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Major histocompatibility complex class I involvement in the rejection of allogeneic erythrocytes in rainbow trout (*Oncorhynchus mykiss*)

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Abstract
Major histocompatibility complex genes are thought to be involved in allogeneic graft rejection but not many reports are available on their functional analysis in fish. Analysis of available sequences of MHC genes suggests functions in antigen presentation similar to those found in higher vertebrates. In mammals, the MHC class I and class II molecules are major determinants of allogeneic graft rejection due to their polymorphism in conjunction with their antigen presenting function. In fish, MHC class II molecules are found to be involved in rejection of allogeneic scale grafts. The present study was designed to investigate the involvement of MHC class I molecules in allograft rejection. Erythrocytes were collected from donors of rainbow trout expressed different MHC class I alleles, stained with two dyes, mixed and grafted to the recipients that were of the same sibling group as the donors. The grafts were rejected by allogeneic recipients and the MHC class I linkage group was the major determinant for the rejection.

Key words: MHC class I, Rainbow trout, Allogeneic graft, Erythrocyte, Fluorescent dye

Introduction

The major histocompatibility complex (MHC) contains a tightly linked cluster of genes, which encode cell membrane glycoproteins, the MHC class I and MHC class II molecules. They appear to be intimately involved in a variety of immunological processes including the restriction of antigen recognition by lymphocytes, the acquisition of the T cell repertoire, and the co-operative interaction among subsets of mononuclear leucocytes. Endogenous antigens are thought to be degraded into peptide fragments that bind to MHC class I molecules within the endoplasmic reticulum, while exogenous antigens are processed into peptide fragments within endosomal compartments and bind to MHC class II molecules (Germain 1999).

Allogeneic MHC molecules can induce both antibodies as well as cytotoxic T -cells directed against the graft (Auchincloss *et al.* 1999). It is generally accepted that mammalian T cells recognize a complex of MHC and endogenous peptide ligands via

TCR (Wang *et al.* 1998). The interaction of TCR with a complex of foreign MHC molecules and foreign peptides results in acute graft rejection. Chronic rejection occurs when the MHCs of the graft donor and the graft recipient are identical but foreign peptides are complexed with the MHC of the grafted cells (Janeway and Travers 1995).

MHC class I and II genes have been identified in many fish (McConnel *et al.* 1998, Hashimoto *et al.* 1999) and are supported to be involved in allograft rejection. Though allograft rejection was studied in many fish species (reviews: Manning and Nakanishi 1996, Nakanishi *et al.* 2002), very rare works with the identification of MHC gene function in allorejection were published. So far only one study in the Gila top minnow (*Poeciliopsis o. occidentalis*) was reported where MHC class II allele-matched scale grafts were better accepted by the recipient than non-matched allografts (Cardwell *et al.* 2001). Functional analysis of MHC class I is also scarce and inconclusive (reviewed in Nakanishi *et al.* 2002).

Only one classical MHC class I locus in rainbow trout, (*Oncorhynchus mykiss*) *Onmy-UBA*, with very low homology between alleles was identified so far (Hansen *et al.* 1996, Shum *et al.* 2001, Aoyagi *et al.* 2002, Xia *et al.* 2002). Different lineages are distinguished for the different domains, and they co-exist in various combinations at the *Onmy-UBA* locus (Aoyagi *et al.* 2002). In rainbow trout, the lineages Sal-MHCIa*A to –K were distinguished (Hansen *et al.* 1999, Shum *et al.* 2001, Aoyagi *et al.* 2002, Xia *et al.* 2002).

Fish erythrocytes are nucleated as like other lower vertebrates such as birds and amphibians (Delany *et al.* 1987, Flajnik and Du Pasquier 1988) and have been shown to express MHC class I molecules on their surface. The purpose of the present study was to investigate the involvement of MHC class I locus in allogeneic graft rejection by excluding involvement of MHC class II. Since erythrocytes do express only MHC class I and do not express MHC class II, erythrocytes were collected from the unsensitized MHC class I characterized rainbow trout and grafted it to the allogeneic DD and DF siblings to determine the rejection of grafted erythrocytes *in vivo*. The involvement of MHC class I to allogeneic erythrocyte graft rejection was clearly demonstrated.

Materials and methods

Experimental fish

Rainbow trout (Donaldson strain) stock were maintained in a flowing water system at the National Research Institute of Aquaculture (NRIA), Nikko branch, Japan and used in the present study. The fish were first investigated for determining the Sal-MHCIa lineages using the RT-PCR system described by Xia *et al.* (2002). After characterization, the broodstock, which expressed sequences belonging to the lineages Sal-MHCIa*D and F were selected. Sequence analyses (data not shown) demonstrated that all the brood fish express both the sequences *Onmy-UBA* *701 and *4901, which are classified into the Sal-MHCIa lineages D and F, respectively (Xia *et al.* 2002). Eggs from a *701/*4901 female were collected and fertilized with the sperm of a *701/*4901 male,

resulting in sibling offspring. During the eyed-egg stage, the siblings were transported to the Tamaki branch of the NRIA and kept in 30l tanks supplied with aerated running spring water at 15 °C. The fish were fed twice a day *ad libitum* with commercial trout dry pellets. When the fish became approximately 40-60 g, they were used in the experiment. For identifying the fish, they were tagged at dorsal fin with a plastic anchor tag using a Tagging Gun (103-XL, Bano'k, Tokyo, Japan).

*Detection of Onmy-UBA*701 and *4901 expression*

The adipose fin samples of the rainbow trout were collected and total RNA was isolated using TRIzol Reagent (Gibco BRL, Life Technologies, Grand Island, U.S.A.) following the manufacturer's recommendations. For RT-PCR amplification the 'RT-PCR high-PLUS' kit (Toyobo, Osaka, Japan) was used. The RT-PCR reaction mixtures were formulated following the manufacturer's suggestions, with 2.5 mM $Mn(OAc)_2$, 1 μM of each primer and 0.5 μg total RNA.

The *Onmy-UBA**701 and *Onmy-UBA* *4901 fragments were amplified following the methods of Xia *et al.* (2002) and Sarder *et al.* (2003). The primers used for amplification of the *Onmy-UBA**701 fragment are specific for sequences belonging to lineage Sal-MHCIa*D and can not amplify *Onmy-UBA**4901. Similarly, the primer set used for amplification of *Onmy-UBA* *4901 fragment were specific for sequences belonging to lineage Sal-MHCIa*F and can not amplify *Onmy-UBA* *701. The conditions for both RT-PCR amplifications were: First 60 °C for 30 min, then 94 °C for 2 min, then 35 cycles (94 °C for 1 min, 60 °C for 1.5 min) and finally 60 °C for 7 min. All parents and siblings used in this study were analyzed with both RT-PCRs.

The two parents of the siblings were analyzed with an RT-PCR system for amplification of 'full-length' *Onmy-UBA* fragments encoding the whole protein. The primers *pG-LPf*, 5'-GTATTATCTTGCTGGTGCTGGGAA (forward), binding to the leader peptide region, in conjunction with primer *pG-3'UTRr*, 5'-TTATGTTCTTGAGAAGTTCCTCTTC (reverse), binding to the 3'UTR, can amplify most *Onmy-UBA* alleles discovered thus far (Xia *et al.* 2002). The RT-PCR mixtures were set up as described above, and the amplification schedule was: First 60 °C for 98 min, then 94 °C for 2 min, then 35 cycles of (94 °C for 1min, 55 °C for 5 min), and finally 55 °C for 7 min.

Sequence analysis

Full-length *Onmy-UBA* fragments were cloned into the vector pGEM-T Easy (Promega Corporation, Wisconsin, U.S.A.). The nucleotide sequences were determined by the dideoxychain termination method using a 'CEQ Dye Termination Cycle Sequencing Kit' (Beckman Coulter Inc., California, U.S.A.) and suitable primers. Sequence analysis was conducted with an automated sequencer (CEQ 2000 DNA analysis system, Beckman Coulter.Inc.). For each fish at least three *Onmy-UBA**701 and three *Onmy-UBA**4901 clones were analyzed to exclude PCR errors. Comparison of deduced amino acid sequences was performed using the program 'Multiple alignment' of

GENETYX version 10.1 (Software Development Co. Ltd, Tokyo, Japan) computer software.

Staining of erythrocyte grafts

Fish erythrocyte grafts were stained with PKH67-GL (Green Fluorescent-cell Linker Kit, Sigma, Saint Louis, U.S.A.) and PKH26-GL (Red Fluorescent-cell Linker Kit, Sigma) by following manufacturer's instructions with some modifications. Blood (100 µl) was drawn from the caudal vessel of anaesthetized fish into a syringe containing an equal volume of MEM/FBS (Eagle's minimal essential medium plus 10% foetal bovine serum, pH adjusted to 7.2) with 2% heparin solution. The blood was centrifuged at 250 g for 5 min at 4 °C for crude separation of leukocytes (buffy coat) from erythrocytes. The erythrocyte cells were drawn from the bottom of the pellet, and washed once with MEM (MEM without FBS, pH adjusted to 6.95). The erythrocytes were resuspended in 1 ml of diluent C (supplied with the Sigma staining kits) and then 1 ml of freshly prepared 0.8 µM PKH67-GL (in diluent C) or PKH26-GL was added. Five min after incubation, an equal volume of MEM/FBS was added to stop the staining reaction. The cells were then washed twice with MEM/FBS and finally resuspended in MEM/FBS. Staining of cells was verified by fluorescence microscopy. At the end of staining, the green- and red-stained erythrocytes were mixed together in an approximately equal ratio with a final concentration of approximately 10^7 total erythrocytes/ml. Leukocyte contamination in the erythrocyte grafts was less than 1%.

Determination of survival of the erythrocyte graft

Approximately 400 µl of the green- and red-stained erythrocyte mixture was injected into the caudal vessel of anaesthetized recipients weighing 40-60 g and maintained them in tanks with regular feeding. For determining survival of the grafts at different time points after grafting, 100 µl of blood was collected from the caudal vessel of anaesthetized recipients. These blood samples were examined by fluorescence microscopy.

Results

*Expression of Onmy-UBA *701 and *4901 lineages*

The siblings used in the experiments were produced from the same parents who expressed only sequences from the lineages of Sal-MHCIa*D (701) and *F (4901). Because the parents were 701/4901, the allelic segregation in the siblings were 701/701(DD), 701/4901(DF) or 4901/4901(FF) in a Mendelian fashion (Fig. 1). This was confirmed by use of RT-PCR amplification reactions specific for sequences of lineages Sal-MHCIa*D and *F. Nucleotide sequence analysis for full length *Onmy-UBA* fragments of the parents of the siblings demonstrated that there was low homology between the *Onmy-UBA**701 and *Onmy-UBA**4901 lineages (data not shown).

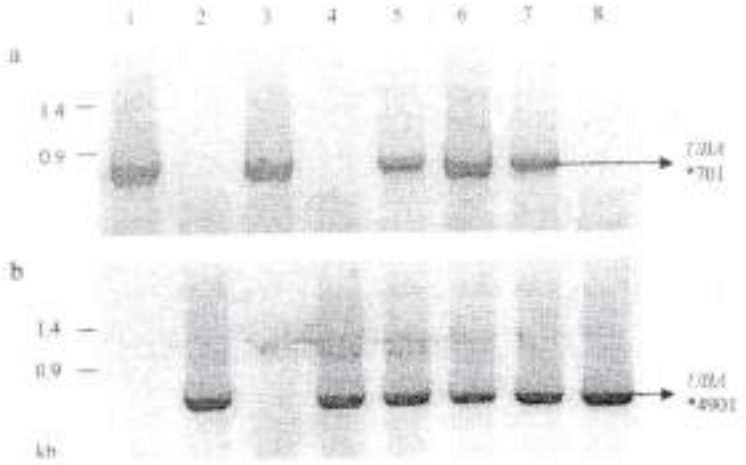

Fig. 1. RT-PCR amplification reactions specific for sequences of lineages Sal-MHCIa*D (a) and F (b). Eight individuals (1-8) of the sibling group were analyzed. The allelic sequences *UBA**701 and *4901 had been determined for the sibling parents. The data (a plus b) indicate that fish 1 and 3 express *UBA**701, fish 2, 4 and 8 express *UBA**4901, and fish 5, 6 and 7 express both alleles.

Survival of autologous grafts

In order to assess the potential toxic effects of the two stains on erythrocyte grafts, an experiment was carried out using four fish not belonging to the same sibling group. Erythrocytes were collected from the four fish separately and stained with either the red or green dye. The red and green stained cells were mixed and re-injected into the same donor fish. To determine the survivability of the stained erythrocytes, blood samples were collected from fish at days 21, 28 and 42 after grafting and checked under fluorescence microscope. Analysis showed that the red and green stained erythrocytes equally survived (Fig. 2a) and the intensity of the stain was quite strong even after 42 days of grafting.

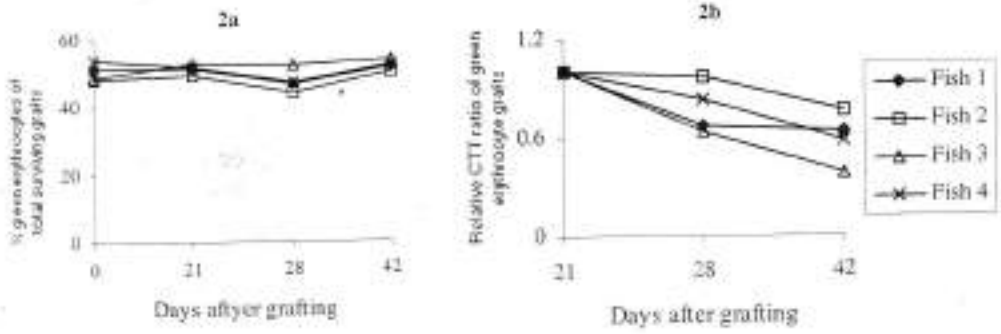

Fig. 2. The graphs (2a) show similar survival of green compared to red autologous erythrocyte grafts. The different symbols indicate four different individuals. The injected graft mixtures contained 51, 48, 49 and 54% green erythrocytes respectively. The graphs (2b) compare the survival of green grafts to the total erythrocytes in the host with the CTT (compared to total) ratio at day 21 arbitrarily assigned a value of 1.

Survival of allogeneic erythrocytes in vivo

Two experiments were carried out to compare the survival of *Onmy-UBA* matching and mismatching allogeneic erythrocytes. In the first experiment, erythrocytes collected from a DD and an FF individual were differently stained and injected to the recipients DD3, DD6, DD8, DD9, DF11, DF19, DF21 and DF23. Similarly, in the second experiment erythrocytes from a DD and an FF were stained and grafted to the recipients DD10, DD14, DD22, DD24, DF1, DF4, DF13 and DF15. In both the experiments donors did not include in the recipient groups. It is necessary to mention that there was no many FF fish other than donors, that's why DF siblings were chosen as recipients instead of FF. To observe the survivability of the grafts the fish blood were sampled at days 7, 14, 21, 28 and 35 after grafting in the first experiment. While fish blood were sampled at 7, 14, 28 and 35 days after grafting in the second experiment. The survival results of the matched and mismatched grafts of the first and second experiments are presented in Fig. 3a and Fig. 4a, respectively. In Fig. 3a, mismatched grafts showed significantly ($p<0.05$) lower survival in all DD recipients. The survival of FF grafts in all DF recipients were significantly ($p<0.05$) lower than those of DD grafts. Although, the experiment continued for 35 days all the DD and the DF23 recipients died before ending the experiment. In Fig. 4a, mismatched grafts had lower survival in all DD recipients except DD22, which died just after first sampling (7 days). All the DF recipients except DF4 showed equal survival for both matched and mismatched grafts. The DF4 recipient demonstrated only 7% survival of mismatched grafts. The survival of the MHC class I-matched grafts was significantly ($p<0.05$) higher than that of the MHC class I-mismatched grafts in all DD recipients in both experiments at all the sampling points.

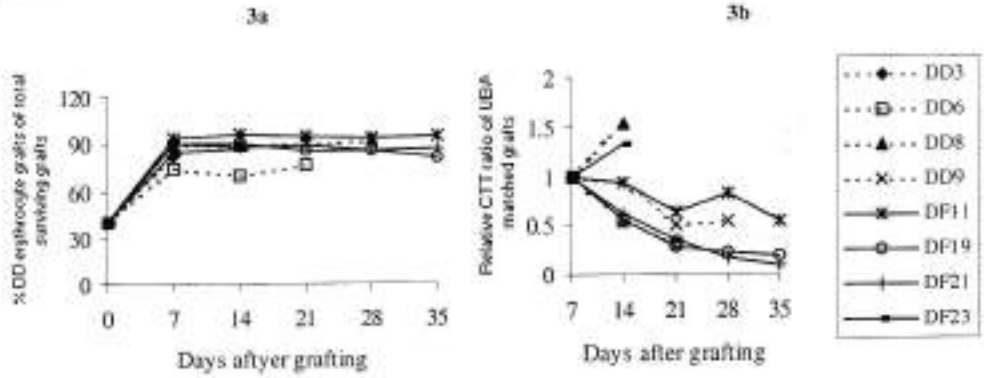

Fig. 3. MHC class I matching is important for the survival of erythrocyte grafts. The injected graft mixture contained 40% DD erythrocytes. Survival of erythrocyte grafts was determined at days 7, 14, 21, 28 and 35 after grafting. Graph indicators at the right apply to both (3a) and (3b) graphs and refer to the recipients. The (3a) graphs indicate the survival of the DD grafts as a percentage of the total surviving grafts (red plus green). The (3b) graphs compare the survival of the MHC class I matched grafts to the total erythrocytes in the host with the CTT (compared to total) ratio at day 7 arbitrarily set to value 1.

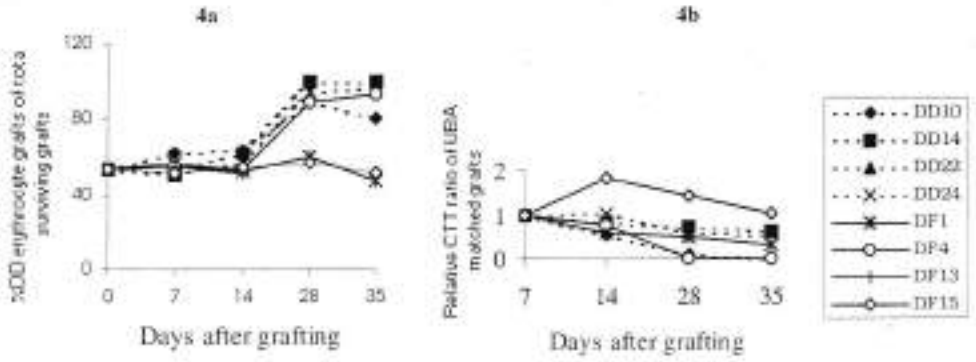

Fig. 4. Survival of MHC class I matched grafts *in vivo*. The injected graft mixture contained 54% DD erythrocytes. Survival of erythrocyte grafts was determined at days 7, 14, 28 and 35 after grafting. Graph indicators at the right apply to both (4a) and (4b) graphs and refer to the recipients. The (4a) graphs indicate the survival of the DD grafts as a percentage of the total surviving grafts (red plus green). The (4b) graphs compare the survival of the MHC class I matched grafts to the total erythrocytes in the host with the CTT (compared to total) ratio at day 7 arbitrarily set to value 1.

Discussion

Data presented in this paper describes the involvement of classical MHC class I locus in the rejection of allogeneic erythrocyte grafts in rainbow trout. In the allogeneic experiments, all DD recipients except DD3 (experiment 1) and DD22 (experiment 2) (died after 7 days of grafting) demonstrated better survival of MHC class I- matched grafts compared to those of MHC class I-mismatched grafts (Fig. 3a and 4a). In the first allogeneic experiment, all the DF recipients rejected the FF allogratfs significantly faster than those of DD allografts. In the second allogeneic experiment, three out of four DF recipients showed equal survival of both allogeneic DD and FF grafts, but the DF4 recipient showed very fast rejection of FF allografts compared to the DD grafts. The overall analysis of results showed that the MHC- mismatched grafts were rejected by all eight DD recipients, five out of eight DF sibling recipients rejected FF grafts significantly faster than those of DD grafts, which was a clear indication of the involvement of MHC class I- linkage groups in the rejection of allogeneic erythrocytes.

The equal survival of FF grafts in DF recipients or faster rejection of FF grafts by DF recipients in allogeneic experiments predicts some assumptions other than the MHC class I linkage group involvement in the allorejection. The first assumption might be the toxic effects of certain stains on erythrocytes that caused high mortality of FF grafts in some DF recipients but it is not convincingly acceptable. Because, the autologous grafts stained with the red and green stains as allogeneic graft staining demonstrated equal survival of both red and green stained grafts (Fig. 2a). The another assumption could be the involvement of phenotypic differences in the immune system of the recipients. Individual phenotypical differences are commonly observed in the fish found immune system (Yoshinaga *et al.* 1994, Alcorn *et al.* 2002).

When compared the survivability of stained grafts with the total harvested erythrocytes in the allogeneic experiments (Fig. 3b and 4b), it was observed that most of the recipient fishes showed similar pattern of survivability as found in the autologous grafts (Fig. 2b), only DD10 and DF4 had profound reduction of stained grafts compared to the total cells, which could be resulted from the loss of big amount of blood from the body due to sampling error. It is wise to mention here that based on the experimental procedures of the two allogeneic experiments, another few more experiments involving DD and FF recipients were conducted and similar rejection responses were observed (Sarder *et al.* 2003).

Only one classical MHC class I sequence is expressed per haploid rainbow trout genome (Shum *et al.* 2001, Aoyagi *et al.* 2002, Xia *et al.* 2002). The homology between many Onmy-UBA allomorphs (proteins encoded by alleles) is very low. For example, the amino acid identity between the $\alpha 1$ and $\alpha 2$ domains of *Onmy-UBA**701 and *4901 is only 42% and 56% respectively (data not shown).

The low homology between the *Onmy-UBA**701 and *4901 allomorphs may have influenced the nature of the cytotoxic response to the erythrocyte allografts. There are two logical effector cell candidates, cytotoxic T -cells and natural killer (NK) cells. These cell types share many characteristics and can under certain conditions even kill the same targets (Yokoyama 1999). In mammals, cell-mediated killing of allografts is primarily performed by cytotoxic T -cells, but dependent on the type of graft tissue NK cells can play a limited role (Manilay and Sykes 1998, Auchincloss *et al.* 1999). NK cells play a more important role in xenograft rejection (Manilay and Sykes 1998, Auchincloss *et al.* 1999). While both cell types have MHC class I binding receptors, MHC binding activates T cells but inhibits NK cells. In fish no cytotoxic T cells or NK cells have been clearly distinguished, but indications for identification of both cell types exist. Genes for the probable T -cell markers TCR (Hawke *et al.* 1999) and CD8 (Hansen and Strassburger 2000) genes, and a possible NK marker in channel catfish (a ligand for MAb CC41, Shen *et al.* 2002) have been identified.

Some *in vitro* cell-mediated cytotoxicity assays for rainbow trout have shown a need for sensitization to detect allograft killing (Fischer *et al.* 2003), reminiscent of T cell activity, while other studies showed spontaneous killing of allogeneic targets by leukocyte populations from some rainbow trout individuals, reminiscent of NK activity (Yoshinaga *et al.* 1994). Cells similar to cytotoxic T cells and cells similar to NK cells

seem to exist in fish and they are capable of killing allogeneic targets. If antibody independent cytotoxicity has played an important role in the erythrocyte graft rejections observed, this was probably mediated by cytotoxic T cells and not NK cells. If antibodies were involved in the graft rejection, NK cells may have killed the allografts by means of antibody-dependent cell-mediated cytotoxicity (ADCC, Yokoyama 1999).

From the above discussion it is concluded that the *Onmy-UBA* classical MHC I lineages are thought to be determinant of rejection of allografts and the present study demonstrated MHC class I involvement in the rejection of allogeneic erythrocytes. However, more studies need to identify the kinds of immune response involving allograft rejection and also to determine some other possible factors, such as blood groups and influence of low homology between MHC class I lineages.

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References

Alcorn, S.A., A.L...Murray and R.J. Pascho, 2002. Effects of rearing temperature on immune functions in sockeye salmon (*Oncorhynchus nerka*). *Fish Shellfish Immunol.*, 12: 303-334.

Aoyagi, K., J.M. Dijkstra, C. Xia, I. Denda, M. Ototake, K. Hashimoto and T. Nakanishi, 2002. Classical MHC class I genes composed of highly divergent sequence lineages share a single locus in rainbow trout (*Oncorhynchus mykiss*). *J. Immunol.*, 168: 260-273.

Auchincloss, H, M. Sykes and D.H. Sachs, 1999. Transplantation Immunology. *In*: Fundamental Immunology (W.E. Paul ed.). Lippincott-Raven publishers, Philadelphia. pp. 1175-1236.

Cardwell, T.N., R.J. Sheffer and P.W. Hedrick, 2001. MHC variation and tissue transplantation in fish. *J. Hered.*, 92: 305-308.

Delany, M.E., W.E. Briles and R.W. Briles, 1987. Cellular expression of MHC glycoproteins on erythrocytes from normal and aneuploid chickens. *Dev. Comp. Immunol.*, 11: 613-624.

Fischer, U., K. Utke, M. Ototake, J.M. Dijkstra and B. Köllner, 2003. Adaptive cell-mediated cytotoxicity against allogeneic targets by CD8-positive lymphocytes of rainbow trout (*Oncorhynchus mykiss*). *Dev. Comp. Immunol.*, 27: 323-337.

Flajnik, M.F. and L. Du Pasquier, 1988. MHC class I antigens as surface markers of adult erythrocytes during the metamorphosis of *Xenopus*. *Dev. Biol.*, 128: 198-206.

Germain, R.N., 1999. Antigen processing and presentation. *In*: Fundamental Immunology (W.E. Paul). Lippincott-Raven publishers, Philadelphia. pp. 287-340.

Hansen, D.J., P. Strassburger and L. Du Pasquier, 1996. Conservation of α_1 domain within the teleostean world, MHC class I from the rainbow trout *Oncorhynchus mykiss*. *Dev. Comp. Immunol.*, 20: 417-425.

Hansen, D.J., P. Strassburger, G.H. Thorgaard, W.P. Young and L. Du Pasquier, 1999. Expression, linkage, and polymorphism of MHC-related genes in rainbow trout, *Oncorhynchus mykiss*. *J Immunol.*, 163: 774-786.

Hansen, J.D. and P. Strassburger, 2000. Description of an ectothermic TCR coreceptor, CD8 alpha, in rainbow trout. *J. Immunol.*, 164: 3132-3139

Hashimoto K., K. Okamura, H. Yamaguchi, M. Ototake, T. Nakanishi and Y. Kurosawa, 1999. Conservation and diversification of MHC class I and its related molecules in vertebrates. *Immunol Rev.*, 167: 81-100.

Hawke N.A., J.A. Yoder and G.W. Litman, 1999. Expanding our understanding of immunoglobulin, T-cell antigen receptor, and novel immune-type receptor genes: a subset of the immunoglobulin gene superfamily. *Immunogenetics*, 50: 124-133.

Janeway, C.A. and P. Travers, 1994. Immunobiology. Current Biology Ltd., London/Garland Publishing Inc., New York.

Manilay, J.O. and M. Sykes, 1998. Natural killer cells and their role in graft rejection. *Curr. Opin. Immunol.*, 10: 532-538.

Manning, J.M. and T. Nakanishi, 1996. The specific immune system: cellular defenses. *In*: The Fish Immune system (G. Iwama and T. Nakanishi eds.). Academic Press, San Diego. pp. 159-205.

McConnell T.J., U.B. Godwin and B.J. Cuthbertson, 1998. Expressed major histocompatibility complex class II loci in fishes. *Immunol Rev.*, 166: 294-300.

Nakanishi, T., U. Fischer, J.M. Dijkstra, S. Hasegawa, T. Somamoto, N. Okamoto and M. Ototake, 2002. Cytotoxic T-cell function in fish. *Dev. Comp. Immunol.*, 26: 131-139.

Sarder, M.R.I., U. Fischer, J.M. Dijkstra, I. Kiryu, Y. Yoshiura, T. Azuma, B. Köllner and M. Ototake, 2003. The MHC class I linkage group is a major determinant in the *in vivo* rejection of allogeneic erythrocytes in rainbow trout (*Oncorhynchus mykiss*). *Immunogenetis*, 55: 315-324.

Shen, L., T.B. Stuge, H. Zhou, M. Khayat, K.S. Barker, S.M. Quiniou, M. Wilson, E. Bengten, V.G. Chinchar, L.W. Clem and N.W. Miller, 2002. Channel catfish cytotoxic cells: a mini-review. *Dev. Comp. Immunol.*, 26: 141-149.

Shum, B.P., L. Guethlein, L.R. Flodin, M.A. Adkinson, R.P. Hedrick, R.D. Nehring, R.J. Stet, C. Secombes and P. Parham, 2001. Modes of salmonid MHC class I and II evolution differ the primate paradigm. *J. Immunol.*, 166: 3297-3308.

Wang, W., S. Man, P.H. Gulden, D.F. Hunt and V.H. Engelhard, 1998. Class I-restricted alloreactive cytotoxic T lymphocytes recognize a complex array of specific MHC-associated peptides. *J. Immunol.*, 160 : 1091-1097.

Xia, C, I. Kiryu, J.M. Dijkstra, T. Azuma, T. Nakanishi and M. Ototake, 2002. Differences in MHC class I genes between strains of rainbow trout (*Oncorhynchus mykiss*). *Fish. Shellfish Immunol.*, 12: 287-301.

Yokoyama, W.M., 1999. Natural killer cells. *In*: Fundamental Immunology (W.E. Paul ed.). Lippincott-Raven publishers, Philadelphia. pp. 575-603.

Yoshinaga, K, N. Okamoto, O. Kurata and Y. Ikeda, 1994. Individual variations of natural killer activity of rainbow trout leucocytes against IPN virus-infected and uninfected RTG-2 cells. *Fish Pathology*, 29: 1-4.

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Hashimoto K., K. Okamura, H. Yamaguchi, M. Ototake, T. Nakanishi and Y. Kurosawa, 1999. Conservation and diversification of MHC class I and its related molecules in vertebrates. *Immunol Rev.*, 167: 81-100.

Hawke N.A., J.A. Yoder and G.W. Litman, 1999. Expanding our understanding of immunoglobulin, T-cell antigen receptor, and novel immune-type receptor genes: a subset of the immunoglobulin gene superfamily. *Immunogenetics*, 50: 124-133.

Janeway, C.A. and P. Travers, 1994. Immunobiology. Current Biology Ltd., London/Garland Publishing Inc., New York.

Manilay, J.O. and M. Sykes, 1998. Natural killer cells and their role in graft rejection. *Curr. Opin. Immunol.*, 10: 532-538.

Manning, J.M. and T. Nakanishi, 1996. The specific immune system: cellular defenses. *In*: The Fish Immune system (G. Iwama and T. Nakanishi eds.). Academic Press, San Diego. pp. 159-205.

McConnell T.J., U.B. Godwin and B.J. Cuthbertson, 1998. Expressed major histocompatibility complex class II loci in fishes. *Immunol Rev.*, 166: 294-300.

Nakanishi, T., U. Fischer, J.M. Dijkstra, S. Hasegawa, T. Somamoto, N. Okamoto and M. Ototake, 2002. Cytotoxic T-cell function in fish. *Dev. Comp. Immunol.*, 26: 131-139.

Sarder, M.R.I., U. Fischer, J.M. Dijkstra, I. Kiryu, Y. Yoshiura, T. Azuma, B. Köllner and M. Ototake, 2003. The MHC class I linkage group is a major determinant in the *in vivo* rejection of allogeneic erythrocytes in rainbow trout (*Oncorhynchus mykiss*). *Immunogenetis*, 55: 315-324.

