope for increasing fish/prawn production integrating with rice. This practice will help to optimize land use without degradation, a suitable ecosystem.

More than 50 small indigenous species of fishes (SIS) play an important role in the national diet of Bangladesh which may be brought under culture and management. The small indigenous species of fishes (SIS) of Bangladesh are generally considered to be those fishes which grow to a length of about 25 cm or 9 inches (Felts *et al.* 1996, Hossain and Afroze 1990). In the past, these small indigenous fish species were abundantly available in rivers, beels, jheels, canals, haors, baors, ditches and flood plains of Bangladesh (Jhingran and Talwar 1991, Shafi and Quddus 1982, Ahmed 1984) and had a low market value. But now a days, these species have gradually been disappearing from the systems which in turn severely affecting the biodiversity of our ecosystem. Malnutrition and protein deficiency of the rural people can be eliminated and their economic conditions can be improved through cultivation of small indigenous fish species (SIS) separately or in combination with carps in rice fields. It is expected that the result of the present study be of greatly benefit the rice-fish farming communities and will be of great use for scientists to undertake fruitful research programme in future.

Materials and methods

Site selection and land preparation

The experiment was conducted at the Agronomy Field Laboratory, Bangladesh Agricultural University, Mymensingh. The facility consists of 12 experimental plots, each comprising an area of 160m^2 and rectangular in shape. Small water channels (0.70m width and 0.30m depth) were made between the plots to supply water to them. Embankments (0.70m height and 0.50m width) were made surrounding the experimental areas. A common inlet and outlet was provided on the dykes of each plot to regulate water depth of 0.3-0.4m. A small ditch was constructed in the middle position in each plot covering an area of 1.5 m^2 with 0.50 m depth. The width and depth of the trenches were 40cm and 30 cm, respectively, which were almost similar to the practice of the farmers in Indonesia (dela Cruz 1992) and in Bangladesh (Mazid *et. al.* 1992). Wetland preparation was followed in the experiment, which is practiced in most

tropical countries (Singh *et. al.* 1980). All the experimental plots were fertilized with triple super phosphate (TSP) and murate of potash (MP) at the rate of 150 kg/ha and 75 kg/ha respectively recommended by BRRI (1999).

Experimental design

The experiment was laid out in a randomized block design (RBD) with four treatments *viz.*, Treatment-I (T_1), Treatment-II (T_2), Treatment-III (T_3) and Treatment-IV (T_4) and randomly assigned in each with three replications Viz., R_1, R_2 and R_3. *A. mola* was stocked with *M. rosenbergii* in T_1, with *M. rosenbergii* and *B. gonionotus* in T_2 and with *M. rosenbergii* and *C. carpio* in T_3. Treatment IV (T_4) was kept as control i.e., without fish. The stocking densities given were 16000/ha for *A. mola* and 9000/ha for *M. rosenbergii* in T_1; 10,800/ha for *A. mola*, 7,600/ha for *M. rosenbergii* and 2,700/ha for *B. gonionotus* in T_2 and 10,800/ha for *A. mola*, 7,600/ha for *M. rosenbergii* and 2,700/ha for *C. carpio* in T_3.

Rice transplantation and management

In rice-fish culture system, high yielding varieties (HYV) that have medium height, resistant to insects, diseases and require less growing time (105-125 days) than the most local varieties (160 days) are recommended for rice-fish culture (Singh *et al.*, 1980 and dela Cruz. *et al.*, 1992). For this purpose seedlings of BR-10 were transplanted in the experimental plots on the 2nd August 2003. Alternate row spacing of 35 cm and 15 cm was followed in the experiment for transplanting rice seedling, which is recommended by Hossain *et al.* (1990). The plant to plant distance given was 20 cm. Water level was kept as low as 2-4 cm up to 12 days after transplanting to allow the rice seedlings well established and to initiate tillers growth. The urea was top dressed in three equal doses at 16, 45 and 65 days of rice seedling transplantation. No pesticide was applied to the crop during the experimental period.

Stocking of fingerlings and their management

The fish fingerlings were stocked 27 days after transplanting of rice seedlings and prawn juveniles were stocked 30 days after transplanting of rice seedlings in the experimental plots. Low-cost supplementary feed comprising of rice bran and oil cake at 1:1 ratio were supplied once daily at the rate of 3% of body weight of fish. Water depth were maintained 0.3 to 0.4m throughout the study period. Observation of fish health were done in each sampling date.

Study of water quality parameters

During the study period, the values of water temperature, dissolved oxygen, pH, chlorophyll-a, nitrate-nitrogen and phosphate-phosphorus were recorded fortnightly. Water temperature and dissolved oxygen were measured directly in the field with the help of a Celsius thermometer and a digital electronic DO meter (YSI, MODEL 58). Water pH was measured with the help of an electrical pH meter (JENWAY, MODEL 3020).The concentration of nitrate-nitrogen (mg/l) and phosphate-phosphorus (mg/l) of

water samples were determined in the laboratory after filtering the water samples taken from rice field by using spectrophotometer (HACK DR 2000) and reagent pillow nitrover-3 and phosver-3. Chlorophyll-a (μg/l) was measured from the filter papers (Whatman GF/C) used for filtering the water samples. The filter papers were dissolved in 10 ml acetone and kept overnight, then centrifuged and made ready for the analysis of Chlorophyll-a. Later Chlorophyll-a was determined by using a spectrophotometer (Milton Roy Spectronic, Model 1001) at 664 and 750 wavelengths.

Harvesting of rice and fish

The rice was harvested on the 30th November 2003 after 120 days of transplantion. The grains and straw were then cleaned and sun dried for three days. Then the weights of dried grain and straw were recorded separately (mt/ha). The fishes and prawns were harvested immediately after harvesting of rice. The collected fishes/prawns from the plots were counted and the number was recorded separately species wise and plot wise to determine the growth. The length and weight of fish were recorded separately with the help of measuring scale and a portable electronic balance (Model OHAUS CT1200-S). The gross and net yield of fish for each treatment was determined.

Collection and preparation of soil samples

Soil samples were collected from each unit plot at a depth 0-15 cm from the surface in two installments, first before application of fertilizer and finally after harvest of fish. After removing weeds, plant roots, stubble's etc., the samples were air dried and ground to pass through a 2 mm mesh size sieve. Then these cleaned ground soil samples were stored in clean plastic container separately treatment wise for subsequent physical and chemical analysis using standard methods of Pipers (1950), Jackson (1962), Black (1965) and Page *et al.* (1989).

Data analysis

All the data collected during the experiment were recorded and preserved in computer. The data obtained in the experiment were analyzed statistically using analysis of variance (ANOVA). The mean values were compared to Duncan's Multiple Range Test (Gomez and Gomez 1984). SPSS 11.5 statistical package was used for analysis of all data.

Results and discussion

Water quality parameters in rice fields

The values of water quality parameters of the present study *viz.*, water temperature, dissolved oxygen (DO), pH, nitrate-nitrogen, phosphate-phosphorus and chlorophyll-a have been shown in Table 1. Water temperature in the rice fields fluctuated between 26.75-29.56 °C under different treatments of the present study. Almost similar ranges of water temperature were reported by Ali (1992), Ghosh (1992), Uddin (1998),

Chowdhury (1999), Mondol (2001) and Das (2002) in their studies in rice fields were 27-40.1 °C, 27-29 °C, 21.9-33 °C, 27-31.20 °C, 26.90-29.60 °C and 25.32-32.04 °C, respectively.

Table 1. Mean values (±SE) of water temperature, dissolved oxygen, pH, NO_3-N, PO_4-P and Chlorophyll-a under four different treatments. Dissimilar alphabets exhibit significant differences ($P < 0.05$)

Treatment	Parameters					
	Temperature (°C)	DO (mg/l)	pH	NO_3-N (mg/l)	PO_4-P (mg/l)	Chlorophyll-a (µg/l)
T_1	28.33±0.44 (26.75-29.50)	4.10±0.03 (3.91-4.44)	7.21±0.08 (6.84-7.50)	1.89±0.06 (1.65-2.20)	0.60±0.01 (0.48-0.72)	18.34±0.5^b (15.55-21.10)
T_2	28.34±0.44 (26.83-29.52)	4.15±0.03 (3.96-4.30)	7.17±0.07 (6.80-7.43)	1.81±0.06 (1.51-2.10)	0.56±0.03 (0.32-0.73)	18.22±0.4^b (15.98-20.43)
T_3	28.33±0.45 (26.74-29.56)	4.07±0.02 (3.88-4.27)	7.20±0.08 (6.83-7.54)	1.86±0.05 (1.61-2.05)	0.56±0.03 (0.41-0.71)	18.34±0.3^b (16.60-20.02)
T_4	28.32±0.43 (26.85-29.52)	4.11±0.03 (3.95-4.40)	7.16±0.08 (6.78-7.42)	1.84±0.05 (1.67-2.00)	0.77±0.03 (0.64-1.00)	25.47±1.9^a (20.60-30.12)

In the present study, the dissolved oxygen contents of water were found to range between 3.88-4.44 mg/l which are almost similar to the range of dissolved oxygen contents obtained by Ghosh (1992), Chowdhury (1999), Mondol (2001) and Das (2002) in rice fields were 3.2-4.5 mg/l, 3.8-4.5 mg/l, 3.42-4.26 mg/l and 3.2-4.65 mg/l, respectively. The pH values of water in the present study found to range between 6.78-7.54, which are almost close to the neutral value indicating suitable condition for fish culture. This range of pH values are more or less similar to the ranges of pH values obtained by Ali (1990), Ghosh (1992), Uddin (1998), Chowdhury (1999), Mondol (2001) and Das (2002) were 6.53-7.08, 7.1-8.0, 6.7-7.8, 5.63-8.20, 5.80-6.90 and 6.75-8.30, respectively. The range of nitrate-nitrogen values recorded by Ali (1992) and Whitton *et al.* (1988) were 0.22-0.23 mg/l and 0.006-0.05 mg/l respectively. But the range of it (1.51-2.20 mg/l) obtained in the present study was higher than those of the above statements. One of the possible causes of higher values of nitrate-nitrogen might be due to fertilization with urea that was practiced in the experimental plots. However, the ranges of nitrate-nitrogen values recorded by Ghosh (1992), Chowdhury (1999), Mondol (2001) and Das (2002) ranged from 0.02-2.60 mg/l, 1.43-3.16 mg/l, 1.60-2.83 mg/l and 1.60-3.22 mg/l respectively were more or less close to the values obtained in the present study. The ranges of phosphate-phosphorus concentrations obtained in the present study was 0.32 to 1.00 mg/l which is almost similar to the values recorded by Ghosh (1992), Mondol (2001) and Das (2002) in rice fields were 0.03-0.99 mg/l, 0.27-0.98 mg/l and 0.51-1.23 mg/l, respectively. The range of chlorophyll-a values (15.55-30.12 µg/l) obtained in the present study was found to lie within the ranges recorded by Uddin (1998), Chowdhury (1999), Mondal (2001) and Das (2002) were 14.7-55.1 µg/l, 10.1-41.0 µg/l, 15.99-26.19 µg/l and 24.11-33.31 µg/l, respectively. Among all the treatments significantly higher concentration of Chlorophyll-a was recorded in T_4 (without fish)

than T_1, T_2 and T_3 (with fish), which might be attributed to grazing pressure of fish on phytoplankton. Except chlorophyll-a other water quality parameters did not show any significant differences among the treatments.

Growth of fish and prawn

The growth rate of *A. mola* by average, net and percentage of increase did not show any significant differences among the treatments (Table 2). However, the average growth rate of *A. mola* by length and weight recorded in T_1, T_2 and T_3 were 6.59cm and 2.58g, 6.61cm and 2.56g and 6.71cm and 2.58g, respectively which are higher than the growth rates 5.30cm and 1.55g and 6.20cm and 1.90gm obtained by Chowdhury (1999) and Das (2002), respectively. Mondal (2001) reported that the average growth rate of *A. mola* was 6.7cm and 2.7gm which is similar to the present study. The growth rate attained by *M. rosenbergii* 15.17cm and 38.47g in T_1, 15.14cm and 38.40g in T_2 and 15.33cm and 38.77g in T_3 by average increase and 7.52cm and 34.30g in T_1, 7.58cm and 34.26g in T_2 and 7.7cm and 34.54g in T_3 by net increase in length and weight, respectively. Whereas, by percentage of increase it was found to attain 98.30%, 100.26% and 100.92% in length and 822.54%, 811.85% and 816.55% in weight in T_1, T_2 and T_3, respectively. There was no significant differences in growth rate of *M. rosenbergii* among the treatments. The growth recorded for *B. gonionotus* were 17.91cm and 81.61g in T_2 by average increase in length and weight respectively, and by net increase they were 11.11cm and 75.27g and the percentage of increase were 163.38% and 1187.22% in length and weight respectively. The growth rate of *B. gonionotus* by average, net and percentage of increase in length and weight recorded by Chowdhury (1999) were 17.03cm and 71.66g, 11.33cm and 66.3g and 197.85% and 1246.35% respectively which are more or less close to the findings of the present study. But the growth rate 17.30cm and 75.5g, 10.60cm and 69.40g and 158.21% and 1137.70% respectively reported by Das (2002) were less than the growth rate obtained in the present study. The growth rate of this fish 38.0g and 54.42g respectively reported by Akhteruzzaman *et al.* (1993) and Uddin *et al.* (2001) were also less than the growth rate obtained in the present study. But the growth rate of this fish (95-115g) recorded by Hossain *et al.* (1988) was higher than the growth rate of the present study. The growth rate observed for *C. carpio* was 22cm and 178g by average increase and 13.59cm and 169.59g by net increase in length and weight respectively. The growth rate of this fish recorded by Mondal (2001) was 20.3cm and 184.7g and by Das (2002) were 21.70cm and 170.0g respectively by average increase in length and weight which are more or less close to the growth rate obtained in the present study. But the growth rate (63g and 122.67g) recorded by Akhteruzzaman *et al.* (1993) and Chowdhury (1999) were far less than the growth rate recorded in the present study.

Table 2. Growth rate, survival rate and production of fish under three different treatments. Dissimilar alphabets exhibit significant differences ($P < 0.05$)

Treatment	Species Stocked	Number/ha		Average Weight (g)		Weight gained		Survival rate	Production (Kg/ha)	
		Stocked	Harvested	Initial	Final	Net Increase	(%) increase		Gross	Net
T_1	*A. mola*	16,000	6,240	0.79±0.02	2.58±0.01	1.79±0.01	226.58	39	16.10	11.17
	M. rosenbergii	9,000	5,400	4.17±0.08	38.47±1.5	34.29±1.38	822.54	60	207.7^{a}	185.2^{a}
Total		**25,000**	**11,640**						**223.8^{c}**	**196.4^{c}**
T_2	*A. mola*	10,800	4,104	0.80±0.03	2.56±0.04	1.76±0.02	220.0	38	10.51	7.22
	M. rosenbergii	7,600	4,332	4.22±0.04	38.46±1.4	34.26±1.41	811.85	57	166.6^{b}	148.4^{b}
	B. gonionotus	2,700	2,187	6.34±0.05	81.61±0.8	75.27±0.76	1187.22	81	178.48	164.62
Total		**21,100**	**10,623**						**355.7^{b}**	**320.4^{b}**
T_3	*A. mola*	10,800	4,644	0.77±0.01	2.58±0.03	1.81±0.04	220.0	43	11.98	8.41
	M. rosenbergii	7,600	4,028	4.23±0.07	38.77±0.7	34.54±0.77	816.55	53	156.2^{b}	139.1^{b}
	C. carpio	2700	1,755	8.41±0.02	178.0±1.9	169.59±1.93	2016.53	65	312.39	297.63
Total		**21,100**	**10,427**						**480.5^{a}**	**$445.^{2}$**

Survival rate of fish and prawn

The survival rate of *A. mola* recorded for T_1, T_2 and T_3 were 39%, 38% and 43%, respectively (Table 2). The survival rates of this fish recorded by Chowdhury (1999), Mondal (2001) and Das (2002) were almost similar to the survival rates of the present study and values obtained by them were 42% and 37%, 35% and 42%, and 44%, 42% and 37%, respectively. Survival of mola did not show any significant differences among the treatments. The survival rates of *M. rosenbergii* recorded in the present study were 60%, 57% and 53% T_1, T_2 and T_3, respectively. Haroon and Alam (1992) recorded the survival of *M. rosenbergii* as 70.74-82.81% which are higher than the survival rate obtained in the present study; whereas, in another experiment the survival rate (53.90-70.24%) recorded by Haroon and Alam (1992) were is more or less similar to the survival rates recorded in the present study. Survival of prawn did not show any significant differences among the treatments. The survival rate recorded for *B. gonionotus* and *C. carpio* were 81% and 65% respectively. Survival rate of *B. gonionotus* recorded by Rahman *et al.* (1995), Akhteruzzaman *et al.* (1993), Chowdhury (1999) and Das (2002) were 65%, 68%, 62% and 69% respectively in their study, was lower than the survival rate recorded in the present study. Survival rate of *C. carpio* (58% and 63%) recorded by Mondal (2001) and 61% by Das (2002) were almost similar to the survival rate of this fish recorded in the present study. Survival rate of this fish 53% and 55% recorded by Akhteruzzaman *et.al.*(1993) and Chowdhury (1999), respectively were slightly lower than the same recorded in the present study, whereas, the survival rate (81.06%) reported by Islam *et al.* (1998) was much higher than the present study.

Production of fish and prawn

Among all the treatments the significantly higher gross (480.54 kg/ha) and net (445.17 kg/ha) production of fish were obtained in T_3 in combination with *A. mola, M. rosenbergii* and *C. carpio* and the lowest (223.84 kg/ha and 196.39 kg/ha) were recorded in T_1 in combination with *A. mola* and *M. rosenbergii.* In another treatment T_2 gross (355.64 kg/ha) and net (320.21 kg/ha) production of fish were obtained in combination with *A. mola, M. rosenbergii* and *B. gonionotus.* The gross and net production of *A. mola* obtained significantly higher in T_1 (16.10 kg/ha and 11.17 kg/ha) than in T_2 (10.51 kg/ha and 7.22 kg/ha) and in T_3 (11.98 kg/ha and 8.41 kg/ha), respectively (Table 2). The production of newly produced fry was not counted here, as they were too small to be harvested. The gross production of *A. mola* (12.6 kg/ha, 8.03 kg/ha and 9.11 kg/ha) recorded by Chowdhury (1999) was more or less close to the production obtained in the present study. Whereas, the gross production of *A. mola* (25.88 kg/ha, 26.88 kg/ha and 29.23 kg/ha) recorded by Das (2002) were higher than the production obtained in the present study. The gross and net production of *M. rosenbergii* were 207.74 kg/ha and 185.22 kg/ha in T_1 was significantly higher than 166.61 kg/ha and 148.41 kg/ha in T_2 and 156.17 kg/ha and 139.13 kg/ha in T_3, respectively. The production of *M. rosenbergii* in rice field recorded by Haroon and Alam (1992) 217-265 kg/ha/230 days was similar to the production obtained in the present study. The gross and net production of *B. gonionotus* recorded in the present study was 178.48 kg/ha and 164.62 kg/ha respectively.

The gross production of this fish recorded by Islam *et al.* (1998), Chowdhury *et al.*(2001) and Uddin *et al.*(2001) were 184.17 kg/ha, 175.21 kg/ha and 142.8 kg/ha respectively which is more or less similar to the production obtained in the present study. The gross and net production of this fish (260.48 kg/ha and 239.43 kg/ha) recorded by Das (2002) was higher than the production obtained in the present study. The gross and net production of C. *carpio* obtained in the present study were 312.39 kg/ha and 297.63 kg/ha, respectively which were found to lie within the range 300-747 kg/ha recorded by Yuchang and Yixian (1988) from the stocking of *C. carpio* in rice fields. Whereas, the yield of this fish recorded by Khan *et al.* (1997) and Chowdhury (1999) were 233.49 kg/ha and 252.94 kg/ha respectively, which are less than the production obtained in the present study. On the other hand, Mondal (2001) and Das (2002) recorded higher production of this species (523.16kg/ha and 518.50kg/ha respectively) than the production of the present study.

Production of rice grain and straw

In the present study, the yield of grain and straw were found to differ significantly ($P<0.05$) between the treatments with fish and without fish. The highest yield of grain 6.22mt/ha and straw 7.84mt/ha were obtained in T_3 where *A. mola*, *C. carpio* and *M. rosenbergii* were stocked together and the lowest production of grain 5.12mt/ha and straw 6.31mt/ha were recorded in T_4 where no fish was stocked (Table 3).

Table 3. Average yield of grain and straw in different treatments. Dissimilar alphabets exhibit significant differences ($P< 0.05$)

Treatments	Average yield (mt/ha)		% of increase over the control	
	Grain	Straw	Grain	Straw
T_1	6.06 ± 0.05^{ab}	7.60 ± 0.18^{ab}	11.81	9.83
T_2	5.90 ± 0.06^{b}	7.25 ± 0.09^{bc}	9.41	4.77
T_3	6.22 ± 0.05^{a}	7.84 ± 0.07^{a}	14.76	13.29
T_4	5.12 ± 0.29^{c}	6.31 ± 0.43^{c}		

The increased yields of both grain and straw in the treatments with fish than without fish (control) indicate that the introduction of fish in rice fields improves the yield of grain and straw. These findings agree with the findings of Purba (1998), Chowdhury (1999), Uddin *et al.*(2000), Mondal (2001) and Das (2002) who also obtained significant difference in the yield of grain and straw between the treatments with and without fish in rice fish culture. The yield of rice grain and straw obtained in the present study is more or less similar to the yield of the same recorded by Das (2002). But the yield of rice grain and straw recorded by Chowdhury (1999), Mondal (2001), and Uddin *et al.* (2001) were less than the yield of the same obtained in the present study. However, the yield of grain recorded by Haroon and Alam (1992), Gupta and Mazid (1993) and Kohinoor *et al.* (1993) in their experiments on rice fish culture were more or less close to the same obtained in the present study. The yield of grain and straw were

found to increase in the treatments with fish from that of control (without fish) by about 11.81%, 9.41% and 14.76% in grain and 9.83%, 4.77% and 13.29% in straw in T_1, T_2 and T_3 respectively and infestation of pest did not occur during the study period. These findings are in conformity with the findings of Hora and Pillay (1962), dela Cruz *et al.* (1992), Lightfoot *et al.* (1990), Chowdhury (1999), Uddin *et al.* (2000) and Das (2002). Mazid *et al.* (1993) and Kumah *et al.* (1996) also stated that introduction of fish in the rice fields reduces the infestation of insects and weeds by feeding upon them and thereby improves the yield of rice.

Availability of nutrients in soil

The initial (before rice transplantation) and final (after the harvest) values of soil pH did not show any significant difference ($P<0.05$) (Table 4). No significant difference was observed in the initial values of organic matter, nitrogen, phosphorus and potassium among the four treatments, but the final values of them showed significant ($P<0.05$) differences between the treatments with fish and without fish. The significantly higher concentrations of nutrients in the soil with fish than without fish in all the treatments clearly indicate that the introduction of fish in the rice fields improved the nutritional status of soil. These findings of the present study are in conformity with the findings of Middendrop (1985), Xu and Gua (1992), Guant *et al.* (1993), Uddin (1998) and Chowdhury (1999). They stated that the introduction of fish in the rice fields stimulates the activities of microorganisms, increases the availability of organic matter by faeces and increases the release of nutrients for better growth of rice. However, the values of soil nutrients obtained in the present study are more or less close to values of the same recorded by Uddin (1998), Ashrafuzzaman (1999), Mondol (2001) and Das (2002).

