Vol. 6(1)
July 2002

ISSN: 1026-6690

Bangladesh Journal of Fisheries Research

Bangladesh Fisheries Research Institute

Bangladesh Journal of Fisheries Research

Aims and scope: The *Bangladesh Journal of Fisheries Research* is published by Bangladesh Fisheries Research Institute (BFRI). The Journal accepts original research articles, scientific notes and review papers on different aspects of fisheries including, but not limited to, fresh and brackishwater aquaculture, mariculture, reproductive physiology & genetics, nutrition & feed, pollution & disease, post-harvest technology & quality control, and aquatic environment. Priority will be given on the articles covering one of the following fields.

Environmental aspects of fisheries resources
Biodiversity conservation
Openwater management
Policy or status paper on national interest

Publication information: The *Bangladesh Journal of Fisheries Research* is published half-yearly in two issues (January and July). The subscription rate for each annual volume is: (a) individual- BDT 500 (overseas US$ 30), (b) institution- BDT 1000 (US$ 60). The Journal is also available in exchange for publications of learned societies, research institutes and universities. All payments should be made in favour of the *Bangladesh Journal of Fisheries Research*, and all correspondence addressed to the Director General, Bangladesh Fisheries Research Institute, Mymensingh-2201, Bangladesh. Fax : (+880-91-55259). E-mail: dgbfri@bdonline.com

Bangladesh J. Fish. Res., 6(1), 2002: 1-9

Feed utilization and wastage in semi-intensive pond culture of mahseer, *Tor putitora* (Ham.)

M.S. Islam*, S. Dewan, M.G. Hussain[1], M.A. Hossain[1] and M.A. Mazid[1]
Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
[1]Bangladesh Fisheries Research Institute, Mymensingh 2201, Bangladesh
*Present address & correspondence: Division of Applied Biosciences, Faculty of Fisheries
Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

Abstract
Mahseer, *Tor putitora* with 12.75, 12.11, and 12.02g of initial weight were fou 1 to attain a net weight gain of 12.0 kg, 11.5 kg, and 11.4 kg respectively in pond-1 (commercial feed), pond-2 (farm-made feed), and pond-3 (farm-made feed), respectively against 78.2 kg, 70.3 kg, and 68.1 kg feed fed. Gross energy contents in fish were 1359.3 Kcal/kg, 1281.5 Kcal/kg and 1266.6 Kcal/kg, respectively in pond-1, pond-2, and pond-3 against 3630.4, 3876.9 and 3570.5 Kcal/kg energy in the feed fed. Only 9.4%, 10.5% and 13.7% of the protein, and 8.9%, 3.4% and 3.3% of the lipid fed to fish were converted into muscle respectively in pond-1, pond-2 and pond-3. It was observed that the higher the protein content in feed, the lower the rate of conversion in muscle; the same was also true for lipid. It is supposed that feed derived wastes contribute potentially to water quality deterioration and eutrophication. Lower feed conversion, higher nitrogenous and phosphatic concentrations and higher plankton biomass in the ponds are all supportive to this observation.
Key words: Feed utilization, Feed wastage, *Tor putitora*

Introduction

An effective feeding regime is one of the key factors affecting the goals of a fish production unit. Considerable research on fish nutrition have so far been done but information regarding the proportion of supplied feeds that is actually utilized and transformed into fish muscle, the proportion lost in one way or other is not adequate or complete. At the same time, production of wastes from feeds and the role of feed wastes on pond ecology and productivity are also important considerations.

An effective feeding regime must take into account the composition of feed, its digestibility, feeding rate and frequency, method of preparation and supplying all of which affect feed consumption and utilization (De Silva and Davy 1992, Chiu *et al.* 1987, Das and Ray 1989). Moreover, a fishery manager must be aware of the ecological implications related to feeding of aquatic animals. Even if feeds are appropriately formulated and fed, the amount of feeds that is actually utilized and retained in fish body is very low, not exceeding around 40% of the ingested feeds (Bergheim and Bratten 2000). The remaining portions are lost in the environment as waste, fish fecal matters,

excretory products etc. and increasing feed losses are associated with higher FCR values (Das and Ray 1989) resulting not only in poor production but also ecological complications. Leaching of nutrients from feed contributes further to increase FCR values.

Waste management in aquaculture has been difficult for a number of reasons. Prediction of feed intake and optimum feeding level, collection of wastes and rapid dispersion of wastes into surrounding water are all complicated to a considerable extent. Therefore, feed waste contributes a relatively large proportion of total waste output in many aquaculture operations (Cho *et al.* 1994). In recent years, attentions have been paid to reduce feed losses and production of wastes from feeds. Improved diet quality and feeding regimes have contributed significantly in reducing feed losses and feed derived wastes. The present study was undertaken to observe the pattern of utilization of feeds supplied to aquaculture ponds of mahseer, *Tor putitora*.

Materials and methods

Formulation and preparation of feeds

Rice bran (33%), mustard oil cake (20%), soybean meal (15%), fishmeal (5%), blood meal (17%), wheat flour (6%), casein (2.5%), vitamin and mineral premix (0.25%) and common salt (1.25%) were used as ingredients for preparing feed 'B'. Feed 'C' was prepared with only the conventional ingredients such as rice bran (50%), mustard oil cake (20%), soybean meal (15%) and wheat flour (15%). All the feeds were supplied as pellets of 3-4 mm size. Among the three feeds, feed A was an commercial feed, purchased from SABINCO feed industry. The other two feeds (feed B and C) were prepared at the Laboratory. Feed 'C' was prepared completely from locally available ingredients. The feeds were different in proximate composition as well as in the total energy content.

Proximate analysis of the prepared feeds and experimental fish

The experimental diets were analyzed for proximate composition using the methods described in AOAC (1980). Nitrogen Free Extract, which was considered as soluble carbohydrate, was determined by 'substracting method' according to Castell and Tiews (1980).

Pond preparation, stocking and management

The selected ponds were drained, renovated and cleaned of aquatic vegetation. Lime (limestone, $CaCO_3$) was then applied by spreading over the bottom at the rate of 250 kg/ha. The bottom was then ploughed and left to dry for about a week. Ponds were then filled with water at a depth of about 1.5 meter and a more or less same depth was maintained for the whole experimental period. Cowdung at the rate of 1000 kg/ha and urea and TSP both at the rate of 25 kg/ha were applied 1 week prior to stocking. Over-

wintering fingerlings were stocked in the experimental ponds at the density of 32 fish per decimal ($40m^2$).

Feeding

Feeds in the form of pellets (3-4 mm) were supplied on a tray once every day between 09.00 and 10.00 h at the rate of 6% of the body weight of the fish. The tray was cleaned every day before placing the feed on it and any uneaten feed was carefully observed and recorded. About 40% of the fishes were sampled fortnightly to adjust the feed requirement on the basis of the weight gained by the fish.

Water quality criteria

Important nutrients of water such as nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N), ammonia nitrogen (NH_3-N) and phosphate phosphorus (PO_4-P) were monitored fortnightly between 09.00 and 10.00 hour on each sampling day. The concentrations of the nutrients were used as an index of eutrophication (Cho *et al.* 1994).

Water samples were filtered through glass fibre filter paper (Whatman GF/C) and treated for nutrient analyses. Clean white PVC plastic bottles of 250 ml were used to collect water samples for nutrient analyses. Standard methods and procedures were followed during sample collection and care was taken to avoid contamination. Nitrate nitrogen, nitrite nitrogen, and ammonia nitrogen were determined by a HACH water analysis kit (DR/2000, direct reading spectrophotometer). Phosphate phosphorus was measured by a spectrophotometer (Milton Roy Spectronic, model 1001 plus) following the method described by Stirling (1985).

Plankton study

Quantitative estimates of phytoplankton and zooplankton was taken fortnightly as an index of the extent of eutrophication resulting from waste feeds. Depth integrated samples of ten liters of water were passed through plankton net (mesh size 0.04 µm) to get a 50 ml sample. The sample was preserved immediately in small sealed plastic bottles with 5% buffered formalin. Plankton were enumerated following the simple method described by Vollenweider (1985) using a Sedgwick-Rafter cell (S-R cell). The slide was left for 15 minutes to allow the plankton to settle and then all plankton cells and colony forming units were counted using a binocular compound microscope (Swift M-4000) in 10 random fields from each sample. The plankton density (number of cells per litre of water sample) was estimated using the following formula:

$$N = (P \times C \times 1000) / L$$

Where,

N = the number of plankton cells or units per litre of original water

P = the number of plankton counted in10 fields

C = the volume of final concentrate of the sample in ml

L = the volume of water sample in litre

Estimation of growth and feed utilization parameters

The growth and feed utilization were calculated in terms of the feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER), and the gross and net production of the fish per unit area. For calculation of FCR, the dry weight of the feed was obtained by using a correction for the analyzed moisture content of the diet. The FCR was calculated after Castell and Tiews (1980) as follows:

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

For calculation of FCR, the dry weight of the feed was obtained by using a correction for the analyzed moisture content of the diet. However, for calculation of the FCR, the amount of feed supplied was taken into account rather than the actual amount of feed fed by the fish and the amount lost in the environment.

The SGR is the instantaneous change in weight of fish calculated as the percentage increase in body weight per day over any given time interval. The SGR was calculated after Brown (1957) as follows:

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

Where,
W_1 = The initial live body weight (g) at time T_1 (day)
W_2 = The final live body weight (g) at time T_2 (day)

The protein efficiency ratio (PER) was calculated according to Steffens (1989) and Wu and Dong (2002) as follows:

$$\text{Protein efficiency ratio (PER)} = 100 \times (W2 - W1)/Dp$$

Where,
W2 and W1 = final and initial wet weight (g) of the fish.
Dp = dry protein intake (g).

The gross energy content of the diet and fish muscle was calculated according to Hossain *et al.* (2001) using a Bomb Calorie meter. The gross and net yield of fish for each treatment was determined by multiplying the average weight of fish by the total number and was expressed as production in kg/ha.

Estimation of waste discharge

The estimation of the waste discharge was done by using the simple equations given by Einen *et al.* (1995) as follows:

$$\text{Waste discharged} = \text{nutrient fed} - \text{nutrient gain} \qquad (1)$$

Where,

nutrient fed = ration fed (g) × nutrient in feed (g g^{-1} diet) (2)
nutrient gain = growth (g) × nutrient in fish (g g^{-1} diet) (3)

Results

The formulation of the experimental feeds and their proximate composition are shown in Table 1 and Table 2 respectively. Feed B was highest in crude protein content followed by feed A. The highest protein content (28.3%) in feed B was attributed to the protein sources used particularly fishmeal and casein.

Table 1. Formulation of the supplementary feeds (feed 'B' and feed 'C')

Ingredients	Composition (%)	
	Feed 'A'	Feed 'B'
Blood meal	17	-
Mustard oil cake	20	20
Rice bran	33	50
Fish meal	5	-
Soybean meal	15	15
Wheat flour ('atta')	6	15
Casein	2.5	-
Salt (common salt)	1.25	-
Premix (Embavit fish premix)	0.25	-

Table 2. Biochemical composition of the supplied feeds and experimental fish

Pond No.	Composition (%)											
	Moisture		Crude protein		Crude lipid		Ash		Crude fibre		NFE*	
	Feed	Fish	Feed	Fish	Feed	Fish	Feed	Fish	Feed	Fish	Feed	Fish
1	11.83	76.18	27.71	17.87	6.85	3.01	16.08	1.69	12.24	0.66	37.12	0.59
2	7.40	76.59	28.30	18.26	12.03	2.44	15.42	1.43	16.46	0.61	27.79	0.67
3	10.52	78.49	21.32	15.90	13.13	3.07	11.79	1.23	15.74	0.68	32.62	0.63

Water quality and plankton

Mean values of nitrate, nitrite, ammonia nitrogen and phosphate phosphorus and the total plankton counts are shown in Table 3. Total number of phytoplankton varied between 1,254 x 10^3 and 2,255 x 10^3 (mean = 1,916 ± 220 x10^3) in pond 1, 1,372 x 10^3 and 2,239 x 10^3 (mean = 1,805 ± 192 x10^3) in pond 2 and 1,247 x 10^3 and 2,444 x 10^3 (mean = 1,945 ± 231 x 10^3) in pond 3. Total number of zooplankton varied between 24 x 10^3 and 42 x 10^3 (mean = 34 ± 4 x 10^3) in pond 1, 23 x 10^3 and 31 x 10^3 (mean = 29 ± 2 x 10^3) in pond 2 and 27 x 10^3 and 38 x 10^3 (mean = 31 ± 2 x 10^3) in pond 3.

Table 3. Range and mean (±SD) values of water quality criteria and plankton counts

Pond No.	Nitrate (mg/l)	Nitrite (mg/l)	Ammonia (mg/l)	Phosphate (mg/l)	Plankton (No. of cells/l)
1	0.95-1.85 (1.48±0.46)	0.01-0.03 (0.025±0.01)	0.1-1.48 (0.53±0.50)	0.47-2.22 (1.22±0.56)	1254×10^3-2255×10^3 ($1916 \pm 221 \times 10^3$)
2	1.05-1.70 (1.41±0.29)	0.016-0.038 (0.025±0.01)	0.11-1.35 (0.45±0.46)	0.40-2.66 (1.31±0.65)	1372×10^3-2239×10^3 ($1805 \pm 192 \times 10^3$)
3	1.0-1.57 (1.26±0.2)	0.008-0.022 (0.019±0.01)	0.12-1.23 (0.64±0.48)	0.05-2.75 (1.37±0.73)	1247×10^3-2444×10^3 ($1945 \pm 231 \times 10^3$)

Growth and feed utilization

Results of different growth parameters of fish in different treatments at the end of the experiment are shown in Tables 4. The net increase by length and weight recorded were 8.3 cm and 76.7 g in pond 1, 8.1 cm and 68.3 g in pond 2, and 8.6 cm and 69.6 g in pond 3 respectively. The trend of fortnightly average increase in length and weight showed that the growth rate was more or less rapid at the beginning and then slowed down towards the end of the experiment in all the treatments. The FCR, SGR and PER values were respectively 5.26, 0.56 and 0.55 in pond 1; 5.28, 0.75 and 0.58 in pond 2; and 5.43, 0.55 and 0.79 in pond 3.

Table 4. Growth and feed utilization parameters

Feed fed	Production (kg/ha)		Mean length gain (cm)	Mean wt. gain (g)	FCR	SGR (% day)	PER
	Gross	Net					
Feed A	599.25	497.25	8.31	76.69	5.26	0.5647	0.553
Feed B	638.78	541.90	8.11	68.31	5.28	0.7527	0.578
Feed C	567.40	471.24	8.61	69.62	5.43	0.554	0.786

Table 5 shows the mass balance of the amount of different ingredients of feed fed and the amount converted to respected ingredients of fish muscle. Comparative energy contents between feeds and fish muscles are also shown. Only 15.35%, 16.36%, and 16.74% of the supplied feeds were converted into harvestable components respectively in pond-1, pond-2, and pond-3. Muscle protein of 2.04 kg, 2.09 kg and 1.99 kg were obtained against 21.7 kg, 19.9 kg and 14.5 kg protein supplied, which, in case of lipid were 0.48, 0.29 and 0.31 kg against 5.4, 8.5 and 8.9 kg supplied to the fish which means that only 9.4%, 10.5%, and 13.7% of the protein and 8.9%, 3.4%, and 3.3% of the lipid fed to fish were converted into muscle respectively in pond-1, pond-2, and pond-3. Calculated gross energy contents in fish were 1359.3 Kcal/kg, 1281.5 Kcal/kg and 1266.6 Kcal/kg by supplying respectively 3730.4 Kcal/kg, 3876.9 Kcal/kg, and 3770.5 Kcal/kg. These values, in addition to those obtained in FCR, PER and SGR clearly indicate very poor feed utilization and conversion.

Table 5. Estimated mass balance of the amount of feed fed and the amount fish produced

Feed fed	Feed A	Feed B	Feed C
Total feed fed (kg)	78.2	70.3	68.1
Crude protein fed (kg)	21.7	19.9	14.5
Crude fat fed (kg)	5.4	8.5	8.9
Crude ash feed fed (kg)	12.6	10.9	11.7
Crude fibre fed (kg)	9.6	11.6	10.7
NFE fed (kg)	29.0	19.6	22.2
Gross energy (Kcal/kg) in feed	3730.4	3876.9	3770.5
Feed retained in fish body			
Total net biomass (kg)	12.0	11.5	11.40
Protein gain (kg)	2.04	2.09	1.99
Lipid gain (kg)	0.48	0.29	0.31
Ash gain (kg)	0.19	0.12	0.15
Crude fibre gain (kg)	0.09	0.08	0.08
NFE gain (kg)	0.08	0.07	0.07
Gross energy (Kcal/kg) in fish	1359.3	1281.5	1266.6

Discussion

It is evident that the performance of the fish in all ponds was very poor in terms of growth and feed utilization. Two major factors are supposed to be responsible; one, a major portion of the supplied feeds might not be taken by fish and, therefore, lost to the environment and the other, the digestibility of the feeds were poor which resulted in poor feed utilization even though the feeds were eaten. Asgard *et al.* (1998) reported that losses of feeds to the environment depend upon a number of factors such as feed formulation (balance of nutrients between that supplied and that required), nutrient digestibility, feed supplying methods including the ration size associated with feed intake and loss etc. In order to maximize feed utilization, fish should be fed by methods that allow feeding to satiation, but do not waste feed.

Considering the above-mentioned factors, there were many possibilities for a major part of the feeds supplied in the present experiment to be lost in the environment. The results of the nutrient analyses of water as well as that of plankton study also give logical support to this point (Palmer 1980). Very high concentration of nitrogenous (particularly ammonia nitrogen) and phosphatic nutrients in water in the present study are most likely to come from decomposition of uneaten feeds (Kissil and Lupatsch 1992, Ackefors and Enell 1994, Axler *et al.* 1996). Part of this may also be contributed by undigested feeds, fecal wastes and excretion products (Bergheim and Braaten 2000). Very high loading of wastes resulted from feed loss was also reported by NCC (1990).

The lower growth rates might also be associated with lower appetite and inefficient food utilization. The causes might be, among others, higher ration size, poor digestibility and wastage of feed. Andrews and Stickney (1972), Reddy and Katro (1979), and Das and Ray (1989) observed increasing trends of FCR values with increasing ration

size. Ghosh *et al.* (1984) obtained FCR values 1.5, 2.92, and 4.29 by feeding common carp with supplementary feeds at the rate of 2%, 4% and 6% of body weight. De Silva and Davy (1992) stated that digestibility of fish plays an important role in lowering the FCR value by efficient utilization of food which, in turn, depends on daily feeding rate, its frequency and the type of food used (Chiu *et al.* 1987).

The ultimate source of wastes in any fish culture unit is feeds and feeding. Therefore, control and reduction of fish culture wastes can best be achieved through an effective nutritional approach focussing on feeds and feeding. Such a nutritional approach should include four main points; these are careful selection of ingredients based upon digestibility, balanced feed formulation to ensure maximum feed utilization, avoidance of excess nutrients, and an effective and strict feeding regime (Cho 1992 and Cho *et al.* 1994).

A further more critical analysis and discussion may be done from the waste production point of view according to Boyd (1999). From the FCR (the amount of feed in kg which results in the production of 1 kg of fish) values shown in Table 4, it may be concluded that 4.26, 4.28, and 4.43 kg of waste is generated in the production of 1 kg of fish each in treatment 1, 2, and treatment 3 respectively. But a more careful analysis of the relationship between feed input, fish production and waste generation reveals a very high loading of wastes in aquaculture ponds. For example, feed 'A' contained 88.7% dry matter and 11.83% water. Fish in treatment 1, on the other hand, contained 13.82% dry matter and 76.18% water. Thus in the production of 1 kg of fish with 5.26 kg of feed (FCR of 5.26), 4.64 kg dry matter in feed yields only 0.14 kg dry matter in fish. Therefore, in the production of 1 kg fish dry matter the dry feeds required is as high as 33.14 kg. Thus the dry matter conversion ratio is only 33.14 (33.14 kg dry feed, 1 kg dry fish). Therefore, the ratio of fish to wastes of 1:4.26 based on the usual method of estimating feed conversion ratio is an apparent ratio. But the true ratio based of dry matter conversion is 1: 32.14 in treatment 1 with feed 'A'. Similarly, in treatment 2 and treatment 3, the dry matter conversion ratios were 36.46 and 22.59 and the ratios of fish to wastes were 1: 35.46 and 1: 21.59 with feed 'B' and feed 'C' respectively.

References

Ackefors, H. and M. Enell, 1994. The reuse of nutrients and organic matter from aquaculture systems in Noradic countries. *J. Appl. Ichthyol.*, 10(4): 225-241.

Andrews, J.W. and R.R. Stickney, 1972. Interactions of feeding rates and environmental temperature on growth, food conversion, and body composition on channel catfish. *Trans. Am. Fish. Soc.*, 101(1): 94-99.

AOAC, 1980. Official Methods of Analysis. Association of Analytical Chemists, 13th edition, Washington DC. 1018 pp.

Asgard, T., M. Hillestad, E. Austreng and I. Holmefjord, 1998. Can intensive aquaculture be eco-friendly? Akvaforsk & BioMaras, Trondheim, Norway, 28pp.

Axler, R., C. Larsen, C. Tikkanen, M.C. McDonald, S. Yokom and P. Aas, 1996. Water quality issues associated with aquaculture: A case study in mine pit lake. *Water Environment Research*, 68 (6): 995-1011.

Bergheim, A. and B. Braaten, 2000. Waste production trends in intensive cold water fish farming. Accepted paper for Conf. Aquac. 3rd Mill., 20-25 Feb. 2000. Bangkok, Thailand

Boyd, C.E., 1999. Management of shrimp ponds to reduce the eutrophication potential of effluents. *Global Aquaculture Advocate*, 2(6): 12-12.

Brown, M.E. (eds.), 1957. Experimental studies on growth. *In*: The physiology of fishes. Vol. I. Academic Press, New York. pp. 361-400.

Castell, J.D. and K. Tiews (eds.), 1980. Report of the FIFAC, IUNS and ICES working group on the standardization of methodology in fish nutrition research, Hamburg, Federal Republic of Germany, 21-23 March, 1979. FIFAC Technical Paper. 26 pp.

Chiu, Y.N., N.S. Sumagaysay and M.G.S. Sastrillo, 1987. Effect of feeding frequency and feeding rate on the growth and feed efficiency of milkfish, *Chanos chanos* (Forskal) juveniles. *Asian Fisheries Science*, 1: 27-31.

Cho, C.Y., 1992. Feeding systems for rainbow trout and other salmonids with reference to current estimates of energy and protein requirements. *Aquaculture*, 100: 107-123.

Cho, C.Y., J.D. Hynes, K.R. Wood and H.K. Yoshida, 1994. Development of high-nutrient-dense, low-pollution diets and prediction of aquaculture wastes using biological approaches. *Aquaculture*, 124: 293-305.

Das, I. and A.K. Ray, 1989. Growth performance of Indian major carp *Labeo rohita* (Ham.) on duckweed incorporated pelleted feed: A preliminary study. *J. Inland Fish.*, 21(1): 1-6.

De Silva, S.S., and F.B. Davy, 1992. Fish nutrition research for semi-intensive culture system in Asia. *Asian Fisheries Science*, 5: 129-144.

Einen, O., I. Holmefjord, T. Asgard, C. Talbot, 1995. Auditing nutrient discharges from fish farms: theoretical and practical considerations. *Aquaculture Res.*, 26: 701-713.

Ghosh, S.K., B.K. Mandal and D.N. Borthakur, 1984. Effects of feeding rats on production of common carp and water quality in paddy-cum-fish culture. *Aquaculture*, 40 (2): 97-101.

Hossain, M.A., U. Focken, and K. Becker, 2001. Evaluation of an unconventional legume seed, *Sesbania aculeata*, as a dietary protein source for common carp, *Cyprinus carpio* L. *Aquaculture*, 198: 129–140.

Kissil, G.W. and I. Lupatsch, 1992. New approach to fish seed in Israeli mariculture as a result of environmental constraints. *Isr. J. Aquacult. Bamidgeh*, 44 (4): 125 p.

NCC, 1990. Fish Farming and the Scottish Freshwater Environment. Nature Conservancy Council, Edinburgh.

Palmer, C.M., 1980. Algae and Water Pollution. Castle House Publications Ltd., UK.

Reddy, S.R. and S. Katre, 1979. Growth rate and conversion efficiency of airbreathing catfish, *Heteropneustes fossilis* in relation to ration size. *Aquaculture*, 18 (1): 35-50.

Steffens, W., 1989. Principles of Fish Nutrition. Ellis Horwood, Chichester.

Stirling, H.P., 1985. Chemical and biological methods of water analysis for aquaculturists. Institute of Aquaculture, University of Stirling, Stirling. 119pp.

Vollenweider, R.A., 1985. A manual on methods for measuring primary production in aquatic environments. IPB Handbook No. 12, 3rd edition. Oxford. Blackwell.

Wu, L. and S. Dong, 2002. Effects of protein restriction with subsequent realimentation on growth performance of juvenile Chinese shrimp (*Fenneropenaeus chinensis*). *Aquaculture*, 210: 343–358.

(Manuscript received 18 October 2000)

Bangladesh J. Fish. Res., 6(1), 2002: 11-18

Early developmental stages of *Nandus nandus* (Ham.)

M. Das*, M.A.K. Tarafder and S. Pal
Department of Aquaculture, Bangladesh Agricultural University
Mymensingh 2202, Bangladesh
* Corresponding author

Abstract
In order to study the early developmental stages of *Nandus nandus* an experiment was conducted, where eggs and milt were obtained from the laboratory reared *N. nandus* by stripping after 15 hours of 150 mg/kg body weight of carp PG extract injection. Then the eggs were fertilized in the laboratory and subsequent developmental stages were studied. First cleavage (two cell), four cell, eight cell, sixteen cell and multi cell stages were found 30, 50, 70, 105 and 160 minutes after fertilization respectively. Morula, early gastrula, middle gastrula, late gastrula and yolk plug stages were found 5, 8, 9, 11 and 13 hours after fertilization respectively. Hatching occurred within 20±2 hours after fertilization, and larvae were measured 1.60 mm in diameter. After one hour of hatching two melanophore bands were found at the caudal region of the body of the larvae. Eyes were first observed in 10 hours, pectoral and pelvic fin buds appeared in 22 hours and well developed in 38 hours old larvae. Mouth cleft and brain lobes were visible when the larvae were 34 and 38 hours old respectively. Myomeres partially appeared in 16 hours, which were clearly visible in 74 hours old larvae. Larvae started wandering and searching for food after 56 hours of hatching. The yolk sac was completely absorbed when the larvae became 62 hours old.
Key words: Early developmental stages, *N. nandus*

Introduction

Nandus nandus locally known as 'Nondoi', 'Meni' or 'Veda' is a common freshwater small carnivorous fish (Mustafa *et al.* 1980) of Bangladesh. It was commonly found in natural water bodies of the country. The fish is going to be rarely available and now a days is considered to be an endangered or threatened species. The species should be protected from being extinct. In view of this the species should be studied thoroughly to take measures for its available in quantities in the natural water bodies of Bangladesh. In view of this, proper domestication techniques should be developed and for this, biological data on the species should be as complete as possible.

Embryonic and larval stages of a fish are the most delicate part of their life. Fish early life stages are especially sensitive to stress and some times succumbed to death in mass due to the unavailability of appropriate quality and quantity of larval food especially at the first feeding. For successful rearing what should be done and when should be done is very important. Although the species have been studied for collecting

data on disease and parasites (Chandra and Golder 1987, Golder *et. al.* 1987, Golder and Chandra 1987), on the food and feeding habits and fecundity (Mustafa *et al.* 1980), on the predatory behaviour and fecundity (Akther 1999, Das *et. al.* 2001) and on laboratory rearing of *N. nandus* from young to sexual maturity (Das and Zamal 2000), no published information about early development of the species is available. So it was felt necessary to study and characterize its various stages of embryonic and larval development to understand the biological clock of the species, identify the early life history stages and to detect first feeding time. These biological information will allow us to take appropriate measures in evolving culture techniques of the species.

Materials and methods

The experiment was conducted from August'99 to August'00 in the laboratory of the Department of Aquaculture, Bangladesh Agricultural University, Mymensingh. Eight glass aquaria each having size of 60 × 35 × 30 cm were used to stock the brood fish for obtaining experimental embryo and larvae. Six whole glass aquaria each having size of 30 × 30 × 25 cm were used as egg incubation chamber and subsequently as larval rearing tank. Early developmental stages of *N. nandus* were studied up to 96 hours starting from egg fertilization.

The aquaria were cleaned properly and filled with fresh tap water for the experiment. Sixteen experimental fish of *N. nandus* (8 male + 8 female) were used from a previous experiment conducted by Das and Zamal (2000). Two fish were reared in each aquarium feeding live prawns (*Macrobrachium lamarrei*) once a day (10 am) as has been reported by Das and Zamal (2000). Maturity of fish was first perceived by observing body colour and ejection of eggs and milt by means of gentle pressure on the abdomen of the female and male respectively. The dark brown colour and even size of the eggs also confirmed ripening of the eggs. To obtain fertilized eggs six pairs of mature fish were kept in different aquaria at 10 am July 14'00. 150 mg/kg of carp PG solution was administered intramuscularly on the dorsal region above the lateral line just beneath the base of the dorsal fin at 5 p.m. Injected breeders were kept in pairs in each aquarium. At 6 a.m. of the next morning fishes (2 pairs) started spawning. To observe the exact time of fertilization, male and female fishes of the remaining four pairs were stripped. Ovulated ova were collected on an enamel plate and milt was collected in a glass capillary tube. A drop of milt was poured on the ova. Ova and milt were mixed thoroughly and small amount of water was added to enhance fertilization and water hardening of the eggs. The fertilized eggs on plate were allowed to remain undisturbed for five minutes on the top of the table. The eggs thus obtained were delicately washed several times with tap water and finally transferred in aquaria for incubation. The fertilized eggs were incubated at ambient temperature 28-29°C in the aquaria. Aeration was given to the water of aquaria to keep the oxygen concentration at high level.

Sample was collected (five eggs) randomly from the aquaria every 5 to 10 minutes interval till the completion of morula and then after every one hour interval up to hatching. The larval sample (five larvae) was collected right from hatching up to 96

hours after hatching at hourly interval for first 22 hours and then every 6 hours, as the development stages did not vary very much. Samples were preserved in 70 percent ethanol for further study. Early developmental stages were studied under a stereomicroscope (Olympus SZH10) and an ocular micrometer was used to measure the eggs and larvae. Three individuals from each of the samples were examined for confirmation of developmental stages and the timing of development. Individuals were temporarily stained with methylene blue for clear observation and early stages of *N. nandus* were drawn by hand using a Camera Lucida (Olympus 306681) setting on stereo microscope.

Results

Present study was performed to find out the developmental clock of *N. nandus* for early developmental stages. The stages of embryonic and larval development of *N. nandus* with relation to the time period after fertilization and hatching respectively and characteristic features of each of stages are shown in Tables 1 and 2, and Figs. 1 (A-O) and 2 (A-Q). First cleavage (two cell), four cell, eight cell, sixteen cell and multi cell stages were found 30, 50, 70, 105 and 160 minutes after fertilization respectively. Morula, early gastrula, middle gastrula, late gastrula and yolk plug stages were found 5, 8, 9, 11 and 13 hours after fertilization respectively. Starting of heartbeat was first found at the age of 16 hours. Hatching occurred within 20±2 hours after fertilization, and larvae were measured 1.60 mm in diameter. After one hour of hatching two melanophore bands were found at the caudal region of the body of the larvae. Eyes were first observed in 10 hours, pectoral and pelvic fin buds appeared in 22 hours and well developed in 38 hours old larvae. Mouth cleft and brain lobes were visible when the larvae were 34 and 38 hours old respectively. Myomeres partially appeared in 16 hours, which were clearly visible in 74 hours old larvae. Larvae started wandering and searching for food after 56 hours of hatching. The yolk sac was completely absorbed when the larvae became 62 hours old.

