Vol. 2(1)
January 1998

ISSN : 1026-6690

Bangladesh Journal of Fisheries Research

Bangladesh Fisheries Research Institute

Bangladesh Journal of Fisheries Research

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

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

Bangladesh J. Fish. Res., 2(1), 1998 : 1-8

Evaluation on propagation of common carp (*Cyprinus carpio* Lin.) with hormonal and natural stimuli

K.K. Ahmed[1,*] and M.A.K. Chowdhury

Asian Institute of Technology, Bangkok, Thailand
[1]Fisheries Research Institute, Riverine Sub-Station, Rangamati-4500, Bangladesh
**Corresponding author*

Abstract

The success of breeding of common carp (*Cyprinus carpio*) using hormonal inducement and environmental stimuli was evaluated considering different sex ratios, and natural and artificial substrates. A total of 18 females (weighing 250 to 2200g) divided into 6 treatments were investigated. A successful spawning was observed in all the treatment groups, only 66.66% female responded successfully to LHRH-A combined with dompheridone and 83.33% female in natural stimuli. Females induced with LHRH-A and dompheridone found prompt ovulation than that of natural stimulation. A significant variation ($F=7.45$, $P< 0.05$) was found among the different treatment groups. The number of eggs released appear to depend on body weight ($t=15.72$, $P<0.05$), sex ratio ($t= 7.96$, $P< 0.05$) and percentage of ovulated females ($t=5.34$, $P< 0.05$). Although environmentally stimulated females released more eggs than injected female ($t=5.18$, $P< 0.05$) but their survival rate was similar ($t= 1.77$, $P< 0.05$). Comparison between the two approaches under the conditions of AIT hatchery shown that both are suitable for spawning induction in common carp. However, environmental stimulation is advantageous because of the less labor and lower cost required for ovulation.

Key words : Common carp, Propagation, Hormone

Introduction

Carps are among the commercially desirable and popularly cultured species in Asian and the Indo-pacific region. The fish common carp (*Cyprinus carpio*) is a very widely cultured species among Cyprinid because of its high tolerance to environmental fluctuation. This fish originated from Central Asia (Jhingran *et al.* 1988) and now spread over almost all the sub-tropical and tropical countries of the world.

There is a wide range of breeding practice of common carp e.g. natural, semi-artificial and complete artificial condition. The special characteristic of this species is its adhesive egg which is usually attached to a substrate. Effective breeding program is an essential requirement to viable commercial operation of a hatchery. Complete intervention of the reproductive cycle is a prerequisite for the development of an efficient breeding program (Gjerde 1986). Intensified

management of breeding fish is required both in reducing contamination and maximizing the demand of seed production. At the higher stage of breeding technology, fertilization rate and quality of egg also increased e.g. subsequently rate of egg hatching was enhanced to about 95% under controlled environment (Dwevedi 1986).

For semi-artificial practice the environmental stimulation was practiced by taking nominal measures like substrate application, keeping male and female together, creating required dark photo period and maintaining ambient water temperature. Isolation of eggs, hormonal treatment etc. are also used together with environmental stimulation. Now-a-days the most common and popular hormonal treatment is 'Linpe method', which is based on the GtH released by a super active analogue of piscine gonadotropin releasing hormone (sGNRHa) combine with dopamine receptor antagonist (Peter *et al.* 1988).

The main objective of the present study was to evaluate the breeding performances of common carp using different hormonal and natural stimuli.

Materials and methods

Broodstock management

Initially broodfish were kept in a earthen pond having water area of 0.2 ha with other species. At the advent of breeding season, the brood fish were checked for observing gonadal condition and manipulated them for better management aspects. To provide plentiful sustained supply of plankton and benthic organisms, brood ponds were fertilized at recommended dose using inorganic and organic fertilizers. Three weeks prior to spawning externally matured 57 female and 48 male carps were segregated and kept to a net cage ($10x10x2m^3$ size) suspended into the pond. Pelleted feed was administered two times daily at the rate of 1-2.5% body weight of the total biomass during the reared period. Fertilization of the pond of suspended cage was done two times (1st week and 4th week) at that period. One day prior to propagate, 18 females and 27 males were finally selected examining their stage of ripeness among the collected brood and transferred them to octagonal breeding tanks having $3.75m^2$ surface area with a flow through system. Ripe females were recognized by palpating the females abdomen which is bulging and soft to touch and swollen genital papilla. Males, on the other hand deep-pit-like vent and more slender body and ooze out milt when gently pressed in the body. The selection rate for female and male fish was 31% and 57% respectively.