Table 4. Concentration of nutrients in soil before rice transplantation and at harvest. Dissimilar alphabets exhibit significant differences ($P< 0.05$).

Treatment	pH		Organic matter (%)		Total N (%)		P (ppm)		K (ppm)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
T_1	6.60 ±0.04	6.65^{a} ±0.02	1.318 ±0.08	2.588^{ab} ±0.04	0.060 ±0.01	0.170^{a} ±0.01	11.077 ±0.65	13.218^{a} ±0.17	58.55^{a} ±0.78	79.431^{a} ±0.88
T_2	6.58 ±0.01	6.54^{b} ±0.02	1.252 ±0.13	2.642^{a} ±0.06	0.075 ±0.01	0.166^{a} ±0.01	11.138 ±0.24	13.576^{a} ±0.39	56.662^{ab} ±0.99	77.265^{a} ±1.38
T_3	6.63 ±0.06	6.60^{ab} ±0.03	1.328 ±0.01	2.257^{b} ±0.16	0.064 ±0.01	0.156^{a} ±0.02	10.585 ±0.18	12.594^{a} ±0.76	54.465^{b} ±0.63	74.642^{a} ±2.65
T_4	6.53 ±0.05	6.56^{ab} ±0.02	1.292 ±0.07	1.904^{c} ±0.03	0.068 ±0.01	0.114^{b} ±0.01	10.714 ±0.33	11.074^{b} ±0.23	55.286^{b} ±0.75	62.466^{b} ±2.14

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Characterisation of neurons in the pedal ganglia of the green-lipped mussel, *Perna canaliculus*, using antibodies raised against neuropeptides and neurotransmitters involved in gastropod egg-laying behaviour and bivalve reproduction and spawning

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Abstract
To characterise central neurons in the pedal ganglia of both male and female green lipped mussel, *Perna canaliculus* immunohistochemical techniques were used. Mollusc antibodies were used against neuropeptides and neurotansmitters known to control reproduction and spawning. Anti-ELH and anti-APGWamide showed very strong immunoreactivity in small type of neurons. Anti-5-HT and anti-DA immunoreactivity was mostly in large type of neurons. The labelled neurons are consistent with descriptions of neurosecretory cells implicated in the control of reproduction and spawning on the basis of earlier histological staining techniques used in this species. The use of selective immunological markers for peptides and amines appears to be a promising tool for further characterisation of neurosecretory cells, and to isolate and characterise neuropeptides and other biologically active materials involved in the control of reproduction in *Perna canaliculus*.

Key words: *Perna canaliculus*, Antibodies, Neurons

Introduction

The existence of neurosecretory cells has been demonstrated mainly through histological work. The application of the Gomori methods, based on the reactions with protein-bound cysteine, appeared to be rather specific for neurosecretory cells. As far as mussels are concerned, a number of descriptive studies of the different types of neurosecretory cells have been published (Illanes-Bucher 1979). Although these classical staining techniques are a recognised method to identify neurosecretory cells, they have a limited ability to describe the functional properties of those identified neurosecretory cells. To overcome this limitation, a number of more recent studies have been done using

immunocytochemistry to characterise neurosecretory materials in the bivalve central nervous system (Croll *et al.* 1993). These studies indicate the presence of neurotransmitters and numerous neuropeptides in the nervous system of those bivalves. However, knowledge of neurotransmitters and neuropeptides in the green-lipped mussel lags far behind that of other bivalves and gastropod molluscs.

The presence of APGWamide-like immunoreactivity has been demonstrated within central neurons of the scallop *Pecten maximus* (Jegou *et al.* 1993). APGWamide is well-studied in gastropod molluscs, where it appears to play an important role in the control of male reproduction. APGWamide may also function as a neurotransmitter within the central nervous system of *Lymnaea stagnalis,* inhibiting the activity of certain neuroendocrine cells (Croll *et al.* 1991) and co-ordinating the activity of different populations of penial motorneurons. Indeed, APGWamide is involved in myoactive and copulatory behaviour (De Lange *et al.* 1997a), and it has effects on central neurons involved in control of egg-laying behaviour and metabolism (Croll *et al.* 1991). Neurosecretory cells controlling egg-laying have been identified and well characterised in the pulmonate, *Lymnaea stagnalia,* and the opisthobranch, *Aplysia californica.* The ovulation hormones in both *Lymnaea* and *Aplysia* are formed within larger preprohormones which produce a number of additional neuropeptides, each of which may regulate particular aspects of ovulation and egg-laying behaviour (Brussaard *et al.* 1990). The preprohormones in *Lymnaea* and *Aplysia* have similar structural organisations despite the fact that the lineage of the two genera is thought to have diverged about 350 million years ago (Moore and Pitrat 1960). The positions of identified cells in several species together with previous histochemical and ultrastructural studies (Roubos and Van Den Ven 1987) support the hypothesis of homologous neurons. The peptides immunoreactive to antisera specifically directed against caudo-dorsal cell hormone (CDCH) and caudo-dorsal cell protein (α-CDCP or β-CDCP) have been detected in the central nervous system of *Sarcophaga bullata* (Diptera), *Leptinotarsa decemlineata* (Coleoptera), *Locusta migratoria* and *Periplaneta americana* (Orthoptera) (Theunis *et al.* 1990).

Further investigations indicate that the egg-laying preprohormone is relatively conserved across a wide range of molluscan classes (Nambu and Scheller 1986). Using this antibody, as well as in antibody raised against CDCH, it has also been shown that neurons in the bivalves *Mytilus edulis*, *Mya arenaria* and *Placopecten magellanicus* contain a similar vitellogenic factor (Croll *et al.* 1993). These selective immunological markers, therefore, suggest that related peptides may be involved in the egg laying of various gastropods and bivalve molluscs (Cummuns *et al.* 2000).

So far information no studies have been attempted to date to locate and identify the neurons containing neurotransmitters or egg-laying hormones in the green-lipped mussel using immuno-histochemistry. In the present study, these deficiencies are addressed by providing a detailed description of the distribution of serotonin (5-HT), dopamine (DA), APGWamide, and egg-laying hormone (ELH) within the pedal ganglia of the green-lipped mussel, *Perna canaliculus.* The immunocytochemical trials have been

carried out to tentatively identify neurosecretory cells involved in reproduction in bivalve molluscs to examine whether the results can be generalised across the class.

Materials and methods

Collection of mussels, fixation and dissection of ganglia

The green-lipped mussels, *Perna canaliculus,* were collected from an exposed rocky shore at Purihurihu Point, near Blueskin Bay, in the South Island of New Zealand. Collection of ganglia of both sexes for immunohistochemistry was done shortly after transporting the mussels to the laboratory of the Department of Physiology at the University of Otago, Dunedin, New Zealand. The pedal ganglia were collected from both sexes. Individual tissues were placed gently in the bottom of an aluminium foil boat containing pre-cooled Tissue-Tek™ O.C.T. compound and then the foil boat was filled with O.C.T. compound. The tissue was snap frozen by partial immersion of the foil boat into isopentane cooled in liquid nitrogen. Individual tissues were preserved at –70°C for sectioning.

Antibodies used for immunohistochemistry

Four antisera were used in this study, all produced in rabbits: (i) Anti-ELH was raised against a synthetic peptide representing the N-terminal fragment (ISINQDLKAITDML) from the egg laying hormone of *Aplysia*. This antibody was produced by G. T. Nagle and J. E. Blankenship (University of Texas Medical Branch), and its characterisation and specificity were described by Ram *et al.* (1998), (ii) Anti-APGWamide (Chemicon International, Inc. 28835 Single oak Drive, Temecula, CA 92590), (iii) Anti-Dopamine (Chemicon International, Inc. 28835 Single Oak Drive, Temecula, CA 92590), and (iv) Anti-Serotonin was obtained from Deptartment of Zoology, University of Otago, Dunedin, New Zealand. The unlabelled goat anti-rabbit secondary antibody was obtained from Cappel Research Products (Durham, North Carolina) and the peroxidase-antiperoxidase complex employing rabbit antibodies was obtained from Sigma Chemical Co. (Mississauga, Ontario).

Immunocytochemistry protocol

Serial sections (two sets - one for experimental and another for control) were cut at 10 μm in a cryostat at –18°C and approximately 8-10 sections were mounted on each slide for immunohistochemistry. The dried sections were fixed for 10 minutes in freshly prepared 4% paraformaldehyde and were washed in PBS. Primary antiserum were then applied and left overnight at 4°C. Antiserum dilutions of between 1:400 and 1:100 were used in an immunodiluent (ID) solution of 2% normal goat serum (Sigma Chemical Co.) and 0.2% Triton X-100 (Sigma Chemical Co.) in PBS.

Next day, secondary antibody was added to all slides after washing in PBS and was left for an hour at room temperature. The secondary antiserum was diluted 1:200 in ID. After another several washes in PBS the slides were kept for another one-hour incubation in peroxidase-antiperoxidase diluted 1:400 in ID. After incubation, slides

were washed off again in PBS and were developed for 2-3 minutes using diaminobenzidine (DAB)- hydrogen peroxide. Slides were dehydrated in graded ethanols washed in xylene, and mounted in DPX. One set of serial sections from each ganglion was processed as described above, with the elimination of the incubation in primary antibody as a negative control. Slides were viewed through an Olympus BX50 Microscope and photographed digitally.

Results

The localisation of neurons containing different neuropeptides and neurotransmitters in the pedal ganglia of the green-lipped mussel, *Perna canaliculus*, was examined imunohistochemically and is shown in Fig. 1 . The labelled neurons and fibres produced by anti-ELH, anti-APGWamide, anti-5-HT and anti-DA in the pedal ganglia are shown in Fig 2.

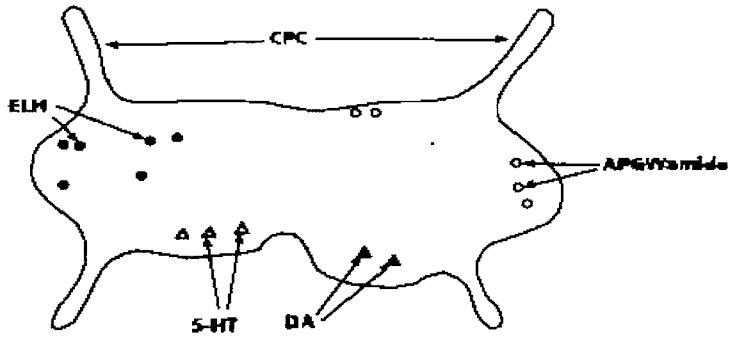

Fig. 1. Schematic representations of anti-ELH immunoreactivity (black circles) and anti-5-HT immunoreactivity (white triangles) on the left side and anti-APGWamide immunoreactivity (white circles) and anti-DA immunoreactivity (black triangles) on the right side in the pedal ganglia of *Perna canaliculus*. All descriptions are bilaterally symmetric in the ganglia. CPC - cerebral-pedal connective.

In the present study, immunocytochemical trials were carried out with antibodies raised against neurotransmitters and peptides including serotonin (5-HT), dopamine (DA), Ala-Pro-Gly-Trp-NH_2 (APGWamide) and egg-laying hormone (ELH), substances that have biological action in other molluscan species. The localisation of neurons containing serotonin in the central nervous system and the gonad of the scallop, *Patinopecten yessoensis*, was examined immunohisto-chemically (Matsutani and Nomura 1984) and these authors suggested that 5-HT be involved in the mechanism of spawning in *Patinopecten yessoensis*.

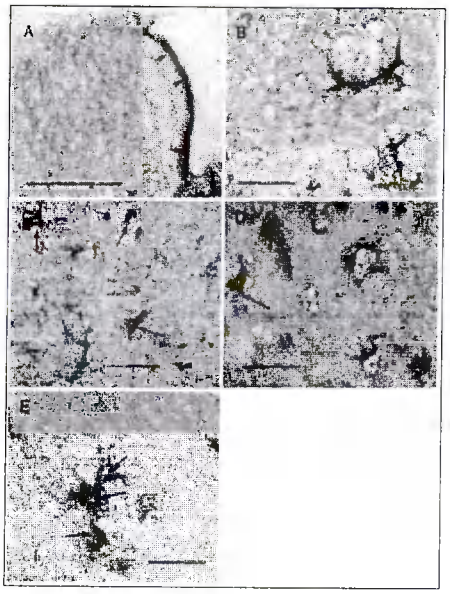

Fig. 2. Immunoreactivity in the pedal ganglia of green lipped mussel, *Perna canaliculus*. (A) Periphery of the ganglia (arrows) showing strong immunoreactivity to anti-ELH in a ripe female. Scale bar 50 μm. (B) Small cells (arrows), cell periphery and nerve fibres (arrowheads) showing immunoreactivity to anti-ELH in a spawned female. Scale bar 20 μm. (C) Several small cells (long arrows), nerve fibres (arrowheads) showing positive immunoreactivity to anti-APGWamide in a ripe male. Scale bar 20 μm. (D) Large cells (long arrow), the cell periphery of the cell body (arrowheads) and inclusion materials (short arrows) showing immunoreactivity to anti-5-HT in a spawned female. Scale bar 20 μm. (E) A few large cells (long arrows) and the inclusion materials (arrowheads) showing immunoreactivity to anti-DA in a spawned male. Scale bar 20 μm.

Antibodies raised against anti-ELH produced positive immunoreactivity in a few small cells, the connective sheath and in fibres in the pedal ganglia. The periphery of the pedal ganglia produced very strong anti-ELH immunoreactivity (Fig. 2A). Only the small cells showed immunoreactivity (Fig. 2B). These cells were not concentrated within any single region of the pedal ganglia but rather a few weakly stained cells were scattered throughout.

The size and location of anti-APGWamide labelled cells were similar to that of anti–ELH labelled cells in the pedal ganglia. Anti-APGWamide immunoreactive cell bodies were located along the peripheral margin and in the neuropile region (Fig. 2C). The pedal ganglia also contained numerous immunoreactive fibres throughout the ganglia. The larger cells revealed no immunoreactivity (Fig. 2D).

Anti-5HT produced light immunoreactivity throughout the pedal ganglia. A few large neurons and fibres in the neuropile revealed moderate immunoreactivity in the pedal ganglia of males (Fig. 2E). They were located in the cortex near the periphery of

the ganglia. Only the periphery of a few small cells showed positive immunoreactivity in the pedal ganglia. Nerve fibres were found to produce light immunoreactivity.

The peripheral sheath of the pedal ganglia and a few neurons produced anti-DA immunoreactivity. Large cells with different shapes and sizes were found with different staining intensities. The periphery of a few large cells produced strong immunoreactivity (Fig. 2F). These neurons were randomly scattered throughout the pedal ganglia.

Discussion

The present study presents the first immunocytochemical description of various monoamines and neuropeptides in the central nervous system of *Perna canaliculus*, which might be involved in controlling reproduction. Antibodies raised against ELH, APGWamide, 5-HT and DA stained relatively large numbers of cell bodies, fibres, axons and connective sheaths in the pedal ganglia of *P. canaliculus*. It is possible that endogenous peptides unrelated to reproduction cross-reacted with the antibodies used in this study. The results of several studies, however, support the hypothesis that some of the labelled neurons contain peptides homologous to those involved in gastropod ovulation. First, the antibodies have already been shown to be highly specific for ovulation related peptides in other molluscs (Theunis *et al.* 1990, Van Minnen *et al.* 1992); they apparently do not react with any of the numerous other well characterised and evolutionarily conserved peptides within the gastropods (e.g., Van Minnen and Schallig 1990, Kerkhoven *et al.* 1993) and bivalves (Stefano and Martin 1983, Vitellaro-Zuccarello and DeBasi 1988). Second, antibodies raised against both ELH and APGWamide labelled in a few cells and the fibres in the same positions in pedal ganglia of bivalves. Such findings are consistent with the possibility that the immunoreactive peptides are synthesised within a single preprohormone, as occurs in gastropods (Croll *et. al,* 1993). However, it must be noted that labelling in all regions was not co-localised, thus suggesting that immunoreactive peptides are not necessarily synthesised together by every cell. Finally, the several immunoreactive cells in this study are very similar to those described as possible neurosecretory cells involved in bivalve reproduction (Illanes-Bucher 1979, Mahmud and Mladenov 1998).

In the present study, some of the neurons in the cerebral, pedal and visceral ganglia were labelled with anti-ELH, anti-APGWamide, anti-5HT and anti DA. According to the size of the immunoreactive neurons in these ganglia, there were two distinct groups. Small cells were mostly located near the periphery of the ganglia with a few in the neuropile region. Large cells were mostly located between the peripheral edge of the ganglia and neuropile region. The small cells exhibited strong immunoreactivity with both anti-ELH and anti-APGWamide in all three ganglia of *P. canaliculus*. Similar patterns were observed in other studies in bivalves and gastropods. Neurosecretory staining in 'a' cell in *Mytilus edulis* was reported to correlate with the reproductive cycle (Illanes-Bucher and Lubet 1980, Lubet and Mathieu 1978, Mathieu and Van Minnen 1989). The studies in gastropods by Hahn (1990) in *Haliotis discus hannai* and by Upatham *et al.* (1998) in *Haliotis asinina* established that the secretion from certain cells

in the ganglia of *Haliotis* spp. are correlated with vitellogenesis, gametogenesis or spawning. The injection of ganglionic homogenates caused spawning in green-lipped mussel. Therefore, the labelling of small cells by both anti-ELH and anti-APGWamide in the pedal ganglia of the green-lipped mussel, *Perna canaliculus*, is strong evidence for the presence of ovulation and reproduction hormone(s).

The anti-5-HT and anti-DA immunoreactive neurons were lightly stained and located in well-defined locations in the pedal ganglia in *P. canaliculus* (Fig. 1). These neurons perhaps correspond to cell types 'C' and 'D' as shown in the previous study (Mahmud and Mladenov 1998). The labelled cell by both anti-5HT and anti-DA indicates the presence of neurotransmitters/monoamine(s) in these cells. The presence of 5-HT and DA-like substances has also been previously reported in *M. edulis* (Mathieu and Van Minnen 1989). Although, the neurosecretory cell types 'C' and 'D' did not show any substantial changes in colour intensity with changes in gonad development and spawning (Mahmud and Mladenov 1998), the presence of neurotransmitters 5-HT and DA in these cells indicate that they might have other modulating or physiological functions in this species which need to be evaluated.

While the present study was based upon the hypothesis that peptides controlling reproduction might be evolutionarily conserved between gastropods and bivalves, it must also be considered that spawning and external fertilisation of bivalves are very different from in gastropods in terms of copulation and subsequent egg-laying behaviour (Croll *et al.* 1993). Therefore, even though related peptides might be involved in reproduction within both taxa, details of their distribution and mechanisms of actions are bound to vary. Their abundance should be investigated seasonally and correlated with stage of reproduction in order to determine which processes or mechanisms they are involved in. In the present study, samples from both mature and spawned mussels showed immuno-reactivity. The sampling protocol (small number of samples) in the present study does not allow assigning such physiological roles to these cells. However, results indicate that the ganglia of this mussel contain substances antigenically similar to peptides known to control reproduction in other molluscs. The present immunocytochemical study identifies unique population of cells containing neuropeptides and neurotransmitters, which are the likely candidates responsible for different aspects of reproduction and spawning in *P. canaliculus*. Although, the labelling of cells with anti-ELH, anti-APGWamide, anti-5HT and anti-DA does not necessarily confirm any physiological functions at this stage but it does indicate the presence of a preprohormone with ovulation factors and neurotransmitters. Further experimentation is needed to identify different hormones/amines in these cells and to assign their exact role in the reproductive functions of the green-lipped mussel, *P. canaliculus*.

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Effects of gamma radiation on nutritional and microbial quality of *Pampus chinensis* (Euphrasen 1788)

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Abstract
To evaluate the efficiency of gamma radiation in combination with low temperature Chinese pomfret, *Pampus chinensis* were preserved by the treatment of different doses of gamma radiation (3, 5 and 8 KGy) at freezing temperature (-20°C) during 90 days of storage period. Quality assessments for fish were carried out at an interval of 15 days during the storage period. Quality assessments were done by organoleptic, chemical (Total Volatile Nitrogen, TVN and Trimethylamine, TMA) and microbiological (Total Bacterial Count, TBC and Total Mould Count, TMC) evaluation. From the analysis of all parameters, maximum shelf-life was observed for irradiated (8 KGy) sample. It remained acceptable up to 75 days and that was the highest duration among 4 types of samples.

Key words: *Pampus chinensis*, Gamma radiation, Nutritional and microbial quality

Introduction

Chinese pomfret, *Pampus chinensis* is very rich in nutritional properties and very popular high valued marine fish species in Bangladesh. Since fish is the main source of our animal protein intake as well as a valuable source of foreign currency, its shelf-life extension is very much needed. Although some protein is removed from the fish during storage, irradiation did not affect the quality of the proteins including myoglobin, as indicate by the spectral characteristics of the melt water. Food irradiation is a process exposing food to ionizing radiations such as gamma rays emitted from the radioisotopes ^{60}Co and ^{137}Cs, or high energy electrons and X-rays produced by machine sources. Depending on the absorbed radiation dose, various effects can be achieved resulting in reduced storage losses, extended shelf life and improved microbiological and parasitological safety of foods. Irradiation of the main commodities such as tuber and bulb crops, stored grains, dried ingredients, meats, poultry and fish, or fruits has an enormous literature evolved during the past 60 years (Molins 2001). Recent research and development directed more on irradiation of minimally processed fresh produce and

cook-chill foods, where our own laboratories are also involved (Farkas 2001a). In the year 2002, the estimated volume of these products treated by irradiation was approximately 90,000 ton (Rubio 2003). Several USDA agencies are collaborating to make irradiated meat and poultry available for use also in the National School Lunch Program of the USA (Murano 2003) and the Food Safety and Inspection Service (FSIS) of the USDA develops an educational program on irradiated meat and poultry. In the above context, the present study was undertaken to determine the effect of radiation on the rate of spoilage of the preserved fish at low temperature (-20°C) and also the feasibility of radiation and consumers acceptance of preserved fish.

Materials and methods

Preparation of samples

All investigations were carried out in the laboratory of Food Processing & Preservation Division, Institute of Food & Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Savar, Dhaka. Specimen fish, Chinese pomfret, (*Pampus chinensis*) commonly known in 'Rup Chanda' was selected for the study. Fresh pomfret were collected from the Malibag Bazar, Dhaka. Fishes were divided into the 4 groups: sample A: [control], sample B: irradiated [3KGy], sample C: irradiated [5KGy], sample D: irradiated [8KGy]. All the samples were preserved at -20°C. Sample A was kept at -20°C without irradiation. Sample B, C and D will be subjected to irradiate in 3, 5 and 8 KGy panoramic Co-60 source supplied by the Atomic Energy of Canada Ltd.

Analytical methods

Fishes were first beheaded, degutted, washed and drained. Then only the muscles were collected for biochemical composition. Biochemical composition i.e. protein, lipid, ash and dry matter were determined by the methods described by AOAC (1975). Total volatile nitrogen (TVN) and Trimethyleamine (TMA) were estimated by "Conway dish" technique.

Calcium was determined by precipitating it as calcium oxalate and titrating the solution of oxalate in dilute sulfuric acid against standard potassium permanganate, $KMnO_4$. Phosphorus was estimated by measuring cotorimetric procedure.

Organoleptic analysis

Sensory evaluation for the detection of freshness or shelf-life of the stored fish and consumer's acceptance was performed with high degree of reliability by organoleptic evaluation following the method of Peryam and Pilgrim (1957) and Miyanchui *et al.* (1964). Quality assessment of preserved sample were studied at an interval of 15 days up to 90 days .