Shen, L., T.B. Stuge, H. Zhou, M. Khayat, K.S. Barker, S.M. Quiniou, M. Wilson, E. Bengten, V.G. Chinchar, L.W. Clem and N.W. Miller, 2002. Channel catfish cytotoxic cells: a mini-review. *Dev. Comp. Immunol.*, 26: 141-149.

Shum, B.P., L. Guethlein, L.R. Flodin, M.A. Adkinson, R.P. Hedrick, R.D. Nehring, R.J. Stet, C. Secombes and P. Parham, 2001. Modes of salmonid MHC class I and II evolution differ the primate paradigm. *J. Immunol.*, 166: 3297-3308.

Wang, W., S. Man, P.H. Gulden, D.F. Hunt and V.H. Engelhard, 1998. Class I-restricted alloreactive cytotoxic T lymphocytes recognize a complex array of specific MHC-associated peptides. *J. Immunol.*, 160 : 1091-1097.

Xia, C, I. Kiryu, J.M. Dijkstra, T. Azuma, T. Nakanishi and M. Ototake, 2002. Differences in MHC class I genes between strains of rainbow trout (*Oncorhynchus mykiss*). *Fish. Shellfish Immunol.*, 12: 287-301.

Yokoyama, W.M., 1999. Natural killer cells. *In*: Fundamental Immunology (W.E. Paul ed.). Lippincott-Raven publishers, Philadelphia. pp. 575-603.

Yoshinaga, K, N. Okamoto, O. Kurata and Y. Ikeda, 1994. Individual variations of natural killer activity of rainbow trout leucocytes against IPN virus-infected and uninfected RTG-2 cells. *Fish Pathology*, 29: 1-4.

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Effects of different dietary levels of vitamin E on the breeding performance of *Heteropneustes fossilis* (Bloch)

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Abstract
Experiments on the study of different dietary levels of vitamin E on the growth and breeding performance of *Heteropneustes fossilis* brood fish were carried out in two phases. The first phase consisted of studying its ovarian development and the second phase on breeding performance. Sixty female fishes were stocked in twelve experimental chambers of a raceway. The effects of four dietary vitamin E levels *viz.* 0 (served as control), 50, 100 and 200 mg/kg feed, on the somatic growth, ovarian development of brood fish and on their breeding performance were studied. Each treatment had three replications. It was observed that body growth in terms of length and weight was best with 0 mg vitamin E/kg feed and 200 mg vitamin E/kg of feed gave poorest result. The gonado-somatic index and fecundity, however, was highest in the fish fed with 100 mg vitamin E/kg of feed. In case of breeding performance such as ovulation rate, fertilization rate, hatching rate and survival rate, the best result was obtained with 200 mg vitamin E/kg of feed. The overall result of this experiment indicates that 200 mg vitamin E/kg of feed is the best vitamin E dose for *H. fossilis* brood and vitamin E content has a positive impact on ovarian development.

Key words : *H. fossilis*, Vitamin E, Breeding performance

Introduction

Air breathing catfishes such as shingi (*Heteropneustes fossilis*) and magur (*Clarias batrachus*) are potentially important culture species and need to be taken under aquaculture system. These catfishes together comprise a handsome percentage of total fish catch and bulk of the catch of these fishes comes from the wild population. As major part of their production depends on natural source, over fishing together with environmental degradation have posed threat to their very existence.

Among these live fishes, shingi is a popular indigenous air-breathing catfish. This species has got many qualities that make it a perfect candidate for pond culture. It grows rapidly and attains marketable size within one growing season (Islam 1989). It breeds in natural shallow waters during monsoon season usually after heavy shower when the adjoining area of ponds and other depressions get inundated.

In spite of having many qualities such as high digestibility of protein, presence of vitamin, iodine and fat in muscle, very little attempt has been made to promote its breeding and culture of shingi. In our country farmers are dependent on natural sources for collection of fry. Therefore, proper techniques of induced breeding and larval rearing for production of fry in commercial scale are needed to be developed. For the supply of quality seeds in sufficient numbers the brood fish must be of good quality. So in case of brood stock management, there should be regular supply of balanced food for their growth and development. Although some works have previously been conducted on its induced breeding and fry rearing but the techniques available have not been standardized to recommend at farmer's level. More comprehensive works need to be carried out if its culture has to be popularized. In the present work, some attempts have been made to focus on the brood stock nutrition of shingi for their growth and development. For the initiation of study on the nutrition it is necessary to determine whether spawning and egg quality are influenced by nutritional quality of brood stock diets or not. Vitamin E plays an important role in reproductive physiology in fish as it does in birds and mammals (Watanabe 1985). Considering the above realities, the present investigation was undertaken to achieve the objectives such as i) to study the effects of different dietary levels of vitamin E on growth of fish and ovarian development and ii) to study the effects of different dietary levels of vitamin E on breeding performance such as ovulation rate, gonado-somatic index, fertilization and hatching rates of eggs and survival of larvae.

Materials and methods

In order to observe the effect of different dietary levels of vitamin E on growth, gonadal development and breeding performance of *H. fossilis*, two experiments were carried out in two phases. In the first phase, brood fish were reared and maintained by providing different dietary levels of vitamin E and in the second phase, breeding performance of the reared broods were investigated.

Experimental sites

The first experiment was carried out in a raceway. The raceway was divided into thirty equal sized chambers where each of the chambers was 183x102x100 cm^3 in size and separated from other by netted frames. The raceway was facilitated with inlet and outlet system which allowed the renewal and removal of water when needed. Since *H. fossilis* is bottom dwelling and prefers shade, raceway bottom was filled with 4 cm mud and some water hyacinths were kept suspended in the chamber. The second experiment was conducted in the Wet Laboratory of Faculty of Fisheries.

Collection and stocking of broods

About 100 females of *H. fossilis* were locally collected and kept in three fiberglass tanks for acclimatization. After three days of conditioning, five similar sized fishes were

stocked in each raceway chamber. Initial length and weight of the fish were recorded. The physico-chemical parameters such as temperature, dissolved oxygen and pH of the raceway water were monitored on regular basis to ensure that the water quality remained suitable for the broods.

Experimental design and feed formulation

Twelve raceway chambers were divided into four groups, which corresponded to four experimental treatments and each of the treatment had three replications. Feeds were formulated for different treatments using four different levels of vitamin E such as 0 (served as control), 50, 100 and 200 mg/kg feed. For preparing feed, finely ground and sieved fish meal, sesame meal, soybean meal, mustard oil cake, rice bran and wheat flour, and vitamin mineral premix (Evit tablet) were used. The proximate composition of the ingredients was determined following the standard methods given by Association of Official Analytical Chemists (AOAC 1980) (Table 1). The formulation of the experimental diets is shown in Table 2. To maintain an approximately 40% protein level in the feed, required amount of ingredients were mixed and converted into pellets by using a hand machine. These pellets were dried and stored in plastic bag with heat sealing and kept in a refrigerator. The formulation of all the experimental diets was same and they differed from each other only by the amount of vitamin E added, hence there was no variation in protein percentage among the diets.

Table 1. Proximate composition of dietary ingredients (% dry matter basis)

Ingredients	Dry matter	Protein	Lipid	Ash	Nitrogen free extract (NFE)[1]
Fish meal	91.66	66.50	8.91	15.58	9.01
Mustard oil cake	91.28	34.43	6.99	10.10	48.48
Soybean meal	90.14	49.53	1.52	8.33	40.62
Sesame meal	91.82	22.77	8.09	18.17	50.97
Rice bran	91.35	14.92	4.38	12.31	68.39
Wheat bran	89.83	14.0	3.97	4.98	77.05

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

Table 2. Formulation (%) of experimental diets

Ingredients	Inclusion level (%)			
	Feed -1	Feed-2	Feed-3	Feed-4
Fish meal	40.0	40.0	40.0	40.0
Sesame meal	15.0	15.0	15.0	15.0
Soybean meal	13.88	13.88	13.88	13.88
Mustard oil cake	13.88	13.88	13.88	13.88
Rice bran	6.12	6.12	6.12	6.12
Wheat bran	6.12	6.12	6.12	6.12
Wheat flour	4.0	4.0	4.0	4.0
Vitamin mineral premix	1.0	1.0	1.0	1.0
Vitamin E	0 mg	50 mg	100 mg	200 mg

Feeding and sampling

The brood fishes were fed two times a day up to satiation. The unused foodstuffs, debris and faeces were removed from the chambers by draining out water. Sampling of fish was done fortnightly. During sampling all the fishes from each chamber were caught by scoop net and their lengths and weights were measured. Growth of the fish were determined by following ways:

Length gain (cm) = Mean final length – mean initial length

Weight gain (g) = Mean final weight – mean initial weight

$$\text{Specific growth rate, SGR (\% day)} = \frac{\log_e W_2 - \log_e W_1}{T_2 - T_1} \times 100 \text{ (after Brown 1957)}$$

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

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

Estimation of gonado-somatic index and fecundity of brood fish

One fish from each chamber was collected and total length and weight was taken separately. The fish were dissected and ovaries were removed and weighed. The gonado-somatic index was calculated by using the following formula:

$$\text{Gonado-somatic index (GSI) (\%)} = \frac{\text{Gonad weight (g)}}{\text{Body weight (g)}} \times 100$$

Fecundity of fish was determined by following gravimetric methods (Lagler 1952). The dissected ovaries were preserved at 5% formalin and three samples (0.1 to 0.5 g) were taken from the anterior, central and posterior regions of each ovary. The samples were weighed and the eggs were counted from each sample. Fecundity was determined by applying following formula:

$$\text{Fecundity (F)} = \frac{\text{N x Gonad weight (g)}}{\text{Sample weight (g)}}$$

where, F is the fecundity and N is the number of eggs in sample.

Induced breeding

Two females from each replication of each treatment were selected and induced with PG extract injection. Mature males of *H. fossilis* were collected from ponds and kept in separate fiberglass tanks. The amount of PG to be required was determined by

$$\text{Weight of required amount of PG (mg)} = \frac{W_{ib} \times 7.0}{100}$$

where, W_{ib} represents total body weight of the fish to be injected and 7.0 represents the rate of PG in mg to be injected/100 g body weight of females.

The total volume of the PG extract to be prepared was calculated by the following formula:

$$\text{Volume of extract (ml)} = \frac{W_b \times 0.5}{100}$$

where, 0.5 represents the volume of the PG extract in ml to be injected/100 g body weight.

The weighed PG was homogenized with distilled water and the homogenate was centrifuged. The supernatant (PG) was taken in a 1.0 ml graduated hypodermic syringe and injected intramuscularly to the fish near dorsal fin. After injection each female was kept separately for ovulation. The males did not receive any inducing agent.

The females were checked for ovulation hourly beginning from 6 hrs post injection and continued up to 12 hrs of injection. As soon as the females ovulated the eggs were collected by stripping and placed in a clean fertilization tray. The milt was collected from the male by dissecting out the testes and macerating them in 0.85% sodium chloride solution. The fertilization was done by mixing the sperm suspension with eggs using a feather and then a little water was added to the egg-sperm mixture.

Incubation and hatching of the fertilized eggs

A portion of fertilized eggs from an individual female of each treatment was homogenously spread on plastic bowls (15 cm diameter). All the incubation bowls received gentle shower and adequate aeration. Soon after fertilization when embryonic development started, the fertilized eggs looking blackish or greenish in colour were counted for respective females. After completion of hatching, the number of newly hatched larvae of each bowl was counted by siphoning them out.

Larval rearing

From the second day of hatching, the larvae were provided with live feed. They were reared for seven days to observe the effect of vitamin E on their survivability as the larvae produced from the broods were maintained under different dietary levels of vitamin E. Twelve plastic bowls each of 10l capacity were divided into four groups corresponding to four treatments and each of the bowls was stocked with 80 larvae as a stocking rate of 8 larvae/l. Continuous water flow of nearly equal rate was maintained in all the bow. Tubificid worms were used as feed and administered twice a day *ad libitum*. At the end of the experiment, the total number of larvae was counted and the length and weight were measured by random sampling of 10 fry in each bowl.

Statistical analysis

The specific growth rate, gonado-somatic index, fecundity, ovulation rate, fertilization rate, hatching success and survival rate of larvae up to eight days from first feeding were tested using one-way analysis of variance (ANOVA). Duncan's Multiple Range Test (DMRT) was also applied to identify significant difference ($P<0.05$) between means.

Results

Effects of vitamin E on growth and ovarian development

The growth, in terms of length and weight gain of the brood fish of *H. fossilis* fed with different dietary vitamin E content have been found satisfactory in all the treatments but the highest growth was observed in T1 whereas the lowest performance was in T4. Fish in treatment 2 showed better performance than those in T3. The specific growth rate (SGR) is presented in Fig. 1 where SGR was highest in T1 followed by T2, T3 and T4. There was no significant difference ($P>0.05$) between the SGRs of fish in T1 and T2, and between T3 and T4, respectively. However, SGR of fish in T1 and T2 were significantly ($P<0.05$) better than those of T3 and T4.

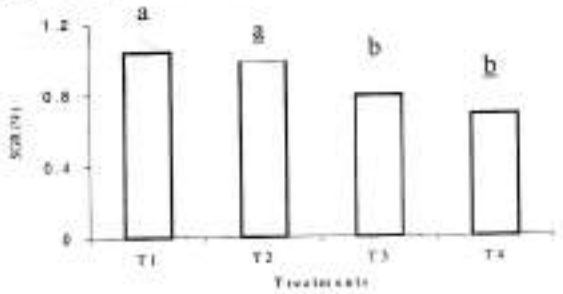

Fig. 1. Specific growth rate (SGR %) of *H. fossilis* brood fish under different treatments. Bars with different letters indicate significant difference ($P<0.05$).

Gonado-somatic index is very important parameter for understanding gonadal development. Results of GSI presented in Fig. 2 showed that fish in T3 produced highest GSI followed by T1, T2 and T4. However, there was no significant difference ($P>0.05$) among the GSI values in different treatments. In case of fecundity, it was found that the number of ovum/g body weight of fish was highest in T3 and lowest in T4 (Fig. 2). No significant difference was observed among the treatments for fecundity. The water quality of the raceway was in suitable range during the rearing period of broods (Table 3).

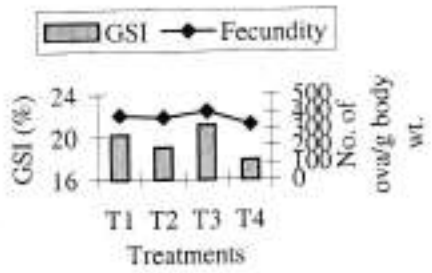

Fig. 2. GSI and fecundity of *H. fossilis* brood fish under different treatments.

Table 3. Physico-chemical parameters of the raceway water where the *H. fossilis* brood fish reared

Sampling number	Parameters		
	Temperature (°C)	Dissolved oxygen (ml/l)	pH
1st	28.5	6.0	7.0
2nd	29.0	6.3	7.2
3rd	28.0	6.0	7.0
4th	30.0	7.0	7.5
5th	29.5	6.5	7.5
6th	30.0	7.0	7.0

Effects of vitamin E on breeding performance

Induced breeding

The breeding performance of female brood fish, fed with different levels of vitamin E, in terms of ovulation percentage, fertilization and hatching rates of eggs are shown in Fig. 3. Brood fish fed with control feed (no vitamin E) in T1 and fish fed with 50 mg vitamin E containing feed in T2 demonstrated higher ovulation than that of fish fed with 100 mg and 200 mg vitamin E containing feed in T3 and T4 respectively. No significant differences were observed between the ovulation rate of fish of T1 and T2, and between T3 and T4. However, ovulation rate of broods in T1 and T2 were significantly better than those in T3 and T4. The fertilization rate of eggs was found significantly ($P<0.05$) higher in T4 followed by T1, T3 and T2 (Fig. 3). Similar results were obtained in hatching of eggs produced by females in different treatments and T4 showed significantly ($P<0.05$) higher hatching rate than that of T1, T3 and T2 (Fig. 3).

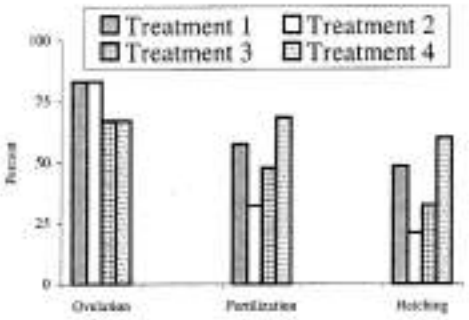

Fig. 3. Ovulation rate of females, fertilization and hatching rates of eggs *of H. fossilis* under different treatments. Bars with different letters indicates significant differences ($P<0.05$) in the respective parameters.

Larval rearing

Average initial length and weight of one day old *H. fossilis* larvae of different treatments were 4.5 ± 0.50 mm and 2.8 ± 0.29 mg respectively. After seven days of feeding with Tubificid worms, the average increment of length and weight of larvae were 4.7 ± 0.46 mm and 2.4 ± 0.35 mg respectively. The larvae of all the treatments showed good survival but T4 had highest survival rate than those of the rest. However, no significant difference was observed between the survival rates of the larvae among different treatments (Fig. 4).

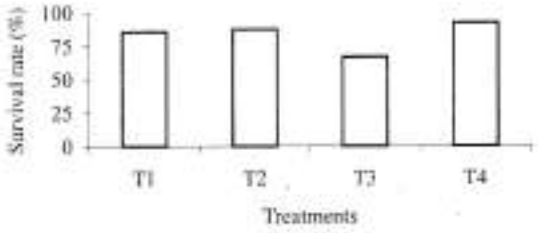

Fig. 4. Survival rate of *H. fossilis* larvae under different treatments.

Discussion

Breeding performance of fish depends on the quality of brood maintenance and the gonadal development of fish. The ultimate goal of the present research was to find out if there was any positive impact of vitamin E on the gonadal development and breeding performance of the brood fish of *H. fossilis*. The results obtained in the experiment provided a clear indication that there was a positive correlation between dietary vitamin E level and breeding performance of the fish.

It is generally agreed that for producing quality brood, they should be maintained in a good environment with proper diet. Here all the experimental brood fishes were maintained in a raceway where water temperature, dissolved oxygen and pH were found to be in the desirable range as reported by Boyd (1979) and Rahman *et al.* (1982). Therefore, there was no evidence of adverse effect of water quality on the existence and growth of female broods. However, it was observed that fish in the treatment 1 which was provided feed with no vitamin E had best growth rate, whereas fish in T4 fed highest level of vitamin E had poorest growth performance. This result was coincided with Dube and Trung (1993) who reported the best growth in terms of length and weight increment of gold fish with a vitamin E content of 50 mg/100 g of diet and the least growth with 200 mg vitamin/100g of diet. The somatic and gonadal growths of *H. fossilis* were antagonistic in nature, that is, with the increased rate of gonadal development the rate of somatic growth slows down. Similar phenomena were also reported in other fish species (Purdm 1976, Utter *et al.* 1983, Malison 1985).

In the events of fertilization rate, hatching rate and survivability of larvae of *H. fossilis*, a positive impact of vitamin E was observed and the highest level of vitamin E containing feed (T4) yielded best results. Takeuchi *et al.* (1981) conducted an experiment with brood fish of Ayu, *Plecoglossus altivelis* and observed better hatchability and survivability of larvae with 3.4 mg vitamin E/ 100 g of diet. Sanchai-Sutjaritvongsanon (1987) reported that a mixture of 35% of fishmeal, 30% soybean, 20% corn meal, 15% rice bran and 10 mg/kg BHT plus 100 mg/kg vitamin E was suitable for stimulating gonad development and spawning of gold fish. Although larvae in all the treatments were fed with Tubificid worms highest survival was found in T4. It could be resulted from the influence of vitamin E as the mother of larvae fed with high-level vitamin E diet and vitamin E could be incorporated into the eggs during oogenesis. King (1985) reported that the presence of vitamin E in the diet of rainbow trout had a significant effect on the final levels of alpha-tocopherol in eggs than fish deprived of vitamin E. During egg development, alpha-tocopherol was slowly, but efficiently transferred from the yolk to the developing embryo. Mortalities during egg development were inversely related to the alpha-tocopherol content of the eggs.

Stocking density is recognized as an important factor which directly affects the growth, survival and production of fish (Backiel and Le Cren 1978). Generally highest stocking density results in the reduction of growth and survival (Sarder *et al.* 1991) and increase food conversion ratio, together with severe competition of food and space. During the larvae rearing, 8 larvae/l was stocked and good survival was found in all the treatments. This is an agreement with the work of Mollah (2001) where optimum stocking density of *H. fossilis* larvae was between 10 and 20 per/l. Tubificid worms were used for larvae rearing which was recommended by Haque and Barua (1989) as best live feed for first feeding of *H. fossilis* larvae. Tubificid worms were also reported suitable live feed for nursing the *H. fossilis* larvae (Gheyas 1998) and for other indigenous and exotic catfish of similar nature (Yasmin and Mollah 1997, Mollah *et al.* 1998).

In the current research work, mainly six parameters were assigned to study the gonadal development and breeding performance such as gonadosomatic index, fecundity, ovulation percent, fertilization rate, hatching rate and survivability of larvae. The gonado-somatic index and fecundity were highest in the fish provided with 100 mg vitamin E/kg feed, whereas for the rest of above mentioned parameters a dose of 200 mg/kg feed proved to be best. Though vitamin E has a positive impact on breeding performance of *H. fossilis* and other fish species, the present research is probably the first ever work of its nature in Bangladesh. Therefore, the preliminary success obtained through this work can serve as an important base for future research on this topic.

References

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

Backiël, T. and E.D. Le Cren, 1978. Some density relationship for population parameters. *In:* the biological basis of freshwater fish production (S.D. Gerking ed.). Black Well Scientific Publications, Oxford. pp. 27-36.

Boyd, C.E., 1979. Water Quality Management for Pond Fish Culture. Elsevier Sci. Publ. Co. Amsterdam-Oxford, New York. 318 pp.

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

Dube, K. and D.V. Trung, 1993. Effect of vitamin E on growth and survival of gold fish (*Carassius auratus*). *Proc. Natl. Acad. Sci. India-B-Biol. Sci.*, 63(4): 437-444.

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

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

Islam, M.A., 1989. Nana deshe machher chash. Bangla Academy, Dhaka. pp.105-121.

King, I.B., 1985. Influence of vitamin E in reproduction in rainbow trout (*Salmo gairdneri*). *Deis. Abst. Int PT. B-Sci. and Eng.*, 46 (2): 185.

Lagler, K.F. , 1952. Studies in Fresh-Water Fishery Biology. Ann Arbor, Michigan, 199 pp.

M.F.A. Mollah, 2001. Development and/or refinement of induced breeding and fry production technique of singhi (*Heteropneustes fossilis*) and pangas (*Pangasius pangasius*). Bangladesh Agricultural Research Council, Dhaka. 6 pp.

M.R.I. Sarder, G.U. Ahmed, M.F.A. Mollah and M.S. Haq, 1991. Effects of stocking density on the growth of African catfish, *Clarias gariepinus* fry. *Bangladesh J. Fish.*, 14 (1-2): 37-40.

Malison, J.A., 1985. Growth promotion and the influence of sex-steriods on sexually related dimorphic growth and differentiation in yellow perch (*Perca flavescens*). *Can. J. Fish. Aquat. Sci.*, 43: 26-35.

Mollah, M.F.A., M.M.R. Khan and G.S. Haylor, 1998. Effects of different feeds on growth and survival of African catfish (*Clarias gariepinus*). *Bangladesh J. Agril. Sci.*, 25 (2): 159-162.

Purdom, C.E., 1976. Genetic technique in flat fish culture. *J. Fish. Res. Board Can.*,33: 1088-1093.

Rahman, M.S., M.Y. Chowdhury, M.A. Haque and M.S. Haq, 1982. Limnological studies of four ponds. *Bangladesh J. Fish.*, 2-5(1-2): 25-35.

Sanchai-Sutjaritvongsanon, 1987. Level of vitamin E in growth of an Indian major carp, Catla (*Catla catla*). *J. Indian. Fish. Assoc.*, 24 : 91-96.

Takeuchi, M., S. Ishii and T. Ogiso, 1981. Effect of dietary vitamin E on growth, vitamin E distribution and mortalities of the fertilized eggs and fry in ayu (*Plecoglossus altivelis*). *Bull. Tokai Reg. Fish. Res. Lab.*, 104: 111-122.

Utter, F.M., O.W. Johnson, G.M. Thorgaard and P.S. Robinoivitch, 1983. Measurement and potential applications of induced triploidy in Pacific salmon. *Aquaculture*, 35: 125-135.

Watanabe, T., 1985. Importance of the study of brood stock nutrition for further development of aquaculture. *In*: Nutrition and Feeding in Fish, (eds. C.B. Eowey, A.M. Mackie and J.G. Bell), Academic Press, London. pp. 395-413.

Yasmin, A. and M.F.A. Mollah, 1997. Rearing of African catfish (*Clarias gariepinus*) larvae with live and prepared feeds. *Bangladesh J. Train. and Devt.*, 10(1&2) : 181-186.

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Comparison of two hormone preparations on the reproductive performance of air breathing catfish *Clarias batrachus* (Lin.)

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Abstract
Two hormone preparations *viz.* Human Chorionic Gonadotropin (HCG) and pituitary gland (PG) suspension were compared for their comparative efficacy on the breeding performance of a air breathing catfish *Clarias batrachus*. It was found that HCG induced fish gave better ovulation response than PG. Both fertilization and hatching of eggs were significantly ($p<0.01$) higher in HCG treated fish than PG. On all consideration, HCG was found more suitable for induced breeding of *C. batrachus* over PG.

Key words: HCG, PG, *Clarias batrachus*, Breeding

Introduction

Among the indigenous cat fishes *Clarias batrachus* is an important fish species in Bangladesh. The fish is highly esteemed for its high market value and delicate taste. The population of this catfish is currently declining due to various man made and natural reasons. Availability of fry and fingerlings is the basic requirement of fish culture, but it is very difficult to collect the fry and fingerlings of this fish from natural sources. Therefore, artificial breeding with hormone manipulation is the only way to mass produce the fry of this species for culture.

Hormones are widely used for artificial breeding of fish, however, hormones are scarce in Bangladesh. Therefore, appropriate methods should be developed to make optimum use of hormones. To control life cycle of fish species some steps have to be followed, these include maintenance of brood stock for the production of eggs and sperm, rearing of larvae and raising of fish to marketable size (Richter *et al.* 1985). However, brood stock kept in captivity seldom reproduce spontaneously thus artificially induced breeding with the help of hormones is common practice in aquaculture. Different hormone preparations are used for this purpose including fish pituitary gland (PG) and Human Chorionic Gonadotropin (HCG). Ahmed *et al.* (1981 and 1985), Khan *et al.* (1975), Rahmatulla *et al.* (1983), Thakur *et al.* (1985) provided basic information on induced breeding of *C. batrachus* with a view to mass production of seeds. During the

past decades, successful breeding of fish had been attained with HCG either alone or with PG. The synchronization of egg production by brood stock fish can be achieved by hormone manipulation, this greatly facilitates planned breeding and culture programmes in fish farms. In the present study two hormone preparations HCG and PG were compared for their comparative efficacy on the breeding performance of *C. batrachus*.

Materials and methods

The study was carried out in a commercial fish breeding farm, Madina Fisheries and Hatchery Complex located at Dohar, Dhaka, Bangladesh. Thirty matured female catfish *Clarias batrachus* were collected from hatchery's own brood ponds. Twenty fish were selected for the experiment. The surplus 10 fish was kept as a backup in case of mortalities and escapes. Furthermore, 10 males were used from the fish farm's own brood stock to provide milt for fertilization. After proper acclimatization, the fishes were divided into two groups having 10 females in each groups and designated as T1 and T2. The two hormone preparations were collected from local market. The dry PG (pituitary gland of carp) in vial is available in market and generally used for carp breeding in Bangladesh. The HCG was produced by a commercial pharmaceutical company (Sumach: Infar, India) and HCG solution was prepared according to the prescriptions of brochure supplied by the manufacturing company (Table 1). Dry HCG powder (30 i.u./mg) was dissolved in distilled water. After homogenizing, the solution was centrifuged for 5 minutes at 4,000 rpm. The clear upper fluid was decanted in a sterile 30 ml flask and used immediately. HCG was diluted in such a way that 1 ml solution corresponds to 1000 i.u. The PG solution was prepared by homogenizing the dry PG in a tissue homogenizer and centrifuged in a centrifuge machine at 4,000 rpm. The upper clear fluid is used for injection purpose. Ten fully matured male brood fish were used for sperm collection. The fish were dissected and the sperm collected from the testes by squeezing out the milt. On mid June 2000, all experimental female fish were arranged into two groups of 10 females with a mean body weight of 119 ± 5 g and 118 ± 5 g, respectively. Each experimental female was injected with one of the two hormone preparations. The suspension was injected into dorsal musculature just below the dorsal fin. One group (T1) was injected with 400 i.u. HCG/100 g body weight and the other group (T2) was injected with 15 mg PG/ 100 g body weight.

Table 1. Source and method of hormone (HCG and PG) administration

Name of hormone	HCG	PG
Producer	Infar Pharmaceutical Company Ltd.	Anonymous
Dosages	400 i.u. / 100 g body weight.	15 mg /100 g body weight
No. of injection	Two	Two
Interval between injections	6 hour after 1st injection	6 hour after 1st injection
Route of administration	Intramuscularly	Intramuscularly

In each case total dose of hormone was divided into two injections and given at 6 hour interval. After injection, the females were individually housed in 200-l trays. A latency period of approximately 18 hours (Table 1) was allowed after which the fish were stripped for egg. The eggs from each individual female was collected in plastic bowl and fertilized with milt. Approximately 100 eggs from each female was incubated in 20-l plastic bowl fitted with inlet and outlet for constant water supply. In all 20 plastic bowls was used for egg incubation. Water temperature was maintained at 27±1°C. After about 26 hours of incubation all egg samples were thoroughly checked, dead eggs were counted per plastic bowl and removed, leaving the hatched larvae behind. The percent ovulation was calculated by the formula: Percent ovulation = No. of fish ovulated x 100 / Total no. of fish injected. The fertilization rate was calculated by following formula: Percent fertilization = No. of fertilized eggs x 100 / Total no. of eggs (fertilized + unfertilized). The hatching percentage was calculated after hatching. The hatching percentage was calculated as [(Number's of eggs in sample - Dead eggs) / Number of eggs in sample x 100. Data were analyzed by using the computer based SPSS program.

Results and discussion

The result presented in Table 2 shows that both experimental groups of fish did not differ significantly in body weight at the start of the experiment, thus it was possible to compare the groups without restrictions. The ovulation response showed (Fig. 1) a significant difference between the PG and HCG treated groups. A higher percentage of fish (100%) was induced by HCG in comparison to PG (80%). The eggs of the HCG treated group were brownish in color and the PG treated groups were yellowish brown in color and contained some white dead eggs. The fertilization rate of HCG treated group was 80.07±2.58% and that of PG treated group was 70.01±1.43% (Table 2). In respect of hatching rate, HCG treated group resulted in 70.45 ±3.60 % hatching, while it was only 60.10 ± 3.21% in PG treated group. It appears from the result that HCG worked better in all respect than PG for artificial breeding of cat fish.