Experimental design

The breeding design is shown in Table 1. In this experiment, the number of treatment was six comprising mainly the effect of injection, male-female ratio, and the substrate. In treatment I and II fish were hormone injected with plastic

substrate in each case but only difference was sex ratios. As the same way treatment III and IV comprised of natural leaves as substrate and fish were also hormone injected with different sex ratios. On the other hand, in treatment V and VI fish were tried to induce environmentally without injection using same substrate in different sex ratios. Amount of substrate was same (3 kg) in each treatment group.

Table 1. Experimental design

Treatments	Experimental unit	Stimulation	Substrate	Sex ratio (F:M)
I	Octagbnal tank	Hormonal	Plastic	1:1
II	,,	Hormonal	Plastic	1:2
III	,,	Hormonal	Natural leaves	1:1
IV	,,	Hormonal	Natural leaves	1:2
V	,,	Environmental	Plastic	1:1
VI	,,	Environmental	Plastic	1:2

Hypophysation

Two types of hormones were used, these are Suprefact (LHRHa) and Motilium (Dompheridone). Only females were injected an intramuscular injection one time with a dose of 10mg suprefact and 10 mg motilium per kilogram of female. Before injection, stock solution was prepared diluting 1 ml of suprefact to 10 ml solution.

Breeding

Injected and non-injected broodfish of both sexes are transferred to the shallow octagonal concrete tanks ($2.0x2.0x0.25m^3$) in the hatchery for breeding and manipulating them in six tanks considering the sex ratios and substrate mentioned in design. The breeding tank is filled to about 0. 18m of freshwater. Spawning nests made of tree leaves and plastic materials are placed in the tank bottom spreading properly. A gentle flow of water is supplied in the breeding tank from biofiltered recirculating water system. Courtship behavior like swimming in pair smoothly, chasing females by the male and jumping little up the surface of the water was found before spawning. The presence of bubbles, milky water and fishy smell were the indications that spawning took place. As per expectation hormone induced breeders spawned the following morning within 6.00-8.00 hrs after injection but environmentally stimulated breeders were bred after 24 hrs (30.00-31.00 hrs after injection) than usually expected. The fish are allowed to spawn completely about 4-6 hrs after breeding start then

they are removed and records were kept of the weight of individual females and the total weight of the eggs released. The breeders then transferred to the small circular tank for further observation. Utilizing the natural adhesiveness of common carp eggs, approximately 80-90% of fertilized eggs were attached to the substrate. Number of eggs were counted by weighing one sample of substrate where eggs are attached and to weighing total substrate of individual treatment, the fecundity was determined. Eggs were treated with malachite green by dipping the eggs attached to the substrate into the bucket filled with malachite green solution (2 ppm) to prevent bacterial and fungal infection before transferring them to the incubation tank. Substrate with fertilized eggs attached are transferred to the small hapa, made of mosquito net placed within small circular tank for incubation and primary nursing, where for sufficient oxygen, biofiltered recirculatory water with aeration systems were maintained. Quality of eggs, egg size and percentage of viable eggs were examined and on set of larval developmental stages were also observed regularly within 48-59 hrs. Ten hours after hatching, the substrates was removed from the hapa. Hatchling rate of eggs was determined to count the hatchlings in a sample of particular amount of water and by measuring the total volume of water of individual hapa. Survival rates of hatchling are recorded 48 hr after hatching through counting of hatchling in a sample measuring about 20 ml of water .