Total Bacterial Count (TBC) and Total Mould Count (TMC)

Total bacteria and mould count were estimated by the pour plate technique in petridishes. Viable colonies that developed on the plates after incubation at 37°C and

30°C in an incubator were count by colony counter (Gallenhamp colony counter, England). Finally the bacterial and mould count were obtained multiplying the number of colonies with dilution factor. The count was expressed as cfu/gm.

Results and discussion

Bio-chemical composition

The arerage moisture content of *Pampus chinensis* was 78.08±0.1% (Table 1). Rubbi *et al.*, (1987) stated that the biochemical composition and mineral contents of the fish analyzed on wet-weight basis showed that moisture of fresh fish varied from 72.1 % - 83.6 % with an average 77.64 %, fat content varied from 0.8 % - 15 % with an average 1.95 % and the protein and ash contents varied from 11.9 % - 21.9 % and 0.8 % - 5.11 % respectively (Table 1). The average protein content was determined in this study 15.75±0.4 %. Chandrasheker and Deosthale (1993) observed that a wide variation exist in protein of freshwater fishes and it were 13.5% to 17.3%. Akande *et al.*, (1991) estimated the lipid content of mackerel *(Scomber scombrus)* was 13.2% of fish. Akande *et al.* (1991) estimated the ash content of mackerel *(Scomber scombrus)* which was 1.2 % per gm of fish. The distribution of mineral contents in the ash samples was also found to vary considerably for calcium 7.2-363 mg/100gm, phosphorus 11.9-170 mg/100gm.

In the present study, mineral content such as calcium and phosphorus were 200 mg per 100 gm and 14O mg per 100 gm of fish muscle respectively (Table 1). Rubbi *et al.* (1989) found that calcium content of Chapila *(Gudushia chapra)* was 117.9 mg, Foli *(Notopterus notopterus)* was 220 mg, Koi *(Anabas testudinus)* was 300 mg and Kachki *(Corica suborna)* was 359 mg. According to Rubbi *et al.* (1987) the phosphorus content of Koi *(Anabas testudinus)* was 75.2 mg, Bele *(Glossogobius gaiuris)* was 75.2 mg, Chapila was 143 mg, Foli and Kachki were 153.9 mg and 172.30 mg respectively.

Table 1. Bio-chemical composition of Chinese pomfret, *Pampus chinensis*

Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Calcium (mg/100gm)	Phosphorus (mg/100gm)
77.50	16.79	1.92	4.25	190	160
78.20	14.75	2.15	3.90	210	140
78.56	15.73	1.79	2.76	200	120
Av. 78.08±0.1	Av. 15.75±0.4	Av. 1.95±0.16	Av. 3.63±0.2	Av. 200 ±4.4	Av. 140±4.4

Protein and lipid content at different anatomical position of controlled and irradiated samples shown in (Tables 2, 3) were determined to observe the effect of radiation on protein and lipid. For that reason samples were taken from both dorsal and ventral position.

Table 2. Protein (%) at different anatomical position of fresh *Pampus chinensis*

Position	3KGY	5KGY	8KGY
Dorsal	15.85	16.10	14.45
Ventral	15.25	15.20	15.80
Average	15.55	15.65	15.12

Table 3. Lipid (%) at different anatomical position

Position	3KGY	5KGY	8KGY
Dorsal	6.32	5.66	4.43
Ventral	4.65	5.84	5.12
Average	5.49	5.75	4.77

Organoleptic evaluation

Organoleptic scores of control fish come down 8.49 to 3.20 stored at -20°C respectively. Whereas in the some storage temperature and storage period the organoleptic scores of irradiated fish were come down from 8.74 to 4.00, 8.95 to 5.50 and 9.0 to 6.5, respectively in 3, 5 and 8 KGY (Table 4). Muscle of live fishes is more or less sterilized but after death autolytic, bacterial and other changes occur (Huss 1986). The physical changes could be perceived with sense organs.

The organoleptic scores were gradually decreased with the progress of storage period. Only irradiated samples stored at -20° C temperature showed the acceptable score up to 90 days. The quality of control sample was gradually decreasing during storage. The effect of radiation on fresh fish during 90 days of storage was studied by (Ghaly *et al.* 2000) and no undesirable changes in the sensory properties of the fish compared with control samples were found.

Table 4. Organoleptic scores of *Pampus chinensis* during storage period at -20°C

Storage period (days)	Control	Irradiated 3KGY	Irradiated 5KGY	Irradiated 8KGY
0	8.49	8.74	8.95	9.0
15	8.24	8.41	8.66	8.5
30	7.33	7.66	7.30	8.0
45	6.49	7.48	7.49	8.0
60	4.91	5.91	6.66	7.5
75	3.66	5.00	5.24	7.0
90	3.20	4.00	5.50	6.5

Total Volatile Nitrogen (TVN)

Regarding the shelf-life of control and irradiated Pomfret stored at -20°C, the TVN values were found to increase gradually from the moment of preservation. The initial TVN values for control and irradiated fishes were 4.5 and 3.0 (3 KGY), 2.5 (5 KGY) and 1.5 (8 KGY) mg nitrogen per 100 gm respectively (Table 5). It was observed that TVN value exceeds 30 mg nitrogen per 100 gm of muscle, the fish become unacceptable. Ota (1985) also found that the total volatile nitrogen increased with the increase of time during spoilage and all of them suggested that 30 mg nitrogen per 100 gm of fish muscle should be taken as the upper limit for acceptability.

Table 5. Total Volatile Nitrogen (TVN) (mg N/1OO g) in control and irradiated *Pampus chinensis* during storage period at -20°C

Storage period (days)	Control (mgN/100g)	Irradiated 3KGY	Irradiated 5KGY	Irradiated 8KGY
0	4.5	3.0	2.5	1.5
15	5.6	3.2	2.9	2.0
30	7.5	4.1	3.5	2.7
45	10.6	5.2	3.9	3.1
60	16.8	5.9	4.3	3.5
75	24.6	6.3	4.8	4.2
90	32.5	7.1	5.3	4.9

At the beginning of storage period, TVN value was comparatively higher in control sample than other samples. This value rapidly increased in control sample than irradiated samples which were storage at -40°C. From the present investigation it was found that the fish samples which were stored at -20°C were acceptable almost 90 days in case of irradiated samples. Hossain *et al.* (2001) experimented on effect of radiation on whole and degutted fish *(Rastrelliger kanagurta)* at low temperature (0°C) and found that the acceptable TVN limit of degutted control and irradiated samples were 28 and 35 days respectively. It was lower than the present investigation because of low temperature. At the 0 day he reported the initial TVN value was 2.3 mg nitrogen per 100 gm of fish.

Trimethylamime (TMA)

At the beginning of storage period, TMA value was comparatively higher in control sample 7.5 (Table 6) than other samples. This value rapidly increased in control sample than chemical treated and irradiated samples which were at -20°C. During storage period this value were found to increase gradually as 7.5 mg % to 55 mg % in control, 6 mg % to 45 mg % in irradiated (3 KGY), 5 mg to 27.5 mg % in 5 KGY and 4 mg to 18.7 mg % in 8 KGY sample stored at -20°C.

Table 6. Trimethylamine (TMA) (mg N/lOO gm) in controland irradiated *Pampus chinensis* during storage period at -20° C

Storage period (days)	Control (mgN/lOOgm)	Irradiated 3 KGY	Irradiated 5KGY	Irradiated 8KGY
0	7.5	6.0	5.0	4.0
15	13.0	10.0	7.5	6.0
30	20.0	17.5	10.0	7.5
45	22.5	17.5	14.5	10.5
60	27.5	25.5	20.0	12.7
75	42.0	37.5	22.5	14.3
90	55.0	45.0	27.5	18.7

Hossain *et al.* (2001) reported the effect of irradiation on the shelf-life extension of mackerel and the TMA values of these sample indicated that the increase of TMA values in irradiated sample were significantly less. Hossain *et al.* (2000) also reported on the potassium-sorbet effectiveness on *Labeo rohita* and found that the TMA values of these sample indicated that the acceptable of degutted treated fish was 26 days which was more similar to the present investigation.

Total Bacterial Count (TBC)

At the beginning of storage period total bacterial counts were affected by the radiation. The initial period of storage control sample was shown maximum ($1.3x10^4$ cfu/gm) bacterial colony and irradiated samples were shown minimum (00 cfu/gm) bacterial colony. At 90 days of observation (Table 7) shown that the bacteria were gradually increased as $2.1x10^5$ cfu/gm in control sample stored at -20°C, $2.3x10^4$ cfu/gm in 3 KGY, $6.7x10^3$ cfu/gm in 5KGY and $3.5x10^3$ cfu/gm in 8KGY sample stored at -20°C. Control samples contained more bacteria than those of irradiated samples.

Table 7. Total bacterial count (TBC) (cfu/gm) in control and irradiated *Pampus chinensis* during storage period at -20° C

Storage period (days)	Control	Irradiated 3KGY	Irradiated 5KGY	Irradiated 8KGY
0	$1.3X10^4$	$2.1X10^2$	00	00
15	$2.5X10^4$	$2.7X10^3$	00	00
30	$3.2X10^4$	$4.1X10^3$	$1.0X10^3$	00
45	$2.9X10^4$	$3.7X10^3$	$2.3X10^2$	$1.5X10^2$
60	$3.5X10^4$	$1.4X10^4$	$5.4X10^3$	$1.3X10^3$
75	$3.8X10^4$	$1.8X10^4$	$6.1X10^3$	$2.8X10^3$
90	$2.1X10^5$	$2.3X10^4$	$6.7X10^3$	$3.5X10^3$

Hossain *et al.* (2001) experimented the radiation effect of mackerel fish *(Rastrelliger kanagurta)* at low dose. He found that the irradiated degutted sample remained acceptable upto 29 days at 4°C. In the present investigation, TBC were higher than that of above mentioned findings but this result more supported to the preseent findings. Lee *et al.* (2001) reported that irradiation significantly affected bacterial count at the dose of 5 KGy, shelf life was enhanced effectively by suppression of microbial growth and proliferation.

Total Mould Count (TMC)

At the beginning of storage period, there were no moulds in the irradiated samples. Both control and irradiated samples were examined and total mould counts were affected by the radiation. The initial period of storage, control samples were shown maximum ($1.1x10^3$cfu/g) mould colony and irradiated samples were shown minimum ($1.2x10^2$ cfu/g) mould colony in 8 KGy. At the 90 days observation (Table 8) shown that the mould were tremendously increased as $3.1x10^5$ cfu/g in control samples, $5.3x10^3$ cfu/g in 3 KGY, $3.8x10^4$ cfu/g in 5 KGY and $3.5x10^4$ cfu/g in 8 KGY sample stored at -20°C. Control samples contained more mould than those of irradiated samples.

Table 8. Total mould count (TMC) (cfu. gm) in control, and irradiated Chinese pomfret, *Pampus chinensis* during storage period at -20°C

Storage period (days)	Control	Irradiated 3KGY	Irradiated 5KGY	Irradiated 8KGY
0	$1.1X10^3$	$3.0X10^2$	$2.0X10^2$	$1.2\ X10^2$
15	$1.5X10^3$	$3.2X10^2$	$2.5X10^2$	$1.5\ X10^2$
30	$1.5X10^4$	$3.5X10^3$	$3.1X10^2$	$2.0\ X10^2$
45	$1.9X10^4$	$3.9X10^3$	$3.5X10^3$	$2.5\ X10^3$
60	$2.3X10^4$	$4.5X10^3$	$4.0X10^3$	$3.2\ X10^3$
75	$2.8X10^4$	$4.9X10^3$	$3.5X10^4$	$2.6\ X10^4$
90	$3.1\ X10^5$	$5.3\ X10^3$	$3.8\ X10^4$	$3.5\ X10^4$

Hossain *et al.* (2001) are reported that low temperature and radiation inhibited the rapid organoleptic degradation of fish during the long time preservation. Extension of shelf-life at radiation doses 5 KGy and 8 KGy did not bring about any notification. From the biochemical composition analysis, the contents of moisture, protein, lipid, ash, calcium and phosphorus were estimated as 78 %, 15.75 %, 1.95 %, 3.63 %, 0.20 % and 0.14 % respectively. In this view, Chinese pomfret should be treated as a highly nutritious fish. To extend the shelf-life of selected species, Chinese pomfret, (degutted) were treated with gamma radiation (3, 5 and 8 KGy) and stored at low temperature (-20°C) for 90 days. For determining the shelf-life extension of these fish sample some parameters like organoleptic score, total volatile nitrogen (TVN),

trimethylamine (TMA), total bacterial count (TBC), total mould count (TMC) were used in every 15 days of interval. So, from the present study, it can be concluded that irradiation (8 KGy) in combination with -20°C temperature is the best method for long time preservation of fresh fish.

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Culture feasibility of silver barb (*Barbodes gonionotus* Bleeker) and GIFT with shrimp (*Penaeus monodon* Feb.) in brackishwater pond

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Abstract
An experiment was conducted to understand the culture feasibility of sliver barb (*Barbodes gonionotus*) and GIFT (Genetically Improved Farmed Tilapia) with shrimp (*Penaeus monodon*). There were three different treatment (T) combinations: (T_1) shrimp (10,000/ha) and silver barb (10,000/ha), (T_2) shrimp (10,000/ha) and GIFT (10,000/ha), and (T_3) shrimp (10,000/ha). Shrimp, after 120 days of culture, attained an average weight of 23.77g in T_1, followed by T_3 (23.70g). The highest average weight was recorded in T_2 (24.93g). The specific growth rate (SGR) of shrimp was 6.9%, 6.94% and 6.9% for T_1, T_2 and T_3, respectively. The SGR for the *B. gonionotus* and GIFT was 2.56% and 4.26%, respectively. The final weight of silver barb was 69.75g and that of GIFT was 161.83g. Survival of shrimp was higher (65.50%) in T_2, followed by T3 (59.97%) and T_1 (57.03%). Survival rate of silver barb (58.10%) was lower compared to that of GIFT (78.43%). Sporadic and scanty mortality of silver barb with a symptom of blind-red-protruded eye, swollen belly and body lesion was observed. Production of shrimp was higher of 284.05 kg/ha in monoculture, followed 162.47 kg/ha in concurrent culture with silver barb and 136.77 kg/ha culture with GIFT. In spite of similar stocking density of *B. gonionotus* and GIFT in T1 and T2, respectively, the production of GIFT was higher (1272.95 kg/ha) than that of silver barb (402.72kg/ha). Survival, final weight and production rates of shrimp among the treatments were found insignificant while total production of shrimp/fish was found to vary significantly ($P<0.01$). Benefit cost ratios (BCR) were 1.04:1.0, 1.32:1.0 and 2.05:1.0 in the T_1, T_2 and T_3, respectively. Results indicate that, concurrent culture of *B. gonionotus* and shrimp will be less prospective in comparison to culture of GIFT and shrimp in brackishwater environment with a salinity range of 9 to 14‰.

Key words: *Penaeus monodon*, *Barbodes gonionotus*, GIFT, Brackishwater

Introduction

Traditional shrimp farming in the South-west region in Bangladesh is an age-old practice. At present about 0.17 million ha (Karim 2005) of tidally inundated lands are being utilized under such traditional culture system, which is locally known as *gher*

fishery. The major cropping pattern is the mono-crop of tiger shrimp (*Penaeus monodon*), cultured in a salinity range of 10-25 ppt in dry season (Krishna 1991). Present average production of shrimp in traditional system is not exceedingly 190 kg/ha/year (Rouf 2006). The successive culture in mono-crop system in *ghers* often results in crop loss due to mortality problem. The crop diversification instead of mono-crop shrimp culture practice may be an effective tool in controlling or diminishing shrimp mortality especially due to ecological and disease factors. On the other hand the cultural of *P. monodon* with any suitable short-grown fish species may help to increase or enhance the production in shrimp farms with an environment friendly situation. Silver barb (*Barbodes gonionotus*) an exotic fish in the country. It is a fast growing species widely cultured in freshwater and grow well in low salinity (Yang and Fitzsimmons, 2007) however, reported to survive in a wide range of salinity 0-17‰ (Hossain *et al.* 1999); eat a variety of foods. It grows on relatively low quality diet; largely vegetable protein (Hossain *et al.* 1994) and its seed are available throughout the country. Tilapia (*Oreochromis sp*) is another fish with an omnivorous and best-known detritivorous species (Bowen, 1981) and generally feeds on algae and minute animals. As GIFT (Anon 1998) has significantly higher growth performance (36-81%) than local strains of *O. niloticus* and has tolerance to a wide range of salinity, this improved strain of tilapia may be another suitable candidate for brackishwater aquaculture. So far, few or no work has been done on culture potentiality of silver barb and GIFT in shrimp *ghers* in Bangladesh and data were not available. This paper describes the culture feasibility of *B. gonionotus* and GIFT with shrimp (*P. monodon*) in brackishwater earthen ponds.

Materials and methods

The experiment was conducted during March to June 2006 at the Brackishwater Station of the Bangladesh Fisheries Research Institute, Paikgacha, Khulna. Six ponds of 2000 m^2 each in the pond complex were selected. The ponds were prepared by sun drying followed by liming with CaO @ 250 kg/ha and by fertilizing with mustard oil cake (MOC), di-ammonium phosphate (DAP) and urea @ 50, 20, and 10 kg/ha, respectively. A nursery enclosure of 40m^2 with fine meshed nylon-net was set up in each pond for nursing shrimp post larvae. After 7 days, the ponds were filled up to a depth of 80cm with tidal water through a feeder canal and left for about a week period for suitable water conditions.

Hatchery produced of *P. monodon* post larvae with an average weight of 0.006g were stocked in the nursery enclosures and nursed to grow juvenile. Silver barb (*B. gonionotus*) fingerling with an average weight of 3.24g and GIFT fry with an average weight of 0.98g were directly stocked to the ponds in March 2006 under three different treatments. The species combination and stocking density in Treatment-1 was *P. monodon* and *B. gonionotus* at 10,000/ha each; in Treatment-2 was *P. monodon* and GIFT at 10,000/ha each and in Treatment-3 was *P. monodon* at 20,000/ha. The juvenile *P. monodon* were allowed to spread over the entire pond at the 14th day, by up-folding the nylon net of nursery enclosure.

Shrimp and fish were fed with a prepared feed (containing 30% protein level). Percent composition of different ingredients of feed was fishmeal (29%), MOC (15%), rice bran (30%), soybean meal (16%), wheat flour (9%) and vitamin premix (0.1%). Feed application was done at the rate of 5~3% bw/day, twice at dawn and dusk.

Routine sampling was done during the experimental period. After 120 days of culture, both shrimp and fishes were harvested completed by dewatering the ponds and the growth and production were estimated.

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

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

$$SGR\% = \frac{Final\ weight\ (g) - Initial\ weight\ (g)}{Initial\ weight\ (g)}$$

Results and discussion

The physicochemical parameters of the pond water are shown in Fig. 1. It reveals that, water temperature varied from 25 to 33^{0}C (Fig. 1b) and salinity ranged from 8.0 to 14‰ during the study period (Fig. 1c). Water transparency was recorded to vary from 17.0 to 60.0 cm (Fig. 1e) and depths from 62.5 to 90cm (Fig. 1f). There was no significant difference in physico-chemical parameters among the treatments. Concentration of plankton population and their variation are shown in the Fig. 2. Phytoplankton population was found within 8000 to 16000 No./litre (Fig. 2a) while zooplankton was found within 1000 to 9000 no./l (Fig. 2b). Benthic population was monitored and has been shown in the Fig. 2c. Amphipods was the major benthic population as well insect larvae was found with a ranged between 40/m^2 to 2540/m^2. Values of different water quality variables were found suitable for *P. monodon* and GIFT in agreement with Grey (1990) and Jung and Co (1988); however, salinity range of the present study (8.00-14.00‰) seems to be less suitable for *B. gonionotus*. Yang and Fitzsimmons (2007) reported better growth and survival of *B. gonionotus* when stocked in low salinity (range 0-5‰) with shrimps in Vietnam and Thailand.

Variations in growth rate of shrimp under different treatments are shown in Fig. 3. No significant growth variations of shrimp among the treatments were observed. Growth of silver barb seems to be slower than that of GIFT (Fig. 4). The slower growth of *B. gonionotus* might be due to salinity or unknown factors. However, Hossain *et al.* (1994) reported the growth 112-114g for *B. gonionotus* in the salinity at 5-6 ppt., which is lower than the present study. Shrimp attained its higher average weight in T-2 (24.93g), where shrimp was stocked with GIFT, followed by 23.77g with silver barb (T-1) and 23.70g with shrimp monoculture (T-3). The total weight gain for shrimp was estimated 4154.0 of T-2, higher than that of 3960.0 and 3949.0 of T-1 and T-3, respectively (Table 1).

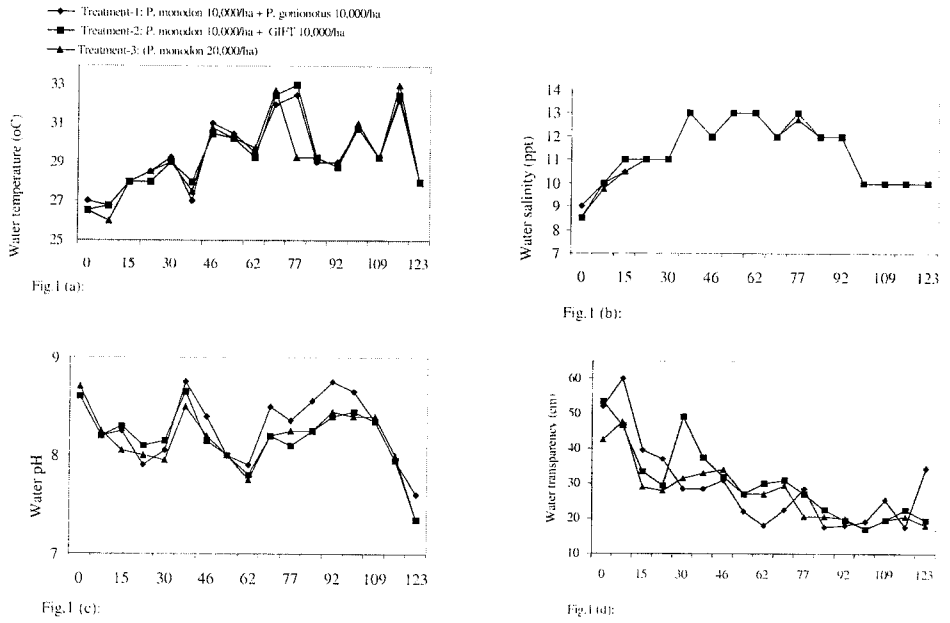

Fig. 1. Physico chemical parameter (a) water temperature, (b) salinity, (c) pH, and (d) Transparency in different ponds.