Table 1. Embryonic development of *Nandus nandus* in the laboratory

Phase	Stage No.	Fig. No.	Time after fertilization (hr : min)	Temp. (°C)	Mean total diameter (mm)	Characteristic
Unfertilized eggs	I	1.A	0	28.5	0.6	Eggs spherical, brownish-yellow, demersal and slightly adhesive
Fertilized eggs	II	1.B	0	29	0.8	Eggs slightly adhesive, spherical, demersal and brownish-yellow.
		1.C	0:15	29	0.8	Blastodisc formed at animal pole
Blastulation	III	1.D	0:30	29	0.9	Start of 1st cleavage which was restricted to small disc of cytoplasm at animal pole, dividing blastodisc into two blastomeres
	IV	1.E	0:50	29	0.9	The second division of the two blastomeres resulted four blastomeres
	V	1.F	1: 10	29	0.9	8 blastomeres formed
	VI	1.G	1: 45	29	0.9	16 cell seen
	VII	1.H	2:55	29	0.9	Multiple cell was visible

Morula	VIII	1.1	5:00	29	1.00	Cap-like structure of blastomeres was visible at the animal pole which gradually increased in size by time
Gastrulation	IX	1.J	8:00	29	1.00	Blastomeres started invading the yolk by spreading over the yolk in the form of a thin layer
	X	1.K	9:00	29	1.00	Germinal ring was visible which occupy about half of the yolk by blastoderm
	XI	1.L	11:00	29	1.00	Blastoderm covered 3/4th of the yolk. Embryo shield was visible
Yolk plug stage	XII	1.M	13:00	29	1.10	The yolk invasion completed. The head and tail ends become differentiated
Starting of heart beat	XIII	1.N	16:00	29	1.30	Both tail and head-end were clearly visible. Heart was beating
Just before hatching	XIV	1.O	19±2	29	1.60	Twisting movement become more vigorous and the embryo ruptured the egg capsule, started hatching

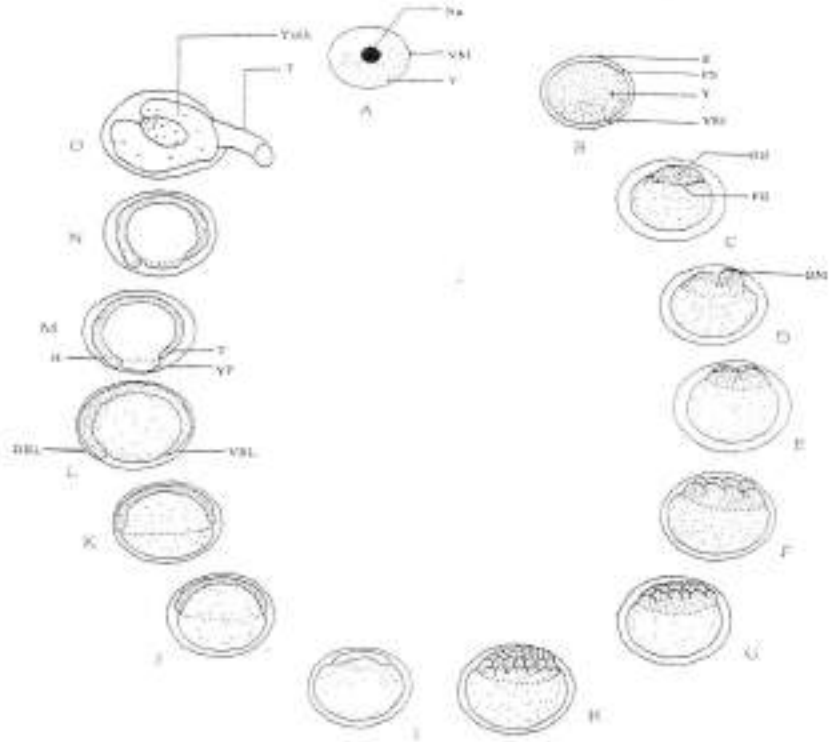


Fig. 1. Embryonic development of *N. nandus* in different time interval.
(A) Unfertilized egg, 0.6 mm in diameter, just after release (B) Fertilized egg, 0.8 mm in diameter, just after fertilization (C) Blastodisc formation, 0.8 mm in diameter, 15 minutes after fertilization (D) Two celled stage.

0.9 mm in diameter, 30 minutes after fertilization (E) Four celled stage, 0.9 mm in diameter, 50 minutes after fertilization (F) Eight celled stage, 0.9 mm in diameter, 70 minutes after fertilization (G) Sixteen celled stage, 0.9 mm in diameter, 105 minutes after fertilization (H) Multi celled stage, 0.9 mm in diameter, 175 minutes after fertilization (I) Morula stage, 1.00 mm in diameter, 5 hours after fertilizatinn (J) Early gastrula, 1.00 mm in diameter, 8 hours after fertilization; (K) Middle gastrula, 1.00 mm in diameter, 9 hours after fertilization; (L) Late gastrula, 1.00 mm in diameter, 11 hours after fertilization (M) Yolk plug stage, 1.1 mm in diameter, 13 hours after fertilization (N) Organogenesis, 1.1 mm in diameter, 16 hours after fertilization (O) Hatching stage,1.6mm, 19±2hours after fertilization.

Table 2. Larval development of *Nandus nandus* in the laboratory

Stage No.	Fig. No.	Age (h)	Mean TL (mm)	Characteristic
I	2.A	0	1.8	Larvae were slender, transparent showing internal organs. The larvae with oval shaped, brownish colored yolk sac.
II	2.B	1	1.9	Body of the larvae yellowish in colour. Yolk sac still remained attached to the body. Two vertical melanophore bands were appeared at the caudal region.
III	2.C	5	2	Body of the larvae was more transparent. Head and body laterally compressed. Yolk sac partially decreased.
IV	2.D	6	2.1	Three melanophore bands were appeared. More melanophores appeared on head, around and/or on the yolk sac. One melanophore band appeared at brain region.
V	2.E	8	2.14	The yolk sac partially reduced. No change in melanophores distribution.
VI	2.F	10	2.19	Yolk sac reduced. Eyes and anus became slightly visible. Intestine was visible.
VII	2.G	14	2.25	Vertical melanophore bands were very much prominent. Melanophores were forming a slander band above the eye around the yolk sac and/or on the yolk sac.
VIII	2.H	16	2.38	Yolk sac slightly decreased. Myomeres were partially visible. Melanophore concentration increased.
IX	2.I	22	2.80	The eyes became pigmented and dark in colour. External melanophore appeared dorsally on head. Myomeres were partially visible. Yolk sac had become thin. Pelvic and pectoral fin-bud appeared.
X	2.J	28	2.92	Prominent pectoral and pelvic fin-bud appeared. Myomeres were slightly visible.
XI	2.K	34	3.10	The colours of larvae were changed to yellowish black. Mouth cleft formed.
XII	2.L	38	3.18	The eyes were increased in size and had become densely pigmented. Mouth cleft became more prominent. Brain lobe was visible. Pectoral and pelvic fin fold well developed.
XIII	2.M	44	3.25	Mouth cleft easily distinguished. Opercula fold appeared. Brain lobe clearly distinguished.
XIV	2.N	50	3.28	The yolk sac very much reduced. Myomeres became more developed. The body became more pigmented.
XV	2.O	56	3.32	Pectoral fin bud became more pronounced. Eyes were fully pigmented. The jaws became well distinguished. The larvae started wandering here and there in search of food.
XVI	2.P	62	3.34	Brain lobe clearly visible. The yolk sac was completely absorbed and the larvae had started feeding.
XVII	2.Q	74	3.38	Myomeres clearly visible and counted 18 post-anal and 5 pre-anal. Larvae were blackish transparent in colour.

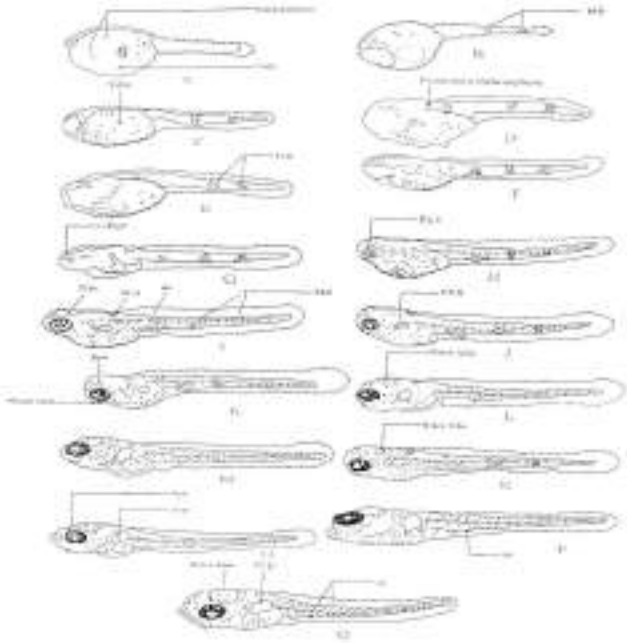


Fig. 2. Larval developmental of *N. nandus* in different time interval.
(A) Hatchling, 1.8 mm in TL (Just after hatching) (B) One hour old larvae,1.8 mm in TL (C) Five hours old larvae, 1.9 mm in TL (D) Six hours old larvae, 2.1 mm in TL (E) Eight hours old larvae,2.14 mm in TL (F) Ten hours old larvae, 2.19 mm in TL (G) Fourteen hours old larvae, 2.25 mm in TL (H) Sixteen hours old larvae, 2.38 mm in TL (I) Twenty two hours old larvae, 2.80 mm in TL (J) Twenty eight hours old larvae, 2.92 mm in TL (K) Thirty four hours old larvae, 3.10 mm in TL; (M) Forty four hours old larvae, 3.25 mm in TL (N) Fifty hours old larvae, 3.28 mm in TL (O) Fifty six hours old larvae, 3.32 mm in TL (P) Sixty two hours old larvae, 3.34 mm in TL and (Q) Seventy four hours old larvae, 3.38 mm in TL.

Discussion

Diagnostic bright reddish colour with black strip was observed in the dermis/skin of male *N. nandus* at the month of April. Female was comparatively dull in colour and their abdominal region was found swollen up. Genital aperture of female fish was found protruded. Eggs and milt were extruded by gentle pressure on the abdomen of the female and male respectively. The mature fish were injected with carp pituitary at the dose of 150 mg/kg body weight (Pal, 2000). The fecundity of *N. nandus* varied from 4858 to 15286. In the present study, the colour of unfertilized eggs of *N. nandus* was found brownish yellow. The findings agree with the findings of Akther (1999) for the same species. Das and Das (1999) found yellowish brown eggs in case of *Notopterus notopterus* and the intensity of that coloration was found varied with the variation of the sources of fish used. The colour of the egg is the complex result of the species specificity, type and amount of food and the inhabiting environment.

Average diameter of unfertilized eggs of *N. nandus* was 0.6 mm immediately after fertilization and 0.8 mm after water hardening. The two cell stage, four cell stage, eight cell stage and 16 cell stage was found 31, 50, 70 and 95 minutes after fertilization. The diameter of the eggs did not change (Table 1) after water hardening although so many physiological and developmental activities were going on. According to Rahman (1975) in case of *Anabus testudineus* same series of stages appeared after 15, 20, 45 and 97 minutes of fertilization respectively. In the present study morula, gastrula and yolk plug stages were found 5, 8-11 and 13 hours after fertilization. The study revealed that heartbeat was observed at 16 hours after fertilization. Whereas Rahman (1975) observed the same in 16.5 hours in case of *A. testudineus* which strongly supports the findings of this experiment. Generally closely related species are also close in their biological clock (Hoar and Randal, 1988). In *N. nandus* hatching was observed 19±2 hours after fertilization. Information is not available on this aspect of the species but several authors (Chakrabarty and Murty 1972, Thakur 1980) observed incubation period of fertilized eggs of some fishes lied between 18-32 hours.

The length of the newly hatched larvae of *N. nandus* was found to be 1.8 mm. Rahman (1975) found the length of the fresh hatchling in case of *A. testudineus* was 1.9 to 2 mm which was more or less similar to the present study. From this study it was found that the pectoral and pelvic fin bud appeared in 22 hours old larvae. Whereas, in case of *A. testudineus* fin buds were found in 14 hours after fertilization (Rahman 1975). This difference might be due to the species variation. Myomeres partially appeared in 16 hours old larvae but in 74 hours old larvae myomeres were clearly visible and counted 18 post-anal and 5 pre-anal. Whereas, Kohinoor *et al.* (1997) found the greater number of thirty six to forty myomeres at newly hatched larvae in case of *O. pabda* of which ten were pre-anal. In this context it can be said that the muscle arrangement is strictly own by the species. Mouth cleft of *N. nandus* had appeared in 34 hours old larvae. The brain lobe was appeared in 38 hours old larvae in the present experiment. Kohinoor *et al.* (1997) found mouth cleft in 12 hours old larvae in case of *O. pabda*. The larvae started feeding at 56 hours after hatching. Barua (1990) found *C. batrachus* larvae started feeding on the 4th day. The larvae started feeding before the completion of yolk absorption keeping required nutrition in hand in case of emergency. The same phenomena of starting external feeding keeping a part of internal food in the yolk sac was reported by Das (1995) for *Carassius auratus* larvae. Conservation is of evolutionary importance for the perpetuation of the species.

In the present experiment it was observed that the yolk sac fully absorbed in 62 hours old larvae. Rahman (1975) and Kohinoor (1997) reported complete yolk absorption in 144 hours old larvae of *A. testudineus* and 48 hours old *O. pabda* larvae at room temperature, which were not similar to the present study, perhaps due to the species variation. The timing of first feeding have evolutionary value and depends on the availability of natural food and synchronized activities of dependable communities in nature. Perhaps those determine the different species to act differently (Nikolsky 1963).

The present experiment provides information on early developmental stages of *N. nandus*, first feeding time for larval rearing enriching the knowledge of biology and

ecology of the fish. The knowledge will help sustainable development of culture as well as management technology of *N. nandus* to protect the species form being extinct. Developments of culture as well as management technology of *N. nandus* will play a substantial role in the overall nutrition of rural people of Bangladesh as it had been playing for long time.

References

Akther, N., 1999. Study of predatory behaviour and fecundity estimation of laboratory reared *Nandus nandus* (Hamilton). M.S. Thesis, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh. 58 pp.

Barua, G., 1990. Gonadal development and fry rearing of *Clarias batrachus* (Linn.). Ph. D. Thesis, Department of Fisheries Biology and Limnology, Bangladesh Agricultural University, Mymensingh. pp. 205-241

Chakrabarty, R.D. and D.S. Murty, 1972. Life history of Indian major carps. *Cirrhina mrigala* (Ham.), *Catla catla* (Ham.) and *Labeo rohita* (Ham.). *J. Inland Fish. Soc. India*, 4: 132-161.

Chandra, K.J. and M.I. Golder, 1987. Effects of helminth parasites on a freshwater fish *Nandus nandus*. *Environ. Ecol.*, 5: 333-336.

Das, M., 1995. The energetics of stress tolerance in the early life stages of the goldfish *Carassius auratus* L. Ph. D. Thesis, Institute of Aquaculture, University of Stirling, Scotland. 210 pp.

Das, M. and D.R. Das, 1999. A study on the fecundity of feather back, *Notopterus notopterus* (Pallas) from two different sources (pond and beel). *Bangladesh J. Aquaculture*, 21: 53-60.

Das, M., N. Akther, M.A.K. Tarafder and S. Pal, 2001. Predatory behaviour of a perch, *Nandus nandus* (Ham.). *Bang. J. Fish. Res.*, 5(2): 197-200.

Das, M. and N. Zamal, 2000. Domestication of an endangered fish species *Nandus nandus* (Ham.). I. Laboratory rearing of young fish up to sexual maturity. *Bang. J. Fish. Res.*, 4: 135-140.

Golder, M.I. and K.J. Chandra, 1987. Infestation of *Isoparorchis hypselobagri* on the host fish *Nandus nandus*. *Environ. Ecol.*, 5: 337-341.

Golder, M.I., K.J. Chandra and A.K.A. Rahman, 1987. Helminth parasitism in *Nandus nandus* (Hamilton). *Bangladesh J. Fish.*, 10: 11-22.

Hoar, W.S. and D.J. Randall, 1988. *Fish Physiology*, Vol. XI. The physiology of developing fish. Part A. Eggs and Larvae. Accademic Press, London.

Kohinoor, A.H.M., M.S. Islam, D.A. Jahan and M.G. Hussain, 1997. Embryonic and larval development of *Ompok pabda* (Hamilton). *Progress. Agric.*, 8: 37-41.

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: 205-210.

Nikolsky, G.V., 1963. *The Ecology of Fishes*, Accademic press, London. 352pp.

Pal, S., 2000. Artificial breeding of laboratory reared *Nandus nandus* (Hamilton) by using carp pituitary gland. M.S. Thesis, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh. 58 pp.

Rahman, M.M., 1975. Developmental and life history of *Anabas testudineus* (Bloch) (Perciformes: Anabantidae). *Bangladesh J. Zool.*, 3: 11-16.

Thakur, N.K., 1980. Notes on the embryonic and larval development of an air-breathing catfish *Clarias batrachus* (Linn.). *J. Inland Fish. Soc. India*, 12: 30-43.

(Manuscript received 6 June 2001)

Bangladesh J. Fish. Res., 6(1), 2002: 19-28

Early developmental stages of two *Secutor* species (Family: Leiognathidae) collected from the Bak-khali river estuary of the Bay of Bengal, Bangladesh

M. M. Islam, M. M. Haque* and M. A. Hossain[1]
Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
[1]Bangladesh Fisheries Research Institute, Mymensingh, Bangladesh
*Corresponding author

Abstract
Early developmental stages of two *Secutor* species, *Secutor insidiator* (Bloch) (11.9-36.0 mm standard length, SL) and *Secutor ruconius* (Hamilton-Buchanan) (14.0-33.0 mm SL) collected by ichthyoplankton net from the Bak-khali river estuary of the Bay of Bengal, Cox's Bazar, Bangladesh are described and illustrated. All of the fins with supporting spines and rays were present in the smallest collected sizes of both species. With growth of the specimens, significant changes in melanophore patterns were found. *S. insidiator* is similar to *S. ruconius* in having upward protracting mouth parts and body colouration, but can be distinguished easily by its more elongate body shape (body depth 38-47% of SL compared with 46-52% of SL in *Secutor ruconius*). Both the species occurred round the year from August 1998 to July 1999. The surface water temperature and salinity during the study period varied from 22.0-32.5 C and 10-37 ppt respectively.
Key wards: Early ontogeny, Bay of Bengal, *Secutor*

Introduction

Members of the family Leiognathidae are small fishes (Jones 1985), shoaling, bacterially bioluminescent and characterized by having silvery, laterally compressed bodies, highly protrusible jaws and locking median fin spines (Seigel 1982); top of head with bony ridges and nuchal crest; single dorsal fin with 8 spines and 16 rays, and anal fin with 3 spines and 14 rays; first dorsal and anal spines minute, second spines usually longest; body colour silvery, upper half of the body usually with some dusky patterning (Haneda and Tsuji 1972). Leiognathids are commonly known as slipmouths, silverbellies, dollarfish and ponyfish, which give the head a horse-like appearance with extremely protractile mouthparts (Jones 1985).

Leiognathids are distributed throughout the coastal waters of the tropical and subtropical Indo-Pacific, from the Red Sea and the eastern coast of Africa through India, Indonesia, Australia, Japan to the Pacific Islands, as far east as Tahiti and Hawaii (Jones 1985), and constitute one of the important fisheries of India (Balan 1963), Bangladesh

(Hussain 1971), Thailand (Kuhlmorgen-Hille 1968), and parts of the Philippine Archipelago (Tiews and Caces-Borja 1965).

The family Leiognathidae is composed of three genera (*Leiognathus, Gazza* and *Secutor*) with at least 30 nominal species (Seigel 1982). According to James (1975), 17 leiognathid species occur in the Indian waters. Of these, 13 were placed under the genus *Leiognathus* Lacépède, two under the genus *Secutor* Gistel and two under the genus *Gazza* Rüppell.

Mito (1966) described *L. rivulatus* larvae from the Seto Inland Sea, Japan. Kinoshita (1988) described the larvae of *L. rivulatus, L. nuchalis* and an unnamed species from Japanese waters. The last one was collected from around Ryukyu Islands, Japan and supposed to be *G. minuta*. Haque and Ozawa (1995) described early ontogeny of three leiognathids, *Leiognathus rivulatus, L. nuchalis* and *L. elongatus* from Kagoshima Bay, Japan. Hussain (1971) reported the occurrence of 4 leiognathids as adults, *Leiognathus equulus, L. bindus, Secutor ruconius* and *Gazza minuta* in commercial catches in the Bay of Bengal, Bangladesh. Rahman *et al.* (1995) noted some common adult characters of *Leiognathus brevirostris* collected from the marine waters of Bangladesh. There is no comprehensive study on the taxonomy of leiognathids in the coastal waters of Bangladesh.

In the present study, the early developmental stages of *S. insidiator* and *S. ruconius* collected from the Bak-khali river estuary of the Bay of Bengal, Cox's Bazar, Bangladesh were studied.

Materials and methods

The specimens used in this study were collected from the Bak-khali river estuary of the Bay of Bengal, Cox's Bazar, Bangladesh. The samples were collected monthly during day time at high tide from 3 selected stations from August'98 to July'99.

A cylindrical-conical type ichthyoplankton net (General Oceanics, USA) made up of nylon (500 μm in mesh size, 50 cm in diameter, 2.5 m in length) was used for collection of fish larvae and early juveniles. For taxonomic study some larger specimens (juveniles) were taken from local shrimp fry collectors.

A circular metallic frame was used at the mouth of the net and a specially designed plastic pocket was used at the cod-end. The net was towed on board a mechanized boat (speedboat) at a speed of about 2 knots per hour using 20 m rope lengths (10 minutes at each station). A sinker was hanged with the bridle of the net. A General Oceanics digital flow-meter (Model 2030) was set at the center of the mouth of the net to get data necessary for computation of the volume of water filtered. Surface water temperature and salinity were recorded during each sampling by using a Celsius thermometer and a hand refractometer respectively.

After collection, the samples were immediately preserved in approximately 5% buffered formalin in seawater. In the laboratory the fish larvae were sorted out under magnifying glass and were preserved in 70% ethyl alcohol.

The collected specimens were examined under a stereo-microscope (Olympus, SZH10 Research Stereo) and were measured using an ocular micrometer. Some specimens were temporarily stained with methylene blue for clear observation of spines and fin rays. For diagnosis and measurement of the specimens Leis and Trnski (1989) was followed. All of the specimens were measured as standard length (SL).

Results

Secutor insidiator Bloch

Identifying characters

Most of the adult characteristics of *S. insidiator* were given by Fischer and Whitehead (1974), James (1975) and Jones (1985). The present specimens were identified by the following characters: dorsal fin with 8 spines and 16 rays, anal fin with 3 spines and 14 rays, and pelvic fin with one spine and 5 rays; body depth 38–47% of SL in specimens of 12–36 mm SL, body depth increases with length; mouthparts protracts upwards; lower jaw ascends at an angle of approximately 85–90^0 when mouth closed; ventral profile of body more convex than dorsal profile.

Early ontogeny

The sizes of the collected specimens were 11.9–36.0 mm SL. All the fins with supporting spines and rays were developed in the smallest collected size of 11.9 mm SL (Fig. 1A). Significant changes occurred in melanophores with the increase of standard length (Fig. 1).

Body form

Body was moderate to deep (38–47% of SL) and strongly compressed laterally, dorsal profile showing a concavity on top of head and is less convex than ventral profile (Fig. 1A-G). Body depth was 3.1-4.0 times of the length of second dorsal spine and 3.86–5.0 times of the length of second anal spine. Pointed snout, snout length 36–50% of HL (when mouth protracted); snout length increased with the increase of SL. Mouth small, when protracted forms a tube directed upward (Fig. 1A-G). Lips were broad and thin. Lateral line was developing in the smallest sized specimens of 11.9 mm SL (Fig. 1A), at first the line showed a slight concavity, later running slightly less convex to dorsal profile, extended posteriorly almost up to the base of the caudal fin at the size of 28 mm to 36 mm SL (Fig. 1F, G). Eyes were round and moderate in size (eye diameter, ED 25–33% of HL). Moderate gut (pre anal length, PAL 39–48% of SL). Lower jaw ascended at an angle of approximately 85^0–90^0 when mouth was closed, in all of the collected sizes (Fig. 1A–G).

Teeth

Teeth minute, numerous, arranged in irregular rows, in a villiform band.

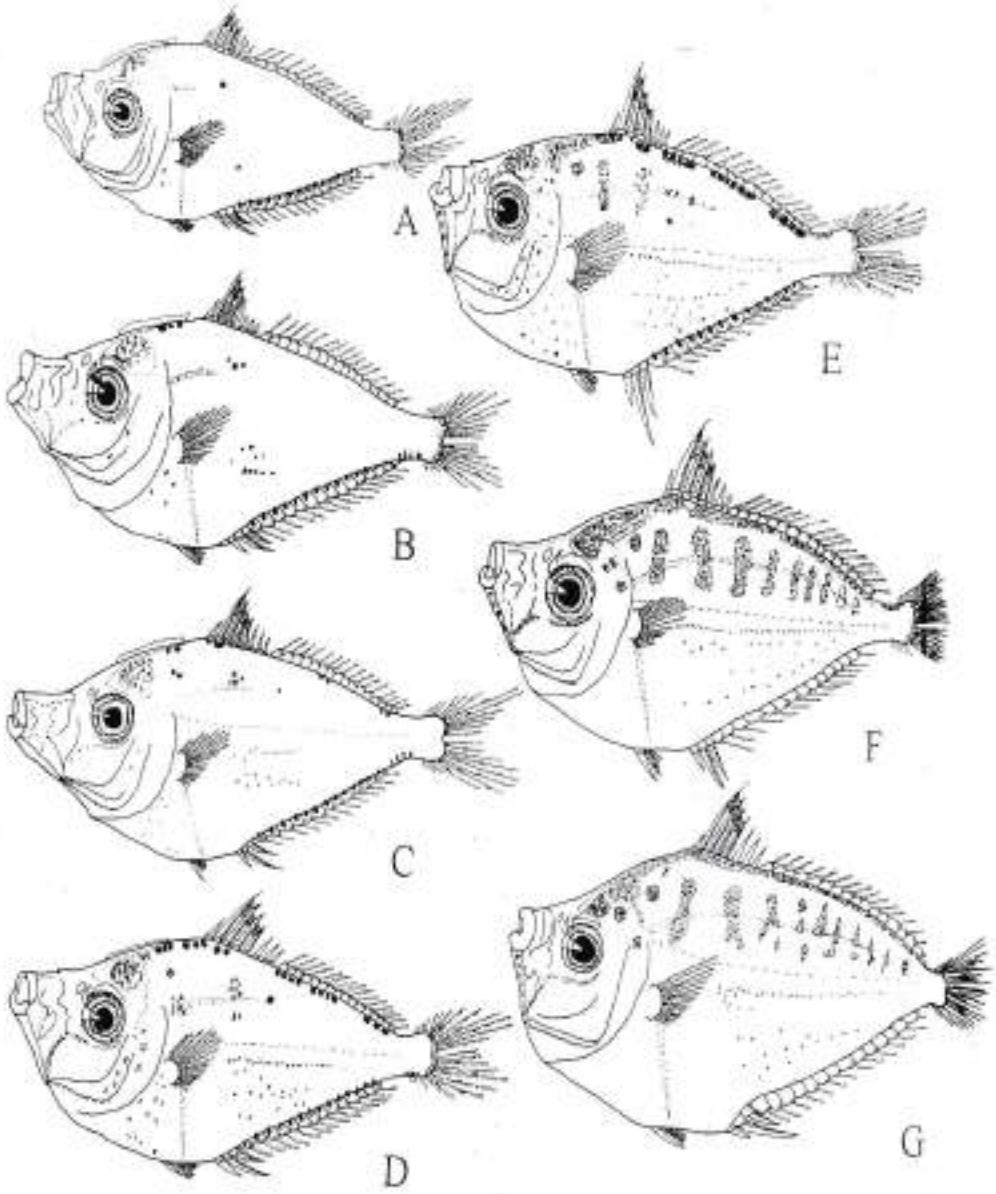


Fig. 1. Early developmental stages of *Secutor insidiator*. A, 11.9 mm SL; B, 13.9 mm SL; C, 16.0 mm SL; D, 18.0 mm SL; E, 21.2 mm SL; F, 28.0 mm SL; G, 36.0 mm SL.

Head spination

No significant head spination was observed in the collected specimens of 11.9–36.0 mm SL. One small spine was found on head, immediately above the eye on its front border. Preopercle with an obtuse angle, its lower margin finely serrated in all of the collected specimens (Fig. 1A–G).

Fin formation

All of the fins with supporting spines and rays were completed in the smallest collected size of 11.9 mm SL specimens. Dorsal and anal spines weak, compressed. Second dorsal and anal spine lengths increased with the increase of SL. Second to fifth dorsal spines and, 2nd and 3rd anal spines showed sculpture along the posterior margin in all collected specimens (11.9–36.0 mm SL). Third and fourth dorsal spines and third anal spine were anteriorly serrated, about 1/3 of their length from the base.

Pigmentation

One stellate melanophore appeared along lateral line behind head at the size of 11.9 mm SL, the number increased gradually with size, coalesced and a heavily pigmented vertical band was formed. Similarly some other bands were formed, the number was one at 18 mm SL, 2 at 21 mm SL, 9 at 28 mm SL and 10 at 36 mm SL. A few melanophores were developed along dorsal fin base at about 15 mm SL, the number increased with growth and became heavily pigmented in largest specimens. Scattered melanophores were observed on brain and nape of the smallest collected specimens and became heavily pigmented in the larger specimens. Melanophores observed on gular region in the specimens of ≥20 mm SL. Pigments appeared on preopercle at about 14 mm SL, the number increased with growth up to 21 mm SL, and later decreased with growth. In specimens of ≥28 mm SL no melanophores were observed on preopercle. Melanophores were also observed on abdomen and mid-ventral region of the tail. Along the median line a series of dot-shaped melanophores was formed from the upper angle of pectoral base to the opposite end of the soft dorsal in specimens of >20 mm SL, these melanophores were started to develop from the size of about 14 mm SL. A black curved band of melanophores was developed from lower margin of eye to the posterior angle of lower jaw, pigmentation increased with the increase of SL (Fig. 1A-G). The upper 1/3 portion of the spinous dorsal membrane between 2 to 5 spines were black at all of the collected specimens (membrane between 5 to 8 spines were damaged). Inner side of pectoral base was black. Black spots were observed along the edge of the lower half of the gill opening from the anterior base of the pectoral (observed by opening up the opercle using forceps). Melanophore patches were present dorsally on caudal peduncle in 21.2-36.0 mm SL specimens. Fourteen melanophores, one at the base of each of the anal fin rays were present at the ventral contour of tail in the specimens of 11.9-21.2 mm SL. Melanophores were also present ventrally on the caudal peduncle (13.9-16 mm SL; Fig. 1B-C), on caudal base and on caudal fin at all of the collected sizes (Fig. 1A-G).

Secutor ruconius Hamilton- Buchanan

Identifying characters

Fischer and Whitehead (1974), James (1975) and Jones (1985) described the adult characters of *S. ruconius*. The present specimens were identified by the following characters: dorsal fin with 8 spines and 16 rays, anal fin with 3 spines and 14 rays, and pelvic fin with one spine and 5 rays; body depth 46–52% of SL in specimens of 14–33 mm SL, body depth increases with length; mouth parts protracts upwards; lower jaw ascended at an angle of approximately 85–90° when mouth was closed; ventral profile of body more convex than dorsal profile.

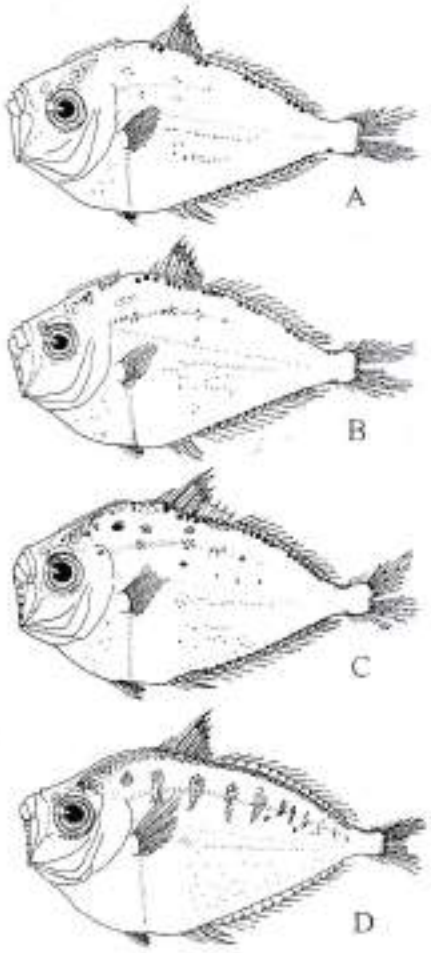


Fig. 2. Early developmental stages of *Secutor ruconius*. A, 14.0 mm SL; B, 15.6 mm SL; C, 17.0 mm SL; D, 33.0 mm SL.

Early ontogeny

The sizes of the collected specimens were 14-33 mm SL. All the fins with supporting spines and rays were completed in the smallest collected size of 14 mm SL. Significant changes occurred in melanophores with the increase of standard length.

Body form

Body deep (46-52% of SL in specimens of 14-33 mm SL) and compressed laterally, dorsal profile rising from snout with a concavity and is less convex than the ventral profile. Body depth, BD was 2.96-3.42 times of the length of 2nd dorsal spine and 3.8 times of the length of 2nd anal spine and remained similar up to the largest specimen. Pointed snout, snout length 32-37% of HL (when mouth is closed), snout length decreased with increased SL. Mouth small, when protracted forms a tube directed upwards (Fig. 2A-D). Lips were broad and thin. Lateral line extended to about middle of the body from behind head at the size of 14 mm SL, at first the line showed a slight concavity, later less convex to dorsal profile, extended posteriorly to the base of caudal (Fig. 2A-D). Eyes were round and large in size (35-37% of HL). Lower jaw ascended at an angle of approximately 85°-90° when mouth was closed (Fig. 2A-D).

