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Induction of mitotic and meiotic gynogenesis and production of genetic clones in rohu, *Labeo rohita* Ham.

M.G. Hussain, S.C. Mahata, M.S. Rahman, M.B. Tanu, M.A Mazid and M.S. Islam

Freshwater station
Bangladesh Fisheries Research Institute
Mymensingh-2201, Bangladesh

Abstract

Studies were undertaken to produce genetic clones derived from all homozygous mitotic gynogenetic individuals in rohu, *Labeo rohita* Ham. In view of this, attempts were made to interfere with the normal functioning of the spindle apparatus during the first mitotic cell division of developing eggs using heat shocks, there by leading to the induction of mitotic gynogenetic diploids in the F_1 generation. Afterwards, viable mitotic gynogenetic alevins were reared and a selected mature female fish was used to obtain ovulated eggs which were fertilized later with UV-irradiated milt. Milt was diluted with Cortland's solution and the sperm concentration was maintained at 10^8/ml. The UV- irradiation was carried out for 2 minutes at the intensity of 200 to 250 $\mu W/cm^2$ at 28± 1^0c. The optimal heat shock of 40^0c for 2 minutes applied at 25 to 30 minutes a.f. was used to induce mitotic gynogenesis in fisrt (F1) generation and at 3 to 5 minutes a.f. to induce meiotic gynogenesis in the second (F2) generation. The results obtained are presented and the light they shed on the timing of the mitotic and meiotic cell division in this species is discussed.

Key words : *L. rohita*, Gynogenesis, Genetic clones

Introduction

Highly inbred strains of fish are advantageous for commercial and research purposes and have been identified as having great potential to the development of aquaculture (Komen *et al.* 1991, Hussain *et al.* 1993). Gynogenesis is useful for rapid improvement of genetic characters, producing inbred (clonal) lines compared with the traditional methods of sib-mating for upto 10 to 20 generations (Purdom and Lincoln 1973). Clonal lines are supposed to be very valuable products for improvement of fish stocks (Han *et al.* 1991). In view of

this, meiotic gynogens have been produced in many species of fish but are not completely homozygous due to recombination between non-sister chromatids (Purdom 1959, Hussain *et al.* 1994). This occurrence is undoubtedly absent in case of the production of mitotic gynogens induced by the suppression of first mitotic cleavage and thereby the resulting progeny are homozygous at every gene locus. Therefore, it is very advantageous for producing clonal lines in the subsequent generation(s) with unique gene combination Although earlier reports are available on meiotic gynogenesis in fish, the production of mitotic gynogens was first successfully initiated by Streisinger *et al.* (1981) and the technique has since been applied to a number of fish. Despite having produced mitotic gynogens, until now clones have only been successfully produced in zebra fish (Streisinger *et al.* 1981), medaka (Naruse *et al.* 1985), common carp (Komen *et al.* 1991), ayu (Han *et al.* 1991) and nile tilapia (Hussain 1992). Successful diploid gynogenesis, both mitotic and meiotic using methods of UV-irradiation of sperm and various physical and chemical shock treatments have been reported by many authors (for reviews, see Komen *et al.* 1991 and Hussain 1994). But until recently, no such report is available on the induction of mitotic gynogenesis and subsequent production of clones in Asian carps. Therefore, the present work was undertaken and the trials were aimed at the production of mitotic gynogens as a first step and secondly meiotic gynogens for the development of clonal lines in *Labeo rohita* Ham.

Materials and methods

Origin of broodstock and induced breeding

The gonadal materials, eggs and sperm, used in this study were obtained from different mature broods of *L. rohita* maintained under hatchery programmes of the Freshwater Station, Bangladesh Fisheries Research Institute, Mymensingh, Bangladesh. The broods were induced with carp pituitary extracts through intramuscular injections (twice, six hourly) and after 11 to 12 hours the milt and eggs were collected by stripping.

UV-irradiation of milt

The milt was kept under refrigerated condition at 4^0c for a few minutes. The milt was then diluted at 1 :100 to 1:200 times with refrigerated physiological saline solution, Cortland's, solution with pH 7.2 to 7.5 (Wolf 1963) and samples from this were checked for motility of sperm. Then the concentration of sperm from samples were determined and maintained at a concentration of about 10^8 ml^{-1} by further dilution. The diluted milt was spread out on to a plastic petridish and a fine film was made with a thickness of about 0.5-1.0 mm. This sperm solution was exposed to ultraviolet (UV) irradiation under a short wave UV-lamp (Model UVBGL-58; Multiband-254/366 NM). The intensity of UV-irradiation was

optimized at 200-250 μW/cm^2 applied for 2 minutes at 28±1^{0}c and the irradiated sperm was kept in a refrigerator controled at 4^{0}c. The irradiated sperm was then used to inseminate the eggs. Based on the preliminary experiment these parameters were fixed for subsequent trails.

Induction of gynogenesis (both mitotic and meiotic)

All the treatment batches of eggs were fertilized with UV-irradiated milt except the control eggs which were fertilized with untreated, diluted milt. The recently stripped eggs fertilized with UV-irradiated sperm were poured into an incubator jar of 6 litre capacity with a water flow rate at 1 litre/minute at a temperature of 26±1^{0}c for normal development. Heat shocks designed to interfere with the first mitotic cell division of these eggs were initiated from 10 minutes up to 50 minutes a.f. to determine the window at which treatment can properly be applied. The experimental parameters used to interfere with the first mitosis were at a late heat shock of 40^{0}c for 2 minutes, applied at 20 to 40 minutes after fertilization with 2.5 minutes intervals. These parameters were also used as optimal for the retention of second polar body in producing meiotic gynogens by early shocks, 3 to 5 minutes a.f.

Egg incubation and karyotyping

The temperature for incubation of eggs was always maintained at 26±1^{0}c. The rates of fertilization, hatching success of eggs and survival of spawn were recorded. The hatching of normal larvae was considered as the primary criterion for estimating the success of induced diploidization. The karyotypes of samples from treated and untreated groups were determined by the techniques as described by Hussain and Mcandrew (1994).

Production of genetic clones

The survivors of two batches of the induced mitotic gynogens attained an average weight of 1.8 and 1.9 kg in their second year of life and they were all female. Among them, only a single mature female was selected and this brood was used in the production of induced meiotic gynogens. The protocol for induction of meiotic gynogenesis is described above.

Results and discussion

Table 1 presents the rates of fertilization, hatching and survivals of different treated and control batches obtained in the production of mitotic gynogens in the first (F_1) and Table 2 for clones through meiotic gynogens in the second (F_2) generation, respectively. No survival was observed in non-shocked groups using UV-irradiated milt (Haploid gynogens) in which the embryos were found to be deformed and abnormal in structure, the karyological investigation reveals them

to be all haploid (n=25) whereas the karyotypes were found to be diploid (2n=50) in both meiotic and mitotic gynogens. Heat shocks of 40°c for 2 minutes, which were optimal for the production of meiotic gynogenetics when applied at 3 to 5 minutes a.f. were also found to induce mitotic gynogenetics when applied between 25 to 30 minutes a.f. The event of first mitotic cell division was observed from 25 to 30 minutes and continued up to 35 tp 40 minutes a.f. at 26±1°c. These results would support that the heat shock application was properly applied between 25 to 30 minutes a.f. for the production of mitotic gynogens. This result is very similar to that of Nagy (1987) and Komen *et al.* (1991) for common carp, *Cyprinus carpio* where they induced mitotic gynogens by heat shocks at 40°c for 2 minutes applied at a window of 28 to 30 minutes a.f. The persent results are also consistent of with those of Mair *et al.* (1987) and Hussain *et al.* (1993), where they found optimal heat shock parameters for the production of mitotic gynogens in tilapia, *Oreochromis niloticus L.* to be at 41°c for 3.5 minutes applied at the window of 28 to 35 minutes and /or 27.5 to 30 minutes a.f., respectively. In this study, the rates of fertilization, hatching and survivals up to yolk sac resorption stage were found to be comparatively much lower in both mitotic gynogens in the first and meiotic gynogens (clones) in the second generation than those of normal control groups. These low survivals of mitotic and meiotic gynogens might be due to their extreme homozygosity. Komen *et al.* (1991) found mitotic gynogens of common carp *(C. carpio)* being survived at 5 to 15%, whereas our results are somewhat lower at 2.5 to 4.8%. Survival rates of inbred individuals in fish may be species - specific.

Table1. Observation on the production of mitotic gynogenetics in rohu, *L. rohita*

Trial No.	Parameters	Normal Control (%)	Haploid Control (%)	Mitotic Gynogens (%)	Mitotic Gynogens (%)
	Fertilization	85	85	87	80
1	Hatching	46	02	05	07
	Viability at one week age	37	0	3.4	4.8
	Fertilization	100	97	97	90
2	Hatching	92	0.9	2.5	3.7
	Viability at one week age	35	0	1.67	2.5

In this study, late heat shocks at either side of 25 to 30 minutes produced no survival : most of the treated eggs and embryos died befor hatching and the rest deformed embryos having haploid syndrome. This would strongly suggests that the survivors (diploids) of treated baches with in the window of 25 to 30

minutes a.f. were viable mitotic gynogens. In other trials, the same heat shocks between 25 to 30 minutes a.f. were failed in producing mitotic gynogens. However, the undeveloped and less developed (comparatively slow moving) embryos produced from batches treated with this window for both successful and unsuccessful cases were karyologically revealed to be haploids and / or haploid/diploid mosaics. Hussain *et al.* (1993) and Hussain (1995) observed high frequencies of haploid/diploid mosaics on either side of optimal window of heat shocks. He also opined that the heat shocks at the extremes of the effective window and or sub-optimal shocks could result in the production of haploid /diploid mosaics. Our data would suggest that the protocol for the induction of mitotic and meiotic gynogenesis in *L. rohita* using heat shock parameters need to be critically optimized as the developmental asynchrony within a single batch of eggs results in haploids, mosaics and diploids apperaing together. Our investigation is in furture progress and we plan to establish different clonal lines in the near future which can probably be used in the production of hybrid vigour in this species.

Table 2. Observation on the production of F_2 'Clone' through meiotic gynogenesis using F1 brood from mitotic gynogens of rohu, *L. rohita*

Trial No	Parameters	Normal Control	Haploid Control : UV=200 µW cm^{-2} (	Haploid Control : UV=250 µW cm^{-2}	Mitotic gynogens i.e., clone
		(%)	%)	(%)	
1	Fertilization	98	76	-	68
	Hatching	92	0	-	5
	Survival at one week age	84	0	-	66
2	Fertilization	87	62	-	64
	Hatching	82	2	-	2
	Survival at one week age	84	89	-	66
3	Fertilization	98	-	72	74
	Hatching	92	-	0	12
	Survival at one week age	84	-	0	66
4	Fertilization	87	-	67	62
	Hatching	82	-	0	2
	Survival at one week age	84	-	0	66

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Mixed culture of fishes in seasonal ponds through fertilization and feeding

M.A. Hossain*, M. Ahmed[1], M. Kamal[1] and M. N. Islam[1]

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

Abstract

A study on mixed culture of mirror carp (*Cyprinus carpio* Lin.), tilapia (*Oreochromis niloticus* Lin.), silver carp (*Hypophthalmicthys molitrix* Val.) and Thai sharpunti (*Puntius gonionotus* Bleeker) in the ratio of 1:2:2:5 was conducted in 12 seasonal mini ponds (30 m^2 each) for 105 days. There were six treatments each with two replicates and each pond was stocked with a total of 100 fish. Rice bran and mustard oil cake were used as supplemental feed either in combination or alone in presence or absence of fertilizer. Fertilizers were used in the form of organic, inorganic or both. The best growth performance of mirror carp, tilapia and Thai sharpunti was obtained in treatment III which received both fertilizer (organic + inorganic) and rice bran while the highest growth of silver carp was obtained in treatment VI receiving only inorganic fertilizer. However, the overall best production (2450 Kg/ha) and economic return for the culture period was obtained in treatment VI followed by treatment III. The results are discussed in the light of water quality parameters.

Key words : Mixed culture, Seasonal ponds, Fertilizer, Feeding

Introduction

Against the backdrop of declining fish catches from open waters in Bangladesh and increasing malnutrition, excellent opportunities exist for small-scale aquaculture development in rural areas, where majority of households have ponds or ditches. These water resources are presently either unutilized or underutilized. Small farmers constitute the bulk of the population in Bangladesh and there is urgent need to improve the efficiency of utilization of limited resource base of these small farmers. Most farmers in rural areas have access to water bodies such as seasonal miniponds, ditches and canals which retains

water for 5 to 6 months (from June - November). Farmers can effectively utilize these water areas for fish culture either for their subsistence or as commercial enterprises.

To minimize the growing gap between demand and supply of fish low-cost feed ingredients and fertilizers should be used for increased production of fish. Mixed culture of several fast growing species such as mirror carp (*Cyprinus carpio* Lin.), red tiliapia (*Oreochromis niloticus* Lin.), silver carp (*Hypophthalmicthys molitrix* Val.) and Thai sharpunti (*Puntius gonionotus* Bleeker) of different feeding habits and behaviour can best utilize the above mentioned water bodies. Mixed culture can even show symbiotic effects, when one species improves the environmental conditions and food supply for others. Thus, the present study was undertaken to study the growth of some fast growing species in seasonal miniponds and to observe the effects of different fertilizers and supplemental feed on growth of fish in mixed culture.

Materials and methods

Experimental system

The experiment was carried out for a period of 105 days during August to November'94 in 12 earthen mini-ponds of size 30 m^2 each. The ponds were prepared by draining out water during summer. For convenience the ponds were numbered as 1 to 12. During the experiment the water depth was maintained at a maximum of 1.2 m using fine meshed PVC over flow pipe on the bank fixed 1.2 m above the pond bottom. Ponds were divided into six duplicated treatments as shown in Table 1

Table 1. Composition of various treatments used in the present study

Treatment No.	Treatments	Pond No.	Rate of application
I	Without feed and fertilizer	1,2	–
II	Rice bran + Mustard oil cake (1:1)	3,4	5% of fish body wt. daily
III	Fertilizer (Inorganic + Organic) + Rice bran	5,6	50g Urea + 50gTSP + 2.5kg cow-dung weekly; rice bran 5% of fish body wt. daily
IV	Fertilizer (Inorganic +Organic)	7,8	50g Urea + 50g TSP + 2.5 kg cow-dung weekly
V	Fertilizer (Organic)	9,10	5.0 Kg cow-dung weekly
VI	Fertilizer (Inorganic)	11,12	100g Urea + 100g TSP weekly

Inorganic= Urea and TSP (triple super phosphate)
Organic = Cow-dung

Stocking and sampling of experimental fish

Fingerlings of mirror carp, red tilapia, silver carp and Thai sharpunti were collected from Freshwater Station, Fisheries Research Institute (FRI), Mymensingh. Each of the pond was stocked with a total of 100 fish comprising mirror carp, red tilapia, silver carp and Thai sharpunti at the ratio of 1:2:2:5 with a mean initial weight of 8.2, 12.0, 12.8 and 2.2g respectively. Fortnightly sampling of at least 20% of the fish was done using a small seine net to ascertain fish body weight and also to adjust feeding rate. At the end of the experiment harvesting was done by total draining out of the ponds.

Water quality parameters

The water quality parameters such as temperature, pH, dissolved oxygen (DO), carbon dioxide, and alkalinity were monitored weekly. Water samples were collected from the ponds and measured for temperature (Hand held mercury thermometer) and pH (Corning pH meter), other parameters like dissolved oxygen, carbon dioxide, and alkalinity were measured by titrametric method (APHA 1981).

Plankton estimation

The plankton samples were collected using a 25 micron mesh plankton net and studied using a Sedgewick-Rafter cell and a binocular microscope (Olympus model BH-2). Planktons were grouped into phyotplankton and zooplankton. Planktons were expressed numerically per litre of water.

Statistical analysis

One way analysis of variance (ANOVA) was used for statistical analysis of the growth data followed by Duncan's Multiple Range Test to determine the significance of variation among the treatment means. Standard error (± S.E) of treatment means were calculated from the residual mean square in the analysis of variance.

Economic analysis

A simple economic analysis was carried out to estimate the net profit generated from this type of operation. The cost of different inputs was based on the Mymensingh whole sale retail market price (1994) for various inputs. However, the cost of leasing the ponds was not included. The average selling price of fish was considered at Tk. 50/-per Kg. An additional 7.5% on the top of total inputs has been included towards operating cost (ADCP 1983).

Results

Water quality parameters

The ranges of water quality parameters in different ponds monitored during the study period were: temperature 27-35^{0}C; pH 6.1-8.8; carbon dioxide 5.0-6.9 mg/l; alkalinity 47.5-105.0 mg/l. The DO content during early morning (06.00 h) varied between 1.3-3.3 mg/l while in the afternoon it varied between 6.0-13.4 mg/l.

Plankton

The mean abundance of plankton with their different groups has been shown in Table 2. Phytoplanktonic population was mainly composed of Bacillariophyceae, Chlorophyceae, Cyanophyceae and Euglenophyceae and the zooplankton population consisted of Crustaceans and Rotifera. Chlorophyceae was found to be the most dominant phytoplankton group. Group Euglenophyceae ranked second and Cyanophyceae ranked third in respect of total count. Bacillariophyceae was the least abundant plankton and its mean abundance varied from $32x10^4$ to $66x10^4$/l.

The zooplankton population was represented by only two planktons *viz.* Crustaceans and Rotifera. The mean abundance of crustacean varied from $8x10^4$ to $14x10^4$/l while the abundance for Rotifera varied from $2x10^4$ to $9x10^4$/l. However, the overall mean abundance of plankton (phytoplankton + zooplankton) varied between $347x10^4$ to $621x10^4$/l with treatment VI showing the highest.

Growth performance of fish

The growth performance of mirror carp, red tilapia, silver carp and Thai sharpunti in terms of initial weight, final weight, weight gain, specific growth rate (SGR), survival rate and total production are shown in Table 3. Among the treatments the weight gain of mirror carp was significantly ($P<0.05$) the highest in treatment III receiving fertilizer (inorganic and organic) and rice bran.

Similarly, the highest growth of red tilapia was observed in treatment III. However, there was no significant differences ($P>0.05$) between the growth of red tilapia in treatment II (rice bran + mustard oilcake) and treatment VI (inorganic fertilizer).

Table 2. Group wise mean plankton ($x10^4$) count in per litre of water in different treatments

		Treatments					
A.	Phytoplankton	I	I	II	V	V	VI
	Bacillariophyceae	35	32	43	40	39	66
	Chlorophyceae	112	127	113	171	114	227
	Cyanophyceae	92	06	152	130	116	156
	Euglenophyceae	97	152	155	128	132	158
B.	Zooplankton						
	Crustacea	9	9	11	12	14	8
	Rotifera	2	4	8	8	9	6
	Total	347	400	482	489	85	621

Silver carp attained the highest growth among the experimental fish. The highest ($P<0.05$) growth of silver carp was attained in treatment VI (inorganic fertilizer) followed by treatments III, II and IV, V and I.

The highest growth of Thai sharpunti was obtained with treatment III followed by treatments II, VI, IV, V and I. However, there was no significant difference ($P>0.05$) between the growth of Thai sharpunti in treatments IV (inorganic + organic) and V (organic).

The specific growth rate (SGR) of mirror carp was between about 2 and 3 except in treatment I (1.41). The highest ($P<0.05$) SGR value was obtained with mirror carp in treatment III followed by treatment VI, II, V, IV and I. On the other hand, the SGR of red tilapia in different treatments were comparatively low which ranged between 1.28 to 1.93. The SGR of silver carp was significantly ($P<0.05$) highest in treatment VI (inorganic fertilizer). There was no significant differences ($P>0.05$) between the SGRs of silver carp in treatments II and III. The SGR values of silver carps ranged between 1.61 to 2.65. The SGR values of Thai sharpunti in different treatments ranged between 1.73 to 2.72.

The survival rate of various species in different treatments were fairly high (Table 3). There was no significant difference ($P>0.05$) between the survival rates of mirror carp in different treatments which ranged between 75 and 90%. In general, red tilapia showed the highest survival rate among all treatments. Tilapia in treatments II, III and VI showed 100% survival. There was no significant differences ($P>0.05$) between the survival of silver carp in different treatments which ranged between 77.5 to 90%. The survival rate of Thai sharpunti varied between 86 to 95% with treatment III showing the highest survival rate (Table 3).