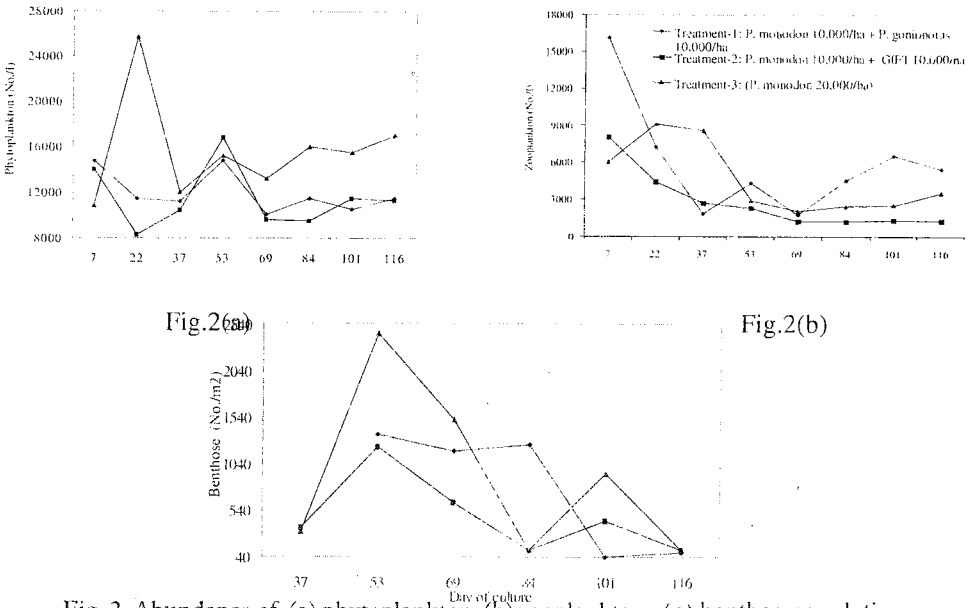

Fig. 2. Abundance of (a) phytoplankton (b) zooplankton, (c) benthos population in different treatments.

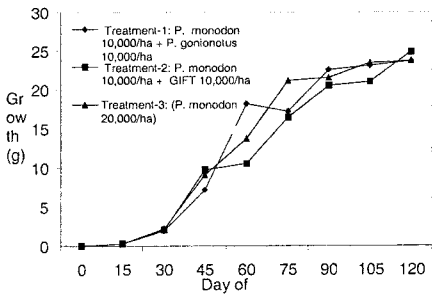

Fig. 3. Comparative growth of *P. monodon* in different treatment of culture

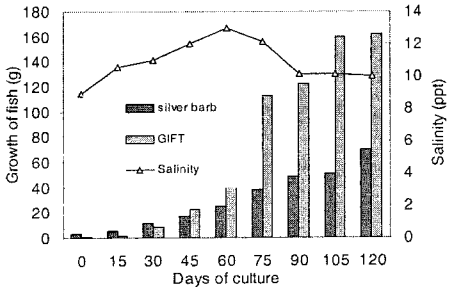

Fig 4. Salinity of water and growth of *B. gonionotus* and GIFT during the culture period.

Table 1. The growth performance and production of *Penaeus monodon, Barbodes gonionotus* and GIFT in different cultural treatments

Cultural treatments	Stocking No./ha	Initial wt. (g)	Final wt. (g)	TWG	SGR (%)	Survival (%)	Prod. (kg/ha)	Total prod. (kg/ha)	FCR
Treatment -1									
P. monodon	10,000	0.006 ±0.001	23.77 ±3.15	3961	6.90	57.03 ±7.67	136.77 ±36.23		2.62
B. gonionotus	10,000	3.24 ±1.13	69.75 ±12.11	21	2.56	58.10 ±2.05	402.73 ±41.24	539.50[b]	
(Concurrent culture)									
Treatment -2									
P. monodon	10,000	0.006 ±0.001	24.93 ±1.17	4154	6.94	65.50 ±9.77	162.47 ±13.22		2.29
GIFT	10,000	0.96 ±0.31	161.83 ±8.56	164	4.26	78.43 ±8.87	1272.95 ±210.17	1435.42[a]	
(Concurrent culture)									
Treatment -3									
P. monodon	20000	0.006	23.7 ±0.20	3949	6.90	59.97 ±11.84	284.05 ±53.68	284.05[b]	3.49
(mono culture)									

** P>0.01

The specific growth rate (SGR) of shrimp was 6.9%, 6.94% and 6.9% in Treatments 1, 2 and 3, respectively. However, the SGR for the *B. gonionotus* and GIFT was 2.56% and 4.26%, respectively (Table 3). The final weight of silver barb was 69.75g and that of GIFT was161.83g. The survival of shrimp was higher (65.50%) in Treatment-2, followed by Treatment-3 (59.97%) and in Treatment-1 (57.03%). The survival of *B. gonionotus* was

58.10%, which is lower than that of 78.43% of GIFT. Sporadic and scanty mortality of silver barb with a symptom of blind-red-protruded eye, swollen belly and body lesion was observed. Production of shrimp was higher of 284.05 kg/ha in monoculture, followed 162.47 kg/ha in concurrent culture with silver barb and 136.77 kg/ha in concurrent culture with GIFT. In spite of similar stocking density and ratio of silver barb: shrimp and GIFT: shrimp, production of GIFT (1272.95kg/ha) was higher than that of silver barb (402.72 kg/ha). The survival, final weight and production of shrimp were insignificant. The total production was significant ($P<0.01$).

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Performance of genetically improved farmed tilapia (GIFT) under mono and mixed culture with silver barb (*Barbodes gonionotus*) in south-west Bangladesh

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Abstract
A participatory on-farm trial was carried out to evaluate the production performance of GIFT (genetically improved farmed tilapia) strain of *Oreochromis* sp., either alone or with silver barb (*Barbodes gonionotus*), in six rain-fed freshwater ponds of coastal area. There were two treatments; (i) GIFT alone at a stocking density of 24,700/ha (T_1) and (ii) 1:1 combination of GIFT and silver barb (T_2). Each of the treatments had three replications. A significantly ($p<0.05$) higher production of 4306.14 kg/ha of GIFT was obtained in monoculture, compared to the total production of 3480.38 kg/ha of GIFT and silver barb. In the 1:1 combination treatment, the production of GIFT (2036.06 kg/ha) was also higher than that of silver barb (1444.32 kg/ha). The results reveal that GIFT monoculture can performe better in rain-fed seasonal freshwater ponds of south-west coastal areas.

Key words: GIFT, Silver barb, coastal freshwater ponds

Introduction

The inter-tidal flat plain areas of the south-west coastal regions (specially Satkhira and Khulna) of Bangladesh are usually used for shrimp, *Penaeus monodon* culture. The culture cycle continues from February to mid August when water salinity remains 6-17 ppt. Now, shrimp culture is severely threatened by a number of problems especially by mass mortalities due to various causes of disease. Besides tidally-fed shrimp culture areas, there are a large number of rain-fed seasonal freshwater ponds in the region, where water remains at least 1-1.5 meter for 4-6 months in a year and even more in some areas. People normally use these water bodies for their household activities. From the aquaculture perspective, these water bodies have a great potentiality.

Genetically improved farmed tilapia (GIFT) and silver barb (*Barbodes gonionotus*) are two important short cycle cultured fish species. Introducing the culture of GIFT and silver barb in coastal region may help the coastal people to their poverty alleviation. Considering the potentiality of small pond/ditches into aquaculture practice in coastal

region, the present study was designed to evaluate the performance of GIFT under mono and mixed culture with silver barb in the rain-fed seasonal ponds in coastal areas of Bangladesh.

Materials and methods

Pond preparation

Six rain-fed freshwater ponds of various sizes (155-410 m^2, average depth 1.5 m) were selected in Gopalpur, Saral and Bandikati villages of Paikgacha Upazilla, Khulna for this study for the duration of April to September. Ponds were prepared by application of lime to @ 250 kg/ha filling up with nearby freshwater sources and rain water. Three days after application of lime, ponds were fertilized with cattle manure @ 750 kg/ha. Inorganic fertilizers - urea @ 25 kg/ha and TSP @ 30 kg/ha were applied after five days of applying organic fertilizer.

Stocking & management

After three days of fertilization, when water became greenish, three ponds were stocked with only GIFT (24,700 nos./ha) (Treatment-1) and other three ponds were stoked with GIFT (12,350 nos./ha) + Silver barb (12,350 nos./ha) (Treatment-2). The average body weight of the stocked GIFT and silver barb was 0.37g and 3.79g, respectively. Fishes were fed with rice bran at the rate of 5~3% of the total biomass. After stocking, ponds were fertilized with urea and TSP @ half of the initial dose at fortnight interval.

Sampling

The ponds were sampled at fortnightly intervals to assess growth and condition of fish and feeding was adjusted on the estimated fish biomass in ponds. Physico-chemical parameters of water *viz.*, temperature, pH, dissolved oxygen and transparency were determined fortnightly according to the standard procedure and methods (APHA 1992). The quantitative abundance of plankton was estimated fortnightly, using a Sedgewick-Rafter counting cell, following the method of Stirling (1985). After six months of rearing, all fishes were harvested by draining out the pond water and growth, survival and production of fishes were estimated.

Statistical analysis

Data were analyzed for one-way ANOVA and any difference at 5% level of significance using the statistical package of STATGRAPHICS *Version* 7.

Results and discussion

Water quality parameters in all culture ponds throughout the experimental period are shown in Table 1. The water temperature of the culture ponds was recorded from 29

to 33 °C . pH of the ponds varied from 7.2-8.4 during the experiment. Water temperature in shallow and small fish ponds of Bangladesh has been found to range from 26-35°C, with the maximum in May to August, and to follow air temperature closely with a small variation (Rahman *et al.* 1982, Hossain *et al.* 1997).

Table 1. Mean (±SD) water quality parameters of ponds in different treatments

Parameters	Treatment 1	Treatment 2	Significance level
Water temperature (°C)	29.34 ± 0.27	29.21± 0.39	NS
pH	7.91 ±1.37	8.2 ± 0.88	NS
Dissolve oxygen (mg/l)	5.50 ± 0.13	5.92 ± 0.11	NS
Transparency (cm)	28.12 ± 1.16	28.76 ±1.07	NS
Phytoplankton (× 10^3 cells/l)	53.65 ± 2.10	49.23 ± 1.75	NS
Zooplankton (× 10^3 cells/l)	12.55 ± 1.52	9.94 ± 1.45	NS

NS= Not Significant at 5% level.

The dissolved oxygen of 3.5-8.5 mg/l and transparency of 25-35 cm were recorded throughout the culture period. A similar trend in fluctuation of 3.2-9.7 mg/l (Haq *et al.* 1994), 2.0-7.2 mg/l (Wahab *et al.* 1995), 3.2-8.5 mg/l (Grag and Bhatnagar 2000) of dissolved oxygen has been reported in fish ponds. Boyd (1982) suggested that a transparency between 15- 40 cm is suitable for fish culture which is very close to the observations of the present study. Values of all observed water quality variables were found congenial for fish culture. There was no significant difference ($p>0.05$) in physico-chemical characteristics of water among different treatment ponds.

In T1, the mean value of phytoplankton concentration was $53.65\pm2.10\times10^3$ cells/l, while in T2 the abundance was slightly lower at $49.23\pm1.75\times10^3$ cells/l. Shah *et al.* (2004) recorded a concentration of phytoplankton and zooplankton ranging from 29.03 to 31.81×10^3 cells/l and 6.32 to 7.09×10^3 cells/l, respectively in his experiment. Wahab *et al.* (1995) recorded the abundance of phytoplankton ranging from 2×10^5 to 8×10^5 cells/l and that of zooplankton from 2×10^4 to 2×10^5 cells/l in their study. Haque *et al.* (1998) recorded phytoplankton and zooplankton abundance of $3.78\pm0.15\times10^4$ cell/l to $50.64\pm1.29\times10^4$ cells/l and 4.91± 0.8 to $6.16\pm0.8\times10^4$ cells/l, respectively in their study. Compared to the observation, the plankton abundance was lower in the present study and this might be due to the lower quantity of fertilizer used.

Monthly growth performance of GIFT and silver barb under two treatments during the experiment is shown in Fig. 1. Throughout the study period, GIFT in monoculture showed higher growth than mixed culture with silver barb. GIFT reached an average final weight of 195.5 g in monoculture (T1) and 190 g in mixed culture (T2). There was no significant difference ($p>0.05$) between the treatments. The highest weight gain (195.13 g) of GIFT was attained in mono culture but when a one way ANOVA was run, the difference was not significant ($p>0.05$) statistically. In mixed culture system, the average final weight of silver barb was 144.5 g and the weight gain was 140.71 g (Table 2). Hossain *et al.* (1997) recorded average weight gain of *O. niloticus* 78.8 g in mixed culture system with mirror carp, silver carp and silver barb for 105 days.

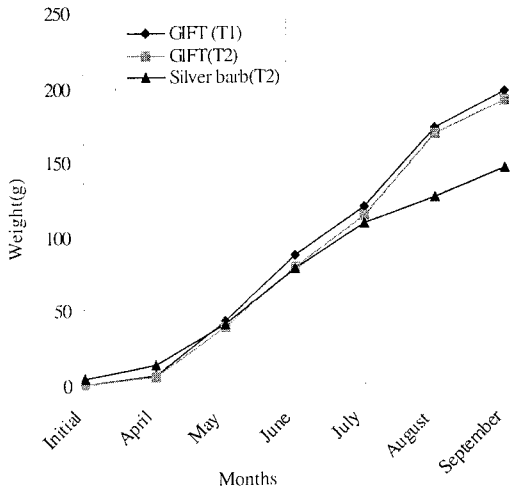

Fig. 1. Monthly growth (g) performances of fishes under two treatments.

Table 2. Growth and production of GIFT under mono and mixed culture system

Treat-ment	Fish species	Average initial weight (g)	Average final weight (g)	Average weight gain (g)	SGR (% day)	Survival (%)	Production (kg/ha)	
							Species wise	Total
T1 (Only GIFT)	GIFT	0.37± 1.12	195.5± 3.13	195.13	2.37	89.17	4306.14	4306.14 ± 11.72[a]
T2 (GIFT + silver barb)	GIFT	0.37± 1.12	190± 2.18	189.63	2.36	86.77	2036.06	3480.38 ± 9.67[b]
	Silver barb	3.79± 2.32	144.5± 3.19	140.71	2.02	80.93	1444.32	

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

In monoculture system, GIFT showed higher growth compared to the mixed culture with silver barb. The specific growth rate (SGR) of GIFT was 2.37 (%/day) and 2.36 (%/day) in mono and mixed culture, respectively. There was no significant difference ($p>0.05$) between the two treatments. In mixed culture system, SGR of silver barb was

comparatively lower (2.02 %/day) than the GIFT. Shah *et al.* (2004) recorded more or less similar SGR of 2.41 %/day of GIFT in mixed culture with silver barb.

The survival rate of GIFT was 89.17 % in monoculture (T1) and 86.77 % in mixed culture (T2). There was no significant difference ($p>0.05$) between survival rates of GIFT in two treatments. Hossain *et al.* (1997) observed 87.5-100% survival of GIFT when studied mixed culture with silver barb, mirror carp and silver carp in seasonal ponds. Shah *et al.* (2004) recorded survival rate of 93.52% and 87.57 % of GIFT and silver barb, respectively in mixed culture system.

Total yield of fish (4306.14 kg/ha) was significantly ($p<0.05$) higher in mono culture system (Table 2). Mazid (2002) reported the production of GIFT of 2,500-3,000 kg/ha in seasonal ponds within 5-6 months, which is lower than the present study. In mixed culture system, GIFT perform better position in the production (2036.06 kg/ha) and the total production was observed 3480.38 kg/ha. In mixed culture, the production of GIFT was higher than that of Shah *et al.* (2004), where production of GIFT was 1442.90 kg/ha in mixed culture with silver barb. The production of silver barb was 1606.53 kg/ha in mixed culture system (T2). Wahab *et al.* (1996) observed 5294 to 5670 kg/ha/yr production of silver barb in the polyculture with carps. Hossain *et al.* (1997) recorded total production of 2233 kg/ha/105 days in mixed culture of silver barb with nile tilapia, mirror carp and silver carp in seasonal ponds.

The cost-benefit from GIFT monoculture and mixed culture with silver barb is shown in Table 3. While estimating the cost of production, variable costs of only lime, feed, fertilizer and fingerlings were taken into consideration. Cost of production was Tk. 119,176.50/ha and Tk. 106,508.44/ha in monoculture and mixed culture, respectively. All the variable costs were remained same in both treatments except silver barb fingerling cost for T2. The gross benefit in monoculture amounted to Tk. 2, 58,368/ ha, leaving a net benefit of Tk 139,191.50/ha while gross benefit from mixed culture (T2) amounted to Tk. 179,936.40/ha with a net benefit of Tk. 73,427.96/ha showing a higher profit per hectare than that of mixed culture (T2).

Table 3. Cost and benefits per hectare from mono and mixed culture system of GIFT and silver barb

Input	Treatment 1 (Only GIFT)		Treatment 2 (GIFT + silver barb)	
	Quantity (kg)	Cost (Tk.)	Quantity (kg)	Cost (Tk.)
A. Cost				
Lime	250	1,750.00	250	1,750.00
Cattle manure	750	1,500.00	750	1,500.00
Inorganic fertilizer	357.50	4,697.50	357.50	4,697.50
Fingerlings-GIFT Silver barb	24,700	24,700.00	12,350 12,350	12,350.00 9,262.50
Rice bran	14,421.50	86,529.00	12,824.74	76,948.44
Total cost		119,176.50		1,06508.44
B. Benefits				

Marketable size fish				
GIFT (Tk.60/kg)	4,306.14	258,368.00	2,036.06	122,163.60
Silver barb Tk 40/kg			1,444.32	57,772.80
Gross benefit		258,368.00		179,936.40
Net benefit (B-A)		139,191.50		73,427.96

From the present results, it may be concluded that GIFT monoculture in freshwater ponds in coastal area is better than mixed culture with silver barb, due to their high growth performance. It is also suggested that GIFT can be a potential fish species to be commercially introduced into culture system to get higher production from the rain-fed seasonal freshwater ponds of coastal area.

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Emigration dynamics of three species of penaeid prawn from backwaters and tidal ponds of Cochin, India

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Abstract
Emigration of three species of penaeid prawn from backwaters and tidal ponds were studied. Considerable diel, tidal, lunar and seasonal fluctuations were observed in emigration process, which was almost nocturnal. Rate of emigration and composition of emigrants varied with time of migration. Large pulses of emigration always coincided with spring tides with major peak during new moon. Seasonal variation was observed with peak emigration of *Penaeus indicus* during monsoon months and that of *Metapenaeus dobsoni* and *Metapenaeus monoceros* during pre-monsoon. Rate of emigration was relatively large from shallow tidal ponds. It correlated directly with the prevailing environmental conditions and juvenile density. Instantaneous rate of emigration was also large in seasonal ponds. The basic stimulus for emigration is the urge for sexual maturation. Coupled with it ecological changes in the habitat have been causing various patterns in migration.

Key words: Emigration dynamics, Penaeid prawn, Brackishwater

Introduction

Penaeids mature and spawn in the open ocean. Early post larvae migrate to shallow coastal waters and backwaters for feeding and nursing. After the nursery phase in the estuarine area they migrate back to sea for their next phase of life; maturation and spawning . The migrating prawns support an extensive artisanal fishery in open backwaters and adjacent tidal ponds. Different authors have investigated the causal stimuli behind penaeid emigration and several factors have been discussed to explain the same (Garcia 1977, Dall 1981, Garcia and Le Reste 1981, Staples and Vance 1986, Laubier, 1989, Benfield *et. al.* 1990). Most of them considered physico-chemical changes in the habitat, lunar periodicity and tidal cycles act as the major stimuli, that evoke emigration in prawns, Barring some information on seasonal shrimp migration based on filter net fishery by Menon (1951), George (1974) and Anon (1982), penaeid emigration was very little understood from Indian waters. So a study was carried out during 1992-2001 to understand more on the causative factors and behaviour of penaeids during their emigration form tidal ponds and backwaters. This paper describes in detail the dynamics of emigration of penaeids from tidal ponds and backwaters and the causal stimuli.

Material and methods

The study was carried out in two each of perennial ponds, deep and shallow seasonal ponds (SPI and SPII) and open backwater sites. Filter nets are 4.5 to 5.0 m long tapering conical nets, made of strong cotton or nylon threads with 0.4 mm meshed code end. Emigration was studied by monitoring the prawn catch in filter nets at fortnightly intervals. Data collections were designed to coincide with different tidal and lunar phases. Biology of the species in the catch and physico-chemical parameters of the tidal ponds and backwaters were also monitored.

Catch details were collected directly on sampling day and from farm registers for the remaining period. Species composition, length, weight and biology of major species were studied. A total of 11,276 specimens of *Penaeus indicus,* 19,782 *Metapenaeus dobsoni* and 2,871 *Metapenaeus monoceros* were used in the study. Number of prawns emigrated from unit pond area was derived from catch and length-weight data. Instantaneous rate of emigration was estimated as the fraction of total prawns recruited into a habitat during the year that emigrated during the same year. Size at emigration was derived from probability curve using length frequency distribution of emigrating population. Multiple regression and analysis of variance were carried out to evaluate and quantify the influence of various physico-chemical and biological factors on emigration. Paired observations were evaluated for significance by hypothesis test for means. Standard procedures were followed in the biological studies and statistical analysis.

Results

Emigrating penaeid population was dominated by *M. dobsoni* followed by *P. indicus* and *M. monoceros.* In perennial ponds, *M. dobsoni* represents 72.2 to 76.6% of the total emigrants and in seasonal ponds between 78.6 to 82.6%. *P. indicus* represents16.7 to 20.8% of the total emigrating population in perennial ponds and 10.9 to 15.4% in seasonal ponds; where as *M. monoceros* represents 4.1 to 4.9% and 3.6 to 5.5% respectively. In backwaters, *M. dobsoni* represents 72.6 to 88.4%, *P. indicus* 10.7-22% and *M. monoceros* 1.4 to 4.4 % of total emigrating population.

Diel periodicity

Emigration occurred only during night hours. Rate of emigration varied during night hours, depending on the time of ebb tide (Table 1). More recruits emigrate, when ebb tide occurred during early hours of the night than late hours. Preference for early hours of night for emigration was more strong in *M. monoceros* (P=0.012, T-5.6421, DF-5), with 60-73% of the total emigration during that time. In *P. indicus* 57.9 to 63.9% (P=0.001, T-=5.6190, DF-5) and in *M. dobsoni* 52.4 to 63.3% (P=0.004, T-4.2381, DF-5) of the emigration occurred during early hours of the night.

Table 1. Dial, tidal and lunar influence on the emigration rate (x 1000 no/ha of habitat area/year) of penaeid prawns (*EN-early hours of night, LN-late hours of night, NM-new moon, FM-full moon, NP-neap tide, SP-spring tide*)

	Time of emigration		Tide phase		Lunar phase	
Species	*EN*	*LN*	*NP*	*SP*	*NM*	*FM*
P. indicus	112-198	64-136	3-5	177-325	104-176	76-154
M. dobsoni	825-945	593-677	40-45	1380-1545	761-919	638-692
M. monoceros	48-72	21-39	1-2	79-98	47-63	27-43

Tidal and lunar periodicity

Emigration varied with tidal and lunar phases, with peaks during spring tides of new and full moon (Table 1). It occurred exclusively during spring tide periods representing 97.2 to 98.8% of the annual emigration in different species. Distinct variation was observed in emigration between new and full moon phases, with large peaks during new moon phase. Hypothesis test for means showed significant variation in emigration between moon phases (P=0.002, T- 8.1146, DF-5). Response of species to lunar stimuli for emigration vary, with strong preference in *M. monoceros* towards new moon phases (P=0.003, T-=7.8437, DF-5). New moon emigration in the species accounted 55.8 to 68.4% of the total emigration. In *P. indicus* it was 53.2 to 59.1% (P= 0.04, T-7.0656, DF-5) and in *M. dobsoni* 50.9 to 59% (P=0.007, T-=3.7581, DF-5).