Teeth

Teeth minute, numerous, arranged in irregular rows, in a villiform band.

Head spination

No significant head spination was observed in the collected specimens of 14-33 mm SL. One small spine projected backward on head, immediately above the eye on its front border. Preopercle with an obtuse angle, its lower margin finely serrated in all of the collected specimens of 14-33 mm SL (Fig. 2A-D).

Fin formation

All of the fins with supporting spines and rays were completed in the smallest collected size of 14 mm SL specimens (Fig. 2A). Dorsal and anal spines weak, compressed. Second to fifth dorsal spines and, second and third anal spines sculptured along the posterior margin in all of the 14-33 mm SL specimens. Third and fourth dorsal spines and third anal spine were anteriorly serrated, about 1/3 of their length from the base. Caudal deeply forked in smallest to the largest sized collected specimens.

Pigmentation

Some stellate melanophores appeared dorsally (upper and lower side of the lateral line) from behind the head at the size of 14 mm SL, the number increased with size, coalesced and a heavily pigmented vertical band was formed. Similarly some other bands were formed, the number was one at 17 mm SL and 10 at 33 mm SL. A few melanophores were developed along dorsal fin base at the size of 14 mm SL, the number increased with growth and became heavily pigmented in largest specimens. Scattered melanophores were observed on head above brain and nape of the smallest collected

specimens and became heavily pigmented in the larger specimens. Melanophores observed on gular region in specimens of ≥15.6 mm SL. Melanophores were also observed on abdomen and mid-ventral region of the tail. Dot-shaped melanophores were developed from the upper angle of pectoral base along the median line, these melanophores were observed up to the half of the soft dorsal fin length in smallest collected specimens, and up to the posterior most end of the soft dorsal in specimens of 33 mm SL. A curved band of melanophores was developed from lower margin of the eye to the posterior angle of the lower jaw (Fig. 2A-D). The upper 1/3 portion of the spinous dorsal membranes between 2 to 5 spines was black in all of the collected specimens (Fig. 2A-D). Black spots were developed along the edge of the lower half of the gill opening from the anterior base of the pectoral (observed by opening up the opercle using forceps). Melanophores were developed dorsally on caudal peduncle in 15.6 to 33 mm SL specimens. Ventral contour of tail bear about 14 melanophores, one on each of the anal fin ray base from 14–17 mm SL (Fig. 2A-C). Pigments were also present ventrally on the caudal peduncle in 14–17 mm SL specimens, on caudal fin base and on caudal fin in all of the collected specimens (Fig. 2A-D).

In the present study, early stages of *Secutor* species were found to occur round the year during the sampling period (August 1998 to July 1999) with maximum number in April 1999 (12.42 individuals/100m^3) and the minimum number in November 1998 (2.18 individuals/100m^3). During the study period the water temperature and salinity varied from 22.0-32.5°C and 10-37 ppt respectively. The occurrence of early juveniles throughout the year with maximum abundance in April (when temperature and salinity were 29.5°C and 34 ppt respectively) and minimum abundance in November (temperature and salinity were 27°C and 28 ppt respectively) reveals that *Secutor* spp. may spawn round the year in the coastal waters of Bangladesh and their main spawning season is during the spring.

Discussion

The present specimens were confirmed to be under the genus *Secutor* by having: dorsal fin with 8 spines and 16 rays, anal fin with 3 spines and 14 rays, pelvic fin with 1 spine and 5 rays; mouth parts protracted upward; lower jaw ascends an angle of 85-90° when mouth is closed; ventral profile more convex than dorsal profile; and body proportions and pigmentation different from other leiognathids.

Juveniles of *S. insidiator* resemble with those of *Secutor ruconius* in having upward protracting mouthparts and similar body colouration, but can be distinguished by its more elongate body shape than *S. ruconius*. In the present study body depths were found to be increased with standard length, and similar sized specimens of the two *Secutor* species differed in their body depths. The body depth of *S. insidiator* were 41, 41, 42 and 47% of SL at the size of 13.6, 16, 18 and 36 mm SL respectively. On the other hand body depth of *S. ruconius* were 46, 48, 50 and 52% of SL at the size of 14, 15.6, 17 and 33 mm SL respectively. Similar phenomena were observed in adults of *Secutor* species by James

(1975) and Jones (1985). Body depth of *Secutor* species were found to differ between specimens collected from different regions (James 1975, Jones 1985). Therefore, care should be taken in distinguishing different *Secutor* species if it is done only on the basis of body depth. In *S. ruconius*, second dorsal and anal spines were more elongate than *S. insidiator*.

In the present study the *Secutor* species possessed deep blotch of melanophores at the upper one-third portion of the dorsal fin membrane between 2 to 5 spines. James (1975) reported similar blotch between 2 to 5 spines in *S. ruconius* and between 2 to 6 spines in *S. insidiator*. Bal and Rao (1990) also reported black blotch between 2 to 6 spines in *S. insidiator* which is not consistent with the present study. It might be due to the partial damage of the dorsal fin or fin membranes in most of the present specimens. Some other leiognathids, e.g. *Leiognathus brevirostris*, *L. daura* and *L. splendens* also bear similar black blotch on dorsal fins (James 1975) but they can be differentiated by body proportions, mouth shape and other melanophores. Among the two *Secutor* species melanophores on preopercles were found in 14-21 mm SL specimens of *S. insidiator* but were absent in *S. ruconius*.

In the present study some of the smaller sized specimens of the both species possessed supraoccipital crest without spines at its degenerating stage and which is already degenerated in the largest specimens. Kinoshita (1988), Leis and Trnski (1989) and Haque and Ozawa (1995) described and figured different head spines during early life history stages of different leiognathid species. They also found degeneration of head spines in late larval or early juvenile stages. The present specimens were larger than those described by the above authors and possibly most of the head spines have been degenerated in our specimens.

During the entire study period from August 1998 to July 1999 *Secutor* species were occurred almost round the year with maximum number in April 1999 and the minimum number in November 1998. Water temperature and salinity in April 1999 were found to be 29.5°C and 34 ppt respectively and in November 1998 were 27°C and 28 ppt respectively. The occurrence of early juveniles throughout the year with maximum abundance in April and minimum abundance in November indicate that *Secutor* species may spawn round the year in the coastal waters of Bangladesh and their main spawning season is possibly during the spring. Fujita (1960) reported the spawning season of one leiognathid species, *Leiognathus nuchalis*, from late spring to early summer (middle of May to end of July) in Japan. Balan (1963) reported that *Leiognathus bindus* in Calicut coast of India spawns only once in a year and its spawning period is shorter from December to the end of February), but James and Badrudeen (1975) reported that *L. brevirostris*, another species of the same family, in the Palk Bay and the Gulf of Mannar, India spawns throughout the year with intense spawning in May/June and October/November but individual fish spawns two times in a year. Therefore, it seems that some species of the family Leiognathidae may spawn round the year but some may not, and it may depend on the local environmental condition. However, to confirm the spawning season of *Secutor* species, it is necessary to study the gonadal development of

the species and survey the early life history stages more intensively, especially pre-larval and early larval stages.

Acknowledgements

A financial grant from International Foundation for Science (IFS), Sweden (Research Grant Agreement No.-A/2649-1) for this research to the second author, is gratefully acknowledged.

References

Bal, D.V. and K.V. Rao, 1990. Marine Fishes of India. Tata McGraw-Hill Publishing Company Limited, New Delhi. 472 pp.

Balan, V., 1963. Biology of the silver belly, *Leiognathus bindus* (Val.) of Calicut coast. *Indian J. Fish.*, 10(1, A): 118-134.

Fischer, W. and P.J.P. Whitehead (eds.), 1974. FAO species identification sheets for fishery purposes: Eastern Indian Ocean-fishing area 57 and Western Central Pacific-fishing area 71. Food and Agriculture Organization of the United Nations, Rome. Vol. II, Plate LEIOG.

Fujita, S., 1960. Egg development and prelarval stages of a silver-belly, *Leiognathus nuchalis* (Temminck et Schlegel). *Bull. Japan. Soc. Sci. Fish.*, 26 (11): 1091-1094.

Haneda, Y. and F.I. Tsuji, 1972. The luminous organs of two species of leiognathid fishes recently found in Ambon, Indonesia. *Sci. Rep. Yokosuka city Mus.*, 19: 7–11.

Haque, M.M. and T. Ozawa, 1995. Ontogenetic larval characters of three leiognathid species in Kagoshima Bay, Southern Japan. *Japan J. Ichthyol.*, 42 (3): 137-146.

Hussain, M.M., 1971. The commercial fishes of the Bay of Bengal. BFDC, Chittagong, Bangladesh. 60 pp.

James, P.S.B.R., 1975. A systematic review of the fishes of the family Leiognathidae. *J. Mar. Biol. Assoc. India*, 17 (1): 138-172.

James, P.S.B.R. and M. Badrudeen, 1975. Biology and fishery of *Leiognathus brevirostris* (Valenciennes) from the Palk Bay and the Gulf of Manner. *Indian J. Mar. Sci.*, 4: 50-59.

Jones, G., 1985. Revision of the Australian species of the fish family Leiognathidae. *Aust. J. Mar. Freshwat. Res.*, 36 (4): 559-613.

Kinoshita, I., 1988. Family Leiognathidae. *In*: An atlas of the early stage fishes in Japan (ed. M. Okiyama). Tokai Univ. Press. Tokyo. pp.483-485. (in Japanese).

Kuhlmorgen–Hille, G., 1968. An illustrated field key to the fish family Leiognathidae in the Gulf of Thailand. Marine Fish. Lab., Bangkok, Contribution No. 12: 1-7.

Leis, J. M. and T. Trnski, 1989. The larvae of Indo-Pacific shore fishes. New South Wales Univ. Press, Australia. 371 pp.

Mito, S., 1966. Fish eggs and larvae. *In*: Illustrations of the marine plankton of Japan (ed. S. Motoda) Vol. 7. Soyo-sha, Tokyo. p 74. (in Japanese)

Rahman. A.K.A., M.G. Khan, Z.A. Chowdhury and M.M. Hussain, 1995. Economically important marine fishes and shell fishes of Bangladesh. Department of Fisheries, Matshya Bhaban, Dhaka, Bangladesh. p. 18. (excluding plates)

Seigel, J.A., 1982. Median fin-spine locking in the pony fishes (Perciformes: Leiognathidae). *Copeia*, 1982 (1): 202-205.

Tiews, K. and P. Caces-Borja., 1965. On the availability of the Family Leiognathidae in Manila Bay and San Miguel Bay and on their accessibility to controversial fishing gears. *Philip. J. Fish.*, 7: 59-86.

(Manuscript received 11 February 2001)

Bangladesh J. Fish. Res., 6(1), 2002: 29-34

Culture suitability of two exotic catfishes (*Clarias gariepinus* and *Pangasius hypopthalamus*) with an indigenous catfish (*Heteropneustes fossilis*)

Nargis Sultana[1] and M.F.A. Mollah*
Department of Fisheries Biology & Genetics, Bangladesh Agricultural University
Mymensingh 2202, Bangladesh.
[1]Department of Fisheries, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1703
*Corresponding author

Abstract
Culture experiment of African catfish (*Clarias gariepinus*) and Thai pangas (*Pangasius hypopthalamus*) with indigenous stinging catfish (*Heteropneustes fossilis*) was conducted in the laboratory. The study was conducted for two experiments, where *C. gariepinus* and *P. hypopthalamus* were used separately with *H. fossilis* for a duration of 21days with three feeding treatments, viz. Tubificid worms (T_1), SABINCO feed (T_2), and no supplemental feed (T_3). In experiment 1, the initial length and weight of 4.4cm and 0.60g of *C. gariepinus* became 6.74cm and 2.33g when fed Tubificid worms, 7.07cm and 2.84g when fed SABINCO feed in the treatment without supplemental feed the final length and weight were 3.67cm and 0.31g at the end of 21 days of trial. The final length and weight of *H. fossilis* reached 4.55cm and 0.53g from the initial 3.3cm and 0.25g under the treatment fed Tubificid worms while those fed SABINCO feed showed a length and weight of 4.37cm and 0.45g respectively. However, both the initial length and weight were reduced to 2.85cm and 0.12g respectively in the treatment without supplemental feed. In experiment 2, the initial length and weight of 4.37cm and 0.57g of *P. hypopthalamus* became 5.57cm and 0.57g when fed Tubificid worms, 4.85cm and 0.82g when fed SABINCO feed in the treatment without supplemental feed the final length and weight reduced to 3.95cm and 0.34g at the end of 21 days of trial. The final length and weight of *H. fossilis* reached 5.19cm and 0.82g from the initial 3.25cm and 0.20g under treatment fed Tubificid worms while those fed SABINCO feed showed the final length and weight of 4.93cm and 0.70g respectively. And both the initial length and weight were reduced to 3.07cm and 0.04g respectively in the treatment without supplemental feed. No predatory effect of *C. gariepinus* and *P. hypopthalamus* on *H. fossilis* was observed in the experiments.
Key words: *C. gariepinus*, *P. hypopthalamus*, *H. fossilis*, Feeding, Predation

Introduction

To mitigate the shortage of fish, 13 exotic high yielding and fast growing varieties of fish, which are disease resistant and well adapted to the prevailing environmental

conditions, were introduced in Bangladesh. Among them two of the catfishes – *C. gariepinus* and *P. hypopthalamus* are predators. According to Mollah *et. al* (1995) *C. gariepinus* is a passive predator in nature and *C. gariepinus* had no predatory effect on the fingerling of carp species (*Labeo rohita*). On the other hand, Alam (1998) observed that *C. gariepinus* and " *sutchi* (later changed to *P. hypopthalamus*) have predatory effect on *Barbodes gonionotus*. It, therefore, seems that predatory fishes have got some species specificity and their predation habits depend upon many factors, viz. size and density of prey fishes, extent of hunger of predator species, body shape of the prey, relative body depth of the prey in relation to mouth gap of the predator (Hoyle and Keast 1987, Paszakowski and Tonn 1994, Das *et. al.* 1999) and so on. In this context, the present experiment was conducted to determine the culture suitability of two exotic catfishes, viz. *C. gariepinus* and *P. hypopthalamus* with an indigenous catfish – *H. fossilis*. This will ultimately provide information about the compatibility of culturing these species with the indigenous one.

Materials and methods

The fry of *C. gariepinus* used in this experiment were obtained from the fish artificially bred by using a mixture of 500 IU HCG and 2 g PG extract/kg body wt. On the other hand, *H. fossilis* fry were produced upon treating the female at the rate of 7mgPG/100g body wt. of fish. The fry of *P.hypopthalamus* were collected from Bangladesh Fisheries Research Institute, Mymensingh. Preys and predators were acclimatized for ten days before starting the experiment.

In both the experiments, there were three treatments (T) and each of the treatments had three replications. Fish in T_1 and T_2 were fed with Tubificid worms and SABINCO feed (Starter-I), respectively and no supplemental feed was provided in T_3. Proximate composition of the feed used are presented in Table 1. For each experiment nine aquaria of size 91cm x 25cm x 30cm were used with a water depth of 15cm. In both the experiments, the ratio of predator and the prey was maintained at 1:2. The fishes were fed twice a day at their satiation level. They were considered satiated when they stopped searching food and assembled at the corner.

Eight fry of *C. gariepinus* of length 4.4 cm weighing 0.6g and sixteen fry of *H. fossilis* of length 3.3cm weighing 0.25g were used for each replicated treatment in experiment 1. On the other hand, eight fry of *P. hypopthalamus* of length 4.37cm and weight of 0.57g and sixteen fry of *H. fossilis* of length 3.25cm and weight of 0.20g were used for each replicated treatment in experiment 2.

The experiments were conducted for a period of 21 days. Weight and length of fish were recorded at 7 days intervals. Mortality if any was also recorded at that time.

Table 1. Proximate composition of Tubificid worms* and SABINCO feed (Starter-1)** on dry weight basis

Elements	Tubificid worms	SABINCO feed (Starter-1)
Crude protein (%)	63.82	39
Crude lipid (%)	28.84	3
Ash (%)	7.95	18
Fibre (%)	--	6

*Source: Mollah and Ahamed (1989) ** Source: SABINCO (without date)

Results and discussion

In experiment 1, the growth in terms of length and weight of *C. gariepinus* was the highest in treatment T_2 where the fishes were fed with SABINCO feed (Stater-1) than those in T_1 where Tubificid worms were fed to the fishes. The growth of the fishes was found to decrease in treatment T_3 where no supplemental feed was provided (Table 2). Similar findings were also reported by Alam (1998), where the highest weight gain was observed in *C. gariepinus* fed with SABINCO feed. In the study of Degani *et al.* (1989) it was revealed that feed containing 40% protein favoured to gain the highest growth in *C. gariepinus*. Madu and Tsumba (1989) also reported better growth in *C. anguillaris* fed with feed containing 40% crude protein than those with lower or higher protein contents.

Table 2. Effects of different feed on the growth parameters and mortality rate of predator (*Clarias gariepinus*) and prey (*Heteropneustes fossilis*) and the predation rate of *C. gariepinus*

Parameters	Treatments		
	T_1 (Tubificid worms)	T_2 (SABINCO feed)	T_3 (Without feed)
Initial length (cm)			
Clarias gariepinus	4.4	4.4	4.4
Heteropneustes fossilis	3.3	3.3	3.3
Final length (cm)			
Clarias gariepinus	6.74[a]	7.07[a]	3.67[c]
Heteropneustes fossilis	4.55[a]	4.37[a]	2.85[b]
Gain in length (cm)			
Clarias gariepinus	2.34[a]	2.67[a]	-0.73[b]
Heteropneustes fossilis	1.25[a]	1.07[a]	-0.45[b]
Initial weight (g)			
Clarias gariepinus	0.60	0.60	0.60
Heteropneustes fossilis	0.25	0.25	0.25
Final weight (g)			
Clarias gariepinus	2.33[a]	2.84[a]	0.31[b]
Heteropneustes fossilis	0.53[a]	0.45[a]	0.12[b]

Gain in weight (g)			
Clarias gariepinus	1.73[a]	2.24[a]	-0.29[b]
Heteropneustes fossilis	0.28[a]	0.19[a]	-0.13[b]
Mortality rate (%)			
Clarias gariepinus	Nil	Nil	Nil
Heteropneustes fossilis	Nil	Nil	5.0
Predation rate (%)	Nil	Nil	Nil

Values in the same row having same superscript are not significantly different ($p<0.01$)

On the other hand the growth performance of *H. fossilis* was the best in treatment T_1 that received Tubificid worms than those in T_2 and T_3, receiving SABINCO feed and no supplemental feed, respectively (Table 2). Similar finding was observed by Gheyas (1998) where the growth performance of *H. fossilis* was the best when fed with Tubificid worms. Haque and Barua (1989) also reported the highest growth and survival of *H. fossilis* with Tubificid worms, followed by the feed with zooplankton and beef liver. It, therefore, seems that different species have different preference for food. During the present study *C. gariepinus* was observed to prefer SABINCO feed to Tubificid worms while the case was reverse for *H. fossilis*.

In experiment 2, the growth in terms of length and weight of *P. hypopthalamus* was the highest in treatment T_1, receiving Tubificid worms. On the other hand the growth of *P. hypopthalamus* decreased in T_3, where fishes were not provided with feed. The increase in growth with Tubificid worms might have resulted due to higher content of protein in Tubificid worms (about 64%) than SABINCO feed (39%).

Kamarudin *et al.* (1987) also found that the higher the protein percentage in the feed the higher the growth rate of *P. sutchi* fingerlings. Similar finding was reported by Seidel *et al.* (1980) where Atlanic silversides cultured on artificial diet with less protein percentage showed poor growth than that of supplied with live brine shrimp nauplii having more percentage of protein.

The growth of *H. fossilis* was the highest in treatment T_1, where fishes were provided with Tubificid worms and decreased in T_3, where no feed was provided during the period of the experiment (Table 3). Haque and Barua (1989) also reported the highest growth and survival of *H. fossilis* fed with Tubificid worms followed by those fed with zooplankton and beef liver. In another study of BFRI (1997) live Tubifex showed the best performance when fed to *H. fossilis* in terms of growth and survival and live zooplankton showed the poorer performance than that of Tubificid worms.

Table 3. Effects of different feed on the growth parameters and mortality rate of predator (*P. hypopthalamus*) and prey (*H. fossilis*) and the predation rate of *P. hypopthalamus*

Parameters	Treatments		
	T_1 (Tubificid worms)	T_2 (SABINCO feed)	T_3 (Without feed)
Initial length (cm)			
Pangasius hypopthalamus	4.37	4.37	4.37
Heteropneustes fossilis	3.25	3.25	3.25
Final length (cm)			
Pangasius hypopthalamus	5.57^a	4.85^b	3.95^c
Heteropneustes fossilis	5.19^a	4.93^a	3.07^b
Gain in length (cm)			
Pangasius hypopthalamus	1.02^a	0.48^b	-0.42^c
Heteropneustes fossilis	1.94^a	1.68^a	-0.18^b
Initial weight (g)			
Pangasius hypopthalamus	0.57	0.57	0.57
Heteropneustes fossilis	0.20	0.20	0.20
Final weight (g)			
Pangasius hypopthalamus	1.18^a	0.82^b	0.34^c
Heteropneustes fossilis	0.82^a	0.70^a	0.04^b
Gain in weight (g)			
Pangasius hypopthalamus	0.61^a	0.25^b	-0.23^c
Heteropneustes fossilis	0.62^a	0.51^a	-0.16^b
Mortality rate (%)			
Pangasius hypopthalamus	Nil	Nil	Nil
Heteropneustes fossilis	Nil	Nil	4.0
Predation rate (%)	Nil	Nil	Nil

Values in the same row having same superscript are not significantly different ($p<0.01$).

Neither *C. gariepinus* nor *P. hypopthalamus* were found to predate on *H. fossilis*, in experiment 1 and 2, respectively. This might be due to the size of the prey and the species specificity of the predator. Merron (1993) also observed that the predation of *C. gariepinus* is related to the size and abundance of prey. Ahmed *et al.* (1991) reported that there is no predatory effect of *C. gariepinus* on *Catla catla* but they have a little predation *L. rohita* fry. On the other hand, Mollah *et al* (1995) found that there was no predation of *C. gariepinus* on *L. rohita*. Ermolin (1981) also observed that there exits a linear relationship between the length of predator and length of prey. Here in this experiment, perhaps *H. fossilis* is not a preferred prey for the exotic species used due to its size or ability to avoid predation. *H. fossilis* a first swimmer, has toxic spine and the size of the prey fish used is perhaps larger to be taken by the predator. However, according to Alam (1998) *P. sutchi* has predatory effect on *B. gonionotus*.

The results clearly indicate that predatory species do not predate on all the species, because of their species specificity and predation also depends on the size of prey. It was suggested that *C. gariepinus* and *P. hypopthalamus*, can be cultured with *H. fossilis* in a water body.

References

Alam, S.M.R., 1998. Investigation on predatory effect of *Clarias gariepinus* and *Pangasius sutchi* on *Barbodes gonionotus*. M.S. Thesis, Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh, 57 pp.

Ahmed, G.U., M. Karim, M.F.A. Mollah and R.I. Sarder, 1991. Predation of carp fingerlings by African magur. Paper presented to the Workshop on Magur Culture. Organized by DOF, MOFL, GOB and Institutional Strengthening in the Fisheries Sector, FAO/UNDP Project—BGD/87/045:16-17 February 1991.

BFRI (Bangladesh Fisheries Research Institute), 1997. Breeding and culture of commercially important catfish in Bangladesh. Progress Report, 1996-97. 6 p.

Das, M., N. Sultana, M.H. Alamgir, M.A. Hossain and M.S. Islam, 1999. Predation by *Channa striatus* (Bloch) on *Rana tigrina* (Daudin), *Puntius gonionotus* (Bleekeer) and *Labeo rohita* (Hamilton) in the laboratory. *Bangladesh J. Fish. Res.*, 3(2): 123-129.

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

Ermolin, V.P., 1981. Nutrition of the channel catfish in Cherepets' reservoir. Rybnoe Khozyistvo, Moscow 50-51.

Gheyas, A.A., 1998. Studies on cold shock induced gynogenesis and artificial breeding performance in *Heteropneustes fossilis* Bloch. M.S. Thesis, Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh. 132 pp.

Haque, M.M. and G. Barua, 1989. Rearing of shingi (*Heteropneustes fossilis* Bloch) fry under laboratory condition. II. Feeding and growth of fry. *Bangladesh J. Fish.* 12(1): 67-72.

Hoyle, J.A. and J.A. Keast, 1987. The effect of prey morphology and size on handling in a piscivore, large mouth bass (*Micropterus salmoides*). *Can. J. Zool.*, 65: 1972- 1977.

Kamarudin, M.S., R.A. Rahman, Z.A. Aizam, S.S. Siraj and R.I. Hutagalung, 1987. Effects of four different diets on weight gain, growth, specific growth rate, feed conversion ratio and protein efficiency of *Pangasius sutchi* (Fowler) fingerlings. Proc. Tenth Annual Conference of the Malaysian Society of Animal Production, Genting Highlands, Pahang, Malaysia. pp. 192-196.

Madu, C.T. and T.T. Tsumba, 1989. Dietary protein requirement of mudfish (*Clarias anguillaris*) fingerlings: The optimum crude protein level for the diet of mudfish fingerlings in an outdoor rearing system. Annu. Rep. Natl. Inst. Freshwat. Fish. Res. (Nigeria). pp. 104-109.

Merron, G.S., 1993. Peck hunting in two species of catfish, *Clarias gariepinus* and *C. ngamensis* in the Okavango Delta, Botswana. *J. Fish. Biol.*, 43(4): 575-584.

Mollah, M.F.A. and M. T. Ahamed, 1989. A note on preliminary study of culture of Tubificid worms. *Bangladesh J. Fish.*, 12(2): 91-95.

Mollah, M.F.A., M.M. Hossain and G.U. Ahmed, 1995. Predation of African catfish on Indian major carp. *Bangladesh J. Training and Dev.*, 8: 82-84.

Parker, R.R., 1971. Size selective predation among juvenile salmonid fishes in an British Columbia inlet. *J. Fish. Res. Bd. Can.*, 28: 1503-1510.

Paszakowski, C.A. and W.M. Tonn, 1994. Effect of prey size, abundance and population structure on piscivory by yellow perch. *Trans..Am. Fish. Soc.*, 123: 855-865.

Seidel, C.R., P.S. Schauer, T. Katayama and K.L. Simpson, 1980. Culture of Atlantic silversides fed on artificial diets and brine shrimp nauplii. *Bull. Jap. Soc. Sci. Fish.*, 46(2): 237-245.

(Manuscript received 27 September 2001)

Bangladesh J. Fish. Res., 6(1), 2002: 35-41

In vitro phagocytic study of blood leucocytes and peritoneal macrophages of walking catfish *Clarias batrachus* (Lam.) against *Aeromonas hydrophila* and *Escherichia coli.*

M. Mamnur Rashid*, M. Sardar and M.R. Islam[1]
Department of Aquaculture, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
[1]Department of Pathology, Bangladesh Agricultural University, Mymensingh
*Corresponding author

Abstract
In observation of *in vitro* phagocytic activity against *Aeromonas hydrophila* isolate 34k (a virulent form) and *Escherichia coli* (an avirulent bacteria) of neutrophil- and monocyte-like cells of walking catfish *Clarias batrachus* showed phagocytosis. Neutrophils and monocytes phagocytized the avirulent form of bacterial isolate more than the virulent one. Other blood leucocytes did not show phagocytosis. Peritoneal macrophage of the fish were separated by glycogen elicitation and the macrophages were being adhered on plastic cover slips for studying their *in vitro* phagocytic activity. Most of the cells were alive after adherence and showed phagocytosis against the virulent and avirulent bacteria. The percent phagocytosis and phagocytic index were higher against the avirulent *E. coli* than the virulent *A. hydrophila*.
Key wards: *in vitro* phagocytosis, *Clarias batrachus*, Leucocytes, Bacteria

Introduction

Like human, fish blood plays an important role in disease resistance because of the antibodies in the serum and other soluble substances like lysin, a growth and enzyme inhibitor that have protective function and inhibit the growth of micro-organisms by neutralizing the enzymes on which the pathogens depend. The phagocytic cells like neutrophil and monocyte that are present in the circulation are being capable of migration to inflammatory sites via blood. They are avidly phagocytic for a wide variety of infectious agents like virus, bacteria, yeast etc. However, both cells and soluble factor of blood plays an important role in the defense mechanism of fish during the adult and earlier stage of infection. A variety of cells are involved in the non-specific defense mechanism. One of the primary defense mechanism against infecting agent is the role of neutrophils and macrophages as well as monocyte like cells that phagocytize the microorganisms and kill them (Mamnur Rashid 1997).

As for the function of leucocytes, phagocytosis of the foreign material is one of the most important mechanism which protects the body from infection. There are some works about phagocytosis of fish leucocytes. Watson *et al.* (1963) conducted an experimental infection by bacteria in gold fish and found that neutrophils, eosinophils

and macrophages containing bacteria were accumulated at the site of infection. Ellis (1977) observed that in plaice, monocytes and macrophages engulfed colloidal carbon particles. However in plaice, Ferguson (1976) reported that thrombocytes, rarely neutrophils and monocytes showed carbon uptake. The present study reports the phogocytosis of virulent and avirulent bacteria by blood leucocytes (monocytes and neutrophils) and peritoneal macrophages.

Materials and methods

Experimental fish

Clarias batrachus of about 70-80 g body weight were used to observe the activity of their phagocytic cells. They were acclimatized in aquaria containing tap water for 7 days prior to the experiment with aeration and feeding at alternative day with SABINCO fish feed. Seventy percent water was changed everyday. The fish were not fed during the experiment.

Bacteria

Aeromonas hydrophila 34k, a virulent isolate and *Escherichia coli*, an avirulent bacteria, were used. These bacteria were cultured on TSA plates at 25°C for 48 hrs. A suspension of 50 mg/ml of both the bacteria were prepared in PS for using in whole blood and 10 mg/ml for using in peritoneal macrophage suspension.

Staining procedure

Routine method of staining was performed for staining of blood smear on glass slide and adherent macrophages on cover slip by Wright and Giemsa stain (Chinabut *et al.* 1991).

Isolation of peritoneal macrophages

Fish were injected intra-peritoneally with 2.5 ml of 2.5% w/v glycogen (Olivier *et al.* 1992) in PS. After 3 days peritoneal macrophage were collected following procedure of Mamnur Rashid (1997). As much blood was taken out as possible with a syringe with anticoagulant from the caudal vein to avoid possible contamination of peritoneal macrophage with red blood cells. Abdominal region of the fish was disinfected with 70% ethanol-cotton and 5-8 ml of calcium and magnesium free Hanks balanced salt solution (HBSS, Sigma Chemical Co. USA: pH 7.2) was injected intraperitoneally with a 26 gauge needle. After 10 min the peritoneal macrophage were collected after making a small incision on the ventral position of the abdomen with one ml sterilized pipette in 15 ml plastic centrifuge tube. The abdomen was rinsed with another 5-8 ml of HBSS and peritoneal macrophage suspension was collected. After centrifuging the tube at 1500 rpm for 15 min the supernatant was discarded and the peritoneal macrophage was resuspended in minimal essential medium with Earle's salt and L-glutamine (MEM, GIBCO laboratories, USA). The peritoneal macrophage-MEM suspension was

centrifuged again for avoiding the effect of glycogen and the supernatant was discarded. The cell suspension was then passed through 26 gauge needle for several times to release any possible clot of the cells. Viability of the cells was checked by staining with 0.2% trypan blue and counting in haemocytometer. Almost 100% of the cells were alive at this stage. Cell concentration was adjusted to 4.5×10^5 cells/mm^3.

Phagocytosis in whole blood culture

One milliliter of blood was aseptically withdrawn from caudal vein of walking catfish with a syringe containing one drop of anticoagulant (3.6% sodium citrate) as before. The blood was divided into two allocates of 0.5 ml into two vials. About 50 µl suspension (50 mg/ml) of *Aeromonas hydrophila* 34k and *E. coli* were added to each vial separately. This mixture of blood and bacteria were incubated under shaking condition at 20°C for 2 h. Smears were prepared from the vial of blood and stained with Wright and Giemsa stain.

Phagocytosis in separated peritoneal macrophages

The peritoneal macrophage suspension was supplemented with 0.1% fetal calf serum (FCS) in MEM and was seeded on to several sterilized 10 × 18 cm plastic cover slips in 50 mm plastic petridish under moist condition and incubated for 2 h to allow adherence of the macrophages. The cover slips were then rinsed gently with phosphate buffered saline (PBS) to wash out non-adherent cells and about 200–300 µl of 0.1% FCS in MEM was added again. Adherence was checked by microscopic observation. About 20 µl of above prepared *A. hydrophila* 34k and *E. coli* suspension were then added separately to each cover slip on the adherent macrophages, at a dose of 2.6×10^5 CFU/cover slip. Control cover slips containing adherent macrophages were not inoculated with bacteria. All works were done in clean bench "Edge-Gard Hood" (Baker Company Inc. Germany). At 30 min, 60 min and 90 min post inoculation time the cover slips were rinsed with PBS, dried, stained with Wright's and Girmsa stain, fixed on a glass slide by mounting its down surface with Canada balsam (Laba chemie, India) and the upper surface, where the cells were adhered, were mounted again in Canada balsam under another cover slip. Slides were observed under oil immersion lens at × 1000 magnification. The percent phagocytosis was calculated according to the following formula after counting at least 100 phayocytic cells either phagocytizing or not (Mamnur Rashid 1997).

