Vol. 1 (1)
January 1997

ISSN : 1026-6690

Bangladesh Journal of Fisheries Research

Bangladesh Fisheries Research Institute

Bangladesh Journal of Fisheries Research

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

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

Preface

Bangladesh Fisheries Research Institute (FRI) was established under an ordinance of the Government of the Peoples' Republic of Bangladesh in 1984 as a national institute for Carrying out and coordinate fisheries research in Bangladesh. The Institute was reorganized and upgraded through 'Bangladesh Fisheries Research Institute (Amendment) Act, 1996' in line with the structure of the National Agricultural Research Institutes. Keeping in view of immense potential of the fisheries sector in fulfilling the national protein requirement and emancipating rural economic development, the institute was mandated to undertake appropriate research programmes with sound and scientific base to maximize fish production through aquaculture and scientific management of the country's fisheries resources. FRI's broad spectrum of activities cover research and training on scientific fisheries thus enabling it to address the constraints facing promotion of sustainable development of the sector.

The overall research and planning activities of the Institute are carried out in close cooperation and collaboration with various regional and international institutes and agencies involved in fisheries R & D activities. The Institute also maintains liaison with national Universities/ Institutes of the country for implementing collaborative research and manpower development. Thus, FRI as a nodal institution provides leadership in fisheries sector R & D activities. The institute plays an important role in planning and carrying out appropriate research to evolove new and appropriate technologies to disseminate to the farmers, entrepreneurs and scientific communities in order to contribute to sustainable resource development.

Considering the significant accomplishment of FRI in generating technical information through a strong feed-back system in the overall research approach of the Institute, it is indeed necessary to publish a scientific journal for documentation of findings for the propose of scientific use by researchers and development workers working in this field. Keeping this in view, FRI has initiated

to publish regularly a journal in the title of **Bangladesh Journal of Fisheries Research.**

The publication of BJFR marks another milestone in the brief history of the Institute. The Journal will serve as an effective mechanism for the dissemination of fisheries research and other relevant information to scientists of not only this Institute but also other institutions at home and abroad. The maintenance of the journal should be the role of the scientists, researchers, development workers who we wish may find the journal useful. Thanks to those who submitted manuscripts. We invite more manuscripts and also personal as well as institutes' subscription.

Dr. M.A. Mazid
Director General

Bangladesh J. Fish. Res., 1(1) : 01-08
Bangladesh Fisheries Research Institute

Disinfection of aquarium effluents by chlorination and UV treatment

M.N. Islam[1,*], M.A.R. Faruk[1] and V. Inglis

Institute of Aquaculture, University of Stirling, Scotland, UK
[1] *Department of Fisheries Technology*
Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

[*] *Corresponding author*

Abstract

The study was conducted to investigate the efficacy of chlorine and UV irradiation in disinfecting aquarium effluent. A non-agglutinating, avirulent strain of *Aeromonas salmonicida* (NCIMB 1102) was used as the test organism. Effluents from a fish tank were inoculated with a suspension of test organisms and subsequently treated with different concentrations of hypochlorite and UV irradiation separately and simultaneously. When used alone, 1.0 ppm hypochlorite reduced the viable cell count from 6.5 log to 3.0 log within 20 minutes of contact period. On the otherhand, when used in combination with UV irradiation only 0.5 ppm hypochlorite exerted the same bactericidal effect within the same contact period as was observed with 1.0 ppm hypochlorite alone. This result indicated that required dose of disinfectant for the disinfection of aquarium effluents can be considerably reduced when it is used in combination with UV irradiation.

Key words : Disinfection, Effluent, Chlorination, UV

Introduction

Effluents from aquacultural activities are a potential source of microorganisms pathogenic to fish and shellfish in the wider environment. Scientific laboratories working with the infected fish and pathogens in particular, are high risk activities since many of these species survive well in the aquatic environment. In order to prevent the possible spread of such pathogens in the environment, disinfection of waste water is of utmost importance (Austin and Austin 1993).

Chlorine is a strong oxidizing agent and rapidly penetrates microbial cells and kills the microorganism. Death results from the chemical reaction of hypochlorous acid with the enzyme triphosphate dehydrogenase which is essential to life process of the cell (White 1972). Chlorine, at a concentration of

1.0 to 0.2 mg/ml, has been demonstrated to efficiently remove bacterial pathogens like *Aeromonas salmonicida* and *Yersinia ruckeri* from natural lake waters within one minute (Wedmeyer and Nelson 1977) whereas infectious hematopoietic necrosis virus (IHNV) and infectious pancreatic necrosis virus (IPNV) were more resistant and required 0.7 to 1.0 /mg ml for inactivation.

Fish pathogenic bacteria, in general appear to be sensitive to UV light and unlike halogen disinfectants UV irradiation of water does produce undesirable by-products toxic to fish (Oliver and Carey 1976). Doses of 1.5 to 3.4 mWs/cm^2 have been reported to inactivate 99.9% of *Vibrio salmonicida*, *V. anguillarum*, *Yersinia ruckeri* and *A. salmonicida* (Sako and Sorimachi 1985). High intensities, *viz*, 4 to 10 mWs/cm^2 were required for human pathogenic and water quality indicator bacteria like *Salmonella*, *Escherichia coli* and *Vibrio* sp. (Chang *et al.* 1985 and Harris *et al.* 1987).

Combined application of a chemical disinfectant and UV irradiation may result in increased efficacy with lower levels of chemicals. Several investigators have expressed interest in the combined organic compounds in effluents and for enhanced disinfection (Venosa *et al.* 1984 and Glaze *et al.* 1991). Combining halogens and UV have not received the attention in water treatment studies. But in a recent studies it was shown that UV and chlorine based disinfectant had a synergistic effect (Liltvet and Landfald 1995, unpublished data).

The aim of this study was to investigate the disinfection efficiency of chlorine and UV irradiation in aquarium effluent and to determine if simultaneous application of chlorine/UV in these increased the efficacy.

Materials and methods

Test organism and growth procedure

A non-agglutinating, avirulent strain of *Aeromonas salmonicida* (NCIMB 1102) was used as a model bacterium in this study because virulent strain of this organism is highly pathogenic to salmonid fish causing septicemia and abundant in both fresh water and marine environment in the colder region. The bacteria were grown at 22°C for 3 days in tryptone soya agar (TSB, Unipath, Basingstoke). Cells were harvested by centrifugation at 2500 rpm for 10 minutes and the resultant pellet was resuspended in 0.85% sterile saline solution. The suspension was further diluted to give a concentration of approximately 10^8 cfu /ml.

Preparation of disinfectant and neutralizing solutions

Chlorine stock solutions were prepared from a sodium hypochlorite solution (14% w/v available chlorine). Appropriate volumes were added to distilled deionized water to obtain 10, 20, 30 and 40 ppm free chlorine. Stock solutions

(11, 22, 33 and 44 ppm) of a neutralizing solution for hypochlorite were prepared by dissolving appropriate quantities of sodium thiosulfate in distilled water. Four separate stock solutions of hypochlorite were prepared so that when 0.5 ml was added to 9.5 ml of the test sample the required concentration range of 0.5 to 2.0 ppm was attained. Similarly, since 1 gm of active chlorine is effectively removed by 2 gm of thiosulfate (Hom 1971), stock solutions of sodium thiosulfate from 11 to 44 ppm were prepared so that when 1 ml was added to the 10 ml test sample there will be a concentration ratio of 2:1.

Fish tank and UV assembly

Twenty five rainbow trout (100-150 gm each) were introduced in a tank of 100 gallon capacity and held for several days with standard feeding and husbandry. The tank was fitted with a pipe in the middle to discharge the effluent into sump tank (100 gallon plastic tank) mounted on the floor below the fish tank. The sump tank had two overflow pipes mounted near the top of the tank but below the level of the fish tank outlets. A submersible pump with integral float switch was mounted inside the sump tank to pump water through the UV unit (Aquarium sterilizer-Model 30) and then to waste. A side arm with valve was also present to control the rate at which water was pumped from the sump.

Water sample preparation

The effluent from the fish tank was collected from the sump tank in a sterile conical flask and brought to the laboratory. The water contained dissolved and suspended organic matter from feed pellets, faecal materials and other waste. The sample was heated at 80°C for 10 min in a water bath to kill the indigenous bacteria and was subsequently cooled to 7°C.

Hypochlorite treatment

Heat treated and cooled water sample was inoculated with the previously prepared bacterial suspension to obtain a suspension of approximately 10^7 cells/ml. From this suspension 9.5 ml aliquots were taken in 4.5 cm diameter sterile plastic disposable petri plates and 0.5 ml stock solutions of different concentrations of hypochlorite were added. Petri plates with the samples were immediately put in an ice bath (ice-water temperature 7°C) placed over a magnetic stirrer. After the required contact periods with stirring 1.0 ml of stock solution of different concentrations of neutralizing solution was added to each sample. After neutralizing for 1 min samples were subjected to total viable count according to drop count method (Miles and Misra 1938). The experimental procedure has been summarized in Fig. 1.

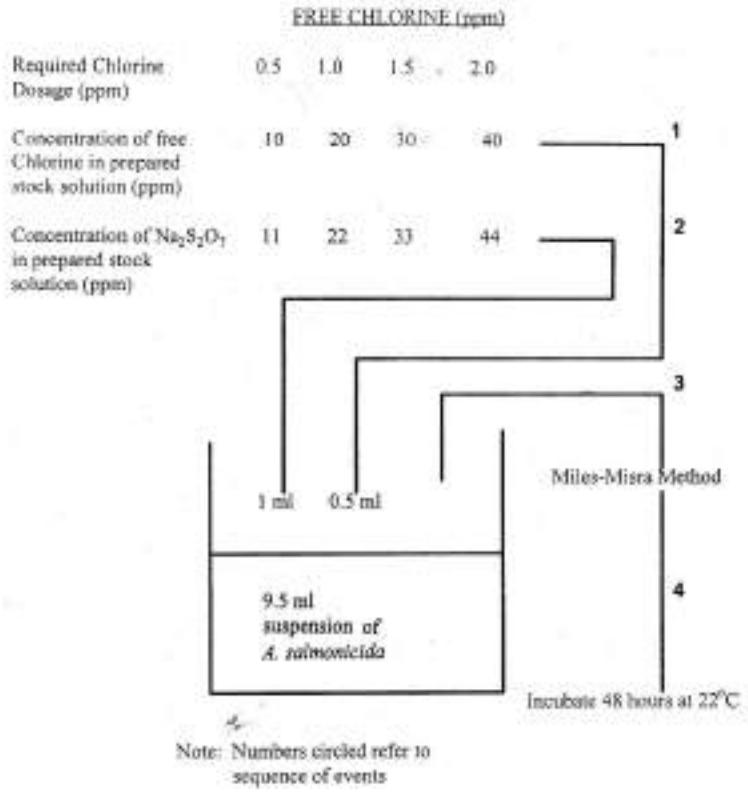


Fig. 1. Experimental procedure to assess bactericidal effect of sodium hypochlorite.

Combined UV-hypochlorite treatment

Effluent in the sump tank with indigenous and added bacterial inoculum was treated with calculated amount of hypochlorite solution to give the desired concentration of chlorine and mixed quickly and thoroughly by vigorous agitation. After selected time intervals (10, 20 and 30 min) the effluent water was passed through a UV unit by using a submersible pump. UV treated water sample was collected from the discharge point and quickly dechlorinated by sodium thiosulfate and subjected to total viable count (Miles and Mirsa 1938). Temperature of the aquarium effluent was around 7°C.

Results

Fig. 2 shows the logarithmic number of colony forming units (cfu) of bacterial cells/ml versus contact time of bacteria with different concentration of chlorine. A general trend in the loss of viability of the cells was observed with the

increase of contact time as well as concentration of chlorine. With 0.5 ppm chlorine, one log reduction was achieved within 5 minutes of contact period, thereafter the reduction was slow and gradual. There was a more rapid and massive reduction of bacterial number at the 1.0 ppm free chlorine concentration level. At this concentration two log reduction could be achieved within 5 minutes and three log reduction in 15 minutes, which is equal to 99.9% reduction in the viable count. The rate of reduction was rapid during the first 5 minutes with 0.5 ppm and the first 15 minutes with 1.0 ppm chlorine but after that, the slope of the curve levelled off. With 1.5 ppm and 2.0 ppm the number of viable cells fell below the level of detection within 5 minutes, which means the .99,9995% reduction in the cell number could be achieved in 5 minutes with 1.5 ppm chlorine under this experimental condition.

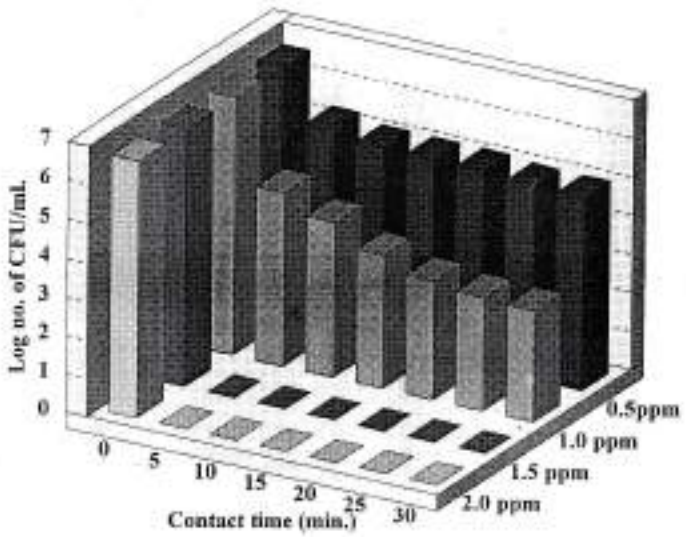


Fig. 2. Loss in viable count of chlorine exposed *Aeromonas salminicida* in aquarium effluent.

Fig. 3 shows the logarithmic number of colony forming units versus UV treatment and combined UV + hypochlorite treatment for different contact periods. With UV treatment alone a log value of 6.35 of starting suspension was reduced to 4.80 which means a reduction by more than 1.5 log. With a combined treatment of UV and 0.5 ppm chlorine log value was reduced to 3.0 within 20 minutes of contact time. This effect is identical with the effect of 1.0 ppm chlorine alone Showing that required chlorine alone showing that required chlorine. concentration is only 50% when it is used in combination with UV treatment. In the UV study test organisms were a mixed population because

indigenous bacterial flora of the aquarium effluent were not eliminated by treatment as was done when only hypochlorite was used. This was necessary to see the combined effect of UV and chlorine in the actual aquarium system.

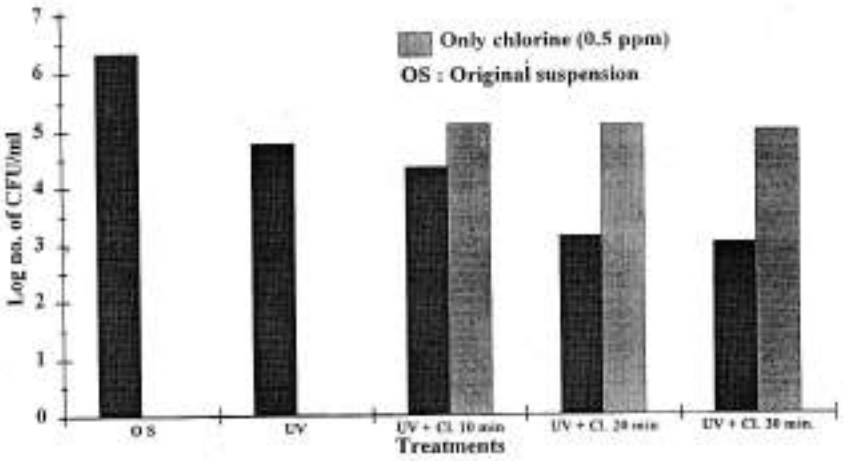


Fig. 3. Effect of UV, chlorine (0.5 ppm) and combined UV/chlorine (0.5 ppm) treatments on the viability of bacteria in aquarium effluent.

Discussion

From the experimental result of the present study it is apparent that freshwater effluents from laboratory based aquarium systems could be effectively disinfected with low doses of chlorine. The efficiency of disinfectant of a bacterial suspension in the effluent is proportional to the initial hypochlorite dose and contact time, therefore, complete destruction may be difficult with lower doses.

Incomplete oxidative destructivc of bacterial cells has been suggested to arise because of chlorine reacting with other substances (White 1972). Polprasert and Rajput (1984) demonstrated the reduction of chlorine residual and colicidal efficiency by increasing the suspended solid concentration in the chlorinated effluent.

The composition of effluents from different sources may vary considerably, depending on operational practice. Compared to commercial aquacultural activities the effluents used in this study had a low concentration of suspended matters. Doses and contact times used in this study can be compared to treated surface waters which are usually satisfactorily chlorinated with dose of 0.5 to 1.0 mg/l. Higher doses upto 5 mg/l are required for purification with high levels of

suspended matter or for the complete oxidation of ammonia and organic matter (Hom 1971). It is quite obvious then that chlorine demand will vary in different situations and depend on the nature of the organism in the effluent, nature and condition of water and temperature.

A combined treatment of UV and chlorine may increase the effectiveness of chlorination in destroying bacteria in the effluents. The present study showed that required chlorine concentration could be reduced by 50% when used in combination with UV treatment. Effectiveness of UV treatment of waste water is generally reduced due to protective effects to bacteria by particulate matter in the water (Qualls and Johnson 1983, Whitby and Palmater 1993). Such protection is a limiting factor in disinfection efficiency. Every effort should be made to reduce the quantity of suspended matter by filtration prior to disinfection.

References

Austin, B. and D.A. Austin, 1993. *Aeromonadaceae representatives. In*: Bacterial Fish Pathogens: Disease in Farmed and Wild Fish. Ellis Horwood Ltd., England. pp. 86-150.

Cheng, J.C.H., S.F. Ossof, D.C. Lobe, M.H. Dorfman, C.M. Dumais, R.G. Qualls and J.D. Jonson, 1985. UV inactivation of pathogenic and indicator microorganisms. *J. Appl. Enviorn. Microbiol.*, **49**: 1361-1365.

Glaze, W.H., J.W. Kang and S.S. Zigler, 1991. Treatment of hazardous waste chemicals using advanced oxidation process. *Proceedings of the 10th Ozone World Congress*. International Ozone Association, Zurich. pp. 261-278.

Harris,.D., V.D. Adams, D.L. Sorensen and M.S. Curtis, 1987. Ultraviolet inactivation of selected bacteria and viruses with photoreactivation of the bacteria. *Water Res.*, **21**: 687-692.

Hom, L.W., 1971. Kinetics of chlorine disinfection in an ecosystem. *Proceedings of the National Specialty Conference on Disinfection* (ed. L.W. Hom), Manchester, England. pp. 515-537.

Miles, A.A. and S.S. Mirsa, 1938. The estimation of the bactericidal power of the blood. *J. Hygiene.*, **38**: 732-749.

Oliver, B.G. and J.H. Grey, 1976. Ultraviolet disinfection, an alternative to chlorination. *J. Water Poll. Control Fed.*, **48**: 2619-2624.

Polprasert, C. and V.S. Rajput, 1984. Study on chlorine disinfection of pond effluent. *Water Res.*, **18**: 513-518.

Qualls, R.G. and J.D. Johnson, 1983. Bioassay and dose measurement of UV disinfection. *J. Appl. Environ. Microbiol.*, **45**: 872-877.

Sako, H. and M. Sorimachi, 1995. Susceptibility of fish pathogenic viruses, bacteria and fungus to ultraviolet irradiation and the disinfectant effect of UV-ozone water sterilizer on the pathogens in water. *Bull. Natl. Res. Inst. Aquaculture,* **8**: 51-58.

Venosa, A.D., A.C. Petrasek, D. Brown, H.L. Sparks and D.M. Allen, 1984. Disinfection of secondary effluent with ozone/UV. *J. Water Poll. Control Fed.*, **56**: 137-142.

Wedmeyer, G.A, and N.C. Nelson, 1977. Survival of two bacterial fish pathogens (*Aeromonas salmonicida* and Enteric Redmouth Bacterium) in ozonated, chlorinated and untreated waters. *J. Fish. Res. Board Can.*, **34**: 429-432.

Whitby, G.E and G. Palmateer, 1993. The effect of UV transmission, suspended solids and photoreactivation on microorganism in waste water treatment with UV. *Water Sc. Technol.*, **27**: 379- 386.

White, G.C., 1972. Handbook of Chlorination. Van Nostrand-Reinhold, New York, USA. 135 pp.

(Manuscript received 4 July 1996)

Bangladesh J. Fish. Res., 1(1) : 09-16
Bangladesh Fisheries Research Institute

Nursery rearing of *Macrobrachium rosenbergii* (de Man) using hapa-nets : effects of stocking density

M.J. Alam, M.E. Hoq, D.A. Jahan and M.A. Mazid

Freshwater Station, Bangladesh Fisheries Research Institute
Mymensingh-2201, Bangladesh

Abstract

An experiment was conducted in two phases for 45 days each to study the effects of six stocking densities (phase-I: 100, 200 and 300 PL/m^2 and phase-II: 250, 500 and 750 PL/m^2) on growth and survival of *Macrobrachium rosenbergii* postlarvae (PL) in nylon hapa-nets (1.8 m x 1.8 m x 1.4 m) installed in a pond. Stocking densities of 100, 200 and 300 PL_{10}/m^2 resulted in similar ($P < 0.05$) body length (47 - 48 mm) and survival rate (84 - 88%), while body weight (0.62 g) in PL with 300/m^2 was significantly lower than that (0.70 g) in PL with 100 and 200/m^2. The growth (body length 47 mm and weight 0.64 g) and survival (84%) of PL stocked at 250 PL/m^2 density were significantly higher ($P < 0.05$) than that of PL at 500 and 750/m^2. Besides the variation in growth and survival in PL at six test stocking densities, a sharp increase in body weight of PL was observed beginning at the 4th week of rearing.

Key words : *M. rosenbergii*, Stocking density, Hapa-net

Introduction

Nursery rearing of newly raised *M. rosenbergii* postlarvae (PL) for 1-3-month period, prior to stocking in the grow-out pond, is an important step in freshwater prawn aquaculture. Though direct stocking of 1-4-week old PL to grow-out pond is practiced by many farmers elsewhere (Alston 1989), incorporation of nursery rearing into both traditional and non-traditional prawn culture systems has significant effects in order to improve survival, grow-out inventory control and ultimately to optimize the production (Malecha 1986). Prawn nursery is also useful particularly, in a country like Bangladesh, where climatic conditions and intermittent water availability restrict the length of pond growing season and prevent continuous culture to market size (New 1990).

For successful prawn nursery operation, commercial nursery operators must need to know the effects of factors such as stocking density of PL, habitat

complexity and, feeds and feeding schedule. A number of published reports on prawn nursery systems (Sandifer and Smith 1977, Smith and Sandifer 1979, Smith *et al.* 1983, Heinen and Mensi 1991) are available, but there have been no research reports, except Angell (1994), on prawn nursery system in Bangladesh conditions. The present study was designed to optimize the stocking density of prawn PL, aiming at resulting in higher survival and growth, in hapa-net nursery system. Once the standard density is known, research and development efforts would be focused on reducing the cost of production and improving the yield, using appropriate feeds and feeding management.

Materials and methods

Preparation and installation of hapa-net

Nine nylon hapa-nets of 0.3 mm mesh size measuring 1.8 x 1.8 x 1.4 m^3 each were installed in a 0.4 ha pond at the Freshwater Station of Fisheries Research Institute, Mymensingh. During hapa installation, the pond was under carp culture and as it was not possible to make the pond complete drained-out and sun-dried due to periodical raining, most of the fishes were harvested by repeated netting. Monoammonium sulphate (21-0-0) and lime were applied in a 1:10 ratio at the rate of 1.5 kg/decimal. Underground water was supplied through central drainage system and the water depth ranged from 85 - 90 cm during the study period. Only bamboo poles were driven into the pond bottom and used to make the supporting frame for hapa-nets. The upper and lower corners of each hapa-net were fastened with bamboo poles in such a way that the nets remained suspended in the water column without touching the pond bottom about 10 cm above the pond bottom. At each hapa-nets, a few dry coconut leaves were positioned horizontally in the water column to serve as shelters as well as substrates for the prawn PL. A feeding tray (1.2 m x 1.2 m) made of plastic mosquito screen sewn to a wire frame was provided with each hapa-nets.

Stocking of PL

Phase-I : Three stocking densities such as 100, 200 and 300 PL/m^2 were assigned in a randomized block design with three replications for each. Ten-day old *M. rosenbergii* PL were collected from a nearby prawn hatchery and transported to the pond site using oxygenated plastic bags. The PL were acclimated to pond conditions and stocked at 17.00 h in the hapa-nets according to the assigned densities. The initial body length (BL) of 9.6 mm and body weight (BW) of 8 mg were recorded by random measuring of PL (n = 100).

Phase-II : Three stocking densities of 250, 500 and 750/m^2 of *M. rosenbergii* PL_{12} (BL = 10.5 mm and BW = 9 mg) with three replications for each were

tested. Transportation and stocking procedures were similar to that followed in the Experiment I.

Feeding to PL

The prawn were fed with a commercial shrimp feed (Saudi-Bangla nursery feed-1 and 2) at the rate of 25% of body weight during 1st week, 15% during 2nd week and 10% during rest of the culture period. The proximate composition of the feed is given in Table 1. The amount of feed was adjusted based on the estimated progressive gain in total biomass and observations of leftover feed on feeding trays. The daily ration was equally apportioned into two feedings: one at 8.00 h and another at 17.00 h. The feed was spreader on the feeding trays, which were hung close to the bottom of hapa-nets. The feeding trays were washed thoroughly prior to each feeding.

Table 1. Proximate composition (dry matter basis) of prawn feed

Parameters	% composition
Moisture	12
Crude protein	40
Crude lipid	5
Crude fibre	6
Ash	18

Source : Saudi Bangla Fish Feed Ltd., Dhaka, Bangladesh

Data recording and analysis

For the estimation of increment in growth, total BL (from tip of the rostrum to end of the telson) and BW of randomly sampled prawn (n = 50) were measured from each hapa-nets once in a week. The survival rate was estimated finally at the end of study by direct counting. Water quality parameters i.e. temperature, dissolved oxygen (DO), pH, ammonia and alkalinity were recorded weekly using a HACH Kit (Model FF-2). The water quality parameters were within the acceptable limits for prawn culture in ponds (Table 2). The growth and survival data were subjected to a one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) at 5% level of significance using a PC equipped with statistical package (Statgraphics, Version 4.0).

Table 2. Mean values of water quality parameters during the experiment (phase-I and phase-II)

Water quality parameters	Days from the initiation of experiments						
	1	7	14	21	28	35	42
Temperature (^{0}C)	29.5	28.0	28.8	28.6	27.8	27.9	28.0
DO (mg/l)	4.0	5.0	6.1	4.2	4.0	7.0	4.0
pH	8.0	8.0	8.5	8.5	8.0	8.0	8.0
Ammonia (mg/l)	0.2	0.2	0.2	0.4	0.3	0.3	0.2
Alkalinity (mg/l)	170	160	146	139	140	141	155

Results and discussion

The production data of 45-day nursery rearing of *M. rosenbergii* PL at different stocking densities are given in Table 3. The BL, BW and survival rates (SR) were inversely related to stocking densities of 250 - 750 PL/m^2, though were not to 100 - 300 PL/m^2. In the phase-I experiment, *M. rosenbergii* PL stocked at 250/m^2 attained significantly higher ($P < 0.05$) BL (46.8 ± 1.27 mm), BW (635.3 ± 0.72 mg) and SR (84.3 ± 3.7%), while those at 750/m^2 had the lowest values. In the phase-II experiment, all the test stocking densities resulted in similar ($P < 0.05$) average gain in BL (47 - 49 mm) and SR (84 - 88%). The final body weight attained by PL at 100 and 200 PL/m^2 were significantly higher ($P < 0.05$) than that at 300 PL/m^2. The rate of progressive increase in body weight was also higher in PL with 100 - 300/m^2, though a sharp increase in body weight was observed in PL at each stocking density beginning at the 4th week of nursery rearing (Fig. 1).

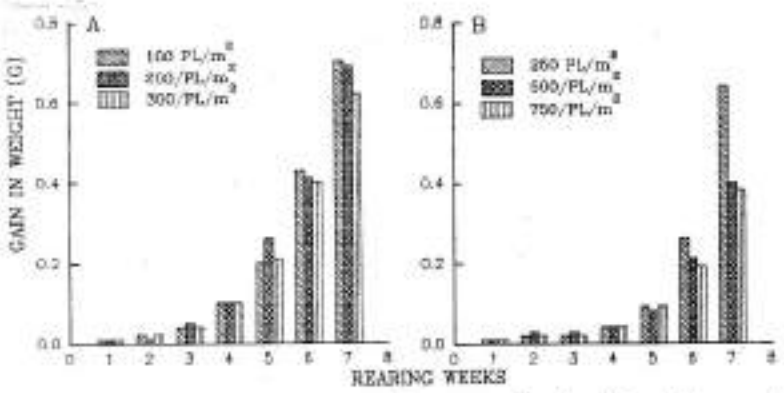


Fig. 1. Weight gain in *M. Rosenbergii* PL at different stocking densities in hapa-net nursery system (A : pahse-1; B : pahse-2).

Table 3. Growth and survival of *M. rosenbergii* PL at different stocking densities in hapa-net nursery for 45 days

Stocking density (PL/m^2)	Mean body engtth (mm)	Mean body weight (mg)	Mean survival rate (%)
Phase-I			
750	37.0 ± 0.86^b	379.6 ± 0.94^b	60.9 ± 4.0^b
500	39.2 ± 0.92^b	403.1 ± 1.03^b	72.8 ± 7.5^b
250	46.8 ± 1.27^a	635.3 ± 0.72^a	84.3 ± 3.7^a
Phase-II			
300	47.7 ± 0.58^a	624.8 ± 0.80^b	85.1 ± 4.0^a
200	48.5 ± 1.01^a	697.4 ± 0.68^a	83.8 ± 5.2^a
100	47.0 ± 1.59^a	702.6 ± 0.21^a	88.2 ± 4.2^a

Means with the same superscripts are not significantly different ($P < 0.05$)

The results obtained at different test stocking densities indicate that a stocking density within the range of 100 - 300 PL/m^2 may results in a desirable growth of 47 - 49 mm BL, 635 - 702 mg BW and 84 - 88% SR for a hapa-net *M. rosenbergii* nursery rearing system. The final body lengths attained by prawn at each stocking density (Table 3) are higher than those are desired in stocking the grow-out ponds. Stocking of ponds with 25 mm juvenile prawn is suggested to reduce initial losses and allow a more predictable pond survival rate (Ling 1967). The final body weights of 0.6 - 0.7 g obtained with 100 - 300 PL/m^2 in the present experiment are also comparable with 0.4 - 0.9 g (Smith *et al.* 1983), 0.4 - 0.6 g (Heinen and Mensi 1991) final weight in postlarval prawn reared for 60 - 90 days at variable stocking densities of 500 - 1000 PL/m^2. MacLean and Ang (1994), however, reported a final weight gain of 1.75 g with 63% SR for 38 days rearing of prawn PL in net enclosures, but the stocking density was only 10 PL/m^2.

Besides growth, final survival rate of *M. rosenbergii* PL is a major factor to be considered in nursery rearing, as the internal rate of return (IRR) in prawn nursery has been reported very sensitive to variations in survival (Angell 1994). Several studies in elsewhere (Sandifer and Smith 1975, Willis *et al.* 1976, Smith and Sandifer 1979a, Kneale and Wang 1979, Saha *et al.* 1989, Heinen and Mensi 1991) have shown that prawn PL stocked at rates between 100 - 700/m^2 for 45 - 60 days of nursery systems may result in final survival rates of 60 - 80%. Though Smith *et al.* (1983) recorded about 90% survival of prawn PL at stocking densities of 1000 - 1500/m^2 in a an enclosed nursery system, only 28 - 37%

survival has been reported (Angell 1994), for nearly the same range of stocking density, in a cage nursery system in Bangladesh condition. Nursery rearing of *M. rosenbergii* PL in earthen ponds is limited (Williams and Berrigan 1977). Though Saha *et al.* (1989) reported 52% survival at the stocking density of 175 PL/m^2 in 30 days of rearing in earthen ponds and a desirable mean final weight of 1 - 2 g after 40 - 90 days may be achieved in earthen pond nursery (Corbin *et al.* 1986), the survival rate is not always known due to problems in complete harvesting of benthic juvenile.

The fact is that the stocking density and survival of prawn PL are highly variable and depend on nursery systems, rearing conditions and management practices including feeds and feeding. The results of the present experiment reveal that the nursery of prawn in hapa-nets has the advantages of reducing mortality and full recovery of prawn juvenile. The higher growth and survival of prawn juvenile might also be influenced by introducing the artificial habitat in nursery compartment (Smith *et al.* 1979b) and multiple feeding (McSweeney 1977) with food having 40% dietary protein (Table 1). It has been reported that *M. rosenbergii* postlarvae may need a minimum dietary protein level of about 30 - 35% (D'Abramo and Reed 1988). Though feeding prawn PL once daily has been found to result in good survival, growth and yields of postlarval *M. rosenbergii* (Heinen and Mensi 1991), juvenile prawn activity patterns lend support for the need of multiple feeding (Corbin *et al.* 1986). The multiple feeding is also necessary to achieve better feed utilization and to prevent the accumulation of uneaten food. However, another experiment is necessary on the best formulation of food using locally available ingredients and on the out-door feeding schedule as well.