Seasonal pattern

Emigration rate varied over the season (Table 2). Significant species by variation was also observed in seasonal emigration. Emigration of *P. indicus* was low during post-monsoon (October – January); thereafter it increased gradually to a small peak in April. It decreased marginally in May and then increased to the large peak by August. Post-monsoon emigration accounted 8.5 to 10.6% of the total emigration, pre-monsoon 29.1 to 31.2 and monsoon 58.1 to 62.5%. Ingression-emigration relationship showed that the resultant juvenile population from post-monsoon recruits emigrate during March-May and that from pre-monsoon recruits during June-September resulting in two waves of emigration.

Table 2. Seasonal variation in the species composition (%) of emigrants

Months	*P. indicus*	*P. monodon*	*M. dobsoni*	*M. monoceros*
Jun	30.84	1.29	65.60	2.27
Jul	33.99	0.72	60.62	4.67
Aug	44.62	0.00	53.65	1.73
Sep	28.46	0.00	70.34	1.20
Oct	10.65	0.00	88. 65	0.70
Nov	7.14	0.00	90.50	2.36
Dec	4.45	0.00	95.55	0.00
Jan	5.63	1.26	91.91	1.20
Feb	11.15	0.00	86.44	2.41
Mar	15.20	0.96	77.09	6.75
Apr	14.64	1.69	77.56	6.11
May	21.94	1.08	72.57	4.41

Emigration of *M. dobsoni* and *M. monoceros* were low during post-monsoon, there after it increased gradually to a large peak by April. It declined in May and again increased to a small peak by August. In the former post-monsoon emigration accounted 14.8-19.1% of the total emigration, monsoon 21.6-32.3% and pre-monsoon 52.9 to 59.3%. Juvenile population from post-monsoon recruits produced a major wave of emigration during March-May and pre-monsoon recruits during July-August. In *M. monoceros* pre-monsoon emigration accounted 50.4 to 63.4, monsoon 32.2 to 46.1 and post-monsoon 3.6 to 4.4% of the total emigration. Juvenile population from post-monsoon recruits emigrated during March-April and pre-monsoon recruits during June-August with respective peaks in April and July.

Statistical tests showed that seasonal variation in emigration directly correlated ($P=0.03$ to 0.05) with juvenile abundance in all species, whereas prevailing environmental conditions have no significant influence. Among environmental factors salinity described maximum (48.3 to 54.8%) variation in emigration.

Emigration rate (E)

Emigration was high from seasonal ponds and low from perennial ponds (Table 3). While emigration rate of *P. indicus* was 2,725 no/ha/month from the perennial ponds, it varied between 3,439 and 5,003 no/ha/month from the seasonal ponds. It was high during the monsoon and low during the post-monsoon months. *M. dobsoni* had an emigration rate of 12,410 no/ha/month from perennial ponds and varied between 23,952 and 24,519 from seasonal ponds. It was low for the species during monsoon and high during pre-monsoon months. Emigration of *M. monoceros* was 762 no/ha/month in perennial ponds and between 1,526 and 1,573 in seasonal ponds. It was relatively low during late monsoon and early post-monsoon and high during pre-monsoon months.

Instantaneous rate of emigration (y) is small for all species in perennial ponds and large in seasonal ponds (Table 3). A large value indicated that recruits left the nursery habitats after a short stay and a small value indicated a prolonged stay.

Table 3. Average emigration rate (E) (no/month/ha of habitat area) and instantaneous rate of emigration (y) of penaeid recruits from tidal ponds

Species	Perennial pond		Seasonal pond (SPI)		Seasonal pond (SPII)	
	E	y	E	y	E	y
P. indicus	2,725	1.653	5,003	3.303	3,439	3.534
M. dobsoni	12,410	2.838	23,952	3.920	24,519	5.197
M. monoceros	792	2.174	1,573	2.763	1,526	3.239

Size and age of emigrants

Size and age of the emigrants from perennial ponds were large and from seasonal ponds and backwaters were small (Table 4). Emigrating populations were characterised by unimodal size distribution. Their size and age at first emigration were large from perennial ponds and small from shallow seasonal ponds and backwaters. Size of the emigrants fluctuated over the season with small during August-September and large during March-April. Proportion of small emigrants with size smaller than the size at first emigration were large in the catches from tidal ponds during July-September. In open backwaters small prawns emigrated round the year with large proportion during June and July. They constituted 6.8 to 9.7 % of the annual emigrants from perennial ponds, 23.8 to 30.3% from seasonal ponds and 36.8% from backwaters.

Table 4. Size and age structure of penaeid emigrants from tidal ponds and backwaters

Habitat/ Species	Size range (mm)	Modal Class (mm)	Mean Length (mm)	Mean age (months)	Mean weight (g)	Size at first emigration	Age at First emigration
P. indicus							
Perennial	38-172	100-110	103.9	3.59	8.4	95.8	3.15
Seasonal I	38-148	90-100	100.2	2.74	7.9	86.4	2.15
Seasonal II	38-143	80-90	92.7	2.21		85.5	85.5
Backwater	9-137	80-90	83.6	2.89	6.5 4.7	-	-
M. dobsoni							
Perennial	36-105	65-70	66.6	3.4	2.0	62.1	3.0
Seasonal I	32-90	60-65	60.8	2.5	1.6	57.6	2.3
Seasonal II	32-89	55-60	58. 5	2.3	1.5	55.2	2.1
Backwater	27-86	55-60	58.7	2.9	1.5	-	-
M. monoceros							
Perennial	37-120	80-85	79.9	3.1	3.6	78.1	3.0
Seasonal I	34-103	65-70	68.9	2.1	3.1	62.0	1.8
Seasonal II	33-98	65-70	65.5	2.0	2.7	63.6	1.9
Backwater	26-99	65-70	60.42	2.1	2.0	-	-

Statistical evaluation showed that size of the emigrants depends mainly on the habitat conditions ($P=0.0274$ for *P. indicus;* 0.0253 for *M. dobsoni* and <0.01 for *M. monoceros*). It correlated directly with depth, spread area of the habitat and strength of water exchange. Seasonal variations in the size of emigrants was influenced by prevailing environmental conditions, which described 93.4% of the observed variations ($P<0.01$). Salinity described the maximum variation.

Discussion

Considerable similarities were observed in the emigration of penaeids, despite species specific variability on different aspects. Several biotic and abiotic factors are observed to influence and modify emigration. Preference of species for different ecological conditions modifies the composition of emigrants over space and time. Recruits stay for quiet long periods in habitats with stable environment. As perennial ponds offer stable environment, emigrants from that habitat have relatively large size and age and also small value for instantaneous rate of emigration.

Shrimps in general, are active at night and take refuge or stay buried in sediments during day. Reflecting this active rhythm, emigration is almost nocturnal in penaeids. Such diel variation in penaeid emigration has been demonstrated by earlier workers (Garcia 1977, Staples and Vance 1986, Vance 1992, Vance and Staples 1992). After low profile activities during day, prawns become more active by dusk in search of food and to meet other biological requirements. If ebb tide coincides this period more prawns will emigrate. With the advancement of time during night, their activities subside and so relatively few recruits emigrate, if ebb tide occurs late in the night. Biological activities and diurnal rhythm may vary for species and produce species specific variation in emigration.

Prawns emigrate at ebb phase of high tides, with large emigration during spring tides. The most widely accepted explanation for this is strong water currents during spring tide resulting in increased water displacement (Le Reste 1978, Lhomme 1979, Subramaniam 1990, Vance and Staples 1992, Vance 1992, Dumas 2006). Present observations also indicate considerable influence for tide height and current speed on emigration rates. Since, tide and lunar phases of the region are synchronous, spring tides always coincide with new and full moon phases and made it difficult to separate the lunar influence from tidal influence. However, prevailing light levels at the time of emigration will produce variation in emigration. Being nocturnal, activity of prawns will be intense if nights become dark and so large emigrations at new moon phase (Staples and Vance, 1986).

In nature many factors trigger migration in shrimps (Garcia 1977) and may interact as is the case with regard their influence on behaviour (Vance 1992, Vance and Staples 1992). Shrimps being highly sensitive to the environment in which they live, many attribute considerable influence for ecology in eliciting emigration. Some considered declining water temperature as the driving force in emigration (Garcia and Le Reste 1981, Coles and Greenwood 1983, Benfield *et.al.* 1990, Manzano-Sarabia *et.al.* 2007). Seasonal variation in temperature during the present study was very narrow, to have any direct influence on emigration. However, low temperature always coincided with declining salinity and other

ecological conditions and so, some synergistic effect with other factors can be expected.

It was seen that, except during periods of extreme habitat disturbances and environmental instability, prawns emigrate selectively, after attaining certain size and developing secondary sex characters only. So it can be assumed that, it may be the biological instincts, which is set in the animals and become active at certain stages of their life, to have most ideal environment to suit their metabolic/physiological requirement is the driving force in emigration. As most of the emigrants were with well-developed secondary sex structures, the biological instinct can be presumed to be the urge for sexual maturation. Coles and Greenwood (1983) and Subramaniam, (1990), suggested onset of sexual maturity coupled with environmental changes as migratory stimuli in prawns. So urge sexual maturation can be considered as the basic stimuli for emigration, whereas factors like habitat environment, competition and predation have only interactive role in modifying patterns and timings.

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Distribution, biology and behaviour of the giant trevally, *Caranx ignobilis* – a candidate species for mariculture

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Abstract
Fishery and biology of the giant trevally, *Caranx ignobilis* exploited along the Tuticorin coast of Tamilnadu were monitored during 2001-2006. Fishery occurred round the year with peak landings during April-August. Spawning and recruitment occur almost round the year with peak during November-December. Young ones are abundant in shallow coastal waters and as grows, they move to deeper waters. Growth parameters, L_∞ and K are estimated respectively as 143.6 cm and 0.69/year and 't_0' as –0.0242 year. Estimates show that they grow fast and attain 73, 108, 126 and 134 cm in total length by first, second, third and fourth year respectively. Their weight increment is also fast and attains 5.5 kg, 16.8 kg, 25.9 kg and 33.7 kg respectively during the period. Stock assessment indicated that the stock at present is over exploited and under heavy fishing pressure. Rearing trial in aquarium tank showed that they are compatible to confined rearing conditions. Based on the distribution and biology of the species, their mariculture potential is discussed.

Key words: *Caranx ignobilis*, Mariculture, Fishery

Introduction

Giant trevally is the largest and fast growing carangid available in the Indian waters. They had wide distribution throughout the Indian Ocean and central Pacific. They support round the year fishery and constitute 10% of the total carangid production of the region. However, reports and information on the fishery and biology of the species is very limited. Present study was aimed to generate more biological information for scientific exploitation and management of the resource.

Report on carangid maricuture is very limited, except that of Yellowtail, *Seriola quinqueradiata* cultured in Japan. This is the only finfish species that contributes significantly to marine aquaculture production and its farming is restricted to Japan. Total production of cultured yellowtail was 162,000 ton in 1989 and contributes 90-95% of the total finfish mariculture production of Japan. This paper discusses some aspects of the biology of giant trevally with special reference to their potential as a candidate species for mariculture.

Materials and methods

Catch, effort and size composition data of giant trevally, *Caranx ignobilis* by different gears were monitored during 2001-2006. Food and feeding habits of the species were studied to develop their diet matrices. Growth and recruitment patterns were studied using monthly length frequency distribution of the species in catch. Growth parameters were estimated following ELEFAN and Shepherd's model using ICLARM's FiSAT software (Gayanilo and Pauly 1997) and also through model progression model (Bagenal 1955, Pauly 1982, 1983). Age of the fish at zero length (t_0) was estimated as that in Bertalanffy (1934).

Natural mortality (M) was estimated from the empirical formula proposed by Pauly (1980) and total mortality (Z) from catch curve (Pauly 1984) using FiSAT software (Gayanilo and Pauly, 1997). Exploitation rate (E) was estimated from the equation; E = F/Z as given by Beverton and Holt (1957) and Ricker (1975); where, F is the fishing mortality rate. Length-weight relationship was estimated as in Sparre (1986) following linear analysis by converting the length and weight data in to log values and was subjected to covariance analysis. Maximum sustainable yield (MSY) was estimated as in Corten (1974).

Results

Fishery

Giant trevally was exploited from the entire marine habitat, right from shallow estuaries and coastal waters to deeper waters during different stages of their life, by almost all gears operating along the coast. Juveniles and young ones from estuaries and shallow coastal waters were exploited by mini-trawls, small meshed gillnets and shoreseines. Medium sized fishes were exploited from relatively deeper areas by trawls and large meshed gillnets and large adults from deep, rocky and seaward reef grounds by hooks and line.

Giant trevally formed 10% of total carangid landings of Tuticorin. 648 tons were landed annually during 2001-2006 (Table 1). Trawls contribute 22% of their annual catch, gillnets, 24.5 % and hook & line, 52.8%. About 62% of the carangid catches in the hooks and line is by this species alone. Fishery occurred round the year, with peak during April-August. Catch rate was also high during this period.

Size composition

Mini trawls and shoreseines landed 7.0 to 16 cm fishes. Large meshed gillnets and trawls landed small to sub-adults of 14-45 cm, whereas hooks and line landed sub-adults and adults of varying size from 29 to 136 cm. Annual mean size of the species in hooks and line catch varied between 50 and 52.3 cm.

Table 1. Annual landings (in tons) of giant trevally, *C. ignobilis* by the major gears at Tuticorin during 2001-2006

Period	Gillnet	Trawl	Hook and line	Other gears	Total of the year
2001	154	30	411	4	599
2002	314	44	151	6	515
2003	170	108	439	12	729
2004	74	296	382	7	759
2005	93	213	429	1	736
2006	146	156	239	8	549
Average	159	141	342	6	648

Length-weight relationship

Length-weight relationship were estimated from log values of length and weight and is expressed by the formula, $W = aL^b$, where, W is weight in g, L total length in cm, 'a' constant and is estimated as 0.0279 and 'b' length coefficient, 2.842. Covariance analysis of the relationship showed significance at 5% level.

Growth

Growth parameters, L_{oo} and K were estimated respectively as 143.6 cm and 0.69/year and 't_o' as -0.0242 years. Growth of the fish can be described by von Bertallanffy equation as;

$$Lt = 143.6\ [1 - e^{-0.69\ (t\ +\ 0.024155)}]$$

This relation shows that the species grows to 25 cm in total length in three months and 44 cm in six months (Table 2). Length and weight of the one year old fish is estimated as 73 cm and weight of 5.5 kg respectively. It is 108 cm and 16.8 kg respectively for two-year-old fish and 126 cm and 25.9 kg for three-year-old fish. Age of the largest fish (136 cm) recorded in the catch, is estimated as 4.2 years.

Table 2. Estimated growth in length (cm) and weight (kg) of giant trevally by the end of different quarters of their life

Year of growth	Particulars	I Quarter	II Quarter	III Quarter	IV Quarter
Ist year	Length	24.6	43.6	59.4	72.8
	Weight	0.255	1.272	3.813	5.46
IInd year	Length	84	93.4	101.4	108.1
	Weight	8.21	11.11	14.01	16.8
IIIrd year	Length	113.7	118.4	122.4	125.8
	Weight	19.4	21.8	24.0	25.86

Recruitment pattern and juvenile abundance

Recruitment pattern and abundance of young ones in fishery along the coast suggest that this species spawn and young recruits enter the stock almost round the year with main spawning peak during November- December and a small peak in March-April (Fig 1).

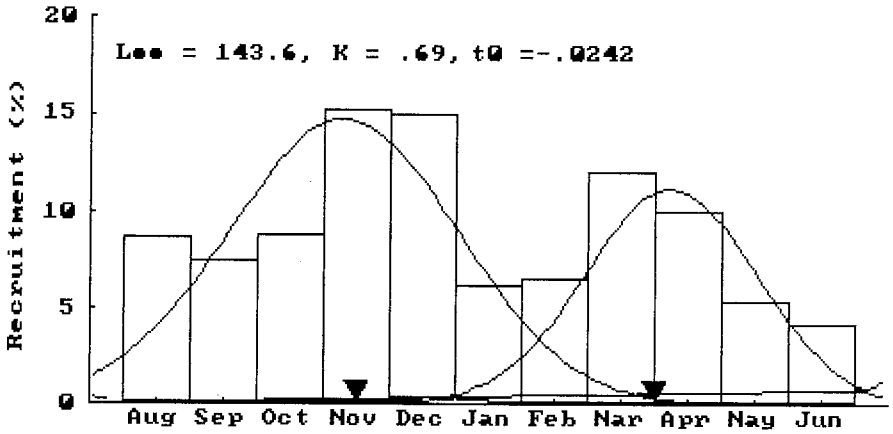

Fig 1. Recruitment pattern of the giant trevally, *C. ignobilis* along Tuticorin coast

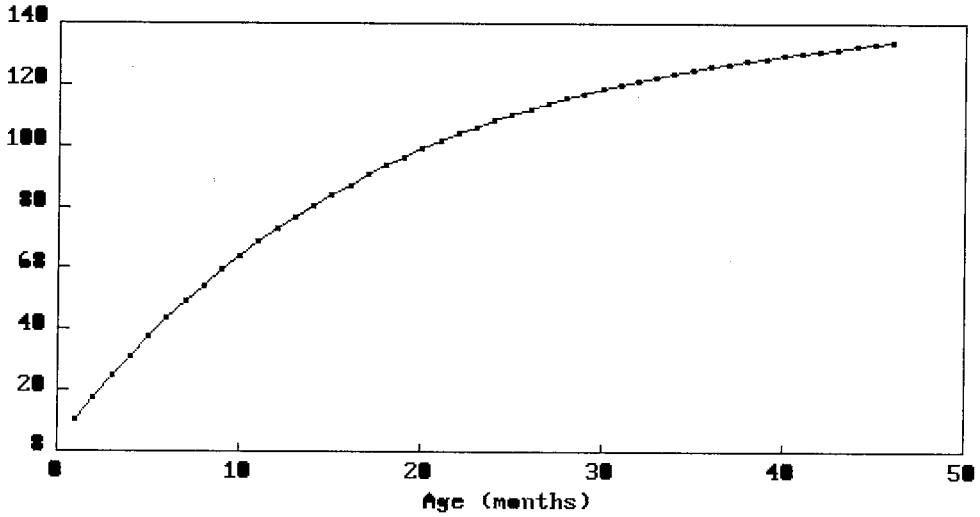

Fig. 2. Growth curve of giant trevally, *C. ignobilis* from Tuticorin region (size in cm)

Spatial distribution of different size groups of the species as indicted by catch in different gears established that, small juveniles move shallow coastal waters and estuaries for feeding. Juveniles were very often encountered in estuarine areas as small shoals. As grows, they move to deeper waters and larger adults were caught mainly from deep rocky beds or seaward reef areas.

Food and feeding habit

Gut content analysis shows that they are carnivorous in feeding. Young ones in shallow coastal waters feed mainly on juvenile of sardines, anchovies and other fin fishes, prawns, crab stars and amphipods. Food of large fishes shows wide variation from individual to individual. Major components of their food are *Decapterus* sp., other carangids, silver bellies, thread fin breams, goat fishes, lizard fishes, crabs and prawns.

Stock assessment

Fishing and total mortality of the species was estimated as 3.18 and 1.88. Estimate of exploitation rate (E) was 0.59 and Emax, 0.52. Average production during the period is 648 ton, more than the estimated maximum sustainable yield (MSY) of 571 ton. These estimates shows that the stock is over exploited and under heavy fishing pressure. However, biomass estimates indicates the existence of a good spawning stock biomass of 503 ton and standing stock biomass of 703 ton.

Consumer acceptability and market price

Carangids of the genera *Caranx* have good consumer acceptability due to their quality flesh without inter-muscular bones. Good local demand prevails for small and medium fishes and from distant urban markets for medium and large sized fishes. They generally fetch Rs. 100-150/kg at landing centres and Rs 120-200/- in the retail markets depending on the season and size of the fish.

Response to confinement

Juveniles collected from shore seine catches were acclimated and reared in 3 x 2 x 1.5 feet aquarium tanks with coral sand and dead coral for refuge for 20 days. Except during the initial phase of stocking, they mostly remain calm during the trial. They were fed with chopped anchovies and sardines. They have shown good feeding response with immediate food acceptance.

Discussion

In the recent years fishing efforts has increased considerably and fishing area was extended to distant waters. As a result, yield of giant trevally increased gradually over the years. Estimates of exploitation rates and MSY showed that the resource is under heavy fishing pressure. With persistent demand for large quality fishes, there are dangers of further increase in fishing pressure over the resource. This necessitates scientific attention to sustain the production at optimum level. Workable alternatives to meet the increasing demand for highly sought after resources is natural stock enhancement through captive breeding, sea ranching of juveniles and mariculture. Since large fishes are being caught by gillnets and hooks and line, chances of getting live mature fishes onboard fishing vessels is relatively high. This provides ample scope for stripping ripe fishes onboard and sea ranching fertilized eggs to augment natural stocks.

Fishing and natural spawning pattern indicated that availability of mature fishes for captive breeding and seed production trials may not be limiting.

Growth estimate showed that they grow faster compared to many other pelagic fin fishes of the Indian waters. Their weight increment is also very fast. Recruitment pattern and abundance of young ones in shallow coastal and estuarine areas suggest that young fishes for capture based rearing trials will be available almost round the year. Moreover distribution of juveniles in the estuarine areas and their response during rearing trials clearly demonstrated that they can be conditioned for confined atmosphere. Since they readily accept locally available trash fishes, in different forms, feeding also may not be a problem. These findings were further supported by the recent report of Giant trevally farming in land based system on small scale in Hawai, (Gefrosh, 2004). The present work and other available information established that this species possess several characters which are considered ideal for a potential mariculture species.

Reliable information on carangid aquaculture is not available, except for that of yellow tail, *S. quinqueradiata* in Japan. World's largest fish production per unit culture area is obtained from their culture. Giant trevally appears to grow faster in the Indian seas than yellowtail in Japan seas. Though, no attempts had been made so far to test their mariculture potential, the present biological and behavioural observations suggested that they are suitable for mariculture.

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Beel fishery and livelihood of the local community in Rajdhala, Netrakona, Bangladesh

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Abstract
Baseline survey and Participatory Rural Appraisal (PRA) during January 2003 to December 2004 on the fishing community revealed that unregulated fishing, use of destructive fishing gears, poaching of fishes, difficulties encountered in enforcing fisheries regulation and the helplessness of fishers to find alternative sources of income during banned fishing period (June to October) were the major management problems. CBFM (Community Based Fisheries Management) system as an alternative management strategy has been introduced to ensure active participation of the target group-the poor fishers living around the *beel* who were previously deprived to get access to the *beel*. Establishing a leasing system for controlled access, ensuring greater user-group participation through equitable distribution of all resource benefits among members, attempting to enforce penalties for illegal fishing linked with surprise checks to enforce management regulations are some of the recent steps taken by the BMC (*Beel* Management Committee). Chapila fish intake by the community was 31.25 g/head/day before stocking the *beel* by carp fingerlings. After stocking, they consumed chapila as fish protein from 8.33 g to 20.8 g/head/day during the fishing season (November to May) indicating that due to introduction of carp fingerlings, chapila production has been decreased in 2003-2004. About 77.5% families around the *beel* were found to be dependent directly and/or indirectly on chapila and other indigenous fishes of the *beel* for their livelihood, through fishing, marketing and other activities like net and boat preparation and nets mending etc. Particularly fishers' families were found to face serious problem during non-fishing period like June to October for their livelihood. Analyzing the present research result it was also observed that other than declination in biodiversity, the fishing pressure on promising chapila of the *beel* was found high and that is why the production of chapila has also been decreased. To get sustainable chapila production from the beel, it is suggested to ensure successful spawning and recruitment as juveniles, and hence the chapila should be undisturbed during its breeding period from March to July, and fishing pressure on the same species needs to be reduced for obtaining sustainable fish production.