The phagocytic index was calculated by counting at least 100 bacteria that were phagocytized by certain number of phagocytic cells/macrophages and expressed by the following formula (Mamnur Rashid 1997):

$$\text{Phagocytic index} = \frac{\text{Total no. of phagocytized bacteria}}{\text{No. of phagocytic cells phagocytizing bacteria}}$$

$$\% \text{ Phagocytosis} = \frac{\text{No. of phagocytic cell phagocytizing bacteria}}{\text{Total no. of phagocytic cells counted}} \times 100$$

Results

Phagocytosis in whole blood culture

The results of *in vitro* phagocytosis of walking catfish leucocytes in whole blood culture were summarized in Table 1. Lymphocyte like and thrombocyte like cells showed no phagocytosis. Neutrophil like and monocyte like cells showed active phagocytosis (Fig. 1 a and b). The phagocytic cells engulfed a higher number of the avirulent bacteria *E. coli* than the virulent form *A. hydrophila* 34k (Fig. 1c).

Table 1. Phagocytosis of different cells of peripheral blood of *C. batrachus* against avirulent *E. coli* and virulent *A. hydrophila* 34k

Blood cells	Phogocytosis against bacteria	
	E. coli	*A. hydriphila* 34k
Lymphocyete	–	–
Thrombocyte	–	–
Neutrophil	++	+
Monocyte	++	+

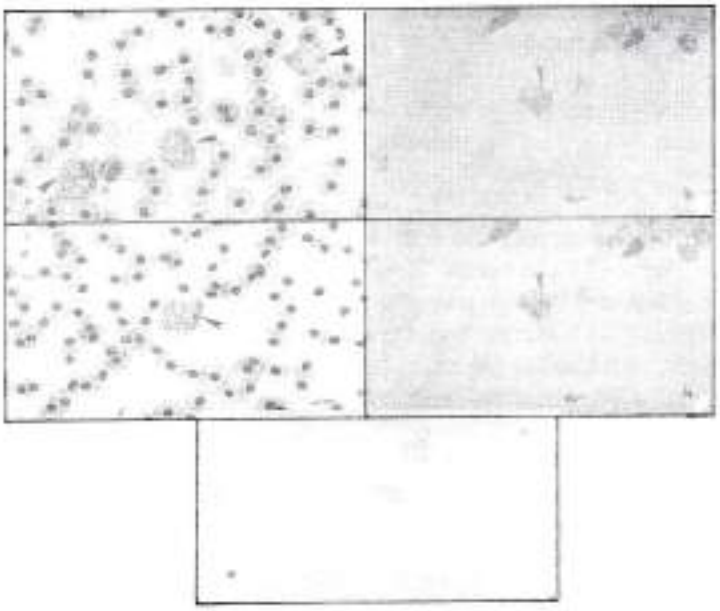


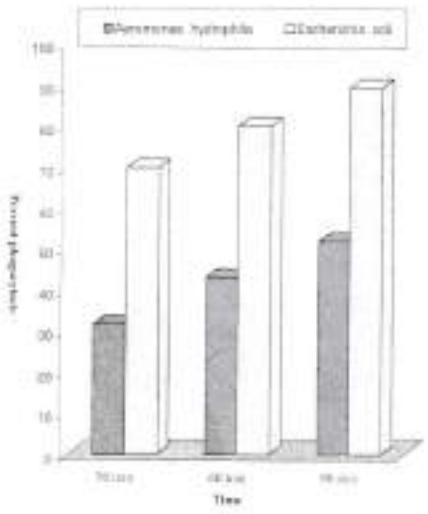
Fig.1. Photomicrograph of stained blood smear of whole blood culture with bacteria showing a. phagocytosis of monocyte like cell (arrow) against *A. hydrophila* b. phagocytosis of neutrophil like cell against *A. hydrophila*; thrombocyte like cell shows no phagocytosis (arrow) c. phagocytosis of monocyte like cell (arrow) against *E. coli* d. photomicrograph showing peritoneal macrophage of walking catfish phagocytizing *A. hydrophila* after 30 min incubation (arrow), e. photomicrograph showing peritoneal macrophage of walking catfish phagocytizing *E. coli* after 60 min incubation.

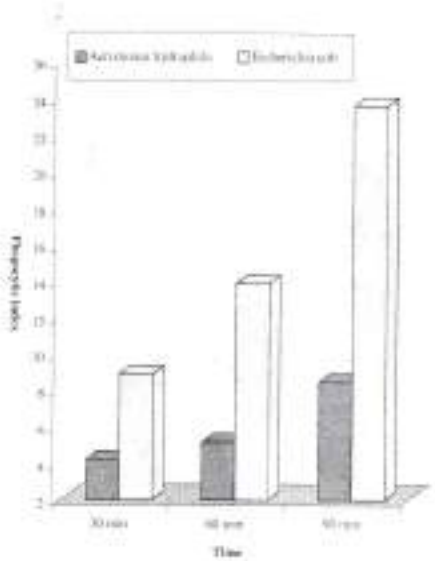
Phagocytosis in separated peritoneal macrophages

Peritoneal macrophages that adhered on cover slips were mostly phagocytic in nature. The phagocytic cells showed weak phagocytosis against the virulent *A. hydrophila* 34k than the avirulent *E. coli* (Figs.1 d and e). The percent phagocytosis and phagocytic index of the isolated peritoneal macrophage against virulent *A. hydrophila* isolate no. 34k and avirulant *E. coli* at different time of post inoculation have been shown in Table 2. Both percent phagocytosis and phogocytic index increased with time. The virulent form (*A. hydrophila* 34k) showed 31.66 % phagocytosis at 30 min, 43.33% at 60 min and 52% at 90 min; whereas the avirulent form (*E. coli*) showed 70%, 80.33% and 89.66 % at the above times respectively. Similarly, the phagocytic indices of *A. hydrophila* 34k were 4.2, 5.13 and 8.43 whereas that of *E. coli* were 8.88, 13.87 and 23.56 at 30 min, 60 min and 90 min post inoculation respectively (Figs. 2 and 3).

Table 2. Change in percent phagocytosis and phagocytic index of isolated peritoneal macrophages by time against virulent *Aromonas hydrophila* 34k and avirulent *Escherichia coli* bacteria

Bacteria	Parameters	Time (post incubation)		
		30 min	60 min	90 min
Aromonas hydrophila 34k	% phogocytosis	31.66	43.33	52.00
	Phogocytic Index	4.20	5.13	8.43
Escherichia coli	% phogocytosis	70.00	80.33	89.66
	Phogocytic Index	8.88	13.87	23.56





Discussion

In whole blood culture, lymphocytes, thrombocytes and erythrocytes did not show phagocytosis but neutrophil like and monocyte like cells showed active phagocytosis. Higher number of avriulent *E. coli* bacteria were engulfed by them than the virulent *A. hydrophila* 34k. Roberts (1978) mentioned that the monocytes in fishes have been observed to take up foreign particles. Finn and Nielson (1971) and Mamnur Rashid *et al.* (1997) reported migration and phagocytosis of nutrophils and macrophages in bacterial inflamation experiments in rainbow trout and Japanese flounder *Paralichthys olivaceus* respectively. Weinreb and Weinreb (1969) and Watson *et al.* (1963) also found those cells highly phagocytic to bacteria. These findings are similar to the leucocytic activity found in this study. Kusuda and Ikeda (1987) found that monocytes and nuetrophils are active phagocytic cells. They also found thrombocytes to be weakly phagocytic but lymphocyte and erythrocyte were not. Van-Furth *et al.* (1972) observed lymphocytes as being non-phagocytic and having no developmental relationship to phagocytic cell. These reports agrees with the present result. Phagocytosis by thrombocytes were not apparent in the present investigation although many workers found the phagocytic nature of the cell (Ferguson 1976, Ahmad and Banerjee 1984). The thrombocytes may not be able to neutralize the extra cellular product of bacteria which resist phagocytosis (Plumb 1994). Phagocytic cells are important in non-specific immunity because of their ability to engulf and digest foreign material, thus they are important in a variety of bacterial diseases (Blazer 1991). Interaction of peritoneal macrophage as well as phagocytic cell with bacterial agents have been studied for *A. salmonicida* in salmon *Oncorhynchus mykiss* (Graham *et al.* 1988), in rainbow trout *S. gairdneri* (Sakai 1984) and in Atlantic salmon *S. salar* (Olivier *et al.* 1992). It was shown by the present study that the virulent form of *A. hydrophila* were more resistant against phagocytosis than the avriulent bacteria *E. coli*. This result is supported by the findings of Mamnur Rashid (1997) in Japanese flounder *Paralichthys olivaceus*. The higher phagocytizing rate observed at the longest incubation time of 90 min were commonly found (Daly *et al.* 1994, Mamnur Rashid 1997). Phagocytic index and percent phagocytosis were also observed to be higher in case of avirulent *E. coli* and with longer time incubation than in virulent *A. hydrophila* and with shorter time incubation. These results agree with the study of Mamnur Rashid (1997) and Anisworth and Dexiang (1990).

From the results of this study it may be appropriate to conclude that the blood leucocyte plays an important role in primary defense mechanism because of their engulfing nature against foreign particles.

Acknowledgements

Thanks are due to Prof. M.A. Baki and M.A.H.N.A. Khan of Dept. of Pathology Bangladesh Agricultural University, Mymensingh, Bangladesh for technical assistance and valuable suggestions.

References

Ahmad, M. and V. Banerjee, 1984. Leucocytes of *Clarias batrachus* with special reference to annual cycle. *J. Zool. Soc.*, 36 (1-2): 56-64.

Ainsworth, A. J. and C. Dexiang, 1990. Differences in the phogocytosis of four bacteria by channel catfish neutrophils. *Dev. Com. Immunol.*, 14(2): 201-209.

Blazer, V. S., 1991. Piscine macrophage function and nutritional influence: A review. *J. Aquat. Anim. Health*, 3: 77-86.

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.

Daly, J.G., A.R. Moore and G. Olivier, 1994. Bacterial activity of brook trout (*Salvelinus fontinalis*) peritoneal macrophages against avirulent strain of *Aeromonas salmonicida*. *Fish Shellfish Immunol.*, 4: 273-285.

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

Ferguson, H. W., 1976. The ultra structure of plaice leucocytes. *J. Fish Biol.*, 8: 139-142.

Finn, J. P. and N. O. Nielson, 1971. Inflammatory response in rainbow trout. *J. Fish Biol.*, 3: 463-478.

Graham, S., H. Jeffries and J. Secombes, 1988. A novel assay to detect macrophage bactericidal activity in fish: Factors influencing the killing of *Aeromonas salmonicida*. *J. Fish Dis.*, 11: 389-396.

Kusuda, R. and Y. Ikeda, 1987. Studies on classification of eel leucocytes. *Nippon Suisan Gakkaishi.*, 53(2): 205-209.

Mamnur Rashid, M., 1997. Studies on *Edwardsiella tarda* infection in Japanese flounder *Paralichthys olivaceus*. Doctoral thesis. 149 pp. Graduate school of Biosphere Sciences. Hiroshima University. Japan.

Mamnur Rashid, M., T. Nakai, K. Muroga and T. Miyajaki, 1997. Pathogenesis of experimental edwardsiellosis in Japanese flounder *Paralichthys olivaceus*. *Fish. Sc.*, 63 (3) : 384-387.

Olivier, G., A. R. Moore and J. Fildes, 1992. Toxicity of *Aeromonas salmonicida* cells to Atlantic salmon *Salmo salar* peritoneal macrophages. *Dev. Com. Immunol*, 16: 49-61.

Plumb, J. A., 1994. Health Maintenance of cultured fishes. CRC Press, London. 254 pp.

Roberts, R. J. (ed.), 1978. Fish Pathology. Bailliere Tindall, London. 467 pp.

Sakai, D. K. ,1984. Opsonization by fish antibody and complement in the immune phagocytosis by peritoneal exudate cells isolated from salmonid fishes. *J. Fish Dis.*, 7: 29-38.

Van-Furth, R., Z. A. Cohn, J. G. Hirsch, J. H. Humphrey, W. G. Spector, and H.L. Langevoort, 1972. The mononuclear phagocytic system: A new classification of macrophages, monocytes and their precursor cells. *Bull. Wld. Hlth. Org.*, 46: 845-852.

Watson, L.J., I. L. Shechmeister and L. L. Jackson, 1963. The haematology of gold fish (*Carassius auratus*). *Cytologia*, 28: 118-130.

Weinreb, E. L. and S. Weinreb, 1969. A study of experimentally induced endocytosis in a teleost. I. Light microscopy of peripheral blood cell response. *Zoologica*, 54: 25-34.

(Manuscript received 14 October 1999)

Bangladesh J. Fish. Res., 6(1), 2002: 43-52

Monogenean gill parasites of Indian major carps from different fish farms of Mymensingh, Bangladesh

K.J. Chandra* and M.S. Jannat[1]
Department of Aquaculture, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
[1] Thana Fishery Extension Officer, Joypurhat, Bangladesh
* Corresponding author

Abstract
All together 148 fish host samples of *Labeo rohita, Cirrhina mrigala, Catla catla* and *Labeo gonius* were collected from different fish farms of Mymensingh. The gill monogeneans were then dislodged from the gill under dissecting microscope and fixed in ammonium picrate. Five species of *Dactylogyrus* namely, *Dactylogyrus mrigali, D. chauhanus, D. yogendrai, D. labei* and *D. kalyanensis* were recovered from sampled fishes. All the parasites were studied and redescribed, and reported for the first time from Bangladesh. The present investigation established *Catla catla* as a new host of *D. labei*.
Key words: Monogenean fluke, Gill parasites, Indian major carps

Introduction

Monogenea is one of three orders of the class Trematoda is known to infect the external surfaces of both freshwater and marine fishes. They usually infect gills, skin, fins, mouth cavity and cause damage of host tissue by their anchors, hooks and sucker during their feeding and they particularly infect fry and fingerlings in nursery ponds (Tripathi 1957). Hoffman (1967) stated that some dactylogyrids cause great damage to gill filaments of carps and goldfish in hatcheries.

No attention has been paid by the workers to study the freshwater monogeneans in Bangladesh. There is so far only a published work on this group of parasite (Mohanta and Chandra 2000). Thus with a view to enrich our knowledge the present investigation was undertaken. During the general survey of freshwater monogeneans of Mymensingh region authors investigated Indian major carps *Labeo rohita, Cirrhina mrigala, Catla catla* and *Labeo gonius*, and found them infested with five gill parasitic monogeans. They are therefore, described and reported for the first time from Bangladesh.

Materials and methods

A total of 148 fishes of Indian major carps- 45 samples of *Labeo rohita*, 38 samples of *Catla catla*, 38 samples of *Cirrhina mrigala* and 27 of *Labeo gonius* were collected from different fish farms and nurseries of Mymensingh district. Live fish were collected from

BFRI (Bangladesh Fisheries Research Institute), Fish Seed Multiplication Farm, Maskanda and private fish farms of Shambhugonj, Digarkanda and Mymensingh. Only the *Labeo gonius* were collected from fish-traders of Shambhugonj.

The collected fishes were carried live to the Fish Disease Laboratory by a water containing bucket. Fishes were then killed by a blow on the head. Both the opercula of the fish were cut by a scissors to remove the gills and dissected gills were placed in petridish containing clean water. Gills containing petridish was placed under dissecting microscope and observed the gill filaments to find out parasite. The live monogeneans were gently rubbed to dislodge from the gill filaments by the help of a bent needle and forceps. The monogeneans were removed and picked out by using a fine pipette to a small drop of water on a clean slide and covered with clean cover slip. Afterwards, a round circle was marked to confine the monogeneans by a marker pen. A small drop of ammonium picrate was introduced beneath the cover slip to fix and clean the worm. The corners of the cover slip were sealed with sialant to prevent it from moving. Preserved monogenetic trematodes were then studied under microscope and their size, shape and chitinoid structure were noted. Figures of the hard parts of flukes were drawn with the aid of a Camera lucida. Measurements were done with help of Oculomicrometer which was adjusted with stage micrometer and the microscope. Terminology used following Gussev (1976). For parasitic infestation ecological terms were used after Margolis *et al.* (1982). All measurements are shown in millimeters unless otherwise stated.

Results and discussion

Infestations

During the study period a number of monogenean flukes were recovered from gill filaments of 4 species of major carps. They were five different species under the genus *Dactylogyrus*. The list of host and their parasites are presented in Table 1.

Table 1. List of hosts and their parasites recovered

Host	Parasites
Labeo rohita	1. *Dactylogyrus labei* Musselius and Gussev, 1976
Catla catla	1. *D. kalyanensis* Musselius and Gussev, 1976 2. *D. labei* Musselius and Gussev, 1976
Cirrhina mrigala	1. *D. mrigali* Gussev, 1976 2. *D. chauhanus* Gussev and Musselius, 1976 3. *D. yogendrai* Gussev and Musselius, 1976
Labeo gonius	1. *D. labei* Musselius and Gussev, 1976

A total of 36 fishes of *Labeo rohita* were infested out of 45 examined. From the infested host, 120 monogeneans were collected. The prevalence was 80%, mean intensity 3.33 and abundance 2.67. The minimum prevalence was found in *Catla catla* was 63.16%,

the mean intensity was 3.96 and the abundance was 2.50, where as the highest prevalence found in *Cirrhina mrigala* was 86.84%, mean intensity was 3.09 and abundance 2.68. The prevalence in *Labeo gonius* was found 66.67%, the mean intensity was 4.17 and the abundance was 2.78. Parasitic infestation though was the highest in *C. mrigala*, mean intensity and abundance was the highest in *L. gonius*. The infestation of monogeneans in different hosts are shown in Table 2.

Table 2. Prevalence (%), mean intensity and abundance of monogenetic trematodes recorded from sampled fishes

Host	No. of host fish		No. of parasites collected	Prevalence %	Mean intensity	Abundance
	Examined	Infested				
Labeo rohita	45	36	120	80.00	3.33	2.67
Catla catla	38	24	95	63.16	3.96	2.50
Cirrhina mrigala	38	33	102	86.84	3.09	2.86
Labeo gonius	27	18	75	66.67	4.17	2.78

Description of the monogeneans

Dactylogyrus mrigali Gussev 1976 (Fig. 1)

Forty seven specimens were collected from gill filaments of *C. mrigala*, out of which five specimens were measured. The flukes are small to moderate in length 0.37-0.51, width is 0.091-0.120. Haptor is well demarcated from body.

Anchors have well-developed roots and recurved point. Their total length is 0.037-0.039 and the inner root 0.010-0.014, outer one 0.002-0.003, point is 0.008 and main part is 0.027-0.032. Dorsal bar has considerably curved backward medial part, saddle shaped, its length and width are 0.023-0.028 and 0.006-0.009 respectively. Ventral bar is 5 ray shaped, its size is 0.033-0.037 x 0.015-0.017. Marginal hooks have handle poorly demarcated from pivot and projected heel of hooklet. Their total length is 0.017-0.024, hooks of 6th pair are the shortest and those of 4th pair are the longest.

The copulatory complex is composed of slightly curved tube and accessory piece. Tube is slender narrowing towards end with bubble like inflated initial part. Total length of copulatory complex is 0.029-0.031. Vaginal armament dextral, it has shape of curved tube, it is 0.012 - 0.016 long, its diameter is about 0.002.

Remarks: *Dactylogyrus mrigali* was first reported by Gussev, 1976 from *Cirrhina mrigala* from the water bodies near Lucknow and from a hybrid *C. mrigala* x *Labeo rohita* in Bhavanisagar water reservoir in India.

The present specimens coincide the detail morphology with *Dactylogyrus mrigali* Gussev (1976) and hence has been identified as *D. mrigali*. However, the size of the present specimens slightly differ from the previously described one. The present one slightly smaller than the Indian specimens, (which may be due to the geographical or

ecological effect on the worms in a new environment) the present specimens also coincide to *D. mrigali* Gussev (1976) in morphology of anchors, dorsal and ventral bar, and hooks. Present report indicates the availability of this worm in *Cirrhina mrigala*, also in Bangladesh.

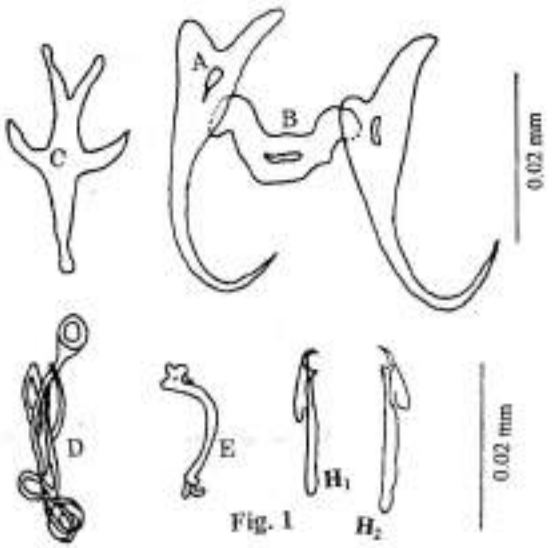


Fig. 1

Fig. 1. Hard parts of haptor and copulatory complex of *Dactylogyrus mrigali*
A. Dorsal anchor B. Dorsal bar C. Ventral bar D. Copulatory complex
E. Vaginal armament H_1 and H_2 Marginal hooklets

Dactylogyrus chauhanus Gussev and Musselius 1976 (Fig. 2)

Nineteen specimens of this fluke could be collected from 38 *Cirrhina mrigala* examined. Four specimens were measured for description. The flukes are small, body length is 0.36-0.42 and body width 0.099-0.100. Eyespot is absent. Anchors with well-developed roots and greatly straightened (opened). The anchors is sabre-like (a heavy sword with a curved blade) point. Anchors bear wing and removed from the basal part to the point. Their total length is 0.044 -0.049, length of main part is 0.040-0.042, inner root is 0.009-0.012, outer root is 0.004-0.005. Only one dorsal bar almost straight, with mass widened lateral ends and a little widened middle part, its size 0.004-0.005 × 0.025-0.026. Hooks thin, with well demarcated handle and its pivot, with projecting round heel of hooklet. Their total length are 0.016-0.022.

The copulatory complex consists of a tube and accessory piece. Tube is thin, faintly narrowing towards the ends, spirally curved, has 2.5 - 3.5 spires, with bubble-like widened initial part. Accessory piece has a shape of claw-like formation disposed near the end of tube. The size of copulatory complex 0.021 - 0.030 × 0.010 - 0.012, diameter of initial part is 0.005 - 0.007. Vaginal tube is straight in the beginning and a wavy in the rest part. Its length is about 0.011 - 0.013, diameter is 0.002.

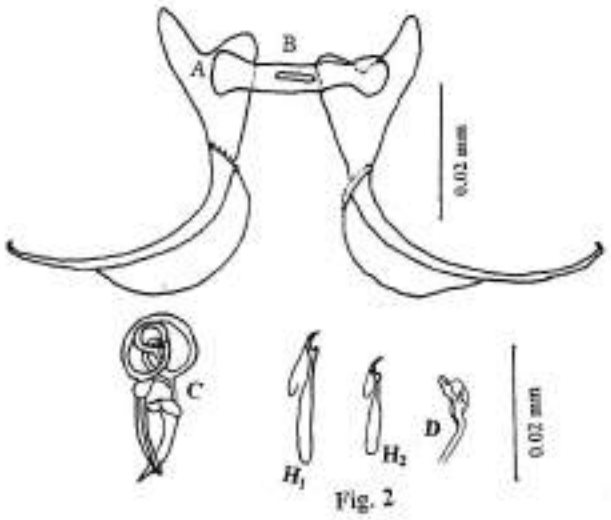


Fig. 2

Fig. 2. Hard parts of haptor and copulatory complex of *Dactylogyrus chauhanus*
A. Dorsal anchor B. Dorsal bar C. Copulatory complex
D. Vaginal armament H_1 and H_2 Marginal hooklets

Remarks: Gussev and Musselius (1976) first described this monogenean from the water bodies in the region of Lucknow from the host *Cirrhina mrigala*. It was named in honour of Dr. B. S. Chauhan, *Dactylogyrus chauhanus*. Simultaneously it was described from Calcutta and Bhawanisagar reservoir, and also fish from Kalyani, West Bengal, India. The present specimens collected from the same host fishes in Bangladesh coincide the detail morphology with *D. chauhanus* (Gussev and Musselius 1976). Present report indicates the availability of this worm in *Cirrhina mrigala* also in Bangladesh.

Dactylogyrus yogendrai Gussev and Musselius 1976 (Fig. 3)

Only 8 specimens were collected from the gill filaments of *C. mrigala* and 3 of them were measured. The flukes are relatively small 0.58-0.64 in length, 0.10-0.13 in width. Eye spot is absent.

Anchors have well-developed inner root, small outer root and small recurved point. Their total length is 0.041-0.048, inner root is 0.012-0.013, outer root is 0.001-0.002, point is 0.008-0.009 and the main part is 0.037-0.041. There is only one dorsal bar, which is almost straight, with thickened lateral butts and with wavy anterior and posterior edges; its size is 0.004-0.006 x 0.027-0.029 and wings are present in both anchors. The marginal hooks have clearly marked handle and its pivot and projected rounded heel of hooklet. The total length of marginal hook is 0.024-0.025.

The copulatory complex consists of a tube and an accessory piece. The tube is thin and spirally curved, with 3.5-4.5 spires. It is almost cylindrical, small and gradually narrowing towards the end. Tube has bubble-like widened initial part and its posterior edge has a cavity. Diameter of the initial part is 0.006. Its size is 0.025-0.029 x 0.009-0.011. Diameter of first spire is 0.010-0.012. Accessory piece has the shape of triangular frame, which disposes near end-spire of tube. The frame has a pitch fork-like appendix with claw-like ends and joins initial part of tube by means of elastic ligament. Vaginal armament has shape of tube, straight in the beginning and wavy in the rest part. Its length is 0.020-0.021 and diameter is about 0.001.

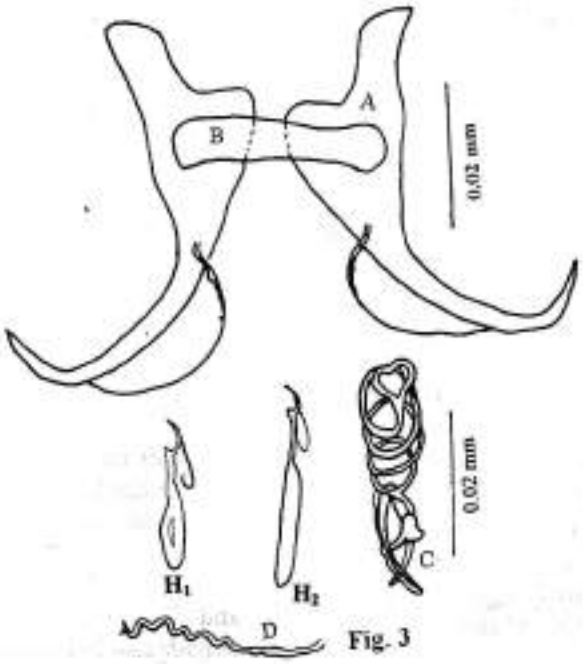


Fig. 3

Fig. 3. Hard parts of haptor and copulatory complex of *Dactylogyrus yogendrai*
A. Dorsal anchor B. Dorsal bar C. Copulatory complex,
D. Vaginal armament H_1 and H_2 Marginal hooklets

Remarks: *Dactylogyrus yogendrai* was first reported by Gussev and Musselius, (1976) from gill filaments of *Cirrhina mrigala* from the waterbodies near Lucknow. This species was also reported from Calcutta in *C. mrigala* and *Labeo rohita*. They also recorded it from Bhawanisagar reservoir and from fish farm, Kalyani, West Bengal, India. It was named in honour of Dr. Yogendra R. Tripathi, an eminent parasitologist of India. The present specimen was also collected from the gill filament of *Cirrhina mrigala* but not found in *Labeo rohita* in Mymensingh district. It coincides with the detail morphology with *D. yogendrai* Gussev and Musselius 1976. However, the size of the present worm differs slightly from the previous forms. It is slightly longer than the Indian specimens, as it has been only collected from *C. mrigala*. It has also minor variation in measurements in the chitinoid elements of haptor.

Dactylogyrus labei Musselius and Gussev 1976 (Fig. 4)

A total of 78 specimens were recovered from *L. rohita, L. gonius* and *C. catla*. Nine specimens were measured for description. The flukes are small. The length of body is about 0.38-0.48, maximum width is about 0.007-0.012. Eyespots are lacking.

Anchors are large, distinct, with well-developed roots and recurved point. Anchors have wings. Their lengths is 0.036-0.038, length of main part is 0.027-0.032, inner root is 0.010-0.017, outer root is0.002-0.007. Length of point is 0.012-0.013. There are two bars - dorsal and ventral (additional) one. Dorsal bar is without posterior process, its size is 0.004-0.006 x 0.021-0.025. Ventral bars is without lateral process, its size is 0.003-0.005 x 0.022-0.023. Seven pairs and two types of hooks are present. Marginal hooks with projecting heel of hooklet and handle, slightly demarcated from its pivot. Their length are 0.012-0.017.

Copulatory complex is composed of a tube an accessory piece. The tube is S- shaped, slender, with bubble-like inflated initial part. In front of it, there is a free lying chitinoid piece. Its length along curve is 0.040 - 0.064. Vaginal tube dextral, it is cylinder-like; end plate is not thin but thick, 0.021-0.033 in length.

Remarks: *Dactylogyrus labei* was first reported by Musselius and Gussev (1976) from *Labeo rohita, L. calbasu* from water bodies in the region of Lucknow, also *L. rohita* from the Kalyani fish farm, West Bengal, India. The present species coincides with the detail morphology of *D. labei* Musselius and Gussev 1976 and hence has been identified as *D. labei*. However, the size of the present species differs from the previously described one. It is slightly larger than the Indian specimens collected from *L. rohita*. The specimens collected from *Catla catla* also show comparatively larger body and copulatory complex. However, specimens from *Labeo gonius* are comparatively smaller body and chitinoid structure than the other specimens collected from *Labeo rohita* and *Catla catla*. This is the first report of *D. labei* from *L. gonius* from Bangladesh and also from a new host *Catla catla*. Present investigation indicates the availability of this worm in *L. rohita, L. gonius* and *Catla catla* in Bangladesh.

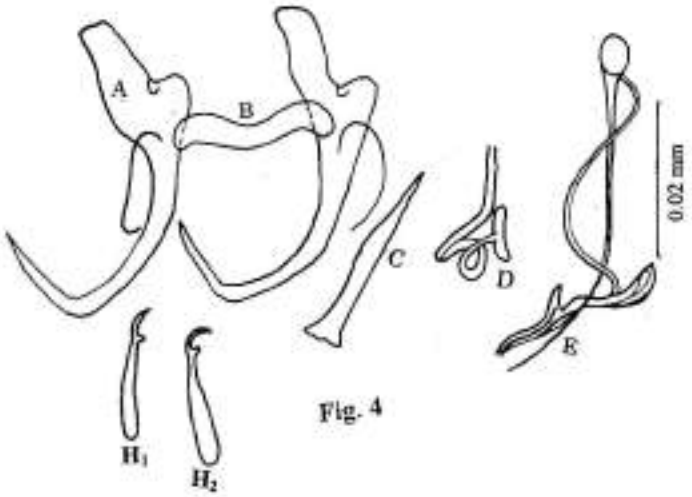


Fig. 4

Fig. 4. Hard parts of haptor and copulatory complex of *Dactylogyrus labei*
A. Dorsal anchor, B. Dorsal bar C. Ventral bar D. Vaginal armament,
E. Copulatory complex H_1 and H_2 Marginal hooklets

Dactylogyrus kalyanensis Musselius and Gussev 1976 (Fig. 5)

Only 3 specimens could be collected from the gill filaments *C. catla* and they were measured. The fluke is moderate in size. Its body length is 0.51-0.65 and the greatest body width is 0.012-0.091. Two pair of scattered eye granules are present, the anterior pair is disposed before the rounded pharynx, and the posterior pair is in the front part of the pharynx. Among the eye granules, the posterior pair is larger in size.

Anchors are prominent, large but thin with long inner curved root. They are rarely protruding outer root. Their main part has a typical widening in the place of attachment of wing, which is considerable removed from the base part to the point. The point is sharply recurved, almost straight. Total length of anchor is 0.069-0.078, length of main part is 0.046-0.050, inner root is 0.031-0.032, the outer root not more than 0.003, and the point is 0.025-0.026. The only purely connective dorsal bar is small dumb-bell shaped with round widened lateral termination. Its size is 0.005-0.007 x 0.024-0.025.

