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Histological distribution and ultrastructure of exocrine pancreas in Indian major carp (*Labeo rohita* Ham.) and its alteration in aflatoxicosis

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Abstract
The distribution pattern of exocrine pancreas in *Labeo rohita* besides its general location along the course of intestinal mesentery was studied. It is evenly distributed within the liver around portal vessels and also within the spleen near a blood vessel. On ultrastructure, two cell types of different degrees of staining intensities containing abundant rough endoplasmic reticulum, mitochondria, pre-zymogen and zymogen granules were marked. During aflatoxicosis, the mesenteric pancreas and hepatic pancreas were mostly affected revealing necrotic changes to acini. The zymogen granular activities were markedly reduced. Ultrastructurally, the rough endoplasmic reticulum were fully dilated and formed whorled pattern. The damage to the exocrine pancreas might be affecting digestive enzymes' secretion which may be one of the cause of aflatoxin-induced anorexia in fish.

Key words: *Labeo rohita*, Histology, Ultrastructure, Aflatoxin, Exocrine pancreas

Introduction

The distribution of pancreatic tissue varies considerably with species and also even within a single species. The most common sites for it are in the mesentery of pyloric caeca as scattered islands of secretory tissue interspersed among the fat cells, as an external layer around the hepatic portal vein and sometimes, in the subcapsular area of the spleen . The acinar pancreatic tissue in a highly active exocrine organ, which produces digestive enzymes like lipase, amylase, trypsinogen and chymotrypsniogen. These enzymes are stored in brightly staining eosinophilic granules within the acini. The enzymes on their release after the damage to acini cause damage to surrounding tissues (Roberts 1989). In spite of these functions, this organ is yet to be studied for its distribution in many species and their alterations in various diseases. The normal fine structure of exocrine acini in Indian major carp are yet to be elucidated.

Aflatoxins are a group of extremely toxic metabolites produced by some strains of the ubiquitous fungi *Aspergillus flavus, A. parasiticus* and *A. nomius* grown on agricultural products under suitable conditions of temperature and moisture. Aflatoxins are best

collected at the end of the experiment. The tissues were preserved in 10% phosphate-buffered formalin and processed for light microscopy using standard method.

Transmission electron microscopy (TEM)

Similarly, the tissues were collected from 5 randomly selected fish from all the groups immediately after anaesthetization with MS 222 at the end of the experiment, sliced and minced (1 mm^3) in chilled fixative (3% glutaraldehyde in phosphate buffer, pH 7.2) and fixed in fresh fixative solution for 24 hour at 4°C. They were washed in 3 changes of phosphate buffer solution for 30 min at 4°C, dehydrated in graded series of acetone and infiltrated in araldite resin (CY-212, Polysciences, Inc, Warrington). Semithin (1-2µm) sections from randomly selected blocks of each liver were cut, stained with toluidene blue for 30 seconds and examined. Ultra thin sections (60-70 nm) from not less than 3 selected blocks of each tissue were cut, mounted on copper grids and stained with aqueous uranyl acetate and lead citrate before examining under JEOL TEMSCAN-100 CX II analytical electron microscope at 60 kv.

Results and discussion

The distribution pattern of exocrine pancreas of healthy normal fish which had not received any treatment was studied. Like other species, the pancreas was scattered in the mesentery of pyloric caeca surrounded by fat cells. Besides, it was also abundant around periportal vein as if it follows the major portal vein tract (Fig.1). Surprisingly, it was also noticed in the subcapsular splenic tissue closely associated with splenic ellipsoids, red pulp and white pulp (Fig.2). Similar locations of spleniic tissue have been recorded in few species of fish earlier (Roberts 1989). On semithin sections, intensely-stained as well as lightly-stained cell types, both packed with secretory granules were marked (Fig. 3). The two cell types were distinctly differentiated on their staining character. Abundant rough endoplasmic reticulum, glycogen, mitochondria, free ribosomes, lysosomes, rounded nucleus with nucleolus along with two types of cytoplasmic granules (one deeply osmiophilic, i.e zymogen, and the other is poorly osmiophilic, i.e prezymogen) were observed on electron microscopy (Fig. 4) in the acinar cells. The control group fish had almost similar structures on light and electron microscopy without any marked differentiation.

Fig. 1. Liver of normal rohu showing the exocrine Pancreas (arrow) around the portal vein (H & E X197).

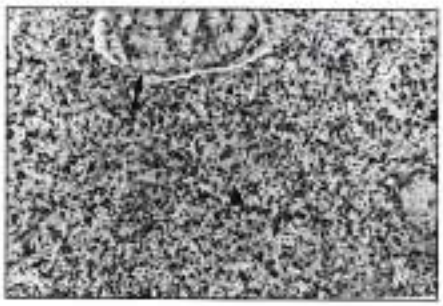

Fig. 2. Cross section of normal spleen revealing exocrine pancreas (arrow) & splenic ellipsoids (arrow head) (H & E X315).

Fig. 3. Semithin section of control mesenteric pancreas showing normal cytoplasmic granularity (Toluidene blue X800).

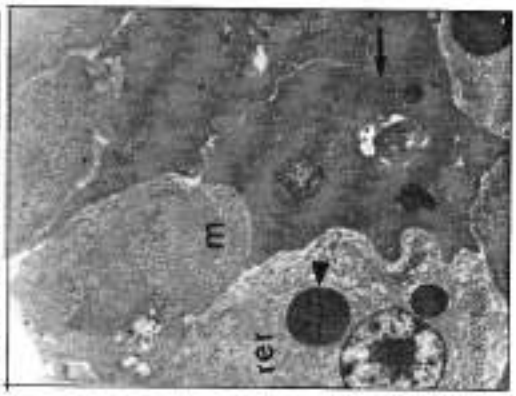

Fig. 4. TEM section of normal pancreatic acinar cell of fish with abundant rough endoplasmic reticulum (rer), mitochondria (m), nucleus with nucleolus, prezymogen (arrow) and zymogen (arrow head) granules within two cell types (EM mag. X4350).

The aflatoxin-treated fish showed dose-related changes in the liver, spleen and exocrine pancreas. On necropsy, pale yellowish, enlarged and mottled liver were the most common findings in toxin-treated fish. The spleen was mildly congested. Similarly, Chavez-Sanchez *et al.* (1994) observed subcapsular focal congestion and hepatomegaly in tilapia fed with 3 ppm of aflatoxin for 25 days. There was no gross abnormality in the pancreatic tissue of toxin-treated fish.

On histology, the liver developed clear preneoplastic nodule to hepatocellular adenoma and there was massive lymphocytolysis in the spleen of toxin-treated fish which has been described in detail in our earlier study (Sahoo *et al.* 2000). The acini of pancreas located within the liver were mostly affected and found to be necrotic. The mesenteric pancreas also had necrotic acini (Fig.5) and congested blood vessels in the vicinity (Fig.6). Jantrarotai *et al.* (1990) also observed acinar necrosis during acute aflatoxin-toxicity in channel catfish. On the contrary, Chavez-Sanchez *et al.* (1994) marked pancreatitis in aflatoxin-fed Nile tilapia. The greater degree of damage to periportal pancreas might be due to its location as because liver is the main target organ of aflatoxicosis. Ashley (1965) reported that rainbow trout force-fed with high doses of aflatoxins had hypertrophic acinar cells and at times markedly desquamated and with focal hyperemia in the visceral fat. There was also marked reduction of cytoplasmic granules observed in semithin sections (Fig.6) indicating the decreased activity of pancreas which might be indicative of rendering less synthesis of pancreatic digestive enzymes there by hampering digestion and subsequent anorexia as observed in our earlier study (Sahoo *et al.* 1998).

Fig. 5. Necrotic acini of exocrine pancreas in aflatoxin -treated fish (H & E X197).

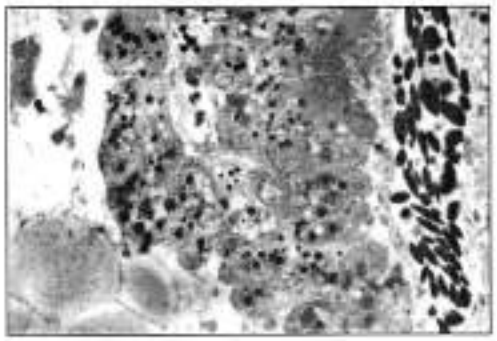

Fig. 6. Semithin section of aflatoxin-treated exocrine pancreas. Note decreased cytopla -smic granularity and engorged blood vessels in the periphery (Toluidene blue X1000).

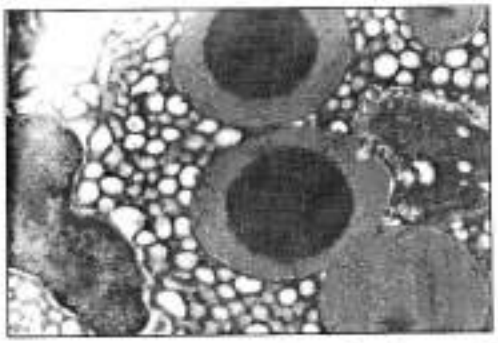

Fig. 7. TEM section of aflatoxin-treated pancreatic acini with dilated rough endoplasmic reticulum, condesed nucleus and another type of granules with osmiophilic core (Mag. X10,800).

On electron microscopy, the rough endoplasmic reticulum were markedly dilated (Fig. 7), sometimes forming whorled-pattern. The dilated rough endoplasmic reticulum was also characteristic in the AFB1-induced hepatocellular neoplasms of rainbow trout (Nunez *et al.* 1991). The granules were mostly of prezymogen stage and many granules with osmiophilic core (Fig.7) were also found. The nuclear chromatin was somewhat condensed with poorly defined nucleolus and irregular shaped nucleus was marked before death. The typical changes observed on TEM might be indicative of toxicosis and exposure to carcinogens (Ghadially 1982).

In conclusion, the present study established the distribution pattern of exocrine pancreas in rohu. This study also showed that aflatoxin-induced pancreatic damage may be one of the important factor of reduced feed intake and growth in aflatoxin exposed fish.

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Binding of radio-iodinated gonadotropin to the ovary of exotic carp (*Cyprinus carpio* Lin.)

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Abstract
Heterologous murrel gonadotropic hormone (m-GtH) binds to common carp oocyte plasma membrane and enhances steroid secretion. With increasing concentration of radio-labelled hormone the receptor binding is also found to increase linearly up to a certain concentration and then decrease. The [^{125}I] murrel GtH binding characteristics to a preparation of common carp ovarian plasma membrane shows saturability with high affinity. Scatchard plot analysis gave dissociation constant (K_d) of 0.81 X 10^{-9}M and maximum binding capacity (MBC) of 22.05 f mole/mg protein.

Key words: *Cyprinus carpio*, Radio-iodinated gonadotropin, Ovary

Introduction

Hormones, the messenger molecules of the body that help coordinating the actions of various tissues, produce a specific effect on the activity of cells, remote from their point of origin. Initial step for any hormone action is its binding to specific receptor which leads to cellular activation. So for hormone action analysis number, affinity and characteristics of binding sites of hormone receptor is considered. As a peptide hormone, GtH has its receptor in the plasma membrane of gonadal tissue. After its binding with specific receptor GtH helps in steroid secretion which regulate gonadal growth, maturation and ovulation or spermiation. For artificial spawning of fish LH-RH analogue or HCG are commercially used because the biological activity of piscine gonadotropin can be shared by LH or HCG but not by FSH (Yamazaki and Donaldson 1968, Sundararaj *et al.* 1976, Pickford *et al.* 1972, Nayyar *et al.* 1976, Mukherjee and Bhattacharya 1982) which indicates the specific receptor similarity of mammals and fish.

The present concept of Gonadotrophic hormone (GtH) action suggests that the target cell responsiveness is a function of availability of hormone specific receptor as well as the efficiency by which the hormone-receptor interaction is mediated within the target cell. For a seasonal breeder the number of receptor varies with the cyclic activity of gonad. During the preparatory, pre-spawning and spawning phase of a fish the receptor numbers increase consecutively whereas in post-spawning phase very low number or absence of receptors has been found (Manna and Bhattacharya 1993). During oogenesis

there are developmental changes of GtH-receptor properties in the ovarian follicles of Amago salmon (Kanamori *et al.* 1987). Specific binding (SB) of radio-iodinated fish GtH binding sites have been demonstrated in the ovaries of chum salmon, *Oncorhynchus keta* (Van der kraak and Donaldson 1982, Van der kraak 1983), amago salmon, *O. rhodurus* (Kanamori *et al.* 1987) and in the testis of goby, *Glossogobius olivaceus* (Aida and Ishii 1989).

The in-vitro binding studies have been observed in the catla, murrel and climbing perch but no work on common carp, *Cyprinus carpio* has been reported yet. So, the present study was concentrated on determining the binding pattern of heterologus GtH to the ovarian tissue of exotic carp, *Cyprinus carpio*.

Materials and methods

Experimental animal

Adult female, *Cyprinus carpio var. communis* (Lin.) was used in the binding experiments. The fishes weighed between 800-1200 g and were 28-30 cm in length. The experiment was conducted for a period of four months between July and October'97 in the Laboratory of the Department of Zoology, Bisva-Bharati University, India.

Preparation of oocyte membranes

The European carp, *Cyprinus carpio* were collected alive from the market and killed by decapitation in a cold room (4-6°C) and ovaries were removed surgically from the abdominal region. Ovaries were immediately placed in a sterile petri-plate containing ice cold fish oocyte culture medium, as described earlier by Mukherjee and Bhattacharya (1982). With the help of fine scissors and non-serrated forceps meso-ovarian cutting was cut from posterior to anterior region and tunica albuginea and germinal epithelium was removed very cautiously resulting in loose and free suspended mass of oocyte within the medium.

After isolation, oocytes were washed three or four times with chilled oocyte culture medium. Oocytes were then homogenized (1g/5ml) very gently at 0-4°C in sodium-phosphate buffer (0.01 M, pH 7.5) in a teflon coated glass homogenizer for about 5 minutes under ice. The homogenate was passed through double layered sterile cheese cloth to remove fat and cell debris and subjected to centrifugation in a refrigerated centrifuge at 1000g (3431 rpm) for 10 minutes.

Oocyte plasma membrane was then prepared using the method of Birnbaumer and Swartz (1982). The protein content of membrane preparation was measured according to the method of Lowry *et al.* (1951) using bovine serum albumin (BSA) (Fraction V, Sigma Chemical Co., St. Louis, A) as the standard.

Hormones

Purified murrel gonadotropic hormone (mGtH) was supplied by Endocrinology Laboratory, Department of Zoology, Visva-Bharati University, Santiniketan, India

specific binding (SB), which was done by subtracting the non specific binding (NSB) from the total binding (TB). Fig. 1 shows that specific binding of [^{125} I] murrel GtH increased with increasing concentration of oocyte membrane preparation. Binding increased linearly from 1 ng to 4 ng of [^{125} I] murrel GtH and reached saturation at 5 ng of iodinated murrel GtH under the present incubation system. The data from saturation experiment was used for scatchard plot analysis. It could be seen from Fig. 2 that the maximum binding capacity (B-max) of oocyte membrane preparation was 22.05 f mole/mg protein and high affinity GtH binding sites with a K_d of 0.81 X 10^{-9} M.

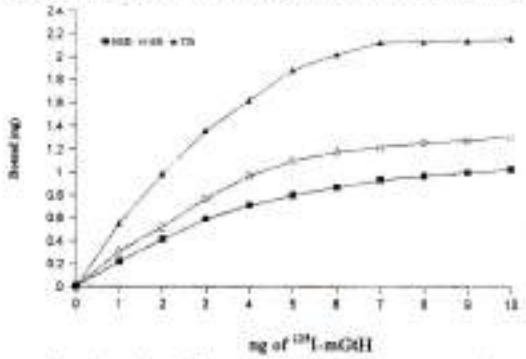

Fig. 1. Displacement curve showing the effect of increasing concentration of murrel gonadotropin (^{125}I-mGtH) on its binding to common carp oocyte membrane preparation.

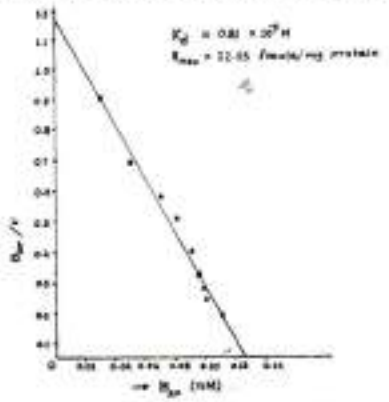

Fig. 2. Scatchard plot of ^{125}I-labelled merrel gonadotropin (^{125}I-mGtH) binding to *Cyprinus carpio* ovary. Scatchard plot analysis of mGtH was made from the data presented in Fig. 1.

Discussion

Hormone acts via its binding with the receptor and therefore the function of a hormone depends on its binding characteristics with the receptor. To understand the biological activity of hormone it is important to know the pattern of hormone receptor complex which imparts biological activity.

In the present investigation we used heterologous radio-labelled piscine GtH (^{125} I-murrel GtH) for receptor binding assay with fairly pure plasma membrane preparation from common carp oocyte. Standardization of common carp GtH receptor binding assay

was followed as published by Manna and Bhattacharya (1993). A linear increase in receptor binding with increasing concentration of radiolabelled hormone was obtained and a scatchard analysis of the plot revealed that the affinity of receptor binding was high ($K_d = 0.81 \times 10^{-9}$ M). The value obtained was compared with the results of published by other workers. Jamaluddin and Bhattacharya (1986) observed heterologous binding of catla GtH to murrel oocyte plasma membrane receptor and obtained maximum binding capacity of 6.27 f mole/mg protein of murrel ovary but Manna and Bhattacharya (1993) obtained a different result because of the use of homologous GtH and oocyte preparation ($K_d = 0.86 \times 10^{-10}$ M). In our result we obtained maximum binding capacity (MBC) of 22.05 f mole/mg protein of common carp oocyte.

Binding experiments were conducted with fairly pure plasma membrane preparation where the amount of receptor/mg tissue taken was more than the crude homogenate preparation as was used by majority of the investigators (Van der kraak and Donaldson 1982). Therefore, due to the receptor enriched plasma membrane preparation maximum binding capacity (MBC) has increased considerably (22.05 f mole/mg protein) while 0.88 f mole/mg of tissue was observed for trout testes (Schlaghecke 1983) and 0.24 f mole/mg of tissue for goby testes (Aida and Ishii 1985).

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Niche measures and feeding strategies of *Barbodes gonionotus* Bleeker and *Oreochromis* spp. from a ricefield in Bangladesh

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Abstract
Intra-and interspecific virtual niche measures and feeding strategies of *Barbodes gonionotus* and *Oreochromis* spp. were studied from a ricefield in Bangladesh. Appropriateness and ease of interpretation of different indices were evaluated. Small sizes of both species had a relatively wider dietary breadth and used many of the resource categories available to them than the large size groups, though none were generalistic feeder. The dietary overlap of large *B. gonionotus* on the small was greater than the reverse, but biologically insignificant. While the dietary overlap of large *Oreochromis* spp. on the small was significantly greater. Interspecific dietary width was relatively broader for *B. gonionotus* than *Oreochromis* spp. and overlap of *B. gonionotus* on *Oreochromis* spp. was significantly greater than the reverse. Evidence of significant intraspecific dietary overlap between the two sizes of tilapia reflects strong competition and cautions for mixed-size stocking in rice-fish system. Besides, there seems fewer opportunities for habitat segregation between *B. gonionotus* and *Oreochromis* spp., due to the significant interspecific dietary overlap of the former on the latter in rice-fish system. Tilapia specialized on periphytic detrital aggregate while silver barb tended to consume aquatic macrophytes and molluscs. Small sizes of *B. gonionotus* should be preferred for rice-fish integration over the *Oreochromis* spp. due to their broader niche width and pronounced ontogenetic dietary shifts with the aging of the stock.

Key words: Silver barb & tilapia, Intra- & interspecific niche indices, Rice-fish system

Introduction

Concurrent rice-fish farming is being practiced in most of southeast Asia, stocking one or many species of fishes with little or no knowledge of resource partitioning, foraging habits and strategy, dietary breadth and overlap. Therefore, fish yield is generally low.

Dietary overlap is affected by food availability, competition, and the size of the fish, among other factors. Though fish may broaden their dietary breadth when food resources are scarce, food items may remain sufficiently partitioned for competition to be avoided (Keast 1977, Keast and Fox 1990). Intraspecific niche overlap decreases with ontogenetic shifts in diet, i.e. differences in habitat utilization by young and adults and increasing disparity in size (Pen *et al.* 1993, Esteves and Galetti 1995).

The silver barb or tawes, *Barbodes gonionotus* Bleeker (formerly *P. javanicus*) and the tilapias [*Oreochromis mossambicus* (Peters) and *O. niloticus* (Linnaeus)] are widely stocked in the southeast Asian rice-fish systems (dela Cruz *et al.* 1992, Fernando 1993, Haroon *et al.* 1994). These two fishes are exotic in Bangladesh and are very popular because of their rapid growth and ability to thrive in seasonal waterbodies which are not suitable for the indigenous carps (Gupta and Rab 1994, Haroon *et al.* 1994, Kohinoor *et al.* 1994, Miah *et al.* 1994, Wahab *et al.* 1995). No reports are available on their dietary breadth, overlap and feeding strategy in rice-fish systems be it Bangladesh or in other Asian countries.

The present study was undertaken to examine and compare the intra- and interspecific dietary breadth, the degree of diet similarity, overlap and feeding strategy of two size groups of *B. gonionotus* and *Oreochromis* spp.(*O. mossambicus* x *O. niloticus* natural hybrid) and a single size group of *B. gonionotus* and *Oreochromis* spp., stocked in ricefields. Different niche measures are compared and evaluated for appropriateness, ease of biologically significant interpretation and explanation. Similar comparative work on dietary breadth, degree of diet similarity, overlap and feeding strategy of two size groups of *B. gonionotus* and *Oreochromis* spp. were done earlier by Haroon and Pittman (1998b) in the pond environment.

Materials and methods

The present work comprises three experiments carried out in an experimental ricefield at the Riverine Station, Chandpur of the Bangladesh Fisheries Research Institute. Two size categories (around 6 and 12 cm, total length) of *B. gonionotus*, were examined in the ricefield during April 1994 (Experiment *I*), similarly two size groups of *Oreochromis* spp. were investigated during July 1995 (Experiment *II*) and during September 1995 (Experiment *III*) a median size range (falling within 6 and 12 cm) of both *B. gonionotus* and *Oreochromis* spp. were examined in the ricefield. The fish and sub-surface plankton were sampled every 3 h for 48 h in the intraspecific trials and for 24 h in the interspecific study for gut contents and available resources analysis. We have categorized the fishes in size classes, simply because fish farmers look first for the fish size rather than the weight when selecting any stock.

Field preparation, fish stocking and sampling

Experiment I: Rice (transplant Aman: *Paijam* - a local variety of *Oryza sativa*) was planted in an experimental field of 166 m^2 (having a refuge canal of 1.0 m breadth and 0.5 m depth, on one side of the plot) according to farmers traditional practice (Haroon *et al.* 1989). Once the tillers reached a height of 0.5 m and field conditions were as close as

possible to those of a natural wet-season ricefield (water depth 0.25 m in the paddy field and 0.7 m in the refuge), two sizes of *B. gonionotus* juveniles procured from the Riverine Station's hatchery were stocked at a total density of 7.0 juveniles m^{-2} (581 individuals of each size). The small fishes were 4.5-5.52 cm (mean 4.85 cm) and 1.28-2.72 g (mean 1.72 g) and the large fishes were 11.54-13.4 cm (mean 12.64 cm) and 21.44-32.36 g (mean 27.81 g) at stocking. Prior to the experiment the fish had been fed a supplemental feed composed of 40% rice bran, 40% wheat bran and 20% fishmeal at 2-5% of body weight (bw) day^{-1}. Before stocking in the experimental ricefield the fishes were kept in a flow-through system for 48 h to empty their gut contents. Two days after stocking, 10 fishes of each size were sampled every 3 h for a further 48 h with a knotless hapa net (3x2x1.5 m, mesh 0.5 cm). A total of 320 fishes (160 of each size) were collected.

Experiment II: Paddies were prepared in the following year in the same way in the same field, and conditions as close as possible to the natural wet-season ricefield were established. Two size categories of *Oreochromis* spp. juveniles procured from the Riverine Station's other nursery ponds were stocked in the ricefield at a total density of 7.0 juveniles m^{-2} (581 individuals of each size). The small fishes were 4.9-8.0 cm (mean 6.82 cm) and 1.92-8.15 g (mean 5.02 g) and the large fishes were 10.3-13.8 cm (mean 12.0 cm) and 17.74-46.2 g (mean 27.84 g) at stocking. Prior to the experiment the fish had been fed a similar supplemental feed at the similar rates as for *B. gonionotus*. Stocked *Oreochromis* spp. had the same pre-treatment as *B. gonionotus* prior to release in the ricefield. A similar sampling regime was followed and a total of 320 fishes (160 of each size) were collected for gut analysis.

Experiment III: The same ricefield preparation and fish treatment was used in the final interspecific experiment when both species were stocked together. In this case a single size of both *B. gonionotus* and *Oreochromis* spp. were stocked at a total density of 7.0 juveniles m^{-2} (581 individuals of each species). The silver barbs were 5.1-10.1 cm (mean 7.1 cm) and 2.5-14.39 g (mean 4.86 g) and the tilapias were 7.5-10.2 cm (mean 8.53 cm) and 8.11-16.66 g (mean 10.48 g). A similar sampling regime was followed and a total of 320 fishes (160 of each species) were collected for gut analysis.

Gut analysis

Fishes were checked immediately after capture for regurgitation (if seen, the fish was replaced), and preserved in 10% buffered formalin until examined. Each fish was measured for total length (mm), and weighed ($\pm$ 0.001 g) within two weeks after collection and no correction factor for fixation was used.

As *B. gonionotus* lacks a well-defined stomach, only the anterior portion of the digestive tract lying between the esophagus and the first major curve of the small intestine was dissected out (Haroon and Pittman 1997) as digestion is less advanced in this portion and food items remain mostly identifiable. Silver barb have an intestine usually 2-3 times their body length (Sattar 1987). For *Oreochromis* spp. the anterior portion of the digestive tract lying between the esophagus and the first major bend of the small intestine, just after the stomach was dissected out (Haroon and Pittman 1998a). Tilapia have a relatively long and coiled intestine up to 14 times the body length

(Edwards 1987), although food digestion and assimilation is completed in the first half of the intestine (Bowen 1981).

Each gut or stomach was blotted uniformly with tissue paper, opened longitudinally and gut or stomach contents were transferred to a petridish or vial with a standard 10 ml of distilled water. Food items of animal origin were usually counted under a dissecting microscope, but in the case of tiny items and items of plant origin the gut or stomach contents were well mixed, one ml was sub-sampled by a digital Finn pipette to a Sedgwick-Rafter counting cell (1000 mm^3, 50x20x1 mm) and 100 randomly chosen cells out of 1000 were examined under an inverted microscope. Three such sub-samples were enumerated per fish. All organisms were identified to the genus level (Prescott 1962, Ward and Whipple 1978) and percentage abundance was used for calculating the proportion of each food item in the gut or stomach (Windell and Bowen 1978, Bowen 1983). Only fishes with food in their gut or stomach were considered for calculation of the proportion of each food item.