Table 3. Growth, survival and production of fish in different treatments during the study period

	Treatments						
	I	II	III	IV	V	VI	± S.E
Initial wt.(g)							
a) Mirror carp	8.2	7.9	7.5	8.1	8.2	7.8	± 0.21
b) Red tilapia	12.1	12.2	12.0	12.4	12.1	12.3	± 0.35
c) Silver carp	12.5	12.8	12.9	12.7	13.0	12.6	± 0.23
d) Thai sharpunti	2.2	2.0	2.2	1.9	2.2	2.1	± 0.15
Final wt.(g)							
a) Mirror carp	36.1	110.5	150.5	87.9	150.1	118.0	± 0.79
b) Red tilapia	46.4	82.3	90.8	72.6	52.2	86.2	± 1.24
c) Silver carp	67.6	132.4	144.0	118.0	75.5	202.6	± 2.05
d) Thai sharpunti	13.5	33.5	38.4	18.9	17.5	24.6	± 0.58
Weight gain (g)							
a) Mirror carp	27.9	102.6	143.0	9.8	96.9 1	10.2	± 0.87
b) Red tilapia	34.3	70.1	78.8	60.2	40.1	73.9	± 1.14
c) Silver carp	55.1	119.6	131.1	105.3	62.5	190.0	± 2.14
d) Thai sharpunti	11.3	31.6	36.2	17.0	15.3	22.5	± 0.60
Specific growth rate (SGR)							
a) Mirror carp	1.41	2.51	2.92	2.27	2.43	2.59	± 0.03
b) Red tilapia	1.28	1.82	1.93	1.68	1.39	1.89	± 0.03
c) Silver carp	1.61	2.23	2.30	2.12	1.68	2.65	± 0.02
d) Thai sharpunti	1.73	2.68	2.72	2.19	1.97	2.34	± 0.07
Survival rate (%)							
a) Mirror carp	75	85	75	85	90	75	± 6.12
b) Red tilapia	87.5	100	100	97.5	95	100	± 1.40
c) Silver carp	77.5	85	85	87.5	80	90	± 3.23
d) Thai sharpunti	86	94a	95	87	88	90	±1.73
Total production (Kg/ha/105 days)	900	2133.3	2333	1683.3	1300	2450	

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

Standard error of treatment mean calculated from the residual mean square in the analysis of variance

Total production of fish shown in Table 3 which ranged between 900 and 2450 Kg/ha for the experimental period of 105 days. Treatment VI showed the highest production (2450 Kg/ha) followed by treatments III, II, IV, V and I. A

simple cost benefit analysis showed that treatment VI generated the highest net profit (TK.99,960/-)/ha/105 days followed by treatments III, II, IV, V and I (Table 3).

Discussion

The basic principles of polyculture involves the idea that when compatible coinhabiting species of different feeding habits are cultured in the same pond, all the food niches are utilized without detriment to one another. Environmental parameters exert an immense influence on the maintenance of a healthy aquatic environment and production of sufficient fish food organisms. The water quality parameters measured in different treatments throughout the experimental period were found to be more or less similar and all of them were within the acceptable ranges for fish culture. Lakshmanan *et al.* (1971) made a fortnightly observations on quality parameters in composite culture of Indian major carps for one year and recorded pH value of 6.0 - 9.3 ; total alkalinity value of 19.4 - 78.2 mg/l.

Dewan *et al.* (1991) in a study with Indian and Chinese carp in polyculture found a surface temperature of 30.2 - 34.0°C and a pH range of 6.6 - 8.8 which is more or less similar to the present study. The range of DO values recorded in the present study in the early morning was very low (1.6 - 3.0 mg/l). However, the range of DO values during afternoon was quite high ranging from 6 - 13.6 mg/l. The low DO level in the early morning might be due to the consumption of DO by fish biomass since no oxygen is produced by photosynthesis during night and utilization of oxygen for decomposition of organic matter in pond bottom.

The ranges of alkalinity observed in all treatments during study period were good. Moyle (1946) reported that pond and lakes with a range of total alkalinity of 40.0 - 90.0 mg/l are of medium to highly productive. Bhuiyan (1970) also stated that the total alkalinity of medium productive water ranged from 25 - 100 mg/l. The range of alkalinity values in the present study varied between 47.5 and 105.0 mg/l. Hence, the ponds are said to be medium to highly productive.

The phytoplankton population composed of four groups e.g. Bacillariophyceae, Chlorophyceae, Cyanophyceae and Euglenophyceae reflected usual composition in the tropical fish pond (Dewan *et al.* 1991, Wahab *et al.* 1994). The highest plankton abundance was recorded in treatment VI receiving only inorganic (Urea + TSP) fertilizers followed by treatment IV receiving both organic and inorganic fertilizers. The plankton population in treatment III (inorganic + organic fertilizer + rice bran) was more or less similar to that of treatment IV. The plankton abundance in different treatments recorded in the present study was much lower than that reported by Dewan *et al.* (1991) and Wahab *et al.*(1994). This might be due to the fact that rate of fertilization used by Wahab *et al.* (1994) was 2 to 3 times higher than those used in the present

study while the stocking density of fish (33,000/ha) in the present study was much higher than those (15,000/ha) used by Wahab *et al.* (1994). No plankton bloom was observed in experimental ponds during the study period except that in treatment VI which showed a slight bloom. However, this bloom diminished within one or two days. The zooplankton population was represented by two groups viz. Crustaceans and Rotifera. The overall abundance of zooplankton was low. However, treatments IV and V receiving organic fertilizer showed slightly higher abundance of zooplankton.

The highest weight gain of silver carp was attained in treatment VI receiving higher dose (200g/week/pond) of inorganic fertilizer. Treatment VI also showed the highest total production (2450 Kg/ha) among all the treatments. The overall production ranged from 900-to 2450Kg/ha in different treatments for a culture period of 105 days. Lakshmanan *et al.* (1971) reported a production ranging from 2230 to 4209 Kg/ha/yr in a 7 species composite culture of Indian and Chinese carps with the application of fertilization and supplemental feeding. Murty *et al.* (1978) reported a net fish yield of 2275.37 Kg/ha/yr with fertilization alone as against an yield of 3558.58 Kg/ha/yr with fertilization and feeding.

A number of factors can affect the amount of available natural food. One of the major determinant is, of course, the productivity of the pond, whether natural or induced by inorganic or organic fertilizers. The inter-species association in polyculture can also affect the production of natural food, through competition among the species and its availability to the fish. Hepher (1989) reported that silver carp at high densities (1300 and 2600/ha) in polyculture with bottom feeding fish (common carp, 1000/ha; tilapia hybrid, 1500/ha) grew better than silver carp stocked at the same density in monoculture, although the concentration of algae was higher in the later. Milstein *et al.* (1985) explained this by overgrazing on the larger algae and zooplankton in the monoculture ponds, which gave rise to the development of large number of very small algae. In the polyculture systems, the bottom feeding fish by their burrowing action cause an "up welling" of nutrients. This stimulates the production of large number of algae to the advantage of silver carp, which therefore, grew better in presence of bottom feeding fish.

The growth of mirror carp, red tilapia and Thai sharpunti were the highest ($P<0.05$) in treatment III but the overall production of fish was second highest (2333 Kg/ha) in treatment III receiving supplemental feed (rice bran) and fertilizer (Inorganic + Organic). The use of fertilizer in combination with supplementary feeding in pond systems may be advantageous because it permits the use of higher fish and shrimp stocking densities and facilitates faster fish and shrimp growth. For example, Sinha (1979) reported fish production (Indian and Chinese carp polyculture) in fresh water ponds in India to be 1053 Kg/ha/yr with no fertilizer or feed inputs, 1398-2303 Kg/ha/yr with organic and inorganic fertilizer inputs 3314-4000 Kg/ha/yr with supplementary feed inputs (mixture of rice bran

oilcake; 1:1) and 4244-5506 Kg/ha/yr with both fertilizer and supplementary feed inputs. Furthermore, the operating costs of fish production are reduced by use of fertilizer (manure) and feed inputs (Shang and Costa-piere 1983).

The growth of red tilapia and Thai sharpunti in treatment II ranked second and mirror carp and silver carp ranked third among the treatments. The overall production of fish in treatment II receiving supplemental feed (mustard oilcake + rice bran) also ranked third (Table 3). In supplemental feeding aquaculture, feeding itself through the accumulation of residues and faeces has manuring effect and increases the amount of available natural food in the pond. The contribution of natural food organisms within semi-intensive pond farming systems can not be under emphasized. For example, Szumiec (1969) estimated that the contribution of natural food as 30% of the food ration for common carp in a supplementary feeding schedule. The overall lower growth and production of fish in treatment II compared to treatment VI (Inorganic fertilizer) in the present study might be due the low production of natural food in treatment II compared to treatment VI.

The overall low growth and production of fish in treatment V receiving organic manure in treatment V may be due to the fact that although manures are considered largely as indirect feed, they are expected to be far less effective than that of direct ones, since at every transition in trophic level in a recycling process, about 90% of the energy and nutrients become unavailable (Wohlfarth and Schroeder 1979). On the contrary, Zhu *et al.* (1990) reported an increased net yield of 10.2 Kg/ha/day in Chinese integrated farm ponds as a result of manure application.

The mean survival rate for various fish in different treatments in the present study varied between 82 and 91% which were higher than the survival rates reported by Wahab *et al.* (1991) for Indian major carps in polyculture where supplemental feed (mustard oilcake + rice bran, 2:1) was given. Lakshmanan *et al.* (1971) observed similar survival rate of 80% with seven species composite culture of Indian and Chinese carps in which ponds were fertilized with both organic and inorganic manures at short interval and fish were fed daily with a mixture of mustard oilcake and rice bran.

The result of the present study demonstrated that in a composite culture system growth of silver carp was markedly enhanced by the application of inorganic fertilizer. However, growth of other species (mirror carp, Thai sharpunti and red tilapia) were higher when receiving supplemental feed and fertilizer. The result of the present study suggests that above mentioned species can successfully be reared in seasonal ponds.

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Impact of chapila (*Gudusia chapra* Ham.) on growth of carps in polyculture

M.A. Hossain[1,*], S.M. Rahmatullah, M.S. Islam, A.K.M.A. Kabir[1], M.S. Islam[1] and S. Dewan

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

Abstract

The impact of chapila (*Gudusia chapra*) on the growth of carps was determined through introducing the fish in polyculture. A net average production with and without chapila were obtained at 467.11 and 889.54 kg/ha respectively ($P<0.05$) without affecting the survival of carps ($P>0.05$). The highest level of dietary overlap occurred between chapila and catla followed by chapila and rohu ($P<0.05$). The present study revealed that chapila reduces the net production at 47.49% in carps polyculture.

Key words : Carps polyculture

Introduction

Polyculture management techniques are based on the relationships between organisms at different levels of the food chain and the environment (Halver 1984, Hepher *et al.* 1989). Food and feeding habits of fishes are the prerequisites to the understanding the interspecific relationships for proper management of an ideal fishery system. For a better utilization of the food available in different strata and zones of an aquatic environment and maximizing production depends on the selection of appropriate fish species (Jhingran 1975). A knowledge of food and feeding habits would thus help in the selection of species for polyculture by ensuring maximum production through utilization of all available potential food in the waterbodies with minimum competition. To achieve adequate knowledge of the food and feeding behaviours and the extent of food competition between coinhabiting fish species, evaluation of dietary overlap are of great importance (Wahab and Ahmed 1992).

Therefore, considering the above facts, the present study was undertaken to observe the growth and determine the feasibility of culturing chapila in polyculture system by evaluating the production and dietary overlap among selected species (chapila, rohu, catla and mrigal).

Materials and methods

The experiment was conducted for a period of five months July to November, 1995 in six ponds of 800 m^2 with an average depth of 1.5m. The ponds were fertilized with urea and triple super phosphate (TSP) @ 25kg/ha respectively, after three days of liming @ 250 kg/ha. Three ponds were stocked with Indian major carps viz., rohu, catla and mrigal at the ratio of 4:3:3 at a stocking density of 6000/ha and the rest three ponds were stocked with the same manner plus 25% (1500/ha) of the total number with chapila under the treatment I and II, respectively.

Twenty fish of each species were randomly sampled fortnightly to know the growth and for adjustment the feeding on the basis of standing crop. All the ponds were fertilized fortnightly with Urea and TSP @ 25 kg/ha. The fishes were fed with supplementary feed with rice bran and mustard oil cake (2:1) daily at 3% of the total body weight. Routine sampling for water quality parameters such as DO, p^H, transparency and temperature were monitored weekly between 8.0-9.0 AM.

Five fishes were sacrificed and plankton sample collected through plankton net from each of the three ponds of the treatment II fortnightly. The preserved samples of plankton from ponds water and stomach content were investigated through a binocular microscope (X10) using a Sedgewick-Rafter Cell (Model 550, Fisons) following a standard method (APHA 1985) for counting plankton and identified upto genus level according to Prescott (1962) and Bellinger (1992). The dietary overlap among *G. chapra, L. rohita C. catla* and *C. migrala* were determined using Schoener's Index equation given by Schoener (1970).

Results and discussion

The overall mean values of each water quality parameters between the two treatments showed insignificant differences ($P>0.05$) except Secchi readings ($P<0.05$). The summarized data of growth of fish in the two Treatments are presented in Table 1. The results showed that the overall mean values for length and weight of carps under the two Treatments were found to be much higher in Treatment I over Treatment II owing to the absence of chapila. The weight basis data varied significantly ($P<0.05$) in rohu between the treatments i.e. with and without chapila and the other two for catla and mrigal were found to be almost higher which is also due to absence of chapila. Comparatively higher values of coefficient of correlation and regression were found in length-weight

relationships for carps in Treatment I than that of treatments II. Thus the results indicates higher growth, healthy and relative robustness of carps cultured without chapila than that of with chapila. The reflection of these results can also be seen in the data given in Table 2 where net production was 90% higher ($P<0.01$) in the polyculture of carps without chapila (Treatment I) over that of with chapila (Treatment II). It also be noted that chapila reduces the net production of carps to 47% in polyculture compared to that of without chapila. No significant differences ($P>0.05$) was found in survival rates of carps between the two treatments.

Table 1. Length-weight relationships and t-tests between mean values for rohu, catla and mrigal under the two treatments

Species	Treatment	Mean		t-statistic	Correlation between length and weight		
		Length (cm)	Weight (g)		Correlation coefficient	Regression coefficient	Intercept
Rohu	T_1	14.364 ± 4.899	89.754 ± 59.573	2.745*	0.97691	11.87884	-80.86872
	T_2	10.233 ± 3.004	31.426 ± 22.694		0.97936	7.39718	-44.27228
Catla	T_1	14.932 ± 5.359	116.594 ± 73.256	1.932^{NS}	0.97579	13.33853	-82.57959
	T_2	11.893 ± 4.027	61.683 ± 43.554		0.92677	9.25999	-51.53552
Mrigal	T_1	15.623 ± 6.072	111.640 ± 86.286	1.442^{NS}	0.96762	13.74926	-103.16920
	T_2	13.292 ± 5.253	62.957 ± 53.029		0.96660	9.75750	-66.74218

NS = Non significant at 0.05 level (P>0.05)

* *= Significant at 0.05 level (P<0.05)*

Where, $t_{0.05}$ (16) = 2.120 and $t_{0.01}$(16) = 2.921

Table 2. Species wise survival rate, yield and production of two treatments during the period of experiment

Treatment	Species	Survival rate (%)	Yield (kg/ha)	Production (kg/ha)	
				Gross	Net
T_1	*L. rohita*	60.50	255.60	926.34	889.54
	C. catla	80.22	312.66		
	C. mrigala	80.00	358.08		
T_2	*L. rohita*	67.33	71.01	512.93	467.11
	C. catla	72.22	132.72		
	C. mrigala	80.00	234.07		
	G. chapra	Not estimated	75.13		

Estimates of dietary overlap based on calculation of schoener's index were summarized in Table 3. The highest level of dietary overlap occurred between chapila and catla followed by chapila and rohu and the lowest level occurred between chapila and mrigal ($P<0.05$).

Table 3. Dietary overlap between chapila and Indian major carps, catla, rohu and mrigal in different fortnight

Group	Schoener Index		
	chapila/catla	chapila/rohu	chapila/mrigal
Bacillarioplyceae	0.79	0.58	0.60
Chlorophyceae	0.70	0.41	0.43
Cyanophyceae	0.60	0.71	0.47
Euglanophyceae	0.53	0.63	0.52
Zooplankton	0.24	0.33	0.25
Phytoplankton	0.66	0.58	0.51
Zooplankton	0.24	0.33	0.25

The branchial mesh size of chapila is nearly related to silver carp (Alam 1995, Rahmatullah 1992). Dewan *et al.* (1991) stated that the greatest dietary overlap were observed for catla-silver carp. There is a remarkable significant effect on the growth of catla and rohu by silver carp (Matin. 1995). Hence, due to smaller branchial mesh size of chapila it appears to dominant for food competition and greatest dietary overlap occurred for chapila-catla and chapila-rohu.

Conclusions

Therefore, there is a clear indication in this study that the chapila does not suite in polyculture with Indian major carps because the fish is a strong filter feeder and heavily compete for food with catla and rohu.

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Studies on growth and mortality of *Cyprinus carpio* (Lin.) in floating ponds

M.A. Hashem[1], M.A. Quddus* and M.S. Khan[1]

Department of Agricultural Statistics
Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[1]*Thana Fisheries Officer, Directorate of Fisheries*
**Corresponding author*

Abstract

Growth and mortality rate of *Cyprinus carpio* (Linnaeus) under five different dietary conditions were studied in fifteen floating net cages in ponds of the Bangladesh Agricultural University Campus, Mymensingh. Growth rate was found to vary under different dietary conditions. The feed with mixture of 25% rice bran, 5% wheat bran, 30% linseed oil cake and 40% water hyacinth leaf meal exhibited the highest growth rate. The gain of log of body weight per unit increase of log of total length was significant. Significant survivals of the fishes was found.

Key words : Floating cage, Supplemental feed

Introduction

Fish culture in floating cages is yet to be popularized in Bangladesh. One of the major constraints behind intensive fish farming in Bangladesh is the multi-ownership of ponds which can be solved through use of floating cages for fish culture. Among the various reasons, lack of knowledge in the selection of appropriate species, preparation of low-cost balanced diets and determination of appropriate stocking density are still the major set backs for the development of floating cage fish culture (Mollah *et al.* 1987). The experiments so far done in Bangladesh indicate that it can registrar spectacular increase over the same under conventional pond farming (Aminul Hoque 1978). Fish culture in cages could be developed by improving stocking density, feeding methods, selection of species and regulating the culture cycle for maximum profitability (Sodikin 1977).

Cyprinus carpio is a phytophagous fast growing species, well suited to farming in ponds and lakes. In the present study the authors attempted to

establish the growth condition of this species by obtaining net gain, relationship between total length and body weight and condition factor. Also an attempt was undertaken to know the mortality rate. All of the above knowledge are very important for the scientific culture and management in floating ponds. The present study reports the growth response of carpio to various supplementary diets in floating net cages. With the above considerations in mind an attempt has been made to culture *Cyprinus carpio* (Lin.) in floating ponds using supplemental feeds.

Materials and methods

Experiment was carried out for five different dietary conditions (Treatment I : rice bran 35% + wheat bran 30% + linseed oil cake 15% + fish meal 20%, Treatment II : rice bran 25% + wheat bran 17% + linseed oil cake 28% + fish meal 10% + ipil-ipil leaf meal 20%, Treatment III : rice bran 10% + wheat bran 10% + linseed oil cake 35% + ipil-ipil leaf meal 45%, Treatment IV : rice bran 25% + wheat bran 17% + linseed oil cake 28% + fish meal 10% + water hyacinth leaf meal 20%, and Treatment V : rice bran 25% + wheat bran 5% + linseed oil cake 30% + water hyacinth leaf meal 40%) for the period of six months in floating ponds made of synthetic netting materials of mesh size 5.0 mm fitted to a bamboo frame. About 25% protein level was maintained in each treatment.

The experiment was carried out for a period of six months in a pond of 3 hectares. Size of each cage was 1 m x 1 m x 2 m. Three hundred fry of *C. carpio*. belonging to the same age group and more or less similar sizes were stocked in 15 cages. The initial length was 7.36 cm and the initial weight was 5.94 gm. They were conditioned in a portable small plastic pool for 24 hours before being released in the floating ponds. Fishes were stocked at the rate of $20/m^3$ in each net cage. Feed was supplied once (morning) in 24 hours at the rate of 5% of total body weight of fishes throughout the experimental period. Twenty percent of the fishes in each pond were supplied at random using hand net and their lengths and weights were measured. Sampling was carried out at an interval of 15 days.

Total length and body weight relationship was established using the formula outlined by Doha and Dewan (1967) :

$W = aL^n$,

where, W = weight, L = length, 'a' = constant, and 'n' = an exponent. The calculation of co-efficient of condition (K) was done by using the formula $K = W/L^3$ (Doha and Dewan 1967). t-statistic was used by using the formula given by Quddus and Dewan (1988). The technique of analysis of variance was used to see the effect of various treatment combinations.

Results and discussion

The original data reveals that the maximum average gain in length (6.07 cm per six months) and in weight (24.52 gm per six months) was investigated by applying the diet with mixture of rice bran 25%, wheat bran 5%, linseed oil cake 30% and water hyacinth leaf meal 40%. The production of *C. carpio* in per cubic meter waterbody was obtained due to different supplementary diets. The Treatment I gave the maximum productivity (371.45 g/m^3) and Treatment IV gave the minimum productivity (371.45 g/m^3). The results regarding cumulative growth (in gram) of *C. carpio* due to the effect of various treatments had been presented in Fig. 1 which shows that the cumulative growth increases gradually due to effect of all the treatments. This figure also reveals that the Treatment I and Treatment II had better effect on fish growth than that of the Treatment III and Treatment IV at the end of the study period.

