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Nutrient digestibility coefficients of diets with varying energy to protein ratio for Japanese flounder, *Paralichthys olivaceus*

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Abstract

A laboratory trial was conducted in a sea water recirculatory system to study the nutrient digestibility coefficients of diets with varying energy to protein ratios in Japanese flounder *Paralichthys olivaceus*. Six different experimental diets with two protein levels (45 and 55%) having six different energy to protein ratio of 87, 90, 94, 107, 110 and 114 were formulated using white fish meal and casein as protein sources. The results of the study showed that the apparent protein digestibility (APD) value ranged between 90.59 to 91.61% and there was no significant differences ($P > 0.05$) between the APD values of diets 1, 2, 3, 4 and 6. The apparent lipid digestibility (ALD) values of diets ranged between 88.24 to 90.18%. The apparent energy digestibility (AED) values ranged between 80.55 to 87.52% with diet 3 producing significantly the highest AED value. In general, except in diet 1 the ALD and AED values increased with the increase of dietary lipid at both protein levels. The results of the present investigation indicated that Japanese flounder can efficiently digest the dietary nutrients at varying energy to protein ratios.

Key words: Nutrient digestibility, Energy-protein ratio, Japanese flounder

Introduction

One of the most important aspects in the evaluation of the effectiveness of a feed is the determination of its digestibility. This measures the ability of the fish to digest and absorb the nutrients it is fed. Besides, the measurement of the digestible crude protein, the determination of digestibility of the organic matter or lipid and energy content of feed is also important.

Measurement with chromic oxide as an exogenous indicator is the most frequently used method of digestibility determination (NRC 1983). But faecal collection methods pose serious technical problems in obtaining correct value. For example, faeces have been collected by several methods including dissection (Nose 1967), faecal stripping (Inaba *et al.* 1962) and suction (Windell *et al.* 1978a), collection in special chamber (Cho and Slinger 1979), settling of faeces (Law 1984) and continuous filtration of effluent water (Choubert *et al.* 1979). In the present study, a specially designed floating net cage

with tank underneath was used to collect faeces by sedimentation.

Japanese flounder (*Paralichthys olivaceus*) is one of the most important aquaculture species in Japan and production of cultured flounder ranked fourth among marine cultured fish in Japan (Kikuchi *et al.* 1997). The fish is highly carnivorous and needs higher dietary protein for growth. The growth of Japanese flounder with different protein sources has been studied (Kikuchi *et al.* 1997). The present study was undertaken to determine the nutrient digestibility coefficients of various diets with different energy to protein ratio for Japanese flounder (*P. olivaceus*).

Materials and methods

Experimental system

The experiment was conducted in Abiko Research Laboratory, Chiba, Japan in a specially designed faeces collection system. The floating net cages made of HDPE (high density polyethylene) were set in a 2000*l* volume sea water recirculating system fibre glass tank. The water depth in the tank was maintained at 0.6 m. There were 6 net cages (30×45×30 cm) set in the tank. Each of the floating net cages were fitted with a conical bottom rectangular perspex glass tank which served as faecal settling tank.

Experimental procedure

About one year old juvenile Japanese flounder (*P. olivaceus*) were obtained from the Abiko Research Laboratory. These fish were originally collected from a commercial hatchery (Anagawa Shokusan) in Mie Prefecture (average wt. 1.0g) and transported to Abiko Research Laboratory in Chiba Prefecture. The fish were reared in a 2000*l* volumes sea water tank with a commercial diet used for Japanese flounder (Higashimaru Foods Inc) at 20°C until the start of the experiment.

Since there were 6 faeces collection tanks, the faeces collection experiment was run twice to have replicate values. Eight fish with mean body weight of 105-107g were stocked in each floating net cages. Faeces were collected twice - morning and evening for two weeks in each run. Natural light-dark was maintained and the water temperature was kept at 20±1°C. Adequate oxygen supply was ensured by artificial aeration. The pH of water was maintained between 7.0 and 8.0 by occasional addition of $NaHCO_3$. The salinity was maintained at 35ppt. In order to adjust the salinity of rearing water, freshwater was added corresponding to evaporated volume.

Experimental diets

Six experimental diets were formulated using white fish meal (Nippon formula Feed Ltd.) and casein (New Zealand Milk Products) as the main protein sources (Table 1). The fish meal contained 68.8% protein, 13.9% lipid and 17.3% ash on dry matter basis. The casein contained 92.0% protein. The diets were formulated at two protein levels (55 and 45%) and each with three energy levels to have six different energy to protein ratio of 87, 90, 94, 107, 110 and 114. Pollack liver oil was used as lipid source to maintain the desired energy levels in different diets. Pollack liver oil was obtained from Riken

Vitamin Co. Ltd. Vitamin and minerals premixes were obtained from Nippon Formula Feed Mfg. Co. Ltd. The energy to protein ratio was calculated as gross energy per kg/crude protein (%) in diet. Chromic oxide was used at 0.5% level as an external marker for nutrient digestibility study. All the feedstuffs except the pollack liver oil were processed in a mixer after grinding and were formed into spheres of 4mm diameter using a twin extruder with addition of tap water and pollack oil. The prepared diets were dried in an air dryer at 25°C. These diets were stored at -35°C until use.

Table 1. Formulation of experimental diets with different ratios of energy to protein for Japanese flounder

Ingredient (%)	Diet No.					
	1	2	3	4	5	6
White fish meal	52.1	52.1	52.1	42.5	42.5	42.5
Casein	22.3	22.3	22.3	18.2	18.2	18.2
Potato starch	8.5	8.5	8.5	8.5	8.5	8.5
Pollack liver oil*	3.0	7.0	11.0	7.0	12.0	17.0
Cellulose	8.1	4.1	0.1	17.8	12.8	7.8
Mineral mixture**	2.8	2.8	2.8	2.8	2.8	2.8
Vitamin mixture**	2.7	2.7	2.7	2.7	2.7	2.7
Chromic oxide	0.5	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100	100

**Riken Vitamin Co., Ltd. (Feed oil Ω)*
***Nippon formula Feed Mfg. Co., Ltd.*

Feeding and faeces collection

Fish were acclimated to the experimental system for 7 days before the start of the experiment. Fish were fed to satiation twice a day on each of the experimental diets. Faeces collection started 4 days after feeding to allow evacuation of all previously ingested materials. After feeding the fish, specially designed faeces collection tanks were attached beneath each of the net cages with hooks so that the faeces voided in net cages could be settled at the conical bottom of the attached tank. Before collecting faeces, the attached tanks were unhooked carefully and the settled faeces were pipetted out in centrifuge tubes. Then the faeces were collected by centrifuging in a refrigerated centrifuge at 5,000 rpm for 15 minutes. The collected faeces were stored at -35°C until sufficient amount was collected. At the end of the experiment, the collected faeces were dried in a vacuum freeze dryer before biochemical analysis was done.

Analytical methods

The proximate composition of the feed ingredients, feed and faeces samples were analysed according to AOAC (1980) with NFE (nitrogen free extract) being determined by difference. The gross energy content in feed and faeces samples were analysed by a bomb calorimeter (Shimadzu, autocalculating bomb calorimeter, Model CA-4P). Chromic oxide was determined by using the wet-digestion method of Furukawa and Tsukuhara (1966). Differences in digestibility coefficients were tested for significance

($P<0.05$) by Duncan's Multiple Range Test (Duncan 1955).

Results

The results of the proximate composition analysis of the experimental diets are shown in Table 2. Crude protein, lipid and energy content ranged between 45.4 to 56.5%, 9.8 to 20.3% and 485 to 532 Kcal/100g respectively. Protein, lipid, energy and chromic oxide content in faeces of fish fed experimental diets are shown in Table 3. The protein content was highest (19.12%) in faeces of fish fed diet 3 whilst fish fed diet 4 produced the lowest faecal protein (11.82%). Similarly, lipid content in faeces of fish fed diet 6 was highest and that of diet 1 was the lowest. The energy content in faeces of fishes fed different diets ranged between 263 to 305 kcal/100g

Table 2. Analysed proximate composition of the experimental diets (% dry matter basis)

Ingredients (%)	Diet No.					
	1	2	3	4	5	6
Dry matter	97.0	97.3	97.0	96.8	97.0	96.9
Crude protein	56.5	56.3	56.3	45.4	46.0	46.3
Crude lipid	9.8	14.9	17.2	12.7	16.0	20.3
Ash	11.2	11.5	11.6	9.5	9.8	9.7
Crude fibre	8.5	6.0	3.0	17.5	13.1	8.2
NFE*	14.0	11.3	11.9	14.9	15.1	15.5
Chromic oxide	0.46	0.47	0.49	0.46	0.46	0.45
Gross energy (Kcal/100gdiet)	492	510	532	485	508	530
GE/CP**	87	90	96	107	113	114

**Nitrogen free extract calculated as 100-% (moisture + protein + lipid + ash + crude fibre)*
*** Gross energy in per kg diet/crude protein (%)*

Table 3. Nutrient and chromic oxide contents in fish faeces fed diets with different energy to protein ratio

Components	Diet No.					
	1	2	3	4	5	6
Protein (%)	16.41	17.00	19.12	11.82	13.06	15.19
Lipid (%)	3.38	5.33	6.76	4.19	5.36	7.81
Energy (Kcal/100g)	263	279	266	265	293	305
Chromic oxide (%)	1.59	1.63	1.96	1.29	1.39	1.60

The apparent nutrient digestibility of protein, lipid and energy of the experimental diets are shown in Table 4. The results of the present study showed that all the nutrients under investigation were well digested by the Japanese flounder. The apparent protein digestibility (APD) values ranged between 90.59 to 91.61% and there was no significant difference ($P>0.05$) between the APD values of diets 1,2,3,4 and 6. However, diet 5 showed significantly the lowest APD value (90.59%). The apparent lipid digestibility

(ALD) values of different experimental diets ranged between 88.24 to 90.18%. There was no significant difference ($P>0.05$) between the ALD values of diets 1, 2, 3 & 4; 2, 5, & 6 and 4 & 5 respectively. The apparent energy digestibility (AED) values ranged between 80.55 to 87.52% with diet 3 producing significantly the highest AED value. There was no significant difference between the AED values of diets 1, 2, & 6 and 4 & 5 respectively.

Table 4. Apparent nutrient digestibility coefficients of diets with different energy to protein ratio in Japanese flounder

Components	Diet No.					
	1	2	3	4	5	6
Protein	91.60^{a*} (±0.28)**	91.51^{a} (±0.13)	91.61^{a} (±0.05)	90.72^{ab} (±0.16)	90.59^{b} (±0.25)	90.74^{ab} (±0.72)
Lipid	90.04^{a} (±0.18)	89.70^{ab} (±0.11)	90.18^{a} (±0.06)	88.24^{c} (±0.23)	88.90^{bc} (±0.27)	89.67^{ab} (±0.85)
Energy	84.54^{b} (±0.53)	84.25^{b} (±0.71)	87.52^{a} (±0.08)	80.55^{c} (±0.04)	80.91^{c} (±1.20)	83.73^{b} (±1.17)

**Figures in the same row having the same superscripts are not significantly different ($P>0.05$)*

*** Values in the parentheris are standard deviation of treatment means.*

Discussion

The result of the present study indicated that the protein, lipid and energy of the experimental diets with different energy to protein ratio were well digested by Japanese flounder. The APD value of all the diets are above 90%. This better digestibility might be related with the higher level of white fish meal used as dietary protein source. According to NRC (1983) fish can digest up to 95% of the protein in fish meal. However, this value can decrease to 80 to 85% depending on the origin and processing of the fish meal used (Ogino and Chen 1973). The fish meal used in the present study was prepared by low temperature processing. Sato (1999) reported slightly higher APD values of 95.1 to 96.6% in Japanese flounder (*P. olivaceus*) fed diets with 55 to 66% protein having different energy to protein ratios. The APD values obtained in the present study are similar to the APD value of white fish meal protein (90.7%) reported for rainbow trout by Smith *et al.* (1995). Kabir *et al.* (1998) also reported a similar APD value of 89.40% in rainbow trout fed a diet containing 50% protein.

In general, higher APD values were obtained in diets with higher protein contents. These results are in agreement with similar studies measuring APD in other species (Austreng and Refstie 1979, De Silva and Perera 1984, Hajen *et al.* 1993). The usual explanation offered has been that the proportion of metabolic faecal nitrogen decreases with the rise in protein content in the diet (Nose 1967, Ogino and Chen 1973, Jauncey 1982). It is also possible that an adaptation of digestive enzymes to the level of protein in the diet contributes to increasing the level of nitrogen (i.e. protein) digestibility when the level of protein in the diet increases (Kawai and Ikeda 1973).

The ALD values obtained in the present study are slightly lower than that of Sato

(1999) who observed ALD values of 90.1 to 95.7% with smaller sized (79g) Japanese flounder fed diets containing 55 to 60% protein. Ohta and Watanabe (1998) also reported crude lipid digestibility of 87.9 to 97.1% in rainbow trout fed 49% protein containing extruded pellet diet. However, Hossain *et al.* (1992) reported 89.96% and 93.24% digestibility of lipid in fish meal and soybean meal diet respectively for Tilapia (*Oreochromis mossambicus*) which are within the range of the values obtained in the present study.

The AED values of different experimental diets in the present study ranged between 80.55 to 87.52% and these values are higher than some reported values (74%, Windell *et al.* 1978b for rainbow trout) but lower than reported by others (91%, Cho *et al* 1982, 91.5%, Smith *et al.* 1980, 89.3%, Smith *et al.* 1995) for the same species. Sato (1999) also reported slightly higher AED values of 85 to 95.4% in Japanese flounder fed diets containing 55 to 66% protein. However, the values obtained in the present study are more or less similar to the value reported for grass carp (83%, Law 1986). The slightly lower AED values obtained in the present study with Japanese flounder compared to that of Sato (1999) might be related to the smaller size of the fish and higher level of dietary protein used.

The lower AED values obtained with different diets compared to diet 3 in the present study might be related to the higher level of cellulose in those diets. Hilton *et al* (1983) suggested that a higher dietary crude fibre content may accelerate the rate of passage of digesta through the intestinal tract thus reducing the digestibility of energy and protein. Smith and Lovell (1973) also reported that fibre is negatively correlated with digestibility of nutrients. The best nutrient digestibility obtained with diet 3 containing 55% protein and a energy to protein ratio of 96 supports the findings of Utsumi (1999) who observed better growth and feed utilization in Japanese flounder fed diet with similar protein and energy levels. The results of this study demonstrated that Japanese flounder can efficiently digest the dietary nutrients with varying energy to protein ratios.

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References

AOAC, 1980. Official Methods of Analysis (ed. W. Worwitz). Association of Official Analytical Chemists, 13th edition, Washington, D. C.

Austreng, E and T. Refstie, 1979. Effect of varying protein level in different families of rainbow trout. *Aquaculture*, **18**: 145 –156.

Cho, C. Y and S. J. Slinger, 1979. Apparent digestibility measurement in feedstuffs for trout. *In*: World Symp. on Finfish Nutrition and Fish Feed Technology, Vol. II (eds. J. E. Halver and K. Tiews). Heinemann, Berlin, pp. 239-247.

Cho, C.Y., S.J. Slinger and H.S. Bayley, 1982. Bioenergetics of salmonid fishes: Energy intake,

expenditure and productivity. *Comp. Biochem. Physiol.*, **3**(B): 25-41.
Choubert, G., J. De La None, and P. Luquet, 1979. Continuous quantitative automatic collector for fish faeces. *Prog. Fish-Cult.*, **41**: 64-67.
DeSilva, S.S. and M.K. Perera, 1985. Digestibility in *Sarotherodon niloticus* fry: Effect of dietary protein level and salinity with further observation on variability in daily digestibility. *Aquaculture*, **38**: 293-306.
Duncan, D. B., 1955. Multiple range and multiple F tests. *Biometrics*, **11**: 1-42.
Furukawa, A and H. Tsukahara, 1966. On the acid digestion method for the determination of chromic oxide as an index substance in the study of digestibility in fish feed. *Bull. Jpn. Soc. Sci. Fish.*, **32**: 502-233.
Hajen, W.E., D.A. Higgs, R.M. Beames, B.S., Dosanjh, 1993. Digestibility of various feedstuffs by post-juvenile chinook salmon (*Oncorhynchus tshawytscha*) in sea water: Measurements of digestibility. *Aquaculture*, **112**: 333-348.
Hossain, M.A., N. Nahar, M. Kamal and M.N. Islam, 1992. Nutrient digestibility coefficients of some plant and animal proteins for tilapia (*Oreochromis mossambicus*). *J. Aqua. Trop.*, **7**: 257-265.
Inaba, D, C. Ogino, C. Takamatsu, S. Sugano, and H. Hata, 1962. Digestibility of dietary components in fishes. 1. Digestibility of dietary proteins in rainbow trout. *Bull. Jpn. Soc. Sci. Fish.*, **28**: 367-371.
Jauncey, K., 1982. The effect of varying dietary protein level on the growth, food conversion, protein utilization and body composition of juvenile tilapias (*Sarotherodon mossambicus*). *Aquaculture*, **27**: 43-54.
Kabir, N.M.J., K.L. Wee, and G. Maguire, 1998. Estimation of apparent digestibility coefficients in rainbow trout (*Oncorhynchus mykiss*) using different markers 1. Validation of microtracer F-Ni as a marker. *Aquaculture*, **167**: 259-272.
Kawai, S. and S. Ikeda, 1973. Studies on digestive enzymes of rainbow trout after hatching and the effect of dietary changes on the activities of digestive enzymes in the juvenile stage. *Bull. Jpn. Soc. Sci. Fish.*, **39**: 819-823.
Kikuchi, K., T. Sato, T. Furuta, I. Sakaguchi and Y. Deguch, 1977. Use of meat and bone meal as protein source in the diet of Japanese flounder. *Fish. Sci.*, **63** (1): 29-32.
Law, A. T., 1984. Nutritional study of jelawat, *Leptobarbus hoevenii*, (Bleeker) fed on pelleted feed. *Aquaculture*, **41**: 227-233.
Law, A.T., 1986. Digestibility of low cost ingredients in pelleted feed by grass carp (*Ctenopharyngodon idella*). *Aquaculture*, **51**: 97-103.
Nose, T., 1967. On the metabolic faecal nitrogen in young rainbow trout. Bull. Freshwater fish. Res. lab. (Tokyo) **17**: 97-105.
NRC (Nutritional Research Council), 1983. Nutrient requirements of warm water fishes and shellfishes. National Academy of Sciences, Washington D. C. 102 p.
Ogino, C. and M.S. Chen, 1973. Protein nutrition in fish. III. Apparent and true digestibility of dietary protein by young carp. *Bull. Jpn. Soc. Sci. Fish.*, **39**: 649-651.
Ohta, M. and T. Watanabe, 1998. Effect of feed preparation methods on dietary energy budgets in carp and rainbow trout. *Fish. Sci.*, **64**(1): 99-114.
Sato, T., 1999. Development of formulated feed for juvenile Japanese flounder. Ph.D Thesis, Dept. of Aquatic Biosciences, Tokyo University of Fisheries, Tokyo. 134pp
Smith, B. W. and R. T. Lovell, 1973. Determination of apparent digestibility in feeds for channel catfish. *Trans. Am. Fish. Soc.*, **102**: 831-835.
Smith, R.R., M.C. Peterson, and A.C. Allred, 1980. Effect of leaching on apparent digestion coefficients of feedstuffs for salmonids. *Prog. Fish-Cult.*, **42**: 195-199.

Smith, R.R., R.A. Winfree, G.W. Ramsey, A. Allred and M. Petterson, 1995. Apparent digestion coefficient and metabolizable energy of feed ingredients for rainbow trout, *Oncorhynchus mykiss*. *J. World Aqua. Soc.*, **26**(4): 432-437.

Ustumi, R., 1999. Optimum dietary energy to protein ratio for young Japanese flounder, *Paralichthys olivaceus*. B.Sc. Thesis, Dept. of Marine Science and Resources, Nihon University, Setagaya, Tokyo 154, Japan. 17pp.

Windell, J. T., J. W. Fottz, and J. A. Sakokon, 1978a. Methods of faecal collection and nutrient leaching in digestibility studies. *Prog. Fish-Cult.* **40**: 51-55.

Windell, J.T. , J.W. Foltz and J.A. Sakokon, 1978b. Effect of fish size, temperature and amount fed on nutrient digestibility of a pelleted diet by rainbow trout, *Salmo gairdneri*. *Trans. Am. Fish. Soc.*, **107**(4): 613-616.

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Effects of animal and plant protein diets on growth of Indian major carp (*Labeo rohita* Ham.) fingerlings

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Abstract
An experiment was conducted with *Labeo rohita* fingerlings in an indoor static fish rearing water system of glass made aquaria. Five experimental diets A, B, C, D and E were formulated containing 33% dietary protein level in five treatments each having two replicates containing 12 fingerlings of mean total initial weight of 13.00$\pm$ 0.2g. Sixty days of feeding trial in this experiment showed that fish fed on diet 'A' containing fish meal and diet 'E' containing mixed plant sources protein had significantly highest and lowest growth respectively. However, no significant difference of growth was found in fish fed on diets C and D containing meat and bone meal, and mix of animal protein source diets respectively. The result showed that the apparent protein digestibility (APD) of diets 'A' and 'E' had significantly best and least values respectively. Food conversion ratio (FCR) and protein efficiency ratio (PER) ranged between 1.37 to 2.17 and 1.38 to 2.18 respectively. On the basis of observed FCR and PER diets 'A' and 'E' produced significantly highest and lowest growth respectively.

Key words: *Labeo rohita*, Protein feed

Introduction

Fish meal has been the well recognized major source of protein in commercial fish feeds elsewhere in the world. Unfortunately, use of fish meal as the sole source of protein in fish feed is not feasible in Bangladesh because of its high price and unavailability for the farmer. Moreover, the quality of fish meal available in Bangladesh is below standard having less protein content and higher non-protein nitrogen with rancid odour. Therefore, it is necessary that alternative protein source or sources of cheaper values of fish feeds are to be selected for boosting up the aquaculture production. In this aspect protein concentrate, or meat and bone meals of animal origin can be concerned as the alternative of fish meal supplement in the diet of fish. Moreover, the nutritive value of these feed ingredients are approximately similar to that of fish meal. Therefore, conventional agriculture by-products, like, oil cakes and the bran of cereals and legumes etc. may also be considered as easily available and cheaper viable fish feed ingredients for raising Indian major carps.

In feed formulation and manufacture, it is essential to have a knowledge of nutrient digestibility of various feed stuffs used in formulating fish feed so that effective

substitution of one ingredient for another may be achieved. In Bangladesh, rohu (*Labeo rohita*) has been cultured at farmers level and this fish species is important because of its seed availability, faster growth rate, acceptability by consumers and good market value. However, the nutritional requirements of the fingerlings is yet to be understood for successful culture in Bangladesh. Considering the above aspects, this experiment was designed to study the feeding and nutritional aspects of rohu fish fed on different formulated pelleted animal and plant protein diets and thus to observe various aspects of its growth parameters.

Materials and methods

The feeding trial of the experiment was conducted in static indoor water system in the laboratory of the Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh for a period of sixty days during July through September'98. In this purpose ten glass aquaria of size 90x30x30cm^3 having a capacity of 81L each were used as experimental tank. Tap water was the source of water in the aquaria during experimental period. An adequate level of dissolved oxygen in each aquarium was maintained through artificial aeration by using aquarium air pump (Daivo pump NS 6200). A natural photoperiod of day-night length was maintained throughout the experimental period. The water temperature ranged berween 27 and 29°C during the experiment.

Induced bred fingerlings of Indian major carp, rohu (*Labeo rohita*) were collected from a local fish farm of Mymensingh district. They were then transferred in a round plastic pool of 290L capacity water as stocking tank with artificial aeration. The fishes in the stocking tank were given a prophylactic treatment with 0.5% NaCl dip for 20 minutes followed by a methylene blue bath of 0.5 ppm for 3 days. During acclimatization, the fish fingerligs were fed on formulated pelleted diet containing 33% crude protein at a rate of 1% body weight as maintenance ration. The fish feed ingredients selected to prepare the experimental diets were fishmeal, protein concentrate, meat and bone meal, mustard oil cake, sesame cake, soybean meal, rice bran and wheat flour. Alpha-cellulose, carboxymethyl cellulose and chromic oxide were used as filler, binder and marker of the diets respectively. Before formulation of diets all the selected dietary ingredients were subjected to proximate analysis. Five iso-nitrogenous diets were formulated to contain 33% crude protein from various combination of the different selected ingredients of animal and plant sources (Table 1). Diet A contained fish meal as the sole source of protein, whereas diets B, C, D and E contained protein concentrate, meat and bone meal, mix of 'fish meal, protein concentrate, meat and bone meal' and mixed plant based protein respectively as major sources of dietary protein (Table 1). Required amount of vitamin and mineral premixes (Rhone-Poulenc, Bangladesh) were added in each of the diets during mixing of ingredients for making pellets. The five experimental diets were prepared as pellets by extruding it through 1 mm diameter of diece of a pelleting machine (Alexander work, GKM, Germany). The pellets were dried at 70°C for overnight and kept in plastic bottle for subsequent analysis and uses.

Table 1. Formulation of different experimental diets (33% crude protein level, dry weight basis) and proximate composition of the diets

Ingredients	Diet No.				
	A	B	C	D	E
Fish meal	43			13.6	
Protein conc.		52		17.2	
Meat & bone meal			46.8	15.9	
Mustard oil cake				13	
Soybean meal					49.5
Sesame cake					10.5
Rice bran					15
Wheat flour	30	30	30	30	10
Carboxy methyl cellulose	2	2	2	2	
Testing salt	0.5	0.5	0.5	0.5	0.5
Vit. premix	1	1	1	1	1
Chromic oxide	0.5	0.5	0.5	0.5	0.5
Cellulose	19	10.4	16.2	16	
Soybean oil	4	3.6	3	3.3	
Proximate composition					
Dry matter	92.92	93.3	92.55	93.13	92.28
Crude protein	33.34	33.38	33.08	33.8	33.36
Crude lipid	11.53	10.57	10.96	11.33	12.6
Ash	9.65	15.83	15.89	12.6	13.53
NFE[1]	45.48	40.22	40.07	42.27	40.51
Gross energy (Kcal/g)[2]	4.35	4.06	4.07	4.22	4.25

[1]*Nitrogen free extract calculated as 100-%(moisture+crude protein+lipid+ash)*
[2]*Gross energy calculated after smith(1971) and Pike and Brown (1967)*

Five treatments were scheduled for the experiment. Each treatment had two replicates. Uniform size of 12 rohu fingerlings (1.0±0.1g) from the acclimation tanks were randomly selected, weighed on a precision balance and thereby a mean total initial weight of 13.0±0.2g was found and distributed in each aquarium. The experimental tanks were designated as replicate A1, A2; B1, B2; C1, C2; D1, D2; and E1, E2 (as two replicates for one treatment) for the fish of five treatments to be reared and fed on diets A, B, C, D and E respectively. The proximate composition of the five diets are shown in Table 1. The water quality parameters such as dissolved oxygen, pH and temperature were monitored weekly throughout the experimental period. The fishes were fed with the formulated diets up to satiation level twice daily at 6 hours interval between 09.00 and 15.00 hours. Faecal matters in the aquaria were cleaned by siphoning before feeding started in the morning. For the growth responses of fish in each replicate group the bulk weight was recorded at every 10^{th} day until the feeding trial was finished.

Faeces were collected during last two weeks of the experimental period to study the protein digestibility of feeds. Chromic oxide in the diets and faeces was determined by using wet digestion techniques of Furukawa and Tsukahara (1966). Apparent Protein

digestibility (APD) of experimental diets were then calculated using the formula of Maynard and Loosli (1969).

At the start of the experiment 50 fingerlings from the stocking tank was randomly collected, sacrificed and treated for initial proximate composition analysis of the experimental fish. The proximate composition of the dietary ingredients, diets, faeces and the fish samples were analyzed according to standard procedures given in AOAC (1990). Statistical analysis of the data were done by one way analysis of variance (ANOVA) and Duncan's Multiple Range Test (Zar1984) to observe the significance of variation between the treatment means.

Results

Percent weight gain of *L. rohita* fed on five different diets A, B, C, D and E during 60 days feeding trial is shown in Figure 1. The highest and the lowest percent growth were observed in fishes fed the diet A containing fish meal and the diet E containing plant protein ingredients respectively. The growth and feed utilization of *L. rohita* in response of different formulated diets are presented in Table 2. Maximum mean percent weight gain during the 60 days of feeding trial was observed in the group of fish fed on diet A which contained fishmeal as a source of dietary protein. The mean percent weight gain of fishes fed on diet A, B, C, D and E were 100.53, 81.90, 73.87, 79.25, 63.85% respectively. Fish fed diet A produced significantly ($p<0.05$) the highest (1.16) specific growth rate (SGR) value than the others. However, there was found no significant differences ($p>0.05$) between the SGR value of fish fed diets C and D.

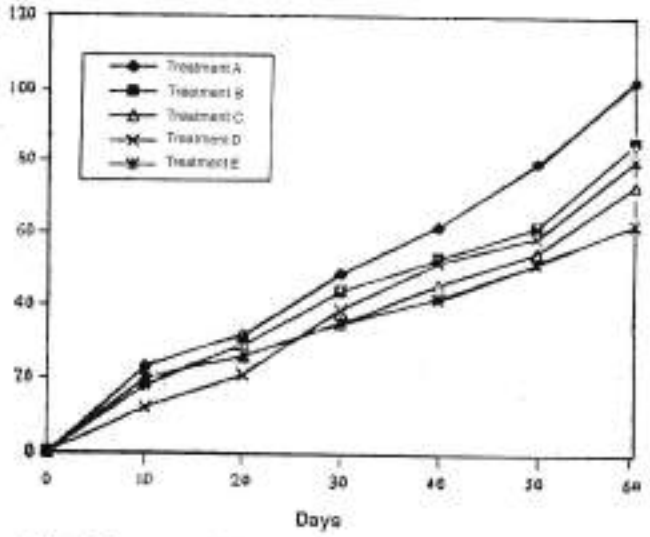

Fig. 1. Percent weight gain of *L. rohita* fed on different diets A, B, C, D and E during 60 days feeding trial

Table 2. Growth and food utilization by *L. rohita* fed five experimental diets for 60 days of feeding trial

Parameters	Treatments				
	A	B	C	D	E
Initial weight (g)	13.1	13.05	13.05	13.2	13.00
Final weight (g)	26.26	23.73	22.69	23.66	21.30
Weight gain	13.16	10.68	9.64	10.46	8.30
% weight gain	100.53a	81.90b	73.87bc	79.25bc	65.8c
Amount of feed fed during experiment (g)	18.08	18.08	18.08	18.08	18.08
Specific growth rate (SGR)	1.16a	1.00	0.92bc	0.97bc	0.82c
Food conversion ratio (FCR)	1.37a	1.69b	1.88bc	1.72bc	2.18c
Protein efficiency ratio (PER)	2.18a	1.76b	1.60bc	1.71bc	1.37c
Apparent net protein utilization (ANPU%)	47.12a	26.01b	16.43bc	15.83bc	12.9bc
Apparent protein digestibility (APD%)	81.70a	78.66ab	69.96bc	72.53bc	75.53bc

**Figures in the same row having the same superscripts are not significantly different ($p>0.05$)*

The lowest FCR value in treatment A was significantly different ($p<0.05$) from other values and therefore was the best FCR value among the diets. The diet A had significantly different ($p<0.05$) FCR value than the diets B, C, D and E. However, no significant differences ($p>0.05$) were found between the apparent net protein utilization values of diets C, D and E (Table 2). It is seen that the ANPU value with fish fed ranged from 12.93 to 47.12% (Table 2) showing significantly higher ($p<0.05$) in the diet A than those of diets B, C, D and E. The PER values ranged from 1.37 to 2.18 having significantly ($p<0.05$) highest and lowest in the fish fed on the diets A and E respectively (Table 2). Significantly different ($p<0.05$) and highest APD value was obtained with diet A containing fish meal, while the diet D and E showed no significantly different ($p>0.05$) values. However, the comparatively lower APD value was obtained with diet C containing protein concentrate. Proximate carcass composition of fish at the start and at the end of the experiment is shown in Table 3. Fish fed on the diet A produced the highest lipid content during growth trial while the fish fed on the diet C gained the lowest lipid content. However, fish fed on diet A had highest carcass protein content followed by diets D, B, E and C respectively.