Key words: *Beel*, Livelihood, CBFM, *Gudusia chapra*

Introduction

The total inland areas of Bangladesh for capture and culture fisheries are 4.05 million ha and 0.29 million ha which cover 93.31% and 6.69% of the total area for inland fisheries. Among the inland capture fisheries the *beels* (natural depressions/reservoirs) alone cover an estimated areas of 0.11 million ha which is 2.63% of total area for inland fisheries (BBS 1997). At present the production from the inland culture fisheries is in satisfactory level but actually there is no strategic management system for inland capture fisheries, which is vast and potential. The average annual rate of production from *beel* (600-700 kg/ha) can be increased manifolds (DoF 2001). Among the vast inland fishery resources, *beels* have the tremendous potential for development. Soil and water of *beels* are very productive and rich in flora and fauna. *Beels* are very good natural habitats of large and small indigenous fishes of different food and feeding habits. Many of the fish, prawn and other species move into the inundated areas of the *beels* from the adjacent rivers and canals to feed, breed and grow during the monsoon. Poor villagers living around the *beels* harvest the fish almost round the year without any prior investment. Large portions of rural households are engaged in part-time fish capture from the floodplain/*beels* (Hughes *et al.* 1994). In Bangladesh there has been a considerable effort to enhance fish production in openwaters through release of large quantities of hatchery-raised native and exotic carps (DoF 1995). Many experts are of the opinion that release of hatchery produced fingerlings of native and exotic carps might contaminate the genetic quality of the fish species of the *beels*. Thus, it has become a burning issue to ascertain whether stocking floodplains with hatchery produced carp fingerlings had any adverse impact on resident fish species or not.

To maintain biodiversity and to improve the socio-economic condition of the fishermen, different approaches have been undertaken to ensure sustainable and increased fish production from open water. As a part of these works, the Community Based Fisheries Management (CBFM) project which is being implemented by the Department of Fisheries (DoF), Government of the peoples Republic of Bangladesh in collaboration with World Fish Center (former ICLARM) and Caritas. Under the CBFM project NGO Caritas is supporting fishery management in Rajdhala beel of Purbadhala Upazila under the District Netrakona in co-ordination with World Fish Center.

Among the small indigenous fishes chapila, *Gudusia chapra* plays an important role in nutrition and livelihood of rural poor. In recent years, the catches of chapila have declined due to various reasons but still chapila is the most dominant species among the indigenous small species. The poor community prefers to buy the small fish chapila because they are less priced and they can satisfy all the family members by a small quantity, creating the opportunity to offer more than one whole fish each of the family members. Moreover, this small fish is rich in vitamins and minerals in addition to protein, which are especially needed for children and pregnant women. If the downward trend of chapila catch from the *beels* continues year after year then the *beel* adjacent poor communities will be nutritionally deprived. The present level of contribution of chapila in nutrition among the *beel* adjacent communities and what may happen if the catch falls

has not yet been studied. Therefore, the contribution of chapila in homestead nutrition of the associated communities and its importance as a livelihood option need to be assessed. In the view of the above, the present study was conducted to understand the fishery of Rajdhala *beel*, management system and biodiversity of the *beel* and livelihood of the fishers around the beel.

Materials and methods

The beel

The Rajdhala *beel* at Purbadhala, Netrakona with an area of about 53 ha (24°70´ to 25°80´ N and 90°29´ to 90°48´ E) is an important reservoir with rich ichthyofauna. The beel is an oval shaped depression capable of retaining about 4.7 to 7.7 m water throughout the year. From early 1996 Caritas, a non-government organization organized the *beel* under the CBFM system. ICLARM (International Center for Living Aquatic Resources Management, currently World Fish Center) provided technical support to the fishers' groups to introduce CBFM activities in the beel.

Catch composition data and other informations were collected from the main fish-landing site of the beel and also from Caritas officials and BMC personnel. On sampling dates, records were taken from the catches of 10 out of 14 crafts and the total weight of the catches. Regular observations were made during the two years and length-frequency data of chapila was collected on each occasion. Information on the status of the fishery and the socio-economics of the fishing community was gathered through interviewing 35 out of 83 active and part-time fishermen during January 2003-December 2004.

Data on monthly income and other informations like the impacts of chapila on homestead nutrition and livelihood of the poor community and additional information on the organization and operation of the fishery were collected by using Participatory Rural Appraisal (PRA) tools (Huda 1999), questionnaire fill up and also by direct interviewing the participants attended in the meeting of the fishermen's co-operative society.

PRA was conducted in several occasions during March-April 2003 and again during March-April 2004 at Rajdhala Beel, Purbadhala, Netrakona dividing the four villagers into two groups each time.

Results and discussion

Present status of management

Rajdhala beel gets connected to the *Kangsho* and the *Dhalai* rivers through two narrow canals, which become dry during the dry season, and in rainy season the fishers use bamboo made fencing so that the beel fishes cannot escape into the river. Once indigenous fishes were abundant in the beel, which have been declined due to various man made and natural causes.

Presently, the Rajdhala beel is being managed by the Department of Fisheries (DoF) in partnership with World Fish Center and Caritas. DoF was responsible for negotiating through the Ministry of Fisheries and Livestock (MOFL) for the transfer of the waterbody from the Ministry of Land (MoL) to DoF. DoF has the big responsibility in the development of CBFM system in the Rajdhala *beel* and also the responsibility for conducting surveys to monitor and assess the development and impacts of CBFM system in the *beel*. The partner NGO Caritas has the main responsibility of helping the fishing community to strengthen their organization and develop alternative income generating sources through training, education, credit facilities etc. World Fish Center provides technical assistance to other partners, particularly for research activities and surveys through GO-NGO collaboration. Actually, the CBFM arrangement is a GO-NGO supported direct fishermen's participatory system through which the target group i e. the poor fishers can achieve greater equity, empowerment and socio-economic uplift. The main aim of CBFM system is to develop shared responsibility between the government and local resource users for sustainable use of openwater resources. CBFM system comprises both biological and social management:

Biological management: After taking decision, BMC (*Beel* Management Committee) released 111,102 fingerlings of 10-12 cm size weighing 1,878 kg of six species viz; catla (*Catla catla*), rohu (*Labeo rohita*), mrigal (*Cirrhinus cirrhosus*), common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*) and pangas (*Pungusius hypohpthalmus*) in the year 2001. Although, at the beginning, they decided not to stock silver carp due to its food competition with most dominant species chapila, *G. chapra* but in the year 2003, unknowingly they released silver carp fingerlings with the above mentioned carp fingerlings. At the same time to get quality fingerlings and to reduce the stocking cost BMC raised fingerlings from *Dhani* (size 1.0-2.0 cm) to fingerlings (size 10-12 cm) in the nursery ponds by their own management system. Eel grass *Vallisneria* sp, a kind of submerged weed is a major problem for fishing because of its tremendous production; the fishers could not set their nets properly for fishing. Therefore, BMC people stocked fingerlings of grass carp at the rate of 5 fingerlings per decimal to control the submerged weed, the eel grass.

Social management: Social management consisted of team guarding and team harvesting by rotation under the BMC executive body of 11 members for the management. Fishing was done two times during 24 hours (day and night) in the fishing season depending on the decision made by the BMC. The benefit was distributed as 40% to member of fisher groups, 40% to repay loan for lease money and 20% as reserve money deposited in BMC account. Caritas provided IGP (Income Generating Program) loan during the lean season (banned period of fishing) to each group member to increase the income at that time. A maximum of 185 fishing days a year was during the lease period and the fishing rate was 151 kg/fishing day and the maximum yield obtained was 529 kg/ha. The maximum rate of harvest on stocking was 20.5% while average harvesting

rate was 10.03%. The contributions of resident and non-resident species were 55.84% and 44.16% by weight, respectively during 1997-2001.

Implementation of Fish Act: Fishers are now more aware of protecting their own resources. They received training from Caritas regarding how to protect the environment. BMC discouraged to catch undersized stocked fish and strictly prohibited use of destructive fishing gears like *current jal*. BMC takes necessary steps like imposing fine or canceling the membership, in case of any violation of rules. BMC maintains 3 to 6 months banned period (closed fishing season) during the breeding season (June-October).

Catch composition: In the catch composition of resident species, the most dominant species chapila, *G. chapra* alone contributed 38.9 % by weight (2001-2002). Negative impact on the catch of major resident species chapila was due to the stocking of silver carp and catla having the same food habit and hence food competition. Overfishing of that particular species might also be responsible for this declination. During PRA sessions in the year 2003, the participants reported to catch the following 15 types of fishes other than the stocked major carps in the *beel*; chapila (*Gudusia chapra*) 21.74%, punti (*Puntius* spp.) 10.87% guji (*Sperata seenghala*) 16.30%, tengra (*Mystus tengara*.) 5.43%, chela (*Salmostoma* spp.) 5.43%, chanda (*Chanda* spp.) 7.61%, chingri (*Macrobrachium* spp.) 5.43%, boal (*Wallago attu*) 7.61%, gazar (*Channa marulius*) 4.34%, Shol (*Channa striatus*) 2.17%, gutum (*Lepidocephalus guntea*) 2.17%, chirka (*Mastacebelus* spp.) 3.26%, kankila (*Xenentodon cancila*) 1.09%, baila (*Glossogobius guiris*) 5.43% and meni (*Nandus nandus*) 1.09%.

Poverty alleviation: Impact studies with baseline survey on the poverty alleviation during 2001-2002 showed that there is a remarkable change in the socio-economic condition of the fishers. The wall and roof of main house made of tin had increased from 0 to 30% and from 40% to 90%, respectively. About 73% of the fishers (33% male and 39% female) had received education under adult literacy program provided by the NGO Caritas Bangladesh. All boys and 97% girl children of the fishers' family belonging to age group 5-14 were found to be school going. Twenty five percent of the families had their own hand-tube-well, and watershed latrine facilities had increased from 8% to 92%. The fisher community reported that normally there was no food deficit while the occasional deficit decreased from 52% to 20%. Sixty eight percent families were in break-even and 12% in surplus food condition. All the fishers' families were reported to consume fish most of the days in a week.

Fishery data: Among the different small species of the *beel*, chapila Gudusia chapra, a self-recruiting species was the most abundant which contributed 23.34% to the total catch in the year 1996-1997 (Islam and Thompson 1999). The annual fish production of the beel was reported to vary from year to year e.g., about 13.4 tons in the year 1997-1998, 8.4 tons in 1998-1999 and 5.95 tons in the year 2002-2003 (Fig. 1), and this

reduction was mainly due to the fluctuation in catch of chapila (Islam and Thompson 1999; personal communication). The failure of optimum chapila recruitment and subsequent reduction in catches were possibly due to insufficient spawning success. Over exploitation of spawning stock during the peak spawning season, and failure of larval survival due to environmental causes and insufficiency of food items could also be responsible for this decline. Increased spawning-stock, resulting from increased harvest restrictions are expected to boost recruitment proportionally through expanded egg deposition (Goodyear 1985). However, the survival of eggs and larvae, and recruitment as juveniles will depend on environmental conditions and availability of suitable food items (Goodyear and Christensen 1984 and Uphoff 1989).

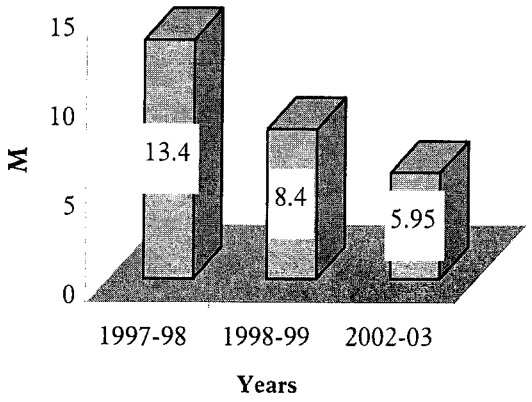

Fig. 1. Total fish production (mt) in Rajdhala *beel*.

Contribution of chapila in the livelihood of beel adjacent poor community

Socio-economic conditions of the fisher had improved much due to empowerment and equal distribution of benefit through the introduction of CBFM concept. The social dignity of the fishers had increased. Adoption of CBFM concepts had also brought in many improvements, like stocking of carp fingerlings in the *beel*, increased awareness about the importance of conservation of resources, decrease in dependence on the *beel* for livelihood due to more and more involvement in other income generating activities, decreased dependence on chapila for protein supply. The *beel* beneficiaries had received loan and other facilities like training, education etc. Chapila fish intake by the community was 31.25 g/head/day before stocking the *beel* by carp fingerlings. After stocking, they consumed chapila as fish protein from 8.33 g to 20.8 g/head/day during the fishing season (November to May) of the year 2003-2004.

About 77.5% families around the *beel* were found to be dependent directly and/or indirectly on chapila and other indigenous fishes of the *beel* for their livelihood, through fishing, marketing and other activities like net and boat preparation and nets mending etc. Particularly fishers' families were found to face serious problem during non-fishing period like June to October for their livelihood.

Major causes of loss of fish biodiversity in Rajdhala beel

The conflicts between conservation and commercial interests were found to cause the delay in adequate and timely implementation of appropriate conservation measures and that is causing continued losses of biodiversity. The major causes for the loss of fish biodiversity are as follows:

1) Loss of habitats due to siltation and reduction of water area. Siltation in the connecting channel *Kumarkhali khal* that connects the *beel* with the river *Dhalai* preventing migratory species entering into the *beel* i.e. loss of connection with the nearby rivers and other water bodies (canals, floodplains, lowlands, rice-fields etc.) preventing migration of fish into the *beel.*
2) Overfishing of particular *beel* resident chapila *G. chapra* using fine meshed gears.
3) Stocking of the exotic species silver carp and catla with other indigenous carps that caused serious competition for food with resident species chapila *G. chapra* due to overlapping feeding habit that is compelling the dominant resident species chapila to decrease gradually.
4) Siltation of migratory routes is causing threat to numerous local populations of migratory fishes e.g. *Sperata aor*, *Mystus cavasius*, *Notopterus notopterus* etc.
5) Decomposition of very dense submerged weed *Vallisneria* sp. causing mass mortality of resident species after every 2-3 years.
6) Use of *katha* (a kind of fishing device) and increased fishing pressure continuously reducing the predatory and high valued resident fish.
7) Previous management practices that were mostly responsible for the species erosion:
   a) Excess fishing pressure to recover the lease money by an individual lessee.
   b) Poisoning to eradicate predators, as a result it destroyed small resident species.
   c) Use of destructive fishing gears to increase the catch.
   d) Indiscriminate use of pesticides and insecticides in the *beel* adjacent agricultural croplands that was down in the *beel* and caused declination of fish biodiversity.

Some problems regarding Rajdhala beel fishery

There is no easy source of income for the fishers during the restricted fishing period (June to October) in the *beel*. Those who depend only on the *beel* fishery face severe problems for their livelihood. Sometimes conflicts arise about fishing with local non-fisher agricultural farmers (such as anglers, monofilament current net users etc.) and *beel* management (BMC) people. Lease (*Ijara*) value of the *beel* is too high. Moreover, 3% income tax and 15% VAT on lease money are a burden for the poor fishers. It is very difficult for the poor fishers to manage this huge amount of money. Again, the increase

of lease value in successive years has made it more difficult. Frequent poaching of fishes using destructive monofilament current nets and other destructive devices. Almost half of the fishers have no jobs except fishing and it is very difficult to get enough loan facilities for alternative income generation. Fingerlings (mainly major carps) for stocking in the *beel* are not always easily available. Occasional mortality of chapila and other species occurred since 2001 mostly during the months of October to December. This was possibly due to agricultural pollution, or water quality deterioration due to decomposition of massively grown submerged aquatic vegetation (*Vallisneria* sp. *Najas* sp. etc.) or due to other unknown causes.

To understand the *beel* management system and its biodiversity, species composition of aquatic organisms were studied through monthly field study round the year, where, a total of 14 species of non-resident fish (Table 1) and 44 species of resident fish were identified of which 30 were common, 9 rare and 5 highly endangered (Table 2). Seven species of fish were also recorded which were thought to be locally extinct. Among the other flora and fauna 21 species of aquatic weeds, 3 species of prawns, 6 species of mollusks, 6 species of aquatic arthropods, 4 species of amphibians and 4 species of reptiles were identified during monthly regular investigation.

The number of resident species (44) as recorded in the present survey is an indicator of biodiversity in Rajdhala *beel* compared with 48 species in floodplains in southwest Bangladesh (Flood Action Plan-17 1995) and 43 species recorded in 1992 to 1995 in Chanda *beel*, Gopalganj and BSKB *beel* in Khulna and Narail (Hossain *et al.* 1999).

Table 1. List of non-resident species recorded in Rajdhala *beel* during the study period

1	Cyprinidae	Catla	Indian major carp	*Catla catla*	Stocked
2	"	Rui	"	*Labeo rohita*	"
3	"	Mrigal	"	*Cirrhinus cirrhosus*	"
4	"	Kalibaus	Kalbasu	*Labeo calbasu*	"
5	"	Goinna	Kuria labeo	*Labeo gonius*	"
6	"	Silver carp	Chinese carp	*Hypophthalmichthys molitrix*	"
7	"	Grass carp	"	*Ctenopharyngodon idellus*	"
8	"	Carpio	Common carp	*Cyprinus carpio* var. *communis*	"
9	"	Mirror carp	"	*Cyprinus carpio* var. *specularis*	"
10	"	Bighead carp	Exotic carp	*Aristichthys nobilis*	"
11	"	Rajpunti	Minor carp	*Barnonymus gonionotus*	"
12	Cichlidae	Nitlotica	Cichlid fish	*Oreochromis niloticus*	Non-stocked
13	"	Tilapia	"	*O. mossambicus*	"
14	Siluridae	Thai pangas	Thai catfish	*Pungusius hypohpthalmus*	Stocked

Among the 12 stocked species in the *beel* (Table 1), two species such as Catla (*Catla catla*) and Silver carp (*Hypophthalmichthys molitrix*) were found competing for food with chapila (*G. chapra*) as their major food items are the same.

Table 2. List of resident species recorded in Rajdhala *beel* during the study period

Sl No.	Local name	English name	Scientific name	Comment
1	Guzzia/Guji	Giant river catfish	*Sperata seenghala*	Common
2	Gulsha	Catfish	*Mystus cavasius*	Endangered
3	Tengra	"	*Mystus vittatus*	Common
4	Buzuri tengra	"	*Mystus tengara*	"
5	Koi	Climbing perch	*Anabas testudineus*	"
6	Khalisha/Boicha	Goramy	*Colisa sota*	"
7	Khalisha	"	*Colisa fasciatus*	"
8	Ranga khalisa	"	*Colisa lalius*	Endangered
9	Bamosh	Freshwater eel	*Anguilla bengalensis*	"
10	Lal chanda	Glass-perch	*Parambassis ranga*	Common
11	Nama/Lamba chanda	Elongated glass perchlet	*Chanda nama*	"
12	Kakila/Kaikka	Needle fish/gars	*Xenentodon cancila*	"
13	Gol chanda	Glass-perch	*Chanda beculis*	Rare
14	Taki/Lati	Snakehead	*Channa punctatus*	Common
15	Shoal	Snakehead murrel	*Channa striatus*	"
16	Gajar/Gajal	Giant snakehead	*Channa marulius*	"
17	Telotaki/Cheng	Asiatic snakehead	*Channa orientalis*	"
18	Magur	Walking catfish	*Clarias batrachus*	"
19	Chapila	River shad/herring	*Gudusia chapra*	"
20	Kachki	Shad/Herring	*Corica soborna*	"
21	Gutum	Loach	*Lepidocephalus guntea*	"
22	Mola	Barb/Mola carplet	*Amblypharyngodon mola*	"
23	Jatputi	Spot fin swamp barb	*Puntius sophore*	"
24	Kanchan punti	Rosy barb	*Puntius conchonius*	"
25	Tit punti	Barb	*Puntius ticto*	"
26	Darkina	"	*Rasbora daniconius*	"
27	Choto darkina	"	*Esomus danricus*	"
28	Narkeli chela	Minnow/Barb	*Salmostoma bacaila*	"
29	Kanpona	Top-minnow	*Aplocheilus panchax*	"
30	Baila/Bele	Goby	*Glossogobius guiris*	"
31	Shing	Stinging catfish	*Heteropneustes fossilis*	"
32	Chikra/Guebaim	Stripped spiny eel	*Macrognathus pancalus*	"
33	Shal baim	Tire-track spiny eel	*Mastacembelus armatus*	Rare
34	Tara baim	One stripe spiny eel	*Macrognathus aculeatus*	"
35	Bheda/Meni	Mud perch	*Nandus nandus*	Common
36	Chital	Knife fish	*Chitala chitala*	"
37	Foli	Bronze featherback	*Notopterus notopterus*	Rare

38	Napit koi	Perch	*Badis badis*	"
39	Batasi	River catfish	*Neotropius atherinoides*	"
40	Boal	Freshwater shark	*Wallago attu*	Common
41	Madhu pabda	Catfish	*Ompok pabda*	Endangered
42	Kani/Boali pabda	"	*Ompok bimaculatus*	"
43	Tepa/Choto patka	Puffer fish	*Tetraodon cutcutia*	Common
44	Kuchia	Mud eel	*Monopterus cuchia*	Rare

In spite of the introduction of the culture-based practice by fishers in Rajdhala *beel* regularly with carp fingerlings, the contribution of wild fish (46.14%) to total income was considerable because the most dominant natural species *G. chapra* that was highly abundant in the *beel*. The catch of chapila is rapidly declining may be due to shortage of available food for chapila resulted through stocking of exotic silver carp and indigenous catla. In addition, overfishing of chapila by using fine-meshed net (*Kaporer jal*) may also be equally responsible for this decline. The non-piscine biodiversity of Rajdhala *beel* comprises 3 species of prawns, 6 species of mollusks, 6 species of arthropods, 4 species of amphibians and 4 species of reptiles (Table 3).

Table 3. List of non-piscine organisms recorded in Rajdhala *beel* during the study period

Group	Local name	English name	Scientific name	Comment
Prawns	Gura icha	Small prawns	*Macrobrachium lamerril*	Common
	Chatka chingri	"	*M. malecolmnsonii*	"
	Galda chingri	Giant freshwater prawn	*M. rosenbergii*	Endangered
Mollusks	Baro shamuk	Apple snail	*Pila globosa*	Common
	Chakti shamuk	Small snail	*Planorbis sp*	"
	Choto shamuk	Small snail	*Viviparus bengalensis*	Common
	Lamba shamuk	Long bivalve	*Lamillidens tuberculatus*	Common
	Lamba jhinuk	Long bivalve	*Lamillidens marginalis*	Rare
	Gol jhinuk	Round bivalve	*Corbiculata*	"
Arthropods (Aquatic insects)	Kakra	Crab	*Potamon sp*	Common
	Katua poka	Giant water bug	*Belostoma sp*	"
	Choto poka	Water bug	*Abedus sp*	"
	Lamba poka	Ranatra	*Ranatra sp*	"
	Ghurni poka	Water scorpion	*Nepa sp*	"
	Gerris	Water strider	*Gerris sp*	"
Amphibians	Kotkoti bang	Cricket frog	*Rana cyanophlyctis*	"
	Sona bang	Indian bull frog	*Rana tigerina*	"
	Gecho bang	Tree frog	*Rhacophorus*	"
	Kuna bang	Common toad	*Bufo melanostictus*	"
Reptiles	Maitya shap	Water living snake		Common
	Dhura shap	Water living snake	*Naja naja*	"
	Gui shap	Lizard	*Varanus bengalensis*	"
	Kochchop	Tortoise	*Kachuga*	Rare

Serious decline in the catch of freshwater giant prawn *Macrobrachium rosenbergii*, two species of bivalves *Lamillidens marginalis* and *Corbiculata* sp. and a reptile *Kachuga tecta* (Kachchop) in the recent years is probably due to lack of preventive measures and awareness.