Hooks (7 pairs) are small in comparison with anchors, with well-developed widened handle and with protruding tongue-shaped heel of hooklet. Two types of hooks and the shortest hooks of 6th and 7th pair have a small pin shaped handle. Hooks are 0.013-0.018. Copulatory complex is composed of a tube and an accessory piece. Tube is very long and thin narrowing to is termination, looped, with bubble-shaped initial part. The size of the copulatory complex is 0.029-0.037 × 0.039-0.040, diameter of the initial part is 0.008 -

0.011. Accessory piece has a shape of shield connected with initial part by elastic pivot. Vaginal armament has shape of a thin tube rolled up into a clew.

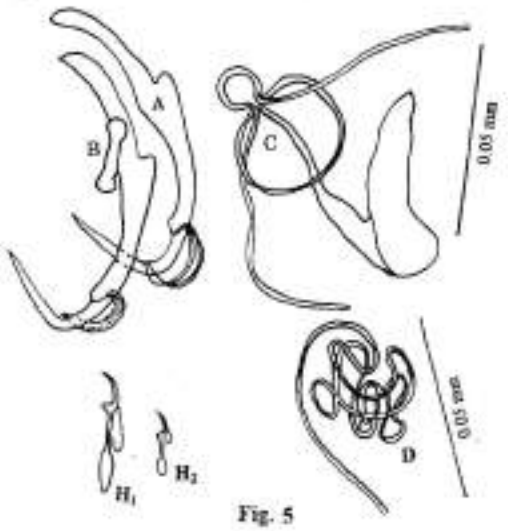


Fig. 5

Fig. 5. Hard parts of haptor and copulatory complex of *Dactylogyrus kalyanensis*
A. Dorsal anchor B. Dorsal bar C. Copulatory complex,
D. Vaginal armament H_1 and H_2 Marginal hooklets

Remarks: *Dactylogyrus kalyanensis* was first reported by Musselius and Gussev (1976) from *Catla catla* from Kalyani fish farm, West Bengal, India. The present species is very similar to *D. kayanensis* Musselius and Gussev 1976. Except in certain measurements the morphology of anchor, hooks, bars, and copulatory complex are also similar. It is slightly smaller in body size but the anchor, inner root and outer root is larger than the previous specimens. This species is reported for the first time from Bangladesh.

Acknowledgements

The authors thank the authorities of BFRI, Farm Manager, Fish seed multiplication farm, Maskanda, Mymensingh for providing fish samples for the study. Grateful acknowledgments are due to the Head, Department of Aquaculture, BAU, Mymensingh for providing laboratory facilities.

References

Gussev, A. V., 1976. Freshwater Indian Monogenoidea. Principles of Systematics, analysis of the world faunas and their evolution. *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.

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 adhoc Committee of the American Society of Parasitologists). *J. Parasit.*, 68 : 131-133.

Mohanta, S. K. and K. J. Chandra, 2000. Monogeneans infestations in Thai silver barb (*Barbodes gonionotus* Bleeker) and their adaptations in Bangladesh waters. *Bangladesh J. Fish. Res.*, 4: 147-155.

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

(Manuscript received 29 March 2001)

Bangladesh J. Fish. Res., 6(1), 2002: 53-58

Inactivation of luminous *Vibrio* spp. by free chlorine

T. Jawahar Abraham*, R. Palaniappan[1] and K. Dhevendaran[2]
Fisheries College & Research Institute, Tamil Nadu Veterinary & Animal Sciences University
Tuticorin 628 008, Tamil Nadu, India
[1]SPK College, Manonmaniam Sundaranar University, Alwarkurichi 627 412, Tamil Nadu, India
[2]Department of Aquatic Biology and Fisheries, University of Kerala, Trivandrum 695 007, India
*Present address and correspondence: Department of Fishery Pathology & Microbiology
Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences
P.O. Krishi Viswa Vidyala, Mohanpur 741 252, West Bengal, India

Abstract
In vitro inactivation of penaeid shrimp larval pathogens, *Vibrio harveyi* and *V. splendidus* biovar 1, by free chlorine and the influence of organic matter on the bactericidal activity of chlorine were assessed. More than 5 log unit (>99.99%) reduction in luminous bacteria from ≥ log 6.00/ml within the first 60 sec of exposure to free chlorine at 1 ppm level was observed. Chlorine was ineffective at <50 ppm levels to inhibit luminous *Vibrio* spp in the presence of 0.1% peptone as interfering organic agent. These results revealed that luminous bacteria are highly susceptible to chlorine but the bactericidal activity of chlorine is affected by organic substance.
Key words: *Vibrio* spp., Chlorine

Introduction

Hatchery production and rearing of penaeid shrimp larvae require good quality water (Lavilla-Pitogo *et al.* 1990). Water treatment in shrimp hatcheries usually consists of sedimentation and filtration. However, in cases where microbial contamination is a problem, such physical treatment is inadequate and some form of disinfectant is needed. Chlorine is a powerful disinfectant that has long been used to control microorganisms in water. Laboratory and field studies demonstrated the biocidal effect of chlorine on viral (LeBlanc and Overstreet 1991) and bacterial pathogens (Sako *et al.* 1988, Pascho *et al.* 1995) of aquaculture importance. Vibriosis caused by luminous *Vibrio* spp., particularly *Vibrio harveyi*, is a problem to shrimp hatchery operations globally (Lavilla-Pitogo *et al.* 1990, Karunasagar *et al.* 1994, Mohney *et al.* 1994, Abraham *et al.* 1997a). The purpose of this work was to examine the *in vitro* inactivation of penaeid shrimp larval pathogens such as *V. harveyi* and *V. splendidus* biovar 1 by chlorine and the influence of organic matter on the bactericidal activity of chlorine under laboratory condition.

Materials and methods

Test organisms

Two species of luminous bacteria *viz.*, *Vibrio harveyi* SW_{57} and *V. splendidus* biovar 1 SW_1, isolated from penaeid shrimp hatchery source water (Abraham *et al.* 1997b) were used.

Chlorine solution

A commercial preparation of sodium hypochlorite containing available chlorine concentration of 20 mg/ml, as determined by the standard iodometric titration method (APHA/AWWA/WEF 1995), was used. This solution was diluted in seawater (salinity 35 ppt), aged for more than 3 months and filtered, to provide 1-100 ppm free chlorine (target) concentrations when mixed with the inoculum.

Interfering substance

This was used to test the influence of organic substance on the efficacy of target chlorine levels. Peptone at 0.1% (w/v), dissolved in aged seawater, was used as interfering substance. The medium was adjusted to pH 7.8 and sterilized at 121°C for 15 min. Half strength seawater made from 35 ppt aged seawater was used as diluent.

Neutralizing solution

A 0.01 mol/l sodium thiosulphate solution prepared in half strength-aged seawater was the neutralizing solution.

Culture medium and preparation of cell suspensions

Complex seawater (CSW) medium, with or without 1.5% agar, containing 75% aged seawater, 25% distilled water, 0.5% peptone, 0.3% yeast extract and 0.3% glycerol was used for the growth and maintenance of luminous *Vibrio* spp. The pH of the medium was 7.80. Cell suspensions of *V. harveyi* and *V. splendidus* biovar 1 was prepared separately as described in Abraham *et al.* (1997a).

Susceptibility of luminous bacteria to free chlorine

These experiments were designed to reproduce luminous bacterial inactivation, but not intended to simulate natural environments. Luminous bacterial inactivation was done in Erlenmeyer flasks containing 100 ml of sterile aged seawater (pH 8.0). Sodium hypochlorite solution was added to these flasks to get a concentration of 1, 5 and 10 ppm of free chlorine separately. The flasks were then inoculated with *V. harveyi* SW_{57} and/or *V. splendidus* biovar 1 SW_1 at $10^6 - 10^7$ cells/ml levels and incubated at 30±1°C. The numbers of bacteria in each flask were determined after 1 min, 30 min and 24 h of exposure to chlorine and 24 h after neutralization. The effect of neutralizing agent, 0.01

mol/l sodium thiosulphate in half strength seawater, on luminous bacteria was evaluated by incubating the cells in neutralizing solution for 30 min. The numbers of bacteria before and after exposure to neutralizing agent were determined.

Enumeration of luminous bacterial counts (LBC) was done by spread plating on CSW agar and/or by 5 tube most probable number (MPN) technique using CSW broth. Before enumeration, the residual chlorine present in one ml each of the samples drawn from chlorine treated flasks were neutralized by vigorous agitation in sterile 9 ml of 0.01 mol/l sodium thiosulphate solution (10^{-1} dilution). Subsequent ten fold serial dilutions were made in sterile half strength seawater. All the plates and tubes, after inoculation, were incubated at 30±1°C for 24-72 h. Aliquots from CSW broth were streaked on to CSW agar and incubated for 48 h at 30±1°C to record MPN value. Bacterial numbers were recorded as counts/ml. All experiments were repeated at least 3 times and the percentage survival calculated.

Results and discussion

Aquaculture is a large consumer of chlorine products. The use of chlorine has been recommended to eliminate shrimp pathogens in hatcheries (Baticodos and Pitogo 1990, LeBlanc and Overstreet 1991, Lewis *et al.* 1992) and as a disinfectant and sanitary agent for fish tanks, raceways, utensils, contaminated equipments and effluents at 200 mg/l level (LeBlanc and Overstreet 1990, Pascho *et al.* 1995). The results presented in Table 1 showed that both *V. harveyi* and *V. splendidus* biovar 1 reacted in a more or less identical way to the bactericidal effect of chlorine. A reduction of more than 5 log unit (>99.99%) from >log 6.00/ml was achieved within the first 60 sec of exposure to free chlorine at 1 ppm level in the absence of any interfering agent, which indicated that luminous bacteria are highly susceptible to free chlorine. At 1 and 5 ppm levels, luminous bacterial populations were completely eliminated and no recovery was possible even after neutralization with 0.01 mol/l sodium thiosulphate and enrichment in CSW broth. Similar chlorine effect was reported for luminous bacteria (Baticados and Pitogo 1990), *V. anguillarum* and *V. ordalii* (Sako *et al.* 1988), *Renibacterium salmoninarum* (Pascho *et al.* 1995). The mechanisms of chlorine inactivation vary among microorganisms and are a result of general oxidation of reduced chemical species. The populations of *V. harveyi* (log 6.778/ml) and *V. splendidus* biovar 1 (log 6.873/ml) exposed to thiosulphate neutralizer for up to 30 min failed to show any difference from the respective counts of log 6.781/ml and log 6.872/ml determined before exposure. The neutralizer was, therefore, assumed to exert no effect on results.

Table 1. Effect of sodium hypochlorite on the growth (counts/ml) of *Vibrio harveyi* and *V. splendidus* biovar 1 in sterile seawater

Treatment / Exposure time	*Vibrio harveyi* SW_{31}		*V. splendidus* biovar 1 SW_1	
	1 ppm	5 ppm	1 ppm	5 ppm
Before chlorination	2.50×10^8	4.45×10^8	2.20×10^8	2.80×10^7
After chlorination				
1 min	1.00×10^1	$<1.00 \times 10^1$	1.00×10^1	<1.00
30 min	<1.00	<1.00	<1.00	<1.00
24 h	<1.00	<1.00	<1.00	<1.00
After neutralization				
24 h	<1.00	<1.00	<1.00	<1.00

Results on the interference of organic substance on the bactericidal activity of free chlorine, as shown in Table 2, revealed that chlorine is ineffective at <50 ppm level to inhibit luminous bacteria (*V. harveyi* and *V. splendidus* biovar 1) in the presence of 0.1% peptone. No bactericidal effect was seen at 1 ppm level. At 5-20 ppm levels, the LBC reduced slightly immediately after chlorination and then increased to ≥ log 8.00 cells/ml in 24 h. Chlorine effect was apparent at 50 ppm level. Chlorination and oxidation reaction between amino group and chlorine are most likely responsible for this effect. These results corroborate with the findings of earlier studies (Sae-Oui *et al.* 1987, Chanratchakool 1995). According to Chanratchakool (1995), the minimum effective concentration of hypochlorite to inhibit *V. harveyi* and other *Vibrio* spp was 2-8 ppm and 2-16 ppm of active chlorine, respectively. About 16 ppm of active chlorine was required for disinfection of water from the shrimp farm containing > log 4.00 organisms/ml. Sae-Oui *et al.* (1987), however, reported that *V. harveyi* could be completely killed by treating with calcium hypochlorite at 20-30 ppm. Nevertheless, Karunasagar *et al.* (1995) using microcosm experiments demonstrated that chlorination would not kill *V. harveyi* present in sediments and, therefore, repopulation of the system occurs immediately after dechlorination. The results of the present *in vitro* study also confirmed that chlorine treatment would not eliminate shrimp larval pathogens such as *V. harveyi* and *V. splendidus* biovar 1 in a system where particulate and suspended organics are present.

Table 2. Interference of 0.1% peptone on bactericidal effect of sodium hypochlorite: effect on luminous bacteria

Treatment / Exposure time	Counts / ml					
	1ppm	5ppm	10ppm	20ppm	50ppm	100ppm
Vibrio harveyi SW_{55}						
Before chlorination	$5.60x10^6$	$7.80x10^6$	$9.00x10^6$	$6.00x10^6$	$4.75x10^6$	$5.20x10^6$
After chlorination						
1min	$5.60x10^6$	$6.90x10^6$	$8.00x10^6$	$2.80x10^5$	$1.50x10^1$	<1.00
30 min	$5.65x10^6$	$7.30x10^6$	$8.30x10^6$	$1.30x10^7$	<1.00	<1.00
Vibrio splendidus biovar 1 SW_1						
Before chlorination	$4.30x10^6$	$4.70x10^6$	$7.50x10^6$	$6.10x10^6$	$8.00x10^6$	$5.00x10^6$
After chlorination						
1min	$4.25x10^6$	$3.45x10^5$	$4.50x10^6$	$1.50x10^5$	$1.00x10^1$	<1.00
30 min	$4.30x10^6$	$3.55x10^5$	$4.65x10^6$	$6.10x10^5$	<1.00	<1.00

The counts of *Vibrio harveyi* SW_{55} and *V. splendidus* biovar 1 SW_1 were increased after 30 min in 1,5,10 and 20 ppm levels. No growth of luminous bacteria was seen in 50 and 100 ppm levels even after 24 h.

It is worth mentioning here that sodium hypochlorite was demonstrated to be effective at relatively high concentrations, ≥50 ppm, and for longer period of time for the control of baculovirus in shrimp mariculture facilities (Lewis *et al.* 1992). In shrimp grow-out systems, chlorine disinfection is being followed in the reservoirs, although with variable success, to inactivate the causative agent of white spot viral disease and other bacterial pathogens. As majority of the components of source water of the shrimp aquaculture systems are particulate and suspended organics, these components must, therefore, be thoroughly removed by mechanical filtration or by sedimentation for effective hatchery disinfection protocol.

References

Abraham, T.J., R. Manley, R. Palaniappan and K. Dhevendaran, 1997a. Pathogenicity and antibiotic sensitivity of luminous *Vibrio harveyi* isolated from diseased penaeid shrimps. *J. Aquacult. Tropics*, 12: 1-8.

Abraham, T.J., R. Palaniappan and K. Dhevendaran, 1997b. Epibiotic infestation of luminous bacteria in penaeid shrimp *Penaeus indicus* (H Milne Edwards) larvae. *Indian J. Mar. Sci.*, 26: 209-212.

APHA/AWWA/WEF, 1995. Standard methods for the examination of water and wastewater. 19th edition (eds. A.D. Eaton, L.S. Clesceri and A.E. Greenberg). American Public Health Association / American Water Works Association / Water Environment Federation. pp. 4-36 - 4-39.

Baticados, M.C.L. and C.L. Pitogo, 1990. Chlorination of seawater used for shrimp culture. *Bamidgeh*, 35: 128-130.

Chanratchakool, P., 1995. Effects of calcium hypochlorite and benzal konium chloride on some shrimp pathogens and microflora - a preliminary study. *Asian Shrimp News*, 23: 3.

Karunasagar, I., R. Pai, G.A. Malathi and Indrani Karunasagar, 1994. Mass mortality of *Penaeus monodon* larvae due to antibiotic resistant *Vibrio harveyi* infection. *Aquaculture*, 128: 203-209.

Karunasagar, I., S.K. Otta and Indrani Karunasagar, 1995. Effect of chlorination on shrimp pathogenic *Vibrio harveyi*. Abstract, World Aquaculture Society, pp. 193.

Lavilla Pitogo, C.R., M.C.L. Baticados, E.R. Cruz-Lacierda and L.D. De la Pena, 1990. Occurrence of luminous bacterial disease of *Penaeus monodon* larvae in the Philippines. *Aquaculture*, 91: 1-13.

LeBlanc, B.D. and R.M. Overstreet, 1991. Efficacy of calcium hypochlorite as a disinfectant against the shrimp virus *Baculovirus penaei*. *J. Aquatic Animal Health*, 3: 141-145.

Lewis, D.H., W.M. Charanza and M.T. Omran, 1992. Baculovirus-impregnated filter paper method for assessing disinfection protocol in shrimp mariculture facilities. *J. Aquatic Animal Health*, 4: 69-72.

Mohney, L.L., D.V. Lightner and T.A. Bell, 1994. An epizootic of vibriosis in Ecuadorian pond reared *Penaeus vannamei* Boone (Crustacea: Decapoda). *J. World Aquaculture Soc.*, 25: 116-125.

Pascho, R.J., M.L. Landolt and J.E. Ongerth, 1995. Inactivation of *Renibacterium salmoninarum* by free chlorine. *Aquaculture*, 131: 165-175.

Sae-Oui, D.A., A. Tansutapanich and L. Ruangpan, 1987. *Vibrio harveyi*: luminous bacteria causing disease in *Penaeus merguiensis*. Technical Paper No. 6. Brackishwater Fisheries Division. Department of Fisheries, Thailand. p. 11.

Sako, H., N. Ishida, Y. Maeno and M. Sorimachi, 1988. Bactericidal activities of five disinfectants on *Aeromonas salmonicida*, *Vibrio anguillarum* and *V. ordalii*. *Fish Pathology*, 23: 219-229.

(Manuscript received 10 September 2001)

Bangladesh J. Fish. Res., 6(1), 2002: 59-68

Diel feeding patterns, rate of gastric evacuation and foods of Indian sandwhiting, *Sillago sihama* in Mulki estuary, west coast of India

T.S. Annappaswamy, H.R.V. Reddy* and K.S. Venkatesha Moorthy
University of Agricultural Sciences, College of Fisheries, Mathsyanagar, Mangalore 575 002, India
*Corresponding author

Abstract
Diel feeding chronology of sandwhiting, *Sillago sihama* was examined from stomach collections taken during the months of April, July and December'99 in Mulki estuary along Dakshina Kannada coast, India. Significant differences in mean stomach content weight were found between several consecutive 3 hour periods with peak fullness occurring in early morning and evening hours. The rate of gastric evacuation of natural food (crustacea, polychaetes and fish) was measured in the field was best described by an exponential model, with an estimated evacuation time of 8.0 h at a temperature of 28.5 ±1.2°C. Stomach content analysis indicated that this species is a carnivore on a wide range of benthic, epibenthic and planktonic prey. The principal food items of *S. sihama* were crustaceans, polychaetes and fish. Fishes less than 100 mm TL preferred mainly crustaceans while larger ones depends on polychaetes, crustaceans and fish. The feeding activity of *S. sihama* was influenced by tidal cycle.
Key words: Diel feeding chonology, Gastric evacuation, *Sillago sihama*

Introduction

Fishes belonging to the family Sillaginidae (Order: Perciforms) commonly known as whitings/lady fish have a wide distribution in the tropical regions. Eight species belonging to the family sillaginidae have been reported from India (Mckay 1976, Dutt and Sujatha 1980). Of the eight species, the Indian sandwhiting, *Sillago sihama* is a highly esteemed table fish in coastal Karnataka locally known as 'Kane meenu'. This species has a great potential for mariculture because of its faster growth rate and high market price. The possibility of culturing this species has been reported by James *et al.* (1976) and Dhulkhed and Ramamurthy (1977).

Although the detail food habits of juvenile whitings in estuarine waters have been examined in several studies (Chacko 1949, Radhakrishnan 1957, Krishnamurthy 1969, Gowda *et al.* 1988), there are little information on dietary rhythm of juvenile whitings. The purpose of the present study is to describe the diel cycle of feeding, gastric evacuation rate and feeding habits of whitings in the tropical estuarine environment.

Materials and methods

Diel feeding pattern

Experiments were conducted in Mulki estuary, during pre-monsoon (April'99), monsoon (July'99) and post-monsoon (December'99) seasons. Sampling were done during 6 different 24-h sampling periods at different locations in the estuary using cast net and seine net. Eight to twelve collections were made during each 24 h sampling period, although not all were successful in catching juvenile sandwhitings. Water temperature was recorded during each sampling.

The sandwhitings were sorted from the catch and their total length and weight were measured to the nearest mm and nearest 0.1g respectively after removal of excess water. The stomach contents were excised and the contents weighed to the nearest 0.01g and preserved for later analysis. Fullness code between 0 (empty) and 5 (full distended stomach) was assigned to each stomach at the time of weighing as a measure of feeding intensity. In addition the stomach contents were also expressed as a % of body weight.

Estimation of evacuation rate

Juvenile whitings (size range: length, 7.5–13.5 cm) were collected from Mulki estuary during early monsoon. Approximately 50 fish were placed in each of four nylon net hapas [(# 200 μm), 2m x 1m x 1 m)] fixed in a nearby brackishwater pond. They were fed on natural feed (polychaetes, crustaceans, molluscan and fish meat) for 2-3 weeks before being used in the experiment.

Prior to experiment, fish were starved for a 12 hour period to ensure empty stomachs and then fed on natural feed for 20 minutes, later fish were transferred to food free hapas. A random subsample of 16 fish (not more than 4 fish from each hapa) were sacrificed immediately after feeding and the stomach contents and fish were weighed as stated earlier and percentage of food recovered was determined. This process was continued every 2 hour interval until most of the stomachs sampled were empty. Dry weights were determined by placing the contents in the pre weighed aluminum pans into an 80°C oven until they achieved a constant weight.

Linear, exponential and square root models (Jobling 1981 and 1986) were used to describe the depletion of stomach contents with time in the evacuation experiment. The co-efficient of determination (R^2) was used to evaluate the goodness of fit of the models. The data were statistically analyzed following One way ANOVA and Duncan Multiple range test.

Qualitative and quantitative analysis

Fortnightly samples were collected from Mulki estuary using cast nets and seine nets to carry out stomach content analysis during April'99 to March'00. The stomachs with food contents were routinely examined under a low power stereo dissector microscope or where necessary, under high power magnification. The occurrence method (Hynes 1950) was used to quantify the diet, the number of stomachs in which

each food type occurred was expressed as a % of the total number of stomachs containing food.

Results

Diel feeding pattern

The results of the diel feeding activity are presented in Fig. 1a and 1b. To test for discontinuity in feeding, the sampling times were grouped into eight successive 3 h intervals after adjusting for minor differences in day-length between the sampling periods. The stomach weight / body weight ratios were found to be significantly different (One way ANOVA) over the eight intervals tested (Fig. 1a). Similar significant differences were noted among stomach fullness code over the diel periodicity (Fig. 1b). Feeding indices were generally found to be high during early morning (5.30 - 6.30 h) and evening (17.30-18.30h) hours with less percentage of empty stomachs. Fullness decreased after dawn and dusk hours. The tidal cycle had impact on its feeding intensity. The feeding activity of whitings increased or decreased *vis -a- vis* tidal fluctuation.

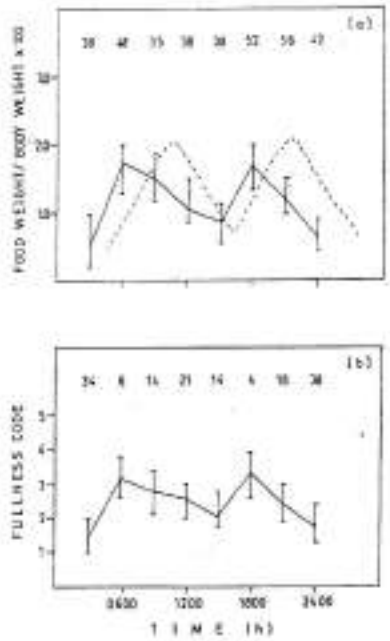


Fig. 1. The relationship between time at day and (a) the amount of food in the stomachs of sandwhitings expressed as % of wet body weight and (b) fullness code.
Data are means (±S.D) placed at the mid point of each 3 h interval. The number of stomachs examined in each time period is given at the top of (a) and the percent of empty stomachs at the top of the (b). * [--------- indicates tide in Fig. 1(a)] .

Gastric evacuation rate

The mean wet and dry weight proportions of the initial meals recovered from the stomachs clearly decreased with time (Fig. 2). Although the linear model gave a significant fit for both wet and dry relationships, F-test for linearity indicated that a non-linear function was more appropriate for both relationships. The exponential model had the highest coefficients of determination for both wet and dry weight relationships of the three models tested (Table 1). This model also yielded fairly close approximation of the initial meal size. These fishes required about 8.0 h to almost completely evacuate the stomach contents (Fig. 2). The values of the instantaneous rates of evacuation (r) and the times to various percentages of stomach fullness for the exponential model are given in Table 2.

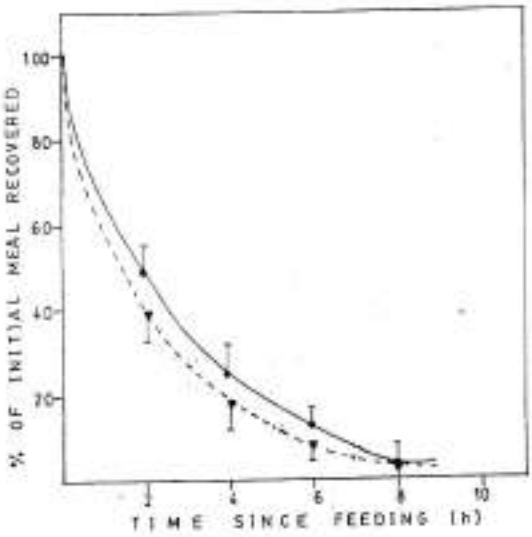


Fig. 2. Wet weight and dry weight recovered from the stomachs of sandwhitings at each sampling interval.

Table 1. Regression coefficients for the depletion curves obtained using different models

Food Condition	Model	a (±S.D)	r (±S.D)	R^2	% intercept
Wet	Linear	83.87±7.46	0.191±0.073	0.86	83.87
	Exponential	4.669±0.431	0.007±0.001	0.98	109.84
	Square root	9.416±1.021	0.016±0.010	0.97	88.66
Dry	Linear	79.064±9.13	0.188±0.036	0.80	79.06
	Exponential	4.613±0.837	0.006±0.001	0.99	100.79
	Square root	9.047±0.993	0.016±0.011	0.93	81.85

Table 2. The instantaneous rate of evacuation (r) and times to various stages of evacuation for the exponential model

Food Condition	r (h^{-1}) (±S.E)	Time (h) to % evacuation		
		50	75	90
Wet	0.007 (0.001)	1.87	3.52	5.70
Dry	0.006 (0.001)	1.94	3.88	6.41

Composition of the food

The results of the monthly stomach content analyses is given in Table 3. The most frequently occurring components in food of *S. sihama* were a wide range of crustacean, polychaetes and fish in the order of their abundance. Polychaetes belonging to 5 genera *viz., Glycera, Diopatra, Pectinaria, Nerieis and Dendronerieis* were recorded. Fish were not identified owing to their advanced state of digestion. Semidigested matter constituted major portion of the stomach content. Apart from this sand particles and miscellaneous items were also recorded.

Seasonal variation in food and feeding intensity

Crustaceans were recorded in all the months with peak in June followed by July and lowest in November. Among crustaceans shrimps and crabs remaining dominated. Polychaetes were recorded in almost all the months with peak during March and lowest in January. Fish were found to be dominant next to crustacea and polychaetes and recorded in all the months. Presence of sand particles and miscellaneous items in the stomachs indicates their bottom feeding nature.

Relationship between food and fish size

Percentage occurrence of food types in the diet of 20 mm size groups is shown in Fig. 3. In the smaller size groups (<100 mm) of *S. sihama,* crustaceans especially copepods, juvenile shrimps constituted major diet. In larger size groups (>100 mm) crustaceans and fish were recorded in various proportion in different size groups. Polychaetes recorded in all the larger size groups and showed increasing trend with increase in size. As the size increased the proportion of semidigested matter decreased. Sand particles and miscellaneous matter were recorded in almost all the size groups in lesser proportions.

Table 3. Monthly percentage composition of different items of diet recorded in the stomachs of sandwhitings, *Sillago sihama*

	Month												
	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Total
No. of Fish Examined	90	110	120	105	127.0	140.0	121.0	117.0	112.0	101.0	119.0	95.00	1357
No. of stomachs with food	51	70	88	57	49	41	28	37	52	45	45	50	613
Food Item													
I Crustacea	24.08	19.96	33.05	30.43	29.40	17.74	13.82	10.54	16.53	12.41	24.03	15.14	20.59
Shrimp remains	8.25	11.53	10.5	15.10	15.15	9.00	4.15	5.75	8.00	4.85	12.27	9.87	9.54
Penaeus sp	1.60	1.53	0.70	4.30	0.50	1.14	0.38	-	0.70	0.73	1.75	2.15	1.96
Metapenaeus sp	2.25	-	2.18	3.75	3.75	-	1.23	-	0.75	0.60	1.75	1.50	1.48
Acetes sp	-	-	-	-	-	-	-	-	0.38	0.75	0.50	-	0.15
Crabs and their larvae	10.18	6.10	18.43	6.15	8.87	7.25	7.38	4.45	6.20	4.50	7.43	3.60	7.55
Mysids	0.85	-	0.25	-	-	-	-	-	-	0.50	-	0.50	0.18
Amphipods	0.25	-	-	-	-	-	-	-	-	-	-	0.25	0.04
Copepods	0.70	0.80	1.00	1.13	1.13	0.35	0.68	0.34	0.50	0.48	0.33	0.75	0.68
II Polychaetes	13.58	14.55	18.58	16.83	26.47	16.80	17.77	23.39	22.49	9.21	12.29	32.63	18.72
III Fish	5.49	13.06	7.47	2.01	0.98	0.55	1.38	2.03	3.82	1.73	2.09	5.21	3.82
IV Semidigested Matter	40.81	38.84	29.36	41.51	35.53	46.77	52.25	55.46	49.57	70.23	52.37	36.68	45.79
V Sand particles	14.50	11.50	8.02	8.20	4.83	14.91	7.76	6.92	4.69	4.20	5.86	8.36	8.31
VI Miscellaneous Items	1.55	2.09	3.54	0.98	2.80	3.24	7.05	1.68	2.91	2.24	3.37	1.89	2.78
Nematodes	-	0.30	0.58	-	0.40	0.73	0.35	0.25	0.25	0.38	-	0.23	0.32
Molluscan shell pieces	0.53	1.35	1.55	0.65	1.43	1.71	2.08	0.75	1.01	1.38	1.76	1.18	1.28
Seaweeds	0.40	-	0.74	0.14	-	0.48	0.35	-	-	-	-	-	0.18
Diatoms	0.63	0.44	0.68	0.19	0.60	0.33	0.58	0.28	0.38	0.49	0.13	0.59	0.44
Unidentified Matter	-	-	-	-	0.38	-	3.70	0.40	0.75	-	1.25	-	0.59

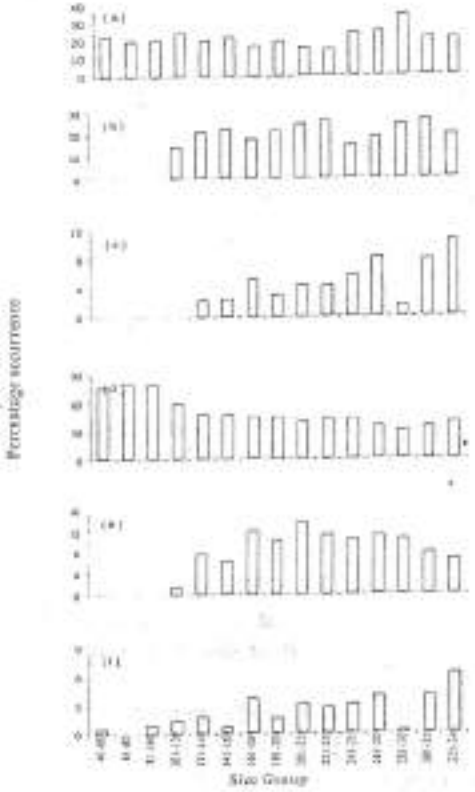


Fig. 3. Percentage occurrence of major food items in the stomachs of 20 mm size groups of *S. sihama*. (a) Crustacea (b) Polychaetes (c) Fish (d) Semidigested matter (e) Sand particles (f) Miscellaneous matter.