Table 3. Carcass composition of experimental fishes at the end of feeding trial (% dry matter basis)

Treatments	Initial	A	B	C	D	E
Moisture (%)	91.00	94.00	94.30	94.80	94.7	94.2
Protein (%)	68.13	69.32	68.41	68.01	68.80	68.19
Lipid (%)	7.6	11.46	10.71	10.02	10.03	10.17
Ash (%)	24.27	19.22	20.88	21.97	21.17	21.64

Discussion

In this experiment 33% crude protein level was scheduled in the formulated diet which was very near to dietary protein of 34% for the same fingerlings *of Labeo rohita* (Khan *et al.* 1991). Silva and Gunasekera (1991) conducted an experiment on the economically optimal growth of *Labeo rohita* comprising with 31% crude protein diets. In another study, significantly improved growth of *Labeo rohita* at 29% of protein level (Mohanty and Swamy 1996) was reported. Therefore, to get an optimal growth response of the fish species, selection of 33% crude protein level in the diet of this experiment was a justified amount.

Growth performance of experimental fish was recorded at every 10th day. The best growth was obtained with fish fed on diet 'A' containing fish meal whereas there were no significant difference among the growth of fish fed on diet C and diet D containing meat and bone meal, and fish meal plus protein concentrate plus meat and bone meal respectively. Similarly, better growth performance was observed in *Cyprinus carpio* var. *Communis* fed on 50% fish meal and 44% rice bran diet and having no significant change of growth with other diets containing plant protein sources (Manissery *et al.* 1988).

It is shown in Fig. 1 that the growth performance of fingerlings fed on diet 'A' exhibited a significantly high increase all along the experimental period. On the contrary, fish fed on diet C and D did not have any significant change on growth, whereas, least growth was found in fish fed on plant source protein (Diet E). Such least growth might be due to the fact that fish fed on diet 'E' containing plant sources had least digestible protein. Similar study was observed by Ferraris *et al.* (1986). Other Plant source protein like *Leucaena leucocephala* leaf meal was the least digestible ingredient (10-40%).

The FCR in the present study ranged between 1.37 and 2.18%. The diet A showed significantly least FCR value than others. The FCR in the present study are lower than those reported by Ahmed *et al.* (1983) and Rangacharyulu *et al.* (1991) with Indian major carp *Labeo rohita*. The FCR in the present study are a little higher than those reported by Biswas (1997) with *Puntius gonionotus*. The ANPU values reported by Hossain *et al.* (1994) and Biswas (1997) for *P. gonionotus* fed diets containing various levels of oil seed meals.

The apparent protein digestibility values (APD) were fairly high which ranged between 69.96 to 81.70%. The fish meal based diet A in the present study produced the highest APD value (81.70). The meat and bone meal based diet C produced the lowest APD value (69.96). According to NRC (1997) carp digest up to 90% of fish meal protein. However, this value may be decreased depending on the origin and processing of fish meal involved (Ogino and Chen 1973). In this study comparatively higher amount of carcass lipid was found in fish fed on diet A containing fish meal (Table 3). Similar result of lipid increment was obtained with *P. gonionotus* fed on different diets containing treated and untreated Soybean meal (Chisty 1997). Biswas (1997) also obtained similar trend of increase of carcass lipid content in *P. gonionotus* fed on similar diets containing fish meal, meat and bone meal, protein concentrate, mix of animal protein and mixed plant protein diet.

Considering the different growth parameters the result of the present study demonstrated that fish meal based diet (Diet A) plays a significant role in growth performance in *L. rohita* among the different diets, whereas, plant based dietary protein produced the lowest growth. From the economic point of view, it can be concluded that the diet B containing protein concentrate as a major protein source may be considered as a substitute of fish meal diet.

References

Ahmed, G.U., A.K.M.A. Haque, M.A. Islam and M.M. Haque, 1983. Intensive culture of *Labeo rohita* (Ham.) in floating ponds with special references to different stocking densities. *Bangladesh J. Fish.*, **6**(1-2):11-17.

AOAC (Association of Official Analytical Chemists). 1990. Official Methods of Analysis of the Association of Analytical Chemists. 15th edition (ed. K. Helrich). Published by the Association of Official Analytical Chemists, Inc., Suite, 400, Arlington, Virginia, 2: 685-1298.

Biswas, M.R., 1997. Animal and plant protein diets in the growth of *Puntius gonionotus* fingerlings. M.S. thesis submitted to Department of Fisheries Technology. Bangladesh Agricultural University, Mymensingh. 61 pp.

Chisty, M.A.H., 1997. Use of treated and untreated soybean meal as fish mea substitute in the diet of Thai sarputi (*Puntius gonionotus*) in the seasonal ponds. M.S. Thesis. Bangladesh Agriculture University, Mymensingh. 77 pp.

Ferraris, R. P., M.R. Catacutan, R.L. Mebelin and A.P. Jazul, 1986. Digestibility in milkfish, *Chanos chanos* (Forsskal): Effects of protein source, fish size and salinity. *Aquaculture*, **59**(2): 93-105

Furukawa, A. and H. Tsukahara, 1966. On the acid digestion method for determination of chromic oxide as an indicator substance in the study of digestibility in fish. *Bull Jap. Soc. Sci. Fish.*, **32**:502-506.

Khan, M.A., A.K. Jafri, M.F. Anwar, M.A. Hasan and M. Erfanullahh, 1991. Nutritional efficiency of oilcakes in diets compounded for fingerlings of Indian major carp *Labeo rohita* (Ham.). Proc. of National Symposium on Horizons in Freshwater Aquaculture. 23-25 January, pp. 73-77.

Hossain, M. A., S.A. Chowdhury, M. Kamal and M.N. Islam, 1994. Evaluation of oilseed meals as dietary protein source of Thai sharpunti, *Puntius gonionotus* (Bleeker). *Bangladesh J. Zool.*, **22**(1): 79-88.

Manissery, J.K., B.S.N. Gowda, K.M. Shankar and J.J. Bharghese, 1988. Growth of common carp (*Cyprinus carpio* var. Communis) fed on feed incorporated with *Cassio tora* leaves. *Indian J. Anim. Sci.*, **58**(6): 737-740.

Mohanty, S. N. and D.N. Swamy, 1986. Enriched conventional feed for Indian major carps. Proceedings of the First Asian Fisheries Forum, Manila, Philipphines, 26-31 May, 1986.

NRC (National Research Council), 1977. Nutrient requirement of warm water fishes. National Academy of Sciences. Washington, D.C., 78 pp.

Ogino, C. and M. S. Chen, 1973. Protein nutrition in fish, III. Apparent and true digestibility of dietary protein in carp. *Bull. Jap. Soc. Sci. Fish.*, **39**: 649-657.

Pike, R.L. and M.L. Brown, 1967. Nutrition: an integrated approach. John Willey and Sons. New York. 542 pp.

Moynard, A.L. and K. J. Loosli, 1969. Animal nutrition. 5th Edition. McGraw Hill, New York.

Rangacharyulu, P. V., S. Sarker, S.N. Mohanty, K.M. Das and P.K. Mukhopadhayay, 1991. Growth and protein utilization in rohu, *Labeo rohita* under different feeding levels, National Symposium on new Horizons in Fresh water. *Aquaculture*, **91**: 95-97.

Silva, S. S. De. and R.M. Gunasekera, 1991. An evaluation of the growth of Indian and Chinese major carps in relation to the dietary protein content. *Aquaculture*, **92**: 237-241.

Smith, R. R., 1971. A method for measuring digestibility and metabolizable energy of fish. *Prog. Fish. Cult.*, **33**:132-134.

.Zar, J. H.,1984. Biostatistical Analysis (Second edition). Prentice Hall International, Inc New Jersey. 718 pp.

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Effect of different feeds on growth, survival and production of African catfish (*Clarias gariepinus* Burchell)

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Abstract
An experiment was conducted to study the effect of different feeds on growth survival and production of African catfish (*C. gariepinus*) in six cemented tanks (3 m^2 each) over a period of 120 days. Three different feeds namely Feed A (Saudi-Bangla fish feed, 33.43% protein), Feed B (formulated feed, 40.12% protein) and Feed C (chicken raw intestine, 59.58% protein) were applied to treatments I, II and III respectively. Each of the tanks was stocked with 24 fry with mean initial body weight of 2.56 ±0.06 g. Feeds were supplied to the fish *ad- libitum* daily in two instalments. Significantly highest weight gain was obtained in treatment III, however, survival rate was low compared to other treatments. The feed conversion ratio (FCR) values ranged from 2.52-6.4. Survival rate of fish varied between 83 and 96%. Treatment II yielded the highest (5000 kg/ha/120 days) production with the highest survival rate of fish. On the basis of survival rate and production, it is suggested that the formulated feed (Feed B) is suitable for the culture of *C. gariepinus* in cemented tanks.

Key words: *Clarias gariepinus*, Feed

Introduction

The development of quality fish feed is a key to the success in commercial fish culture. A large number of indigenous raw materials mainly poultry by-product meal, blood meal, various oilcakes, cereal by-products, leaf meals etc. are available in the country (Akand *et al.* 1991). These raw materials can be used in developing supplemental feed for rearing and culture of different fish species (Bhadra *et al.* 1997).

African catfish, *Clarias gariepinus* is very popular for aquaculture in a number of African, Asian and European countries (Huisman and Rechter 1987). This popularity stems from several characteristics of the fish, which includes wide distribution, ability to tolerate poor water quality conditions, adaptability to overcrowding, extremely high yields and good response to the artificial feed. For the good result of this fish production, good quality artificial feed is essential and requires protein level of 35-45% in feed (Degani *et al.* 1989). The aim of the present investigation was to evaluate the suitability of different feeds for African catfish (*C. gariepinus*).

Materials and methods

The experiment was carried out over a period of 120 days in cemented tanks, belonging to Department of Aquaculture, Bangladesh Agricultural University, Mymensingh during the month of August to December'95.

Six cemented tanks each of size 3 m^2 were used for the growth trial. For convenience of study, the tanks were numbered as 1, 2, 3, 4, 5 and 6. Water level in each tank was maintained 1.00 m throughout the experimental period by adding freshwater from a deep tubewell. In each tank, earthen plate was hung from a pipe just half metre below the surface for supplying the feed daily.

The tanks were divided in to three treatments namely I, II and III each having two replicates. Three different feeds namely Feed A, Feed B and Feed C were applied to the treatments I, II and III respectively. *C. gariepinus* fry used in the present study were obtained from Faculty of Fisheries, BAU, Mymensingh. Fish fry of 2.56±0.06 g size were randomly distributed to the tanks at the rate of 8/m^2. Fish, during the experimental period, were fed up to satiation twice daily.

Feed A was the pelleted catfish feed collected from Saudi-Bangla Fish Feed Ltd. Bhaluka, Mymensingh. Feed B was prepared with the combination of various indigenous ingredients i.e. fishmeal 40%, blood meal 10%, mustard oilcake 30%, rice bran 10%, wheat flower 3.5%, powder milk 1.5%, table salt 0.5%, vit. & mineral premix 3%, casein 0.5% and molasses 1%, maintaining 40% crude protein level. Prior to preparation of Feed B all the dietary ingredients were subjected to proximate analysis and the results are presented in Table 1. All the collected ingredients were ground finely with a mortar and sieved to be passed through 0.5 mm mesh. All the ingredients were mixed thoroughly and adequate amount of water was added in it. Then, the diet was prepared by using pelleting machine (Hobart Mixture Machine Model A 200). The resultant pellets were then sun dried for 2 days and kept in an air tight polyethylene bag in a deep freezer for further use. Feed C was chopped chicken intestine (raw) bought from BAU Co-operative market.

Table 1. Proximate composition of the protein sources (% dry matter basis) used

Ingredients	Composition					
	Dry matter	Protein	Lipid	Ash	Crude fibre	NFE*
Fish meal	90.60	50.47	20.98	21.81	1.24	5.50
Blood meal	95.52	90.02	1.40	6.48	-	2.1
Mustard oil cake	85.55	30.33	13.44	9.73	12.12	34.68
Rice bran	90.44	10.94	17.23	21.81	23.09	26.93
Wheat floor	90.07	17.78	3.9	1.6	1.12	75.60

** Nitrogen free extract (NFE) calculated as*
100- %(moisture + protein + lipid + ash + crude fibre)

All the feeds were analysed for proximate composition using standard method given in AOAC (1980) and the results are presented in Table 2.

Table 2. Proximate composition (% dry matter basis) of experimental feeds

Feeds	Composition					
	Dry matter	Protein	Lipid	Ash	Crude fibre	NFE*
Feed- A	88.33	33.43	8.53	13.49	12.80	31.75
Feed- B	89.70	40.12	10.25	8.30	9.3	32.03
Feed- C	22.71	59.58	17.78	6.79	ND	15.85

** Nitrogen free extract (NFE) calculated as 100- %(moisture +protein + lipid + ash + crude fibre)*
ND = Not determined

Fortnightly temperature and DO were measured by DO meter (YSI, model 58, USA), pH was recorded by pH meter (Jenway, model 3020, UK) and NH_3-N was estimated according to phenol-hypochloric method (Stirling 1985).

Sampling of fish during feeding trial was done fortnightly by using cast net and 40% of total population from each tank were caught and then length and weight of individual fish were measured carefully. The water of the tanks were replenished fortnightly by draining the water of the tanks for removing unused feed and faeces.

The one-way analysis of variance (ANOVA) was used to determine the suitability of different feeds on the growth of fish. This was followed by Duncan's New Multiple Range Test (Duncan 1955) to identify the level of significance of variation among the treatment means. Standard errors (±SE) of treatment means were calculated from the residual mean square in the analysis of variance.

Results

The values of water quality parameters *viz.* water temperature, dissolved oxygen, pH and NH_3-N are presented in Table 3. Water temperature of the experimental tanks was found to vary from 17.5-30°C. The highest temperature was recorded in the treatment II and lowest was recorded with the treatment I. The range of dissolved oxygen values of water was 4.7 - 8.7 mg/l, highest in treatment II and lowest in III. pH of water was recorded around neutral 6.5-7.8. The values of NH_3-N varied from 0.03-0.59 ppm, highest in treatment II and lowest in I.

Table 3. Ranges of water quality parameters in different treatments during the experiment

Parameters/ Treatments	I	II	III
Temperature (°C)	17.8-29.9	18.0-30.0	17.5-29.2
Dissolved oxygen (mg/l)	5.1-7.5	5.7-8.7	4.7-7.8
pH	6.5-7.3	6.7-7.2	6.8-7.8
NH_3-N	0.05-0.52	0.03-0.59	0.04-0.58

The growth performance of *C. gariepinus* in terms of weight, specific growth rate (% per day), feed conversion ratio (FCR), survival rate and total production are shown in Table 4.

Table 4. Growth, survival and production of fish in different treatments during the study period

Parameters/ Treatments	I	II	III	±SE[1]
Initial weight (g)	2.6[a]	2.6[a]	2.5[a]	0.01
Final weight (g)	64.1[b]	65.4[b]	72.1[a]	0.47
Weight gain (g)	61.5[b]	62.8[ab]	69.6[a]	0.17
Specific growth rate (% per day)	2.67[b]	2.69[b]	2.80[a]	0.03
Feed conversion ratio (FCR)	2.5[b]	3.1[ab]	6.4[a]	0.34
Survival rate (%)	88[a]	96[a]	83[a]	5.62
Production (kg/ha/120 days)	4500[a]	5000[a]	4800[a]	3.15

[1]*Standard error of treatment means calculated from the residual means square in the analysis of variance.*
[2]*Figure in the same row having same superscripts are not significantly different (P>0.05).*

The weight gain was significantly (P>0.05) highest (69.60 g) in treatment III and lowest (61.50 g) in treatment I. The specific growth rate (% per day) of fish in different treatments varied between 2.67 and 2.80. Significantly (P<0.05) the highest value was obtained in treatment III and lowest in treatment I. The mean feed conversion ratio (FCR) values in different treatments varied from 2.5 to 6.4 with treatment III producing significantly (P<0.05) the poorest (6.4) FCR. However, significantly better FCR (2.5) was obtained in treatment I followed by treatment II (Table 4). The survival rate of fish did not vary significantly (P>0.05) among the treatments. However, the highest survival rate of fish (96%) was observed in treatment II and the lowest (83%) in treatment III. The highest fish production (5000 kg/ha/120 days) was obtained in treatment II followed by treatment III & I respectively. However, there was no significant difference (P>0.05) in total fish production among the treatments.

Discussion

African catfish can tolerate temperatures as low as 6°C and as high as 50°C (Babiker 1984). During the period of investigation, water temperature varied from 17.5-30°C. Mollah (1984) found highest growth of *C. gariepinus* at the temperature of 28-30°C whereas Henken *et al.* (1986) found best result of *C. gariepinus* at temperature ranges from 24-29°C. The range of water temperature recorded during the study period was quite fluctuating which showed minimum value of 17.5°C in December. The ranges of dissolved oxygen values recorded in the present study were 4.7-8.7 mg/l. Lakshmanan *et al.* (1967) reported the dissolved oxygen content of water ranging from 6.7-8.3 ppm were at satisfactory level of fish production. Dissolved oxygen contents of water in the present investigation were within the productive range (Dewan *et al.* 1991). The ranges of pH values recorded in the present study are more or less similar to that reported by Dewan *et al.* (1991) and Lakshmanan *et al.* (1967). Ammonia-nitrogen (NH_3-N) is toxic for fish and above a certain level in water it can cause mortality in fish. In culture condition lower the value of NH_3-N, the better the quality of water for fish (Alabaster and Lloyd 1980). Chen (1988) suggested to bring down ammonia-nitrogen content in pond water to less

than 1 ppm. In the present study, the NH_3-N content was 0.03-0.59 ppm, which was acceptable for fish culture.

Growth rate of *C. gariepinus* was highest in treatment III receiving chopped chicken intestine (Feed C). The increasing trend of mean weight gain in fish was obtained during first two months of culture period and there was gradual decline in growth increment until harvesting. This decline may be due to decrease in temperature of water. Average weight gain by fish in the present study was higher than that the growth (53 g) recorded by Hecht and Appelbaum (1987) in *C. gariepinus* fed fish meal based diet (40.56% protein) whereas growth of 494.3 g recorded by Tangtrogpairos *et al.* (1988) in 141 days of rearing of *C. gariepinus* was much higher with artificial diet containing 45% protein. The lowest growth performance of fish fed Feed A might be due to the fact that this feed was less acceptable to *C. gariepinus*. Similar result was obtained by Bhadra *et al.* (1996) in *C. gariepinus*. In the present study, the growth of fish increased with the increase of dietary protein level and recorded highest growth with Feed C containing 59.58% protein, which was similar to that reported by Sanaullah *et al.* (1986).

The specific growth rate (% per day) of fish in all treatments was unsatisfactory. As water temperature has direct effect on the growth and metabolism of fish, fish during the culture period of last two months showed minor increment in growth until harvesting. However, the highest SGR (% per day) value was found in treatment III because feed contained 59.58% crude protein level. Unlike this, Bhadra *et al.* (1997) obtained satisfactory SGR value (6.42-7.43) of *C. gariepinus* fingerlings with formulated feed (49% protein).

Feed conversion ratio is a measure of diet efficiency. The more suitable the diet the less feed is required to produce a unit weight gain i.e. lower feed conversion ratio. FCRs of all the treatments ranged from 2.5 to 6.4. The FCR values obtained in treatment III are more or less similar to that reported by Bolock (1973) for *C. lazera* with supplementary feed.

The survival rate of fish fed with different feeds ranged from 83 to 96%, with highest value in treatment II receiving Feed B. The similar survival rate (96%) of *C. gariepinus* was recorded by Polling *et al.* (1988) with supplementary live foods.

The total production of fish obtained in the present study ranged from 4500-5000 kg/ha/120 days. The highest production of fish was obtained in treatment II might be due to the greater survival of fish fed with formulated feed. However, the production obtained in the present study was lower (97,000 kg/ha/yr) than that reported by Viveen *et al.* (1985) in *C. gariepinus* fed with artificial diet (45% protein).

References

Akand, A.M., M. Soeb, M.R. Hasan and M.G. Kibria, 1991. Nutritional requirements of Indian major carp, *Labeo rohita* (Hamilton). Effect of dietary protein on growth, food conversion and body composition. *Agriculture International*, 1: 35-43.

Alabaster, J.S. and R. Lloyd, 1980. Water quality criteria for freshwater fish. Butterworths, London, UK. 271 pp.

AOAC, 1980. Offical Methods of Analysis. (ed. W. Horwitz). *Association of Official Analytical Chemists*, 13th edition, Washington, D.C. 1018 pp.

Babiker, M.M., 1984. Aspects of the biology of the catfish *Clarias lazera* related to its economic cultivation. *Hidrobiologia*, **110**: 295-304.

Bhadra, A., M.A. Hossain, M. Kamal and M.A. Rahman, 1996. Growth and survival of African catfish (*Clarias gariepinus*) on different formulated feed and live food mixtures. *Progress. Agric.*, 7(1) 47-51.

Bhadra, A., M.A. Hossain, M. Kamal and G.U. Ahmed, 1997. Growth and survival of African catfish (*Clarias gariepinus*) on formulated feed. *Bangladesh J. Agril. Sci.*, **24**(1): 123-129.

Bolock, A.R.E.I.., 1973. Rearing of the Nile catfish *Clarias lazera* to marketable size in Egyptian experimental ponds, Institute of Oceanography and Fisheries Academy of Scientific Research and Technology. Cairo, Egypt, pp. 612-620.

Brown, M.E., 1957. The Physiology of Fishes. Vol. I. Academic Press Inc., New York. 447 pp.

Chen, L.C., 1988. Aquaculture in Taiwan. Fishing News Books, London. 273 pp.

Degani, G., Y. Ben-Zvi and D. Levanon, 1989. The effect of different protein levels and temperature on feed utilization, growth and body composition of *Clarias gariepinus* (Burchell 1822). *Aquaculture*, **76**: 293-301.

Dewan, S., M.A. Wahab, M.C.M. Beveridge, M.H. Rahman and B.K. Sarkar, 1991. Food selection, electivity and dietary overlap among planktivorous Chinese and Indian major carp fry and fingerlings grown in extensively managed rain-fed ponds in Bangladesh. *Aquacult. Fish. Managt.*, **22**: 277-294.

Duncan, D.B., 1955. Multiple range and multiple F-Tests. *Biometrics*, **11**: 1-42.

Hecht, T.S. and S. Appelbaum, 1987. Notes on the growth of Israeli sharptooth catfish (*Clarias gariepinus*) during the primary nursing phase. *Aquaculture*, **63**: 135-204.

Henken, A.M., M.A.M. Machiels, W. Deeker and H. Hogendoorn, 1986. The effect of dietary protein and energy content on growth rate and feed utilization of the African catfish, *Clarias gariepinus* (Burchell 1922). *Aquaculture*, **58**: 55-74.

Huisman, E.A. and C.J.J. Richter, 1987. Reproduction, growth, health control and aquaculture potential of the African catfish, *Clarias gariepinus* (Burchell 1922). *Aquaculture*, **63**: 1-14.

Lakshmanan, M.A.V., B.R. Bhuyan, S.R. Krishna and N. Bau, 1967. Survival and growth of cultivated fishes in Assam ponds. *Indian J. Fish.*, **14**: 1-23.

Mollah, M.F.A., 1984. Effect of water temperature on the growth and survival of catfish (*Clarias macrocephalus*) larvae. *Indian J. Fish.*, **31**(1): 66-73.

Polling, L., H.J. Schoonbee, J.F. Prinsloo and A.J.B. Wiid, 1988. The evaluation of live feed in the early larval growth of the sharptooth catfish, *Clarias gariepinus*. *Water SA.*, **14**(1): 19-24.

Sanaullah, A.A.S.M., M.A. Mazid, M.A. Rahman, S. Gheyasuddin and S.C. Chakraborty, 1986. Formulation of quality fish feeds from indigenous raw materials and their effects on the growth of catfish, *C. batrachus*. *Bangladesh J. Fish.*, **9**(1-2): 39-46.

Stirling, H.P., 1985. Chemical and biological methods of water analysis for aquaculture. Institute of Aquaculture, University of Stirling. KF9 4LA, Scotland, 119 pp.

Tangtrongpairos, M., S. Luanprida and S. Nukhwan, 1988. Breeding and nursing of African sharptooth catfish, *C. gariepinus*. *Thai Fisheries Gazette*, **41**(6): 535-544.

Viveen, W.J.A.R., C.J.J. Richter, P.G.W.J. Van Oordt, J.A.L. Jansen and E.A. Huisman, 1985. Manual for the culture of the African catfish (*Clarias gariepinus*). Directorate General for International Technical Cooperation, the Hogue, The Netherlands. 93 pp.

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Development of low-cost feed for culture of giant freshwater prawn (*Macrobrachium rosenbergii* de Man)in ponds

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Abstract
An experiment was conducted for 105 days in 12 earthen mini ponds of each 30 m^2 size. Five different experimental diets containing 32% protein were formulated and prepared using fishmeal, shrimp meal, soybean meal, mustard oil cake, sesame meal, wheat bran and rice bran. A commercial shrimp diet (SABINCO starter-III) was assigned to treatment six and considered as the control. Prawns were stocked at the rate of 2.5 fry/ m^2 and feed twice daily at the rate of 10% at the beginning and reduced to 8% for the last two months. The results of the experiment showed that prawn fed diets 1, 2, and 6 (control) showed significantly ($P<0.05$) highest weight gain among the dietary groups, while prawn fed diet 5 showed significantly lowest weight gain. The FCR values of diets ranged between 3.06 to 4.85. Prawns fed diet 1 and 6 showed significantly higher SGR, survival (%) and production among the dietary groups. The survival (%) of the prawns ranged between 46.6 to 66.6% and the production ranged between 304.5 to 563.3 kg/ha/105 days. The result of the study showed that diet containing 30% fishmeal, 5% shrimp meal, 5% soybean meal, 10% mustard oil cake, 10% sesame meal, 20% wheat bran, 18% rice bran, 1% oyster shell and 1% vitamin premix may be recommended for monoculture of *M. rosenbergii*.

Key words: *M. rosenbergii*, Monoculture, Low-cost feed

Introduction

Although fish culture in ponds and tanks are known in Bangladesh for centuries, freshwater prawn farming has not been expanded widely compared to that of the carp farming. Among the factors that have slowed the expansion of freshwater prawn farming the scarcity of prawn fry (PL), unavailability of formulated pelleted diet and high price of the diet are considered as the main factors. In recent years some hatcheries and fish feed industries have been developed which are contributing effectively to reduce scarcity of prawn fry and the unavailability of the pelleted diet. But the pelleted rations are quite expensive. As a result most of the farmers of Bangladesh are not interested to culture freshwater prawn in their ponds, though the economic value and the market demand of freshwater prawn, *M. rosenbergii* is much higher than that of carp. However, the present

trend shows that freshwater prawn farming is becoming a significant and expanding industry (New 1991).

There is a great potential or successful monoculture of freshwater prawn in numerous ponds of Bangladesh. Now-a-days the production of prawn from natural sources are decreasing day by day. So, scientific prawn culture should be introduced in freshwater ponds. Although some works have been done on the rearing of *M. rosenbergii* with different supplemental feeding and stocking densities (Khan *et al.* 1984, Humayun *et al.* 1986, Mazid *et al.* 1989, Islam *et al.* 1990 and Hoq *et al.* 1996); most of them are on polyculture. Therefore, the present study was undertaken to develop a suitable low-cost diet from indigenous sources for monoculture of *M. rosenbergii* in ponds.

Materials and methods

The experiment was conducted in 12 earthen rain-fed mini ponds of each 30 m^2 size situated behind the Fisheries Faculty Building, Bangladesh Agricultural University (BAU) Campus, Mymensingh during the month from July to October'98. The water depth was maintained to maximum of 1.2 m using fine meshed PVC overflow pipe (2 inches diameter) on the bank fixed at 1.2 m above the pond bottom. All the ponds were of similar size, depth, basin configuration and the bottom type including water supply facilities from a deep tubewell. Undesirable species in the ponds were eradicated by repeated netting followed by application of rotenone (20 g/40m^2). Liming was performed at the rate of 1 kg/40m^2.

For formulation of experimental diets, different feed ingredients such as fish meal, shrimp meal, soybean meal, mustard oil cake, sesame meal, wheat bran, rice bran, oyster shell and vitamin premixes were collected from Dhaka and Mymensingh local markets. The fish meal was of German origin. These ingredients were analysed for their proximate composition (Table 1).