Plankton

Five 1-l samples of surface to sub-surface (0.02 m) water were taken from different areas of the ricefield (refuge canal, middle and extreme end of the field) every 3 h, prior to fish sampling, filtered through a 15 μm mesh plankton net, washed into plastic jars and made up to a standard 200 ml volume with 5% buffered formalin. Once well settled, plankton were concentrated in a standard 50 ml volume and preserved until examination. Three 1 ml sub-samples were examined from each plankton sample and the proportion and identification of each food item were done in the same way as for gut or stomach content.

Niche measures

Diet breadth indices were calculated with Levin's modification of Simpson's diversity index B and B_n (Hurlbert 1978, Keast 1978, Easton *et al.* 1996).

$$B_x = 1/\Sigma (p_{xi}^2) \quad (1a)$$

$$B_y = 1/\Sigma (p_{yi}^2) \quad (1b)$$

where B_x and B_y are the dietary breadth (Shannon and Weaver information statistic) of x and y respectively (two different size classes or species), p_{xi} and p_{yi} are the proportions, out of all those resources used by x or y, that consists of food items in resource state i. B value varies from 1.0, when the population uses one resource state exclusively, to equal to R, when the population uses all resource categories in equal proportions. R is the number of food categories.

$$B_{nx} = 1/[R\Sigma p_{xi}^2] \quad (2a)$$

$$B_{ny} = 1/[R\Sigma p_{yi}^2] \quad (2b)$$

In Eq. 2a and 2b, the index (reciprocal of Simpson's diversity index) is normalized by R. Except for normalization other notations are the same as Eq. 1a and 1b. Conversely, B_n value ranges from 1/R, when the population uses one resource state exclusively, to 1.0, when the population uses all resource states in equal proportions.

Feinsinger *et al.* (1981) stated that niche breadth, as defined by Levin (1968, cited in Keast 1978), Hurlbert (1978) and others, is simply a special case of sample similarity and proposed for using Czekanowski's *PS*, the proportional similarity index rather than Schoener's (1970) index.

$$PS_x = 1\text{-}0.5\ \Sigma\ |p_{xi} - q_i| \quad (3a)$$

$$PS_y = 1\text{-}0.5\ \Sigma\ |p_{yi} - q_i| \quad (3b)$$

where p_{xi} and p_{yi} are respectively the proportion of resource items in category i out of all items used by x or y and q_i is the proportion of i items in the resource base available to the population. Values for *PS* ranges from 1.0 for the broadest possible niche (when a population uses resources in proportion to their availability) to a minimum for the narrowest possible niche (when a population is specialized exclusively on the rarest resource state and consequently bypasses all other items).

Diet overlap indices were calculated with Levin's α_{xy} and α_{yx} (after Keast 1978, Wallace 1981) and Schoener's α (Schoener 1970). The Levin's dietary overlap indices are represented by α_{xy} (overlap of x on y) and α_{yx} (overlap of y on x). Those are:

$$\alpha_{xy} = \Sigma\ (p_{xi} \cdot p_{yi}) / \Sigma\ p_{yi}^2 \quad (4a)$$

$$\alpha_{yx} = \Sigma\ (p_{xi} \cdot p_{yi}) / \Sigma\ p_{xi}^2 \quad (4b)$$

where p_{xi} and p_{yi} are similar as described for Eq. 2a and 2b. Values of α_{xy} or α_{yx} range from 0 to slightly over 1.0 and measures respectively the overlap of x on y or the reverse.

Schoener's overlap (1970) index is denoted by α.

$$\alpha = 1\text{-}0.5\ (\Sigma|p_{xi} - p_{yi}|) \quad (5)$$

where p_{xi} and p_{yi} are similar as described for Eq. 2a and 2b. Schoener's α index varies from 0 representing no overlap to 1.0 reflecting complete overlap between the x and y It is one of the least objectionable indices available (Wallace 1981, Martin 1984, Knight and Ross 1994) and widely used.

All these indices have been calculated from discrete counts, as animals choose resources item by item rather than joule by joule (Feinsinger *et al.* 1981), and compared to evaluate appropriateness and ease of biological interpretation. We have followed Zaret and Rand (1971), Wallace (1981), Martin (1984), Pen *et al.* (1993) in considering values of dietary overlap indices above the arbitrary level of 0.60 as representing a biologically significant overlap.

Feeding strategy

Amundsen *et al.*'s (1996) modified approach of Costello's (1990) method was used for graphical analysis of feeding strategy. This is based on a two-dimensional representation where each data point represents the frequency of occurrence (F_i) and the prey-specific abundance ($\%P_i$) of a food category. Mathematically F_i and $\%P_i$ can be described by the equations:

$$F_i = (N_i/N) \quad (6)$$

$$\%P_i = (\Sigma S_i/\Sigma S_{ii}) \times 100 \quad (7)$$

where N_i is the number of predators with prey type i in their stomach, N is the total number of predators with food in stomach, S_i is the stomach content comprised of prey type i (in number, weight or volume), and S_{ii} is the total stomach content in only those

predators with prey type *i* in their stomach. Only unidentifiable digested food items were excluded from the analysis.

Results

Intraspecific niche measures

B. gonionotus: Both sizes consumed relatively large amounts of macrophytes, 0.39 (proportion as fraction of 1.0) in small fish and 0.23 in large fish (Table 1). Zooplankton were more important to small than to large fish (0.26 vs. 0.02), as were insects (0.09 vs. 0.05). Of the microalgae, *Spirogyra* and *Oedogonium* (both filamentous green algae) were consumed in large amounts by small fish (>0.25). Large fish consumed only *Spirogyra* and *Cladophora* in small amounts. *Cypris* of the ostracods were consumed by both sizes. Molluscs were found only in the gut of large fish (0.60) as small bits of shell and muscle rather than the whole animal (Table 1).

Table 1. Resource availability and use by two sizes of *Barbodes gonionotus* (4.5-5.52 cm and 11.54-13.4 cm TL) during 26-28 April 1994, two sizes of *Oreochromis* spp. (4.9-8.0 cm and 10.3-13.8 cm TL) during 19-21 July 1995, and by a single size category of both *B. gonionotus* (5.1-10.1 cm TL) and *Oreochromis* spp. (7.5-10.2 cm TL) during 19-20 September 1995, in a ricefield from Bangladesh. (Unid.= unidentified; empty guts or stomachs were not included in the calculation)

Resource category	Intraspecific						Interspecific		
	Fraction available	*B. gonionotus* Fraction used by small[1]	large[2]	Fraction available	*Oreochromis* spp. Fraction used by small[3]	large[4]	Fraction available	Fraction used by *B. gon*[5]	*O. sp.*[6]
Chlorophyceae									
Ankistrodesmus				0.0487					
Closterium	0.0024	0.0007			0.0012		0.0144	0.0003	0.0044
Pleurotaenium	0.0045	0.0008					0.0348	0.0028	
Pediastrum	0.0041			0.0084		0.0002			
Scenedesmus	0.2305			0.0374	0.0001				
Sphaerocystis	0.2461								
Volvox							0.1208	0.13	0.0029
Oedogonium	0.0021	0.1295							
Ulothrix	0.001								
Spirogyra	0.0045	0.1261	0.0184		0.0014				
Cladophora		0.0063	0.0208						
Pithophora					0.0345				
Rhizoclonium					0.0721	0.0003		0.0032	
Cyanophyceae									
Anabaena				0.0026		0.0005	0.1084	0.0208	
Merismopedia	0.193			0.1464					

Bacillariophyceae									
Coscinodiscus	0.0019								
Cyclotella	0.0079								
Gomphonema	0.0005								
Melosira				0.0185	0.0019	0.0012			
Surirella	0.001								
Synedra	0.001								
Euglenophyceae									
Phacus	0.004			0.0037					
Unid. Macrophytes		0.392	0.227					0.2845	0.0049
Ciliata									
Vorticella	0.0001								
Rotifera									
Brachionus							0.0074	0.0004	0.0012
Euchlanis	0.001								
Keratella				0.0037					
Lecane	0.0002	0.0011					0.0078		
Monostyla	0.002								
Platyias							0.1005		
Polyarthra	0.0033			0.0156			0.0639		
Crustacea									
Alona				0.0089					
Bosmina				0.0031	0.0033				
Cyclops	0.1350	0.236	0.0181	0.0344	0.0005		0.1011	0.0371	
Daphnia				0.00778	0.0002				
Diaptomus	0.0180	0.0069	0.0045	0.4292			0.1527		
Diphanosoma				0.0057			0.0066		
Moina	0.0052	0.0054		0.0052	0.0007		0.0139	0.0111	
Polyphemus	0.0026							0.0420	0.0017
Unid. egg					0.0069				
Unid. naupili	0.1272	0.0067		0.205	0.0082	0.0047	0.2136	0.0127	0.0041
Unid. *Mysis* larv							0.0045		
Ostracoda									
Cypris	0.0001	0.0002		0.0157	0.0014	0.0012	0.0078	0.0238	0.0006
Unid. insects remain		0.0883	0.0568		0.0339			0.1222	0.0051
Unid. molluscs remain			0.5977					0.0116	
Detrital aggrega					0.837	0.9917		0.3380	0.9768
Silt and sand			0.0566						
Total	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

[1] n= 108, [2] n = 74, [3] n = 90, [4] n = 39, [5] n = 55 and [6] n = 35; only fishes with food in the stomachs were considered.

Dietary breadth values were a little wider in the small sizes (B= 3.99, B_a = 0.12) than the larger (Table 2). PS indices also reflect that both sizes of *B. gonionotus*. are using some

resource items exclusively, though small ones have relatively broader selection of prey items, about 5 times greater niche width (PS= 0.155) than the larger ones (Table 2).

Intraspecific dietary overlap of large fish on small (α_{xy}= 0.41) was much greater than the reverse (α_{yx}= 0.24), but biologically insignificant in both ways. Schoener's index (α= 0.33) also supports this trend of biologically insignificant intraspecific dietary overlap (Table 2).

Table 2. Intraspecific virtual diet breadth and overlap indices* of two sizes of *Barbodes gonionotus* (4.5-5.52 cm and 11.54-13.4 cm TL) during 26-28 April 1994 and two sizes of *Oreochromis* spp. (4.9-8.0 cm and 10.3-13.8 cm TL) during 19-21 July 1995 in a ricefield from Bangladesh (x= small size, y= large size; B_x and B_y= dietary breadths of x and y; B_{sx} and B_{sy} = dietary breadths of x and y; PS_x and PS_y= proportional similarity index of x and y respectively; α_{xy} = overlap of x on y, α_{yx}= overlap of y on x and α= Schoener's overlap index)

Fish species	Niche indices								
	Czekanowski's		Levin's						Schoener
	PS_x	PS_y	B_x	B_y	B_{sx}	B_{sy}	α_{xy}	α_{yx}	α
B. gonionotus	0.155	0.03	3.99	3.37	0.12	0.07	0.24	0.41	0.33
Oreochromis spp	0.02	0.015	1.42	1.02	0.05	0.04	0.84	1.18	0.85

* *diet overlap values >0.60 are shown in bold and are considered to be biologically significant, Zaret and Rand 1971.*

Oreochromis spp.: Both sizes of tilapia fed mainly on the periphytic detrital aggregate (PDA), 0.84 in small and 0.99 in large (Table 1) and showed an overall avoidance for zooplankton, microalgae and consumed no aquatic macrophytes. Of the zooplankton, small fish preferred crustacean eggs and nauplii, and randomly consumed *Bosmina*. Large fish avoided all adult crustaceans and their eggs but consumed a few nauplii. Rotifers were avoided by both sizes while *Cypris* of ostracods were consumed by both sizes. Insects were consumed (0.03) by the small fish only (Table 1).

Niche width values were marginally broader in the small fish (B= 1.42, B_n= 0.05) than in the large fish (B= 1.02, B_n= 0.04) (Table 2). Czekanowski's PS indices also confirm this trend of dependence on single or few selective food items (PS= 0.02 in the small, PS= 0.015 in the large), discriminating others. Dietary breadth of *Oreochromis* spp. is relatively narrower than that of *B. gonionotus*.

Dietary overlap indices revealed that both sizes of tilapia had biologically significant intraspecific overlap (α_{xy}= 0.84, α_{yx}= 1.18) to each other, exclusively for the PDA. The overlap strength of large sizes on the smalls was about 1.4 times greater than the reverse. Schoener's index (α= 0.85) also revealed biologically significant intraspecific dietary overlap between the sizes (Table 2).

Interspecific niche measures

B. gonionotus fed on the PDA but in lesser proportion (0.34) than the *Oreochromis* spp. as well as feeding on aquatic macrophytes (0.28), *Volvox* (0.13) of the green algae and insects (0.12). *Oreochromis* spp. fed exclusively on the PDA (0.98) avoiding blue-green algae, most of the green algae, adult crustaceans and molluscs (Table 1).

Interspecific dietary width (Table 3) was little higher for *B. gonionotus* (B= 4.35, B_a= 0.21 and PS= 0.21) than *Oreochromis* spp. (B= 1.05, B_e= 0.05 and PS= 0.02). *Oreochromis* spp. display exclusive specialization for PDA while *B. gonionotus* have many alternative preferences in addition to PDA.

Table 3. Interspecific virtual diet breadth and overlap indices* of a single size category of both *Barbodes gonionotus* (5.1-10.1 cm TL) and *Oreochromis* spp. (7.5-10.2 cm TL) during 19-20 September 1995, in a ricefield from Bangladesh (x = *B. gonionotus* and y = *Oreochromis* spp)

Fish species	Niche indices					
	Czekanowski's	Levin's				Schoene
	PS	B	B_a	α_{xy}	α_{yx}	α
B. gonionotus	0.21	4.35	0.21	1.46		0.36
Oreochromis spp.	0.02	1.05	0.05		0.35	

** diet overlap values >0.60 are shown in bold and are considered to be biologically significant, Zaret and Rand 1971.*

Interspecific dietary overlap of *B. gonionotus* on *Oreochromis* spp. (α_{xy}= 1.46) was more than 4 times greater and biologically significant than the reverse (α_{yx}= 0.35). Schoener's index (α= 0.36) indicated that the interspecific dietary overlap between this size range of *B. gonionotus* and *Oreochromis* spp. was biologically insignificant (Table 3).

Intraspecific feeding strategy

Most of the small individuals of *B. gonionotus* consumed moderately dominant food items, occasionally including items with low specific abundance and low occurrence reflecting mixed feeding strategy. Nonetheless, some individuals showed moderate specialization (individual level) for aquatic insects while others showed moderate population specialization for aquatic macrophytes and *Cyclops* of the crustaceans (Fig. 1a). Most of the large individuals of *B. gonionotus* consumed dominant food items as well as rare food items have been consumed occasionally by some individuals (Fig. 1b). However, some large ones showed individual specialization on *Cyclops* (crustaceans) and aquatic insects while others showed population specialization for molluscs and aquatic macrophytes.

All small *Oreochromis* spp. had been feeding on PDA, but small proportions of other food types were also included occasionally. A few showed individual specialization on certain food items like, crustacean eggs and aquatic insects (Fig. 1c). Similarly, all large *Oreochromis* spp. were found feeding on PDA but small proportions of other food items were also consumed occasionally by some individuals (Fig. 1d).

Interspecific feeding strategy

In case of *B. gonionotus* (interspecific study) most of the prey were of rare occurrences (Fig. 1e). The most important food items were consumed by more than half of the fish, but their average contribution to the gut contents of these fishes was low. In addition,

some individuals showed specialization for *Anabaena*, a blue-green algae (Fig. 1e). In contrast, *Oreochromis* spp. was found feeding exclusively on PDA but a few individuals also consumed other food items in small proportions (Fig. 1f).

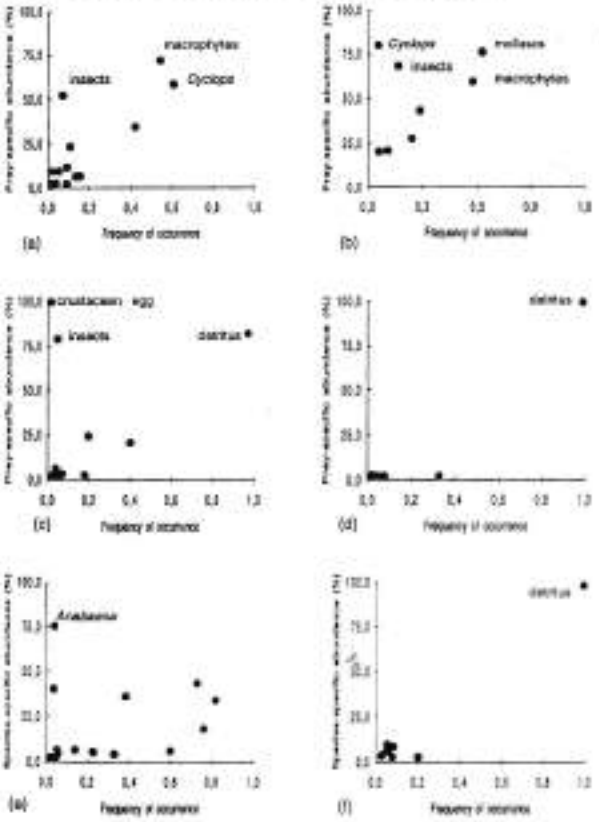

Fig.1. The feeding strategy diagram: prey-specific abundance plotted against frequency of occurrence of different food items in the diet of two sizes of *Barbodes gonionotus* (4.5-5.52 and 11.54-13.4 cm TL) during 26-28 April 1994, two sizes of *Oreochromis* spp. (4.9-8.0 and 10.3-13.8 cm TL) during 19-21 July 1995, and by a single size category of both *B. gonionotus* (5.1-10.1 cm TL) and *Oreochromis* spp. (7.5-10.2 cm TL) during 19-20 September 1995, in a ricefield from Bangladesh (Amundsen *et al.*'s 1996 modified approach to Costello's 1990 method). (**a**) *B. gonionotus*, 4.5-5.52 cm TL (n = 108); (**b**) *B. gonionotus*, 11.54-13.4 cm TL (n = 42); (**c**) *Oreochromis* spp., 4.9-8.0 cm TL (n = 90); (**d**) *Oreochromis* spp., 10.3-13.4 cm TL (n = 39); (**e**) *B. gonionotus*, 5.1-10.1 cm TL (n = 55) and (**f**) *Oreochromis* spp., 7.5-10.2 cm TL (n = 35). The black dots represent different food items (only the important items are labeled on the figures).

Discussion

The small size groups of both species had a wider dietary niche than the large individuals. Large fish increased their specialization on certain food items (on aquatic macrophytes by *B. gonionotus* and on PDA by *Oreochromis* spp.) and narrowed down their niche width with increasing size and competitive ability (Haroon and Pittman 1997,

Interspecific dietary width (Table 3) was little higher for *B. gonionotus* (B= 4.35, B_n= 0.21 and PS= 0.21) than *Oreochromis* spp. (B= 1.05, B_n= 0.05 and PS= 0.02). *Oreochromis* spp. display exclusive specialization for PDA while *B. gonionotus* have many alternative preferences in addition to PDA.

Table 3. Interspecific virtual diet breadth and overlap indices* of a single size category of both *Barbodes gonionotus* (5.1-10.1 cm TL) and *Oreochromis* spp. (7.5-10.2 cm TL) during 19-20 September 1995, in a ricefield from Bangladesh (*x* = *B. gonionotus* and *y* = *Oreochromis* spp)

Fish species	Niche indices					
	Czekanowski's	Levin's				Schoene
	PS	B	B_n	α_{xy}	α_{yx}	α
B. gonionotus	0.21	4.35	0.21	1.46		0.36
Oreochromis spp.	0.02	1.05	0.05		0.35	

** diet overlap values >0.60 are shown in bold and are considered to be biologically significant, Zaret and Rand 1971.*

Interspecific dietary overlap of *B. gonionotus* on *Oreochromis* spp. (α_{xy}= 1.46) was more than 4 times greater and biologically significant than the reverse (α_{yx}= 0.35). Schoener's index (α= 0.36) indicated that the interspecific dietary overlap between this size range of *B. gonionotus* and *Oreochromis* spp. was biologically insignificant (Table 3).

Intraspecific feeding strategy

Most of the small individuals of *B. gonionotus* consumed moderately dominant food items, occasionally including items with low specific abundance and low occurrence reflecting mixed feeding strategy. Nonetheless, some individuals showed moderate specialization (individual level) for aquatic insects while others showed moderate population specialization for aquatic macrophytes and *Cyclops* of the crustaceans (Fig. 1a). Most of the large individuals of *B. gonionotus* consumed dominant food items as well as rare food items have been consumed occasionally by some individuals (Fig. 1b). However, some large ones showed individual specialization on *Cyclops* (crustaceans) and aquatic insects while others showed population specialization for molluscs and aquatic macrophytes.

All small *Oreochromis* spp. had been feeding on PDA, but small proportions of other food types were also included occasionally. A few showed individual specialization on certain food items like, crustacean eggs and aquatic insects (Fig. 1c). Similarly, all large *Oreochromis* spp. were found feeding on PDA but small proportions of other food items were also consumed occasionally by some individuals (Fig. 1d).

Interspecific feeding strategy

In case of *B. gonionotus* (interspecific study) most of the prey were of rare occurrences (Fig. 1e). The most important food items were consumed by more than half of the fish, but their average contribution to the gut contents of these fishes was low. In addition,

some individuals showed specialization for *Anabaena*, a blue-green algae (Fig. 1e). In contrast, *Oreochromis* spp. was found feeding exclusively on PDA but a few individuals also consumed other food items in small proportions (Fig. 1f).

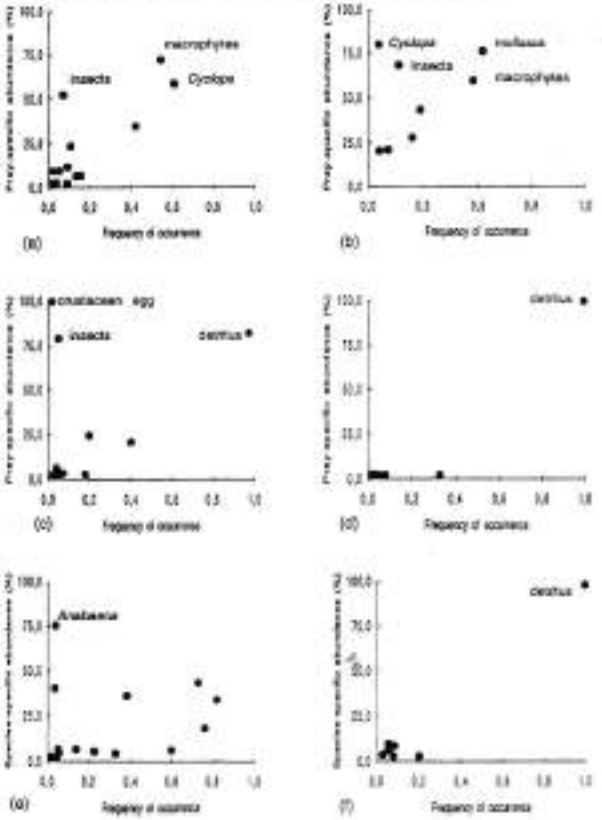

Fig.1. The feeding strategy diagram: prey-specific abundance plotted against frequency of occurrence of different food items in the diet of two sizes of *Barbodes gonionotus* (4.5-5.52 and 11.54-13.4 cm TL) during 26-28 April 1994, two sizes of *Oreochromis* spp. (4.9-8.0 and 10.3-13.8 cm TL) during 19-21 July 1995, and by a single size category of both *B. gonionotus* (5.1-10.1 cm TL) and *Oreochromis* spp. (7.5-10.2 cm TL) during 19-20 September 1995, in a ricefield from Bangladesh (Amundsen *et al.*'s 1996 modified approach to Costello's 1990 method). (**a**) *B. gonionotus*, 4.5-5.52 cm TL (n = 108); (**b**) *B. gonionotus*, 11.54-13.4 cm TL (n = 42); (**c**) *Oreochromis* spp., 4.9-8.0 cm TL (n = 90); (**d**) *Oreochromis* spp., 10.3-13.4 cm TL (n = 39); (**e**) *B. gonionotus*, 5.1-10.1 cm TL (n = 55) and (**f**) *Oreochromis* spp., 7.5-10.2 cm TL (n = 35). The black dots represent different food items (only the important items are labeled on the figures).

Discussion

The small size groups of both species had a wider dietary niche than the large individuals. Large fish increased their specialization on certain food items (on aquatic macrophytes by *B. gonionotus* and on PDA by *Oreochromis* spp.) and narrowed down their niche width with increasing size and competitive ability (Haroon and Pittman 1997,

1998a and 1998b). The interspecific dietary niche was wider for the silver barbs than the tilapias, indicating greater specialization and less pronounced ontogenic dietary shifts in tilapia.

Insignificant intraspecific dietary overlap between the two sizes of barbs reflects a resource partitioning according to size or ontogenetic shift in diet. Resource partitioning may also occur in time, since small barbs are feeding most actively around midday while large barbs are more active near dusk and after dawn (Haroon and Pittman 1997). By contrast, both sizes of tilapia display peak feeding activity around midday (Haroon and Pittman 1998a), suggesting that temporal, spatial and habitat overlap will affect the resource utilization and the growth of tilapia in mixed-size rearing.

There was neither a high within-phenotype (generalization) nor high between-phenotype (specialization) contribution to the niche width for the silver barbs. Small silver barbs showed a mixed feeding strategy, with varying degrees of specialization and generalization for different food items. The population of silver barbs indicated specialization for certain food items and the dietary width was relatively narrow (PS= 0.16). Large silver barbs also showed a mixed feeding strategy with some individuals specializing on certain food types and a population specialization on other food types, and the niche width was narrower than that of small (PS= 0.03). When reared with tilapia, the silver barb showed a relatively high within-phenotype contribution to the increased niche width (PS= 0.21), indicating more generalized feeding strategy.

Small tilapia demonstrated individual specialization for crustacean eggs and aquatic insects in addition to population specialization for PDA, giving a narrow niche breadth (PS= 0.02). At the population level, large tilapia specialized exclusively on PDA and hence the niche width was narrowest (PS= 0.015). When reared with barbs, tilapia displayed a similar feeding strategy and narrow niche breadth.

Our results suggest that an ontogenetic shift in diet occurs more strongly in silver barb (Haroon and Pittman 1997) than in tilapia (Haroon and Pittman 1998a). The various sizes of a single species may occupy several trophic units depending on their ontogenetic progression in diet (Eggold and Motta 1992), suggesting that optimal stocking strategies for *B. gonionotus* should consider a changing resource utilization with the aging of the stock.

B_a is more useful than B as the former incorporates the number of food categories available while the latter does no. The use of Czekanowski's PS reveals more information about the ecological determinants of dietary breadth as it simultaneously incorporates the availability and use of the particular resource category. For example, the values of 0.16 and 0.03 for PS indices of small and large silver barb respectively, 0.02 and 0.015 for PS indices of small and large tilapia respectively and 0.21 and 0.02 for PS indices of silver barb and tilapia respectively are easily interpreted - of all the food categories available 1/6.45 (0.16) and 1/33.3 (0.03) part of foods were obtained respectively by the small and large silver barb, while 1/50 (0.02) and 1/66.7 (0.015) part of foods were obtained respectively by the small and large tilapia. Similarly,1/4.8 (0.21) and 1/50 (0.02) part of the food items were obtained by the silver barb and tilapia, respectively.