Water quality parameters

Temperature and dissolved oxygen content of incubation tanks were monitored during the incubation period with the help of a oxygen meter.

Statistics

Analysis of Variance (one way ANOVA) was used to compare the significant differences among the treatment groups and to find out the least differences between the treatments, the critical analysis (CD) was done. Also Students t-test was used to compare the difference between factors involved.

Results and discussion

Tables 2 and 3 shows the overall performance of common carp breeders subjected to induce spawning during this investigation. Eight out of twelve and five out of six-females responded successfully to LHRH-A combined with dompheridone (66.66%) and environmental stimulation (83.33%) respectively. The overall success was 77.22%. Initial egg release occurred after 6 hrs interval in all injected females. Although most of the hormone treated female spawned successfully within the expected period but the percentage of success was lower than that of the environmentally stimulated females. Due to sub-optimal conditions in the fish pond and lack of certain environmental cues, fish can not spawn always at all in captivity or response in hypophysation even if their ovaries have completed successful vitellogenesis.

Table 2. Spawning performance of common carp

Treatments	Hour of treatment	Hour of initial egg release	Spawning ratio	Body weight of female before / after spawning	Fecundity (No of eggs)	Fertilization rate(%)
I	0.45am	8.00am	1/3	2.05/1.90	96,922	88.32[b]
II	0.45am	7.00am	1/3	3.20/2.90	176,761	87.60[b]
III	0.45am	7.00am	3/3	2.55/2.30	22,670	81.60[c]
IV	0.45am	7.00am	3/3	2.65/2.40	113,505	89.70[b]
V	0.45am	6.00am	2/3	2.65/2.30	249,309	91.00[b]
VI	0.45am	6.00am	3/3	2.65/2.20	338,559	83.00[a]

Means followed by same superscript letters vertically are not significantly different (p< 0.05)

Yaron and Levavi-Zermonsky (1986) cited that, vitellogenesis in the Israeli population of the common carp is completed by February, when follicle diameter reaches 1mm, however, at this time of year not all female carp will ovulate in response to either hypophysation or GnRH+MET (Drori unpublished). Another most important factor is the selection of suitable female carp including examination of abdomen softness by palpation or measuring fish maximal circumference (Rothbard 1981, Horvath and Tamas 1984). These method, though easy to perform, adopting accurately require much skill and experience. The eggs retained in unspawned females may be of poor quality also (Rothbard 1981, Horvarth and Temas 1984) and the rate of processes involved in oocyte final maturation and ovulation in fish depends on the ambient temperature (Yaron 1995).

Table 3. Hatching performance of common carp

Treatments	Hatching time (hours)	Total hatching number (after 8 hours)	Hatching rate (%)	Number of hatchling at harvest	Survivability (after 8 hours)
I	48	64,800	75.70	24,400	37.65
II	50	137,472	88.78	53,028	38.57
III	52	17,200	92.97	400	2.33
IV	51	84,811	83.30	69,799	82.30
V	59	144,599	64.00	51,875	36.00
VIa	59	109,951	39.00	30,800	28.00
VIb	59	56,201	20.00	1,120	2.00

The fecundity for injected and non-injected females varied between 0.02 to 0.17 and 0.25 to 0.34 million respectively with an average range of 0.02-0.34 million. A significant variation ($F=7.45$, $P<0.05$) was observed among the treatments in respect of number of eggs released by the females. Production of eggs in treatment-VI differ significantly ($P< 0.05$) from all other treatments groups and no variation was found among the rest of the treatments except treatment-III. Due to lack of replications in each treatment and some systems error in on set of experiments, it could not be able to find out the suitable treatment and factors responsible for more success of ovulation. Even than, an attempt was made here to find out the extent of difference between the treatment groups considering individual factor effect. A comparison of pairing one or two males with each female indicated that fecundity was consistently higher when two males present ($t=7.96$, $P<0.05$). The number of eggs released appear to depend on the body weight ($t=15.72$, $P<0.05$) of the fish used in these experiment and oocyte maturation and ovulation (100% success) in common carp were potentiated when natural leaves used to serve as spawning substrate for them. In present experiment, number of eggs production by the environmentally stimulated female were significantly higher ($t=5.18$, $P<0.05$) than that of the injected female and survival rate was similar ($t= 1.77$, $P<0.05$). A significant variation ($t=5.34$, $P<0.05$) of the percentage of ovulated females with respect to eggs released was observed also. Although 100% ovulation success performed by the females in treatment-III, but their fecundity and average survival of hatchling from eggs were remarkably low. The causes of lower number of egg production is unknown but a tremendous drop in average survival of hatchling was done due to insufficient oxygen (Table 4) in the system as air stone was out of order at the period of incubation. On the other hand, in treatment-VI, about half of the total eggs were kept in the same tank along with the substrate and another half transferred to the tank V as the same manner with the parents for incubation. In treatment-V, the lowest hatchling at harvest indicate cannibalistic nature of common carps.