Table 1. Proximate composition of the feed ingredients used (% dry matter basis)

Ingredients	Dry matter	Protein	Lipid	Ash	Crud fibre	NFE[1]
Fish meal	90.12	56.00	14.82	24.10	2.22	2.86
Shrimp meal	92.40	60.10	13.14	22.13	2.14	2.49
Soybean meal	91.26	48.02	18.48	7.36	7.10	19.04
Mustard oilcake	89.12	32.05	12.61	10.40	11.64	33.3
Sesame meal	90.21	28.11	9.42	15.62	22.41	24.44
Wheat bran	89.24	14.24	5.60	6.24	15.26	58.66
Rice bran	90.44	12.40	8.82	10.48	16.34	51.96

NFE[1] = Nitrogen free extract calculated as- 100 - %(moisture + protein + lipid + ash + crude fibre)

Five experimental diets were formulated to contain 32% protein (Table 2.). Diets were also formulated to be isoenergic as far as possible to maintain similar energy levels. A SABINCO shrimp diet (starter-III) from Saudi-Bangla Fish Feed Ltd, Bhaluka, Mymensingh was used as the control. All the collected ingredients were ground finely and sieved through 0.5 mm mesh. After sieving all the required amount of dry

ingredients along with vitamin premix were weighed according to the formulae of experimental diets. The well mixed ingredients were then put into the pellet mill for the preparation of pelleted feed of size 3 mm.

Table 2. Formulation of experimental diets used in Monoculture experiments of *M. rosenbergii*

Ingredients	Diet- 1	Diet- 2	Diet- 3	Diet- 4	Diet- 5
Fish meal	30.0	25.0	20.0	15.0	10.0
Shrimp meal	5.0	5.0	5.0	5.0	5.0
Soybean meal	5.0	10.0	15.0	20.0	25.0
Mustard oilcake	10.0	10.0	12.0	12.0	12.0
Sesame meal	10.0	10.0	12.0	12.0	12.0
Wheat bran	20.0	20.0	20.0	20.0	20.0
Rice bran	18.0	18.0	14.0	14.0	14.0
Oyster shell	1.0	1.0	1.0	1.0	1.0
Vitamin premix[1]	1.0	1.0	1.0	1.0	1.0
Total	100.0	100.0	100.0	100.0	100.0

[1] *"Embavit" fish premix obtain from Rhone-Poulenc (Bangladesh)*

About 0.3 g size PL of *M. rosenbergii* were collected from Chandpur which were originally collected from Dhakatia river. These PLs were brought to Mymensingh in oxygen bags. Ponds were divided into six treatments viz., 1,2,3,4,5 and 6 each having two replicates. *M. rosenbergii* PL of 0.3±0.02g size were stocked at the rate of 2.5 larvae/m^2 in the ponds. Prawns were fed at the rate of 10% of body weight at the beginning. The feeding rate was reduced to 8% of the body weight for the last two months. Fortnightly random sampling was done using seine net to monitor growth of prawn and to adjust the feeding rate. Water quality parameters such as temperature, dissolved oxygen and pH were monitored fortnightly and the ranges were : temperature 28.7 to 31.5°C, dissolved oxygen 3.0 to 6.1mg/e and pH 6.8 to 8.4.

The proximate composition of feed ingredients and experimental diets were analyzed according to the methods given in Association of Official Analytical Chemists (AOAC 1980). One way analysis of Variance (ANOVA) was used to determine the effects of feed on the growth of prawn. This was followed by Duncan's New Multiple Range Test (Duncan 1955) at 5% level of significance to observe any difference among treatment means. Standard error (± SE) of treatment means were calculated from the residual means square in the analysis of variance.

A simple economic analysis was performed to estimate the net profit from monoculture system of prawn. The cost of production was based on the Mymensingh whole sale market price (1998) for the inputs used. The cost of prawn larvae was : Tk. 4/PL. The cost of feed ingredients was: (a) fish meal: Tk. 30/kg, (b) shrimp meal: Tk. 40/kg, (c) soybean meal: Tk. 12/kg, (d) sesame meal: Tk. 7/kg, (e) mustard oilcake: Tk. 6/kg, (f) rice bran: Tk. 4/kg, (g) wheat bran: Tk. 5/kg, (h) oyster shell: Tk. 7/kg and (i) vitamin premixes: Tk. 160/kg. The sale price for prawn was assumed as on average Tk.

250/kg. The cost of leasing ponds was not included in the total cost. An additional 7.5% on total cost was included as operational cost according to ADCP (1983).

Results

The proximate composition of experimental diets including the control are shown in Table 3. There was slight variation in protein, crude fibre, and NFE content. The protein content in different experimental diets varied between 32.10% and 33.25%. The lipid content in different diets varied between 9.41% and 11.09% with diet 5 showing the highest lipid (11.09%) content. This might be due to the higher soybean meal content in diet 5 as soybean meal originally contained high lipid content (18.48%). The ash content in different diets varied between 13.20% and 14.31%. In the control diet (SABINCO) the protein, lipid and ash content were 35.10, 8.79 and 12.57% respectively.

Table 3. Analysed proximate composition of the experimental diets used in *M. rosenbergii* monoculture experiment (% dry matter basis)

Components	Diets					
	1	2	3	4	5	6 (SABINCO)
Dry matter	90.29	91.12	90.49	90.31	90.19	90.57
Protein	33.05	32.10	32.50	33.25	32.40	35.10
Lipid	9.41	10.35	10.54	10.41	11.09	8.79
Ash	14.31	14.13	14.10	13.20	13.48	12.57
Crude fibre	11.10	10.80	11.05	11.28	11.30	9.36
NFE[1]	32.13	32.62	31.81	31.86	31.73	34.18
G.E. (kJ/g)[2]	16.73	16.98	16.97	17.10	17.14	17.30
Cost (Tk./kg)	18.19	17.29	16.49	15.59	14.69	45.00

NFE[1] = Nitrogen free extract calculated as- 100 - %(moisture + protein + lipid + ash + crude fibre)
G.E.[2] - Gross Energy.

Growth performance of prawn in different treatments in terms of mean weight gain (g) and total length (cm), specific growth rate (SGR %day), feed conversion ratio (FCR), survival and total production (kg/treatment) are shown in Table 4. The prawns fed on diets 1,2 and 6 (SABINCO) showed significantly higher mean total length. The significantly lowest total length was found in treatment 5. The prawns fed on diets 1, 2 and 6 (control) showed significantly ($P<0.05$) higher weight gain among the dietary groups. The significantly lowest growth was found in the treatment 5.

The specific growth rate (SGR) values of prawns ranged between 4.32 and 4.50 with diets 1, 2 and 6 exhibiting the higher SGRs. There was no significant difference ($P>0.05$) between the SGR values of diets 4 and 5. The mean feed conversion ratio (FCR) values varied between 3.11 and 4.85. The lowest FCR value (3.06) was observed with diets 1, 2 and 6. Diet 5 showed the highest FCR value (4.85). Survival (%) of prawns were estimated after total harvesting by draining out the ponds. The survival (%) of prawns varied between 46.60 and 66.6 % with diets 1, 2 and 6 showing significantly the higher survival. The lowest survival rate (46.6%) was recorded with prawns receiving diet 5.

Table 4. Growth performance of *M. rosenbergii* in monoculture fed on experimental diets

Parameters	Diets 1	2	3	4	5	6	± S.E.
Mean initial length (cm)	5.0	5.0	5.0	5.0	5.0	5.0	-
Mean final length (cm)	15.0^a	15.1^a	14.3^{ab}	13.8^{bc}	13.0^c	15.1^a	± 0.28
Mean initial weight (g)	0.3^a	0.3^a	0.3^a	0.3^a	0.3^a	0.3^a	-
Mean final weight (g)	33.8^a	33.1^a	29.5^b	28.1^c	26.0^c	34.0^a	± 0.75
Weight gain (g)	33.5^a	32.8^a	29.2^b	27.8^c	25.7^e	33.7^a	± 0.75
SGR (%day)	4.50^a	4.48^a	4.37^b	4.32^b	4.24^c	4.50^a	± 0.02
FCR	3.06^a	3.23^a	3.92^b	4.14^b	4.85^c	3.11^a	± 0.13
Survival (%)	66.6^a	64.0^a	54.6^b	52.0^b	46.6^c	65.3^a	± 0.99
Production (kg/treatment)	1.69^a	1.59^b	1.21^c	1.10^d	0.91^e	1.67^a	± 0.03
Production (kg/ha/105 days)	563.3^a	529.6^b	403.2^c	365.3^d	304.5^e	557.3^a	± 2.26

** Superscript (s) in the same row indicate no significant difference.*
SGR = Specific growth rate (% day)
FCR = Food conversion ratio.

Total production of prawn in terms of kg/treatment ranged between 0.91 and 1.69 kg in different treatments. Prawns fed on diets 1 and 6 resulted in significantly ($P<0.05$) higher production. The lowest production (0.91 kg) was obtained with treatment 5. The production ranged between 304.5 and 563.3 kg/ha/105 days.

A simple cost and return analysis was performed to estimate the net profit derived from monoculture of *M. rosenbergii* in ponds with formulated pelleted diets (Table 5). The highest net profit (Tk. 51,443/ha/105 days) was obtained in 1 and a loss of profit of Tk. 28,433/ha/105 days was observed in treatment 6. Treatments 3, 4 and 5 also resulted in loss in profit.

Table 5. Economic analysis of prawn production in monoculture experiment

Investment (Tk.)	Treatments I	II	III	IV	V	VI
i) Lime, fertilizer and rotenone	9.00	9.00	9.00	9.00	9.00	9.00
ii) Cost of fry	225.00	225.00	225.00	225.00	225.00	225.00
iii) Feed cost	94.07	88.80	78.22	71.00	64.83	233.72
Operational cost*	24.60	24.21	23.41	22.88	22.41	35.08
Total cost	352.67	347.01	335.63	327.88	321.24	502.80
Gross income (Tk.) from sale	507.00	477.00	302.50	275.00	227.50	417.50
Net profit (Tk./treatment)	154.33	129.99	- 33.13	- 52.88	- 93.74	- 85.30
Net profit (Tk./ha/105 days)	51443	43330	-11043	- 17627	- 31247	- 28433

Sale price : Average Tk. 250/kg
** Operational cost is considered as 7.5% of total cost (ADCP, 1983)*
*** Leasing cost for pond is not included.*

Discussion

The physico-chemical parameters observed in the present study were within the suitable range for fish culture. Only exception was the sudden drop of dissolved oxygen at early morning in the later part of the experiment causing few prawn mortality. During late September, the dissolved oxygen level in some experimental ponds dropped to 1.0 *mg/l* in the early morning (at 4.00 a.m.) with cloudy sky. This resulted in mortalities of few prawns. Similar type of prawn mortalities were also reported by Mazid and Mahmood (1994) and Humayun *et al.* (1986) in *M. rosenbergii* in monoculture ponds with supplemental feed. Wulff (1982) reported that juveniles of freshwater prawn could tolerate minimum oxygen levels of 1.0-1.5 *mg/l* and suggested not to allow the prawns at such levels for long time.

The stocking densities of prawn in the present study was 2.5/m^2. Thangdurai (1991) reported that a stocking density of 3/m^2 was optimum in India feeding on a combination of groundnut cake, rice bran and fish flesh. By investigating the potential of *M. rosenbergii* culture in Malaysia obtained 977 kg/ha/158 days cycle with 32.4% survival, when prawns were stocked at 10/m^2 and feed 30% protein ration at 20, 10 and 5% body weight daily for the first, second and subsequent months respectively (Ang, 1990).

Daniels *et al.* (1995) reported a higher survival rate of 73.7 to 81.9% with *M. rosenbergi* fed a specially formulated diet in earthen ponds. The production of prawn in the present study range between 304.5 to 563.3 kg/ha/105 days which are higher than that of Mazid and Mahmood (1994) but lower than that of Ang (1990).

Law *et al.* (1990) suggested that copra cake, soybean meal and wheat flour (which are low-cost ingredients in the Malaysia) were good sources of nutrients for prawn having examined their digestibility in 30 or 40% protein feeds with juveniles and adults. Generally, adult prawns could digest these ingredients and shrimp and fish meals, better than juveniles. Durairaj *et al.* (1992) noted an improved growth rate when prawns in manured ponds were fed a pelleted feed containing shrimp head meal (20%) and fish meal (10%) rather than a conventional feed ratio 1:2:1 groundnut oilcake, rice bran and trash fish but the trial was unreplicated.

The survival rate, individual weight and feed conversion ratio of the diet 1, 2 and 6 of the present study (65.3%, 33.6g and 3.13:1 respectively) were more or less similar to that of Tidwell *et al.* (1993). Who reported that the survival, individual weight and feed conversion ratio of 78%, 42g and 2.9:1 respectively in the monoculture of *M. rosenbergii* using formulated diet containing 32% protein.

The maximum yield (1.69 kg/treatment) in the present study was obtained in treatment 1. A simple economic analysis also revealed that treatment 1 could generate maximum net profit of Tk. 51,443 per hectare in 105 days. The total production in treatment 6 (using SABINCO diet) was more or less similar to that of treatment 1, but the net profit of treatment 6 was negative. This was due to the higher cost of SABINCO diet used in treatment 6. The loss in profit in other treatment (3, 4, and 5) may be due to the higher mortality of prawn or low stocking density used which decreased the total production.

In the present study, prawns fed diets, 1, 2 and 6 (SABINCO diet) attained the higher weight gain. There was no significant ($P>0.05$) difference between the weight gain of prawn fed diets 1, 2 and the control diet 6. Considering, the growth performance of prawn and the price of the experimental diet-1 (Tk. 18.19/kg) compared to that of SABNCO shrimp diet (Tk. 45/kg), diet-1 (containing 30% Fish meal, 5% Shrimp meal, 5% Soybean meal, 10% Mustard oilcake, 10% Sesame meal, 20% Wheat bran, 18% Rice bran, 1% Oyster shell and 1% Vitamin premix) may be recommended for monoculture of *M. rosenbergii* in ponds. However, further study should be carried out to find out an optimum stocking density of *M. rosenbergii* for monoculture in ponds.

References

ADCP (1983). Fish feeds and feeding in developing countries. Aquaculture Development and Co-ordination Programme. ADCP/REP/83/18. UNDP/FAO, 97pp.

Ang., K.J., 1990. Evaluation of the production potential of *M. rosenbergii* in monoculture in Malaysia. In. Proceedings of the 2nd Asian Fisheries Forum, 17-22 April, 1989, Tokyo, Japan (eds. R. Hirano and I. Hanyu). Asian Fisheries Society, Manila, Philippines. pp. 103-106

AOAC (Association of Official Analytical Chemists). 1980. Official methods of Analysis of the Association of Official Analytical Chemists, W. Horwitz (Editor). 13th edition, Washington, D.C. 1018 pp.

Daniels, W.H., L.R. D'Abramo, M.W. Fonderen and D.D. Martin, 1995. Effects of stocking densities and feed on pond production characteristics and revenue of harvested freshwater prawns *Macrobrachium rosenbergii* stocked as Size-Graded juveniles. *J. World. Aquacult. Soc.* 26(1): 38-47.

Duncan, D.B., 1995. Multiple Range and Multiple F-Tests. *Biometrix*, 11: 1-42.

Durairaj, S., Chandrasekharan, G. and R. Umamaheswary, 1992. Experimental culture of giant freshwater prawn in Tamil Nadu. *In*: Freshwater prawns (ed. E.G. Silal). Kerala Agricultural University, Thrissur, India. pp. 176-179.

Hoq, M.E., M.M. Islam and M.M. Hossain, 1996. Polyculture of freshwater prawn (*Macrobrachium rosenbergii*) with Chinese and Indian carps in fermer's pond. *J. Aqua. Trop.*, 11: 135-141.

Humayun, N.M., A.K.M.N. Alam, and M.A. Islam, 1986. Culture and production of *M. rosenbergii* in a freshwater pond with *Catla catla*. *Bangladesh J. Fish.*, 9(1-2): 1-7.

Islam, S.K., Y.S.A. Khan and M.J.U. Chowdhury, 1990. Semi-intensive culture of giant freshwater prawn, *Macrobrachium rosenbergii* (de Man). *J. Asiat. Soc. Bangladesh Sci.* 16(2): 81-85.

Khan, Y.S.A., A.K. Bhuiyan, S.I. Begum and M.A. Islam, 1984. Effect of different foods on the survival of growth of the juvenile *Macrobrachium rosenbergii* (de Man 1987) in indoor aquarium. *Ctg. Univ. Studies*, Pt. II, 81(1): 55-60.

Law A.T., K.S. Chin, K.J. Ang and M.S. Kamaruddin, 1990. Digestibility of low cost ingredients in pellleted feeds by *Macrobrachium rosenbergii*. *In*: Proceedings of the 2nd Asian Fisheries Forum, 17-22 April 1989, Tokyo, Japan (eds. R. Hirano and I. Hanyo). Asian Fisheries Society, Manila, Philippines. pp. 333-336

Mazid, M.A. and Y. Mahmud, 1994. Growth performance of giant freshwater prawn *Macrobrachium rosenbergii* in monoculture in ponds with supplemental feed. *In*: The third Asian Fisheries Forum (eds. L.M. Chou, A.D. Munro, T.J. Lam, T.W. Chen, L.K.K. Cheong, J.K. Ding, K.K. Hooi, H.W. Khoo, V.P.E. Phang, K.F. Shim and C.H. Tan). Asian Fisheries Society, Manila, Philippines. pp. 1010-1012.

Mazid, M.A., Y. Mahmud, J.U. Miah and S.U. Ahmed, 1989. Effect of different stocking densities on the growth and survival of *Macrobrachium rosenbergii* with supplemental feed. *Bangladesh J. Fish.* (in press).

New, M.B., 1991. Turn of the millennium aquaculture: navigating troubled waters of riding the crest of waves. *World Aquacult.* 22: 28-49.

Rey, I., 1989. Projects integrals and acuicultura, la experiencia del Hulla. In Memories Segunda Reunion Red Nacoinal de Acuicultura, Septiembra, 19888, Nevia, Colombia. pp. 121-132.

Thangadurai, A.J., 1991. Experimental culture of freshwater prawn, *Macrobrachium rosenbergii* in ponds. *In: Proceedings of Biannual Symposium on New Horizons in Freshwater Aquaculture, 23-25 January 1991, Bhubaneswar, Orissa, India*. Central Institute of Freshwater Aquaculture, Bhubaneswar, India. pp. 7-8.

Tidwell, J.H., C.D. Webster, D.H. Yancy and L.R. D'Abramo, 1993. Partial and total replacement of fish meal with soybean meal and distillers' by products in diets for pond culture of the freshwater prawn (*Macrobrachium rosenbergii*). *Aquaculture*, **118(1-2)**: 119-130.

Wulff, R.E., 1982. The experience of freshwater prawn farm in Honduras, Central America. *In*: Giant prawn farming (ed. M.B. New) Elsevier. pp. 445-448.

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Domestication of an endangered fish species *Nandus nandus* (Ham.): I. Laboratory rearing of young fish up to sexual maturity

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Abstract
An experiment was conducted for rearing of Meni, *Nandus nandus* in laboratory condition for seven months with the objective to select appropriate feed for the species and to develop a rearing technique of the species up to the stage of sexual maturation. Different trials were conducted using artificial feed (35.5% protein), dead fresh kachki (*Corica soborna*), dead fresh prawn (*Macrobrachium lamarrei*) and live prawn (*Macrobrachium lamarrei*). The provision of bottom sediment did not significantly influence the growth of fish. Between dead fresh kachki and dead fresh prawn, the fish preferred dead fresh prawn. The fish was found to be reluctant to take dead fresh kachki and prawn as food unless they became very hungry. The fish was found actively feeding on live prawn. The FCR of the prawn as food for *N. nandus* was found to be 2.5. From the study, it was observed that in laboratory rearing *N. nandus* preferred live prawn as food than artificial feed, dead fresh kachki and dead fresh prawn. The fish fed on live prawn became sexually matured (eggs or white milt extruded by gentle pressure on the abdomen of the fish) in the laboratory at the end of the experiment.

Key words: Endangered species, *Nandus nandus*, Laboratory rearing

Introduction

Nandus nandus (Hamilton) is a common freshwater perciform fish of Bangladesh. It is locally known as 'Meni' or 'veda'. It was once abundantly available in pond, canals, beels, floodplains and other open and closed waters of all over Bangladesh. Now it is rarely found in the market. Recently, it is considered as an endangered or threatened species.

Considering consumer's preference and market value and to preserve the biodiversity, this species should be protected from being extinct. In view of this, its biological study and domestication is earnestly needed. For the domestication its rearing technique, brood maintenance, breeding, fry rearing are to be developed. Until recently not so much works have been done on this fish except some parasitological studies (Dwivedi 1978, Mukherjee *et al.* 1982, Chowdhury *et al.* 1983, Chandra *et al.* 1987, Golder *et al.* 1987). Mustafa *et al.* (1980) studied food and feeding habits and fecundity of *N.*

nandus in Bangladesh. He reported that the fish was predominantly carnivorous. He reported that the fecundity was ranged from 7381 eggs for a fish with body length of 9.7 cm to 46222 eggs for a fish with body length of 13.5cm. The species has not been tried yet to rear or culture either in the laboratory or in the field condition experimentally and therefore no information is available on its rearing technique. Successful rearing and breeding of 'Meni' in farm condition would be tremendously helpful in conserving the genepool of the fish and, at the same time, it would play a substantial role in the overall nutrition of the rural people of Bangladesh. Therefore, laboratory rearing of *N. nandus* has been attempted to select the appropriate feed for the species (*N. nandus*) for laboratory rearing and develop a rearing technique in the laboratory until sexual maturity.

Materials and methods

The present study was conducted from October'98 to April'99 in the laboratory of the Department of Aquaculture under the Faculty of Fisheries, BangladeshAgricultural University, Mymensingh. Eight glass aquaria each having size of 60x35x30cm were used to conduct the experiment. The aquaria were marked as number 1 to 8 for convenience of the study. A set of 4 aquaria (1 to 4) was provided with sandy layer. The rest 4 aquaria were of bared bottom. All the aquaria were filled with fresh tap water almost up to the top level.

Experimental fish and acclimation

A total of 75 experimental *N. nandus* of "0" age group were collected from a fish trader which were caught by using bana and scoop net from a beel called Gopadmarchar near by the Bhramaputra river, under Mymensingh Sadar Thana. The fish caught form the beel were kept in a 50 liter aluminum pot and brought alive to the laboratory as early as possible. Fish were placed immediately into the aquaria in the laboratory. Three aerators (Daivo 8400) were fitted to supply oxygen in the aquaria. The fish were treated with 1% salt solution (dip) as a prophylactic treatment. No feed was supplied to the fish on first two days of the experiment. Then the dead kachki (*Corica soborna*) was supplied as food and the fish were acclimatized for seven days.

Stocking density

A total of eight fish were kept in each aquarium. From the collected fish a total of 64 fish were used in this experiment. The average body length and weight of the fish were 6.56 ± 0.62 cm and 4.10 ± 0.88 g respectively.

Experiment- I

An experiment with two treatments *viz.* artificial feed (containing 35.5% protein) and dead fresh fish kachki (*C. soborna*) were used in two replicate aquaria of two types having sandy bottom and bared bottom. In all the experiments, feed was supplied once a day at morning (9.00am). Feeding duration was for fifteen minutes every day. During

the experiment the water was aerated by using aerators continuously to maintain dissolved oxygen concentration at saturation level.

Experiment-II

Following the results of the 1st experiment another experiment with two treatments *viz.* kachki (*C. soborna*) and dead fresh prawn (*Macrobrachium lamarrei*) were supplied as food in quadruplicate aquaria for another one month. Feeding time and duration were maintained as per 1st experiment.

Rearing

Based on the findings of the 1st and 2nd experiments, which showed that growth of fish was not satisfactory with artificial feed, dead fresh kachki and dead fresh prawn, a four months rearing experiment was conducted by feeding live prawn. In rearing about one third of total water of each of the aquarium were changed in every morning and food (live prawn, 12 in each aquarium) was supplied once a day (9.00am) for fifteen minutes. Uneaten remaining prawn (if any) were removed from the aquarium. Total number of prawn eaten in each aquarium was counted and recorded properly. Any faeces on the bottom of the aquarium was removed by siphoning. Initial individual body weight (g) and total length (cm) and their average were measured and recorded. Monthly sampling was done to record the average body weight (g) and total length (cm) gain. The weight (g) of live prawn fed were also recorded for calculation of FCR.

Maturity observation

Impirical observations of body color and body shape of fish were made in order to ascertain sexual maturity fortnightly intervals starting from 1st February'99. Maturity of fish were first perceived by observing the eggs or milt of fish by means of stripping process during the April'99. Dissections of fish were also made in order to know maturation stage and the maximum ripeness of eggs at the mid May'99.

Analysis

Statistical analysis of growth data in experiment -I and experiment-II were performed using the two way ANOVA and one way ANOVA respectively by using the programme, Stategraphices (version 7). Moisture content of prawn was determined by following the standard method (AOAC 1980). The moisture content of the prawn was found to be 75.68%. The FCR (food conversion ratio) of prawn was calculated for *N. nandus* using the following formula -

$$\text{FCR} = \frac{\text{Amount of dry food (g)}}{\text{Live weight gain (g)}}$$

For FCR calculation, the dry matter content of prawn was determined.

Results and discussion

Prior to the start of experiment, the fish (*N. nandus*) have been adjusted into the aquaria supplied with dead fresh kachki as food. It was observed that at the beginning period fish took supplied food in small quantities and it was increased day by day and settled at the end of a week.

Experiment- I

In Experiment- I, artificial feed was used in duplicate in two sets of aquaria and dead fresh kachki was used in duplicate in two sets of aquaria also. ANOVA analysis indicated insignificant differences ($P>0.5$) for difference in both bottom conditions of aquarium and feed. Although there were insignificant differences in growth for both bottom condition and feed, the growth was better for the fish fed on kachki than the fish fed on artificial feed.

It was also observed that the fish supplied with artificial feed seems to be reluctant to response to the supplied artificial feed. On the contrary, fish supplied with kachki seems to be active at the time of feed supply. In the natural environment the principal food items of *N. nandus* consists of prawn, small fish, fish fry, chironomid and insect larvae (Mustafa *et al.* 1980). Perhaps the habit of taking fish as food influenced the response after offering familiar food item (fish) in case of kachki but not in the case of artificial feed.

Experiment-II

From the Experiment-I, it was evident that the bottom condition does not have any effect. So bottom soil was removed and the fish were supplied with two types of food in experiment II. Dead fresh kachki and dead fresh prawn were supplied as food in quadruplicate aquaira. Dead fresh prawn was supplied in lieu of artificial feed as it was found that fish refused to have artificial feed. Mustafa *et al.* (1980) reported that *N. nandus* was predominantly carnivore. Its food item were mainly prawns (31.76%) small fish (22.05%) fish fry (14.22%) chironomid and insect larva. It was observed that fish of the aquaria where kachki were supplied took their food spontaneously. But later on it was observed that fish refused to take kachki and just caught hold of kachki and immediately throw it out from the mouth by spitting and the fish was found feeling uneasy and also seems to be weak. Fish of the rest aquaria where dead fresh prawn were supplied had their food in optimum quantities at the first spell of a few days. As the time progressed fish were found to change their feeding habit. At the later stage of this experiment fish reduced having prawn like the fish those fed on kachki.

A summary of the analysis of variance of the effect of food on growth of fish indicated significant ($p< 0.05$) differences in growth of fish due to the variation of food organisms. Higher weight gain was found in treatments where dead fresh prawn was used. *N. nandus* fed on prawn exhibited better growth but the growth rate was not so satisfactory, which may be due to the temperature decline of the environment and also may be due to the other unknown factors.

Rearing

With the hope that live prawn will perform better than the dead fresh prawn, live prawn were supplied in each of the aquarium for rearing experiment. And it was observed that famished fish resumed taking food and improved their condition and subsequently, their weight was increased. The pattern of increase in weight is presented in Fig. 1. It was observed frequently that once a fish took sufficient prawn (more than one) in a day then remained starved for the subsequent day even sometimes days together.

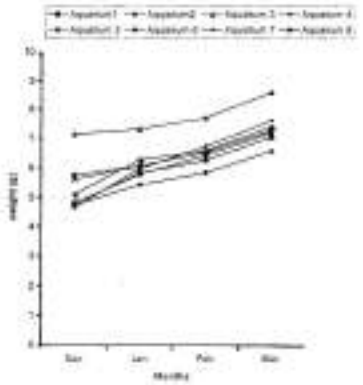

Fig. 1. Growth curve of *N. nandus* during the rearing experiment from December to March'99.

The FCR value of prawn for *N. nandus* was calculated 2.5 (Table 1). The result indicated that live prawn, as food is very useful for the growth and rearing of *N. nandus* in the laboratory. The famished fish became shiny in color, robust in appearance and found more active in movement specially after feeding the live prawn as food.

From the above discussion it is evident that *N. nandus* preferred live prawn as food than dead fresh kachki and prawn as well as artificial feed. Mustafa *et al.* (1980) reported that *N. nandus* was predominantly carnivore. Its food item were mainly prawn (37.6%), small fish (22.05%), fish fry (14.22%), chironomid and insect larvae. It can be said that *N. nandus* is a highly carnivorous fish which prefers live food as food and the fish can be cultured in the laboratory up to the stage of sexual maturity by providing live food for future plan of action in breeding and fry rearing.

Table 1. Growth performance of *N. nandus* during the rearing period of 4 months fed with live prawn

No. of Fish	Mean Initial Weight (g)	Mean Final Weight (g)	Mean Weight gain (g)	Dry weight of prawn fed (g)	Total weight gain (g)	FCR
64	5.62	7.37	1.75	278	111.6	2.5

Maturity observation

Maturity observations of fish were done by means of impirical observation, stripping process and dissection of fish. At the start of being maturity stage color of male fish became brightly reddish and black striped. Abdominal region of female fish was swollen up and the female was comparatively dull in color. Genital aperture of female fish was found protruded in breeding season. Stripping was done for fish of both the sexes (male and female) to test the maturity. If a few eggs or white milt extruded by gentle pressure on the abdomen the fish was considered matured.

Dissection of male fish as well as female fish was done. Testes and ovary of male and female fish were found under the digestive tract in the ventral cavity. Brown eggs were also seen inside ovary of female fish when the ovarian tissue was dissected. In male tubular white testes and in female spindle shaped ovary were dissected out. Fish were found fully matured during last week of May and the eggs were found to be of even size. All 64 fish were found sexually matured of which 42 were male and 22 were female (Table 2). Which indicate that in the natural population the sex ratio of *N. nandus* was more or less 2: 1 (male : female). From this study it was evident that for laboratory rearing of *N. nandus*, the fish preferred live prawn as food than artificial feed, dead fresh kachki and dead fresh prawn. Laboratory rearing of *N. nandus* up to the full maturation was successfully achieved in this experiment.

Table 2. Maturation of *Nandus nandus* in the laboratory by feeding live prawn

Initial No. Stocked	Total No. Male Matured	Total No. Female Matured
64	42	22

References

AOAC, 1980. Official Methods of Analysis (ed. W. Horwitz). Association of Official Analytical Chemists. 13th edition, Washington, D.C. 1018 pp.