PS measures most accurately the actual area of intersection between two frequency distributions and is therefore more robust than Levin's B or B_e and Ivlev's (1961) electivity indices. It has often been used to measure niche overlap (Colwell and Futuyma 1971). Feinsinger *et al.* (1981) concluded that PS is more appropriate than Schoener's (1970) index for measuring the degree to which an animal's diet is specialized for testing hypotheses on foraging tactics. PS values will either change as the resource spectrum changes if the particular species or size being considered discriminates against resource items in other categories, or maintain a similar value if the same selectivity over time is found regardless of changes in resource states (Hurlbert 1978, Petraitis 1979, Feinsinger *et al.* 1981). Another consideration is that, niche breadth measures the variability in resource use while the conceptual basis for variation in niche breadth is resource selectivity by the individuals (Petraitis 1979).

In investigating the available resources, we sampled only plankton, which contained mostly minute forms. Had we taken benthos and macrovegetation into account, the dietary breadth indices with PS would have been more robust and different. However, because of the use of absolute differences between the resource use and availability in Equations 3a and 3b, there is a PS value for a certain resource item even though that particular food item was not ingested by the species or sizes concerned.

The interspecific dietary overlap between the silver barb and the tilapia was biologically insignificant with Schoener's index. However, Levin's overlap indices revealed that the dietary overlap of the silver barb on the tilapia is much greater and biologically significant while the reverse is not significant. Here lies the significance in the use of Levin's dietary overlap indices over the Schoener's.

For future wok on niche measures, the appropriate index would seem to be Czekanowski's PS (Eqns. 3a and 3b) for the niche breadth and Levin's α_{jv} (Eq. 4a) and α_{js} (Eq. 4b) for the niche overlaps. Although Schoener's index is widely used, we agree with Wallace (1981) and Martin (1984) that it is the least objectionable of the indices only when resource availability data are not present.

In general, an observed biologically significant dietary overlap (>0.60) is considered indicative of competition, which may not always be the case. The existence and intensity of competition can only be ascertained by comparing actual to virtual dietary overlap of the competitors. The existence of competition would be certain if virtual overlap exceeds the actual overlap value (Colwell and Futuyma 1971). Niche breadth and overlap, when measured under natural conditions, are called 'actual' metrics, while 'virtual' niche breadth and overlap are the corresponding values measured in the absence of competition among species. It is the condition under which data are collected, rather than the method of calculation. Hence, our metrics are virtual niche measurements as we exploited natural conditions as closely as possible in the absence of other competitors. Such clarification between actual and virtual measurements is essential when expressing niche overlap, which by any means is a measure of competition.

In the present study resource availability and use estimated by counting discrete individuals seemed adequate. Discrete counts are more apt to explain the degree of discrimination than are values calculated from energy contents of items (Feinsinger *et al.* 1981, Wallace 1981, Martin 1984). However, these measurements are vulnerable to bias due to patchiness of the plankton and to digestion rates of particular items (Strauss 1979).

As our present study on the two sizes of *B. gonionotus*, two sizes of *Oreochromis* spp. and between *B. gonionotus* and *Oreochromis* spp. were separately done these indices are not directly comparable towards either small sizes of *B. gonionotus* versus small or large sizes of *Oreochromis* spp. or large sizes of *B. gonionotus* versus small or large sizes of *Oreochromis* spp. However, interspecific niche measures estimated with a single median size range of *B. gonionotus* and *Oreochromis* spp., as shown in Table 3, gives an indication of what degree of overlap may be expected.

Significant interspecific dietary overlap of silver barb on tilapia cautions for mixed-species stocking in the same system. It seems that there are less opportunities for habitat segregation between the *B. gonionotus* and *Oreochromis* spp. when stocked together in a rice-fish system. Haroon and Alam (1992) reported poor yield of tilapia with mixed-stocking of silver barb and tilapia in concurrent rice-fish culture in Bangladesh. Mixed rearing of barbs and tilapia in the same habitat will likely result in a suppression of the growth of tilapia, due to their high degree of specialization on items with low nutrient value and their dietary overlap with barbs which have a broader niche width.

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Feeding metabolism in an Indian major carp (*Catla catla* Lin.) fed on different protein diets

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Abstract
Feeding metabolism in an Indian major carp, *Catla catla* fingerlings of 10.8±0.56g was investigated in a flow-through water recirculating system. The metabolic energy loss in resting metabolism and feeding metabolism were determined by the indirect method of oxygen consumption followed by multiplication by suitable oxycalorific coefficient. This was done in four metabolic chambers of a respirometer system. Ten fish fingerlings of mean total weight of 109.5, 110.4 and 112.8g/chambers respectively each in two experimental runs of three treatments a, b and c were used. The mean resting metabolic rate during unfed condition showed no significant variation in different treatments. The fish in three treatments a, b and c fed on diets containing 28, 33 and 38% crude protein had significantly different ($p<0.05$) post-fed SDA magnitude of 497.7, 638.7 and 735.5 mgO_2/chamber/day having an equivalent energy loss of 12.68, 14.68 and 15.86 KJ respectively. The SDA co-efficient in three treatments a, b and c were 14.95, 19.00 and 22.36% respectively whereas, respiratory energy - 'R' as % of mean total ingested energy in three treatments were 26.93, 31.17 and 34.74% respectively showing a significant increase ($p<0.05$) with increase of protein.

Key words: Feeding metabolism, protein diets, *Catla catla*.

Introduction

The metabolic heat energy loss associated with the digestion and transformation of ingested food into metabolisable is known as specific dynamic action (SDA). SDA can represent a major component of respiratory energy budget. To determine the food ration and growth performance of fish, it is necessary to know the specific dynamic action which may range from 4 to 45% of the ingested energy and generally approaching 30% of the gross energy ingested. In poikilotherms SDA has been observed as a post-prandial increase in the rate of oxygen consumption expressed as mgO_2 (then converted into KJ or Kcal). Following food ingestion the oxygen consumption rate in fish increases and then gradually declines back to its resting level (Jobling 1980). The principal component of SDA effect - the total magnitude, the peak increased level and the duration (Jobling 1980). The "magnitude" is the sum of excess metabolic heat production expressed as mgO_2 (or expressed into Kcal) induced by the ingested meal, integrated from the time

metabolism first rises to the peak value and falls back to the base is determined by plotting the curve of increased oxygen consumption after feeding against time until it subsides to the prefeeding rates (resting rate) and then intergrating the area beneath the curve. SDA is expressed as a percentage of the total caloric value of the food ingested (Hill 1976) in metabolic term of SDA coefficient (Smith *et al.* 1978). Whereas, SDA duration is the time during which the oxygen consumption by the fed fish is above resting metabolism.

Estimation of the metabolic expenditure associated with feeding are usually obtained from laboratory experiments using different types of respirometer where the motor activity of fish inside the respirometer (metabolism chamber) chambers may be controlled as much as possible (Chakraborty *et al.* 1992b). The increased oxygen consumption for a fed fish with a defined ration in the chambers may be measured accurately over a defined period of time. The SDA magnitude is dependent on so many factors among which the quantitative and qulitative aspects of dietary protein is important. The SDA coefficient of a particular fish in response to particular diet is very important in fish culture where they must be taken into consideration when constructing energy budgets involved in feeding and growth during holding on growing period.

Till to date, there have been no reported work on post-fed oxygen consumption (specific dynamic action -SDA) in Indian major carp, *Catla catla*. The aim of the study was to investigate the feeding metabolism - SDA in an Indian major carp, *Catla catla* fed on different protein diets.

Materials and methods

Healthy fingerlings of Indian major carp, *Catla catla* (10-12g) from a single stock was collected in August 1996 from local fish farm. Acclimation of the fingerlings was made for 15 days in large plastic pool with continuous aeration at 28 ± 1^{0}C.The fingerlings were given prophylectic treatment with NaCl (3%) dip for 10 minutes and 0.5mg/L methylene blue bath for 24 hours. Faecal matter produced by fingerlings were removed everyday morning and evening. The pool water was partly (about 40%) replaced with fresh aerated water every morning. The fingerlings were not fed on first two days of acclimation. From the third day they were given pelleted diet containing fish meal, mustard oil cake, wheat flour and bran etc. having 30% dietery protein at the rate of 1% as maintenance ration. Normal dark- light period was maintained throughout the study period.

The experiment was conducted in metabolism chamber in a flow through water recirculatory system in laboratory of Fisheries Technology Department. Measurement of respiration rates in unfed, fed and post-fed condition were done in system of metabolism chambers according to the design made by Chakraborty (1992).

Three experimental diets A, B and C containing 28, 33 and 38% crude protein levels respectively were prepared by using fish meal, mustard oil cake, duck weed, rice bran and wheat bran (Table 1).The diets prepared as pellets were sun dried for 1 day followed by oven drying at 70^{0}C for over night. The proximate composition of these dry experimental diets are given in Table 1. The diets were fed at a known ration to three

treatment groups of fish in metabolism chambers. Each of three treatments had two replicates each having ten fishes of 10.8±0.56g fish in metabolism chambers. The metabolism chambers were marked as M_1, M_2, r and M_3 where the chamber "r" was used as reference and had no fish in it. A constant water flow- rate of 30 L/h through the flow meter was maintained in all metabolism chambers during direct monitoring of oxygen consumption by an oxygen probe kept in cuvette. After the first day in unfed condition for acclimation in the chambers, the fish fingerlings were subjected to measure resting metabolic rate for the following two unfed days over 24 hours period. The 24 hours measurement of the oxygen consumption (directly measured by oxygen probe of oxygen meter in the cuvette) in each three group of fish were measured as resting rate and the values were expressed as mg O_2/kg/h (Chakraborty *et al.* 1999).

Table 1. Formulation and proximate composition of experimental diets prepared from various ingredients

Ingredients	Diet (s)		
	A	B	C
Fish meal	20.00	35.00	50.00
Mustard oil cake	21.50	17.50	18.50
Duck weed	21.00	22.00	16.00
Rice bran	25.00	18.00	8.00
Wheat bran	10.00	5.00	5.00
Chromic oxide	0.50	0.50	0.50
Vitamin and Mineral premix*	2.00	2.00	2.00
Proximate composition (% dry matter basis)			
Parameters			
Dry matter	95.16	93.48	93.77
Moisture	4.84	6.52	6.23
Crude protein	28.08	33.82	37.29
Crude lipid	8.27	7.83	10.66
Ash	16.11	18.70	21.91
NFE**	42.20	32.53	23.41
Gross energy value (kJ/g)***	21.15	21.31	21.74

**Rhone Poulenc, Bangladesh*

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

**** Calculated by Bomb calorimeter (Gallenkamp)*

After 3 days, the fishes in metabolism chambers a, b and c were given known amount of diets having either 18, 33 or 38% crude protein. Feeding of fish started at 9.00 am and continued up to 4.00 pm. The pellets which were left uneaten and entered into faecal column collected, dried. The uneaten weight were subtracted from the amount offered to fish and the value was treated as the amount of diet ingested. Alternate hourly feeding of fish continued according to their response during feeding period. Twenty four hours hourly monitoring of oxygen consumption as mg O_2/kg/h of the post-fed fish group of fish in each metabolism chambers was recorded for alternate 13 days as days 3, 5, 7, 9, 11, 13. 15, 17, 19, 21, 23, 25 and 27 for each replicates.

The proximate composition of the diets, fish and faeces were analysed according to standard procedure given in AOAC (1980). The gross energy content of fish, feeds and faeces were determined by Bomb Calorimeter (Gallankamp, Automatic Adiabatic Bomb Calorimeter). The gross energy loss through metabolism - respiration (R) was determined indirectly by measuring oxygen consumption (Brafield and Llewellya 1982) of fish in the chambers. The values thus obtained were multiplied by the calculated Q_{ox} (oxycalorific co-efficient of respective substrate) value of individual diet which were 14.15, 14.01 and 13.87 J/mgO_2 respired by fish fed on diet A, B and C respectively.

Oxygen consumption by fish in the chambers was directly measured as mg/L by using an oxygen meter (Check:mate, oxygen probe) and calculated by the following formula:

$$\text{Oxygen consumption, mg } O_2\text{/ kg/ h} = \frac{(O_2\text{Sat} - O_2\text{out}) \times \text{water flow rate (L/h)} \times 100}{\text{wt. of fish (g) in chamber}}$$

where, O_2 Sat = Dissolved Oxygen (DO) in the reference chamber

O_2 out = Dissolved Oxygen (DO) in the out let of metabolism chamber containing fish.

The values thus obtained were multiplied by the respective Q_{ox} of 14.15, 14.01 and 13.87 J/mgO_2 for fish fed on diet A, B and C respectively to convert the values into energy.

Statistical analysis of the study was done by analysis of variance (ANOVA) to compare treatment means using the statistical package of Minitab (Ryan *et al.* 1985).

Results

Oxygen consumption of unfed *Catla catla* in different treatments showed no significant variation ($p>0.05$) for resting metabolism (Chakraborty *et al.* 1999). The mean oxygen consumption of fish during resting metabolism over 24 hours period were 151.66 ± 4.86, 153.91 ± 6.23 and 150.26 ± 4.38 mgO_2/kg/hr for treatments a, b and c respectively considering oxycalorific value of 13.56 (Brafield and Llewellyn 1982). These values were recalculated and found 5.40, 5.52 and 5.52 KJ/chamber/day in treatment a, b and c respectively.

Figure 1. shows the post-feeding oxygen consumption over resting rate of *Catla catla* fed on 28, 33 and 38% dietary protein. In all cases oxygen consumption started to increase after the feeding commenced and continued for several hours to a level to reach the highest (peak) and then started to decrease to the level of resting rate showing no further effect of feeding on respiration. Fish feeding at 38% dietary protein, the mean oxygen consumption over 24 hrs period were found a much higher value having a longer duration than those found with 28 and 33% protein diet (Fig. 1). Various aspects of oxygen consumption by fish in three treatments fed on diets A, B and C containing 28, 33 and 38% dietary protein respectively is shown in Table 2. The mean daily oxygen consumption (R) over 24 hrs in fish fed on 28, 33 and 38% protein diet in treatment a, b and c was found 341, 394 and 419 mgO_2/kg/h respectively. These values were found significantly different ($p<0.05$) from one another and found to increase with an increase

of dietary protein (Table 2). The higher mean peak value with a larger SDA magnitude was obtained in fish fed on 38% dietary protein. Table 2 also shows that the peak oxygen consumption by fish fed on diet A, B and C was 565.5, 657.5 and 696.0 $mgO_2/kg/h$ respectively with percent increase over mean resting rate (peak mean) of 273, 327 and 363 respectively. These values increased significantly ($p<0.05$) with the increase of dietary protein. Mean SDA duration was quite high with values of 21 to 22 plus hours in fish fed on the three diets.

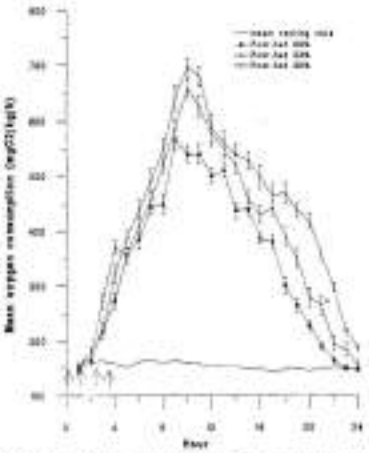

Fig. 1. Post feeding oxygen consumption over resting rate of *C. catla* fed on 28, 33 and 38% dietary protein.

Table 2. Different aspects of specific dynamic action (SDA) in fish in three treatment groups A,B and C fed on28, 33 and 38 % dietary Protein (N = 2)

Treatment	Total fish wt.(g) in metabolism chambers	Mean ration (g/day)	Mean resting rate ($mgO_2/kg/h$)	Peak oxygen consumption ($mgO_2/kg/h$)	Peak mean (% increase over mean resting rate)	Time to reach the peak
A	109.50	2.19	151.66	565.50	272.87	7
B	110.40	2.21	153.90	657.50	327.19	8
C	112..80	2.10	150.26	6996.00	363.20	8
Treatment	Duration (h)	Mean metabolism (mgO_2 /kg/h)	SDA magnitude (mgO_2/chamb/day)	Mean daily energy intake (KJ/day)	SDA Coeff (%)	"R" as % of "C^"
A	21	341.00	497.59	47.09	14.95	26.93
B	22+	394.10	638.72	47.10	19.00	31.17
C	22+	419.40	736.53	45.65	22.36	34.74

The post feeding effect in fish fed on 38% dietary protein still had some effect which continued beyond the scope of measurement due to data recording time over 24 hours cycle. However, this amount being an insignificant was overlooked. The mean SDA magnitude in post fed fish in treatment a, b and c over 24 hours was recorded as 498, 639 and 737 mgO_2/chamber/day respectively (Table 2). When recalculated the mean

metabolism (R) in fish in each chamber fed on 3 diets was found significantly different ($p<0.05$) having the values of 896, 1048 and 1143 mgO_2/chamber/day and showed an equivalent energy loss of 12.68, 14.68 and 15.86 KJ/chamber/day respectively (Table 2). Whereas, the equivalent energy of SDA magnitude was 7.04, 8.95 and 10.21 KJ/chamber/day in fish fed on diet A, B and C respectively with resulting SDA coefficients of 14.95, 19.00 and 22.36% in fish in treatment a, b and c respectively showing a significant increase with the increase of dietary protein (Table 2). Total daily metabolic expenditure including resting metabolic rate - R, as % of total ingested energy (consumption, C^) was 26.93, 31.17 and 34.74% which was found directly dependent on the dietary protein content (Table 2). The regression model drawn for the relationship between energy in consumption- C^ and energy lost in metabolism, R as % of C^ is expressed by the following metabolical model :

$Y = 5.1737 + 0.781 X$ ($r^2 = 0.9976$)

Where, Y = Energy lost in metabolism, R as % of ingestion C^.

X = Dietary protein level (28 to 38% range).

Discussion

There have been reports about the values of oxygen consumption for resting metabolism in different fish species and a large variation is evident (Hamada and Maeda, 1983, Chakraborty *et al.* 1992) depending upon some factors like size of fish, water temperature, fish species etc. Thus, Kausch (1969) found that 10 ± 0.5 g size young carp, *Cyprinus carpio* had resting metabolic rate of 80, 136 and 214 mgO_2/kg/h at 10, 15 and 20°C respectively whereas, Huisman (1974) showed that *Cyprinus carpio* of 31-47g and 2-16g had resting metabolic rate of 48 and 83 mgO_2/Kg/h respectively. However, Chakraborty *et al.* (1992) obtained mean resting rate for unfed common carp of 70 ± 10g size as 152 mgO_2/Kg/h. Therefore, this experiment had showed a quite reasonable value among the reported values for resting metabolic rate.

Fig. 1 shows that the post-prandial oxygen consumption in response to feeding of different diets containing 28, 33 and 38% crude protein increased with increase of dietary proetin. Similar results were obtained in blue gill, *Lepomis machrochirus* by Pierce and Wissing (1974); in *Cyprinus carpio* by Chakraborty *et al.* (1992b). The time to reach the peak oxygen consumption in this study varied from 7 to 8 hours after feeding and was not significantly affected by the energy intake and dietary protein content (Table 2). In the study with largemouth bass, *Micropterus salmoides* Tandler and Beamish (1981) observed that maximum oxygen uptake reached within 2 to 4 hours of feeding whereas, Chakraborty *et al.* (1995) obtained this time to reach the peak value by *C. carpio* with 1 to 7 hours after feeding. Jobling and Davies (1980) noted that the peak level of oxygen consumption in plaice, *Pleuronectus platessa* reached before satiation and concluded that the processes of producing the SDA effect are limited by cellular metabolism. Hamada and Ida (1973) reported two peaks in post-fed common carp, one 3-4 hours after feeding and the other after 5-7 hours after feeding which was dependent to the amount of food intake. The duration of elevated metabolic rate is variable between fish species under different experimental condition. Present study shows a small difference in duration (h)

of elevated metabolic of 21 to >22 hours (Table 2). Similarly, the SDA duration ranged between 10 to 19 hours, 12 to 19 hours and 10 to 21 hours in common carp fed on 20, 35 and 50% dietary protein respectively which was dependent on ration size but not dietary protein content (Chakraborty 1992). This study showed the similar findings that there was no significant variation in duration of SDA effect of fish fed on different protein diets. Tandler and Beamish (1981) showed that the rate of oxygen uptake remained elevated which was positively related to energy ingested, negetively related to body weight and unrelated with protein content of the diet in bass (*Micropterus salmoides*). Soofiani and Hawkins (1982) obtained similar results for juvenile cod, *Gadus morhua*. LeGrow and Beamish (1986) working with rainbow trout *Salmo gairdneri* found that protein content in the diet does not significantly influence the duration of elevated metabolism. SDA magnitude in this study was found clearly related to protein content in the diets (Table 2) and increased significantly with increase of dietary protein.Chakraborty *et al.* (1992b) obtained that SDA magnitude was related to both energy intake from varying dietary protein content. In largemouth bass, the SDA magnitude increased linearly with the protein content of the diets (Tandler and Beamish, 1980). SDA coefficient values in this study with *C. catla* fed on 28, 33 and 38% dietary protein were 14.95, 19.00 and 22.36% respectively (Table 2). Cho *et al.*, (1976), Jobling and Davies (1980), Tandler and Beamish (1980) obtained the SDA co-efficient values of 8.0-12.0% in *Salmo gairdneri* , 10.0-18% in *Pleuronectes platessa* and 5.1-17.5% in *Micropterus salmoides* respectively. Similarly, Medland and Beamish (1985) and Chakraborty *et al.* (1992b) obtained the SDA co-efficient as 8.5-33.7% in *Salmo gairdneri*, 8.99-15.94% in *Cyprinus carpio* respectively. Noted that SDA co-efficient is dependent on the protein content of the diet in rainbow trout. LeGrow and Beamish (1986) and Chakraborty *et al.* (1992b) found that SDA co-efficient in rainbow trout and common carp respectively increased with the protein content of the diet as has been obtained in this study with *Catla catla*.

In the present study the oxygen consumption was considered as total energy of metabolism comprising resting, feeding and restricted active metabolism and was designated as 'R'. Whereas, SDA was determined by subtraction between 'R' and 'mean resting metabolism value' for feeding metabolism. However, this study showed a significant ($p<0.05$) increase in metabolic rate with increase in dietary protein (Table 2). Similarly, Chakraborty *et al.* (1992b) obtained the values of 'R' as 27.92% to 30.51% of energy in 'C^' which showed were increasing with the increase of dietary protein in *Cyprinus carpio*.

From the model developed for % energy lost in 'R' is of importance for aquaculturist because, the post-feeding oxygen requirement in the intensive culture system can be calculated where the protein levels (and if ration levels) are known. This is also important in energy budgeting to determine the amount of energy wasted or expended as 'SDA' or 'R'.

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Effects of duckweed (*Lemna minor*) as dietary fishmeal substitute for silver barb (*Barbodes gonionotus* Bleeker)

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Abstract

A 60-day long growth trial was conducted to evaluate the suitability of duckweed *Lemna minor* as dietary fish meal substitute for silver barb (*Barbodes gonionotus* Bleeker). Five iso-nitrogenous diets were formulated to contain 35% protein and each treatment had three replicates with 15 fish in each aquarium with a mean initial weight of 1.5 ± 0.2 g. Duckweed was used in the experiment to replace 10, 20, 30 and 35% of the dietary fish meal in diet 2, 3, 4 and 5 respectively. Fish meal was used as the sole source of protein in control diet (Diet 1). Fish were fed three times daily at satiation level. In terms of growth, food conversion and protein utilization, the control diet and diet containing 17.07% duckweed showed the best ($P<0.05$) performance followed by diets containing 34.14%, 51.21% and 59.24% duckweed. Fish fed diets containing higher levels of duckweed had higher carcass moisture and lower lipid content compared to the control diet. Histopathological examination revealed abnormalities in the liver of fish fed diets containing higher inclusion of duckweed. It was noted that 10% of the dietary fish meal protein could be replaced by duckweed (*L. minor*) in the diet of silver barb (*B. gonionotus*).

Key words: *B. gonionotus*, Duckweed, Fish meal

Introduction

Duckweed are small floating aquatic plants, widely available in Bangladesh and consists of four genera *viz. Lemna, Spirodela, Wolffia* and *Wolffiella* among which about 40 species have been identified so far (Journey *et al.* 1991). In Bangladesh, it can easily be grown abundantly with minimum cost, made available in much cheaper than other alternative plant protein sources. Fresh duckweed is widely used as fish feed but only a few reports are available on the use of dry duckweed as fish feed.

Silver barb, *Barbodes gonionotus* is one of the best suited species for bringing the unutilized or underutilized water bodies under intensive culture. This fish was also found to feed well on supplemental feed (Hussain *et al.* 1987). The fish can grow fast at high stocking densities (Sipitekhiat and Leenannod 1984).

The present investigation was designed to evaluate the suitability of duckweed as dietary fishmeal substitute for silver barb (*B. gonionotus*) fingerlings.

Materials and methods

A static indoor rearing system was used for the growth trial. Rectangular glass aquaria of 55 L capacity containing about 50 L of water was used as experimental tanks. Tap water was supplied in the aquaria during the experimental period and aeration was provided to maintain the adequate oxygen level. Induced bred fingerlings of silver barb (*B. gonionotus*) were obtained from a stocking pond adjacent to the Faculty of Fisheries at the Bangladesh Agricultural University (BAU) Campus, Mymensingh. Prior to the start of the experiments, the fingerlings were acclimated to the laboratory condition for seven days.

Five iso-nitrogenous diets were formulated to contain 35% protein to evaluate duckweed (*L. minor*) as dietary fish meal substitute for silver barb (*B. gonionotus*). The fish meal was prepared in the laboratory by grinding small dry fishes of mixed origin and seived to pass through 0.5 mm mesh. Duckweed were collected locally from a fish pond in a village adjacent to BAU campus. After collection, duckweed were dried in the sun and ground into powder to pass through a 0.5 mm seive. Before formulating the test diets, all the ingredients were subjected to proximate analysis and the results are presented in Table 1. Then the various ingredients were mixed together in required quantities according to formulations as shown in Table 2.

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

Protein sources	Dry matter	Protein	Lipid	Ash	C. fibre	NFE*
Fish meal	96.55	65.44	10.00	20.39	1.19	2.48
Duck weed	93.48	20.50	7.02	14.41	13.47	44.60

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

Table 2. Formulation of experimental diets

Ingredients (%)	Diet No.				
	1(Control)	2	3	4	5
Fish meal	53.48	48.13	42.78	37.43	34.76
Duck weed	-	17.07	34.14	51.21	59.24
Cod liver oil	-	-	1.00	1.50	1.50
Soybean oil	5.00	4.00	3.00	1.50	1.00
Starch	28.00	23.00	13.58	2.86	1.00
Vitamin premix[1]	1.00	1.00	1.00	1.00	1.00
Mineral premix[1]	2.00	2.00	2.00	2.00	1.00
CMC[2]	2.00	2.00	2.00	2.00	1.00
α-Cellulose	8.02	2.30	-	-	-
Chromic oxide	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00

1 *According to Hossain and Jauncey (1989)* [2]*Carboxymethyl cellulose of high viscosity.*

Adequate amount of water was added to moisten the mixture and the mixture was extruded through 1 mm diameter die of a pelleting machine (Hobart Mixture machine A 200). All the diets were subjected to proximate analysis and the results are presented in Table 3.