Conclusions

The results of the present study show that nursery rearing of *M. rosenbergii* postlarvae in hapa-nets at densities as high as 300/m^2 may offer benefits of rearing of sufficient numbers of postlarvae to allow them to grow to a desirable stocking size and to survival, and to faster turnover for both the hatchery and nursery operators. The present study also indicates the possibility of holding and rearing prawn postlarvae, either produced in hatchery or caught from nature in late season (September - October), at high stocking density over the winter period for stocking at the beginning of the next grow-out season.

Acknowledgements

The project is supported by the Government of Bangladesh funding (project code: 01-020-9596). The authors are thankful to Dr. M.G. Hussain, Chief Scientific Officer, Freshwater Station, Fish... Research Institute, for providing the research facilities.

References

Alston, D.E., 1989. *Macrobrachium* culture: a caribbean perspective. *World Aquaculture*, **20**(1): 18-23.

Angell, C.L., 1994. Cage nursery rearing of shrimp and prawn fry in Bangladesh. *BOBP/WP/92*, Madras, India. 16 pp.

Corbin, S.J., M.M. Fujimoto and T.Y. Iwai, Jr., 1986. *Feeding practices and nutritional considerations for Macrobrachium rosenbergii culture in Hawaii. In*: J.P. McVey (ed.) CRC Handbook of Mariculture, Vol. 1. CRC Press, Inc. Boca Raton, Florida. pp. 391-412.

D'Abramo, L.R. and L. Reed, 1988. Optimum diets protein level for juvenile freshwater prawn *Macrobrachium rosenbergii*. *J. World Aquaculture Soc.*, **19**(1): 25A (Abstract No. 64).

Heinen, J.M. and M.J. Mensi, 1991. Feeds and feeding schedules for indoor nursery culture of post larval freshwater prawn *Macrobrachium rosenbergii*. *J. World Aquaculture Soc.*, **22**(2): 118-127.

Kneale, D.C. and J.K. Wang, 1979. A laboratory investigation of *Macrobrachium rosenbergii* nursery production. *Proc. World Mariculture Soc.*, **10**: 359-365.

Ling, S.W, 1967. Methods of rearing and culturing *Macrobrachium rosenbergii* (de Man). *FAO Fisheries Report*, **357**: 607-619.

MacLean, M.H. and K.J. Ang, 1994. An enclosure design for feeding and fertilization trials with the freshwater prawn *Macrobrachium rosenbergii* (de Man). *Aquaculture*, **120**: 71-80.

Malecha, R.S., 1986. *Commercial pond production of the freshwater prawn, Macrobrachium rosenbergii, in Hawaii. In*: J.P. McVey (ed.) CRC Handbook of Mariculture, Vol. 1. CRC Press, Inc. Boca Raton, Florida. pp. 231-259.

McSweeney, E.S., 1977. *Intensive culture systems. In*: V.A. Hamson and H.L. Goodwin (eds.). Shrimp and Prawn Farming in the Western Hemisphere. Dowden, Hutchinson and Ross, Stroudsburg, PA. pp. 255-260.

New, M.B., 1990. Freshwater prawn Aquaculture: a review. *Aquaculture*, **88**: 99-143.

Saha, S.B., M.S. Shah and M.V. Gupta, 1989. A preliminary study on the rearing of post-larvae of *Macrobrachium rosenbergii* (de Man) in nursery ponds. *Bangladesh J. Life Sci.*, **2**: 47-49.

Sandifer, P.A. and T.I.J. Smith, 1975. Effects of population density on growth and survival of *Macrobrachium rosenbergii* reared in recirculating water management systems. *Proc. World Mariculture Soc.*, **6**: 43-53.

Sandifer, P.A. and T.I.J. Smith, 1977. Intensive rearing of postlarval Malaysian prawns (*Macrobrachium rosenbergii*) in a closed cycle nursery system. *Proc. World Mariculture Soc.*, **8**: 225-235.

Smith, T.I.J. and P.A. Sandifer, 1979a. Development and potential of nursery systems in the farming of Malaysian prawns. *Proc. World Mariculture Soc.*, **10**: 369-384.

Smith, T.I.J. and P.A. Sandifer, 1979b. Observations on the behavior of the Malaysian prawn, *Macrobrachium rosenbergii* (de Man) to artificial habitats. *Marine Behavior and Physiology*, **6**(2): 131-146.

Smith, T.I.J., W.E. Jenkins and P.A. Sandifer, 1983. Enclosed prawn nursery systems and effects of stocking juvenile *Macrobrachium rosenbergii* in ponds. *J. World Mariculture Soc.*, **14**: 111-125.

Williams, S.A. and M.E. Berrigan, 1977. Effects of stocking size and density on growth and survival of *Macrobrachium rosenbergii* (de Man) in ponds. *Proc. World Mariculture Soc.*, **8**: 251-264.

Willis, S.A., R.W Hagood and G.T. Eliason, 1976. Effects of four stocking densities and three diets on growth and survival of postlarval *Macrobrachium rosenbergii* and M. *acanthurus*. *Proc. World Mariculture Soc.*, **7**: 655-665.

(Manuscript received 5 January 1997)

Bangladesh J. Fish. Res., **1**(1) : 17-24
Bangladesh Fisheries Research Institute

Larval ontogeny of a percichthyid fish, *Synagrops philippinensis* (Günther) in Kagoshima Bay, Southern Japan

M.M. Haque[1,*] and T. Ozawa

Faculty of Fisheries, Kagoshima University, 4-50-20 Shimoarata, Kagoshima-890, Japan
[1]*Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh*

[*]*Corresponding author*

Abstract

The larval ontogeny of a developmental series (1.2-8.3 mm body length, BL) of *Synagrops philippinensis* from Kagoshima Bay, Southern Japan is described and illustrated. The yolk was completely absorbed in larva of ≥1.5 mm BL. Notochord flexion commenced at about 3.5 mm BL and was completed by about 4.0-4.5 mm BL. *S. philippinensis* larvae were distinguished from their congeners based on melanophore patterns, head spination and fin spines and rays. Larvae of 7.5-8.3 mm BL were characterized by anteriorly serrated pelvic spine, two anal spines, nine inner preopercular spines and no melanophore on lateral side of the caudal peduncle; 7.0 to 7.5 mm BL larvae by the above characters except serration on pelvic spine; and yolk-sac, preflexion, flexing and postflexion larvae up to 7.0 mm BL by unique melanophores on lower lobe of pectoral finfold/fin.

Key words : *Synagrops philippinensis*, Larval ontogeny

Introduction

The genus *Synagrops* is the most speciose in the family Percichthyidae with more than 10 species (Schultz 1940, Kotthaus 1970, Garzon and Acero 1986), and is distributed in tropical and subtropical waters of Western Atlantic Ocean, Gulf of Mexico, Caribbean sea, Suriname, the Philippines, Indonesia, Australia, India, Japan, the Western Pacific, the Hawaiian Islands, the Indian Ocean and South Africa (Fujii 1983, Mochizuki 1984, Mochizuki and Sano 1984).

The fishes of the genus *Synagrops* are characterized with the following: body elongated, laterally compressed, covered with large deciduous cycloid scales; eyes large, upper side of the head with muciferous cavities, mouth large and oblique; a narrow band of villiform teeth in the jaws, on the vomer and palatine

bones, with the addition of a pair of canine teeth in the upper jaw, and a series of similar teeth in the lower; opercle with two flat spines, margins of subopercle and interopercle finely serrated; two separate dorsal fins, the first with VIII-X spines and the second with one spine and 8-10 softrays; anal with II-III spines and 6-9 softrays; pectoral with 15-18 softrays; and pelvic with one spine and five softrays (Fujii 1983, Goode and Bean 1984, Mochizuki and Sano 1984, Leis and Trnski 1989).

According to Hatooka (1993), the following five species occur around Japan: *S. japonicus* (Steindachner et Döderlein), *S. philippinensis* (Günther), *S. spinosus* (Schultz), *S. serratospinosus* (Smith et Radcliffe) and *S. analis* (Katayama). In Kagoshima Bay, Imai and Nakahara (1969) reported *S. japonicus* and *S. philippinensis* as adults. The body shape and colour of *S. philippinensis* resemble those of *S. japonicus* and *S. analis*, but the former is easily distinguishable by the following characters: anteriorly serrated pelvic spine, anal fin with two spines and seven softrays and 16 rays in pectoral fin (Mochizuki 1984).

Konishi (1988) reported very briefly some characters of a 10.7 mm BL larva of *S. japonicus* and a few larvae larger than 2.8 mm BL of *S. philippinensis*. During the ichthyoplankton survey in Kagoshima Bay, among the *Synagrops* species only *S. philippinensis* larvae were identified and it was found that the descriptions of Konishi (1988) are now considered to be incomplete with some characters being not illustrated that may be of diagnostic value for species identification. In this paper, the early ontogeny of *S. philippinensis* is described including important diagnostic characters.

Materials and methods

The specimens used in this study were collected from Kagoshima Bay fortnightly or monthly from October '83 to December '93 on board the R/V "Shiranami" (1.5 tons) of the Laboratory of Fisheries Biology, Kagoshima University, Japan, principally from 14 fixed stations (Fig. 1).

A cylindrical-conical type net (1.3 m in diameter, 4.5 m in length and 0.51 mm in mesh size) was towed in step hauls using 50 and 100 m rope lengths (5 min for each) at a speed of about 2 knots. A flowmeter (TSK) was set at the centre of the mouth of net to get data necessary for computation of the volume of water filtered.

Each set of collections in the middle and inner parts, usually required a full day, was termed a cruise. In each month, samples were collected during daytime just after the middle of each month normally in two consecutive days, one day for nine fixed stations of the middle part and on the other day for five fixed stations of the inner part (Fig. 1). In addition, from November '83 to October '84, an additional cruise was made just after the beginning of each month. Due to bad weather condition and/or mechanical problems, sometimes

it was not possible to collect the samples. In total, 1556 collections from 121 cruises were made for the study.

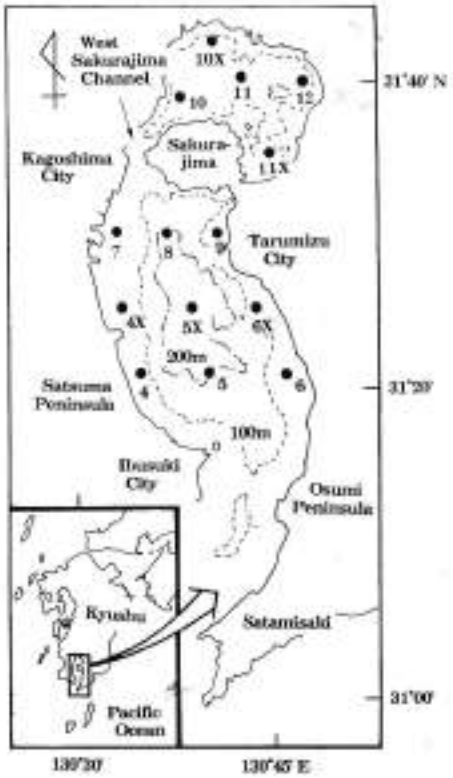


Fig. 1. Ichthyoplankton sampling stations in Kagoshima Bay.

On board, the samples were cleaned of any twigs, leaves, large ctenophores and coelenterates and were immediately preserved in approximately 5% buffered formalin in sea water. In the laboratory, fish larvae were sorted out under a magnifying glass and were preserved in 70% ethyl alcohol. A total of 3656 (1.2-8.3 mm BL) *S. philippinensis* larvae were identified after Konishi (1988), and Leis and Trnski (1989). To identify the smallest larvae (the yolk-sac bearing ones, about 1.2-1.4 mm BL) from other ichthyoplankton in the samples, a developmental size-series from the largest available specimen to the smallest one was sorted out tracing the external morphological characters discernible on the largest to the smallest specimens. The series was linked together by myomere counts, pigment patterns, head spination and in larger larvae and juvenile specimens, counts of other meristic characters (fin spines, rays etc.).

All of the *Synagrops* larvae collected from the bay were classified as *S. philippinensis* with the following diagnostic characters: ≥7.5 mm BL larvae and early juveniles with anteriorly serrated pelvic spine, two anal spines, nine inner

preopercular spines, and no melanophore on lateral side of the caudal peduncle; 7.0 to 7.5 mm BL larvae with all of the above characters except serration on pelvic spine; and yolk-sac, preflexion, flexing and postflexion larvae up to 7.0 mm BL by melanophores on lower lobe of pectoral finfold/fin.The larvae were examined under a dissecting microscope and measured using an ocular micrometer. Some larvae were temporarily stained with methylene blue for clear observation of spines and finrays. For definitions and measurements Leis and Trnski (1989) was followed. Body lengths (BL) were notochord length in larvae prior to the completion of notochord flexion and standard length thereafter.

Results

Early ontogeny

The yolk was not completely absorbed in the smallest larvae (1.2-1.4 mm BL) of the present samples (Fig. 2A). Notochord flexion was found to commence at about 3.5 mm BL and completed at about 4.0-4.5 mm BL (Fig. 2D). All of the finrays became clear at about 8.0 mm BL (Fig. 2G).

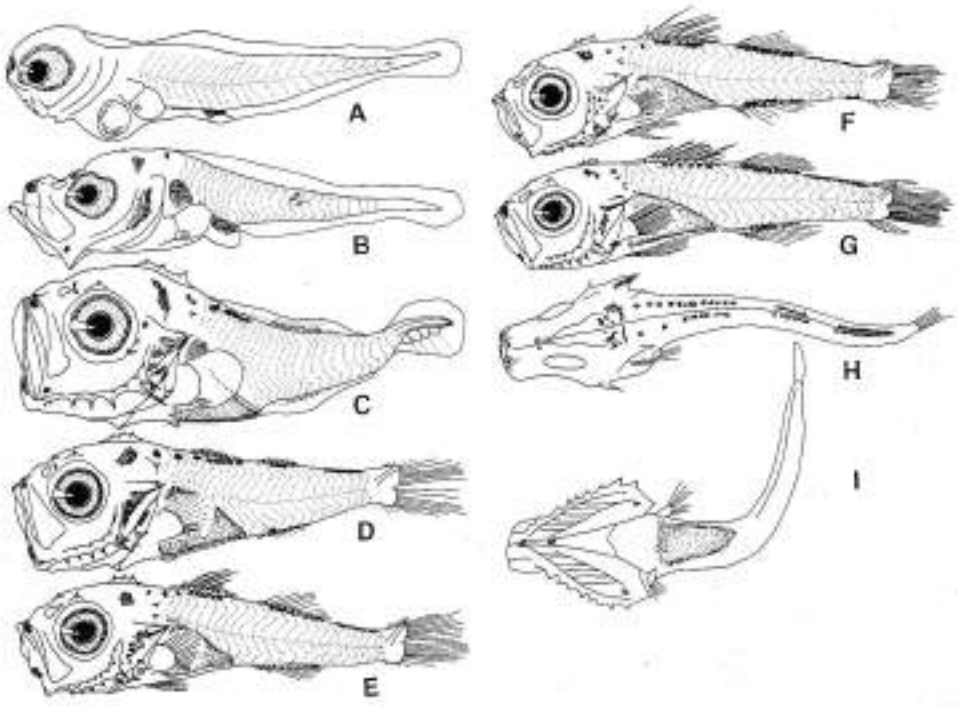


Fig. 2. Larvae of *Synagrops philippinensis*. A, 1.4 mm BL; B, 2.1 mm BL; C, 3.4 mm BL; D, 4.2 mm BL; E, 5.5 mm BL; F, 7.0 mm BL; G, 8.3 mm BL; H, 6.1 mm BL (dorsal view); I, 5.5 mm BL (ventral view)..

Body form

Body was found to be elongated and compressed laterally (Fig. 2). Head was large (about 30-35% of BL). The snout was short, initially concave but became rounded at the beginning of flexion. Eyes were round and large in preflexion and flexing larvae (about 35% of HL) (Fig. 2A-D) and moderate in postflexion larvae and early juveniles (about 30% of HL). Mouth was large, remarkably oblique and was almost vertical when closed. The maxilla almost reached the horizontal line from the anterior edge of pupil. A short projection was present at the tip of lower jaw in larvae of ≥7.0 mm BL (Fig. 2F-G).

Teeth

Minute villiform teeth developed on both jaws at about 2.1 mm BL, and were found to remain small throughout early ontogeny. Large canine teeth were not developed even in the largest collected specimen.

Head spination

Small to moderate spines developed on preopercle, supraoccipital crest, supraocular ridge, supracleithrum, maxillary, subopercle, interopercle, posttemporal in this order (Fig. 2). A strong opercular spine developed at about 3.2 mm BL (Fig. 2C). The larvae less than 2.0 mm BL were devoid of head spination. Three smooth spines appeared on the outer margin of preopercle at about 2.1 mm BL (Fig. 2B) and increased in number up to 11 of different sizes with development, decreasing upwards and anteriorly with the largest spine at the angle, and the number of anterior spines was more than the posterior spines (Fig. 2C-G). On the inner margin of preopercle three small spines appeared at about 2.5 mm BL, increased to a fixed number of nine almost equal in size at about 7.0 mm BL (Fig. 2G). A single spine of supraoccipital crest appeared at about 2.1 mm BL (Fig. 2B) and increased in number to mostly 3 or rarely 4 with development. Supraocular ridge with single spine appeared at about 3.0 mm BL and remained the same in larger specimens (Fig. 2C-G). A small spine developed on supracleithrum at about 3.0 mm BL and increased into two in specimens beyond about 5.5 mm BL (Fig. 2C-G). Two maxillary spines, one very small and one a little larger, appeared at about 3.0 mm BL. A subopercular spine and an interopercular spine appeared at about 3.5 mm BL (Fig. 2C-D), and the number of subopercular spine became two at about 6.0 mm BL. A small posttemporal spine appeared at about 7.5 mm BL. A frontal spiny ridge on each lateral side appeared at about 4.2 mm BL (Fig. 2D). All the spines and ridges progressively degenerated after the beginning of juvenile stage.

Fin formation

Completion of fin development in *S. philippinensis* occurred in the sequence as follows: caudal and first dorsal (almost simultaneously), second dorsal, anal and pelvic (almost simultaneously), and pectoral.

Pectoral finfold with rudimentary rays became visible in larvae of about 2.0 mm BL. Rudiments of hypural elements like triangular thickening, anlage of dorsal and anal fins and pelvic bud as slight swellings on either side of the gut developed almost simultaneously just prior to or at the beginning of caudal flexion at about 3.2-3.5 mm BL (Fig. 2C). Principal rays, 9 + 8 of caudal fin and IX spines of first dorsal were the first to complete at about 5.5 mm BL. The second dorsal fin (one spiny and nine softrays) completed with the modification of the anteriormost finray into spine at about 6.0 mm BL. Followed by anal (two spiny and seven softrays) and pelvic fins (one spiny and five softrays) almost simultaneously at 6.2-6.5 mm BL. The serration at the anterior edge of pelvic spine appeared at about 7.5 mm BL (Fig. 2G). The pectoral finrays (16 rays) developed at about 8.0 mm BL.

Pigmentation

The larvae of *S. philippinensis* were moderately pigmented. The major characteristic pigments were on the lower lobe of pectoral fin, fore and hindbrain, nape, base of the first dorsal fin, on the gut and surface of gas bladder (Fig. 2).

The smallest larvae (1.2-1.4 mm BL) had melanophores on the tip of lower jaw, angle of lower jaw, forehead, on oil droplet, lower lobe of pectoral finfold and on gut (Fig. 2A). The melanophores on the lower lobe of pectoral fin were present in larvae up to 7.0 mm BL (Fig. 2F). At about 1.7-2.0 mm BL, melanophores appeared on fore and hindbrain, opercle, nape, chin and gas bladder, and those on forehead in yolksac larvae moved on the tip of upper jaw (Fig. 2B). At about 3.5 mm BL, melanophores appeared between the mandibles (Fig. 2C-G, 2I) and those on opercle increased in number and spread throughout the opercle. The melanophores on hindbrain intensified and those on nape extended posteriorly as a double row (Fig. 2C, 2H) to the base of first dorsal fin anlage. At about 4.2 mm BL, melanophores appeared at the bases of second dorsal (2D, 2H) and anal fins and dorsally on caudal peduncle (Fig. 2D). At about 5.5 mm BL, melanophores appeared ventrally on caudal peduncle, those on angle of lower jaw disappeared and those on tip of lower jaw reduced to a minute dot shape, became internal and sometimes not discernible. At about 7.0 mm BL, melanophores on opercle reduced in number and intensity, those on forebrain and lower lobe of pectoral fin became faint, and those on caudal peduncle dorsoventrally increased in number and intensity. Beyond 7.5 mm BL melanophores on forebrain and lower lobe of pectoral fin disappeared (Fig. 2G).

Discussion

Considering the area of collection of samples for the present study (Kagoshima Bay, Southern Japan), *Synagrops* larvae could be one of the two resident species as adult in the area (Imai and Nakahara 1969): *S. japonicus* or *S. philippinensis*. The presence of anteriorly serrated pelvic spine and two anal spines (characteristics of adult *S. philippinensis*) and smooth preopercular spines in the largest specimens collected eliminated the chances for them to be *S. japonicus*, having smooth pelvic spine and serrated preopercular spines (Konishi 1988), in the identification of the larvae. The adults of *S. analis* and *S. serratospinosus* bear anteriorly serrated pelvic spine. *S. analis* has three anal spines in contrast with two in *S. philippinensis*, and *S. serratospinosus* has 17 pectoral finrays and distinctly serrated second spine of anal fin, second spine of first dorsal fin and spine of second dorsal fin in contrast with 16 pectoral finrays and smooth dorsal and anal spines in *S. philippinensis* (Mochizuki 1984). These characteristics confirms identification of the present specimens as *S. philippinensis*.

Some of the pigmentation of *S. philippinensis* larvae described here are similar to those illustrated by Konishi (1988). The melanophores on the lower lobe of pectoral fin were not described or illustrated and the head spination (e.g., preopercular, supraocular and supraoccipital) were not clearly described in Konishi (1988) which may be the useful diagnostic characters for the species.

In this study, the yolksac bearing larvae of *S. philippinensis* were identified with two melanophores on lower lobe of pectoral finfold, 25 myomeres and elongated body shape. Preflexion, flexing and postflexion larvae up to 7.0 mm BL were identified with melanophores on lower lobe of pectoral fin, smooth preopercular spines, only one supraocular spine and three rarely four spines on supraoccipital crest. Larvae of 7.0-7.5 mm BL with nine small inner preopercular spines, in addition to above characters except melanophores on pectoral fin; and those of ≥7.5 mm BL and early juveniles in addition to above characters with anteriorly serrated pelvic spine and two anal spines which are characteristic to adults.

Acknowledgements

The authors would like to thank the former students of the Laboratory of Fisheries Biology, Faculty of Fisheries, Kagoshima University who helped in the collection of the larvae. The first author is grateful to the Ministry of Education, Science and Culture (Monbusho), Japan for awarding a Scholarship.

References

Fujii, E., 1983. *Percichthyidae. In*: Fishes Trawled off Suriname and French Guiana (eds., T. Uyeno, K. Matsuura and E. Fujii). Japan Marine Fishery Resource Research Centre, Tokyo. pp. 295-298.

Goode, G. B. and T. H. Bean, 1984. Oceanic Ichthyology: A Treatise on the Deep-sea and Pelagic Fishes of the World. Part-1 (Text). Narendra Publishig House, Delhi-110006, India. 553 pp.

Hatooka, K., 1993. *Family Percichthyidae. In:* Fishes of Japan with Pictorial Keys to the Species. (ed. T. Nakabo), Tokai Univ. Press, Tokyo. pp. 594-599. (in Japanese)

Imai, S. and K. Nakahara, 1969. *A list of fishes in Kagoshima Bay. In*: Report of the Fauna and Flora for the Establishment of Marine Park in Kagoshima Bay. Kagoshima Prefecture. Special Report, Kagoshima Prefectural Authority, Japan. pp. 65-78. (in Japanese)

Garzón, J. F. and P. A. Acero, 1986. Notes on the fish *Synagrops trispinosus* (Perciformes: Acropomatidae) from the Colombian Caribbean. *Japan. J. Ichthyol.*, **33**(3): 316-318.

Konishi, Y., 1988. *Acropoma japonicum, Doederleinia berycoides, Synagrops japonicus, Synagrops philippinensis, Malakichthys wakiyae. In:* An Atlas of the Early Stage Fishes in Japan. (ed. M. Okiyama), Tokai Univ. Press, Tokyo. pp. 406-411. (in Japanese)

Kotthaus, A., 1970. Fische des Indischen Ozeans. Ergebnisse der ichthyologischen Untersuchungen während der Expedition des Forschungsschiffes "Meteor" in den Indischen Ozean, Oktober 1964 bis Mai 1965. A. Systematischer Teil, VIII, Percomorphi (2) "Meteor" Forch. Engebnisse, Reihe D, (6): 56-75.

Leis, J. M. and T. Trnski, 1989. The Larvae of Indo-Pacific Shore Fishes. New South Wales Univ. Press, Australia. 371 pp.

Mochizuki, K., 1984. *Family Percichthyidae. In:* The Fishes of the Japanese Archipelago (Text). (eds. H. Masuda, K. Amaoka, C. Araga, T. Uyeno and T. Yoshino), Tokai University Press, Japan. pp. 123-126.

Mochizuki, K. and M. Sano, 1984. A new percichthyid fish *Synagrops trispinosus* from the Caribbean Sea and its adjacent waters. *Japan. J. Ichthyol.*, **31**(1):1-4.

Schultz, L. P., 1940. Two new genera and three new species of cheilodipterid fishes, with notes on the other genera of the family. *Proc. U. S. Natn. Mus.*, **88** (3085): 403-423.

(Manuscript received 20 July 1996)

Bangladesh J. Fish. Res., 1(1) : 25-30
Bangladesh Fisheries Research Institute

Comparative studies on growth of fry of GIFT and existing strain of Nile tilapia (*Oreochromis niloticus* L.)

R. Sultana[1], A.H.M. Kohinoor*, M.S. Islam , M.A. Mazid and M.G. Hussain

Freshwater Station, Bangladesh, Fisheries Research Institute
Mymensingh-2201, Bangladesh
[1] *Dept. of Fisheries Biology & Genetics, BAU, Mymensingh*
* *Corresponding author*

Abstract

Comparative study on growth of fry in nursery system of Genetically Improved Farmed Tilapia (GIFT) and Existing strain of Nile tilapia (*Oreochromis niloticus*) was performed. The trials were conducted in a series of hapa for two months. The initial mean weight of GIFT and Existing strains of tilapia were 1.03 and 1.12g, respectively and the stocking density for both the strains was maintained at 150/m^3. Fishes were fed with supplementary feed 31.29% of protein level. After two months the final cumulative mean weight of GIFT and Existing strain were observed to be 8.38 and 5.51 g, respectively. The net gain for weight of GIFT and Existing strain were estimated to be 666% and 368% and the mean survival were 95.75% and 81.25%, respectively. The GIFT strain showed significantly ($P<0.05$) higher net gain in growth in weight and also higher ($P<0.01$) survival than that of Existing strain.

Key words : *O. niloticus*, GIFT, Hapa-net

Introduction

Tilapia culture is increasing significantly in Asia particularly in China, Indonesia, Philippines, Srilanka, Thailand and Vietnam and elsewhere in the Indian subcontinent and Pacific region countries. Tilapia has been dubbed the "Aquatic Chicken" (Maclean 1984) of which the most widely farmed stock is the Nile tilapia (*Oreochromis niloticus*). Nile tilapia are widely recognized as one of the most important tilapia species for farming in a wide range of aquaculture systems from single small scale waste fed fish ponds to intensive culture systems (Pullin 1985).

The introduction of tilapia in Bangladesh from Thailand was first initiated in 1954 with *O. mossambicus* (Ahmed 1956) and later with *O. niloticus* (Rahman 1985) with a hope that it would make a significant contribution in fish production but the attempt was not successful because of very little efforts were made to understand the culture management by the farmers. Fisheries Research Institute

again brought a fresh batch of *O. niloticus* from Thailand in 1987 and developed low input and low-cost culture technologies.

Recently the International Centre for Living Aquatic Resources Management (ICLARM) has developed after four years of research, the 'Genetically Improved Farmed Tilapia' known as GIFT strain through several generations of selections involving eight different pure breed Nile tilapia, *O. niloticus*, strain. In on-farm trials, the GIFT fish grew, on an average of 60% better in growth and 50% in survival than a normal farmed breeds. The GIFT strain has been introduced in Bangladesh from Phillippines in July 1994 through International Network on Genetics in Aquaculture (INGA) under ICLARM.

Fisheries Research Institute has, therefore, initiated GIFT strain evaluation research in connection with the implementation of DEGITA Bangladesh project with the objective to compare the performances in growth and survival between GIFT and Existing strain, *O. niloticus* in nursery conditions.

Materials and methods

The study was conducted at the Fisheries Research Institute under its Freshwater Station for a period of two months (January to March'95). Eight nylon net hapas (mesh size of 2.5 mm), 3.0 m^3 each (2.0 x 1.5 x 1.0 m^3) were set in a small well prepared 1000 m^2 pond with bamboo poles in two columns. Four hapas under treatment-I were stocked with fry of GIFT (1.02 ± 0.12 g) and the rest four hapas under treatment-II with existing Nile tilapia (1.11 ± 0.03 g) at a stocking density of 150 fry/m^3.

A prepared supplemental feed consisted of ingredients of rice bran (25%), wheat flour (30%), mustard oil cake (15%) and fish meal (30%) with crude protein level of 31.19% were supplied to the fishes twice a day at 8% (according to Guerrero 1987) of the total body weight.

Thirty fry from each hapa were sampled at fortnightly intervals to assess the growth and feeding ration was adjusted on the basis of estimated weight of fish biomass. Water samples were taken at weekly intervals between 6:00 and 7:00 a.m. from inside the hapas during the trial and analysed for assessing some environmental parameters *viz*. water temperature, pH and dissolved oxygen (DO).

Statistical analysis with student's t-test was incorporated on the data to see whether the two strains show any differences or not i.e., to identify the level of significance in differences, if any, in growth patterns and survival during experimental period.

Results

The data of growth in length and weight, fortnightly gain, net gain and daily gain as mean values of GIFT and Existing strain of Nile tilapia (*O. niloticus*) are

given in Table 1. The initial mean length and weight of GIFT and Existing strains were 3.75 and 3.79 cm and 1.03 and 1.12 g, respectively. After 60 days, the final cumulative mean growth in length and weight were recorded at 7.59 ± 0.77 and 6.44 ± 0.21 cm and 8.39 ± 1.87 and 5.51 ± 0.29 g. The net gain for length and weight were estimated to be 102 & 70% and 666 & 368%, respectively. The mean survival rate of GIFT and Existing strains of tilapia were 95 and 81%, respectively.

Table 1. Average cumulative growth of GIFT and existing strain of tilapia (*Oreochromis niloticus*) in terms of increase in length (cm) and weight (g) and fortnightly gain in percentage (in parenthesis) over a period of 60 days

Growth parameter	Treatment	1st sampling	2nd sampling	3rd sampling	4th sampling	5th sampling	Net gain	Daily gain
		3.75	4.94	5.36	7.08	7.59	3.83	0.06
	I	±0.14	±0.20	±0.26	±0.60	±0.77		±0.01
		(31.84)	(8.63)	(32.21)	(7.27)	(102.41)		
Length (cm)								
		3.79	4.37	4.78	5.79	6.44	2.64	0.04
	II	±0.10	±0.26	±0.26	±0.22	±0.21		±0.00
			(15.70)	(9.44)	(21.40)	(11.37)		
		1.02	2.59	3.52	6.72	8.38	7.35	0.07
	I	±0.12	±0.31	±0.63	±1.60	±1.86		±0.03
		(155.25)	(36.87)	(89.43)	(89.08)	(666.17)		
Weight (g)								
		1.11	1.80	2.70	4.18	5.50	4.39	0.07
	II	±0.01	±0.31	±0.63	±0.18	±0.28		±0.00
		(61.46)	(56.39)	(56.05)	(31.58)	(368.46)		

On the sequential fortnightly estimated mean values of length and weight of both the strains, the student t-statistic indicates insignificant differences ($P>0.05$) between the two strains in only the initial sampling whereas significant differences ($P<0.05$ and $P<0.01$) were found in all other sampling, with an exception for weight ($P>0.05$) in the 3rd sampling. GIFT strain showed significantly higher ($P<0.05$) net gain and daily gain in growth (length and weight) and also higher ($P<0.01$) survival than that of Existing strain [Table 2(a) & 2(b)].