Table 2. Comparison of two groups of *Clarias batrachus* injected with two hormone preparation

Treat-ments	Replications	Fish wt. (g)	Doses	Ovulation responses (%)	Fertilization rate (%)	Hatching rate (%)
T_1	R_1	120	HCG 400	+	78.50	71.44
	R_2	115	i.u/100g	+	81.25	69.22
	R_3	125		+	76.25	63.25
	R_4	122		+	82.12	74.26
	R_5	115		+	80.10	71.26
	R_6	110		+	79.50	75.23
	R_7	125		+	82.50	67.12
	R_8	114		+	84.15	68.23
	R_9	112		+	80.15	71.25
	R_{10}	123		+	76.25	73.26
Mean		118.10	400	100	80.07	70.45

Treatments	Replications	Fish wt. (g)	Doses	Ovulation responses (%)	Fertilization rate (%)	Hatching rate (%)
T_1	R_1	110	PG 15	+	70.50	63.21
	R_2	125	mg/100g	+	69.25	60.15
	R_3	114		+	71.30	54.26
	R_4	120		+	68.35	59.21
	R_5	112		-	-	-
	R_6	123		+	69.55	61.23
	R_7	120		+	68.80	64.27
	R_8	120		+	72.35	57.29
	R_9	125		+	70.70	61.23
	R_{10}	125		-	-	-
Mean		119.4	15	80	70.01	60.10

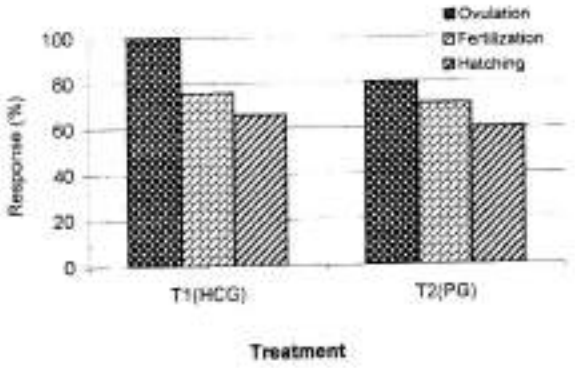

Fig. 1. Comparative performance of HCG and PG on ovulation response, fertilization and hatching of eggs of *Clarias batrachus*.

Statistical analysis showed a significant difference in respect of fertilization between HCG and PG treated group ($t = 9.85$, $df = 16$; $p < 0.01$) (Table 3). t test performed for hatching shows a significant difference ($t = 6.34$, $df = 16$; $p < 0.01$) between HCG and PG treated group. Thus in respect of ovulation, fertilization and hatching, the response of HCG treated fish is much higher than PG treated fish.

Table 3. t-test for fertilization and hatching of eggs of *Clarias batrachus*

Parameters	df	t	Performances
Fertilization	16	9.85	Significant
Hatching	16	6.34	Significant

Ahmed *et al.* (1985) worked with HCG and PG on reproductive performance of walking catfish opined that HCG is appropriate hormone for breeding of *C. batrachus*.

The present observation is in good agreement with the results of that study. Mustafa *et al.* (1986) studied on the efficacy of HCG and PG, and found HCG more effective with higher percentages of ovulation, fertilization and hatching than PG. This particular finding also closely agrees with the findings of the present study. Richter *et al.* (1985) found similar response in induced breeding of *C. gariepinus*. Devraj *et al.* (1972) described induced spawning using pituitary glands of marine catfish. They allowed the fishes to spawn naturally but observed that only few fishes spawned successfully. Mollah and Tan (1983) found HCG as effective agent in breeding *C. macrocephalus*. Khan and Mukhopadhyay (1975) observed successful spawning using carp pituitary extract in glass aquaria, but they observed poor rate of hatching. Ahmed *et al.* (1981) studied the spawning of *C. batrachus* in aquarium conditions and they observed repeated mating at small intervals with a few eggs released at each matings. They also noted that control fishes, which received no injection did not respond to spawning.

The present experiment clearly showed that under the given conditions the mode of action of inducing hormone to stimulate reproductive response of *C. batrachus* is in favour of HCG. Therefore, from the results of the experiment, it may be concluded that under the given condition HCG has clear advantage over PG for induced breeding of *C. batrachus*. The results showed that the use of HCG in induced breeding of *C. batrachus* could give better yield if proper induced breeding technology in the farmer level can be disseminated.

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References

Ahmed, A.T.A, G. Mustafa, K.R. Islam and A. Hai, 1981. Observations on the induced spawning of *Clarias batrachus*. *Bangladesh J. Agri.*, 6(1): 1-6.

Ahmed, K., G. Mustafa, S. Ali and M. Shajahan, 1985. Induced spawning of magur fish, *Clarias batrachus* (L.) by stripping method in a plastic bowl hatchery. *Bangladesh J.* Zool., 13(1): 19-24.

Devaraj, K.V., T.J. Varghese and G.P.S. Rad, 1972. Induced breeding of freshwater catfish, *Clarias batrachus* (Linn.) by using pituitary glands from marine catfish. *Curr. Sci.*, 41(25): 868-870.

Mollah, M.F.A and E.S.P. Tan, 1983. HCG - induced spawning of the catfish (*Clarias macrocephalus*, Gunther). *Aquaculture*, 35: 239-247.

Khan, H.A. and S.K. Mukhopadhyay, 1975. Production of stocking material of some air breathing fishes by hypophysation, *J. Inland Fish. Soc.* India., 7: 156-16 1.

Mustafa, G., K. Ahmed and M. Shajahan, 1986. Effect of H.C.G. (Human Chrionic Gonadotropin) and P.G. (Pituitary Gland) on induced spawning of the catfish, *Clarias batrachus* (Linn.). *Dhaka University Studies*, 34 (2): 159-165.

Rahamatulla, S.M., M.A. Islam, M.M. Hossain, M.M. Ali and A.K.M.N. Islam, 1983. Experiment on the induced breeding of *Clarias batrachus* (Linn.) by pituitary hormone injection. *Bangladesh J. Aquaculture*, 2-5.

Richter, C.J.J. and B.C. Cattel, 1985. A new way to standardize fish breeding. *Fish Farming*, 12: 14-15.

Thakur, N.K. and P. Das, 1985. Synopsis of biological data on magur, *Clarias batrachus* (Linnaeus, 1758). Bull. No. 41. CIFRI. Barrackpore, India. 82 pp.

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Species interaction between carp species in polyculture system

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Abstract
A three months experiment was conducted to study the species interactions of two carp species in polyculture system under supplemental feeding. Four species of fishes such as silver carp (*Hypophthalmichthys molitrix*), mrigal (*Cirrhina cirrhosus*), catla (*Catla catla*) and common carp (*Cyprinus carpio*) were cultured in four different combinations each containing two species. The combination of silver carp and mrigal in treatment 1, and silver carp and common carp in treatment 2 resulted better growth and production than other two treatments of different combinations of catla and common carp, and catla and mrigal.

Key words: Polyculture, Carp, Species interaction

Introduction

Inland water resources of Bangladesh is considered to be one of the richest in the world both in area and potential for fisheries development (Rahman 1989). We are fortunate to have a vast area of inland water resources such as rivers, beels, canals, ponds and estuaries, from where we get over 72% of total fish production. But the fish production in inland waters decreasing rapidly due to over exploitation of fish resources, adverse effect of flood control structures on the fish habitat, filling of rivers bottom by silt, indiscriminate fishing, and use of over dosage of fertilizers, chemicals and insecticides in agricultural lands, and discharge of industrial pollutant in waterways etc.

In many areas of the country, between seven and nine species of carps of both exotic and native origin are being stocked in the hope of enhancing pond production. Fish farmers are often disappointed at the harvesting time when they find that their most valuable fish like catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhina cirrhosus*), have not grown well and sometimes do not even attain marketable size. Chinese carps like silver carp grows fast, but often face a significantly lower market price than either catla or rohu. In recent studies, Dewan *et al.* (1991), Wahab *et al.* (1991) and Wahab and Ahmed (1992) have clearly indicated that dietary overlap between silver carp and native species, catla and rohu was very high, and the growth and production of the later ones are significantly reduced in polyculture. So an evaluation of other exotic fish with native species based polyculture should be made to understand their effects on the pond

ecology and growth of fish in polyculture. In Israel, Hepher *et al.* (1989) observed fish-fish and fish-environment relationships by stocking common carp and silver carp together in polyculture. Such studies with common carp and native carp for the development of a sustainable polyculture in Bangladesh are long overdue.

Keeping the above facts in mind, the present experiment was undertaken to study the interaction and growth performances of Indian major carps *viz.* catla (*Catla catla*) and mrigal (*Cirrhina cirrhosus*) with exotic carps *viz.* silver carps (*Hypophthalmichthys molitrix*) and common carp (*Cyprinus carpio*).

Materials and methods

Study area

The experiment was carried out in eight experimental ponds, situated at the northern side of the Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh.

Preparation of ponds

Embankment of ponds were first repaired and all the aquatic weeds were eradicated manually and mechanically. Then rotenone (20 g/40m^2) was applied to kill all the types of unwanted aquatic organisms including insects and predators. The ponds were sun dried for 10 days, and then limed at the rate of 1.0 kg/40m^2. Cowdung was also used at the rate of 8.0 kg/40m^2. The lime and cowdung were mixed thoroughly with pond bottom soil. The ponds were serially numbered from 1 to 8 for convenience of study.

Pond facilities

The size of the ponds used in this experiment was not equal (Table 1). It varied from 56 to 116 m^2. The water depth was maintained to a maximum of 1.50 m using PVC and overflow pipes on the bank fixed at 1.50 m above the pond bottom, so that excess water could be drained out.

Table 1. Treatments, area of ponds and number of different fishes released

Treatments	Pond number	Area (m^2)	Stocking ratio/pond (1:1/40m^2)
T_1	1	116	Silver carp – 87, Mrigal – 87
	2	101	Silver carp – 76, Mrigal – 76
T_2	3	98	Silver carp – 75, Common carp – 75
	4	56	Silver carp – 42, Common carp – 42
T_3	5	103	Catla – 77, Common carp – 77
	6	112	Catla – 84, Common carp – 84
T_4	7	103	Silver carp – 77, Common carp – 77
	8	56	Silver carp – 42, Common carp – 42

Collection and preparation of feed

Good quality rice bran, wheat bran and soybean oil were used as feed ingredients in this experiment, which were collected from the local market of Mymensingh town. The ingredients were ground into fine particles and then sieved through a sieve of 0.1 mm mesh. All the ingredients were analyzed for their proximate composition and the results are shown in Table 2. The required amount of wheat bran and rice bran were mixed thoroughly with soybean oil at the ratio of 4:4:1. Then the dough were prepared adding certain amount of water. From these dough, several small balls were made before throwing into the pond to feed the fish. The proximate composition of the experimental diet is shown in Table 3.

Table 2. Proximate composition of feed ingredients used in the experiment (% dry matter basis)

Ingredients	Dry matter	Crude protein	Crude lipid	Ash	Crude fibre	NFE[1]
Rice bran	90.44	12.40	8.82	10.48	16.34	51.96
Wheat bran	89.24	14.24	5.60	6.24	15.26	58.66

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

Table 3. Proximate composition and cost of experimental diet used in different treatments

Components	Diet (%)
Dry matter	90.10
Moisture	9.90
Protein	13.80
Lipid	9.08
Ash	9.15
NFE[1]	57.25
Cost (Tk/kg)	5.00

Experimental Design and Procedure

Different species of fish of different feeding habits were stocked to record their interactions, which ultimately focused in respect to feed utilization and their growth. Four species of carps *viz.* catla (*C. catla*), mrigal (*C. cirrhosus*), silver carp (*H. molitrix*) and common carp (*C. carpio*) were used as experimental species in the polyculture system. Ponds were divided into four treatments *viz.* T_1, T_2, T_3 and T_4, each having two replicates. Each treatment was provided with fishes of two species. Complete layout of the experimental system is shown in Table 1. The mean initial weight of catla, mrigal, silver carp and common carp were 1.90, 5.80, 2.20 and 5.81g, respectively. In all the treatments two species were released at the ratio of 1:1. In treatment 1, silver carp and mrigal in ponds 1 and 2, in treatment 2, silver carp and common carp in ponds 3 and 4, in treatment 3, catla and common carp in ponds 5 and 6, and in treatment 4, silver carp and common carp in ponds 7 and 8 were released at the rate of 15,000 fry/ha.

The supplemental feed weighing 5% of total fish body weight was provided two times a day, at 9.00 am and 5.00 pm in all the treatments. For every day feeding, the total amount of feed was divided into two equal volume and each half was applied to the pond as small dough in three places.

Sampling

Fortnight sampling was done randomly using a seine net to observe the fish growth, health condition and also to adjust the feeding rate. Growth of fish in each sampling was measured by weighing the fish using top pan balance. At the end of the experiment, fishes were harvested by seine net followed by draining out of pond water.

Water quality parameters

Some selected important parameters such as temperature, dissolved oxygen and pH of water in the ponds were recorded between 9.00 – 10.00 am in the morning during the study. The temperature and dissolved oxygen of the pond water were determined by DO meter (YSI 58) and pH was recorded by pH meter (Jenway 3020).

Results and discussion

The supplemental feed provides around 20% protein (Table 2) and rest of the protein requirement of fish is supplemented by the live food grown during culture. Fishes of two different species were released in this polyculture system assuming efficient utilization of pond resources, distribution of grazing pressure among feeding niches and levels, and feed wastes from one species can be fed by another (Milstein 1990, Islam *et al.* 1993).

Water quality recorded just before stocking fish and during experiment were within the acceptable ranges for fish culture that agrees with the findings of Islam *et al.* (1993). Water temperature was favourable for the culture system during the experiment. Water transparency was decreased after release of fish fry in the ponds, which might be due to growth of phytoplankton, and little turbidity due to fish movement. Dissolved oxygen and pH were decreased due to decomposition of organic matter after use of feed and other organic matter in the ponds and respiration of fish (Wahab *et al.* 1995).

The results of the present study in the cases of treatments 1 and 2 showed that the growth performance of silver carp was better when cultured with mrigal than cultured with common carp (Table 4). Though both mrigal and common carp are occupants of benthic niche of the ponds, but their growth varied which might be due to species variation, availability of food according to choice and feeding frequency. Similar results observed in the cases of treatments 3 and 4 (Table 5). But the growth of catla in treatment 3 was slightly higher than the same in treatment 4. However, the variations of fish growth were due to the interactions among species (Hepher *et al.* 1989, Dewan *et al.* 1991). In addition, it may be explained that the bottom feeders of benthic niches might have eaten away excess detritus from the pond as food which ultimately improved the environment for herbivorous fishes like catla and silver carp (Islam *et al.* 1993). Again,

mrigal and common carp stirred up the mud which helped recirculation of nutrients from bottom into the water column and enhanced the development of phytoplankton and increased algal production - the major food for catla and silver carp (Milstein 1995a and b, Islam *et al.* 1993). In treatments 3 and 4, total biomass of fishes were not increased satisfactorily. The combination of catla and common carp, and catla and mrigal gave almost similar results. It might be due to scarcity of enough food and algae, which agrees with the findings of Dewan *et al.* (1991).

Table 4. Weight of different fish species in Treatments 1 and 2 in various sampling dates

	Treatment 1				Treatment 2			
Pond	Pond 1		Pond 2		Pond 3		Pond 4	
Fish species	H.M.	C.M.	H.M.	C.M	H.M.	C.C1	H.M.	C.C1
Initial Wt.	2.20	5.80	2.20	5.80	1.90	5.80	1.90	5.80
	±0.2	±0.40	±0.20	±0.40	±0.25	±0.40	±0.25	±0.40
Sampling date	13.64	13.60	14.41	13.50	27.30	16.03	9.51	15.14
15 days	±0.84	±1.52	±2.56	±1.14	±2.68	±1.35	±1.32	±1.59
30 days	40.47	19.79	44.05	16.03	70.13	36.55	33.12	34.70
	±5.16	±2.97	±4.13	±1.85	±1.07	±3.80	±3.73	±2.65
45 days	61.80	27.01	71.37	24.57	80.62	60.61	51.56	17.09
	±2.31	±3.00	±6.01	±3.90	±5.83	±5.62	±2.69	±1.92
60 days	75.37	39.35	97.20	41.80	122.20	59.25	83.00	39.00
	±6.65	±1.95	±5.67	±3.93	±7.5	±3.58	±3.82	±3.37
75 days	160.00	63.20	153.57	40.00	152.00	92.80	125.16	49.80
	±9.63	±6.95	±7.18	±5.42	± 7.2	±4.01	±4.63	±2.28
90 days	165.51	68.15	183.18	62.93	154.67	95.32	172.29	126.11
	±10.57	±5.86	±11.5	±8.71	± 7.9	±7.17	± 8.13	±11.41

H.M.= *Hypophthalmichthys molitrix*, C.M.= *Cirrhina cirrhosus*, C.C1= *Cyprinus carpio*

Table 5. Weight of different fish species of treatments 3 and 4 in various sampling dates

	Treatment 3				Treatment 4			
Pond	Pond 5		Pond 6		Pond 7		Pond 8	
Fish species	C.C.	C.C1.	C.C.	C.C1	C.C.	C.M	C.C.	C.M.
Initial Wt.	1.90	8.50	1.90	8.50	1.90	5.80	1.90	5.80
	± 0.25	± 0.40	±0.40	±0.40	±0.25	±0.40	±0.25	±0.40
Sampling date	13.92	19.64	13.93	18.78	12.96	12.09	6.18	7.59
15 days	±1.81	±1.33	±0.71	±1.47	±4.33	±1.78	±1.14	±2.11
30 days	35.11	17.41	25.42	18.92	23.21	30.05	16.47	14.21
	±3.80	±0.62	±3.02	±2.05	±3.05	±2.01	±1.78	±5.12
45 days	43.87	26.72	37.71	31.37	38.60	30.82	24.39	35.46
	± 6.56	±3.23	±3.66	±3.17	±4.02	±2.84	±5.01	±4.83
60 days	63.50	37.60	51.40	49.33	42.00	42.77	47.00	38.75
	± 4.57	±4.52	±2.19	±5.26	±6.05	±4.20	±5.35	±4.50
75 days	78.20	37.00	71.00	53.33	76.00	70.83	60.88	62.00
	±5.69	±4.09	±3.46	±5.66	±2.00	±3.18	±0.43	±2.46
90 days	81.38	52.27	67.75	61.39	79.64	84.40	80.57	93.14
	±6.77	±10.11	±0.72	±5.33	±3.91	±6.77	±1.51	±7.63

H.M.= *Hypophthalmichthys molitrix*, C.M.= *Cirrhina cirrhosus*, C.C.= *Catla catla*, C.C1= *Cyprinus carpio*

From the results, it is concluded that the combination of silver carp and mrigal, and the combination of silver carp and common carp gave better growth and production than other two combinations.

References

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

Hepher, B., A. Milstein, H. Leventer and B. Teltsch, 1989. The effect of fish density and species combination on growth and utilization of natural food in ponds. *Aquacult. Fish. Managt.*, 20: 59-71.

Islam, M.M., M. Das and S. Dewan, 1993. Effects of supplementary feed on the grass carp (*Ctenopharyngodon idella*). *Bangladesh J. Agric. Sci.*, 20(2): 323-329.

Horwitz, W. (ed.), 1984. Official Methods of Analysis of the Association of Official Analytical Chemists (14th edn.). Association of Official Analytical Chemists, Washington D.C., USA, 1018 pp.

Milstein, A., 1990. Fish species interactions. EIFAC/FAO symposium on Production enhancement in still water pond culture. 15-18 May 1990. Prague, Czechoslovakia.

Milstein, A., B. Hepher and B. Teltsch, 1995a. Principal component analysis of interactions between fish species and ecological conditions in fish ponds: I Phytoplankton. *Aquacult. Fish. Manag.*, 20: 59-71.

Milstein, A., B. Hepher and B. Teltsch, 1995b. Principal component analysis of interactions between fish species and ecological conditions in fish ponds: II Zooplankton. *Aquacult. Fish. Manag.*, 16: 319-330.

Rahman, A.K.A., 1989. The Freshwater Fishes of Bangladesh. Zool. Soc. Bangladesh, Dhaka. 352 pp.

Tripathi, S.D., D.N. Mishra, 1986. Synergistic approach in carp polyculture with grass carp as a major component. *Aquaculture*, 54(1-2): 157-160.

Wahab, M.A., M. Begum and Z.F. Ahmed, 1991. The effects of silver carp introduction I the polyculture of major Indian carps. *Proc. BAU Res. Prog.*, 5: 429-437.

Wahab, M.A. and Z.F. Ahmed, 1992. The effects of planktivorous carps species combination on food organisms and electivity indices in the fish ponds. *BAU Res. Prog.*, 6: 427-437.

Wahab, M.A., Z.F. Ahmed, M.A. Islam, M.S. Huq, S.M. Rahmatullah, S.J. de-Groot and R.J. Roberts, 1995. Effects of introduction of common carp, *Cyprinus carpio* (L) on the pond ecology and growth of fish in polyculture. *Aquaculture Res.*, 26(9): 619-628.

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Use of crude salt in rearing of freshwater giant prawn, *Macrobrachium rosenbergii* (de Man) larvae

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Abstract
Three different types of culture media: (i) 100% brine (B_{100}), (ii) 75% brine and 25% crude salt ($B_{75}CS_{25}$), and 50% brine and 50% crude salt ($B_{50}CS_{50}$) were tested to evaluate the possible use of brackishwater reconstituted from the crude salt for the production of *M. rosenbergii* post-larvae. The production rate of 25.26±0.20 Pl/ℓ with a corresponding survival rate of 84.20±0.66% was significantly higher ($P<0.05$) for the larvae reared on B_{100} than that of 22.10±0.57 Pl/ℓ with a corresponding survival rate of 73.68±1.89% on $B_{50}CS_{50}$. Larvae cultured on $B_{75}CS_{25}$ did not show any significant difference ($P<0.05$) in production as well as in survival of post-larvae than that on B_{100}. The result shows that, for rearing of prawn larvae, use of brine can be replaced up to 25% without any undue reduction in production of post-larvae. However, the production as well as survival rate of post-larvae with 50% replacement ($B_{50}CS_{50}$) is also appreciable. It is assumed that the mineral constituents of natural seawater might have some triggering effects on prawn larvae in closing their larval cycle.

Key words: *M. rosenbergii*, Larvae, Crude salt

Introduction

The life cycle of giant freshwater prawn, *Macrobracium rosenbergii* is being completed both in fresh- and brackish-water. The prawn larvae require 12-14 ppt brackishwater for the development of different larval stages to post-larvae. Larvae developed in brackishwater areas migrate to freshwater, shortly after metamorphosis to the post-larval stage, where they grow and get matured.

Since the breakthrough in closing *M. rosenbergii* larval cycle in captivity, this is being practiced with the conventional techniques (Ling 1969), where brackishwater of about 12 ppt is prepared diluting the seawater. The requirement of seawater for production of *Macrobrachium* larvae restricts the establishment of prawn hatcheries along with the seashore and/or coastal belt, limiting sufficient and easy supply of post-larvae for stocking upper inland freshwater ponds. Though establishing prawn

hatcheries in inland areas may solve such problem, there is difficulty and added cost in trucking seawater from its source to the inland hatcheries.

Now a day, instead of seawater, trucking of concentrated brine solution from the coastal salt beds and its use in rearing of *M. rosenbergii* larvae either in large or in small backyard hatcheries have been found economical and effective (Yambot and Vera Cruz 1986, Pramanik and Halder1996). However, collection as well as transportation of brine solution from the salt pens to inland regions far away from of coastal areas is again a problem. This bottleneck may be minimized if the *M. rosenbergii* larval rearing medium can be instantly prepared using salt. Though Yambot and Vera Cruz (1986) made conjectures about the use of brackishwater reconstituted from sea salt in production of *M. rosenbergii* larvae, there has been no any thorough investigation on it so far. The purpose of the present experiment was to find out the effects of brackishwater reconstituted from crude salt and its different combinations with diluted brine solution in rearing of *M. rosenbergii* larvae in captive conditions.

Materials and methods

Three treatments having different compositions of brine and crude salt water (Table 1) were tested in rearing of *M. rosenbergii* larvae. Each treatment had three replications and assigned into a completely randomized design.

Table 1. Compositions of brine and crude salt solution used in rearing of *M. rosenbergii* larvae

Treatments	Notations	% composition	
		Brine solution	Crude salt solution
I	B_{100}	100	-
II	$B_{75}CS_{25}$	75	25
III	$B_{50}CS_{50}$	50	50

Concentrated brine (=100 ppt) was collected from a salt pan of Cox's Bazar, the south-eastern coastal district of Bangladesh, while crude salt were collected form a salt refinery factory located in Narayangonj district and transported to the prawn hatchery at Freshwater Station (FS) of Bangladesh Fisheries Research Institute (BFRI), Mymensingh. The crude salt was diluted with underground freshwater and left for overnight to settle. The diluted clear supernatant crude salt solution was pumped into a fiberglass tank. The brine and crude salt water was then diluted separately to bring the salinity level at 12 ppt and kept under vigorous aeration a period of 24-h. Prior to subsequent use of prepared brackishwater media, the aeration was stopped and allowed the suspended materials to be settled down. The supernatant brine and crude salt water was then pumped into nine rectangular fiberglass tanks (100 cm x 75 cm x 65 cm) at the required volume for the preparation of 300ℓ larviculture medium of different test compositions (Table 1). The larval rearing tanks were placed under semi-transparent roofing and provided with constant aeration. The salinity of larviculture water was

maintained at 12 ppt throughout the experimental period. A fresh stock of both brine and crude salt-water medium of 12 ppt salinity was maintained for exchange of larviculture water during the course of experiment.

Female prawn bearing gray coloured egg were collected from brood ponds of FS pond complex and kept in the fiberglass tank containing brackishwater of 6 ppt salinity. To obtain a larval batch with synchronized development, larvae from a single overnight spawning were stocked randomly into each larviculture tank at the density of 30 larvae/ℓ of culture medium.

Larvae in all the treatments were fed, up to the 4th day of rearing, with newly hatched *Artemia*, maintaining an approximate constant concentration of 3 nauplii per ml of culture medium. Afterwards larvae were fed with egg custard four times a day at 08.30, 11.30, 14.30 and 17.00 h followed by *Artemia* naupli at 18.00 h. Prior to every *Artemia* feeding, aeration was stopped and uneaten food particles were siphoned out. Egg custard was prepared according to the method given by Ang and Cheah (1986). Approximately 10 g powdered milk, 5 ml water and a whole chicken egg were blended. Ten drops of red food colouring (Bush Boaken Allen London E17 5 QP) were mixed and steamed for about 10-15 minutes. The prepared egg custard was stored in a refrigerator for not more than three days. Prior to daily feeding, the egg custard was passed through sieves (0.225- 0.600 μm Endecotts BS410) to obtain an appropriate particle size for the growing larvae. The particle size of egg custard used for feeding larvae at different stages is given in Table 2.

Table 2. Particle size of egg custard for feeding *M. rosenbergii* larvae

Stage of larvae	Size of food particle (mm)
II - IV	0.23
V - VIII	0.43
IX – post-larvae	0.60

About 25-50% of the total water volume of each larval rearing tank was siphoned out once in every 72 hrs and gradually replaced with fresh medium to maintain the volume of 30ℓ. Any fluctuation in salinity level and temperature of both fresh and larval rearing media was minimized at the time of each exchange. Ammonia-nitrogen (NH_3-N), pH, temperature and salinity of larviculture water were monitored periodically using LaMotte Ammonia Test Kit, pocket pH Meter (pH Scan 1™), maximum-minimum centigrade thermometer and ATAGO S/Mill Refractometer (8810), respectively.

The effectiveness of different test larviculture media was assessed on the basis of progressive development in larval stages and production of post-larvae at the termination of the experiment. Ten larvae from each tank were randomly sampled daily for the first week, at every alternate day for the second week and every third day for the rest of the experimental period. The larval stages were identified under a binocular microscope following the descriptions given by Uno and Kwon (1969). Progressive

development of larvae was determined by calculating the mean larval stage (MLS) at each sampling day using the following formula given by Lovett and Felder (1988):

$MLS = \sum (SxP_S)$

where, S is the larval stage number, and

P_S is the proportion of larvae at stage S.

The experiment was terminated when more than 95% of the larvae in all tanks metamorphosed to post-larvae. At the termination, total number of post-larvae was counted directly to determine the production rate. Post-larvae (n = 25) were randomly taken from each replicate tank to measure individual total length (from the tip of the rostrum to the end of the telson) and wet body weight.

Data were analyzed by one-way ANOVA using SAS linear model procedures (SAS, 1985). Differences among means were analyzed for significance using Duncan's Multiple Range Test (DMRT) at the 5% probability level. Percentage data were normalized by arcsine transformations (Zar 1984) prior to statistical analysis.

Results

Production of *M. rosenbergii* post-larvae (Pl) under various brine replacement treatments is presented in Table 3. The production rate of 25.26±0.20 Pl/ℓ with a corresponding survival of 84.20±0.66% obtained for larvae cultured on the 100% brine solution (B_{100}) was the highest, but not significantly different ($P<0.05$) to that of 24.49±0.71 Pl/ℓ with a corresponding survival rate of 81.68±2.36% for larvae cultured on 75% brine plus 25% crude salt solution ($B_{75}CS_{25}$). In contrast, the treatment 50% brine plus 50% crude salt solution ($B_{50}CS_{50}$) resulted in significantly lower ($P<0.05$) production of 22.10±0.57 Pl/ℓ. The results indicate that 25% of brine water could be replaced with crude salt solution without any undue reduction than in production rate of post-larvae with brine alone.

Table 3. Production of *M. rosenbergii* post-larvae reared on different combinations of brine and crude salt solution for a period of 33 days

Treatments	Production of post-larvae (mean±SD)[1]						
	Larvae stocked		Total post-larvae	Post-larvae/ℓ	% Survival	Growth of post-larvae	
	Total	Per liter				Length (mm)	Weight (mg)
B_{100}	9000	30	7578±59.02[a]	25.26±0.20[a]	84.20±0.66[a]	9.39±0.52[a]	8.84±0.49[a]
$B_{75}CS_{25}$	9000	30	7351±212.78[a]	24.49±0.71[a]	81.68±2.36[a]	9.32±0.42[a]	8.40±0.39[a]
$B_{50}CS_{50}$	9000	30	6630±170.23[b]	22.10±0.57[b]	73.68±1.89[b]	8.84±0.48[a]	8.20±0.43[a]
			F=26.19	*F=26.26*	*F=26.16*	*F=0.25*	*F=7.29*

[1]Values not sharing common superscript letter are significantly different ($P<0.05$).

The development of the larvae, expressed as the mean larval stage (MLS) is presented in Table 4. The MLSs were similar ($P<0.05$) with B_{100}, $B_{75}CS_{25}$, $B_{50}CS_{50}$ up to the day 6 of the rearing period, showing mean larval stages of 3.37±0.03, 3.23±0.15 and

Table 4. Mean larval stages of *M. rosenbergii* larvae under different combinations of brine and crude salt solutions[1]

Treatments		Mean larval stages													
		Elapsed days													
		2	3	4	5	6	7	9	11	13	16	19	22	25	28
B_{100}	Mean	1.57^{b}	2.13^{a}	2.48^{a}	2.97^{a}	3.37^{a}	3.93^{c}	4.60^{b}	5.42^{b}	6.33^{b}	7.41^{b}	9.00^{b}	10.17^{a}	11.22^{a}	11.77^{a}
	SD	0.06	0.06	0.10	0.03	0.03	0.15	0.20	0.19	0.15	0.09	0.10	0.21	0.04	0.06
$B_{75}CS_{25}$	Mean	1.50^{ab}	2.08^{a}	2.47^{a}	2.83^{a}	3.23^{a}	3.70^{b}	4.40^{b}	5.33^{b}	6.33^{b}	7.37^{b}	8.97^{b}	10.07^{a}	11.07^{a}	11.70^{a}
	SD	0.05	0.10	0.12	0.12	0.15	0.10	0.10	0.06	0.15	0.25	0.15	0.12	0.15	0.10
$B_{50}CS_{50}$	Mean	1.40^{a}	2.05^{a}	2.40^{a}	2.87^{a}	3.30^{a}	3.47^{a}	4.20^{a}	5.00^{a}	6.03^{a}	7.03^{a}	8.57^{a}	9.93^{a}	10.93^{a}	11.63^{a}
	SD	0.10	0.13	0.07	0.25	0.17	0.02	0.10	0.17	0.06	0.06	0.15	0.12	0.15	0.12

[1]Mean values in each column not sharing a common superscript letter are significantly different ($P<0.05$).