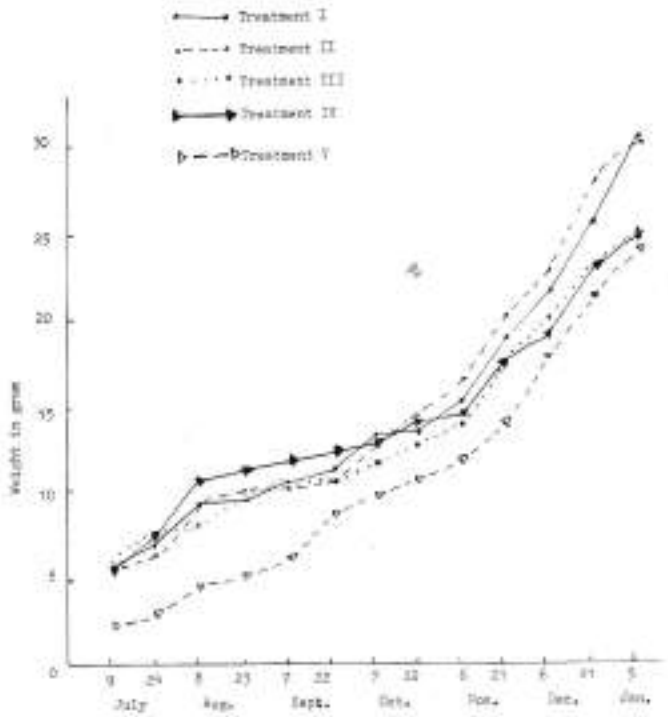

Fig. 1. Average cumulative growth of *Cyprinus carpio* (Lin.) in terms of increase in weight under same stocking rates over a period of six months.

Analysis of variance indicated that gain in length under different mixture of diets was significant (F = 27.00) at 1% level of probability and that gain in weight

was significant at 5% level of probability. The t-values of the Table 1 reveals that the relationship between log of total length and log of body weight obtained under different dietary conditions were significant. This results agreed with the result of Barua *et. al.* (1988). Statistical test also reveals that the Treatment I and Treatment II were significantly better effect on fish growth than the other treatments. Moreover, Treatment V exhibit highly significant increase of log of body weight with the increase of per unit log of body length (Table 1). This result occurred mainly due to application of higher rate of linseed oil cake, ipil-ipil leaf meal and water hyacinth leaf meal in replace of fish meal.

Table 1. T-values and Co-efficient of condition of *Cyprinus carpio* under different dietary conditions

Treatment s	t-value	Co-efficient of condition	
I		Range	Mean
	25.76*	1.09 to 1.92	1.59
II	26.14*	1.28 to 1.99	1.62
III	46.79**	1.32 to 1.95	1.74
IV	20.21*	04 to 1.92	1.74
V	45.04**	1.36 to 2.13	1.69

The ranges and mean values of condition factor in Table 1 shows that the conditions for fishes under same stocking rates were more or less similar. Doha and Dewan (1967) and Islam *et al.* (1978) reported that condition factor of *Tilapia mossambica* (Peters) was 1.69. Islam *et. al.* (1978) also reported that the condition factor of *Oreochromis niloticus* ranges from 1.66 to 1.88, which agree well with the findings of the present study. This agrees with the findings of Viola (1975) who reported severe decrease in growth of carps in cages when two-thirds of fish meal of control ration containing 15% of fish meal were replaced by soybean meal.

Mortality of fishes among different treatment groups as recorded during investigation period is presented in Table 2.

Table 2. Survival and mortality of fingerlings at same stocking rates after six months of rearing

Treatments	Replication	Stocking rate		Survival/pond		Mortality/pond	
		per m^3 of	pond	No	%	No	%
I	1	20	20 s	19	95.00	1	5.00
	2	20	20	20	100.00	0	0
	3	20	20	18	90.00	2	10.00
	Mean			19	95.00	5.00	
II	1	20	20	18	90.00	2	10.00
	2	20	20	18	90.00	2	10.00
	3	20	20	20	100.00	0	0
	Mean		18.67	93.33	1.33	6.67	
III	1	20	20	20	100.00	0	0
	2	20	20	20	100.00	0	0
	3	20	20	20	100.00	0	0
	Mean			20	100.00	0	0
IV	1	20	20	19	95.00	1	5.00
	2	20	20	18	90.00	2	10.00
	3	20	20	20	100.00	0	
	Mean			19	95.00	1	5.0
V	1	20	20	18	90.00	2	10.00
	2	20	20	19	95.00	1	5.00
	3	20	20	15	75.00	5	5.00
	Mean			17.33	86.67	2.67	13.33

The highest mortality (13.33%) was exhibited for Treatment V and no mortality was recorded for Treatment III. The highest mortality may be due to the effect of higher rate of water hyacinth leaf meal or other factors (fry condition, size, transportation, environmental condition etc.). However, the total growth of fish due to the effect of this treatment is the best. This contradiction of higher growth and mortality within the same treatment group may be overcome through a further study with a large number of replications. F-statistic indicated that there was no significant variation among the mortality of *C. carpio* due to different dietary conditions. It is recommended that in future, effects of variation in the amount of animal protein on the growth of fishes should be studied with management cost. It is further suggested that investigation with cheaper and easily available animal protein feed such as frog meal, slaughter house waste, etc., and a little amount of protein from plant sources, such as water hyacinth

leaf meal and ipil-ipil leaf meal, may be used in the feed trial of fishes in floating ponds.

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Some aspects of association and development of *Lytocestus indicus* Moghe in catfish *Clarias batrachus* (Lin.)

K.J. Chandra, K.Z. Islam and R. Wootten[1]

Department of Aquaculture
Bangladesh Agricultural University, Mymensingh - 2202, Bangladesh
[1]Institute of Aquaculture, University of Stirling , FK9 4LA, Scotland, U.K.

Abstract

Some aspects of association and development of the caryophyllid cestode *Lytocestus indicus* Moghe, 1925 infecting the catfish *Clarias batrachus* (Linn.) from the Kailla Beel of Mymensingh, Bangladesh were studied. About 33.14% of *Clarias batrachus* were infected with a mean intensity of 3.75, mean density 1.25. The infection was not found throughout the year. Two seasonal occurrence of this cestode were observed, one in April-May and the other in August-September. However, maturation period of the worm coincided with the maturation of the host. The worm was found attached to the wall of the intestine of the host. At the site of attachment tissue layers were compressed due to machanical injuries. Prevalence and mean intensity of infection increased with length groups. No variation in infection was significantly observed in different sexes of the host examined.

Key words : *C. batrachus, Lytocestus indicus*

Introduction

Seasonal cycle in prevalence and maturation have been observed in few species of caryophyllid cestode in fishes (Calentine and Fredickson 1965, Kennedy 1968, 1969, Zaman and Leong 1988). *Lytocestus indicus* a caryophyllid, was first recorded by Moghe in 1925 from *Clarias batrachus* (L.). Since then, it has been reported in this catfish from other South East Asian countries. Ahmed and Sanaullah (1976) first reported this helminth in Bangladesh . Later they (Ahmed and Sanaullah 1977, Sanaullah and Ahmed 1979) also studied the associations, distribution and pathogenecity of the parasite with other helminths from the same host. As partial information on seasonal association of their species were provided by Rashid *et al.* (1983) and

Zaman and Leong (1988), this paper therefore gives a detailed account of infection and developmental stages of *Lytocestus indicus* in its final host the *Clarias batrachus* of Kailla Beel in Mymensingh.

Materials and methods

Fish samples were collected from the Kailla Beel, Mymensingh by cast net during October '94 through September '95. Twenty specimens of *Clarias batrachus* were targeted to sample in every month. They were brought in a container with water. Total length and sex of the hosts were noted. The host fishes were classified into three groups on the basis of their total length. The fish were opened ventrally by a sharp scalpel. Whole gut was removed, measured and opened in physiological saline. Position of the *Lytocestus indicus* was noted by measurement of the gut, small worms were searched by scraping out mucus using dissecting microscope. All the worms were collected, released in water and counted. Some worms were fixed in A.F.A. stained in Alum carmine, dehydrated in alcohol grades and prepared permanent slides for determining the state of maturity. The worms were classified into four groups on the basis of their development (immature- early larval stage, sex organs not developed; maturing- sexual organs distinct and developing; mature- sexually mature, not fertilized; gravid- gravid worms containing fertilized eggs in them). Data on the number, size and developmental state of worms collected from each fish were recorded and analyzed.

Results

Seasonal occurrence in prevalence and intensity of infection

A total of 175 specimens of *Clarias batrachus* were examined during the period from October '94 to September '95 and 218 *Lytocestus indicus* were collected from 58 fishes. This presented an overall prevalence of 33.14% and intensity 3.75 worms.

The occurrence, prevalence and intensity of *L. indicus* in *C. batrahus* is shown in Table 1. The worm occurred throughout the year but in varying intensities. Over the period of 12 months, the prevalence was higher during December-September and varied from 2.70 to 55.56%. However, maximum prevalence was in August-September when 55.56% fishes examined harboured the worm. The infection was minimum in October-November when 2.70% of the examined fish were infected. In other period the prevalence ranges between 15-50%.

The highest intensity (an average of 5.54 worms per infected host) was recorded in April-May. The lowest intensity was recorded (only one parasite per host) during October-November. In the remaining period the intensity varied between 1.62 to 3.1.

Table 1. The occurrence of prevalence and intensity of *Lytocestus indicus* in *Clarias batrachus*

Sampling period	No. of fish examined	No. of fish infected	Prevalence (%)	Mean intensity per infected fish	Mean density per examined fish	Variance / Mean
Oct/Nov	37	01	2.70	1.00	0.03	1.00
Dec/Jan	37	13	35.14	3.92	1.37	4.58
Feb/Mar	20	03	15.00	1.62	0.25	2.05
Apr/May	29	13	44.83	5.54	2.48	5.46
Jun/Jul	16	08	50.00	3.25	1.62	2.53
Aug/Sep	36	20	55.56	3.10	1.72	2.54
Total	175	58	33.14	3.75	1.25	4.51

Infection in relation to sex and size of the host

Data relevant to the host-sex and distribution of *L. indicus* is given in Table 2. Out of 92 males and 83 females; 28 male and 30 female fishes were infected. The ratios differ and it is evident that parasites burden in females is heavier than that of males. But statistically no significant variation in intensity were observed between male and female fishes ($T_{0.10}$=1.564 with d.f.10). The prevalence was not found significant among different sex fishes ($T_{0.10}$=0.682 with d.f.10). The level of infection in male and female fish fluctuated in different months. In June-July and August-September highest infection (50%) were recorded in males and females highest infection (62.50%) were recorded in August-September. However, lower infection was found in females in October-November. Thus both the prevalence and intensity of infection appeared higher in females. But in June-July males and females were infected to the same level.

The results of host-size relative data in *L. indicus* are shown in Table 3. All size groups of fishes were infected and largest size group also appeared to be more susceptible. The decline of prevalence are found in smaller fishes. But the prevalence was not found significant among different size groups of fishes (F_5=3.17 with d.f.2,10). However, there was a relationship between the mean parasite burden and the size of the fish. It varies significantly (F_3=7.83 with d.f.2,10) with the size groups. It is evident that the fish accumulate more parasites as they grow in size.

Table 2. The occurrence of prevalence and intensity of *L.Indicus* in male and female *C. batrachus*

Sampling period	No. of fish examined		No. of fish infected		Prevalence (%)		Mean intensity		Mean density	
	M	F	M	F	M	F	M	F	M	F
Oct/Nov	17	20	00	01	-	05.00	-	1.00	-	0.10
Dec/Jan	20	17	06	07	30.00	41.81	3.17	4.57	0.95	1.88
Feb/Mar	11	09	01	02	09.00	22.22	1.00	2.00	0.09	0.44
Apr/May	14	15	06	07	42.86	46.67	3.83	6.71	1.64	3.33
Jun/Jul	10	06	05	03	50.00	50.00	3.00	3.67	1.50	1.83
Aug/Sep	20	16	10	10	50.00	62.50	2.30	3.90	1.15	2.43
Total	92	83	28	30	30.43	36.14	2.89	4.57	0.88	1.65

Table 3. occurrence of *Lytocestus indicus* in three size groups of *C. batrachus*

Length groups (cm)	No. of fish examined	No. of fish infected	Prevalence (%)	Mean Intensity
<22.5	56	15	26.79	3.13
22.5-23.5	61	18	29.51	3.39
> 23.5	58	25	43.10	4.40

The developmental stages of L. indicus

The development and maturation of the worm has been studied by the monthly distribution of immature and mature worms. The pattern of variation in infection of the population structure of the worm in the host are shown Fig.1. All four groups were not present in October-November, February-March and August-September. However, in these months reproduction did not take place. Maturing and mature worms make up the larger part of the population during most part of the year may be due to the greater duration of these two stages. Recruitment indicated by the presence of immature worm in the fish gut appears to take place from December to September. However, after September recruitment does not take place. The presence of higher proportion of larval worms in December-January indicates that the peak recruitment occurred during this months. Seasonal occurrence in the arithmatic mean of larvae (immature) and gravid worms are shown in Fig. 2. The mean number of immature worm shown a seasonal pattern with maximum abundance in autumn (April-May) and no larvae in October-November. The increase of mean number of larvae in autumn is however, to be an effect of highest number of gravid worms in late summer.

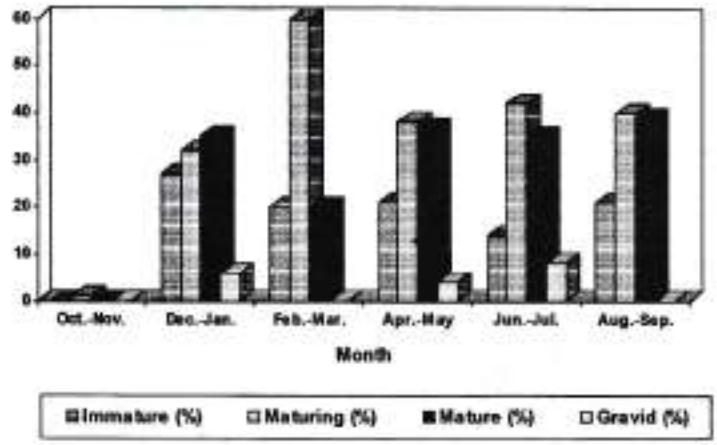

Fig. 1. Seasonal occurrence of immature, maturing, mature and gravid worm of *Lytocestus indicus in Carias batrachus.*

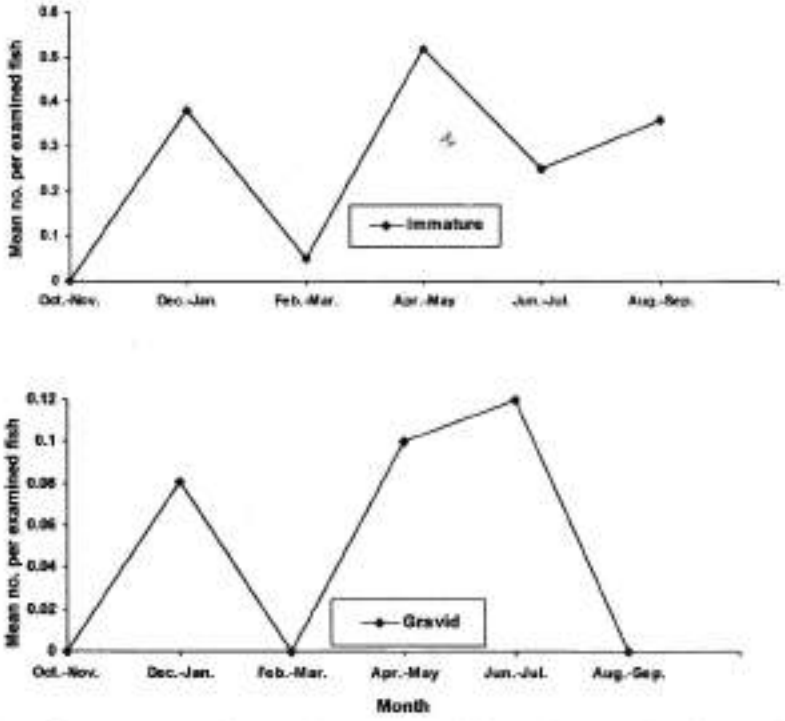

Fig. 2. Seaonal occurrence in the arithmatic mean of larvae (immture) and gravid worms of *Lytocestus indicus* in *Clarias batrachus.*

Discussion

The caryophyllid cestode, *Lytocestus indicus* was first recorded in *Clarias batrachus* by Moghe (1925, 1931). Since then various species of this genus *L. parvulus* Furtado 1963, *L. birmanicus* Lynsdale 1956, *L. filiformis* (Woodland) Fuhrman and Baer 1923, *L. fossilis* Gupta 1961 and *L. logicollis* Ramadevi 1973 have been described from silurid fishes from Indian subcontinent. Chandra and Khatun (1993) described *Pseudocaryophyllaeus heteropneustus* a new cestode from *Heteropneustes fossilis* from Mymensingh.

Seasonal occurrence in prevalence and intensity of *L. indicus* were found to be higher in August-September. Satpute and Agarwal (1980) found the similar seasonal infection in *C. batrachus* in six different tanks at Raipur, India. Niyogi *et al.* (1982) also reported very high incidence in intensity and density during March to August in *C. batrachus* of five species of caryophyllids, *viz. Lytocestus indicus, Pseudocaryophyllaeus indica, Djombangia penetrans, Introvertus raipurensis* and *Lucknowia indica.* Recruitment of these species of worms corresponded with the spawning season, when the fish were under tremendous stress, both hormonal and environmental. Agarwal (1985) mentioned that the infection was heavy during spawning of fish from March to August being recruitment season. Ahmed *et al.* (1985) also observed the highest rate of infection in *C. batrachus* by *L. indicus, Bovienia serialis* and *Pseudocaryophyllaeus indica* in rainy season.

In relation to the prevalence and level of infection were found to be relevant in different sexes. The present study indicated that the caryophyllid cestode, *L. indicus* showed higher infection in female than male hosts. Similar observations were made by Niyogi *et al.* (1982) for *L. indicus, P. indica, D. penetrans, I.raipurensis* and *L.indica*. Present finding is also the agreement of Skorping (1980) who reported significant difference in prevalence and mean intensity in male and female hosts. Sanaullah and Ahmed (1978) although did not find conspicuous variation in infection, it was observed that female hosts were more infected by caryophyllid cestodes.

Size differences were found to relevant to the prevalence and infection of *L. indicus*. Higher infection was significantly observed in larger groups. Distribution of the worm with respect to host size was similar to that observed for *Caryophyllaeus* and *Echinorhynchus* (Kennedy 1968, Awachie 1965). Prevalence and intensity of infection generally increased with host size up to a point and then declined (Stromberg and Crites 1975).

In the present study the infection of the parasite showed an overdispersion (variance > mean) distribution (Table 1). Stromberg and Crites (1975) stated that no precise mathematical functions could be defined for prevalence and intensity of infection of parasites in case of overdispersed population. Although the parasite *L. indicus* was present in the parasite throughout the year, there appeared a seasonal infection, invasion and maturation. The highest prevalence

occurred during August-September. This could be attributed to several factors including a large volume of parasites (maturing, matured) retained in the host for a longer duration. It also appeared that not all the parasites acquired maturity during summer months. Higher level infection was maintained from April to September. The observation on the maturation of *L. indicus* revealed that the fish becomes infected throughout the year, the period of infection appeared to be December-January and the large number of immature worms receive during these months to grow. Sanaullah and Ahmed (1978) suggested that the water temperature never falls below 4°C, even in the winter, thus making a suitable environment for infection by caryophyllid cestodes.

Conclusions

Lytocestus indicus is one of the most important caryophyllid cestodes of *C. batrachus* at Mymensingh. A seasonal cycle of its infection is found in the host. Spawning season of fish, water temperature and rainfall, availability of infected food organisms may be associated factors for its invasion, development and maturation. Winter period (December-January) appeared to be the recruitment period for this worm. In case of heavy infection it can cause serious injuries by its scolex penetration to fish intestine. Severe intestinal lesions, infiltration of leukocytes and hypertrophy and hyperplasticity of submucosa were observed by several authors (Ahmed and Sanaullah 1979, Agarwal 1985). Further works are therefore necessary for understanding the life cycle pattern, histopathology and its control measures.

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Survival of antibiotic resistant *Pseudomonas* strains in different types of water

M. S. Islam and M. B. R. Chowdhury

Department of Aquaculture
Bangladesh Agricultural University
Mymensingh- 2202, Bangladesh

Abstract

A study was conducted to investigate the survival of five *Pseudomonas* strains resistant to antibiotics in different types of water. The selected *Pseudomonas* strains were designated as strain P_1(CT-29), strain P_2(CT-25), strain P_3(CT-36), strain P_4(CT-20) and strain P_5(CT-27) which were only recovered from farmed fishes. Six types of water viz., distilled water, saline water, tap water, deionised water, pond water and river water were used. Among these experimental waters, river water was found to be the most suitable for long-term survival of these strains. Deionised water did not support survival of all these *Pseudomonas* strains. Pond water, tap water and distilled water were moderately suitable for strain P_1 and strain P_4. Saline water was also found to be highly suitable for long-term survival in case of the strain P_3 and moderately suitable for normal survival of strain P_2 and strain P_5.

Key words : *Pseudomonas*, Water

Introduction

Disease is the abnormal condition of body and mind which is expressed with certain symptoms. It affects the normal health condition and causes retardation of growth, abnormal metabolic activities and death. Fish disease is the interaction among host (fish), active pathogen and aquatic bio-ecological stress. Bacterial fish disease is one of the major limiting factors to fish culture and production in Bangladesh. In an investigation of the fish farms in Bangladesh, it was found that many farmed fishes suffered from disease seemed to be caused by bacterial pathogens (Chowdhury 1993) A number of investigation about the bacteria, *Pseudomonas* spp. in various organs of fish and water have been carried in the world and a few information is available in our country about this bacteria. *Pseudomonas* sp. is an important genus among the bacterial fish pathogen, for example *Pseudomonas fluorescens* is one of the major fish

pathogens which is widely distributed throughout the world in freshwater fish. Environmental water condition is undoubtedly important for persistence of bacterial pathogen. Any water which supports long-term survival may contribute to an easy out break of disease (Chowdhury and Wakabayashi 1990). Considering the importance, survival of five selected antibiotic resistant *Pseudomonas* strains in different types of water was investigated in the present study.