Table 3. Proximate composition of the experimental diets (% dry matter basis)

Parameters	Diet No.				
	1	2	3	4	5
Dry matter	91.86	91.93	92.68	93.09	92.05
Crude protein	33.88	33.85	34.67	34.28	34.39
Lipid	10.34	9.85	11.65	10.33	10.31
Ash	13.01	14.61	15.44	16.12	17.98
Crude fibre	7.05	6.48	6.98	8.08	8.03
NFE[1]	35.72	35.21	31.26	31.19	29.29
Gross energy (kcal/g)[2]	4.26	4.20	4.25	4.10	4.03
P/E ratio[3]	79.53	80.59	81.57	83.60	85.33

[1] *Nitrogen free extract = 100-% (Crude protein + lipid + ash + crude fibre).*
[2] *Gross energy calculated after Jauncey and Ross (1982).*
[3] *Protein to energy ratio in mg protein /Kcal of total energy.*

The uniform sized fingerlings of silver barb were randomly distributed at the rate of 15 fish in each replicate with initial mean weight of 15±0.02 g. The fish were fed at satiation level with the formulated diets three times daily at 4 hourly intervals between 09.00 and 17.00 hours. In order to maintain good water quality, about half of the water in each tank was changed every day throughout the experimental period. Faeces were collected during the last two weeks of the experimental period for the study of protein digestibility of diet. Water quality parameters such as temperature, dissolved oxygen and pH were monitored weekly and the ranges were: temperature 27-30°C, dissolved oxygen 6.2 - 8.3 mg/l and pH 6.8 - 7.8.

Feed ingredients, experimental diets and fish sample were analysed for their proximate composition (Horwitz 1980). The chromic oxide content of the experimental diet and faeces was determined after Furukawa and Tsukahara (1966). The histological study of different organs were done according to the procedure and methods described by Luna (1968). Specific growth rate (SGR), weight gain (%), food conversion ratio (FCR), protein efficiency ratio (PER), apparent net protein utilization (ANPU) and apparent net protein digestibility (APD%) were calculated after Castell and Tiews (1980). Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (Duncan 1955) to test the significance of variation among the treatment means.

Results

In term of growth, food conversion and protein utilization, control diet and diet 2 (containing 17.07% duckweed) showed significantly (P>0.05) best growth performances among the experimental diets. Fish fed diet containing 59.24% duckweed produced significantly (P>0.05) the lowest growth performance. The growth performances of silver barb fingerlings during the experimental period are presented in Table 4.

Table 4. Growth and feed utilizition of *B. gonionotus* fed experimental diets

Parameters	Diets					
	1	2	3	4	5	± SE[2]
Initial wt. (g)	1.48[a]	1.50[a]	1.51[a]	1.52[a]	1.49[a]	0.02
Final wt.(g)	5.14[a]	5.06[a]	4.52[b]	3.65[c]	3.35[d]	0.038
Weight gain (g)	3.66[a]	3.56[a]	3.01[b]	2.13[c]	1.86[d]	0.034
% Weight gain	247[a]	237[b]	199[c]	140[d]	125[e]	2.46
SGR (% day)	2.07[a]	2.02[a]	1.83[b]	1.46[c]	1.35[d]	0.016
FCR	1.86[a]	1.92[a]	2.32[b]	3.21[c]	3.66[d]	0.045
PER	1.59[a]	1.54[a]	1.28[b]	0.91[c]	0.79[d]	0.033
ANPU (%)	25.53[a]	25.05[a]	19.42[b]	13.76[c]	11.69[d]	0.164
APD (%)	90.42[a]	88.60[b]	85.26[c]	81.24[d]	80.10[d]	0.43

[1] *Figures in the same row with same superscripts are not significantly different (P>0.05).*
[2] *Standard error of treatments calculated from the residual mean square in the analysis of variance.*

The highest SGR value was obtained from the control diet and diet 2 containing 17.07% duckweed (Table 4). The FCR values ranged between 1.86 to 3.66 with diet 1 and 2 producing significantly (P<0.05) the lowest FCR. The PER values ranged between 0.79 to 1.59. Diet 1 and 2 produced higher PER values of 1.59 and 1.54, respectively. There was no significant difference (P>0.05) between the ANPU values of control diet and diet containing 17.07% duckweed. The ANPU values of different diets ranged between 11.69 to 25.53%. Control diet produced significantly the highest APD value and diet 5 containing 59.24% duckweed produced the lowest APD value. In general, the APD value decreased with the decreasing level of fish meal in the diets.

Histological examination of control diet showed no evidence of any degenerative changes in any organs. Plate 1a shows the sections of liver of fish fed control diet. The most noticeable microscopic changes were recorded in the liver of the fish fed diets with higher levels of duckweed (59.24 %). The striking lesions of liver was the appearance of fat changes which was characterized by the presence of empty spaces in the hepatocytes (Plate 1b). There were also focal accumulation of leukocytes and haemmorhagic lesions in the midzonal area of hepatic lobules (Plate 2).

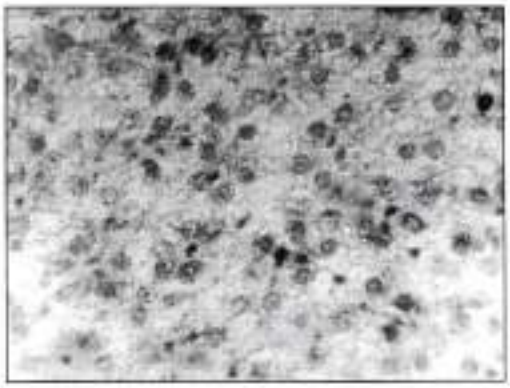

Plate 1a. Section of fish liver from control diet (400 times) showing no evidence of fatty changes.

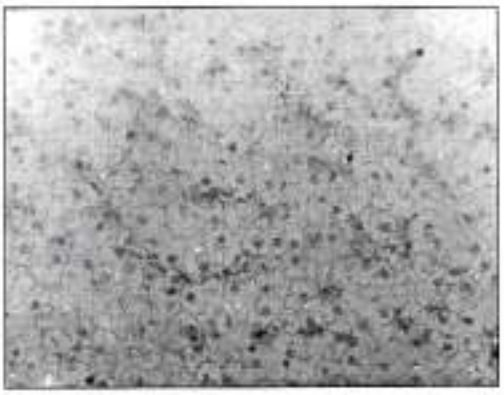

Plate 1b. Showing fatty changes in the cytoplasm of hepatic cells (400 times) in liver of fish fed diet containing 59.24% duckweed.

Plate 2. Section of liver showing focal accumulation of leukocytes in the hepatic lobules of fish fed diets (H & E x 400).

Proximate carcass composition of the initial fish and fish sample at end of experiment is shown in Table 5. The highest carcass protein (15.46%) content was obtained in control diet followed by diets 2, 3, 4 and 5 duckweed. In general, there was a decrease in carcass protein and lipid content with the increase of duckweed levels.

Table 5. Proximate carcass composition of the fish at the start and at the end of the experiment (% fresh matter basis)

Parameters	Initial	Final diets				
		1	2	3	4	5
Moisture	78.46	74.17	75.56	75.99	76.84	77.12
Crude protein	14.06	15.46	15.43	15.02	14.56	14.40
Lipid	4.88	6.18	5.48	4.65	3.90	3.68
Ash	2.66	3.31	3.21	3.43	3.69	3.87

Discussion

In the present study, control diet and diet containing 17.07% duckweed showed better performance in term of growth, food conversion and protein utilization in comparison to other diets. Diets containing 34.14%, 51.21% and 59.24% duckweed resulted poor growth performance. The poor growth performances are also substantiated by the histological examination where kidney and liver of fish fed diet containing higher levels of duckweed revealed degenerative changes in the liver.

The findings of the present study indicate that duckweed is comparatively less successful in replacing fish meal protein in comparison to other plant protein sources. Hossain *et al.* (1994) reported that up to 50% of the fish meal protein in *P. gonionotus* diet could be replaced by plant proteins (mustard oilcake and sesame meal) without affecting the growth performance.

In contrast to the present findings, Okoye and Mbagwu (1985) observed higher FCR i.e. poor growth performance of *Sarotherodon galilaeus* fingerling fed diet containing 33% crude protein with 10% duckweed. Attempts by Devaraj *et al.* (1981) to incorporate 40% *Lemna* powder with ricebran, oilcake and ragiflour in common carp diet resulted similarly poor growth and food utilization.

The reason for comparatively better growth of *B. gonionotus* fed on diet containing 17.07% duckweed may be due to the combination of plant protein and animal protein which gave a good nutritional balance. A mixture of plant and animal proteins is much more efficient than that of a single source of protein (Cho *et al.* 1974, Meske and Pruss 1977). Hossain and Jauncey (1990) also reported that use of different protein sources in combinations can prevent a high inclusion level of any single anti-nutritional factor in the diet and can also be a means of compensating for essential amino acid deficiency in any single protein source.

The significantly lower growth of *B. gonionotus* observed with diet containing 34.14%, 51.21% and 59.24% duckweed may be due to the decreased level of fish meal and higher level of duckweed inclusion. The growth responses for fish fed diets containing increasing level of duckweed may presumably be due to the presence of antinutritional factors, low essential amino acids (EAAs) and polyunsaturated fatty acids, although no reports are available on the toxic substances present in duckweed. Although the EAAs content of the experimental diets were not analysed, another possible cause of retard growth may be that diets containing higher levels of duckweed were deficient in some of the EAAs. Duckweed is reported to contain very low amount of EAA (Hossain 1996).

In this study, the apparent protein digestibility (APD) decreased with the higher level of duckweed inclusion. Plant protein appears to be basically less digestible than animal protein (Singh and Pandey 1980). The APD values obtained from this study ranged from 80.10 to 90.42% (Table 4). The fishmeal based control diet in the present study produced the higher APD value (90.42). According to NRC (1977) carp can digest up to 95% of fish meal protein. However, the value may be decreased to 80-85%, depending on the origin and processing of fish meal (Ogino and Chen 1973). The lower APD values obtained in the diets containing duckweed may be due to low digestibility of

plant protein. Hasan *et al.* (1990) also reported lower digestibility of diets with increasing level of dietary water hyacinth and leucaena meal in *Labeo rohita*.

From the histological evidences in the livers of the fish fed higher levels of duckweed it is assumed that an unidentified toxic component in the higher levels of duckweed is responsible for the degenerative changes.

In the present study, the diets containing higher level of duckweed inclusion produced significantly ($P<0.05$) the highest carcass moisture and lower lipid content (Table 5). Hassan and Edwards (1991) reported that feeding duckweed to tilapia had a profound effect on carcass composition i.e. increase in carcass moisture and decrease in carcass lipid content. Similar results have been reported with higher level of dietary plant protein inclusions in common carp (Hossain and Jauncey 1989) and rainbow trout (Yurkowski *et al.* 1978).

Therefore, considering the findings of this study and the availability, cost and abundance of duckweed in Bangladesh, it may be used as an alternative protein source for silver barb but the inclusion level should not exceed 20% duckweed on dry matter basis.

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Larval development of a mangrove crab (*Perisesarma bidens* De Haan) (Crustacea: Brachyura: Sesarminae)

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Abstract
Larval development of *Perisesarma bidens* (De Haan) was investigated in laboratory conditions. Morphology of all larval stages and 1st crab stage was described and illustrated in detail, and compared with other species of sesarmid crabs. The zoeal morphological features of *P. bidens* are almost similar to other species of *Sesarma* in lacking a pair of lateral spines on carapace.

Key words: Mangrove crab, *Perisesarma bidens*, Morphology

Introduction

Sesarmid crabs are one of the brachyuran, which show various degrees of adaptation to semi-terrestrial existence, ranging from the sea-bound and intertidal preference terrestrial life. Many brachyuran crabs, particularly the members of Ocypodidae and Grapsidae (Sesarminae) have achieved the transition from life in the marine environment to terrestrial and freshwater habitats (Hartnoll 1988). While most of these species need to return to the sea in order to reproduce, others show abbreviated larval development as an adaptation to their life on land or in freshwater (Powers and Bliss 1983, Rabalais and Gore 1985, Zimmerman and Felder 1991).

The mangrove rearing sesarmid crab, *Perisesarma bidens* (De Haan), is widely distributed in the Indo-West Pacific region, the Bay of Bengal to the Andamans, Malay archipelago, Hong kong, Formosa, Korea and Japan (Sakai 1976, Aiyun and Siliang 1991). In Japan, this species mainly occurred from Tokyo Bay to Kyushu and the Ryukyu Islands (Miyake 1983). The present species generally live in burrow constructed in the edges or within the forests or in the reed marsh higher than ordinary high water mark. This crab commonly occurred in Okinawan mangal areas (Watanabe 1993).

Larval development of this crab is not well known. Watanabe (1993) described the population structure and feeding habits of it from Okinawa Island, but not investigated its larval development. The larval development of several sesarmid crabs have been described by many workers in the past (Terada 1976, Lago 1993, Mia and Shokita 1996 and 1997, Cuesta *et al.* 1999). But until now, the larval development of *P. bidens* have not been described. The objectives of the present study are to provide the detail

morphological descriptions of all the larval stages including megalopa and 1st crab of *P. bidens*, and to compare them with other species of sesarmid crabs.

Materials and methods

An ovigerous female of *Perisesarma bidens* (16.2 mm in carapace length and 19.3 mm in carapace width) was captured from the mouth of west bank of Nuha river in Manko estuary, Naha, Okinawa Island, Japan on 16 April'99, and placed it in a plastic holding tank. Then the crab was brought to the wet laboratory of the Department of Chemistry, Biology and Marine Science, and reared in a plastic trough containing seawater of 27±1‰ salinity with moderate aeration. During the experimental period, water temperature ranged from 21.3 to 23.5°C.

Hatching occurred on the morning of April 21. Among the hatched larvae, most photo positive zoeae were transferred to 10-liter capacity plastic bowls that were covered with black paper sheet outside and reared under the same conditions as mother. The water was also aerated and renewed 50% daily. The newly hatched nauplii of brine shrimps, *Artemia salina* were supplied to fed the larvae. The minced meat of clam with brine shrimps also supplemented to fed megalopa and 1st crab daily.

Everyday several numbers of larvae were preserved in 50% ethylene glycol solution for the identification of larval stages. The larvae were dissected under a binocular stereomicroscope. Drawings and measurements were performed with the aid of a profile projector and a dissecting microscope. At least 10 specimens of each stage were measured and dissected. Measurements were taken for the zoeal stages include the distance between the tips of dorsal and rostrum spines for total length (TL), from the base of rostral spine to the posterior margin of carapace for carapace length (CL) and maximum distance across the carapace for carapace width (CW). For the megalopa and 1st crab, distance between the tip of anterior margin to the posterior margin of carapace for CL and maximum distance across the carapace for CW. All measurements were taken following Konishi and Shikatani (1998). Specimens of all larval stages and 1st stage of *P. bidens* have been deposited in the Marine Science Laboratory of the University of the Ryukyus, Okinawa, Japan.

Results

The larvae of *P. bidens* hatched out of about 6.30 pm on 21 April'99 and reached to 1st crab stage after passing through 4 zoeal stages and one megalopa stage. Measurements of typical features are summarized in Table 1. Segmentation and setation of appendages of zoeae and megalopa are listed and compared with other species in Table 2 and 3, respectively. The major characteristics of each zoeal stages, megalopa stage and 1st crab stage are described in detail below.

Table 1. Measurements of morphological features of *Perisesarma bidens larvae*

Characters	Larval stages				Megalopa	1st crab
	ZI	ZII	ZIII	ZIV		
TL (mm)	0.78±0.05	0.95±0.02	1.08±0.11	1.39±0.13		
CL (mm)	0.45±0.07	0.51±0.02	0.62±0.05	0.64±0.01	0.95±0.01	1.85±0.04
CW (mm)	0.32±0.01	0.37±0.03	0.42±0.01	0.46±0.08	0.71±0.06	1.69±0.21
LD (day)	3.5±0.03	4.0±0.1	3.8±0.21	3.6±1.01	6.5±1.02	8.9±1.13

TL- total length, CL- carapace length, CW- carapace width, LD- larval duration.

Table 2. Distinguishing characters among the zoeae of *Perisesarma bidens*, *Sesarma guttatum**, *S. intermedia*** and *S. erythrodactyla***

Stages and appendages	*P. bidens*	*S. guttatum*	*S. intermedia*	*S. erythrodactyla*
Zoea I				
Maxillule: Setae on basal & coxal endites	6,5	5,6	5,5	5,5
Maxilla: Setae on basal and coxal endites	5+5, 3+5	5+3, 5+3	4+5, 3+5	4+5, 3+5
Zoea II				
Maxillule: Setae on basal & coxal endites	7,5	7,6	7,5	7,6
Maxilla: Setae on basal and coxal endites	5+5, 3+5	5+4, 5+3	4+5, 3+5	4+5, 3+5
Zoea III				
Maxillule: Setae on basal & coxal endites	7,7	7,6	7,5	7,6
Maxilla: Setae on basal and coxal endites	5+5, 3+5	5+5, 5+3	4+5, 3+5	5+5, 3+5
Setae on scaphognathite	12	11	11	12
Zoea IV				
Maxillule: Setae on basal & coxal endites	11,8	11,7	10,6	11,7
Maxilla: Setae on basal and coxal endites	5+6, 3+7	6+5, 7+4	5+5, 4+5	6+7, 4+5
Setae on scaphognathite	19	17	19	21

* *Lago (1993)*, ** *Terada (1976)*

Table 3. Distinguishing characters (setal arrangement) among the megalopa of *Perisesarma bidens*, *Sesarma guttatum**, *S. intermedia*** and *S. erythrodactyla***

Appendages	*P. bidens*	*S. guttatum*	*S. intermedia*	*S. erythrodactyla*
Antenna				
Segments	8	8	9	8
Setae	10	13	13	7
Maxillule				
Endopod	7	6	6	6
Basal and coxal endites	15, 11	18,11	18,12	17,12
Maxilla				
Endopod	0	3	0	5
Basal and coxal endites	12, 12	18, 15	12, 15	13, 12
Scaphognathite	32+2	36+2	35+2	29+2
1st maxilliped				
Endopod	4	3	4	7
Exopod	3, 3	3, 3	3, 3	3, 4
Epipod	4	5	3	4
Basal and coxal endites	12, 9	11, 8	11, 7	9, 7
2nd maxilliped				

Endopod	0, 1, 3, 6	0, 1, 4, 7	0, 1, 3, 7	0, 1, 5, 9
Exopod	1, 6	1, 7	1, 5	1, 7
3rd maxilliped				
Endopod	9, 6, 3, 6, 9	8, 6, 2, 5, 7	9, 8, 4, 3, 7	8, 5, 3, 4,6
Exopod	1, 3	1, 5	1, 5	1,5
Epipod	9	24	15	12

* *Lago (1993)*, ** *Terada (1976)*

Morphology of zoeal stages

Carapace (Fig. 1. A-D): Carapace smooth, inflated, no lateral spine, rostral spine relatively shorter than dorsal spine, which is all zoeal stages. Carapace increased in size in each moulting. Eyes sessile in zoea I but stalked or movable in zoeae II to IV.

Antennules (Fig. 2. A-D): Zoea 1 bear 3 equal size of broad flat terminal aesthetes. Zoeae II to III bear 4 broad flat terminal aesthetes and small seta. Zoeae IV has 4 broad flat terminal aesthetes and 3 small setae of which 2 were subterminal, small endopod bud appeared. Antennules also increased in size in each moulting.

Antennae (Fig. 2. E-H): Spinous process well developed, bearing 2 rows of denticles, exopod with 2 short setae in all zoeal stages. Endopod developed in zoea III, well developed and shorter than spinous process in zoea IV.

Mandibles (Fig. 2. I-L): Incisor and molar processes differentiated in zoea I, incisor processes with 4 teeth, molar process subcylindrical in zoea II, incisor processes with teeth on one side, unarmed on opposite, molar process denticulated in zoea III, incisor processes with 3 teeth on one side, masticatory surface of molar process gradually flattened, no palp in zoea IV.

Maxillules (Fig. 3. A-D): Endopod 2 segmented, distal segment with 4 terminal plus 1 subterminal hairy spine and 1 seta on inner of proximal segment which was constant in all zoeal stages. Basal and coxal endites of zoeae I to IV bear 6,5; 7,5; 7,7 and 11,8 hairy setae, respectively. One additional plumose seta was present at the base of endopod that was also constant in zoeae II to IV.

Maxillae (Fig. 3. E-H): Endopod unsegmented but bilobed with 3+2 hairy spines in all zoeal stages. Basal and coxal endites of zoeae I to IV also bilobed and bearing 5+5, 3+5; 5+5, 3+5; 5+5, 3+5 and 5+6, 3+7 hairy setae, respectively. Scaphognathite with 4 soft plumose setae on distal margin, apical process tapering with fine setae in distal half in zoea I, 5 soft plumose setae on distal margin, apical process flattened, distally rounded with 3 plumose setae in zoea II, 8 plumose setae on distal margin and 4 on apical process in zoea III, 19 long soft plumose setae in zoea IV.

1st maxillipeds (Fig. 4. A-D): Basis with 10 medial setae in all zoeal stages. Endopod 5 segmented with 2, 2, 1, 2, 5 setae in zoea I to II, 2, 2, 2, 2, 5 in zoea III and 2, 3, 2, 2, 5 in zoea IV from proximal to distal segments. Endopod 2 segmented, distal segment with 4, 6, 8 and 10 plumose natatory hairs in zoeae I to IV, respectively.

2nd maxillipeds (Fig. 4. E-H): Basis with 4 medial setae in all zoeal stages. Endopod 3 segmented with 0, 1, 6 setae in all zoea stages. Endopod 2 segmented, distal segment with 4, 6, 8 and 10 plumose natatory hairs in zoeae I to IV, respectively. Small but segmented 3rd maxilliped (Fig. 5.I) bud appeared in zoeae IV.

Endopod	0, 1, 3, 6	0, 1, 4, 7	0, 1, 3, 7	0, 1, 5, 9
Exopod	1, 6	1, 7	1, 5	1, 7
3rd maxilliped				
Endopod	9, 6, 3, 6, 9	8, 6, 2, 5, 7	9, 8, 4, 3, 7	8, 5, 3, 4,6
Exopod	1, 3	1, 5	1, 5	1,5
Epipod	9	24	15	12

* *Lago (1993)*, ** *Terada (1976)*

Morphology of zoeal stages

Carapace (Fig. 1. A-D): Carapace smooth, inflated, no lateral spine, rostral spine relatively shorter than dorsal spine, which is all zoeal stages. Carapace increased in size in each moulting. Eyes sessile in zoea I but stalked or movable in zoeae II to IV.

Antennules (Fig. 2. A-D): Zoea 1 bear 3 equal size of broad flat terminal aesthetes. Zoeae II to III bear 4 broad flat terminal aesthetes and small seta. Zoeae IV has 4 broad flat terminal aesthetes and 3 small setae of which 2 were subterminal, small endopod bud appeared. Antennules also increased in size in each moulting.

Antennae (Fig. 2. E-H): Spinous process well developed, bearing 2 rows of denticles, exopod with 2 short setae in all zoeal stages. Endopod developed in zoea III, well developed and shorter than spinous process in zoea IV.

Mandibles (Fig. 2. I-L): Incisor and molar processes differentiated in zoea I, incisor processes with 4 teeth, molar process subcylindrical in zoea II, incisor processes with teeth on one side, unarmed on opposite, molar process denticulated in zoea III, incisor processes with 3 teeth on one side, masticatory surface of molar process gradually flattened, no palp in zoea IV.

Maxillules (Fig. 3. A-D): Endopod 2 segmented, distal segment with 4 terminal plus 1 subterminal hairy spine and 1 seta on inner of proximal segment which was constant in all zoeal stages. Basal and coxal endites of zoeae I to IV bear 6,5; 7,5; 7,7 and 11,8 hairy setae, respectively. One additional plumose seta was present at the base of endopod that was also constant in zoeae II to IV.

Maxillae (Fig. 3. E-H): Endopod unsegmented but bilobed with 3+2 hairy spines in all zoeal stages. Basal and coxal endites of zoeae I to IV also bilobed and bearing 5+5, 3+5; 5+5, 3+5; 5+5, 3+5 and 5+6, 3+7 hairy setae, respectively. Scaphognathite with 4 soft plumose setae on distal margin, apical process tapering with fine setae in distal half in zoea I, 5 soft plumose setae on distal margin, apical process flattened, distally rounded with 3 plumose setae in zoea II, 8 plumose setae on distal margin and 4 on apical process in zoea III, 19 long soft plumose setae in zoea IV.

1st maxillipeds (Fig. 4. A-D): Basis with 10 medial setae in all zoeal stages. Endopod 5 segmented with 2, 2, 1, 2, 5 setae in zoea I to II, 2, 2, 2, 2, 5 in zoea III and 2, 3, 2, 2, 5 in zoea IV from proximal to distal segments. Endopod 2 segmented, distal segment with 4, 6, 8 and 10 plumose natatory hairs in zoeae I to IV, respectively.

2nd maxillipeds (Fig. 4. E-H): Basis with 4 medial setae in all zoeal stages. Endopod 3 segmented with 0, 1, 6 setae in all zoea stages. Endopod 2 segmented, distal segment with 4, 6, 8 and 10 plumose natatory hairs in zoeae I to IV, respectively. Small but segmented 3rd maxilliped (Fig. 5.I) bud appeared in zoeae IV.

Pereiopods (Fig. 1. C-E): Present as small bud in zoea III, elongated and segmented in zoeae IV.

Abdomens and telsons (Fig. 5. A-D): Five somites plus telson in zoeae I and II, while 6 somites plus telson in zoeae II and IV. Somites 2 and 3 with a pair of spines on dorsolateral margins, former directed anteriorly and the latter posteriorly, posterolateral margin on somites 2 to 5 produced moderately in all zoeal stages. One pair of lateral spines presents on 2 to 5 somites in each stage. Telson bifurcated, each fork moderately widened, slightly longer than basal portion, bearing minute hairs on inner margins, posterior margin with 3 pairs of setae. Somites 2 and 5 with posterolateral pleopod buds in zoea III, pleopods well developed but unsegmented in zoea IV, Somites 6 with buds of uropods in zoeae III and IV. Structure of telson unchanged in all zoeal stages.

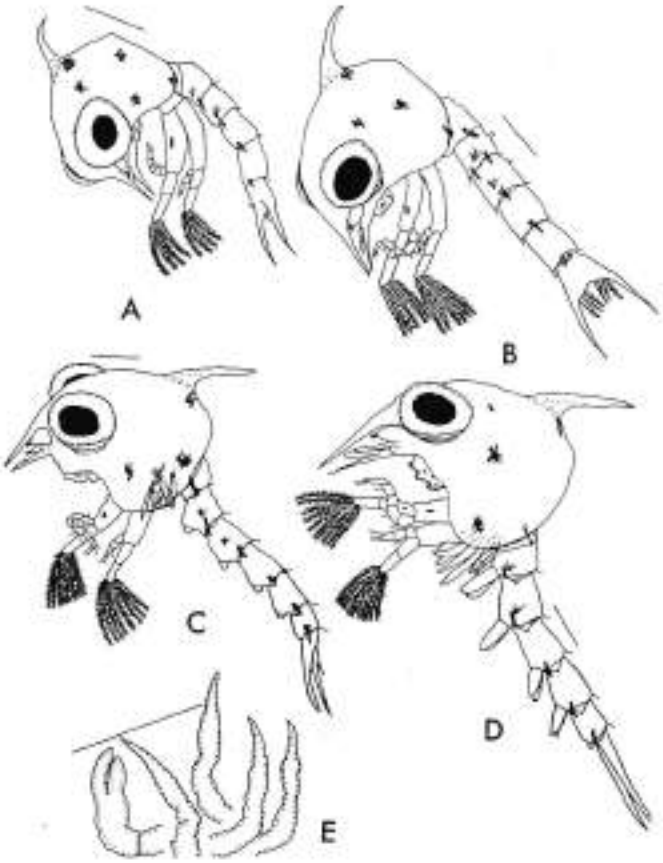

Fig. 1. *Perisesarma bidens* (De Haan). Zoeal stages: A- zoea I; B- zoea II; C- zoea III; D- zoea IV; E- pereiopods rudiment of zoea IV (scale bars= 0.2 mm).