3.30±0.17, respectively. From the day 7 to day 19, the MLS values were significantly lower with the treatment $B_{50}CS_{50}$ but afterwards those were similar ($P<0.05$) ranging from 10.17 to 11.77, 10.07 to 11.70, 9.93 to 11.63 for the treatments B_{100}, $B_{75}CS_{25}$, and $B_{50}CS_{50}$ respectively. The overall MLS data indicate that the development of larvae reared in 100% of brine (B_{100}) and in 75% of brine and 25% of crude salt ($B_{75}CS_{25}$) was apparently faster throughout the experimental period than that of larvae reared in 50:50 parts of brine and crude salt ($B_{50}CS_{50}$). On the 7th day of hatching, the treatment $B_{50}CS_{50}$ resulted in the highest of 60% larvae of stage III and the lowest of 5% of stage V (Fig. 1). Similarly, on the 16th day, the treatment $B_{50}CS_{50}$ also resulted in the highest percent composition of larvae at stages VI and VII, but the lowest of that at stage VIII. However, on that 25th day of hatching, the percent composition of stages in larvae reared in 50:50 brine and crude salt solution was quite comparable with that of larvae reared in other two test media (Fig. 1).

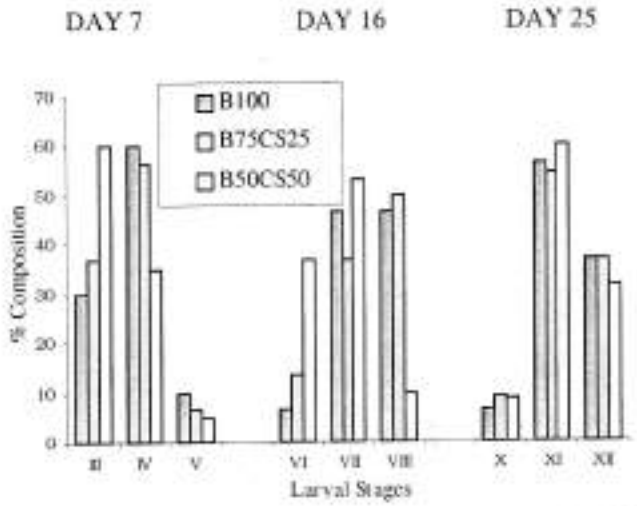

Fig. 1. Percent composition of *M. rosenbergii* larval stages on day 7, 16 and 25 post-hatch under different combinations of brine and salt solutions.

Discussion

A preliminary experiment was conducted to evaluate the possibilities of using (i) 100% crude salt (CS_{100}), (ii) 75% crude salt and 25% brine ($CS_{75}B_{25}$), and (iii) 50% crude salt and 50% brine ($CS_{50}B_{50}$) in rearing of *M. rosenbergii* larvae. The larval rearing technique was similar, as followed in the present experiment. The percentage survival rates of larvae to post-larvae under different treatments used in the preliminary study are given in Table 5. No larvae in 100% crude salt metamorphosed to post-larvae. They started to die from the day 5, the mortality rate increased gradually and all were died at

day 12. The larvae were found to stop taking food, become whitish, and to aggregate at the bottom of the tank from the early days of rearing period. With the culture medium of 75% crude salt and 25% brine, only 7.05% of stocked larvae metamorphosed to post-larvae. However, 50% crude salt and 50% brine resulted in a quite significant rate of survival (57.85%) of larvae to post-larvae. The result of the preliminary study indicated that neither the 100% crude salt water nor its 25% of replacement with brine could be used in hatchery production of *M. rosenbergii* post-larvae. A similar opinion was made by Yambot and Vera Cruz (1986), though they reported the survival rate of *M. rosenbergii* post-larvae ranging from 2.88% to 11.48% with an average of 6.71%, while they used a combination of sea salt, deionized freshwater and *Chlorella* culture (greenwater). Probably this percentage of survival rate was obtained due to an advantageous use of greenwater in larviculture (Cheah and Ang 1979). Based on the results of the preliminary study, the present experiment was designed, keeping in mind that 50% of brine could be replaced with crude salt solution in *M. rosenbergii* hatchery operation.

Table 5. Percentage survival of *M. rosenbergii* from larvae to post-larvae in the preliminary trial under different combinations of crude salt and brine solution

Treatments	Survival (%)			Treatment mean (%)
	Replication-1	Replication-2	Replication-3	
CS_{100}	-	-	-	-
$CS_{75}B_{25}$	4.37	10.65	6.14	7.05
$CS_{50}B_{50}$	43.58	71.13	58.83	57.85

The temperature, pH and NH_3-N varied from 26 to 33°C, 7.8 to 8.6 and 0.06 to 0.12 mg/ℓ, respectively and were similar in all larval rearing tank throughout the experimental period. Water quality parameter levels were within the optimum range for rearing of *M. rosenbergii* larvae in captivity (New and Singholka 1985, Ang and Cheah 1986). The production data of *M. rosenbergii* larvae obtained under different combinations of brine and crude salt (Table 3) reveal that 25% of brine solution could be replaced with crude salt solution without any undue reduction in per unit yield of *M. rosenbergii* post-larvae. Though the average production of 22.10±0.57 Pl/ℓ with 50% replacement of brine (T-III) was significantly lower, but higher than that of 11.93 Pl/ℓ (Islam and Khan 1990), 10.22 Pl/ℓ (Adisukresno *et al.* 1982), 9.5 – 18.9 Pl/ℓ (Lee, 1982), 7.56 Pl/ℓ (Yambot and Vera Cruz 1986), while the authors used seawater either in static or in closed recirculatory system. Pramanik and Halder (1996), who used 100% of brine solution in a closed recirculatory backyard hatchery system, reported a production rate of 25 Pl/ℓ at a stocking density of 40 larvae/ℓ. This production rate is comparable with 22.10±0.57 – 25.26±0.20 Pl/ℓ obtained in the present experiment with either 100% brine or with different combinations of brine and crude salt solution (Table 3). It indicates that the either brine alone or up to 50% replacement of brine with crude salt solution can be used as suitable as of using seawater in rearing of *M. rosenbergii* larvae.

The results of our preliminary study and that of Yambot and Vera Cruz (1986) indicate that the sea salt alone cannot be used in rearing of *M. rosenbergii* larvae up to post-larvae. Similar to that observed in the preliminary study, Yambot and Vera Cruz (1986) recorded a substantial mortality of larvae within five days after stocking. Though the authors suspected improper acclimatization of larvae prior to stocking as one cause of larval mortalities with sea salt alone, but this might not be so. It is presumed that as *M. rosenbergii* is apparently evolving "out of the sea" (Johnson 1960), as it requires a salinity of 10-14‰ in closing the larval cycle (Ling 1962) . Therefore, it is plausible that the complex nutrient and mineral constituents of the natural seawater regulate the physiological growth and survival of larvae. The ions that make up the salt content of natural seawater are shown in Table 6.

Table 6. Constituents of seawater (after Ingmanson and Wallace 1985)

Substrate	Symbol	‰ seawater	% total weight of salt
Chloride	Cl^-	18.980	55.04
Sodium	Na^+	10.556	30.61
Sulphate	SO_4^{-2}	2.649	7.68
Magnesium	Mg^{+2}	1.272	3.69
Calcium	Ca^{+2}	0.400	1.16
Potassium	K^+	0.380	1.10
Bicarbonate	HCO_3^-	0.140	0.41
Bromide	Br^-	0.065	0.19
Boric acid	H_3BO_3	0.026	0.07
Stornium	Sr^{+2}	0.013	0.04
Fluride	F^-	0.001	0.00
Total		34.482‰	99.99%

The salt in seawater is not the same as in regular table salt. When table salt (NaCl) dissolved in water, it breaks up into Na^+ and Cl^- ions with an equal amounts of positive and negative ions (Ingmanson and Wallace 1985). Therefore, most of the ions except Cl^- and Na^+ are lost in the process of preparation salt from seawater. This non-presence of a number trace minerals in brackishwater reconstituted by salt might be a cause of not supporting the normal survival and growth of *M. rosenbergii* larvae. The production data of prawn post-larvae in the present experiment of supplementing salt made culture media with 50% of brine (naturally concentrated seawater) further prove that the ionic constituents of natural seawater might have significant triggering effects on growth and survival of *M. rosenbergii* larvae.

It is interesting to note that in the early rearing period, with a mean larval stage of 3.23 – 3.37 at day 6, the growth of larvae were not significantly affected by 50% supplement of salt media with brine (Table 4). However, from day 7 to 19, the larvae reared on $CS_{50}B_{50}$ showed significantly lower development in terms of MLSs (Table 4). Fig. 1 shows that on day 7 and 16, the highest number of larvae were at stage III and VII, respectively. The progressive larval development (Table 3) and occurrence of larvae at

different stages (Fig. 1) towards the end of the rearing period for the treatment $CS_{50}B_{50}$ are more or less similar to that for treatments B_{100} and $B_{75}CS_{25}$. These results amply demonstrate that the minerals of natural seawater not only might have some effect on growth of larvae but are also required particularly at the mid-stages of larval development.

The overall results of the study supports the findings of Yamboot and Vera Cruz (1986) and Pramanik and Halder (1996) that brine can effectively be used in rearing of *M. rosenbergii* larvae. There is also the possibility of using brackishwater reconstituted from crude salt for more economically rearing of prawn larvae. However, the crude salt media need to be replaced or supplemented by at least 50% with brine for any undue reduction in production of freshwater prawn post-larvae. The stocking density of prawn larvae in the present culture condition is also a factor to be taken into consideration. With an increasing stocking density of 40 - 60 larvae/ℓ, Pramanik and Halder (1996) reported a decreased survival rate of 63% to 47%. As a stocking density of 30/ℓ in the present experiment resulted in the survival rate of 74% to 84%, this stocking rate can be used for better production of post-larvae.

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References

Adisukresno, S., G.L. Escritor and K. Mintardio, 1982. Mass production of *Macrobrachium* post larvae in the Brackishwater Aquaculture Development Centre, Japra, Indonesia. *In*: Giant Prawn Farming (ed. M.B. New). Elsvier, New York. pp. 143-156.

Alam, M.J., S.H. Cheah and K.J. Ang, 1991. Use of coloured egg custard for larval rearing of the giant freshwater prawn *Macrobrachium rosenbergii* (de Man). *Bangladesh J. Fish.*, 13(1&2): 63-72.

Ang, K.J. and S.H. Cheah, 1986. Juvenile production of the Malaysian giant freshwater prawn (*Macrobrachium rosenbergii* de Man) using modified static modified greenwater system. Proc. Int. Conf. Dev. Manage. Trop. Living. Aquat. Resources, Serdang, Malaysia, 2-5 Aug. 1983. UPM Publ., Serdang, pp. 141-144.

Cheah, S.H. and K.J. Ang, 1979. Preliminary trials on juvenile *Macrobrachium rosenbergii* production under modified static "green water" conditions. *Pertinika*, 36: 69-71.

Ingmanson, E. and W.J. Wallace, 1985. *Oceanography: An Introduction*. Wordsworth Publishing Company, California.

Islam, K.S. and Y.S.A. Khan, 1990. Mass production of post larvae of *Macrobrachium rosenbergii* (de Man) at the Prawn Hatchery and Research Centre, Cox's Bazar, Bangladesh. *Bangladesh J. Zool.*, 18(1): 53-59.

Johnson, D.S., 1960. Some aspects of the distribution of freshwater organisms in the Indo-Pacific area and their relevance to the validity of the concept of an oriental region in zoogeography. *Proc. Cent. Bicent. Congr. Biol.*, Singapore, 2-9 Dec., 1958. Singapore, pp. 170-181.

Lee, C.L., 1982. Progress in developing standardized system for producing of juvenile *Macrobrachium rosenbergii* (de Man) at Mardi, Malacca. *In*: Giant Prawn Farming (ed. M.B. New). Elsvier, New York, NY, pp. 129-142.

Ling, S.W., 1962. Studies on the rearing of larvae and juveniles and culturing adults of *Macrobrachium rosenbergii* (de Man). *FAO/IPFC Curr. Aff. Bull.*, 35: 1-11.

Ling, S.W., 1969. Methods of rearing and culturing *Macrobrachium rosenbergii* (de Man). *FAO Fish. Rep.*, 57(3): 607-619.

Lovett, D.L. and D.L. Felder, 1988. Evaluation of the rotofer *Brachionus plicatilis* as a substitute for *Artemia* in feeding of larvae of *Macrobrachium rosenbergii* (de Man). *Aquaculture*, 71: 331-338.

New, M.B. and S. Shingholka, 1985. Freshwater Prawn Farming. A manual for the culture of *Macrobrachium rosenbergii*. *FAO Fish. Tech. Pap.*, 1/FIRI/T225, 118pp.

Pramanik, M.W.A. and G.C. Halder, 1996. Development of backyard *Macrobrachium* hatchery system and larval rearing technique in different stocking density. *J. Inland Fish. Soc. India*, 28(1): 21-27.

SAS, 1985. SAS/STAT Guide to Personal Computers Version 6, SAS Inc., Cary, NC, USA.

Uno, Y and C.S. Kwon, 1969. Larval development of *Macrobrachium rosenbergii* (de Man) reared in the laboratory. *J. Tokyo Univ. Fish.*, 55(2): 179-190.

Yambot, A.V. and E.M. Vera Cruz, 1986. Larval rearing of *Macrobrachium rosenbergii* (de Man) in brine solution and sea salt. *In*: The First Asian Fish. Forum (eds. J.L. Maclean, L.B. Dizon and L.V. Hosillos). Asian Fisheries Society, Manila, Philippines. pp, 185-188.

Zar, J.H., 1984. Biostatistical Analysis, 2nd edn. Prentice-Hall, Englewood Cliffs, NJ, 717 pp.

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Studies on the food habits of three species of Mastacembelidae

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Abstract
To identify the food habits of three species of Mastacembelidae namely *Mastacembelus armatus, Mastacembelus pancalus* and *Macrognathus aculeatus,* the gut content analysis was performed by three methods i.e. occurrence method, points method and index of fullness method. All three species were found to consume prawn, molluscs, insects, earth warm, debris and plant materials. *M. armatus* and *M. pancalus* were found to feed mainly on animal food items and 84.68% of different types of animal food were taken by *M. armatus* and 62.72% by *M. pancalus. M. aculeatus* was found to consume 44.86% of different types of animal food items, 53.51% of debris and plant materials which indicated that this fish feeds almost equally on animal and plant food. Analysis of the food habits showed that both *M. armatus* and *M. pancalus* are carnivore in nature with higher feeding preference for animal food namely prawn, crabs, fishes, molluscs etc. On the other hand, *M. aculeatus* is an omnivore in nature feeding almost equally on animal and plant food.

Key words: Food, Food habit, Mastacembelidae

Introduction

Food and feeding habit of fishes has a great significance in aquaculture practice. It helps to select such species of fishes for culture which will utilize all the available potential food of the water bodies without any competition with each other but will live in association with other fishes. This will allow the best utilization of the food sources of water body and will give an optimum yield. Food and feeding habits of fish vary with the time of day and season of the year. Food consumption of fishes is influenced directly or indirectly with changes of temperature, pH, light and dissolved oxygen of water (Keast 1968). However, analysis of stomach contents is a method for determining the food and feeding habits of fishes by which we can easily find what the fish take as food.

The freshwater eels such as *Mastacembelus pancalus* and *M. aculeatus* are also called small indigenous fish species but *M. armatus* is relatively larger than those species. These fishes are the inhabitants of rivers, canals, beels, ponds and inundated fields (Rahman 1989). These are now considered as endangered species. Considering the consumers preference, nutritional value and market preference and to preserve the biodiversity, *Mastacembelus* spp. should be protected from being extinct. However, for

developing culture technologies, biological studies of these species are indispensable. Very little attempt has been made in the country to promote their breeding and culture. Therefore, a research work was carried out to identify the food habits of *M. armatus, M. pancalus* and *M. aculeatus* to generate base-line information for facilitating the future aquaculture practice of these species.

Materials and methods

Collection and preservation of fish sample

The fishes were collected from the Brahmaputra river on several occasions. Immediately after collection, the whole alimentary canal of each fish was dissected and preserved in 10% formalin to prevent further digestion of food.

Laboratory studies and stomach analysis

In the laboratory, the stomach content (lying between the esophagus and intestine) was taken out from the preserved vial into a petridish with the help of fine forceps. The method practiced was similar to the methods followed by MaComish (1967), McKechnie and Fenner (1971) and Dewan (1973). For the qualitative and quantitative analyses of different food items eaten by fish, several methods have been used. The commonly used methods ar: i) numerical method, ii) weight method, iii) volumetric method (index of fullness method), iv) points method and v) percentage of frequency of occurrence method. In the present study percentage of frequency of occurrence method, points method and index of fullness method were used.

Counting procedure

To study the gut content each stomach was analysed separately. The stomach of individual fish was cut open and removed on to a petridish with the help of very fine forceps. The percentage of occurrence of a particular food item was calculated on the basis of the following formula:

$$\text{Percentage of occurrence of a food type} = \frac{\text{Number of gut where the food occurred}}{\text{Total number of gut analysed}} \times 100$$

To apply the points method all the food items eaten by the species were identified. Then the volume of the stomach contents of each fish was estimated by observation and recorded on an absolute scale and points were allotted to each stomach according to the volume of its contents.

The stomach with largest volume was allotted 100 points, and each of the stomachs as examined was then rated in one of the following point categories; 0, 3, 6, 12, 25, 50 and 100 points, according to volume of the food present. The categories were based on inspection and estimation of stomachs of all categories made from extra stomach and was used in relating absolute volumes to assigned point values. Then the occurrence of each

and every food item in the individual stomach was recorded and point allotted for every food item.

In case of Index of fullness method, the index of fullness of the stomach was recorded irrespective of the size of the stomach of the fish using '0' for empty; '1' for one fourth full; '2' for half full, '3' for three fourth full and '4' for full stomach.

Results

The gut contents of 45 fishes of each species of Mastacembalidae was performed by three methods namely frequency of occurrence method, points method and index of fullness method. It is well known that no single method is adequate for analysis of stomach contents of food. The total length ranges of the fishes studied were 30 to 56 cm for *M. armatus*, 10 to 16 cm for *M. pancalus* and 17 to 26 cm for *M. aculeatus*.

Types and amount of food taken by the three species

The major food items found in the stomach content analysis of 45 fishes from each species showed that the fish fed on a variety of food items. The food types recorded are prawns/shrimps, plants, small fishes, small crabs, molluscs, insects, earth worms, fish eggs, debris, soil and 'others' which include unidentified items.

Food habit in M. armatus determined by frequency of occurrence and points methods

The gut analysis in *M. armatus* (Table 1) shows that prawn is the most frequently ingested food item (obtained in 77.78% fish) followed by debris (40%), small fish and plant (each 22.22%) and then molluscs (20%) which indicated that the fish fed mainly on animal food. Some other types of food were also identified but in much less frequency, e.g. earth worm (8.89%), small crab (6.67%), insect (2.22%), fish egg (2.22%) etc.

Analysis by points method which reflects the percent contribution of a particular type of food in total volume of gut content shows (Table 1) that also prawn is the most important food type (36.58%) in *M. armatus*. Then comes sequentially the small crab (22.41%), small fish (15.80%), debris (6.31%), plant material (5.48%) and earth worm (4.72%). Table 1 also shows the feeding pattern in different size group in *M. armatus*. It shows that prawn as a food item was very important in all three size groups both according to frequency of occurrence as well as percent contribution in gut content. Occurrences of small fishes and molluscs were highest in Group III which composed of largest size fish. This probably indicates that with increase in size the fish reaches in more advantageous position to predate on or engulf these large live food.

Food habit in M. pancalus determined by frequency of occurrence and points method

Stomach content analysis by percentage of occurrence method in case of *M. pancalus* shows (Table 2) that debris is the most frequently encountered (obtained in 57.78% fish) food item followed by prawn (55.55%), plant material (40%), fish egg (22.22%), earth worm (13.33%) and small insects (11.11%) for this species.

Table 1. Relationship of size and patterns of feeding and average composition of diet of 45 fishes of *Mastacembelus armatus* based on food categories, according to percentage of occurrence and percentage of total points

Items	Percentage of occurrence				Average points per fish				Percentage of total points			
	Group I (30-36) cm	Group II (37-43) cm	Group III (44-56) cm	Average	Group I (30-36) cm	Group II (37-43) cm	Group III (44-56) cm	Average	Group I (30-36) cm	Group II (37-43) cm	Group III (44-56) cm	Average
Prawn	80.00	66.67	86.67	77.78	7.20	6.53	12.53	8.75	59.65	29.51	33.33	36.58
Earth worm	6.66	6.67	13.33	8.89	0.67	0.73	2.00	1.13	5.55	3.30	5.32	4.72
Small fish	13.33	26.67	26.67	22.22	1.87	3.07	6.40	3.78	15.49	13.87	17.03	15.80
Mollusc	13.33	20.00	26.67	20.00	0.53	0.67	2.13	1.11	4.39	3.03	5.67	4.64
Small crab	0	13.33	6.67	6.67	0.93	8.47	6.67	5.36	7.71	38.27	17.74	22.41
Insect	0	0	6.67	2.22	0	0	1.00	0.33	0	0	2.66	1.38
Plant material	0	33.33	33.33	22.22	0	1.20	2.73	1.31	0	5.42	7.26	5.48
Fish egg	0	6.67	0	2.22	0	0.33	0	0.11	0	1.50	0	0.46
Debris	20.00	40.00	60.00	40.00	0	1.13	3.40	1.51	0	5.11	9.04	6.31
Other	6.66	0	13.33	6.66	0.87	0	0.73	0.53	7.21	0	1.94	2.22

* The no. of fish in each group was 15
The average was calculated by taking in consideration the fishes of all 3 groups together

Table 2. Relationship of size and patterns of feeding and average composition of diet of 45 fishes of *Mastacembelus pancalus* based on food categories, according to percentage of occurrence and percentage of total points

Items	Percentage of occurrence				Average points per fish				Percentage of total points			
	Group I (10-11) cm	Group II (12-13) cm	Group III (14-16) cm	Average	Group I (10-11) cm	Group II (12-13) cm	Group III (14-16) cm	Average	Group I (10-11) cm	Group II (12-13) cm	Group III (14-16) cm	Average
Prawn	60.00	53.33	53.33	55.55	5.67	9.80	16.13	10.53	32.09	41.88	38.77	38.22
Earth worm	6.67	13.33	20.00	13.33	2.00	2.47	5.53	3.33	11.32	10.56	13.29	12.09
Small insect	13.33	6.67	13.33	11.11	1.07	1.00	1.40	1.16	6.06	4.27	3.37	4.21
Plant material	26.67	33.33	60.00	40.00	1.80	4.20	7.67	4.56	10.19	17.95	18.44	16.55
Fish egg	26.67	26.67	13.33	22.22	2.07	2.13	2.47	2.22	11.72	9.10	5.94	8.06
Debris	60.00	53.33	60.00	57.78	4.33	3.33	7.40	5.02	24.51	14.23	17.79	18.22
Other	26.67	13.33	20.00	20.00	0.73	0.47	1.00	0.73	4.13	2.00	2.40	2.65

* The no. of fish in each group was 15

The average was calculated by taking in consideration the fishes of all 3 groups together

According to points method the greatest amount is contributed by prawn (38.22%), followed by debris (18.22%), plant material (16.55%) and earth worm (12.09%) which is shown in Table 2. All the size groups of *M. pancalus* show almost similar type of preference for fish egg as food (Table 2). Apart from other food types Group I also shows preference for fish egg as food. Thus 'fish egg' was found in 26.67% of fish in Group I and it contributed about 11.72% of total gut content. On the contrary in only 13.33% fishes belonging to Group III took fish eggs and this food contributed only 5.94% of the gut content.

Food habit in M. aculeatus calculated by frequency of occurrence and points methods

In *M. aculeatus* debris was found in the highest number of fish (82.22%) as well. The second highest frequency of occurrence was of earth worm (37.78%) followed by plant material (26.67%) and prawn (17.78%) (Table 3).

According to points method the debris was found to be the greatest contributor to gut content as well. Earth worm was second (37.35%), followed by plant materials (12.72%) and prawn (7.51%) (Table 3). Unlike two other species 'debris' was the most predominant type of food in all three size groups of *M. aculeatus* (Table 3). The second most important food type was earth worm in all three size groups.

Relationship between fish size and feeding pattern based on average index of fullness and average points for fish

The patterns of feeding in different size groups of fishes in three species of Mastacembelidae are shown in Table 4. To determine the size and pattern of feeding, the total number of fishes- 45 of each species was divided into 3 groups; Group I, Group II and Group III.

Average index of fullness

The value of average index of fullness showed little variations in different size groups. However, a rather higher value of average index of fullness were recorded in size Group I of all the three species with values of 2.3 in *M. armatus*; 2.6 in *M. pancalus* and 1.9 in *M. aculeatus*. The lowest value of average index of fullness was recorded in size Group III for all of the three species i.e. 2 in *M. armatus*; 2.4 in *M. pancalus* and 1.7 in *M. aculeatus*. The values of size Group II was more or less similar among all three species.

Average points per fish

The values of average points per fish showed interesting variations with the increase in size of the fish. The highest values of average points per fish 38 in *M. armatus*, 42 in *M. pancalus* and 30 in *M. aculeatus* were recorded in size Group III. The lowest values of the same were recorded in size Group I. Therefore, the values of average points per fish were found to increase with the increase in size of the fish.

Table 3. Relationship of size and patterns of feeding and average composition of diet of 458 fishes of *Macrognathus aculeatus* based on food categories and according to percentage of occurrence and percentage of total points.

Items	Percentage of occurrence				Average points per fish				Percentage of total points			
	Group I (17-19) cm	Group II (20-22) cm	Group III (23-26) cm	Average	Group I (17-19) cm	Group II (20-22) cm	Group III (23-26) cm	Average	Group I (17-19) cm	Group II (20-22) cm	Group III (23-26) cm	Average
Prawn	6.67	26.67	20.00	17.78	0.20	2.93	1.80	1.64	1.56	12.82	6.03	7.51
Earth worm	33.33	33.33	46.67	37.78	4.73	7.67	12.07	8.16	36.98	33.55	40.41	37.35
Plant material	6.67	46.67	26.67	26.67	0.80	3.53	4.00	2.78	6.26	15.44	13.39	12.72
Debris	86.67	73.33	86.67	82.22	7.06	8.33	11.33	8.91	55.20	36.44	37.93	40.77
Other	0	6.67	6.67	4.45	0	0.40	0.67	0.36	0	1.75	2.24	1.65

* The no. of fish in each group was 15
The average was calculated by taking in consideration the fishes of all 3 groups together

Table 4. Relationship of size and pattern of feeding of three species of the family Mastacembelidae based on average index of fullness and average points per fish

Items	*M. armatus*			*M. pancalus*			*M. aculeatus*		
	Group I (30-36) cm	Group II (37-43) cm	Group III (44-56) cm	Group I (10-11) cm	Group II (12-13) cm	Group III (14-16) cm	Group I (17-19) cm	Group II (20-22) cm	Group III (23-26) cm
Number of fish examined	15	15	15	15	15	15	15	15	15
Number of total points	181	332	564	265	356	624	192	343	448
Average points per fish	12	22	38	18	24	42	13	23	30
Average index of fullness	2.3	2.3	2.0	2.6	2.4	2.4	1.9	1.7	1.7
Average length in cm	33.2	39.1	47.5	10.8	12.5	14.2	16.8	20.6	24.5

Discussion

Prawn was the most preferred live food items of *M. armatus* and *M. pancalus*. On the other hand, for *M. aculeatus* earth worm was the most preferred animal food type. All three species had a considerable amount of debris and plant material in their guts. *M. armatus* consumed 85.99% of different type of animal food items and 14.01% of debris, plant material and others which indicated that the fish fed mainly on animal food (Table 1). The most dominant food item of the fish was prawn (36.58%) followed by small crab (22.41%), small fish (15.80%), earth worm (4.72%), mollusc (4.64%), insect (1.38%) and fish egg (0.46%) among animal food items and debris (7.61%) followed by plant material (5.48%) and others (2.22%) among plant food.

M. pancalus consumed 62.85% of different type of animal food items and the rest 37.42% of plant material, debris and unknown which indicated that the fish fed mainly on animal food. The most dominant food item of the fish was prawn (38.22%) followed by earth worm (12.09%), fish egg (8.06%) and small insects (4.21%) among animal origin and debris (18.22%) and plant materials (16.55%) among plant origin and unknown food (2.65%).

M. aculeatus ingested 44.86% of different type of animal food items and the rest 55.14% of debris, plant materials and others which indicated that the fish fed mainly on plant food. The most dominant food item of the fish was debris (40.77%). The second dominant food item was earth worm (37.35%) followed by plant materials (12.72%), prawns (7.51%) and unknown materials (1.65%).

From the above findings it is evident that both *M. armatus* and *M. pancalus* are carnivore in nature with higher feeding preference for animal food like prawn, crab, fish, mollusc etc. However, they consistently took some plant materials and debris along with their animal food. On the other hand, *M. aculeatus* was omnivore in nature feeding almost equally on food of animal and plant origin. Dewan (1973) studied the food habit

of *M. pancalus* by percentage of occurrence method and volumetric method and supported the above findings.

The determination of food habit was also reported by Saha and Dewan (1979) for *Tilapia nilotica;* Mustafa *et al.* (1980) for *Nandus nandus*; Bisht and Das (1981) for *Puntius ticto, Cyprinus carpio, Tor tor, Nemaechilus rupicola* and *Channa gachua*; Bhuiyan and Rahman (1983) for *Channa gachua*; Nargis and Hossain (1987) for *Anabas testudineus*; Reddy and Rao (1987) for *Mystus vittatus*; Sivareddy and Rao (1989) for *Heteropneustes fossilis*; Choudhury and Thakur (1990) for *Clarias batrachus*; Ahmed *et al.* (1993) for *Nandus nandus, Mystus vittatus* and *Puntius stigma*; Bais *et al.* (1994) for *Channa punctatus*; Dutta (1994) for *Channa punctatus* and Alam (1995) for *Gudusia chapra*. They categorised these fishes either as carnivore or omnivore.

On the other hand, the values of average points per fish increased with the increase in size of fish. The highest values of average points per fish were recorded in size group III. The lowest values of the same were recorded in size group I. Thus from the results of average points per fish, it can be concluded that the amount of food in the stomachs increases with the increase in size of fishes. This might be due to increased size of the stomach as the fish increased in size. These findings agree with the findings of Dewan *et al.* (1977) in *Labeo rohita*. From the above discussion it can be concluded that both *M. armatus* and *M. pancalus* are carnivorous and *M. aculeatus* is omnivorous.

References

Ahmed, A.T.A., M.M. Rahman, G. Mustafa and M. Sanaullah, 1993. A comparative study of food and feeding habits of three species of fish from "Beel Mahmud" Faridpur. *Bangladesh J. Zool.*, 21(1): 11-21.

Alam, M.T., 1995. Food and feeding habit of chapila (*Gudusia chapra*). M.Sc. Thesis. Department of Aquaculture and Management. BAU, Mymensingh. 53pp.

Bais, V., S. Thakur and S.S. Agarwal, 1994. Food and feeding activity of *Channa punctatus* (Bloch). *Freshwater Biol.*, 6: 247-251.

Bhuiyan, A.S. and K. Rahman, 1983. Food and seasonal pattern of feeding of the freshwater snake-headed fish *Channa gachua* (Hamilton). BAAS Proceeding of the 8th Bangladesh Science Conference, Dhaka.