Chandra, K. J. and M. G. Golder, 1987. Effects of helminth parasites on a freshwaters fish *Nandus nandus*. *Environment and Ecology*, 5(2): 33-336.

Chowdhury, M.B.R., A. K. M. Haque and M. A. Islam, 1983. The occurrence of nematode parasite in Nandid fish (*Nandus nandus*) of Bangladesh. *Bangladesh J. Agril, Sci.*, **10**(1): 37-42.

Dwivedi , M. P., 1978. *Coitocaecum orientalis* n. sp.(Coitocaecidae:Trematoda) from a freash water fish *Nandus nandus* . *Rivista di parassitologia*, **39**(2-3): 91-95.

Golder, M.I. and K. . Chandra, 1987. Infestation of *Isoparorchis hypselobagri* on the host fish *Nandus nandus*. *Environment and Ecology*, 5(2): 337-341.

Mukherjee, M., D.P. Haldar, 1982. Observations on the urceolariid ciliates of the genera *Trichodina* and *Tripartiella* in freshwater teleosts. *Archiv fur protistenkunde*, **126**: 4, 419-426.

Mustafa, G., A.T.A. Ahamed and K.R. Islam, 1980. Food, feeding habits and fecundity of a freshwater perch, meni fish. *Bang. J. Ag.*, 5(4): 205-210.

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Observation on the production performance of *Penaeus monodon* with *Liza parsia* under different cropping system

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Abstract
Study was conducted in six ponds each with an area of 0.1 ha in the pond complex of Brackishwater Station, Paikgacha, Khulna from February to October'96, to find out the variation of production rate in two culture system viz., single and double crop of *P. monodon* with *L. parsia*. In treatment T_1,wild fry of *P. monodon* (0.006g) and *L. parsia* (0.20g) collected from neaerby river were stocked at a rate of 40,000 and 10,000/ha, respectively, for a culture period of 120 days. In treatment T_2, the rate was 20,000/ha for bagda fry in 1st and 2nd crop each and 10,000 for parsia fry/ha for an extended period of 225 days. The highest survivality and growth of *P. monodon* and *L. parsia* were 57.08% (1st crop of T_2) and 75.26% (T_2), and 27.08g (1st crop of T_2) and 47.78g (T_2), respectively with a significant variations ($P>0.05$) with other treatment. The net profit (Tk. 93,134) and cost benefit ratio of 1 : 1.76 were also found higher in T_2.

Key words: *P. monodon*, *L. parsia*, Polyculture

Introduction

The culture of shrimp in Bangladesh has been drawing greater attention by fish farmers, particularly in brackishwater areas. In the coastal area of the greater Khulna region having a tropical climate, productive and unpolluted estuarine areas is considered to be a suitable natural habitat for penaeid shrimp culture with other brackishwater fin fishes in polyculture system. The latest estimate of the area of land under shrimp cultivation in Bangladesh is about 140,000 ha (BBS 1996). In these places, shrimp has been traditionally produced and the yield per unit area (150-200 kg/ha) is very low. So, through application of various improved aquaculture practices, production may be enhanced by several folds.

Jingran (1984) denoted some culturable brackishwater fin fishes in India, many of which are applicable to Bangladesh. Polyculture of *Penaeus monodon* and *Liza parsia* could be one of the ideal approaches of farming system while shrimp and mullet would be quite compatible having no territorial conflict and have little or no food competition. The fry of *P. monodon* and *L. parsia* are also available in coastal rivers and mangrove swamps of the greater Khulna region. In traditional shrimp farms of the country only post larvae (pls) of *P. monodon* are stocked in the ghers but other fin fishes that already entered into the ghers

along with the unsieved tidal waters are harvested time to time. Among the fin fishes, *L. parsia* is one of the most common and highly accepted to the local people due to its taste and demand. This type of species of non carnivorous nature is also easily culturable in shrimp growing areas that plays a vital role to keep a friendly environmental condition for both shrimp and fin fishes. With this consideration the study was undertaken to find out a suitable production technique of *P. monodon* with *L. parsia* at different stocking and cropping system.

Materials and methods

The study was conducted at the pond complex of Brackishwater Station, Bangladesh Fisheries Research Institute, Paikgacha, Khulna for a period of nine months from February to October, 1996. Six ponds, each of 0.1 ha were selected for this work with two types of culture treatment having three replications for each treatment. After the completion of pond preparation by mid February '96, tidal water was introduced to a depth of about 50cm for the growth of plankton.

For treatment T_1, wild fry of *P. monodon* (0.006 g) and *L. parsia* (0.20 g) were collected from local rivers and stocked in the 1st week of March at a rate of 40,000 and 10,000 fry/ha respectively. Whereas for the 1st crop of treatment T_2, fry of *P. monodon* and *L. parsia* were stocked at a rate of 20,000 and 10,000 /ha respectively and for 2nd crop of T_2, the *P. monodon* fry were restocked at the same rate as earlier with *L. parsia* those were not harvested with the first crop of *P. monodon*. After 120 days of culture *P. monodon* and *L. parsia* were completely harvested from T_1. In T_2, shrimp of first crop was harvested after 120 days and 2nd crop after 105 days of culture. Whereas *L. parsia* was harvested once after 225 days of culture. A commercial nursery feed Saudi-Bangla (starter-1) was fed twice a day (morning and evening) at of 100% of the body weight for the 1st week and then reduced to 60, 40 and 20% during 2nd, 3rd and 4th week, respectively. A portion of water (only 20% of the pond water) was exchanged during the full moon and new moon. Water quality parameters *viz.* dissolved oxygen, pH, salinity, temperature and transparency were monitored twice a week. After 30 days of rearing, shrimps were fed with locally available ingredients such as fish meal, ricebran, oil cake, wheat bran, oyster shell and vitamin premix (30-32% protein level) at 3% of the total biomass. Inorganic fertilizers were applied depending upon the availability of natural feed in the pond water.

Result and discussion

Table 1 shows that weight gain, survival and production performance of T_2 is better than T_1. The survival rate of *P. monodon* in T_2 was found 57.08% and 53.05% in the 1st and 2nd crop, respectively which was higher than T_1 and for *L. parsia* the rate was 75.26% which was also higher than T_1.

Table 1. Stocking, growth, survival and production performances of *P. monodon* and *L. parsia* under different cropping pattern

Treatments	Crop patterns	Species	Density (nos./ha)	Initial wt. (g)	Final wt. (g)	Survival rate (%)	Production (kg/ha)
T_1	Single	*P. monodon*	40,000	0.006	23.72	47.33	449.37
		L. parsia	10,000	0.200	30.45	55.67	171.05
T_2 (Double)	1st crop	*P. monodon*	20,000	0.006	27.08	57.08	310.34
		L. parsia	10,000	0.200	-	-	-
	2nd crop	*P. monodon*	20,000	0.006	22.07	53.05	234.29
		L. parsia	-		47.68	75.26	361.36

Table 2 shows that all the physioco-chemical parameters are within the range of optimum production level showing a close agreement with the results shown by Quddus *et al.* (1990), Rahman and Bhuiyan (1979). The average salinity was recorded from 16.96 to 7.71 ppt in T_1 and 16.25 to 1.87 ppt in T_2. No remarkable difference was observed in salinity of T_1 and T_2 in 1st crop from March to June but during the 2nd crop of *P. monodon*, a gradual decrease in salinity from 9.54 to 1.87 ppt (July to October) was observed which might act in T_2 as a growth retardation factor for *P. monodon* as mentioned by Das *et al.* (1982) and Chen *et al.* (1988).

Table 2. Average values of physico-chemical parameters of the pond water during the culture period

Parameters	Treatments	Months							
		March	April	May	June	July	Aug.	Sep.	Oct.
Salinity (ppt)	T_1	7.7	12.5	16.0	16.3	-	-	-	-
	T_2	7.7	12.5	15.8	16.3	9.5	5.0	2.0	1.9
Temp. (°C)	T_1	25.5	27.4	29.6	28.9	-	-	-	-
	T_2	25.5	27.4	29.0	28.8	26.3	28.2	31.4	31.3
Transperancy (cm)	T_1	18.9	25.5	22.3	22.8	-	-	-	-
	T_2	21.0	23.5	21.8	21.1	25.8	21.0	21.5	16.0
pH	T_1	8.4	8.3	8.2	7.8	-	-	-	-
	T_2	8.6	8.4	8.3	7.9	7.6	8.0	8.5	8.5
DO (mg/l)	T_1	6.1	4.3	4.1	3.7	-	-	-	-
	T_2	6.5	5.6	5.4	5.5	4.4	5.2	5.2	4.0

The average values of dissolved oxygen (DO) content of T_1 and T_2 were ranged from 3.65 to 6.45 mg/l and which showed no remarkable variation between the treatments.

However, comparatively lower values of DO content in T_1 might be co-related with the higher stocking rates of *P. monodon* fry which was stated by Chen and Ray (1990), where in such cases other than its biological oxygen demand (BOD), a portion of DO may also be utilized by organic decomposition and other metabolism.

From Table 1 the average weight gain of *L. parsia* was recorded as 30.45g (for T_1) and 47.68g (for T_2) after the cultivation of 120 and 225 days, respectively. This indicates that higher growth of *L. parsia* is possible because of longer culture period with available food and low stocking rate of *P. monodon* in T_2.

Total production of *L. parsia* in T_1 (171.05 kg/ha) was lower than T_2 (361.36 kg/ha). The production rate of *P. monodon* in T_1 (449.37 kg/ha) was also significantly lower ($P>0.05$) than T_2 (554.63 kg/ha). Average weight gain of *P. monodon* in T_1 (23.72g) and in T_2 (27.03g and 22.07g) did not show any significant difference.

Finally a comparative economic evaluation of single and double cropping system of *P. monodon* with *L. parsia* was done by using standard economic tools and methods of analysis. Table 3 reveals that the total gross income per ha for T_2 (Tk.215884.20) was higher than T_1 (Tk. 154597.75). The total operating cost was estimated as Tk. 94750.00/ha for T_1 as against Tk. 122750.00/ha for T_2. The net profit of T_2 (Tk. 93134.20) was higher than T_1 (Tk. 59847.75) and cost benefit ratio was also higher in T_2 (1 : 1.76) than in T_1 (1 : 1.63). The economic analysis of this study shows close similarity with the results shown by Yaha (1990) in Malaysia where the farms were mainly dependent on low cost inputs for this type of farming system. Findings also revealed that higher production and unit market price of *P. monodon* and *L. parsia* enhance the culture profitability to a considerable extend.

Table 3. Treatment-wise cost-benifit analysis of *P. monodon* and *L. parsia* under different cropping pattern

Items		Single crop (T_1)	Double crop (T_2)
Input costs (Tk/ha):	Land	11,250	15,000
	Sluice gate and dike repair	8,750	8,750
	Liming	1,250	1,250
	Organic fertilizers	500	500
	Inorganic fertilizers	1,500	2,500
	Seed	42,500	42,500
	Feed	15,000	25,000
	Labour	9,000	18,000
	Miscellaneous	5,000	9,250
Gross benefit:			
Production(kg/ha)-	monodon	449.37	544.54
	parsia	171.05	361.36
Gross income (Tk):	monodon	146,045	190,589
	parsia	8,553	25,295

Total gross income (Tk)	154,598	215,884
Production cost (Tk/ha)	94,750	122,750
Net profit (Tk)	59,848	93,134
Net profit as % of production cost	63.16	75.87
Cost benefit ratio	1.63	1.76

Price of monodon 325-350 Tk/kg, parsia 50-70 Tk/kg
1US$= Tk. 48.00

References

Bangladesh Bureau of Statistics, 1996. Annual report published by Government of the Peoples Republic of Bangladesh.

Chen, J.C., P.C. Liu, Y.T. Lin and C.K. Lee, 1988. Supper intensive culture of red -tailed shrimp *Penaeus penicillatus*. *J. World Aquacult. Soc.*, **19**: 127-131.

Das, N.K., N.M. Chakrabarti and D.D. Halder, 1982. Experiments on mixed prawn farming in brackishwater pond at Kwakdip. *In*: Proceedings of the symposium on coastal aquaculture, Part-1, Chochin, 12-18 Jan. 1980. Marine Biol. Assoc. India. 129-133.

Jingran, A.G. ,1984. The fish genetic resources of India. New Delhi. Indian Council of Agriculture, New Delhi, India.

Quddus, M.M.A., G.B. Sufi., M.A. Kalam and B.S. Nahar, 1990. Culture of bagda shrimp, *Penaeus monodon* Fab. in a brackishwater pond. *Bangladesh J. Sci. Res.*, **8**(2): 155-163.

Rahman, M.A. and A.L. Bhuiyan, 1979. Culture of tiger prawn in brackishwater pond. *Bangladesh J. Agril.*, 1(2).

Yahaya, J., 1990. An economical analysis of brackishwater shrimp pond culture, Johore, Peninsular Malaysia. *In*: The Second Asian Fisheries Forum (eds. R. Hirano and Hanyu). Asian Fisheries Society, Manila, Philippines.

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Monogenean infestations in Thai silver barb (*Babodes gonionotus* Bleeker) and their adaptations in Bangladesh waters

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Abstract
An investigation on the infestation of monogenetic trematodes of *Barbodes gonionotus* was conducted during the period from July'97 to June'98. Host specimens were collected from local fish farms and also from local fish markets of Mymensingh. Samples of *P. nicto* and *P. sarana* were also examined. Two species - *Dactylogyrus lampam* (Lim and Furtado 1986) and *Dacylogyrus siamensis* (Chinabut and Lim 1993) the Thai parasites were recorded from *B. gonionotus* and *D. lampam*, from our indigenous fish *P. sarana*. Two species of *Gyrodactylus* were also recorded from *B. gonionotus*. Both prevalence and intensity of infestation were moderate in *B. gonionotus*. Prevalences were recorded higher in larger fishes and mean intensity in intermediate size group fishes. Infestations were higher in winter months. Adaptations of the foreign parasites in Bangladesh waters, their transmission in local fishes and invasion of the local parasites to this exotic fish have been discussed. Suggestions have also been made to protect the introduction of new species in our waters.

Key words: Monogenetic trematodes, *Barbodes gonionotus*, Adaptations

Introduction

One of the major problems of fish culture is the parasitic infestation and disease. Fish parasites can cause mortalities of fishes in culture operations. They attack fishes and destroy them or make wounds or disease on their flesh, thus making them unedible (Cheng 1964). Moreover, the ecological peculiarities of the parasites are of greatest significance in elaborating a rational complex of central measure against them (Bauer *et al.* 1959). Hence, in order to control fish diseases caused by the parasites, it is essential to study their taxonomy to identify them, their infestations, as well as their effect on hosts.

The group of Monogenea are small to medium sized trematodes which complete the life cycle on one host. The chief organ of attachment is the haptor, which is posterior, mostly parasitic on the gills, some on the body and fins. Some monogenetic trematodes are serious pests in fish culture on occasion. Some dactylogyrids cause damage to the gill filaments of fishes and some gyrodactylids may cause damage to skin and fin (Hoffman 1967).

One of the most important exotic freshwater fish is Thai silver barb *Barbodes gonionotus* which was introduced in Bangladesh waters in 1987 and is getting priority in Bangladesh aquaculture and becoming popular for its rapid growth, delicacy and comparatively cheaper price. The fish is however, known to be infested by a number of monogeneans in its native Thailand (Lim and Furtado 1986, Chinabut and Lim 1993). As the species was imported from Thailand and no quarantine system is still introduced in Bangladesh there are possibilities that the fish carry these parasites to Bangladesh and thus establish in our water.

Considering the above facts, the present investigation was designed to study the monogenetic treamatodes of Thai silver barb *B. gonionotus* and two other local puti *P. sarana* and *P. ticto* of Mymensingh, their infestations, establishment and transmission to our local fishes.

Materials and methods

In total 97 specimens of *B. gonionotus* were investigated out of which 73 specimens were collected from Bangladesh Fisheries Research Institute (BFRI) and others were collected from fish farms of BAU (Bangladesh Agricultural University), Sutiakhali, Shambhuganj and other local fish markets of Mymensingh for examination during July'97 to June'98. Specimens of *P. ticto* and *P. sarana* were collected from different farms of BAU, and fishermen who caught them from different ponds or beels around Mymensingh. In the laboratory fishes were killed by a blow on the head and recorded its total length by a measuring scale. The gills were removed into petri dishes containing water and gently scrapped to dislodge monogeneans. The monogeneans were removed on to clear slides with a fine pipette in a drop of water and covered with cover slip. Ammonium picrate solution was added beneath the cover slip to fix and clear the monogeneans. Four corners of the cover slip were then sealed with sialant to prevent it from moving. The monogeneans were measured and given average in millimeter (mm) followed by the range in parentheses. Prevalence (%) and intensity of infestation were studied following Morgolis *et al.* (1982).

Results

Infestations of monogeneans

In the present investigation two species of *Dactylogyrus* and two species of *Gyrodactylus* could be collected from *B. gonionotus*. They are: *Dactylogyrus lampam* (Lim and Furtado 1986), *D. siamensis* (Chinabut and Lim 1993), *Gyrodactylus* sp. I and *Gyrodactylus* sp. II. From *Puntius ticto* 2 species of dactylogyrids and from *Puntius sarana* *D. lampam* were recorded.

Prevalence (%) and intensity of infestations of the monogeneans in relation to different length groups of *B. gonionotus*, and seasons of the year were presented in Tables 1 and 2. A total of 33 specimens of *B. gonionotus* were infested out of 97 examined. From the infested hosts 117 monogeneans were recovered where prevalence was 34.02% and the mean intensity was 3.5 (Table 1).

The prevalence of different length groups of *B. gonionotus* are 20%, 36.67% and 46.88% for <5, 5-8 and >8 cm fish group. It was observed that larger fishes are more infested than smaller fishes and the intensity of different length groups of *B. gonionotus* were 2.42, 4.36 and 3.47 for <5, 5-8 and >8 cm length group of fish respectively. Intensity of infestation were therefore heavy among the hosts of intermediate length groups than smaller and larger hosts (Table 1). It was observed that smaller and intermediate hosts are more infested than larger hosts and the intensity was higher in smaller fishes and lower in intermediate length group of fishes.

The infestation in *B. gonionotus* exhibited seasonal fluctuation during the study period. Prevalence of parasitic infestation was 36.67% in winter (November-February), 25.00% in summer (March - June) and 32.00% in rainy season (July-October). It was observed that hosts were more infested in winter than other seasons. Mean intensity of the host was also recorded higher in winter months (Table 2).

Table 1. Prevalence (%) and mean intensity of 117 monogenetic trematodes in different length group of *B. gonionotus* during July '97 - June '98

Length group (cm)	No. of host		Prevalence %	Mean intensity
	Examined	Infested		
<5	35	7	20.00	2.42
5-8	30	11	36.67	4.36
>8	32	15	46.88	3.47
Total	97	33	34.02	3.55

Table 2. Prevalence (%) and mean intensity of monogenetic trematodes in *B. gonionotus* in different seasons during July '97 - June '98

Season	No. of host		Prevalence %	Mean intensity
	Examined	Infested		
Nov. - Feb.	60	22	36.67	4.23
Mar. - Jun.	12	3	25.00	1.67
Jul. - Oct.	25	8	32.00	2.38
Total	97	33	34.02	3.55

Description of the monogeneans

***Dactylogyrus lampan* (Lim and Furtado 1986)** (Fig. 1)

A number of specimens from *B. gonionotus* and only two specimens from *P. sarana* were recovered.

Body elongated and medium, 0.345 (0.290 - 0.400) × 0.080 (0.070 - 0.090). Anchors well developed, small outer root with recurved point. Its inner length 0.038 (0.036 - 0.040), outer length 0.032 (0.030 - 0.034), inner root 0.014 (0.012 - 0.016), outer root 0.0035 (0.003 - 0.004), point 0.013 (0.012 - 0.014). Dorsal bar basin shaped and ventral bar V-shaped. Marginal hooks with slight demarcation of handle from pivot. Dorsal bar length 0.0035 (0.003 - 0.004), width 0.0235 (0.022 - 0.025), ventral bar length 0.021 (0.020 - 0.022), width 0.0075 (0.007 - 0.008), hook length 0.025 (0.020 - 0.030), vaginal armament 0.063 (0.054 - 0.072).

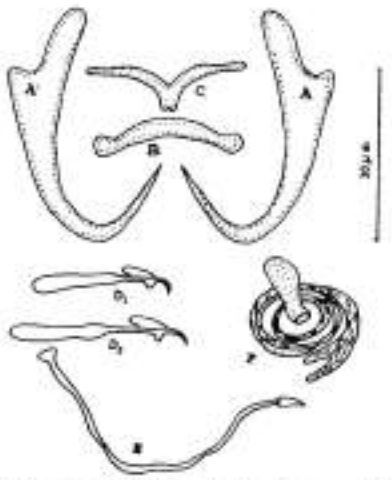

Fig. 1. *Dactylogyrus lampam.* A: Anchors B: Dorsal Bar C: Ventral Bar D_1 & D_2: Marginal hooks E: Vaginal armament F: Copulatory organ.

Comparative measurements of Thai *Dactylogyrus lampam* with Bangladeshi specimens.

	Thai specimen from *B. gonionotus* (mm)	Bangladeshi specimens from *B. gonionotus* (mm)	Bangladeshi specimens from *P. sarana* (mm)
Body	0.547(0.521-0.573) × 0.125(0.104-0.135)	0.345(0.290-0.400 × 0.70-0.90)	0.455(0.430-0.480 × 0.96-0.110)
Inner length	0.32 (0.030-0.034)	0.038(0.036-0.040)	-
Outer length	0.29 (0.027-0.030)	0.032(0.036-0.034)	-
Inner root	0.008 (0.008-0.009)	0.014(0.012-0.016	0.014
Outer root	0.002(0.001-0.002)	0.0035(0.003-0.004)	0.004
Point	0.008 (0.008-0.010)	0.013(0.012-0.014)	0.0014
Dorsal bar length	0.010 (0.009-0.011)	0.0035(0.003-0.004)	0.025(0.024-0.026)
Dorsal bar width	0.025(0.024-0.027)	0.0235(0.022-0.025)	0.004
Ventral bar length	0.023 (0.022-0.025)	0.021(0.020-0.022)	0.014
Ventral bar width	0.008 (0.007-0.009)	0.0075(0.007-0.008)	
Hook length	0.021(0.016-0.026)	0.025(0.020-0.030)	
Vaginal Armament	0.067	0.063(0.054-0.072)	

Remarks: The present specimen agrees with the description of the *Dactylogyrus lampam* (Lim and Furtado 1986) in all characters except in measurements of the body size. This difference may be due to host difference in different geographical area. The specimens collected from *B. gonionotus* and *P. sarana* were comparatively smaller than the specimens from Thai host.

***D. siamensis* Chinabut and Lim 1993** (Fig. 2)

Numerous specimens were collected from *B. gonionotus*.

Moderate to long worm, 0.330 (0.260 - 0.400) × 0.085 (0.70 - 0.100). Anchors are large with well developed inner root with short outer root and very short recurved point. Anchors' inner length 0.032 (0.030 - 0.034), outer length 0.035 (0.034 - 0.036), inner root 0.0125 (0.011 - 0.014), outer root 0.045 (0.004 - 0.005), point 0.002. Dorsal bar saddle-shape and thin 'V' shaped ventral bar present. Dorsal bar length 0.0035 (0.003 - 0.004), width 0.0225 (0.022 - 0.023), Ventral bar length 0.002, width 0.017 (0.016 - 0.018), hooks 0.020 (0.014 - 0.026). Wing observed at the portion of the outer root. Sword like short copulatory tube observed. Copulatory tube length 0.020 (0.016 - 0.018).

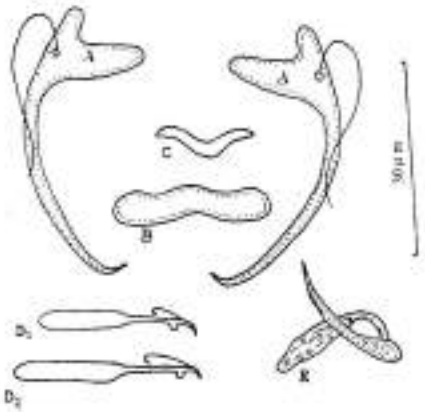

Fig. 2. *D. siamensis*. A: Anchors B: Dorsal Bar C: Ventral Bar D_1 & D_2: Marginal hooks E: Copulatory organ.

Comparative measurements of Thai *Dactylogyrus siamensis* Chinabut and Lim 1993 with Bangladeshi specimens

	D. siamensis Chinabut & Lim1993 (mm)	*D. siamensis* Bangladeshi specimen (mm)
Body	0.396 (0.315-500) × 0.161(0.050-0.080)	0.330(0.260-0.400 × 0.070-0.100)
Inner length	0.029 (0.020-0.042)	0.032(0.030-0.034)
Outer length	0.024 (0.018-0.030)	0.035(0.034-0.036)
Inner root	0.009 (0.008-0.010)	0.0125(0.011-0.014)
Outer root	0.006 (0.005-0.007)	0.0045(0.004-0.005)

Point	0.004 (0.003-0.006)	0.002
Dorsal bar length	0.006 (0.005-0.008)	0.0035(0.003-0.004)
Dorsal bar width	0.028 (0.022-0.035)	0.0225(0.022-0.023
Ventral bar length	0.002 (0.001-0.002)	0.002
Ventral bar width	0.022 (0.020-0.024)	0.017(0.016-0.018)
Hooks	0.019 (0.017-0.022), 0.025(0.024-0.026)	0.020(0.014-0.026)
Copulatory tube length	0.020 (0.018-0.025)	0.017(0.016-0.018)

Remarks: *Dactylogyrus siamensis* was first reported by Chinabut and Lim (1993) from *B. gonionotus* in Thailand. The present specimens also confirm their identity as *D. siamensis* Chinabut and Lim 1993. However, the size of the specimens are smaller than Thai specimens.

***Gyrodactylus* sp. I.** (Fig. 3)

Only one specimen was recovered from the gill filament of *B. gonionotus*.

Body short and cylindrical, 0.339 (0.238 - 0.440) × 0.116 (0.084 - 0.148). Anterior end notched with three pair of head organ. Anchors root long and two round spot present at the side of the inner and outer root. Anchor: inner length 0.045 (0.042 - 0.048), outer length 0.037 (0.034 - 0.040), inner root 0.013 (0.010 - 0.016), outer root 0.005 (0.004 - 0.006). Points nearly half of anchor length. Point 0.019 (0.018 - 0.020). Ventral bar thinner than the dorsal bar. Dorsal bar length 0.004, width 0.018 (0.016 - 0.020), Ventral bar length 0.001, width 0.021 (0.016 - 0.026), marginal hooks 0.021 (0.014 - 0.028).

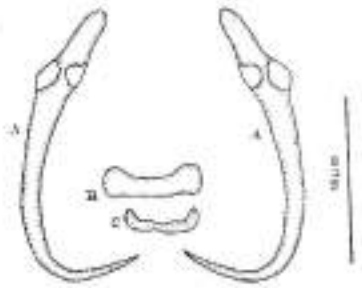

Fig. 3. *Gyrodactylus* sp. I. A: Anchors B: Dorsal Bar C: Ventral Bar.

Remarks: *Gyrodactylus* is popularly known as skin fluke. There are reports of its infection in the fins and opercula. Tripathi (1957) recorded three specimens of *Gyrodactylus* from the fins of a Rohu fry in the fish farms of Lucknow. Condition of the single specimen recorded from the gills, was not good. So, it could not be identified up to species level.

***Gyrodactylus* sp. II.** (Fig. 4)

Twelve specimens could be collected from the gills of *B. gonionotus*.

Body short and cylindrical, 0.410 (0.400 - 0.420) × 0.128 (0.126 - 0.130). Anchor long. Anchor's root curved pointing outwardly and little more than a quarter of total anchor length. Bar short and curved. Testis post ovarian. Anchor: inner length 0.0225 (0.022 - 0.023), outer length 0.021 (0.020 - 0.022), inner root 0.0045 (0.004 - 0.005), outer root 0.005, point 0.0075 (0.007 - 0.008), dorsal bar 0.003, width 0.008, marginal hooks 0.008.

Fig. 4. *Gyrodactylus* sp. II. A: Whole parasite B: Anchor C: Bar.

Remarks: The present specimen is similar to the morphology of anchors, marginal hooks with *Gyrodactylus elegans indicus* (Tripathi 1957) but differs by bar and the length of the parasite. Collection of more specimens is necessary to identify them up to species level.

Discussion

Monogenetic trematodes have been known to be a serious pest for aquaculture particularly to younger culturing species. Some *Dactylogyrus* species cause serious damage to the gill filaments of fishes and thus potential threats to fish culture (Tripathi 1957). But no information is available from Bangladesh on this area. Parasitological aspects of exotic fishes are yet to be investigated. Monogenean infestations are very common in *Puntius* and carps in Indian farms (Gussev 1976,Tripathi 1957) and South East Asian countries (Lim and Furtado 1986, Chinabut and Lim 1993). During the investigation prevalence of this fluke in *B. gonionotus* was quite high and fishes of larger size group were more susceptible. On the other hand mean intensity could be recorded from the intermediate size group fish. Many workers reported different intensity in different size groups of various fishes. However, several authors have noted the correlation between outbreak of monogenean infestations and stocking density (Sarig 1971, Molnar 1971, Johnsen and Jensen 1986). It was also observed in our study that monogenetic infestations were higher in case of the fishes collected from BFRI and BAU fish farm. Parasitic infestations appeared to be higher in winter than summer months. Low temperature seems to be more suitable for monogenean infestation. It could be said

that *B. gonionotus* is moderately susceptible to this group of parasite as 4 species of monogeneans could be recorded from the waters of Mymensingh.

The Thai silver barb *B. gonionotus* have gathered much popularity to Bangladesh people among exotic species. Parasitological investigation have been done in its country of origin (Lim and Furtado 1986, Chinabut and Lim 1993) and 7 species of monogenean trematodes were reported from it. After twelve years of its introduction to Bangladesh only two species of *Dactylogyrus* were found to be established in Bangladesh situation. Other species of this genus might have failed to adapt in the ecological condition of our water. On the other hand two gyrodactylid species attacked the exotic host, where there were no reports of gyrodactylid infestations in Thailand. Hossain (1998) and Nahar (1997) studied the monogeneans infestation of barbs and Indian major carps of Mymensingh area but no dactylogyrids were found to infest this exotic fish. Body physiology and defence mechanism may be the barrier for these local parasites to invade. Gyrodactylids seem to have a wide range of adaptations and invasion including new hosts breaking their immunity.