Table 2(a). t-test for data of nursery trials (length and weight)

Trial No.	Calculated				t-statistics		
	GIFT		Existing		Length	Weight	Degree of freedom
	Length	Weigth	Length	Weight			
1st Sampling	3.75 ±0.14	1.02 ±0.12	3.79 ±0.13	1.11 ±0.01	0.455^{NS}	1.45^{NS}	6
2nd Sampling	4.94 ±0.20	2.59 ±0.31	4.38 ±0.26	1.8 ±0.38	3.324*	3.20*	6
3rd Sampling	5.36 ±0.26	3.52 ±0.63	4.78 ±0. 26	2.70 ±0.24	3.120*	2.40^{NS}	6
4th Sampling	7.08 ±0.60	6.72 ±1.60	5.79 ±0.22	4.18 ±0.18	4.031**	3.12*	6
5th Sampling	7.59 ±0.76	8.38 ±1.86	6.42 ±0.21	5.50 ±0.28	3.242*	3.04*	6

Note : NS - Not-significant at 0.95 confidence limit i.e., P>0.05
** - Significant at 0.95 confidence limit i.e., P<0.05*
*** - Significant at 0.99 confidence limit i.e., P<0.01*
$t_{0.05}$ - 2.447 with d.f. 6
$t_{0.01}$ - 3.707 with d.f. 6

Table 2(b). t-tests for nursery trials (net gain, daily gain and survival rate)

Trial No.	Calculated means		t-statistic	Degrees of freedom
	GIFT	Existing		
Net gain in length	3.83 ±0.76	2.64 ±0.30	2.90*	6
Net gain in weight	7.35 ±1.80	4.39 ±0.28	3.24*	6
Daily gain in weight	0.12 ±0.03	0.07 ±0.00	3.24*	6
Daily gain in length	0.06 ±0.01	0.04 ±0.00	2.90*	6
Survival rate	95.75 ±2.62	81.25 ±2.75	7.61**	6

*Note : *- Significant at 0.95 confidence limit i.e., P<0.05*
*** - Significant at 0.99 confidence limit i.e., P<0.01*
$t_{0.05}$ - 2.447 with d.f. 6
$t_{0.01}$ - 3.707 with d.f. 6

The physico-chemical parameters of water revealed that the values of water temperature, pH and dissolved oxygen ranged from 18.67 ± 1.21 to 25.16 ± 2.29°C, 7.87 ± 0.48 to 8.01 ± 0.25 and 4.05 ± 1.36 to 5.97 ± 1.18 mg/l, respectively during the study period (Table - 3).

Table 3. Physico-chemical characteristics of pond water during the study period

Parameter	January	February	March
Water temperature (°C)	18.67 ± 1.21	21.87 ± 2.47	25.16 ± 2.29
Dissolved oxygen (mg/l)	4.05 ± 1.36	5.84 ± 0.94	5.97 ± 1.18
pH	7.90 ± 0.32	7.87 ± 0.48	8.01 ± 0.25

Discussion

The present study investigates on nursery trial of GIFT strain in comparison to Existing strain of *O. niloticus* in hapas, placed in pond with a view to observe their comparative performances . Apparently literature is rarely available on nursery trial of *O. niloticus* in cages or hapas in pond. Most of the literatures are confined on grow out trial of tilapia in cages and earthen pond. Cruz and Ridha (1989) are probably the only group of workers who provide information on nursery trial of tilapia in floating cages.

To evaluate the two strains of *O. niloticus*, same ecological conditions i.e., same environment was maintained where the fry of GIFT and Existing strains representing similar size were stocked at a density of 150 fish/m^3 and fed a formulated feed containing protein level of 31.29% for 60 days. Thus the GIFT strain was proved to be a significantly fast growing fish ($P<0.05$).

Cruz and Ridha (1989) observed the performances of *O. spilurus* in nursing phase for 68 days in seawater cages. They found no significant differences in mean individual final weight, daily growth rate and survival rate among three stocking densities, but considerable higher yields were obtained when stocked with 400 and 600 fish/m^3 compared with that of 200 fish/m^3. The fry were fed with a diet containing 55% crude protein throughout the experimental period after which they observed the weight of 28.65 -38.61 g. However, much lower final weight attained by both the strains in the present experiment in comparison to that of Cruz and Ridha (1989) could be the effect of sea water.

Whatever might be the stocking densities in nursery system for tilapia in pond cages in different experiments conducted by some authors, the supplemental feeding ration contained 70% rice bran and 30% fish meal at 5 -

20% of body weight (Eknath 1993), 20% ipil-ipil, 40% copra meal and 10% fish meal at 10 - 20% of body weight (Guerrero 1987). On the other hand, the feeding ration in the present experiment was quite low (8 - 10% of body weight) as to why the final body weight attainment of fish was comparatively lower to the others. It is evident from the present experiment that the GIFT strain showed higher ($P<0.01$) survival than existing strain of Nile tilapia. Eknath *et al.* (1993) observed that the introduced African wild strains of tilapia performed better than the most widely farmed Asian strains. The experimental GIFT strain is also a derivative of these better performed strains of tilapia. However, this phenomenon of the better performance of GIFT strain might be the cause of stock improvement through several generation of genetic selection.

References

Ahmed, M.,1956. Transplantation of food fish to East Pakistan. *Pak. J. Sci.*, **8**(4): 167-170.

Cruz, E.M. and M. Ridha, 1989. Preliminary study on the production of the tilapia, *Oreochromis spilurus* (Gunther), cultured in seawater cages. *Aquaculture and Fisheries Management*, **20**: 381-388.

Eknath, A.E., M.T. Melchor, S. Marietta, J.C. Palada-de Vera, A.R.Danting, E.E. Ruben, J.B. Dionisio, H.L. Capili, T.A. Bolivar, A.V. Abella, H.B. Circa, G. Bentsen, T. Bjarne, and R.S.V. Pullin, 1993. Genetic improvement of farmed Tilapias : The growth performance of eight strains of *Oreochromis niloticus* tested in different farm environments. *ICLARM Contribution No. 709.* Elsevier Science Publishers B.V., Amsterdam 111: pp. 171-188.

Guerrero, R.D., 1987. Tilapia farming in the Philippines. Technology and Livelihood Resource Center. Published by National Book Store, Inc., The Philippines.

Maclean, J.L., 1984. Tilapia - the aquatic chicken. *ICLARM Newsletter*, **7**(1): 17p.

Pullin, R.S.V., 1985. Tilapias- "everyman's fish". *Biologist*, **32**(2) : 84-88.

Rahman, A.K.A., 1985. Introduction of exotic fishes in Bangladesh.Paper presented in the seminar on culture of exotic fish in Bangladesh. October 15, 1989. Zool. Soc. Bangladesh. Dhaka University, Dhaka. 14 pp.

(Manuscript received 7 January 1997)

Bangladesh J. Fish. Res., 1(1) 31-40
Bangladesh Fisheries Research Institute

Limnology of Chanda beel

M. A. Ehshan, M. S. Hossain*, M. A. Mazid, M. F. A. Mollah [1], S. Rahman and A. Razzaque

Bangladesh Fisheries Research Institute, Mymensingh-2201, Bangladesh
[1] Dept. of Fisheries Biology & Genetics, BAU, Mymensingh

** Corresponding author*

Abstract

A limnobiological investigation was conducted in Chanda beel over a period of 8 months from June' 95 to January' 96. The floodplain showed temporal spatial and vertical variation in physico-chemical as well as biological conditions. During study period, physico-chemical parameters were with in the suitable range for fish culture. Plankton population was higher in true beel areas. Both phytoplankton and zooplankton showed direct relationship among themselves. Presence of several indication plankton genera showed that the floodplain was eutrophic in nature.

Key words : Floodplain, Phytoplankton, Zooplankton

Introduction

Bangladesh is very rich in water resources. Her floodplains cover an estimated area of 2.833 million ha, while that of beels stand at 0.114 million ha (Mazid *et al.* 1996). These openwater floodplains /beels were once exceptionally rich in fisheries resources, contributing significantly to meet the national nutritional requirements. In recent years, fish production from these sources has alarmingly declined due to various reasons. The floodplain by virtue of their continuous productivity, constitute one of the front-line areas capable of yielding at least 0.5 million MT of fish per year. Floodplain fisheries have received particular attention because of enormous potential with managerial issues critical to sustainable development (DOF 1995). Enormous investigations have been accomplished in various aspects of limnology but in Bangladesh, floodplain still remains a virgin field for such investigation.

Chanda beel having a total area of 10,870 ha (BCAS 1991) at the Faridpur-Madaripur belt of Bangladesh plays an important role in the economy of surrounding people, providing fish and fishery product. Recently government of Bangladesh has taken a massive programme on releasing fingerlings aiming sustainable increase of fish production. This study was undertaken for understanding the ecological status of a natural floodplain beel ecosystem, "the Chanda beel".

Materials and methods

Physico-chemical data and plankton samples were collected fortnightly from nine different spots which were stratified into several categories considering the coverage of area and different habitat conditions. Samples were collected between 08.00 hours and 11.00 hours. Temperature, turbidity, pH and dissolved oxygen were measured with the help of Aquamate (Model WQA-1A-Japan) and conductivity by a conductivity meter (Model 44600 Hach-USA). Rainfall data was collected from local office of Bangladesh Water Development Board.

For plankton study water was collected by Kemmerer water sampler (Model 1904-E-307) from the selected spots. Phytoplankton was sampled after settlement of collected water. To make zooplankton sample, 10 litre water were sampled and subsequent filtration was performed through a 55mm mesh plankton net and concentrated to 20 ml. The filtrates were immediately preserved in 5% formalin. Microscopic identification was performed up to genera. Each sample was stirred smoothly just before microscopic analysis. One ml from agitated sample was then withdrawn with wide mouth graduated pipette and poured in a Sedge-Wick Rafter counting cell. Identification and enumeration of each sample were done by a binocular microscope (Model Olympus CK-2, ULWCD-Japan). The mean of 3 of such estimates was then calculated for each component occurring in the total count. Finally the existing phytoplankyon and zooplankton were expressed in no./ml and no./l, respectively. Monthly quantitative fluctuation and percent composition of various groups were also determined. Qualitative studies were done according to Presscott (1964), Needham and Needham (1972). Statistical analysis was done to find out the deviation of different parameters from the mean and to determine the extent of correlation amongst different parameters.

Results

Depth : Water invaded the Chanda beel in May-June, reached maximum in September and then dropped sharply (Fig 1). In January, there was some rise in water level.

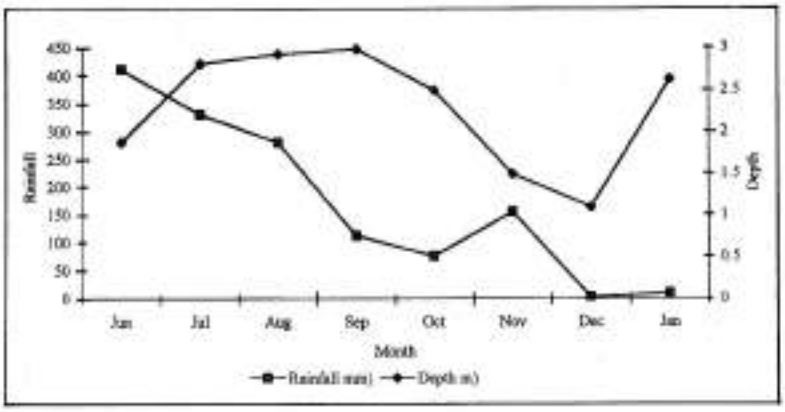


Fig. 1. Monthly fluctuation of rainfall (mm) and water depth (m).

Air temperature **:** Air temperature was the highest in the month of June and the lowest in the month of January. It ranged between 33.67 ^{0}C and 26.6 ^{0}C (Fig 2).

Water temperature **:** It ran almost parallel to ambient air temperature in most parts of the floodplain. The highest temperature (31.7 ^{0}C) was recorded in the month of June and the lowest (25.2 ^{0}C) in January (Fig 2). Air temperature and water temperature showed strong positive (r=0.89) correlation.

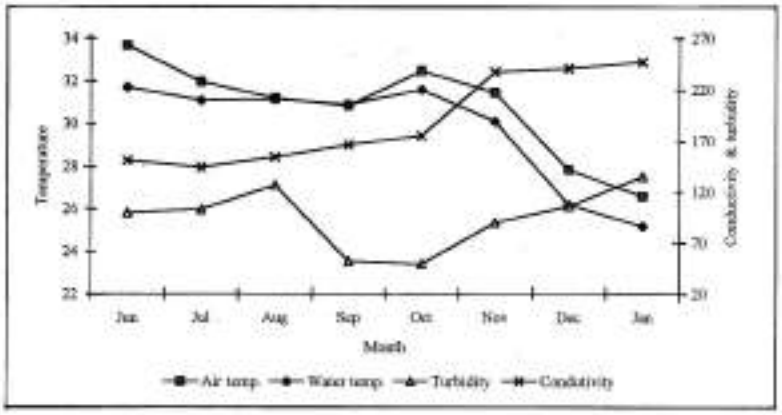


Fig. 2. Monthly fluctuation of air temp. (^{O}C), water temp. (^{O}C), conductivity (µs) and turbidity (ppm).

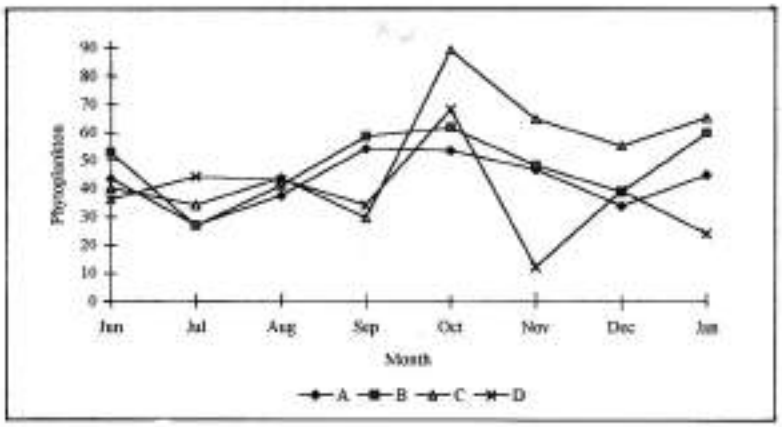
Turbidity **:** The water of Chanda beel was not highly turbid. Water was comparatively more turbid in August and January (Fig 2). Canal water showed more turbid and it was clearly different from the rest parts of the beel. Turbidity showed negative and weak relationship (r=-0.25) with temperature.

Dissolved oxygen **:** Dissolved oxygen (DO) content prevailed around or above 6 ppm. The highest value (6.96 ppm) was recorded in December and the lowest (5.46 ppm) in August. DO content showed negative relationship (r=-0.39) with temperature.

pH **:** The pH values remained around neutral throughout the study period. It showed a sharp rise during July-August. January was the month when the pH value (7.46) was the highest. Seasonal and temporal fluctuation of pH was very conspicuous. pH values were more acidic at the bottom layer than the surface. Relationship of pH with temperature was not significant (r=0.03).

Conductivity **:** Conductivity values remained between 144 ms/cm and 240.63 ms/cm during the study period (Fig 2). It showed a strong negative relationship (r=-0.63) with temperature.

Phytoplankton : The monthly abundance of total phytoplankton varied from 30±10/ml (July) to 66±19/ml (October). The temporal and spatial fluctuation of total number of phytoplankton in different areas are shown in Fig 3. In the area of true beel portion the content of phytoplankton was always relatively high.



(A = Aman paddy area, B=true beel area, C= canal, D=deep waterhyacinth area)

Fig. 3. Temporal and spatial fluctuation of total number of phytoplankton (nos/ml).

A total of 43 genera of phytoplankton were identified. Among these the most abundant genera were *Chlorella, Scenedesmus, Spirogyra, Ulothrix, Aphanocapsa, Anabaena, Lyngbya, Diatoma, Navicula, Synedra, Phacus, Closterium* etc. All phytoplankton genera belonged to five groups and these were Chlorophyceae, Myxophyceae, Bacillariophyceae, Desmid and Protozoa. The percent composition of these groups in different months are shown in Fig 4. Chlorophytes and Myxophytes showed their maximum abundance in June to August whereas Protozoans and Desmids were higher in percent composition during winter.

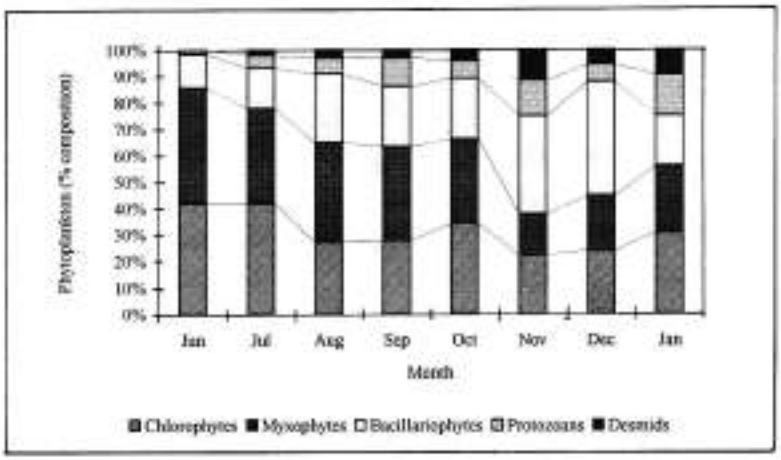
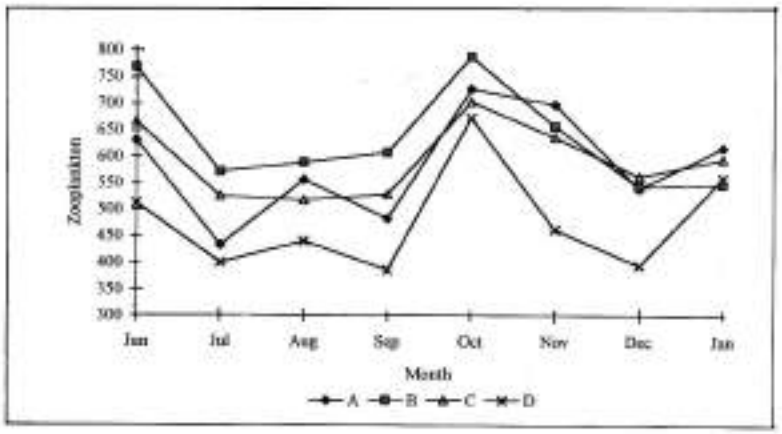


Fig. 4. Temporal fluctuation in percent compostion of various Phytoplankton groups.

Chlorophyceae and Myxophyceae were the most dominant groups followed by Bacillariophyceae, Desmid and Protozoa. Yearly mean phytoplankton population during investigation was 46±10/ml. Relationship of phytoplankton with temperature (r=0.19) and with turbidity (r=-0.22) were not significant.

Zooplankton : The average number of zooplankton fluctuated between 496±90/l and 735±52/l. The temporal and spatial fluctuation of total number of zooplankton (nos/l) in different areas of the floodplain are shown in Fig 5



(A= Aman paddy area, B= true beel area, C=canal, D= deep waterhyacinth area)

Fig. 5. Temporal and spatial fluctuation of total number of zooplankton (nos./l)

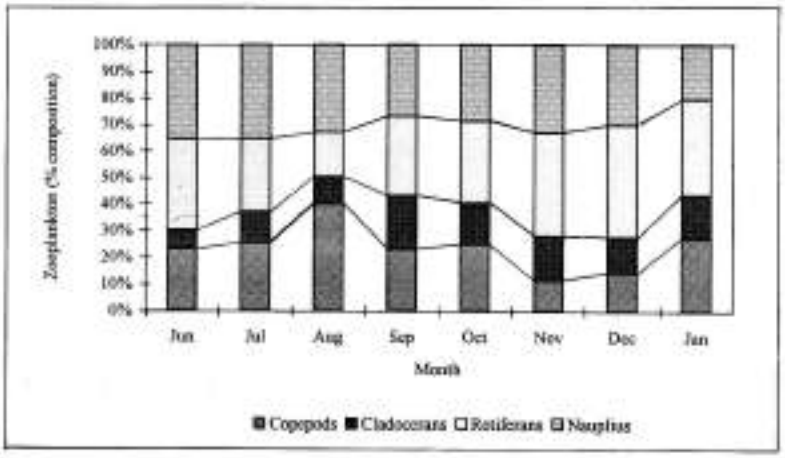


Fig. 6 . Temporal variation in percent composition of various zooplakton groups.

Like phytoplankton, zooplankton population was also relatively high in true beel area. A total of 26 genera of zooplankton were identified. Among these the most abundant genera were *Cyclops, Diaptomous, Bosmina, Daphnia,*

Diaphanosoma, *Sida*, *Moina*, *Brachionus*, *Keratella*, *Lecane* and *Polyarthra*. All zooplankton genera belonged to four groups which are Rotifera, Copepoda, Cladocera and Nauplius. Monthly variation in percent composition of various zooplankton groups is shown in Fig 6. Rotifera and Nauplius were higher in percent composition during winter whereas Copepoda and Cladocera were found to be higher in percent composition during summer. Rotifera was the most dominant group followed by Nauplius, Copepoda and Cladocera.

Yearly mean of zooplankton population was 575±90/l. Relationship of zooplankton with temperature (r=-0.18) and turbidity (r=-0.08) was negative and insignificant. Phytoplankton and zooplankton showed positive correlation (r=0.41).

Discussion

Natural waterbody is an ecosystem with a complicated network of various physico-chemical and biological parameters and its biota.

Depth : Chanda reached in maximum water depth in the month of September. Similar phenomena was observed by Hasanat *et al.* (1996) in the river of Old Brahmaputra. In January there was some rise in water level in the canal due to occasional rain. Minimum depth was registered in winter. Leaching and evaporation were supposed to be important operatives behind lower water depth.

Water temperature : Water temperature is highly synergistic with the air temperature. The water temperature is influenced by the air temperature, hours of sunshine, rainfall, depth of water and overall weather condition.

Chanda showed the highest temperature in June and the lowest temperature was found in the month of January is supported by Mathew (1975). Temperature between surface and bottom water did not differ notably throughout the study period possibly due to flow of water and wave action. Data pertaining to temperature showed that water temperature fairly followed air temperature as it is generally known except few exceptions. Water temperature showed direct relationship with air temperature, also reported by Ismail *et al.* (1984) and Begum *et al.* (1989).

Turbidity : The turbidity of water is caused by silting, microorganisms and suspended organic matter in the water (Hutchinson 1957). Waterbody showed two distinct maxima, one in monsoon i.e. in the month of August and another in dry season. Hussainy (1967) registered the highest water turbidity in the month of August. Ahmed *et al.* (1992) recorded the lowest water transparency in January in Kaptai lake. The turbidity of the canal and rest parts of the beel were

clearly different, canals being more turbid, perhaps due to continuous movement of water.

Dissolved oxygen : As oxygen regulates the most of the vital process of plants and animals, it is the most important factor in both aquatic and terrestrial environment (Rahman 1992). DO content in Chanda was in expected level throughout the study period. The highest values of DO were recorded in winter and the lowest in summer. Similar was recorded by Dewan (1973).

The high concentration of dissolved oxygen content was possibly because of low temperature, low rainfall and low pH. Due to low temperature and low rainfall, the decomposition of organic matter was less with low production of free CO_2 and low consumption of DO. The low DO content during summer and autumn was possibly due to high temperature and heavy rainfall which enhanced the oxidation of organic matter by the consumption of DO and the high production of free CO_2. Annual average DO content was around the optimum productive range 5-7 ppm.

pH : pH is considered as an important factor in fish culture. It indicates the acidity-alkalinity condition of a waterbody. Observed pH value was alkaline in nature with small variation. Ruttner (1953) stated that a eutrophic lake normally maintains alkaline pH. Comparatively low values of pH in monsoon agree with the findings of Banerjea and Roy (1970). The floodplain showed its highest pH values in the month of January and October. pH values had been observed to be more acidic at the bottom water than the surface.

Conductivity : Electrolytic conductivity is the ability of a solution to pass an electric current and the reciprocal of the solution resistivity. It is closely related to the chemical purity of water, the amount of dissolved solids in a solution and the efficiency of various treatments processes. Conductivity was observed to increase during winter months. Similar observation was made by Ahmed *et al.* (1992), and Khondker and Parveen (1992).

Phytoplankton : The productivity of a waterbody has a direct bearing upon the welfare of fish life and the role of plankton in the tropic cycle has been well recognized. During the present study a distinct fluctuation of both phytoplankton and zooplankton in different months as well as seasons was observed. Similar observations were noted by Chowdhury and Sultana (1989) and Mathias (1991) in various habitats.

It is evident from the observation that a variation with the months existed in the phytoplankton standing crop. It varied between 30±10/ml and 60±19/ml. More or less similar was found in some other floodplains (FRI 1995). The phytoplankton bio-mass showed mainly two peaks in the present study, the first peak in October and the second peak in January. Patra and Azadi (1987)

registered two high peaks, one in February and another in August, minimum count was in May.

Chlorophyceae and Myxophyceae were the most abundant group followed by Bacillariophyceae, Protozoan and Desmid. Chlorophytes and Myxophytes showed their maximum abundance in June to August whereas, Protozoans and Desmids was higher in percent composition during winter. Findings of Mathew (1975) that Desmids though represented by several species, formed a very limited part of the plankton, were similar to present investigation. Kaliyamurthy (1974) observed Protozoan plankters were very rare in Pulicat lake in India.

Total number of zooplankton also varied with the months as well as seasons, between 496±90/l and 735±52/l. Like phytoplankton, zooplankton also showed two peaks, first peak in October and second peak in January. Patra and Azadi (1987) found two peaks of zooplankton, one in August and another in February.

Rotifera was the most dominant group followed by Nauplius, Copepoda and Cladocera. Rotifera and Nauplius were higher in percent composition in winter, whereas Copepoda and Cladocera were found to be higher in percent composition in summer. Patra and Azadi (1987) noted that zooplankton was mainly dominated by Copepoda, Cladocera and Rotifera. They added that Copepoda attained the highest peak in June, minimum in December. Ahmed *et al.* (1992) found Rotifera was the most dominant group followed by Copepoda and Cladocera in Kaptai lake.

Both phytoplankton and zooplankton showed direct relationship between themselves. Similar relationship were found by Kaliyamurthy (1974) in Pulikat lake, India; Patra and Azadi (1987) in Halda river. Several plankton obtained in present observation composed of typically eutrophic. *Melosira, Ceratium, Anabaena , Anacystis* etc. are mentioned as indicator of eutrophic nature Mathew (1975).

The physico-chemical parameters of Chanda beel were in the suitable range. Obtained planktons also indicate that this beel is eutrophic in nature. Through stocking with carp fingerlings fish production may be increased in this beel.

References

Ahmed, K.K., G.C. Halder and S.B. Saha, 1992. *Limnological Studies on Kaptai Lake*. Final Report. Fisheries Research Institute, Riverine Sub-station, Rangamati. 36 pp .

Banerjea, S.M. and N.C. Roy, 1970. Observations on some physico-chemical features of the Chilka lake. *Indian J. Fish.*, **13:** 395-429.

BCAS, 1991. *Floodplain Production Monitoring*. Initial Study Report. Bangladesh Centre for Advanced Studies, Dhaka. 7 pp.

Begum, A., G. Mustafa, S. Ali and K.. Ahmed, 1989. Studies on limnology in a minipond and growth of *Tilapia* (=*Oreochromis*) *nilotica*. *Bangladesh J. Zool.*, **17**(1): 35-45.

Chowdhury, A.N.B. and N. Sultana, 1989. Occurrence and seasonal variation of zooplankton in a fish pond in relation to some physico-chemical factors. *Bangladesh J. Zool.*, **17**(2) : 101-106.

Dewan, S. 1973. Investigation into the Ecology of Fishes of a Mymensingh Lake. A Ph.D. thesis submitted to the Department of Aquaculture and Management, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. pp. 191-202.

DOF (Department of Fisheries), 1995. *Project Proforma (revised)*, Third Fisheries Project. Department of Fisheries, GOB, Bangladesh. 126 pp .

FRI, 1995. *Research Progress Report on Limnological Investigation into New Floodplains*. November '95. Fisheries Research Institute, Third Fisheries Project, Santahar, pp. 11-13.

Hasanat, M.A., M.A. Islam, M.A. Khair and M.S. Alam, 1996. *Studies on the Plankton of the river Old Brahmaputra. In*: Souvenir on Tenth Zoological Conference of the Zoological Society of Bangladesh. May 28-30, 1996. 72 p.

Hutchinson, G.E., 1957. A Treatise on Limnology. Vol. 1. Geography, Physics and Chemistry. John Wiley and Sons Inc., New York, USA. 1051 pp.

Hussainy, S.V., 1967. Studies on the limnology and primary production of a tropical lake in India. *Hydrobiologia*, **30**: 335-352.

Ismail, M., A. Rahman, S. Ali, and K. Ahmed. 1984. Studies on limnology and some aspects of biology of *Oreochromies niloticus* (L) in a pond Juran, Dhaka. *Proc. Fourth. Nat. Zool. Conf. Bangladesh* : 97-105.

Kalyiamurthy, 1974. Observation on the plankton ecology of Pulicat lake, *Hydrobiologia*, **45:** 86-95.

Khondker, M.. and L. Parveen, 1992. Study on the physical and chemical limnology of a shallow, hypertrophic artificial lake. *Bangladesh J. Sci. Res.*, **10**(1): 9-16.

Mathew, P. M., 1975. Limnology and productivity of Govindgarh lake, Maddhya Prodesh, India. *J. Inland. Fish. Soc. India*, **11:** 16-24.

Mathias, P.M., 1991. Seasonal trends in the fluctuation of phytoplankton and physico-chemical factors in a tropical lake (Govindgarh lake, Maddhya Pradesh, India) and their interrelationship. *J. Inland. Fish. Soc. India*, **17**(1-2): 11-24.

Mazid, M.A., M.S. Hossain, A. Razzaque, and S. Rahman, 1996. Fishing Methods in the Floodplains and Measure to Control. FRI Publication No. 13. Fisheries Research Institute, Mymensingh, Bangladesh. 15 pp.

Needham, J.G. and R.P. Needham, 1972. A Guide to the Study of Freshwater Biology. Holden-Dey, Inc Cali., 94 (III), USA.

Patra, R.W.R. and M.A. Azadi, 1987. Ecological studies on the planktonic organisms of the Halda river. *Bangladesh J. Zool.*, **15**(2): 109-123.

Presscott, G.W., 1964. How to Know the Freshwater Algae. Brown Co., Inc., Dubupue, Lowa. 272 pp.

Rahman, M.S, 1992. Water Quality Management in Aquaculture. BRAC prokashona, Dhaka, Bangladesh. pp. 71-72 .

Ruttner, F., 1953. Fundamentals of Limnology. Univ. of Toronto Press. Toronto, Canada. 242 pp.

(Manuscript received 2 December 1996)

Bangladesh J. Fish. Res., 1(1) : 41-46
Bangladesh Fisheries Research Institute

A study on damage caused to crustacean and finfish larvae during collection of *Penaeus monodon* (Fab.) postlarvae in the estuaries of Barguna, Bangladesh

S.L. Rahman[1], M.M. Islam*, M.E. Hoq[2], G.C. Haldar[1] and S.U. Ahmed

Brackishwater Station, Bangladesh Fisheries Research Institute
Khulna-9280, Bangladesh
[1]FRI, Riverine Station, Chandpur
[2]FRI, Freshwater Station, Mymensingh
**Corresponding author*

Abstract

A year round investigation in the estuaries of Barguna district revealed that for each *Penaeus monodon* postlarvae (PL), about 37 larvae of other shrimp species, 12 finfishes and 10 macrozooplankters are destroyed during the process of shrimp seed collection. Although abundance of *P. monodon* PL was not recorded throughout the year, a significant number of other shrimp spp., fin fishes including macrozooplankters are being damaged by the shrimp seed collectors. This indiscriminate destruction of aquatic organisms during *P. monodon* PL collection is serious threat to aquatic biodiversity.

Key words : *P. monodon* PL, Finfish larvae, Colossal loss

Introduction

Shrimp exports contribute a very positive impact on the national economy of Bangladesh. Recent expansion of farming areas and the trend of selective stocking of *P. monodon* postlarvae (PL) by farmers has resulted in tremendous demand of seed (PL) of this species. The high demand of *P. monodon* PL has stimulated a large number of people in shrimp seed collection along the coastal belt. Shrimp seed are extensively collected by push net and fixed bag nets, and the seed collectors transfer their catches to earthen bowls, which are carried to the river bank. *P. monodon* PL locally known as "Bagda pona" are then sorted out and the rest are discarded along the dry shore, result in large wastage of both penaeid larvae and other commercially important aquatic organisms.