Bisht, R.S., S.M. Das, 1981. Observation on aquatic insects as food of fishes and the predatory action of some aquatic insects on fish and fish food. *J. Inland. Fish. Soc. India*, 13(2): 80-86.

Choudhury, L.K. and P.K. Thakur, 1990. Seasonal variations in food and feeding habits of an air-breathing catfish, *Clarias batrachus* (Linn.) of the Kosi region (North Bihar). *J. Freshwater Biol.*, 2(3): 275-283.

Dewan, S., 1973. Investigation into the ecology of fishes of Mymensingh lake. Ph.D. dissertation. Bangladesh Agril. Univ., Mymensingh, Bangladesh. 235pp.

Dewan, S., M.M. Ali and M.A. Islam, 1977. Studies on the size and pattern of feeding and fingerlings of three major carps. *viz. Labeo rohita* (Ham.), *Catla catla* (Ham.) and *Cirrhinus mrigala* (Ham.). *Bangladesh J. Agril. Sci.*, 2: 225-228.

Dutta, S.P.S., 1994. Food and feeding habits of *Channa punctatus* inhabiting Gadigarh stream, *Jammu. Freshwater Biol.*, 69: 333-336.

Keast, A., 1968. Feeding of some Great lake fishes at two temperatures. *J. Fish. Res. Bd. Canada.* 25(25): 1199-1218.

MaComish, T.S., 1967. Food habits of big mouth and small mouth buffalow in Lewis and Cark Lakes and Missouri River. *Trans. Am. Fish. Soc.*, 69: 70-74.

McKechnie, R.J. and R.B. Fenner, 1971. Food habits of white sturgeons, *Acipensor transmontanus* in San Pablo and Suisan Bays, California, *Calif. Fish Game*, 57(3): 109-212.

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

Nargis, A. and M.A. Hossain, 1987. Food and feeding habit of koi fish (*Anabas testudineus* Bloch), (Anabantidae : Perciformes). *Bangladesh J. Agri. Sci.*, 12(2): 121-127.

Rahman, A.K.A., 1989. Freshwater Fishes of Bangladesh. *Zool. Soc. Bangladesh,* Dhaka. 364pp.

Reddy, Y.S. and M.B. Rao, 1987. A note on the food of *Mystus vittatus* from the highly polluted Hussain sugar lake, Hydrabad. *Indian. J. Fish.*, 34(4): 484-487.

Saha, S.N. and S. Dewan, 1979. Food and feeding habits of *Tilapia nilotica* (Linnaeus) (Perciformes: Cichlidae). I. Types and amount of food taken by the fish and its size and pattern of feeding. *Bangladesh J. Zool.*, 7(1): 53-60.

Sivareddy, Y. and M.B. Rao, 1989. Studies on the feeding biology of an air-breathing fish *Heteropneustes fossilis* (Bloch.). *J. Indian Fish. Assoc.*, 19: 31-36.

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A comparative study on the effect of commercial fish feeds on the growth of Thai pangas, *Pangasius hypophthalmus*

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Abstract
A 70 day long experiment was carried out to evaluate three commercial pangas feeds available in Bangladesh *viz.* Quality Feeds Ltd. (QF), Aftab Bohumukhi Farm Ltd. (ABF) and Saudi-Bangla Fish Feed Ltd. (SBFF) (designated as treatment T_1, T_2 and T_3) on the growth of Pangas, *Pangasius hypophthalmus*. Each treatment had two replicates using six experimental ponds of each 80m^2 size. The stocking density was 250 fish/80 m^2. At the beginning, the fish were fed twice daily at 15% of their body weight which was gradually reduced to 10 and 6%, respectively for the rest of the period. The ranges of water quality parameters observed were: temperature 29.0°C - 35.1°C, pH 6.85 - 8.52, DO 1.71 - 7.65 mg/l and transparency or Secchi depth 14.5 - 30.0 cm. The mean weight gain of fish was significantly ($P<0.05$) higher in T_3 followed by T_2 and T_1. The specific growth rate (SGR) ranged between 4.09 and 5.06, feed conversion ratio (FCR) values between 1.54 and 1.61 with treatment T_1 showing the lowest FCR. Protein efficiency ratio (PER) values ranged between 2.03 and 2.11. The survival of fish varied between 90.4 and 91.6%. The significantly ($P<0.05$) highest production of fish (kg/ha/70 days) and profit (Tk/ha/70 days) was observe in T_3 (SBFF) followed by T_2 (ABF) and T_1 (QF), respectively. The result of the study showed that on the basis of nutritive value and growth performance of pangas, feed from Saudi-Bangla Fish Feed Ltd. is the best.

Key words: *P. hypophthalmus*, Commercial fish feed

Introduction

With the increasing demand for food fish and the decline in capture fisheries production, aquaculture in Bangladesh is heading towards intensification. This shift from low density to high density culture i.e. traditional to semi-intensive or intensive culture is consequently leading to an unprecedented rise in the demand for feeds. Farmers shift gradually from no feed, through the use of farm-made feeds, to factory-made feeds. The success of intensive and semi-intensive fish culture depends to a large extent on the application of suitable feeds. Fish feeds provide nutrients for optimum fish growth and bring higher economic return to farmers. Fish production as high as 3,700-4,500 kg/ha could be obtained by using semi-intensive polycultue in ponds with supplementary feeding. This demonstrates a real possibility of increasing production

and reveals the potential importance of aquafeeds in Bangladesh (Zaher and Mazid 1993). At present, there are about 25 commercial fish feed industries in Bangladesh. Saudi-Bangla Fish Feed Ltd., Aftab Bohumukhi Farm Ltd., Quality Feeds Ltd. are among the pioneers whose feeds available throughout the country (Pers. Comm. Manager, SBFF).

Feed costs generally constitute the highest single operation cost of semi-intensive or intensive grow-out farming operation (Shang and Costa-pierce 1983). It is essential that the feed provides maximum production efficiency at a minimum cost. The relative importance of growth rate and feed conversion efficiency will depend upon the quality and cost of feed in relation to the market value of the farmed product. The unit cost of various types of feed and cost of fish production using each of this feed as well as the unit profitability of each system of fish production must be compared before one type of feed is selected. It is therefore of great importance to the fish farmers to utilize their investments in feed as optimal as possible.

Thai pangas (*P. hypophthalmus*) is an indigenous fish species of Thailand (Roberts and Vidthayanon 1991). It was introduced in Bangladesh from Thailand in 1989 is particularly important for their fast growth, lucrative size, good taste and high market demand. The species can also be stocked at a much higher density in ponds compared to other culturable species. Tavarutmanegul *et al.* (1979) reported that *Pangasius sutchi* is one of the most suitable catfishes for rearing in ponds and cages (floating ponds).

Only few years back *P. hypophthalmus* was a popular table fish in our country and farmers were economically benefited from pangas farming. But recent years, pangas culture is being depleted because of decreasing market value, increasing feed cost, decreasing feed quality, unavailability of low cost supplementary feeds, lack of proper management and related socio-economic constraints. As government has no legislation over control of feed quality and cost, there is a great possibility that the farmers may be deceived by using the commercial feeds without knowing their nutritive values. Therefore, the present study was undertaken to observe the growth and feed utilization of *P. hypophthalmus* using three different commercial fish feeds available in the market so that the best commercial feed for pangas will be known.

Materials and methods

Three most commonly used Pangas feeds from Quality Feeds Ltd. (QF), Aftab Bohumukhi Farm Ltd. (ABF) and Saudi-Bangla Fish Feed Ltd. (SBFF) were collected from local Mymensingh market. Three categories of feeds e.g. nursery, starter and grower/finisher for QF and ABF Feed and starter-I, starter-II, starter-III and grower-I were used for SBFF.

The experiment was carried out over a period of 70 days in six experimental ponds located in the Hatchery and Field Laboratory Complex of the Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh. The size of each pond was 80 m^2 and average depth was about 1.5 m. There were three treatments *viz* T_1, T_2 and T_3 assigned to QF, ABF and SBFF feed respectively each having two replicates.

The proximate composition of the commercial feeds were analysed and the resuts are shown in Table 1. The fingerlings of Thai pangas, *P. hypophthalmus* were collected from a local fish vendor. All the fish were of same age group having mean length and weight of 8.44±0.04 cm and 6.60±0.04 g respectively. Each pond was stocked with 250 fingerlings. Nursery feeds were applied at the beginning of the trial followed by starter and grower/finisher feeds. The feeds were supplied twice daily morning (9.00 hr) and afternoon (16:00 hr). At the beginning, fish were fed at a rate of 15% of the body weight which was reduced to 10 and 6% for the rest of the period. The feeds were dispersed by hand broadcasting over the ponds. Fortnightly sampling was done to adjust the feeding rate and to observe the health condition of fish. Water quality parameters such as temperature, pH, dissolved oxygen and transparency were measured and recorded weekly throughout the experimental period.

Table 1. Proximate composition (% dry matter basis) of different commercial fish feeds used

Treatments	Type of feed	Dry matter	Crude protein	Crude lipid	Ash	Crude Fibre	NFE*
T_1	Rupali (N)[a]	88.71	31.96	6.06	13.2	11.06	33.47
(Quality	Rupali (S)[b]	90.35	27.21	6.61	14.81	10.37	32.05
Feeds Ltd.)	Rupali (G)[c]	89.50	27.98	6.28	14.07	11.01	36.39
T_2	(S)	90.41	30.82	9.97	11.27	9.61	34.65
(Aftab	(G)	89.47	27.91	9.92	11.27	9.45	37.08
Bohumukhi	(F)	90.32	27.07	9.98	11.99	8.54	38.31
Farm Ltd.)							
T_3	(S-I)	90.06	31.53	7.06	18.84	9.86	29.46
(Saudi-	(S-II)	90.22	32.47	7.90	18.42	10.28	27.90
Bangla Fish	(S-III)	89.53	28.97	7.11	17.29	9.24	33.47
Feed Ltd.)	(G-I)	89.99	28.38	7.83	18.37	9.55	32.27

*Nitrogen free extract (NFE) calculated as: 100- %(moisture + crude protein + crude lipid + ash + crude fibre).
[a]N= Nursery; [b]S= Starter & [c]G= Grower

At the beginning of the experiment ten fish from the stock and at the end of the experiment four fish from each treatment were collected randomly for carcass analysis. The proximate composition of fish carcass and feed were determined in triplicates according to AOAC (1980). Growth performances and feed efficiency were calculated according to Castell and Tiew (1980). One way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) was done to determine the significance of variation among the treatment means.

Results

The crude protein content of the various diets differed slightly. However, all the nursery feeds contained higher protein (27.07-32.47%) than the starter (27.21-28.97%) and grower (27.07-28.38%) feeds. The crude lipid content of ABF were much higher (9.92-9.98%) than those of SBFF (7.06-7.90%) and QF (6.06-6.61%).

The ranges of water quality parameters recorded in different experimental pond did not vary considerably during the experimental period and the values were: temperature 29.0-35.1°C, pH 6.8-8.5, dissolved oxygen 5.71-7.65 mg/l) and transparency 14.5-30.0 cm.

The results of growth performance and food utilization are shown in Table 2. The mean initial weight of 6.63g, 6.62g and 6.55g reached to a mean final weight of 116.3g, 158.68g and 226.83g in treatment T_1, T_2 and T_3 respectively. The significantly ($P<0.05$) highest growth was achieved in treatment T_3 followed by T_2 and T_1. The weight increment of *P. hypophthalmus* in different treatments during the experimental period is graphically shown in Fig. 1.

Table 2. Growth and feed utilization of *P. hypophthalmus* in different treatments during the experimental period

Parameters	Treatments			±SE[1]
	T_1	T_2	T_3	
Mean initial weight (g)	6.63[a2]	6.62[a]	6.55[a]	0.039
Mean final weight (g)	116.3[c]	158.68[b]	226.83[a]	3.706
Weight gain (g)	109.67[c]	152.07[b]	220.28[a]	3.996
% Weight gain (g)	1654.29[c]	2298.56[b]	3362.86[a]	48.349
Specific Growth Rate (SGR % day)	4.09[c]	4.54[b]	5.06[a]	0.032
Food Conversion Ratio (FCR)	1.54[a]	1.61[a]	1.57[a]	0.055
Protein Efficiency Ratio (PER)	2.11[a]	2.03[a]	2.03[a]	0.071
Apparent Net Protein Utilization (ANPU %)	35.14[a]	30.66[c]	32.07[b]	1.490
Survival (%)	90.4[a]	91.2[a]	91.6[a]	1.200
Production (kg/ha/70 days)	3062.01[c]	4282.99[b]	6231.66[a]	-
Net profit (Tk/ha/70 days)	31,004[c]	31,801[b]	34,950[a]	-

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

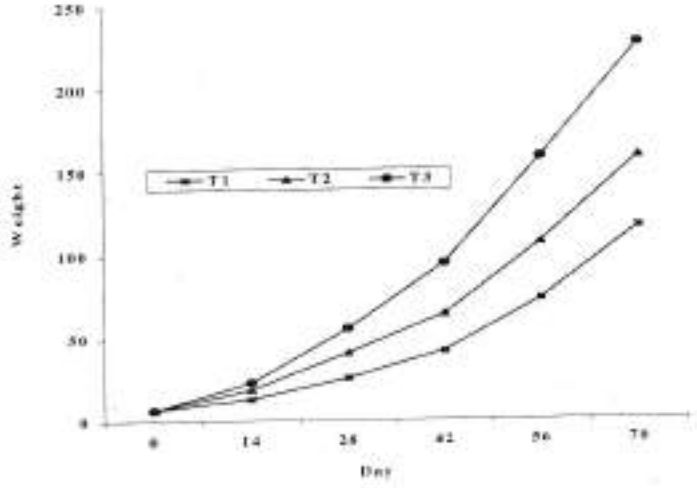

Fig. 1: Weight increment of P. hypophthalmus in different treatments during the experimental period.

The specific growth rate (SGR % day) of fish in different treatments varied from 4.09 to 5.06 with fish in T_3 showing significantly the highest SGR. The survival of fish in different treatments ranged between 90.4 and 91.6%. There was no significant difference in survival rates of fish among the treatments (Table 2).

The mean food conversion ratio (FCR) in different treatments ranged between 1.54 and 1.61 (Table 2). The highest FCR was found in treatment T_2 (1.61) and the lowest in treatment T_3 (1.54). However, there was no significant difference between the FCR values in different treatments. The PER values ranged between 2.03 and 2.11. There was no significant ($P>0.05$) difference in PER values among the treatments. The ANPU% values were 35.14, 30.66 and 32.07% in treatments T_1, T_2 and T_3 respectively. The values significantly ($P>0.05$) differed from each other.

Significantly ($P>0.05$) highest fish production was achieved in treatment T_3 (6231.66 kg) followed by T_2 (4282.99 kg) and T_1 (3062.01 kg) for a period of 70 days. Consequently, significantly ($P<0.05$) highest net profit (Tk.) was also obtained in treatment T_3 followed by T_2 and T_1 respectively (Table 2).

Discussion

The water quality parameters such as temperature, pH, dissolved oxygen, transparency measured in different treatments throughout the experimental period were found to be more or less similar and were within the suitable range for fish culture (Jhingran 1991).

In the present study, the highest weight gain of fish was observed in treatment T_3 receiving SBFF followed by T_2 fed with ABF and T_1 fed with QF. The significantly highest growth of fish in T_3 may be attributed to the better quality of the SBFF which contained average 29% protein. On the other hand, ABF feed contained 28% and QF contained 27% protein. Protein and feed quality as well as mineral contents of SBFF was better than other feeds. Another possible cause for better growth performance of fish in SBFF might be that the quality of protein or the amino acid balance might have been better in SBFF. Level of crude protein and other necessary elements in the diets and mode of feed presentation influence the growth rate of the fish (Khan 1997). Pathmasothy and Jin (1987) reported that the growth rate of fish was lower when fed with pelleted feed having 22% crude protein compared to those having 32% crude protein. Growth rate of *P. pangasius* increased with the increment of protein concentration and the highest growth of fish was obtained at 40% protein level in feed as reported by Rahman (1989). However, Chuapoehuk and Pothisoong (1983) stated that 25% protein containing diet was best for optimum growth of *P. sutchi*.

The SGR values observed in the present study is much higher than the values (3.09 to 3.51) obtained with pangas reared in net cages (Azimuddin *et al.* 1999) and the value (3.34) observed in outdoor concrete tank for *P. sutch.* (Hung *et al.* 1998).

A low FCR value is an indicator of better food utilization efficiency of formulated feed. The high energy diet produced the lowest feed conversion ratio (FCR) and the highest nutrient retention (Hillestad 2001). In the present study, there was no significant

($P > 0.05$) difference in FCR values among the treatments. However, comparatively lower FCR (1.54) obtained in T_1 where fish were fed with QF. The FCR values were higher than the values (1.40) reported by Hung *et al.* (1998) but lower than the values (1.73 to 2.04) stated by Azimuddin *et al.* (1999) for *P. sutchi*. Rashid (1997) reported higher FCR values of 4.45 and 4.67 for *P. sutchi* in cage fed diet containing 29.98% and 29.63% protein respectively. The PER values in the present study is higher than the values reported by Kamrudin *et al.* (1987) for *P. sutchi*. Survivals (%) of fishes in the present study were similar to the values reported by Azimuddin *et al.* (1999).

The total fish production in T_3 reached almost two times higher than T_1 during the same experimental period (70 days) due to higher weight gain of individual fish. The production obtained in this study is much higher than the findings of Ahmed *et al.* (1996) who reported a production of 339.39 kg/ha for *P. pangasius* fed with SBFF for a three months experimental period. This might be due to the fact that *P. hypophthalmus* is a culturable species whereas *P. pangasius* is a riverine fish species. A simple economic analysis showed that treatment T_3 generated maximum net profit of Tk. 34,950/ha/70 days which is due to the higher production of fish in T_3.

The carcass proximate composition of fish was influenced by the feeds from different company (Seikai *et al.* 1997, Austreng and Storebakken 1985). There was a marked increase in lipid content of fish compared to the initial content of fish (Table 3). The carcass lipid content was directly influenced by the dietary lipid content. ABF which contained the highest crude lipid (9.95%) resulted in the highest carcass lipid. An inverse relationship between lipid and moisture contents could be observed as reported earlier (Andrews and Stickney 1972, Garling and Wilson 1976).

Table 3. Carcass composition of the experimental fish at the start and end of the experiment (% dry matter basis)

Parameters	Initial (all fish)	Treatments		
		T_1	T_2	T_3
Moisture	78.83	69.81±1.82	67.4±1.98	67.88±1.44
Crude protein	56.43	49.27±1.94	42.02±2.87	43.98±0.95
	(11.94)*	(14.88±0.59)	(13.70±0.94)	(14.39±0.37)
Crude lipid	18.86	41.91±3.63	48.19±4.3	43.43±4.9
	(3.99)	(12.66±1.10)	(15.71±1.4)	(14.17±1.5)
Ash	18.67	8.54±1.10	8.96±0.60	9.28±1.62
	(3.96)	(2.58±0.33)	(3.03±0.53)	(2.92±0.20)

*Figures in the parentheses indicates the values expressed in % fresh matter basis

In the present study, growth performance, survival (%) and overall production in terms of kg/ha was highest in T_3 receiving SBFF. Therefore, the result of the study suggests that Saudi-Bangla Fish Feed is the best commercial fish feed for mono culture of *P. hypophthalmus* in ponds at a higher stocking density as used in the present study.

References

Ahmed, G.U., M.R.I. Sarder and M.G. Kibria, 1996. Culture feasibility of pangas (*Pangasius pangsius* Ham.) in earthen ponds with different supplemental diets. *Bangladesh J. Fish.*, 19(1-2): 23-27.

Andrews, J.W. and R.R. Stickney, 1972. Interactions of feeding rates and environmental temperature on growth, food conversion and body composition of channel catfish. *Trans. Am. Fish. Soc.*, 101: 94-97.

AOAC, 1980. Official Methods of Analysis. Association of Official Analytical Chemists (W. Horwitz ed.) 13th edition, Washington DC. 988pp.

Austreng, E. and T. Storebakken, 1985. Practical formulation of salmonid diets with emphasis on fat and protein. Actes du Groupe de Travail Franco Norvegien sur l' Aquaculture Proceedings of the Norwegian French Workshop on Aquaculture. IFREMER, Brest France. 342 pp.

Azimuddin, K.M., M.A. Hossain, M.A. Wahab and J. Noor, 1999. Effect of stocking density on the growth of Thai pangas, *Pangasius sutchi* (Fowler) in net cage fed on formulated diet. *Bangladesh J. Fish. Res.*, 3(2):173-180.

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

Chuapoehuk, W. and T. Pothisoong, 1983. Protein requirement of catfish, *P. sutchi* (Fowler). *In*: Proceeding of the Asian fin fish nutrition workshop held in Singapore, 23-26 August, 1993 in "Fishfish Nutriton in Asia" (C.Y. Cho, C.B. Cowey and T. Watanabe eds.). Ottawa, Ontario., IDRAC, Canada. pp. 103-106.

Garling, D.L. (Jr.) and R.P. Wilson, 1976. Optimum dietary protein to energy ratio for channel catfish fingerlings, *Ictalurus punctatus. J. Nutr.*, 106: 1368-1375.

Hillestad, M., 2001. High-energy diets for Atlantic salmon: effect on growth, feed utilization, product quality and recipient loading. *In*: Reservoir and culture based fisheries: biology and management. Proc. of an International Workshop held in Bangkok, Thailand from 15-18 February, 2000. 81pp.

Hung, L., N. Tuan and J. Lazard, 1998. Effects of frequency and period of feeding on growth and feed utilization in two Asian catfishes, *Pangasius bocourti* (Sauvage 1880) and *Pangasius hypophthalmus* (Sauvage 1987). *In*: The biological diversity and aquaculture of clariid and pangasiid catfishes in south-east Asia (M. Legendre and A. Pariselle eds.). Proc. of the mid term workshop of the "catfish Asia Project" Cantho, Vietnam. pp157-166.

Jhingran, V. G., 1991. Fish and Fisheries of India. 3rd edition, Hindustan Publishing Corporation, India. 727pp.

Kamarudin, M.S., R.A. Rahman, Z.A. Azim, S.S. Siraj, and R.I. Hutagalung, 1987. Effect of four different diets on weight gain, growth, specific growth rate, feed conversion ratio and protein efficiency of *P. sutchi* (flower) fingerlings. *In*: Advances in animal feeds and feeding in the tropics. Proc. of the tenth annual conference of the Malaysian society of animal production, Genting Highlands, Pahang, Malaysia, April 2-4, 1987. pp 192-196.

Khan, M.S.R., 1997. Culture of Pangasius sutchi (Flower) in ponds and cages. M.S. Thesis, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh, 62 pp.

Pathmasothy, S and L.T. Jin, 1987. Comparative study of the growth rate and carcass composition of the stripped catfish, *Pangasius sutchi* (Flower) fed with chicken viscera and pelleted feeds in static ponds. *Fish. Bull. Dep. Fish. Malays.* Buktin perikanan Jabatan perikanan Malays. No. 50. 11 pp.

Rahman, A.K.A., 1989. Freshwater Fishes of Bangladesh. Zool. Soc. Bangladesh, Dhaka, 352pp.

Rashid, M.H., 1997. Preparation of a low cost feed for cage culture of pangas Pangasius sutchi (Flower). M.S. Thesis. Department of Aquaculture, Bangladesh Agricultural University, Mymensingh. 46pp.

Roberts, T.R. and C. Vidthayanon, 1991. Systematic revision of the Asian catfish family Pangasiidae, with biological observations an descriptions of three new species. *In*: Proceeding of the Academy of National Sciences of Philadelphia, 143: 97-144.

Seikai, T., T. Takeuchi, and G. Park, 1997. Comparison of growth, feed efficiency, and chemical composition of juvenile flounder fed live mysids and formula feed under laboratory conditions. *Fish. Sci.*, 63(4): 520-526.

Shang, Y.C. and B.A. Costa-pierce, 1983. Integrated agriculture-aquacutlrue farming system-Some economic aspects. *J. World Maruculr. Soc.*, 14: 523-530.

Tavarutmanegul, P., C. Stritongsuk and C. Sasrimahachai, 1979. Induced spawning of pond reared fish by using pituitary hormone injection. Second Inland Aquaculture Training Course (June 11- August 10, 1979). *National Inland Fish. Tech. Pap.* (special), 6: 296-311.

Zaher, M. and M.A. Mazid, 1993. Aquafeed and feeding strategies in Bangladesh. In: Farm Made Aqua Feed. (M.B. New, A.G.J. Tacon and I. Csavas eds.). Proc. of the FAO/AADCP Regional Expert Consultation on Farm Made Aquafeeds. 14-18, December, 1992. Bangkok, Thailand. FAO/AADCP Bangkok, Thailand. 161-180pp.

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Post impoundment changes in the fish fauna of Kaptai reservoir, Bangladesh

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Abstract
Based on the present investigation and reviewing the published and unpublished documents critically, this communication considers the post impoundment changes in the fish fauna of Kaptai reservoir. Investigation reveals that a total of 73 species of fish belongs to 47 genera, 25 families and 2 species of prawn are present in the reservoir. Of them, 31 are commercially important, 6 exotic and 9 species are newly identified.

Key words: Fish fauna, Post impoundment change, Kaptai reservoir

Introduction

Kaptai reservoir is one of the largest man-made freshwater lake in the world (BLP/IDRC 1980), and the largest in Southeast Asia (Fernando 1980). With a surface area of 68800 ha at full supply level (FSL) (Ali 1985) it offers a wide possibilities for the development of fisheries and the enhancement of annual fish production. Before damming on the course of the river Karnaphuli in 1960-61, no investigation was done of its fish fauna.

The fish population of Kaptai reservoir was primarily dependent on the previous riverine stocks that goes back to 36 years after impoundment, so, the scenario of fish fauna exists in this reservoir shows strong resemblance with those of the rivers, haors, beels system of Bangladesh. Even than, changes in the fish faunastic structure in respect to existence and abundance and shifting habitat have been occurred with time from riverine to lacustrine environment due to the formation of dam. In case of every reservoir created through damming where a common phenomena exist that fish those become captivated either it may be adapted itself to the new ecosystem or endangered or dwindled or become extinct from that environment. These phenomena have probably occurred to the history of Kaptai lake fish fauna. From 80s onward a number of species like Chinese carps, Thai punti has introduced. African magur, a carnivorous as well as highly competitive fish has accidentally introduced in the reservoir recently from the culture ponds as those of tilapia escaped from culture cages during 1985. This tilapia like the Chinese carps already adapted them in the reservoir water and contributes subsequently to the total landing. The African magur which recently escaped from the

culture pond are found in the fisher's catch which may be harmful for many indigenous species due to its carnivorous habit.

On the other hand, some species captivated during the closing of the river Karnafuli, have been adapted themselves successfully to the new ecosystem and now-a-days, contributing substantially to the total landings. Some of the fish might have inhabited within the reservoir but not found frequently to the fisher's catches or in the harbors. As a result, a great controversy arisen regarding few rarely found species according to several author's whether they exists till today or not. The investigation is, therefore, undertaken considering the post impoundment changes in the fish fauna of Kaptai reservoir chronologically.

Materials and methods

The present investigation was made over a period of two years from 1995 to 1997. The fish enlisted here were collected mostly from the fisher's catch at major fishing sites (Kattoli, Burighat, Suvalong and Bilaichhori) in the reservoir. A number of specimens were collected from Bangladesh Fisheries Development Corporation (BFDC) pontoon at Rangamati, the main fish landing center where fish were brought out every far off fishing grounds by carrier boats. Local retail markets were also accounted and a few of the listed specimens were collected from the laboratory of Bangladesh Fisheries Research Institute, Riverine Sub-station, Rangamati. Species enlisted here are preserved in the laboratory of Riverine Sub-station. Identification of fish was done following Day (1958), Talwar and Jhingran (1991), Rahman (1989) and Jayaram (1981).

Results and discussion

In this investigation a total of 73 species of fish belongs to 47 genera, 25 families and 2 species of prawns were recorded, of them, 31 are commercially important, 6 exotic and 9 species are newly identified. Most of the species collected are as illustrated in the Table-1 with their present status within the community. Chookder (1966) in an unpublished report mentioned 54 species of fish inhabiting the reservoir area and added that, before construction of the dam the Karnafuli estuary and its upstream was rich in fish fauna. Sandercock (1966) studied first the fish fauna of Kaptai reservoir and enlisted 27 commercially important species. Ahmed and Hasan (1981) prepared a check list of 27 species of fish but they could not able to include very common, abundant and commercially important species inhabiting in the reservoir those even recorded in the daily landing register. While reporting on behalf of the committee for the evaluation of the fisheries management of Kaptai reservoir, Baily (1982) mentioned that the reservoir harbors 58 species of fish without providing any list. As the same way, Hye (1983) also mentioned 58 species belonging to 25 families of which 28 commercially important but failed to include a complete list of the same. ARG (1986) in their final report gave list comprising 49 indigenous and 5 exotic species, mentioning 31 commercially important.

Hafizuddin *et al.* (1989) enlisted 58 species including 5 exotic ones and considered again the same number of commercially important species. Likewise, Halder *et al.* (1989) encounted 66 indigenous and 5 exotic species belonging to 49 genera, 26 families and 10 orders.

Sandercock (1966), in his pioneer work in the history of Kaptai reservoir fisheries mentioned *Silonia silondia, Bagarius bagarius* and *Clupisoma garua* as commercial species and he predicted the future potential of these species in the commercial catches would be hampered as these fish were inhabited in some particular areas of the reservoir and added that among them *S. silondia* was found as rare species during that period. None of the above species was found by the present investigators or by the antecedents (Ahmed and Hasan 1981, ARG 1986, Alamgir *et al.* 1990).