Materials and methods

Bacterial strain

Five bacterial strains were used for survival test. These were as follows:

1. Strain P_1(CT-29)
2. Strain P_2(CT-25)
3. Strain P_3(CT-36)
4. Strain P_4(CT-20)
5. Strain P_5(CT-27)

The source of collection and the resistance level of the selected *Pseudomonas* strains are shown in Table 1.

Table 1. Screened *Pseudomonas* strains used for survival test in different types of water

Strains	Source of collection			Resistant to the antibiotics
	Farm	Fish	Organ	
P_1(CT-29)	BCL	*Clarias* sp.	Liver	C,SXT,E & OA
P_2(CT-25)	DFL	*Catla catla*	Kidney	C,OT,SXT,E & OA
P_3(CT-36)	BCL	*Clarias* sp.	Liver	C,E,S & OA
P_4(CT-20)	DFL	*Catla catla*	Kidney	C,SXT,E,S & OA
P_5(CT-27)	JFF	*Labeo rohita*	Slime	C,SXT,E & OA

BCL *Bangladesh Catfish Ltd.*
DFL *Dhaka Fisheries Ltd. J*
JFF *Jhalak Fish Farm*
P *Pseudomonad isolates*
C *Chloramphenicol (30 µg/disc)*
OT *Oxytetracycline (30 µg/disc)*
SXT *Sulphamethoxazole(25 µg/disc)*
E *Erythromycin (10 µg/disc)*
S *Streptomycin (10 µg/disc)*
OA *Oxolinic acid (2 µg/disc)*

Experimental water

Six different types of water were used as experimental water for survival test of the five selected *Pseudomonas* strains. These were as follows:

1. Distilled water
2. Saline water
3. Tap water
4. Deionised water
5. Pond water (Isha Khan Lake, BAU campus)
6. River water (Brahmaputra river, near BAU campus)

The waters were used for the survival test after sterilization by autoclave. Parameters like dissolved oxygen and pH of the relevant water were recorded before and after autoclaving shown in Table 2.

Table 2. Parameters of experimental water recorded during the sampling period of survival test

Experimental water	Before autoclaving		After autoclaving	
	DO (mg/l)	pH	DO (mg/l)	pH
Distilled water	7.6	7.1	7.2	7.1
Saline water	8.2	7.1	8.3	7.2
Tap water	7.8	7.2	7.7	7.1
Deionised water	7.3	7.1	7.5	7.1
Pond water	8.5	7.4	8.6	7.2
River water	8.9	7.5	8.8	7.4

Procedures of survival test

Individual experimental Pseudomonad isolates were cultured on TSA plate. Then a sample of freshly cultured (18-24 h) inoculum weighing 20-30 mg (cfu) was taken into the sterile test tube containing 3-4 ml of distilled water to make a stock suspension. Then 0.5 ml of suspension was inoculated into 150 ml of sterile individual experimental water from the stock suspension and maintained at 25°C in incubator.

At each time of sampling 0.2 ml of incubated bacterial suspension was taken separately for individual and required ten-fold dilutions were made in sterile relevant experimental water. From required each dilution 0.1 ml of fluid was taken for inoculation on TSA plate and spreaded it by L-shaped sterile glass rod. Then the plates were placed at 25°C in the incubator for 24-36 hours to incubate. After 24-36 hours incubation, the number of colonies were counted by colony counter. Viable number of bacteria were determined at 0 day (immediately after incubation), 1 day, 3 day, 7 day and 10 day until completion of the experiment (Chowdhury and Wakabayashi, 1990). In each circulation duplicate plates were used and average total load of bacteria were counted.

Results

The results of survival test of individual strain are given below :

Survival of Pseudomonas strain P_1(CT-29)

The water of Brahmaputra river supported long-term survival of this strain (Fig. 1). Distilled water and pond water did support moderate survival of this strain. The survival of this strain was declined rapidly in deionised water, where its CFU could not be determined in the end of 3 day experiment. But a gradual decrease in number occurréd in saline and tap water. The highest survival was observed in river water, where the CFU number was almost similar to that of the initial value after 10 days.

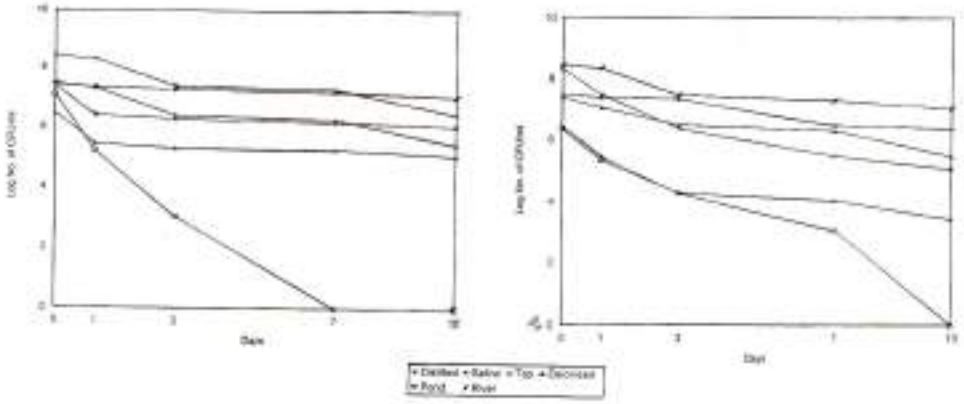

Fig. 1. Survival patterns of *Pseudomonas* strain P_1 (CT-29) in different types of water.

Fig. 2. Survival patterns of *Pseudomonas* strain P_2 (CT-25) in different types of water.

Survival of Pseudomonas strain P_2(CT-25)

The highest survival of this strain was found in river water where the CFU number after 10 days was almost similar to that of the initial value (Fig. 2). A moderate level of survival was observed in saline water with the CFU decreasing to about 10^{-1} of the initial value after 7 days. A gradual decrease of the initial number was observed in distilled water, tap water and pond water respectively. But a gradual decrease in number was observed in deionised water and no colony was detected on the plate culture after 7 days.

Survival of Pseudomonas strain P_3(CT-36)

The highest survival of this strain was found in saline water where the CFU number after 10 days was almost similar to that of the initial value and the water

supported long-term survival of this strain (Fig. 3). A moderate level of survival was observed in river water with the CFU decreasing to about 10^{-1} of the initial value after 7 days. A gradual decrease of the initial number was observed in distilled water, tap water and pond water respectively. A gradual decrease in number was observed in deionised water and no colony was detected on the plate culture after 3 days.

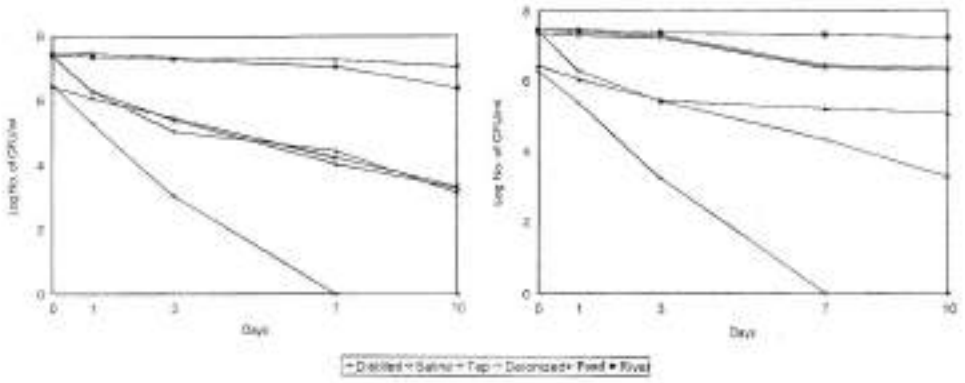

Fig. 3. Survival patterns of *Pseudomonas* strain P_3 (CT-36) in different types of water.

Fig. 4. Survival patterns of *Pseudomonas* strain P_{24} (CT-20) in different types of water.

Survival of Pseudomonas strain P_4(CT-20)

Survival of this strain was found to be the highest in river water where the bacterial number was almost the same as that of the initial value throughout the 10 days experimental period (Fig. 4). Distilled water and tap water also maintained a high survival of this strain, where the bacterial number decreased to less than 1/10 of the initial after 7 days. The bacteria gradually decreased in number in saline water. A moderate level of survival was observed in pond water with the CFU decreasing to about 1/10 of the initial value after 7 days. A gradual decrease in number was observed in deionised water and no colony was detected on the plate culture after 3 days.

Survival of Pseudomonas strain P_5(CT-27)

The highest survival of this strain was found in river water and saline water, where the bacterial number decreased to less than 1/10 of the initial after 7 days experimental period (Fig. 5). A moderate level of survival was observed in distilled water, tap water and pond water with the CFU decreasing to about 2/10

of the initial value after 7 days. A sharp decrease in the number of also occurred in deionised water where no bacteria could be recovered after 3 days.

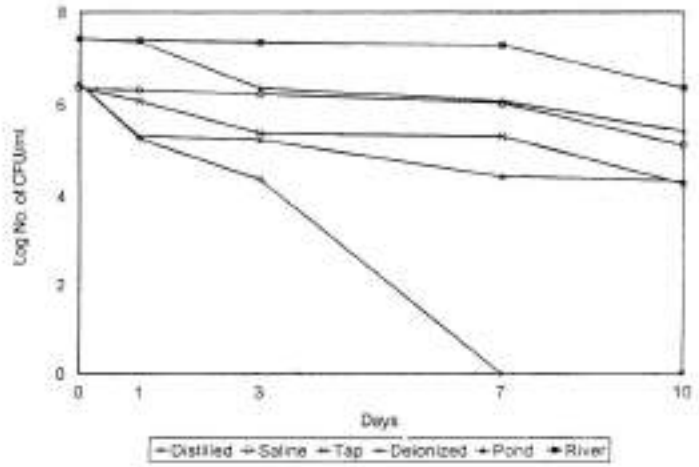

Fig. 5. Survival patterns of *Pseudomonas* strain P_5 9CT-27) in different types of water.

Discussion

Survival of five selected antibiotic resistant *Pseudomonas* strains in different types of water including distilled water, saline water, tap water, deionised water, pond water and river water was important work in the present study. Among the water tested, river water was found to be the most suitable for long-term survival of the five *Pseudomonas* strains followed by pond water. The reasons might be the high concentration of dissolved oxygen and favourable pH of these water bodies. Tap water and distilled water to be supported the survival of the strain P_1 and strain P_4 moderately. Saline water was found to be most suitable for long-term survival of the strain P_3 and moderately suitable for normal survival of strain P_2 and strain P_5. Deionised water was found not to support the survival of either of the strains.

Chowdhury and Wakabayashi (1990) reported that tap water was better than distilled water for the survival of *F. columnaris*. They mentioned that tap water might contain some trace elements which probably helped *F. columnaris* in its long-term survival. The results of the present study was related with the results of other scientists. Wakabayashi and Egusa (1972) demonstrated that high survival of *F. columnaris* in tap water. Muroga and Tatani (1982) reported that growth of *V. anguillarum* in 0.0% NaCl was negative but positive in 0.5-5% NaCl. In the present study, survival of *Pseudomonas* strain P_3 in saline (0.85% NaCl) water was found most suitable than other water. Deionised water may be lacking any such essential elements which was necessary for the survival of *Pseudomonas* strains, may have failed to produce helpful effect. Islam (1996)

found that river water, pond water and saline water were found to be the most suitable for long-term survival of *Pseudomonas* strains.

The present study provides useful information for the fish culturists on the survival of the *Pseudomonas* bacteria (fish pathogens) and with this knowledge, a fish culturist could possibly change the water in order to reduce the incidence of the bacteria. Further studies are necessary to know the pathogenicity of these *Pseudomonas* strains to various species of fish and to find out an appropriate control measures against the recovered pathogen.

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Effect of brood source on the growth of rohu (*Labeo rohita* Ham.) fingerlings reared in glass tanks with formulated diets

M. N. Ahsan[1] and S. C. Chakraborty[*]

Department of Fisheries Technology
Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
[1]Marine Biology Discipline, Khulna University, Khulna, Bangladesh
[*]Corresponding author

Abstract

A 60 day long feeding trial was conducted in an indoor static water system with rohu fingerings (*Labeo rohita* Ham.) originating from wild brood, private and public hatcheries (*denoted as A, B and C respectively*). They were fed on formulated diet having 34% crude protein level using indigenous ingredients. The effect of brood source on growth as well as their responses to formulated diet were observed . On the basis of the observed growth rate, food conversion ratio, protein efficiency ratio, apparent net protein utilization and apparent protein digestibility, fingerling source A showed significantly ($p<0.05$) higher growth, while the sources B and C produced no significantly different ($p>0.05$) in terms of these parameters. The results of the present study demonstrated that the fingerlings of wild source was of best quality in terms of growth and food utilization in comparison to those had the sources from hatcheries.

Key words : *L. rohita*, Fingerlings, Formulated diets

Introduction

The spawn fry or fingerlings of rohu fish (*Labeo rohita* Ham.) one of the most popular cultivable fast growing, non predacious species, are to be collected either from wild source or through making the reproduce in captivity as they do not breed naturally in ponds. The difficulties inherent in the former method are associated with the success of natural spawning but due to various man made and environmental changes the availability of fish seeds in the wild is being affected resulting in a phenomenal growth in number of hatcheries both in public and private sectors. But the hatchery population may be subjected to inbreeding, so the chances of bottleneck effects will be vary common in the hatchery population because of the small size of the brood. The two processes

in combination can very quickly damage a population by loosing genetic variability, lowering production performance and increasing production cost.

Recently other trends have been observed within the hatcheries belonging to the private sectors. Persons having no or little technological knowledge want early and good returns of their investment which leads them towards the mal-practice of inducing immature undersized broods by means of introducing overdoses of pituitary and human chorionic gonadotropin (HCG) hormones. Thus the fingerlings produced are believed to be of inferior quality.

Therefore, the main aim of this study was to assess if there is any effect on the nutritional uptake of fingerlings from different brood sources like wild, public and private hatcheries.

Materials and methods

Experimental fish and acclimation

The experiment was conducted for 60 days from 15 August to 15 October '95 in the laboratory of the Fisheries Technology, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh with rohu (*Labeo rohita*) fingerlings of three different brood sources. Wild fingerlings had source from the old Brahmaputra river were collected from Shutiakhali village of Mymensingh district (denoted as **A**). In this case, local fishermen collected the wild spawn of *L. rohita* from the river Brahmaputra and reared them in local pond at Sutiakhali. When these fish fingerlings became about 3 months old (about 35 to 38g size) they were collected for the experiment. The possible size of wild brood fish of these spawn/fingerlings was not known.

For other group of fish from private hatchery, fingerlings were collected from Puliamari Aquaculture Farm, situated at Shambhuganj of Mymensingh district (denoted as **B**). The broods used in this case were collected from the brood stock of the farm which had their source through artificial reproduction and the spawns were also artificially propagated from under-size brood (1.5 to 2.0 kg) of the farm. In this case, age of the experimental fish was estimated about three months. However, the under-sized brood fish had good health.

The third group of the experimental fish fingerlings had the source from the Fisheries Research Institute (FRI) hatchery of the same district (denoted as **C**). In this case the weight of the healthy brood ranged between 3.0 to 4.0 kg. In this case the broods were used from the brood stock pond of Fisheries Research Institute. The probable origin of this brood fish was from the river Halda of Chittagong. The fingerlings used from this source were also about three months old and had an average size between 35 and 38g. After prophylactic treatment with table salt and 0.5mg/l methylene blue the fingerlings were subjected to acclimation for two weeks in three separate 80 cm diameter plastic pools with 100 Litre water (stocking rate @ 1 fish/l) having continuous aeration. During this

period, they were given semi-purified pelleted diet containing 30% crude protein as maintenance ration.

Formulation and preparation of diets

Fish meal, mustard oil cake, duck weed and rice bran were used as feed ingredients. After proximate analysis (by the method as was followed by AOAC 1980) of each of the ingredients a diet was prepared by adjusting these ingredients in such a way to obtain 34% dietary crude protein level (Table 1). Wheat flour was used as binder as well as source of carbohydrate while "Embavit fish" premix" (Rhone Poulenc, Dhaka) was used for vitamin and minerals supplement. 0.5% chromic oxide was used also in the diet for the digestibility study.

Table 1. Proximate composition of dietary ingredients and experimental diet (% dry wt. basis)

	Components			
Ingredients	Crude protein	Crude lipid	Ash	NFE*
Fish meal	55.30	18.32	26.53	0.25
Duck weed	21.23	3.94	23.22	51.61
Mustard oil-cake	40.12	11.27	9.98	38.63
Wheat flour	12.60	3.50	1.40	82.50
Rice bran	14.17	12.30	17.97	55.56
Experimental diet	33.92	7.83	18.70	39.55

NFE = Nitrogen free extract as calculated as : 100 - %(moisture + Crude protein + Crude lipid + Ash)

Experimental procedure

The experiment was conducted with 9 glass aquaria (45 x 25 X 30cm each) containing 30 litres of water with adaquate supply of aeration to maintain saturated level of oxygen in each aquarium. A triplicate group of 12 uniform sized fish from each of the three treatment groups A, B and C were randomly selected for the feeding trials. The mean initial weight of all fish in the triplicates was 36.80 $\pm$ 0.54g. The experimental feeding regime was started after 24 hours of transferring the fish to the glass aquaria and was as follows: the fish were fed at satiation level daily for 60 days at regular intervals and amount of feed was recorded for subsequent calculation for various growth parameters.

Water from each aquarium was partially changed with clean stored aerated water every morning before feeding. Routine monitoring of dissolved oxygen

(DO), pH and temperature was done by using DO meter (Check mate, Mettler-Toledo Ltd, U.K.) every 10th day before sampling. For digestibility study, any uneaten food or faeces were removed from each aquarium 30 minutes after last feeding by a siphoning technique followed by collection of faeces on the subsequent morning.

Post experimental analysis

All dietary ingredients, diet, faeces and initial and final fish samples were analysed for the determination of moisture, ash, lipid, protein etc. according to the method followed by Association of Official Analytical Chemists (AOAC, 1980). Data collected during the feeding trial and subsequent proximate analysis were used for the determination of various growth parameters. Simple Analysis of variance (ANOVA) was employed to observe the effect of brood source on the growth of fish fingerlings followed by Duncan's New Multiple Range test to identity the level of significance (5%) among the treatment means.

Results and discussion

Proximate composition of experimental diets

The proximate composition of experimental diet is shown in Table 2. Singh *et al.* (1978) found better conversion efficiency of rohu fed on a diet containing 29.5% crude protein level, while Jayaram (1978) observed best growth with a diet having 35% protein level. Therefore selection of 34% dietary protein in this study was based on previous studies for a good growth response in subsequent feeding trials.

Table 2. Formulation of the experimental diet

Ingredients	% dry weight (in gram)
Fish meal	35.0
Duck weed	22.0
Mustard oil-cake	17.5
Wheat flour	5.0
Rice bran	18.0
*Embavit premix	2.0
Chromic oxide	0.5
Total	100.0

*Embavit premix = Vitamin & mineral premix, Rhone poulenc, Dhaka, Bangladesh

Water quality

Physico-chemical parameters of the water used in aquaria were routinely monitored and the ranges were: Temperature 25.8 - 28^{O}C; pH 6.6 - 7.3 and dissolved oxygen 4.4 - 6.5 mg/l. The amount of ammonia was not measured because the water of each aquarium was replaced partially everyday.

Acceptability of the diet

Response of the fish groups to the formulated diet was judged by a subjective behavioural assessment. The fish became adjusted to the experimental diet within the first two or three days and no marked differences between the acceptability of the diet of the fish groups were observed. No mortality was observed in any of the treatment groups during the experimental period.

Growth and food utilization

The effect of fingerlings sources on their growth and food utilization are summarized in Table 3. The highest growth was obtained with fish group A (wild source) followed by C (public hatchery) and B (private hatchery) respectively. However, statistically no significant ($p>0.05$) differences were observed between the fish groups B and C. The highest growth of fish originating from a wild brood has also been reported for silver carp (Vdovichek and Selivanova 1984) and seabass juvenile (Melloti *et al.* 1993). In the present study the specific growth rate (SGR) ranged from 1.13 to 1.33, with source A producing significantly ($P<0.01$) the highest SGR.

The FCR in the present study varied from 1.79 to 2.16 (Table 3) with significantly ($p<0.05$) lower FCR values for fish source A . A significantly ($p<0.05$) higher FCR value for fish source B in this study indicates that the fingerlings originating from the private hatcheries are not efficient converters of supplementary fish to flesh which results in increased production cost. This indication is also supported by the fact that the protein efficiency ratio (PER) of the present study followed this same trend as with FCR ranging from 1.36 to 1.63 (Table 3) with significantly ($p<0.05$) the highest PER produced by fish source A followed by C and B respectively. This may be due to the fact that the fish originating from the wild brood can utilize the dietary nutrients better, maximizing the use of each meal as unlike in the hatcheries a regular food supply is not guaranteed in the wild.