A total of 21 species of aquatic vegetation under 4 types were recorded during the study period mostly dominated by free floating weed *Eichhornia crassipes* and submerged weed *Vallisneria* sp. (Table 4). Everywhere there was floating aquatic vegetation with some exception in the periphery and dense submerged aquatic vegetation *Vallisneria* sp. throughout the bottom with some exception in deeper area.

Table 4. List of aquatic vegetations recorded in Rajdhala *beel* during the study period

Sl No.	Type	Local name/English name	Scientific name	Comment
1	Free floating	Kachuripana/Water hyacinth	*Eichhornia crassipes*	Most common
2	"	Topa pana/Water lettuce	*Pistia stratiotes*	Less common
3	"	Duchweed/Khudi pana	*Lemna minor*	Common
4	"	Water velevet/Kuti pana	*Azolla pinnata*	"
5	Leafy floating	Lal shapla/Red water lily	*Nymphaea rubra*	Common in shallow area
6	"	Shada shapla	*Nymphaea nouchali*	"
7	"	Shaluk/Water lily	*Nymhyaea lotus*	"
8	"	Padda	*Nelumbo nucifera*	"
9	Submerged	Eelgrass/Pataseola	*Vallisneria spiralis*	Common, except in deeper area
10	"	Pond weed	*Potamogeton sp*	Common
11	"	Dholkalmi	*Ipomoea fistulosa*	Common in bank area
12	"	Arail	*Leersia hexandra*	"
13	"	Kachu	*Colocasia esculenta*	"
14	"	Arrow head	*Sagittaria sagitifollia*	"
15	"	Keshordam	*Ludwigia adscondens*	"
16	"	Smart weed/Bishkatali	*Polygonum hydropiper*	"
17	Spreading	Kalmisak	*Ipomoea aquatica*	Common
18	"	Malancha	*Enhydra sp*	"
19	"	Helencha	*Enhydra fluctuans*	"
20	"	Amrul shak	*Oxalis corniculata*	"
21	"	Shushni shak	*Marsilea quadrifolia*	"

Seven species were identified as locally extinct (Table 5) from the interrogation of the fishers and PRA during the gear wise catch observations.

Table 5. List of extinct species recorded in Rajdhala *beel* during the study period

Sl No.	Local name	English name	Scientific name	Comment
1	Ayre	Long-whiskered catfish	*Sperata aor*	Locally extinct
2	Sarputi	Olive barb	*Puntius sarana*	"
3	Gilarchaki	Square head catfish	*Chaca chaca*	"
4	Ek-thuita	Half-beaks	*Dermogeneys pussilus*	"
6	Khaira	Indian glass barb	*Chela laubuca*	"
7	Dhela	Cotio	*Osteobrama cotio cotio*	"
8	Neftani	Indian paradise fish	*Ctenops nobilis*	"

Based on findings of the present study on the prevailing management condition of the *beel* fishery, following general comments can be made for its better management. As the management of openwater common property like *beel* is very difficult due to its size and nature, biological and social management under community based fisheries management may be taken up for effective and sustainable management and to improve the socio-economic conditions of the fishers. Therefore, before taking any steps for developing the beel fisheries (inland openwaters) of the country, following measures should be undertaken:

1) Lease oriented management through MoL should be stopped to maintain biodiversity and conserve the fisheries resources and all beels should be transferred from MoL to MoFL basis to make biological and social management effective.
2) Lease should be given to bonafide fishers who live adjacent to the waterbody and leasing period should be around 10 years.
3) Lease money should be fixed on the basis of biological productivity of the waterbody with reasonable increment rate. Unilateral imposing of 3% income tax and 15% VAT each year on lease money should be relaxed to encourage the poor fishers.
4) As a part of better management, closed fishing season should be maintained strictly for 3-5 months during the peak breeding season of the resident fish species.
5) Use of destructive fishing gears should be strictly prohibited.
6) Commercial banks and other financial institutions should come forward with collateral free special supervisory credit package for fingerling stocking and overall management.
7) CBFM is a unique example of co-management arrangement of GO-NGO-Target fishers where both biological and social management would be done effectively. After a long-term trial it might be developed further as a model management system for other similar beels in the country.

Conservation of fish biodiversity in Rajdhala beel

Biological and social conditions are necessary to be addressed for the protection of fish biodiversity. Biological aspects on biodiversity protection programs should include inventory of genetic resources, setting of conservation priorities, protection of critical habitats and ecosystems, and development of existing propagation and release. For

protection of fish biodiversity of Rajdhala beel the following measures are recommended:

1) Fishing pressure on some resident fish species particularly *G. chapra* should be reduced to sustain its biodiversity.
2) Effective fish sanctuaries should be established in Rajdhala beel. One permanent and two or three temporary sanctuaries can be established. Temporary sanctuary can be fished once in a year and permanent sanctuaries once in every three years.
3) Broods of highly endangered species can be stocked before breeding season.
4) Artificial propagation and stocking in the protected areas with threatened species might help in re-establishing the almost threatened species.
5) Protection of endangered species by extending legal protection to those species. BMC can impose penalty to discourage to catch endangered species.
6) Continuous monitoring of fish, prawns, crabs, snails, bivalves, and other aquatic organisms should be done.
7) Use of destructive fishing gears or devices should be strictly prohibited.
8) Stocking of exotic species should be controlled to maintain resident fish production.
9) Government facilities and loans can be provided for beel fishery management, and for creation of alternative income generating activities.
10) Ban of using current net in the beel should be judiciously executed with the help of Government law enforcing agencies.
11) Fish nursery in beel adjacent lands should be established with the help of NGOs and local associations.
12) Ban should be imposed on the use of pesticides and other harmful chemicals around the beel area.
13) During fishing ban period poor fisherfolk around the beel can be given alternative jobs so that they do not need to catch undersized fishes or brood fishes of any species from the beel. Some of the surrounding non-fishers who are interested to establish farms near the beel using beel facilities through CBFM program/BMC or personally, should be encouraged for creation of alternative sources of income through poultry, goat and cow fattening etc.

The observation in this study indicated that the annual chapila production is decreasing due to stocking of silver carp and catla, over fishing of chapila and use of fertilizers, pesticides, insecticides etc. in the nearby agricultural lands. If the reduction of chapila catch continues in future then there is every possibility that the poor community living around the Rajdhala beel will face nutritional deficiency. If re-stocking of silver carp is stopped and chapila remains undisturbed during peak spawning period, then production of chapila may increase. It was also observed that the beel fishery as a whole and particularly the beel chapila play a pivotal role in various ways on the family nutrition and livelihood of the rural poor community around the beel. Therefore, effective co-management system need to be developed to obtain sustainable fish production from the beel for maintaining fish biodiversity of the beel.

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Socioeconomic assessment of shrimp farming in relation to local livelihoods in the south-west coastal Bangladesh

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Abstract
Nine different categories of stakeholders in shrimp farming industry were assessed to show the socioeconomic impact of shrimp farming in south-west Bangladesh. Among all the stakeholders the shrimp farmer's average own land was 4 ha whereas the seed collectors and *faria's* had lowest amount of average land, 0.1 and 0.5 ha respectively. The shrimp farming positively impacted to the livelihood of stakeholders. Income of the coastal people, sanitation, working facilities of women, employment, health condition and the literacy rate increased due to shrimp farming. On the other hand shrimp farming had negative impact on the rice production, livestock, drinking water supply, and social conflict and violence had increased due to shrimp farming. There were internal conflicts between different stakeholders; the *farias* conflict with the *depot* owners and shrimp farmers, marginal farmers' conflict with the rich shrimp farmers about leasing lands and saline water control, the rice farmers conflicts with the shrimp farmers about agricultural crop production.

Key words: Shrimp farming, Socioeconomic, Livelihood

Introduction

Shrimp farming is not only earnings of foreign exchange but also losses the gross domestic product (GDP) of Bangladesh (Bhattacharya 1999). Shrimp is an industry where a chain of stakeholder is being worked from shrimp PL collection/production to export it into the international market. Among the value chain the relationship between different stakeholders is an important factor for its business, socioeconomic and overall benefits from the product. Sometimes the relation is remained well between some stakeholders, but in most cases the experience of such relationship is very worst. Internal conflict and socioeconomic situation of shrimp farming and existence of "mafia, musclemen and cycles of poverty, debt and dependency in shrimp value chain has been raised a perennial questions of boycott of shrimp product from Bangladesh by the international buyers (Rosenberry 2007b). Conflict between shrimp farmers and rice

farmers is existed in Thailand (Lebel *et al.* 2002), as well as in Bangladesh (Islam *et al.* 2002) due to salinity intrusion, shortages of animal feed (Ahmed 2006). However, relationship between different stakeholders in shrimp value chain has been done indiscriminately and information regarding it is still scares (Hoq *et al.* 1995). In the above context, the present study assessed the socioeconomic impact of shrimp farming and its implications for local livelihoods in south-west coastal Bangladesh.

Methodology

Study area and selection of the shrimp farming stakeholders

The study areas were selected from the south-west part of Bangladesh, mainly in Satkhira and Khulna districts where the majority shrimp farming activities are concentrated. According to their involvement in different activities of the shrimp industry, nine categories of stakeholders were selected for data collections (Table 1).

Table 1. Sampling design and distribution of shrimp farming stakeholders

Sl. No	Stakeholder	Sample size	Description
1	Shrimp farmers	30	Year round only shrimp farming
2	Alternate rice and shrimp-prawn farmers	30	After rice culture; shrimp-prawn culture together
3	Rice farmers (control)	30	Rice farming in shrimp growing area
4	Depot owners	10	Depot: Local shrimp processing factory
5	Depot workers	10	Worker in the shrimp processing factory
6	Shrimp farm laborers	20	The laborers are working either whole day and night or part time
7	Faria-Shrimp traders	10	Buying shrimp from shrimp farm and selling it to depot
8	Land lessors	10	Leasing their land to the rich shrimp farm
9	Shrimp seed collectors	10	They collect the Post Larvae (PL) from the river
Total stakeholders		160	

Simple random sampling techniques were applied for selecting the respondents of rice farmers, shrimp farmers, and alternate rice and shrimp-prawn farmers. For applying the technique, at first the list of rice farmers, shrimp farmers and also the alternate rice and shrimp-prawn farmers were collected from the Upazila Agricultural office and Upazila Fishery Office of the selected upazila and then random number table was used for selecting the sample units. The sample of shrimp farm laborers, depot

owners, depot workers, shrimp seed collectors and land lessors were selected by purposive method.

Questionnaire design and survey of the study area

One draft questionnaire was designed to survey the socioeconomic issues due to shrimp farming and implications on local livelihood. The preliminary survey focused on the shrimp farmers', rice farmers' and other shrimp farming stakeholders' recent socioeconomic conditions. During August 2007 the data were collected by the pre-tested draft questionnaire from the two respondents of each category. Then the questionnaire has been finalized for collecting the necessary data through interview method. The data collection period was from Sept 2007 to February 2008.

The survey method was followed through direct interview from the different stakeholders. For determining the socioeconomic impact of shrimp farming "before and after" and also "with and without" methods were applied. The respondents were asked about what were the socioeconomic situations were before and after the shrimp farming practice at the study areas. The information were also collected about the earlier traditional social structure and livelihoods of shrimp farming stakeholders were changed or not due to shrimp farming. Intra-generational changes in the case of sustainability of livelihood framework such as human, social, physical, natural and financial capital assets were also analyzed by DFID (2000) for determining the impacts of shrimp farming development at the coastal area of Bangladesh. Data was collected through direct observation and transect walk (informal surveys and this participatory studies known as a walk over the transect of an area for the observation and documentation of the similarities and differences of socio-economic and bio-physical features described by PPM&E (2004). Data were collected through interviews (questionnaire) by grouping of all stakeholders in to following groups.

- Group 1 was for three categories such as rice farmers, shrimp farmers and alternate rice and shrimp-prawn farmers and
- Group 2 was for six categories such as *depot* owners, *depot* workers, shrimp farm laborers, *faria*, land lessors and for shrimp seed collectors.

The data were also collected by oral history method. Oral history is an interview method by which the researcher collects about the past events and ways of life. The beginning history of the shrimp farming, the mangroves were present at the shrimp farming area or not and also the agro-ecosystem gradually destroying or not were obtained from the very old aged people at the coastal areas by this oral history method. In this case the respondents were more than sixty years old. The selected respondents were different categories like shrimp farmers, shrimp seed collectors and shrimp farm laborers and land lessors of very near the mangroves region of the coastal area.

Data analysis

The data from the questionnaire were grouped and categorized according to the different stakeholders of the shrimp farming. The whole data were entered into the MS Excel program and in the tabular form in the computer. Mainly the tabular and graphical methods were used for analyzing the data. Independent sample T-test was applied for assessing whether the differences of income from different types of farming activities are significant or not. Most of the data were formed in the histograms, percentage, pie chart and mean value.

Result and discussion

Conflicts between different stakeholders

The internal conflicts were found from the different shrimp farming stakeholders as depicted in the table 2 on the basis of the respondent's reports. Around 81 percent *faria*, shrimp farmers and depot owners reported that they had internal conflicts about buying and selling rate of shrimps in the shrimp farm and depot. Around 78 percent rice producer and shrimp farmers have agreed that they have conflicts about the paddy culture in their area because paddy cannot grow in saline water. The Thai shrimp and rice farmers also have conflicts due to salinity intrusion for shrimp farming (Lebel *et al.* 2002). The shrimp farming is not so profitable for the marginal rice farmers because their land amount is very small even if they want to do shrimp culture they will not get proper saline water from the narrow canals which are controlling by wealthy farmers.

Table 2. Conflicts between different stakeholders

Different stakeholders	Aspects of the conflict	Number of respondents
Faria and shrimp farmers & depot owners, (n=80)	On the selling and buying rate of shrimp in the shrimp farm and depot	65 (81.25)
Rice producer and shrimp farmers (n=90)	The rice producers could not grow rice due to salinity water in the surroundings	70 (77.77)
Marginal farmers and rich farmers, (n=90)	The rich farmers were controlling the saline water from the canal	50 (55.55)
Depot workers and *depot* owners (n=20)	Low salary amount and part-time wages	10 (50)
Livestock producers and shrimp farmers (n=90)	The cow, goat, sheep and buffaloes were decreased due to decreasing of rice field	45 (50)
Land lessors and shrimp farmers (n=40)	Sometimes the poor farmers are bound to lease their land to the rich shrimp farmers.	15 (37.50)
Shrimp farm laborers and shrimp farmers, (n=80)	Low salary amount and part-time wages	20 (25)

Figures in the parentheses indicate percentages of total.

Around 56 percent marginal farmers and rich farmers have been reported that the rich shrimp farmers are controlling the saline water from the canal of the river. Sometimes the rich farmers are selecting the middleman who will buy the shrimp from the marginal farmers cheaply. The clashes are also occurring between the villagers (marginal farmers) and the illegal rich shrimp farmers about the possession of shrimp farms (Rosenberry 2006b). The wealthy land owners have been converted the polders into the extensive shrimp ponds and the marginal farmers have nothing to say against the powerful illegal politicians and powerful market players (Samarakoon 2004). In Bangladesh hundreds of thousands of coastal people have been displaced sometimes the land seizures involving use of force (EJF 2008).

About 38 percent land lessors and marginal shrimp farmers have been claimed about the conflict of leasing land with rich shrimp farmers. The marginal farmers and land lessors want to culture agricultural crops but due to saline water they cannot do it. Even they cannot do the shrimp culture because they do not get enough saline water from the canal due to insist of the rich farmers and finally the land lessors leasing their land to the rich shrimp farmers. So, the poor land lessors are becoming poorer by losing their lands for shrimp farming. The land lessors are not benefited from the shrimp farming at the coastal area (Islam *et al.* 2000).

Problems of shrimp farmers and alternate rice & shrimp-prawn farmers

The problems affected by the shrimp farming and other causes were estimated on the basis of the respondents reports in percentage term (Table 3). All the stakeholders (100 percent) reported about their problems that saline water, excess river siltation destroying shrimp farms, less depth of river and canal, salinity fluctuation, post larvae transportation problem from Coz's Bazar to Khulna, lack of available shrimp seed, virus problem by WSSV, lack of training and irregular electricity. About 75-83 percent stakeholders reported that weak shrimp seed, shrimp farm looting and breaking down of river embankment. Around 60-70 percent stakeholders reported that communication problem, land fertility decreasing, loan problem, connection from river or canal to the shrimp farm and they also about the drinking water problems in the region.

Table 3. Problems of shrimp farmers and alternate rice & shrimp-prawn farmers

Different types of problems	Percentage of respondents reported		
	Shrimp farmers (n=30)	Alternate rice and shrimp -prawn farmers (n=30)	Over all n=60
Proper saline water is not available for shrimp farm	100	100	100
Excess river siltation destroying Shrimps farms	100	100	100
Less depth of river and canal	100	100	100
Salinity fluctuation	100	100	100

PL transportation problem	100	100	100
Lack of available shrimp seed (PL-post larvae)	100	100	100
Virus problem (White spot disease by SEMBV)	100	100	100
Lack of training	100	100	100
Irregular electricity	100	100	100
Unknown for viral contamination	80	86.66	83.33
Weak shrimp seed	83.33	80	81.66
Breaking down of river embankment	80	73.33	76.66
Shrimp farm looting	80	70	75
Loan problems	60	80	70
Communication problems	76.66	60	68.33
Land fertility decrease	53.33	80	66.66
Connection from river or canal to the farm	66.66	60	63.33
Drinking water problems	66.66	53.33	59.99

Problems of rice farmers and land lassoers

Problem of rice farmers and land lassoers has been presented in Table 4. Rice farmers and land lessors had reported that the saline water was harmful for agricultural crop and saline water also decrease their land fertility. The livestock production (cow, goat, buffalo etc.) decrease due to decreasing of agricultural lands for saline water intrusion in their region. About 65-70 percent respondents reported that grazing land decreased, the land lessors became poorer and sometimes they are bound to lease their land to the rich shrimp farmers.

Table 4. Problems of rice farmers and land lessors

Different types of problems	Percentage of respondents reported		
	Rice farmer (n=30)	Land lessors (n=10)	Over all (N=40)
Saline water is harmful for agricultural crop	100	100	100
Land fertility decreasing	100	100	100
Decreasing of livestock- cow, goat, buffalo etc.	100	100	100
Decreasing of grazing land	30	100	70
Bound to lease their land to the rich shrimp farmers	40	100	65
Land lessors becoming poorer	0	100	65
Drinking water problem	76.66	30	56.66
High input price for paddy	63.33	50	53.33
Fuel cost of shallow machine (water pump machine).	80	30	55
Loan problem	80	50	50
Lack of training	70	30	50
Low rate of leasing money	0	100	50

Problems of depot owners, depot workers, farm laborers, faria and fry collectors

According to the table 5 all the *depot* owners reported about the irregular electricity or power problems and for this reason they did not get enough ice from the ice factory. About 30 percent depot owners reported that they did not get enough training for shrimp processing and HACCP. All the depot workers and farm laborers claimed about their low and fixed wage amount and gender wage gap. All the *faria's* problem was that their selling rate of shrimp sometimes was too low in the *depot*.

Table 5. Problems of depot owners, workers, farm labourers, faria and fry collectors

Stakeholders	Problems	% of respondent
Depot owners	Ice problems	100
	Irregular electricity	100
	Lack of training	30
Depot workers & farm laborers	Low wage amount	60
	No part time wages, salary fixed	100
	Gender wage gap	43.33
Faria	Shrimp selling rate lower than buying rate	100
	Drinking water problem	50
	Communication problems	60
Shrimp seed collectors	Govt. ban the seed collection	50
	Resettlement and rehabilitation problems	100
	Low rate of collected PL from river	100

Most of the problems discussed here are due to shrimp farming in the coastal areas, but some other problems of local livelihoods also have mentioned. On the other hand, not all the problems are directly affecting all the stakeholders. According to the table 3 the shrimp farmers and the alternate rice and shrimp-prawn farming stakeholders have reported about their problems that the proper saline water is not present in the river, excess river siltation and the less depth of the canal and rivers, lack of available shrimp seed (post larvae-PL) for their shrimp farming, PL transportation from Cox's Bazar to Khulna is very costly, salinity fluctuation, lack of training and irregular power causes the ice problems which are drastically harmful for shrimp preservations. Power shortage and load shedding are badly affected the shrimp processing units (Rosenberry 2006e) which earns valuable foreign currency for the country. Most of the shrimp hatcheries are in Cox's Bazar region, the shrimp farmers transport the PL from Cox's Bazar to Khulna and Satkhira by helicopters which are very costly. All the stakeholders of shrimp farms and alternate rice and shrimp farms have mentioned the insufficient depth of the connected rivers and canals gradually decreasing due to soil erosion, even in some areas the depth of the canal is less than the shrimp farms depth. The vegetables and rice production also have decreased due to saline water intrusion for shrimp culture in the coastal area. The soil also contaminated by the use of fertilizer and medicine in the shrimp farm of Ecuador (Nolting and Schirm 2003).

All the rice farmers and land lessors have claimed that the land fertility is gradually decreasing due to saline water intrusion in their lands and the paddy culture also extinct from the study areas. The land lessors cannot do anything to their small amount of land because of saline water of large shrimp farms so, they are leasing their lands to the rich and wealthy farmers. All the *depot* owners and *depot* workers have claimed about their ice problems due to irregular electricity or power in their area and they need more training about HACCP in their processing plants. The depot worker and shrimp farm laborers want higher salaries and if they work more than 8 hours they have claimed the part-time wages. The female workers have demanded about the same wage amount as male workers on the daily basis; they don't want any gender wage gap. Around 50 percent of the laborers are women whose are working less amount (US$ 0.71/day) of wages than male worker (US$ 01/day) (Rosenberry 2007j). All the *farias* have claimed that sometimes the selling rate of shrimp in depot is too low.

About half million coastal people are involved for shrimp seed (post larvae or fry) collection from the estuaries. Shrimp fry collection is ecologically destructive because the mesh size of the net of fry collection is very small and for this why all kinds of fishes (wanted and unwanted) are captured by the net. Bangladesh government has banned the post larvae (PL) collection in 2000, but the fry collectors do not obey/care the law because they are very poor and they have no other sources of income. Bangladesh government does not enforce the law maybe because of resettlement and rehabilitation cost for the shrimp fry collectors.

All the shrimp fry collectors have claimed that the faria and shrimp farmers buy the shrimp PL from them by very cheap rate (1000PL /5 $) whereas they buy from the hatchery at higher rate (1000PL /10$). Most of the fry collectors do not want to collect the fry but they have no alternate way for their income. So, they want to get the government help for their resettlement and rehabilitation facilities. The resettlement and rehabilitation systems, credit facilities, poverty are the most important influences of shrimp fry collection from the river.