Some previous investigations gave some information on zooplankton with special reference to penaeid postlarvae (Zafar and Mahmood 1994, Hossain 1984) in estuaries of this country. The first information on colossal loss to zooplankton during shrimp seed collection in the estuarine waters of Chakaria

Sundarban, Satkhira and Khepupara are given by Mahmood (1990). The present year-round study was carried out in two river-estuaries of Barguna with the objectives of identification of seasonal and spatial pattern in crustacean and fin fish larval distribution, although, the main objective was to assess the quantum of damage caused to shell and finfish larvae while collecting *P. monodon* seed.

Materials and methods

Field methods

Samples were drawn from two major rivers *viz.* Baleshawr and Bishkhali of Barguna coastal district during December'92 through November'93. Three stations were selected in each river.

Sample were drawn at fortnightly intervals. A rectangular drag net made of nylon netting (mesh size 1 mm) and bamboo spilt structure(1.6x0.6m) was used for sampling. The net was operated in shallow waters of the river against current for about 10 minutes. Two samples were collected at day time during low and high tides. Immediately after collection samples were preserved in 5% buffered formalin solution. Salinity of water was recorded weekly by direct reading refractometer. Water temperature was measured weekly by a alcohol thermometer.

Laboratory analysis

In the laboratory *P. monodon* PL were identified and separated following Muthu (1978) and Motoh and Buri (1980), and other shrimp and fin fishes larvae including zooplankters were identified into major taxonomic group following Davis (1985), Fischer and Witchead (1974) and George (1969).

Results and discussion

Hydrographic conditions

Average monthly water temperature and salinity values have been shown in Fig. 1. Water temperature ranged between 20.4 to 30.8^{0}C in both the rivers. During the period between July and December salinity attained '0' in both rivers. This lowest salinity may be due to monsoon effects, during other part of the year (January-June) salinity ranged between 1 to 6 ppt with highest in May. Abundance of *P. monodon* PL were probably related with presence of salinity, during '0' salinity period almost no *P. monodon* PL was recorded. Average salinity was lower than the rivers of Patuakhali and Bagerhat districts (FRI 1996).

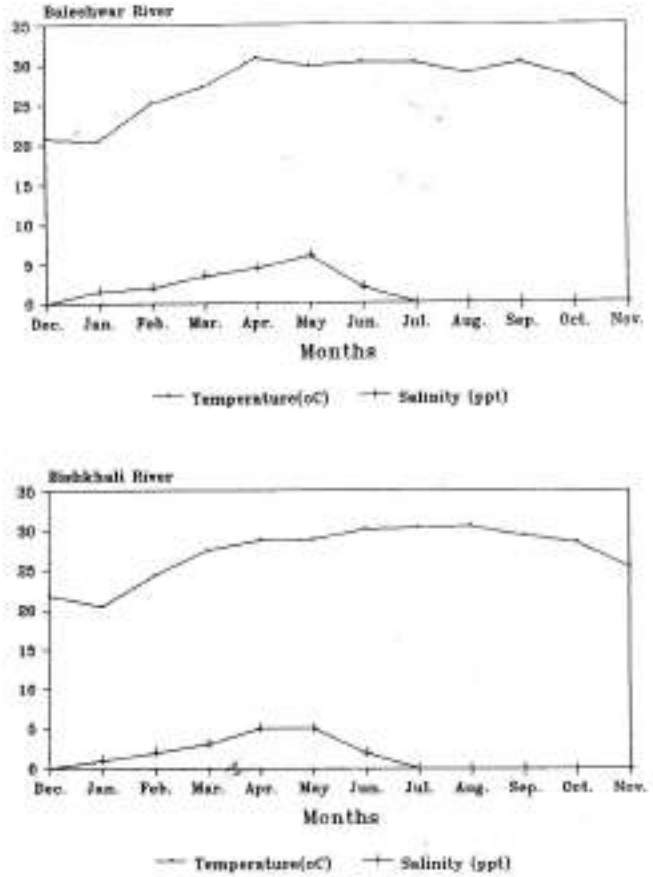


Fig. 2. Monthly distribution of water temperature and salinity in two rivers of Barguna district during study period.

Distribution of individual taxa

Monthly quantitative distribution (individuals/unit effort) of *P. monodon* postlarvae, larvae of other shrimps, finfishes and macrozooplankters in two rivers of Barguna district have shown in Table 1. No *P. monodon* PL was found during the months of July to October when there was no salinity in river. On the other hand, abundance of other shrimp spp. increased during the months of August through January, and the maximum was recorded in October. There was a sudden decrease of other shrimps in November, but the finfish larvae occurred in abundance during this month. There was no uniform pattern in distribution of both finfish larvae and macrozooplankters, their abundance fluctuated from one month to another. Finfish larvae and zooplankters were more abundant in post monsoon period than other time of the year.

Table 1. Monthly distribution (Individual/unit effort)[1] of different taxa in the two major rivers of Barguna

Major taxa	Months												Yearly Total	%
	Dec.	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.		
A. BALESHWAR RIVER														
Penaeus monodon	1	2	3	3	2	2	1	0	0	0	1	1	s6	1.75
Other shrimp spp.	100	42	27	35	22	14	34	19	37	46	155	35	566	61.99
Finfish	16	4	4	16	11	9	7	11	5	7	30	80	200	1.91
Macrozooplankter	9	5	16	20	13	19	12	8	11	7	7	4	131	14.35
Total number	126	53	50	4	48	44	54	39	3	60	192	120	913	100.00
B. BISHKHALI RIVER														
Penaeus monodon	1	2	3	2	3	2	1	0	0	0	0	1	15	1.60
Other shrimp spp.	95	6	36	1	14	21	5	26	35	85	17	0	591	63.21
Finfish	10	7	8	15	12	9	6	18	13	11	20	37	166	17.76
Macrozooplankter	8	6	14	19	26	36	9	7	15	1	2	10	163	17.43
Total number	114	71	61	67	55	68	51	51	63	107	139	88	935	100.00

[1] *Operating a drag net (1.6 X 0.6m) for about 10 minutes as a unit effort*

Composition and dominant taxa

P. monodon PL contributed a small fraction to the total catch composition, 1.75% in Baleshawr and 1.60% in Bishkhali river. Other shrimp spp., finfishes and macrozooplankters showed more or less similar pattern in distribution in the two rivers. Other shrimp species included *P. indicus, Metapenaeus monoceros, M. brevicornis, Macrobrachium* spp. Finfishes included *Lates calcarifer, Setipina phasa, Glossogobius* spp., *Liza* spp. and other macrozooplankters were Isopods, Copepod, *Acetes* sp., Mysids, Alima, and crab larvae etc. Abundance of shrimp PL other than *P. monodon* reflected majority (63.21%) in Bishkhali river followed by Baleshawr river (62%). Finfishes occupied 21.91% and 17.76% in Baleshawr and Bishkhali river respectively. Other zooplankton population occupied only 17.43% in Bishkhali and 14.35% in Baleshawr river.

Relative abundance and colossal loss

Due to high demand of *P. monodon* PL, the number of shrimp fry collectors increased to a great extent in the coastal region of this country. All suitable sites for shrimp fry collection along the coastal rivers are exploited by them using fine meshed nylon nets. This causes a great loss to other aquatic organisms at the early stage of their life cycle. The present attempt to quantify the damage as a result of such exploitation, revealed that on an average the catch composition (%) of *P. monodon* PL were only 1.67%, other shrimps 62.60%, finfishes 19.84% and macrozooplankters 15.89% (Table 2). This observation revealed that for collecting single *P. monodon* PL, 37 other shrimps, 12 finfishes and 10 macrozooplankters are being destroyed by shrimp fry collectors. Mahmood (1990) reported from Chakaria Sundarban, Satkhira and Khepupara estuaries that for fishing single *P. monodon* PL, 14 other shrimp PLs., 21 finfishes and 1631 zooplanktons were killed. The variations in zooplankton population with the present observation might be due to difference in mesh size of the collection net. The microzooplankters could not collected for this study due to large mesh size (1 mm) of the gear used.

Table 2. Average catch composition of *P. monodon*, other shrimp spp., finfishes and macrozooplankters from rivers of Barguna

Major Taxa	Yearly average catch (%)	Number of other species destroyed for each *P. monodon* PL collection
P. monodon	1.67	-
Other shrimp spp.	62.60	37
Finfishes	19.84	12
Macrozooplankters	15.89	10
Total	100.00	59

Composition and dominant taxa

P. monodon PL contributed a small fraction to the total catch composition, 1.75% in Baleshawr and 1.60% in Bishkhali river. Other shrimp spp., finfishes and macrozooplankters showed more or less similar pattern in distribution in the two rivers. Other shrimp species included *P. indicus, Metapenaeus monoceros, M. brevicornis, Macrobrachium* spp. Finfishes included *Lates calcarifer, Setipina phasa, Glossogobius* spp., *Liza* spp. and other macrozooplankters were Isopods, Copepod, *Acetes* sp., Mysids, Alima, and crab larvae etc. Abundance of shrimp PL other than *P. monodon* reflected majority (63.21%) in Bishkhali river followed by Baleshawr river (62%). Finfishes occupied 21.91% and 17.76% in Baleshawr and Bishkhali river respectively. Other zooplankton population occupied only 17.43% in Bishkhali and 14.35% in Baleshawr river.

Relative abundance and colossal loss

Due to high demand of *P. monodon* PL, the number of shrimp fry collectors increased to a great extent in the coastal region of this country. All suitable sites for shrimp fry collection along the coastal rivers are exploited by them using fine meshed nylon nets. This causes a great loss to other aquatic organisms at the early stage of their life cycle. The present attempt to quantify the damage as a result of such exploitation, revealed that on an average the catch composition (%) of *P. monodon* PL were only 1.67%, other shrimps 62.60%, finfishes 19.84% and macrozooplankters 15.89% (Table 2). This observation revealed that for collecting single *P. monodon* PL, 37 other shrimps, 12 finfishes and 10 macrozooplankters are being destroyed by shrimp fry collectors. Mahmood (1990) reported from Chakaria Sundarban, Satkhira and Khepupara estuaries that for fishing single *P. monodon* PL, 14 other shrimp PLs., 21 finfishes and 1631 zooplanktons were killed. The variations in zooplankton population with the present observation might be due to difference in mesh size of the collection net. The microzooplankters could not collected for this study due to large mesh size (1 mm) of the gear used.

Table 2. Average catch composition of *P. monodon*, other shrimp spp., finfishes and macrozooplankters from rivers of Barguna

Major Taxa	Yearly average catch (%)	Number of other species destroyed for each *P. monodon* PL collection
P. monodon	1.67	-
Other shrimp spp.	62.60	37
Finfishes	19.84	12
Macrozooplankters	15.89	10
Total	100.00	59

An estimate was made on the *P. monodon* PL, harvested from rivers of Barguna district revealed that about 1.3 billion *P. monodon* PL were collected during the year 1993, resulted damage of large number of other crustaceans and finfishes during shrimp fry collection. According to Funegaard (1986), about 2000 shrimp fry/net/day were collected by the collectors of Satkhira district in 1982 which were reduced to 200 fry/net/day in 1986- reflected the adverse effect of indiscriminate shrimp fry collection in the coastal region of this country. Immediate steps should be taken to stop this practice to conserve the aquatic biodiversity.

References

Davis, C. C., 1985. The Marine and Freshwater Plankton. Michigan State University Press. 16 pp.

Fischer, W. and P. J. P. Witchead (eds.), 1974. FAO species identification sheets for fishery purposes. Eastern Indian ocean fishing area 57 and western central pacific area 71. Rome, FAO, Pag-Var., Vol.I-IV.

FRI (Fisheries Research Institute), 1996. *Survey and assessment of shrimp fry resources in Bangladesh- Annual report. In*: Research Progress Report (1993-96). FRI, Mymensingh. (unpublished)

Funegaard, P., 1986. Shrimp seed any to sell? Come to Satkhira, Bangladesh. *Bay of Bengal News*, **22**: 2-6.

George, M.J., 1969. Prawn Fisheries of India-II. Systematic taxonomic consideration and general distribution. *Bull. Cent. Mar. Fish. Res. Inst.*, **14**: 5-48.

Hossain, M. A., 1984. Studies on zooplankton communities of the Mathamuhuri river estuary with special reference to shrimp larvae during south west monsoon. *M.Sc. dissertation*. Inst. of Mar. Sci., University of Chittagong, Bangladesh. 38 pp.

Mahmood, N., 1990. An assessment on the quantum of damage caused to the zooplankton while fishing bagda shrimp *Penaeus monodon* fry in Bangladesh estuaries. *Proc. Seventh Zool. Conf. Bangladesh*: pp. 87-94.

Motoh, H. and P. Buri, 1980. Identification of the postlarvae *Penaeus* (Crustacea, Decapoda, Penaeidae) appearing along shore water. Aquaculture Dept. SEAFDEC, Trigbaun, Iliolio, Philippines, *Res. Rep.* **4**(2): 15-19.

Muthu, M. S., 1978. *Larval development : Specific identity of penaeid postlarvae found in brackishwater areas in coastal aquaculture. In*: Marine Prawn Culture, Part-1 : Larval development of Indian penaeid prawns (ed. KNK Kartha). *CMFRI Bulletin*, **28**: 86-90.

Zafar, M. and N. Mahmood, 1994. Occurrence and abundance of penaeid postlarvae of the genera *Penaeus, Metapenaeus and Parapenaeopsis* in the estuarine waters of Satkhira, Bangladesh. *Chittagong University Studies, Part-II: Science*, **18**(1): 39-45.

(Manuscript received 31 December 1996)

Bangladesh J. Fish. Res., 1(1) : 47-52
Bangladesh Fisheries Research Institute

Impact of stocking density on growth and survival rate of mud crab (*Scylla serrata* Froskal)

M. Rani Saha, P. K. Roy and S. U. Ahmed

Brackishwater Station, Bangladesh Fisheries Research Institute
Khulna-9280, Bangladesh

Abstract

A 10-weeks culture trial of mud crab, *Scylla serrata* in brackishwater earthen pond was conducted in different stocking densities. The aim of the experiment was to identify a suitable stocking density for optimum production. There were three treatment as 5000 crablings/ha, 10000 crablings/ha and 15000 crablings/ha of each with three replications. The initial mean weight of crablings were same (5.5 ± 0.13 g). The experimental month was June '95 to August '95. The size of each pond was 500 m^2. To maintain a good water quality water was exchanged in every spring tide. The salinity during the experiment were 2-18 ppt. Prepared feed of about 32% protein consisting fish meal, MOC, rice bran and wheat flour was used at 5% of their body weight. In terms of production, survival rate, growth and carapace width, the stocking density having 10000/ha showed the best ($P<0.05$) performance followed by 5000/ha and 15000/ha.

Key words : *S. serrata*, Stocking density

Introduction

Bangladesh has a coast line of about 480 km and about 628,780 ha of potential mangrove tidal flats (Mac-Nac 1974), where capture and culture of mud crab can be undertaken profitably. Many rivers and tributaries terminating in the Bay have formed an intricate network of cross-channels and creeks in Bangladesh's estuarine area. Mahmood (1977) reported 16 crab species of the coast of Bangladesh. In spite of having a culture favour brackishwater environment a crab fishery has not yet been established in Bangladesh. Literature suggests that the optimum range for better growth of mud crab is 15-30 ppt, and for larval rearing the salinity should above 17 ppt. And the year round occurrence of the larvae in Mathamuhury estuary even at a very low salinity (2ppt) was reported by Ahmed (1991).

Mud crab as a good export item in live condition from Bangladesh has been growing higher demand in world market day by day. In view of the gradual increasing importance of the commodity study was carried out for the

development of culture technology of mud crab at different stocking densities in the brackishwater environment of Bangladesh.

Materials and methods

The experiment was conducted in the brackishwater ponds of Fisheries Research Institute at Paikgacha, Khulna and continued for 70 days. The experimental conditions are given in Table 1. After construction of dykes and gates, the ponds were allowed for sun drying for 15 days. All the ponds were fenced by bamboo slits at about 0.5m deep in the soil to prevent escaping and burrowing of crab. Lime was applied at a rate 125 kg/ha in all the ponds followed by application of cowdung at a rate of 500 kg/ha after 7 days of liming and the ponds were filled by tidal water of Kapotakhya river.

Table 1. Experimental conditions of mud crab (*Scylla serrata*)

Conditions	Recorded data
Experimental period	70 Days
Water source	Kapotakhya river
Experimental months	June-August
Water depth	0.8-0.6M
Water exchange	50%/spring tide
Feeding frequency	5% body weight/day
Pond size	500 sqm
Treatment	Three stocking density
T_1	5000 crabling/ha
T_2	10000 crabling/ha
T_3	15000 crabling/ha
Range of water temperature	29-33^{0}C
Salinity range	2-18 ppt
pH range	8.4-8.6
Dissolved oxygen range	5.6-6.5 ppm

Crablings collected from natural source by trapping, baiting and netting during shrimp seed collection. There were three treatments having three replications of each such as stocking density of 5000/ha crablings (T_1), 10000 crablings/ha (T_2) and 15000 crablings/ha(T_3) respectively. Crablings were acclimated to the laboratory condition for 7 days prior to stock in the experimental ponds.

Formulated feed prepared by fish meal, mustard oil cake, rice bran and wheat flour were fed twice daily at 12 hourly intervals between 06:00 and 18:00 hours at a rate of 5% body weight per day for all treatments. Ingredients of feed and proximate composition are given in Table 2.

Table 2. Formulation and nutritional value of feed used during the experimental period

Composition of ingredients	Proximate composition of ingredients					
	Dry matter	Protein	Fat	Ash	CF	NFE
Fish meal	89.04	56.00	16.00	28.00	-	-
Mustard oil cake	90.14	35.35	16.81	6.36	13.40	8.04
Rice bran	92.45	11.88	10.45	5.40	27.85	44.42
Wheat flour	92.14	12.48	1.32	2.11	2.14	81.95
Percent of nutrient		32.29	12.19	11.81	10.02	33.69

Water exchange by at least 50% was done during the high tides of new and full moon throughout the experimental period. Sampling for growth performance and water quality parameters were done weekly. Specific growth rate, food conversion ratio (FCR), carapace width of crab, total production and survival rate were calculated following the guide lines of European Inland Fisheries Advisory Commission (1980). All crabs were harvested by complete drain out of the pond water. Comparison of treatment means was carried out using one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test ($P<0.05$).

Results

The growth responses and production in three different stocking densities are presented in Table 3 and 4 respectively.

Table 3. Growth responses of *Scylla serrata* crablings at different stocking density over the 70 days experimental period

Treat ments	Mean body weight (g)										
	Culture period (Week)										
	Initial	1	2	3	4	5	6	7	8	9	10
T_1	5.5	8.6	14.5	25.8	36.4	46.2	55.7	68.5	82.1	98.3	113.13
T_2	5.4	8.7	14.0	24.4	35.4	39.0	51.6	63.2	76.4	80.4	93.0
T_3	5.5	8.5	14.1	24.0	32.6	37.1	44.0	53.2	61.7	70.3	73.0

Table 4. Growth, production and survival rate of mud crab during the experimental period

Parameters	Treatments			
	T_1	T_2	T_3	SE ±
Initial weight (g)	5.50	5.40	5.50	0.13
Final weight (g)	113.13[a]	93.0[b]	73.00	2.14
Weight gain (g)	107.63	87.60[b]	67.50[c]	3.66
Specific growth rate (%)	1.88	1.77[b]	1.75[b]	0.03
Initial carapace width (cm)	4.0[a]	2.30[a]	2.40[a]	0.20
Final carapace width (cm)	5.50[a]	5.30[a]	5.00	0.17
Food conversion ratio	2.56[a]	2.18[b]	2.87[a]	0.17
Survival rate (%)	28	31	31	
Production (Kg/ha)	158.4[b]	288.7[a]	338.3[a]	11.1

Figures in the same column with same letters are not significant different (P>0.05)

Differences in the initial weights of the crablings used in three treatment were insignificant but at termination of the experiment the performance differed significantly ($P<0.05$). Growth was more or less similar in the first two weeks, but from the third week a variation in the growth was appeared which was more prominent from the fourth week (Table 3). The best production was recorded in T_3 (338.25 kg/ha) followed by the T_2 (288.7 kh/ha) and T_1 (158.37 kg/ha) respectively. The survival rate of T_3 and T_2 were (31%) same. The performance of specific growth rate was best in T_1 (1.88) followed by T_2 (1.77) and T_3 (1.75) respectively. The change of carapace width during the experiment were homogenous. Though apparently there were slight variation of final carapace width but there were no significant different among three treatments. The lowest weight gain was found in T_3 (67.5 g) followed by T_2 (87.6 g) and T_1 (107.625 g). The food performance in T_2 was best (FCR 2.18) than other two treatments.

Water quality parameters was monitored from each pond throughout the experimental period. The temperature range from 29-33^{0}C, pH 8.4-8.6, salinity 2-18 ppt, dissolved oxygen 5.6-6.5 ppm and transparency 32-40 cm.

Discussion

Although the apparent production of crab in T_3 was highest but not statistically significant different from T_2. The lowest value of food conversion ratio found in T_2 among three treatment indicate low production cost in stocking density having 10000/ha. The same carapace width also confirm the same market price of harvested crab in all treatments. In terms of carapace size the present study reveals, that stocking density having 5000/ha have the highest carapace size which is supported by the finding of Balioao and Gerochi (1981) where author showed relation between highest average carapace size and percentage of survival, so positive impact of low stocking density on carapace size has been assumed by low stocking density. Total production of crab having stocking density of 15000/ha was found higher than 10000/ha, without any significant variation. Higher production obtained from stocking density of 10000/ha than 5000/ha suggest the best stocking density among three treatment which is supported by the statement of Fuad and Hanafi (1991). Chaiyakam and Parnichsuka (1977) also studied the relation among the stocking density, survival rate and production of mud crab. They found survival rate of 57% with a yield of 171.68 kg/rai while stocked with 1 individual/m^2, the survival rate was found low (30%) with increase in yield (224.16 kg per rai). This studies confirmed the present findings. However, the overall total production of crabs in all treatments were found low than recorded by Chaiyakam and Parnichsuka (1977 and 1978), Bensam (1980), Lapie and Librero (1979) which may be due to low feeding rate and short culture period. Although the survival rate of crab in present study were low but was similar to the findings after Raphae (1972). The low survival may in cause low salinity in the last three weeks of the experimental period.

Conclusions

Considering the average weight gain, total production and survival rate of crab, it may be concluded from this experiment that optimum stocking density for the culture of mud crab equal to or greater 1000/ha. More growth trial with longer culture period may be carried out to support of the present results in mono and polyculture of mud crab.

References

Ahmed, M.K., 1991. *Mud crab- a potential aqua-resource of Bangladesh. In*: The mud crab - a report on the seminar convened in Surat Thani, Thailand, 5-8 November 1991. BOBP/REP, **51**: 95-102.

Arshadullah, M.D., 1976. Industrial processing of crab *Scylla serrata* (Forskal) (Decopoda Brachyura) of the Karnaphully estuary, Chittagong, Bangladesh. *M. Sc. Thesis*. Dept. of Mar. Biol., Univ. of Chittagong.

Balio, D.D., E.M. Rodriguez, D.D. Gerochi, 1981. Culture of the mud crab, *Scylla serrata* (Forskal) at different stocking densities in brackishwater ponds. Quarterly Research Report. SEAFDEC Aquaculture Department, **5**(1): 10-14.

Bensam, P., 1980. *Culture experiment on the crab Scylla serrata* (Forskal) *at Tuticorin to assess growth and production. In*: Symposium on Coastal Aquaculture, Cochin, India, 12-18 January 1980. Abstracts. Cochin, Marine Biological Association of India. 97 pp.

Bhuyian, A.L. and N.G Das, 1976. Preliminary study on the taxonomy of crabs (Crustacea Brachyura) of the northeastern part of the Bay of Bengal. Symp. P. Ist Bangla. Conf. 48 pp.

Chong, L.P., 1993. INFOFISH International. **3/93**: 46-49.

Chaiyakam, K., P. Parnichsuka, 1977. Experiments on rearing of mud crab, *Scylla serrata* (Forskal) in bamboo pens. Annual Report–Songkhla Fisheries Station. Department of Fisheries: 91-98.

Chaiyakam, K., P. Parnichsuka. 1977. Experiments on rearing of mud crab, *Scylla serrata* (Forskal) in brackishwater pond and cement pond. Annual Report.– Songkhla Fisheries Station. Department of Fisheries: 72-89. (in Thai).

Cholik, F. and A. Hanafi, 1991. *A review of the status of the mud crab (Scylla sp.) fishery and culture in Indonesia. In*: The mud crab - a report on the seminar convened in Surat Thani, Thailand, 5-8 November 1991. BOBP/REP, **51**: 13-27.

Duncan, D.B., 1995. Multiple range and multiple F-tests. *Biometrics*, **11**: 1-42.

Islam, J., 1977. Brachyura of the Karnafally estuary and north-east part of the Bay of Bengal with special reference to the biology of *Scylla serrata*. *M. Sc. Thesis*. Dept of Mar. Biol., Univ. of Chittagong. 160 pp.

Lapie, L.P., A.R. Librero, 1979. Crab farming in the Philippines: a socio–economic study. Los Banson, Laguna, SEAFDOC-PCARR Research Programme. Research paper series. Socio–economic Survey of the Aquaculture Industry in the Philippines no. 21: 56 pp.

Lijauco, M.M., O.Q. Prospero, E.M. Rodriguez, 1980. Polyculture of milkfish (*Chanos chanos*) and mud crab (*Scylla serrata*) at two stocking densities. Quarterly Research Report. SEAFDEC Aquaculture Department, **4**(4): 19-23.

Mac-Nac, 1974. Mangrove Forest and Fisheries. FAO/UNDP/IOPC/Dev. 74/34: 35 pp.

Mahmood, N., 1977. Crustacean fauna of the Bay of Bengal of the coast of Bangladesh. Paper presented at the Biological Workshop organized by National Commission for UNESCO. 6 pp.

Raphel, Y.I., 1972. *Preliminary report on the brackishwater pond culture of Scylla serrata (Forskal) in Ceylon. In:* Coastal Aquaculture in the Indo-Pacific Region (ed. T.V.R. Pillay). West Bfleet, Fishing News (Books). p.395. (Abstract only)

Sivasubramaniam, K. and C. Angell, 1991. *A review of the culture, marketing and resources of the mud crab (Scylla serrata) in the Bay of Bengal region. In:* The mud crab- a report on the seminar convinced in Surat Thani, *In*: Thailand, 5-8 November 1991. BOBP/REP, **51**: 5-12.

(Manuscript received 14 December 1996)

Bangladesh J. Fish. Res., 1(1) : 53-63
Bangladesh Fisheries Research Institute

Effect of stocking density on growth of tiger shrimp (*Penaeus monodon* Fab.) fed on commercial formulated diets

S. C. Chakraborty, A. K. Paul[1] and M. A. Hossain [2]

Department of Fisheries Technology,
Bangladesh Agricultural University, Mymensingh, 2202, Bangladesh
[1]*Sonali Bank, Barisal Branch, Bangladesh*
[2]*Department of Aquaculture, BAU, Mymensingh*

Abstract

Three different stocking rates in a semi-intensive pilot shrimp project was adopted in duplicates of three treatments designated as T_1, T_2 and T_3 having initial per metre square stocking density of shrimp of 40, 44 and 51 respectively of 0.025 g size post larva. The study was conducted for 84 days. Commercial pelleted diets designated as starter - 1, 2, 3 and grower were fed at a satiation level during the study period with a feeding frequency of 4 to 5 times per day. Feed rationing was based on the survivability, body weight and tray checking. Five to twenty five percent of the pond water was exchanged daily. Sampling was done for growth after every 2nd week. Monthly sampling was done for mortality in the ponds. Mean weight gain of the shrimp in treatments T_1, T_2 and T_3 were 16.96 ± 1.14, 16.04 ± 1.38 and 14.08 ± 1.17 g respectively and T_1 with a low stocking density showed a significantly best growth among the treatments. Total mortality in treatments T_1, T_2 and T_3 were as 30.00, 39.77 and 31.37% respectively. Significantly higher feed conversion ratio (FCR) of 1.87 was obtained with shrimp in treatment T_3 followed by shrimp in T_1 and T_2 with FCR values of 1.70 and 1.41 respectively. A positive correlation of growth and salinity was observed during the study. Total production per unit area was the highest in the treatment T_3 (4928 kg/ha) and followed by T_1 (4747 kg / ha) and T_2 (4251 kg/ ha). The result show significantly negative correlation between individual growth and density.

Key words : *P. monodon*, Stocking density, Formulated diets

Introduction

Bangladesh has about 2.5 million ha of coastal tidal lands, of which 2.2 million ha of lands may be suitable for brackish water aquaculture. Total shrimp production of Bangladesh in 1994 - 95 is reported to be 10,1778 mt of which

47,830 mt comes from inland water, 23,445 mt comes from marine water and 30,503 mt from coastal area of which 80% of total shrimp production comes from natural sources (Paul 1996). Most shrimp farms in Bangladesh are operated on a very extensive basis, relying on natural productivity and have little or no managements. A few farmers are claiming production levels of 900 kg / ha /year with improved cultural practices. This can be achieved to an average production of 4,500 to 7,200 kg/ha / year by encouraging the introduction of more intensive shrimp farming methods.

It is reported that 30,000 ha of potential area at Cox's Bazar is suitable for developing semi-intensive culture farm of Peneaid shrimp (C. P. Shrimp News 1994). Natural tidal fluctuations of this area permit easy entrance and elimination of expected saline water. Depending on the stability of salinity 2 crops per year may be obtained by culturing Peneaid species. Moreover, Peneaid shrimp species may be stocked at a higher density than *Macrobrachium* sp. which return a higher yield in comparison to later. Considering potentials, Peneaid shrimp is much more suitable for semi-intensive culture system. Semi-intensive culture of Penaeid shrimp in this country has developed recently. In semi-intensive culture of penaeid shrimp a good water quality management and proper utilization of supplied food may afford a fruitful result. In this aspect proper food rationing plays an important role. However, high feeding rates results in organic load which alter pond environment leading to mass mortality of shrimp particularly in ponds stocked with high densities (Clifford 1992). The optimum stocking density together with water quality management and feed rationing paves for developing semi-intensive culture of Penaeid shrimp.

For sustainable development of semi-intensive farming system, the study has been undertaken with the objectives to investigate the effect of stocking density on the growth and survival of tiger shrimp (*Penaeus monodon*) using commercial pelleted diets in order to assess the growth performance and feed utilization efficiencies.

Materials and methods

The experiment was conducted between September 1993 and December 1993 in six culture ponds of Beximco Fisheries Ltd. located at Khurushkul, on the bank of the river Bakkhali in the coastal district of Cox's Bazar, Bangladesh. A total area of about 5.5 hectares comprising 3.93 hectares of water area was selected for the study. The farm consisted of 7 ponds, a single reservoir, a network of water discharge and feeder canals. A good water supply and drain-out system was there in the farm where water could be pumped to the reservoir (during spring-tide water entered in the reservoir through the sluice gate via the lift-valve), to get in to the feeder canal. The water in the reservoir is treated to settle the suspended material. A coarse and fine meshed net protected the entrance of undesirable particle and organism to the feeder canal. Discharge of water was maintained by gravitational forces through PVC pipes which was set at three different levels of water for elimination of desired level

water as per requirements. Six paddle wheels for each pond were used for aeration and elimination of waste product.

For the study a total of six ponds comprising two replicates for each of treatment T_1, treatment T_2 and treatment T_3 with stocking densities of 40, 44 and 51 nos. post larvae of shrimps per metre square respectively were selected. Before starting of the experiment, black soil of the pond bottoms were removed followed by sun drying for 7 days. Lime was used at a rate of 250 kg/ha on the bottom and slope of these ponds. The water depth of the ponds were kept up to 1 meter. Urea and Triple-super-phosphate (TSP) at a rate of 7 kg/ha and 4 kg / ha respectively were then sprayed on paddle wheel for production of Phytoplankton and Zoo-plankton. Tea seed cake, at a rate of 8 ppm was applied three days after entering water for killing of unexpected organisms in ponds. After three days, the ponds were ready for stocking shrimp fry .

The fry (PL_{10} to PL_{20}) having mean weight of 0.025 g and mean length of 18.00 mm) of tiger shrimp (*Penaeus monodon*) were collected from "The Niribili private nursery and hatchery, Ltd", Cox's Bazar, Bangladesh. Commercial pelleted diets designated, Starter-1, starter-2, starter-3 and grower obtained from Saudi-Bangla fish feed Ltd., Mymensingh containing 30.57, 38.00, 38.00 and 36.00% crude protein mostly fish meal based respectively were used in the feeding trials (Table 1). Blind feeding was continued 7 days after stocking, considering 10% mortality. Weekly sampling was done for getting mean weight of shrimps in each pond. Feeding frequency varied between 4 - 5 times per day depending on the mean weight of the shrimps. Starter-1 was fed upto 0.1 g mean body weight of shrimp. Starter-2, starter-3 and grower were fed upto 0.5 g, 3 g and 20 g size respectively from the lower limit.