Table 1. Checklist of the fish fauna of Kaptai reservoir

Family/Genus/Species	Vernacular name	Present status
1. SYMBRANCHIDAE		
G- *Monopterus Mc* Clelland	Kuichcha/Kuchey	C
Monopterus cuchia		
2. F- TETRAODONIDAE		
G- *Tetraodon* Linneus		
Tetraodon cutcutia	Potka/Tepa	R
3. F-BELONIDAE		
G- *Xenontodon* Regan		
Xenontodon cancila	Kaikley/Kakila	VC
4. F- HEMIRHAMPHIDAE		
G-*Dermogenys* Van Hasselt		
Dermogenis pussilus	Ek thuta	VC
5. F- CYPRINODONTIDAE		
G- *Aplocheilus* McClelland		
Aplocheilus punchax	Tinchoka/Tekucha	C
6. F- CHANNIDAE		
G- *Channa* Scopoli		
Channa striatus	Shoil	C
Channa puntatus	Cheng/Taki	VC
Channa marulius	Gozar	VC
Channa orientalis	Okol/Raga	FC
7. F- CYPRINIDAE		
G- *Salmostoma* Swainson		
Salmostoma phulo	Chela	VC
Salmostoma bacaila	Chela	VC
G- *Esomus* Swainson		
Esomus danricus	Darkina/ Jhia	FC
G- *Amblypharyngodon* Bleeker		
Amblypharyngodon mola	Mola/Moia	C
G- *Rohtee* Sykes		
H- *Rohtee cotio*	Dhela	C

G- *Labeo Cuvier*	Kalbaush/Kali	
Labeo calbasu	Ghoinna	VC
Labeo rohita	Rui	C
Labeo gonius	Sada Ghoinna/Ghonia	C
Labeo bata	Bata	FC
Labeo angra	Bhanga Bata	R
G- *Cirrhinus (Oken)Cuvier*		
Cirrhinus mrigala	Mrigala/Mirgi/Mirka	C
Chirrhinus reba	Lasu/Bata/Raikh	C
G- *Puntius Hamilton*		
Puntius sophore	Punti	C
Puntius ticto	Tit Punti	VC
G- *Aspidoparia* Heckel		
Aspidoparia jaya	Piali	R
Aspidoparia morar	Piali	R
8. F- COBITIDAE		
G- *Lepidocephalus* Bleeker		
Lepidocephalus guntea	Gutum/Gotey	FC
9. F- CLARIIDAE		
G- *Clarias* Scopoli		
Clarias batrachus	Magur	VC
10. F- SILURIDAE		
G- *Wallago* Bleaker		
Wallago attu	Boal	C
G- *Ompok* Laceped		
Ompok bimaculatus	Pabda	C
11. F- HETEROPNEUSTIDAE		
G- *Heteropneustes* Muller		
Heteropneustes fossilis	Shinghi/Jhial	C
12. F- SCHILBEIDAE		
G- *Ailia* Gray		
Ailia coilia	Baspata/Kajori	C
G- *Pseudeutropius* Bleeker		
Pseudeutropius atherinoides	Batashi	FC
G- *Eutropiichtys* Blleker		
Eutrophichthys vacha	Bacha	VC
13. F- BAGRIDAE		
G- *Mystus* Scopoli		
Mystus aor	Ayre	VC
Mystus cavasius	Gulsha	VC
Mystus bleekeri	Tengra	FC
Mystus vittatus	Tengra	FC
14. F- SISORIDAE		
G- *Gagata* Bleeker		
Gagata youssoufi	Gagtengra	FC
15. F- NOTOPTERIDAE		
G- *Notopterus* Lacepede		
Notopterus chitala	Chital	VC

Notopterus notopterus	Foli /Foloi	C
16. F- ENGRULIDAE		
G- *Setipinna* Swainson		
Setipinna phasa	Phaissha	VC
17. F- CLUPEIDAE		
G- *Gudusia* Fowler		
Gudusia chapra	Chapila	VC
G- *Corica* Hamilton	Katchki/Soborn	
Corica soborna	Khorika	VC
G- *Gonialosa* Regan		
Gonialosa manminna	Bori chapila/chapila	VC
18. F- MASTACEMBALIDAE		
G- *Macrognathus* Lacepede		
Macrognathus aculeatus	Tara baim/Tota	FC
G- *Mastacembelus* (Gronovius) Scopoli		
Mastacembelus armatus	Baim/Bara Baim	FC
Mastacembelus pancalus	Pakal/Guchi	FC
19. F- MUGILIDAE		
G- *Rhinomugil* Gill		
Rhinomugil corsula	Khorsula	VC
20. F- ANABANTIDAE		
G- *Colisa* Cuvier	Lal Kholisha	
Colisa lalius	PataKholisha/Bara	VC
Colisa fasciatus	Kholisha	VC
G- *Anabas* Cuvier & Cloquest		
Anabas testudineus	Koi	FC
21. F- GOBIIDAE		
G- *Glossogobius* Gill		
Glossogobius guiris	Bele/Baila	VC
22. F- NANDIDAE		
G- *Nandus* Cuvier		
Nandus nandus	Bheda/Meni	R
23. F- PRISTOLEPIDAE		
G- *Badis* Bleeker		
Badis badis	Napit Koi	C
24. F- SCIAENIDAE		
G- *Johnius* Bloch		
Johnius coitor	Poa	C
25. F- CENTROPOMIDAE		
G- *Chanda* Hamilton		
Chanda ranga	Chanda	FC
Chanda nama	Chanda	VC

New identification

F- CYPRINIDAE
G- *Danio* Hamilton

Danio sondhii	Bara darkina	FC
G- *Crossocheilus* Van Hasselt		
Crossocheilus latius	Bara darkina	C
G- *Puntius* Hamilton		
Puntius jelus	Tit puti	C
Puntius chola	Puti	C
Puntius conchonius	Kanchon puti	C
F- CENTROPOMIDAE		
G- *Chanda* Hamilton		
Chanda baculis	Chanda	VC
Chanda lala	Chanda	FC
F- COBITIDAE		
G- *Nemachilus* Van Hasselt		
Nemachilus zonanternas	Gutum	FC
F- BAGRIDAE		
G- *Batasio* Blyth		
Batasio tengana	Tengra	R
Species introduced		
F- CYPRINIDAE		
G- *Cyprinus* Hamilton		
Cyprinus carpio	Carpu	C
G- *Puntius* Hamilton		
Puntius gonionotus	Thai punti	FC
G- *Hypophthalmicthys* Bleeker		
Hypophthalmicthys molitrix	Silver carp	FC
G- *Ctenopharyngodon* Steindachner		
Ctenopharyngodon idella	Grass carp	FC
F- CICHLIDAE		
G- *Oreochromis* Smith		
Oreochromis nilotica	Tilapia	C
F- CLARIIDAE		
G- *Clarias* Scopoli		
Clarias gariepinus	African magur	FC
Prawns		
F- PALAEMONDAE		
G- *Macrobrachium* de Man		
Macrobrachium rosenbergii	Golda Chingri	FC
Macrobrachium lamarri	Kucha Chingri	VC

VC: Very common; C: Common; FC: Fairly common; R: Rare

After Sandercock (1966) nobody except Halder *et al.* (1989) mentioned the *B. bagarius* and there remains strong possibility of erroneous identification of this species

because, in our investigation no single specimen was found. There might have been great possibility of existence of this species before and after the construction of the dam and disappeared afterwards.

A check list made by Ahmed and Hasan (1981) for Kaptai reservoir fisheries is questionable where they could not include four very common abundant and commercially important species (*Notopterus chitala, Mystus aor, Channa striatus and Setipinna phasa*) during their study and also interesting to note that they also failed to cite the pioneer work of Sandercock (1966). Moreover, two enlisted species, viz. *Ailiichthys punctatus* and *Mystus seenghala* are again a matter of question because Sandercock (1966), ARG (1986), Alamgir *et al.* (1990) did not mention about *A. punctatus* in their checklist and only Alamgir *et al.* (1990) collected a few specimen for *M. seenghala*. ARG (1986) corrected the name of *A. punctatus* as *Ailia coilia* and mentioned that such erroneous identification might have been occurred due to similar morphology between the two species. Though Halder *et al.* (1989) reported *Ailia coilia* and *M. seenghala* in their checklist but they did not mention the name of *A. punctatus*. In the present investigation none of the two species (*A. punctatus and M. seenghala*) have been found so far. This *A. coilia* is available in the landing during the period of peak water level (November to January).

Hye (1983) and ARG (1986) mentioned the name of *Mystus guleo* and *M. tengra* respectively but Haffizuddin *et al.* (1989) and Halder *et al.* (1989) did not include both of these fish and in this investigation same result is found. The existence of *Ompok bimaculatus* is also controversial according to the works of Sandercock (1966), Ahmed and Hasan (1981), ARG (1986) and Alamgir *et al.* (1990). Because the workers except Sandercock (1966) mentioned *O. bimaculatus* as *Ompok pabda* but in our investigation it was found that *O. bimaculatus* is very common in fishers' catch not *Ompok pabda*. Similar opinion was made by Halder *et al.* (1989) in their checklist for the same reservoir.

Rita rita, described by Halder *et al.* (1989) and Alamgir *et al.* (1990) and *Puntius sarana* (Ahmed and Hasan 1981, ARG 1986 and Alamgir *et al.* 1990) was not found in the present investigation. Halder (1989) reported that *Puntius sarana* was caught in the large numbers at the inception of the reservoir. The species become disappeared from the reservoir and on the other hand, *Puntius gonionotus*, an exotic species introduced here escaping from the research cages accidentally and with the stocked fingerlings, and going to be established within this environment and being found in the normal catch.

At the inception of the reservoir *Tor tor* was found to catch in riffle areas of Karnafuli reservoir at Barkal and of the Kassalong river at Gangaram (Sandercock 1966) but at present, this species found rare in the landings. Meanwhile, the evidence of observing fry and fingerlings of the same through fisher's catch at Barkal immediately after breeding season is claimed by BFRI scientists. This species may be treated as endangered and immediate steps should be taken for the conservation of this highly priced fish by artificial propagation and banning the catch in the reservoir.

Considering the geographical distribution, the *Danio sondhii* and the *Dermogenys pussilus* covers the Kaptai reservoir and south-eastern part of Bangladesh (Talwar and

Jhingran 1991, Rahman 1989), the Kaptai reservoir may be regarded as possible habitat for those rarely found species although they are not still recorded from any other inland waters. From the inception of landing (1965-66) to 1984-85 the contribution of total landing was made by major carps (21%) which have been replaced recently by Clupeids (Kechki+Chapila, 64%). However, adopting proper management techniques and following the conservation measures strictly, can make this reservoir a prominent source for diversified ichthyobiota. Nevertheless, the list of these fish fauna is not exhaustive and it may be believed that there are more species yet to be accounted and identified and those species may be harbored in the remote areas and parts rest in India.

References

Ahmed, B. and S. Hasan, 1981. A check list of the fishes of the Karnafuli reservoir. *Bangladesh J. Zool.*, 9(1: 37-40.

Alamgir, M., S.H. Chowdhuary and A.S. Ahmed, 1990. New records of the ichthyofauna of Lake Kaptai. *Chittagong University Studies*, Part II : *Science*, 14(2).

Ali, L. (ed.), 1985. Proceeding of the National Seminar on Fisheries Development in Bangladesh, 15-19 January 1985, Sponsored by Fisheries and Livestock Division, Ministry of Agriculture, Bangladesh. 41pp.

Aquatic Research Group (ARG), University of Chitagong, Bangladesh. 1986. Hydrobiology of Kaptai Reservoir. FAO/UNDP Final Report No. DP/BGD/79/015/4/FI. 192pp.

Bailey, W. M., 1982. Report of Committee for Evaluation of Fishery Management of Kaptai Lake Report; Bangladesh Fisheries Development Corporation. 14 pp.

BLP/IDRC (Bangldesh Landsat Progrmme/ International Development Research Centre), 1980. Report on the Bangladesh Applied Research and Training Program in Remote Sensing. Bangladesh Landsat Program/ IDRC Research Project. 65pp.

Chokdar, A. H., 1966. Checklist of the fishes of Kaptai Lake (Unpublished). 6pp.

Day, F., 1878. The Fishes of India: being a Natural History of the Fishes Known to inhabit the Seas and Freshwater of India, Burma and Ceylon. Reproduced in 1958 by William Dowson and Sons, London. 778pp.

Fernando, C. H., 1980. The fisheries potentials of man made lake in South-east Asia and some strategies for its optimization. *In*: BIOTROP Anniversary Publication, BIOTROP, pp. 23-28.

Hafizuddin, A. K. M., N. Mahamood and M.A. Azadi, 1989. An addition to the ichthyofauna of Kaptai Lake, *Bangladesh J. Zool.*, 17(1): 29-33.

Halder, G. C., M.A. Mazid, M.K.I. Haque, M.S. Huda and K.K. Ahmed, 1989. A review on the fisheries fauna of the Kaptai reservoir. *Bangladesh J. Fish.*, 14(2): 23-30.

Hye, M. A., 1983. Fishery potentials of Kaptai Lake. *ADAB News*, 10: 2-6.

Jayaram, K. C., 1981. The Freshwater Fishes of India, Pakistan, Bangladesh, Burma and Sri Lanka. Zool. Survey of India, Calcutta; XXII + 475pp.

Rahman, A.K.A., 1989. Freshwater Fishes of Bangladesh. Zoological Soc. of Bangladesh, 364 pp.

Sandercock, F. K., 1966. Chittagong Hill Tracts Soil and Land Use Survey. Vol. 4; Fisheries (Canadian Colombo Plan Project F-475). East Pakistan Agricultural Development Corporation, Karachi, Pakistan. 67pp.

Talwar , P. K. and A.G. Jhingran, 1991. Inland Fishes of India and Adjacent Countries. Oxford & IBH Publishing Co. Pvt. Ltd. , Vol. 1 541 pp.

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Growth performances of three microalgal species in filtered brackishwater with different inorganic media

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Abstract
The growth of three microalgae species, *viz., Nannochloropsis oculata, Tetraselmis chui* and *Chaetoceros muelleri,* which are commonly used in aquaculture, was investigated using three different inorganic nutrient media: (i) Modified Guillard's f/2 medium (ii) Rix Mix medium and (iii) BFRI medium. Each microalgae species was cultured for 24 days in small- scale with initial inoculation density of $17x10^4$ cell /ml in the three media with triplicates. *N. oculata* cultured in modified Guillard's f/2 medium showed superior growth with a mean peak density of $221\pm4.24 \times 10^4$ cell/ ml, to Rix Mix medium ($141\pm 10.54x10^4$ cell/ml) and BFRI medium ($47\pm4.94 \times 10^4$ cell/ml) on the 16^{th} day of culture at stationary phase. Considering the increase in cell density for 20 days of culture in Rix Mix medium, *C. muelleri* was significantly ($P<0.05$) highest than in other two media. *N. oculata* cultured in BFRI medium resulted in the poorest growth with a mean peak increase in density of $84\pm9.19 \times 10^4$ cell/ml in 12 days of culture. However, with an increase in cell density, growth of *T. chui* ($182 \pm 6.26 \times 10^4$ cell/ml) was significantly ($P<0.05$) higher in BFRI medium than in modified Guillard's f/2 medium. The results of the present study suggest that *N. oculata* and *C. muelleri* can be grown very well in both the modified Guillard's f/2 medium and Rix Mix medium. Better growth of *T. chui* can be obtained while culturing either in BFRI and Rix Mix medium. These three nutrient media used in the present study may be useful for microalgae species culture for establishing green-water culture for suitable target zooplankton, and fish and crustacean larvae in marine and brackishwater hatcheries.

Key Words: Microalgae, Brackishwater, Nutrient media

Introduction

Microalgae are of great importance to the commercial culture of bivalves (larvae, juvenile and adults), crustaceans (mostly the early larval stages), zooplankton and to a lesser degree to finfish (larvae and /or adults). The algae are used to feed during mass culture of zooplankton such as rotifers, copepodes and brine shrimp. These zooplankton are the live food for late larval stages of crustaceans and fish. *N. oculata* can be useful in establishing the rotifer, *Brachionus plicatilis* culture protocol because of its high levels of vitamin B_{12} and eicosapentaenoic acid (EPA) content (Okauchi 1991). *C. muelleri* is high in HUFAs and its overall nutritional value is also high (Okauchi 1991). *T. chui,*

though nutritionally inferior to *N. oculata*, may also prove useful as a direct food in culturing organisms that are too small to accept rotifers (Wilkerson 1998).

Growth of phytoplankton in nature is mainly controlled by various environmental factors such as temperature, salinity, irradiance, stratification, water turbulence, etc. (Tomas 1978, Uye and Takamatsu 1990). However, nutrients are also very important environmental factors that influence the growth of any alga (Okaichi *et al.* 1989). Since the objective of growing microalgae in controlled condition is to obtain the highest density in the shortest possible time, utilization of natural seawater or freshwater without enrichment is not expected to yield significant results. Nutrient media used in the microalgae culture include Conway, Modified F or TMRL medium depending on the species cultured (Kongkeo 1991). Microalgae grow best in media with quite different primary and trace nutrient composition than natural seawater (Hoff and Snell 1989). Marine or brackishwater microalgal species require a culture medium with a chemical composition similar to that of seawater. Besides carbon, the requirement of principal nutrients for phytoplankton are nitrogen and phosphorus, in an approximate ratio of 6:1 by weight, respectively. Trace minerals and vitamins (especially B_{12} and thiamin and biotin) can also be added. These are necessary in most axenic cultures (Fulks and Main 1991) with addition of silicate for diatom. The need for microalgae to employ either as direct food for larvae and rotifers or in green water culture is important to consider in light of the results of trials to test growth of some well known microalgae species against varying microalgal culture media.

The effect of the three culture nutrient media, *viz.*, modified Guillard's f/2 medium, Rix Mix medium and BFRI medium on the growth of the three species of microalgae, *viz.*, *Nannochloropsis oculata*, *Tetraselmis chui* and *Chaetoceros muelleri*, which are commonly used in aquaculture, have not yet been studied in prevailing local condition. Considering the need for a thorough investigation with the suitable culture nutrient media for microalgae culture, the present study has been designed with the objective to determine the effect of three selected inorganic nutrient media on the growth performance of above three microalgae.

Materials and method

The culture of the microalgae in these trials was done in a temperature controlled (about 25 °C) microalgal laboratory at the Brackishwater Station of Bangladesh Fisheries Research Institute (BFRI), Paikgacha, Khulna. The culture was maintained in 1.5-liter mineral water bottles with sufficient aeration in axenic condition. The brackishwater of 25 ppt was filtered through 1.0μm cartridge filter to remove particulate and treated with 30 ppm chlorine at the rate of 0.1g/l and dechlorinated by using 0.175 g/l of sodium thiosulphate. As excess of sodium thiosulphate may reduce trace metal availability (Hoff and Snell 1989), the rate of application and period of dechlorination for 30 minutes were maintained properly. Media types tested were modified Guillard's f/2 medium, Rix Mix medium and BFRI medium. The standard nutrient medium used for algal culture was the Medium-F presented by Guillard and Ryther (1962). The Guillard's F medium is

suitable for the growth of most algae and is being used extensively (Fox 1983). The modified Guillard's f/2 medium used in this study was modification from Guillard's F medium for microalgal culture. The Rix Mix medium used by the private laboratory in North Queensland, Australia for stocks and medium size working culture (Braley 2001). The BFRI medium was prepared at the laboratory, which contain similar trace metals and vitamin solution of modified Guillard's f/2 medium. The composition of Rix Mix, BFRI medium and modified Guillard's f/2 medium is shown in Table1. The application rate of BFRI medium and modified Guillard's f/2 medium was 1 ml/L brackishwater and for Rix Mix it was 2 ml/L brackishwater. The application rate of sodium metasilicate was 1 ml to 1-L brackishwater for diatom only. The algal cells were inoculated into the bottles with equal initial concentration ($17x10^4$ cell/ml) for all species of microalgae. Aeration was moderate and the source was aquarium aerators. The bottles were set up approximately 15 cm from the two 'daylight' fluorescent tube light sources with a light intensity of about 2500 lux / m^2/ s.

Table 1. Composition of Rix Mix, BFRI medium and Modified Guillard's f/2 medium

Ingredients	Amount of ingredient(g or ml)		
	Rix Mix	BFRI medium	Modified Guillard's f/2 medium
Thrive *	99g/1-L dw	-	
Ammonium sulphate	27g/1-L dw	10 g/100 ml distilled water (dw)	
Urea	-	23 g/100 ml dw	-
TSP	-	2g/100 ml dw	-
Borax	-	1g/100 ml dw	-
Sodium nitrate	-	-	75g/1000 ml dw
Sodium dihydrogen orthophosphate	-	-	5g/1000 ml dw
Citric acid	-	-	16.8 g/1000 ml dw
Iron (ferric) citrate	-	-	3g/1000 ml dw
Sodium metasilicate	20 g/L dw	20 g/L dw	20 g/L dw
Copper sulphate	-	1g/100 ml dw	1g/100 ml dw
Zinc sulphate	-	2.2 g/100 ml dw	2.2 g/100 ml dw
Sodium molibdate	-	0.6g/100 ml dw	0.6g/100 ml dw
Manganese chloride	-	18g/100 ml dw	18g/100 ml dw
Cobalt chloride	-	1g/100 ml dw	1g/100 ml dw
Multi-Vitamin B	1- tab crushed/1-dw	-	-
Vitamin B_1	-	10g/100ml dw	10g/100ml dw
Vitamin B_{12}	-	0.05g/100 ml dw	0.05g/100 ml dw
Vitamin Biotin	-	0.05g/100 ml dw	0.05g/100 ml dw

*Thrive is common garden fertilizer in Australia having following chemical constituents:

Nitrogen (N) as Nitrate	3.0%	Sulphur (S) as sulphates	0.22%
Nitrogen (N) as ammonia form	2.6%	Copper (Cu) as copper sulphate	0.005%
Nitrogen as urea	21.4%	Zinc (Zn) as zinc sulphate	0.02%
Total Nitrogen (N)	27.0%	Boron (B) as sodium borate	0.005%
Phosphorus (P) as water soluble	5.5%	Manganese (Mn) as manganese sulphate	0.04%
Potassium (K) as potassium nitrate	9.0%	Iron (Fe) as chelated iron	0.18%
Magnesium (Mg) as magnesium sulphate	0.25%	Molybdenum (Mo) as sodium molybdate	0.002%
		Maximum Biuret	0.4%

There were three replications for each treatment i.e. for each nutrient medium. The total culture period was twenty-four days. Counts of algal cells were made at every four days interval using an improved Neubauer Haemocytometer by applying the methodology used by Hoff and Snell (1991). All the data were analyzed statistically using Analysis of Variance (ANOVA) and the significance test among mean values of treatments was done using Duncun's New Multiple Range Test (Zaman *et al.* 1982).

Results and discussion

Nannochloropsis oculata showed the best growth performance in modified Guillard's f/2 medium among the three media tested in this study (Fig. 1). The peak density of *N. oculata* in modified Guillard's f/2 medium was $221 \pm 4.42 \times 10^4$ cell/ml at the 16th day of culture before stationary phase. Lim (1991) found a peak density of 20-25 x 10^6 cells/ml using Walne's medium in 3 L polyethylene bag. In Rix Mix medium the growth increased to the highest density of $185 \pm 9.26 \times 10^4$ cell/ml at the 12th day of culture. BFRI medium showed the poorest growth performance for this species.

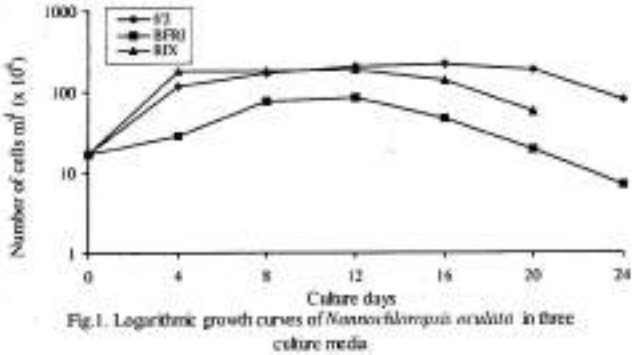

Fig.1. Logarithmic growth curves of *Nannochloropsis oculata* in three culture media

Fig. 1. Logarithmic growth curves of *Nannochloropsis oculata* in three culture media.

Chaetoceros muelleri showed unconventional growth pattern in f/2 and Rix Mix media, but followed the typical growth pattern of microalgae in BFRI medium (Fig. 2). The reason of this is less understood. *C. muelleri* attained the highest density of $321 \pm 5.41 \times 10^4$ cells/ml in Rix Mix medium and about same density ($319 \pm 12.02 \times 10^4$ cells/ml) at the 24th day of culture in modified Guillard's f/2 medium. The maximum cell density of *C. muelleri* ($135 \pm 6.95 \times 10^4$ cells/ml) in BFRI medium was observed at the 12th day of culture. Chen (1991) observed a maximum 200-250 x 10^6 cells/ml of *C. muelleri* in cemented ponds and tanks in large scale using $NaNO_3$:60g/ton,KH_2PO4:4 g/ton, Vit B:100 mg/ton and Vit B_{12}: 0.5 mg/ton.

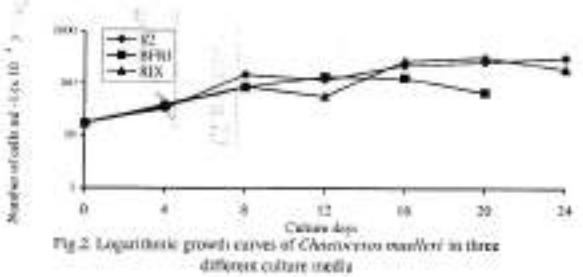

Fig. 2. Logarithmic growth curves of *Chaetoceros muelleri* in three culture media.

Tetraselmis chui was grown reasonably well in both BFRI and Rix Mix medium compared to modified Guillard's f/2 medium (Fig. 3). As water quality deteriorates and algal cells starve with the increase in density, death and breaking of cells occur after exponential growth phase of microalgae (Hoff and Snell 1991, Fox 1983). *T. chui* is less sensitive to environmental stress (Liao *et al.* 1991), which could be responsible for the extended exponential growth phase of this species in all the media. The maximum cell density of $182 \pm 6.26 \times 10^4$cell/ml was with BFRI medium at the final day (24th day) of culture. The highest density of the species with Rix Mix medium was $169 \pm 4.48 \times 10^4$ cells/ml at the final day (24th day), which was close to the maximum value with BFRI medium at the same day. *T. chui* gave reduced growth in modified Guillard's f/2 medium during the entire culture period, reaching a maximum cell density of only $78 \pm 4.24 \times 10^4$ cells/ml at the final day (24th day) of culture. Different microalgal culture test media resulted in varying growth rates of cultured microalgae species (Table 2). Considering the increase in density at the 16th day of culture, in modified Guillard's f/2 medium, the cell growth of *C. muelleri* ($222 \pm 4.24 \times 10^4$ cell/ml) was significantly ($P<0.05$) higher than the other two species. After 16 days, *N. oculata* and *T. chui* were reduced in their growth, but *C. muelleri* increased at the highest density up to the last day of culture.

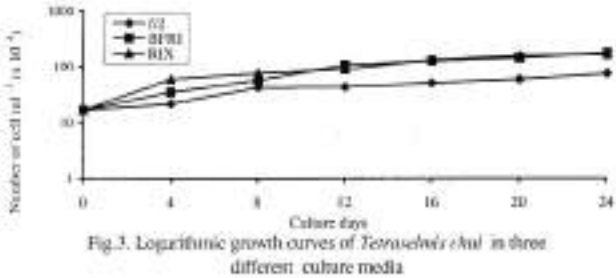

Fig. 3. Logarithmic growth curves of *Tetraselmis chui* in three culture media.

Table 2. The effect of different microalgae culture test media on the growth (cells x 10^4/ml) of three microalgae species. Values are means ± SD from triplicate observations

Culture days	Modified Guillard's f/2 media			BFRI medium			RIX MIX medium		
	'N O	"CM	"""TC	NO	CM	TC	NO	CM	TC
0	17	17	17	17	17	17	17	17	17
4	119±7.07	33±9.19	22±2.82	29±5.65	36±10.06	35±9.89	180±7.28	39±8.20	60±4.59
8	170±1.31	147±12.84	43±6.36	76±4.94	85±4.24	58±7.27	178±4.56	86±5.19	77±6.71
12	206±9.19^a	117±10.60^a	44±8.48^b	84±9.19^b	135±6.95^a	108±10.12^a	185±9.28^a	57±8.31^b	91±4.81^a
16	221±4.42^a	222±4.24^b	52±7.77^b	47±4.94^c	128±11.11^a	129±5.62^a	141±10.54^b	273±9.61^a	134±9.27^a
20	189±11.55^a	262±6.36^b	61±2.12^b	19±4.98^c	67±9.01^a	147±8.18^a	57±6.81^b	321±5.41^a	161±7.61^a
24	79±7.08	319±12.02	78±4.24^b	7±1.41	0±0	182±6.26^a	0±0	191±7.79	169±4.48^a

*NO= *Nannochloropsis oculata*, **TC= *Tetraselmis chui*,***CM= *Chaetoceros muelleri*

Note: Different superscript in the same row for same species indicates significant variation and same superscript in the same row for same species means insignificant at 5 % level of significant.

In BFRI medium, considering the increase in cell density at the 12th day, growth of *C. muelleri* (135±6.95 x10⁴ cell/ml) was significantly (P<0.05) higher than other species, but at the 24th day *T. chui* showed significantly (P<0.05) higher density (182±6.26 x 10^4 cells/ml) than other two species. *N. oculata* and *C. muelleri* concentration decreased after 12 days and reached at minimum level at the last day of culture. At the 12th day of culture in Rix Mix medium, *N. oculata* concentration was significantly (P<0.05) higher (185±9.28 x 10^4 cells/ml) than other two species. After 12 days this species gradually decreased but *T. chui* increased up to the end and *C. muelleri* reached the highest density (321±5.41 x 10^4 cell/ml) at the 20th day and then decreased.

The growth of *N. oculata* reached a higher density of 221±4.42 x 10^4 cells/ml in modified Guillard's f/2 media at the 16th day than that of (141±10.54 x 10^4 cell/ml) in Rix Mix medium. Modified Guillard's f/2 medium promoted significantly (P<0.05) higher growth than other media. Hur (1991) observed the highest cell density of 197.5 x 10^5 of *N. oculata* cultured in F/2 medium, which is much higher than the density obtained in present study and this might be due to the changes in chemical composition of nutrient medium. *N. oculata* grew reasonably well in modified Guillard's f/2 and Rix Mix media among the three media tested in this study.

The highest cell density of *N. oculata* was attained between 12 and 16 days of culture period in three culture media. On the other hand, the cell number of *T. chui* increased up to the 24th day (final) day of culture and this difference might be due to the difference in exponential growth phase of these two microalgal species. *C. muelleri* showed difference in maximum cell density at different days of culture.

In the above view, it may be concluded that, *N. oculata* and *C. muelleri* might be grown very well in both the modified Guillard's f/2 and Rix Mix media. Better growth of *T. chui* might be obtained while growing in BFRI and Rix Mix media. These three microalgal culture media used in the present study may be useful for culture of different algal species to supply as live food for suitable target zooplankton and larvae of fish, crustaceans, etc. in marine and brackishwater hatcheries.

References

Braley, R.D, 2001. Manual for the operation of the hatchery at Brackishwater station, Paikgacha, Khulna. ARMP Report. Winrock International Institute for Agricultural Development. Arkansas. USA. pp. 108.

Chen, J.F., 1991. Commercial Production of Microalgae and Rotifer in china. *In:* Rotifer and Microalgae culture systems (W. Fulks and K.L. Main eds.). The Oceanic Institute, Honolulu. pp. 105-111.

Fox, J. M., 1983. Intensive algal culture Techniques. *In:* CRC Handbook of Mariculture. Vol-1 .Crustacean Aquaculture (J.P. McVey ed.). CRC press , Florida. 15 pp.

Fulks, W. and K.L. Main (eds.), 1991. Rotifer and Microalgae Culture Systems. Proc. of a US – Asia Workshop, Honolulu, Hawaii, 28-31 January 1991, 3-12, The Oceanic Institute, Honolulu. 364 pp.

Guillard, R.R.L. and J.H. Ryther, 1962. Studies of marine planktonic diatoms. 1. Cyclotella nana Hustedt and Detonula confervaces (Cleve) Gran. *Can. J. Microbiol* ., 8: 229-239.

Hoff, F.H. and T.W. Snell, 1989. Plankton Culture Manual. Florida Aqua Farms, Inc. 126 pp.

Hur, B.S., 1991. The selection of optimum phytoplankton species for rotifer culture during cold and warm seasons and their nutritional value for marine finfish larvae. *In:* Rotifer and Microalgae culture systems (W. Fulks and K.L. Main eds.). The Oceanic Institute, Honolulu. pp. 163-173.

Kongkeo, H., 1991. An overview of live feed production systems design in Thailand. *In:* Rotifer and Microalgae culture systems (W. Fulks and K.L. Main eds.). The Oceanic Institute, Honolulu. pp 175-186.

Liao, I.C., K.H. Su and J.H. Lin, 1983. Larval foods for Penaeid prawn. *In*: CRC Handbook of Mariculture. Vol-1. Crustacean Aquaculture (J.P. McVey ed.). CRC press , Florida. pp. 43-69.

Lim, L.C., 1991. An overview of live feeds production systems in Singapoor. *In:* Rotifer and Microalgae culture systems (W. Fulks and K.L. Main eds.). The Oceanic Institute, Honolulu. pp. 187-201.

Okaichi, T., S. Montani, J. Hiragi and A. Husui, 1989. The role of iron in the outbreaks of *Chattonella* red tide. *In*: Red tides: biology, environmental science and toxicology (T. Okaichi, D.M. Andersons and T. Nemoto eds.). Elsever, New York. pp. 353-356.