On the other hand, it is interesting to note that the monogeneans infecting *P. gonionotus* in Thailand were supposed to be carried with the fish in Bangladesh during its introduction and only two species could survive here. Among these two dactylogyrid species, *Dactylogyrus lampam* was found to spread to our local fish *P. sarana*. This particular parasite might have wide range of establishment in our local environment. None of other parasites were recorded from eight species of *Puntius* and two species of carps (Nahar 1997, Hossain 1998). This might be an indication of slow progress of invasion of foreign parasites to our indigenous fishes.

Fish culture is one of the fasinating practice becoming popular throughout the world. Commercial species are being introduced from one country to another. While importing fishes all countries of the world except few like Bangladesh have gone through a check by quarantine. It is therefore, essential for every country to have a prior check before introducing any new fish, otherwise local fishes may be attacked by foreign pathogens /parasites which may cause great loss. For Bangladesh all these should be taken into account and exotic fishes should be introduced with great care.

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References

Bauer, O.N., V.A. Musselius and Y.A. Strelkov, 1959. Disease of Pond Fishes. Pub. Ozdatel's tvo 'Kolos' Moskhova, 177-179.

Cheng, T.C., 1964. The Biology of Animal Parasites. W.B. Saunders Company. Philadelphia and London, pp. 369, 441-442.

Chinabut, S. and L.H.S. Lim, 1993. Seven new species of *Dactylogyrus* Diesing, 1850 (Monogenea) from *Puntius* Hamilton (Cyprinidae) of Thailand. *Raf. Bull. Zool.* **41**(1): 47-59.

Gussev, A.V., 1976. Freshwater Indian Monogenoidae, Principles of Systematics, analysis of the world faunas and their evaluation. *Ind. J. Helm.*, **25/26**: 1-241.

Hoffman, G.L., 1967. Lesions due to internal helminths of freshwater fishes. In the pathology of fishes (eds. W.E. Ribelin and G. Higaki). The University of Wisconsin Press. Madison. Wisconsin, pp. 151-186.

Hossain, M.M., 1998. Studies on the Monogenean Gill Parasites of Three *Puntius* species of Mymensingh. M.S. Thesis 87 pp.

Johnsen, B.O. and A.J. Jensen, 1986. Infection of Atlantic Salmon *Salmo salar* by *Gyrodactylus salaris* in Norwegian rivers. *J. Fish Biol.*, **29**: 233-214.

Lim, L.H.S. and J.I. Furtado, 1986. Sixteen new species of *Dactylogyrus* from the genus *Puntius* Hamilton. (Cyprinidae) *Folia Parasit.* (Praha). **33**:21-34.

Margolis, L., G.W. Esch, J.C. Holmes, A.M. Kuris and G.A. Schad, 1982. The use of Ecological Terms in Parasitology (Report of an ad hoc Committee of the American Society of Parasitologists. *J. Parasit.*, **68**(1): 131-133.

Molnar, K. 1971. Studies on gill parasites of the grass carp (*Ctenopharyngodon idella*) caused by the *Dactylogyrus lamellatus* Achmerow 1952. II Epizootiology. *Acta Vet. Acad. Sci. Hung.*, **21**: 316-375.

Nahar, S. 1997. Monogenean parasites of juvenile carps in nurseries of Mymensingh. M.S. Thesis. 58 pp.

Sarig, S. 1971. The prevention and Treatment of Disease of warm water fishes under subtropical conditions, with special emphasis on intensive fish farming. T.F.H. Publications, Neptune City, N.Y.

Tripathi, Y.R. 1957. Monogenetic trematodes from fishes of India. *Ind. J. Helm.*, **11**: 1-149.

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Cell types in the peripheral blood of waking catfish *Clarias batrachus* (Lin.)

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Abstract
Different types of haematocytes found in the peripheral blood of walking catfish *Clarias batrachus*, have been characterized and identified using morphological, morphometric and cytochemical techniques. These cells are: erythrocytes, reticulocytes, large and small lymphocytes, thrombocytes, monocytes and polymorphonuclear leucocytes (neutrophils).

Key words: *Clarias batrachus*, Peripheral blood, Blood cells

Introduction

Haematological studies in fish have assumed greater significance with the increasing emphasis on pisciculture and even greater awareness on fish diseases, the pollution of natural water resources as well as determination of patho-physiological condition of fish. Many routine haemotological studies already exist in human medicine to assist in providing evidence and possible identification of an abnormality or disease process. Like human and other higher vertebrates both cells and soluble factors of blood play an important role in the defense mechanism of fish during the acute and earlier stage of infection. Many haematological studies were conducted with different fishes in different countries (McCarthy *et al.* 1973, Grilzzle and Rogers 1976, Mahajan and Dheer 1979). Walking catfish or magur *Clarias batrachus* is an important food fish of Bangladesh with high market price. Chinabut *et al.* (1991) and Boomker (1981) reported on various blood cells of walking catfish from Thailand and South Africa respectively. But there is no report of any haematological work on this important fish in this country. Sardar (1999) worked on different aspects of the haematology of apparently healthy and artificially infected *C. batrachus*. This report includes the identification of different cell types found in peripheral blood of walking catfish, *C. batrachus* with their possible classification.

Materials and methods

Experimental fish

Clarias batrachus was selected for its haematological study. In total 78 fish were investigated to know their blood cell types. Thirteen fish were sampled in each month randomly taking only one fish a day. Fish samples were bought from local market of

BAU campus and Mymensingh town. Experimental fish were transported to the laboratory from market in bucket containing water. Average length and weight of the fish were 20.95±0.64 cm and 79.09±6.75g respectively.

Acclimatization of fish

Fish were acclimatized in aquaria containing tap water for 7 days before experiment with aeration and feeding at alternate day with SABINCO fishmeal pellet. Seventy per cent of water was changed every day. The aquaria were set in a room (laboratory II) beside the fish disease laboratory.

Blood collection

Fish were caught gently in a small scoop net avoiding stress, transferred into a bowl containing the same water where they were acclimatized. After anesthetizing the fish with 5 ppm quinaldine (Sigma chemical Co. USA) (Hossain and Shariff 1992), blood samples were collected from the caudal vein with a sterile disposable plastic syringe coated with 3.6% sodium citrate as an anticoagulant (Smith *et al.* 1952). To avoid contamination with mucus and water the area of insertion of syringe was wiped with cotton soaked in 70% alcohol.

Preparation and staining of blood smears

Thin blood films were prepared immediately after collection on previously cleaned glass slide in the following way: a drop of blood was placed on one side of the slide. Another slide was placed in front of the drop in an inclined vertical position making 45° angle towards the drop in such a way that the slide just touches the drop. This slide was then pushed to the other side of the flat slide to prepare a smear. It was then air-dried, fixed in methanol and stained by Wright's and Giemsa's stain (Chinabut *et al.* 1991) in the following way:

- Dried smear was fixed in 100% methyl alcohol for 15 minutes and was air dried again.
- Stained with Wright's stain for 3-5 min.
- Dipped in PBDW for 5-6 min.
- Stained with 1:10 diluted Giemsa's stain in distilled water for 20-30 min.
- Flashed with distilled water to prevent stain-precipitation on slide
- Dipped in phosphate buffer distilled water (PBDW) for 10-20 min.
- Rinsed well in distilled water, blotted, dried and finally mounted in Canada balsam.

It is remarkable that satisfactory result was not obtained when only Wright's or Giemsa's stain was used.

All microscopic studies were done under a compound binocular microscope at different magnifications as and where needed and occulomicrometer was used for measuring the cells.

Results

The following cells were identified from stained smear of walking catfish *Clarias batrachus* peripheral blood: erythrocytes, large and small lymphocytes, thrombocytes, monocytes and polymorphonuclear leucocytes (neutrophilic granulocytes). Their descriptions are given below and they were shown in Fig. 1.

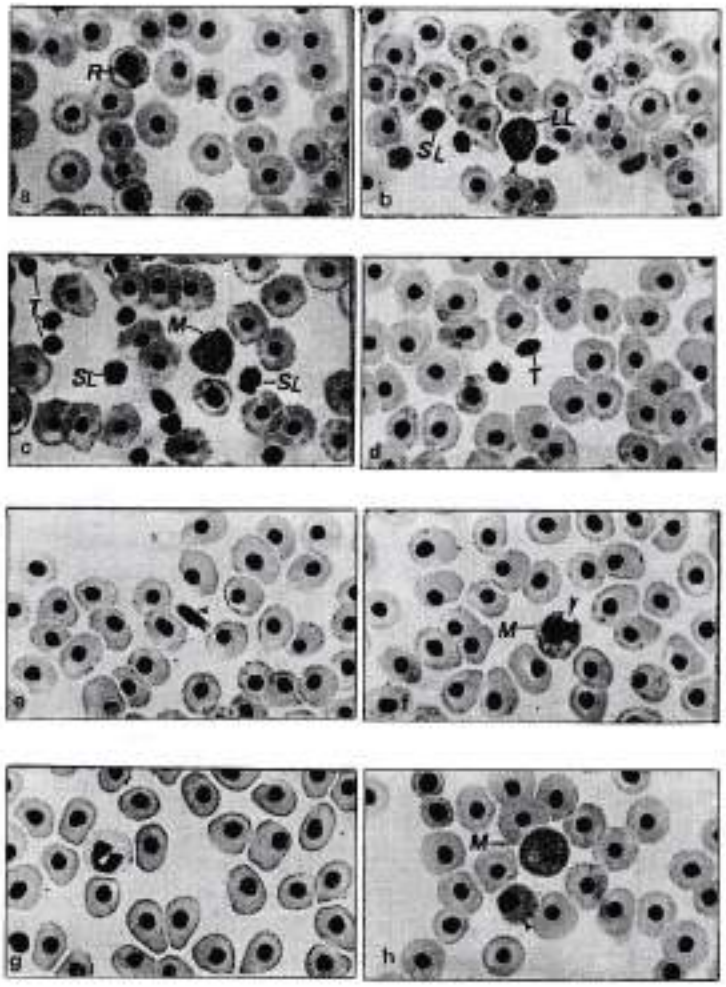

Fig. 1. Photomicrograph of stained blood smear showing a. mature erythrocytes and a reticulocyte (R); b. small lymphocyte (SL) and large lymphocyte (LL) with pseudopod like formation (arrow); c. small lymphocyte (SL), rounded thrombocyte (T) and monocyte like cell (M); d. oval shaped thrombocyte (T); e. elongated form of thrombocyte (arrow); f. monocyte (M) with cytoplasmic vacuoles (arrow); g. neutrophil like cell with bilobed nucleus and h. neutrophil like cell with cytoplasmic granules (arrow) and a monocyte like cell (M) (Wright and Giemsa × 875).

Erythrocytes

The identified mature erythrocytes or red blood cells were oval to round in shape with deeply stained nucleus and abundant cytoplasm. Size of the cells were 9-10 × 12-13 µm and the diameter of the nuclei were 3-4 µm. Nuclei were centrally located, took an auricula purple colour having alternate lighter and darker area, and the cytoplasm was lightly stained of a buff colour no cell inclusions or vacuoles were observed in the cytoplasm of the erythrocytes (Fig. 1a). The immature erythrocytes known as reticulocytes were rarely found in the circulating blood (Fig. 1a). The size of the reticulocytes were about same as that of the mature erythrocytes but the nuclei were larger. The cytoplasm of the reticulocytes were stained a light blue with dark auricula purple coloured nuclei in Wright's and Giemsa's.

Leucocytes

Observed leucocytes or white blood cells were divided into two groups, agranulocytes and the granulocyte depending on the absence and presence of granules in their cytoplasm respectively.

Agranulocytic leucocytes

Following cells were identified as agranulocytic leucocytes.

Lymphocytes: Observed lymphocytes of walking catfish were smaller than the erythrocytes and much more variable in size ranging from 5 to 9 micrometers. These cells were characterized by being round in shape with narrow band of non-granular cytoplasm that stained a light blue colour in Wright's and Giemsa's. The nucleus, tending to be eccentric, occupied more or less ¾ th of the cytoplasm and characterized by very dark staining chromatin of dark purple colour in Wright's and Giemsa's. Lymphocytes with pseudopods were also observed (Fig.1b). The large and small lymphocytes (Fig.1b and c) were determined on the basis of their comparative size: the larger lymphocytes mostly had varied shape- circular to amoeboid form with pseudopod like formation on the cytoplasm. They were 7 to 9 µm in size, whereas the small lymphocytes were 4 to 5 µm in size.

Thrombocytes: Three types of thrombocytes were identified from the stained blood smear of walking catfish: round, oval and elongated (Fig. 1c, d and e). The round thrombocytes resembled and were confused with small lymphocytes in blood smear, and were characterized by a thin rim of cytoplasm around the nucleus. The nuclei were slightly larger than those of erythrocytes. The cytoplasmic region surrounding the nucleus was very small, stained faintly and the cell shape was mainly provided by the nucleus which took a variant auricula purple colour in Wright's and Giemsa's staining. It was very difficult to visualize the cytoplasm of these round thrombocytes because the nucleus occupied almost the whole area of the cell. They were 3-4 micrometers in diameter and were the major type of thrombocytes in the leucocyte population that appeared single or in groups. The average size of the oval shaped thrombocytes were 4-5

× 6-8 μm. The elongated thrombocytes had pale pink cytoplasm at one or both ends of the cell. They were considered immature.

Monocytes: The monocytes were found to be the largest (about 9 to 15 micrometers) of the formed blood elements. The cell shape, although variable, were generally globular. They were often characterized by the presence of pseudopods. Vacoules were found in the cytoplasm ranging from few and small to many and large. Observed nuclei of the monocytes were eccentric and irregular in shape, their cytoplasm took little stain of bluish-gray colour and nucleus was stained of a variant auricula purple colour which was lighter than that of erythrocytic and thrombocytic nuclei (Fig. 1f). The cells were generally seen on the border of blood films surrounded by erythrocytes.

Granulocytic leucocytes

Polymorphonuclear leucocytes: The polymorphonuclear leucocytes that were observed in the peripheral blood of *Clarias batrachus* during the present investigation were thought to be the neutrophils which were found to be large and round in shape and 9-14 μm in diameter with an abundant amount of cytoplasm containing fine granules (Fig. 1h). The cytoplasm stained light blue, whitish or white-gray colour and nucleus, purple with Wright's and Giemsa's. Nuclei were eccentric and oval (Fig. 1g) or bilobed (Fig. 1h) in shape. Basophilic and eosinophilic leucocyts were not found.

Discussion

Cell type of peripheral blood and their identification is very important to haematologists and immunologists for understanding patho-physiological condition of fish and primary defense mechanism associated with leucocytes.

In the present investigation, the following cells of walking catfish *Clarias batrachus* circulating blood were identified: erythrocytes, reticulocytes, lymphocytes (large and small), thrombocytes, monocytes and neutrophils (polymorphoneuclear leucocytes). Chinabut *et al.* (1991) found the same types of cells in the same fish, but they did not classify the lymphocytes. In this study lymphocytes were divided into two groups, large and small, according to their morphology and size. This finding coincides with the identification of Mahajan and Dheer (1979) in the snakehead fish *Channa punctatus*, that is an air breathing fish. Boomker (1981) also found such type of cells in *Clarias gariepinus*.

Mature erythrocytes of *Clarias batrachus* were oval to round with deeply stained centrally located nuclei having abundant cytoplasm. The over all size of the erythrocytes ranged from 9-10×12-13 μm and the diameter of the nuclei were 3-4 μm. Similar observations were made by Chinabut *et al.* (1991) in walking catfish, as 10×11 micrometer cell size and 4-5 micrometer nucleus size. Blaxhall and Daisley (1973) found erythrocyte of brown trout *Salmo trutta* ellipsoidal in shape with centrally located nucleus (cell size 10.8–18.0 × 9 μm). McCarthy *et al.* (1973) also observed the diameter of erythrocyte as 9.4–11.7 × 14.1–17.1 μm in rainbow trout *Salmo gairdneri*.

The nucleus of mature erythrocyte took auricula purple colour and cytoplasm took buff colour that is similar to the observation of Blaxhall and Daisley (1973) in rainbow trout and Mahajan and Dheer (1979) in *Channa punctatus*. In the present study reticulocytes (immature erythrocyte) were also found in the circulating blood of *C. batrachus*. Its morphology and staining characters are same as those observed by Chinabut *et al.* (1991).

Among the leucocyte population, lymphocytes, monocytes and thrombocytes are considered as agranulocyte and the neutrophils are considered as granulocyte due to absence and presence of granules in their cytoplasm respectively (Ellis, 1977). Similar cells were found in the present study as also found by Chinabut *et al.* (1991).

The size of lymphocytes were much variable ranging from 5 to 9 µm, having no granules in cytoplasm and eccentric nucleus occupying about ¾ part of the cell. The large lymphocytes were circular to amoeboid with pseudopod like formation on the cytoplasm that are in accordance with the findings of Mahajan and Dheer (1979). Cytoplasm of lymphocytes were stained light blue colour and nucleus stained dark purple in Wright's and Giemsa's. These results agree with the findings of Chinabut *et al.* (1991) in *C. batrachus* and Mahajan and Dheer (1979) in *C. punctatus*.

Thrombocytes were also found in the peripheral blood of *C. batrachus* that were agranulocytic leucocytes similar to those observed in *C. batrachus* by Chinabut *et al.* (1991). Aydin *et al.* (1997) also found thrombocytes in the peripheral blood of rainbow trout *Oncorhynchus mykiss*. Blaxhall and Daisley (1973) did not find thrombocytes in the circulating blood of *Salmo trutta* might be due to the confusing appearance of thrombocyte (round) with the small lymphocytes (Chinabut *et al.* 1991) and decreasing in number during blood film preparation for their involvement in the clotting process (Grizzle and Rogers 1976).

The thrombocytes were variable in shape - round to elongate, the nucleus was larger than that of erythrocytes and cytoplasm was difficult to visualize because of the fact that most of the area of cytoplasm were occupied by neucleus. The average size of the thrombocyte was 4-5 × 6-8 µm. The average size of thrombocytes observed in other haemotological studies on different fishes *viz*. *C. punctatus* (Mohajan and Dheer 1979), *Salmo gairdneri* (McCarthy *et al.* 1973) and *C. batracus* (Chinabut *et al.* 1991) were 1-6 µm. Nucleus of thrombocyte took auricula purple colour and elongated thrombocyte had pale pink cytoplasm at one or both ends of the cell. These results were similar to that of Chinabut *et al.* (1991) and Mahajan and Dheer (1979).

Monocytes were found to be the largest cells (9-15 µm in diameter) in the peripheral blood of *C. batrachus*. Usually the cells were rounded to irregular having eccentric nucleus and vacuoles in cytoplasm, as also found by Chinabut *et al.* (1991). Grizzle and Rogers (1976) were unable to find such cells in channel catfish *Ictalurus punctatus* blood but Dogen and Sullivan (1969) reported the occurrence of monocytes in the same fish.

In Wright's and Giemsa's staining method cytoplasm of monocyte took bluish gray colour and nuclei were stained auricula purple colour. Mahajan and Dheer (1979) and Chinabut *et al.* (1991) also found the same staining character of monocytes in air breathing fish.

The granulocytic neutorphils, found in blood of *C. batrachus*, were large and rounded in shape (9-14 µm in size). Abundant fine granules were found in the cytoplasm that stained light blue, whitish or whitish gray colour and their neuclei stained dark purple with Wright's and Giemsa's stains. Eccentrically located, oval or bilobed nuclei were found in the present study. Similar characteristics were also found by Chinabut *et al.* (1991) in *C. batrachus*, Mahajan and Dheer (1979) in *C. punctatus* and Blaxhall and Daisley (1973) in *Salmo trutta*.

Catton (1951) had noted that the basophils were generally absent or rarely seen in blood film. Mahajan and Dheer (1979) reported that eosinophils and basophils had not been clearly characterized in *Channa punctatus*. Similarly in the present study no basophilic and eosinophilic leucocytes were found in the peripheral blood of *C. batrachus*.

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References

Aydin, S., S. Celebi and I. Akyurt, 1997. Clinical, hematological and pathological investigation of *Escherichia vulneris* in rainbow trout (*Oncorhynchus mykiss*). *Fish Pathol.*, **32** (1): 29-34.

Blaxhall, P. C. and K. W. Daisley, 1973. Routine haemotological methods for use with fish blood. *J. Fish Biol.*, 5: 771-781.

Boomker, J. , 1981. The haematology and histology of haematopoietic organs of South African fresh water fish. *J. Vet. Res.*, **48**(4): 195-205.

Catton, W.T., 1951. Blood cell formation in certain teleost fishes. *Blood*, 6: 39-60.

Chinabut, S., C. Limsuwan and P. Kitsawat, 1991. Histology of the walking catfish *Clarias batrachus*. Asian Fish Heath Network Co-ordination. Sponsored by International Development Research Center, Canada. 94 pp.

Dogen, C. L. and S. Sullivan, 1969. Haematological effect of apholate on channel catfish (*Ictalurus punctatus*). *Soc. Exp. Biol. Med. Proc.*, **131**: 124-126.

Ellis, A. E. , 1977. The leucocytes of fish: A review. *J. Fish Biol.*, 11: 453-491.

Grizzle, J. M. and W. A. Rogers, 1976. Anatomy and Histology of the channel catfish (*Ictalurus punctatus*). Auburn University Agricultural Experiment Station. Auburn, Alabama., 94 pp.

Hossain, M. A. and M. Shariff, 1992. Effect of copper on humoral immune response in goldfish, *Carassius auratus In:* Disease in Asian Aquaculture (eds. M. Shariff. R. Subasinghe and J. R. Arthur). Fish Health Section, *Asian Fish. Soc.*, Manila, Philippines. pp. 371-377.

Mahajan, C. L. and J. S. Dheer, 1979. Cell types in the peripheral blood of an air breathing fish *Channa punctatus*. *J. Fish Biol.*, 14: 481-487.

McCarthy, D. H., J. P. Stevenson and M. S. Roberts, 1973. Some blood parameters of the rainbow trout (*Salmo gairdneri*, Richardson). *J. Fish Biol.*, 5: 1-8.

Sardar, M., 1999. Studies on the haematological assessment and phagocytic activity of the leucocytes of walking catfish *Clarias batrachus* (linnaeus) against *Aeromonas hydrophila*. M.S.

Thesis. Department of Aquaculture, Bangladesh Agricultural University, Mymensingh, Bangladesh. 117 pp.

Smith, G.C., W.M. Lewis and H.M. Kaplan, 1952. A comparative morphologic and physiologic study of fish blood. *Prog. Fish. Cult.*, **14**: 169-172.

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Pathogenicity of *Aeromonas sobria* to Thai silver barb (*Barbodes gonionotus* Bleeker) and its sensitivity to some antibiotic agents

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Abstract
Five isolates of *Aeromonas sobria*, collected from the diseased fish were selected for detection the pathogenicity following water-born infection method on silver barbs (*Barbodes gonionotus*) at the selected exposure dose 2.5 x 10^8 CFU/ml which was standardized by preliminary test. In the experimental condition lesion and mortality were found in fishes. Among the isolate, Ass_{13}, Ass_{30}, Ass_{31} and Ass_{36} were successfully infected 20-60% fishes. Another isolate Ass_{28} was found non-pathogenic. Drug sensitivity test was performed by six antibiotics *viz.* Oxytetracycline, Oxolinic acid, Chloramphenicol, Sulphamethozazole, Streptomycin, Erythromycin. All the isolates showed variable reaction patterns to antibiotics. Most of the isolates were found sensitive to Oxytetracycline (OT), Oxolinic acid (OA) and Chloramphenicol (C) but resistance to Erythromycin and Sulphamethoxazole (SXT). Isolate Ass_{31} found resistant to Oxolinic acid.

Key words: *Aeromonas sobria*, Pathogenicity, Sensitivity, *B. gonionotus*

Introduction

Disease is one of the most constraining factor in aquaculture of Bangladesh. Both farmed and wild fishes have been found to be affected by various kinds of diseases (Rahman 1997). Common diseases of fresh water fishes are ulcers including Epizootic Ulcerative Syndrome (EUS), Septicaemic disease, tail and fin rot, bacterial gill rot, dropsy, various types of fungal, parasitic and protozoan disease (Chowdhury 1997). Bacteria, one of the major causative agents found to be associated with many diseased fish as primary causative agent and secondary invaders of ulcers and other lesions. Among all other bacteria, Aeromonads are the major pathogens with are widely distributed in farmed fish and water in Mymensingh region (Banu 1996).

Motile members of *Aeromonas* are ubiquitous in fresh water and known to cause haemorrhagic septicemia in both warm and cold water fishes (Wakabayashi *et al.* 1981). The principal motile species of *Aeromonas* are *Aeromonas hydrophila*, *Aeromonas sobria* and *Aeromonas caviae* (Plumb 1994). Many works on *Aeromonas hydrophila* have been done by

the scientists all over the world including Bangladesh and recognized as pathogen to fish (Banu 1996 and Rahman 1997). But *Aeromonas sobria* was not detected as pathogen until this work in Bangladesh. So, the present study was undertaken considering evaluation of pathogenicity of *Aeromonas sobria* to silver barb (*Barbodes gonionotus*).

Materials and methods

Isolation of bacterial isolates

Some Aeromonad isolates were collected from the ulcer affected different fish species of different water bodies during June to December'97. After collection the fish samples were immediately brought to the laboratory. A selective media Aeromonas Agar Base (Oxoid) supplemented with amphecillin. SR 136 E was used for isolation of Aeromonads isolates. It was found to be suitable for specific culture of Aeromonads (Choudhury and Inglis 1994b). Swabs were taken from the lesions kidney and liver of the the affected fish and stricked on the plate containing Aeromonas selective agar by sterile inoculating loop and subsequently pure culture were obtained on TSA media using conventional separation techniques.

Identification of Aeromonas sobria

Primary characterization were carried out upon Cowan and Steel's Manual for the identification of medical Bacteria edited by barrow and Felthaim (1993). For species level identification of *A. sobria* biochemical tests were performed according to barrow and Felthan (1993) and finally confirmed with the Bergesy's Manual for Systematic Bacteriology (Krieg and Holt 1984).

Pathogenicity test for A. sobria isolates

Five *Aeromonas sobria* isolates selected to investigate the pathogenic power to fish. The study was performed by water born infection method. The isolates are listed below :

Aeromonas sobria Isolates	Host fish	Location
ASS_{17}	*Clarias gariepinus* (Fingerling)	Wet laboratory, FF, BAU
ASS_{19}	*C. gariepinus*	Fisheries Bio. and Gen. Lab. FF, BAU
ASS_{20}	*C. gariepinus*	Wet laboratory, FF, BAU
ASS_{31}	*Barbodes goniomotus*	Faculty pond, FF, BAU
ASS_{36}	*C. gariepinus*	Field laboratory, FF, BAU

FF=Faculty of Fisheries.

The experiment was conducted in the wet laboratory of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. Healthy young silver barb collected from an experimental pond under Fisheries Faculty were selected for pathogenicity test. These were further acclimatized to aquarium conditions before use in the experiment. The fish was a exotic carp, *Barbodes gonionotus* weighing 10-15 gm.

Isolates were cultured on the TSA medium by spreading method and incubated at 25 °C for 24 h. The stock suspension of bacteria was prepared in sterile tap water with required amount of bacteria. In 30 l capacity aquarium, 15 l of bacterial suspension was prepared in the tap water with the stock suspension in such way that the bacterial density became 2.5 X 10^8 CFU/ml. This exposure dose of bacteria was selected by a preliminary experiment. Five fishes were exposed to bacterial suspension in aquarium under aerated condition at room temperature (water temperature ranged 24-27 °C). After 24 h of exposure 80% of the bacterial suspension was exchanged with tap water and from the following day 60% of water was exchanged at every 24 h of experimental period. The experimental period was 15 days.

Two replications were set up for the same isolate and the experiments were repeated for confirmation of infection, and thus in total 20 fish were used for single species against the individual bacterial isolate. For each set of experiment control fish were maintained in the same way. Aeration system was controlled by air pump. No feed was applied during the experimental period.

The appearance of lesion and mortality of experimental fish confirmed the infection. The pathogen was confirmed by reisolation of bacteria from the exposed fish. In the experimental condition radish zone appeared on the body surface, tail region, lower jaw and fin base of the fish. Then it terns in to dip hemorhagic lesion and died. Some time fishes were died with out appearing any lesion on the body surface. For these case internal infection was found. Experimental fish with lesion or died fishes were collected with the sterile forcep and container. Then isolation was done by the previous way.

Antibiotic sensitivity test

Sensitivity test were performed by disc dispenser method according to Islam and Chowdhury (1997). The drug discs (Oxoid Ltd.) were Oxytetracycline (30 μg/disc), Chloramphenicol (30 μg/disc), Streptomycin (10 μg/disc) and Oxolinic acid (2 μg/disc) were used to observed the resistance pattern of *A. sobria* isolates.

Results

Among the five isolates of *Aeromonas sobria*, four were capable of causing lesion and mortality in experimental fish. But the appearance of lesion and mortality of silver barb varied from one isolate to another. Four isolates produced lesion in 20-50% fishes and mortality ranged 30-60% and reisolation was positive (Table 1).

Isolate Ass_{17} showed haemorrhagic lesions and mortality in fishes. Lesions were observed at 6th day but the mortality was started at the 8th day of exposure. Appearance of lesion and mortality gradually increased day by day and 60% fishes were died where lesions appeared in 50% of fishes (Fig. 1).

When the fishes were exposed to the bacterial suspension with isolates Ass_{19} the appearance of lesions were observed at 7th day and gradually increased with the increase of time. Mortality first recorded at the 10th day of exposure. Half of the fishes (50%) were died where 30% found to be affected by lesion (Fig. 2). In case of isolate Ass_{31} 20% fishes were died where haemorrhages were observed also in 20% fishes (Fig. 3). Isolates Ass_{36}

also showed lesion and mortality in fish. Mortality began at the 7th day of exposure. Lesion appeared in 30% fishes and 50% mortality were observed (Fig. 4). However, no lesion and mortality was recorded with Ass_{20}.

Table 1. Pathogenicity test of some isolates of *Aeromonas sobria* against silver barb

Aeromonas sobria isolates	No. of fish callenged	Fish				Reisolation
		With lesion		Mortality		
		No.	(%)	No.	(%)	
ASS_{17}	20	10	50	12	60	+
ASS_{19}	20	6	30	10	50	+
ASS_{20}	20	0	0	0	0	Nd
ASS_{31}	20	4	20	4	20	+
ASS_{36}	20	6	30	10	50	+
Control	20	0	0	0	0	Nd

Nd = Not detected, + = Confirmed by reisolation
The data summarizes to repeated trials. In each 10 fishes were used for individual Aeromonas sobria isolates n = 20.