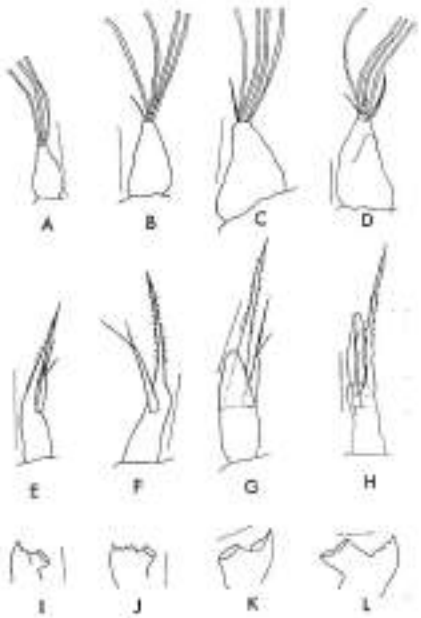

Fig. 2. *Perisesarma bidens* (De Haan). Antennules: A-D, zoea I-IV; Antennae: E-H: zoeae I-IV; Mandibles: I-L, zoeae I-IV (scale bar= 0.1 mm).

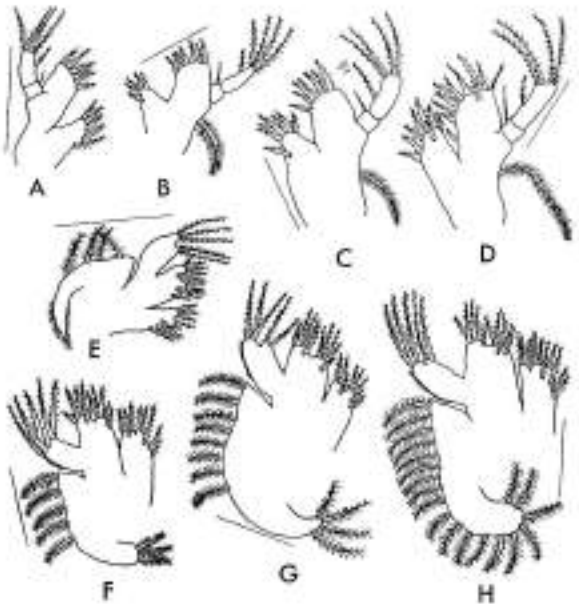

Fig. 3. *Perisesarma bidens* (De Haan). Maxillules: A-D, zoeae I-IV; Maxillae: E-H, zoeae I-IV (scale bar= 0.1 mm).

Fig. 4. *Perisesarma bidens* (De Haan). 1st maxillipeds: A-D, zoeae I-IV; 2nd maxillipeds: E-H, zoeae I-IV; 3rd maxilliped bud: I, zoea IV (scale bar = 0.1 mm).

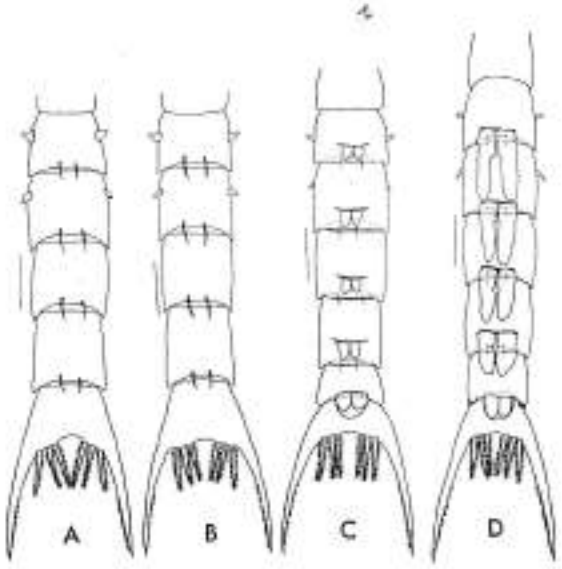

Fig. 5. *Perisesarma bidens* (De Haan). Abdomens: A-B, dorsal view of zoeae I-II; C-D, ventral view of zoeae III-IV (scale bar = 0.1 mm).

Morphology of megalopa

Carapace (Fig. 6.A): Dorsal surfaces smooth, longer than borad, eyes large and stalked.

Antennule (Fig. 6.B): Enlarged base with out seta. Peduncle 2 segmented inner flagellum absent, outer flagellum with 8 aesthetascs and 3 short setae.

Antenna (Fig. 6.C): Peduncle 3 segmented with 0, 1, 1, 5, 2 setae.

Mandible (Fig. 6.D): Cutting edge rounded, palp 2 segmented distal segment with 5 short setae.

Maxillule (Fig. 6.E): Endopod unsegmented, bearing 3 terminal, 3 marginal and 1 lateral setae. Basal and coxal endites with 16 and 11 setae, respectively. Basis with 1 long simple seta.

Maxilla (Fig. 6.F): Endopod simple with out seta. Basal and coxal enditesbilobed with 5+7 and 5+10, respectively. Scaphognathite with 30 plumose setae on marginal and 2 simple setae on blade surface.

1st maxillipeda (Fig. 7.A): Endopod with 3 terminal and 1 subterminal setae, exopod 2 segmented with 3, 3 plumose setae, epipod with 4 long simple setae of moderate length. Basal and coxal endites with 12 and 9 setae, respectively.

2nd maxilliped (Fig. 7.B): Endopod 4 segmented with 0,1,3,6 setae, exopod 2 segmented with 1, 6 plumose setae, short epipod with 4 marginal setae.

3rd maxilliped (Fig. 7.C): Endopod 5 segmented with 9,6,3,6,9 setae, exopod 2 segmented with 1, 3 setae. Basis and coxa fused, bearing 4 hairy setae. Epipod elongated with 9 long plumose setae.

Pereiopods (Fig. 8.F-J): Cheliped of 1st pereiopod was subequal in shape and size, tooth with irregular cutting edge. Second to 4th pereiopods were similar in structure, dactylus tapering and slightly curved inwards with several setae on both surfaces. Dactyls of the 5th pereiopod with 2 log setae.

Abdomen and telson (Fig. 7.C): Six somites, posterolateral margins of each somite rounded, somites 2 to 6 with several setae on posterolateral posterodorsal margins. Functional pleopods (Fig. 8A-D) present on somites 2 to 4, endopod with 3 minute hooks, unsegmented exopod of pleopods 1 to 4 bearing 13,13,13 and 11 plumose natatory setae, respectively. Uropods (Fig. 8E) segmented, bearing 6 natatory plumose setae on distal and 1 on proximal segment.

Morphology of 1st crab

Carapace (Fig. 9A): Carapace as long as board, dorsal surface unsmoth, narrowing anteriorly, eyes large and movable, antenna situated out side of orbit.

Antennule (Fig. 9B): Enlarged basal segment with 9 marginal and 10 lateral setae. peduncle 2 segmented, basal segment 2 setae, inner flagellum absent, outer flagellum with 3 aesthetascs and 1 setae.

Antenna (Fig. 9C): Peduncle 3 segmented with 1, 4, 3 setae, flagellum 5 segmented with 2, 2, 3, 6, 2 setae.

Mandible (Fig. 9D): Cutting edge sharp and almost straight, palp 3 segmented with 3, 4, 15 setae.

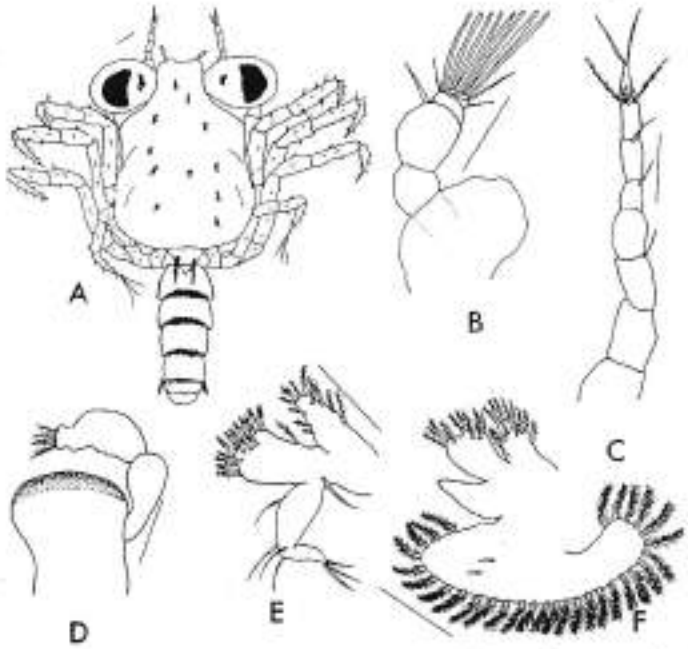

Fig. 6. *Perisesarma bidens* (De Haan). Megalopa: A- dorsal view; B- antennule; C- antenna; D- mandible; E- maxillule; F- maxilla (scale bar= 0.1 mm).

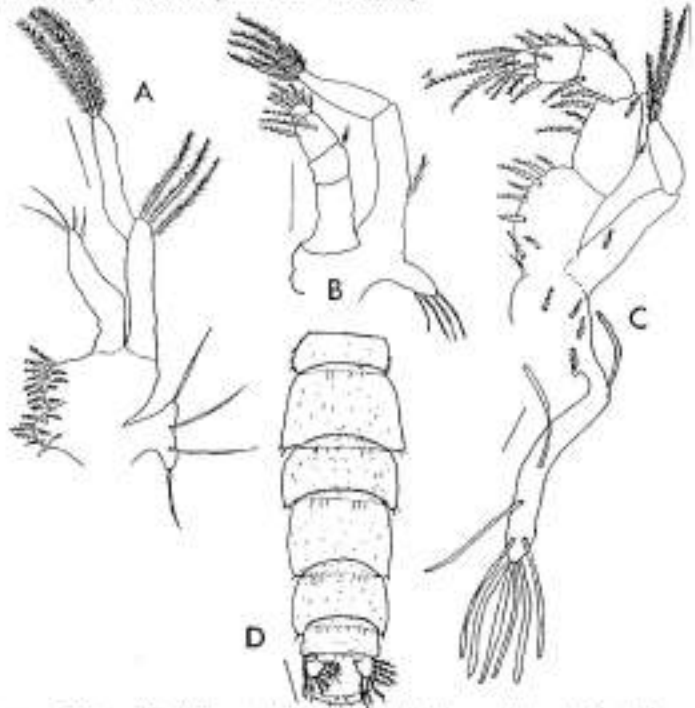

Fig. 7. *Perisesarma bidens* (De Haan). Megalopa: A- 1st maxilliped; B- 2nd maxilliped; C- 3rd maxilliped; D- abdomen (dorsal view) (scale bar= 0.1 mm).

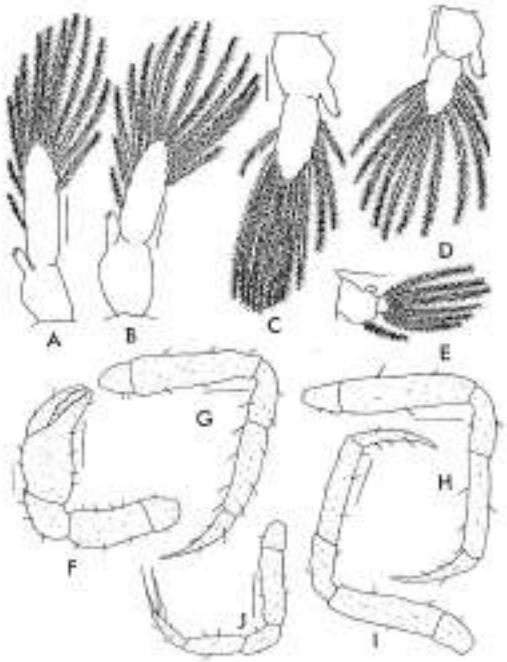

Fig. 8. *Perisesarma bidens* (De Haan). Megalopa: A-D: 1st–4th pleopods; E- uropod; F-J: 1st-5th perciopods (scale bar= 0.1 mm).

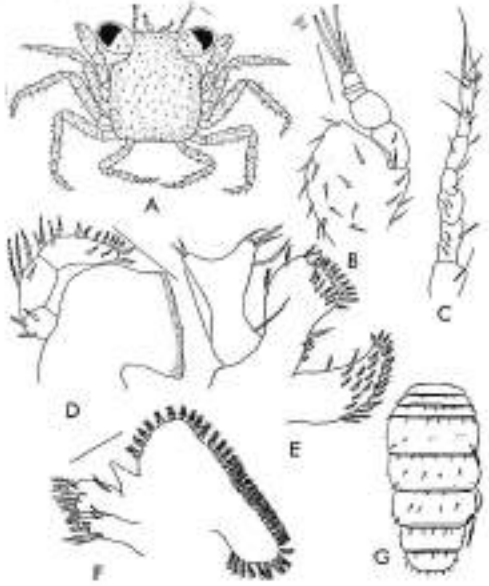

Fig. 9. *Perisesarma bidens* (De Haan). 1st crab: A- dorsal view; B- antennule; C- antenna; D- mandible; E- maxillule; F- maxcilla; G- dorsal view of abdomen (scale bar= 0.2 mm).

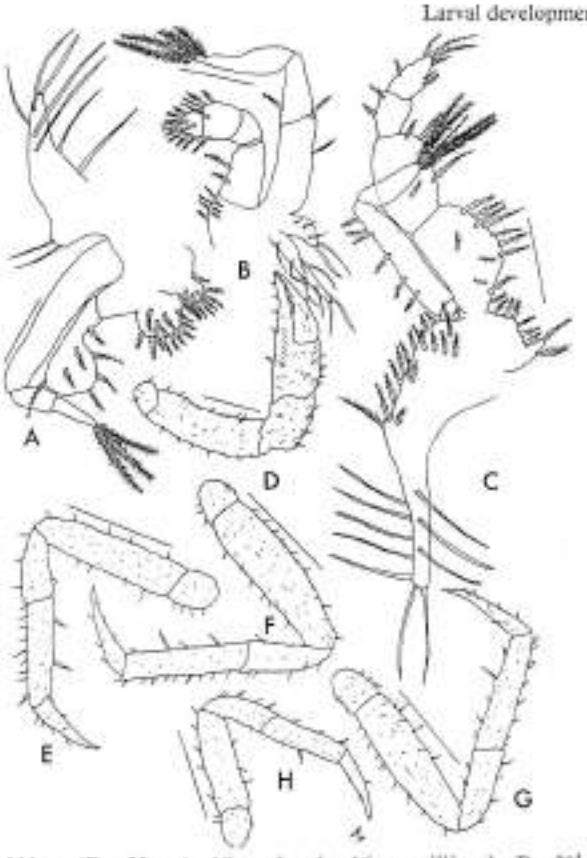

Fig. 10. *Perisesarma bidens* (De Haan). 1st crab: A- 1st maxilliped; B- 2nd maxilliped; C- 3rd maxilliped; D-H: 1st –5th pereiopods (scale bar= 0.2 mm).

Maxillule (Fig. 9E): Endopod unsegmented, Y shaped, bearing 3 plumose and 4 simple setae no margin. Basal and coxal endites with 27 and 28 setae.

Maxilla (Fig.9F): Endopod simple without seta. Basal and coxal endites bilobed with 5+6 and 8+4, respectively. Scaphognathite with 38 hairy setae on margin.

1st maxillipeda (Fig. 10A): Endopod triangular in shape with 5 marginal and 2 lateral setae, exopod 3 segmented, distal segment with long plumose setae, epipod elongated with 9 long plumose setae of moderate length. Basal and coxal endites bilobed with 13 and 9 setae, respectively.

2nd maxilliped (Fig. 10B): Endopod 4 segmented with 2,0,5,10 setae, exopod 3 segmented with 2,1,4 plumose setae, epipod bilobed with 5+7 naked setae. Basis and coxa fused with 7 setae.

3rd maxilliped (Fig. 10C): Endopod wider than in megalopa. Exopod and epipod similar to those of megalopa, but setation of exopod and epipod more complex then in megalopa.

Pereiopods (Fig. 10D-H): Chelipeds equal in size with numerous setae, 2nd to 4th pereiopods similar in structure, pointed, nearly equal to propodus in length, 3rd pereiopod largest, 5th one smallest.

Abdomen and telson (Fig. 9G): Six somites, each somite with several setae on dorsal on surface and lateral margin of telson round with numerous setae. Pleopods and uropods degenerated.

Discussion

The characteristics which are useful for the identifying *Perisesarma bidens* larvae include: the absence of lateral spine on the carapace, the setation patterns of the antennule and the basal and coxal endites of the maxillule and maxilla, the segmentation and setation on the exopods of maxillipeds and the presence of dorsmedian setae on the abdominal segments (Table 2). The following characters that were constant through all the zoeal stages of *P. bidens*, found during the experimental period: a) absent of lateral spine on the carapace, b) the patterns of setae on the endopod of maxillule were 1,5; the protopod of 1st maxilliped were 2, 2,3,3; the same of 2nd maxilliped were 1,1,1,5; the endopod of 2nd maxilliped were 0,1,6; c) the telson belong to A_0 type (Aikawa 1937).

The characters which undergo changes in the development progresses, the following were common to all the specimens examined: a) in the zoea stage I, antenna has rudiment endopod, b) in zoea stage II, a plumose setae appears on the basis of the maxillule to persist through subsequent stages, c) the endopod, basal and coxal endites on the maxilla were bilobed in all zoeal stages, d) the 1st maxillule has setae as 2-2-1-2-5 on the endopod in first two stages, e) natatory setae on the endopod of the 1st and 2nd maxillipeds increase in their number after a formula of 2X (S+1+, where S is the ordinal number of zoeal stages, f) the endopod rudiment of antenna becomes distinct in the final stage, g) plumose seta on the scaphognathite of maxilla increase with the stages.

In order to identify brachyuran zoeae, Aikawa (1929, 1937) proposed the following characters: a) grouping of chromatophores , b) character of the telson, including its armature, c) character of the 2nd antenna, d) presence or absence of spines on the carapace. According to these criteria, the character of the zoeae of *P. bidens* may be summarized as follows: a) chromatophores of dark brown color present on 2nd to 6th abdominal segments, the protopodite of 1st maxilliped, carapace and mandible; d) telson was A_0 type; e) second antenna was B_2 type and d) rostral and dorsal spines present on the carapace but absent in lateral spine.

In order to clarify the specific difference of the larvae of *P. bidens*, some comparisons have been made among the species of some sesarmid crabs, and the information related to the larval development on the basis of the previous studies and the present research are compiled in Table 2. The number of zoeal stages comprised in entire course of development differs according to species, this being 4 in *P. bidens*, and 5 in other three species when compared (Table 2). There were 10 setae on the basal endites of zoea I of *P. bidens*, while 8 in *S. guttatum* (Lago 1993), 9 in *S. intermedia* and *S. erythrodactyla* (Terada 1976). Differences also observed in the patterns of plumose setae on the scaphognathite of maxilla of zoeae III and IV stages (Table 2).

Generic differences in the characters of megalopa of *P. bidens* are compared with other species (Table 3). No remarkable differences were noted among the other three

species of sesarmid crabs, little variation was found in the setation of some appendages: endopod of maxillule bear 7 setae in *P. bidens* while 6 in other three species (Table 3). In maxilla, basal and coxal endites bears 12+12 seate *P. bidens*, 18+15 in *S. guttatum*, 12+15 in and *S. intermedia* and 13+12 in *S. erythrodactyla*, respectively. Differences also observed among these four species when compared in the setal arrangement on the basal and coxal endites of 1st maxillipeds, the endopods of 2nd maxillipeds, and the epipod of 3rd maxillipedd (Table 3).

There is no available information related to the development through 1st crab of *S. guttatum*, *S. intermedia* and *S. erythrodactyl.* The carapace length of 1st crab of *P. bidens* always larger than the other species of sesarmid crabs including *Helice formosensis* (Mia and Shokita 1997), *Helice leachi* (Mia and Shokita 1996) and *Helice Japonica* (Baba and Moriyama 1972) when compared.

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Culture of pearl in freshwater mussels (*Lamellidens marginalis* Lamarck)

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Abstract
A pond trail on pearl culture in freshwater mussels, *Lamellidens marginalis* was carried out for one year in an artificial perennial pond. Four types of foreign particles of indigenous sources, sand, stone, fish eyeball and beads of artificial pearl nucleus were used as nucleus for pearl production. Among the nuclei inserted mussel highest survival rate (72%) was recorded for stone and lowest survival rate (50%) for artificial pearl by nucleus implantation. Highest pearl production rate (%) was recorded for the insertion of stone and lowest for the sand. All nuclei inserted mussel produced pearl accept the mussel which was inserted beads of pearl nucleus for pearl formation. Growth rate (length and weight) was found higher for uninserted mussel than nuclei inserted mussels.

Key words: *L. marginalis*, Pearl culture

Introduction

In Bangladesh, there is a good prospect for commercial pearl production from the joint venture with Japan, China or the countries which are technically developed in this sector. *Lamellidens marginalis* (Lamarck), an important pink pearl producing freshwater mussel is increasing demand in pearl producing countries (Ram 1989). *Placuna*, *Placenta*, *Mytilus*, *Hyriosis* species are found abundantly in Cox's Bazer, Moheshkhali, Sonadia, St. Martin Island of the country (Alam 1994). Despite of the favorable environment, our country could not develop the modern pearl culture techniques, though the pearl culture techniques is easy and simple. Ahmed (1968), Hossain (1983) and Begum *et al.* (1990) did some work on pearl culture from *L. marginalis*. Selection and production of low cost, available and suitable nucleus of indigenous sources for the insertion of the mussel is essential for pearl culture. Production rate, shape, size, colour and quality of pearl and acceptability of the nucleus by the mussel depends on the nucleus. The present studies were conducted to study the pearl culture system in a freshwater mussel *L. marginalis* using different indigenous nucleus materials.

Materials and methods

Experimental pond

The experimental pond was about 200 m^2 and the sources of water were rainfall and ground water from deep-tubewell. The pond was completely independent with an outlet on the western side to discharge excess rainwater. The average water depth of the pond varied from 1.2-1.6 meter. The bottom mud of the pond was silty and muddy with a depth ranged from 15-22 cm.

Pond management

The pond was fertilized with different chemical fertilizers. Urea was applied at the rate of 100 g/40m^2 and triple super phosphate at 50 g/40m^2 in the months from March to October. Lime was applied at the rate of 500 g/40m^2.

Cage materials

The cages of 100 cm x 100 cm x 90 cm (length, breadth and height) were made of steel framework with 1.5 cm mesh sized nylon netting. The cages were closed on all sides by net except the top. Cages were placed in the experimental pond at a water depth of 1 meter. Fifty mussels were kept in each cage.

Selection of foreign particles as nucleus for insertion

Different foreign particles of indigenous sources (1-2 mm diameter) like sand particles, stone (small pebbles), beads of pearl nucleus and dried eyeballs of small fishes were used as nucleus. To manage eyeballs of fishes, small fishes were boiled for half an hour and eyeballs (1-2 mm) were pressed out from soften head and kept at freezer after washing them at 5% alcohol.

Collection of the mussels

Bivalve mussels (*Lamellidens marginalis*) were collected on from the experimental pond, adjacent to the Department of Aquaculture and Management, BAU. These mussels were collected by hand from different depths ranging from 0.6 m to 1.5 m. The mussels were kept in the container and transferred to the laboratory immediately.

Insertion of foreign particles

The large mussels having 8.9 to 12.2 cm in length and 84.5 to 149.6 g in weight were sorted out in the laboratory. Weight and length (L: greatest dimension along the anteroposterior axis) of every specimen was recorded on 1994 and kept for 2 days in the aquaria filled with pond water for conditioning. After 2 days, mussels were transferred in a tray from aquaria and washed in 40% alcohol. Sands and stones were placed at the end of a hypodermic injection needle and inserted them into the epithelial layer at the right side and middle position of the mantle by opening the valve of the mussels with the help of knife and sharpended bamboo pegs. With the help of knife, the valve was first made open and a bamboo peg was inserted to keep the gap wide open. The gap of about 0.5 cm

to 1 cm was made open between the shells. Sands and stones were pushed in a proper position by passing a platinum wire through the hole of the needle. Fish eyeball and beads of pearl nucleus were placed into the proper place with the help of forceps.

Pearl production

After insertion of the foreign particles, mussels were than transferred to the cages placed at the experimental pond. Fifty mussels were kept in each cage with two replication for each different nuclei used. Besides inserted mussels, uninserted mussels were also kept in the cages at same density and replication. Mortality rate were recorded at every four months of intervals. Mussels were finally harvested from the cages after 12 months of the experiment and transferred to the laboratory. Mussels were killed to observe the conditions of pearl development.

To determine the survival rate of mussels, every individual of each cage was counted for the number of alive mussel. Any mussel having its valve open or having the smell of decomposition was treated as a dead one. On the following sampling date every alive individual in each cage was again counted for the number of alive mussel in the same way. Sampling was done at every four months of intervals. The survival rate of each cage in each sampling was expressed in percentage and was calculated using the following formula:

$$S = n/N \times 100$$

Where, S is the survival rate (%) for each sampling, n is the number survived for each sampling, N is the initial number stocking. Finally, at the close of the experiment, the entire stock of mussels of each cage was collected and survival rates (%) were computed from the initial and final data.

To determine the growth rate of mussels, 5 mussels were collected from each cage and the average increase in length and weight of each individual was computed. The growth rate of mussels of each sampling at each cage was expressed in percent and was calculated by using the following formula :

$$G = I/L \times 100$$

Where, G is the growth rate (%/day), I is the increase in shell length (cm) or weight (g), and L is the initial shell length (cm) or weight (g).

Results and discussion

Survival rate

Highest survival rate of 80% was recorded for nuclei uninserted mussel. Among the inserted mussel the survival rates of 72%, 71%, 67% and 50% were recorded for the insertion of stone, fish eyeball, sand and beads of pearl nucleus, respectively (Table 1).

Lower survival in the nuclei inserted mussel than the uninserted mussel might be due to physiological injury or other environmental stress during experimental period.

Begum *et al.* (1990) and Hossain (1983) found similar survival for the nucleus inserted mussel in case of *L. marginalis*. Alagarswami and Qasim (1974) and Bautil and Boulle (1992) also found similar result for *Pinctada fucata* and *P. margaritifera*.

Table 1. Survival rate of mussels with and without insertion of nucleus

Inserted foreign particles	Initial stocking	After 4 months Survival rate (%)	After 8 months Survival rate (%)	Final survival S = n/Nx 100 (%)
Sand	50	80	73	67
Stone	50	84	78	72
Fish eyeball	50	82	76	71
Artificial pearl	50	59	54	50
Without nucleus	50	93	86	80

Growth rate

Highest average growth rate of nuclei uninserted mussel was recorded 0.06%/day. Mussels with sand, stone, fish eyeball and artificial pearl resulted average growth rateof 0.05%/day, 0.05%/day, 0.03%/day and 0.04%, respectively (Table 2).