Discussion

The results of the study suggest that the diel feeding behaviour of juvenile sandwhitings is usually characterized by two main feeding periods. A morning feeding period (dawn) begins before sunrise and an evening period (dusk) around sunset. The activity of feeding was positively correlated with tidal cycle with intensive feeding during high tide. Similarly Gunn and Milward (1985) reported that the feeding activity of *Sillago analis* is limited by the tidal cycle, a factor which may be a form of temporal partitioning. Tidal cycle at the time of feeding probably plays a major role in determining the availability of prey / food items for fish. Odum (1968) also observed a relationship between the state of the tide and feeding intensity in case of *Mugil cephalus*. Al-daham *et al.* (1977) also observed a similar diel feeding periodicity in catfish, *Heteropneustus fossilis* from southern Iraq. They reported that stomach of the

fish contained food almost thoughout the 24 h period with two peaks at 05.00 h and 17.00 h. Brodeur and Pearcy (1987) reported that juvenile coho salmon *Oncorhynchus kisutch* showed two peak feeding periods one in the early morning and the other around dusk. Kim and Kang (1991) reported that the daily feeding activity of rock trout, *Agrammus agrammus* was more intense at sunrise and sunset and it decreased in the late morning at noon and during the night.

The exponential model provided the best fit to data obtained in the experiment with whiting at temperature of 28.5±1.2°C. Although a number of factors including food particle size, food quality, meal size, and in some cases predator size have been shown to affect evacuation rates, temperature appears to be paramount importance (Fange and Grove 1979, Durbin *et al.* 1983). Higher temperature can lead to substantially increased evacuation rates (Brett and Higgs 1970, Tyler 1970). One shortcoming of the exponential model is that the stomach contents would theoretically begins to level off when stomachs are nearly empty, but fullness never reaches zero. This leads to overestimate of the amount of food remaining in the stomach at the later stages of evacuation.

S. sihama is a carnivore feeding on a wide range of benthic, epibenthic and planktonic prey. The principal foods of *S. sihama* are crustaceans, polychaetes and fish. Presence of sand particles and miscellaneous food items indicates its bottom feeding nature (Chacko 1949, Radhakrishnan 1957, Krishnamurthy 1969, Gowda *et al.* 1988). Large number of empty stomachs observed in the monthly samples may be due to disgorging of stomach contents as a result of shock sustained at the time of capture (Pillay 1952, Jayaprakash 1976).

The considerable variation observed in the percentage occurrence of crustaceans, polychaetes and fish may be related to factors such as seasonal variation in abundance of food items, its consumption rate, age of the fish and diurnal variation in feeding. Among crustaceans, shrimps and crabs were dominated in most of the months. The occurrence of amphipods in the stomach indicates the bottom feeding nature of the fish (Radhakrishnan 1957). Polychaetes dominated over all other items of food in March and August to December. According to Bhat (1978) and Ramachandra (1981) the polychaetes are most abundant in the Netravathi-Gurpur and Mulki estuaries during the pre-monsoon and post-monsoon months. Presence of fish in the stomach was also reported by Radhakrishnan (1957) and it was not possible to identify all of them owing to their advanced state of digestion.

Fishes of less than 100 mm TL seem to prefer crustaceans (juveniles of shrimps, crabs and their larvae along with mysids, copepods and amphipods) whereas fishes larger than 100 mm TL preferred polychaetes followed by crustaceans (*Penaeus sp. Metapenaeus sp.* and crabs), other fishes and miscellaneous food items. It is also evident that irrespective of size both smaller and larger fishes prefer larvae, juveniles and adults of shrimp which form a favorite food. A similar change in composition of the diet with age of the fish has been reported by Krishnamurthy (1969). Gunn and Milward (1985) observed size related dietary shifts in *S. sihama*, from predominantly planktonic crustaceans in fish less than 80 mm TL to polychaetes, penaeid and brachyuran crustaceans and molluscs at larger size. Burchmore *et al.* (1988) reported that the

observed variation in the diet were due to fish size and temporal and spatial habitat differences within and among the species.

Acknowledgements

Authors are very much thankful to ICAR (Indian Council of Agricultural Research), New Delhi for their financial support in carrying out this investigation. We also indebted to Dr. S.L. Shanbhogue, Director of Instruction (Fisheries) for the facilities. Authors are also thankful to Mr. A. Praveen for preparing line diagrams

References

Al–daham, N.K., A.L. Sarkar and M.N. Bhatti, 1977. Diet patterns of feeding in *Heteropneustes fossilis* from Southern Iraq. *Trans. Amer. Fish. Soc.*, 106(6): 614- 616.

Bhat, V.B., 1979. Studies on the meroplanktonic larvae with reference to hydrography of Nethavathi – Gurpur estury, Mangalore, Dakshina Kannada. M.F.Sc. Thesis. University of Agricultural Sciences Bangalore.

Brett, J.R. and D. A. Higgs, 1970. Effect of temperature on the rate of gastric digestion in fingerling sockeye salmon, *Oncorhynchus nerka*. *J. Fish. Res. Bd. Can.*, 27: 1767-1779.

Brodeur, R.D. and W.G. Pearcy, 1987. Diel feeding chonology, gastric evacuation and estimated daily ration of juvenile coho salmon, *Oncorhynchus kisutch* (Walbum), in the coastal marine environment. *J. Fish Biol.*, 31(4): 465- 477.

Burchmore, J. J., D. A. Polland, M. J. Middleton, J.D. Bell and B.C. Pease, 1988. Biology of four species of whiting (Pisces: Sillaginidae) in Botany Bay, New South Wales. *Aus. J. Mar. Freshw. Res.*, 39(6): 709- 727.

Chacko, P.I., 1949. Food and feeding habits of the fishes of the Gulf of Mannar. *Proc. Indian Acad. Sci.*, 29(3): 83- 97.

Dhulkhed, M.H. and S. Ramamurthy, 1977. A note on a preliminary experiments on the culture of *Sillago sihama* (Forskal) at Mangalore. *Indian J. Fish.*, 24 (1&2): 229- 232.

Duribin, E. G., A.G. Durbin, R.W. Langton and R.E. Bowman, 1983. Stomach contents of silver hake, *Merluccious bilinearis* and Atlantic cod, *Gadus morhua*, and estimation of their daily rations. *Fish. Bull.*, 8: 437- 454.

Dutt, S. and K. Sujatha, 1980. On the seven species of fishes of the family sillaginidae from Indian waters. *Mahasagar*, 13(4): 371- 375.

Fange, R. and D. Grove, 1979. Digestion. *In*: Fish Physiology Vol. 8. Energetics and Growth (eds. W.S. Hoar, D.J. Randell and J.R. Brett), Academic Press, NewYork. pp. 161- 260.

Gowda, H.H., P.S. Joseph and M.M. Joseph, 1988. Feeding ecology of the Indian sand whiting, *Sillago sihama* (Forskal) inhabiting the Nethavathi – Gurpur estuary. *In*: *The First Indian Fisheries Forum Proceedings*. December, 4-8., Mangalore, Karnataka. Asian Fisheries Society, Indain Branch, Mangalore (India). pp. 263- 266.

Gunn, J.S. and N.E. Milward, 1985. The food, feeding habits and feeding structures of the whiting species *Sillago sihama* (Forskal) and *Sillago analis* (Whitley) from Townsville, North Queensland, Australia. *J. Fish Biol.*, 26(4): 411- 427.

Hynes, H.B.N., 1950. The food of fresh water sticklebacks (*Gasterosteus aculeatus* and *Pygosteus pungitius*) with a review of methods used in studies of the food of fishes. *J. Ani. Ecol.*, 19: 36 - 58.

James, P.S.B.R., T.J. Varghese and K.V. Devaraj, 1976. Some observations on the possibilities of culture of Indain sand whiting *Sillago sihama* (Forskal) in Brackish waters. *J. Inland Fish. Soc. India*, 8: 212- 220.

Jayaprakash, A.A., 1976. A note on a sciaenid, *Johnieops sina* (Cuvier) with three anal spines. *Indian J. Fish.*, 21(2): 569- 570.

Jobling, M., 1981. Mathematical models of gastric emptying and the estimation of daily rates of food consumption for fish. *J. Fish Biol.*, 19: 245- 257.

Jobling, M., 1986. Mythical models of gastric emptying and implications for food consumption studies. *Envi. Biol. Fish.*, 16(1-3): 35- 50.

Kim, C.K. and Y.J. Kang, 1991. Mathematical approaches related to daily feeding activities of rock trout, *Agrammus agrammus. Bull. Korean Fish. Soc.*, 24(5): 315- 326.

Krishnamurthy, K. N., 1969. Observations on the food of sand whiting, *Sillago sihama* (Forskal) from Pulicat lake. *J. Mar. Biol. Assoc. India*, 2 (1&2): 295- 303.

Mckay, R.J., 1976. The fishes of the family sillaginidae from India with a description of a new species. *J. Mar. Biol. Assoc. India*, 18(2): 375- 385.

Odum, W.E., 1968. The ecological significance of the fine particle selection by striped mullet, *Mugil cephalus. Limnol. Oceanogr.*, 13: 92- 97.

Pillay, T.V.R., 1952. A critique of the methods of study of food of fishes. *J. Zool. Soc. India*, 4: 185- 200.

Radhakrishnan, N., 1957. A contribution to the biology of Indian sand whiting, *Sillago sihama* (Forskal). *Indian J. Fish.*, 6(1): 254- 283.

Ramachandra, U., 1981. Studies on the macrobenthos of Mulki estuary, Dakshina Kannada. M.F. Sc. Thesis, University of Agricultural Sciences, Bangalore.

Tyler, A.V., 1970. Rates of gastric emptying in young cod. *J. Fish. Res. Bd. Can.*, 27: 1177- 1189.

(Manuscript received 8 April 2001)

Bangladesh J. Fish. Res., 6(1), 2002: 69-74

Hydrography of the Bay of Bengal during south west monsoon and its significance on oil sardine fishery

K.M. Santosh*, H.R.V. Reddy and Arun Padiyar
Department of Fishery Oceanography, College of Fisheries, Kankanady PO, Mangalor 57002, India
*Corresponding author

Abstract
Hydrographic data collected from east coast of India during 1994 monsoon period revealed that these waters are highly characterized by upwelling especially in the coastal waters with more intensity in the southern part of the region. However, the near surface salinity stratification consequent to high fresh water inflow into the bay was absent in the present study. Oil sardines are directly influenced by hydrographic parameters such as salinity and temperature and stratification of these parameters are the major reasons for non-availability/migration of oil sardine from this region in the earlier years. Considering the recent topographical change in the east coast coupled with hydrological stability an attempt has been made in this paper to give reasonable justification to the reported bumper catches of oil sardines from 1994 on wards in the east coast of India.
Key words: Hydrography, Upwelling, Stratification, Sardine fishery

Introduction

Earlier studies conducted in the shelf waters and adjoining areas of the Bay of Bengal in the east coast of India revealed that these waters are highly influenced by changing wind pattern over the Bay and the prevailing current system coupled with large amount of fresh water discharge in to the coastal waters at different points along the coast. The water quality characteristics of the area have been studied earlier by Murthy and Varadachari (1968), Hastenrath and Lamb (1979), Gopalakrishna and Sastry (1985), Sasmal (1989), Shetye *et al.* (1991) and Suryanarayana *et al.* (1992). According to Ramana (1985) salinity and temperature appear to play a vital role on the appearance and disappearance of oil sardine. Considering this aspect and available data on hydrography, land use and oil sardine landings a comparative study has been made.

Material and methods

The data for the present study pertain to the FORV *Sagar Sampada* cruise No. 121 B from the Bay of Bengal region during 1994 and fish landing data collected and published by CMFRI from 1989 to 1997. Water samples collected at all standard depths from the coastal and off-shore stations up to a maximum depth of 500 m along transects 16°30', 17°30', 18°30' and 19°30' N. Temperature, salinity and dissolved oxygen were obtained

and charts for spatial variation and vertical distribution of these parameters were prepared. The salient features of the study were critically examined with recently fabricated structures in the coastal belts of east coast of India and oil sardine landings of south east coast of India for a period from 1989-1997 (CMFRI landing data).

Results

Along 16°30' (Fig. 1) upwelling features were clearly observed in the upsloping of isotherms from deeper depths to shallower coastal region. The near shore areas were characterized by comparatively lower temperature, higher salinity and lower oxygen values. The top 50 m layer at off-shore stations was found isothermal. In this area below 100 m depth temperature stratification was dominant and salinity remained more or less constant. Upwelling resulted in higher salinities towards the coastline. The upsloping was noticed from depths up to 150 m in the salinity and oxygen fields. Temperature and oxygen values at surface showed an increase towards off-shore stations.

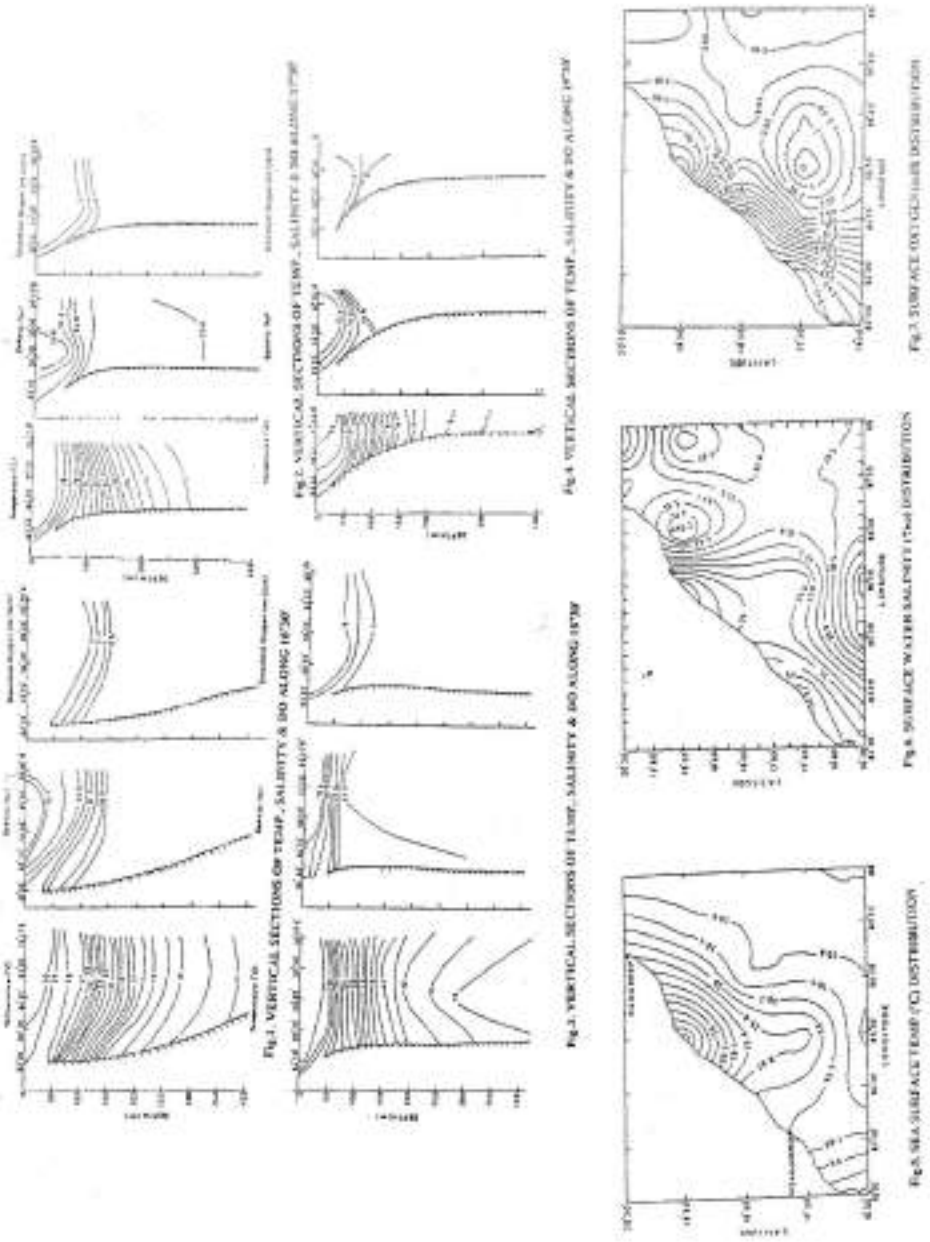
At 17°30' (Fig. 2) upsloping of isotherms was noticed in the shelf region to a maximum depth of about 75 m and below these depths isotherms showed downward tilt indicating downwelling. A low salinity pool was observed outside the shelf at 17°30' N and beyond this zone the salinity showed an increasing trend. The mixed layer found extending up to 40-50 m depth zone in the off-shore stations (both 16°30' and 17°30' N). The presence of higher salinity water near the coast and comparatively low oxygen values near surface layers were the characteristics of upwelling. Salinity gradient was absent below 100 m depth. However, surface temperature values were comparatively high near the coast.

Along the latitude 18°30' (Fig. 3), isotherms showed an upward tilt towards shallow coastal region from a depth of 70 m and below this depth isotherms tilted downward indicating downwelling. Temperature values near the coast were comparatively low and showed an increasing trend towards off-shore region. The top mixed layer temperature was generally high at off-shore stations. While in the coastal stations salinity values were relatively high with low oxygen content.

At latitude 19°30' (Fig. 4), isotherms showed an upward tilt towards the coast with comparatively low temperature values near the coast. Water with higher salinity prevailed at coastal stations, while below 100m depth salinity values remained more or less uniform. Dissolved oxygen values also were high at coastal stations.

Upwelling intensity is observed more in the southern sections from the vertical distribution of isolines. Temperature values at surface were lower in the sections at 16°30' and 19°30'N, while generally higher values were observed at 17°30'N. The temperature (Fig. 5) values near the coast were also low towards northern latitudes. The surface distribution (Fig. 6) indicated the presence of high salinity near the coast and it showed a decreasing trend towards off-shore region. Salinity values near the coast at surface were comparatively lower in the northern latitudes. Surface oxygen values (Fig. 7) were relatively low near the coast and showed gradual increase towards offshore stations at 16°30', 17°30', and 18°30'N. While at latitude 19°30' N oxygen values were high near the coast and decreased towards offshore station. From the salient features of the

study, it was observed that the water quality characteristics did not show any positive influence of river run-off to the bay.



Ground-truth information were gathered from land use data of east coast of India, revealed that many dams were commissioned recently which are close to rivers Krishna and Godavary in the east coast, this will create a blockade to the river discharge, ultimately to the Bay. This may be the possible reason for reduction in river water input in to the Bay. This in turn affects stratification of salinity and temperature.

Oil sardine catches of southeast coast of India for a period of 9 years, i.e. 1989 to 1997 (Table 1) showed gradual increase in catch year after year when compared to southwest cost catches of India. In 1994 it exceeded Southwest coast catches. The catch reported in 1994 in the southeast coast was 43,000 tones and in southwest coast were 3,000 tones. Thereafter tremendous increase in catches were recorded in the southeast coast and in the year 1997 highest catch of about 1,11,500 tones was recorded.

Table 1. Oil sardine landings along Indian coasts (tons)

Area	1989	1990	1991	1992	1993	1994	1995	1996	1997
South -East	21,000	38,000	94,000	38,200	39,000	43,000	37,000	70,000	1,11,500
South -west	2,38,000	2,22,000	1,42,000	66,000	56,000	3000	18,000	39,000	1,11,000

(S.E-Bay of Bengal & S.W-Arabian Sea-CMFRI, Published data)

Discussion

The mixed layer temperature was generally high at all sections, but below the thermocline layer the temperature profile indicated a decreasing trend towards northern latitudes. Salinity value showed an increasing trend towards the bottom at all sections and decreased towards offshore stations.

The general pattern of isotherms indicates upwelling of subsurface waters to the shallow coastal region. The temperature profile below the upwelling zone indicated sinking which is more prominent at 17°30' N and weak at 18°30'N. Upwelling formation was best marked by temperature and salinity distribution near the coast. The upsloping of isolines was noticed from deeper depths in the southern sections than the north. A relatively less saline water was observed in the off- shore stations at all sections (16°30' to 19°30' N). Below the upwelling band salinity remained more or less constant, temperature showed decreasing trend especially towards northern sections. The downwelling features observed in certain sections were indicative of sub surface current prevailing in the area.

From the earlier reports during the southwest monsoon period pertaining to June-August months, it was observed that these waters were under the strong influence of fresh water discharge. However though refers to the beginning of the southwest monsoon season, the water characteristics did not show any positive influence of river discharge to the sea in the present study. The newly commissioned dams near Godavari and Krishna rivers, instead of discharging into the sea, diverting the fresh water flow which reduces stratification.

Ganapati and Murty (1955) reported fall in surface temperature during April-May and July-August. They attributed this to the upwelling of waters from the sub surface layers. Lafond (1954) reported upwelling in some years during summer months. Pronounced upwelling in March-April and July-August at Waltair was noticed. The wind distribution over the Bay of Bengal favors upwelling in the east coast of India during southwest monsoon period and upwelling driven by local wind alone occurs in the western boundary region of the Bay (Suryanarayana *et al.* 1992). Cutler and Swallon (1984) reported that the drifts did not show consistent trend in the bay during May-September, although from September to January the flow was equatorward and from January to May it was poleward. The hydrographic features in the present study clearly indicate the occurrence of upwelling in the section at 16°30' and 18°30' N characterized by presence of low temperature, high salinity and low oxygen. There have been reports of upwelling during this period along the East Coast (Lafond 1957, Murthy and Varadachari 1968). Sasmal (1989) reported that the isotherms of the subsurface layer along 19°N associated with low temperature water in the southern sector at 50 m level indicated upwelling along the coast. Sanilkumar (1995) observed upsloping of isotherms towards the coast off-Visakhapatnam throughout the upper 100 m water column from the observations of R.V. *Gaveshani* cruise in June'86 indicating the extension of upwelling at least to a depth of 100 m. Shetye *et al.* (1991) reported signatures of downwelling below the upwelling band along the western boundary of the Bay of Bengal indicating a southward undercurrent. Similar features were also observed in the present study at 17°30' and 18°30' N. Most of the freshwater influx to the bay occurs during the southwest monsoon. The formation of low salinity plume like structure in the northern latitude observed in the present study is due to the combined effect of upwelled water and low salinity water in the northern latitudes. A similar structure moving southward also was reported by Shetye *et al.* (1991) in their study during the southwest monsoon period. But in contrast to their observation, a near surface salinity stratification consequence of high fresh water inflow in to the bay was not found in the present study.

As far as oil sardines are concerned, they cannot tolerate large fluctuations of salinity and temperature. According to Nathaniel (1988), there is an optimum range of temperature and salinity, which coincided with sardine, catch and whenever salinity and temperature recorded above or below the optimum value, less oil sardine catch was reported. Hence it is evident that sea water temperature, salinity and other oceanographic parameters have a significant bearing on the oil sardine. Maddikery (1981) observed relatively high catches of oil sardine in January and September to November off Gangolli, when surface water temperature ranged from 28.65 to 29.95°C and surface salinity from 32.54 to 33.57 ‰. Bensam (1970) observed high catches of sardine, when the temperature and salinity fluctuated between 27-28°C and 34-35‰.

Usually the stratification in salinity and temperature are more in Bay of Bengal compared to Arabian Sea. This is one of the reasons of non-availability of oil sardines earlier from this region. The reduced stratification of salinity and temperature in the Bay of Bengal is due to the recent topographical changes in the east coast region i.e.

mainly because of new dams. This is one of the major reasons for the appearance of oil sardines in the east coast of India.

Acknowledgements

Authors express their gratitude to Director, Sagar Sampada Cell, Cochin for giving an opportunity to collect data onboard FORV *Sagar Sampada* and Director, Central Marine Fisheries Research Institute (CMFRI), Cochin for the utilization of their published fish landing data for the study.

References

Bensam, P., 1970. On the fluctuations of the oil sardine fishery at Cannanore during 1961-64. *Ind. J. Fish.*, 17(1&2): 132-148.

Cutler, A.N and I.C. Swallon, 1984. Surface currents of the Indian Ocean. Institute of Oceanographic Sciences, Report No. 187, 36 charts, 7pp.

Ganapati, P.N. and V.S.R. Murty, 1955. Preliminary observation on the hydrography and inshore plankton in the Bay of Bengal off Visakhapatnam coast. *Indian J. Fish.*, 2(1): 84-95.

Gopalakrishna, V.V. and J.S. Sastry, 1985. Surface circulation over the shelf off the East Coast of India during the southwest monsoon. *Indian J. Mar. Sci.*, 14: 62-65.

Hastenrath, S. and P.J. Lamb, 1979. Surface climate and atmospheric circulation. Climatic atlas of the Indian Ocean, Part 1 (Wisconsin, Univ. Wisconsin Press). 10pp.

Lafond, E.C., 1954. On upwelling and sinking off the East Coast of India. Andhra Univ. Memoirs, 1: 117pp.

Lafond, E.C., 1957. Oceanographic studies in the Bay of Bengal. Proc. of the Indian Acad. Sci., section B, 46: 1-47.

Maddikery, G.V., 1981. Hydrographic features in the Arabian Sea off Gangolli region, Dakshina Kannada. M.F.Sc. Thesis, Univ. of Agri.Sci., Bangalore. 309pp.

Murthy, C.S. and V.V.R. Varadachari, 1968. Upwelling along the East Coast of India. Proc. Symp. on the Indian Ocean, Bull. of National Inst. of Sci. of India, 38: 80-86.

Nathaniel, D.E., 1988. Studies on some oceanographic features in relation to demersal and pelagic fish catches in the Arabian sea off Mangalore, Ph.D. thesis, Univ. of Agri. Sci., Bangalore., 286pp.

Ramana, T.V., 1985. Studies on the variation of temperature, salinity and some other oceanographic factors in the Arabian Sea off Manjeswar and their possible influence on the oil sardine and mackerel fisheries in the areas. M.Sc. Thesis, Univ. of Agri.Sci., Bangalore., 161 pp.

Sanilkumar, K.V., 1995. Some aspects of oceanic variability in the upper layers of the Bay of Bengal. Ph.D. Thesis, Cochin University of Sci. and Tech. 105pp.

Sasmal, S.K., 1989. Hydrography of northern Bay of Bengal during southwest monsoon. *Mahasagar*, 22(3): 105-112.

Shetye, S.R, S.S.C. Shenoi, A.D. Gouveia, G.S. Michael, D. Sunder and G. Nampoothiri, 1991. Wind driven coastal upwelling along the western boundary of the Bay of Bengal during southwest monsoon. *Cont. Shelf Res.*, 2: 1397-1408.

Suryanarayana, A., C.S. Murthy and D.P. Rao, 1992. Characteristics of the western Bay of Bengal during different monsoon seasons. *Aust. J. Mar. Freshwater Res.*, 43: 1517-1533.

(Manuscript received 8 April 2001)

Bangladesh J. Fish. Res., 6(1), 2002: 75-81

Population dynamics of two jewfishes (*Jhonius argentatus* and *Johnieops vogleri*) in the coastal waters of Bay of Bengal, Bangladesh

H. Rashid*, S. Dewan, M.G. Mustafa[1] and M.M. Haque
Department of Fisheries Management, Bangladesh Agricultural University
Mymensingh 2202, Bangladesh
[1]Marine Fisheries & Technology Station, BFRI, Cox'S Bazar
*Corresponding author

Abstract
Population parameters of *Jhonius argentatus* and *Johnieops vogleri* in coastal waters of Bay of Bengal, Bangladesh were estimated by using FiSAT programme. The von Bertalanffy growth parameters, extreme length (cm) and growth constant K (year $^{-1}$) were found to be 46.50 and 0.59 for *J. argentatus*, and 33.50 and 0.85 for *J. vogleri*. The L_∞ (cm) and Z/K estimates provided by Wetherall plot were 46.694 and 1.791 for *J. argentatus*, and 31.25 and 2.623 for *J. vogleri*. The annual rate of natural (M) and fishing mortality (F) were estimated as 1.12 and 0.78 for *J. argentatus*, and 1.56 and 1.28 for *J. vogleri*. Rate of exploitation (E) was estimated as 0.41 for *J. argentatus* and 0.45 for *J. vogleri*. About 80.04% of *J. argentatus* were found to be recruited during peak pulses (April-May) and 19.96% during lean pulses (October-November) and 85.75% *J. vogleri* during peak pulses (May-July) and 14.25% during lean pulses (September-October). The growth performance index (ϕ') was 3.11 for *J. argentatus* and 1.93 for *J. vogleri*. The total length and body weight relationship was found to be $W = 0.0403\ TL^{2.3723}$ for *J. argentatus* and $W = 0.0907\ TL^{2.3482}$ for *J. vogleri*.
Key words: Population dynamics, *Jhonius argentatus*, *Johnieops vogleri*

Introduction

Jhonius argentatus and *Johnieops vogler*, locally called 'Lal poa' and 'Keti poa', are two most commonly appearing Sciaenid in the coastal waters of Bangladesh. These species live in school, usually close to muddy of sandy-mud bottom and along with 18 other Perciforms found so far in this region they account for about 12.8% of the total demersal fish stock in the EEZ of Bangladesh and 66.5% of the demersal fishes found in the continental shelf within 20 m depth of water (Sarker and Rahman 1991). They inhabit shallow coastal waters upto 100 m depth in the Bay of Bengal. These two species pay an important role in the economy of Bangladesh. Recently salted dehydration of these fishes are being done to export to the foreign countries.

The fishing pressure is increasing day by day in the coastal waters of Bangladesh and the indiscriminate operation of Set Bag Net (SBN) and other detrimental gears in the Cox's Bazar region is hampering the pelagic and demersal fish stocks in the region.

However, information on fishing pressure and sustainable stock position is limited and little information on population dynamics and status of exploitation in the coastal waters of Bangladesh is available.

Utilizing methods of analysis (FiSAT– The FAO-ICLARM Stock Assessment Tools) of length frequency data, growth parameters ($L\infty$, K) of the von Bertalanffy equation, instantaneous mortality rates (Z, M and F), selection pattern (Lc), recruitment pattern and length-weight relationship have been estimated for *Jhonius argentatus* and *Johnieops vogler*. Phi pharm (ϕ') value was calculated to compare ϕ' value of these two species in this region as well as to establish a guideline of growth performance index.

Materials and methods

The study was conducted from November'99 to October'00. Length and weight data were collected for present study from commercial catches of the fishermen operating three types of gears *viz*., gill net, set nag net and long line at Cox's Bazar off Bay of Bengal. Samplings were done monthly and all length-frequency data for each month were pooled and pooled data were entered in computer through ELEFAN 0 program. Total length was measured in cm from the tip of the snout to the tip of the tail for a total of 1975 specimen for *J. argentatus* and 2400 specimen for *J. vogleri*.

FiSAT as explained in detail by Gayanilo *et al.* (1994) was developed mainly for the detailed analysis of length frequency data. Length-frequency based computer programs ELEFAN I and ELEFAN II were used to estimate population parameters. $L\infty$ and K values were estimated by ELEFAN I (Pauly and David 1981, Saeger and Gayanilo 1986). Additional estimate of $L\infty$ and Z/K value was obtained by plotting L - L′ on L (Wetherall 1986 as modified by Pauly 1986).

The growth performance of *J. argentatus* and *J. vogleri* population in terms of length growth was performed based on the ϕ′ index of Pauly and Munro (1984).

$$\phi' = \text{Log}_{10}K + 2\text{log}_{10}L\infty \quad \text{---------- (1)}$$

The ELEFAN II estimated Z from catch curve based on equation as:

$$Z = \frac{K(L\infty - L)}{L - L'} \quad \text{---------- (2)}$$

where L is the mean length in the sample, computed from L′ (upper) and L′ (lower) limit of the smallest length class used in the computation of L (Beverton and Holt 1956). The parameter Z of equation 2 estimated using the routine ELEFAN II (Pauly 1983, Saeger and Gayanilo 1986) which is based on the method of catch curve analysis and an extract solution found using the recursive model, i,e,;

$$\ln(Ni/(-e^{-zj\,dti})) = a - zj + 1 * ti \quad \text{---------- (3)}$$

where dti is the time needed to grow through class i, ti the relative age corresponding to the lower limit of class i, zj is an initial value of Z and Ni is the number of fishes (Pauly 1984). The parameter M was estimated using the empirical relationship derived by Pauly (1980), i.e.;

$$\text{Log10M} = 0.0066 - 0.279\text{Log10}L\infty + 0.6543\text{Log10} + 0.463\text{Log10T} \quad \text{---------- (4)}$$

where $L\infty$ is expressed in cm, T(°C) is the mean annual environment temperature (here it was taken as 28°C). The estimate of F was taken by subtraction of M from Z. An additional estimate of Z value was obtained by ELEFAN II (Jones and van Zalinge 1981). The exploitation ratio E was then computed from expression:

$E = F/Z = F/(F+M)$.

Length-weight relationship

Total length in centimeter and total weight in gram were recorded. The relationship between length-weight was calculated by a computer program followed after Sparre (1985). The intercept (a) and slope (b) of regression line were calculated by using the following formula: $W = a \, . \, L^b$.