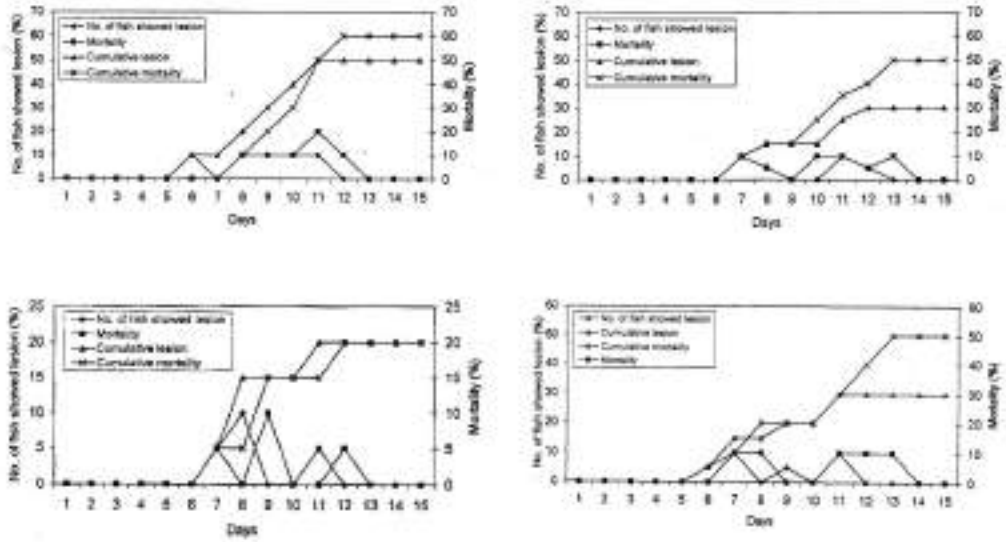

Fig. 1-4. Daily and cumulative percentage of lesion and mortality of silver barb exposed to different isolates.

The *Aeromonas sobria* isolates showed various sensitivity patterns to the six different antibiotics tested (Table 2). Among these Ass_{17}, Ass_{19} and Ass_{16} were highly sensitive to Oxytetracycline (OT), Oxolinic acid (OA) and Chloramphenicol (C) and resistant to Erythromycin (E) and Sulphamethoxazole (SXT).

Table 2. Reaction of *Aeromonas sobria* to some antibiotic agents

Aeromonas sobria Isolates	Response to different antibiotic agents with their zone of inhibition (mm)					
	OT	OA	S	C	E	SXT
ASS_{17}	25	20	17	22	R	R
ASS_{19}	20	15	R	15	R	R
ASS_{20}	± 20	± 12	± 15	± 16	R	± 20
ASS_{8}	15	R	10	17	10	11
ASS_{16}	22	17	R	22	R	10

OT = Oxytetracycline OA = Oxolinic acid C= Chloramphenicol SXT = Sulphamethoxazole
S = Streptomycin E = Erythromycin
± = Confusing zone R = Resistant

Discussion

The experimental infection of five selected *Aeromonas sobria* isolates were performed by bath exposure of fish host to 2.5 X 10^8 CFU/ml bacterial suspension. Four isolates successfully caused infection and mortality in fishes. The results of the present study was correlated with a number of scientists in the world. Rogulska *et al.* (1994) performed artificial subepidermal infection with *Aeromonas sobria*, 10^7 bacterial cells in 0.2 ml of 85% PBS in 2 year old carp fish and found it as pathogen. Xu *et al.* (1985) stated that *Aeromonas sobria* is the causal bacterium of the caudal peduncle disease of grass carp. Panjagua *et al.* (1996) carried out an investigation on pathogenicity of *Aeromonas* strains and found 72.02% of *Aeromonas hydrophila*, 63% of *Aeromonas sobria* isolates were virulent for fish by intramuscular challenge. Sopinska *et al.* (1997) identified *Aeromonas sobria* as pathogen to carp. Gantam *et al.* (1992) carried out an experiment and stated the *Aeromonas sobria* was potentially pathogenic.

The results of drug sensitivity tests were variable to six antibiotics. Most of the isolates were highly sensitive to Oxytetracycline, Oxolinic acid and Chloramphenicol. On the other hand, they were resistance to Erythromycin and Sulphamethoxazole. Banu (1996) observed similar results for Aeromonads isolates. The results partially correlated with the observation of Chowdhury and Baqui (1997). Bornemann (1989) observed that strains of *Aeromonas sobria* are resistant to Ampicilin, 72% to Chlortetracycline, 12% to Kanamycin and 8% to Chloramphenicol.

In the present study, the selected *Aeromonas sobria* isolates under Aeromonad genus were capable of producing ulcerative disease.

References

Banu, G.R., 1996. Studies on the bacteria *Aeromonas* spp. in farmed fish and water in Mymensingh region. M.S. Thesis, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh. 95 pp.

Barrow, G.I. and R.K.M. Feltam, 1993. Cowan and steel's manual for the Identification of Medical Bacteria. Third edition. Cambridge University press. 331pp.

Bornemann, H., 1989. Occurrence of antibiotic resistant strains of *Aeromonas* in farmed carp. *J. Clinical Microbil.*, **12**: 146.

Chowdhury, M.B.R., 1997. Bacterial involvement in fish disease in Bangladesh. Represented at the International Symposium on Disease in marine Aquaculture, October 3-6, 1997. Hiroshima, Japan, Abstract: III-2: 24.

Chowdhury, M.B.R. and V. Inglis, 1994b. Study on the resistance Pseudomonads in the fish ponds of Bangladesh to some antibacterial agents. Proceedings, International Congress on Quality Veterinary Services for the 21st century, pp. 78-83.

Chowdhury, M.B.R. and M.A. Baqui, 1997. Bacterial flora in farmed carp (*Labeo rohita*) in Bangladesh. *In*: Diseases in Asian Aquaculture III (eds. T.W. Flegel and I.H. MacRae). Fish Health Section, Asian Fisheries Society, Manila. pp. 101-105.

Gantam, A.R., S.P. Pathak, P.W. Ramtake and J.W. Bhattacharjee, 1992. Virulence factors in environmental isolates of *Aeromonas* spp. *J. General and Applied Microbiol.*, **38**: 185-189.

Islam, M.S. and M.B.R. Chowdhury, 1997. Status of antibiotic resistant Pseudomonas in farmed fish and water in Bangladesh. *Bangladesh Vet. J.*, **31**: 41-46.

Krieg, N.R. and J.G. Holt (eds.), 1984. Bergeys Manual of Systematic Bacteriology, vokum -1, Willams and Wilkings. 964pp.

Paniagua, C., O. Rivero, J. Anguita and G. Naharro, 1990. Pathogenicity factors and virulence for rainbow trout (salmogaridneri) or mitile *Aeromonas* spp. Isolated from a river. *J. Clinical Microbiol.*, **28**: 350-355.

Plumb, J.A., 1994. Health maintenance of cultured fishes: Principal Microbial Diseases. CRC Press. Boca Raton, Ann Arbor, London, Tokyo. 239 pp.

Rahman, M.M. , 1997. Studies on the bacterial associated with ulcer disease in the farmed and wild fiehes of Mymensingh. M.S. Thesis, Faculty of Fisheries, BAU., Mymensingh., Bangladesh.

Rogulska, A., J. Antychowicz and J. Zelazny, 1994. Haemolytic and proteolytic activity of *Aeromonas hydrophila* and *Aeromonas sobria* as markers of pathogenicity for carp (*Cyprinus carpio* L.). *Medycyna-Weterynaryjna*, **50**: 55-58.

Sopinska, A., A. Grochola and L. Guz, 1997. Pathogenicity and cytotoxicity of *Aeromonas* sp. infection in carp. *Medycyna-Weterynaryjna*, **53**: 394-397.

Wakabayashi, H.K., T.C. Kanai Hsu and S. Egusa, 1981. Pathogenic activities of *Aeromonas hydrophila* biovar hydrophila (Chester) to fishes. *Fish Pathol.*, **15**: 319-325.

Zu, H.H., R.F. Ge and M.L. Xiong, 1985. Studies on the pathogenic bacteria of the "peduncle disease" of grass carp (*Ctenophyrynogodon idellus*) and its relation with other body surface of diseases of fish. *Acta-Hydrobiologica-Sinica*, **10**(1): 39-51.

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Pathology of mixed infections of saprolegniasis-myxosporidiosis in Indian major carp (*Catla catla* Ham.)

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Abstract

An outbreak of saprolegniasis in *Catla catla* in composite carp culture ponds were recorded during winter season. The typical cotton wool growths were observed on whole body surfaces of catla along with sporadic mortality. The fungal invasion was only restricted to skin and no fungal elements were visible in any internal organs after periodic acid schiff staining. On histology, periportal accumulation of mononuclear cells in liver, presence of myxosporidean cysts in antieror kidney, eosinophilic granular cells reaction in submucosa of stomach and intestine, dilated and engorged blood vessels of brain along with sloughing of epidermis and hyperplasia at gill lamellar base were pronounced changes. The possible role of release of *Saprolegnia* toxin in producing internal organs pathology has been discussed .

Key words: Saprolegniasis, Myxosporidiosis, *C. catla*

Introduction

Fungal infections of carp are serious problem in intensive system of aquaculture. Winter saprolegniasis (Winter Kill) is a serious disease affecting pond raised carps that produces clinical signs of growth of conspicuous fungal colonies growing on body surface of the fish. These cotton-wool like lesions are normally white in colour, or discoloured by the accumulation of debris between the fungal hyphae (Jeney and Jeney 1995). The fungal growths on the skin were generally thought to be secondary to primary skin/scale erosion and some predisposing factors. However, recent studies have demonstrated that winter kill of channel catfish resulted from an immunosuppression caused by a rapid decrease in water temperature in presence of zoospores of the oomycete *Saprolegnia* sp. (Bly and Clem 1991, Bly *et al*, 1992 & 1993).

The primary sequela of uncomplicated saprolegniasis is osmotic imbalance due to loss of epithelial integrity (Noga 1993). Histopathological changes described due to saprolegniasis in common carp was mostly restricted to epidermis and dermis, being characterised by degeneration of epidermal cells, focal oedema in dermis and ultimate sloughing of epidermis without any inflammatory reaction at the site of infection (Jeney and Jeney 1995). However, Alvarez *et al.* (1988) found damage to hemopoietic organs

with lymphoid cell degeneration, and vascular alterations with blood vessel enlargement and hypertrophy of sinusoidal endothelial cells of liver in brown trout due to saprolegniasis. Surprisingly, Duran *et al.* (1987) could able to detect extreme increase in liver specific enzymes with slight elevation of muscle enzymes in brown trout infected with saprolegniasis. The above inconsistent findings prompted us to study the damage caused due to saprolegniasis in internal organs of *Catla catla* obtained from pond culture systems during one winter outbreak.

Myxosporidia are also an important group of pathogens known to parasitize fish. Several species of myxosporidia have been found to infect cultivable fishes also in India (Chakravarty 1939 & 1943, Tripathi 1952, Bhatt and Siddiqui 1964, Chaudhuri and Chakravarty 1970, Karamchandani 1970, Seenappa and Manohar 1980, Mishra *et al.* 1984, Dey *et al.* 1988). Sanaullah and Ahmed (1980) from Bangladesh have reported number of cases of mass mortalities of the fingerlings of Indian major carps, *Catla catla* due to *Myxobolus* spp. Infection. They cause great problems in intensive fish culture and seed rearing leading to emaciation, retarded growth and even mass mortality (Hoshina 1952, Tripathi 1952, Dey *et al.* 1988). The enzootic nature of myxosporidiasis in Indian major carp in Orissa, India has been reported, where the pathogen produces infections ranging from sub-clinical and lethal. The fish that survive infection may become lifelong carrier and spread the infection (Mishra *et al.* 1982). Dykova and Lom (1988) reviewed the literature on myxosporeans in intensive culture of carp and concluded that the damage exerted by these species may range from almost non to serious growth impairment or direct mortality, depending on the intensity of infection, of fish condition and environmental factors. This paper describes one of the complicated field outbreaks of mixed infections of saprolegniasis and myxosporidiosis in Indian major carps from one organised freshwater farm.

Materials and methods

During one winter (November to January), it was observed that few of the ponds (0.1 to 0.4 ha area) stocked with three species of Indian major carps, were infested and infected with *Argulus* and *Saprolegnia*. It was also interesting to note that the host specificity of argulosis was restricted mostly to rohu (*Labeo rohita*), and saprolegniasis was only marked in catla. Rohu with argulosis were not included in this study. The affected ponds revealed >90% morbidity of infection during December.

Sporadic mortality was only marked in catla in the ponds few days before netting. During netting, it was observed that most of the catla (80%) were having the saprolegniasis lesions. The pH of the ponds water was varied from 6.3 to 7.7 and temperature was 18 to 24 °C. Diseased fish showing initial, advance lesions of skin ulcers and fungal growths as well as succumbed fish of about 35 numbers were collected during the period mentioned and brought to the laboratory for examination. The fungal specimens were isolated and subjected to wet mount examination with lactophenol cotton blue stain. Subsequently, the fish were anaesthetized with MS 222 (Tricane methane sulfonate, Sandoz) and necropsy was conducted. Internal organs *viz*, liver,

with lymphoid cell degeneration, and vascular alterations with blood vessel enlargement and hypertrophy of sinusoidal endothelial cells of liver in brown trout due to saprolegniasis. Surprisingly, Duran *et al.* (1987) could able to detect extreme increase in liver specific enzymes with slight elevation of muscle enzymes in brown trout infected with saprolegniasis. The above inconsistent findings prompted us to study the damage caused due to saprolegniasis in internal organs of *Catla catla* obtained from pond culture systems during one winter outbreak.

Myxosporidia are also an important group of pathogens known to parasitize fish. Several species of myxosporidia have been found to infect cultivable fishes also in India (Chakravarty 1939 & 1943, Tripathi 1952, Bhatt and Siddiqui 1964, Chaudhuri and Chakravarty 1970, Karamchandani 1970, Seenappa and Manohar 1980, Mishra *et al.* 1984, Dey *et al.* 1988). Sanaullah and Ahmed (1980) from Bangladesh have reported number of cases of mass mortalities of the fingerlings of Indian major carps, *Catla catla* due to *Myxobolus* spp. Infection. They cause great problems in intensive fish culture and seed rearing leading to emaciation, retarded growth and even mass mortality (Hoshina 1952, Tripathi 1952, Dey *et al.* 1988). The enzootic nature of myxosporidiasis in Indian major carp in Orissa, India has been reported, where the pathogen produces infections ranging from sub-clinical and lethal. The fish that survive infection may become lifelong carrier and spread the infection (Mishra *et al.* 1982). Dykova and Lom (1988) reviewed the literature on myxosporeans in intensive culture of carp and concluded that the damage exerted by these species may range from almost non to serious growth impairment or direct mortality, depending on the intensity of infection, of fish condition and environmental factors. This paper describes one of the complicated field outbreaks of mixed infections of saprolegniasis and myxosporidiosis in Indian major carps from one organised freshwater farm.

Materials and methods

During one winter (November to January), it was observed that few of the ponds (0.1 to 0.4 ha area) stocked with three species of Indian major carps, were infested and infected with *Argulus* and *Saprolegnia*. It was also interesting to note that the host specificity of argulosis was restricted mostly to rohu (*Labeo rohita*), and saprolegniasis was only marked in catla. Rohu with argulosis were not included in this study. The affected ponds revealed >90% morbidity of infection during December.

Sporadic mortality was only marked in catla in the ponds few days before netting. During netting, it was observed that most of the catla (80%) were having the saprolegniasis lesions. The pH of the ponds water was varied from 6.3 to 7.7 and temperature was 18 to 24 °C. Diseased fish showing initial, advance lesions of skin ulcers and fungal growths as well as succumbed fish of about 35 numbers were collected during the period mentioned and brought to the laboratory for examination. The fungal specimens were isolated and subjected to wet mount examination with lactophenol cotton blue stain. Subsequently, the fish were anaesthetized with MS 222 (Tricane methane sulfonate, Sandoz) and necropsy was conducted. Internal organs *viz*, liver,

kidney stomach, intestine, spleen, pancreas, brain along with gills and affected skin with muscle were collected and processed for histopathology . The tissues were fixed in 10% phosphate buffered formalin and processed for haematoxylin and eosin staining. The internal organs were also stained with periodic acid- schiff (PAS; Humason 1972).

Results and discussion

The yearlings of catla (weight range, 600-1000g) were affected. Although, most of rohu of the ponds were infested with argulosis, catla were almost free from louse infestation. Rohu are more prone to argulosis in composite carp culture ponds in comparison to catla which has been reported earlier (Dey 1989).

The catla infected with saprolegniasis had cotton wool growth all over the body surface including the head region and gill surfaces. The scales were rough and loosened. Some places, fungi had invaded deep into musculature with sloughed up epidermis. Similar type of lesions were also observed in common carp due to saprolegniasis (Pickering and Willoughby 1982).

On wet mount preparation with lactophenol cotton blue staining, it was confirmed, based on their mycelia and zoo sporangia that the fungi belong to *Saprolegnia* sp. as described by earlier worker (Noga 1993).

On PAS staining, the fungal elements could not be detected in any of the internal organs. On histology, the degenerative changes in epidermis and dermis without any inflammatory reaction could be marked. Pickering and Richards (1980) also described absence or weak inflammatory response at the infected site unless secondary bacterial infections complicate the process during saprolegniasis. There was massive accumulation of mononuclear cells around the portal vessel of liver (Fig.1) with mild degenerative changes of hepatocytes. The increase in the liver-specific enzyme levels in serum of brown trout due to saprolegniasis as observed previously by Duran *et al.* (1987) might have occurred due to damage to the liver. Surprisingly, the anterior kidney revealed myxosporidian cysts in the haemopoietic areas (Fig. 2). However, the haemopoietic organs did not reveal any degenerative changes as observed in brown trout (Alvarez *et al.* 1985). The innocuous myxosporidean cysts were also observed in apparently healthy Indian major carps kidneys in many instances earlier, particularly when the fish were infected with other organisms (Kumar *et al.* 1986, Sahoo *et al.* 1998). The degree of damage to kidney and other organs due to myxosporidiosis depends on the intensity of infection in fish and environmental conditions, mostly (Dykova and Lom 1988). Thus, the presence of myxosporidean cysts in kidney only further confirmed enzootic and carrier nature of the pathogen in Indian major carp in this region as reported earlier (Mishra *et al.* 1982). The submucosa of the stomach and intestine revealed massive eosinophilic granular cell reaction (Fig. 3) which might be indicative of degree of stress in fish. The pial vessels present in periphery to the ventricles in the brain were profusely dilated and engorged (Fig. 4) . Although, Alvarez *et al.* (1988) marked enlarged blood vessels in trout saprolegniasis, the changes could be marked in blood vessels of haemopoietic tissue. The heart, spleen, pancreas and other internal

organs were devoid of any marked alteration. Other than these changes, there was hyperplasia of epithelial cells at the base of primary lamellae in the gills. However, neither the myxosporidean cyst nor the fungal elements could be marked in the gill. The cause of hyperplastic reaction could not be determined.

Fig.1. Liver showing periportal accumulation of mononuclear cells (H&E X200).

Fig.2. Myxosporidian cyst (arrow) in the anterior kidney (H & E X 400).

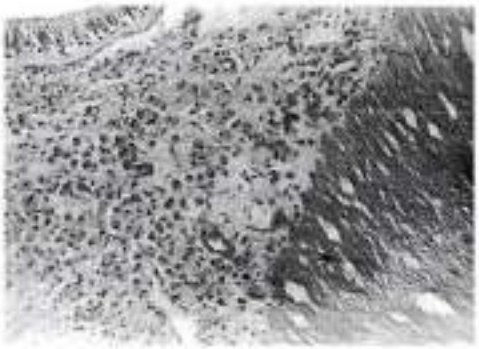

Fig. 3. Submucosa of stomach revealing massive reaction of eosinophilic granular cells (H & E X 400).

Fig.4. Dilatation and engorgement of pial blood vessels in brain (H & E X 200).

The changes observed in many of the organs might be adding the osmoregulatory problems further there by causing death of the fish ultimately. As the fungal elements could not be traced in any internal organs on PAS reaction, the observed changes may be due to release of some toxic factors by the fungus itself at the infection site, which might have reached in other organs through blood circulation. The changes in the vasculature as observed earlier and also in this experiment further added to the role of release and transport of some toxic materials by the fungi. On the contrary, according to Pickering and Willoughby (1982) there is no evidence that pathogenic saprolegnia strains produce any toxins that might be transmitted systematically. However, further studies on these aspects are warranted to confirm the cause of damage to internal organs due to saprolegniasis.

In the present study, the occurrence of myxosporidean cysts indicated the enzootic nature of this pathogen in Indian major carps in this state, and saprolegniasis could further proved to be season-specific pathogen, occurring particularly in winter due to fall of temperature leading to immunosuppression.

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References

Alvarez, F., B. Razquin, A. Villena, P. Lopez Fierro and A. Zapata, 1988. Alterations in the peripheral lymphoid organs and differential leukocyte counts in *Saprolegnia*-infected brown trout, *Salmo trutta*. *Vet. Immunol. Immunopath.*, 18: 181-193.

Bhatt, V.S., W.A. Siddiqui, 1964. Four new species of myxosporidia from the Indian freshwater fish, *Ophiocephalus punctatus* (Bloch). *J. Protozool.*, **11**: 314-316.

Bly, J.E. and L.W. Clem, 1991. Temperature- mediated processes in teleost immunity: *In vitro* immunosuppression induced by *in vivo* low temperature in channel catfish. *Vet. Immunol. Immunopath.*, **28**: 365 - 377.

Bly, J.E., L.A. Lawson, A.J. Szalai and L.W. Dlem, 1993. Environmental factors affecting outbreaks of winter saprolegniosis in channel catfish, *Ictalurus punctatus* (Rafinesque). *J.. Fish Dis.*, **16**: 541-549.

Bly. J. E., L. A. Lawson, D.J. Dale, A.J. Szalai, R.M. Durborow and L.W. Clem, 1992. Winter saprolegniosis in channel catfish. *Dis. Aquat. Org.*, **13**: 155-164.

Chakravarty, M. M., 1939. Studies on myxosporidia from fishes of Bengal with a note on the myxosporidian infection in aquaria fishes. *Arch. Protistenk.*, **92**: 169-178.

Chakravarty, M. M., 1943. Studies on the myxosporidia from common food fishes of Bengal. *Proc. Ind..Acad. Sci.*, **18B**: 21-35.

Chaudhuri, S.R., M.M. Chakravarty, 1970. Studies on the myxosporidia (Protozoa: Sporozoa) from the food fishes of Bengal. Three new species from Ophiocephalus punctatus (Bloch). *Acta Protozool*, **8**: 167-173.

Dey, R.K., 1989. Histopathology of Argulosis in the Indian major carp, *Labeo rohita*. *Proc. Nat. Sem. Freshwat. Aqua.*, : 126-127.

Dey, R.K., D. Kumar and B.K. Mishra, 1988. Tissue level reaction s in the Indian major carp, *Catla catla* (Ham.), due to *Myxobolus* sp. Infections. *Asian Fish. Sci.*, **1**: 117-122.

Duran, A., L.B. Rodriguez Apracio, A. Reglero and J. Perez Diaz, 1987. Changes in Serum enzymes of saprolegnia infected brown trout, *Salmo trutta* L. *J. Fish Dis.*, **10**: 505 -507.

Dykova, I. And J. Lom, 1988. Review of pathogenic myxosporeans in intensive culture of carp (*Cyprinus carpio*) in Europe. *Folia Parasitologica*, **35**: 289-307.

Hoshina, T., 1952. Notes on some myxosporidian parasites on fishes of Japan. *J. Tokyo Uni. Fish.*, **39**: 69-89.

Humason, G. L., 1972 Animal Tissue Techniques. W.H. Freeman and Co, San Francisco. pp. 166-167.

Jeney, Zs. and G. Jeney, 1995. Recent achievements in studies on diseases of common carp (*Cyprinus carpio* L.). *Aquaculture*, **129**: 397 -420.

Karamchandani, S. J., 1970. A new species of Myxobolus from the gill cavity of Labeo bata (Hamilton). *J. Ind. Fish. Soc., India*, **2**: 170-171.

Kumar, D., B. K. Mishra and R. K. Dey, 1986. Dropsy in *Catla catla caused* by mixed infection of *Aeromonas hydrophila* and Myxosporidian sp. *Aqua. Hungarica (Szarvas)*, **V**: 107-112.

Mishra, B. K., D. Kumar and R. K. Dey, 1984. Observations on the efficacy of some drugs against renal myxosporidiasis of Indian major carp. *Veterinarski Arhiv*, **54**: 211-215.

Mishra, B. K., R.K. Dey, D. Kumar, K. Suresh, 1982. Observations on renal myxosporidiosis in Indian major carp. Abstract. *Proc. Symp. Dis. Finfish Shellfish*, College of Fisheries , Mangalore,1-3 March, 1982.

Noga, E.Z, 1993. Water mold infections of freshwater fish: recent advances. *An. Rev. Fish Dis.* **3**: 291-304.

Pickering, A.D and L.G, Willoghby , 1982. *Saprolegnia* infections of salmonid fish. In: Microbial diseases of fish (ed. R. J. Roberts). Academic Press, London. pp. 271- 298.

Pickering, A.D. and R. H. Richards, 1980. Factors influencing the structure, function and biota of the salmonids epidermis. *Proc. Royal Soc. Edinb.*, **79 B**: 93 -104.

Sahoo, P. K., S. C. Mukherjee, A. K. Jain and Ashok Mukherjee, 1998. Light and ultrastructural changes in opisthonephros of rohu, *Labeo rohita* during acute and subchronic aflatoxin B_1

toxicity (Abstract). Fifth Asian Fisheries Forum and International Conference of Fisheries and Food Security beyond the Year 2000, Chiang Mai, Thailand

Sanaullah, M., and A. T. T. Ahmed, 1980. Gill myxoboliasis of major carp, Bangladesh. *J. Fish Dis.*, **3**: 349-354.

Seenappa, D. and L. Manohar, 1980. *Myxobolus vanivilasae* n. s. parasitic in *Cirrhina mrigala* (Hamilton). *Proc. Ind. Acad. Sci.*, **89**: 485-491.

Tripathi, Y. R., 1952. Studies of parasites of Indian fishes. Protozoa: Myxosporidia together with a check list of parasitic protozoa described from Indian fishes. *Rec. Ind. Mus.*, **50**: 63-88.

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Population dynamics of the ribbon fish, *Lepturacanthus savala* (Cuvier 1829) from the north-eastern part of the Bay of Bengal

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Abstract
Population parameters of *Lepturacanthus savala* from the trawl catches in the north-eastern part of the Bay of Bengal, Bangladesh were investigated based on length frequency data, using compleat ELEFAN computer program. The asymptotic length (L_∞) and growth constant (K) were estimated to be 106.50 cm (total length) and 0.80/year respectively. Based on these growth parameters, the total mortality (Z) was estimated to be 1.89. The estimated values for natural mortality (M) and fishing mortality (F) were 1.08 and 0.81 respectively. The estimated value for the exploitation rate (E) using the length converted catch curve was 0.43. The recruitment pattern showed two peaks per year. The estimated sizes of *L. savala* at 25, 50 and 75 percent probabilities of capture were 57.49, 60.39 and 63.28 cm respectively. The estimated length weight relationship for combined sex was $W = 0.00093\ TL^{2.97}$

Key words: Population dynamics, *L. savala*, Bay of Bengal

Introduction

Lepturacanthus savala (F. Trichiuridae) is the most abundant and commercially important fish in the Bay of Bengal. It is locally known as "Chori Mach". It forms about 2.5% of the total demersal trawl catch (Lamboeuf 1987) and account for 0.83% of the estimated total shrimp trawl production for 1989-90 (Mustafa and Khan 1992). They feed on teleostean fishes, shrimps, other crustacean and cephalopods (Mustafa and Begum 1994). Dried fish is very popular all over the country and also exported to the other countries like U.K., Singapore, Middle-East and SriLanka.

There is no published information on population dynamics of *Lepturacanthus savala* in Bangladesh. In the present study, the population parameters of *L. savala* were estimated to assess its stock in the north-eastern part of the Bay of Bengal.

Materials and methods

Collection of data

Length frequency data of *L. savala* were collected from trawler catches in the north-eastern part of the Bay of Bengal during the period October 1996 to September 1997 by making regular fortnight field visits to the major fish landing centres in Firingibazar, Chittagong. More than eight trawlers of Sea Resources Group Companies Ltd. Bangladesh were sampled randomly. On each sampling day, total lengths of 100-200 fish obtained by random sampling were measured to the nearest 0.1cm using a measuring scale.

Analysis of data

Monthly length frequency distribution of *L. savala* for each month was analysed using compleat ELEFAN computer program (Gyanilo *et al.* 1989). The program was also used to estimate the parameters of the von Bertalanffy growth equation. The fitting of the best growth curve was based on the ELEFAN I program, which allows the line to pass through the maximum number of peaks of the length frequency distribution. With the aid of the best growth curve the growth constant (K) and the asymptotic length ($L\infty$) were estimated. Additional estimate of $L\infty$ and Z/K value were obtained by plotting $\bar{L}$ minus L' on $\bar{L}$ (Wetherall 1986 as modified by Pauly 1986), i.e.

$L - L' = a + bL'$

where, $L\infty = - a/b$ and $Z/K = - (1 + b)/b$

where $\bar{L}$ is defined as the mean length, computed from L' upward, in a given length-frequency sample while L' is the limit of the first length class used in computing a value of $\bar{L}$.

The growth performance of *L. savala* population in terms of length growth was compared using the index of Pauly and Munro (1984). i.e.

$\phi' = \text{Log}_{10} K + 2 \text{Log}_{10} L\infty$. (Where $L\infty$ is the asymptotic length in cm and K is the growth constant per year).

The instantaneous total mortality coefficient (Z) was estimated using the length converted catch curve method which has been incorporated into the compleat ELEFAN computer program (Gayanilo *et al.* 1989). Natural mortality rate (M) was estimated using Pauly's empirical relationship (Pauly 1980) i.e.

$\text{Log}_{10} M = - 0.0066 - 0.279 \text{Log}_{10} L_\infty + 0.6543 \text{Log}_{10} K + 0.4634 \text{Log}_{10} T$

where L_∞ is expressed in cm and T, the mean annual environmental water temperature in °C (here it was 28°C).

Fishing mortality (F) was obtained by subtracting M (natural mortality) from Z (total mortality) and exploitation rate (E) was obtained from F/Z (Gulland 1971). Recruitment pattern was obtained by backward projection on the length.

Using Pauly's empirical equation for theoretical age at length zero (Pauly 1979) a very approximate estimate of theoretical age at length zero was obtained. The equation used as follows:

$\text{Log}_{10} (-t_0) = - 0.3922 - 0.2752 \log_{10} L\infty - 1.038 \log_{10} K$

The recruitment pattern was also derived using the compleat ELEFAN computer program (Gayanilo *et al.* 1989).