Table 1. Proximate composition of the commercial diets (% dry matter basis) used in the feeding trials (obtained from Soudi-Bangla Fish Feed Ltd, Bangladesh)

	FEED			
Components	Starter-1	Starter-2	Starter-3	Grower
Dry matter	81.50	87.40	87.50	88.00
Crude protein	37.50	43.47	43.42	40.90
Crude lipid	3.44	3.27	3.47	4.04
Ash	22.08	20.02	16.00	13.63
Crude fibre	4.29	4.34	6.28	4.68
[1]Nitrogen free extract (NFE)	32.70	28.90	0.83	5.75
[2]Gross energy (Kcal/g)	3.76	3.91	4.01	4.13

[1] *Nitrogen free extract as 100 - %(Moisture + crude protein + crude lipid + Crude fibre + ash)*

[2] *The energy value of the feeds calculated as adopted by Dare and Edwards (1975) considering protein =5.5 Kcal/g;lipid = 9.45 Kcal/g and carbohydrate = 4.2 Kcal/g*

For feeding, 1 m^2 hanging-feeding-tray platforms for each 1600 m^2 water area were set at 2 to 3 meters far from the bank by a pair of bamboo pole and a strong plank. Total daily ration allocated for feeding tray was divided by the no. of trays per pond and spread out on the tray during feed broadcast. The unfed food on trays were collected, pooled and dried for each pond and used to adjust subsequent feed ration. Gut contents of shrimp were checked for under feeding and full feeding.

Routine water quality measurement was done during the experimental period. Among these, dissolved oxygen was measured by YSI 57 DO meter before sun rise and at 4.00 p.m. daily. Temperature, pH, salinity and transparency (secchi-disc method) were measured once daily and ammonia was measured (by field method) monthly. Depending on the requirements water was exchanged about 5 to 25% daily and water depth was maintained 1 meter up to 30th day of culture and up to 1.75 meters for the rest. At the primary culture stage a small amount of water was exchanged for maintaining expected phytoplankton and zooplankton level which are natural food for fry and also a controlling factor of "lab lab" and dissolved oxygen. Fertilization was done as per requirements for maintaining transparency at expected level of 30 to 40 cm. Lab lab was collected by scoop net immediately after floating. Lime was applied in the pond water, 30 days after stocking at a rate of 5 ppm and continued weekly for rest of the culture period. Paddle wheels of 2 HP in each pond were used for occasional agitation upto 20 days of culture period. Sampling by cast net was done after every 2nd week of stocking. One hundred shrimps were weighed and measured for each pond. For estimation of mortality, cast net was operated randomly in 5 to 7 pre-selected places and the captured shrimps were counted. The area covered by the sampling cast net was measured and compared by some terrestrial casting. Efficiency of cast net was considered at 80% (Pers. com. Uddin, Farm Manager, Beximco Fisheries Ltd., Cox's Bazar). Total stocking was then determined by using following formula.

$$\text{Estimated stock} = \frac{\text{Mean No. of shrimp per capture X Total area of pond}}{\text{Measured mean area covered by cast net}} \text{ X } 0.8$$

Then survivability was calculated. Initial harvesting was done by cast net and finally by lantern net and hand picking. During harvesting, mean weight and number of harvested shrimps were recorded and finally survivability was calculated.

The experimental diets, carcass composition of shrimps under the experiment were analyzed for proximate analysis by the method described in AOAC (1980). For initial carcass analysis of shrimp a group of significant number of fry (weighing about 20g fry from the initial stock) were sacrificed at the beginning of the experiment, and used for proximate analysis for moisture, crude protein, crude lipid and ash, and considered as initial carcass

composition. The final carcass composition of harvested shrimp samples from each replicate was dried in oven at 105°C for 24 hours and ground by meat grinder to be analyzed in the same process.

The following nutritional performances were measured from the recorded data.

(a) Weight gain (%) = [(final weight - initial weight)/initial weight] X 100

(b) Specific growth rate (SGR %/day) = [(ln final weight - ln initial weight)/time (in days)] X 100where, ln = natural log, log base 2.303

(c) Food conversion ratio (FCR) = dry food fed/live weight gain

(d) Protein efficiency ratio (PER) = live weight gain/protein fed

(e) Apparent net protein utilization (ANPU) = protein retained/protein ingested) X 100

One-way analysis of variance (ANOVA) was done to find the significant difference among the treatment means followed by the Duncan's new multiple range test using minitab (Rhyan & Joiner 1985) as package on microcomputer.

Results

The water quality in respect of temperature, dissolved oxygen, salinity, transparency, ammonia etc. have been shown in Table 2. Temperature during the experimental period in different period in different treatments T_1, T_2 and T_3 ranged between minimum value of 24.4 and maximum value of 29.5^{0}C having no significant variation in weekly temperature. Similarly, dissolved oxygen ranged from 4.28 to 5.88 mg/l in the morning and 7.45 to 8.98 mg/l in the evening showing no significant variation ($p>0.05$) on weekly observation among the three treatments (Table 2). Salinity varied within a range between 17.2 and 25.8 ppt. having no significantly different ($p>0.05$) values within weekly observation. The mean pH values in different weeks in the treatments T_1, T_2 and T_3 were 7.78 to 8.69, 7.87 to 8.73 and 7.72 to 8.82 respectively and did not vary on weekly values. Transparency of the pond water varied from 31.71 cm in T_2 at the start to 72.0 cm at the end (Table 2). Ammonia was always below 0.002 mg / l$^-$.

Table 2. Water quality parameters in respect of temperature, dissolved oxygen, salinity, pH and transparency of the different treatments during experimental period

Treatment	Temperature(^{0}C)				Dissolved Oxygen (mg /l)			Salinity (ppt)		pH		Transparency (cm)	
	Mini-	Maxi	Mini	Maxi	Mini	Maxi	Mini-	Maxi	Mini-	Maxi	Mini-	Maxi
T_1	24.5	29.5	4.3	5.6	7.6	9.0	17.4	25.8	7.8	8.7	32.2	51.8
T_2	4.4	29.4	4.7	5.9	7.5	9.0	17.2	25.4	7.9	8.7	31.7	72.0
T_3	24.5	29.5	4.3	5.8	7.5	9.0	17.5	25.5	7.9	8.7	32.7	45.7

The mean body weight gain of shrimps during the experiment in different treatments are presented in Fig 1. Significantly higher ($p<0.05$) growth was observed with shrimp in treatment T_1 (lowest stocking density) followed by treatment T_2 and T_3 respectively. The nutritional parameters of shrimp, *P. monodon* measured in various treatment groups during experimental period are shown in Table 3. Specific growth rate (SGR) of shrimp in treatments T_1, T_2 and T_3 were 3.41, 3.38 and 3.31 respectively and were not significantly different ($p>0.05$) whereas, significantly different ($p<0.05$) values of food conversion ratio (FCR) of the treatments T_1, T_2 and T_3 were 1.70, 1.41 and 1.87 respectively (Table 3).

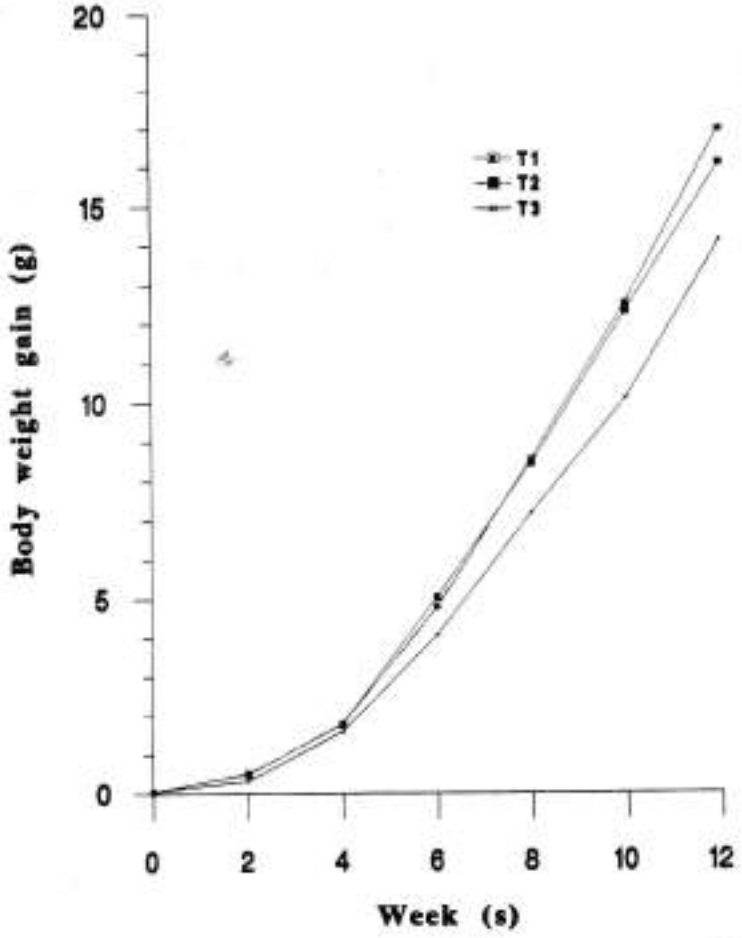


Fig. 1. Weight gain of *Penaeus mondon* in three treatment groups fed on different diets during 12 weeks of experimental period.

Table 3. Different growth parameters of *Penaeus monodon* in various treatment groups during 84 days of feeding trial

Parameters	Treatments		
	T_1	T_2	T_3
Initial mean weight (g/No.)	0.025	0.025	0.025
Final mean weight (g/No.)	16.96 (±1.14)	16.04 (±1.38)	14.08 (±1.17)
Mean weight gain (g/No.)	16.93^a (±0.45)	16.02^b (±0.35)	14.05^c (±0.65)
Specific growth rate (SGR)	3.41^a (±0.07)	3.38^a (±0.04)	3.31^a (±0.05)
Food conversion ratio (FCR)	1.70^a (±0.04)	1.41^b (±0.14)	1.87^c (±0.06)
Protein efficiency ratio (PER)	1.58^a (±0.05)	1.91^b (±0.12)	1.43^c (±0.08)
Apparent net protein utilization (ANPU) (%)	26.42^a	31.84^b	23.88^c
Survivability (%)	70.00	60.23	68.63
Production (Kg/ha)	4747	4251	4928

Different superscripts in the same row repogsent significant difference ($p<0.05$) of mean values

The food conversion ratio (FCR) values were calculated ignoring the effects of natural diets but on the basis of fed supplemented dry pellets only. The protein efficiency ratio (PER) of the treatment T_2 was significantly higher ($p<0.05$) (1.91) than other treatments (Table 3). Apparent net protein utilization (ANPU) values by the shrimp in different treatments fed various diets were 26.42, 31.84 and 23.88 for T_1, T_2 and T_3 respectively.

Survival rate was estimated twice during the experimental period and finally after harvesting. A massive mortality was occurred in the treatment T_2 just after stocking. Survivability was highest (70%) in treatment T_1 followed by T_3 (68.63%) and T_2 (60.23%).

Although, final mean weight-gain of individual shrimp in the treatment T_1 was the highest (16.96 g) (Table 3), total production per hectare was not the highest in this treatment due to its lower stocking density. However, the production calculated on the basis of final harvesting were 4747, 4251 and 4928 kg ha^{-1} in the T_1, T_2 and T_3 treatments respectively.

Discussion

Salinity in the range of 15 to 25 ppt were usually considered more suitable for *P. monodon* grow-out (Boyd 1989). Chiu (1988) also reported that the

optimum range of salinity for *P. monodon* farming should be within 20 to 25 ppt. The salinity in this experiment, was found within this limit which indicates a favourable condition for growth and survival of *P. monodon*.

Liao and Murai (1986) reported that the oxygen respiration rate of *P. monodon* remained constant at dissolved oxygen (DO) concentrations above 3.0 to 4.0 mg/l. In the present study dissolved oxygen (DO) measured in the morning (before sun rise) in the treatments were within the safe level (Table 2).

In the present study pH values in ponds were not below 7.72 (Table 2). This shows a reciprocal relationship between the salinity and pH values. Nakra (1994) stated that the optimum range of secchi disc reading should be between 30 and 60 cm during juvenile stage, above and between 25 and 40 cm to the sub-adult and final stage. In this study among the mean transparency level of three treatments, the mean value in the treatment T_2 was above the optimum level of juvenile shrimp. Massive mortality during the experimental period in the treatment T_2 might be due to the low productivity (Nakra 1994). However, the transparency in treatment T_3 was optimum from beginning of the stocking period and a greater survivability in that treatment might be due to optimum natural productivity.

The initial stocking density of the treatments in the present study were much higher in comparison to the stocking density practiced at other farms of the Cox's Bazar coast (Pers. comm. with other farm Managers). The stocking densities of the treatment T_1, T_2 and T_3 finally reduced to 28, 26.5 and 35 per metre square respectively due to uneven and massive mortality. However, the individual growth achieved by shrimp in the treatment T_1 was the highest (16.96g). Roy (1992) recorded pond water temperature in the winter season 20-30°C and in the summer season 25-32.5°C in his experiment, conducted at Aquaculture Farm Ltd., Cox's Bazar. Nakra (1994) stated the optimum range of temperature for the Black Tiger Shrimp (*P. monodon*) was between 28°C-30°C. Chiu (1988) also pointed out the temperature range of 25-32°C was the optimum for *P. monodon* farming. Therefore, lower individual weight of *P. monodon* in this study might be affected by the temperature below the optimum level (Apud *et al*. 1985).

In spite of these, individual weight gain by the shrimp of treatment T_1 was highest and followed by T_2 and T_3 (Table 3). It might be due to the lower density, maintained from initial stocking in the treatment T_1. Significant difference of individual weight gain was observed in the treatment T_3 (14.08) compared to T_1 (16.96) and T_2 (16.04). It may be explained as the stocking density of treatment T_3 was much higher (51/m^2) than T_1 (40/m^2) and T_2 (44/m^2) treatments. Wyban (1987) showed a negative co-relation between stocking density and growth. Similarly, values of 3.41, 3.38 and 3.31 for specific growth rate (SGR) found in treatments T_1, T_2 and T_3 might also be due to the effect of stocking density.

Chanratchakool *et al.* (1993) stated that the total FCR varies depending on the stocking density; quality of feed and the size at which the shrimps are harvested, but ideally it should not be higher than 2. They also stated that weekly FCR varies over the production cycle and between populations, but as a rough guide it should be between 1 and 1.5 in the early stages of the cycle and 1.5 and 2.5 in the later stages. Table 3 showed the FCR values of three treatments which were not greater than ideal value, but inspite of higher stocking density in the treatment T_2 FCR was lower than treatment T_1. It might be due to the higher mortality in the T_2 treatment which lowered the stocking density of treatment T_2 in comparison to T_1. The values of FCR in all the treatments indicates that the feeds (Starter - 1, 2, 3 and grower) were in category of good quality diets as because the mean FCR values were 1.70, 1.41 and 1.87 in T_1, T_2 and T_3 respectively. Similar result was obtained by Sedgwick (1979) with shrimp. He reported that FCR increased with level of rations fed and with the mean weight of the prawn as they grew.

It may be explained that growth was affected by the increase of total biomass due to higher stocking density which ultimately decreased the efficiency of food conversion with higher FCR value. From Fig. 1 it is seen that individual weight gain in shrimp in treatment T_3 was lower than those in T_1 and T_2 from the beginning of culture but FCR was higher than other two treatments. According to Sedgwick (1979) it seemed to be logical. He found that greatest efficiency of food conversion was achieved with relatively poor growth rate and he suggested to sacrifice some conversion efficiency to take full advantage of growth rate.

Apparent net protein utilization (ANPU) was the highest in the treatment T_2 (31.84) followed by T_1 (26.42) and T_3 (23.88). Protein efficiency ratio value was also highest in treatment T_2 (1.91) followed by T_1 (1.58) and T_3 (1.43). The ANPU and PER values in the present study indicates the better utilization of dietary protein.

Result of the study showed that stocking density affected the individual weight gain of shrimp but mortality may not be correlated with the stocking density. Therefore, similar experiment should be conducted with lower stocking density to compare the growth efficiency and mortality. Considering the disease problem of neighboring farms it was also recommended that farms should be situated at a reasonable distance from each other for avoiding entrance of polluted water-discharge.

References

Ali, S.A., 1982. Relative efficiencies of pelleted feed compound with different animal proteins and the effect of protein level on the growth of prawn *Penaeus indicus*. *Proc. Symp. Coastal Aquaculture*, **1**: 321-328.

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

Apud, F.D., J.H. Primavara and P.L. Torres Jr, 1985. Farming of prawn and shrimps (3rd edition). *Aquaculture Extension Manual No. 5*. Tigbaun, Ilollo, Philippines, SEAFDEC Aquaculture Department. 67 pp.

Boyd, C.E., 1989. Water quality management and aeration in shrimp farming. *Fisheries and Allied Aquaculture Departmental Series No. 2,* Auburn University, Alabama. 83 pp.

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

Chanratchakool, P., J.F. Turnbull and C. Limsuwan, 1993. Health Management in Shrimp Ponds. Published by Aquatic Animal Health Research Institute, Department of Fisheries, Kasetsart University Campus, Bangkok.

Chiu, Y. N., 1988. *Considerations for planning prawn farm projects. In*: Technical Consideration for the Management and Operation of Intensive Prawn Farms. (eds. Y.N. Chiu. L. M. Santos and R.O Juliano) U.P. Aquaculture Society, Iloilo City, Philippines. pp. 25-28.

Clifford, H. C., 1992. *Marine shrimp pond management: A review. In*: Technical Bulletin (ed. J. Wyban). *Proc. Special Session on Shrimp Farming*. World Aquaculture Society, Baton Rouge, LA, USA.

C. P. Shrimp News, 1994. *A successful shrimp farm in Bangladesh.* A Bimonthly Publication of C.P. Aquaculture Business Division. 12th Fir. C .P. Tower. 313 Silom Bangrak, Bangkok 10500. Thailand. **2**(4).

Liao, I.C. and T. Murai, 1986. Effects of dissolved oxygen, temperature and salinity on the oxygen consumption of grass shrimp, *Penaeus monodon*. *In*: J.L. Machlean, L.B. Dizon and L. V. Hosillos (eds.). The First Asian Fisheries Forum. Asian Fisheries society, Manila, Philippines. pp. 641-646.

Nakra, M., 1994. Prawn Farming Manual. Published by Shri G. A Raju, General Manager for the Waterdase Ltd., Ananthapuram, Village, Nellore 52344, India. 113 pp.

Paul, S. K., 1996. Chingri: Adhunik Chash Projukti O Upakulyo Paribesh Babosthapona. Published by Monika Paul, 22 Elephant Road, Dhaka -1205. 289 pp. (in Bangla)

Roy, A. K. 1992. Semi-intensive culture of tiger shrimp, *Penaeus monodon* with artificial diet. *M.Sc. Thesis,* Dept. of Fisheries Technology, Bangladesh Agricultural University, Mymensingh. 63 pp.

Ryan, B.F. and B.L. Joiner, 1985. Minitab Handbook. Duxbury Press, Boston. 379 pp.

Sedgwick, 1979. Effect of ration size and feeding frequency on the growth and food conversion of juvenile *Penaeus merguiensis* de Man. *Aquaculture,* **16**: 279-298.

Wyban, J.A., C.S. Lee, V.T. Sato, J.N. Sweeney, and W.K. Richards Jr, 1987. Effect of stocking density on shrimp growth rates in manure fertilized Ponds. *Aquaculture,* **61**: 23-32

(Manuscript received 9 July 1996)

Bangladesh J. Fish. Res., 1(1) : 65-72
Bangladesh Fisheries Research Institute

Digestible protein and energy value of fish meal, dextrin, fish oil and soybean oil for Thai sharpunti (*Puntius gonionotus* Bleeker)

M. A. Hossain, M.M. Rahman[1] and S. C. Chakraborty[1]

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

Abstract

A laboratory trial was conducted to determine the digestible protein and energy value of fish meal, dextrin, fish oil and soybean oil for Thai sharpunti (*Puntius gonionotus* Bleeker). A reference diet containing 35% protein was formulated in which fish meal was the sole source of protein. Five test diets were formulated using reference diet and individual test ingredients (fish meal, dextrin, fish oil and soybean oil). Each treatment had three replicates with 15 fish per replicate. Fish were fed twice daily at the rate of 5% of their body weight. The result of the study indicated that the dietary protein in both reference and test diets were well digested and the apparent protein digestibility (APD) values of test diets ranged between 82.81 and 85.99%. The APD value of fish meal protein was 88.05%. The apparent digestible energy (ADE) value for the test ingredients ranged between 70.79 and 85.80% with soybean oil having the highest and fish meal the lowest value. The ADE values calculated in terms of Kcal/g of ingredients were 3.68, 3.22, 4.38 and 4.44 Kcal/g for fish meal, dextrin, fish oil and soybean oil respectively.

Key words : *P. gonionotus*, Protein, Energy, Fish meal

Introduction

In feed formulation and manufacture, it might be useful to have an understanding on the digestibility of main ingredients of a diet as well as the whole diet. It appears that the ingredients in question could be treated as a diet and usual digestibility determination method could be used to determine its digestibility. This is not always possible. For example, the ingredient by itself might not behave in the same way as it would as a component of a compounded diet. Alternately, the fish may not ingest the ingredients itself. Ingredients with low protein can not be used as a single protein source in a diet.

Again, for determination of optimum digestible protein to energy ratio, digestibility of protein and energy of each of the ingredient is necessary.

The method that is presently used for estimating digestibility of an ingredient was first introduced by Cho *et al.* (1974) in which a reference diet and a test diet is used. Test diet is prepared by mixing 30% or 20% of the ingredients to be tested with the reference diet. The present study was undertaken to determine the digestible protein and energy value for fish meal, dextrin, fish oil and soybean oil for Thai sharpunti (*Puntius gonionotus* Bleeker).

Materials and methods

The experimental system used in the study consisted of 15 glass aquaria of 55 l capacity. All the aquaria were kept on 1 m high platform to facilitate better observation and accessibility. An adequate level of dissolved oxygen in each aquarium was maintained through artificial aeration.

Fry of Thai sharpunti, *P. gonionotus* (Bleeker) were collected from Freshwater Station, Fisheries Research Institute (FRI), Mymensingh. After receiving fry were given prophylactic treatment with 0.5 ppm $KMnO_4$ solution for 30 minutes. Before starting the experiment, fish fry were acclimated to the experimental system for one week. The fry were fed with formulated feed containing 35% protein during acclimation period.

There were five treatments each with three replicates. Uniform sized fingerlings of Thai sharpunti were randomly distributed at the rate of 15 fish per aquarium with a mean initial weight of 4.5g. Water in each aquarium was changed partially twice daily during the removal of uneaten food or faeces.

For formulation of experimental diets, fish meal was collected from Saudi Bangla Fish Feed Ltd, Bhaluka, Mymensingh which was originally imported from Singapore. Cod liver oil was used as fish oil (Seven Seas, British Cod Liver Oils Ltd, England). Good quality soybean oil was collected from Mymensingh local market. Dextrin, alpha-cellulose and carboxymethyl cellulose were obtained from Sigma Chemical Company, England. Mineral and Vitamin premixes (Embavit Fish Premix) was collected from Rhone Poulenc (Bangladesh).

Prior to the formulation of diet, the fish meal was analysed and the proximate composition (% dry matter) was protein 65.18%, lipid 11.24%, ash 21.51%, crude fibre 0.50% and nitrogen free extract 1.57%. A basal or reference diet was formulated using fish meal as the sole source of protein (Table 1). Test diets were formulated using reference diet and individual test ingredients (Table 2). Formulated diets contained 0.5% chromic oxide to study protein digestibility. Diets were prepared by using a Hobart pellet mill (Hobart A200). Diets were subjected to proximate composition analysis and the results are shown in Table 3.

Table 1. Composition of basal or reference diet

Ingredients	Percent of ingredients
Fish meal	53.70
Dextrin	33.30
Alpha-cellulose	10.00
Binder (CMC)[1]	2.00
Vitamin-mineral premix (Embavit)[2]	1.00
Total	100.00

[1] *Carboxymethyl cellulose (high viscosity)*
[2] *Obtained from Rhone poulenc (Bangladesh)*

Table 2. Formulation of test diets

Ingredients	Diets (%)				
	Reference	Fish meal	Dextrin	Fish oil	Soybean oil
Basal mixture	99.50	79.67	9.67	89.50	89.50
Fish meal	-	19.83	-	-	-
Dextrin	-	-	19.83	-	-
Fish oil	-	-	-	10.00	-
Soybean oil	-	-	-	-	10.00
Chromic oxide	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00

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

Components	Diets				
	Reference	Fish meal	Dextrin	Fish oil	Soybean oil
Dry matter	96.32	96.62	95.92	6.12	7.08
Protein	38.57	42.22	30.94	34.73	32.45
Lipid	4.09	5.26	4.12	12.55	14.70
Ash	13.71	17.55	11.34	13.12	12.94
Crude fibre	9.38	8.46	7.79	9.20	9.48
NFE[1]	34.25	26.51	45.81	30.40	30.43
Chromic oxide	0.49	0.49	0.50	0.48	0.48
Gross energy (Kcal/g)	4.49	4.49	4.56	5.20	5.18

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

Fish were fed experimental diets in the morning (at 9.00 h) and afternoon (at 17.00 h) daily at the rate of 5% of their body weight.

Faeces collection started four days after feeding to allow evacuation of all previously ingested material. Uneaten food or faeces were removed from each aquarium by siphoning after 30 minutes of feeding. Faeces were collected separately for each replicate twice daily in the morning and afternoon for four weeks. Collected faeces were dried in an oven at 60°C and kept in air- tight container for subsequent chemical analysis.

The water quality parameters such as temperature, pH and dissolved oxygen were monitored weekly and the ranges were temperature 27.5-32°C; pH 6.8-7.4 and dissolved oxygen 6.2-7.4 mg/l.

Proximate composition of the dietary ingredients, diets and faeces were analysed according to AOAC (1980). Energy content in feed and faeces were analysed by a Adiabatic Bomb Calorimeter. Chromic oxide content was determined following the wet-digestion technique of Furukawa and Tsukahara (1966). Estimates of the apparent nutrient digestibility of experimental diets were derived from the following equation (Maynard and Loosli 1969).

Apparent nutrient digestibility

$$=100 - \left(100 \times \frac{\%\ \text{Chromic oxide in feed}}{\%\ \text{Chromic oxide in faeces}} \times \frac{\%\ \text{nutrient in feed}}{\%\ \text{nutrient in faeces}}\right)$$

The apparent nutrient digestibility of the feed ingredients were estimated using the following equation (Cho *et al.* 1982).

$$\frac{100}{20}\left(\frac{\text{digestibility coefficient}}{\text{of test diet}} - \frac{80}{100}\ \frac{\text{digestibility coefficient}}{\text{of reference diet}}\right)$$

Statistical analysis of the data was performed by analysis of variance (ANOVA) followed by Duncan's New Multiple Range Test (Duncan 1955).

Results

The proximate composition of the experimental diets are shown in Table 3. The protein, lipid and energy content in different diets ranged between 32.45 to 42.22%, 4.09 to 14.70% and 4.49 to 5.20 Kcal/g respectively.

The protein, energy and chromic oxide content in faeces of fish fed experimental diets are shown in Table 4. The protein content was highest in faeces fish of fed reference diet whilst fish fed reference + soybean oil diet produced the lowest (14.14%) faecal protein.

Table 4. Protein, energy and chromic oxide content in faeces of fish fed experimental diets

	Diets				
	Reference diet	Ref. + Fish meal	Ref. + Dextrin	Ref. + Fish oil	Ref. + Soybean oil
Protein (%)	18.05	18.10	16.79	17.19	14.18
Chromic oxide (%)	1.57	1.53	1.58	1.44	1.44
Energy (Kcal/g)	4.44	4.48	4.44	4.48	4.44

The apparent protein digestibility (APD) and apparent digestible energy (ADE) value of the test diets are shown in Table 5. There was no significant difference (P>0.05) between the APD values of reference diet, reference + fish meal diet and reference + soybean oil diet. But these values were significantly (P>0.05) higher than those of reference + dextrin diet and reference + fish oil diet.

Table 5. Apparent protein digestibility and digestible energy value of experimental diets

	Diets				
Reference diet	Ref. + Fish meal	Ref. + Dextrin	Ref. + Fish oil	Ref. + Soybean oil	± S.E[2]
APD(%) 85.10[a][1]	85.89[a]	82.82[b]	82.81[b]	84.82[a]	0.36
ADE(%) 68.50[a]	70.36[a]	69.19[a]	70.08[a]	70.23[a]	0.53

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

[2] *Standard error of treatment mean calculated from the residual mean square in the analysis of variance*

There was no significant (P>0.05) differences between the ADE values of reference diet and test diets which ranged between 68.50 and 70.36%. The APD value of fish meal and ADE value of test ingredients such as fish meal, dextrin, fish oil and soybean oil are shown in Table 6. There was no significant difference (P>0.05) between the ADE of fish oil and soybean oil. But there was significant (P<0.05) difference between the ADE value of fish meal (74.69%) and dextrin (70.79%). The digestible energy (DE) values of fish meal, dextrin, fish oil and soybean oil calculated in terms of Kcal/g of the ingredients are also shown in Table 6. These values were 3.68, 3.22, 4.38 and 4.44 Kcal/g for fish meal, dextrin, fish oil and soybean oil respectively.

Table 6. Apparent protein digestibility (APD) and apparent digestible energy (ADE) values for test ingredients

	Ingredients				
	Fish meal	Dextrin	Fish oil	Soybean oil	± S.E[2]
APD(%)	88.05	-	-	-	-
ADE(%)	74.69[c1]	70.79[d]	84.30[a]	5.80[a]	0.48
DE (Kcal/g)	3.68[c]	3.22[a]	4.38[a]	4.44[a]	0.03

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

[2] *Standard error of treatment means calculated from the residual mean square in the analysis of variance*

Discussion

The protein, lipid and NFE content varied between experimental diets due to the variation in the amount of test ingredients mixed with the reference diet. The ranges of water quality parameters monitored during the study period were well within the limit for fish life and could not have hampered the growth of fish (Jhingran 1983).

The result of the present study indicated that the dietary protein in both reference and test diets were well digested. The APD value of the reference diet in the present study is 85.10%. This value is similar to the value reported by Law (1984) and Khan (1994) for reference diet consisting of fish meal, soybean, copra cake, maize and rice bran for jelawat (*Leptobarbus hoevenii*) and tropical catfish (*Mystus nemurus*). The APD values of the test diets ranged between 82.81 to 85.99%. The high APD values obtained in the test diets may be due to the fact that all the test diets contained more than about 80% of the reference diet and in reference diet fish meal was the only source of dietary protein. According to NRC (1977) carp can digest up to 95% of the protein in fish meal. However, the value can decrease to 80-85% depending on the origin and processing of the fish meal used (Ogino and Chen 1973). Brown *et al.* (1985) reported an APD value of 86% for fish meal in channel fish.

In the present study, there was no significant (P>0.05) difference between the ADE value of reference diet and test diets and the values ranged between 68.50 and 70.36%. Khan (1994) reported an ADE value of 78.5% for a reference diet for *Mystus nemurus* which is higher than the value obtained in the present study (68.50%). However, ADE (68.50%) value obtained in the present study is similar to the value of 69.41% reported by Law (1984) for Jelawat (*L. hoevenii*).

The APD value of fish meal was calculated from the formula by Cho *et al.* (1982) used to calculate apparent digestibility value for test ingredients. The APD value of fish meal in this study is 88.05% which is slightly lower than the APD value of fish meal (90.81%) reported by Law (1986) for jelawat (*L. hoevenii*) but

higher than the value reported by Khan (1994) for catfish (*M. nemurus*). Nandeesha *et al.* (1991) reported a higher APD value of 90.40% for fish meal in *Catla catla* using fish meal as 30% of the reference diet. On the other hand, Hasan *et al.* (1990) reported a somewhat lower APD value of 79% for fish meal in *Labeo rohita*.

The ADE value for fish meal in the present study is similar to the value (74%) reported for rainbow trout by Windell *et al.* (1978) but lower than the value obtained by Smith *et al.*(1980) 95% and Cho *et al.* (1982) 91% for the same species. Khan (1994) also reported a somewhat higher ADE value (77.88%) for fish meal in catfish (*M. nemurus*).

The ADE value of fish oil and soybean oil were significantly higher than ADE of fish meal and dextrin (Table 6). The higher ADE value of fish oil and soybean oil might be related to the high lipid digestibility of fish oil and soybean oil. Hossain *et al.* (1992) reported 89.96% and 93.24% digestibility of lipid in fish meal and soybean meal diet respectively for tilapia (*Oreochromis mossambicus*). Singh (1991) reported a lipid digestibility of 92.10 to 98.10% for feedstuff and pelleted feed of plant origin *Cirrhinus mrigala* yearlings. He also reported a lipid digestibility of 87.10 to 96.70% for conventional and unconventional feedstuff of plant origin in grass carp *(Ctenopharyngodon idella)*

The ADE value of dextrin was the lowest (70.79%) which might be related to the digestibility of dextrin as carbohydrate source in fish feed. Hasting (1969) reported a digestion coefficient of 74.8% for dextrin as carbohydrate source in rainbow trout diet.