Results and discussion

Growth parameters

Growth parameters of von Bertalanffy growth formula were estimated as $L\infty = 46.5$ cm and $K = 0.59$ per year for *J. argentatus* and $L\infty = 33.5$ cm and $K = 0.85$ per year for *J. vogleri* (Fig. 1). For these estimates through FiSAT the response surface (ESP/ASP) were 0.151 for main line (solid line) and 0.131 for secondary line (dotted line) in case of *J. argentatus*. In case of *J. vogleri* the ESP/ASP were 0.136 for main line (solid line) and 0.114 for secondary line (dotted line). The t_0 value was taken as 0. The $L\infty$ and K values for *J. argentatus* (50.0 cm and 0.72 $year^{-1}$) reported by Shahanaz (1996) were close to the values of the present study. Whereas, $L\infty$ and K values for *J. argentatus* reported by Ashraful (1998) were 46.1 cm and 0.86 $year^{-1}$ respectively from the Bay of Bengal.

Estimation of $L\infty$ and Z/K

The modified Wetherall (1986) plot analysis incorporated in the FiSAT yielded the regression line $Y = 16.73 + (-0.358)*X$ and $r = 0.972$ for *J. argentatus* and $Y = 8.62 + (-0.276)*X$ and $r = 0.996$ for *J. vogleri*. Based on these points from 21.5 cm show a good linear relationship and that points of lengths below 43.5 cm smoothly approach the extended line from which $L\infty = 46.69$ cm and $Z/K = 1.791$ were obtained in case of *J. argentatus* and also from 21.5 cm show a good linear relationship and that points of lengths below 29.3 cm smoothly approach the extended line from which $L\infty = 31.25$ cm and $Z/K = 2.623$ were obtained in case of *J. vogleri* (Fig.2).

The growth performance index (ϕ') obtained were 3.11 and 1.93 for *J. argentatus* and *J. vogleri* respectively.

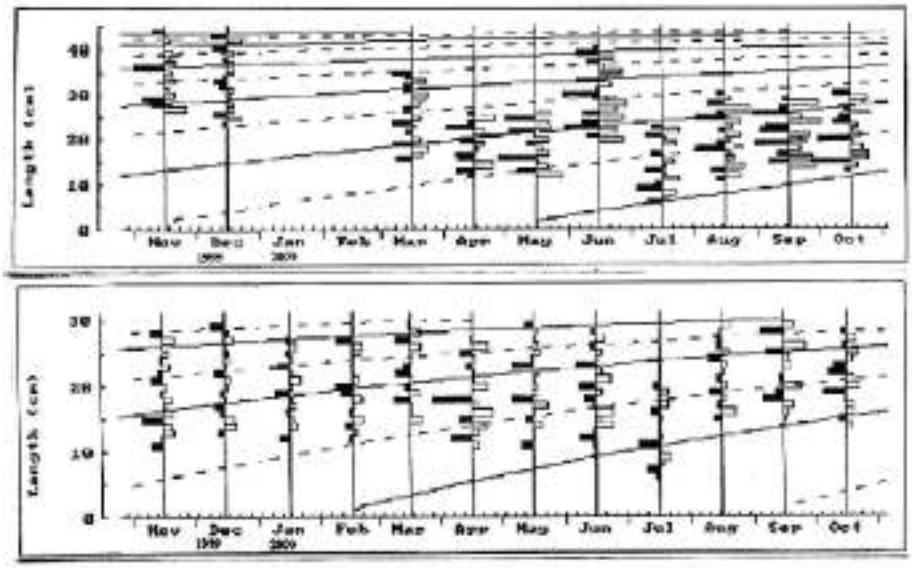


Fig. 1. Growth curve superimposed over the restructed length-frequency data of *Jhonius argentatus* (a) and *Johnieops vogleri* (b) from the Bay of Bengal.

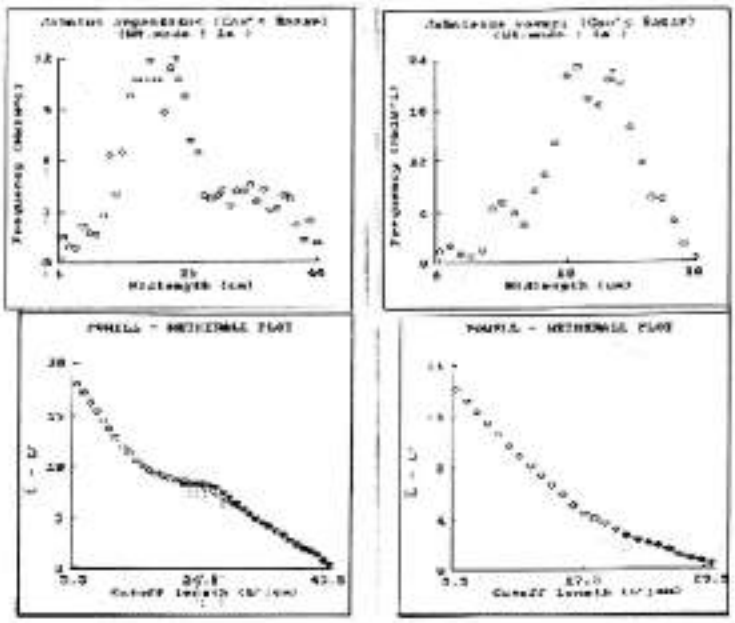


Fig. 2. Estimation of L∞ and Z/K using the methods of Wetherall for *Jhonius argentatus* (a) (L∞=46.69 cm and Z/K=1.791) *and Johnieops vogleri* (b) (L∞=31.25 cm and Z/K=2.623).

Mortality

The mortality rates M, F and Z were found to be 1.12, 0.41 and 1.90 for *J. argentatus* and 1.56, 0.45 and 2.84 for *J. vogleri* respectively. Fig. 3 presents the catch curve utilized in the estimation of Z. The darkened circles in the figure represent the points used in calculation Z via least squares linear regression. The correlation co-efficient for the regression was 0.964 for *J. argentatus* and 0.975 for *J. vogleri.*

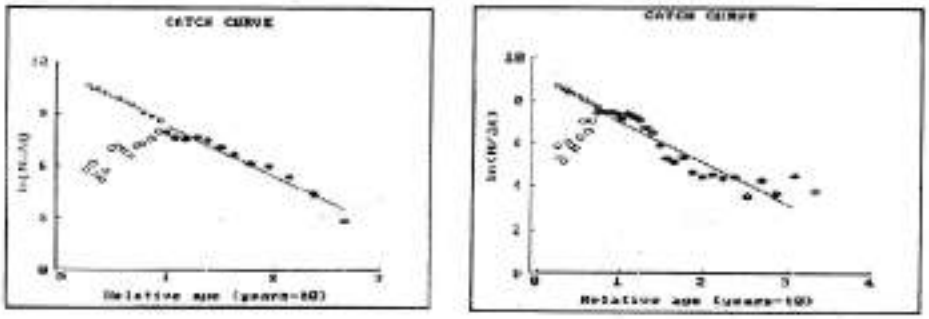


Fig. 3. Length-converted catch curve of *Jhonius argentatus* (a) and *Johnieops vogleri* (b).

Exploitation rate

The exploitation rate E was estimated from the Gulland's (1971) equation E=F/F+M. Thus from the range of values F and F+M it can be shown that the rate of exploitation, E was 0.41for *J. argentatus* and 0.45 for *J. vogleri.*

Recruitment pattern

The recruitment pattern determined through FiSAT (Fig. 4) suggested that annual recruitment consists of two uneven seasonal pulses one in April-May (peak recruit) and other in October-November (lean recruit) in *J. argentatus* and May-June (peak recruit) and September-October (lean recruit) in *J. vogleri.* It appears from original pattern of recruitment with superimposed normal distribution that *J. argentatus* is recruited 80.04% during peak pulses and 19.96% during lean pulses and *J. vogleri* is recruited 85.74% during peak pulses and 14.25% during lean pulses.

Length-weight relationship

In the present study 244 specimen of *J. argentatus* were measured where total length varied from 6.00 to 44.00 cm and the body weight varied from 7.00 to 795.00 g during one year samples. On the other hand, 218 specimen of *J. vogleri* were measured where total length was between 6.00 and 28.00 cm and the body weight was between 7.00 to

245.00 g. From the regression analysis of the length and weight the relationship was found to be $W = 0.0403\ L^{2.5723}$ in *J. argentatus* and $W = 0.0907\ L^{2.3482}$ in *J. vogleri*.

The value of 'b' in this study was lower than 3 in both the fishes. The equation shows that the fishes increased in weight a power lesser than the cube of length i.e., their growth was allometric.

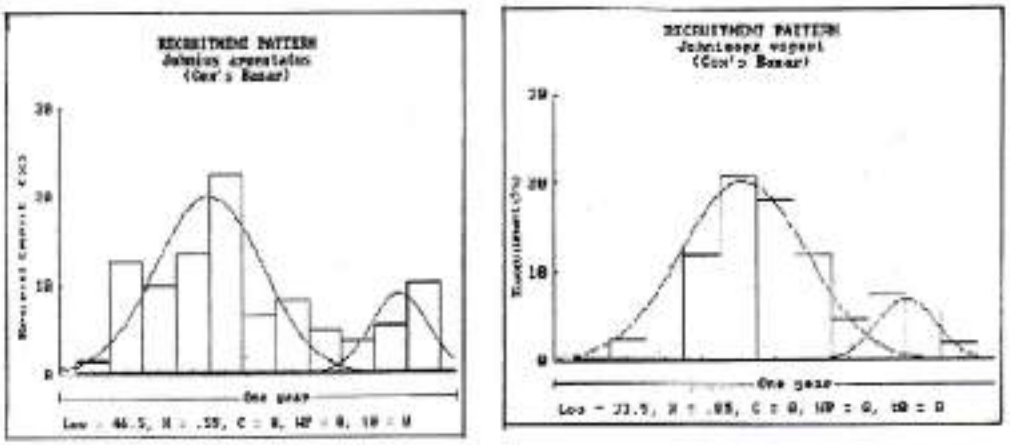


Fig. 4. Recruitment pattern showing recruitment season for Jhonius argentatus and Johnieops vogleri.

References

Ashraful, H.A., 1998. Population dynamics of five commercially important marine fishes in north-eastern part of the Bay of Bengal. M.Sc. Thesis, Inst. of Marine Sciences, University of Chittagong, Chittagong, Bangladesh, 131 pp.

Beverton, R.J.H. and S.J.Holt, 1956. A review of method for estimating mortality rates in fish population, with special references to sources of bias in catch sampling. Rapp. P-V. Reen. *Cons. Inst. Explor. Mer.*, 140: 67-83.

Gayanilo, F.C.Jr., P. Sparre and D.Pauly, 1994. The FAO-ICLARM Stock Assessment Tools (FiSAT) User's Guide. FAO Computerized Information Series (Fisheries) No. 6. Rome, FAO. 186pp.

Gulland, J.A., 1971. The fish resources of the Oceans, West byfleet, Survey, Fishing News Books, for FAO 255.

Jones, R. and N.P. van Zalinge, 1981. Estimation of mortality rate and population size for shrimp in Kuwait waters. *Kuwait Bull. Mar. Sci.*, 2: 273-288.

Pauly, D., 1980. On the interrelationship between natural mortality growth parameters and mean environmental temperature in 175 fish stock. *J. Cons.Int.Explor.Mer.*, 39(3): 175-192.

Pauly, D., 1983. Some simple methods for the assessment of tropical fsih stocks. *FAO. Fish. Tech. Pap.*, 235: 52.

Pauly,D.,1984. Fish population dynamics in tropical waters: a manual for use with programmable calculator. *ICLARM Stud.Rev.*, 8: 325.

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 growth comparisons in fish and invertebrates. *Fishbyte,* 2(1): 21.

Pauly, D. and M.L.Soriano., 1986. Some practical extentions to Beverton and Holt's relative yield-per-recruit model, p149-495. In: First Asian Fisheries Forum (eds. J.L. Maclean, L.B. Dizon and L.V.Hosillos), Asian Fisheries Society, Manila, Philippines.

Saeger, J. and Jr.F.Gayanilo, 1986. A revised graphics orientated version of ELEFAN 0, I and II basic programs for use on HP 86/87 microcomputers. Tech. Rep. *Dept. Mar. Fish. Tech. Rep.,* 8: 1-233.

Sarker, M.N. and A.K.A. Rahman, 1991. Jew fish (Sciaenidae: Perciformes) fishery in the Bay of Bengal. *Bangladesh J. Zool.,* 19(2): 161-166.

Shahanaz, B., 1996. ELEFAN based population dynamics of silver jew, *Johnius argentatus* (Fowler 1933) from Bay of Bengal, Bangladesh. M.Sc. Project, Department of Zoology, University of Chittagong, Chittagong, Bangladesh. 33 pp.

Sparre, P., 1985. Introduction of tropical fish stock assessment. CCP/INT/392/DEN, FAO. Manual-1. 384pp.

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

(Received on 19 April 2001)

Bangladesh J. Fish. Res., 6(1), 2002: 83-88

Selective biochemical studies in a freshwater prawn, *Macrobrachium nobilli* (Crustecea: Palaemodinae)

P. Mariappan*, P. Balamurugan and Chellam Balasundaram
Department of Animal Science, Bharathidasan University, Tiruchirapalli 620024, India
*Corresponding author

Abstract
Calcium and phosphorous contents of abdomen and cheliped muscles of juvenile, male and female *Macrobrchium nobilii* were determined from field collected samples. In all the three groups calcium concentration was higher in chelipeds while the phosphorous content was more in abdomen muscles than in the chelipeds. However between three groups the calcium content varied significantly both in the abdomen and cheliped muscles ($P<0.001$) while the phosphorous content differed ($P<0.05$) only in abdomen muscles.
Key words: Calcium, Phosphorous, *Macrobrchium nobilii*

Introduction

A knowledge on the proximate composition (Ash, moisture, macro and micro nutrients and energy) of a animal chosen for aqualculture is essential for not only selecting an ideal stock but also to formulate an ideal diet (Davis *et al.* 1992, Davis and Gatlin 1996). Available literature on the micro and macro nutrient composition of various and even commercially important decapods indicate their inter and intra-species variations and even within species with developmental stages (Boyd and Teichert-Coddington 1995). However there is a paucity of information on mineral content of freshwater crustaceans and its requirement for normal growth. Crustaceans obtain the required minerals for growth from the environment either by ionic exchange across the gill membrane or from the ingested water through gut absorption (Chuang 1995). A dietary sources of some minerals is necessary to ensure normal growth since the periodic exudation of heavily mineralized exoskeleton leads to loss of some minerals despite their reabsorption in premolt period (Greenaway 1985).

Materials and method

Macrobrchium nobilii (Henderson and Mattai 1910) were collected from river Cauvery, near Tiruchirapalli and transported to the laboratory with enough aeration in large plastic container and used for the study immediately. Tissue samples were obtained

from the muscle of abdomen and chelipeds in juvenile, male and female. A known quantity of sample was dried at 70°C in a hot air oven for 24 hrs and reweigh to quantify the moisture content of the sample (Passoneau and Williams 1953). To find out the ash content, the dried samples were shed at 550-600°C for 4 hrs in a muffle furnace. The left out inorganic constituents in the form of ash was then weighed (Huner *et al.* 1990). Calcium and phosphorous content of the tissue samples were estimated by drying the samples in a hot air oven at 70° C to a weight constancy and then digested (Van Loon 1985) in acid-washed test tubes with a mixture of concentrated nitric and perchloric acid. The samples were slowly boiled to dryness on a hot plate and allowed to cool to room temperature. The died samples were-re dissolved in concentrated HCL and de-ionized water to quantify the calcium and phosphorous content using the UV 1604 Shimadzu atomic spectrophotometer (Fiske and Subbarow 1925, Mendez *et al.* 1998). Variations in the moisture, ash, calcium and phosphorous contents among juvenile, male and female were analyzed through one way ANOVA. The variations in proximate composition between the abdomen and chelipeds muscle in each group was tested through Student's "t" test (Zar 1996).

Results and discussion

The moisture content of abdominal muscle of juvenile is 72.±121.31% which differs from that of cheliped (59.01± 1.85%) (Table 1). A similar trend is also found between the muscle of abdomen and cheliped of male ($p<0.01$, t 7.46) and female (t 7.99, $t_{0.01}$ 2.78, df = 4). To find out the variations between the moisture content of the samples obtained from the three groups, ANOVA was performed which indicates that there is a significant difference between the three groups also ($p<0.05$) (Table 2). But no such relationship is found in the moisture content of chelipeds (Table 2). Ash content (%) was more in male chelipeds (40.57±0.91) than in female (37.06±0.39) and juvenile (16.67± 0.55). The Ash content of abdomen muscle of three groups are 07.37±0.27 for juvenile, 10.29±0.12 for male and 8.64±0.28 for female which very significantly between the three groups ($p<0.05$) (Table 2), however no such difference ($p>0.05$) is observed in chelipeds of these groups. Between abdomen and cheliped the variation in ash content is statistically highly significantly within the groups ($p>0.001$, t 24.61 for juvenile, 33.06 for male and 32.38 for female, $t_{0.001}$ 8.64, df = 4). Higher amount of ash content in male cheliped is due to the heavier mineralization since they possess robust chela.

Table 1. Moisture, ash, calcium and phosphorous content (%) in the abdomen and chelipeds of juvenile, male and female *Macrobrachium nobilii*

Sample	Juvenile	Male	Female
		Moisture (%)	
Abdomen Muscle Chelipeds	72.12±1.32 59.01±1.85	78.17±1.07 61.18±2.01	79.39±1.18 58.72±1.87

	Ash (%)		
Abdomen Muscle Chelipeds	07.37±0.27 16.67±0.55	10.29±0.12 40.57±0.91	08.64±0.28 37.06±0.39
	Calcium (%)		
Abdomen Muscle Chelipeds	00.49±0.03 05.11±0.31	00.72±0.05 12.79±0.48	00.47±0.03 10.62±0.16
	Phosphorous (%)		
Abdomen Muscle Chelipeds	01.62±0.09 00.97±0.13	01.13±0.09 00.97±0.13	01.38±0.11 00.96±0.06

Table 2. ANOVA to find out the validity of relationship between the studied parameters in three chosen groups of *Macrobrachium nobilii*

Variation	SS	Df	MS	F
	Moisture- abdomen muscles			
Total	79.12	14		
Between Groups	42.10	02	21.05	6.82*
Error	37.02	12	03.09	
	Moisture-Chelipeds			
Total	80.46	14		
Between Groups	06.24	02	03.12	0.5^{NS}
Error	74.22	12	06.18	
	Ash-Abdomen muscles			
Total	25.41	14		
Between Groups	21.91	02	10.96	37.5***
Error	03.51	12	00.29	
	Ash-Chelipeds			
Total	715.7	14		
Between Groups	705.23	02	352.62	404.5***
Error	010.46	12	000.87	

*** Statistically highly significant (P<0.001) ** Statistically significant (P<0.05) NS- not significant (P<0.05)

Calcium content in the abdomen and cheliped muscle, is significantly differ between the groups (Table 3). The variation in the calcium content between abdomen and chelae also differ significantly within each group ($p<0.0011$, t 14.46 for juvenile, 25.17 for male and 61.44 for female, $t_{0.001}$ 8.61, df= 4). In juvenile *Macrobrchium nobilii* the percentage content of phosphorous is higher in abdominal muscle (1.62±0.09) than male (1.13±0.9) and female (1.38±0.11). The phosphorous content of juvenile, male and female cheliped muscle did not vary significantly ($P>0.05$). However in the abdominal muscle there is a

significant difference ($p<0.05$) in phosphorous content (Table 3). But within the groups there is a significant variation between abdomen and cheliped muscle observed only in juveniles ($p<0.05$).

Table 3. ANOVA to find out the validity of relationship between the studied parameters in three chosen groups of *Macrobrachium nobilii*

Variation	SS	Df	MS	F
Moisture- abdomen muscles				
Total	3.20	14		
Between Groups	2.16	02	1.08	12.51**
Error	1.03	12	0.09	
Moisture-Chelipeds				
Total	180.07	14		
Between Groups	172.73	02	86.37	141.25***
Error	007.34	12	00.61	
Ash-Abdomen muscles				
Total	6.99	14		
Between Groups	3.51	02	1.75	6.05*
Error	3.48	12	0.29	
Ash-Chelipeds				
Total	1.12	14		
Between Groups	4.81	02	2.41	0.03^{NS}
Error	1.12	12	0.09	

*** Statistically highly significant ($P<0.001$) ** Statistically Highly significant ($P<0.01$)
* Statistically significant ($P<0.05$) NS- not significant ($P<0.05$)

Crustaceans require high amount of minerals since there is a significant loss of there minerals during ecdysis (Huner *et al.* 1990) through the loss is partially compensated by minerals obtained from food. Hence it is recommended to include seven minerals (calcium, copper, magnesium, phosphorous, potassium, selenium and zinc) in crustacean diets (Davis and Gatlin 1996), of these calcium and phosphorous play a major role since they contribute per se to the structural components of hard tissues like exoskeleton. Apart from this, calcium is also essential for muscle function, proper nerve impulse transmission and act as a cofactor for enzymatic process (National Research Council 1993). Phosphorous is a component of a variety of organic phosphates, such as nucleotides, phospholipids, coenzymes, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Inorganic phosphates serves as buffers to maintain normal pH of intra and extra cellural fluids (Zubay 1983). Through various decapods obtain environmental calcium for their normal growth, dietary calcium also plays a supplementary role in

mineralization of exoskeleton (Deshimaru *et al.* 1987, NRC 1993) and the interactions between calcium and phosphorous has also evaluated (Brown 1995).

Unlike calcium, phosphorous concentration is very low in natural sources and hence its proper incorporation in the crustacean diet plays a major role in their nutrition (Lall 1991, Mayeaux 1988). Lochnam *et al.* (1992) reported the vital requirement of supplemental phosphorous rather than other minerals for *Procambarus clarkii.* Usually incorporation of phosphorous at a concentration of 1-2% of the diet promotes optimum growth among various decapods; however, its proper utilization also depends upon the availability of calcium.

From the available literature, it is known that both calcium and phosphorous levels vary from species to species. For instance calcium content is 0.35% in M. *dayanum* (Paul and Gupta 1995) and 26.6% in *Autacus astacus* (Welinder 1974). The phosphorous content is vary from 0.49% in *Metapeanus* spp. (Dall 1965) to 2% in *A. astacus* (Huner and Lindqvist 1985). For *M. nobilii* the calcium and phosphorous levels fall within this range. The difference in the uptake of minerals in a species is adduced to the availability of these elements in environment, age and sex of the animal (Greenaway 1985).

Acknowledgements

The first author is much grateful to CSIR-India for financial assistance in the from of Senior Research Fellowship (Extended) and Dr. A. Rajendran, Offshore Platform and Marine Electrochemistry Center, Tuticorin for his supports in chemicals analysis.

References

Boyd, C.E. and D. Teichert-Coddington, 1995. Dry matter, ash and chemical composition of pond cultured *Penacus vannamei* and *P. stylirostris. J. World Aquacul. Soc.*, 26: 88.

Brown, P.B., 1995. A review of nutritional research with crayfish. *J Shellfish. Res.*, 14: 561-568.

Chang, E.S., 1995. Physiological and biochemical changes during the molt cycle in decapod crustaceans: an overview. *J. Exp. Mar. Biol. Ecol.*, 193: 1-14.

Davis. D.A. and D.M. Gatlin, 1996. Dietary mineral requirement of fish and marine crustaceans *Rev. Fish. Sci.*, 4: 75-99

Davis, D.A., A.L. Lawrence and D.M. Gatlin, 1992. Mineral requirements of *Penaeus vannamei*: a preliminary examination of the dietary essentiality of thirteen minerals. *J. World Aquacul. Soc.*, 23: 8-14.

Deshimaru, O., K. kuroki, S. Sakamoto and Y.Yone, 1978. Absorption of labeled ^{45} Ca by prawn from sea water. *Bull. Jpn. Soc. Sci. Fish.*, 44: 975-977.

Fiske, C.H. and Y. Subbarow, 1925. The colorimetric determination of phosphorous. *J. Biol. Chem.*, 66: 375-400.

Greenaway, P., 1985. Calcium balance and moulting in the crustacea. *Biol. Rev.*, 60: 425-454.

Henderson, J.R. and G. Matthai, 1990. On certain species of Palaemon from South India. Rec. *Indian. Mus.*, 5: 277-306.

Huner. J.V., H. Kononen and O.V. Lindqvist, 1990. Variation in body composition and exoskeleton mineralization as a function of the molt and reproductive cycles of the noble

crayfish, *Astacus astacus* L. (Decapoda, Astacidae), from a pond in central Finland, Com. *Biochem. Physiol.*, 96: 225-240.

Lall, S.P., 1991. Digestibility, metabolism and excretion of dietary phosphorus. *In*: Nutritional strategies and aquaculture waste (eds. C.B. Cowey and C.Y. Cho). University of Guelph, Ontario. pp. 77-90.

Lochmann, R., W.R. McClain and D.M. Gatline, 1992. Evaluation of practical feed formulation and dietary supplements for red swamp crayfish. *J. World Aquacul. Soc.*, 23: 217-227.

Mayeaux, M.H., 1988. Nutrient requirement studies with the red swamp crayfish, *Procambarus clarkii*: phosphorus and lysine. MS thesis, Texas A & M University, College Station, Texas.

Mendez L., B. Acosta and I. Recotta, 1988. Mineral concentrations in muscle and hepatopancreas of newly caught wild and hatchery-exchausted spawners of Pacific white shrimp, *Penaeus vannamei*. *J. Appl. Aquacul.*, 8: 17-26

Passoneau, J.V. and C.M. Willimas, 1953. The molting of Cecropia. *J. Exp. Biol.*, 30: 545-560.

National Research Council (NRC), 1993. Nutrient Requirements of fish. National Academy Press, Washington D.C.

Van Loon, J.C., 1995. Selected methods of trace metal analysis: biological and environmental samples. Wiley-Interscience, Toronto, Canada. pp 1-357.

Welinder, B.S., 1974. The crustacean cuticle-I. Studies on the composition of the cuticle. *Comp. Biochem. Physiol.*, 47: 779-787.

Zar, J.H., 1996. Biostatistical Analysis. Prentice Hall, Upper Saddle River, New Jersey, USA.

Zubay, G.L., 1983. Biochemistry. Addision-Wesley, Reading, UK.

(Manuscript received 16 August 2001)

Bangladesh J. Fish. Res., 6(1), 2002: 89-97

Flavour components of some processed fish and fishery products of Japan

Mohammad Abul Mansur*, Mohammad Ismail Hossain[1], Hitoshi Takamura and Teruyoshi Matoba[2]

Department of Food Science and Nutrition, Faculty of Human Life and Environment, Nara Women's University, Nara 630-8506, Japan

[1]Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh 2202

[2]Graduate School of Human Culture, Nara Women's University, Nara 630-8506, Japan

*Present address and correspondence: Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

Abstract

A study was conducted to examine the flavour components of some processed fish and fishery products of Japan by gas chromatography-mass spectrometry (GC-MS). In brief the method was to absorb the headspace volatiles at 70°C into the fused silica fibre of the needle of the solid phase micro extraction fibre. The absorbed components were injected to the GC-MS. The components were identified by computer matching with library database as well as by authentic standard components. In general the number of flavour components were higher in the processed fish and fishery products (except frozen prawn) than that of the raw fish and prawn. The concentration (quantity) of the flavour components in processed fish and fishery products was much higher than that of the raw fish and prawn. Smoked salmon and baked salmon possessed double number of flavour components than that of the raw salmon. Smoking resulted the highest number of flavour components followed by baking (grilling) and canning, surimi products (kamaboko and chikuwa), drying and lastly salting. However, freezing and frozen storage resulted loss of flavour components in prawn.

Key words: Flavour components, Backing, Canning, Kamaboko, Salting, Smoking

Introduction

Processed fish and fishery products are characterized by their specific taste, flavour and sometimes by texture, which in general are referred to as 'sensory attributes'. Sensory attributes of the processed fish and fishery products are important criteria for consumers preference. It is more important if such processed fish and fishery products are eaten without any treatment or cooking. In such cases flavour is the most important attribute of the product. Fishery science and technology need to retain the original flavour of fish as well as to make the processing and product development perfectly so that the consumers can find these types of products with their desired flavour. Thus flavour of processed fish and fishery products are important for the consumers for their

good dietary satisfaction as well as for the fishery industries to get a good market share and consumers acceptance.

The flavour of processed fish and fishery products differ with the processing technology. Even such difference exist although prepared from the same species of fish. The same result may take place with the difference of size, area of fish catch, season of catch, storage condition etc. Therefore the experiment and research on processed fish and fishery products are necessary to specify and identify the flavour components of such processed fish and fishery products.

The early studies on the flavour chemistry of fish were on the identification of flavour components of a particular species of fish (Jones 1961, Ikeda 1980). Some investigations had been done on the quantification of flavour components (McGill *et al*, 1974). Some studies have been done on the relationship between the fat oxidation and the flavour of fish (Lea 1953, Yu *et al.* 1961, Aitken and Connell 1979, Forss 1960, Badings 1973, Meijboom and Stroink 1972). A few studies have been done on the identification of flavour components of pickled fish (Josephson *et al.* 1983). Some studies are done on the origin of fish flavour (Pokorny *et al.* 1987, Lindsay 1990). Despite such studies there is a remarkable lack of literature on the flavour components of processed fish and fishery products although such processed fish and fishery products have a long traditional history in every country, community and nation. The purpose of this study was to identify the flavour components of the processed fish and fishery products of Japan. Such data are not available in the literature (Lindsay 1990). The results of this study are expected to contribute to fill up the gap of literature / data in Fisheries Science.

Material and methods

Source of experimental materials

Smoked salmon, dried horse mackerel, salted pacific mackerel, canned sardine, canned tuna meat, kamaboko and chikuwa were bought from a departmental store at Nara city of Japan. Baked salmon was bought from a fish shop. Tiger prawn was bought form a fish shop at Nara city in chilled condition which after bringing to the laboratory was frozen at -20°C in the deep freeze chamber of a laboratory refrigerator. The experimental materials were bought with few days interval (as fresh materials) immediately before the experiments were conducted instead of buying all items together and storage in the laboratory except frozen tiger prawn.

Sample preparation

For smoked salmon, dried horse mackerel, salted pacific mackerel, canned sardine, the muscle was separated by scissor, forcep, knife and cut into small pieces from at least three samples. Canned tuna meat, kamaboko, and chikuwa were cut into small pieces directly as they do not contain skin or shell. Frozen tiger prawn was thawed at room temperature in the laboratory inside a polyethylene packet. After thawing shell was

removed and the muscle was cut into as small pieces as the grains are. For all of the experimental materials at least three specimen were used for sample preparation.

Extraction of headspace volatiles

Immediately after sample preparation 5 g of experimental material was weighed in 20 ml vial (Perkin Elmer) and it was sealed with teflon lined rubber septum to make the vial air tight. This vial containing the sample was heated in an automated headspace sampler at 70°C for 30 minutes to allow the volatile flavour components evaporate from the sample but remain in the vial. The needle of the SPME (Solid Phase Micro Extraction) fibre holder (Spelco) was pierced through the septum and the flavour components were extracted to SPME fused silica fibre (Carboxen-PDMS) for 5 minutes. The fused silica fibre of the needle of SPME was then retracted and the needle was taken out of the vial. Before the extraction of each sample's flavour components the SPME fused silica fibre was conditioned by thermal desorption in GC column through the injection port of the GC-MS. Such blank analysis was done to make sure that the fibre does not contain any other volatile component before the extraction of sample's flavour components. In some cases it was necessary to do blank analysis twice or thrice to make the SPME fused silica fibre free from any component.

Gas Chromatography-Mass Spectrometry (GC-MS)

The flavour components extracted into the fused silica fibre of SPME needle were injected and thermally desorbed for 5 minutes to the capillary column DB 624 (60 m×0.322 mm ID, 1.80 μm film thickness) through the injection port of GC-MS (Shimadzu QP 5050A). The desorbed components were subjected to GC-MS analysis under standard conditions. The mass spectrum of each peak of GC was analysed by the Mass Spectrometer and the components were identified by computer matching of mass spectra of the components with those of the data stored in the mass spectral data base (NIST). In each case the component of highest possibility is reported. Result of each experiments were checked in a subsequent set of experiments.

Analytical conditions

Capillary column DB 624 (60 m×0.322 mm ID, 1.80 μm film thickness) was used. Helium was used as carrier gas. The analytical conditions were as follows;

Oven temperature 40°C, Oven equilibration time 3 minutes, Injection temperature 280°C, Interface temperature 230°C, Column pressure 35.0 (KPa), Column flow 1.5 (ml /min) and linear velocity 30.7, split ratio 25, total flow 40.0 (ml /min), carrier flow 40.0 (ml/min). Mass range (40-350 m/z). Scan interval (0.50 sec), threshold (5000), scan speed 1000 amu/ sec.

Confirmation of results

To confirm the results of these experiments another set of experiments was conducted by the standard authentic components (Nacalai Tesque). The experimental methods and analytical conditions were same as for the processed fish and fishery products of this research study except heating at 70°C for 30 minutes. The results obtained from GC-MS analysis by using authentic components were compared with those of the previous results to confirm the findings of this research as well as to sort out the unusual components and peaks resulted from unknown source etc.