The fertilization rate was found more of less similar for all the treatment groups and ranged between 81.60 to 91.00%. Lin *et. al.* (1986) observed from several experiments that fertilization rates of ovulated oocyte for common carp were consistently high (90%), hatchling rates were consistently high (90%) and that fry were normal. A wide range of survival rate (2.00-82.30%) of hatchling in all treatment groups after 48 hours observation found poor survival rate in both approaches, may be due to some unsuitable condition for incubation of eggs in the shallow tank with insufficient oxygen is responsible for poor hatching rate and survivability of hatchlings.

Table 4. Temperature and dissolved oxygen content in incubation tank

Treatments	Hours	Temperature (^{o}C)		Dissolved Oxygen (mg/l)	
		Feb. 25 1996	Feb. 26 1996	Feb. 25'96	Feb. 26'96
I	8.00	27.5	25.7	7.2	6.8
	16.00	32.0	31.7	5.5	7.1
II	8.00	27.5	25.7	6.6	6.3
	16.00	32.0	31.7	5.6	7.0
III	8.00	27.5	25.7	1.5	0.4
	16.00	32.0	31.7	0.9	1.1
IV	8.00	27.5	25.7	6.8	7.0
	16.00	32.0	31.7	7.1	7.4
V	8.00	27.5	25.7	6.7	7.1
	16.00	32.0	31.7	7.0	7.1
VI	8.00	27.5	25.7	7.7	7.5
	16.00	32.0	31.7	7.4	7.4

In this experiment adopting 'Linpe method', induced ovulation and spawning has proven to be successful with common carp. Utilizing low doses of dompheridone plus sGnRH-A analogue may be considered as the cost effectiveness of the 'Linpe method' but less labor and lower cost required for hypothalamic manipulations through environmental stimuli. On the other hand, 'Linpe method' has advantages than other hormonal induced method in terms of cost effectiveness of labor and reduced stress on broodstock as fish generally have to be handled only once to give a single set of injections. Comparison between the two approaches under the present physical facilities available shown highly effective and reliable technique in induced ovulation and spawning of cultured freshwater fish .

Acknowledgements

The authors are indebted to Dr. D.C. Little, Associate Professor, School of Environment, Resources and Development, Asian Institute of Technology for providing facilities, encouragement and above all critically going through the manuscript.

References

Bromage, N.R. and R.J. Roberts (eds.), 1995. Broodstock Management and Egg and Larval Quality. Blackwell Science Ltd , Oxford , UK .

Cooperative Team for Hormonal Application in Pisciculture, 1977. New highly effective ovulating agent for fish reproduction . *Sci . Sin.*, **20** : 469-474.

Dwevedi, B. and A.K. Reddi, 1996. Fish breeding in a controlled environment. *Aquaculture*, **74** : 1-10.

Gjerde, B., 1986. Growth and production of fish and shell fish. *Aquaculture*, **57** : 37-55.

Horvath, L. and G. Tamas, 1984. Propagation and intensive larval rearing of common carp and Asian herbivorous fishes and tench. *In* : Special methods in pond fish husbandary (ed. J.E. Halver). Akademiai Kiado, Budapest, Halver Corporation , Seattle, WA. pp. 1-54 .