Table 2. Growth rate (in weight) of mussels with and without insertion of nucleus

Inserted foreign particles	Average weight of the mussels		Increase in weight (g)	Average growth (g/day)
	Initial weight (g)	Final weight (g)		
Sand	110.4	132.3	21.9	0.05%
Stone	108.8	128.5	19.7	0.05%
Fish eyeball	114.6	128.5	13.9	0.03%
Artificial pearl	112.4	128.8	16.4	0.04%
Without nucleus	110.2	135.5	25.3	0.06%

Growth rate of nuclei uninserted mussels was recorded highest might be due to without disturbance of mussels however growth rate of nuclei inserted mussel might be hampered for physiological stress, operational injury and hazards. Chellam (1988) and Amin (1977) found the similar results in their experiments in case of *Pinctada fucata* and *Corbicula japonica* respectively.

Pearl production

Mussels which were inserted artificial pearl did not produce any pearl due to ejection of all nuclei. However other mussels which were inserted sand, stone and fish eyeball produced pearl. The pearl production was recorded highest (72%) in case of stone and lowest (67%) in case of sand (Table 3).

Table 3. Survival rate of mussels and pearl production rate using different foreign particles as nucleus

Foreign particles use as nucleus	No of survived mussel	Total no. of pearl	Pearl production rate (%)
Sand	67	67	67
Stone	72	72	72
Fish eyeball	71	71	71
Artificial pearl	50	-	-

Pearl production was highest in case of stone nuclei, might be due to its acceptability by the mussels. Alagarswami (1974) and Hossain (1983) reported the more or less similar results in their experiment in case of *Pinctada fucata* and *L. marginalis*, respectively.

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Water quality management on the enhancement of shrimp (*Penaeus monodon* Fab.) production in the traditional and improved-traditional ghers of Bangladesh

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Abstract
On-farm research on enhancement of *P. monodon* production through water quality management was carried out in five ghers of Paikgacha, Khulna. Based on the prevailing condition of the ghers, lime in the form of $CaCO_3$, urea and TSP were used as the major inputs to minimize the soil-water acidity and to ensure the availability of natural food particles in the water bodies. Exchange of water at required level also practised for the qualitative improvement of culture water. Ghers of varying sizes showed that water quality management and fertilization have a positive impact on production performance of *P. monodon* (61.59% increment) that yielded an average production of 385.43kg/ha/crop against the present traditional rate of 238.50 kg/ha/year.

Key words: *P. monodon*, Water quality, Traditional farming

Introduction

The present shrimp farming area of Bangladesh covers an area of 130 000ha out of which approximately 80% area are under traditional and improved traditional farming system (Anonymous 1996). Traditional shrimp farming is characterized by low lying coastal water flooded areas that allows to enter shrimp, finfish and seeds of other species through tidal wave action and grows there up to marketable size. Though a significant development in the traditional shrimp gher operation through releasing of shrimp seed at a particular density (1.0-1.5/m^2) observed during the last few years in many ghers, where from an production of 175-250 kg/ha is obtainable, but due to the lack of proper management of soil-water of the gher and shortage of natural food particle in the gher water, culture species neither grows healthy nor attain a considerable size for marketing in the stipulated time. Among the soil-water parameters, low pH value and absence of natural food particles in the water body are mostly responsible for low growth performance. Other required factors like dissolve oxygen content, water salinity, water temperature, water depth etc. also plays significant role in shrimp production. But in the prevailing improved traditional shrimp culture system of the country these factors are not taken into consideration by the farmers. Many of these problems has arisen as

result of undesirable farming practices. Despite the nature of the current problems, it is possible to increase profits and reduce environmental damage through the application of currently available shrimp farm management and production techniques (Chanratchakool *et al.* 1995). In this context, the present study was undertaken to find out the appropriate shrimp farm management techniques with a view to obtain higher production.

Materials and methods

Preparation of ghers

The study was carried out in five selected ghers of Paikgacha. Construction of dikes and gates were completed by the farmers as per instruction and then allowed to exposed the drain out wet field by sun light for about fifteen days. Based on the level of acid content gher bottom was flushed 3-5 times with tidal water to minimize the acidity. Lime were also applied depending upon the pH level of the soil. Cow dung and mustard oil cake (MOC) were applied at the rate of 500 kg and 100 kg/ha, respectively. Inorganic fertilizers, like, triple super phosphate (TSP) and urea were also applied at the rate of 30 kg/ha (TSP:U=3:1). After 4 to 5 days of fertilization, ghers were filled with water up to a depth of about 15-20 cm and then after one week, the depth of each gher was finally maintained at about 90 cm on an average.

Stocking of P. monodon seed

P. monodon postlarvae from local rivers of average 0.006g were stocked at a rate of 1.5 to 1.75/m^2. Depending upon the unavailability of local seeds farmers sometime used imported seeds from Thailand, India, Taiwan and Indonesia (Table 1). In all the cases, PL were screened before stocking by high rate of aeration in 100 ppm formalin solution for about thirty minutes.

Table 1. Culture management of experimental ghers

Farm no. & area (ha)	Culture period (days)	Water depth (m)	Stocking density (nos./ha)	Initial wt. (g)	Final wt. (g)	Gain in wt. (g)	Survival rate (%)	Production (kg/ha)
1. 46.7	150	1.2	17500*	0.006	43.5	43.49	52.2	399.66
2. 4.0	135	1.1	15000		44.7	44.69	59.7	400.29
3. 24.0	148	1.25	17500		44.0	43.99	58.3	448.91
4. 6.9	130	1.0	15000		45.0	44.99	49.6	334.80
5. 3.3	130	1.15	15000		44.9	44.89	51.0	343.49

* *Local seed 80% with imported seed 20%*

Water management

Water quality parameters such as dissolve oxygen (DO), pH, salinity, water temperature, transparency, un-ionized ammonia, hydrogen sulphide and alkalinity content were monitored weekly basis. Based on the prevailing soil-water condition and as per need, fortnight water exchange were done by tidal flushing during new and full moons followed by application of chemical fertilizers at a rate of 15kg/h (TSP:U=1:3). To keep the pH and alkalinity of water at a standard level, lime in the form of $CaCO_3$ was applied as per requirement (150-150 kg/ha).

Growth data recording

For the calculation of growth performance average weight of shrimp were recorded by fortnight sampling. The final growth and survival rate was calculated at the end of the culture period. Major portion of the shrimps were harvested after 120-150 days of rearing. But partial harvest of the marketable size shrimps before the stipulated period were encouraged to enhance the growth of smaller ones by increasing space and feed for the individuals of the remaining stock.

Results and discussion

Because of variation in water quality and management techniques of a shrimp farm, a great variation in production rate (238 kg/ha for improved traditional farms and 2500 kg/ha for semi-intensive farms) and survival rate (35-65%) can be observed (Anonymous 1994). It is beyond doubt that water quality management plays a vital role to promote the productivity of a shrimp farm directly. A healthy environmental condition and importance of entire management practice at different level from site selection to better production performance is crucial (Boyd 1995). As application of many production oriented management techniques require major. Among the important water quality parameters of gher, the major difference in pH, alkalinity and water transparency was recorded which were probably because of the direct effect of water management of the ghers under study (Table 2). The monthwise water quality parameters shows that temperature, salinity and dissolve oxygen content of the water of all the ghers are in good form which might also be similar for the traditional ghers, because these parameters are mostly controlled by natural environmental condition which indicates that management of a shrimp farm effectively require an understanding of the relationship between the shrimp and the environment. Interactions of some particular components, such as, oxygen, alkalinity, pH, dissolve nutrients and solid wastes are vitally responsible to produce such environment in a water body. Within a production pond, conditions which are less than perfect for culture are more commonly found than conditions which is directly responsible for shrimp death and cause low survival and poor production.

Table 2. Water quality parameters during 5 months culture period in experimental ghers

Parameters	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5
Temperature (°C)	22.0-32.0	25.0-32.0	23.5-31.5	24.5-31.5	23.5-32.0
Transperancy (cm)	26.0-30.2	24.0-27.8	24.0-28.0	23.0-26.5	23.0-26.5
pH	7.8-8.2	7.5-8.2	7.2-8.2	7.8-8.2	7.7-8.1
Salinity (ppt)	12.5-22.5	13.0-21.0	13.5-21.5	12.5-21.0	13.0-22.0
DO (ppm)	5.0-7.0	6.0-8.5	6.0-8.3	7.5-8.2	7.0-8.0
Alkalinity (ppm) (as $CaCO_3$)	100-325	125-300	156-330	100-350	122-320
Un-ionised Ammonia (ppm)	0.06-0.09	0.05-0.08	0.05-0.09	0.05-0.09	0.06-0.08
H_2S (ppm)	0.03-0.04	0.02-0.03	0.02-0.03	0.02-0.03	0.02-0.03

In the experimental ghers, this sorts of problems were overcame by occasional water exchange and lime application. As these treatments are not implied in the traditional ghers, as a result, this condition harm the shrimp, reduce the productivity and increase susceptibility to disease (Chanratchakool *et al.* 1998). Another cause of low production in the shrimp ghers of south east Asia is undesirable predator fishes. Because in the traditional shrimp farming system, most of the predators directly killed and feed on mullet and shrimp (Anonymous 1976).

The natural breakdown of toxic substances and wastes in shrimp ghers are performed by bacteria and plankton. This process are affected by the amount of oxygen present in water, temperature and water movement. If wastes are produced faster than the rate of breakdown, accumulation of waste substances will occur in the pond water. If the situation persists, this can lead to undesirable rearing condition (Chanratchakool *et al.* 1998) which is common in traditional and improved traditional shrimp farms of the country and which was minimized in the experimental ghers by required level of water exchange. Production data reveals that, considerable higher survival, growth and production of *P. monodon* can be achieved by keeping the environment of the gher suitable for shrimp farming (Table 1). Because in such a situation the main objective of the water management was stand to avoid lethal conditions and to provide adequate culture condition. As the traditional shrimp culture system in our country is a continuos process and in many cases extended up to 240 days or more, so there has been every possibilities of producing ammonia and hydrogen sulphide at toxic level in the bottom soil. In such case ammonia may produce by the excretion from the shrimp and decomposition of nitrogen containing organic materials, whereas, hydrogen sulphide produce under anaerobic conditions in sediments with high level of organic materials where reduced iron compounds are also present. Prior to culture, as the ghers under experiment were dewatered, exposed to sun light for several days and limed, and during culture operation, water exchange was done properly to a required level to keep the culture condition and water quality of the gher ideal, so in no case, unionised ammonia and hydrogen sulphide found to exceed to toxic level.

In traditional farming, in course of reducing the salinity level toward almost fresh (1-0 ppt) due to dilution by rain water at the end of the culture period (from later part of July to September), shrimp shell become soften because of low alkaline nature of the water where the presence of carbonates/bicarbonates are poor. Acid leached from the pond soil breaks down carbonates and bicarbonates reducing the water alkalinity and this process may continue until there is little or no carbonate or bicarbonate left in the gher water. Application of cabonate lime in the experimental ghers supplemented the level range 100 - 350 ppm (Table 2) against minimum required level of 80 ppm of carbonates and bicarbonates and helped to maintain the level of water alkalinity.

Production and water quality parameters of experimental and some traditional ghers shows that the low stocked traditional ghers with continuos long culture period has low survival and production compare to that of well managed shrimp ghers under study (Table 3). So management of water quality is vitally important for two basic reasons, such as, it will help to direct the farmer to maintain optimum environmental condition within the water body that will help to maximize growth and survival (Paul and Khondoker 1996) and the other is the maintenance of a good water quality that will eliminate most of the disease related problems of the particular water body (Tareen and Farmer 1983)

Table 3. Comparison of production performances and water quality management in the experimental and the traditional ghers

Culture practices and water management	Experimental gher (n= 5)	Traditional gher (n=10)
Culture period (days)	139	110-240
Farm area (ha)	3.9-46.7 (av. 16.98)	3.0-100 (av. 22.8)
Application of fertilizers, lime	Applied	Not applied
Stocking rates (PL/m^2)	1.7	1.0
Exchange of water	Done, as per required	Done, only during harvesting
Transparency (cm)	24-30	Above 30
pH	7.5-8.0	5.5-7.0
Alkalinity (ppm)	100-350	60-225
Initial wt. (g)	0.006	0.006
Final wt. (g)	44.42	32
Survival rate (%)	54.16	38.0
Yield (kg/ha/crop)	385.43	238.50

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Biodiversity in floodplains with special reference to artificial stocking

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Abstract
A five years investigation on fish biodiversity in connection with artificial stocking was conducted in three south-western floodplains of Bangladesh from 1992 to 1996. The ten top most available and ten most rarest fish species were identified. *Puntius sp.*, *Channa punctatus*, *Mystus sp.*, *Anabas testudineus*, *Ambaris sp.*, *Colisha sp.* and *Macrobrachium sp.* etc. were the most common available species. On the other hand, *Mystus aor*, *Notopterus chitala*, *Clupisoma garua*, *Aplocheilus panchax*, *Ctenopharyngodon idella* etc. were the most rarest species. However, the most abundant and the rarest fish species behaved differently in different floodplains in different years. Shannon diversity index was used to assess the extent of diversity in different years. The study revealed that the artificial stocking programme, to some extent, influenced the biodiversity in floodplains.

Key words: Fish biodiversity, Floodplain, Shannon weaver index, Artificial stocking

Introduction

The world wide loss of biodiversity is widely accepted as a major problem, yet it is poorly documented, because of our knowledge of the taxonomy of most organisms is scant (Moyle and William 1990. Though loss of aquatic species is occurring rapidly, aquatic organism have received comparatively little attention to the conservation biologists (Allendorf 1988). A rich diversity of fish species are critical to the ecology and sustainable productivity of the floodplains. While tremendous genetic diversity is embodied 500 fish species which inhabit Bangladesh's inland, estuarine and coastal waters ever little substantive data on the ecology of these species is not available to say something significantly (Nuruzzaman 1993).

In early sixties the open-water fisheries contributed about 90% of the total fish production which in the recent years has drastically dropped to 49% (Mazid and Hossain 1995). The decline is due to habitat degradation of aquatic ecosystems through over exploitation of fisheries resources with increasing population pressure, adverse effects of natural and man-made catastrophes including human interventions through construction of flood control embankments, drainage systems, sluice gates, conversion of inundated land to crop land thereby reducing water area, siltation etc. Under this situation, it is really important to take necessary steps so that floodplains may remain to the harmony with the environment. However, since 1992 government of Bangladesh had

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taken a massive fingerling stocking program in some selected floodplains aiming fish production augmentation from the floodplains under the Third Fisheries Project. Stocked species were *Labeo rohita*, *Catla catla*, *Cirrhinus mrigala*, *Cyprinus carpio*, *Hypopthalmicthyes molitrix* and *Puntius gonionotus*. Present investigation is worthwhile because, it is an important task to ascertain whether stocking floodplains with carp fingerlings had any adverse impact upon resident fish species or not. This study deals with the status of fisheries diversity and its dynamics in three floodplains of Bangladesh during 1992 to 1996 in relation to artificial stocking.

Materials and methods

The study was conducted from June'92 to December'96 in three major floodplains of Bangladesh. These are the Chanda beel of Faridpur-Gopalgonj depression, the Halti beel of Pabna-Natore depression and the BSKB (Basukhali-Salimpur-Kola-Barnal) beel of Khulna-Narial depression. Each floodplain possesses distinctive features. The BSKB is completely closed system floodplain and regulated by several sluice gates. The Halti is semi-open while the Chanda beel is an open system floodplain. The area of Chanda, Halti and BSKB are 10,870ha; 16,770ha, respectively (BCAS 1991). All these beels are prone to monsoon flooding and remain flooded for periods between four and seven months depending on the severity of flood in different years. During the dry season, however, the water area of the beels shrinks to a negligible amount and most of the dried-up land is cultivated with various crops. To accomplish the investigation a weekly data collection schedule was maintained. Data was collected from the fishing spots and peripheral fish landing centres, which are usually called *para* or *gala*. Catch composition by number for individual gear was recorded through the examination of catch for the respective gear. In order to data collection most of the gears i.e., more than 23 types of gears, both selective and non-selective were covered in which only 11 were of selective type. A total of 5,55,769 specimens were sampled in the present study of which 2,18,052; 1,71,985 and 1,65,732 samples belonged to Chanda, Halti and BSKB respectively.

The Shannon weaver index (H') was used to measure the extent of diversity by combining aspects of species richness and evenness. It is perhaps the most commonly used diversity index in ecology. Goswami (1985) used Shannon weaver index to assess zooplankton diversity in coastal waters of India. The formula for Shannon diversity index is: $H' = -\Sigma P_i \log_2 P_i$

Where P_i (the proportional abundance of the ith species) = (n_i / N)

n_i = number of individuals recorded of ith species

i = is the species reference

N = total of individuals in the sample

($\log_2 = 1.442 \log_e$).

Inferences can be drawn on the basis of the H' values calculated. The lesser values will be the lower diversity and vice-versa. For statistical analysis, using Shannon weaver index student's t-test was performed to find out significance of stocking.

Results and discussion

Top ten most available and ten rarest species in different years in different floodplains have shown in Table 1. Total number of fish species caught in the Chanda, Halti and BSKB beels were 47, 55 and 49 nos., respectively. *Puntius sp.* (both *P. stigma* and *P. ticto*) was the most available fish species in all the beels during the study period except 1993 and 1994 in the BSKB beel. Several fish species were found to be exclusive for particular floodplain and as well as some other were not found in other/s floodplain. Such as *Anguilla bengalensis*, *Mystus bleekery*, *Colisha chuna*, *Nemacheilus botia*, *Hilsha ilisa*, *Awaous grammepomus*, *Cirrhinus reba*, *Botia dario*, *Rita rita*, *Silonia silondia* and *Channa gachua* were not available in the Chanda beel; *Anguilla bengalensis*, *Rhotee cotio*, *Nandus nandus*, *Awaous grammepomus*, *Badis badis* and *Channa gachua* were not available in Halti beel; and *Anguilla bengalensis*, *Ailia coila*, *Colisha chuna*, *Nemacheilus botia*, *Rhotee cotio*, *Labeo gonia*, *Corica soborna*, *Awaous grammepomus*, *Badis badis*, *Silonia silondia*, *Botia dario*, *Rita rita*, *Labeo bata* and *Danio deverio* were not available in the BSKB beel. These species can be treated as *species at risk*. Total 67 species of fish were recorded in three floodplains under study. Ali (1998) enlisted three locally extinct fish species of Chanda beel *viz.*, *Puntius sarana*, *Rasbora elenga* and *Anguilla bengalensis*. He added that *Notopterus chitala*, *Labeo calbasu*, *Mystus aor*, *Gudusia chapra*, *Oreochromis mossambica*, *Euthropiichthys vacha* were rare in the same floodplain.

Table 1. Top most available and the rarest ten fish species in different floodplains during 1992-96

Status	Chanda beel		Halti beel		BSKB beel	
	Scientific name	Local name	Scientific name	Local name	Scientific name	Local name
A1	*Puntius* spp.	Punti	*Puntius* spp.	Punti	*Channa punctatus*	Taki
A2	*Macrobrachium* spp.	Chingri	*Mystus* spp.	Tengra	*Puntius* spp.	Punti
A3	*Colisha faciatus*	Kholisha	*Ambasis* spp.	Chanda	*Anabas testudineus*	Koi
A4	*Mystus* spp.	Tengra	*Glossogobius giuris*	Baila	*Labeo rohita*	Rui
A5	*Nandus nandus*	Bheda	*Channa punctatus*	Taki	*Heteropneustes fossilis*	Shing
A6	*Channa punctatus*	Taki	*Cirrhinus reba*	Raikhor	*Channa striatus*	Shoal
A7	*Xenentodon cancila*	Kakila	Chela spp.	Chela	*Cirrhinus mrigala*	Mrigel
A8	*Glossogobius giuris*	Baila	*Mastacembelus pancalus*	Gochi	*Mystus* spp.	Tengra
A9	*Heteropneustes fossilis*	Shing	*Colisha faciatus*	Kholisha	*Mastacembelus armatus*	Bain
A10	*Channa striatus*	Shoal	*Corica soborna*	Ketchki	*Colisha faciatus*	Kholish
R10	*Labeo calbasu*	Kalibasu	*Bagarius bagarius*	Baghair	*Clupisoma garua*	Ghaura
R9	*Ctenopharyngodon idella*	Grass carp	*Clarias batrachus*	Magur	*Pseusomopina atherinoides*	Batashi
R8	*Labeo gonia*	Ghonia	*Silonia silondia*	Shilong	*Cirrhinus reba*	Raikhor
R7	*Eutropiichthys vacha*	Bacha	*Notopterus chitala*	Chital	*Awaous grammepomus*	Nandi baila
R6	*Gudusia chapra*	Chapila	*Tetradon cutcutia*	Tepa	*Nandus nandus*	Bheda

R5	*Tilapia* spp.	Tilapia	*Labeo bata*	Bata	*Mystus bleekeri*	Gulsha
R4	*Corica soborna*	Ketchki	*Hilsa ilisha*	Hilsa	*Guduria chapra*	Chapila
R3	*Notopterus chitala*	Chital	*Notopterus notopterus*	Pholi	*Mystus aor*	Aire
R2	*Mystus aor*	Aire	*Aplocheilus panchax*	Kan-pona	*Notopterus chitala*	Chital
R1	*Clupisoma garua*	Ghaura	*Ctenopharyngodon idella*	Grass carp	Badis badis	Napit koi

A= Available, R= Rare

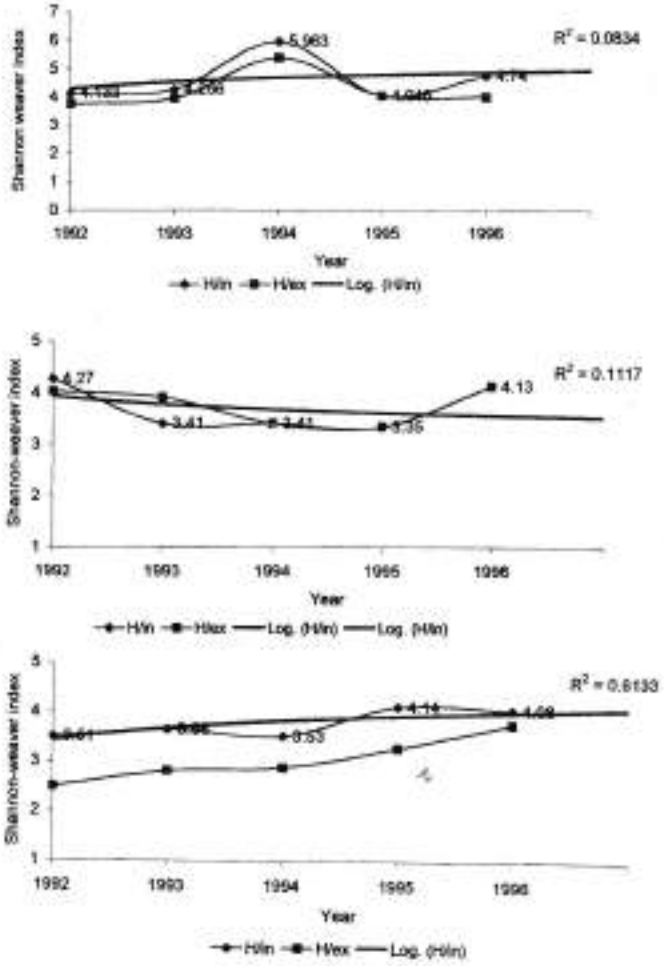
Yearly variation of Shannon weaver index and its trend line of the Chanda, Halti and BSKB beels are shown in Figs. 1, 2 and 3, respectively. Table 2 represents stocking and non-stocking years and Shannon diversity index for those years. Chanda beel showed the highest diversity index (H'=5.96), this was the consecutive third year of stocking, when the maximum number (43) of fish species were also caught (Fig. 1). In 1995, the index was found to be lower values (H'=4.05) seems due to non-stocking effects. Halti beel showed the highest diversity index (H'=4.27) in the year 1992. After 1993 it showed decreasing trend might be due to desist on stocking program from 1994 (Fig. 2). The BSKB beel showed the diversity index (H'=4.14) in 1995 when total number of fish species were also the highest (43). Perhaps continuous stocking program made this positive trend. It can be mentioned that among three study floodplains only in BSKB beel, stocking program was continued up to 1996. Student t-test using Shannon weaver index showed significant impact (at 1% level) of stocking in Chanda beel and BSKB beel. In case of Halti beel it was not derived because stocking program in this floodplain was held only first two years. Shannon weaver index was also derived by Rao *et. al.* (1991) in Chambal river, India. They found that species diversity was drastically reduced due to industrial effluents.

Table 2. Shannon weaver index in different years

Beels	1992		1993		1994		1995		1996	
	H'_{in}	H'_{ex}	H'_{in}	H'_{ex}	H'_{in}	H'_{ex}	H'_{in}	H'_{ex}	H'_{in}	H'_{ex}
Chanda	4.13	3.76	4.27	3.95	5.96	5.39	4.05*	4.05	4.74	4.01
Halti	4.27	4.03	3.94	3.41	3.41*	3.41	3.35*	3.35	4.13*	4.13
BSKB	3.51	2.49	3.66	2.82	3.53	2.89	4.14	3.30	4.08	3.79

* *No artificial stocking*

Present investigation reveals that the overall performance of *C. carpio*, *L. rohita* and *Catla catla* was satisfactory and they are suitable for floodplain stocking. But it was not clearly understood whether there was any adverse effect of stocked species, especially of exotic carp on any specific resident species or group of species or not. Jhingran (1997) commented transplantation of exotic fish into open water as a subject of controversy. He also stated that, without any knowledge of production potential of the floodplain *ad hoc* stocking could be considered as a wasteful exercise.



Figs. 1-3. Yearly variation of Shannon-weaver index of Chanda, Halti and BSKB beels and their trend lines.

Conclusions

The overall diversity index of fish in the Chanda and Halti beel were found to be lower after stopping the stocking, while diversity index showed increasing trend in BSKB beel as stocking program was continued. The findings of the present study reflect a primary picture of fisheries biodiversity of the three floodplains. Although it can be concluded that, stocking program had an impulsive impact on fish biodiversity but impact of stocked species on non-stocked resident species was not clearly understood in the present study. Food habit and food competitions are very crucial factors with respect to survivability in case of artificial stocking. So it is suggested that, cautious and scrupulous study should be conducted before such open-water stocking to find out the

competition and interaction between resident and stocked species. Special attention and further studies also should be continued on presently recorded rare species.

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Some aspects of population dynamics of juvenile hilsa shad (*Tenualosa ilisha* Ham.) from the Meghna river, Bangladesh

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Abstract
Population dynamics of the juvenile hilsa shad (*Tenualosa ilisha*) in the nursery ground of the Meghna river have been studied on the basis of the length cohort analysis of 8023 specimens. The growth parameters viz; asymptotic length (L_∞), curvature character (K) and initial time(t_0) were found to be 30.69 cm, 1.2 yr^{-1} and 0.45 yr^{-1} respectively. Curvature parameter indicate that jatka is a fast growth performer. The natural, fishing and total mortality were found to be 1.37 yr^{-1}, 1.41 yr^{-1} and 2.78 yr^{-1} respectively. Survival rate (S) was found to be 6.2%. A small difference was found between the age at first capture (T_c) and the recruitment age (T_r). Stocks of jatka seems to be overexploited and need to be conserved.