Significant differences ($p<0.01$) between the apparent net protein utilization (ANPU) values of fish sources A and B and that of A and C were observed while the values for B and C showed no significant ($p>0.05$) difference. This can be explained from the fact that the amount of dietary protein for fish is determined not only by the requirements for its maintenance and growth, but also by its utilization capacity for this purpose. From the above statement it is

clear that the protein utilization capacity of fish of wild origin is high. Because the relationship between anabolic capacity as rate of protein synthesis and growth potential in a number of species is dependent on the genetic strain which tells that protein synthesis displayed by individuals of rapidly growing strains can be greater than recorded for individuals from slow growing strain (Jobling, 1994).

Table 3. Summary of growth parameters of rohu fish (*L.rohita*) collected from different brood sources fed on formulated diet for 60 days

Treatment groups	Growth parameters								
	Initial wt (g)	Final wt (g)	Weight gain (g)	Weight gain(%)	SGR (%day)	FCR	PER	ANPU %	AOD %
A (Wild brood)	37.07^{a} (±0.10)	82.19^{a} (±1.46)	45.12^{a}	121.27^{a}	1.33^{a} (±0.06)	1.79^{a} ±0.09)	1.63^{a} (±0.08)	27.98^{a} (±0.16)	84.54^{a} (±0.05)
B Private hatchery	37.01^{a} (±0.08)	72.93^{b} (±0.46)	35.92^{b}	97.05^{b}	1.13^{b} (±0.02)	2.16^{b} (±0.03)	1.36^{b} (±0.01)	23.72^{b} (±0.31)	82.04^{b} (±0.84)
C Public hatchery	36.38^{a} (±0.04)	73.54^{b} (±0.65)	37.16^{b}	102.14^{b}	1.17^{b} (±0.02)	1.99^{C} (±0.02)	1.47^{C} (±0.01)	24.29^{b} (±0.21)	82.40^{b} (±0.19)

Figures in the same column having the same superscripts are not significantly different (P>0.05) from each other

Fish group A showed significantly ($p<0.05$) the highest apparent protein digestibility (APD) value while no significant ($p>0.05$) difference was observed between the APD values of B and C. APD values in this study was slightly lower than that of Jayaram and Shetty (1980) who reported a protein digestibility of 91.89% in rohu fish. Fish meal used in this study was prepared by grinding marine fishes of mixed origin in which the non-protein nitrogen content could be fairly high. This might have contributed to the lower protein digestibility of fish meal based diet in the present study. The findings of the present study clearly reveals that fingerlings originated from wild brood (A) are able to digest supplementary protein to the maximum thereby producing the highest yield.

Initially no significant ($p>0.05$) differences among the proximate composition of the fingerling sources were observed (Table 4). Source A significantly ($p<0.05$) produced the highest protein (17.59) and lowest moisture content (75.63%) whilst source C produced significantly ($p<0.05$) the lowest protein (16.82%) and the highest moisture content (76.13%). However, final lipid content of the treatment groups showed no significant ($p>0.05$) difference due to the differences in their brood origin (Table 4).

Table 4. Initial and final carcass composition of experimental fishes (% fresh matter basis)

Parameters	Initial			Final		
	A	B	C	A	B	C
Moisture	80.16	80.23	80.28	75.63	76.13	76.01
Crude protein	12.80	12.70	12.63	17.59	16.82	16.97
Crude lipid	2.98	2.95	2.99	3.14	3.30	3.22
Ash	3.45	3.44	3.48	3.56	3.63	3.59
NFE*	0.61	0.68	0.62	0.08	0.12	0.21

* *NFE = 100 - %(Moisture + Crude protein + Crude lipid + Ash)*

The results of the present study indicate that fingerlings of the wild origin are of superior quality in terms of of growth and food utilization in comparison to those originating from hatcheries. This may be due to the fact that the hatchery operators do not maintain large populations of quality brood stock to keep the population abated from inbreeding and genetic drift. To determine this further investigation on genetic and histopathological condition of fingerlings of different brood origin should be carried out.

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Effects of some selective supplemental feeds on the survival and growth of catfish (*Clarias batrachus* Lin.) fry

M.A. Rahman, A. Bhadra, N. Begum and M.G. Hussain
Freshwater Station, BFRI, Mymensingh -2201, Bangladesh

Abstract

Feeding experiments were conducted for 21 days to study the effect of live food (*Tubifex sp.*) and three prepared supplemental feeds on the growth and survival of 13-day old magur (*C. batrachus*) fry. It was observed that the growth of fry varied significantly ($p< 0.05$) with different diets. The best growth was shown by the fry fed with *Tubifex* sp. followed by those fed with the diet containing yeast (30%), milk powder (30%) and chicken eggs (30%). The poorest growth rate was given by the fry fed on yeast (45%) and fish meal (45%). There was no significant difference in survival rates and condition factors among the fry fed with live food and prepared feeds.

Key words : *C. batrachus* fry, Supplenental feeds

Introduction

Clarias batrachus locally known as magur, is a fish of great demand and attracted the attention of farmers for its high market value in Bangladesh. Until recently, the supply of its fry was dependent on natural sources which was one of the limiting factor towards catfish farming. To overcome this problem, attempts have been made by many workers to induce magur to breed using hormones (Rahmatullah *et al.* 1983, Mollah 1987). But till now, feed for successful rearing of catfish fry still exists as the main impediment for it's intensive farming. Varghese *et al.* (1973), Thakur (1978), Munnet (1979), Sanaullah *et al.* (1986) and Rahman *et al.* (1987) provided some basic information on the feeding of *C. batrachus* fry. However, development of suitable feed for rearing of *C. batrachus* fry is essential to ensure reliable and regular supply of quality fry at farmer's level. It is, therefore, deemed important to study the efficacy of few selective feeds for the rearing of *C. batrachus* fry.

Materials and methods

The study was conducted in 12 steel trays of equal size (105x45x14 cm deep) for a period of 21 days at Freshwater Station, Bangladesh Fisheries Research Institute, Mymensingh.

Each of the tray was filled with 20 liters of tap water and continuous water flow was maintained. There was a provision to pass out the excess water through an outlet of trays after maintaining the mentioned volume of water. Water flow rate was 0.2 l/min. The trays were stocked with 13-day old *C. batrachus* fry having an average initial length of 2.12 ± 0.12 cm and weight of 0.079 ± 0.016 g. Sixty fry were stocked in each tray. Four supplemental feeds were tested and they were designated as four treatments each with three replications. Treatment I (T_1) was live food (*Tubifex sp*), treatment II (T_2) was prepared with 30% egg + 30% milk powder + 30% yeast, treatment III (T_3) with 45% milk powder + 45% yeast and treatment IV (T_4) with 45% yeast + 45% fish meal. In all the prepared diets 8% wheat flour + 1% vitamin premix and 1% fish oil were used as common ingredients. Feeding was done three times daily (at 8:00, 16:00, 22:00 hours) upto satiation. The fry were considered to be satiated when they stopped feeding or searching for food. After half an hour of feed supply the uneaten food particles and faeces were removed by siphoning. The dead fishes were removed as soon as they were detected. Twenty percent of the stocked fish in each tray were sampled at weekly interval and length, weight and survival rate were recorded. The mean gain both in weight and in length and survival rate as obtained from different treatments were recorded.

Proximate analyses of the supplemental feeds tested were done according to AOAC (1980) and the results were shown in Table 1. The data obtained from the experiment were analyzed in Randomized Block Design and ANOVA were performed. Duncan's New Multiple Range Test at $\alpha = 0.05$ were also employed for further analysis of the results.

Results

The data on different growth parameters and survival rate of catfish fry fed on different supplemental diets are presented in Table 2 which shows that the supplemental diets tested had significant influence on the growth and survival of *C. batrachus* fry. The highest gain both in length and in weight was given by the fish fed on live supplemental feed (T_1), whereas the lowest was recorded in case of T_4 (yeast 45%+ fish meal 45%). T_2 and T_3 gave similar growth rate which was significantly lower than that of T1 but significantly higher than T_4. Percentage of weight gain and specific growth rate followed the same trend like length and weight gain. There was no significant variation in the condition factor as obtained from the four treatments, T_1 and T_4 gave the same average condition factor. Average survival rates as obtained from different treatment groups ranged from 70.03 to 78.37% but there was no significant ($P>0.05$) difference between the survival rates of different groups.

Table 1. Composition and proximate analyses of the test diets

	Diets			
	I	II	III	IV
Ingredient				
Tubifex sp.	100			
Yeast	-	30	45	45
Milk powder	-	30	45	-
Fish meal	-	-	-	45
Chicken eggs	-	30	-	-
Wheat flour	-	8	8	8
Vitamin premix	-	1	1	1
Cod liver oil	-	1	1	1
Proximate analyses: (% dry matter basis)				
Crude protein	68.29	44.56	40.23	41.27
Lipid	24.62	22.74	18.43	14.35
Ash	4.85	5.16	6.05	19.74
Moisture	84.77	47.46	35.92	49.18
NFE	2.24	27.52	35.28	24.58
Energy (Kcal/100g)	628.9	580.6	547.0	470.2

Table 2. Growth parameters and survival of *C. batrachus* fry fed on different diets

Paramenters	Treatment groups			
	I	II	III	IV
Length gain (cm)	1.96±0.10^{a}	1.33±0.11^{a}	1.23±0.08^{b}	0.70±0.12^{c}
Weight gain (g/fish)	0.51±0.01^{a}	0.25±0.01^{a}	0.23±0.03^{b}	0.11±0.01^{c}
Percentage weight gain	641.67±14.43^{a}	312.50±12.50^{b}	287.50±33.07^{b}	141.67±7.22^{c}
Specific growth rate (%/day/fish)	9.54±0.09^{a}	6.75±0.14^{b}	6.44±0.40^{b}	4.20±0.14^{c}
Condition factor	0.87±0.05	0.81±0.10	0.82±0.01	0.87±0.08
Survival rate (%)	78.37±4.48	70.03±7.18	73.17±4.49	76.67±3.35

Proximate analysis of the supplemental feeds showed highest protein and lipid content in diet of T_1 and the lowest in diet of T_3 (Table 2). Ash content was the highest in T_4 and the lowest in T_1. Nitrogen free extract was the highest in T_3 and the lowest in T_1 where moisture content was the highest.

Discussion

In the present observation, best growth and survival rate of *C. batrachus* fry was obtained from fed with live *Tubifex* sp. (T_1) agree with the findings of Alam and Mollah (1988) and Haque and Barua (1989). The diet containing fish meal (45%) and yeast (45%) (T_4) showed the lowest growth rate but better survival rate than the T_2 and T_3. Feeds containing baker's yeast are more acceptable to *C. batrachus* fry (Alam and Mollah 1988).

In contrast, Uys and Hecht (1985) reported that *Clarias gariepinus* larvae fed with 69.8% dried torula yeast (*Candida utilis*) and 23.3% brown fish meal having protein content of 55.4% which showed significantly better growth response than live food like zooplankton. The present observation is probably due to the poor quality of fish meal in diet IV. However, growth performance of the fry fed on preapared diets were not good in comparsion to the live food. The poor growth attained by the fry fed on prepared diets may be due to the inadequate acceptability of the feed to the fry. Low digestibility of feed might also caused such type of inferior growth.

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Culture feasibility of African catfish (*Clarias gariepinus* Lin.) fry in glass tank and synthetic hapa system using supplemental diets

G.U. Ahmed, M.F. Islam, M.N.A. Khan, M.M. Haque and M.G. Kibria

Faculty of Fisheries Bangladesh Agricultural University Mymensingh-2202, Bangladesh

Abstract

An experiment was carried out with 10 days old *Clarias gariepinus* fry over a period of 42 days to determine the effects of different feeds on growth and survival of African catfish fry in glass tanks. The experiment was designed into four treatments each having three replications. Thus treatment 1 (T_1) was named as Tank Tubifex (TT) and treatment 2 (T_2) as Tank Sabinco (TS), treatment 3 as Pond Tubifex (PT), and treatment 4 (T_4) as Pond Sabinco (PS). Live *Tubifex* (protein levels 64.48%) was supplied to treatments 1 and 3 and rest of the treatments were supplied Sabinco starter-1 (protein levels 40.13%). The highest and the lowest growth in total length and weight were 12.90 cm, 18.77 g and 6.17 cm, 4.04 g recorded from the treatments 3 and 2, respectively. Growth of catfish fry under treatment 3 in terms of both length and weight were significantly higher ($P<0.01$) than those of the other treatments. However treatment 2 showed the significantly lowest ($P<0.01$) growth performance among the various treatments. The highest survival rate (92%) was also obtained with treatment 3. *Tubifex* proved to be the best larval feed in respect of growth and survival rate.

Key words : African catfish fry, Glass tanks, Hapa, Supplemental diets

Introduction

Catfish is one of the very delicious and highly priced fishes which can be cultured in haors, baors, beels, jheels, canals and ponds. It has been drawing the attention of more and more fish culturists in Bangladesh day by day. Once easily available in the nature, the fish has, in the recent times, become scarce because of many adverse changes in their natural breeding and growing habitats. For this reason, catfish fry is very rare in nature. In Thailand *Clarias* meat is well known for its palatability (Tongsanga *et al.* 1963). Similarly, *Clarias* is in great market demand in the Philippines as a food fish (Carreon *et al.* 1973). *Clarias* is also fetching high market price in Bangladesh. African catfish (*C.*

gariepinus) was introduced in Bangladesh in December 1989 from Thailand. The fish seemed to adapt well in Bangladesh condition and was successfully bred for the first time in our country by Mollah and Karim (1990). The authors reported that the fecundity of African catfish is immensely higher (50,000-150,000) and breeding season is much longer (March - November) in Bangladesh.

Understanding of food and feeding habits of fishes are the prerequisites for effective management of a certain fishery. The food and feeding habits of fish vary with time of day, season, species, size of fish, ecological factors and with different food substances present in the waterbody. Information of daily food consumption by the fish fry under natural conditions is insufficient and scanty. To gain a better growth and survival of fish fry live feed is highly essential. Natural food provides a substantial availability of the protein and other essential nutrients required by fish. *Tubifex* have been reported to be an important live food for the larvae of many commercially viable fishes (Jhingran 1975). The tubificids worms are also used throughout the world, including Bangladesh, as food for the aquarium fish. Few attempts have been made in rearing *Clarias* larvae with only live food such as *Tubifex* sp. (Mollah and Nurullah 1988) and *Artemia* nauplii (Bairage *et al.*, 1988). Alam and Mollah (1988) found significantly higher survival rate and 10 times more growth of catfish (*C. batrachus*) larvae fed tubificids when compared with formulated dry fed. Mollah (1991) found similar growth rate in *C. batrachus* and *C. gariepinus* larvae. Although works on larval feed have been carried out but till today there has been lacking a suitable rearing technique, especially in the pond system. Considering the above facts the present study has been undertaken to determine a suitable rearing technique of African catfish (*C. gariepinus*) fry using supplemental feed.

Materials and methods

The proposed research work was carried out in a rectangular pond of size 0.006 hac. Twelve cultural basins were selected each having a size of 60 X 32 X 33 cm. The effective size of basins were maintained at 60 X 32 X 20 cm each having 10 liters of water. Six basins were glass aquaria which were set in the laboratory and the rest were synthetic hapa having mesh size of 1.00 mm. A bamboo frame was made and placed in the pond where all the synthetic hapa were fixed with the frame in such a position that 20 cm of the structure of each synthetic hapa remained below water. A small opening was kept at one top-corner of each hapa for providing feed and sampling of fry. The larvae were reared up to 10 days in metal trays and fed *Tubifex* and other prepared feed. the experiment was designed into four treatments each having three replications. In treatments 1 and 2 glass tanks were used where *Tubifex* and Sabinco starter feed were supplied having a protein level of 64.48% and 40.31% (Table 1). In treatments 3 and 4 synthetic hapa were used providing similar supplemental feed. Ten days old African catfish fry of initial total length of 2.90 ± 0.01 cm and

weight of 0.30 ± 0.01 g were released at same stocking densities i.e. 50 individuals per experimental basins. Fry were acclimatized with experimental pond water in plastic bowl and then stocked in the synthetic hapa and glass aquaria at 1700 h on 7 July 1995. The larvae of treatments 1, 2, 3 and 4 were fed two times a day at 0800h and 1600h respectively. Feeds were supplied in excess of satiation. Two third of water from each aquarium was changed once daily in the morning before feeding. A weekly record of water quality parameters such as pH, dissolved oxygen and temperature were also maintained. The parameters were determined by pH meter (Jenway microprocessor pH meter, model no. 2050). Digital DO meter (Jenway oxygen meter, model no. 3050) and a celsius/centigrade thermometer respectively. Plankton samples were collected from the rearing pond at every 7 days interval. Ten litres of water samples were passed through the plankton net for filtration of plankton. Two plankton samples were taken at every sampling day. Two clear white plastic bottles were used for plankton preservation. The bottles had a capacity of 200 ml. Five percent formalin was used for preservation of plankton samples. The number of phytoplankton was expressed as units per liter. In that case the colonial as well as the filamentous algae each were treated as a single unit. The water of zooplankton was expressed as cells per liter. The number of larvae died in a day were recorded carefully. All the fry were counted during sampling period from both tanks and hapa. However, a final count was made at the end of the experiment.

Table 1. Proximate composition (% dry weight) of *Tubifex* sp. (after Jhingran, 1975) and Sabinco starter-1

Feed	Moisture	Crude protein	Lipid	Ash	Nitrogen free extract
Tubifex worms	----------	64.48	16.00	7.28	15.04
Sabinco starter-1	9.70	40.31	4.28	14.40	----------

The data obtained in present experiment were analyzed statistically to see whether the effects of different feed on growth (length and weight) and health condition of fry were significant. The mean values were compared according to Duncan's New Multiple Range test at the 0.01 probability level.

Results

The maximum and minimum gain in length were 12.90 cm and 6.17 cm in the treatments 3 and 2 respectively. The highest and lowest growth in weight were 18.77 g and 4.04 g in the treatments 3 and 2 respectively during the experimental period. Table 2 shows the data related to growth parameters of *C. gariepinus* under different treatments during the experimental period. The length

gain of catfish fry under treatments 1 and 3 were significantly higher (P<0.01) than those of the treatments 2 and 4. Similar trend was also observed in case of weight gain. In both cases, better result gain was shown by the catfish fry under treatments 1 and 3 where natural feed was supplied.

The average survival rate in the treatments 1, 2, 3 and 4 were 87%, 56%, 92% and 70% respectively. The survivability of catfish fry under treatment 3 was higher when compared to those of the others. However, the lower survivability was shown by the fish under treatment 2.

The values of water temperature are also shown in Tables 3 and 4 respectively. Temperature of the experimental glass tank and synthetic hapa were found to range from 26.7°C to 29.6°C and 27.5°C to 30.5°C, respectively. The highest pH values of experimental glass tank and synthetic hapa were 8.08 and 7.76 and the lowest pH values of those were 6.80 and 6.20, respectively. The values of dissolved oxygen (DO) are also shown in Tables 3 and 4. Irregular fluctuations in the concentration of DO were observed. The range of dissolved oxygen (DO) values in the experimental glass tank and synthetic hapa (pond) were 4.95 to 5.60 m/l and 5.50 to 6.50 mg/l respectively.

Table 2. Growth parameters of *C. gariepinus* fry under different treatments during the experimental period

Parameters	TT (T_1)	TS (T_2)	PT (T_3)	PS (T_4)
Initial length (cm)	2.90^a	2.90^a	2.80^a	2.80^a
Final length (cm)	10.45^b	6.17^d	12.90^a	7.89^c
	±0.151	±0.364	±0.212	±0.276
Length gain	7.55^b	3.27^d	10.10^a	5.08^c
Initial weight (g)	0.30^a	0.30^a	0.29^a	0.29^a
Final weight (g)	10.74^b	4.04^d	18.77^a	6.28^c
	±0.580	±0.248	±0.249	±0.305
Weight gain	10.44^b	3.74^d	18.48^a	5.99^c
Ratio (g/cm)	1.028^b	0.655^d	1.455^a	0.796^c
Specific growth rate (SGR)	8.52^b	6.19^d	9.93^a	7.32^c
Survival rate (%)	87	56	92	70

Length - $T_1 > T_3 > T_4 > T_2$ Weight - $T_3 > T_1 > T_4 > T_2$ Ratio - $T_3 > T_1 > T_4 > T_2$ Survival- $T_3 > T_1 > T_4 > T_2$

Six planktonic groups consisting 20 genera were identified from experimental pond during the study period (Table 5). Four groups of phytoplankton and two groups of zooplankton were found. Fourteen genera of phytoplankton belonging to Chlorophyceae (7), Cyanophyceae (4), Euglenophyceae (2) and Bacillariophyceae (1) were found. Six genera of zooplankton were also identified belonging to crustacea (3) and rotifera (3).