Huet, M., 1978. Text Book of Fish Culture, Breeding and Cultivation of Fish. Fishing News Books Ltd., England. pp. 111-150.

Jhingran, V.G. and R.S.V. Pullin, 1988. A Hatchery Manual for the Common, Chinese and Indian Major Carps. ADB/ ICLARM, Manila, Phillipines. 191 pp.

Lin , H.R., G.V.D. Kraak, J.Y. Liang, C. Peng, G.Y. Li, L.Z. Lu, X.J. Zhon, M.L. Chang and R.E. Peter, 1986. The effects of LHRH analogue and drugs which block the effects of dopamine of gonadotropin secretion and ovulation in fish cultured in China. *In* : Aquaculture of Cyprinids (eds. R. Billard and J. Marcel). INRA Paris, France. pp. 139-140.

Peter, R.E., 1982. Nature, localization and actions of neurohormone regulating gonadotropin secretion in teleosts. *In* : *Proceedings of the International Symposium on Reproductive Physiology of Fish* (eds. C.J.J. Fichter and H.J Th. Goos). Wageningen, Netherlands. pp. 30-39.

Peter, R.E., J.P. Chang, C.S. Nahorniak, R.J. Omeljaniuk, M. Sokolowska, S.H. Sheh and R. Billard, 1986. Interactions of catecholamines and GnRH in regulation of gonadotropin secretion in teleost fish. *Prog . Horm . Res.*, **42** : 513- 548 .

Peter R.E., H.R. Lin, and G.V.D. Kraak, 1988. Induced ovulation and spawning of cultured freshwater fish in China : Advances in application. *Aquaculture*, **74** : 1-10.

Rothbard. S., 1981. Induced reproduction in cultivated cyprinids-the common carp and group of Chinse carps. I. The technique of induction, spawning and hatching. *Bamidgeh*, **33** : 103-121 .

Wohlfarth , G.W., 1984. Common carp *In* : Evaluation of domesticated animals (ed. I.L. Maso). Longman, London and New York. pp. 375-80.

Yaron. Z., 1995 . Endocrine control of gametogenesis and spawning induction in the carp. *Aquaculture*, **129** : 49-73.

Yaron Z. and B. Levavi-Zermonsky, 1986 . Fluctuations in gonadotropin and ovarian steroids during the annual cycle and spawning of the common carp. *Fish . Physiol. Biochem.*, **2** : 75-86.

(Manuscript received 19 June 1997)

Bangladesh J. Fish. Res., 2(1), 1998 : 9-14

Polyculture of gulsha (*Mystus cavasius* Ham.) with rajpunti (*Puntius gonionotus* Bleeker) and silver carp (*Hypophthalmichthys molitrix* Val.) in earthen ponds

M. Anwar Hossain, A.H.M. Kohinoor and M.G. Hussain

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

Abstract

Culture of gulsha (*Mystus cavasius*) with rajpunti (*Puntius gonionotus*) and silver carp (*Hypophthalmichthys molitrix*) was undertaken to assess the growth and production potential of these species under polyculture system. Fingerlings of gulsha, rajpunti and silver carp were stocked at a density of 18,000, 10,000 and 4,000/ha respectively. Two treatments were tested in this experiment. Treatment-I was conducted with rice bran and mustard oil cake and treatment-II with rice bran and duck weed. All the ponds were fertilized with urea and TSP at fortnightly intervals. After six months' rearing, the gross production was estimated to be 3,582 and 3,125 kg/ha from treatment-I and treatment-II respectively. Total yield showed non-significant differences ($P > 0.05$) between the treatments.