Key words: Juvenile hilsa, Population dynamics, Meghna river

Introduction

Jatka is the juvenile stage of the hilsa shad. It is the most exploited fishery in Bangladesh water of rivers, estuaries and the coast. As an open water fishery, its exploitation is a crucial phase for sustainable yield. Recruitment is a continuous biologically renewable process in the open water system. If by any means, the recruitment process impaired the adult population will decline in the long run. Biomass is regenerated both from recruitment and tissue growth. Nonetheless, it is necessary to have established clear and strong relationship between the adult stock and the recruiting young to obtain a sustainable yield from any fishery (Cushing 1968). Therefore, jatka fishery has a significant effect on hilsa production year after year. The growth parameters differ from species to species and also vary from stock to stock within the same species and age (Pauly 1980a). The aim of this investigation is to provide practical guidelines to understand the impacts of juvenile hilsa population as a capture fishery.

Materials and methods

Length-frequency data collection

Length-frequency data of 8023 individuals of jatka were collected from the nursery ground of the Meghna river in and around Chandpur during December 1993 through

April 1996. Length-frequency data were collected both by experimental fishing as well as from commercial catches. In each month adequate number of length-frequency data were randomly collected. Total length (cm) was measured from the tip of the snout to the posterior most margin of the caudal fin. Experimental fishing was done by a beach seine net (150m x 18m, 0.75cm mesh) manually hauled by 12 people. Commercial catches were done by a large seine net (400m x 30m, 0.75cm mesh) employing about 60 fishermen for hauling.

Data analysis

Length-frequency data were analyzed by using the Microstat and Excel-statistica computer software packages program. Growth parameters of jatka fishery viz; asymptotic length (L_∞), curvature parameter (K) and initial time (t_o) when jatka begins to grow just after hatching, were estimated on the basis of the Von-Bertalanffy growth analysis by using the following growth equation model;

$L(t) = L_\infty [1-e^{-K(t-t_o)}]$.....(i)

A linear regression analysis was done between the two variables X and Y using the formula;

$Y=a+bX$;.....(ii)

Considering $\Delta L/\Delta t$ as X and mean length [L'(t)] as Y. Asymptotic length of jatka was calculated by the formula;

$L_\infty = -a/b$.....(iii)

Mean length [L' (t)] was converted by the formula of $-\ln[1-L'(t)/L_\infty]$ and was denoted as Y_1 variable. In this case the independent variable (X_1) was denoted as assumed cohort age (t). By the regression analysis between X_1 and Y_1 variables, Curvature parameters (K) was calculated as K=slope, b_1 and the initial age as $t_o = -a_1/b_1$. After determining the values of L_∞, K and t_o length data converted into age data by using the Inverse Von-Bertalanffy growth equation (1938);

$t(L) = t_o - 1/k * \ln [1-L'(t)/L_\infty]$.....(iv)

The growth pattern of jatka was determined by plotting length against age.

Total mortality

Total mortality was calculated by the "linearized length- converted catch curve" method. The linearized length converted catch curve formula was;

$\ln C(L_1,L_2)/t(L_1,L_2) = a - Z*t[(L_1+L_2)/2]$.....(v)

Where $t=1/K*\ln[(L_\infty-L_1)/(L_\infty-L_2)]$, $X = t[(L_1+L_2)/2] = t_0-1/K*\ln[1-(L_1+L_2)/L_\infty]$ and $Y=\ln C(L_1,L_2)]/ t(L_1,L_2)$, $C(L_1,L_2)$ is the number of fish caught, L_1 and L_2 were the lower and upper limit of each length class, `a' was the intercept and the slope b (-Z) was the total mortality.

Natural and fishing mortality

Natural mortality (M) for jatka fishery was calculated by Pauly's empirical formula (Pauly 1980 b) through various combinations of L, K and average annual temperature(T°c). Here annual temperature was considered only for the period of November to April when Jatka is available in the riverine nursery ground. Natural mortality was calculated by the following formula;

$$M= 0.8*\exp[-0.0152-0.279*\ln L_\infty +0.6543*\ln K+ 0.463*\ln T] \quad \text{.....(vi)}$$

Fishing mortality rate (F) was derived by subtracting the natural mortality from the total mortality, since $Z=M+F$, where Z, M and F were the instantaneous rate of total, natural and fishing mortality.

Exploitation rate

The rate of exploitation of jatka in the Meghna river was calculated by the formula;

$$\text{Exploitation rate, } E=F/Z \quad \text{.....(vii)}$$

Results

Length-frequencies of 8023 specimens of jatka from the riverine nursery grounds of the Meghna river were analyzed and shown in Fig.1.

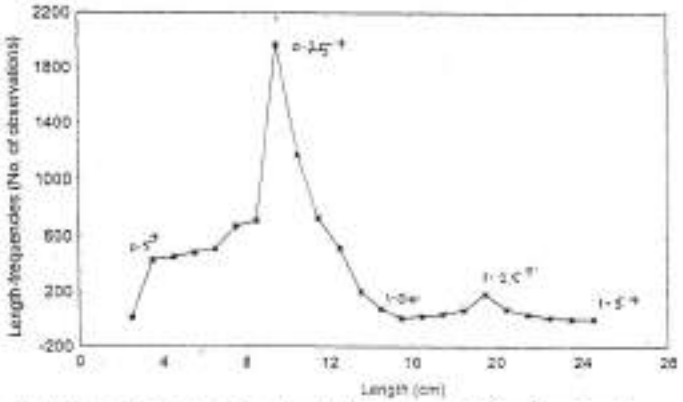

Fig. 1. Length cohort analysis of jatka population for riverine nursery ground of the Meghna river.

Length-frequency analysis revealed that the length cohorts of jatka population were <2, <9, <15, <19 and <22 cm respectively (Fig.1). Cohort assumed age (t), cohort mean

length [*ΔL/Δ t)], growth rate *(ΔL/Δt) and individual mean length [L(t)] were shown in Table 1. Through a regression analysis between Y (ΔL/Δt) and X (L(t)), the intercept a and slope b were found to be 36.22 and -1.18 respectively. According to the Von-Bertalanffy growth equation asymptotic length (L_∞) of jatka was 30.69 cm, cohort mean length [L(t)] converted into -ln [1-L(t)/ L_∞] denoted as Y_1 and assumed cohort age (t) denoted as X_1 were put into a simple regression. The values of curvature parameter K and initial time t_0 were found as 1.2 yr^{-1} and 0.45 yr (Table 2).

Table 1. Growth rate of *Tenualosa ilisha* as a function of cohort age

Age t years	Δt	Cohort mean length cm	ΔL(t)	Growth rate L(t+Δt)-L(t)/t= ΔL/Δt	L(t+Δt)+L(t)/2= L'(t)
0.5⁺	0.25	<2	7.0	28	5.5
0.75⁺	0.25	<9	6.0	24	12
1.0⁺	0.25	<15	4.0	16	17
1.25⁺	0.25	<19	3.0	12	20.5
1.5⁺		<22			

N=4, x= 13.75 a (intercept) = 36.22, b= (slope) = -1.18

Table 2. Calculation of Curvature parameter (K) and Initial time (t_0), (using L_∞ = 30.69 cm)

Cohort age t (year) (X)X_1	Cohort mean length L(t) cm	-In[1-L(t)/ L_∞] (Y)Y_1
0.5⁺	2	0.06
0.75⁺	9	0.35
1.0⁺	15	0.67
1.25⁺	19	0.97
1.5⁺	22	1.26

a_1 (intercept)= -0.54, b_1= (slope)= 1.2yr^{-1}, (Curvature parameter) and initial time t_0= $-a_1/b_1$= 0.54/1.2= 0.45 yr.

The values of curvature parameters are indicating that jatka is a fast growth performer. The growth rate of jatka was calculated by using Inverse Von-Bertalanffy growth equation. The length of jatka was converted into age (t). Growth is the change of absolute increase in length and weight with respect of age. The instantaneous rate of increase in length with respect of age ΔL(t) has been estimated by the Von-Bertalanffy growth equation. The growth curve of jatka was shown in Fig.2.

The growth pattern indicating that the instantaneous rate of growth in the younger phase of life was found much higher. Jatka attain up to 28 cm within 2 years. But 7-15 cm attain within one year. It was also found that maximum peak catch attain with 8-12 cm size groups (Fig. 1).

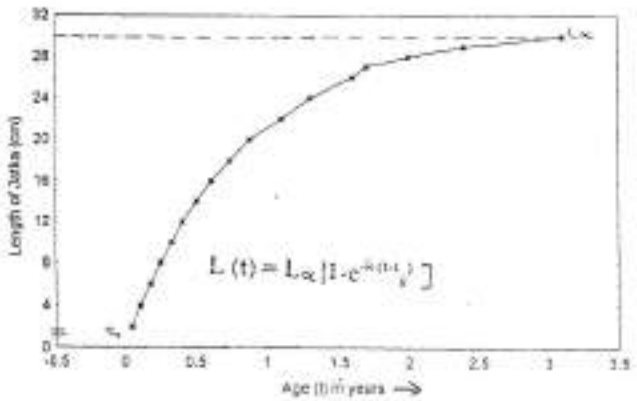

Fig. 2. Growth curve of jatka from the riverine nursery ground.

Total mortality rate (Z)

The instantaneous rate of total mortality was estimated by means of the length converted catch curve method. The total mortality rate (Z) was found to be 2.78 yr^{-1}, which indicating a high value. The length converted age based catch curve was shown in Fig.3.

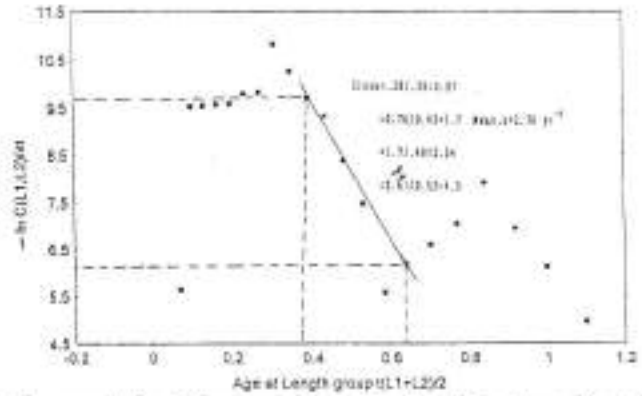

Fig. 3. Length Converted Catch Curve used for estimation of total mortality of jatka fishery.

The descending part of the catch curve was not calculated due to too close to L_∞ and hence it was avoided. The high Z value for jatka fishery was not a good sign to obtain a sustainable yield. From the Z value it was calculated that the survival rate of jatka fishery in the riverine nursery ground was $S = e^{-z} \times 100 = e^{-2.78} \times 100 = 6.2\%$, which was very low, which indicating that the hilsa population catch will be seriously hampared in the long run.

Natural and fishing mortality

The natural mortality was calculated by the Pauly's empirical formula (1980b) and it was shown in Table 3. Where L_∞ = 30.69 cm and K= 1.2 yr^{-1} were used in the calculation.

Mean natural mortality M was found to be 1.37 yr^{-1} was found. The instantaneous rate of fishing mortality as derived from the values of Z and M, was obtained F=Z-M=1.41yr^{-1}.

Table 3. Natural mortality calculated by Pauly's empirical formula (1980b) for various ambient water temperature (month-wise, during the jatka season)

		Ambient water temperature (T°C)					
L_∞	K	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
30.69	1.2	23	19	17	20	22	24
	M=	1.45	1.33	1.28	1.39	1.36	1.48

*Mean M= 1.37yr^{-1}, M= 0.8*exp [-0.0152-0.279*ln L_∞ +0.6543 lnK+0.463lnT°C]*

Exploitation ratio

Exploitation ratio, E = 0.51 has been estimated on the basis of the relationship E=F/Z, which tends to overexploitation for the jatka fishing. Tends of overexploitation is not a good sign for any open water fishery.

Discussion

Length-frequency analysis of jatka population reveals that major share of jatka fishery in the riverine nursery ground were caught between the sizes of 8-12 cm in November to April and the peak were 9-10 cm size group. In the riverine nursery ground, the abundance of jatka was found from November to April and peak in March in each year (Rahman *et. al.* 1995) which supports the present findings.

The first capture age (T_c) was calculated as T_c =0.5 yr (6 months), and the recruitment size was 8-9 cm, so the recruitment age was T_r=0.70 yr (8.4 months). It was seen that the differences between T_c and T_r was very small (2.4 months). The results for Tr and T_c showed that T_c age was smaller than T_r age, which was not true for the proper management. Therefore, to obtain a sustainable hilsa population, T_r age must be less than T_c age. Beverton and Holt (1957) reported that in the open water system the fish population are affected by the natural mortality during the ages between T_r and T_c. But in case of jatka fishery in the Meghna river T_r to T_c period suffers from both by natural (M) and fishing mortality (F). So, it can be said that due to fishing pressure at the recruitment phase, the total mortality might be high for jatka population.

A stock is supposed to be optimum when E_{opt}=0.51 but when E value is more than 0.5, the stock of a fish population is overfished (Gulland 1965). So, it appears that the stock of jatka fishery tends to cross the overfished level in the riverine nursery ground of the Meghna river.

Csirke and Sharp (1984) opined that if any population affected by high mortality in their recruitment phase the population might be seriously hampered in the long run. So, as the fishing pressure on the jatka fishery was on the way to cross the optimum fishing condition, it is urgently needed to control fishing at their recruitment phase.

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Studies on the post-mortem changes in shrimp and prawn during ice storage: I. Organoleptic and physical changes

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Abstract
The organoleptic characteristics such as appearance, textural condition, colour and odour indicated that the *M. rosenbergii* stored in ice for 5-6 days were acceptable for processing in the industry while *P. monodon* under similar ice storage condition were acceptable for 8-9 days. In both species, samples stored in headless condition in ice had longer shelf life than that of stored in head-on condition.

Physical changes were evaluated by determining expressible moisture and breaking strength of sample of muscles. The expressible moisture increased continuously in both samples with the lapse of storage period. The expressible moisture increased up to around 44% in 4-5 days of ice stored *M. rosenbergii* muscle while it was around 40% in 8-9 days ice stored *P. monodon*. At the end of 9 days of ice storage, the expressible moisture content in *M. rosenbergii* increased up to 60%, while it was up to 47% in *P. monodon* after 11 days of ice storage. The breaking strength declined from 0.78 kg/cm^2 to 0.53 kg/cm^2 in tiger shrimp after 8 days of ice storage, while in case of immediately killed prawn, the breaking strength of muscle was 0.8 kg/cm^2 which declined to 0.43 to 0.35 kg/cm^2.

Key words: *Penaeus monodon, Macrobrachium rosenbergii*, Ice storage, Quality change

Introduction

In Bangladesh, considerable quantity of post-harvest loss of prawn and shrimp are reported to occur at different stages of handling and transportation. The major source of raw material supply is various categories of shrimp farms located in the coastal belt of Khulna, Bagerhat, Satkhira and Cox,s Bazar area. The collection of raw material passes through a number of steps and finally delivered to the market and exporting industries using road, rail and water transport (Uddin and Das 1994). It takes usually 12-44 hours to transport the shrimp in iced condition from shrimp farm to the processing plants. Depending on the supply of raw material, in the industry, the shrimps after beheading are stored in the processing plants for about another 2-3 days particularly when the raw material supply is abundant compared to per day capacity utilization of the plants.

A considerable information is available on the organoleptic and physical changes in shrimp during ice storage but mostly on the species from colder region. However, very

little is known on the giant freshwater prawn and marine tiger shrimp of the tropical region. An essential prerequisite for designing the infrastructure for handling, transportation and marketing is to know how long each particular commercial species can be kept in ice condition. This paper reports the results of the organoleptic and physical changes of freshwater and marine water shrimp during ice storage.

Materials and methods

Materials

Giant freshwater prawn (*Macrobrachium rosenbergii*) and marine tiger shrimp (*Panaeus monodon*) were used for the study. Live fresh water giant prawns were collected from the local market of Bangladesh Agricultural University Campus, Mymensingh. They were caught by the cast net from the nearby Brahmaputra river by fishermen and transported to the market in live condition. While the tiger shrimp were obtained in lots from farms of Khulna region in live condition and transported to the Laboratory. It took about 18– 24 hours from catch point to destination before start the subsequent experiment.

Experimental condition

The samples (*M. rosenbergii* average size 25/kg, *Penaeus monodon* average size 22/kg) were obtained in lots several times from March to October'98. The samples of each species were divided into two groups, head-on and headless conditions and stored separately in ice in an insulated box. At selected time interval, a desire number of samples were used to assess the degree of freshness by evaluating organoleptic and physical changes.

Organoleptic assessment

The organoleptic methods used in this study is based on the existing procedure of the Fish Inspection and Quality Control Service (FIQC) of the Department of Fisheries (DOF), the Government of Bangladesh which is a modified version of Multilingual Guide to freshness grade described by Howgate *et al.* (1992). Six members panel were constituted to evaluate the organoleptic quality changes of giant fresh water prawn and tiger shrimp on the basis of odor, texture, color (with shell), color of flesh and general appearance of shrimp. The quality was evaluated by grading the shrimp using the score from 5 to 25 in case of fresh shrimp and 4 to 20 in case of boiled shrimp. The grade defined in terms of the total number of points were: 22 to 25 considered as very good or excellent, 19-21 good, 14-18 acceptable, 8-13 bad and 5 to 7 very bad condition in case of raw shrimp. In case of boiled shrimp, the score point 18-20 considered as very good/excellent, 14-17 good, 11-13 acceptable, 7-10 bad and 4 to 6 very bad.

Expressible moisture test

The expressible moisture test was determined according to Saban *et al.* (1987). At selected time interval, the samples were taken from the container and cut into a number of pieces. About 1 g of muscle was placed between double layer of filter paper No. 102 and passed at 1 kg/cm^2 for 3 minute. Decrement of weight was measured and the ratio of decrement to the original weight was defined as expressible moisture (%) in the following formula:

% of expressible moisture = W_1-W_2/W_1 x 100

Where, W_1 = Weight of the shrimp muscle before compression.

W_2 = Weight of the shrimp muscle after compression.

Textural test

Texural test was determined according to Nakayama *et al.* (1993) with some modification. The shell of shrimp was removed and cut into equal pieces of 2 cm from near the middle portion of the shrimp body. The puncture test was done by measuring breaking force of the shrimp muscle against the penetration of a ball type plunger. The cutting muscle was placed on the pan of an electronic balance and pressed by a spherical plunger (6 mm diameter) over it until penetrate into it. The force (in gram) required to break the shrimp muscle by the plunger was recorded from the balance display window.

Results

The organoleptic quality changes in tiger shrimp and fresh water prawn during ice storage are shown in the Tables 1 and 2, respectively for fresh shrimp and Table 3 for cooked boiled tiger shrimp. The organoleptic characteristics such as appearance, textural condition, colour and odour judged by panel members indicated that the shrimp in a lot were acceptable condition in term of commercial standard for processing in the industry up to 5th days of ice storage. On the other hand, when the shrimp obtained from the same lot and stored in ice in headless condition were organoleptically acceptable condition up to 6th days in ice. That is keeping time of headless shrimp in ice can be increased one day more than that of head-on shrimp.

Table 1. Changes in organoleptic qualities of different days of ice stored head-on giant freshwater prawn

Stored period or days	Organoleptic qualities	Number	Overall quality
0 day	Fresh bright shining and irridescent. Firm consistent and elastic texture. With characteristics of white colour of flesh. Odour and colour of shell is natural	25	Very good
1st day	Slight loss of brightness. Moderately soft and some loss of elastic texture. Slight change in colour of flesh and shell. Odour is neutral.	20	Good
2nd day	Slight loss of brightness. Some softening texture. Slight pink colour of flesh and shell. Neutral odour.	19	Good
3rd day	Slight dullness and loss of brightness. Some softening texture. Slight	18	Acceptable

	pink colour of flesh and shell. Slight sour odour.		
4th day	Definite dullness and loss of brightness. Some softening texture. Pink colour of flesh and borwnish red of shell. Slight sour odour.	14	Acceptable
5th day	Definite dullness and loss of brightness. Soft and watery texture. Pink colour of flesh and shell is discolour. Ammonical odour.	10	Bad
6th day	General appearance dull. Soft and watery texture. Dull/discolour of flesh. Shell discolour. Rotted odour.	7	Very bad
7th day	General appearance dull. Soft and juicy texture. Dull/discolour of flesh. Shell discolour. Rotted odour.	5	Very bad
8th day	General appearance is dull. Soft and juicy texture.	5	Very bad
9th day	Dull/discolour of flesh. Shell discolour. Rotted odour.	5	Very bad

Table 2. Changes in organoleptic qualities of different days of ice stored head-on tiger prawn

Stored period or days	Organoleptic qualities	Number	Overall quality
1st day	Fresh bright shining and irridescent. Firm consistent and elastic texture. Colour of flesh is white. Odour and colour of shell is natural	25	Very good
2nd day	Slight loss of brightness. Moderately soft and some loss of elastic texture. Slight pink colour of flesh and shell-odour is neutral.	20	Good
3rd day	Slight loss of brightness. Some softening texture. Slight pink colour of flesh and shell-odour is neutral.	19	Good
4th day	Slight dullness and loss of brightness. Some softening texture. Flesh and shell is slight pink colour and odour is slight sour.	18	Acceptable
5th day	Definite dullness and loss of brightness. Some softening texture. Flesh and shell slight pink colour. Odour is slight sour.	16	Acceptable
6th day	Definite dullness and loss of brightness. Some softening texture. Slight pink colour of flesh. Shell is brownish red and odour is slight sour.	15	Acceptable
7th day	Definite dullness and loss of brightness. Some softening texture. Pink colour of flesh and shell is brownish red. Odour is slight sour.	14	Acceptable
8th day	Definite dullness and loss of brightness. Some softening texture. Pink colour of flesh and shell is brownish red. Odour is slight sour.	14	Acceptable
9th day	General appearance is dull. Texture is soft and watery. Flesh is pink colour and shell is brownish red. Odour is ammonical.	11	Bad
10th day	General appearance is dull. Texture is shoft and watery. Flesh pink colour and shell is discolour. Odour is rotted	8	Bad
11th day	General appearance is dull. Texture is soft and juicy. Flesh is dull/discolour. Shell is also discolour. Odour is rotted	5	Very bad

Table 3. Changes in organoleptic qualities of different days of ice stored boiled head-on tiger shrimp

Stored period or days	Organoleptic qualities	Number	Overall quality
1st day	Difficult to remove the shell. Natural smell while chewing. Odour is natural and texture is very good.	20	Very good
2nd day	Difficult to remove the shell. Neutral smell while chewing. Odour is neutral and texture is good.	17	Good
3rd day	Slight difficult to remove the shell. Neutral smell while chewing. Odour is neutral and texture is good.	19	Good

4th day	Slight difficult to remove the shell. Slight sweet odour while chewing. Odour is neutral and texture is good.	14	Good
5th day	Slight difficult to remove the shell. Slight sweet odour while chewing. Odour is neutral and texture is slightly hard.	13	Acceptable
6th day	Slight difficult to remove the shell. Slight sweet odour while chewing. Odour is slight sour and texture is slightly hard	12	Acceptable
7th day	Slight difficult to remove the shell. Sour odour while chewing. Odour is slightly sour and texture is slightly hard.	11	Acceptable
8th day	Easy to remove the shell. Sour odour while chewing. Slight sour odour. Texture is slightly hard.	9	Bad
9th day	Easy to remove the shell. Sour odour while chewing. Odour is ammonical and texture is very hard.	7	Bad
10th day	Easy to remove the shell. Rotted odour while chewing. Sour odour. Soft and watery texture	5	Very bad

Similar studies were also conducted with tiger shrimp either head-on or headless condition. The head-on samples were acceptable condition up to 8 days and the headless shrimp for 9 days. The changes in organoleptic characteristics of ice stored tiger shrimp were also judged upon boiling based on fishy, flavours and odours. The results obtained upon cooked were more or less similar to that of the results obtained judging organoleptic qualities in iced condition.

Fig. 1 shows the changes in expressible moisture (EM) of head-on giant tiger shrimp during ice storage. The EM of shrimp muscle gradually increased during ice storage. Organoleptically the shrimp were found acceptable condition up to 8 days of ice storage and the expressible moisture content at that time was recorded around 41%. However, at the end of 11 days of ice storage the expressible moisture content increased to around 47% and organoleptically the samples were rejected with objectionable odour.

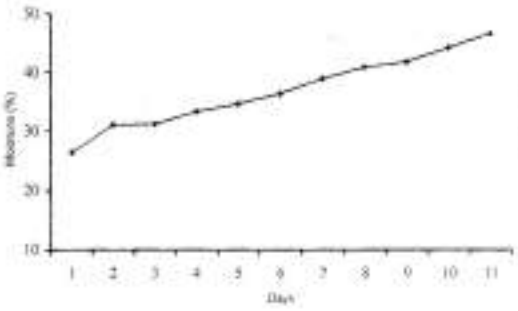

Fig. 1. Changes in expressible moisture (EM) content of tiger shrimp (head-on) during ice storage.

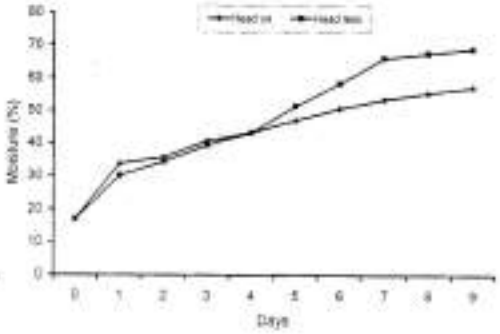

Fig. 2. Changes in expressible moisture (EM) content of freshwater giant prawn (head-on and headless) during ice storage.

Similar studies were also conducted with giant fresh water prawn either in head-on or headless conditions. The expressible moisture of live prawn immediately after killed was around 17% (Fig. 2.). A rapid increase in expressible moisture content was found

after one day both in head-on or head less condition. Organoleptically the freshwater prawns were acceptable condition up to 4-5 days and the expressible moisture content increased up to around 44%. At the end of 9 days of storage expressible moisture was 69% for head less sample and head on about 57%. At this stage, both the samples were already rotten and not fit for consumption. The result of the present study indicated that expressible moisture content up to around 44% was upper limit for organoleptically acceptable condition both in head on and head less prawn.

Studies were also conducted on the changes in breaking strength in shrimp muscle slice. The textural test of head-on giant tiger shrimp was done with one day old ice stored shrimp. The shrimp showed obvious sign of fresh organoleptic characteristics and the breaking strength of muscle at this stage was 0.78 kg/cm^2. Then the breaking strength declined gradually with the increase of storage period. After 8 days of storage when the sample reached the upper limit for organoleptically acceptable condition, the breaking force decreased down to 0.53 kg/cm^2 together with considerable loss of expressible moisture. At the end of the 11 days of ice storage when the shrimps were rejected by organoleptic assessment, the breaking strength decreased to 0.42 kg/cm^2. In fresh water prawn the breaking strength of immediately killed shrimp muscle was 0.8 kg/cm^2. Organoleptically fresh water prawn stored in ice either head-on or headless condition were acceptable for 4 to 5 days and the breaking force during the period dropped to 0.43 to 0.35 kg/cm^2.

Discussion

The shelf life of *P. monodon* and *M. rosenbergii* determined by various organoleptic and physical aspects varied greatly between two species. The available reports suggest that the shelf life of shrimp/prawn during ice and frozen storage varies from species to species, chemical composition and ambient temperature in which they are kept (Takada *et al.* 1988, Santoso *et al.* 1992, Yamagata and Low 1995). The results of the present study demonstrated that the quality of shrimp for shrimp for export by seafood industry could be maintained in ice 5 to 6 days for freshwater shrimp and 8 to 9 days on brackishwater shrimp either head-on or head less conditions after catch. Spots on the shell, offensive sulphide smell and loose shell were the reasons commonly attributed for spoilage as reported for *P. monodon* by Fonseka and Ranjini (1994).