Table 3. Water quality parameters (temperature, pH, dissolved oxygen) from rearing pond

Parameters	Sampling							
	Initial	1st	2nd	3rd	4th	5th	Final	Mean
Temp.	28.00	28.20	30.50	30.00	30.00	28.30	27.50	28.92
pH	7.75	7.76	7.00	6.20	7.20	7.00	6.88	7.11
DO	6.50	6.10	6.40	5.50	6.50	6.30	5.60	6.13

Table 4. Water quality parameters (temperature, pH, dissolved oxygen) from laboratory glass tank

Parameters	Sampling							
	Initial	1st	2nd	3rd	4th	5th	Final	Mean
Temp.	26.70	27.50	27.80	27.00	28.50	29.00	26.80	27.62
pH	8.08	8.08	7.00	6.80	7.20	7.20	7.13	7.35
DO	5.50	5.00	5.30	5.60	4.95	5.20	5.40	5.57

Table 5. Generic status of plankton (phytoplankton and zooplankton) in the experimental pond

A. Phytoplankton	
1. Chlorophyceae	*Pediastrum, Chlorella, Glococystis, Tetraedron, Volvox, Ulitrix*
2. Cyanophyceae	*Irocystis, Merismopedia, Chlrococcus, Coelastrum,*
3. Euglenophyceae	*Aphanocapsa*
4. Bacillariophyceae	*Euglena, Phacus*
B. Zooplankton	*Navicula*
1. Crustacea	*Cyclops, Diaptomus, Diaphyanosoma.*
2. Rotifera	*Filinia, Bracionus, Polyarthra*

Discussion

Effects of natural (*Tubifex*) and artificial (Sabinco Starter-1) feeding conditions on the growth of African catfish (*C. gariepinus*) fry in glass tanks and synthetic hapa (pond) were investigated in this experiment. Growth and survival rate of catfish fry were significantly higher in the treatment provided with live food (treatments 1 and 3) when compared with the treatment provided with formulated food (treatments 2 and 4). The maximum gain in length and weight were 10.10 cm and 18.48 g respectively which was obtained in the treatment 3 where live feed was supplied. The minimum gain in length and weight i.e. 2.27 and 3.74 g were obtained respectively in the treatment 2 where artificial formulated feed was supplied. This was possibly due to more affinity of catfish fry to *Tubifex*. This result coincides with the findings of different authors. Alam and Mollah (1988) reported *C. batrachus* larvae fed on live feed (*Tubifex* sp.) to exhibit significantly superior growth than artificial feeds. Hashim *et al.* (1993)

observed better results supplemented with live *Tubifex* than those reared without supplemental diets. Polling *et al.* (1988) used zooplankton, *Artemia* and trout fry starter meal (dry food) as a feed for *C. gariepinus* larvae over 12 days of experiment. Among the supplied food the highest growth was recorded from fish fry supplied with *Tubifex* which was followed by zooplankton, *Artemia* and dry feed. Mollah and Nurullah (1988) successfully reared *C. batrachus* larvae with live feed (*Tubifex* sp.).

Protein levels of *Tubifex* was 64.48% and Sabinco starter-1 was 40.31% in the treatments, 1, 3 and 2, 4 respectively. Henken *et al.* (1986) reported that crude protein requirement of *C.* varied from 41.2 to 43.6 which were depended on temperature. Chuapoehuk (1987) carried out experiment with 7 diets containing 20, 25, 30, 35, 40, 45 and 50% protein each of which was used to feed 200 walking catfish (*C. gariepinus*) fry kept in circular concrete tanks for 60 days. He found that 30, 35 and 40% protein gave excellent growth but the diet containing 30% protein produced optimum growth. Degani *et al.* (1989) and Madu and Tsumba (1989) reared catfish fingerling in an outdoor rearing system with feeds of different protein levels. They found that 40% crude protein gave better result than lower and higher. Mollah and Hossain (1990) reported that 39.5% protein appeared suitable for rearing of *C. batrachus*. According to Cruz and Laudencia (1976) the crude protein requirement for *C. batrachus* was 37.72%.

Water quality parameters did not show any significant difference in both the culture systems and hence did not influence the growth and survival of catfish fry. However, water quality parameters remained in the suitable range of tropical fish culture and the mean value of temperature, pH and DO for *Clarias* culture were 30.88^{0}C, 8.34 and 5.9 ppm. The values of temperature and pH for experimental pond coincided with the findings of Viveen *et al.* (1985). Mollah (1984), Britz and Hecht (1987) and Haylor and Mollah (1994) observed that 30^{0}C is the favourable temperature of *Clarias* larvae rearing. Henken *et al.* (1986) reported that the growth rate of African catfish at 29^{0}C is higher than at 24^{0}C. The pH value was in alkaline range in the pond which was suitable for fry rearing. Tarnchalanukit *et al.* (1983) indicated that the improved water quality in ponds allows greater growth and survival of *Clarias*.

Survival rate was significantly higher in the treatments provided with *Tubifex* and with the net cage systems when compared with culture in glass tanks. The survival rate was 92% in case of live feed whereas it was 56% when formulated feed was supplied. The highest survival rate was noticed in the pond conditions where natural feed was supplied. The survival rate of 70% obtained from similar environmental condition applying artificial feed could also be considered satisfactory. This results coincide with the findings of Alam and Mollah (1988) who reported that the survival rate (80.2%) obtained with artificial feed containing 56% fish meal, 90% bakers yeast and 14% wheat flour was comparable to those

fed *Tubifex* sp. (91.5%). Kestemont and Statmans (1992) reported that from an initial body weight of 1.86 mg at hatching, *Phoxinus phoxinus* larvae reared to about 30 mg in 4 weeks time and survival rate was higher than 96%. On the other hand the dry feed was not suitable for the *Phoxinus phoxinus* larvae where mortality rate was increased.

According to Ahmed (1994), *Tubifex* alone was a suitable feed which is very difficult to obtain throughout the year. The author further mentioned that considering the survival rate (75%) obtained and protein content (52%) in Sabinco starter-1 feed, it could be recommended as an alternative artificial feed for commercial production of African catfish especially when *Tubifex* is not available. In both the cultural basins, growth and survival of catfish fry were significantly increased with the live feed, *Tubifex*. Till now commercial production of *Tubifex* has yet to be developed. So, we have to depend on a particular season to have required quantity of *Tubifex*. On the other hand, artificial feed like Sabinco starter-1 is available throughout the year. Moreover growth and survival of catfish fry on this feed was not too low. Thus, this feed could be considered as an alternative to natural feed for the large scale production of African catfish fry.

As a cultural basin, net cages fixed in waterbodies might be better when compared with culture in aquaria. Net cage system, are less labour intensive, low cost showing a higher growth performances in the present experiment. Thus for rearing of young catfish fry a net cage system set would be preferable. However, more research works are necessary in order to make a final conclusion about the cultural system for African catfish fry.

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Effect of decapsulation on viability and hatching performance of *Artemia* cysts at different salinity levels

S.U. Ahmed, M.A. Rahman[1], M.N. Islam[1], and M. Kamal [1]

Brackishwater Station, BFRI, Khulna-9280, Bangladesh
[1]Department of Fisheries Technology , BAU, Mymensingh

Abstract

Artemia cysts were produced from the traditional solar salt works of Bangladesh through different fertilization treatments were tested for viability and hatching performance in different forms, such as processed and preserved, processed and decapsulated and unprocessed and undecapsulated. Decapsulated cysts performed maximum hatching (86.0%) in 20 ppt salinity during 48 hours of incubation. The hatching percentage by the unprocessed and undecapsulated cysts were very low (12.0 – 18.7%) in all the tested salinity grades.

Key words : *Artemia*, Decapsulation, Hatching, Salinity

Introduction

While feeding *Artemia* nauplii to fish and prawn larvae, the main constraint has been found to be the imperfact separation of nauplii from the hatching debris like empty shells, which often carry a heavy bacterial load (Shelbourne 1964, Gilmour *et al.* 1975). The unhatched cysts were also observed to carry a bacterial load after 24 hours of incubation (Wheeler *et al.* 1979). Such cysts upon ingested by the predator larvae cause blockage in their elimentary canal (Herald and Rackowicz 1951, Morris 1956, Stults 1974). Unhatched cysts and empty shells may also lead to serious infection in the culture system, leading to considerable mortality of the cultured larvae (Shelbourne 1964, MacFarlane 1969). Gunther and Catena (1980) reported infection of *Artemia* nauplii by pathogenic bacteria (*Vibrio* spp.) which also got transmitted to the predator larvae. A new method of feeding the *Artemia* cysts directly to the prawn larvae by decapsulating (removing of the outer chorion layer) them by sodium or calcium hyphochloride (decapsulation reagent) was found to be very useful, as it caused no harm to the viability of the embryo and also acted as a disinfectant

(Sorgeloos *et al.* 1977). Hatching efficiency and output of nauplii from decapsulated cysts were found higher than those from the untreated cysts (Bruggeman *et al.* 1980, Vanhaecke and Sorgeloos 1983). However, the effect of decapsulation has not been tested for all the strains in different environments. So it bears immense importance to find out the effect of decapsulation of different forms of locally produced *Artemia* cysts on viability and hatching performance.

Materials and methods

Artemia cysts were produced using the strain of Great Salt Lake (GSL), Utah, USA, in the *Artemia* production ponds (APP) of the modified solar salt works of Bangladesh by various feeding / manuring treatments. Treatments were T_1 (Urea and TSP at a rate of 25 and 20 kg / ha / week respectively), T_2 (dried powdered and sieved chicken manure at a rate of 125 kg / ha 3 - 4 days interval in the first year), T_3 (same as T_2 applied in the second year) and T_4 (double the rate of application of T_3 of second year). Initial fertilization was done before five days of *Artemia* nauplii release and the quantities were different than the regular ones, such as for T_1, Urea + TSP = 50 + 20 kg / ha, for T_2 and T_3, chicken manure 500 kg / ha and for T_4, chicken manure 1000 kg / ha.

Hatching of both decapsulated and undecapsulated cysts were tested in different hatching conditions :

- Hatching of the cysts in different salinity media : 10, 20, 30 and 40 ppt.
- Observation of the hatching performances of the decapsulated cysts at different time intervals, such as 24, 36 and 48 hours.
- Hatching of the cysts in different comparable forms like processed and preserved (not decapsulated), processed and decapsulated and unprocessed and undecapsulated (preserved).

The number of nauplii produced per 100 full cysts were counted and the hatching percentage was calculated following Sorgeloos *et al.* (1986) :

$$\text{Hatching percentage (HP)} = \frac{\bar{n} \times 100}{c^{\circ}}$$

Where, c° = mean number of cysts after one hour of initial incubation.
$\bar{n}$ = mean number of nauplii.

First produced *Artemia* cysts were processed according to Sorgeloos *et al.* (1986). The steps of processing were : a. size separation with brine, b. density separation in brine, c. washing in freshwater, d. density separation in freshwater and e. drying.

Cysts were decapsulated following the techniques described by Sorgeloos *et al.* (1977) and Bruggeman *et al.* (1979 and 1980).

The cysts hydrated up to 2 hours in fresh water at ambient temperature ranging between 25 - 28°c (hydration time increased with decreasing temperature and increasing salinity). For hydration, 1 g dried cysts wereprovided

per 20ml of water and aeration was made at a rate of 0.51 / min. Prolonged hydration prior to hypochloride treatment was avoided because it could drastically affect the hatching rate and efficiency of the decapsulated cysts.

Commercial grade sodium hypochloride (NaOCl) was diluted to 50% with sea water and 40% sodium hydroxide and was used for decapsulation. NaOH was added to increase p^H above 10.0.

Hydrated cysts were transferred to the decapsulation reagent (15 ml for 1 g cyst) and stirred well continously with a glass rod for about 10 minutes. Temperature was maintained below 40^0C by keeping the decapsulation container in cold water bath. Cysts were decapsulated within 15 minutes which were immediately filtered out on a 120 µm mesh cloth.

After complete dissolution of the chorions, the decapsulated cysts were filtered off and excessively washed on a 120 µm screen with tap water until no more chlorine smell was noticed. Hypochloride residues adsorbed into the decapsulated cysts were deactivated by dipping the cysts in 0.1N HC1 for several times.

This deactivation lasts less than one minute and then cysts were again washed with tap water. Hypochloride residues were detected by putting some decapsulated cysts in a small amount of diluted starch-iodine reagent (i.e. starch, potassium iodide, sulphuric acid and water).

After the completion of the deactivation and washing procedures, cysts were drained on a 120 µm sieve and transferred into a saturated brine solution at a rate of 1 g (decapsulated cysts) / 10 ml. Since upon incubated in brine, dehydrated cysts were releasing water so that brine had to be renewed after each hour of incubation. Settled cysts in the bottom were filtered through 120 µm screen. Cysts were then poured in fresh brine solution and kept in refrigeration for future use.

Results

Processed and preserved cysts

The cysts produced in T_3 showed highest hatching percentage (78.3% ± 4.0) in 48 hours of incubation for processed and preserved form in natural day light. The cysts of the same source offered 60.0% ±1.7 and 75.7% ±2.5 in 24 and 36 hours of incubation, respectively (Table 1). In this experiment, the cysts from all the four sources ($T_1 - T_4$) showed their best hatchability (72 – 78%) at 20 ppt salinity media. The lowest hatchability was recorded at 40 ppt salinity compare to other concentrations in 24, 36 and 48 hours of incubation. The second highest percentage (75.7%) of hatching was observed in 36 and 48 hours of incubation for the cysts of T_3 at 20 ppt and for the cyst of T_1 at same salinity.

Table 1. Percentage (mean ±SD, n = 5) of hatching of processed and preserved cystts (in natural day light)

Incub. period	Treatments	Salinity of hatching media (ppt)			
		10	20	30	40
24 hr	T_1	50.3 ±3.1	60.7 ±3.5	45.0 ± 3.0	33.7 ±2.1
	T_2	55.3 ±5.7	58.0 ±5.0	40.0 ± 5.7	32.0 ±3.0
	T_3	55.3 ± 1.2	60.0 ±1.7	50.7 ± 0.6	45.0 ±1.7
	T_4	45.0 ± 2.6	62.7 ± 0.6	50.3 ± 2.3	38.7 ± 1.5
36 hr	T_1	65.0 ± 2.6	72.0 ± 3.0	51.3 ± 2.1	40.3 ± 1.2
	T_2	65.7 ± 3.2	70.3 ± 3.8	45.7 ± 3.5	40.3 ±3.2
	T_3	64.3 ± 2.1	75.7 ± 2.5	58.7 ± 1.5	47.7 ± 2.1
	T_4	58.3 ±1.5	70.0 ± 1.7	57.3 ± 3.2	42.7 ± 0.6
48 hr	T_1	65.3 ± 1.2	75.7 ± 1.5	55.0 ±2.3	45.0 ± 1.7
	T_2	75.0 ± 3.6	75.3 ± 4.2	55.0 ± 7.2	48.7 ±5.7
	T_3	70.3 ± 1.5	78.3 ± 4.0	62.0 ± 4.4	50.7 ± 0.6
	T_4	56.3 ± 1.5	72.7 ± 0.6	54.0 ± 1.7	38.7 ± 1.5

Processed and decapsulated cysts

In case of processed and decapsulated cysts, the highest hatching percentage value (86.0% ± 4.6) was also offered by the cysts produced through T_3 in 48 hours of incubation. In 36 hours of incubation, the cysts of same source offered 84.0% ±3.5 hatchability (Table 2) which is the second highest hatching value for that particular batch. Maximum hatchability by the cysts of all sources found at 20 ppt salinity followed by 10 ppt in 36 and 48 hours of incubation. Gradual decrease in hatching percentage with the increase in salinity concentration of the hatching media was observed in all the cases. The lowest percentage of hatching (but not poor) mostly in between 57 - 67% was observed at 40 ppt salinity in 36 and 48 hours of incubation.

Unprocessed and undecapsulated cysts

In this trial, cysts from all the sources showed very minimum percentage of hatching at all the salinity grades (10, 20, 30 and 40 ppt) and in all incubation periods (24, 36 and 48 hours). Minimum hatching percentage (12.0% ±0.0) recorded for the cyst produced through T_3 at 40 ppt salinity in 36 hours of incubation and the highest percentage (18.7%) was recorded for the cyst of T_1 at 10 and 20 ppt salinity (in 48 hours of incubation) and for the cyst produced through T_3 at 20 ppt salinity in 48 hours of incubation (Table 3). However, from 20 ppt to higher concentrations, decreasing in hatching percentage of the cysts from all sources has been observed.

Table 2. Percentage (mean ±SD, n = 5) of hatching of processed and decapsulated cysts (in natural day light)

Incub. period	Treatments	Salinity of hatching media (ppt)			
		10	20	30	40
24 hr	T_1	55.0 ± 3.6	59.3 ± 4.9	53.0 ± 7.5	47.3 ± 5.9
	T_2	58.3 ±3.2	68.0 ±3.5	60.0 ± 2.0	56.0 ± 2.6
	T_3	57.0 ±5.2	63.3 ±3.1	52.0 ± 2.0	50.3 ±3.5
	T_4	53.3 ±0.6	73.0 ±2.0	59.0 ±2.6	47.0 ±2.0
36 hr	T_1	73.7 ±7.6	78.0 ±2.1	65.3 ±2.1	63.0 ±4.0
	T_2	71.0 ±2.6	77.0 ±2.6	68.7 ±3.1	64.0 ±2.0
	T_3	68.0 ±4.0	84.0 ±3.5	63.0 ±3.6	58.7 ±4.6
	T_4	59.0 ±1.0	77.7 ±4.2	67.3 ±3.2	51.7 ±1.2
48 hr	T_1	59.0 ±2.3	80.0 ±8.7	69.0 ±4.0	67.3 ±2.1
	T_2	76.7 ±9.1	81.3 ±1.2	70.3 ±1.2	66.0 ±0.6
	T_3	74.4 ±2.3	86.0 ± 4.6	64.0 ±3.5	60.0 ±1.7
	T_4	65.0 ± 2.0	79.7 ± 3.2	67.3 ± 2.1	57.7 ± 1.2

Discussion

Among all the cysts, the decapsulated ones offered highest hatchability (86.0% ±4.6) at 20 ppt salinity in 48 hours of incubation. The variation in percentage of hatching of the cysts of first two forms, such as processed and preserved and preocessed and decapsulated of all treatments were found very minimum. Hatching percentage of the cysts of these two forms was very good in 10 and 20 ppt salinity for the cysts of all sources. But for other concentrations (30 and 40 ppt) hatching was found to decrease gradually. Least hatching percentage was recorded for the cysts of unprocessed and undecapsulated cysts. Finding of higher hatchability at 20 ppt in two forms is in disagreement with the findings of Versichele and Sorgeloos (1980). According to them, maximum hatchability can be obtained at sea water (35 ppt) or at 5 ppt concentration. As cyst production, its quality and hatching performance is strongly determined by environmental conditions (Browne *et al.* 1984), nature and availability of food particles in culture environment (Lavens *et al.* 1986), but not by the gene type (Browne *et al.* 1984). So it is not unlikely to evolve such information on hatching of the same cysts in new environment of culture and feeding. Versichele and Sorgeloos (1980) and Lavens and Sorgeloos (1984) studied the influence of abiotic and/or biotic factors on the hatchability of *Artemia* cysts and found a direct correlation with the environmental factors, which is supported by the present findings also. Cysts produced through different treatments, such as chemical fertilizer and organic manure did not show any remarkable variation in hatching, which means that in all the

treatments, feeding quality has been maintained properly. Because food quality available to the reproducing adults appears to be a parameter of primary importance in determing the hatching quality of encysted offered by two types of under quality feeding to the population (Lavens and Sorgeloos 1984). However, detail information regarding optimum set of abiotic conditions for the hatching of a particular strain is still lacking (Sorgeloos 1980).

Table 3. Percentage (mean ±SD,n = 5) of hatching of unprocessed and undecapsulated cysts (in natural day light)

Incub. period	Treatments	Salinity of hatching media (ppt)			
		10	20	30	40
24 hr	T_1	15.7 ±0.6	17.0 ± 1.0	15.3 ± 0.6	13.0 ±1.0
	T_2	16.7 ±0.6	16.7 ±0.6	15.3 ±0.6	13.7 ±0.6
	T_3	16.3 ±3.0	17.3 ±1.2	16.0 ±1.0	13.0 ±1.0
	T_4	15.3 ± 0.6	16.0 ± 1.0	14.0 ± 1.0	12.0 ±0.6
36 hr	T_1	16.0 ±1.0	17.0 ±1.7	14.3 ±0.6	13.3 ± 0.6
	T_2	16.0 ±1.0	17.0 ±1.0	15.7 ± 1.5	14.3 ± 0.6
	T_3	18.0 ± 1.0	17.0 ± 1.0	15.3 ±0.6	12.0 ±0.0
	T_4	17.0 ±1.0	16.7 ±0.6	13.3 ±0.6	13.0 ±1.0
48 hr	T_1	17.7 ± 1.5	18.7 ± 0.6	15.7 ± 0.6	15.0 ±1.0
	T_2	16.7 ± 0.6	18.0 ± 0.0	16.0 ± 1.0	15.3 ±0.6
	T_3	18.3 ±1.2	18.7 ±0.6	16.0 ± 1.0	13.3 ± 0.6
	T_4	17.3 ± 1.2	17.0 ± 1.0	14.0 ± 1.0	13.3 ± 0.6

Less amount of hatching percentage as offered by the unpreserved and undecapsulated cysts in quite normal. The main causes of which as identified were : degradation of cysts by hydration, non-wintering of cysts at least for few weeks and infection of the cysts by pathogenic bacteria etc. As the cysts were not processed and preserved and not decapsulated, so the state of quality of the cysts remains in a matter of doubt. Because removal of intra-cystic water from the cysts and reduced to less than 10% is a vital factor for preventing embryonic metabolism and long term presrvation (Voronov 1974, Sorgeloos *et al.* 1976, Dempster and Hanna 1956, Clegg 1962 and 1967, Vanhaecke and Sorgeloos 1982) and wintering of cysts at -4^0C (stop metabolism) or at -25^0C (for 32 weeks) is a process of diapause deactivation of the cysts of GSL origin (Lavens *et al.* 1986) and / or a treatment with 3% H_2O_2 for 15 minutes could offer a better hatchability (Mathias 1937, Bogatova and Shmakova 1980, Bogatova and Erofeeva 1985). Therefore, the absence of these attempts in the above forms of cysts, quality was undoubtedly degraded because of embryonic metabolism and / or infection that caused mortality to the cysts embryo.