Key words : Polyculture, *M. cavasius*, *P. gonionotus*, *H. molitrix*

Introduction

Mystus cavasius locally known as gulsha is an indigenous small fish once available in flood plains, swamps and canals of Bangladesh. It is a great favorite of consumers and, therefore, has a great demand fetching high price in the market. As the catches of the fish have drastically declined from open waters like rivers, beels, haors etc. in recent years due to various ecological changes in the inland water bodies, the fish is now sold at an exorbitant price in the market. Keeping this in mind to increase its production, seed production technology through artificial propagation was developed by the Fisheries Research Institute (Akhteruzzaman *et al*. 1991). Very little information is, however, available on the culture aspect of gulsha. Kohinoor and Hussain (1994) observed that monoculture of gulsha is economically not viable. While polyculture maximizes production, it depends on selection of appropriate fish species for better utilization of the food available in different strata and zones of a given aquatic environment.

Gulsha is carnivorous in nature and feeds on crustaceans, protozoans, insect larvae, small fishes and debris (Akhteruzzaman *et al*. 1991). On the other hand, rajpunti is herbivorous and feeds mainly on soft aquatic plants,

grasses, algae and some invertebrates (Phaohorm 1980, Srisuwantach 1981). Silver carp is principally filter-feeding, planktivore fish (Khan and Siddique 1973, Pillay 1990). Keeping their different feeding habits and niches in mind the present study was undertaken to determine the growth and culture potential of gulsha with rajpunti and silver carp in the polyculture system.

Materials and methods

A six-month experiment was initiated in November'95 to April'96 at the Freshwater Station of the Fisheries Research Institute, Mymensingh. The ponds were prepared by draining and limed at the rate of 250 kg /ha. Three days after liming, ponds were filled with ground water and fertilized with cattle manure at the rate of 1,000 kg/ha. Five days later, inorganic fertilizers, urea and TSP, were applied at the rate of 8 and 17 kg/ha respectively. Three days after the application of inorganic fertilizers, stocking was done with gulsha (2.73 g), rajpunti (5.85 g) and silver carp (3.75 g) at a stocking density of 18,000; 10,000 and 4,000/ha respectively.

Two treatments, each with three replicates were tested. Rice bran (60%) and mustard oil cake (40%) were used at the rate of 5% of body weight in treatment-I. While only rice bran along with duck weed were used each at 3% of body weight in treatment-II. Subsequent to stocking, all the ponds were fertilized regularly at fortnightly intervals with urea and TSP at the rate of 8 and 17 kg/ha/month. The ponds were sampled at fortnightly intervals to assess the growth and condition of fish, and feeding was adjusted on the basis of estimated fish biomass in the ponds.

Physico-chemical parameters such as temperature, transparency, DO, hardness, pH, and ammonia of water were monitored at weekly intervals while biological parameters on productivity at fortnightly intervals.

All the ponds were completely harvested after six months' rearing first by netting and later by draining the ponds.

Results and discussion

The summarized data of water quality parameters between the two treatments showed non-significant differences ($P>0.05$) except pH and are furnished in Table 1. Phytoplankton population mainly comprised four major groups, Chlorophyceae, Cyanophyceae, Bacillariophyceae and Euglenophyceae and zooplankton comprised two groups, Crustacea and Rotifera (Table 2). Both phytoplankton and zooplankton population were high in treatment-I than in treatment-II ($P> 0.05$).

Table 1. Comparison of physico-chemical parameters of pond water of the two treatments

Parameters	Treatment-I	Treatment-II	t- Statistics
Water temperature (°C)	21.38 (±4.18)	22.04 (±3.90)	0.57 NS
Transparency (cm)	27.60 (±6.75)	30.67 (±8.72)	1.37 NS
P^H	8.10 (±0.27)	7.85 (±0.34)	4.58 *
DO (mg/l)	3.91 (±0.84)	3.85 (±0.59)	0.29 NS
Total hardness (mg/l)	150.33 (±21.82)	152.75 (±23.53)	0.26 NS
NH_3 (mg/l)	0.03 (±0.01)	0.04 (±0.03)	1.46 NS