The result of the present experiment indicated that fish meal, fish oil, soybean oil and dextrin used as fish feed ingredients have been well digested by Thai sharpunti as dietary protein, lipid and carbohydrate source respectively. The digestible energy values in terms of Kcal/g of the ingredients are 3.68, 3.22, 4.38 and 4.44 Kcal/g for fish meal, dextrin, fish oil and soybean oil respectively.

Acknowledgements

The authors would like to acknowledge the financial support of the ODA-BAU link project in carrying out the research work.

References

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

Brown, P.B., R.J. Strange and K.R. Robbins, 1985. Protein digestibility co-efficient for yearling channel catfish fed high protein feedstuffs. *Prog. Fish. Cult.*, **47**: 94-97.

Cho, C.Y., H.S. Bayley and S.J. Slinger, 1974. Partial replacement of herring meal with soybean meal and other changes in a diet for rainbow trout (*Salmo gairdneri*). *J. Fish. Res. Board Canada.*, **31**: 1523-1528.

Cho, C.Y., S.J. Slinger and H.S. Bayley, 1982. Bioenergetics of salmonid fishes: Energy intake, expenditure and productivity. *Comp. Biochem. Physiol.*, **73**(B): 25-41.

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

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

Hastings, W.H., 1969. *Nutritional score. In:* Fish in Research (eds. O. W. Neuhans and J.E. Halver). Academic press. New York. pp. 263-292.

Hossain, M.A., N. Nahar, M. Kamal and M.N. Islam, 1992. Nutrient digestibility coefficients of some plant and animal proteins for tilapia (*Oreochromis mossambicus*). *J. Aqua. Trop.*, **7**: 257-265.

Hasan, M.R., M. Moniruzzaman and A.M. Omar Farooque, 1990. Evaluation of leucaena and water hyacinth leaf meal as dietary protein sources for the fry of Indian major carp *Labeo rohita* (Hamilton). *In* : R. Hirano and I. Hanyu (eds.) *The Second Asian Fisheries Forum*, Asian Fisheries Society, Manila, Philippines. pp. 275-278.

Jhingran, V.G., 1983. Fish and Fisheries of India. Hindustan Publishing corporation. Delhi, 666 pp.

Khan, M.S., 1994. Apparent digestibility coefficient for common feed ingredients in formulated diets for tropical catfish, *Mystus nemurus* (Cuvier and Valenciennes). *Aquacult. Fish. Manage.*, **25**: 167-174.

Law, A.T., 1984. Nutritional study of Jelawat, *Leptobarbus hoevenii* (Bleeker), fed on pelleted feed. *Aquaculture*, **41**: 227-233.

Law, A.T., 1986. Digestibility of low cost ingredients in pelleted feed by grass carp (*Ctenopharyngodon idella*). *Aquaculture*, **51**: 97-103.

Maynard, L.A. and J.K. Loosli (eds.), 1969. Animal Nutrition. 6th edition. McGraw Hill Book Company. 613 pp.

Nandeesha, M.C., G.K. Srikanth, P. Keshavanath and S.K. Das, 1991. *Protein and fat digestibility of five feed ingredients by an Indian major carp, Catla catla (Ham.). In:* S.S. De Silva (ed.) Fish Nutrition Research in Asia. Special Publication No. 5. Asian Fisheries Society. Manila, Philippines. pp. 75-81.

NRC, 1977. Nutrient Requirements of Warmwater Fishes. National Academy of Science, Washington, D. C. 78 pp.

Ogino, C. and M.S. Chen, 1973. Protein nutrition in fish III. Apparent and true digestibility of dietary protein in carp. *Bull. Japan Soc. Sci. Fish.*, **39**: 649-651.

Smith, R.R., M.C. Peterson and A.C. Allred, 1980. Effect of leaching on apparent digestion coefficients of feedstuffs for salmonids. *Prog. Fish. Cult.*, **42**: 195-199.

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

(Manuscript received 27 December 1996)

Bangladesh J. Fish. Res., 1(1) : 73-82
Bangladesh Fisheries Research Institute

The 66 k-Da protein identified as a light meromyosin is involved in the setting of surimi

A.K.M. Nowsad Alam[1,*], and E. Niwa

Faculty of Bioresources, Mie University, Kamihama 1515, Tsu, Mie 514, Japan
[1] *Department of Fisheries Technology, Bangladesh Agricultural University Mymensingh 2202, Bangladesh*

[*] *Corresponding author*

Abstract

The 66 kilo-Dalton (kDa) protein split off from the crosslinked myosin heavy chain (CMHC) formed due to the setting of Alaska pollack surimi, frozen-storage of Pacific cod flesh, and vinegar-curing of Pacific mackerel mince was identified as a light meromyosin (LMM). Puncture and stress-relaxation tests showed that the actomyosin subunits (AMS) of Alaska pollack surimi, upon setting at 30°C, transformed into gel, although the elasticity of this gel was very low when compared to the gels from surimi or actomyosin (AM). Electrophoretic studies showed that the band due to LMM in the gel from AMS gradually disappeared with the progress of setting but higher molecular weight polymer did not form. The intensity of the bands due to other myosin sub-fragments decreased a little. The findings suggest that at setting temperature, LMM of MHC molecule leads to an unfolding resulting in an intramolecular aggregation through non-covalent interactions, and thus plays a significant role in the crosslinking of MHC.

Key words : Surimi, Light meromyosin, Non-covalent aggregation

Introduction

In the manufacturing process of kamaboko, a viscous water-washed and salt-ground fish paste (surimi paste) is usually heated at a low temperature (0-40°C) prior to cooking at a higher temperature (80-90°C). This process of heating at low temperature, called "setting", is performed to improve the elasticity of kamaboko. The surimi paste turns to an elastic, semi-transparent gel, called set gel or "suwari" in Japanese, at this time. During setting myosin heavy chain (MHC) of fish myosin crosslinks to form high molecular weight crosslinked myosin heavy chain (CMHC) (Lee *et al.*, 1990, Numakura *et al.* 1985, Numakura *et al.*1990).

In our previous experiments (Nowsad *et al.* 1993a, Nowsad *et al.* 1993b), it was observed that the CMHC was formed during the setting of Alaska pollack surimi by the aggregation of MHC and 66 kilo-Dalton (kDa) protein through weak hydrophobic and hydrogen bonds, from the fact that the CMHC split into MHC

and 66 kDa protein upon repeated extraction with concentrated urea and electrophoresis. Some low molecular weight protein components including 66 kDa protein were also split off from the CMHC formed during frozen-storage of Pacific cod flesh and dehydration of Alaska pollack surimi (Niwa *et al.* 1993). The results suggested that the 66 kDa protein might play an important role in the crosslinking of MHC. In this study, the 66 kDa protein was tentatively identified as a light meromyosin (LMM) and the role of this MHC subfragment in the setting of surimi was examined.

Materials and methods

SA-grade (Super-A grade) unsalted frozen surimi of Alaska pollack from Golden Alaska Seafoods Inc., Seattle, WA, U.S.A. was used. Pacific cod Gadus macrocephalus and Pacific mackerel *Pneumatophorus japonicus* were purchased from a retail fish store in Tsu city, Japan. Both trypsin (bovine pancreas) and trypsin inhibitor (soybean) were obtained from Sigma Chem. Co. U.S.A.

Extraction of AM

Mince of Alaska pollack surimi and dorsal and lateral muscles of Pacific cod were washed three times with 5 volumes of 3.38 mM NaH_2PO_4-15.5 mM Na_2HPO_4 (pH 7.5), followed by centrifugation at 6,000 x g for 10 min. AM was extracted for 3 h with 5 volumes of 0.8 M KCl-3.38 mM KH_2PO_4-20.5 mM Na_2HPO_4 (pH 8.0), followed by a dilution precipitation with water. One part of AM was concentrated by dialyzing against polyethylene glycol # 20,000 and the other part was dissolved in 0.5 M KCl-0.05 M Tris-maleate buffer (pH 6.2) for subsequent digestion by trypsin. All the procedures were done at 4°C.

Preparation of AM subunits containing 66 kDa protein (AM(S))

AM was digested with trypsin at an enzyme myosin ratio of 1 : 100 (w/w) at 10°C for 2 h at an AM concentration of 15 mg /ml in 0.5 M KCl-0.05 M Tris-maleate buffer (pH 6.2) according to the method of Tsuchiya and Matsumoto (1975). The reaction was terminated by the addition of a 3 fold higher by weight of soybean trypsin inhibitor solution. The digest was dialyzed against distilled water overnight, centrifuged at 30,000 x g for 30 min and dehydrated by dialyzing against polyethylene glycol # 20,000. All the products were used within a week.

Setting, frozen-storage, and vinegar-curing

Thawed surimi, AM, and AM(S) from Alaska pollack were, respectively, ground together with 3 % NaCl, 1 % sterilizer (Ueno Fine Chem. Co., Japan, Solmighty), and varied amount of water to maintain a final moisture content of 88

% by a mortar for 10 min at 4°C. The resulting pastes were stuffed into glass tubes (10 mm in inner diameter, 45 mm in length), set in a water bath at 30°C after wrapping both ends by parafilm (American National Can, Greenwich, CT. U.S.A.) and cooled in running tapwater.

For frozen-storage, 1 g of Pacific cod mince kept in a stoppered Erlenmeyer flask was stored in a freezer at -20°C for 30 days and thawed at room temperature before the preparation of sample for electrophoresis. For vinegar-curing, 1 g of Pacific mackerel paste ground with 3 % NaCl was stuffed into cellophane tube and cured in 5 % acetic acid at 4°C for 48 h.

Measurement of elasticity

a. Puncture test

Puncture test of the set gels sliced into 15 mm height was done by using a rheometer (Fudoh Kogyo Co., Japan, NRM 2010J-CW) with a spherical plunger (5 mm in diameter) at a table speed of 6 cm/min.

b. Stress-relaxation test

The same rheometer with a flat plunger (20 mm in diameter) was used to carry out the compression stress-relaxation test. The gel of the same shape was compressed at a table speed of 30 cm/min with a constant strain of 0.1. Stress-relaxation curves recorded for five min after compression were analysed by a four-element mechanical model where two sets of Maxwell's model were connected each other in parallel. An instantaneous elastic modulus of this mechanical model (G0), the elastic modulus of a spring coil of the Maxwell's model showing the longer relaxation time (G1), the modulus of another Maxwell's model showing the shorter relaxation time (G2), the viscosity of a dashpot constructing the former model (h1), and the viscosity of a dashpot of the latter model (h2) were obtained by the progressive approximate method (Tobolsky and Murakami 1959) through the following equation:

$$P(t) = e0 \left(\sum_{i=1}^{n} Gi\ e^{-t/ti}\right)$$

where, $P(t)$ = stress, e_0= constant strain, t = time, G_i = elastic modulus of i-th element, ($G_o = G_1 + G_2 + \ldots\ldots + G_n$, the instantaneous elastic modulus); and $T_i = \eta i / G_i$, η is the viscosity of the i-th element.

Sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE of the gels, frozen-stored flesh, vinegar-cured meat, AM and AM(S) were carried out as reported previously (Nowsad *et al.* 1993a) after dissolving in 8 M urea- 2 % SDS-2 % 2-mercaptoethanol-20 mM Tris-HCl (pH

8.0). AM and digested AM were dissolved in 0.5 M KCl-0.05 M Tris-maleate (pH 6.2), dialyzed against the same buffer and electrophoresed as above (op cit).

Extraction of CMHC

CMHC remained at the top of the disc gel after the SDS-PAGE was extracted in 8 M urea-2 % SDS-2 % 2-mercaptoethanol-20 mM Tris-HCl (pH 8.0) by the method described previously (Nowsad *et al.* 1993a).

Results and discussion

In Fig.1, SDS-PAGE patterns are shown for Alaska pollack surimi set at 30°C for 10 h (A), Pacific cod mince frozen-stored at -20°C for 30 days (B) and Pacific mackerel paste cured in acetic acid for 48 h (C). Three main bands were observed in all the discs - at their top as CMHC, at 205 kDa as MHC and just below 45 kDa as actin (Ac), similarly as in the case we described before (Nowsad *et al.* 1993a, Niwa *et al.* 1993). The intensity of MHC bands decreased with the progress of setting of Alaska pollack surimi, frozen- storage of Pacific cod mince and vinegar-curing of Pacific mackerel paste and in correspondence, the intensity of CMHC bands increased in all the cases. The bands for Ac did not change. The results supported the views that with the progress of either setting or frozen-storage or vinegar-curing MHCs were crosslinked to higher molecular weight CMHC.

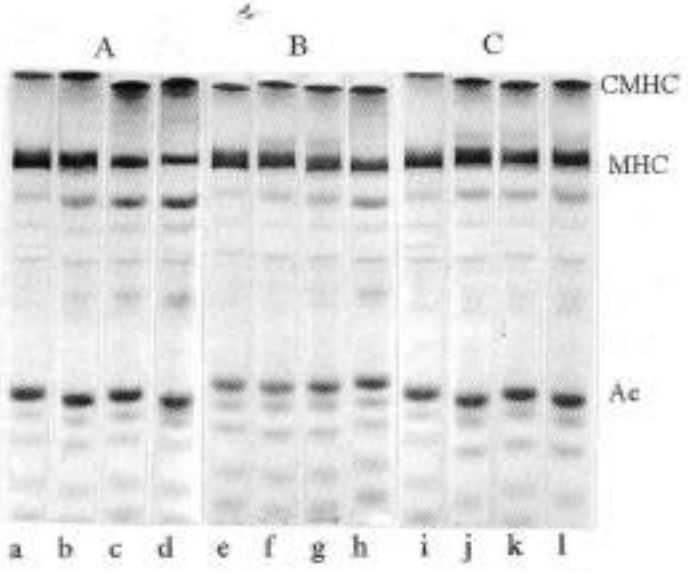


Fig.1. SDS-PAGE patterns for setting (A), frozen-storage (B) and vinegar-curing(C) of fish pastes. a, salt-ground Alaska pollack surimi; b, that set at 30°C for 1 h; c, that set for 5 h; d, that set for 10 h; e, Pacific cod mince; f, that frozen-stored at -20°C for 10 d; g, that stored for 20 d; h, that stored for 30 d; i, Pacific mackerel paste; j, that vinegar-cured for 12 h; k, that cured for 24 h; l, that cured for 48 h.

Fig. 2 shows the SDS-PAGE pattern for AM, digested AM, and CMHC extracts. CMHCs were extracted from the discs d, h and l for the setting of Alaska pollack, frozen-storage of Pacific cod and vinegar-curing of Pacific mackerel, respectively. These CMHC extracts were electrophoresed to the discs o, p and q of Fig. 2, respectively. In all of these three discs it was seen that CMHCs formed by the above treatments, were dissociated to MHC and 66 kDa protein subunit. The disc m was for AM extracted from Alaska pollack and the disc s was that from Pacific cod. The disc M was for the molecular weight marker. However, by the digestion with trypsin, MHC molecules of AMs from both Alaska pollack and Pacific cod were cleaved into 170, 145, 87, 78, and 66 kDa subfragments (disc n and r). Each mobility was in good agreement with that of the tryptically digested abalone myosin (Asakawa and Azuma 1990) in which the first (170 kDa) was assigned to an intra-cleavage of MHC, the third (87 kDa) to myosin subfragment-1 (S-1), and the last (66 kDa) to LMM. Chan *et al.* (1993) purified LMM from cod and herring myosin at a molecular weight of 66 kDa. During prolonged digestion by a-chymotrypsin and trypsin, thermally acclimated carp myosin was cleaved into various subfragments, where a number of protein bands corresponding to the molecular weight from 66 to 70 kDa were indentified to be the LMM (Watabe *et al.* 1992). From the comparison of these results, 66 kDa subfragment split off from CMHCs could also be assigned to LMM, although it was still obscure whether the LMM was formed after the aggregation of MHC or from MHC before its aggregation and then entangled with the parent MHC molecule to form CMHC.

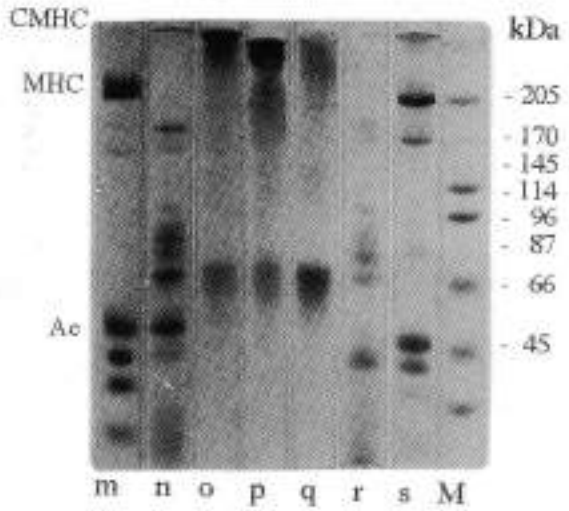


Fig. 2. SDS-PAGE patterns for AM, tryptically digested AM and CMHC extracts. m, AM of Alaska pollack; n, that digested by trypsin; o, CMHC extract from setting; p, CMHC extract from frozen-storage; q, CMHC extract from vinegar curing; r, tryptically digested AM of Pacific cod; s, AM of Pacific cod; M, molecular weight marker.

Several studies (Chan *et al.* 1993, Samejima *et al.* 1981, Sano *et al.* 1990, Taguchi *et al.* 1987) have demonstrated an active involvement of LMM in the MHC aggregation phenomenon, where most of the works have been done with purified LMM from different fish sources. The dynamic viscoelastic behaviour and turbidity studies of isolated carp heavy meromyosin (HMM) and LMM suggested that the initial gel formation was attributed mainly to LMM at 30-44°C (Sano *et al.* 1990). However, the purification process can affect the stability of protein (Otani *et al.* 1983, Park and Lanier 1989). It is important to determine the gelling characteristics of model protein not only in the purified form but also in a less purified system (Beas *et al.* 1991). Therefore, in order to understand the role of MHC subunits including LMM in surimi gelation, AM(S) from Alaska pollack was used in the present study. The objective was to understand the influence and interactions of these subunits in the crosslinking phenomenon in a condition of set gel which more or less resembled set gel of AM, where MHC was completely cleaved by a prolonged digestion with the enzyme but the subfragments were not isolated or separated.

Fig. 3 shows the elasticity of set gel in respect of puncture test. Both surimi and AM showed increased setting ability at 30°C as their breaking strength and breaking deformation increased rapidly at the initial stage and then gradually till 6 h of setting. On the other hand, the paste of AM(S) although transformed into gel, the breaking strength and breaking deformation were very low and reached a constant after an increase till 4 h. The elastic modulii and viscosities of these gels are presented in Fig. 4. G0, G1, G2, h1, and h2 rapidly increased in the gels from surimi and AM till 6 h of setting, but slowly increased in the gel from AM(S). The increment of elasticity in the gel from AM(S) was very small when compared to the strong elasticity of surimi and AM. Nevertheless, the results confirmed that the gelling characteristics of MHC also persisted in its subfragments. The influence of Ac in this gelation could be nullified, since it has been demonstrated from the turbidimetric and electrophoretic studies that actin does not involve in the gelation and protein crosslinking phenomena (Samejima *et al.* 1969).

Several studies (Chan *et al.* 1993, Samejima *et al.* 1981, Sano *et al.* 1990, Taguchi *et al.* 1987) have demonstrated an active involvement of LMM in the MHC aggregation phenomenon, where most of the works have been done with purified LMM from different fish sources. The dynamic viscoelastic behaviour and turbidity studies of isolated carp heavy meromyosin (HMM) and LMM suggested that the initial gel formation was attributed mainly to LMM at 30-44°C (Sano *et al.* 1990). However, the purification process can affect the stability of protein (Otani *et al.* 1983, Park and Lanier 1989). It is important to determine the gelling characteristics of model protein not only in the purified form but also in a less purified system (Beas *et al.* 1991). Therefore, in order to understand the role of MHC subunits including LMM in surimi gelation, AM(S) from Alaska pollack was used in the present study. The objective was to understand the influence and interactions of these subunits in the crosslinking phenomenon in a condition of set gel which more or less resembled set gel of AM, where MHC was completely cleaved by a prolonged digestion with the enzyme but the subfragments were not isolated or separated.

Fig. 3 shows the elasticity of set gel in respect of puncture test. Both surimi and AM showed increased setting ability at 30°C as their breaking strength and breaking deformation increased rapidly at the initial stage and then gradually till 6 h of setting. On the other hand, the paste of AM(S) although transformed into gel, the breaking strength and breaking deformation were very low and reached a constant after an increase till 4 h. The elastic modulii and viscosities of these gels are presented in Fig. 4. G0, G1, G2, h1, and h2 rapidly increased in the gels from surimi and AM till 6 h of setting, but slowly increased in the gel from AM(S). The increment of elasticity in the gel from AM(S) was very small when compared to the strong elasticity of surimi and AM. Nevertheless, the results confirmed that the gelling characteristics of MHC also persisted in its subfragments. The influence of Ac in this gelation could be nullified, since it has been demonstrated from the turbidimetric and electrophoretic studies that actin does not involve in the gelation and protein crosslinking phenomena (Samejima *et al.* 1969).

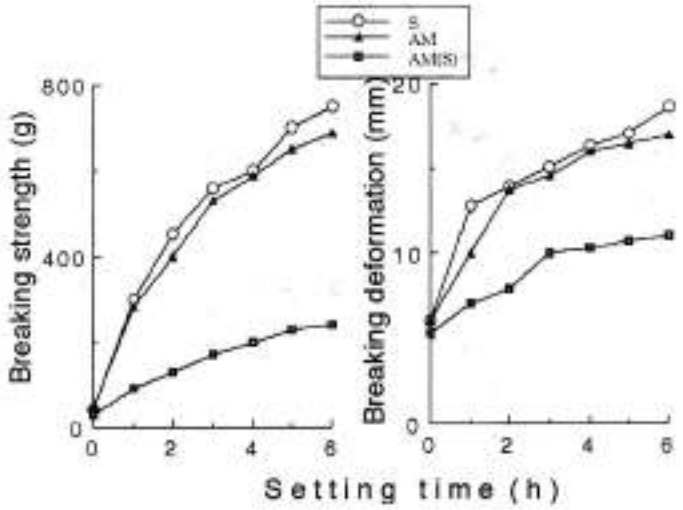


Fig. 3. Breaking strength and breaking deformation of the gel prepared from surimi (S), actomyosin (AM) and actomyosin subunits containing 66 kDa protein [AM(S)] from Alaska pollack.

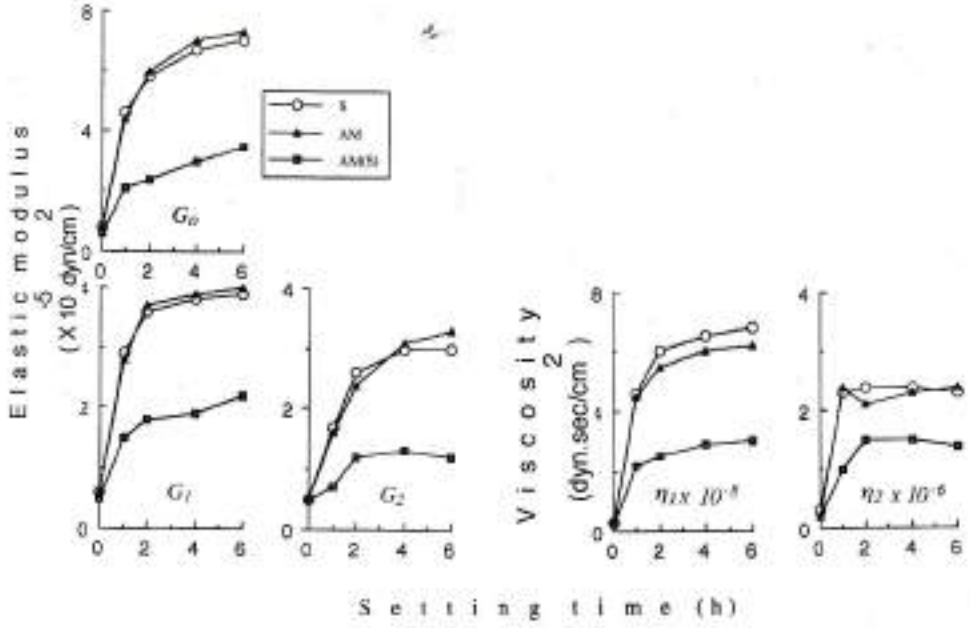


Fig. 4. Compression stress-relaxation of the gel prepared from surimi, AM and AM(S) of Alaska pollack.

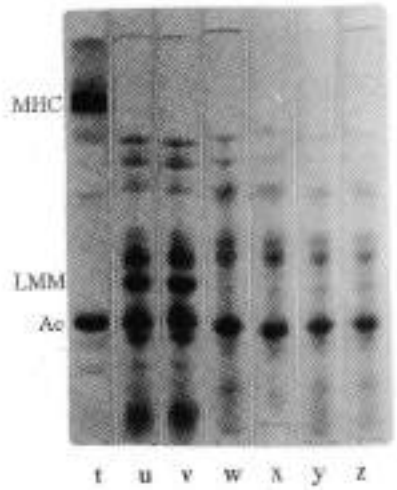


Fig. 5. SDS-PAGE patterns for the gel prepared from the AM(S) pastes. t, AM of Alaska pollack; u, AM(S); v, salt-ground AM(S); w, that set at 30°C or 1 h; x, that set for 2 h; y, that set for 4 h; z, that set for 6 h.

Fig. 5 shows the SDS-PAGE pattern for the gel prepared from the paste of AM(S). Due to prolonged digestion, MHC was completely cleaved and most of the other higher molecular weight subunits like HMM and S-1 split into smaller units, but LMM remained intact. The role of MHC subunits in the low temperature gelation had been studied by many authors (Chan *et al.* 1993, Samejima *et al.* 1981, Sano *et al.* 1990, Taguchi *et al.* 1987). However, as mentioned earlier, the objective of this study was to find whether the 66 kDa protein, tentatively identified as a LMM, could go crosslinking in a less purified form. It was observed from the electrophoretic study that during setting at 30°C the band due to LMM disappeared (discs w, x, y, z) and the intensity of the bands due to other MHC subfragments gradually decreased. However, high molecular weight polymer was not formed. Since the fact that only intermolecular interactions would lead to formation of high molecular weight polymers (Chan *et al.* 1992), the polymerization occurred among MHC subfragments in this study as observed due to their disappearance during setting would be intramolecular. Furthermore, the polymerization of subunit proteins during setting was predominantly non-covalent, since the enzyme transglutaminase, responsible for the occurrence of covalent crosslinking, might be washed away during AM extraction and digestion process, as had been explained previously (Nowsad *et al.* 1994). The demonstration of non-covalent crosslinking by simple SDS-PAGE system is very often precluded because SDS readily breaks hydrogen, hydrophobic and electrostatic bonds (Chan *et al.* 1992). However, from the results that the intensity of the band due to LMM during setting decreased more among the subfragments in the present experimental condition, it was presumed that LMM involved more in the crosslinking process at the temperature around 30°C. Samejima *et al.* (1981) mentioned that the tail

portion of myosin rod in rabbit myosin was apparently responsible for the formation of a gel network, presumably by hydrophobic interaction. The same authors also reported such entanglement of protein molecules at temperatures around 30-35°C in the setting of fish paste. Gill and Conway (1989) studied chymotryptic cleavage of thermally aggregated cod myosins and concluded that the initial stages of thermal crosslinking of myosin were mediated primarily by the HMM-S-2 and LMM region of heavy chain, i.e., by the myosin tail rather than the head. Chan *et al.* (1992) reported the involvement of both HMM and LMM in the thermal crosslinking of cod and herring myosin and explained a mechanism which expressed the view that an initial aggregation might be occurred by the unfolding and interaction at 30-40°C range and further aggregation might be mediated by the interaction of LMM to form clusters of aggregates at 40-55°C. The results of the present study apparently differ from that of Chan *et al.* (op cit) since LMM was mostly crosslinked within one hour of setting at 30°C. In fact, larger myosin subfragments like HMM and S-1 were mostly cleaved into smaller ones in this study. Therefore, the exact influence of these subfragments can not be understood and explained from these results. Nonetheless, it seems that MHC molecule may lead to an unfolding of the super helix of the tail at setting temperatures, exposing hydrophobic residues to the polar environments, resulting in the intramolecular crosslinking through non-covalent bonds among the subfragment proteins that cleaved from the tail portion of the molecule. This may be further entangled with the intermolecular crosslinking of MHC itself and locked up in CMHC at the top of the SDS-PAGE disc gel which could split off again from CMHC in concentrated urea, as we explained elsewhere (Nowsad *et al.* 1993).

References

Asakawa, T. and N. Azuma, 1990. Proteolytic digestion of myosin from abalone Haliotis discus smooth muscle. *Nippon Suisan Gakkaishi*, **56:** 297-305.

Beas, V.E., J. R. Wagner, M. C. Anon and M. Crupkin, 1991. Thermal denaturation in fish muscle proteins during gelling: effect of spawning condition. *J. Food Sci.*, **56:** 281-284.

Chan, J. K., T. A. Gill and A. T. Paulson 1992. Cross-linking of myosin heavy chains from cod, herring and silver hake during thermal setting. *J. Food Sci.*, **57:** 906 - 912.

Chan, J. K., T. A. Gill and A. T. Paulson, 1993. Thermal aggregation of myosin subfragments from cod and herring. *J. Food Sci.*, **58:** 1057-1069.

Gill, T. A. and J. T. Conway, 1989. Thermal aggregation of cod *(Gadus morhua)* muscle proteins using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide as a zerolength cross-linker. *Agric. Biol. Chem.*, **53:** 2553-2562.

Lee, N., N. Seki, N. Kato, N. Nakagawa, S. Terui and K. Arai, 1990. Gel forming ability of myosin heavy chain in salted meat paste from threadfin bream. *Nippon Suisan Gakkaishi*, **56:** 329-336.

Niwa, E., S. Suzuki, A.K.M.A. Nowsad and S. Kanoh, 1993. Lower molecularfish proteins separated from the band around that of cross-linked myosin heavy chain. *Nippon Suisan Gakkaishi,* **59:** 1013-1016.

Nowsad, A.K.M.A., S. Kanoh and E. Niwa, 1993a. Electrophoretic behavior of crosslinked myosin heavy chain in suwari gel. *Nippon Suisan Gakkaishi,* **59,** 667-671.

Nowsad, AKM.A., S. Kanoh and E. Niwa, 1993b. Aggregation of myosin heavy chain while grinding surimi and setting its paste. *Nippon Suisan Gakkaishi,* **59:** 1957.

Nowsad, AKM. A., S. Kanoh and E. Niwa, 1994. Setting of transglutaminase-free actomyosin paste prepared from Alaska pollack surimi. *Fisheries Science,* **60:** 295-297.

Numakura, T., N. Seki, I. Kimura, K. Toyoda, T. Fujita, K. Takama and K. Arai, 1985. Cross-linking reaction of myosin in the fish paste during setting (SUWARI). *Nippon Suisan Gakkaishi,* **51:** 1559-1565.

Numakura, T., I. Kimura, K. Toyoda and T. Fujita, 1990. Temperature-dependent changes in gel strength and myosin heavy chain of salt-ground meat from walleye pollack during setting. *Nippon Suisan Gakkaishi,* **56:** 2035-2043.

Otani, O., S. Hikichi, K. Nishita, T. Sekii and K. Arai, 1983. A loss of actin from shellfish myofibrils and fish myosin B during wash-treatment. *Nippon Suisan Gakkaishi,* **49:** 415-424.

Park, J. W. and T. C. Lanier, 1989. Scanning calorimetric behavior of tilapia myosin and actin due to processing of muscle and protein purification. *J. Food Sci.,* **54:** 49-51.

Samejima, K., Y. Hashimoto, T. Yasui and T. Fukazawa, 1969. Heat gelling properties of myosin, actin, actomyosin and myosin-subunits in a saline model system. *J. Food Sci.,* **34:** 242-245.

Samejima, K., M. Ishioroshi and T. Yasui, 1981. Relative roles of the head and tail portions of the molecule in the heat-induced gelation of myosin. *J. Food Sci.,* **46:** 1412-1418.

Sano, T., S. F. Noguchi, J. J. Matsumoto and T. Tsuchiya, 1990. Thermal gelation characteristics of myosin subfragments. *J. Food Sci.,* **55:** 55-58.

Taguchi, T., M. Ishizaka, M. Tanaka, Y. Nagashima and K. Amano, 1987. Protein-protein interaction of fish myosin fragments. *J. Food Sci.,* **52 :** 1103-1104.

Tobolsky, A.V. and K. Murakami, 1959. *J. Polymer Sci.,* **40** : 443-446, (cited by Iso, N., H. Mizuno, T. Saito, F. Ohzeki and C. Y. Lin, 1983. An analysis of the stress-relaxation curve obtained from a sample of raw meat sample. *Nippon Suisan Gakkaishi,* **49:** 949-952).