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Role of transglutaminase-mediated cross-linking of protein in the temperature-dependent setting of Alaska pollack surimi

Alam AKM Nowsad[1,*] and E. Niwa

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

Abstract

During the low temperature setting of fish paste, myosin heavy chain (MHC) is polymerized to cross-linked myosin heavy chain (CMHC), which is considered to occur by the action of endogenous transglutaminase (TGase). In this study the contribution of TGase on the setting of Alaska pollack surimi at different temperatures was studied. Alaska pollack surimi was ground with 3 % NaCl, 30 % H_2O and with or without ethylene glycol bis(β-aminoethylether) N, N, N',N'-tetraacetic acid (EGTA), an inhibitor of TGase. Among the pastes without EGTA, highest TGase activity was observed at 25°C but breaking force of the gel set at 25°C was lower than that set at 30°, 35°, and 40°C. Addition of EGTA (5 m mol/kg) to the paste suppressed TGase activity at all setting temperatures from 20° to 40°C. Gelation of the pastes and cross-linking of MHC on addition of EGTA were suppressed completely at 20° and 25°C, partially at 30° and 35°C, and not at all at 40°C. The findings suggested that during the setting of Alaska pollack surimi TGase mediated cross-linking of MHC was strong at around 25°C but the thermal aggregation of MHC by non-covalent bonds was strong at above 35°C. Setting of surimi at 40°C and cross-linking of its MHC did not involve TGase.

Key words : Surimi, Transglutaminase, Myosin heavy chain

Introduction

Mechanically deboned water-washed fish flesh is called surimi. Now-a-days, some very popular analog or fabricated food products like crab-leg, beef, scallop or shrimp analogs are produced from surimi along with Japanese style traditional *kamaboko* products. In the manufacturing process of *kamaboko* or analog

products, the surimi is first ground with salt (2 - 3 %) and water (25 - 50 %) and then heated at a low temperature (0 - 40°C) before further heating at cooking temperature (80-90°C). The process of heating the surimi paste at low temperature is called setting or *suwari* in Japanese. The setting is generally performed to improve the elasticity of the final products, where salt-ground paste turns to an elastic and semi-transparent gel, called *suwari* gel. It was recently found that a myosin heavy chain (MHC) band disappeared in a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) pattern of surimi paste from Alaska pollack with the progress of setting; consequently, the bands due to macromolecular cross-linked myosin heavy chain (CMHC) appeared (Itoh *et al.* 1980, Lee *et al.* 1990, Numakura *et al.* 1985, Numakura *et al.* 1990). It was also found that a Ca^{+2} dependent enzyme, transglutaminase (TGase) existed in the surimi paste (Seki *et al.* 1990) and this enzyme mediated e - (g-glutamyl)lysine bond was formed during setting (Kimura *et al.* 1991). From these findings, it has been presumed that an enzymatically catalyzed cross-linking of MHC by covalent bonds plays the major role in the formation of protein network structure in *suwari* gel during setting.

TGase is also found to induce setting in some other easy-setting species, like hoki (Kimura *et al.* 1991) and sardine (Tsukamasa *et al.* 1993) at low temperature around 30°C. However, in our previous investigations, we observed that at 30°C setting proceeded in the actomyosin paste from which TGase was completely removed (Nowsad *et al.* 1994a) and in fish flesh pastes in which TGase was totally inactivated by the addition of SH reagents, such as *P*-chloromercuribenzoic acid (PCMB), iodoacetic acid (IAA) and N-ethylmaleimide (NEM) (Nowsad *et al.* 1994b, 1994c, 1994d). Further more, when CMHC was extracted from the top of the electrophoresed disc in the presence of concentrated urea and electrophoresed again, the band for MHC reappeared (Nowsad *et al.* 1993). These results concluded that CMHC was also formed by the aggregation of MHC through weak bonds such as hydrogen and hydrophobic bonds. The contribution of TGase in the elasticity of *suwari* gel from easy-setting species was found to be around 40 % and that from hard-setting species was almost nil or negligible (Nowsad *et al.* 1995). However, on the other hand, Tsukamasa *et al.* (1993) very recently suggested that the contribution of TGase to the low temperature setting was more important than that of non-covalent protein-protein interactions, from the findings that both the gelation of sardine myofibril sol and e-(g-glutamyl)lysine cross-link formation in it were completely suppressed at 25°C by the addition of EGTA, another inhibitor of Ca^{+2} dependent TGase. It is assumed that such discrepancy in results has arisen from the difference in setting temperatures within the same species in addition to the difference of species as we observed in easy-setting and hard-setting species (Nowsad *et al.* 1995). The TGase-mediated cross-linking of MHC may be temperature-specific and intense at relatively low temperature below 30°C.

In this experiment the role of TGase to the setting of Alaska pollack surimi at various temperatures was investigated. EGTA was added to the surimi paste to offset and compare the influence of TGase on setting and cross-linking of MHC.

Materials and methods

Suwari gel

Alaska pollack *Theragra chalcogramma* frozen surimi (Alaska Ocean Seafood, Anacortes, WA, U.S.A., SA-grade, unsalted) was thawed at 4°C, minced (3-mm-hole) and ground together with 30 % water, 3 % NaCl (in surimi weight), and EGTA (5 m mol/kg of surimi (Tsukamasa *et al.* 1993)) in a hand mortar for 10 min in a cold room at 4°C. The resulting surimi paste was stuffed into polyvinylidene chloride casing (2.8 cm in diameter), set in water baths at variable temperatures (20, 25, 30, 35, 40°C) for 1, 3, and 5 h, and cooled in running tap water. Control gels were prepared without EGTA keeping other conditions constant.

Physico-chemical measurements of suwari gel

pH of *suwari* gel was measured by putting a gel slice (1 mm in thickness) in a hollow chamber of the electrode (Horiba Ltd., Twin Compact pH Meter, B-112).

Suwari gel was sliced into 2 cm in thickness and subjected to a puncture test using a rheometer (Fudoh Rheo Meter, NRM-2010J-CW) with a spherical plunger (5 mm in diameter) at a table speed of 6 cm/min.

TGase activity measurement

Surimi mince with EGTA (5 m mol/kg) and without EGTA were incubated at different temperatures after grinding with 3 % NaCl, 2.5 mM monodansylcadaverine (MDC), and 30 % water and the activity of TGase was measured as described by Wan *et al.* (1992). Protein concentration was measured according to Umemoto (1966) after precipitating with an equal volume of 10 % trichloroacetic acid.

SDS-PAGE of suwari gel

A small piece of *suwari* gel (0.5 g) was solubilized in 9.4 ml of 8 M urea-Tris-HCl buffer (Numakura *et al.* 1985) by heating in boiling water for 1.5 min and stirring overnight at 24°C. After centrifugation at 30,000 x *g* for 30 min, the supernatant was filtered (Toyo Roshi Co., No. 1) and the filtrate was used as SDS-PAGE sample. SDS-PAGE was carried out using a vertical disc gel system as described previously (Nowsad *et al.* 1993). Densitometry of the disc gel and the calculation of the amount of subunit proteins were done as described before (Nowsad *et al.* 1993).

Results and discussion

Alaska pollack surimi showed a peak setting ability at 30°C as the breaking force of the gel without EGTA increased almost linearly in proportion to setting time up to 5 h as presented at the left of Fig. 1. The breaking force increased in proportion to the rise of setting temperature from 20°C, showed its maximum value at 30°C and then gradually decreased at 35°-40°C. During setting at 20°C, however, gelation did not occur till 3 h. In the gel set at 25°C, only a little gel strength was recorded up to 1 h. On the other hand, when EGTA at the rate of 5 m mol / kg was ground with the surimi mince, the paste did not transform into gel during setting at 20° and 25°C as shown at the right of Fig. 1, suggesting that EGTA suppressed the gelation completely at these temperatures. This result is quite consistent with that of Tsukamasa *et al.* (1993). However, when the setting temperature was raised further, gelation of this paste began to occur. The breaking force of the EGTA added gels increased in proportion to the rise of setting temperature. Maximum value was observed in the gel set at 40°C. However, the breaking force was very little at 30°C.

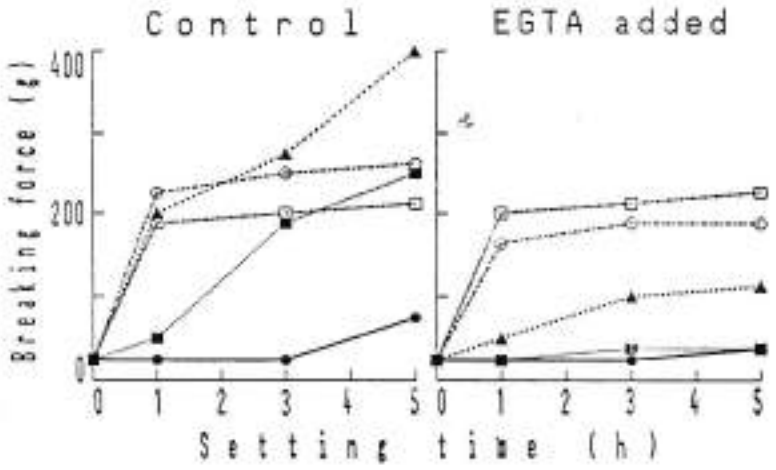

Fig. 1. Changes of breaking force in the control and EGTA added gels set at various temperatures. Setting temperatures (°C) -O-: 20, - -: 25, -Δ-: 30, -O-: 35, - -: 40

The pH of both the pastes with or without EGTA was more or less constant (range: 6.8-6.9, data not shown), suggested that the muscle pH was not affected by the addition of EGTA.

TGase has been considered essential in this setting (Seki *et al.* 1990, Kimura *et al.* 1991, Tsukamasa *et al.* 1993). Therefore, the activity of TGase in the gels

without EGTA and with EGTA was examined. The results are presented in Table 1. Highest TGase activity was observed in the gel without EGTA at 25°C, followed by that at 30°, 20°, 35°, and 40°C. Although the activity was high at 25°C, the breaking force was lower in this gel compared to the gels set at 30°, 35°, and 40°C. During setting at 35° and 40°C, TGase activity reduced to 0.32 - 0.35 n mol MDC / mg protein, a 25 % of the activity showed at 25°C after 5 h. But the *suwari* gels at these temperatures were stronger than that at 25°C. On the other hand, no TGase activity was observed in the gels with EGTA added at any setting temperature, suggesting that the action of TGase was completely inhibited by the addition of EGTA, and according to Tsukamasa *et al.* (1993), formation of e-(g-glutamyl)lysine cross-links was also inhibited in such gels. However, the surimi paste lost its stickiness and transformed into gel at 30°, 35°, and 40°C. This gelation did not occur due to TGase-mediated cross-linking of MHC. Non-covalent protein-protein interactions as we described before (Nowsad *et al.* 1994a, Nowsad *et al.* 1994b, Nowsad *et al.* 1994c, Nowsad et al. 1994d) might be important for this gelation.

Table 1. TGase activity of the paste with or without EGTA added*

Temperature	Without EGTA		With EGTA	
(°C)	Incubation time (h)			
	1	5	1	5
20	0.35	1.14	0.0	0.0
25	0.70	1.56	0.0	0.0
30	0.70	1.25	0.0	0.0
35	0.32	0.35	0.0	0.0
40	0.16	0.32	0.0	0.0

*TGase activity was expressed in terms of nmol MDC imcorporated into 1 mg surimi protein

The changes of protein subunits during the gelation of the pastes without and with EGTA at different temperatures were investigated electrophoretically, the SDS-PAGE patterns have been shown in Fig. 2. In the gels without EGTA at all temperatures (top row), as setting progressed, the intensity of MHC decreased and that of CMHC increased. That meant, MHC gradually cross-linked to CMHC in proportion to setting time at all setting temperatures examined. However, the degree of such cross-linking was very slow and the lowest at 20°C but fast and the highest at 25°C, followed by 30°, 35° and 40°C.

On the other hand, when EGTA was added to the pastes, the intensity of MHC was unchanged in the gels set at 20° and 25°C but gradually decreased in the gels set at 30°, 35°, and 40°C. However, a gradual concomitant increment of CMHC was observed in the latter three gels. This meant that the cross-linking of MHC was stopped in the EGTA added gels set at 20° and 25°C, but again proceeded at the temperatures above 30°C.

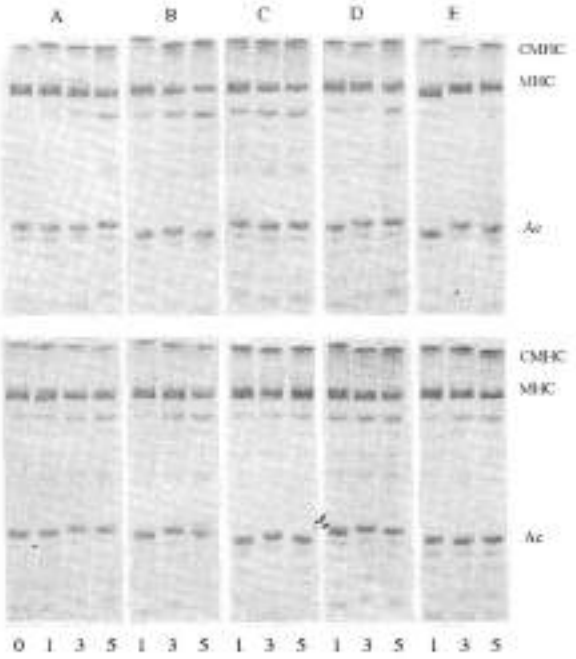

Fig. 2. SDS-PAGE patterns for control (top) and EGTA added (bottom) gels. Numbers in X axis indicate setting time in hour. Letters at the top indicate setting temperature (°C): A, 20; B, 25; C, 30; D, 35; E, 40

The electrophoresed discs were scanned densitometrically to calculate the changes in the amount of various subunit proteins. The per cent decrease of MHC and increase of CMHC have been shown in Fig. 3. As can be seen at the top-left, the amount of remaining MHC was high in the gel set at 20°C, followed by that at 40°, 35°, 30°, and 25°C, suggesting that higher amount of MHC was cross-linked in the gels in reversed order. At the bottom-left, the percent increment of CMHC in these gels also followed the similar reverse order. In case of EGTA added gels (right-top and -bottom), although the amounts of MHC and CMHC were virtually constant at 20° and 25°C, they began to change at higher setting temperatures. Much decrement of MHC and greater formation of CMHC were found at 35°C. However, the formation of CMHC was very little at

30°C which corresponded well with its low breaking force. The effect of EGTA in the gels set at 20° and 25°C was in good agreement with that of Tsukamasa *et al.* (1993), because gelation and MHC cross-linking were completely suppressed here with the inhibition of TGase activity. But, it is interesting that when setting temperature of such paste was raised from 25°C to 40°C, MHC began to cross-link again in proportion to both temperature and time. The results suggest that this cross-linking of MHC was occurred not by the action of TGase but by non-covalent bonds.

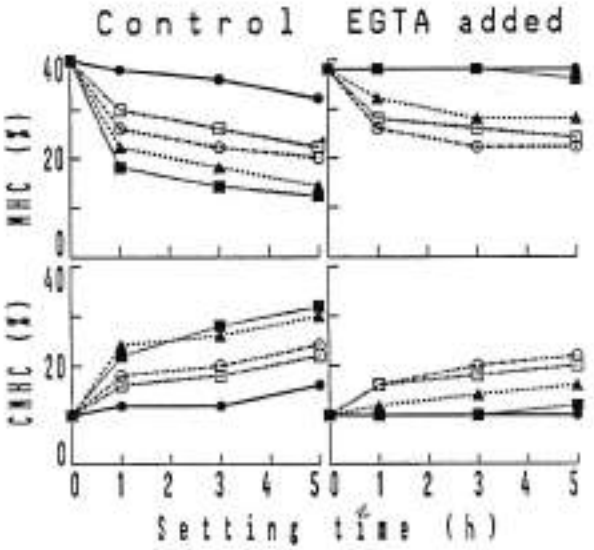

Fig. 3. Changes of MHC and CMHC in the control and EGTA added gels at various temperatures.

Cytosolic TGase showed its optimum activity at 25°C. The breaking force of the *suwari* gels set at the temperatures above 25°C was higher than that at 25°C. Therefore, TGase was not only factor that promoted setting. When the paste with added EGTA was incubated at 25°C, TGase activity was inhibited. This inhibition brought about a complete suppression of surimi gelation and cross-linking of MHC at this temperature. But with the same paste at the elevated setting temperatures, although TGase was inactive, gelation of the paste and the cross-linking of MHC occurred. At 40°C, the rate and extent of gelation and MHC cross-linking were almost similar in both control and EGTA added gels. The fact suggests that addition of EGTA could not suppress setting and cross-linking at 40°C. Therefore, by the addition of EGTA gelation of surimi paste and the cross-linking of MHC were suppressed completely at 20° and 25°C, partially at 30° and 35°C but not at all at 40°C. The results indicate that setting at 40°C was not involved with a TGase-mediated cross-linking at all, although the control gel showed a little TGase activity at this temperature. On the other hand, the highest increment of breaking force was recorded in the gels without EGTA at 30°C, a transitional temperature where both TGase and non-covalent protein

interactions might work together. However, this non-covalent protein aggregation phenomenon may be inactive in Alaska pollack below 30°C where TGase-mediated cross-linking is strong, but well active at elevated temperatures around 35°C where TGase-mediated cross-linking is weak. On the other hand, the extent of non-covalent aggregation of MHC varies significantly with the variation of species as reported before (Nowsad *et al.* 1995).

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Quality aspects of some exportable dried fishery products of Bangladesh

M.N.A. Khan, M.A. Hossain[1], M. Kamal and M.N. Islam

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

Abstract

A study was conducted to evaluate the quality aspects of eight exportable dried fishery products of Bangladesh. The products were evaluated by examining organoleptic properties, water reconstitution behaviour, microbiological and biochemical aspects. The water reconstitution rate was faster in ribbon fish and bombay duck, and slower in other fishes and air bladder and shark fin products. Organoleptic and physical characteristics in respect of colour, odour, texture, insect infestation and broken pieces of the products revealed that all of the products were either in excellent or acceptablecondition. Total viable bacterial load ranged from 0.95 x 10^4 CFU/g to 1.8 x 10^4 CFU/g in dried products. Coliform bacteria was absent in all the dried fishery products. The results of moisture, crude protein, lipid and ash content of the products ranged from 16.20 to 23.51%, 58.37 to 82.86%, 0.17 to 14.44% and 0.58 to 9.23%, respectively. Non-protein nitrogen (NPN) contents were in the range of 0.104 to 2.372% and the pepsin digestibility values were of 88.41 to 94.23%. The results of the study suggested that the exportable dried products were of good quality and hygienically safe.

Key words : Quality aspect, Dried fishery products

Introduction

Fishery industry of Bangladesh is mainly involved with the processing of high value items such as frozen shrimps, dried and salted dehydrated fishery products. Bangladesh stepped into a new era of sophisticated industrial processing of fish. Dried processed fishery products have occupied a key position in the exportable fishery items in Bangladesh. However, the process of drying fish is mainly performed by the households of the artisanal fishermen who are mostly illiterate. There are frequent complaints from the consumers

about the quality of the products. Lack of proper amenities like proper handling during loading and unloading, time and exposure of the fish to the high environmental temperature and, besides, insufficient knowledge about scientific and hygienic methods of handling from time of catch until it is processed into finished products contribute significantly to the loss of quality. The major problems associated during the storage of dried and salted-dehydrated processed fishery products are infested by the fly and insect larvae during drying and storage which deteriorate the products before consumption (Ahmed *et al.* 1979).

Very little is known on the quality aspects of exportable dried fishery products of Bangladesh. In order to get sufficient information for expansion of export market, a study was undertaken to the quality aspects of eight exportable dried fishery products of Bangladesh. The products were selected on the basis of their economic significance and export potential.

Materials and methods

Eight different dried exportable fishery products were investigated, the products were processed from the marine fishes caught by the artisanal fishermen. The species were: chinese pomfret (*Stromateus chinensis*), silver jew fish (*Johnius argentatus*), bombay duck (*Harpodon nehereus*), white grunter (*Pomadasys hasta*), dog fish shark (*Scoliodon sorrakowah*), red snapper (*Lutianus johnii*), ribbon fish (*Trichiurus haumela*) and Indian salmon (*Polynemus indicus*). The products were obtained from processing industry of Cox's Bazar and brought to the Fisheries Technology Laboratory of Bangladesh Agricultural University, Mymensingh in air-tight polythene bag.

The characteristics such as colour, odour, texture, broken pieces and insect infestation of the products were evaluated organoleptically.

To study the water reconstitution behaviour, 5 g of fish flesh was kept soaked in 1 litre of water at 30°C for 150 minutes and in hot water at 80°C for 60 minutes with occasional stirring. Water was drained off through a fine mesh nylon sieve. All the flesh were transferred to the strainer and extraneous water was wiped off by a piece of blotting paper and the flesh was weighed again. By the given soaking time, flesh could reabsorb maximum amount of water. Results were expressed in terms of weight of water absorbed by 5 g of the sample.