Saban, *et al.* (1987) found that the expressible moisture increased gradually with the lapse of storage time irrespective of temperature. The largest change was observed for the specimens stored at –20°C. It increased from 22% to around 30% after 3 months and then decreased to 18-22% after 9 months irrespective of storage temperature adopted.

The breaking force in head on muscle was comparatively higher than that of head less samples throughout the storage period. Organoleptically giant fresh water prawn stored in ice either head-on or head less condition was acceptable for 4 to 5 days and the breaking force during the period dropped to 0.43 to 0.35 kg/cm^2. A negative correlation between the textural changes and expressible moisture content either in fresh water prawn or marine shrimp was established where expressible moisture increased with the

decrement of breaking force. The breaking force for giant fresh water prawn immediately after death was 0.8 kg/cm^2 which decreased to around 0.6 kg/cm^2. On the other hand, initial expressible moisture content of the fresh water shrimp was 16% which increased to around 30% during the same period. At the end of the storage period the breaking force decreased from 0.8 kg/cm^2 to 0.28-0.31 kg/cm^2 while expressible moisture increased from about 16% to 69% either in head-on or head less condition. Nakayama *et al.* (1993) reported that the breaking strength of stressed and unstressed fish muscle decreased sharply within 16 and 31 hrs after death respectively.

Conclusions

The organoleptic characteristics indicated that the freshwater prawn were found acceptable condition in term of commercial standard for processing up to 5 days in head-on and 6 days in headless condition during ice storage. On the other hand, marine tiger shrimp were found acceptable condition up to 8 days in head on and 9 days in headless during similar storage.

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Studies on the post-mortem changes in shrimp and prawn during ice storage: II. Biochemical aspects of quality changes

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Abstract
Studies were conducted on biochemical changes in *P. monodon* and *M. rosenbergii* during ice storage. At the end of 10 days of ice storage, moisture and protein content of freshwater prawn slightly decreased from 78.34 to 77.35% and 18.46 to 17.10, respectively, while lipid and ash content slightly increased. The moisture, crude protein, lipid and ash content of one day ice stored tiger shrimp samples were 78.07, 18.06, 1.3 and 1.29% respectively. The protein composition of fresh water prawn immediately after killed were 36.51% sarcoplasmic, 44.63% myofibrillar, 8.12% stroma and 6.44% alkali soluble protein. At the end of 10 days of ice storage, sarcoplasmic and storma protein slightly decreased while there was little or no changes observed in myofibrillar and alkali soluble protein. In case of one day ice stored tiger shrimp, the composition of protein were 35.32% sarcoplasmic, 46.29% myofibrillar, 7.86% stroma protein and 7.08% alkali soluble protein. At the end of 10 days in ice, sarcoplasmic protein decreased from 35.32% to 32.16% while there was slight change in other protein fractions. The TVB-N value of 1 day ice stored shrimp was 10.5 mg/100g of sample. It increased gradually with the lapse of storage period and at the end of 10 days storage in ice, the value increased up to 60 mg/100g sample. The tiger head on shrimp in ice storage were found organoleptically acceptable condition for 8 days and at that time the TVB-N values were 32.2 mg/100g which is slightly above the recommended limit for TVB-N for export.

Key words: *Penaeus monodon*, *Macrobrachium rosenbergii*, Ice storage, Quality change

Introduction

Fish and shellfish muscle protein is known to consist of sarcoplasmic myofibrillar, alkali soluble and stroma protein fractions and the extractability of muscle protein varies from species to species and according to the post-mortem changes (Mauruyama and Suzuki 1968, Hashimoto *et al.* 1979 and Suzuki and Watabe 1987). Preservation of shrimp in the ice or other refrigerated media is an important way of delaying biochemical changes or in other words, preventing deterioration from spoilage. It is well known that protein and other components of shrimp are generally labile and denature or degrade very quickly. Because of influence of chemical composition on keeping quality, it is important to determine the proximate chemical composition (moisture, fat, protein,

and ash) of the samples, although chemical composition varies with season and fishing ground. To assess the potentialities of effective utilization of shrimp for industrial purposes and also to determine the changes during storage it is necessary to know the composition of material under investigation.

It is well known that a variety of chemical compounds or groups of compounds accumulate in post-mortem fish flesh. These chemical compounds are intermediaries or end products of biochemical changes occur in the muscle of fish after they have died or result from the action of exogenous bacterial enzymes released by the bacteria. The amount formed can be used as an index of spoilage. It is, therefore, of interest to see the changes in pH and TVB-N values during the ice storage. There has been an extensive series of studies on the value of pH and TVB-N as a measure of fish spoilage but very little is known the freshwater prawn and marine tiger shrimp of Bangladesh.

Materials and methods

Samples

Giant freshwater prawn (*Macrobrachium rosenbergii*) and marine tiger shrimp (*Panaeus monodon*) were used for the study. Live fresh water giant prawns were collected from local market of BAU, Mymensingh. They were caught by the cast net from the nearby Brahmaputra river by fishermen and transported to the market in live condition. While the tiger shrimp were obtained in lots from coastal farms in live condition and transported to the Laboratory, Department of Fisheries Technology, BAU, Mymensingh. It took about 18–24 hours from catch point until start of the experiment.

Biochemical analysis

For muscle pH, two grams of peeled shrimp muscles were homogenized with 10 ml distilled water in a blender and the pH was measured using a pH meter (Corning Model 250).

Fresh ice stored shrimp were used for the study of protein fractionation. After removing the shell, twenty grams of muscle was fractionated by a procedure described by Hashimoto *et al.* (1979). All the operations were performed at 3–4°C as quantitatively as possible. The protein obtained after fractionation was determined by Kjeldahl method. TVB of the samples were determined according to the method described in European Commission (1997). Proximate analysis such as moisture, ash, lipid and crude protein were carried out according to the methods given in AOAC (1980).

Results

The changes in proximate composition of freshwater giant prawn and tiger shrimp are presented in Table 1. The initial moisture, protein, lipid and ash content of freshwater prawn were 78.34, 18.46, 1.8 and 1.15%, respectively. At the end of 10 days of ice storage, moisture and protein contents slightly decreased from 78.34 to 77.35% and

18.46 to 17.05%, respectively while lipid and ash contents slightly increased. For convenient of calculation, the protein, lipid and ash contents were calculated on dry weight basis. On moisture free basis, protein contents decreased considerably from 85.22 to 75.27%. There is little or no change in lipid content while ash content slightly increased.

Table 1. Changes of proximate composition of tiger shrimp and giant freshwater prawn during ice storage

Name of species	Storage period in ice (day)	Moisture %	Protein %	Lipid %	Ash %
Macrobrachium rosenbergii	0	78.34	18.46 (85.22)*	1.8 (8.31)*	1.15 (5.30)*
	10	77.35	17.05 (75.27)*	1.9 (8.38)*	1.42 (6.25)*
Penaeus monodon	1	78.07	18.06 (82.35)*	1.3 (5.92)*	1.29 (5.80)*
	10	77.96	16.85 (76.45)*	1.35 (6.25)*	1.69 (7.66)*

**Results in parentheses expressed as dry wt basis.*

Proximate composition of tiger shrimp was also investigated with one day old ice stored samples. The moisture, crude protein, lipid and ash content of the samples were 78.07, 18.06, 1.30 and 1.29%, respectively. At the end of 10 days of storage, there was little or no change of moisture content. During the storage on wet weight basis, protein content decreased from 18.06 to 16.85 while on moisture free basis, it decreased considerably from 82.35 to 76.45%. There is little change in lipid and ash content either in wet weight and dry weight basis.

The changes in protein fraction of giant fresh water prawn and tiger shrimp are presented in Table 2. The composition of fresh water prawn immediately after killed were 36.51% sarcoplasmic, 44.63% myofibrillar, 8.12% stroma and 6.44% alkali soluble protein. At the end of 10 days of ice storage, some changes in composition were occurred in protein fraction. Sarcoplasmic and stroma protein slightly decreased while there was little or no changes occurred in myofibrillar and alkali soluble protein.

Table 2. Changes of protein fraction of tiger shrimp and giant fresh water prawn during ice storage

Name of species	Storage period in ice (day)	Sarcoplasmic protein (%)	Myofibrillar protein (%)	Stroma protein (%)	Alkalisoluble protein (%)
Macrobrachium rosenbergii	0	6.74 (36.51)*	8.24 (44.63)*	1.50 (8.12)*	1.19 (6.44)*
	10	5.82 (34.13)*	7.52 (44.10)*	1.28 (7.50)*	1.14 (6.68)*

Penaeus monodon	1	6.38 (35.32)*	8.36 (46.29)*	1.42 (7.86)*	1.28 (7.08)*
	10	5.42 (32.16)*	7.64 (45.34)*	1.23 (7.29)*	1.19 (7.06)*

**Results in parentheses expressed percentage of total protein.*

Similar studies were also conducted with one day old ice stored tiger shrimp. The composition of protein was 35.32% Sarcoplasmic, 46.29% myofibrillar, 7.86% stroma protein and 7.08% alkali soluble protein. Some changes in composition was occurred during ice storage. At the end of 10 days in ice, sarcoplsmic protein decreased from 35.32% to 32.16% and slight changes also occurred with other protein fraction. In both experiments, the protein compositions in various fraction were almost similar where sarcoplasmic protein ranged 35.32 – 36.51%, myofibrillar 44.63 – 46.29%, stroma 8.12 – 7.85% and alkali soluble protein 6.44 – 7.08%.

Early post-mortem changes in prawn were associated with a drop in pH from 6.95 to 6.33 within an hour after death but the pH increased again during the latter phases. Fig. 1 shows the pH changes in fresh water prawn during extended ice storage for 7 days both in head-on and headless conditions. In both cases, the pH increased gradually with the lapse of storage period and at the end of 7 days of storage, the pH increased from 6.88 to 8.18 in headless and from 6.89 to 8.3 in head-on prawn.

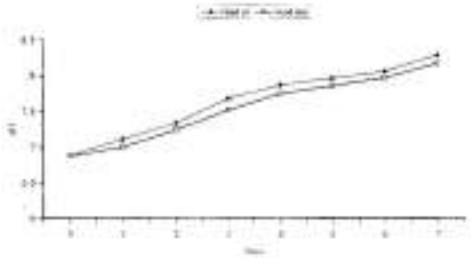

Fig.1. Changes in pH of giant freshwater prawn (head-on and headless) during ice storage.

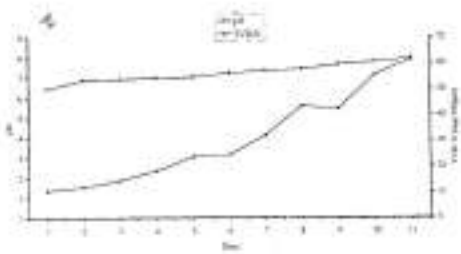

Fig.2. Changes of pH and TVB-N of tiger shrimp (head-on) during ice storage.

Studies were also conducted on the changes in pH and TVB-N values of head-on tiger shrimp during similar ice storage (Fig. 2). The pH of the samples measured 1 day after ice storage was 6.44. The pH increased slowly and at the end of 10 days of ice storage, it reached to 7.86. The TVB-N value of one day ice stored shrimp was 10.5 mg/100g of sample. It increased gradually with the lapse of storage period and at the end of 10 days storage in ice, the value increased up to 60 mg/100g sample.

Discussion

In both the experiments, an inverse relationship existed between moisture and lipid content so that some of these two constituted approximately 80%. The decrease in crude protein during 10 days of ice storage in both experiments is due to the formation of free drip accompanied by some sarcoplasmic protein. Tar (1965) reported that some loss of organic nitrogenous constituents, largely sarcoplasmic protein and inorganic salts with free drip are probable contributing factor of such loss of protein contents in chilled fish. A slight increase either in lipid or ash content during storage period could be explained by individual variation since lipid content varies greatly even within the same species. The values of proximate composition obtained in fresh water giant prawn and marine water shrimp were within the similar range reported by Babbitt *et al.* (1974) for shrimp, although proximate composition varies greatly from species to species and within the same species depending on size, sex, season and feeding habit. They reported the composition of shrimp were 78.3% moisture, 19.22% protein, 1.28% lipid and 1.77% ash content. The results obtained in the present study indicated that both freshwater prawn and tiger shrimp contained higher amount of sarcoplasmic protein and lower myofibrillar protein than that reported for teleost fishes (Shimizu and Shimidu 1960). The available reports suggest that extractability of muscle proteins varies from species to species (Shimizu *et al.* 1976) The sarcoplasmic protein are reported to extractable from ordinary muscle, even in water but in pelagic fishes, the amount of extractable proteins in the ordinary muscle reported to be increased rapidly with the increase of ionic strength of homogenate (Suzuki and Watabe 1987). However, the results of both experiments indicated that the shorter period of shelf life of shrimp/prawn during ice storage is probably related to loss of sarcoplasmic protein with free drip.

The decline in pH in early post-mortem muscle is the gradual hydrolysis during the first few hours of glycogen to lactic acid. The decline in pH also accompanied by the natural post-mortem stiffening called rigor-mortis. The available reports suggest that the generation of basic nitrogenous compound like TMA and ammonia due to bacterial action gradually rises the pH during the period after rigor-mortis has passed off. A good relationship between changes in pH and organoleptic qualities of prawn was observed where the quality gradually decreased with the increase of pH. The values above 7.5 seems to indicate spoilage. Melanosis, offensive sulphide smell and loose shell were the reasons commonly attributed for rejection.

However, the present study revealed that the head on tiger shrimp was organoleptically acceptable condition for 7 days. At that time the TVB-N value was 32.2 mg/100g which is slightly above the recommend limit for TVB-N value of exportable shrimp. The TVB-N values of 25 mg/100g is recommended for import of marine products (Cobb *et al.* 1973, Reilly and Dangla 1984, Connell 1995). According to Connell (1995) the value of 35-40 mg TVB are usually regarded as the limit beyond which whole chilled fish can be considered spoiled for most uses. The available reports suggest that the fin fish such as cod, haddock, eel and sea pike, the upper limit of 30mg TVB-N/100g is considered for acceptability.

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Scientific Notes

Fecundity and sex-ratio of Thai silver barb *Barbodes gonionotus* (Bleeker)

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Abstract
The fecundity and sex- ratio of *Barbodes gonionotus* were studied. The fecundity of 99 gravid females varied from 18001 (total length 197 mm and body weight 72 g) to 42034 (total length 187 mm and body weight 159 g). The mean fecundity was 24959.23 ± 6961.48 (for mean total length 210.50± 17.26 mm, mean body weight 118.16±37.34g, mean ovary length 70.21±27.30 mm, mean ovary weight 13.66± 7.12 g and mean ovary breadth 15.4 ±2.79 mm). The relationship between fecundity (F) and other parameters such as total length, total body weight, ovary length, ovary weight and ovary breadth were studied. The fish was highly fecund and the number of eggs produced was more or less directly proportional to other different lengths.

Key words: Fecundity, *Barbodes gonionotus*, Sex- ratio

Thai silver barb *Barbodes gonionotus* is commonly known as Thai sharpunti or rajpunti. This fast growing fish was introduced in Bangladesh from Thailand in 1977. Works on the fecundity of different fishes have been reported in our country, e.g. *Puntius* species (Mustafa *et al.* 1982), *Channa* species (Bhuiyan and Rahman 1984), *Anabas testudineus* (Nargis and Hossain 1988), *Colisa fasciata* (Bhuiyan *et al.*, 1995), *Oreochromis nilotica* (Bhuiyan and Afrose 1996), *Nandus nandus* (Hossain *et al.*, 1997) etc. But published information is not available on the fecundity of rajpunti in Bangladesh. The present study was undertaken to study the fecundity and sex- ratio of *B. gonionotus*. The relationships between the fecundity and different lengths i.e. total length, total body weight, ovary length, ovary weight were established.

A total of 274 specimens of *B. gonionotus* were collected at random from different places in Rajshahi city of which 130 females were identified. The gravid females were easily identified by their swollen abdomens. All collected specimens were preserved in 5% formalin. Total length and total body weight of the specimens were recorded. After dissection the gonads were taken out and then moisture was thoroughly wiped from the ovaries with blotting paper and weighed by a sensitive pan balance. The average total length and the average breadth of the ovaries were also recorded by a measuring scale

and a divider. Gravimetric method was used for the estimation of fecundity (Lagler 1956).

Data from 99 gravid females of *B. gonionotus* showed that the fecundity varied from 18001 (for a fish with length 197 mm and body weight 72g) to 42034 (for a fish with total length 182 mm and total body weight 159g). The largest fish with a total length 240 mm and body weight 169g showed the fecundity as 4105. The smallest sized fish in the sample with a total length 180mm and body weight 68g showed the fecundity as 17090. The mean fecundity of gravid females was recorded as 24959.23 ± 6961.48 eggs for a fish with a mean total length of 210.5 ±17.26 mm and mean body weigh of 118.16 ± 37.34g. The observed mean total length of ovary 70.21 ± 27.3 mm with mean total weight of ovary was 13.66 ± 7.12 and with mean breadth of ovary was 15.4 ± 2.19 mm.

The relationships between fecundity (F) and (i) total length (TL), (ii) total body weight (TW), (iii) ovary length (OL), (iv) ovary weight (OW), (v) ovary breadth (BO) were as follows:

i. $F = -54633.89 + 378.11$ TW ($r = 0.937$).
ii. $F = 4478.902 + 173.317$ TW ($r = 0.929$).
iii. $F = 9221.05 + 224.13$ OL ($r = 0.880$).
iv. $F = 11759.39 + 956.84$ OW ($r = 0.988$).
v. $F = 9962.20 + 2267.62$ BO ($r = 0.909$).

Therefor, in *B. gonionotus* the relationships between fecundity and total length, with total body weight, with ovary length, with ovary weight and with ovary breadth were found to be strongly correlated. This fish was highly fecund and the number of eggs produced was more less directly proportional to the total length, total body weight, ovary length, ovary weight and ovary breadth. Variation of fecundity among the same sized fish was noticed, individual physiology of the fishes and their surroundings may be the controlling factors for such variation (Hossain *et al.* 1997).

Sex-ratio

During the study period, it was observed that out of 274 specimens, 144 were males and 130 were females. The total male and female ratio was 52.55: 47.44 or 1: 0.90. It was observed from the Table 1 that the males were predominant during the months of February, April-June, October-December, while the females were predominant in the rest of the months. The chi- square test shows that the male and female distribution in the natural population is significantly different at the 5% and 1% level of significance.

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Table 1. The male and female ratio of *Barbodes gonionotus*

Months	Total fish	Percentages		Sex-ratio Male:Female
		Male	Female	
February'94	30	63.33	36.66	1:0.58
March	26	46.15	53.84	1:1.16
April	20	80.00	20.00	1:0.25
May	21	71.42	28.57	1:0.40
June	23	52.17	47.82	1:0.91
July	20	45.00	55.00	1:1.22
August	20	40.00	60.00	1:1.50
September	25	44.00	56.00	1:1.27
October	19	63.15	36.84	1:0.58
November	20	70.00	30.00	1:0.42
December	20	60.00	40.00	1:0.66
January'95	30	13.33	86.66	1:0.65
Total	274	52.55	47.44	1:0.90

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Scientific Notes

Toxicity of malathion to silver barb (*Barbodes gonionotus* Bleeker) fingerlings

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Abstract
Static bioassays were performed to observe the toxic effect of malathion to *Barbodes gonionotus* at 0.0 to 20.0 ppm concentrations. Malathion at 5.0 ppm was harmless to *B. gonionotus* and concentration above 6.0 ppm were found to be lethal. Malathion at 2.06 ppm was safe for the *B. gonionotus*.

Key words: Toxicity, Malathion, *B. gonionotus*

Thai silver barb or rajpunti (*Barbodes gonionotus*) was introduce to Bangladesh from Thailand has shown considerable promise as a culturable species in ponds, ditches and other seasonal water bodies with low cost inputs at low level of management. The fish is becoming popular in paddy-fish culture systems also. The main problem of widespread culture of *B. gonionotus* in rice field is the indiscriminate use of insecticides for control of insect pests. Pesticides may cause extensive damage to different vital organs of fishes (Gosh and Dutta 1985) and also cause degradation of aquatic ecosystem which gradually reach disastrous limits and thus become a potential killer of fish population (Mckim *et al.* 1974).

A good number of literature's has been reported on the toxicity of agrochemicals to varieties of cultivable fishes (Ghosh and Dutta 1985, Haque 1989 and Hoque *et al.* 1993), with little information is available on the toxic effects of malathion to fishes. Since malathion has become a regular part of pest management in rice field, therefore, it is essential to evaluate the toxic effect of malathion to *B. gonionotus* with a view to formulate recommendation for the safe use of this pesticide in rice-cum-fish culture practice in Bangladesh.

The study was conducted at Freshwater Station, Bangladesh Fisheries Research Institute, Mymensingh, during the period during August'98. Twenty six aquaria each of 90 litre capacity were used for this purpose. Two aquaria were kept as control. Healthy rajpunti of three months old were acclimatized to the laboratory condition by keeping in holding tank before bioassay experiment. Initially a few screening tests were performed to find out the mortality range of *B. gonionotus*. The fishes were supplied mixed plankton food collected with a plankton net (mesh size, 150 µm).

Malathion chemically known as O, O-dimethyl phosphorodithioate of diethyl mercaptosuccinate with 57% active ingredient was collected from local BADC dealer and tested at 0.0, 2.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 12.0, 16.0 and 20.0 ppm concentrations with three replications. Ten fishes of uniform size (7.6±1.0 cm, 6.3±1.9g) were placed to each aquarium at least 24 hour prior to the addition of the pesticide to water. Relative toxicity of malathion to rajpunti was determined by means of standard 96-h semi-static bioassay (APHA, 1975) method. Initially the observations were made at 3 hourly intervals and after 24 hours, the interval time was 6 hours. The behaviours of the fish was observed regularly at 3 hours interval. Dead fishes were recorded and subjected to a binomial formula after Ward and Parrish (1982):

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

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

B = Lowest toxin concentration in which all organisms died.

Safe level of concentrations were calculated using the following formula,

$C = 48h\ LC_{50} \times 0.3/S^2$

Where C = Harmless concentration and S = 24h LC_{50}/48h LC_{50}

Toxicity to fish depends on a number of factors such as species, age and health of fish, concentration of chemicals, exposure period and physico-chemical characteristics of water. During the period of investigation temperature of water was 28.0 $\pm$ 0.5 while dissolved oxygen content varied between 4.3 to 5.5 ppm. The total hardness of the water was in the range of 108 - 110 ppm and pH was fairly stable (8.0 $\pm$ 0.3). The physico-chemical parameters of the water were within the desirable range for fish (Boyd 1979).

Per cent mortality of the test fish at different concentrations are shown in Table 1. It can be seen from table 1 that the mortality of fish increased gradually with concentrations of toxin. Malathion below 5.0 ppm was ineffective to kill any fish within 96 hours. Malathion at 9.0 ppm killed all the *B. gonionotus* in 24 hours. Low rates of mortality were observed in *B. gonionotus* when exposed to 6.0 ppm of malathion for more than 72 hours. Mortality was found to increase as the dose of the malathion was increased. Total mortality within 3 hours (3h LC_{100}) was observed at 12.00 ppm. Nine ppm of malathion killed all the fishes in 24 hours whereas diazinon and sumithion caused total mortality (24h LC_{100}) at 6.00 ppm and 5.00 ppm respectively on more or less same size *B. gonionotus* (Hoque *et al.* 1993). Lower than 5.0 ppm concentration did not cause any mortality. The safe level of this insecticides was found to be 2.06 ppm for *B. gonionotus*. In the same species the safe level is 1.63 and 1.42 ppm for diazinon and sumithion respectively (Hoque *et al.* 1993). Sharama *et al.* (1981) reported that malathion was safe for *Clarias batrachus* at 2.72 ppm concentration, whereas it was 1.12 ppm for *Labeo rohita* of 10.5 cm size at temperature of 21.0°C (Rahman 1989). These variations appeared to be related with the differences in species of fish.

Median lethal dose concentration (LC_{50}) of malathion were estimated to be 13.85, 9.16, 8.36, 7.07, 7.07, 6.70 and 6.52 ppm for 1, 3, 6, 12, 18, 24 and 48 hours of exposure respectively (Table 2). The LC_{50} value of malathion for 96h exposure was found to be 56

ppm for *Heteropneustes fossilis* (Ghosh and Dutta 1985), 4 ppm for *Chana punctatus* (Dubale and Shah 1979) and 0.35 ppm for *C. striatus* (Choudheri *et al.* 1984), which are air breathing fishes. LC_{50} value of malathion for some non-airbreathing fishes like fatheads, blue gills, gold fish and guppies was 23.0, 0.09, 0.045 and 0.84 ppm (Pickering *et al.* 1962). LC_{50} does for 48h in *Tilapia mossambica* was reported to be 5.54 ppm (Sailatha *et al.* 1981). The 24h LC_{50} for *B. ticto* was 4.0 ppm and *B. daniconius* was 6.0 ppm (Singh and Sahai 1984).

Table1. Cumulative percentage mortality of rajpunti to malathion at different concentrations

Doses (ppm)	1h	3h	6h	12h	18h	24h	48h	72h	96h
0.0 - 5.0	0	0	0	0	0	0	0	0	0
6.0	0	0	0	10	10	10	10	10	10
7.0	0	0	0	10	30	30	30	30	30
8.0	0	10	20	50	70	70	70	70	70
8.5	0	10	30	60	80	90	100	100	100
9.0	0	40	60	70	90	100	100	100	100
10.0	0	100	100	100	100	100	100	100	100
12.0	0	100	100	100	100	100	100	100	100
16.0-20.0	100	100	100	100	100	100	100	100	100

Table 2. Values of LC_{50} of malathion at varying time intervals

H	A	B	LC_{50}
1	12.0	16.0	13.85
3	7.0	12.0	9.16
6	7.0	10.0	8.36
12	5.0	10.0	7.07
18	5.0	10.0	7.07
24	5.0	9.0	6.70
48	5.0	8.5	6.52

H= Time passed during the experiment (in hour)
A= Highest toxin concentration (ppm) in which none of test organisms died
B= Lowest toxin concentration (ppm) in which all test organisms died
LC_{50}= Calculated median lethal concentration (ppm) of malathion against rajpunti calculated from Binomial test $LC_{50} = (AB)^{1/2}$ of Ward and Parrish (1982)

After application of malathion in the aquarium the fish exhibited various signs of distress. At high concentrations an initial period of excitation was noted. Fish swam rapidly and opercular rate increased. A severe reaction involved in fin extension and temporary body curvature. Similar reaction was observed by Hoque *et al.* (1993). Most of the fish came to the surface water showing sign of suffocation with in 10 to 15

minutes. Gradually they lost equilibrium, became paralyzed and finally settle down to the bottom of the aquarium and remained to same position till death. Similar reactions were also observed in tilapia, *Oreochromis nilotica* (Haque 1989) and in *B. gonionotus* (Hoque *et al.* 1993).

In aquatic medium the organophosphorus insecticides enter into the blood stream of fishes cause rupture of the gill epithelium, hemolyse the red blood corpuscle and destroys the nerve impulses by inhibiting the cholinesterase (O'Biren 1967). This inhibition results in the apparent death of the organisms. The rate of inhibition is related to the degree and duration of exposure.

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