Results

The flavour components identified in processed fish and fishery products in this investigation are listed in Table 1. Corresponding chromatograms are shown in Figs 1& 2. The number and concentration of the flavour components of processed fish and fishery products were obtained to be higher except in frozen prawn than those of the raw fish and prawn identified in our previous investigations. Among the 26 components identified in the present research study majority were aliphatic hydrocarbons (alkane, alkene, cyclic hydrocarbons); some were carbonyl compounds (aldehydes, ketone); some were alcohols, an organic acid and two were aromatic compounds according to their molecular structure. The flavour components may also be grouped according to their molecular weight. Most of them were of molecular weight less than 100, some are of molecular weight between 100 and 150; and a few above this figure. Some of the flavour components were originally present in the raw fish while the rest of the components were formed during processing. In general processed fish and fishery products possessed higher number of flavour components and the concentration of each flavour components in processed fish and fishery products are much higher than those of the raw fish expect frozen prawn. The concentration of each flavour component of the processed fish and fishery products are shown in Table 1 as peak area (total number of ions).

Smoking of salmon resulted the highest number of flavour components followed by baking of salmon (grilled salmon) and canning of sardine, surimi products (kamaboko and chikuwa), drying of horse mackerel, salting of pacific mackerel. However, freezing and frozen storage of prawn caused the loss of flavour components.

Discussion

Among the identified flavour components of processed fish and fishery products majority were originally present in raw fish and prawn which was identified in our previous investigation. Some more flavour components were identified in processed fish and fishery products which may be the result of processing except in freezing and frozen storage (Lindsay 1990). The concentration of the flavour components of processed fish and fishery products was comparatively much higher. Two reasons may lay behind

Table 1. List of the flavour components (with their quantity in terms of peak area as $\times 10^5$) identified in the processed fish and fishery products of Japan (Corresponding chromatograms are shown in Figs. 1 and 2)

No.	Component name	Retention time	Smoked salmon	Baked salmon	Dried horse mackerel	Salted Pacific mackerel	Canned sardine	Canned tuna meat	Frozen tiger prawn	Surimi products	
										Kamaboko	Chikuwa
1	Ethanol	3.15	80			17		4490		10640	11792
2	Trimethylene oxide	4.27	210								6
3	Propanal	4.29		270	294	570					
4	Acetic acid, anhydride	4.62	326	789	319						
5	2-methyl-Pentanal	4.64									3
6	Acetone	4.67					898	801	1736		
7	Dimethyl sulfide	4.86					496	954			
8	2-methyl-Propanal	8.7	250	319			161	327			
9	2-Butanone	13.23	228	624	57		314	907			
10	Ethyl acetate	13.95								497	553
11	3-methyl-Butanal	20.06	449	256			226				154
12	2-methyl-Butanal	21.4					239				
13	2-ethyl-Furan	24.2					681				
14	1-Butanol	24.29						324			
15	1-Penten-3-ol	26.45	641			1321					
16	Cyclopentanol	26.61		241	783		184			265	
17	Cyclobutanemethanol	26.62						122			
18	Toluene	31.58	311							1701	
19	Octane	32.37		24							
20	Hexanal	34.45	388	579	759	949	68	122		1821	494
21	Cyclopentanone	34.7	85								
22	Ethylbenzene	36.63	28.7								
23	Nonanal	37.75									32
24	1-Hexanol	37.86			15	28				82	
25	2-Heptanone	28.42								75	
26	Heptanal	28.65	213	524	363	108				146	53

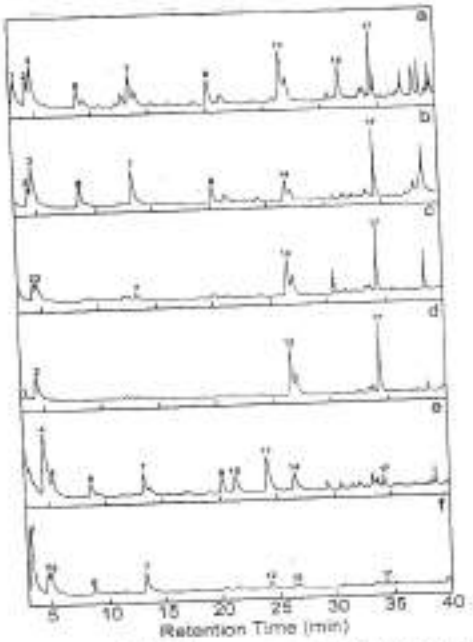


Fig. 1. GC-MS Chromatograms of the flavour components of (a) smoked salmon, (b) baked salmon, (c) dried horse mackerel, (d) salted pacific mackerel, (e) canned sardine, (f) canned tuna meat.

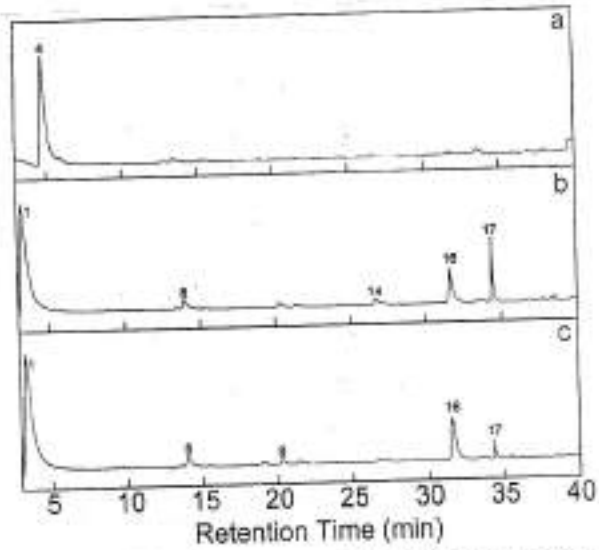


Fig. 2. GC-MS Chromatograms of the flavour components of (a) frozen tiger prawn, (b) kamaboko, (c) chikuwa.

this fact. One is that such components are further increased as a result of biochemical pathways of protein and fat of fish (Pokorny 1980). Another reason may be the concentration (quantity per unit mass) of such flavour components was found to be much higher in GC-MS analysis because the moisture content is normally reduced during processing which resulted a higher concentration of flavour components in the final product. Any one or both of the reasons are responsible for such phenomenon except during freezing and frozen storage.

During the process of smoking and baking of salmon the predominant cause of higher number of flavour components in the final product is the deposition or settling of smoke components to the fish. Biochemical changes due to slightly higher temperature may also partially contribute to the production or formation of such flavour components (Josephson and Lindsay 1987). Three undesirable components were detected in smoked salmon and baked salmon. The undesirable components are octane, ehtylbenzene and toluene. These are graded as undesirable because their role in human body or their biofactors are not known. Neither the muscle nor the skin of raw salmon contain octane, ehtylbenzene and toluene. During our previous investigation on raw fish and prawn it was found that the muscle and skin of raw salmon do not contain octane, ehtylbenzene and toluene. It appears that the smouldering by the use of special type of wood, wood shave, saw dust, straw and acceleration of smouldering by the use of octane produced a considerable fraction of smoke components of toluene, ethylbenzene and octane which continuously settled on fish during 'fish smoking' and 'fish baking' process. Ehtylbenzene and toluene may be resulted from the thermal degradation of materials (wood, straw, saw dust) used for smouldering.

During the process of canning some flavour components formed as a result of nonenzymic browning reactions during heat processing step of canning. Such enzymic activities may resulted the changes in protein and fat which finally formed some flavour components. It is also possible that some flavour components were formed during heat processing step of canning due to the effect of heat on the ingredients used in canning e.g. oil, tomato sauce, (Pokorny 1980). However the possibility of such contribution of ingredients to flavour of canned fish used in the present investigation is soybean oil because the experimental material was canned sardine with soybean oil.

In the dried horse mackerel the flavour components were formed probably as a result of oxidation of fat as well as enzymic hydrolysis of the original components of fish e.g. protein, fat. The drying process of horse mackerel is sun drying for only 3-5 days. Sometimes antioxidants are used during drying to prevent high degree of oxidation. Short period of drying results soft texture compared to the complete drying of fish by 7-10 days. In the completely dried fish the number of flavour components are usually higher than that of the dried horse mackerel, used in the present study, which is partially dried.

Similar type of result obtained in surimi based products e.g. kamaboko and chikuwa. During mincing of fish after bone separation and during texture formation steps of surimi products the enzymic hydrolysis of the original components of fish e.g. protein, fat resulted the formation or biogeneration of flavour components in surimi products. A

certain degree of oxidation may also be responsible for the phenomenon. Thermal condition may accelerated retro-aldol degradation of unsaturated aldehydes which lead to altered flavour in these products (Joseophson and Lindsay 1987).

In case of salted pacific mackerel (Shio saba) the number of flavour components was comparatively less than the expectation. Because the pacific mackerel is fatty fish and salting process should give rise to the production or formation of large number of flavour components. But the salted pacific mackerel used in the present investigation was salted in slightly different but modern way. It was realized that the sample bought from departmental store was salted at chilling temperature and ratio of salt : fish was about 1: 20 (1 part of salt for 20 parts of fish), and the process continued only for 2-3 days at chilling temperature (0-4°C). This is why the number of flavour components was comparatively less than the expectation. The reason behind the formation of flavour components in salted pacific mackerel may be the oxidation of fat. Prokorny (1980) has reported such browning reactions of oxidized fat.

In almost all of the processing and storage technique the process resulted an increase in the number and concentration of flavour components. However the opposite type of result was obtained in freezing and frozen storage of prawn. Freezing and frozen storage resulted loss of two flavour components (Dimethyl sulfide and hexane). However, another component (acetone) was identified in frozen stored prawn after thawing at room temperature during the present investigation. The concentration of acetone was also much higher in thawed prawn than that of the fresh raw prawn. Loss of flavour components during freezing and frozen storage of prawn may be the condensation of volatile flavour components due to low temperature (-20°C) and leaching out during thawing. The high concentration of acetone in frozen stored and subsequently thawed prawn indicates that this component may be further formed either during storage or during thawing.

From the results obtained in this research study it can be concluded that some flavour components are formed during processing and storage of fish except freezing. Such phenomenon may be influenced by the differences in processing technique, storage technique etc.

Acknowledgements

This research was carried out by the financial support of JSPS (Japan Society for the Promotion of Science), Ministry of Education, Japan.

References

Aitken, A. and J. J. Connell, 1979. Fish. *In*: Effects of Heating on Foodstuffs (ed. R.J. Pristly), Applied Science Publishers, Essex. pp. 238-245.

Badings, H.T., 1973. Fishy off-flavours in autoxidised oils. *J. American Oil Chemists Soc.*, 50: 334-339.

Forss, D.A., E.A. Dunstone and W. Stark, 1960. Fishy flavour in dairy products. *J. Dairy Res.*, 27: 211-216.

Ikeda, S., 1980. Other organic components and inorganic components. *In*: Advances in Fish Science and Technology (ed. J. J. Connell). Fishing News Books, London. pp. 111-123.

Jones, N.R., 1961. Fish Flavours. *In*: Proceedings of the Falvour Chemistry Symposium. Campbell Soup Company. Camden, New Jersey. pp. 61-79.

Josephson, D.B. and R.C. Lindsay, 1987. Retroaldol degradations of unsaturated aldehydes: Role in the formation of C4-heptanal from t_2, C6-nonadienal in fish, oyester, and other flavours. *J. Food Sci.*, 64: 132.

Josephson, D.B., R.C. Lindsay and D. A. Stuiber, 1983. Identification of compounds characterizing the aroma of fresh whitefish (*Coregonus clupeaformis*). *J. Agricul. Food Chemistry*, 31: 326-330.

Lea, C.H., 1953. Recent developments in the study of oxidative deterioration of lipids. *Chemistry and Industry* (London), 49: 1303-1309.

Lindsay, R. C., 1990. Fish Flavours. *Food Reviews International*, 6(4): 437-455.

McGill, A.S., R. Hardy, J.R. Burt and F.D. Gunstone, 1974. Hept-cis-4-enal and its contribution to the off-flavour in cold stored cod. *J. Sci. Food and Agricul.*, 25: 1477-1481.

Meijboom, P.W. and T.B.A. Stroink, 1972. 2-trance, 4-cis, 7-cis-Decatrienal, the fishy off flavour occurring in strongly autoxidised oils containing linolenic acid or 3, 6, 9 etc fatty acids, *J. American Oil Chemists Soc.*, 49: 72-79.

Pokorny, J., W. Janitz, I. Viden, J. Velisek, H. Valentova and J. Davideck, 1987. Reaction of oxidised lipids with protein. Part 14. Aldolization reaction of lower alkanals in presence of nonlipidic substances. *Die Nahrung.*, 41: 63-70.

Yu, T.C., E.A. Day and R.O. Sinnhuber, 1961. Autoxidation of fish oils, I. Identification of volatile monocarbonyl compounds from autoxidised salmon oil. *J. Food Sci.*, 26: 192-195.

(Manuscript received 13 August 2001)

Bangladesh J. Fish. Res., 6(1), 2002: 99-106

Investment in fish seed multiplication farms in Bangladesh: Evidences of an attractive business

M. Tofazzal H. Miah*, S. Siddique, R.H. Sarwer and M.A. Mazid[1]
Department of Agricultural Economics, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
[1]Bangladesh Fisheries Research Institute, Mymensingh 2001
*Corresponding author

Abstract
This study mainly evaluated the profitability of Fish Seed Multiplication Farms (FSMFs) having hatchery, nursery and hatchery-cum-nursery located in the districts of Jessore, Jhenidah and Narail in Bangladesh. The general findings of the study were that the investment in FSMFs with hatchery, nursery and hatchery-cum-nursery was highly profitable business. The results clearly indicated that the investment on hatchery was the most profitable than those of nursery and hatchery-cum-nursery operations from the viewpoints of individual investors. The results of sensitivity analysis suggested that the investment in nursery farm was a risky business with 20 per cent increase in operation and management as well was production costs or 20 per cent reduction in benefits if other things remaining the same. It was also evident from the study that the investors of FSMFs had currently been facing some crucial problems, which among others are: problems of inbreeding, shortage of brood fish, incidence of diseases, unavailability of certain inputs and lack of credit.
Keywords: Fish seed, Benefit-cost ratio, Internal rate of return

Introduction

Fish culture under scientific management with hatchery produced seeds is relatively a new gesture in Bangladesh, which at the very beginning was fully dependent on seeds collected from rivers, estuaries and coastlines. Production of fish seeds in hatcheries through induced breeding initiated in the country in 1967. In the last decade, fish culture under improved management expanded rapidly. The major input in culture fishery is quality fish seed and the expansion and development of aquaculture production depend mainly on the availability of seed. In order to ensure the supply of fish seed it is essential to establish hatchery. For this purpose, the Department of Fisheries (DOF) established 110 Fish Seed Multiplication Farms (FSMFs) covering almost all the districts of Bangladesh. Private FSMFs have currently been established in different areas to the country which are also producing fish seeds and competing with government FSMFs.

However, timely supply of fish of seeds high yielding species is a precondition for fish culture in inland water bodies. FSMFs can ensure this service. The supply of stockable fish seeds does not depend only on the collection of spawn from the hatcheries

and the natural sources. But it also depend of the survivability of spawn to fingerlings in the nursery ponds. So importance of fish seed farming in the country cannot be ignored. The shortage of fish seed has been identified by various agencies as the main constraint for aquaculture development in Bangladesh. A few empirical studies (Ali *et al.* 1982, Islam and Dewan 1987) observed that pond fish production was suffering due to shortage of fish seeds. An economic study is therefore very essential of FSMFs possessing either hatching or nursery facilities or both together to understand their profitability, the production status and possibilities of increasing more production. As such no study has yet been undertaken in this regard, the present study was a moderate attempt to determine the profitability of FSMFs and problems related to fish seed business.

Methods

The present study was based on primary data of a sample survey of FSMFs under the management of private ownership. On the basis of easy accessibility and high concentration of fish seed farms, Jessore and Jhenidah districts were selected for this study. Three categories of FSMFs such as: (a) FSMFs with hatchery (b) FSMFs with nursery pond and (c) FSMFs with hatchery-cum-nursery pond were investigated in this study. Out of 47 pre-selected FSMFs, 17 were hatchery, 10 were nursery and 20 were hatchery-cum-nursery.

Three sets of questionnaires for each of the selected categories of FSMFs were prepared separately to collect relevant information. The survey of the present study covered the whole production period of 1998. The formal survey, however, was conducted during the months from March to May'99. The data so collected were then coded and data entry and analysis were done to obtain results useful in the project appraisal calculations. The whole analysis of course was done on per farm basis.

Methods of appraisal

The method of project appraisal suggested by Gittinger (1994) was followed, since it is widely used by the World Bank and also many other donor and planning agencies, for evaluating agricultural projects (Miah and Hardaker 1988). This study was limited only to financial analysis.

Most investments accrue benefits and incur costs in the future as well as in the present. The time streams of costs and benefits can vary considerably among projects. For this reason, costs incurred and benefits derived in different periods must be reduced to some common point in time, before they can be compared with each other (Riodan 1980, Miah and Hardaker 1988). In the financial analysis, all costs and benefits were determined in domestic currency using farm-gate prices.

This appraisal, however, is based on the actual field level data, rather than the planned level utilization and/or recommendations of the concerned aquaculture experts regarding the selected FSMFs. Three discounting measures namely: (i) benefit-cost ratio

(BCR), (ii) net present value (NPV) and (iii) internal rate of return (IRR) were employed in the study.

Benefits of FSMFs

The items considered under the benefits of different categories of FSMFs are as follows:

a. Gross value of production includes mainly the values of spawn and/or fingerlings of the concerned fish seed farms,
b. Return from sale of unproductive and/or old brood fish after every five years,
c. The salvage values of the concerned capital items and/or durable tools and equipment were estimated considering the views of the concerned experts, traders and their ultimate users. Salvage values of these assets have been added to the benefit stream at the last year of the project life and/or at the end of the productive life of the concerned equipment.

Cost of FSMFs

The cost of FSMFs can broadly be classified into the following heads:

a. Investment costs,
b. Operation and maintenance (O & M) costs, and
c. Production costs.

Investment cost of the selected FSMFs included cost of farm structures and building, cost of shallow tube-well (STW), cost of others tools and equipment, cost of brood fish and cost of re-excavation of ponds.

O & M costs involve cost of human labour, cost of fuel and dewatering cost, cost of electricity, cost of repairing and spare parts. These were essential for all categories of FSMFs to produce fish seeds and continuing the farm business.

Production costs associated with fish farming was calculated by taking into considerations the cost of humar labour, feed, fertilizers, chemicals, lime and miscellaneous cost and cost of hormone was also included for hatchery and hatchery-cum-nursery farms for induced breeding. On the other hand, cost of stocking of spawn was included for FSMFs with nursery and hatchery-cum-nursery.

Discount rate

The result of benefit-cost analysis is highly sensitive to the discount rate. The choice of an appropriate discount rate, therefore, plays a vital role in the appraisal of project. The selection of discount factor has become more different in developing countries because of various imperfections and distortion in capital market. However, the available literatures (Miah and Hardaker 1988, Gittinger 1994) suggest that the opportunity costs of capital in most developing countries vary between 8 to 15 percent. In this study 14 percent discount rate was chosen for the appraisal of fish seed farming projects. Other researches (Kabir 1995, Islam and Miah 1999) have also used this rate.

Results and discussion

The appraisal results of the evaluation based on the opinion of individual investors of hatchery, nursery and hatchery-cum-nursery farms are presented in this section.

Financial analysis

The calculations of financial appraisal the FSMFs were based on the following general and technical assumptions:

General assumptions:

a. A typical model of FSMFs with hatchery, nursery and hatchery-cum-nursery having 1.91 ha. 2.5 ha and 2.92 ha farm sizes, respectively and there will be no change in farm size throughout the project life.
b. All brood fish have been purchased in cash for the purpose of induced breeding for every five years.
c. It was assumed that farmers stocked all different species of brood fish at a time and the species combinations and ratio would remain the same.
d. The rate of stocking of brood fish in different ponds was assumed 1709-1870 Kg/ha depending upon the condition of fish and ponds.
e. Brood fish mortality in every five years was assumed to be nil and 5 percent were not used for breeding purpose, and spawn production was assumed not to be affected by these reasons.
f. Per unit prices of the concerned inputs and outputs are given and constant during the whole project life.

Technical assumptions

a. The most productive age of brood fish was assumed to range from 1 to 7 years for the concerned species and the same brood fish was used for induced breeding up to 7 years of age.
b. The size ranges of different species used by the model FSMFs for induced breeding were assumed 1.5 kg Rohu, 3.0 kg Mrigal, 2.5 kg Silver carp, 2.5 kg Grass carp, 2.0 kg Mirror carp, 2.0 kg Carpio and 0.5 kg Thai sarpunti.
c. The nursery owners assumed to buy 4 days old spawns, stock the spawns in the nursery pond for 2-3 months to raise up to 2-3" and sell out to the buyers.

In can be seen from Table 1 that the investments on all categories of FSMFs are profitable business. It is evident from the table that BCRs of the three categories of farms are more than the unity and NPVs are also positive at the selected discount rate and all these investments yields much higher IRR than the possible opportunity costs of capital.

Table 1. Result of financial analysis of FSMFs

Discounted measures	FSMFs with hatchery	FSMFs with nursery ponds	FSMFs with hatchery-cum-nursery
BCR at 14%	1.49	1.17	1.22
NPV at 14% (Tk 10^3)	1808.67	310.10	849.16
IRR (percent)	80.0	41.0	56.0

Source: Adopted from Siddique (1999).

It is evident from the above table that all the selected FSMFs are attractive to individual investors considering the real world situation. It is also evident from the study that FSMFs with hatchery will bring a higher profit than the FSMFs with nursery ponds and FSMFs with hatchery-cum-nursery ponds.

Sensitivity analysis

The results of sensitivity analysis show how the value of the investment criteria changes with the changes in the value of any variable in the discounted cash flow analysis. The profitability of these small-scale fisheries farm projects may be sensitive, as expected, to O & M costs, production costs and gross benefit of the project. Two factors were, therefore, taken into consideration for sensitivity analysis of FSMFs such as: (i) reducing existing benefits (other than salvages values of the concerned equipment) at the rate of 10 and 20 percent, and (ii) if O & M and production costs increase at the rate of 10 and 20 percent.

Under the changed circumstances, the financial analysis has been reworked separately in this section to see what happen in the profitability of FSMFs.

Table 2. Result sensitivity analysis of FSMFs considering 10 percent increase in O & M and production cost

Discounted measures	FSMFs with hatchery	FSMFs with nursery ponds	FSMFs with hatchery-cum-nursery
BCR at 14%	1.37	1.08	1.13
NPV at 14% (Tk 10^3)	1518.94	150.48	526.85
IRR (percent)	66	27	38

Source: Adapted from Siddique (1999).

It is evident from the results presented in Table 2 that BCRs of all the selected FSMFs are greater than unity, NPVs are positive and IRRs are higher than the opportunity cost of capital (14 percent). This implies that if O & M and production cost would increase at the rate of 10 percent, while benefits and other costs would remain the same, investment of FSMFs would still be profitable.

Table 3. Result sensitivity analysis of FSMFs considering 10 percent increase in O & M and production cost

Discounted measures	FSMFs with hatchery	FSMFs with nursery ponds	FSMFs with hatchery-cum-nursery
BCR at 14%	1.28	0.99	1.04
NPV at 14% (Tk 10^3)	1229.20	-10.04	204.53
IRR (percent)	54	13	23

Source: Adapted from Siddique (1999).

Table 3 shows that BCRs of hatchery and hatchery-cum-nursery farms are still greater than unity, NPVs are positive and IRRs are higher than the opportunity cost of capital. This situation also yields more profits to the investors of hatchery and hatchery-cum-nursery farms. On the other hand, the nursery farm could not make any profit at this changed situation. This implies that if O & M and production costs of nursery farms increase by 20 percent then it becomes a risky to invest on nursery farms.

Table 4. Result sensitivity analysis of FSMFs considering 10 percent increase in Gross profit

Discounted measures	FSMFs with hatchery	FSMFs with nursery ponds	FSMFs with hatchery-cum-nursery
BCR at 14%	1.34	1.06	1.10
NPV at 14% (Tk 10^3)	1267.27	106.84	392.81
IRR (percent)	58	24	33

Source: Adapted from Siddique (1999).

Table 4 indicates that BCRs of all categories of farms are greater than unity, NPVs are positive and IRRs are still higher than the opportunity cost of capital. This implies that if benefits would decrease at the rate of 10 percent while all costs would remain the same, investment on all categories of farm projects would still be profitable.

Table 5. Result sensitivity analysis of FSMFs considering 20 percent decrease in gross profit

Discounted measures	FSMFs with hatchery	FSMFs with nursery ponds	FSMFs with hatchery-cum-nursery
BCR at 14%	1.19	0.94	0.98
NPV at 14% (Tk 10^3)	725.43	-97.41	-63.54
IRR (percent)	38	4	11

Source: Adapted from Siddique (1999).

It can be seen from Table 5 that BCR of hatchery farm is greater than the unity, NPV is positive and IRR is higher than the opportunity cost of capital, while nursery farm and hatchery-cum-nursery are making loss at 14 percent discount rate. This implies that if gross benefit would decrease at the rate of 20 percent, while all costs remain the same, investment on only hatchery farm would still be profitable and nursery farm is more loser than the hatchery-cum-nursery. It can therefore be concluded that the BCR, NPV and IRR are highly sensitive to change in benefits of FSMFs.

Although the selected FSMFs are found highly profitable considering the real world situation, but the results of sensitivity analysis clearly hint that O & M and production cost and gross benefits, as expected, have a strong influence on the opportunity of hatchery, nursery and hatchery-cum-nursery farms.

Problems of owners of FSMFs

Fish seed production through artificial propagation or induced breeding is relatively a new practice in Bangladesh. This section indicates the major problems facing the farmers in conducting fish seed farm business. For the sake of convenience the problems and constraints faced by the selected owners of FSMFs have been categorized under three groups such as: (i) technical (ii) economic and (iii) social.

Technical problems are related to production techniques and technologies such as breeding, lack of brood fish and its management problem, non-availability of various inputs, attack of diseases infestation, insufficient water in dry season and lack of scientific knowledge and technology.

Economic problems and constraints are related to such financial considerations are lack of capital or institutional credit, problems of selling spawn or marketing facilities and high price of various inputs.

Some social problems were also faced by all categories of owners of FSMFs such as: theft of brood fish, poisoning of ponds and problems of getting pond.

The farmers put forward some suggestions for resolving these problems, which included implementation of government rules, fixation of prices of key inputs, providing social, moral and scientific education and training to the producers and improving marketing facilities.

Policy implication and conclusions

The fisheries sector must make a significant contribution and could contribute more than any development sector of Bangladesh in the form of income, employment, human nutrition and foreign exchange earning to the national economy. Several policy recommendations as emerged from the results of this study which are highlighted below:

Present level of institutional credit of fisheries is not sufficient to meet the demand for credit of the owners of FSMFs. Since these farms are profitable, financial

institutions should come forward to provide required credit to the genuine farmers for establishing new FSMFs.

- In order to meet the demand for brood fish for induced breeding production of brood fish through scientific management should be increased.
- For overcoming the inbreeding problem, the hatchery owners can exchange brood fish among their hatcheries. It is advisable to set up "brood bank" for successful hatchery operation in terms of quality of seeds.
- Government should take positive steps to train up the concerned interested people on modern methods of brood fish rearing, hatchery and nursery management.
- More emphasis should be given on nursery pond management.
- An effective mechanism for information exchange between the farmers and researchers has to be developed and maintained.

The present evaluation provides some useful information for farmers, researchers and decision-markers regarding the economic as prospects of fish seed production. The findings of the study, however, are based on the data collected from a specific area of Bangladesh. These findings should, therefore, be interpreted cautiously if any greater generalizations are sought for different regions with distinct topographies of the country. Nevertheless, fish farmers from the similar region, who have enough money and resources should come forward to invest in FSMFs, since these are highly profitable and attractive business to the investors.

References

Ali, M.H., A. Akbar and M.H. Rahman, 1982. 'Utilization of fish ponds in Mymensingh District. *Bangladesh J. Agricul. Econ.*, 5 (1 and 2): 103-114.

Dillon, J.L. and J.B. Hardaker, 1993. Farm Management Research for small Farmer Development, FAO Farm systems Management Series 6, FAO Rome

Gittinger, J.P., 1994. Economic Analysis of Agricultural projects, 2nd Edn. John Hopkins University Press, Baltimore.

Islam, M.S. and M.T.H. Miah, 1999. Socioeconomic Review of DOF/NGO Fisheries Schemes, A Consultancy report submitted to the United Nations World Food Programme, Dhaka.

Islam, M.S and S. Dewan, 1987. An economic analysis of pond fish production in some areas of Bangladesh, Research report No. 11, Bureau of Socioeconomic Research and Training, BAU, Mymensingh.

Kabir, M.H., 1995. An economic study of subsidized private dairy farming in a selected area of Bangladesh, MS Ag Econ Thesis, Bangladesh Agricultural University, Mymensingh.

Miah, M.T.H. and J.B. Hardaker, 1988. Benefit-cost analysis of deep and shallow tube-well project in Tangail district in Bangladesh. *Bangladesh J. Agricul. Econ.*, 11 (1): 29.

Riordan, P.O., 1980. An economic evaluation of alternative programs to control brucellosis in Canadian Cattles. *Canadian Farm Economics*, 15(3): 1-6.

Siddique, S., 1999. An economic analysis of fish seed multiplication farms in some selected areas of Bangladesh, MS Ag Econ. Thesis, Bangladesh Agricultural University, Mymensingh.

(Manuscript received 6 August 2000)

Notes for Authors

Bangladesh Journal of Fisheries Research considers English-language research papers, scientific notes and review articles related to all aspects of fish & fisheries science for publication. Materials must be original, unpublished work and not under consideration for publication elsewhere.

Preparation of manuscripts: Manuscript must be typed, double-spaced on one side of A4 (30 x 21 cm) paper with at least 2.5 cm margin on each side. Each manuscript should have a cover page bearing the full title, name(s) of author(s) and academic address(es), address of correspondence and a short running title of not more than 30 characters. The second page will include the full title and a concise Abstract of not more than 250 words, followed by up to five key words. Beginning from the third page, the manuscript should be arranged in the following sequences: Introduction, Materials and methods, Results, Discussion, Conclusions (if necessary), Acknowledgements (if necessary) and References. Each table and figure should be on separate page. Placement of tables, figures and photographs should be marked in the text. Scientific notes should be written with an Abstract, but not dividing up into other conventional sections. Review article on particular, on fairly wide topics of current interest, either invited or agreed with the Editor, should have an Abstract and not exceed 30 pages of type-scripts.

References: The list of references should be arranged alphabetically according to the surname of the first author. Common examples are given below:

(Book) Pillay, T.V.R., 1990. Aquaculture: Principles and Practices. Fishing News Books, Oxford. 575 pp.

(Book/ proceedings chapter) Wong, C.S., F.A. Whitney and W.K. Johnson, 1992. Application of different types of marine experimental enclosures to study the pathways and fate of chemical pollutants. *In*: Marine ecosystem enclosed experiments (eds. C.S. Wong and P.J. Harrison). International Development Research Centre, Ottawa. pp. 174-185.

(Journal article) D'Silva, J., K. Ahmed and B. Das, 1995. Resource utilization by beneficiaries in pond fish farming. *Bangladesh J. Zool.*, 23(1) : 71-76.

Tables: Tables should be numbered in Arabic numeral in the order of their mention in the text. To prepare the tables, use only horizontal lines, no vertical or diagonals should be used. Brief explanatory footnotes, if required, should be indicated with lower case superscript letters and typed at the bottom of the tables.

Figures: Figures should be numbered in Arabic numerl. Original line drawings on tracing papers or laser printed computer drawings, not requiring additional artwork and type- setting but withstandable up to 40% reduction, should be submitted. Photographs should be selected only to illustrate something that cannot adequately be displayed in any other manner, they should be in original enlargements in the form of glossy black and white prints.

Units and symbols: Metric and internationally accepted physical measurement units should be used. The 24-hour clock should be used as time of day, eg. 13.45 h, not 1.45 pm.

Submission: For the first time, two copies of the manuscript can be sent to The Editor, *Bangladesh Journal of Fisheries Research*, Bangladesh Fisheries Research Institute, Mymensingh-2201, Bangladesh. Fax: 880-91-55259, E-mail: fsbfri@bdonline.com. Papers will be reviewed by one or more experts in the relevant discipline and evaluated by the Editorial Board for publication. Any paper deemed to be of inadequate quality or inappropriate format for the Journal may be returned to author(s) without review. The cover letter must be signed by all authors in case of multiple authorship.

Printing charge: Taka 1000 will be charged from in-country author(s) for accepted paper.

Proofs: Proofs will be sent to the corresponding author and should be returned to the Editor within 7 days of receipt. Alterations in proofs other than the correction of typesetter's errors are discouraged.

Reprints: Author(s) will receive 10 free reprints of the published article.

Bangladesh J. Fish. Res., **6**(1), 2002

Contents