Tsuchiya, T. and J. J. Matsumoto, 1975. Isolation, purification and structure of carp myosin, HMM and LMM. *Nippon Suisan Gakkaishi,* **41:** 1319-1326.

Watabe, S., G. C. Hwang, M. Nakaya, X. F. Guo and Y. Okamoto, 1992. Fast skeletal myosin isoforms in thermally acclimated carp. *J. Biochem.,* **3:** 113-122.

(Manuscript received 21 July 1996)

Bangladesh J. Fish. Res., 1(1) : 83-90
Bangladesh Fisheries Research Institute

An objective method to monitor and quantify texture of salted herring during ripening in barrels at 4°C

M.A. Mansur[1,*] and W.F.A. Horner

International Fisheries Institute, The University of Hull , Hull, England
[1] *Department of Fisheries Technology*
Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[*] *Corresponding author*

Abstract

Changes in the texture (elastic nature) of the flesh of barrel salted herring during the ripening process at 4°C have been monitored. The method employs the analysis of stress-relaxation curves after compression to half of the sample thickness on an Instron Model 1112. The parameter 'T/P' for each sample represents the reciprocal of the gradient of a line connecting P and $T_{0.368p}$. This parameter characteristic of each sample's texture was calculated as the ratio of 'T/P' where, T is the relaxation time and is defined as the time required for a stress at constant strain to decrease to 1/e of its original value, where 'e' is the base of natural logarithms (2.7183). Since 1/e = 0.368, the relaxation time is the time required for the force to decay to 36.8% of its original value. P is the peak height of the curve (i.e. the force value at the maximum height). This method was adopted from the bakery industry for testing the degree of gluten development in bread dough. The 'T/P' values obtained over the course of ripening for differently treated salted-herring in barrels ranged between 1 and 12. The trends in 'T/P' value, during ripening period for the different samples, appeared to be parallel changes in texture perceived by sensory observation (subjective measurement), although the heterogeneous nature of the samples gave standard deviations, about the replicate sample mean, around 5%. The method appears promising as an objective measure for monitoring this aspect of the textural quality of barrel salted-herring through ripening if reproductibility of test results can be improved by more careful standardization of sample preparation and test protocol.

Key words : Salted herring, Texture, Ripening

Introduction

The concept of texture is wide and its definition varies from discipline to discipline. Even in the same discipline, eg. in food science, the definition of texture can vary from one product to another. The textures of cheese, meat and crisp will certainly be defined in different ways. What constitutes an ideal texture

in any given fish product has rarely been defined, although most sensory schemes, available for organoleptic testing of such products, require that some assessment of texture be made (Bourne 1982). Texture is an important attribute when the degree of maturation/ ripening of a particular sample of barrel-salted herring is being assessed. In general, the textural attributes of a fishery product are essential factors in the determination of its overall quality (Borresen 1986).

The 'ripening' process for heavily salted (1 part salt : 4 parts fish) herring, whereby the salt dissolves in the aqueous extract from the fish in a sealed barrel, involves a gradual change in sensory characteristics over a period of 12 months or more at 4^{O}C. A major factor in this change is textural. Initially, the fish retains its elastic, raw texture but becomes firmer due to exosmosis to the forming 'blood-pickle' and inward diffusion of salt. Subsequently, a gradual tenderisation occurs, ideally to the point, coinciding with optimum flavour maturity, where the flesh is firm enough to retain its integrity but is neither too rubbery nor too ready to disintegrate to a palate-clinging paste on mastication.

The desired textural development, during ripening, is characterized rheologically by an increase followed by decrease in firmness superimposed by a gradual loss of elasticity. Subjectively, over short periods of ripening, these textural changes are difficult for taste panels to perceive, let alone quantify. Objectively, mechanical devices with graphical printout-linked attachments and programmes to represent the variety of our textural perceptions of food during biting and mastication have been employed.

The extent to which components of our perception of food texture can be separately quantified in this way and, moreover, related back to taste panel scores, has been the subject of many years of debate amongst researchers in this area of investigation. Howgate (1977) mentioned the instruments used by earlier researchers to monitor the texture of raw and cooked fish as it is modified by normal processing methods, but the relationships between data obtained from such objective texture assessment methods and texture data from sensory panel tests on fish, in its whole or filleted form, seemed tenuous. Borderias *et al.* (1983) found better correlation between instrumental and sensory data with fish mince. This has also been the case for those like Lee and Chung (1989) and Knudsen *et al.* (1987) assessing the texture of surimi.

Performing instrumental texture analysis on fish mince rather than natural flesh overcomes the problem of data variability due to the heterogeneity of the flesh, but relating data thus obtained to the changes perceived by the sensory panel over the ripening period may prove difficult. A prospective purchaser of fish may assess the freshness of raw fish subjectively by squeezing the flesh between finger and thumb. The imprint so made would disappear instantaneously with the release of this pressure if the fish was fresh but remain for a moment or longer if the fish was not so fresh. This could provide a clue to the sort of instrumental device to be sought for an objective assessment of texture.

A device which represents our initial perception of food texture (as we first bite through food) is the shear cell which, linked to a scale, records extent and manner of the food's resistance to an increasing cutting force. This has been used extensively to quantify the degree of toughness or tenderness particularly in meat products (Bourne 1982). Whilst toughness/tenderness is a parameter of relevance (as it appears, subjectively, to follow a particular pattern of development in the ripening of salt-herring) it could not be used to estimate the ripening stage reached in an unknown sample. This is because the maximum/minimum value characteristically occurs during the ripening process rather than initially or terminally.

Elasticity, however, is partly responsible for the undesirable `raw herring' perception at the beginning of the ripening period; whilst the lack of it is partly responsible for the similarly undesirable textural `pastiness' of the over-ripened product. Elasticity is a crucial characteristic of bread dough texture and a method of quantifying this parameters, by recording the relaxation time from the peak to a standard fraction of the peak stress on the stress-relaxation curves, as obtained from an Instron, has been published by Frazier *et al*. (1973).

In spite of the physical dissimilarity of the food materials, with respect to shape regularity and structural homogeneity, this methodology was adapted to obtain stress-relaxation time data on the fillets taken from different batches of salt-herring as they ripened. As the relaxation curves appeared typical of an exponential decay (Fig. 1) the relaxation times from the peak of the compression/ relaxation trace to 0.368 (=1/e) of the peak force value were recorded. Each fillet was compressed to 50% of its thickness at a standard position. As different fillet thicknesses in the replicates led to different peak heights, the data was presented as a series of graphs of relaxation time divided by peak force (T/P) against ripening time.

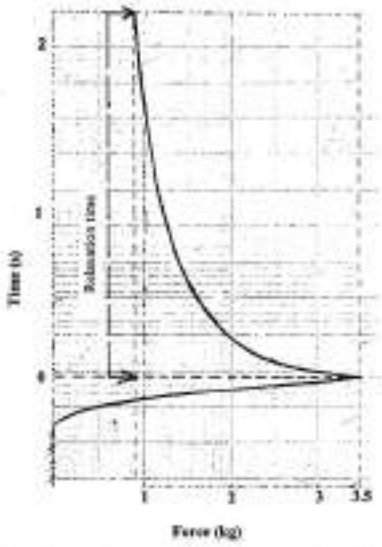


Fig. 1. Instron recorder trace for compression and relaxation of the sample.

Materials and methods

Herring from North Sea of composition shown in Table 1 were salted in polypropylene barrels and stored at 4°C to ripen for 12 months as follows :

1. Whole fish + New salt
2. Whole fish + Used salt
3. Gipped fish + New salt
4. Gipped fish + Used salt

Table 1. Composition and initial characteristics of herring caught from North Sea in July'92

Parameter	Result
Moisture	63%
Fat	17%
Protein	19%
pH	6.33
Histamine	0.29 mg/100 g
Temperature	0.2°C
Average weight	231 g (165 - 290)*
Average length	27 cm (22 - 30)*
Colour	Silvery
TVB-N	9 mg/100 g
FAN	52 mg/100 g
PO	8.4 m eq/kg oil
TBARS	23 m eq MA/kg oil

* *Range is shown in parenthesis*

The ratio of salt to fish was 1:4 (i.e. 1 part salt to 4 parts fish). The salt concentration in the herring during the study period was 11% two weeks after salting, rising to 16% after 12 months of ripening.

Small sections of samples from the fillets were prepared from the dorsal, skinless muscle at 3 month intervals through the ripening period. The samples were of approximately 1 cm^2 surface area. These small pieces of samples were kept chilled until the experimental work.

Instrument setting

An Instron Model 1112 was used in the present study with the following set up -

50 kg load cell
Chart speed : 50 cm/min
Cross head speed : 10 cm/min

The probe attachment was a brass cylinder of 14 mm diameter.

Determination

During determination the samples were taken into a stainless steel dish and placed on the load cell table of the Instron. The diameter of the metal dish was much less than the diameter of the load cell (load cell diameter was 15 cm; metal dish diameter was 8 cm). The sample was then subjected to compression between parallel plates i.e. load cell table and the cross head. The crossead control was programmed to stop its downward movement when the sample was compressed to 50% of its thickness. The sample was then allowed for relaxation at constant deformation. The compression and stress relaxation was traced on a continuous chart at a chart speed of 50 cm/min. For each determination at least 10 replicates were carried out because of the non-homogeneous nature of the sample. During determination the sample temperature was between 8 and 10°C.

Calculation

The parameter characteristic of each sample's texture was calculated from the relaxation curve as the ratio of - T/P

Where, T is the relaxation time and is defined as the time required for a stress at constant strain to decrease to 1/e of its original value, where 'e' is the base of natural logarithms (2.7183). Since 1/e = 0.368, the relaxation time is the time required for the force to decay to 36.8% of its original value (Bourne, 1982). P is the peak height of the curve (i.e. the force value at the maximum height). From the relaxation curves $T_{0.368}$ were determined and the texture parameter was calculated (T/P).

Values of T/P were taken as the texture parameter characterizing each relaxation curve and plotted against the time of ripening in each of the differently prepared batches of herring.

Results and discussion

Results of the present study have been shown in the Figures 2.1 to 2.2. Before discussing the results the following texture-affecting phenomena should be taken into consideration which are occurring simultaneously during the ripening of salted herring :

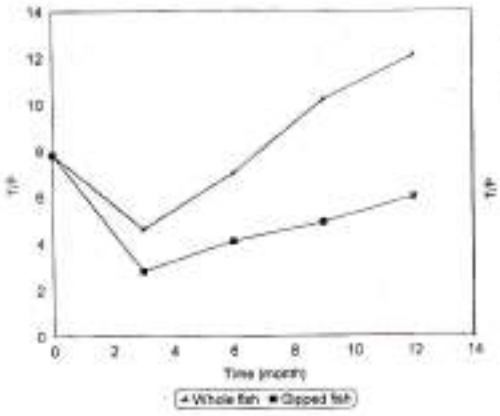


Fig.2. Changes in texture of salted herring during the ripening in barrels at 4°c using new salts.

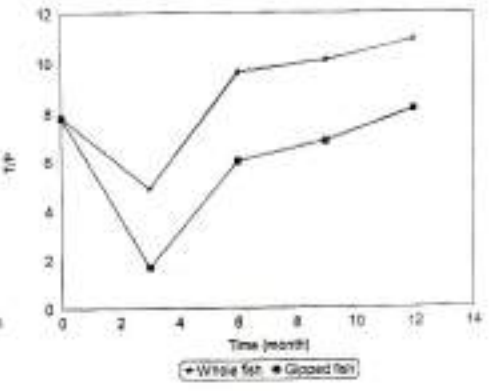


Fig.2. Changes in texture of salted herring during the ripening in barrels at 4°c using used salts.

At the beginning of the process -

(i) Salt is diffusing into the fish flesh causing it to stiffen, that is to become firmer but not more or less elastic;

(ii) Water is diffusing into the brine until there is osmotic equilibrium between the aqueous medium in the fish and the surrounding brine - this will similarly cause the flesh to become firmer without affecting its elasticity;

(iii) Removal of water from the protein gel matrices might cause the proteins to aggregate and exclude even more water - this might result in increased elasticity in the same way as a gelatin gel becomes more rubbery the greater the concentration of gelatin;

(iv) On the other hand, penetration of Na^+ and Cl^- ions into such matrices would favour greater retention of water.

The following phenomena take place during the ripening process:

(v) Autolytic activity, very gradually proceeding in such high ionic concentrations, includes proteolysis which would reduce elasticity progressively until the flesh eventually liquefied as occurs in the ambient temperature production of fish sauce;

(vi) Some of the products of the ripening process, like free fatty acids - the products of lipolysis, and the products of oxidative scission of the unsaturated hydrocarbon chains of glyceride, can attack proteins denaturing them, or even causing breaks in their chains;

(vii) Other breakdown products, like formaldehyde have been implicated in the formation of cross-links between protein molecules which would increase their firmness and affect elasticity.

The concept of sensory textural attributes (hardness, softness, toughness, tenderness) may also be taken into consideration for the better understanding of the results of present study. The definitions of these sensory textural attributes had been developed by the International Organization for Standardization, Standard 5492/3, 1979 (Bourne 1982).

Hardness is the product textural attribute which infers that, it displays substantial resistance to deformation or breaking.

Softness, on the other hand, infers that it displays only slight resistance to deformation.

These attributes which are the opposite to one another are most frequently used to describe the texture of amorphous, nonfibrous food. For example a ginger biscuit is hard and a banana is soft. `Firmness' is frequently used in place of `hardness' when referring to flesh but has the same meaning. Toughness and tenderness, however, are attributes more frequently used to describe fibrous gel foods like meat and fish.

Toughness infers resilience on mastication. A tough product regains its dimensions quickly and repeatedly on the repeated compression-relaxation cycles of mastication. A tough product is often described as `Chewy' or `Elastic'.

Tenderness, which is the opposite to toughness, infers little resilience to repeated compression-relaxation cycles during mastication. Flesh which is tender does not regain its original dimensions quickly when compressed and is soon broken down to a paste on mastication.

The instrumental texture parameters 'T/P' of the present study, relates to the above definitions. An increase in 'T/P' value determined by Instron tests indicate a loss of resilience or elastic nature in the product and, hence, an increase in tenderness.

From a sensory point of view, the ripening continues until the required mature flavour and tender eating texture has been achieved. This is said to take 12 months at 4°C. The expected textural change during the ripening of salted herring is the tenderisation of the fish flesh. The texture of the properly matured/ripened fish is softer and less elastic than the raw unsalted fish. The process is very slow and the difference is not very great between the raw flesh and the mature/ripened flesh. Such trend of textural change was evident in Instron results. During the periodic sensory observations the flesh of all of the samples show toughening and then gradually becomes tender and the final state was tenderized flesh (Table 2). Such intermediate toughening was also supported by the Instron results which are presented in the Figs. 2.1 and 2.2. The decrease in the value of T/P during the ripening period signifies the increase of firmness of the samples. The reasons might be the phenomena mentioned in points 1 to 7 before.

Table 2. Sensory observation on the texture of salted herring during ripening in barrels at 4°C

Ripening time (Months)	Whole fish + New salt	Whole fish + Used salt	Gipped fish + New salt	Gipped fish + Used salt
3	Similar to raw herring	Similar to raw herring	Similar to raw herring	Similar to raw herring
6	Tough	Tough	Tough	Tough
9	Slightly tender	Slightly tender	Slightly tender	Slightly tender
12	Tender	Tender	Tender	Tender

At the end of the ripening process all of the samples were found to be tender and less elastic (instrumental) than the original unsalted fish. The salted whole fish were found to be more tender than the gipped fish when the process of ripening was complete. This was obvious both by Instron results (Figs. 2.1 and 2.2) and sensory observations (Table 2), during the study period. It is likely to be

due to the difference in the degree of proteolysis taking place during the ripening process, i.e. the influence of proteolytic enzymes present in the gut.

Results of the present study using the compression and relaxation of the sample under certain experimental conditions show that it permits the quantification of texture change of a particular set of samples during the ripening of salted herring. These objective results correlated with those of sensory observations. Comparing the results of the present study, there does not seem to be any appreciable significance to textural development in the use of either new or used salt.

This method for objectively monitoring changes in texture of salted-herring during ripening appeared promising in that the trends identified by sensory observations were, to some extent, vindicated instrumentally. With careful preparation of sample, standard presentation of each sample to the Instron probe and standard test temperature, the standard deviation of the replicate sample mean for the same salted herring sample typically remained around 5% of the mean T/P value (with extremes of 1.3% and 12.5%).

Acknowledgements

The fish was kindly supplied and prepared by Alexander Buchan Ltd, Denholm Seafood Group, Peterhead, Scotland. Use of laboratory facilities and instruments of the Department of Food Science, Leeds University, UK is also acknowledged. The suport by ODA of British Government and partial support of EC AIR Project CT 93-1141 is highly acknowledged.

References

Borderias, A.J., M. Lamua and M. Tejada, 1983. Texture analysis of fish fillets and minced fish by both sensory and instrumental methods. *J. Fd. Technol.*, **18**: 85-95

Borresen, T., 1986. Fish texture. A discussion paper submitted to WEFTA Working Group on Analytical Method for Fishery Products. April, 1986.

Bourne, M.C., 1982. Food Texture and Viscosity : Concept and Measurement. Academic Press, New York. pp. 44-106.

Frazier, P.J., F.A. Leigh-Dugmose, N.W.R. Daniels, P.W. Russell Eggitt and J.B.M. Coppock, 1973. The effect of Lipoxygenase action on the mechanical development of wheat flour doughs. *J. Sci. Food Agric.*, **24**: 421-436.

Howgate, P., 1977. *Aspects of fish texture. In :* Sensory Properties of Foods, G G Birch, J G Breenran, K J Parker (eds.). Applied Science Publishers Ltd., London. pp. 249-269.

Knudsen, L.B., T. Borresen, J. Nielsen, 1987. Textural parameters in compression testing of a gel made from fresh and frozen fish mince. *J. Texture Studies*, **18**: 261-271.

Lee, C.M. and K.H. Chung, 1989. Analysis of surimi gel properties by compression and penetration tests. *J. Texture Studies*, **20**: 363-377.

(Manuscript received 1 September 1996)

Bangladesh J. Fish. Res., 1(1) : 91-99
Bangladesh Fisheries Research Institute

Postmortem changes in hilsa fish-I. Studies on rigor-mortis, typical yields and protein composition of dark and white muscle of hilsa fish (*Tenualosa ilisha* Ham.)

M.E. Haque[1], M. Kamal[*] K. Ahmed[1], M.A.R. Faruk and M.N.A. Khan

Department of Fisheries Technology
Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[1]*Bangladesh Fisheries Research Institute, Mymensingh*
[*]*Corresponding author*

Abstract

Hilsa (*Hilsa ilisha*) caught by gill net were immediately killed by cranial spiking. Three fish were kept in ice (0°C) and three other at room temperature (33°C) to follow development of rigor mortis and changes in muscle pH. The rest were frozen stored at -20°C. Rigor started 15 minutes after death in all fish and reached full rigor (100%) state in 2 and 4 hours respectively in fish kept at 33 °C and (0 °C). The fish at 33°C deteriorated 16 hours after while in full rigor but those at 0°C lasted 26 hours of death without deterioration. Freshly caught hilsa had a muscle pH around 7 which decreased with time rapidly at 33°C and slowly at 0°C. The relative proportion of protein fraction in white and dark muscle of fish stored at 0°C and -20°C were also studied. The proportion of dark muscle was 30.34% of the white muscle. White muscle in fish at 0°C was found to contain 32.0% sarcoplasmic, 57.6% myofibrilla, 9.4% alkali-soluble and 1.1% stroma protein whereas these proteins in dark muscle were 29.9%, 58.4%, 9.8% and 1.9% respectively. The protein fractions of white muscle in frozen-fish were found 27.6% sarcoplasmic, 64.7% myofibrilla, 6.0% alkali-soluble and 1.7% of stroma protein whereas they were 30.6%, 58.6%, 8.9 and 1.9% for dark muscle. Some changes occurred in protein composition during frozen storage. The relative amounts of sarcoplasmic, alkali soluble and stroma protein fractions decreased while myofibrilla fraction increased in frozen condition. This may be attributed to drip loss of soluble protein during thawing.

Key words : *T. ilisha*, Rigor-mortis, White and dark muscle, Protein composition

Introduction

Rigor-mortis is the result of important physical and biochemical changes in muscle occurring in an animal soon after death. The physical changes are those which concern with appearance, texture and stiffening of the body. There are three stages of rigormortis-pre-rigor, in-rigor and post-rigor. The time involved in

each stage of development, duration and subsequent resolution of rigor-mortis depends on many factors such as species, catching method, handling of fish, temperature and the physical condition of fish. Although it is generally accepted that the onset and duration of rigor-mortis is more rapid at high temperatures, it has been observed in certain tropical fish that the biochemical changes and the rigor-mortis, may actually be stimulated at 0°C compared with 22°C (Poulter *et al.* 1981). The state of rigor and changes in pH influence the keeping quality of fish.

It is well known that changes in muscle pH largely depend upon the state of rigor. The muscle pH of pelagic fishes such as sardine and mackerel decreases very quickly after death, easily reaching below 6, in contrast to bottom fish (Ishikawa *et al.* 1979, Fukuda *et al.* 1984). Pelagic fishes such as hilsa usually have high metabolic rate and it is of interest to see if it would follow a similar rigor-mortis process as that of bottom tropical fishes.

In most fish, the myotomal musculature consists mainly of two homogenous populations of muscle fibers: the white or ordinary muscle and the dark muscle. Most fish muscle tissue is white but depending on species, many fish contain a certain amount of dark tissue of a brown or reddish colour. The proportion of dark to white muscle is known to vary with the length of fish. The chemical composition of dark and white muscle is different. The dark muscle is generally higher in lipid content and lower in moisture and crude protein content than the ordinary muscle, (Kanoh *et al.* 1986). The chemical composition of fish muscle varies depending on developmental stage, season, fishing ground and schools. Major research effort in the area of biochemistry has been to identify, isolate and characterize the muscle proteins. Muscle proteins are generally classified on the basis of solubility. On these basis fish muscle protein consists of myofibrillar, sarcoplasmic, alkali soluble and stroma protein fractions. While considerable information on the muscle type and its protein composition are available for the fish from the cold and temperate waters such as sardine, tuna, mackerel, etc. very little is known about the fish from tropical water. In order to develop new techniques of processing technology of a value added product, adequate information on the yields and characteristics of muscle protein of the fish is very important.

The present study deals with the rigor-mortis, typical yields and protein composition of dark and white muscle of hilsa fish (*Hilsa ilisha*)

Materials and methods

Fresh hilsa (*Tenualosa ilisha*), average body weight and length, 700-800g and 30-32cm respectively, were caught by gillnet from the river Meghna near Chandpur. Six live hilsa were killed by cranial spiking immediately after capture on board of a vessel. Three of those fish were kept in ice (0°C) in an insulated

box and the other at room temperature (33°C) to follow rigor-mortis development and changes in muscle pH.

The remaining fishes were brought to the laboratory of Fisheries Technology Department, Bangladesh Agricultural University, Mymensingh in iced condition in an insulated box and frozen stored at-20°C for subsequent studies.

Rigor index

"Rigor index" of the fish was measured essentially according to Bito *et al.* (1983) and used as a parameter of rigor tension. Briefly, the fish was placed on a horizontal table with half of its body (tail part) kept out of the table (Fig. 1). At selected time intervals, rigor index was calculated by the following equation:

$$\text{Rigor index (\%)} = \frac{D_0\text{-}D}{D_0} \times 100$$

Where D_0 and D represent the distances of the base of caudal fin from horizontal line of the table immediately after catch of the fish and at subsequent storage periods respectively.

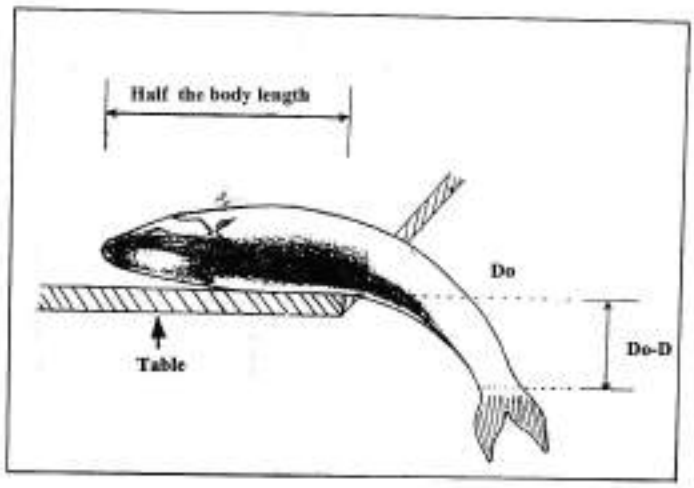


Fig. 1. Determination of "rigor-index" of fish.

Measurement of muscle pH

pH value of the muscle homogenate prepared by blending 10g of fish muscle with 40 ml chilled water was measured by Corning pH meter.

Typical yields and determination of protein fraction

Fresh ice-stored and frozen-stored hilsa fishes were used for the study. Frozen fishes were partially thawed in ice. Transversal sections through the trunk representing the layout and relative distribution of the dark and ordinary muscles from fresh *Hilsa ilisha* are shown in Fig. 2. The fish were weighed individually

and different body parts - head, edible flesh, scale, fin, intestine, egg, gills, skin and bone were separated with the help of a sharp knife, weighed and expressed in percentage of the total weight. For study of the protein composition, after removing the head and caudal fin, the specimen were filleted laterally into two parts. The white and dark muscles were carefully excised to minimize cross contamination of fibre type. The ordinary muscle was dissected from the dorsal part, while the dark myotomal muscle from the whole trunk under the lateral line. Twenty grams of each muscle were fractionated into various protein fraction following the procedure Hashimoto of *et al*. (1979) All the operations were done at 3-4° The protein obtained after fractionation was determined by Biuret method and non-protein nitrogen of TCA extract by Kjeldahl method.

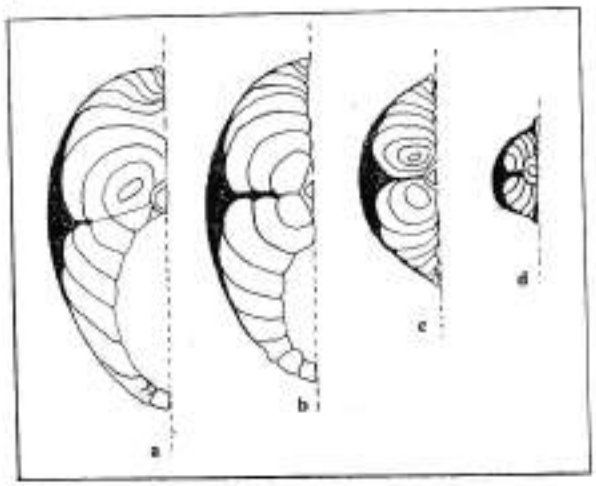


Fig. 2. Transversal sections through the trunk of the dark and ordinary muscles from fresh hilsa fish.

Results and discussion

Progress of Rigor-mortis and changes in muscle pH

Rigor-index of hilsa fish stored at room temperature (33°C) and in ice (0°C) are shown in Fig. 3.1 and 3.2. Rigor started in all fish within 15 min after death but the progress of rigor was faster in fish stored in ice (0°C) than in those stored at room temperature. The fishes stored in ice attained full rigor (100%) 2 hrs after death, whereas the rigor index of the fishes stored at room temperature reached about 85% in 2 hours and attained full rigor (100%) 4 hrs after death.

Hilsa fishes stored at room temperature were discarded after 16 hrs of death while in full-rigor conditions after emitting an offensive odour. At this stage the fishes were found to be organoleptically unacceptable. On the other hand, the fishes stored in ice remained in full rigor up to 26 hrs and then started to relax from rigor. They relaxed to about 50% after 52 hrs of death without showing any perceptible sign of spoilage.

It is well known that pelagic fishes usually have very high metabolic rates. Correspondingly, the post-mortem progress rate of hilsa was found to be extremely high in the present study compared to those of bottom fishes reported by others (Poulter *et al.* 1981, Iwamoto *et al.* 1985, 1987 and 1988, Iwamoto and Yamanaka 1986). The time involved in each stage of the rigor development, duration and subsequent resolution of rigor-mortis depend on many factors such as species, size, catching method, handling of the fish, temperature and the physical condition of the fish. Although it is generally accepted that the onset and duration of rigor-mortis is more rapid at high temperatures, it has been observed in hilsa that rigor-mortis was actually stimulated at 0°C compared with 33°C as reported for some other tropical fishes (Poulter *et al.* 1981). Hilsa kept at 33°C spoiled 16 hours after death when they were still in full rigor condition. The off-odour was first detected in the area around the abdominal cavity. This phenomenon is due to enzymatic activity in the gut which caused degradation of belly which might happen within few hours of capture at high temperature.

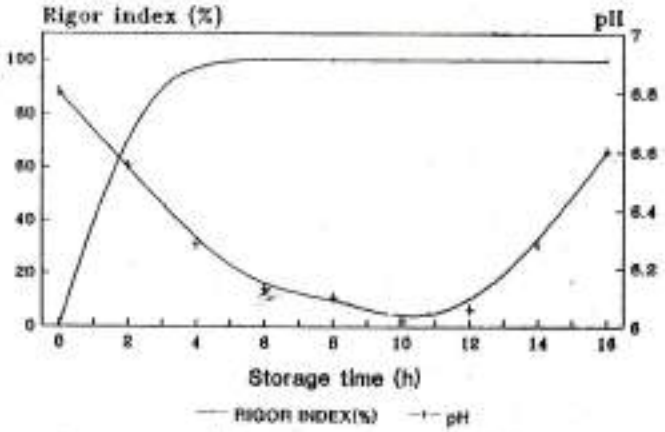


Fig. 3.1. Rigor-mortis progress and change in muscle pH of hilsa fish during storage at room temperature.

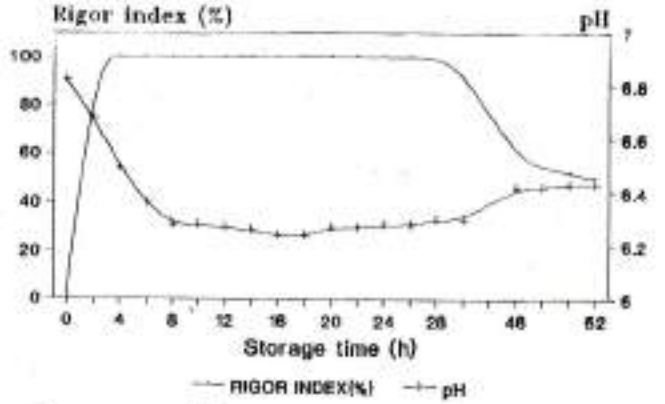


Fig. 3.2. Rigor-mortis progress and change in muscle pH of hilsa fish during ice-storage in an insulated box.

pH change

Freshly killed hilsa had a muscle pH around 7.0 which tended to decrease with time irrespective of temperature but the decline in pH was faster at room temperature (33°C) than at 0°C (Fig. 3.1 & 3.2). At room temperature the muscle pH decreased rapidly from around 7.0 to 6.02 in 10 hrs, while in iced condition it decreased slowly from around 7.0 to 6.28. After 10 hrs of storage at room temperature the pH began to increase steadily up to pH 6.6 after 16 hrs when the fish were discarded. At this stage fishes were reddish in colour and emitted an offensive odour.

On the other hand, the fish stored in ice remained at ultimate pH of 6.24 until 30 hrs of storage and then it increased slowly with time. The fish muscle attained a final pH value of 6.43 after 52 hrs of storage when the fish relaxed about 50% from rigor. The decreasing trend in muscle pH is similar to that reported for catla (*Catla catla*) where muscle pH fell much faster in fish held at room temperature (25°C) than at 0°C (Faruk *et al*. 1994). In general, when the rigor is quicker and the duration is shorter, the values of pH decline much faster than where rigor develops slowly and lasts longer. This change in pH is attributed to the accelerated turnover of ATP at high temperature (Watabe *et al*. 1991). Scopes (1974) reported that the rate of pH fall in post-mortem muscle is entirely due to ATPase activity.

Typical yields and protein composition

Table 1 shows that the proportion of dark to white muscle of hilsa was 30.34%. The results obtained from the present study is more or less similar to those reported for pelagic fishes such as tuna, sardine and mackerel. These fishes have a large amount of superficial dark muscle and some of them, such as tuna have developed deep-seated dark muscle along with the superficial, while other pelagic fishes of relatively small size, such as sardine and mackerel have only superficial dark muscle (Suzuki and Watabe 1987). The proportion of dark to white muscle varies with activity of the fish (Love 1970). The proportion of dark to white muscle of sardine and mackerel is reported to be 20-30% (Hashimoto *et al*. 1979, Suzuki and Watabe 1987) and is known to vary with the length of the fish and from species to species. It is generally accepted that the dark muscle is used for the slow and continuous swimming of fish, where as the white muscle is called upon for a quick burst of activity. The red colour of the dark muscle is due to the high content of hemoproteins, hemoglobin (Hb), myoglobin (Mb) and cytochrome C. Among these, the content of Mb is the highest, followed by Hb (Suzuki and Watabe 1987).