Impact of shrimp farming on different stakeholders

Shrimp farming has positive and negative impacts along the south-west coastal area of Bangladesh. The shrimp farmers and farm laborers, seed collectors, feed mill owners and mill workers, *faria*, local processing plants (*depot*) and large processing plants owners and plant workers, alternate rice and shrimp-prawn farmers and the shrimp seed collectors have been benefited from the shrimp farming (Fig 1). Employment opportunities, socioeconomic situation, education, available food intake, communication and health condition are increasing due to shrimp farming practice in the coastal area. On the other hand the shrimp farming is negatively affected for the land lessors, fish farmers, rice farmers and marginal farmers. The social conflicts and social violence have increased, freshwater fish, livestock production, agricultural crops have decreased due to shrimp farming.

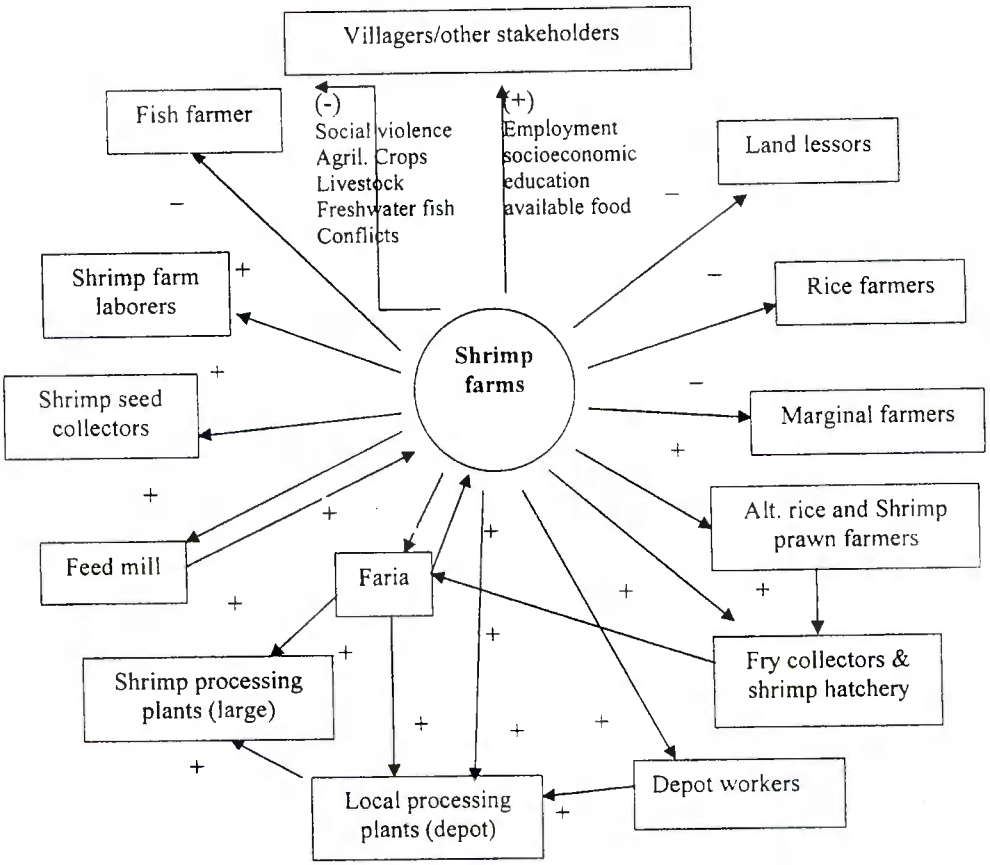

Fig. 1. Schematic diagram of relationship of different shrimp farming stakeholders

Solution suggested by the stakeholders

The survey has been conducted to identify the solutions and recommendation about the problems of shrimp farming by stakeholders in the southwest costal area of Bangladesh (Table 6). All the shrimp farmers and alternate rice and shrimp-prawn farmers have suggested about the well dredging of connected river and canal because they need more saline water. They want more shades and high embankments for cyclone & flood protection. Almost every year the cyclone and floods are attacking to the coastal people. In November 2007 during the period of data collection about 3167 coastal people have died and 10,000 shrimp farms were washed away by the cyclone (Rosenberry 2008a).

Most of the rice farmers and land lessors are marginal. They want to rear domestic animals but there is not enough grazing lands also the rice farming area is reduced in

the coastal areas due to saline water intrusion for shrimp farming. All the land lessors have reported that the new shrimp farm should be restricted or even that all shrimp farming should be banned from their region. Most of the land lessors have lost their lands to the rich shrimp farmers though they are getting a tiny amount of leasing money. The land lessors want to get higher amount of leasing money which should be at fixed rates, and that they want an administrative (public) management for the leasing systems.

Table 6. Suggestion of different stakeholders

Stakeholders	Suggestions	% of respondents
Shrimp farmers and alternate rice and shrimp-prawn farmers (n=60)	Well dredging of connected river and canal	100
	Shade for cyclone & flood protection	100
	More training for the shrimp farming stakeholders	100
	Viral disease specialist in the shrimp farm area	100
	Govt. hatchery and supply healthy, strong PL	100
	High embankment for flood & cyclone protection	88
	Ensure - connection of shrimp farms to the rivers or canals	83
	Mangroves destroyers should be punished by the govt. rules	60
	Shrimp farms should be banned where mangroves may grow	60
	Loan to marginal farmers and other stakeholders	54
Rice farmers and land lessors (n=40)	New shrimp farm registration should be restricted	83
	Grazing land should be fixed for livestock production	72
	Administrative management for land leasing system	64
	Shrimp farm should be postponed from this area	60
	Land leasing amount should be higher and fixed rated	60
Depot owners (n=10)	Continuous electricity supply in the coastal area	100
	Antibiotic testing equipment in the processing plant	100
	Training on HACCP in the processing plant	100
	Shrimp farmers should supply shrimp directly to depot	100
Depot workers and shrimp farm laborers (n=30)	Part-time wages if it is more than 8 hours	100
	Daily wages should be higher	100
	Should ensure the availability of drinking water	54
	No gender wage gap	50
Faria (n=10)	Selling and buying rate should be negotiated (for fixed rate)	100
	Communication	100
Shrimp seed collectors (n=10)	Resettlement & rehabilitation of shrimp seed collectors	100
	River PL selling rate should be higher	70

Electricity or power supply is the main problem for local processing factory (*depot*) owners. Maximum time the power is absent due to load shedding for this reason they cannot get enough ice and sometimes their shrimp are spoiled so, they want continuous electricity supply especially to the processing plants area. Antibiotic testing equipment

is necessary in the shrimp processing plant. The shrimp processing plants owners are sending their shrimp samples to Singapore for antibiotic testing (Rosenberry 2006g) which is very expensive for them, so they suggest antibiotic testing equipment in their processing plants area. They also suggest more training about HACCP (hazard analysis and critical control point) in the shrimp processing plants and the shrimp farmers should supply their shrimps directly to the depot.

Bhattacharya (1999) has proposed some solutions for shrimp farming in Bangladesh such as effluent charge on pollutants of water, mixed rice-shrimp and clear land zoning, licensing of shrimp farms, mandatory mangrove development, a ban on shrimp catch by trawlers, strengthening of property rights, and a rationalizing of current laws. He also has emphasized that "in Bangladesh, a major requirement is that all stakeholders, especially local communities must be involved in the decision making process." The indigenous and local knowledge holders should be included in co-management committees regulating practices and service delivery on designated lands or in designated communities and these committees could make decision regarding biological resources, environment, natural resources, land-use planning, fisheries and oceans, agriculture, health or any other sectors (The Crucible Group 2001). For increasing the production rate farmers training is essential. Education and experience can provide the increasing rate of production and shrimp farming sustainability mentioned by Saengnore and Lebel (2001).

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Quality assessment of rotating and solar tunnel dried marine fish product

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Abstract
Studies were conducted to evaluate the quality aspects of marine dried fish i.e. silver jew fish (*Johnius argentatus*), Bombay duck (*Harpodon nehereus*) and ribbon fish (*Trichiurus haumela*) products produced in rotating and solar tunnel dryers. On the basis of organoleptic characteristics such as colour, odour, texture, broken pieces, insect infestation and overall quality, four member panels of experts evaluated the quality of the dried products obtained from both rotating and solar tunnel dryers and all the products were found in acceptable quality. Reconstitutions properties of samples were in the range of 51.05 to 98.75% for the dried fish produced in rotating dryer, while 24.64 to 76.76% for dried fish produced in solar tunnel dryer. The highest reconstitution rate was found in dried silver jew fish and lowest in ribbon fish produced in rotating dryer. On the other hand, the highest reconstitution was observed in dried silver jew fish and lowest in dried Bombay duck produced in solar tunnel dryer. Proximate composition such as moisture, crude protein, lipid and ash content of the dried fish muscles produced in rotating dryer ranged from 16.36% to 19.1%, 62.35% to 67.37%, 6.37% to 10.75% and 7.00% to 8.05% , respectively and in solar tunnel dried fish products, they were in the range of 14.05% to 19.71%, 57.64% to 69. 21%, 6.92% to 15.40%and 7.69 % to 8.80 %, respectively. The TVB-N values of dried fish products obtained from rotating dryer were in the range of 15.02 to 19.05 mg/100g, while in solar tunnel dried fish products, the values were in the range of 15.46 to 19.21 mg/100g. The results of the studies indicated that dried fish produced from both rotating and solar tunnel drier were acceptable quality in terms of organoleptic and food quality aspects.

Key words: Rotating dryer, Solar tunnel dryer, Marine dried products

Introduction

Sun drying is a low cost traditional method of fish preservation and the dried products play an important role particularly in providing nutrition of the poor and economically disadvantaged people. About 20% of the marine artisanal catch is sun dried in Bangladesh and consumed mainly by the rural people (Coulter and Disney 1987). Most of the traditional sun dried products available in the market are poor in quality and not suitable for human consumption (Kamruzzaman 1992, Khan 1992, Saha 1999, Reza *et.*

al. 2005). The major quality problems associated with sun dried fish products are insect infestation during drying, improper storage, contaminations, use of insecticides and spoilage. The quantitative losses through spoilage and insect attack on fish being dried fish have been estimated to 10-35% in the marine areas (Doe *et al.* 1977, Ahmed *et al.* 1978). In order to improve the quality of traditional dried fish products, a solar tunnel dryer constructed with locally available materials was found very suitable for drying of silver jew fish at temperature of 45 to 52°C for 5 days without showing any infestation, oxidative rancidity, spoilage and contamination (Bala and Hossain 1998, Bala and Mandal 2001, Reza 2002, Reza *et. al.* 2006). The solar dryer is a suitable technology which can be widely used for producing high quality dried fishes with the following advantages over traditional drying: (i) low production cost with less energy (ii) insecticide free high quality dried fish products (iii) no chance of insect infestation and free from animal and dust contamination (iv) faster drying due to greater speed of the air over the fish and the larger the surface area of fishes during drying and (v) the technology can be easily adopted at low-cost using locally available materials. However, solar dryer can not effectively be used in bad weather conditions, particularly during rainy season when sunlight is not sufficient for drying. There is a demand from the marginal processors for developing a dryer which can be used 24 hours round the year. The rotating dryer is a suitable technology widely used in Japan for producing high quality dried fish in all weather conditions having lot of advantages: (i) no chance of insect infestation and free from any sort of contamination (ii) production of high quality products and (iii) faster drying due to greater speed of the air over the fish and the larger the surface area of fishes during drying. It is therefore, the present study was undertaken to compare the quality dried fish produced in a solar tunnel dryer and rotating dryer by determining organoleptic, physical and biochemical aspects.

Materials and methods

Sample collection

Three fresh marine fish samples namely silver jew fish (*Johnius argentatus*), Bombay duck (*Harpodon nehereus*) and ribbon fish (*Trichiurus haumela*) were collected from a local fish market of Chittagong and transported to the laboratory of Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh in an insulated box in ice (1:1).

Organoleptic quality assessment

The evaluation of organoleptic quality of dried fish is based on the method currently used by Fish Inspection and Quality Control (FIQC) of Department of Fisheries (DoF), Government of Bangladesh with slight modification. Representative whole sample of dried products were taken on a tray to assess the organoleptic characteristics such as colour, odour, texture, broken pieces and insect infestation by a four member panels of experts constituted in the Department of Fisheries Technology, BAU, Mymensingh.

Physical study (Water reconstitution properties)

Five gram of dried fish muscle sample put into a nylon net and allowed to soak in one liter of water in water bath at different temperature for a period of maximum of 60 minutes with occasional stirring. When the samples were taken out from the water bath, water was drained off through a coarse nylon net. All the muscle fleshes were then transferred to the strainer and extraneous water was wiped off by a piece of blotting paper. Then the fleshes were weighed again. The gain of moisture for each sample was calculated as percent reconstitution. The reconstitution behavior was measured in water temperature at 20°C, 40°C, 60°C and 80°C. Reconstitution percentage was measured at 15 minutes intervals for each temperature.

Evaluation of nutritional quality

Proximate composition analysis of moisture, ash, lipid and crude protein were carried out according to the standard methods given in AOAC (1980). Total volatile base nitrogen was determined according to the method described in the Official Journal of European Commission (EC 1997).

Results and discussion

Organoleptic characteristics

The results on organoleptic characteristics of dried products obtained from the present study are summarized in the Table 1. The colour of dried silver jew fish, Bombay duck and ribbon fish produced in rotating dryer were brown yellowish to shiny silvery with little difference among the three species. Texture of solar tunnel dried fish products was more harder than those obtained from rotating dryer and not easily flexible with characteristic odour. No infestation or broken pieces were found around the products and the overall quality of all three dried fish products was good. On the other hand, the colour of solar tunnel dried silver jew fish, Bombay duck and ribbon fish was also brown to silver colour with little difference among the three species. The odour was very natural and the textural characteristics were firm and flexible in all of the samples. The products were from insect infestation and no broken pieces were found around the products. The overall quality of all the products was also good.

Water reconstitution properties

The reconstitution properties of rotating dried and solar tunnel dried marine fish muscles were assessed at a wide range of temperatures after soaking in water for 15 to 60 minutes and the results are presented in Table 2. The results shows that incase of solar tunnel dried fish, at 20°C, reconstitution level was in the range of 24.64% to 36.49% after 15 min of soaking with minimum in Bombay duck and maximum in silver jew fish. After soaking for 60 min, the highest level of reconstitution properties was in the range of 41.51% to 45.65% with minimum in ribbon fish and maximum in Bombay duck. Reconstitution capacity of dried fish samples increased considerably with increase in temperature and duration of time. When the samples were allowed soak at 80°C, for a

period of 60 min. reconstitution level was in the range of 66.04% to 76.76% with minimum value recorded for Bombay duck and the maximum value for ribbon fish.

Table 1. Organoleptic characteristics of solar tunnel and rotating dried marine fish products

Dried fish	Organoleptic characteristics of Solar tunnel dried products	Organoleptic characteristics of Rotating dried products	Overall quality
Bombay duck	• Brown colour • Characteristic odour • Firm and flexible texture • No infestation • No broken pieces found	• Brown yellowish colour • Characteristics odour • Firm and flexible texture. • No infestation • No broken pieces found.	Excellent
Silver Jew fish	• Yellowish silvery colour • Characteristic odour • Firm and flexible texture • No infestation • No broken pieces found	• Yellowish silvery colour • Characteristics odour • Firm and flexible texture. • No infestation • No broken pieces found.	Excellent
Ribbon fish	• Silvery colour and shining • Characteristic odour • Firm and flexible texture • No infestation • No broken pieces found	• Silvery colour shining • Characteristics odour • Firm and flexible texture. • No infestation • No broken pieces found.	Excellent

Table 2. Reconstitution percentage of solar tunnel and rotating dried products at different temperature and time interval

Dried fish	Soaking temperature (°C)	Soaking time in minutes for solar dried fish				Soaking time in minutes for rotating dried fish			
		15	30	45	60	15	30	45	60
Bombay duck	Room temp. (20°C)	24.64	31.16	40.58	45.65	53.00	70.94	75.21	80.19
	40	35.45	49.09	60.00	61.82	60.68	73.00	80.00	85.03
	60	42.11	53.50	58.49	63.16	62.96	76.54	81.48	88.01
	80	44.34	55.66	58.77	66.04	66.95	77.97	86.44	91.53
Silver jew fish	Room temperature	36.49	39.72	42.06	44.86	60.26	67.43	80.34	91.53
	40	45.56	53.89	60.62	62.18	61.02	78.64	86.64	94.44
	60	46.91	55.15	62.37	66.49	70.70	82.17	91.08	96.95
	80	48.00	58.22	66.67	74.22	82.22	88.89	91.67	98.73
Ribbon fish	Room temperature	26.04	30.18	37.36	41.51	51.05	56.18	60.02	62.12
	40	33.96	49.06	56.98	60.38	55.56	60.11	63.05	65.01
	60	42.47	52.40	60.96	68.49	60.19	67.02	70.15	75.08
	80	46.46	56.56	66.66	76.76	66.18	72.00	78.03	85.05

On the other hand, when dried fish samples obtained from rotating dryer were allowed to soak in water at 20°C, dried samples showed reconstitution level in the range of 51.05% to 60.26% after 15 min of soaking with minimum in ribbon fish and maximum in silver jew fish. After soaking for 60 min, the reconstitution properties were in the range of 62.12% to 91.53% with minimum in ribbon fish and maximum in silver jew fish. Like solar dried products, reconstitution capacity of rotating dried fish samples increased with increase in temperature and duration of time but the increment trend of uptake of water by the rotating dried samples was much slower than those of solar dried products. At 60°C, highest reconstitution of 70.70% in silver jew fish and lowest reconstitution of 60.19% in ribbon fish were found after 15 min soaking and after 60 min soaking the highest and the lowest value of 96.95% and 75.08% were found for silver jew fish and ribbon fish, respectively. Reconstitution level at 80°C for 60 min, the reconstitution capacity ranged from 85.05 to 98.73% with minimum value obtained from ribbon fish and maximum value obtained from silver jew fish.

A close relationship was observed between the reconstitution capacity and physical properties of the samples. The quality of the dried fish is also related to final a_w. At low values, water uptake proceeds more quickly. In properly dried fish the water uptake is more is reported to complete in 3-15 minutes (Sikorski *et al.* 1995). In the present study, solar dried products exhibited slightly less rehydration properties compared to rotating dried fish species which might be due to the denaturation of protein that took place during drying process and cause some sort of damage to the cellular structure in an irreversible manner. Thus little poor reconstitution in solar dried fish compared to rotating dried fish was due to cemented and compact structure of the muscle with few interfibrillar spaces. With a tough and rubbery tissue, water penetrates mostly to the centre of large pieces by diffusion through the protein of the fibre itself and the process is very slow (Connell 1957, Sen *et al.* 1961, Lahiry *et al.* 1961). On the other hand, rotating dried fish products exhibited an enormously rapid initial rehydration rate due to water being carried deep into the pieces by porous structure which absorbed and retained sufficient water by capillary (Jason 1965). Considering the reconstitution ability, it can be stated that dried fish products from rotating dryer were of slightly better quality compared to that of solar tunnel dried products.

Biochemical composition

Data of proximate composition analysis of rotating dryer and solar tunnel dried marine products are shown in Table 3. Initial moisture content of fresh samples of ribbon fish, silver jew fish and Bombay duck was in the range of 78.7 to 85.47% with highest moisture content in Bombay duck and lowest in ribbon fish. Protein, lipid and ash content in fresh samples of these species were in the range of 9.52 to 15.25%, 1.95 to 4.28% and 1.8 to 2.5%, respectively. For better understanding of the data, these values were calculated in dry weight basis. In dry weight basis, protein, lipid and ash content of fresh samples of ribbon fish, silver jew fish and Bombay duck were in the range of 65.90 to 71.60%, 13.42 to 21.30% and 11.27 to 12.44%, respectively.

Table 3. Comparative study of proximate composition of solar tunnel and rotating dried marine fish products

Dried fish	Proximate composition of raw fish				Proximate composition of solar dried fish				Proximate composition of rotating dried fish			
	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
Ribbon fish	78.7	15.25 (71.60)	2.92 (13.71)	2.4 (11.27)	16.19	65.82 (78.53)	10.28 (12.27)	7.94 (9.47)	19.1	63.00 (77.87)	10.75 (13.29)	7.25 (8.96)
Silver jew fish	79.91	13.24 (65.90)	4.28 (21.30)	2.5 (12.44)	19.71	57.64 (71.79)	15.40 (19.18)	7.69 (9.58)	16.36	62.35 (74.55)	11.70 (13.99)	8.05 (9.62)
Bombay duck	85.47	9.95 (68.48)	1.95 (13.42)	1.8 (12.39)	14.05	69.21 (80.52)	6.92 (8.05)	8.80 (10.24)	17.90	67.37 (82.06)	6.37 (7.76)	7.00 (8.53)

*Values within parentheses indicate results on dry matter basis.

Incase of solar dried fish, moisture content was in the range of 14.05 to 19.71% with the highest moisture content was found in silver jew fish and lowest in Bombay duck. Protein ranged from 57.00 to 69.21% with the lowest and highest value being found in silver jew fish and Bombay duck, respectively. Lipid and ash content ranged from 6.90% to 15.40% and 7.69 % to 8.80 %, respectively. On dry weight basis, protein, contents of these species were in the range of 71.9 to 80.52% with highest value in Bombay duck and lowest value in silver jew fish. Lipid content was in the range of 8.05 to 19.18% with highest value in silver jewfish and lowest value in Bombay duck. Ash content of ribbon fish, silver jewfish and Bombay duck was in the range of 9.47 to 10.24%.

In case of rotating dryer, the highest moisture content was found in ribbon fish and lowest in silver jew fish. Protein content ranged from 62.35% to 67.37% with the lowest and highest value being found in silver jew fish and Bombay duck, respectively. Lipid and ash content ranged from6.37% to 11.70% and 7.00% to 8.05% respectively. On dry weight basis, protein contents of these species were in the range of 74.55 to 82.06% with highest value in Bombay duck and lowest value in silver jew fish. Lipid content was in the range of 7.76 to 13.99% with highest value in silver jewfish and lowest value in Bombay duck. Ash content of ribbon fish, silver jewfish and Bombay duck was in the range of 8.53 to 9.62%. An inverse relationship was found between moisture and lipid content as reported by Stansby (1962) where sum of the total approximates near about 80%. The summation of oil and water was not necessarily constant and it frequently ranges from 78 to 85%.The results obtained in this investigation is more or less in agreement with general rule suggested by Stansby (1962). For the better comparison, the data for protein, total lipid and ash content have been recalculated on moisture free basis and shown in the parentheses. An inverse relation also found between the protein and fat contents where the relationship was markedly evident by the data calculated on dry matter basis. Protein contents in dried fish obtained from both rotating and solar tunnel dryer was comparatively higher than those of fresh samples on dry weight basis. On the other hand, on dry weight basis, lipid content of those products was lower than those of fresh samples. During processing it might happen of some losses of lipid this is why slightly poor value recorded on dry weight basis and on the other hand high calculated value was observed in protein content on dry weight basis. There might be individual variation of composition.

The TVB-N values of solar dried fish species of ribbon fish, silver jew fish and Bombay duck were in the range of 15.02% to19.05% with lowest value observed in silver jew fish and highest value in Bombay duck. On the other hand, the TVB-N values of rotating dried fish species were in the range of 15.46 % to19.21% with lowest value in silver jew fish and highest in Bombay duck. The TVB-N values of all the samples were found lower than the recommended value (100 - 200mg/100g) for variety of salted and dried products (Connell 1995).

Solar tunnel dryer and rotating dryer constructed using locally available materials at a low cost were found suitable for production of high quality dried fish products. Rotating dryer was found most effective to run uninterruptedly in all weather conditions.

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