Okauchi, M., 1991. The status of phytoplankton production in Japan . *In:* Rotifer and Microalgae culture systems (W. Fulks and K.L. Main eds.), The Oceanic Institute, Honolulu. pp. 257-273.

Tomas, C.R., 1978. *Olisthodiscus luteus* (Chrysophyceae). 1. Effect of salinity and temperature on growth, motility and survival. *Phycol.*,14: 309-313.

Uye , S. and K. Takamatsu, 1990. Feeding interactions between planktonic copepodes and red-tide flagellates from Japanese coastal waters. *Marine Ecology progress Series*, 59: 97-107.

Wilkerson, Joyce D, 1998. Clownfish: a guide to their captive care, breeding and natural history. Microcosm Ltd, Shelburne, VT 05482. 181pp

Zaman, S.M.H, K. Rahim and M. Howlader, 1982. Simple lessons from biometry, Bangladesh Rice Research Institute, Joydebpur, Dacca, Bangladesh. pp. 85-92.

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Effect of farm made feeds on polyculture of shrimp (*Penaeus monodon*) and three brackishwater finfish species

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Abstract
A study was conducted to evaluate the effects of feed made from locally available ingredients on polyculture of shrimp and three brackishwater finfish species. Hatchery produced post-larvae (PL) of shrimp *Penaeus monodon* (0.005g) were stocked at the rate of 15,000 PLs/ha. Brackishwater finfish species *Liza parsia*, *Mugil cephalus* and *Rhinomugil corsula* of 0.63-1.41g collected from local rivers were stocked at the rate 8,000, 1,000 and 2,000/ha, respectively in four treatments. Shrimp and finfishes were fed four different experimental diets composed of fish meal, mustard oil cake, rice bran, oyster shell power and vitamin premixes at the rate of 3-5% estimated crop/day for 195 days. Among four treatments, *P. monodon* showed comparative better growth in T_4 and T_3. Finfish *L. parsia* showed its better performance in treatment T_2. Species *M. cephalus* and *R. corsula* showed insignificant production. *P. monodon* showed better growth with diet of fish meal and mustard oil cake @ 28.84 and 33.65%, respectively in T_3 and 19.22 and 43.27%, respectively in treatment T_4.

Key words: *P. monodon*, *L. parsia*, *M. cephalus*, *R. corsula*, Feed, Polyculture

Introduction

Brackishwater aquaculture in the south-west part of Bangladesh at the present time is absolutely directed to farming the penaeid shrimp and some cases practiced as polyculture with accidental intruded finfishes. Now- a- days, trend being changing with the farmers to care about polyculture of penaeid shrimp with some selective fish species (Shofiquzzoha *et al.* 2001). Culture practices are extensive in nature and rely on natural productivity of water body (Hoq *et al.* 1994) with little or without management. Traditionally farmers do not use supplemental feed and/or not aware of using any feed to their *gher* (a traditional shrimp farm). In polyculture system, finfish may contribute to the shrimp *ghers* a higher rate of production with shrimp, if a low-cost effective feed from locally available ingredients can be supplement to the stocked animal.

To obtain a sustainable higher production the present experiment was undertaken to observed the effect of feed prepared from locally available ingredients on shrimp *Penaeus monodon* and three non-carnivore brackishwater finfish species in polyculture practice.

Materials and methods

The experiment was carried out during May to October in 12 earthen ponds as four treatments *viz.* T_1, T_2, T_3 and T_4 with three replications each.

Preparation of the pond

The ponds (0.2 ha each) were drained and dried. Soil pH was measured and lime was applied at the rate of 270-300 kg/ha depending on the pH levels of the soil. The water pH was also maintained in the same way. Mustard oil cake as an organic fertilizer was applied at the rate of 100 kg/ha. Inorganic fertilizers like triple super phosphate and urea (ratio 3:1) at the rate of 30 kg/ha were applied. Initially tidal water was allowed to enter up to a depth of about 40-60 cm and awaited for natural feed development then level was finally increased up to 90cm (Ali *et al.* 1999). As per necessity, portion of water (approximately 20%) was exchanged from ponds during the new and full moon.

Stocking

Hatchery produced post larvae (PLs) of shrimp *P. monodon* were stocked (during mid May) at the rate of 15000 PLs ha^{-1} along with fingerlings of *Liza parsia*, *Mugil cephalus* and *Rhinomugil corsula* of collected from local rivers were stocked 8,000; 1,000 and 2,000 fry/ha, respectively in treatments T_1, T_2, T_3 and T_4 (Table 1).

Feeding

Commercial nursery shrimp feed (Saudi-Bangla) Starter-1 was fed the shrimp twice a day at dawn and dusk at the rate of 100% of stocking bio-mass during first week and then the rate were gradually reduced to 60, 40 and 20% in the subsequent 2^{nd}, 3^{rd} and 4^{th} week respectively.

After 30 days, shrimp and fin-fishes were fed with supplement feed made from locally available ingredients. Four diets were formulated using rice bran (RB), fishmeal (FM), mustard oil cake (MOC), wheat flour (WF), oyster shell powder (OS) and animal grade vitamin premixes (Table 2). Feeds were fed twice a day (at dawn and dusk) at the rate of 3-5% of estimated crop. The ingredients RB, FM, WF, OS and vitamin, were proportionally weighted and mix together except MOC. The mixture were added with soaked MOC and was made into dough balls (Wood *et al.* 1991). These balls were supplied in some particular places in ponds as the animals could easily get their feed. Feed supplying often stopped for day or per serve whenever the weather became cloudy or after heavy rainfall.

Sampling and data collection

Water quality parameters i.e., air and water temperature, pH, salinity, transparency were monitored once in a week. After 87th day of culture, harvest was done for *P. monodon* by selective cast netting. Remaining finfish species i.e., *L. parsia*, *M. cephalus* and *R. corsula* those were reared in the same ponds for stipulated 195 days of culture. The final harvest of finfish and rest of shrimp was done by completely drain out the ponds.

Table 1. Stocking density, stocking time, initial and final weight of shrimp *P. monodon* and brackishwater finfish species *L. parsia, M. cephalus* and *R. corsula* used in the study

Species	Stocking rates individual/ ha	Stocking period (months)	Initial length (cm)	Initial wt. (g)	Culture period (days)	Final weight (g)			
						T_1	T_2	T_3	T_4
P. monodon	15,000	Last of May	1.10	0.005	87-195	37.49 (±2.97)	31.19 (±3.15)	38.18 (±2.22)	35.62 (±2.19)
L. parsia	8,000	Mid of May	4.62	1.41	195	19.93 (±8.13)	22.37 (±5.18)	16.02 (±7.45)	16.13 (±6.16)
M. cephalus	1,000	Aug.-early Sep.	4.22	1.22	85	8.37 (±1.60)	7.90 (±0.67)	5.13 (±1.24)	7.32 (±1.16)
R. corsula	2,000	Sep.–mid Oct.	3.94	0.63	45	3.20 (±0.53)	3.28 (±0.64)	4.46 (±1.59)	3.57 (±0.86)

Figures in the parentheses indicated ± standard deviation.

Table 2. Formulation of experimental diets (%)

Ingredients	Diets (%)			
	T_1	T_2	T_3	T_4
Fish meal	48.07	38.45	28.84	19.22
Mustard oil cake	14.42	24.04	33.65	43.27
Rice bran	28.85	28.85	28.85	28.85
Wheat flour	3.85	3.85	3.85	3.85
Oyster shell powder	4.33	4.33	4.33	4.33
Vitamin premixes	0.48	0.48	0.48	0.48
Total	100.00	100.00	100.00	100.00
Calculated protein (%)*	32.90	30.94	29.03	27.03

*Protein level: fishmeal A grade (50.81%), mustard oil cake (30.33%), rice bran (11.88%) and wheat flour (17.78%) (Bhuiyan *et al.* 1989).

Results and discussion

Treatments wise physico-chemical properties of ponds during the experimental period are shown in Table 3. The mean physico-chemical properties of water in the treatment ponds during the experimental period were found suitable except salinity. Water temperature ranged between 27.98 and 28.01±SD °C, pH between 8.29 and 8.46±SD and transparency was between 20.33 and 25.48±SD cm in all treatments however, salinity, which was ranged between 5.97 and 6.09±SD ppt. However, the parameter wise range was found within the limit for shrimp and brackishwater fin-fish culture (Ali *et al.* 1999, Roy *et al.* 1999). The variation was observed for water transparency ranged between 20.33 and 25.48±SD cm was also favorable for shrimp culture (Grey 1990). The transparency/visibility often higher might be due to growth of aquatic weeds and low for turbidity due to suspended particles after heavy rainfall, erosion of dyke due to strong wind action or silt carried by tidal water. However, Hossain (1987) attributed the variation to the differential formation of plankton and qualitative incursion of silt laden in water during tidal exchange.

Table 3. Mean physico-chemical properties of water during the experimental period

Treatments	Water temp. (°C)	Salinity (ppt)	pH	Transparency (cm)
T_1	27.98 (±5.08)	5.97 (±2.99)	8.29 (±0.49)	24.28 (±9.04)
T_2	27.85 (±4.79)	5.91 (±3.22)	8.34 (±0.52)	25.48 (±9.10)
T_3	28.01 (±4.95)	5.91 (±3.26)	8.46 (±0.59)	20.33 (±7.65)
T_4	27.39 (±6.12)	6.09 (±2.88)	8.29 (±0.49)	24.47 (±12.22)

Figures in the parentheses indicated ± standard deviation.

The treatment wise mean production in shrimp fin-fish polyculture was shown in Table 4. Data revealed that, the total production range between 90.5 and 183 kg/ha/195 days in all treatments might be considered as low. Shofiquzzoha *et al.* (2001) obtained 242.68 kg/ha in similar species combination and stocking density while, the animal fed with commercial shrimp feed (30% protein level) for 195 days. Ali *et al.* (2000) formulating feed with fish meal, rice bran, mustard oil cake, wheat bran and vitamin premix (30-32% protein level) and fed at the rate 3% of estimated crop while stocked 40,000 PLs and 10,000 fry/ha for a culture period of 225 days and produced *P. monodon* and *L. parsia* of 449.37 and 171.05 kg/ha, respectively. Roy *et al.* (1999) obtained 231 kg/ha/crop of *P. monodon* for a culture period 149days in monoculture system. The low production obtained in the present study might be due to inaccurate proportions or low efficacy of the food ingredients used in the rations. Lovell (1989) mentioned about dietary protein level of penaeid shrimp ranged 28 to 60% and for *P. monodon* the range were 42 to 46% how eve, values differ on animals size, level of dietary energy, feeding rate and availability of natural food organism. Wood *et al.* (1991) mentioned that, feeds to be water stale for minimum of two hours, raw materials must also be selected in terms of their functional properties or ability to induced water stability, nutritive and as well as an attractants. Hossain *et al.* (2000) emphasized on digestible crude protein, organic matter or lipid and energy content in feed and ingredients.

The treatment wise production of shrimp-fish were calculated (Table 4). Study revealed that, among treatments, growth/production of shrimp *P. monodon* and finfish species *L. parsia* were significant ($P<0.05$). However, species *M. cephalus* and *R. corsula* production as well as total production found insignificant.

Among the four treatments, *P. monodon* showed comparative better performance in treatments T_3 and T_4 (Table 4) where the two of five feed ingredients *viz.*, fishmeal and mustard oil cake were used at 28.84 and 33.65% respectively, in treatment T_3; however, in treatment T_4, it was 19.22 and 43.27% respectively. Finfish *L. parsia* showed its better performance in treatment T_2 (fishmeal 38.45% and mustard oil cake 24.04% were used).

Table 4. Production (kg/ha) of shrimp and finfish in polyculture system

Commodity produced	T_1	T_2	T_3	T_4
Shrimp *Penaeus monodon*	39.51^c	54.08^c	108.25^a	105.00^{ab}
Finfish *Liza parsia*	37.12^c	117.75^a	48.87^c	66.83^b
Finfish *Mugil cephalus*	9.00	7.92	5.59	5.66
Finfish *Rhinomugil corsula*	4.88	3.88	5.47	6.23
(Total finfish)	(51.00)	(129.55)	(59.93)	(78.72)
Total production	90.51	183.63	168.18	183.72

In this study, *P. monodon* showed better growth when fed diet of fish meal and mustard oil cake 28.84 and 33.65% respectively in T_3, and was 19.22 and 43.27% in

treatment T_4 respectively. The results indicated that, it needed to be more decisive of selecting feed ingredients (*e.g.* protein, lipid etc) and/or ratios to producing sustainable production of these species in polyculture system.

References

Ali, M.S., A.FM. Shofiquzzoha and S.U. Ahmed, 1999. Effect of submerged aquatic vegetation on growth and survival of *Penaeus monodon* (Feb.). *Bangladesh J. Fish. Res.*, 3: 145-149.

Ali, M.S., A.FM. Shofiquzzoha and S.U. Ahmed, 2000. Observation on the production performances of *Penaeus monodon* with *Liza parsia* under different cropping systm. *Bangladesh J. Fish. Res.*, 4(2): 141-145.

Grey, C.W., 1990. A guide to shrimp and prawn culture in Bangladesh. BAFRU/ODA, Institute of Aquaculture, University of Stirling, Stirling KFK9 4LA, Scotland. U.K., 49 pp.

Hossain, S.M.Z., 1987. Studies on some physico-chemical parameters of tide fed shrimp ponds. *Bangladesh J. Fish.*, 10: 47-56.

Hossain, M.A., P. Jahan and K. Kikuchi, 2000, Nutrient digestibility coefficients of diets with varying energy to protein ration for Japanese flounder, *Paralichthys olivaceus. Bangladesh J. Fish. Res.* 4(2): 105-112.

Bhuiyan, A.K.M.A, N.N. Begum, M. Begum and M.E. Hoq, 1989. Survey of potential fish feed ingredients of Bangladesh on the basis of their availability and biochemical composition, Final report. Fisheries Research Institute, Mymensingh, Bangladesh. 70pp.

Hoq, M.E., G.C. Halder and M. Begum, 1994. Experimental pond culture of tiger shrimp, *Penaeus monodon* Feb. with various stocking rates and supplemental feeding. *Progress. Agric.*, 5: 55-61.

Lovell, R.T., 1989. Diet and fish husbandary. *In:* Fish Nutrition, Second edition (J.E. Halver, ed.). Academic press, Inc. pp. 519-604.

Roy, P.K., S.U. Ahmed and A.F.M. Shofiquzzoha, 1999. Optimization of stocking density for environmental friendly improved extensive shrimp farming system in south-west part of Bangladesh. *Bangladesh J. Fish. Res.* 3: 137-143.

Shofiquzzoha, A.F.M., M.I. Islam and S.U. Ahmed, 2001. Optimization of stocking rates of shrimp (*P. monodon*) with brackishwater finfish in a polyculture system. *Online J. Biol. Sc.* 1(8): 694-697.

Wood, J., J. Coulter and I. Rajendran, 1991. India's expanding prawn culture industry- where will the raw materials come from? *In:* Proceeding of the symposium on aquaculture production (V.R.P. Sinha and H.C. Srivastava eds.), held in December 1988, under aegis of Hindustan Lever Research Foundation. pp. 257-268.

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Effects of salinity and temperature on the larval development of a sesarmid crab *Neosarmatium trispinosum* Davie (Crustacea: Brachyura: Sesarmidae) from mangrove swamp in Okinawa Island, Japan

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Abstract
The larval development of the semiterrestrial sesarmid mangrove crab *Neosarmatium trispinosum* was studied under laboratory conditions at salinities 0-35‰ and constant temperatures of 20-30°C. The larval development consists of five zoeal stages and a megalopa. Larvae survived to the first crab stage at salinities between 15 and 35‰ with different percentages. At 0, 5 and 10‰, the larvae died within 12-18 hours without moulting to subsequent stages. The highest survival rate was recorded at 20-25‰ and 25-30°C with shortest development duration to the first crab stage ranging from 24-28 days. At the highest salinity (35‰), survival rate was gradually decreased with increasing development duration. There were significant differences ($P<0.01$) found in the development period among the tested salinities. Results of this study suggest that the larvae of *N. trispinosum* develop in estuarine water and recruit to the mangrove swamp at the megalopa stage, where they spend the rest of their lives.

Key words: Mangrove crab, *Neosarmatium trispinosum*, Larval development

Introduction

The crabs of the family Sesarmidae are important components of mangrove ecosystems in the Indo-West Pacific, Africa, the Caribbean and South America. By retaining a large proportion of mangrove leaf-litter within mangrove forests, they profoundly influence the functioning of mangrove ecosystems (Sheaves and Molony 2000). Crabs of the genus *Neosarmatium* are among the largest of the intertidal mangrove sesarmids (Ng *et al.* 1996). Among the recognised species, *N. trispinosum* is an uncommon species found in the mangrove swamps of southern Okinawa Island, Japan. *Neosarmatium trispinosum* builds large characteristic mounds at the entrance to its burrow. This species is also found in the mud flats between low and high tide marks, and mangrove areas (Sakai 1976, Dai and Yang 1991). The crab emerges from its burrow at

night to remove litter from the surface of the mud (Gaddins *et al.* 1986). This species is a major leaf consumer, carrying leaves into its burrow where they are allowed to age and decay prior to consumption (Gaddins *et al.* 1986, Neilson and Richards 1989).

N. trispinosum has long been confused with *N. smithi* (H. Milne Edwards 1853), from which it can be easily separated by the shape and position of the teeth on the upper margin of the dactyl of the male cheliped (Davie 1994). On *N. trispinosum* the three spines are acute, and placed close together in the proximal two-thirds; whereas in *N. smithi* they are truncate, and spaced out over the proximal half (Davie 1994).

Sesarmid crabs are frequent inhabitants of transitional habitats between marine intertidal and adjacent freshwater or terrestrial zones (Anger and Charmantier 2000). In most of these species, however, reproduction depends on an export of the larval stages into the ancestral environment, the sea, and later remigration of megalopae or benthic juveniles into the brackish or semiterrestrial parental habitats (Hartnoll 1988, Anger 1995, Anger and Charmantier 2000).

Salinity and temperature are among the most important physical factors in the life of marine and brackish water organisms (Browne and Wanigasekera 2000). There is often a complex co-relationship between the two factors, where temperature can modify the effects of salinity, thereby changing the salinity tolerance of an organism, and salinity can modify the effects of temperature accordingly (Kinne 1963, Browne and Wanigasekera 2000). Several published documents deal with the effects of a variety of salinity and temperature levels on the larval development of different species of sesarmid crabs (Costlow *et al.* 1960, Anger *et al.* 1990, Schuh and Diesel 1995a,b, Diesel and Schuh 1998, Islam *et al.* 2000, 2002a).

Prior to this study, larval development is known only for *N. fourmanoiri* (Islam *et al.* 2003), *N. indicum* (Islam *et al.* 2002b) and *N. meinerti* (Pereyra Lago 1989, as *Sesarma meinerti)* within the genus *Neosarmatium*. Their larval development consists of five zoeal stages and a megalopa under laboratory conditions. Considering the importance of these species in the mangrove ecosystem, we examined the influence of salinity and temperature on the larval development of *N. trispinosum*.

Materials and methods

An ovigerous *N. trispinosum* crab measuring 34 mm in carapace length and 37 mm in carapace width was captured by hand from a burrow in the Shimajiri mangrove swamp of Miyako Island (26°20′N-26°30′N, 128°10′E-128°20′E), southern Okinawa, Japan. The female was brought to the Laboratory of Fisheries Biology, University of the Ryukyus, Okinawa, and reared in a plastic container (32x18x22 cm) with 16±1‰ salinity, 28.5±0.31°C ambient temperatures and moderate aeration. The female was fed with "Tetra Fin" (small dry fish) and aged mangrove leaves. Seawater was changed daily until the eggs hatched. Hatching occurred after 18 days of rearing. The larvae were reared for mass culture under the same conditions as indicated for the ovigerous female.

The larvae were subjected to eight different salinity levels (0-35‰, by step of 5‰), measured with an Atago Hand Refractometer to the nearest 1‰, and three different

constant temperatures (20, 25 and 30°C). The above salinities were obtained by diluting filtered seawater with dechlorinated tap water. Temperature was controlled by thermostat. Within one day after hatching, the most photopositive and active larvae were selected, and then used for experiments. A 5-ml glass pipette was used to retrieve active larvae from the rearing container (mass culture) and to place them into the one-liter plastic test container containing the test solutions (20 individuals per container). Half of the aerated test water in each container was replaced daily. The larval stage was identified based on the setal number on maxillipedal exopods, under a binocular stereomicroscope.

Newly hatched nauplii of *Artemia* sp. were added daily to each bowl as larval food. In addition, finely chopped meat of the short-necked clam *(Ruditapes philippinarum)* was fed to megalopa. Moulting, survival and development duration were checked for each larval stage daily between 7:00 am and 7:00 pm. Dead larvae were preserved in 50% ethylene glycol solution for later re-identification of stages. Experiments were terminated when all the larvae had moulted to the first crab stage or died. Development duration for larvae at each salinity level was analyzed using a two factor analysis of variance (ANOVA) on the statistical package Minitab 11.12 for Windows.

Results

Larval survival of *N. trispinosum* from hatching through first crab stage occurred over the range of tested salinities (15-35‰), with a slight tendency of higher survival in 25‰ at 25°C, 20‰ at 30°C and 30‰ at 20°C (Table 1). However, 0-10‰ appeared unsuitable, 100% mortality occurring after 12-18 hours exposure. At the time of metamorphosis from the fifth zoea to the megalopa stage, survival was higher in 25‰ at 30°C, 25‰ at 25°C and 30‰ at 20°C than at 15‰ or in full-strength seawater (35 ‰) (Table 1). At 20, 25 and 30°C, development was successful in the salinity ranges were tested (Table 1), but with different survival and different development duration. Development duration are almost similar except for the first zoea at 20°C and fifth zoea at 30°C. Significant differences ($P<0.05$, <0.01) among the development duration of larvae at different salinity levels tested at different temperature conditions was observed (Table 2).

Total development duration required for metamorphosis from first zoea to first crab stage at five different salinities ranged from 28-32 days at 20°C, 24-29 days at 25°C and 25-30 days at 30°C (Fig. 1). Average development duration was 30 days at 20°C, and 27 days at 25 and 30°C in all tested salinities (15-35‰). However, minimum duration recorded 24 days at 25‰ (25°C), 25 days at 20-25‰ (30°C) and 28 days at 25‰ (20°C). Results show that reduced salinity (15‰) or full-strength seawater (35‰) increased development duration significantly when compared with intermediate salinity (20-25‰). Significant differences ($P<0.01$) in the total duration for complete larval development at different salinity levels tested at different temperature conditions was observed (Fig. 1).

Table 1. Development duration and survival rate of *Neosarmatium trispinosum* from hatching to first crab reared under five different salinity conditions at three constant temperatures. Salinity 0, 5 and 10‰ are not included, since no larvae survived these treatments within 12-18 hours after exposure. Number of live individuals is provided in parenthesis. Z = zoea, M = megalopa

Stages	Temp.	15‰		20‰		25‰		30‰		35‰	
	(℃)	*S (%)*	*D (day)*	*S (%)*	*D (day)*	*S (%)*	*D (day)*	*S (%)*	*D (day)*	*S (%)*	*D (day)*
Z-I	20	65.0 (13)	5.0±0.46	80.0 (16)	5.0±0.37	90.0 (18)	4.0±0.34	100.0 (20)	5.0±0.32	85.0 (17)	5.0±0.35
Z-II	20	92.3 (12)	5.0±0.30	81.3 (13)	4.0±0.29	88.9 (16)	4.0±0.26	95.0 (19)	4.0±0.33	88.2 (15)	5.0±0.27
Z-III	20	83.3 (10)	5.0±0.41	84.6 (11)	4.0±0.15	87.5 (14)	4.0±0.28	89.5 (17)	4.0±0.35	66.7 (10)	5.0±0.33
Z-IV	20	80.0 (8)	4.0±0.32	90.9 (10)	4.0±0.33	78.6 (11)	4.0±0.45	88.2 (15)	4.0±0.38	80.0 (8)	4.0±0.27
Z-V	20	62.5 (5)	4.0±0.32	60.0 (6)	5.0±0.29	72.7 (8)	5.0±0.38	86.7 (13)	4.0±0.29	75.0 (6)	4.0±0.29
M	20	20.0 (1)	9.0±0.00	33.3 (2)	8.0±0.00	37.5 (3)	7.0±0.00	61.5 (8)	8.0±0.38	50.0 (3)	8.0±0.35
Z-I	25	80.0 (16)	5.0±0.37	100.0 (20)	4.0±0.32	100.0 (20)	4.0±0.32	100.0 (20)	4.0±0.32	95.0 (19)	5.0±0.33
Z-II	25	81.3 (13)	5.0±0.29	95.0 (19)	4.0±0.33	100.0 (20)	4.0±0.32	85.0 (17)	4.0±0.25	84.2 (16)	4.0±0.26
Z-III	25	84.6 (11)	4.0±0.32	84.2 (16)	4.0±0.26	95.0 (19)	3.0±0.24	82.4 (14)	4.0±0.28	81.3 (13)	4.0±0.29
Z-IV	25	72.7 (8)	4.0±0.35	81.3 (13)	4.0±0.29	100.0 (19)	4.0±0.33	85.7 (12)	4.0±0.21	84.6 (11)	4.0±0.32
Z-V	25	25.0 (2)	4.0±0.00	84.6 (11)	4.0±0.32	94.7 (18)	4.0±0.24	83.3 (10)	4.0±0.33	72.7 (8)	4.0±0.25
M	25	50.0 (1)	7.0±0.00	9.1 (1)	7.0±0.00	55.6 (10)	5.0±0.33	30.0 (3)	6.0±0.41	25.0 (2)	8.0±0.00
Z-I	30	80.0 (16)	4.0±0.37	100.0 (20)	4.0±0.28	100.0 (20)	4.0±0.32	95.0 (19)	4.0±0.17	65.0 (13)	5.0±0.35
Z-II	30	93.8 (15)	4.0±0.27	100.0 (20)	4.0±0.32	95.0 (19)	4.0±0.33	94.7 (18)	4.0±0.30	76.9 (10)	4.0±0.41
Z-III	30	86.7 (13)	3.0±0.29	95.0 (19)	4.0±0.29	89.5 (17)	4.0±0.31	88.9 (16)	4.0±0.37	70.0 (7)	4.0±0.38
Z-IV	30	84.6 (11)	4.0±0.39	100.0 (19)	3.0±0.24	88.2 (15)	4.0±0.38	87.5 (14)	4.0±0.34	71.4 (5)	4.0±0.00
Z-V	30	90.9 (10)	4.0±0.41	89.5 (17)	3.0±0.31	80.0 (12)	3.0±0.00	78.6 (11)	3.0±0.22	60.0 (3)	4.0±0.00
M	30	40.0 (4)	9.0±0.35	76.5 (13)	7.0±0.29	83.3 (10)	6.0±0.33	27.3 (3)	8.0±0.00	33.3 (1)	9.0±0.00

Table 2. Comparison of larval development duration of *Neosarmatium trispinosum* at different salinity and temperature levels
Z = zoea, M = megalopa, NS = not significant

Source	*DF*	*F-value*						*P-value*					
		Z-I	Z-II	Z-III	Z-IV	Z-V	M	Z-I	Z-II	Z-III	Z-IV	Z-V	M
Salinity	4	20.745	12.834	4.564	3.429	4.000	70.940	<0.01	<0.05	NS	NS	NS	<0.01
Temperature	2	22.340	9.626	89.480	3.429	76.000	73.504	<0.05	NS	<0.05	NS	<0.05	<0.05
Sal. x Temp.	8	6.383	5.615	11.268	3.429	16.000	9.402	<0.01	<0.01	<0.01	<0.05	<0.01	<0.01

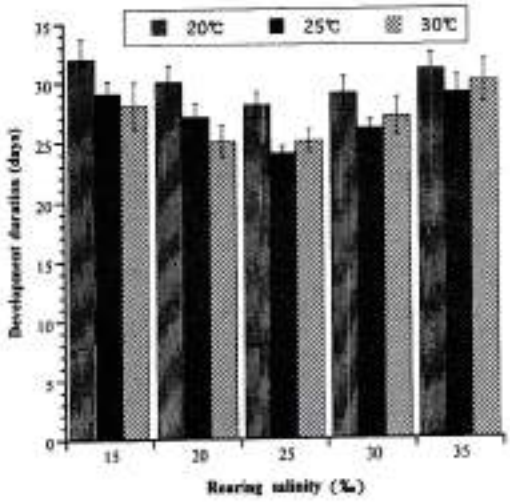

Fig. 1. Development duration to the first crab stage of *Neosarmatium trispinosum* under five different salinity conditions at three constant temperatures. Vertical bars indicate standard deviation. Significant differences 9P<0.01) were found among the salinity levels tested.

Discussion

The larvae of laboratory reared *N. trispinosum* were able to develop successfully through metamorphosis in a wide range of salinities. Compared with the previous observations on its congener *N. indicum* (Islam *et al.* 2002a), however, the larvae in the present study showed a less euryhaline response. A salinity of 20-25‰ at 25-30°C represents a suitable condition for early larval stages and possibly for the megalopa. Hence, the intermediate salinity (20-25‰ at 25-30°C) appears optimum for the larval development of *N. trispinosum*.

At 15‰, *N. trispinosum* larvae had poor survival and delayed development, while Islam *et* al. (2002a) reported for this condition a low mortality and occasionally some survival even at 5 or 10 ‰ in *N. indicum*. Compared with the optimum condition (20- mortality was higher at 15 and 35‰, with an increasing development duration. Development to the first crab stage was completed, with different percentages, at salinities ranging from 15-35‰. This represents a narrow range than in other sesarmid crabs as *Sesarma curacaoense* (Schuh and Diesel 1995a), *Armases miersii* (Schuh and Diesel 1995b), *Perisesarma bidens* (Islam *et al.* 2000) and *N. indicum* (Islam *et al.* 2002a).

Development to the first crab stage in *A. cinereum* was successful at 20.1 and 26.7 ‰ but not at 12.5 and 31 ‰ (Costlow *et al.* 1960) and individuals of *A. ricordi* survived at 15-35‰, but died at 10‰ or lower salinities (Diesel and Schuh 1998), which was similar to the present results. Unfortunately, the above authors and the present study did not test at higher salinities, so that the upper limit of salinity tolerance during the early

development of *A. cinereum, A. ricordi* and *N.* trispinosum remain unknown. In all these species including the present one, the first zoea most probably hatches at low salinity near shore or estuarine waters; subsequent zoeal stages occur in the marine environment, and waters of lower salinities are invaded by the megalopa (Costlow *et al.* 1960, Alvarez and Ewald 1990).

Tolerance of *N.* trispinosum larvae to low salinity decreases in successive stages. The first zoea had high survival rates at 20-30‰, and developed well at 25‰ with a slightly shorter duration. This may reflect an adaptation of the first zoea to regular release in brackishwater and early development in marine water habitats. Laboratory investigation showed that the later zoeal stages of *N.* trispinosum exhibit a slight preference for brackish to marine conditions, where they showed the shortest development and highest survival rates. At 15 and 35‰, they displayed clearly delayed development and increased mortality. This shows that *N. trispinosum* is a species whose development takes place in near shore water.

Results of the present studies revealed that the major part of the larval development of *N. trispinosum* appears to take place in lower estuaries and in coastal oceanic waters, where salinities between ca 20 and 30‰ are found. This salinity range was also found to be optimum for larval development in other brackishwater sesarmid species (Costlow *et al.* 1960, Anger *et al.* 1990, Islam *et al.* 2000, 2002a). The salinity tolerance of other sesarmid larvae decreased during development where the first zoea are released into brackish near shore waters, and subsequent stages occur in the relatively stable conditions in lower estuaries (Schuh and Diesel 1995a,b, Diesel and Schuh 1998, Islam *et al.* 2000, 2002a).