The probabilities of capture by length (Pauly 1984) of *L. savala* were estimated by calculating the ration between the points of the extrapolated descending arm and the corresponding assending arm of the length converted catch curve.

Relative yield-per-recruit Y/R and biomass-per-recruit B/R were obtained from the estimated growth parameters and probabilities of capture by length (Pauly and Soriano 1986). The calculations were carried out using the compleat ELEFAN package developed at ICLARM (Ingles and Pauly 1984).

Length weight relationship was estimated for combined sex using simple linear regression (Zar 1984). For this purpose 352 specimens of *L. savala* were measured and varied from 12.0 cm to 32.0 cm in total length and 25g to 340g in body weight during one year samples.

Results and discussion

Growth parameters

The length range obtained in the fishery was 32-104 cm. In addition, the length range, which contributed significantly to the fishery, was within 56-70 cm. The length frequency distribution of *L. savala* for one year study period are shown in Fig. 1. The best growth curves estimated by the compleat ELEFAN computer program (Gayanilo *et al.* 1989) are shown in this figure. The values for asymptotic length ($L\infty$) and the von Bertalanffy growth co-efficient (K) estimated for the stock were 106.50 cm and 0.80/year respectively. The powell-wetherall plot is shown in Fig. 2. The corresponding estimates of $L\infty$ and Z/K for *L. savala* are 106.90 cm and 2.38 respectively. This additional estimate of $L\infty$ is slightly higher than the estimated through ELEFAN I.

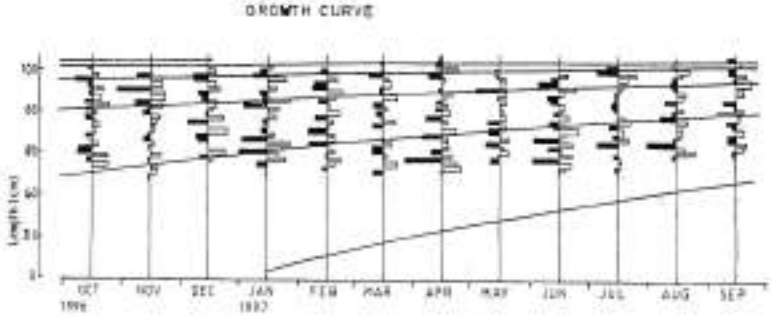

Fig. 1. Monthly length frequency distribution of *Lepturacanthus savala* during the study period with the estimated growth curves.

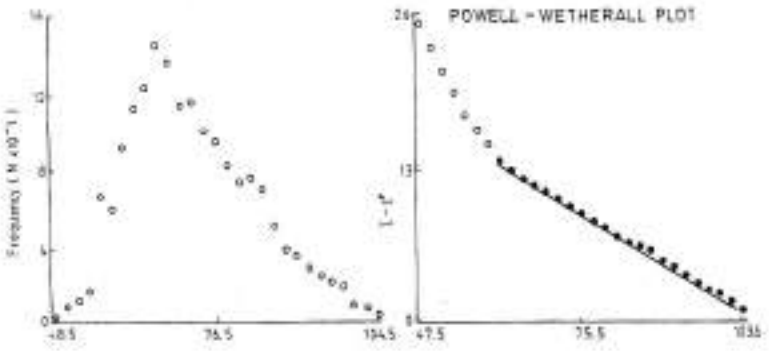

Fig. 2. Powell-wetherall plot of *Lepturacanthus savala* (L∞= 106.90cm and Z/K=2.38).

Mortality and exploitation rate

The length converted catch curve of *L. savala* is shown in Fig. 3. The values for instantaneous total mortality co-efficient (Z), natural mortality co-efficient (M), Fishing mortality co-efficient (F) and the exploitation rate (E) calculated form the data points of figure were 1.89, 1.08, 0.81 and 0.43 respectively. It appears that the stock of *L. savala* of the investigated area is not under fishing pressure.

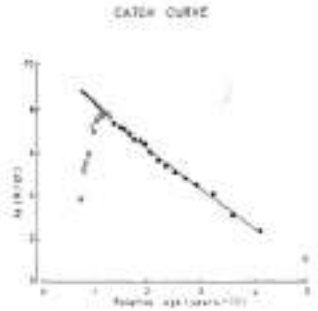

Fig. 3. Length-converted catch curve of *Lepturacanthus savala.*

Theoretical age of length zero (t_o)

The estimated value for t_o using Pauly`s empirical equation (Pauly 1979) was 0.0708/year.

Recruitment pattern

Recruitment pattern of *L. savala* during the study period are shown in Fig. 4. The recruitment pattern showed two peaks, one around in March and the other around in July. The means of two pluses of recruitment are separated by an interval of four months. The first pulse produced 11.39% of the recruits while the other produced 16.56%.

Fig. 4. Recruitment patter of *Lepturacanthus savala.*

Probabilities of capture

The probabilities of capture of *L. savala* is shown in Fig. 5. The estimated sizes of *L. savala* at 25%, 50% and 75% probabilities of capture were 57.49 cm, 60.39cm and 63.28 cm respectively.

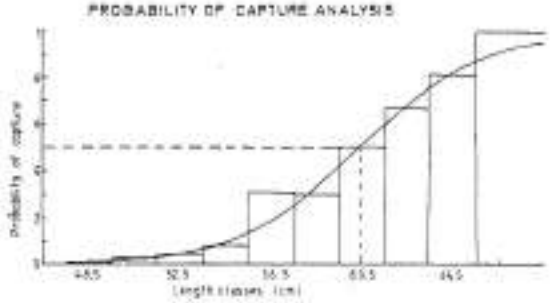

Fig. 5. Probabilities of capture pattern of *Lepturacanthus savala.*

The yield-per-recruit and biomass-per-recruit

The relative yield-per-recruit and biomass-per-recruit were determined as a function of Lc/L∞ and M/K are 0.567 and 1.35 respectively. Fig. 6 shows that the present exploitation rate (E = 0.43) not exceeds the optimum exploitation rate (E_{max} = 0.64).

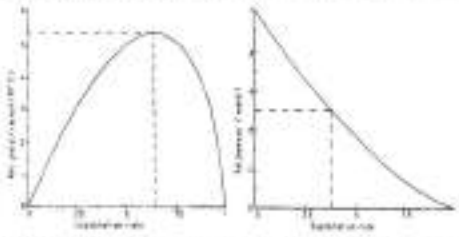

Fig. 6. Relative yield-per-recruit and biomass- per-recruit of *L. savala* (Lc /L∞=0.567, M/K=1.35).

Length-weight relationship

The estimated length weight relationship for the species was Log W = - 3.03 + 2.97 Log TL, in exponential form this equation is $W = 0.00093TL^{2.97}$ The log length and log

weight of the fish linearly related with a co-efficient of correlation ($r = 0.996$, $p = <0.001$, t cal. = 22.08, n = 37 size range).

References

Gulland, J.A., 1971. The fish resources of the ocean west of fleet survey. Fishing News (Books). Ltd, FAO. 225 pp.

Gayanilo, F. C. Jr., M. Soriono and D. Pauly, 1989. A draft guide to compleat ELEFAN. ICLARM Software 2. 70pp.

Ingles, J and D. Pauly, 1984. An atlas of the growth, mortality and recruitment of Philippine fishes. ICLARM Teach. Rep., 13: 127pp.

Lamboeuf, M., 1987. Bangladesh fish resources of the continental shelf, R/V Anushandhani trawling survey result. (Sept. 1984-June, 1986) FI: DP/DGD/80/025, 1: 26pp.

Mustafa, M. G. and M. Begum, 1994. Some aspect of the biology of the ribon fish, *Lepturacanthus savala* in the Bay of Bengal. Proc. Ninth Nat. Zool. Conf. Bangladesh. pp. 23-30.

Mustafa, M. G. and M G. Khan, 1992. Bottom trawl fishery studies on interactive marine fisheries of Bangladesh BOBP/WP/89, 89-105p.

Pauly, D., 1979. Theory and management of tropical multi-species stocks. A review, with emphasis on the South-east Asian Demersal Fisheries. ICLARM Studies and Reviews 1. 35pp.

Pauly, D., 1980. On the inter-relationships between natural mortality, growth performance and mean environmental temperature in 175 fish stocks. J. *Cons. CIEM.* **39**(3): 175-192.

Pauly, D., 1984. Fish population dynamics in tropical waters. A manual for use with programmable calculators. ICLARM Stud. Rev. 8. 325pp.

Pauly, D., 1986. On improving operation and use of the ELEFAN programs. Part II. Improving the estimation of L_∞. *Fishbyte* 4(1): 18-20.

Pauly, D and J. L. Munro, 1984. Once more, on the composition of growth in fish and invertebrates *Fishbyte* 2(1): 21.

Pauly, D and M.L. Soriano, 1986. Some practical extensions to Beverton and Holts relative yield-per-recruit model. *In*: Ist Asian Fisheries Forum (eds. J.L. Maclean, L.B. Dizon and L.V. Hosillos). Asian Fisheries Society, Manila, Philippines. pp. 149-495.

Wetherall, J. A., 1986. A new method for estimating growth and mortality parameters from length frequency data. *Fishbyte*, 4(1): 12-15.

Zar, J.H., 1984. Biostatistical analysis. Prentice-Hall Inc. Englewood Cliffs, N.J. 718 pp.

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A histological study of the spermatogenesis in *Ompok pabda* (Hamilton-Buchanan 1822)

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Abstract
The present study deals with the histological analysis of testicular development in *Ompok pabda*. For the study, male gonads were collected monthwise from January to September at Freshwater Station, BFRI, Mymensingh. From the analysis, 4 stages of sperm formation, namely, spermatogonia, spermatocytes, spermatids and spermatozoa, were distinguished. The percent distribution of spermatozoa was highest in July (about 92%). Maximum GSI value was 1.129 ± 0.271 found in July. By analysing the histology of spermatogenesis it was established that this species breeds once in a year.

Key words: Histology, Spermatogenesis, *Ompok pabda*

Introduction

Ompok pabda belonging to the family Siluridae of the order Siluriformes is a freshwater catfish, locally known as " madhu pabda" , found in beels, haors, baors, flooded waterbodies, ponds, streams and rivers of Bangladesh. Although many researchers of our country as well as of other countries have worked on other commercially important catfish and other teleost fishes, very little attention has so far been paid to *O. pabda* in Bangladesh. Chaudhury (1962) worked on induced breeding and development of *O. bimaculatus*, Hossain *et al* (1991) studied the food and feeding habits of *O. pabda*, Hossain *et al* (1992) worked on the reproduction and fecundity of *O. pabda*, Khanum (1994) studied the Helminth endoparasites in both *O. pabda* and *O. bimaculatus*, Palmer *et al* (1995) studied the histology of seasonal ovarian development in freshwater drum, and Janseen *et al*. (1995) examined the annual ovarian cycle of a flounder, etc., but no work has been reported on histology of spermatogenesis in *O. pabda*.

Considering the economic as well as biological importance of *O. pabda*, an attempt was made to define the successive maturational stages of male gonads histologically for detecting the spawning season, to classify the gonads by measuring the percent distribution of different developmental stages of spermatogenesis at successive months.

Materials and methods

To complete the experiment, studies were conducted from January to September, 1996, at the Freshwater Station, Bangladesh Fisheries Research Institute (BFRI),

Mymensingh. To study the testicular development histologically, the experimental fish (*O. pabda*) which were more or less of same year-class were collected from the same rearing pond.

Before dissecting the fish sample in every month, total body length and body weight were measured. After dissection, the gonads were carefully removed and were cleaned of all surrounding tissues. Before fixing the gonads in Bouin's fluid the gonad weight and the physical features of testes were also observed and noted.
GSI (Gonado Somatic Index) was determined by using the following formula:

$$GSI = \frac{\text{Gonad weight of fish}}{\text{Total weight of fish}} \times 100$$

For the histological investigation, permanent microscopic slides of the successively collected gonads were prepared in the laboratory by microtechnique method. The prepared slides were examined and studied by a compound microscope in laboratory and all the successive stages of spermatogenesis were observed. The percentage of different stages of sperm formation was determined and photomicrography was done.

Results and discussion

General morphology of testes: The male gonad consisting of a pair of testes was situated dorso-laterally to the gut, and these were attached to the dorsal body wall by the mesorchium. In immature stage the testes were thin, whitish thread-like structures, very small and translucent, but they became thick, creamy white, highly coiled and opaque at the mature stage. Maximum mean gonad weight was about 0.22g found in June.

GSI: Monthly variation of GSI in male is presented in Fig.1. The GSI values increased slowly from January to April and from May to July increased highly with a peak in July (1.129 ± 0.271). GSI values began to fall steeply from August.

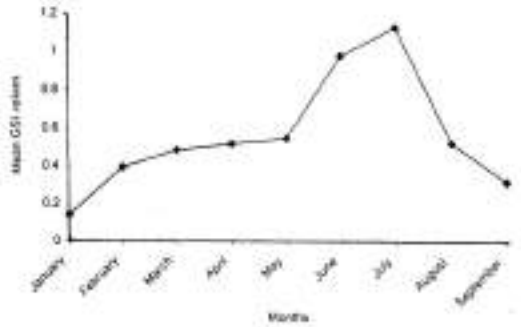

Fig.1. Monthwise changes in mean GSI values of male *O. pabda*.

Histology of gonads

A. Spermatogonia: This is the primary stage of spermatogenesis which is the largest germ-cell in the testis. It was spherical in shape and the cell membrane was clearly seen (Fig. 2A). An oval, slightly basophilic nucleus was present in the centre of the cell. About 65% of this stage was observed in January and only 1-2% in July.

B. Spermatocytes: Spermatogonia transformed into spermatocytes by meiotic division which were also spherical in shape containing a nucleus in the centre. These were smaller in size than the spermatogonia (Fig. 2B). The percent distribution of this stage was 20% in January, but only 1-4% in July.

C. Spermatids: These were spherical shaped in which nucleus was not clearly seen for its dark appearance under the microscope (Fig. 2C). About 15% of this stage was observed in January, but only 3-8% in July.

D. Spermatozoa: These were the functional male gametes which were the smallest cells of all germ cells in testes. They appeared as small black-coloured spots under the microscope. Spermatids transformed into spermatozoa by the process of spermiogenesis (Fig. 2D). This stage was found from February and percent distribution in testes was only 5%, but 75-92% in July.

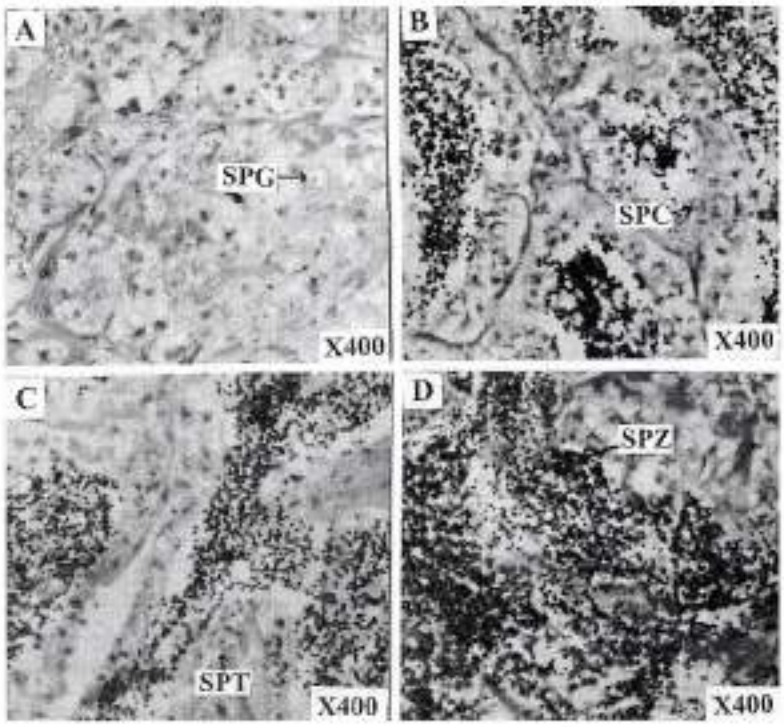

Fig. 2. Different development stages of spermatogensis: A. Spermatogonia (SPG), B. Spermatocytes (SPC), C. Spermatids (SPT), D. Spermatozoa (SPZ).

Average frequency distribution (%) of different developmental stages of sperm formation in testes is presented in the Fig. 3.

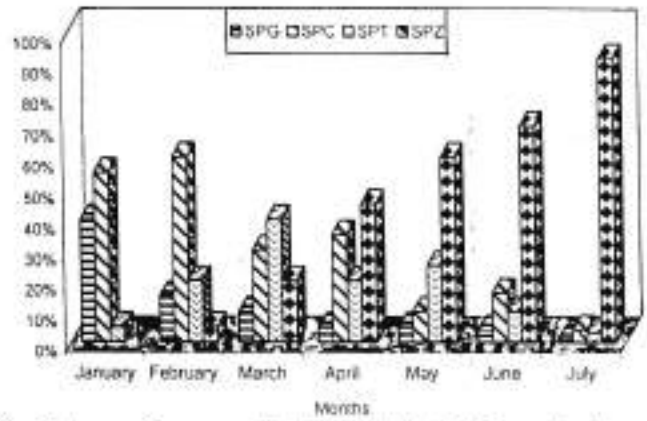

Fig. 3. Average frequency distribution (%) of different development stages of sperm formation in testes of *O. pabda* at successive months.

Testicular development

A. Immature testes: It was very small, whitish, slightly coiled and translucent in which histologically large number of spermatogonia, a few spermatocytes and a very small number of spermatids were present. The mean gonad weight was 0.012g.

B. Maturing testes: It was observed from February to April and histologically it contained 5-45% spermatozoa, 20-40% spermatids, 10-50% spermatocytes approximately. Spermatogonia decreased gradually from 35-5%. It became opaque gradually and creamy white in colour. The mean gonad weight ranged from 0.051g to 0.07g.

C. Mature testes: It was seen from May to July with highest mean gonad weight in June (0.22g). Histologically, it contained 60-92% spermatozoa, and other developmental stages were also seen, but very small in numbers.

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References

Chaudhuri, H., 1962. Induced breeding and development of a common catfish, *Ompok bimaculatus* (Bloch). *Proc. Indian Sci. Congr.* 3: 390-391.

Elorduy-Garay, J. F. and S. Ramirez-Luna, 1994. Gonadal development and spawning of female ocean whitefish, *Caulolatilus princeps* in the Bay of La Paz, B.C.S. Mexico. *J. Fish Biol.*, **44**: 553-566.

Hossain, M. A., A. Taleb and M.H. Rahman, 1992. Reproduction and fecundity of *Ompok pabda* (Ham.). *Bangladesh J. Sci. Res.*, **10**(1): 49-52.

Hossain, M.A., A. Taleb and M.H. Rahman, 1991. Food and feeding habits of *Ompok pabda* (Ham.). *J. Asiat. Soc. Bangladesh (Sci.)*, **17**: 49-52.

Janseen, P. A. H., J.G.D. Lambert, and H.J.T. Goos, 1995. The annual ovarian cycle and the influence of pollution on vitellogenesis in the flounder, *Pleuronectes flesus*. *J. Fish. Biol.*, **47**: 509-523.

Khanum, H., 1994. Endoparasitic Helminth infestation in *Ompok bimaculatus* (Bloch 1794) and *Ompok pabda* (Hamil.-Buchanan 1822) in relation to some of their biological, pathological and biochemical aspects. A Ph.D. thesis submitted to the Department of Zoology, University of Dhaka.

Yamamoto, K. and H. Onozato, 1965. Electron microscope study on the growing oocyte of the goldfish during the first growth phase. Mem. Fac. Fish. Hakkaido Univ. Japan. **13**: 79-106.

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Acute toxicity of chlorpyrifos, cadusafos and diazinon to three Indian major carps (*Catla catla, Labeo rohita and Cirrhinus mrigala*) fingerlings

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Abstract
Fingerlings of three Indian major carps, viz. *Catla catla* (Hamilton-Buchanon), *Labeo rohita* (Hamilton-Buchanon) and *Cirrhinus mrigala* (Hamilton-Buchanon), were exposed to different concentrations of chlorpyrifos (lorsban 10 G), cadusafos (rugby 10 G) and diazinon (basudin 10 G) for a period of 96 h with a view to determine the median lethal concentrations (LC_{50}) values for each of chemicals. Of the tested concentrations, chlorpyrifos at a dose of 6.65 ppm, cadusafos at 2.0 ppm and diazinon at a dose of 8.40 ppm or above induced 100% mortalities within 96 h of exposure. The 96 h LC_{50} values of chlorpyrefos, cadusafos and diazinon were 1.66, 0.72 and 2.10 ppm for *C. catla*, 2.35, 0.72 and 2.97 for *L. rohita* and 2.35, 0.72 and 2.10 ppm *for C. mrigala*, respectively. Pesticide induced behavioural abnormalities observed in the present study included erratic movements, rapid operculum activities, jumping of fish out of the test media, violent spasm and convulsion.

Key words: Toxicity, Chlorpyrifos, Cadusafos, Diazinon, Indian major carp

Introduction

Fish is the most accessible animal protein for majority of the population. Therefore, it is vital that the aquatic environment be used in a sustainable manner, and that the resource base is not destroyed. Unfortunately, presently, fish habitat of Bangladesh is jeopardized by pollution from different sources. Pesticides are one of the notorious causes of environmental pollution because the used pesticides drain off into open waterbodies through rainfall and floods and as a result the aquatic environment obviously gets polluted.

The organophosphorus pesticides are generally much more acutely toxic to vertebrates and are non-persistent. It is the latter quality that brought them onto the agricultural scene to gradually replace the persistent organochlorine, particularly the DDT. About 280 pesticides have so far been registered in Bangladesh for marketing and of them, 80% are organophosphorus.

Catla (*Catla catla*), rui (*Labeo rohita*) and (*Cirrhinus mrigala*) are the common fishes of south-east Asian countries. Carps use paddy fields as their nursing and feeding grounds.

Pesticides effects on fish mortality were observed by Kabir and Begum, 1978; Kabir and Ahmed, 1979; Ponce, 1984; El-Basyouni, *et al.* 1989; Hosny, *et al.* 1989; Samudra, *et al.* 1989; Medina, *et al.* 1991 and Mohd-Zulkifli, *et al.* 1993. Chlorpyrefos, cadosafos and diazinon are very important organophosphorus pesticide widely used in the paddy field. However, no work was carried out with these pesticides on the above mentioned fish species. Therefore, the purpose of the present study was to determine the LC_{50} values of chlorpyrifos (lorsban 10 G), cadusafos (rugby 10 G) and diazinon (basudin 10 G) to three Indian major carps (*C. catla, L. rohita and C. mrigala*) fingerlings at different exposure times during a 96 h exposure period.

Materials and Methods

The insecticides: The empirical formula of chlorpyrifos is $C_9H_{11}Cl_3NO_3PS$. These are a product of O,O-diethyl O-(3,5,6-trichloro-2 pyridyl) phosphorothiate. Lorsban are emulsifiable concentrate and granular product containing 150 g/kg of chloropyrifos as the active ingredient respectively. Chlorpyrifos has a very low solubility in water but is readily soluble in most common organic solvents. These two insecticides are effective for control of a wide range of important insects and certain other arthropod pests.

The empirical formula of cadusafos is $C_{10}H_{23}O_2S_2P$. It is a product of S, S-di-Sec-butyl O-ethyl Phosphorodithioate. It is a granular product containing 100 g/kg of cadusafos as the active ingredient. It is soluble in water and most organic solvents. It is effective to control nematodes and many soil insects.

The empirical formula of diazinon is $C_{12}H_{21}N_2O_3PS$. It is a product of O,O-diethyl O-2-isopropyl-6-6methylpyrimidin-4-yl Phosphorodithioate. It is a granular product containing 100 g/kg of diazinon as the active ingredient. It is effective to control wide range of important insects of rice, sugarcane and vegetables. All the pesticides were procured from the local pesticide dealer.

Test animal: Fingerlings of *C. catla, L. rohita and C. mrigala* averaging 2.95 ± 0.09 g, 2.90 ± 0.08 g and 2.94 ± 0.10 g in body weight and 6.78 ± 0.13 cm, 7.59 ± 0.15 cm and 7.60 ± 0.14 cm in total length respectively were tested in the present study. The length and weight of the fingerlings were recorded by a measuring scale and a five figure digital electrical balance (model, JL-180, Japan). The test fingerlings were acclimatized without feeding in 500 L fibre glass tanks at the density of 0.03 g/l for 3 days at room temperature.

Experimental procedure: The experiments were conducted in a series of glass aquaria of size 30 cm x 60 cm x 28 cm having capacity of 50 litres of water. Aquaria were filled with 35 litres pond water which was free from insecticide contamination. For the test of lorsban 15 G, rugby 10 G and basudin 10 G 0.83, 1.66, 3.33, 6.65, 13.30 and 26.60 and, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0 and 8.0 and 1.05, 2.1, 4.2, 8.4, 16.8, 33.6 and 67.2 ppm concentrations and a control were maintained for *C. catla, L. rohita and C. mrigala* each having three replicates. The required test materials (lorsban, rugby and basudin) were

weighed by a five figure digital electrical balance. Ten acclimatized fingerlings of uniform size were stocked in each aquarium as soon as after mix-up of test materials. Behaviour of test fish fingerling were observed and dead fish were recorded and removed as soon as they were seen. Temperature, dissolved oxygen, carbon-di-oxide, p^H, total alkalinity and hardness of test media were recorded everyday. Temperature and p^H were monitored with a model HI-8314 portable p^H meter. Dissolved oxygen were measured with a oxygen meter (model, HI 9142, England). Total alkalinity and hardness were determined with a Hach water quality test kit (model FF-2, USA). The LC_{50} values of different concentrations and exposure times were calculated with the application of binomial formula of Ward and Parris (1982).

$LC_{50} = (AB)^{1/2}$

where, A= Highest toxin concentration in which none of the test organisms died.

B= Lowest toxin concentration in which all organisms died.

Results and discussion

Experiment with chlorpyrifos

The cumulative mortality percentage of *C. catla, L. rohita and C. mrigala* exposed to different concentrations of chlorpyrefos are presented in Table 1. Hundred percent mortalities occured in all fish groups at the doses of 26.60, 13.30 and 6.65 ppm within the 96 h exposure period. While a dose of 0.83 ppm could not induce any mortality within the same period. Alam *et al.* (1995) reported that 100 % *C. mrigala* fry died at the doses of 1.75 and 1.50 ppm and, 80 %, 80 % and 50 % at the doses of 1.25, 1.00 and 0.75 ppm of diazinon, respectively. Haque (1989) also reported that 100 % *Oreochromis niloticus* died at the doses of 30.0, 27.5, 25.0, 22.5, 20.0, 17.5 and 15.0 ppm of sumithion. The results of the present experiment were very close with the findings of Haque (1989). In the present case, a 30% *C. mrigala* fingerlings died at the dose of 1.66 ppm, on the contrary Alam *et al.* (1995) reported that 100 % *C. mrigala* fry died at the doses of 1.75 and 1.50 ppm of diazinon. The LC_{50} values of chlorpyrifos for *C. catla, L. rohita and C. mrigala* are shown in Table 2. The LC_{50} values of phosphamidon on *Channa striatus* was 10.47 at 96 h (Choudhuri *et al.* 1984) and for *Puntius ticto* the LC_{50} value of malathion was 4.0 ppm (Singh and Sahai, 1984). In the current experiment, the LC_{50} for *C. catla,* and *L. rohita* was 9.41 ppm at 12 h and 4.7 ppm at 36 h and 48 h. The above mentioned results coincided with the findings of Choudheri *et al.*, 1984 and Singh and Sahai, 1984.

Table 1. Cumulative mortality percentage of *C. Catla, L. rohita* and *C. mrigala* fingerlings exposed to different concentrations of chlorpyrifos

Concentrations (ppm)	Cumulative mortality (%)								
	6 h	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h
Catla fingerlings									
Control	00	00	00	00	00	00	00	00	00
0.83	00	00	00	00	00	00	00	00	00

1.66	00	00	00	00	10	23	30	40	40
3.33	00	00	20	40	60	67	70	87	100
6.65	00	27	40	67	87	90	100	100	100
13.30	20	50	80	100	100	100	100	100	100
26.60	100	100	100	100	100	100	100	100	100
Rui fingerlings									
Control	00	00	00	00	00	00	00	00	00
0.83	00	00	00	00	00	00	00	00	00
1.66	00	00	00	00	00	10	20	30	37
3.33	00	00	10	27	40	50	67	70	77
6.66	00	10	30	50	73	87	93	100	100
13.30	10	30	67	83	100	100	100	100	100
26.60	100	100	100	100	100	100	100	100	100
Mrigal fingerlings									
Control	00	00	00	00	00	00	00	00	00
0.83	00	00	00	00	00	00	00	00	00
1.66	00	00	00	00	00	20	23	27	30
3.33	00	00	00	10	37	47	60	67	70
6.65	00	00	13	30	63	80	90	100	100
13.30	10	20	40	67	90	100	100	100	100
26.60	70	100	100	100	100	100	100	100	100

Table 2. The median lethal concentration (LC_{50}) values of chlorpyrifos on *C. Catla, L. rohita* and *C. mrigala* fingerlings at different exposure times

Exposure time (hours)	LC_{50} values (ppm)		
	C. catla	*L. rohita*	*C. mrigala*
6	13.30	13.30	--
12	9.41	9.41	13.30
24	6.64	6.64	9.41
36	4.70	6.64	6.64
48	3.32	4.70	6.64
60	3.32	3.32	3.32
72	2.35	3.32	3.32
84	2.35	2.35	2.35
96	1.66	2.35	2.35

Experiments with cadusafos

The cumulative mortality percentages of *C. catla, L. rohita and C. mrigala* exposed to cadusafos are presented in Table 3. A dose 2 ppm cadusafos caused 100% mortality of catla fingerlings by 96 h of exposure. However, concentrations of 4 ppm caudusafos and diazinon killed 100% rui and mrigal fingerlings within 72 h of exposure. Alam *et al.* (1995) found 80 %, 50 %, 30 % and 10 % mortality of *C. mrigala* fry at the doses of 1.0, 0.75, 0.50 and 0.25 ppm diazinon respectively and thus agree favourably with the result of present study. The LC_{50} values of cadusafos for *C. catla, L. rohita and C. mrigala* are shown in Table 4. The estimated LC_{50} values for *C. mrigala* were 0.72 and 1.0 ppm at 96 and 84 hours respectively. The LC_{50} value of sumithion for *O. niloticus* was 0.87 ppm at

24 hours (Haque, 1989). Alam *et al.* (1995) reported LC_{50} values of 168 and 96 h as 0.739 and 1.002 ppm for the same fish.