Qualitative determination of bacterial flora of dried fishery products was done by dilution technique using nutrient agar (Seely and Vandemark 1972). Test of health hazard microorganism, such as coliform count was conducted by using Levine-EMB agar (DIFCO 1960).

Proximate composition of the samples were determined according to AOAC (1980). The total lipid was, however, determined by the modified method of Bligh and Dyer (Smith *et al.* 1964). Non-protein nitrogen (NPN) was

determined according to the method of Konosu *et al.* (1974). The pepsin digestibility test was done according to AOAC (1980).

Results

Organoleptic evaluation and bacteriological examination of the exportable dried fishery products are presented in Table 1. The dried fishery products had characteristic natural colour (reddish white, yellowish white, slightly transparent and blackish white). Air bladder was of attractive cream colour.

Table 1. Organoleptic observation of exportable dried fishery products

Name of the products	Colour	Odour	Texture	Insect infestation	Broken piece	Overall quality
Chinese pomfret	Characteristic natural colour (reddish white)	Characteristic natural odour	Tough & flexible	No visible sign	Nil	Very good
Bombay duck	Characteristic natural colour (Yellowish white)	Characteristic dried fishy smell	Firm & flexible	No visible sign	Nil	Very good
Indian salmon	Characteristic natural colour (whitish)	Characteristics favour	Tough & flexible	No visible sign	Nil	Excellent
Shark's fin	Blackish white	Characteristic natural flavour	Very tough	No visible sign	Nil	Excellent
Red snapper	Reddish white	Characteristic natural flavour	Firm	No visible	Nil	Good
Air bladder of white grunter	Cream colour & translucent	Characteristic odour	Very tough	No visible sign	Nil	Very good
Ribbon fish	Silvery white	Characteristic smell	Firm	No visiblesign	Nil	Excellent
Silver jewfish	Reddish	Characteristic flavour	Firm	No visible sign	Nil	Very good

The reconstitution behaviour of the dried fishery products soaked in water at 30°C for 120 minutes and in hot water (80°C) for 60 minutes are presented in the Table 2. The reconstitution rate was found higher in ribbon fish (81.8 % at 30°C and 74.9% at 80°C) and lower in shark's fin (19.8% at 30°C and 33.2% at 80°C).

Table 2. Reconstitution rate (%) of the dried fishery products soaked at 30°C for 150 minutes and at 80°C for 60 minutes

Name of the products	Reconstitution rate(%) at 30°C for 150 mins.	Reconstitution rate(%) at 80°C for 60 mins.
Chinese pomfret	55.5	69.8
Bombay duck	80.1	68.1
Indian salmon	64.1	70.4
Shark's fin	19.8	33.2
Red snapper	55.5	84.9
Air bladder of white grunter	45.1	72.6
Ribbon fish	81.8	74.9
Silver jewfish	32.8	43.3

The results of the total plate count and coliform count are shown in Table 3. Total viable bacterial count in the chinese pomfret, bombay duck, Indian salmon, shark's fin, red snapper and the air bladder of white grunter, ribbon fish and silver jewfish were found as 1.8×10^4, 1.3×10^4, 1.5×10^4, 1.1×10^4, 1.1×10^4, 1.0×10^4, 0.95×10^4 and 0.95×10^4, respectively. Coliform bacteria was not detected in any type of dried products.

Table 3. Standard plate count and coliform test of exportable dried fishery products

Name of the products	Standard plate count (CFU/g)	Coliform microorganism (CFU/g)
Chinese pomfret	1.8×10^4	Nil
Bombay duck	1.3×10^4	Nil
Indian salmon	1.5×10^4	Nil
Shark's fin	1.1×10^4	Nil
Red snapper	1.1×10^4	Nil
Air bladder of white grunter	1.0×10^4	Nil
Ribbon fish	0.95×10^4	Nil
Silver jewfish	0.95×10^4	Nil

The proximate composition of the dried products are shown in Table 4. The moisture, crude protein, lipid and ash content in different types of products

ranged between 16.20 to 23.51%, 58.37 to 82.86%, 0.17 to 14.44% and 0.58 to 9.23%, respectively.

Table 4. Proximate composition of exportable dried fishery products

Name of the products	Moisture %	Crude protein %	Net protein %	Lipid %	Ash %
Chinese pomfret	19.78	59.36	58.64	14.44	(5.87
		(74.00)	(73.10)	(18.00)	(7.32)
Bombay duck	20.95	66.86	64.65	5.61	(5.47
		(84.58)	(81.78)	(7.10)	(7.26)
Indian salmon	18.79	68.71	68.06	6.98	6.01
		(84.61)	(83.81)	(8.60)	(7.40)
Shark's fin	22.07	72.64	61.30	0.74	3.98
		(93.21)	(78.66)	(0.94)	(5.11)
Red snapper	23.51	58.37	57.22	11.71	7.22
		(76.31)	(74.80)	(15.31)	(9.44)
Air bladder of white grunter	20.59	82.86	71.09	0.17	0.58
		(104.34)	(89.51)	(0.21)	(0.74)
Ribbon fish	16.20	70.90	69.77	4.67	9.23
		(84.61)	(83.26)	(5.57)	(11.01)
Silver jewfish	20.69	60.56	59.37	13.91	5.91
		(76.36)	(74.86)	(17.54)	(7.45)

** Values in parentheses are on moisture-free basis*

The NPN and pepsin digestibility values have been shown in Table 5. The values of these products were found to vary from 0.104 to 1.883% for NPN and 88.41 to 94.23% for pepsin digestibility.

Table 5. Non protein nitrogen content (NPN) and pepsin digestibility of exportable dried fishery products

Name of the products	NPN content %	Pepsin digestibility %
Chinese pomfret	0.1159	90.13
	(0.1445)	
Bombay duck	0.3541	89.75
	(0.4479)	
Indian salmon	0.1041	94.23
	(0.1282)	
Shark's fin	1.8147	89.75
	(2.3286)	
Red snapper	0.1845	92.71
	(0.2412)	
Air bladder of	1.8837	94.07
white grunter	(2.3721)	
Ribbon fish	0.1815	88.41
	(0.2166)	
Silver jewfish	0.1904	89.11
	(0.2401)	

** Values in parentheses are on moisture-free basis*

Discussion

During organoleptic evaluation, the exportable dried fishery products had characteristic natural colour (reddish white, yellowish white, silvery white, slightly transparent and blackish white). Air bladder was of attractive cream colour. Almost all of the products had no rancid smell and insect infestation. No broken pieces or powdery particles were observed during the study. Based on the observation on organoleptic qualities of dried products, three were found to be excellent, four were very good and one was simply good (Table 1).

The best way of reconstitution is to conserve a porous structure by a suitable method which absorbs and retains sufficient water by capillary. Compressed products absorbed slowly and less completely (Jason 1965). In the present study, the samples of ribbon fish and bombay duck exhibited a rapid initial rate of rehydration which was obviously due to the rapid absorption of water by sufficient porous structure (Jason 1965). According to Schewan *et al.* (1956) the most important requirements of a satisfactory dried fish products are (i) resemblance to fresh fish in flvour and texture, and free from ripened flavours

caused by prolonged bacterial, enzymatic, oxidative and chemical changes, (ii) compactness, (iii) ready and rapid reconstitution, and (iv) retention of good palatability. On the basis of reconstitution ability of these products, ribbon fish, bombay duck and chinese pomfret were better in quality than the rest of the products.

The results of bacteriological study showed that the total bacterial load of these products were comparatively low. The ranges were within the acceptable limit. Generally marine fish contains a high level of NPN and samples with high NPN content contain high bacterial load. But there is a positive relationship between moisture content and bacterial growth in fish. Sen *et al.* (1961) reported that when water content of the fish fell below 25% of the wet weight, bacterial action stopped and when the water content was further reduced to 15%, mold ceased to grow. The present study indicated that moisture content of about 20% in dried fishery products was quite unsuitable for the bacteria and both the moisture content and bacterial load were in acceptable condition. No coliform bacteria was found in the products. Therefore, the products were safe from microbial point of view.

The moisture content of the dried products were comparatively low which ranged, between 16.20 and 23.51%. For better evaluation of the nutritive value of the products, the crude protein contents were corrected for NPN to obtain net protein. As a result, range of net protein content stood at the ranges of 57.22 to 71.09%. It was observed that air bladder of white grunter (82.86%) and shark's fins (72.64%) had the higher amount of crude protein contents; the corrected net protein content stood at 71.09% and 61.30%, respectively. Elasmobranchs are characteristically known to contain higher amount of NPN in the form of urea and other nitrogenous bases and they may contain NPN up to 40% of the total nitrogen (Schewan 1950). But no such information was available for air bladder. This is largely due to the fact that urea retention, unlike in other animals, is a normal physiological process in elasmobranch. There were variations in lipid contents in the teleost fishes which, ranged from 0.17 to 14.44%. On the other hand, shark's fins contained only 0.74% lipid. In the air bladder lipid content was very low because of the presence of high amount of crude protein content. Shark's fin is known to contain very little amount of oil because most of the lipid is generally deposited in the liver (50-70%) (Rahman *et al.* 1978).

The crude protein, lipid and ash contents of dried fishery products on moisture-free basis ranged from 74.00 to 104.34%, 0.21 to 18.00% and 0.74 to 11.01%, respectively. Since the crude protein content was determined on the basis of total nitrogen content, an absurd value of 104.34% was obtained on dry matter basis for the air bladder of white grunter. Its corrected net protein content (excluding NPN) was only 82.86%. It was found that dried products of relatively high protein content had low lipid content and vice-versa. This is in agreement with the findings of Ahmed *et al.* (1979).

In this study it was observed that only the air bladder of white grunter and shark's fin contained higher amount of NPN than that of the other dried fishery products. The elasmobranchs are, however, known to contain a higher NPN than usual tissue mainly in the form of urea and trimethyl amine oxide (TMAO). The NPN content of the marine sharks and rays range from 36 to 50% of the total nitrogen (Kizevetter and Nasedkina 1975). The NPN contents of sharks fin obtained in this study falls within this range. NPN values vary considerably from species to species and even among the individuals of the same species due to various causes such as sex, age, season, feeding habit, spawning cycle etc. (Schewan 1950).

It was also observed that Indian Salmon and air bladder of white grunter showed higher pepsin digestibility. NPN was not considered during the determination of the digestibility. Therefore, the high pepsin digestibility value of the air bladder of white grunter (94.07%) and sharks fin (89.75%) does not represent the actual digestibility value of that products because those products contained considerable amount of NPN, mainly urea. Rahman *et al.* (1978) studied the digestibility of some marine fish meal and found a digestibility value of 82 to 92% with an average of 86%. Compared to the reported values, the pepsin digestibility of the products in the present study may be considered satisfactory.

The result of the present study indicates that the dried products that are exported from Bangladesh are of good quality and free from health hazard microorganisms.

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Occurrence and abundance of *Acetes* shrimps in the Kutubdia channel of Bangladesh coastal water

Mohammad Zafar and M.D. Alam

Institute of Marine Sciences, University of Chittagong
Chittagong-4331, Bangladesh

Abstract

This investigation was carried out from June '89 to May '90 and focuses on the occurrence and abundance of *Acetes* shrimps in the Kutubdia channel. The shrimps of the genus *Acetes* occurred throghout the year in the area of investigation. *Acetes* showed a bimodal peak in occurrence, one in late monsoon season (August - September) and other in premonsoon season (February - March). But the maximum number of *Acetes* shrimps was recorded in March (462 individuals/haul). The *Acetes* population of this channel was dominated by four species, *Acetes erythraeus* (38.50%), *A. indicus* (32.98%), *A. chinensis* (4.48%) and *A. japonicus* (3.32%).

Key words : *Acetes* shrimps, Kutubdia channel

Introduction

The shrimps of the genus *Acetes* are planktonic (Omori 1975) and which are living mainly in the estuarine and coastal waters of tropical and subtropical regions (Omori 1977). These shrimps often become a major component in the diets of shore fishes, large shrimps and shore birds (Omori 1974, Xiao and Greenwood 1993) and play a significant role in the food web of neritic waters, particularly in mangroves and seagrass beds. During certain part of the year, *Acetes* forms conspicuous aggregations near the shore. Such accumulations have been exploited as human food for many years in Asia and Africa. The annual world catch of *Acetes* is estimated to be about 170,000 tons, or about 15% of the total shrimp catch in the world and about 13.5% of the world crustacean fisheries prduction (Omori 1975). Now-a-days *Acetes* shrimps are used as a food in the hatchery operation and nursery ponds for larval rearing (Kungvankij *et al.* 1986).

In the coastal waters of Bangladesh, *Acetes* is one of the abundant group of macrozooplankton (Zafar and Mahmood 1989, Zafar 1995). But information on *Acetes* is not available except one publication on taxonomic description (Mahmood *et al.* 1978). The present investigation is the first of its kind on temporal distribution of *Acetes* shrimps from the Kutubdia channel in south-eastern coastal water of Bangladesh.

Material and methods

Sampling was made between June '89 and May '90. The area of investigation in the Kutubdia channel is situated at Lat. 21°53'36" N and Long. 91°54'54" E which is laterally fed by tributaries of rivers. On the south-eastern side of the channel, the delta of Mathamuhuri river and adjacent Chakaria mangrove forest. Most of the shoreline on the two sides of the channel is covered by poor mangrove vegetation and natural uloo grass (*Imperata cylindrica* and *Urigrass oryza*), with the adjacent land consisting of coastal salt pans being used for salt extraction during dry season and aquaculture afterwards. This channel is a good spawning and nursery ground for a number of commercially important fish and shrimps (Zafar 1994, Zafar *et al.* 1994 and Zafar 1995).

Four stations were selected for hydrobiological samples. Zooplankton samples were collected during new moon period at every month by a triangular push net made of ordinary nylon cloth having a mesh size of 750 µm. The net, which consisted of a flattened conical bag, measured 2.5 m, and had a mouth of 3.6 m. Samples were collected during the spring tides from shallow waters (depth 1. 2 m) by pushing and dragging the net on the bottom for 15 minutes at a time against tide. Samples were immediately preserved in 5% formalin. Concurrently during the sampling time surface water temperature was recorded by a bucket thermometer. Salinity and dissolved oxygen were recorded following standard procedures and pH was recorded by a digital pH meter. Data on atmospheric temperature and rainfall were obtained from the Meterological Department Kutubdia, Bangladesh. In the laboratory the *Acetes* shrimps were identified from zooplankton samples as following the methods and key characters suggested by Omori (1975), Mahmood *et al.* (1978) and Tirmizi and Ghani (1982). Analysis of variance, correlation and regression are used for data analyses.

Results and discussion

Hydrometeorological parameters

Statistical analysis (Kruskal-Wallis one factor ANOVA) shows no significant differences in the four stations among the recorded hydrological parameters. So, only average hydrological parameters are indicated in Table 1.

Occurrence and abundance of Sergestid shrimp, Acetes

The planktonic shrimps of *Acetes* were present in the study area throughout the period of investigation. *Acetes* shrimps showed a bi-modal peak in occurrence of the Kutubdia channel, one in premonsoon season (February - March) and other in late monsoon season (August - September). But the maximum density was recorded in March (462 individuals/haul). The most abundant of *Acetes* shrimps in this channel were *Acetes erythraeus* (38.50%), *A. indicus* (32.98%) (Fig. 1). The *Acetes* shrimps of the Kutubdia channel shows no significant relationship with recorded hydrological parameters. Zafar and Mahmood (1989) reported that *Acetes* shrimps were present throughout the year in the Satkhira estuarine system.

Table 1. Monthly variations of hydrometeorolgical factors in the the kutubdia channel, Bangladesh

Parameters	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
Salinity (%0)	18.00	9.72	10.79	11.00	20.00	25.57	27.53	30.00	29.45	25.00	26.00	25.00
Air Temperature (°c)	32.20	24.50	30.00	27.00	29.00	26.00	19.00	29.00	28.00	29.50	29.00	32.00
Water tenoeratyre (°c)	31.60	24.00	29.00	26.90	27.50	27.00	21.00	27.00	28.50	29.00	28.00	33.00
Dissoved oxhen(ml/ll	3.51	4.71	6.97	6.99	5.28	4.63	8.35	8.34	5.35	3.82	4.97	5.35
pH	7.50	7.14	8.00	7.10	7.16	8.30	8.40	8.22	7.95	7.14	7.90	7.00
Monthly rainfalll (mm)	562.00	722.00	76.00	465.00	512.00	0.00	0.00	0.00	55.60	195.10	62.00	222.00

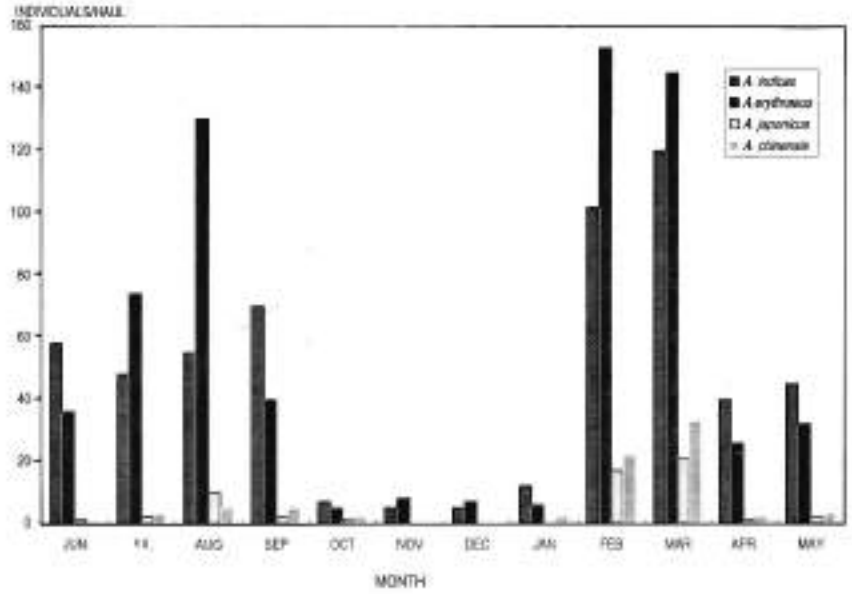

Fig. 1. Abundance of four Acetes shrimps inthe kutubdia channel of Banglsdesh coastal waters.

Temporal distribution of four species of Acetes shrimps

Four species of *Acetes* shrimps in the Kutubdia channel varied seasonally but statistically (Kruskal-Wallis one way ANOVA) their abundance showed no significant difference among the four sampling stations . So, only average abundance of *Acetes* shrimps are shown here.

Acetes indicus

This species was most abundant (120 individuals/haul) in March (Fig. 1). Omori (1977) found that *A. indicus* was distributed from the west coast of India through the Andaman sea, Gulf of Sian and the Java sea, to the South China sea. Mahmood *et al* . (1978) stated the presence of *Acetes indicus* in the Karnafuli estuary throughout the year. Bhattacharya (1988) reported that the upper and the lower lethal temperatures were 35 °C and 13 °C respectively for *A. indicus* . In the Kutubdia channel temperature varied between 21 °C - 31 °C and *A. indicus* were found in lower densities during winter months and maximum in February-March months. Bhattacharya (1988) also mentioned that the large numbers of *A. indicus* occurred when salinity varied from 26.50 %0 to 35%0. In the Kutubdia channel this species were recorded within the salinity range of 9.7%0 to 30%0 and large numbers of *A. indicus* occurred when salinity varied from 25%0 -30%0.

Acetes erythraeus

The maximum density of *A. erythraeus* was in February (153 individuals/haul) and minimum in October (5 individuals/haul) (Fig. 1). This

species has the most extensive geographical distribution in the Indo-west Pacific. Its range extends from the coast of South Africa to the South China sea, through the south and west coast of India, the Malay Archipelago and the Java sea. *A. erythraeus* was also recorded near the Mossman, Australia (Omori 1975). This species appeared in the coastal water of south India during January to April (Nataraj 1947). Le Reste (1970) stated that *A. erythraeus* were found in water where the salinity fluctuates seasonally between 1.5%0 and 35%0 . In the present investigation salinity varied between 9.7%0 and 30%0, but higher density was recorded during spring season.

Acetes japonicus

A. japonicus was first recorded from Kutubdia channel during the present investigation. It was recorded throughout the period of investigation except in November - January. Two peak occurrence of *Acetes japonicus* in the studied area, one peak in August (10 individuals/haul) and another in March (21 individuals/haul) Fig. 1. *Acetes japonicus* were recorded from the coasts of India and from the Andaman sea to the southern Japan (Omori 1977).

Acetes chinensis

It was the third dominant species in the *Acetes* population. The maximum abundance of *Acetes chinensis* was recorded in March (33 individuals/haul) (Fig. 1.) Luo and Zhang (1957) reported the spatial distribution of *A. chinensis* in Liaotung Bay and found that spatial variability led to marked differences in the catches at different localities and they also stated that *Acetes chinensis* was euryhaline. However in the present investigation area, *A. chinensis* was found within the salinity range of 9.7 %0 to 30%0.

Conclusions

It appears from this study that usually the *Acetes* shrimps in the Kutubdia channel have a bimodal peak in occurrence, one in premonsoon season (February - March) and other in late monsoon season (August - September). The abundance of four species of *Acetes* in the Kutubdia channel shows no significant correlation with recorded hydrological parameters . But *Acetes indicus* and *A. erythraeus* shows negative relation and *A. chinensis* and *A. japonicus* positively related with salinity. There is no published work on *Acetes* specially ecology, temporal distribution in the coastal waters of Bangladesh. The present account therefore, constitutes the first report on its from in the Kutubdia channel.

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