The osmoregulation in decapod crustaceans did not change during development from larval hatching through the adult phase (Charmantier *et al.* 1998, Charmantier and Anger 1999). Successful development of *Sesarma curacaoense* from hatching to the end of the first crab stage through metamorphosis occurred in the full salinity range tested (15-32‰), although mortality was significantly enhanced and development delayed at 15‰ (Anger and Charmantier 2000). Our results are most similar with these findings. Larval survival of *A. miersii* was frequently higher at 15-25‰ than in seawater (Anger 1996), while higher mortality occurred at the extremes of 10 and 55‰. The present result is again similar to those observed in *A. miersii.* Lowest mortality and shortest development duration occurred generally at 15-25‰, indicating an optimum at moderately reduced salinities (Anger *et al.* 2000). The optimal salinity required for complete development of each larval stage of *N. trispinosum* varied at ca. 20-30‰. The salinity of the Shimajiri mangrove swamp is nearly 20-25‰, which is lower than seawater and similar to the rearing water. Hence, the present results suggest that the larvae of *N. trispinosum* develop in estuarine water and recruit to the mangrove swamp at the megalopa stage, where they spend the rest of their lives.

Temperature had a strong effect on development duration in *A. miersii* (Schuh and Diesel 1995b). Larvae of *N. trispinosum* reared at 20°C took a longer period to reach the first crab stage than those reared at 25 or 30°C, and they suffered higher mortality at lower salinities. Similar effects have been reported for other sesarmid species reared

under comparable constant laboratory conditions (Costlow *et al.* 1960, Alvarez and Ewald 1990, Schuh and Diesel 1995b). The development duration of *N. trispinosum* at 25°C was intermediate between those observed at 20°C and 30°C, and survival was higher. The results are similar to those of *A. miersii* (Schuh and Diesel 1995b). The conditions of constant temperature and salinity used in this study are obviously not found in the larvae's natural environment, but the ecological significance of our results can be appreciated when the situation in the larval habitat is considered.

In future studies, larvae should be reared under optimum salinity conditions to the megalopa stage and then transferred to different conditions of gradually decreasing salinity. Those tests combined with field conditions, would hopefully show at which developmental stages return from the sea to brackish or estuarine water, and eventually, to freshwater habitats, and where their metamorphosis takes place (Anger *et al.* 1990). Once the complete life cycle is known, this species could become an interesting and suitable model for the studies of larval development in mangrove sesarmid crabs and hence, a model for the development of physiological adaptations in crustacea during the transition from life in the sea to freshwater and terrestrial environments.

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References

Alvarez, Z. and J. Ewald, 1990. Efectos de la salinidad y la dieta sobre el desarrollo larvario de *Sesarma ricordi* (Milne Edwards, 1853) (Decapoda, Grapsidae). *Scientia Marina*, 54: 55-60.

Anger, K., 1995. The conquest of freshwater and land by marine crabs: adaptations in life-history patterns and larval bioenergetics. *J. Expt. Mar. Biol. Ecol.*, 193: 119-145.

Anger, K., 1996. Salinity tolerance of the larval and first juveniles of a semiterrestrial grapsid crab, *Armases miersii* (Rathbun). *J. Expt. Mar. Biol. Ecol.*, 202: 205-223.

Anger, K. and G. Charmantier, 2000. Ontogeny of osmoregulation and salinity tolerance in a mangrove crab, *Sesarma curacaoense* (Decapoda: Grapsidae). *J. Expt. Mar. Biol. Ecol.*, 251: 265-274.

Anger, K., K. Riesebeck and C. Puschel, 2000. Effects of salinity on larval and early juvenile growth of an extremely euryhaline crab species, *Arma3VIN miersii* (Decapoda: Grapsidae). *Hydrobiologia*, 426: 161-168.

Anger, K., J. Harms, M. Montu and C.D. Bakker, 1990. Effects of salinity on the larval development of a semiterrestrial tropical crab, *Sesarma angustipes* (Decapoda: Grapsidae). *Mar. Ecol. Prog. Ser.*, 62: 89-94.

Browne, R.A. and G. Wanigasekera, 2000. Combined effects of salinity and temperature on survival and reproduction of five species of *Artemia*. *J. Expt. Mar. Biol. Ecol.*, 244: 29-44.

Charmantier, G. and K. Anger, 1999. Ontogeny of osmoregulation in the palaemonid shrimp *Palaemonetes argentinus* (Crustacea: Decapoda). *Mar. Ecol. Prog. Ser.*, 181: 125-129.

Charmantier, G., M. Charmantier-Daures and K. Anger, 1998. Ontogeny of osmoregulation in the grapsid crab *Armases miersii* (Crustacea: Decapoda). *Mar. Ecol. Prog. Ser.*, 164: 285-292.

Costlow, J.D., C.G. Bookhout and R. Monroe, 1960. The effect of salinity and temperature on larval development of *Sesarma cinereum* (Bosc) reared in the laboratory. *Biological Bulletin*, 118: 183-202.

Dai, A. and S. Yang, 1991. Crabs of the China Seas. China Ocean Press, Beijing, China. 543 pp.

Davie, P.J.F., 1994. Revision of *Neosarmatium* Ser~ne and Soh (Crustacea: Brachyura: Sesarminae) with descriptions of two new species. *Memoirs of the Queensland Museum*, 35: 35-74.

De Man, J.G., 1889. Uber einige neue oder seltene indopqcifische Brqchyuren. *Zoologische Jahrbuchner, Jena* 4: 409-552.

Diesel, R. and M. Schuh, 1998. Effects of salinity and starvation on larval development of the crabs *Armases ricordi* and *A. roberti* (Decapoda: Grapsidae) from Jamaica, with notes on the biology and ecology of adults. *J. Crust. Biol.*, 18: 423-436.

Giddins, R.L., J.S. Lucas, M.J. Neilson and G.N. Richards, 1986. Feeding ecology of the mangrove crab *Neosarmatium smithi* (Crustacea: Decapoda: Sesarmidae). *Mar. Ecol. Prog. Ser.*, 33: 147-155.

Hartnoll, R.G., 1988. Evolution, systematics, and geographical distribution. *In:* and B.R. McMahon (W.W. Burggren ed.), Biology of the Land Crabs. Cambridge University Press, New York. pp. 6-54.

Islam, M.S., S. Shokita and T. Nagai, 2000. Effects of salinity on the larval development of the mangrove dwelling semiterrestrial sesarmine crab, *Perisesarma bidens* (De Haan). *Crust. Res.*, 29: 152-159.

Islam, M.S., S. Shokita and T. Naruse, 2002a. Effects of salinity on the larval development of the semiterrestrial sesarminid mangrove crab *Neosarmatium indicum* (*A.* Milne Edwards) under laboratory conditions. *Crust. Res.*, 31: 1-8.

Islam, M.S., S. Shokita and N. Shikatani, 2002b. Larval development of the mangrove sesarmid crab *Neosarmatium indicum* (Brachyura: Grapsoidea) described from laboratory reared materials. *J. Crust. Biol.*, 22: 916-937.

Islam, M.S., T. Yarnazaki and S. Shokita, 2003. Larval development of the mangrove sesarmid crab *Neosarmatium fourmanoiri* (Brachyura: Grapsoidea) reared in the laboratory. *Crustaceana*, 76: in press.

Kinne, 0., 1963. The effect of temperature and salinity on marine and brackish water animals. 1. Temperature. *Oceanography and Marine Biology Annual Review*, 1: 301-340.

Milne Edwards, A., 1868. Etudes zoologiques Crustaces des iles Celebes provenant. *Nouvelles Archives du Museum d'Histoire Naturelle*, 4: 173-185.

Milne Edwards, H., 1853. Memoires sur la famille des Ocypodiens, suite. *Annales des Sciences Naturalles*, 20: 163-228.

Neilson, M.J. and G.N. Richards, 1989. Chemical composition of degrading mangrove leaf litter and changes produced after consumption by mangrove crab *Neosarmatium smithi* (Crustacea: Decapoda: Sesarmidae). *J. Chem. Ecol.*, 15: 1267-1283.

Ng, P.K.L., H.C. Liu and C.H. Wang, 1996. On the terrestrial sesarmine crabs of the genus *Neosarmatium* (Crustacea: Decapoda: Brachyura: Grapsidae) from Taiwan. *J. Taiwan Museum*, 49: 145-159.

Pereyra Lago, R., 1989. The larval development of the red mangrove crab *Sesarma meinerti* de Man (Brachyura: Grapsidae) reared in the laboratory. *South African J. Zool.*, 24: 199-211.

Schubart, C.D., J.A. Cuesta R. Diesel and D.L. Felder, 2000. Molecular phylogeny, taxonomy, and evolution of nonmarine lineages within the American grapsoid crabs (Crustacea: Brachyura). *Molecular Phylogenetics and Evolution*, 15: 179pp.

Schuh, M. and R. Diesel, 1995a. Effects of salinity, and starvation on larval development of *Sesarma curacaoense* De Man, 1892, a mangrove crabs with abbreviated development (Decapoda: Grapsidae). *J. Crust. Biol.*, 15: 645-654.

Schuh, M. and R. Diesel, 1995b. Effects of salinity, temperature, and starvation on larval development of *Armases (= Sesarma) miersii* (Rathbun, 1897), a serniterrestrial crab with abbreviated development (Decapoda: Grapsidae). *J. Crust. Biol.*, 15: 205-213.

Serene R., 1973. Notes sur quelques especes de Brachyoures de Nouvelle-Caledonie. *Cahiers Pacifique*, 17: 119-16 1.

Sheaves, M. and B. Molony, 2000. Short-circuit in the mangrove food chain. *Ma. Ecol. Prog. Ser.*, 199: 97-109.

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An empirical study on income and efficiency of pond fish and nursery fish production in some selected areas of Pabna, Bangladesh

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Abstract
The study was designed to determine the costs, returns and relative profitability of pond fish and nursery fish production. In order to attain this objective, a total of 70 producers: 35 producing pond fish and 35 producing nursery fish were selected on the basis of purposive random sampling technique from 6 villages under two Upazilas (Sujanagar and Santhia) of Pabna district. It was estimated that per hectare per year gross cost of pond fish production was Tk 65,918 while gross return and net return were Tk 91,707 and Tk 25,789 respectively. Per hectare per year gross cost of nursery fish production was Tk 87,489 while gross return and net return were Tk 1,39,272 and Tk 51,783 respectively. The findings revealed that nursery fish production was more profitable than pond fish production. Cobb-Douglas production function was applied to realize the specific effect of the factors on pond fish and nursery fish production. It was observed that most of the included variables had significant impact on pond fish and nursery fish production. Out of five variables included in the function, all the variables had positive impact on return from pond fish production but stock value of pond, material cost and pond area had positive impact on return from nursery fish production.

Key words: Economic efficiency, Nursery pond

Introduction

Fisheries sector plays an important role in nutrition, employment and foreign exchange earnings in the economy of Bangladesh. Most of the people in this country depend on fish as main source of animal protein. About 63 percent of animal protein is supplied by fish alone (DoF 2002). It has been estimated that about 1.3 million people are directly employed in this sector. Another 12 million people indirectly earn their livelihood from fisheries related activities. Frozen shrimp, fish and fishery products occupy the third position in the country's exports (5.77% of total foreign exchange earning). In 2000-2001, fisheries sector contributed 5.3 percent to the total GDP of the country (DoF 2002). The country's total production of fish was 17,81,057 tones in 2000-2001 of which 14,01,560 tones were from inland sources and 3,79,497 tones from the marine sources. The growth rate of the production during the last decade on an average

was 7.11 percent. However, the present growth rate is quite encouraging which, in fact, is 7.20 percent (DoF 2002).

In Bangladesh, there are two sources of fisheries- inland fisheries and marine fisheries. The contribution of the inland fisheries is 78.69 percent to the total catch while the marine's contribution is 21.31 percent. Of the different inland water bodies, ponds are the most important for their easy access to fish production and about 79 percent of total production of fish comes from inland sources and of this, about 40 percent comes from ponds. The total area of ponds and ditches is 2,41,500 hectares (DoF 2002). The need for fish seed production is now more increasingly felt than ever before as natural seed collection has been alarmingly depleted due to many natural and man made causes. In Bangladesh, there are about 779 private and 113 public hatcheries. The annual fish seed/fry production is about 2,20,217 kg. The stocking size of fish seed of the country comes from nurseries, both public and private nurseries. In the country, there are about 4,133 private nurseries, with an average area of 1 hectare each and 82 government nurseries covering an area of about 60 hectares. In 2000-2001, both private and public nurseries produced 5,055 million fingerlings (DoF 2002).

In Bangladesh, increased aquacultural production, mainly pond fish production can help to meet the increased domestic demand for fish (20.75 lakh ton/2.075 million tones; FFYP, 1997-2002). In order to meet the shortage of fish, the Department of Fisheries (DoF) and some Non-government Organizations (NGOs) are encouraging people to increase fish production in their surrounding water areas (pond, *haor, baor, beel* etc.). In response to government's initiative for augmenting fish production in the country, people have started to be occupied in different types of fish production activities. The two important activities are raising of fingerlings and production of table fish using different types of technologies popularised by various government and non-government agencies.

The focus of the present study is to provide information about comparative profitability of pond fish and nursery fish production. Moreover, results of production function analysis can indicate which input is used efficiently. Some studies (Islam and Dewan 1987, Khan 1996, Malek 1997, Rahman *et al.* 1997, Rahman *et al.* 1998 and Siddique 1999) were conducted on pond fish production and fish seed/fingerling production based on economic returns. In this context, the present study was aimed to determine the relative profitability and resource use efficiency of pond fish and nursery fish production.

Methodology

The study was carried out from January to November 2000 in selected areas of Pabna district. A set of interview schedules was pre-tested and developed. Data were collected from 6 villages under two Upazilas (Sujanagar and Santhia) of Pabna district. A total of 70 producers: 35 producing pond fish and 35 producing nursery fish were selected on the basis of purposive random sampling technique. In this study, a simple tabular method was followed to illustrate the whole picture of analysis. The sum, mean,

percentage, ratio, etc. were the simple statistical measures employed to show the comparative performance of pond fish and nursery fish production. Relative profitability of pond fish and nursery fish production has been determined on the basis of net return analysis.

Net return analysis

To determine the net returns from pond fish and nursery fish production, gross costs (variable and fixed cost) were deducted from gross returns. An easy principle to determine costs and return was followed to determine the profitability of pond fish and nursery fish production. For this purpose the following equation was used (Dillon and Hardaker 1993).

The equation has been applied for each of the selected producers.

$$\Pi = Pm \bullet Ym + P_b \bullet Y_b - \sum_{i=1}^{n} (Px_i \bullet X_i) - TFC \quad \text{Where,}$$

Π	=	Net return
Pm	=	Price of main product per unit
Ym	=	Total quantity of main product
P_b	=	Price of by-product per unit
Y_b	=	Quantity of by-product
Px_i	=	Price of ith input per unit for producing pond fish / nursery fish
X_i	=	Quantity of the ith input for producing pond fish / nursery fish
TFC	=	Total fixed cost
i	=	1, 2, 3, - - - - - - - , n (number of input)

Functional analysis

To explore the effects of variable inputs both linear and Cobb-Douglas production function models were estimated initially. Data were converted to per farm basis to facilitate the analysis. The results of the Cobb-Douglas models appeared to be superior on theoretical and econometric grounds. So the Cobb-Douglas model was accepted for interpretation. Five independent variables were employed to explain the gross returns from pond fish and nursery fish production in the study areas. Regression analysis (ordinary least squares) method was used to determine the effect of these inputs. A series of regression procedures were carried out to be sure that serious multicollinearity problem did not exist. Cobb-Douglas production function analysis was done taking 35 pond fish farms and 35 nursery fish farms into account separately. The function was specified as:

$$Y = a x_{1i}^{b1} x_{2i}^{b2} x_{3i}^{b3} x_{4i}^{b4} x_{5i}^{b5} e^{Ui}$$

The function was linearised by transforming it into the following double log or log linear form:

$$\ln Y = \ln a + b_1 \ln X_{1i} + b_2 \ln X_{2i} + b_3 \ln X_{3i} + b_4 \ln X_{4i} + b_5 \ln X_{5i} + U_i$$

Where,

Y = Gross return from fish/fingerlings production per farm (Tk);
X_1 = Stock value of pond per farm (Tk);
X_2 = Material cost per farm (Tk);
X_3 = Labour cost per farm (Tk);
X_4 = Pond area (hectare);
X_5 = Depth of pond water (metre);
ln = Natural logarithm;
a = Intercept;
b_i = Production coefficients; and
U = Error term.

Some important inputs like feed, fertilizer and chemicals were included in the model as material input. Furthermore, few important variables like experience in pond keeping, number of ponds, duration of water etc. which might affect pond fish and nursery fish production could not be included in the model due to non-availability of appropriate data for the model.

Results and discussion

Costs and returns

Among the different cost items, cost of fish seed appeared to be the highest and represented 55 and 50 percent of total cost of pond fish and nursery fish production. The average per hectare cost per year amounted to Tk. 59,532 and Tk. 75,267 for pond fish and nursery fish production (Table 1).

Table 1. Annual cost/ha of different items required for pond fish and nursery fish production

Cost items	Pond fish production	Nursery fish production
Fish seed	32,562.79 (54.70)	37,929.59 (50.39)
Feed	5,950.98 (10.00)	2,704.22 (3.59)
Fertilizer	2,120.69 (3.56)	4,443.29 (5.90)
Human Labour	13,121.55 (22.04)	17,895.80 (23.78)
Chemicals	1,431.71 (2.40)	6,056.95 (8.05)
Miscellaneous*	4,344.38 (7.30)	6,237.46 (8.29)
Total	59,532.10 (100)	75,267.31 (100)

Source: Haque 2000 Note: Numbers in the parentheses indicate the percentage of total cost.
Miscellaneous included entertainment, transportation, medicine etc.

Table 2 captures information on cost and return per hectare of pond fish and nursery fish production. Gross returns from pond fish and nursery fish production amounted to Tk.91,707 and Tk.1,39,272 respectively. Gross costs were Tk. 59,532 and Tk.75,267 per hectare of pond fish and nursery fish production. The net returns from pond fish and nursery fish production were computed at Tk. 32,175 and Tk. 64,005 per hectare. Net returns per taka invested were Tk. 0.54 and Tk. 0.85 for pond fish and nursery fish production respectively. The benefit cost ratios (BCR, undiscounted) of pond fish and nursery fish were 1.54 and 1.85 respectively, indicating that production of fish and fingerlings was profitable. A comparison of the net returns from production of pond fish and nursery fish suggested that the net return of nursery fish was higher than that of pond fish.

Table 2. Per hectare annual cost and return from pond fish and nursery fish production

Measures	Pond fish production	Fish nursery operation
Gross return (Tk/ha)	91,706.61	1,39,272.20
Gross cost (Tk/ha)	59,532.10	75,267.31
Net return (Tk/ha)	32,174.51	64,004.89
Net return per Taka invested	0.54	0.85
Benefit cost ratio (BCR)	1.54	1.85

Source: Haque 2000

Factors affecting pond fish and nursery fish production

Fish and fingerlings production in ponds results from the effects of the various inputs employed in the production process. Inputs used in any kind of production may be classified broadly into material inputs and labour inputs. Furthermore, in pond operation there are some inherent characteristics of pond environment and factors that affect its production such as pond area, depth of pond water and these factors can be employed to explain the variation in output of ponds. Accordingly, some crucial inputs have been included to explain the variation in productivity of fish ponds.

Interpretation of results

Estimated values of coefficients and related statistics of the Cobb-Douglas production function for pond fish production and fish nursery operation are shown in Table 3. From the table, the following features emerge:

The function fitted well for pond fish and nursery fish production as indicated by F-values and R^2. The coefficients of multiple determination, R^2 were 0.897 for pond fish

and 0.916 for nursery fish production. R^2 of 0.897 for pond fish indicated that variables included in the model succeeded in explaining about 90% of the total variations in the value of pond fish. On the other hand, R^2 of 0.916 indicated that about 92% variations in output of nursery fish was explained by the explanatory variables included in the model. The F-values of the two equations were highly significant at 1% levels implying that all the included explanatory variables were important for explaining the variations in pond fish and nursery fish output. The sum total of all the production coefficients (production elasticities) of the equations for pond fish and nursery fish production were 1.285 and 0.869. This indicated that the production function exhibited increasing returns to scale for pond fish production while it indicated decreasing returns to scale for nursery fish production.

Interpretation of Coefficients for Individual Variables

Stock value of pond (X_1): The regression coefficients of stock value of pond were positive for pond fish and nursery fish farm and significant at 1 percent and 5 percent levels respectively indicating that each one percent increase in the cost of fry and fingerlings, keeping other factors constant, would increase gross returns by 0.226 and 0.393 percent respectively.

Table 3. Estimated values of coefficient and related statistics of double-log production function model

Explanatory variables	Pond fish production	Nursery fish production
Intercept	4.936	8.212
Stock value of pond (X_1)	0.226***	0.393**
	(0.080)	(0.196)
Material cost (X_2)	0.293**	0.314*
	(0.144)	(0.178)
Labour cost (X_3)	0.226**	-0.377***
	(0.100)	(0.142)
Pond area (X_4)	0.404**	0.699**
	(0.172)	(0.270)
Depth of pond water (X_5)	0.136	-0.160
	(0.207)	(0.322)
R^2 (adjusted)	0.897	0.916
F	60.256***	74.921***
Returns to scale ($\sum$ bi)	1.285	0.869

Note: Figures in the parentheses indicate standard error.
*** Significant at 1% level. ** Significant at 5% level. * Significant at 10% level.

Material cost (X_2): The coefficients of material cost were positive for pond fish and nursery fish farm and significant at 5 percent and 10 percent levels respectively. It

revealed that each one percent increase in the material cost, keeping other factors constant, would increase gross returns by 0.293 and 0.314 percent respectively.

Labour cost (X_3): The regression coefficient of human labour cost was positive for pond fish production and significant at 5 percent level indicating that one percent increase in cost of this input, keeping other factors constant, would increase the gross return of pond fish production by 0.226 percent. On the other hand, the coefficient of human labour cost was negative for nursery fish production and significant at 1 percent level, which implied the indiscriminate and excessive use of this input resulting in inefficiency.

Pond area (X_4): The coefficients of pond area were positive for pond fish and nursery fish production and significant at 5 percent levels. It revealed that each one percent increase in pond area, keeping other factors constant, would increase gross returns by 0.404 and 0.699 percent respectively.

Among the inherent variables, depth of pond water has no significant impact on pond fish and nursery fish production.

Results of the regression coefficients suggest that most of the variables included in the production function were significant in explaining the gross returns from pond fish and nursery fish production. The coefficients of stock value of pond, material input, labour cost and pond area were highly significant for both the practices.

Conclusions

The study reveals that nursery fish production is more profitable than pond fish production. The results of the study indicate that both pond fish and nursery fish production can be increased by efficient reallocation of factor inputs. It is evident from regression coefficients that stock value of pond, material input, labour cost and pond area of both the enterprises emerged as crucial factors. In the case of nursery fish production, labour cost demonstrated negative coefficient, which implied the indiscriminate and excessive use of the resource resulting in inefficiency. It may be suggested that the producers have scope to attain full efficiency by reallocating the resources in both the practices.

References

Dillon, J.L. and J.B. Hardaker, 1993. Farm Management Research for Small Farmer Development. FAO, Agricultural Services Bulletin 41. Food and Agricultural Organization of the United Nations, Rome.

DoF, 2002. Smranika, Motsha Pakkha, 10-24 August, 2002. Department of Fisheries, Fisheries and Livestock Ministry, Dhaka.

GOB, 1998. The Fifth Five Year Plan 1997-2002. Government of the People's Republic of Bangladesh, Planning Commission, Dhaka.

Gujarati, D.N., 1995. Basic Econometrics. 3rd edn. McGraw Hill, Inc., New York.

Haque, M.M., 2000. A Comparative Economic Analysis of Pond Fish Production and Fish Nursery Operation in Some Selected Areas of Pabna District. M.S. Ag. Econ. Thesis, Bangladesh Agricultural University, Mymensingh.

Islam, M. S. and S. Dewan, 1987. An Economic Analysis of Pond Fish Production in Some Areas of Bangladesh. Research Report No. 11, Bureau of Socioeconomic Research and Training, Bangladesh Agricultural University, Mymensingh.

Khan, A.H., 1996. A Comparative Study of Fingerlings and Pond Fish Production in Some Selected Areas of Mymensingh District. M.S. Ag. Econ. Thesis. Bangladesh Agricultural University, Mymensingh.

Koutsoyiannis, A., 1987. Theory of Econometrics. 2nd edn., Macmillan, London.

Malek, A.M., 1997. An Economic Study on Fish Seed Multiplication Farms in Bangladesh. M.S. Ag. Econ. Thesis. Bangladesh Agricultural University, Mymensingh.

Rahman, M.H., M.Z. Ali, A.F.M. Shofiquzzoha and M. Nurullah, 1998. Efficiency of pond fish production in Bangladesh. *Bangladesh J. Agricul. Sci.*, 25(2): 235-239.

Rahman, M.H., A.F.M. Shofiquzzoha, M.Z. Ali and M. Nurullah, 1997. Nursery fish production in Bangladesh: An empirical study on income and efficiency. *Economic Affairs*, 42(1): 23-27.

Siddique, S., 1999. An Economic Analysis of Fish Seed Multiplication Farm in Some Selected Areas of Bangladesh. M.S. Ag. Econ. Bangladesh Agricultural University, Mymensingh.

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Short Note

Eco-biology *of Mastacembelus pancalus* (Ham.) and their distribution in different water bodies

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Abstract
The eco-biological of the spiny eel, *Mastacembelus pancalus* in the river Padma, adjacent flood plains and ponds were influenced by various physico-chemical factors such as water temperature, water transparency, pH, dissolved oxygen, free carbon dioxide and alkalinity. Flood plain areas are the best habitat for the *M. pancalus* with maximum abundance.

Key words: Eco-biological condition, Padma, Flood plains, *M. pancalus*

Mastacembelus pancalus is a common freshwater spiny eel of the family Mastacembelidae locally known as baim. The present study area comprised of the river Padma and flood plain areas is supposed to be ideal for freshwater habitat of lotic typed and lentic typed for the occurrence of *M. pancalus*. The systematic account and studies reported by Karim and Hossain (1972 a & b), Shafi and Quddus (1982), Rahman (1989), Jhingran and Talwar (1991) indicated that this fish remained virtually unstudied.

To establish the eco-biology of *M. pancalus*, fortnightly water samples were collected for a period of one year in the river Padma, adjacent flood plains area (beel) and ponds. The water temperature was recorded with a mercury thermometer, pH by pocket pH meter (WQC-OA, TOA). Dissolved oxygen (DO) and free carbon dioxide (CO_2) were measured following the methods described by APHA (1989) and Welch (1948), respectively. Water current, aquatic vegetation and abundance of the fish were also investigated. The monthly meteorological recorded data on the air temperature, rainfall were collected from the Meteorological Department of Rajshai.

The physico-chemical parameters of the river Padma, flood plain areas and ponds are exhibit maximum and minimum variations according to change of month and season are shown in Table 1, and eco-biological variation in distribution of *M. pancalus* is given in Table 2.

Table 1. The maximum and minimum limit of physio-chemical parameters

Parameters	Limits	Water bodies		
		River	Flood plains	Pond
Air temp.	Maximum	April	April	May
	Minimum	January	January	January
Water temp.	Maximum	May	April	May
	Minimum	January	January	December
Rainfall	Maximum	September	December	September
	Minimum	December	April	December
Transparency	Maximum	April	December	August
	Minimum	August	December	May
pH	Maximum	January	July	April
	Minimum	July	November	August
Dissolved oxygen	Maximum	Oct.-May	August	April
	Minimum	July-Sep.	April	August
Carbon-di-oxide	Maximum	June-Nov.	Feb.-May	June-Dec.
	Minimum	Dec.-May	June-Jan.	Jan.-May

Table 2. Eco-biological distribution of *M. pancalus* in different water bodies

Ecological condition	River	Flood plains	Pond
Soil texture	Sandy and sandy loamy	Mostly sandy loamy	Mostly clay
Water current	Very common	Mostly stagnant, rare in monsoon	Stagnant
Water temp.	13.5-29.8°C	16.7-28.9°C	28-31.4°C
Transparency	0.05-1.24m	0.31-0.43m	0.35-0.46m
Dissolved oxygen	3.15-5.95mg/l	4.23-8.33mg/l	3.19-6.54mg/l
Carbon-di-oxide	2.62-8.41mg/l	2.69-8.66mg/l	2.77-9.83mg/l
pH	6.75-8.1	6.97-7.63	6.83-8.39
Aquatic vegetation	Rare	Rare	Common
Species abundance	Common during monsoon, rare in rest period	Very common	Rare

On the observation, among the 3 areas studied, *M. pancalus* were more abundant in flood plain areas then ponds or river Padma. Among the fishing season winter season and early part of the summer are found as peak period for *M. pancalus* along with other fishes because flood plains becomes calm and water starts to be vacated. It was observed that this species spawn in the monsoon.

Among the different parameters, rainfall is an important factor in the breeding of *M. pancalus* as cloudy day accompanied by the thunderstorm and rain, seen to exercise some influenced on spawning (Saha *et al.* 1957). In the river Padma, the minimum turbidity was found in the monsoon and post monsoon months like June to September due to strong current of water which washed away huge silt in water including many

other suspended matter. From October onwards up to May, water become slowly clear with the maximum values of transparency due to absence of such disturbing matters (Hickman 1979, Hossain 1989).

The calculated mean values of pH were recorded in the Padma indicated neutral or slightly alkaline. In the flood plains and ponds, the pH values were almost same to the river water (Table 1). Among the dissolved gases, in the flood plain areas the average dissolved oxygen (DO) contents is more than the other water bodies (Table 2). The fluctuation in the DO concentration is mainly influenced by the factors like dissolved organic matter, plankton and bottom vegetation, The values of free carbon dioxide show inverse relationship to the oxygen.

The present investigation aiming the physico-chemical condition of three types of water bodies and a comprehensive observation do, however, make it possible to describe which factors play a definite role are in need of more precise analysis.

References

American Public Health Association (APHA), 1989. Standard methods for the examination of water and waste water. American Public Health Assciation Washington DC.

Hickman, M., 1979. Seasonal succession, standing crop and determinants of tile phytoplankton of Ministik lake, Alberta, Canada, *Hydrobiologia*, 64: 105-121.

Hossain, M.A., 1989. On the methods of determining the reproductive cycle in fisheries species. 76th Ind. Sc. Cong. Ass. Madurai, India.

Jhingran, A.G. and P.K. Talwar, 1991. Inland Fishes of India and Adjacent Countries. Oxford & IBH Publishing Co. Pvt. Ltd. New Delhi, India. Vol (1&2): 1158 pp.

Karim M.A. and M.A. Hossain, 1972a. Studies on the biology *of Mastacembelus pancalus* (Ham.) in artificial ponds. Part-1, Natural habitat, distribution, food and feeding habits and economic importance. *Bangladesh J. Biol. Sci.*, 1(2): 10-14.

Karim, M.A. and M.A. Hossain, 1972b. Studies on the biology of *Mastacembelus pancalus* (Ham.) in artificial ponds. Part-II, Sexual maturity and fecundity. *Bangladesh J. Biol. Sci.* 1(2): 15-18.

Saha, K.C., D.P. Sen, A.K.R. Chowdhury and Chakrabarty, 1957. Studies on the factors influencing spawning of Indian major carps in "bundh" fisheries. *Indian J. Fish.*, 4: 284 - 294.

Shafi, M. and M.M.A. Quddus, 1982. Bangladesher Matsaw Sampad. Bangla Academy. Dhaka. 444 pp. (in bengali)

Welch, P.S., 1948. Limnological Methods. McGraw-Hill. Publ., New York. 381 pp.

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