Table 1. Proportion of dark to white muscle of hilsa fish

No. of specimen	10
Total weight of fish (g)	9850
Total muscle (g)	4570
Dark muscle (g)	1063
White muscle (g)	3507
Proportion of dark to white muscle (%)	30.34

Table 2 shows the typical yields of various body parts of hilsa. The yield of edible flesh was 46.4%. The head and viscera were 17% and 6.2% respectively. The yield of edible flesh and other organs of fish varies from species to species. Species having a large head such as cod give much lower yields of dressed fish (54%) than those having a small head and slim contour such as tuna (73%) (Stansby and Olcott 1963). Pelagic fishes such as Pacific herring (*Clupea pallasi*) is the most important fishery of the cold and temperate regions. The yield of edible flesh and organs (head and viscera) as percentage of fresh weight of the herring were reported to be 42-65%, 8-18% and 6-21%, respectively (TDRI 1985).

Table 2. Protein composition of dark and white muscle of hilsa fish

Condition	Muscle	Protein fraction (%)			
		Sarcoplasmic	Myofibrillar	Alkali-soluble	Stroma
Iced fish	Dark muscle	29.90	58.40	9.80	1.90
	White muscle	31.98	57.55	9.37	1.10
Forzen	Dark muscle	30.60	58.60	8.90	1.90
	White muscle	27.60	64.70	6.00	1.70

In the present study, the protein compositions of white muscle of iced-stored hilsa were 31.98% sarcoplasmic, 57.55% myofibrillar, 9.37% alkali-soluble and 1.1% stroma protein and in dark muscle it was 29.9%, 58.4%, 9.8% and 1.9% respectively (Table 2). The protein compositions of frozen-stored hilsa were found to be 27.6% sarcoplasmic, 64.7% myofibrillar, 6.0% alkali-soluble and 1.7% stroma protein for white muscle, while it was 30.6%, 58.6%, 8.9 and 1.9% respectively for dark muscle (Table 2). This is within the range reported for most pelagic fishes (Hashimoto *et al.* 1979, Kanoh *et al.* 1986). Some changes occured in protein composition during frozen storage. As described above, the protein of dark muscle are clearly distinct from those of white muscle in many respects such as dark muscle contained higher percent of stroma protein than the white muscle. The percentage sarcoplasmic, alkali soluble and stroma

protein fraction decreased, while myofibrilla fraction increased when the fish was kept frozen at -20°C. These results indicate that sarcoplasmic and alkali soluble proteins are lost due to drip formation during frozen storage, leading to relative increase of myofibrillar proteins.

In most teleost fishes the amount of extractable protein of various fractions have been reported to be in the range of 20-30% sarcoplasmic, 50-70% myofibrillar, 1-10% alkali-soluble and 1-3% stroma protein (Shimizu and Shimidu 1960). Pelagic fishes have been reported to contain a higher amount of sarcoplasmic proteins and small amount of myofibrillar proteins compared to other teleost. For example, protein composition of the dark muscle from sardine was reported to be 23-29% sarcoplasmic, 62-66% myofibrillar, 6-9% alkali-soluble and 2-3% stroma protein fraction. In ordinary muscle, protein composition was 33-37%, 59-61%, 1-5% and 1-2% in the above order respectively (Suzuki and Watabe 1987). In general,the extractability of muscle proteins from fish varies depending on fish species (Shimizu *et al.* 1976). In most bottom fishes, such as Alaska pollack and lizard fish the whole sarcoplasmic proteins have been reported to be extractable even in water while for small pelagic fishes such as sardine and mackerel the amount of extractable proteins in white muscle increased rapidly as the ionic strength of the homogenate increased up to I=0.05 (Hashimoto *et al.* 1979) where "I" stands for ionic strength.

Conclusions

The following conclusioos can be drawn from the above study.

1 The rigor-mortis of hilsa fish started within 15 min after capture irrespective of temperature. The progress of rigor was faster at 0°C than at 33°C.

2 The decline in muscle pH during rigor-mortis period was faster at 33°C than at 0°C.

3 The proportion of dark to white muscle was 30.34%. The muscle protein of dark muscle are clearly distinct from those of white muscle in many respects such as dark muscle contained more sarcoplasmic and stroma protein than the white muscle. The percentage sarcoplasmic, alkali soluble and stroma protein fraction decreased, while myofibrillar fraction increased when the fish was kept frozen at -20°C.

References

Bito, M., K. Yamada, Y. Mikumo and K. Amono, 1983. Studies on rigor-mortis of fish-I. Differences in the mode of rigor- mortis among some varieties of fish by modified cuttings method. *Bull. Tokai Reg. Fish. Res. Lab.*, **109**: 89-96

Faruk, M.A.R., M. Kamal, S.M. Alamgir, M.N. Islam and M.A. Hossain, 1994. Post-mortem changes in fish I. Rigor mortis progress in two important freshwater fishes of Bangladesh. *Bangladesh Vet.*, **11**(1-2): 26-30.

Fukuda, Y., Z. Tarakita and K. Arai, 1984. Effect of chub mackerel on the freeze-denaturation of myofibrillar proteins. *Bull. Japan Soc. Sci. Fish.*, **50**: 845-852.

Hashimoto, K., S. Watabe and M. Kono, 1979. Muscle protein composition of sardine and mackerel. *Bull. Japan Soc. Sci. Fish.*, **45:** 1435-1441.

Ishikawa, S., K. Nakamurea, Y. Fujii, G. Yamano, T. Sugiyama, K. Shinozaki, K. Tobita and Y. Yamaguchi, 1979. Fish Jelly product (Kamaboko) and frozen minced meat (frozen suriimi) made of sardine III. Influence of the treatment methods for materials just after catch on kamaboko forming ability of sardine meat. *Bull. Tokai Reg. Fish. Res. Lab.*, **99**: 31-42.

Iwamoto, M. and H. Yamanaka, 1986. Remarkable differences in rigor mortis between wild and cultured specimens of the red sea bream. *Bull. Japan Soc. Sci. Fish.*, **52**: 275-279.

Iwamoto, M., H. Ioka, M. Saito and H. Yamanaka, 1985. Relation between rigor-mortis of sea bream and storage temperature. *Bull. Japan Soc. Sci. Fish.*, **51**: 443-446.

Iwamoto, M., H. Yamanaka, S. Watabe and K. Hashimoto, 1987. Effect of storage temperature on rigor mortis and ATP degradation in plaice muscle. *J. Food Sci.*, **52**: 1514-1517.

Kanoh, S., T.S. Suzuki, K. Maneyama, T. Takeway, S. Watabe and K. Hashimoto, 1986. Comparative studies on ordinary and dark muscles of tuna fish. *Bull. Japan Soc. Sci. Fish.*, **52**: 1807-1816.

Lagler, K.F., J.E. Bardach, R.R. Miller and D.R.M. Passino, 1977. Ichthyology (2nd edition). Jihn Wiley and Sons, Singapore. 506 pp.

Love, R.M., 1970. Chemical Biology of Fishes. Academic press, New York. 547 pp.

Poulter, F.G., C.A. Curran and J.G. Disney, 1981. *Chill storage. of sropical and temperate water fish-differences and similarities. In:* Advances in Technology in the Chilling, Freezing, Processing, Storage and Transport of Fish, Especially Under-utilized Species. Intern. Inst. Refrigeration, Paris., pp. 111-123.

Scope, R.K., 1974. Studies with reconstituted muscle glycolytic system. *Biochem. J.*, **142**: 79-86

Shimizu, Y and W. Simidu, 1960. Studies on muscle of aquatic animals XXVIII. Protein composition of fish muscle. *Bull Japan Soc. Sci. Fish.*, **28**: 8p.

Shimizu, Y., S. Karata and F. Nishioka, 1976. Extractability of proteins from fish skeletal muscle at low ionic strengths. *Bull. Japan Soc. Sci. Fish.*, **42**: 1025-1031.

Stansby, E. and H.S. Olcott, 1963. *Composition of fish. In*: Industrial Fishery Technology. (ed. M.E. Stansby). Reinhold Publishing Corporation, Chapman and Hall Ltd. London. pp. 339-340.

Suzuki, T. and S. Watabe, 1987. New processing technology of small pelagic fish protein. *Food Reviews International*, **2**(3): 271-307.

TDRI (Tropical Development and Research Institute), 1985. Fish Handling, Preservation and Processing in the Tropics. Part 1. 140 pp.

Watabe, S., M. Kamal and K. Hashimoto, 1991. Post mortem changes in ATP, creatine phosphate, and lactate in sardine muscle. *J. Food Sci.*, **56**: 151-154.

(Manuscript received 20 November 1996)

Bangladesh J. Fish. Res., 1(1) : 101-110
Bangladesh Fisheries Research Institute

Postmortem changes in hilsa fish-II. Studies on physical and bacteriological changes in iced and frozen stored hilsa fish (*Tenualosa ilisha* Ham.)

K. Ahmed[1], M. Kamal[*], M.E. Haque[1], S.C. Chakraborty, M.N.Islam and M.N. Uddin

Department of Fisheries Technology
Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[1]Bangladesh Fisheries Research Institute, Mymensingh-2201
[*]Corresponding author

Abstract

Studies were conducted to evaluate the quality of hilsa fish during icing and freezing storage at -20°C by determining organoleptic and bacteriological aspects. The fishes stored in ice were organoleptically in acceptable condition for 20 days. The bacterial load in muscles of 4 days ice stored fish was 2.5×10^2 CFU/g which gradually increased up to 1.8×10^3 CFU/g after 20 days when the fishes were organoleptically in acceptable condition. The keeping qualities of different days of ice stored fishes were also evaluated during their subsequent frozen storage at -20°C. Both 4 and 7 days of ice stored fishes were organoleptically in acceptable condition up to 48 weeks but the highest degree of freshness was found for fish stored in ice for 4 days before freezing at -20°C. The result indicates that the longer is the duration of ice storage before freezing, the shorter is the shelf life of the fish. The initial bacterial load prior to freezing of the 4 and 7 days of ice stored samples were 2.5×10^3 CFU/g and 3.8×10^4 CFU/g, respectively which reduced to 2.21×10^2 CFU/g and 2.38×10^2 CFU/g, respectively at the end of the 24 weeks of frozen storage. However, after 40 weeks the bacterial load in the frozen stored sample fell below the detection level.

Key words : *T. ilisha*, organoleptic, Bacteriological changes, Frozen storage

Introduction

Fish is an extremely perishable foodstuff and spoilage of fish begins as soon as fish dies but there are wide differences in the degree of deterioration in fish of different families, and even of the different species in a same family. Post-mortem changes greatly influence the quality of fish depending on the storage

conditions. The changes include physical, biochemical and bacteriological. The physical changes are those which are perceived with the senses, i.e., appearance, odour, texture and taste and these changes are closely related to the freshness of a fish.

It is well known that during post-mortem period bacteria present on the surface and in the guts multiply rapidly and gradually invade the flesh which provide nutrition for their growth and multiplication. The bacterial population in fish greatly influence the quality of fish and fishery products. There are some recommended limits of bacterial load by which the quality of fish and fishery products has been judged under various storage conditions. Investigations on the changes in the bacterial flora in fish during ice storage could provide useful information on the spoilage patter of the fishes.

One of the problems related to the increased utilization of tropical fish is the poor handling, storage and prevention facilities found in many developing countries like Bangladesh. Freezing is one of the means of preserving fish for longer periods and frozen fish have become an important commodity both for domestic and export markets in a number of countries of the world. In some cases, distribution costs for frozen fish may compare favourably with those for large scale distribution of iced fish. However, as with many other fields of fish technology, most of the published data on frozen fish is available from cold and temperate water species and little data is available on the relative frozen storage stability of tropical fish. Poulter (1978) found that textural changes in frozen Indian mackerel *Rastrelliger brachysoma* occurred rather more slowly than would be expected from data on cold water fish. It should be mentioned here that hilsa is the single largest fishery of the country and it has very good domestic and export markets. The fish are exported in the form of frozen state and therefore, scientific knowledge on the quality changes in fish during freezing and subsequent frozen storage will provide a basis for supplying foods of better quality

The papers deals with the physical and bacteriological changes of hilsa fish during ice and frozen storage.

Materials and methods

Hilsha fish (mean body weight 750g) were caught by gill net from the river Meghna by the fishermen. The fish were kept in ice (fish to ice ratio 1:1) in an insulated box immediately after catch on board of a vessel. The fishes were first transported to the Riverine Station of the Fisheries Research Institute, Chandpur for repacking and then finally to the laboratory of Fisheries Technology Department, Bangladesh Agricultural University, Mymensingh in iced condition in an insulated box.

Twenty fishes were selected randomly and stored in ice in an insulated box in the ratio of 1:1 (fish:ice). The box had a number of holes at the bottom to drain

out the melt water. Stowage in the box consisted of a bottom layer of ice, about 5 cm deep, layer of fish sprinkled with ice, and a final top layer of ice again about 5 cm deep.Fresh commercial block ice collected from the local market were crushed into small pieces and used in storage experiment. The required amount of ice was replenished from time to time. The samples were obtained at time interval (0, 4, 8, 12, 16, 20 and 21th day) to assess the quality of fish by organoleptic and microbial studies.

To assess the changes in organoleptic qualities of different days of iced stored fish during frozen storage, thirty specimens of 4 days old ice-stored hilsa were frozen at -20°C in a lot and again 30 specimens of 7 days old ice stored hilsa were frozen in another lot. The shelf life of the fishes frozen in two lots were determined by the organoleptic and bacteriological studies.

Organoleptic assessment

Sensory methods were used for organoleptic evaluation. The guidelines and methods given here are based on the organoleptic characteristics of fish as described by EC freshness grade for fishery products (Howgate *et al.* 1992) as shown in Table 1 and 2, respectively.

Table 1. Determination of defect points

Characteristics of whole fish		Defect characteristics	Defect points	Grade
i) Odour of neck when broken	a)	Natural odour	2	Acceptable
	b)	Faint or sour odour	3	Reject
ii) Odour of gills	a)	Natural odour	1	Excellent
	b)	Faint sour odour	2	Acceptable
	c)	Slight moderate sour odour	3	Acceptable
	d)	Moderate to strong sour odour	5	Reject
iii) Colour of gills	a)	Slight pinkish red	1	Excellent
	b)	Pinkish red or brownish red, some mucus may be present	3	Acceptable
	c)	Brown or gray colour covered with mucus	3	Acceptable
	d)	Bleached; thick yellow slime	5	Reject
iv) General	a)	Full bloom; bright; appearance shining; iridescent	1	Excellent
	b)	Slight dullness and loss of bloom	2	Acceptable
	c)	Definite dullness and loss of bloom	3	Acceptable
	d)	Reddish lateral line; dull; no bloom	5	Reject

v) Eyes	a)	Bulging with protruding lens transparent eye cap;	1	Excellent
	b)	Slight clouding of lens and sunken	3	Acceptable
	c)	Dull, sunken, cloudy	3	Acceptable
	d)	Sunken eye covered with yellow slime	5	Reject
vi) Slime	a)	Usually clear, transparent and uniformly spread but occasionally may be slightly opaque or milky	1	Acceptable
	b)	becoming turbid opaque and milky, with marked increase in amount of slime present i skin	1	Acceptable
	c)	Thick, sticky, yellowish is he greenish in colour	5	Reject
viii) Consistency of flesh	a)	Firm and elastic		Excellent
	b)	Moderately soft and some loss of elasticity		Acceptable
	c)	Some softening		Acceptable
	d)	Limp and floppy		Reject

Table 2. Grading of fresh fish

Grade	Points	Degree of freshness
A	<2	Excellent / Acceptable
B	2 to < 5	Good / Acceptable/
C	5	Bad / Rejected

Bacterial load

Fish muscle were collected from the fish samples aseptically, weighed and finely chopped by a scissor on watch glass. Then the stock suspensions of the muscle were prepared separately by homogenizing 10 g of sample with 90 ml of physiological saline (0.85% NaCl) in a sterile blender jar giving a sample with 1:10 dilution. Total viable bacterial load expressed as colony forming units per gram of fish muscle, gills or intestine (CFU/g) of the representative samples were determined by standard plate count on plate count agar (Hi Media) following consecutive decimal dilution technique. An amount of 0.1 ml of each desired diluted samples were pipetted out and transferred to agar plate and quickly spread on the agar surface by an L-shaped glass rod until the samples were dried out. The plates were incubated at 30°C for 48 h and plates having 30 to 300 colonies were counted to estimate the standard plate count.

Results and discussion

Organoleptic changes during ice and frozen storage

Table 3 shows the changes in organoleptic qualities of hilsha fish during ice storage in an insulated box. The fishes were organoleptically in acceptable condition for 20 days. The pattern of changes in organoleptic quality can be roughly divided into 4 phases corresponding to periods of 0 to 4, 5 to 8, 9 to 12, 13 to 20 days in ice. In the phase I the fishes were just passes the rigor-mortis and there was a very little change in texture. At this stage, the fishes were in excellent condition with natural flavour and odour. In phase II there was a slight decrease in brightness, natural flavour and odour. But in phase III there was considerable loss of characteristic odour and the flesh was neutral but had no off-flavour. In phase IV there was signs of early spoilage with sour off-odour. In the later part of this stage, the fish begins to taste stale, its appearance began to show obvious sign of spoilage and the gills and belly cavity had an unpleasant smell. In the previous study, the shelf life of the hilsa fish obtained from the landing centre at Chandpur was found 18 days in ice in an insulated container (Uddin 1995). However, the pattern of quality changes observed in the present study is almost similar to that reported for the cod fish storage in ice (FAO 1975).

Table 3. Changes in organoleptic qualities of Hilsa fish during ice storage in an insulated box

Days of storage	Organoleptic qualities	Defect points	Grade	Overall quality
0	Fresh, bright appearance; soft and firm texture with characteristics of fresh odour	1.15	A	Excellent
4	Fresh, bright,slightly softer Excellent texture; natural fish odour	1.37	A	Excellent
8	A decrease in brightness; slight loss of natural flavour some slime on surface	2.14	B	Acceptable
12	Considerable loss in brightness; Acceptable surface is covered with slime; softer in texture; some loss n natural flavour	2.57	B	Acceptable

16	Slimy surface and soft texture; considerable loss of flavour and odour; reddish in lateral muscle	2.72	B	Acceptable
20	Fish has dull appearance with blood and slime on surface; Acceptance texture begin to show obvious sign of spoilage. Gills and belly cavity had an unpleasant smell	3.14	B	In the limit of acceptance
21	The fish is putrid by all of the characteristics	5.00	C	Reject

Changes in organoleptic qualities of 4 and 7 days of ice stored hilsa during frozen storage at -20°C are shown in Fig.1. The pattern of changes in organoleptic quality can also be roughly divided into four phases corresponding to periods of 0 to 24, 25 to 40, 41 to 48 and 49 to 60 weeks in frozen storage. In the phase I the fishes were in excellent condition with natural odour and flavour. In phase II, a little changes in odour, flavour, texture and brightness were occurred. But still then, they were in good condition. In phase III there were considerable loss of characteristic odour and texture and the flesh was neutral but had no off-flavour. The fishes were in acceptable condition in this phase. In phase IV fishes lost their original quality. In the beginning of this phase considerable loss of odour and flavour occurred. In the later part of this stage, its appearance and texture already showed obvious signs of spoilage.Although in both conditions the fishes were organoleptically in acceptable condition up to 48 weeks, yet with regard to the potential storage life of frozen fish, it can be said that the highest degree of freshness can be maintained up to 48 weeks for hilsa fish stored in ice for 4 days and subsequently frozen at -20°C. The results also showed that the longer is the duration of ice storage before freezing, the shorter is the keeping quality of the fish. This is in agreement with Partmann (1963) who suggests that the keeping quality of fish can be maintained for a longer period if the fish are frozen before resolution of rigor mortis. It would be noted that the 4 days of ice stored fishes were frozen in-rigor state, while 7 days of iced fish were frozen after resolution of rigor. Kietzmann (1969) has reported that a storage temperature of -28°C is most favourable for fish freezing.

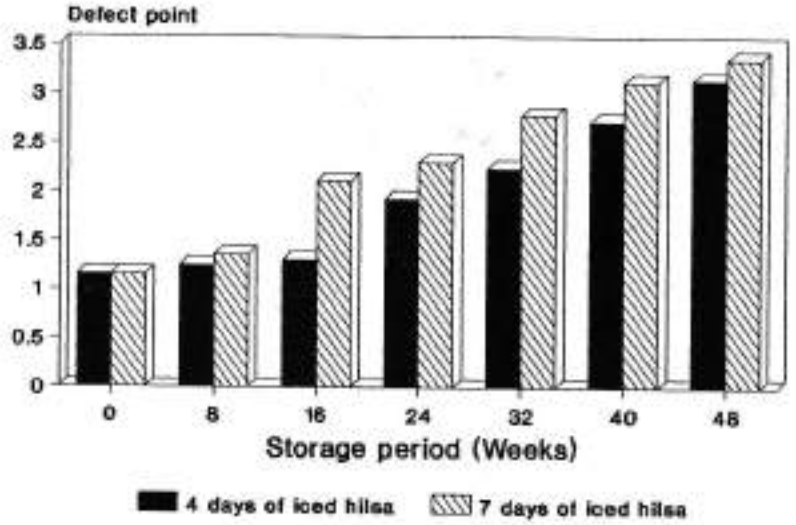


Fig. 1. Changes in organoleptic qualities of 4 and 7 days of ice-stored hilsa fish during storage at 20^0C.

Bacteriological changes during ice and frozen storage

Changes in bacterial load in ice stored hilsa fish are shown in Table 4. The bacterial load of the fish muscle was not determined during first 3 days after capture of the fish since the fishes were transported from the capture sites near Chandpur to Mymensingh in iced condition and it took two days to reach the samples where the actual experiment was started. Bacterial load in muscles of 4 days ice stored fish was 2.5×10^2 CFU/g which increased gradually with the storage period. At the end of 20 days of ice storage bacterial load increased to 1.8×10^5 CFU/g and at this stage the fishes were organoleptically in acceptable condition.

Table 4. Standard plate count of ice-stored hilsa

Days of storage	CFU/g of muscle
4	2.5×10^2
8	9.3×10^3
12	1.7×10^4
16	8.8×10^4
20	1.8×10^5

The increasing number of bacteria in fish muscle during the latter stages of study period indicated the effective penetration of bacteria in fish muscles from intestine, gills and body surface. This period could be linked to a period of gradually accelerating phase associated with organoleptic changes in fish, typically by the loss of characteristics of fresh fish flavour. The continued increasing number in the latter stages could be explained by the the onset phase of exponential growth triggered by post-rigor autolysis of fish which probably provided excellent nutrition for the growth of bacteria. Joseph *et al.* (1988) observed a similar increasing trend in bacterial population from 7.66×10^3 CFU/g to 6.51×10^5 CFU/g after 20 days of ice storage in muscle of ruhu fish (*Labeo rohita*). Shetty *et al.* (1991) also observed a similar trend during storage of oil sardine in chilled sea water.

Table 5. Changes in bacterial load of 4 days and 7 days of ice- stored Hilsa during freezing at -20°C

Storage period	4 days iced hilsa CFU/g of muscle	7 days iced hilsa CFU/g of muscle
Initial	2.5×10^3	3.8×10^4
8 Weeks	3.5×10^2	3.8×10^2
24 Weeks	2.2×10^2	2.4×10^2
40 Weeks	Below the detection level	Below the detection level

Table 5 showing the changes in the bacterial load of frozen stored (-20°C) hilsa fish which were stored in ice for 4 and 7 days prior to freezing. The initial bacterial load of the samples prior to freezing were 2.5×10^3 CFU/g and 3.8×10^4 CFU/g but after 8 weeks of frozen storage the bacterial load were 3.5×10^2 CFU/g and 3.8×10^2 CFU/g in 4 days and 7 days ice stored fish samples respectively. After 24 weeks of frozen storage, the above bacterial load decreased to 2.21×10^2 CFU/g and 2.38×10^2 CFU/g respectively. However, after 40 weeks the bacterial load in the frozen stored sample fell below the detection level. This is to be mentioned that, in healthy live and freshly caught fish, the muscles are sterile, so bacterial contamination is found only on the outer and inner surfaces, gills and intestine of the fish. Formerly, it was often assumed that the bacteria invaded the muscle tissue by way of the vascular tissue or by penetrating the skin. However, histological examinations have shown that in the case of chilled fish only very few bacteria invade the muscle and only at a rather late stage. Microscopical examination of iced whole cod stored for 12-14 days showed that the fillet as such still contained a very limited

number of bacteria. In contrast, the bacteria actually penetrated the flesh via the collagen fibres when the fish were stored at higher temperatures (>+8 °C). (Shewan and Murray 1979). In the present study fish samples were ice-stored almost immediately after catch, therefore, it is expected that only a few bacteria could invade the muscle during the period of 7 days ice storage. Low initial count in the fish samples is the proof of above assumption . Joseph and Solanki (1985) examined the frozen storage characteristics of Elasmobranchs shark (*Scoliodon laticaudus*). They found the total bacterial count (TBC) of the fish sample registered sharp decrease during frozen storage. Garg and Stephan (1985) studied on the frozen storage condition of Raws (*Eleutheronema tetradactylus*). They found that the total plate count decrease was significant after 4 weeks of frozen storage.

Under the usual conditions of storage of frozen foods microbial growth is prevented entirely. During freezing the ice crystals rapture tissue cells or even microorganisms. The initial killing rate during freezing is rapid, but it is followed by a gradual reduction of microorganisms and is referred to as storage death. The number of viable organisms decreases with lengthened time of storage (Frazier and Westhoff 1990). The results of the present study showed the same phenomena the number of viable organisms in the frozen stored hilsa gradually decreased with the increase of storage time.

Conclusions

1 Hilsa fishes stored in ice in an insulated box immediately after capture were organoleptically in acceptable condition for 20 days compared to 18 days for fishes obtained from landing centre under similar storage conditions.

2 The bacterial load in 4 days ice stored fish muscle was 2.5×10^2 CFU/g which increased gradually with the storage period. At the end of 20 days of ice storage bacterial load increased to 1.8×10^5 CFU/g and the fishes were organoleptically in acceptable condition.

3 Regard to the potential storage life of frozen fish, it can be said that the highest degree of freshness can be maintained up to 48 weeks for hilsa fish stored in ice for 4 and 7 days and subsequently frozen at -20^0C but it is clearly indicated that the longer is the duration of ice storage before freezing, the shorter is the shelf life of the fish.

4 The bacterial loads of 4 days ice stored fishes were 2.5×10^3 and 3.8×10^4 CFU/g respectively which decreased drastically and fell below the detection level after 40 weeks of frozen storage.

References

FAO (Food and Agriculture Organization of the United Nations), 1975. Ice in Fisheries. FAO Fisheries Reports. No. 59.

Frazier, W.C. and D.C. Westhoff, 1990. Food Microbiology. 3rd edition. Tata McGraw-Hill publishing company Ltd. New Delhi. 247 pp.

Garg, D.K. and J. Stephen, 1985. *Frozen storage studies of Raws (Eleutheronema tetradactylus).* In: Harvest and Post harvest Technology of Fish (eds. K. Ravindran, N.U. Nair, P.A. Perigreen, P. Madhavan, A.G.G. Pillai, P.A. Panicker and M. Thomas. Society of Fisheries Technologists (India), Cochin, India. pp. 543-544.

Howgate, P., A. Johnston and K.J. Whittle, 1992. Multilingual Guide to EC Freshness Grades for Fishery Products. Torry Research Station, Food safety Directorate, Ministry of Agriculture, Fisheries and Food, Aberdeen, Scotland.

Joseph, A.C. and K.K. Solanki, 1985. *Frozen storage characteristics of Elasmobranchs shark (Scoliodon laticaudus).* In: Harvest and Post harvest Technology of Fish (eds. K. Ravindran, N.U. Nair, P.A. Perigreen, P. Madhavan, A.G. G. Pillai, P.A. Panicker and M. Thomas). Society of Fisheries Technologists (India), Cochin, India. pp. 536-538.

Joseph, J., P.K. Surendran and P.A. Perigreen, 1988. Studies on iced storage cultured ruhu (*Labeo rohita*). *Fish. Technol.*, **25**: 105-108.

Kietzmann, U. 1969. *Evaluation of quality of frozen fish and fish portions.* Freezing and Irradiation of Fish (ed. R. Kreuzer). Fishing News (Books) Ltd. London. pp. 358-360.

Partmann. W., 1963. Post-mortem Changes in chilled and frozen muscle. *J. Food Sci.*, **28**: 15-27.

Poulter, R. G. 1978. *Quality changes in fish from the South China Sea-2. Frozen storage of chub mackerel.* Paper presented at IPFC/FAO Conference on Fish Utilization, Technology and Marketing. Manila, Philippines, March 1978.

Shetty T.S., T.M.R. Setty and C.N. Ravishankar, 1991. Quality changes in Indian oil sardine (*Sardinilla longiceps)* during storage in chilled sea water. *Fish Technol.*, **28**: 51-54.

Shewan, J.M. and C.K. Murry, 1979. *The microbial spoilage of Fish with special reference to the role of psychrophiles.* In: Cold-tolerant Microbes in Spoilage and the Environment (eds. A.D. Russell and R. Fuller). London, Academic press. pp.117-136.

Uddin, M., 1995. Studies on the quality changes in fresh hilsa (*Hilsa ilisha*) during storage in various containers. *M. S. Thesis.* Dept. of Fisheries Technology, Bangladesh Agricultural University, Mymensingh. 87 pp.

(Manuscript received 20 November 1996)

Notes for Authors

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

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

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

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

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

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

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

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

Units and symbols : Metric, and internationally accepted physical measurement units, should be used. The 24-hour clock should be used as time of day, eg. 1345 h, not 1.45 pm.

Submission : For the first time, two copies of the manuscript can be sent to **The Editor, Bangladesh Journal of Fisheries Research**, Bangladesh Fisheries Research Institute, Mymensingh-2201, Bangladesh. Papers will be reviewed by one or more experts in the relevant discipline and evaluated by the Editorial Board for publication. Any paper deemed to be of inadequate quality or inappropriate format for the journal may be returned to authors without review. Submission of final accepted article on a 3.5" disc prepared on PC-compatible computers, along with hard copy print-out, will help rapid publication.

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

Reprints : Author(s) will receive 25 free reprints of the published article.

Contents

Disinfection of aquarium effluents by chlorination and UV treatment
M.N. Islam, M.A.R. Faruk and V. Inglis 1

Nursery rearing of *Macrobrachium rosenbergii* (de Man) using hapa nets : effects of stocking density
M.J. Alam, M.E. Hoq, D.A. Jahan and M.A. Mazid 9

Larval ontogeny of a percichthyid fish, *Synagrops philippinesis* (GÜnther) in Kagoshima Bay, Southern Japan
M.M. Haque and T. Ozawa 17

Comparative studies on growth of fry of GIFT and existing strain of Nile tilapia (*Oreochromis niloticus* L.)
R. Sultana, A.H.M. Kohinoor, M.S. Islam, M.A. Mazid and M.G. Hussain 25

Limnology of Chanda beel
M.A. Ehshan, M.S. Hossain, M.A. Mazid, M.F.A. Mollah, S. Rahman and A. Razzaque 31

A study on damage caused to crustacean and finfish larvae during collection of *Penaeus monodon* (Fab.) postlarvae in the estuaries of Barguna, Bangladesh
S.L. Rahman, M.M. Islam, M.E. Hoq, G.C. Haldar and S.U. Ahmed 41

Impact of stocking density on growth and survival rate of mud crab (*Scylla serrata* Forskal)
M. Rani Saha, P.K. Roy and S.U. Ahmed 47

Effect of stocking density on growth of tiger shrimp (*Penaeus monodon* Fab.) fed on commercial formulated diets
S.C. Chakraborty, A.K. Paul and M.A. Hossain 53

Digestible protein and energy value for fish meal, dextrin, fish oil and soybean oil for Thai sharpunti (*Puntius gonionotus* Bleekeer)
M.A. Hossain, M.M. Rahman and S.C. Chakraborty 65

The 66 K-Da protein identified as a light meromyosin is involved in the setting of surimi
A.K.M. Nowsad Alam and E. Niwa 73

An objective method to monitor and quantify texture of salted herring during ripening in barrels at 4° C
M.A. Mansur and W.F.A. Horner 83

Postmortem changes in hilsa fish- I. Studies on rigor-mortis, typical yields and protein composition of dark and white muscle of hilsa fish (*Tenualosa ilisha* Ham.)
M.E. Haque, M. Kamal, K. Ahmed, M.A.R. Faruk and M.N.A. Khan 91

Postmortem changes in hilsa fish-II. Studies on physical and bacteriological changes in ice and frozen stored hilsa fish (*Tenualosa ilisha* Ham.)
K. Ahmed, M. Kamal, M.E. Haque, S.C. Chakraborty, M.N. Islam and M.N. Uddin 101