Table 3. Cumulative mortality percentage of *C. Catla, L. rohita* and *C. mrigala* fingerlings exposed to different concentrations of cadusafos

Concentrations (ppm)	Cumulative mortality (%)								
	6 h	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h
Catla fingerlings									
Control	00	00	00	00	00	00	00	00	00
0.125	00	00	00	00	00	00	00	00	00
0.25	00	00	00	00	00	00	00	07	13
0.50	00	00	00	00	00	10	27	37	50
1.00	00	00	00	00	10	20	40	60	73
2.00	00	00	10	27	30	60	80	90	100
4.00	10	30	40	60	77	100	100	100	100
8.00	50	100	100	100	100	100	100	100	100
Rui fingerlings									
Control	00	00	00	00	00	00	00	00	00
0.125	00	00	00	00	00	00	00	00	00
0.25	00	00	00	00	00	00	00	10	10
0.50	00	00	00	00	00	00	10	20	37
1.00	00	00	00	00	00	10	23	47	70
2.00	00	00	00	13	30	50	67	80	93
4.00	07	20	37	50	67	87	100	100	100
8.00	40	87	100	100	100	100	100	100	100
Mrigal fingerlings									
Control	00	00	00	00	00	00	00	00	00
0.125	00	00	00	00	00	00	00	00	00
0.25	00	00	00	00	00	00	00	10	07
0.50	00	00	00	00	00	00	00	13	30
1.00	00	00	00	00	00	10	20	40	60
2.00	00	00	00	10	27	47	60	80	87
4.00	00	10	27	40	60	80	100	100	100
8.00	30	60	100	100	100	100	100	100	100

Table 4. The median lethal concentration (LC_{50}) values of cadusafos for *C. Catla, L. rohita* and *C. mrigala* fingerlings at different exposure times

Exposure time (hours)	LC_{50} values (ppm)		
	C. catla	*L. rohita*	*C. mrigala*
6	--	--	--
12	4.0	--	--
24	2.83	4.00	4.00
36	2.83	2.83	2.83
48	2.00	2.83	2.83
60	1.00	2.00	2.00

72	1.00	1.00	1.41
84	0.72	1.00	1.00
96	0.72	0.72	0.72

Experiments with diazinon

The cumulative mortality percentages of *C. catla, L. rohita and C. mrigala* exposed to diazinon are shown in Table 5. Hundred percent *C. catla, L. rohita and C. mrigala* fingerlings died at 67.2, 33.6, 16.8, 8.4, ppm diazinon within 96 h of exposure. Diazinon was reported to cause 100 % mortality in *C. mrigala* at a concentration 1.5 ppm (Alam *et al.*, 1995). However, in the present study 100% mortalities were observed with 96 h of exposure in all three species of fish tested at a dose of 8.4 ppm diazinon. The median lethal concentrations (LC_{50}) values of diazinon for *C. catla, L. rohita and C. mrigala* at different exposure times are presented in Table 6. Bengeri *et al.* (1984) reported the LC_{50} values of dimethyl parathion on *L. rohita* to be 6.34 ppm at 96 h and similarly, the LC_{50} values of diazinon on *C. mrigala* fry were 1.399, 1.308, 1.218 and 1.002 ppm at 24, 48, 72 and 96 h respectively (Alam *et al.* 1995). In present experiments, the LC_{50} values on *C. mrigala* were 16.80, 8.40, 5.94 and 2.10 ppm at 24, 48, 72 and 96 h respectively and, on *L. rohita* the LC_{50} value was 2.97 ppm at 96 h. The results are somewhat lower than that of Bengeri *et al.* 1984 and Alam *et al.* 1995.

Table 5. Cumulative mortality percentage of *C. Catla, L. rohita* and *C. mrigala* fingerlings exposed to different concentrations of diazinon

Concentrations (ppm)	Cumulative mortality (%)								
	6 h	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h
Catla fingerlings									
Control	00	00	00	00	00	00	00	00	00
1.05	00	00	00	00	00	00	00	00	00
2.10	00	00	00	00	10	20	30	40	57
4.20	00	00	00	10	30	40	70	80	100
8.40	00	00	20	37	70	90	100	100	100
16.80	10	40	67	70	90	100	100	100	100
33.60	30	50	80	90	100	100	100	100	100
67.20	100	100	100	100	100	100	100	100	100
Rui fingerlings									
Control	00	00	00	00	00	00	00	00	00
1.05	00	00	00	00	00	00	00	00	00
2.10	00	00	00	00	00	00	10	20	50
4.20	00	00	00	00	10	20	37	60	77
8.40	00	00	07	10	30	60	77	90	100
16.80	00	10	20	40	70	87	100	100	100
33.60	10	20	40	67	100	100	100	100	100
67.20	100	100	100	100	100	100	100	100	100
Mrigal fingerlings									
Control	00	00	00	00	00	00	00	00	00
1.05	00	00	00	00	00	00	00	00	00
2.10	00	00	00	00	00	00	00	10	40

4.20	00	00	00	00	00	10	37	60	87
8.40	00	00	7	10	20	40	77	90	100
16.80	00	10	20	40	60	87	100	100	100
33.60	10	20	40	67	97	100	100	100	100
67.20	100	100	10	100	100	100	100	100	100

Table 6. The median lethal concentration (LC_{50}) values of diazinon for *C. Catla*, *L. rohita* and *C. mrigala* fingerlings at different exposure times

Exposure time (hours)	LC_{50} values (ppm)		
	C. catla	*L. rohita*	*C. mrigala*
6	23.76	33.60	33.60
12	23.76	23.76	23.76
24	16.80	16.80	16.80
36	11.88	16.80	16.80
48	5.94	8.40	8.40
60	4.20	4.20	8.40
72	2.97	4.20	5.94
84	2.97	2.97	4.20
96	2.10	2.97	2.10

Several abnormal behaviour such as rapid swimming, loss of balance, increased operculum activities, jumping out of the test media, violent spasm, convulsion etc. were observed in all fish groups exposed to the pesticides. However, the extent of such abnormalities were dependent on the concentrations of the pestcides.

Water quality in aquaria was measured during the experiments. Temperature and p^H ranged from 24.7 to 29.8° C and 6.4 to 7.8 respectively, monitored with a model HI-8314 portable p^H meter. Dissolved oxygen ranged from 5.8 to 7.6 mgl^{-1}, measured with a oxygen meter (model, HI 9142, England). In case of all experiments, dissolved oxygen was higher in the lower concentrated test media. Meletev *et al.* (1971) reported that fish kept in toxic concentration (pesticides) the oxygen requirement increases 2-3 times. Total alkalinity and hardness, determined with a Hach water quality test kit (model FF-2, USA) were 88.3 ± 5.3 and 80.3 ± 5.1 mgl^{-1} respectively.

References

Alam, M.G.M., S.A.M Al-Arabi, G.C. Haldar, and M.A. Mazid. 1995. Toxicity of diazinon to the fry of Indian major carp *Cirrhina mrigala* (Hamilton). *Bangladesh J. Zool.*, **23** (2): 183-186.

Bengeri, K.V., K.M. Shivaraj, and H.S. Patil. 1984. Toxicity of dimethyl-parathion to freshwater fish *Labeo rohita* and oxygen uptake rate of exposed fish. *Environ. and Ecol.*, **2** (1): 1-4.

Choudhuri, D.K., A.K. Sadhu, and P.K. Mukhopadhyay. 1984. Toxicity of two organo-phosphorus insecticides malathion and phospharnidon to the fish, *Channa Striatus*. *Environ. and Ecol.*, 2(2): 143-147.

El-Basyouni, S.A., M.E. Salama, and A.H. Hosny. 1989. Studies on the effect of some insecticides on certain aquaculture. *J. Agril. Res.*, **15**: 112-124.

Haque, M.Z. 1989. Toxic and sub-lethal effect of the sumithion on *Oreochromis niloticus* (Lin.). *Bangladesh J. Fish.*, **12**(1): 79-85.

Hosny, A.H., M.E. Salama, and S. El-Basyouni. 1989. Studies on the effect of some insecticides on certain aquaculture. *J. Agril. Res.*, **15**: 96-111.

Kabir, S.M.H and N. Ahmed. 1979. Histopathological changes in climbing perch, *Anabas testudineus* (Bloch), due to three granular insecticides. *Bangladesh J. Zool.*, **7**: 21-29.

Kabir, S.M.H. and R. Begum. 1978. Toxicity of three organophosphorus insecticides to shinghi fish, *Heteropneustes fossilis* (Bloch), *Dacca Univ. Stud. B.*, **26**: 115-122.

Medina, M.J.B., S.M.F Calumpang, A.W. Tejada, J.R. Medina and E.D. Magallona. 1991. Fate of endosulfan in a rice-fish ecosystem. College, Laguna (Philippines). 17 p.

Mohd-Zulkifli, A.W. Tejada, and E.D. Magallona. 1983. The fate of BPMC and Chlorpyrifos in some components of a paddy rice ecosystem. *Philippine-Entomologist.* **5** (5-6): 555-565.

Meletev, V. V., A. I. Kanaev and N.G. Dzasokhova. 1971. Water Toxicology. Amerind Publishing Co. Pvt. Ltd. 216 pp.

Ponce, B.R. 1984. Bioassy: the effect of Basudin 20 EC, Thiodan 35 EC, Mipcin and Diagran 5 G on Crucian carp and Nile Tilapia. *CLSU Scientific Journal.* **5-6** (1-2): 65 p.

Samudra, I.M., A. Nugraha and D. Djunaedi, 1989. Residual analysis of carbofuran, diazinon, quinalfos and fonophos from water, soil, rice straw, rice hull and fish. *Buletin* (Indonesia). **3**: 831-840.

Singh, S. and S. Sahai. 1984. Effect of malathion on the mortality and behaviour of two freshwater teleosts. *J. Environ, Biol.*, **5** (1): 23-28.

Ward, G.S. and P.R. Parris. 1982. Manual of Methods in Aquatic Environment Research., Part 6, Toxicity test. FAO Fish. Pap 185: 23 pp.

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Scientific Note

Distribution of *Macrobrachium rosenbergii* (de Man) in three rivers of Paikgacha, Bangladesh

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Abstract
Distribution of postlarvae, juveniles and adults of *Macrobrachium rosenbergii* was studied in three rivers of Paikgachha, Khulna during November'91 to April'94. The adults were found to ascend upstream and the matured ones again return to the estuary to breed. Postlarvae of *M. rosenbergii* prefer a salinity range in between 0.5 to 19.0 ppt and juveniles require a salinity range in between 0.0 to 2.0 ppt.

Key words: *Macrobrachium rosenbergii*, Postlarvae, Physico- chemical parameters

Distribution of the giant freshwater prawn, *Macrobrachium rosenbergii* is limited to the freshwater zone, river mouths and back waters with a salinity range from 0.0 to 20.0 ppt and a temperature range form 25 to 30°C in the tropics and sup-tropics of Indo-Pacific region(Sebastian 1990 and Rao 1991). Though the larvae grow only in brackish water, but the juveniles and adults typically inhabit in the lower salinity and freshwater areas (George 1969). Adult *M. rosenbergii* migrates to the estuary for breeding (Sarvaiya 1990). Distribution of different life stages of this species have been reported from India (Rajyalakshmi and Maheswardu 1986, Rao 1991) and from the river systems of Bangladesh (Patra 1977, Hill and Kibria 1979, Kibria 1983). However, distribution and migration of this species, and their relation to the Physico- chemical factors of the river systems have not been reported so far from Bangladesh. The present work was aimed to report the abundance and distribution of postlarvae, juvenile and adults *M. rosenbergii* in Bangladesh.

The adult prawns were collected once in a month during the period from November' 91 to April'94 from the Shibsha, Kapatakhha and Haria rivers of Paikgachha thana under Khulna district. The specimens were grouped according to size, sex and berried females. The prawns of total length up to 70 mm were considered as juvenile (Rao 1991). Post-larvae were collected from a professional prawn seed collector, once in a month, mostly during the full moon. Post-larvae were collected by push nets made of nylon with 0.6 mm mesh. Total number of post-larvae caught in one day was recorded. All the post-larvae were collected from the river Shibsha.

Salinity, temperature and pH of the river water were recorded during sampling of the prawns. Occurrence of the larvae and adults *M. rosenbergii* from different areas of Bangladesh was recorded from personal communication with different levels of personnel engaged in fishing, fish trading and Deparment of Fisheries.

Physico- chemical parameters

Salinity of the studied rivers fluctuated greatly with the influence of rainfall. During the periods from August to November, salinity ranged from 0.0 to 1.5 ppt, and from February to July it ranged from 6 to 19 ppt (Table 1). The lowest and highest temperature and pH were recorded as 19.5°C (January) and 30.55°C (July), 7.1 (August) and 8.5 (May) respectively (Table 1). Distribution of the adults, postlarvae and juvenile of *M. rosenbergii* is dependent on salinity, water temperature, pH and turbidity. The prawn breeds when all these parameters remain high.

Table 1. Monthly distribution of berried, postlarvae and juvenile *M. rosenbergii* with physico-chemical parameters in Shibsha, Kapatakkha and Haria rivers, Khulna

Months	*M. rosenbergii*			Water parameters		
	Berried	Postlarvae	Juvenile	Salinity (ppt)	Temp. (°C)	pH
Nov.'91	-	-	**	1	23	7.9
Dec.	-	-	-	4	21	8.0
Jan.'92	-	-	-	5	20	8.0
Feb.	*	-	-	8	23	7.8
Mar.	***	*	-	12	26	8.0
Arp.	***	**	-	16	29.5	8.2
May	***	***	-	18	29.5	8.4
Jun	**	***	-	15	30	8
Jul	**	**	-	7.5	30.5	7.5
Aug	**	*	**	2	30	7.2
Sep	*	*	***	0.5	29	7.9
Oct	-	-	***	0	26	8
Nov	-	-	**	1	24	8
Dec.	-	-	-	3	20.5	8
Jan.'93	-	-	-	5	19.5	8
Feb	*	-	-	9	22.5	7.9
Mar	**	*	-	13	27	8
Apr	***	**	-	16	30	8.1
May	***	***	-	19	29	8.5
Jun	**	***	-	14	29.5	8
Jul	**	**	-	6	30.5	7.7
Aug	**	*	**	1.5	30	7.1
Sep	*	*	***	0	29.5	7.8

Oct	-	-	***	0	25	8.1
Nov.	-	-	-	1.5	22.5	8
Dec.	-	-	-	4	21	8
Jan.'94	-	-	-	7	20	7.8
Feb.	*	-	-	9.5	23.5	7.9
Mar.	**	*	-	13.5	26.5	8.1
Arp	***	**	-	17	29.5	8.2

**rare (n= 5-10), **common (n>50), ***very common (n>80)*

Distribution of *M. rosenbergii*

The species was found to breed in studied rivers from February to September with a peak in April to May. The breeding season of *M. rosenbergii* was found to vary from place to place, e.g. January to July at Bagerhat, Khulna; December to July at Hoogly estuary, India (Rao 1976b); July to December at Cochin backwaters, India (Rahan 1967). These reports suggest that the species breeds round the year depending on the availability of the required parameters for the maturation. During the peak breeding season salinity, p^H and temperature of water of the study area were found at high level. The percentage of berried females were low during February-March and July to September (Table 1). The juveniles become available from August to December, and totally absent during the period from April to July. This result confirms that juvenile of *M. rosenbergii* prefers freshwater habitat than saline habitat. However, the berried females were abundant at a wide range of salinity and similarly, the postlarvae were also found to be tolerant to salinity and temperature fluctuation (Table 1).

The adult *M. rosenbergii* are available throughout the year in both the freshwater and brackis water zones of Bangladesh, except the north- west region (Pabna to Dinajpur) and Chittagong Hill tracts. The berried females descend down , only a few population are found up to Faridpur and Comilla during the dry and rainy seasons. The berried females need a salinity range from 5 to 20 ppt and a temperature rang from 26 to 33°C to breed. Distribution of larvae and post- larvae of *M. rosenbergii* were found to be strictly restricted only at the brackishwater zone, with a few population in the Dakatia, Chandpur and the Kapatakkha rivers during the dry and rainy seasons.

Distribution of *M. rosenbergii* at a wide rang of salinity (0.0 to 20 ppt) and temperature ranged from 25 to 33°C was also reported by Sebastian(1990) and Roa (1991). Distribution of the postlarvae, juvenile and adult *M. rosenbergii* was found in the present study is also similar to the distribution of this species as reported by George (1969) and Ling (1969). Bangladesh has a rich population of *M. rosenbergii* throughout her major river systems and the vast estuary.

References

George, M.J., 1969. Genus *Macrobrachium* Bate 1868. *In*: Prawn Fisheries of India. *Bull. Cent. Mar. Fish. Res. Inst.*, **14**: 179 -216

Hill, M.T. and G. Kibria, 1979 Fish stock assessment program, chapter 7. 2nd Annual Report, Karnaphuli Irrigation and Fishery Development Project, Snell Environ. Gr., Lansing, Michigan. 30pp.

Kibria, G., 1983. Bangladesher Chingri Sampad O Chingrir Chash. Jahan printing press, Satkhira, Khulna, 126pp. (in Bangla)

Ling, S.W., 1969. The general biology and development of *Macrobrachium rosenbergii* (de Man). FAO Fish Rep., 57(3): 589-606.

Patra, R.W.R ., 1977. Seasonal abundance and sex- ratio of the natural population of *Macrobrachium rosenbergii* (de Man). *Bangladesh J. Zool.*, 5 (2): 101 -106

Rahman, K., 1967. Observations on the fishery and biology of the giant freshwater prawn, *Macrobrachium rosenbergii* (de Man). *Proc. Symp. Crustacea, Marine Biol. Assoc. India*, Part II: 649- 669.

Rajyalahshmi, T. and G. Maheswardu, 1986. On the culture of *Macrobrachium rosenbergii* (de Man) in Andhra pradesh, India. *J. Indian Soc. Coastal Agric. Res.*, 4(1): 25-41.

Rao, R.M., 1967. Studies on biology of the *Macrobrachium rosenbergii* (de Man) of the Hooghly estuary with notes on its fishery. *Proc. Nat. Inst. Sci. India* 33B(5&6): 252-279.

Rao, K.J., 1991. Reproducetive biology of giant freshwater prawn *Macrobrachium rosenbergii* (de Man) from the lake Kolleru (Andhra pradesh). *Indian, J. Anim. Sci.*, 61(7): 780-787.

Sarvaiya, R.T., 1990. Life history and culture of giant freshwater prawn. *Fishing Chimes*, **10**(2): 13-15.

Sebastian, M.J., 1990. The giant freshwater prawn *Macrobrachium rosenbergii* (de Man). AGG Menon Publ Kerala Agricultural University, Thrissur, India, pp. 1-43.

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Scientific Note

Socio-economic conditions of the pond owners of Demra, Dhaka

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Abstract
On the basis of fish culture status 96 ponds of the study area were classified into 4 categories like wild stock (27%), extensive culture (24%), improved extensive culture (33%) and semi-intensive culture (16%). Percentage of small, medium and large ponds were 38, 44, and 18 respectively whereas education levels below SSC, below Bachelor and above were 43, 38 and 19 respectively and single owners belonged to 54% of the ponds. Per hectare yields of extensive, improved extensive and semi-intensive categories of culture were 1.3, 2.12 and 4.0 metric tons respectively and their net return were 46, 63 and 92 thousand taka respectively. Considering the problems of fish culture, multiple ownership was found to be the most important one.

Key words: Socio-economic, Pond fishery

Fish and fishery resources play a vital role in improving socioeconomic conditions, combating malnutrition, earning foreign currency and creating employment opportunities in Bangladesh. There are 5,277,571 hectare water bodies of which 915,506 hectare ponds which are suitable for fish culture, but most of them remain unused (BBS 1997). If the existing ponds are brought under fish culture by exact planning, proper management and re-excavation, the present fish production level can easily be increased 2 to 3 times to reach the recommended quantity of fish for the people of Bangladesh. Some limited survey type and related works on pond fishery resources have so far been done in Bangladesh. Most of these studies (Rahman and Ali 1986, Khan 1986, Quddus and Akbar 1995), however, focused on socioeconomic aspects of developing pond fish culture. Cost and returns of different management category is included in this study in addition to the above investigation.

A survey of ponds was conducted in Demra area of Dhaka city during July'95 to June'96 through personal visits and interviews following a detail questionnaire. The survey was carried out with the help of Demara Thana Fishery Officer for identification of the actual area boundary and to detect each and every pond. A sample of 96 stocking ponds (5% of the total ponds) were selected randomly. The fish culture status was

categorized into four types vz. (i) wild stock category, (ii) extensive culture category, (iii) improved extensive culture category and (iv) semi-intensive culture category on the basis of amount of inputs used and how inputs used. Cost and return of different management categories of ponds and various problems and their solutions were recorded through interview. Collected data were analysed by objectives of the study. A tabulation plan was developed and statistical measures such as frequency count, percentage distribution, mean, chi-square test etc. were used to analyse the data. Ponds were classified into 4 categories with less than 0.10 ha as small, 0.10-0.25 ha as medium, 0.26-0.50 ha as large and above 0.50 ha as very large. Educational status of pond owners were classified into three categories: (i) below S.S.C. (ii) below Bachelor's and (iii) Bachelor's degree & above. On the basis of number of owners the ponds were classified into five categories (i) single ownership, (ii) 2-3 ownership, (iii) 4-5 ownership, (iv) above 5 ownership, and (v) public/organization ownership. Flooding condition of ponds were as (i) every year flooding, (ii) rarely flooding and (iii) never flooding. The chi-square test was done to find out the interaction of fish culture status with the size of pond, number of pond owners, educational status of pond owners, and flooding condition of ponds.

The total and percentages of the different categories of the socioeconomic factors are shown in the Table 1. Most of the ponds of Demra area were found to be small (38%) and medium (44%) (Table 1). It is a low lying area for which they needed to raise their homestead area. Similar results were also reported by Kaiya (1986) after a survey of pond fishery resources in Mirzapur upazila. The very few larger ponds found in this area were excavated basically in abandoned brick fields and for raising industrial sites. The chi-square test indicates that fish culture status did not depend on pond size.

Considering the ownership of the ponds, it was observed that 34% of the total ponds were under joint ownership, 54% were under single ownership and the rest 12% ponds were public or organizational property. This result contradicts with those of Alkbar and Rahman (1982) who reported that 84% ponds were joint ownership and only 16% were-under single ownership in Mymensingh district. Khan *et al.* (1991) also expressed dissimilar results that about 97.8% of the ponds were under private ownership comprising 28.29% under single ownership and 69.51% under joint ownership in Trisal Upazila. The ponds, under public or organizational property, were not properly utilized for fish culture. So, multiple ownership and public ponds were the most important limiting factor to fish culture. Number of ownership of the ponds affected remarkably fish culture status of the ponds. Multiple ownership affected intensity of fish culture. The chi-square test showed that there was significant relationship between pond-ownership and fish culture status (Table 1). This indicates that the fish culture status is affected by the increase in number of pond owners. Kaiya *et al.* (1987) pointed out that multiple ownership was considered as the major problem by 82.5% of the pond owners.

A majority (95%) of the pond owners showed their interest in fish culture. But the institutional credit facilities and other support such as training and motivation were not sufficient for fish culture. Only 34% pond owners got Bank loan for fish culture. Majority (53%) pond owner's expenditure for fish culture was from own sources (Table 1). The name of loan issuing bank are Sonali Bank, Bangladesh Krishi Bank and Agrani

Bank. It was remarkable observation that there was no NGO help, either training or credit facilities, for fish culture in the survey area. Khan *et al.* (1991) observed that about 38% of the pond owners had invested their personal capital for fish cultivation while 62% of them invested money for fish culture through obtaining loans from Bank (43%), BRDB (8%), UFO (5%), co-operatives (4%) and other organization like BRAC, SARA etc. The range of educational status of pond owners of the survey area was from primary level to master's degree. There was no illiterate pond owners whereas most of the owner's (81%) academic qualification was high school to H.S.C. level. In addition to these, 19% of the owners were higher educated because the study area was in the capital city. The chi-square test statistic indicates that the different categories of fish culture status were significantly depend on educational level of the pond owners.

Table 1. Relationship of fish culture status with pond size, pond ownership, sources of fund, educational status of pond owners and flooding conditions

Parameters	Category	Fish culture status				Total 96	Value of Chi-square
		Wild stocking	Extensive	Improved Extensive	Semi-intensive		
Pond size	a) Small	13	8	8	7	36	8.41
	b) Medium	11	9	17	5	42	(9 d. f.)
	c) Large	2	4	5	3	14	
	d) Very large	-	2	2	-	4	
Pond-ownership	a) Single	4	9	26	13	52	68.49**
	b) 2-3 owners	10	5	2	1	18	(12 d. f.)
	c) 4-5 owners	7	-	2	-	9	
	d) Above 5 owners	5	-	-	1	6	
	e) Public/ Organization	-	9	2	-	11	
Sources of fund	a) Self	15	8	8	6	37	15.20**
	b) Self+Bank loan	-	5	11	8	24	(6 d. f.)
	c) Self+Others	3	2	3	1	9	
Educational status	a) Below S.S.C.	19	8	9	5	41	18.62*
	b) Below Bachelor's	7	11	12	7	37	(6 d. f.)
	c) Bachelor's & above	-	4	11	3	18	
Flooding conditions	a) Every year flooded	19	3	5	-	27	47.31**
	b) Rarely flooded	2	10	7	1	20	(6 d. f.)
	c) Never flooded	5	10	20	14	49	
	Total	26	23	32	15	96	

* *Indicates significant at $p<0.05$*

** *Indicates significant at $p<0.01$*

The number of ponds under fish culture decreased with the increase of incidence of flooding. The Table 1 shows the distribution of ponds under different categories considering the flooding condition. This table reveals that 28% of the total ponds surveyed were found to become flooded every year and 21% were found rarely flooded, whereas 51% of the ponds found to be never flooded. Mollah (1980) observed that only 7% of the ponds got flooded in the rainy season. Kaiya (1986) reported that 53.3% were

found to become flooded every year. It is evident from the statistical analysis that there is a significant relationship between the flooding condition of ponds and the fish culture status. The result indicates that the increase of flood reduces pond fish culture.

Per hectare cost, gross and net return of fish production of different culture categories are shown in the Table 2. Total cost of fish production was highest for semi-intensive category and lowest cost for extensive category. The table also shows that both gross and net returns/ha were highest for semi-intensive and lowest for extensive category. This means that there may be a positive correlation between production cost and gross return, as well as production cost and net return. Table 2 also shows that per hectare yield by improved extensive culture is 61.5% higher than that by the extensive culture. Furthermore, per hectare yield by semi-intensive culture is 19% higher than the improved extensive culture and 3 times higher than the extensive culture. On the other hand, net return of improved extensive culture was 37% higher than the intensive culture. Net return of semi- intensive culture was 46% higher than improved extensive culture and double than the extensive culture. On the basis of the major findings of the study, the following suggestions are made which may help increase the pond fish production and interest of scientific pond fish culture.

Table 2. Average per hectare costs and returns of different management category

Culture and management of fish pond	No of ponds	Av. area (ha)	Cost (Taka) in thousand Taka				Yield / ha (m.ton)	In thousand Tk.	
			Fry	Fertilizer & feed	Labour & Harvesting	Total		Gross return	Net return
Extensive	23	0.28	12	-	6	18	1.3	64	46
Improved extensive	32	0.13	21	13	10	44	2.1	107	63
Semi-intensive	15	0.20	30	58	20	108	4.0	200	92

• *The ponds with wild stock category are excluded here.*

The multiple ownership is a problem for fish culture because the share holders are usually unable to arrive at an unified decision in respect of fish farming. This problem can be solved by leasing out the pond to a person interested in fish culture. Khan (1986) suggested that utilization of joint ownership ponds can be done by (a) co-operatives, (b) leasing to interested person(s), and (c) village organization under which all ponds may be put into productive use. In this case, the share holders will have the lease money distributed among them according to their shares. The public ponds may be utilized by being provided to the co-operatives of rural youths, women, landless labours or private enterprise and by ensuring available funds to them with a reasonable interest. It is suggested that necessary steps to be taken by the Government to provide a minimum level of education and training facilities to the fishermen on the scientific methods of pond fish cultivation. In order to meet the credit needs of the fishermen the commercial and other specialized bank such as 'Agriculture Bank' should offer credit to the pond owners on short term at a reasonable rate of interest. Provision should, although, be

made for adequate diversified and timely delivered loans with strict supervision for their proper utilization.

The responses of pond owners about the problems of fish culture indicates that multiple ownership was the most important problem. Among others, lack of scientific knowledge, scarcity of funds , non-availability of fish fry and incidence of flooding were the major problems indicated by the pond owners. These problems could be overcome, at least in partially through proper training of the pond owners, ensuring of fish fry supply and provision of credit facilities. The incidence of flooding is the important problem which is very hard to be tackled.

References

Akbar, M.A. and M.H. Rahman, 1982. Utilization of Fish ponds in Mymensingh district. *Bangladesh J. Agril. Econ.*, 5(1-2): 103-114.

BBS, 1997. Statistical pocket book of Bangladesh, Statistical Devision, Ministry of Planning, Government of Bangladesh, Dhaka, Bangladesh. 170 pp.

Kaiya, M.K.U., 1986. Survey of pond fishery resources in Mirzapur upazila under Tangail district. An M.Sc. thesis submitted to the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, 52 pp

Kaiya, M. K.U., M.F.A. Mollah and M.S. Islam, 1987. Survey of pond fishery resources in Mirzapur upazila under Tangail district. *Bangladesh J. Fish.*, **10**(1) : 37-43.

Khan, M.S., 1986. Socio-economic factors in the development of pond fisheries. *Bangladesh J. Agril. Econ.*, 9(2) : 127-132.

Khan, M. S., M.A. Quddus and M. A. Islam, 1991. A study of pond fishery resources in Trisal upazila. *Bangladesh J. Ext. Edu.*, 6(1&2) : 55-64.

Mollah, M.F.A., 1980. Survey of pond culture in two villages in Dhaka district. *Bangladesh J. Agril. Sci.*, 7: 39-42.

Quddus, M. A. and M. A. Akbar, 1995. A study on the investment-income relationship of the inland water fishermen. *Bangladesh J. Agril. Sci.*, 22(1): 171-178.

Rahman, M.M., 1986. Small-scale fisheries in Bangladesh: Some socio-economic problems and issues. *Bangladesh J. Agril. Econ.*, 9(2) : 97-110.

Rahman, M.L. and M.H. Ali, 1986. Developing pond fisheries in Bangladesh: Some findings of a study on credit and marketing aspects. *Bangladesh J. Agril. Econ.*, 9(2) : 111-125.

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