*NS = Non significant at 5 % level * = Significant at 5% level*

Table 2. Mean abundance of plankton (units x 10^3/l) in two treatments

Planktons	Treatment-I	Treatment-II	Significance
Phytoplankton			
Bacillariophyceae	2.25	2.00	NS
Chlorophyceae	8.84	8.70	NS
Cyanophyceae	4.82	4.21	NS
Euglenophyceae	2.37	2.47	NS
Total (A)	18.28	17.38	NS
Zooplankton			
Crustacea	0.65	0.73	NS
Rotifera	2.46	1.15	*
Total (B)	3.11	1.88	*
Grand Total (A+B)	21.39	19.26	NS

*NS = Non significant at 5% level * = Significant at 5% level*

The comprehensive data on the stocking density, production and survival of gulsha, rajpunti and silver carp are given in Table 3. The increase in net weight of gulsha, rajpunti and silver carp was 44.1, 137.4, 433.9g and 33.6, 119.8, 380.1g in treatment-I and II respectively. It was found that the increase in net weight of all the fishes was high in treatment-I than in treatment-II. Species-wise monthly growth pattern of the fishes in treatment-I and II is graphically shown in Figure 1. Growth rate of all species was also high in treatment-I than in treatment-II. However, there was no significant difference in the average survival rate of fishes in treatment-I (85%) and treatment-II (81%).

Table 3. Stocking density, culture period, gross production and survival of gulsha with rajpunti and silver carp

Treat-ments	Species	Stocking density/ha	Culture period	Production (kg/ha)		Survival (%)	
				Species wise	Total	Species wise	Av.
I	*M. cavasius*	18,000	6 months	705.56	3,581.78[a]	84	85
	P. gonionotus	10,000		1,243.33		80	
	H. molitrix	4,000		1,632.89		91	
II	*M. cavasius*	18,000	6 months	579.72	3,124.71[a]	74	81
	P. gonionotus	10,000		1,123.05		89	
	H. molitrix	4,000		1,421.94		81	

Figures in the same column with same superscripts are not significantly different (P>0.05)

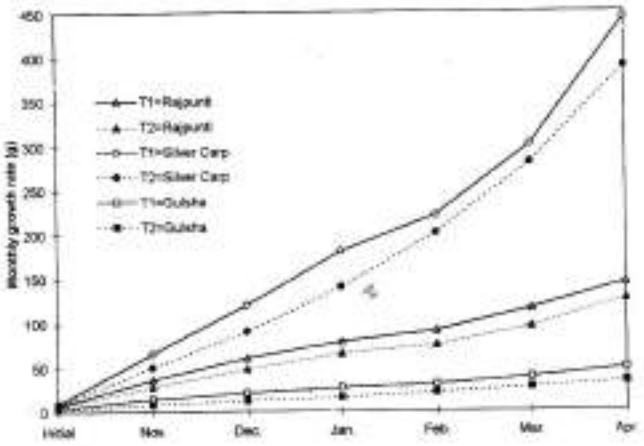


Fig. 1. Showing the monthly growth rate of fishes by average increase in weight.

While gross production did not show significant differences in yield in treatment-I and treatment-II (P> 0.05, F= 5.47), production of gulsha and silver carp was significantly higher (F= 198.30, F= 18.42) in treatment-I over treatment-II which might be due to the application of a nutritionally richer feed in treatment-I. Further, the production of rajpunti did not show any significant differences (F= 6.09) between the two treatments, probably application of duck weed in treatment-II which was utilized by rajpunti alone.

Cost of production and return from this study are presented in Table 4. While, estimating cost of production, variable costs towards lime, feed, fertilizer and fingerlings have been taken into account. As these small ponds are managed by the farmer himself, no labour charges have been taken into consideration. Cost of production in treatment-I and treatment-II was Tk. 79,494 and Tk. 62,788/ha/6 months respectively. While, the net benefit of Tk. 98,846

and 93,892 was obtained from treatment-I and II respectively. Where treatment-I indicating higher profitability in which rice bran and mustard oil cake were used.

Table 4. Cost and return analysis of gulsha with rajpunti and silver carp production per hectare per six months

Item	Treatment-I		Treatment-II	
	Quantity (kg)	Cost (Tk.)	Quantity (kg)	Cost (Tk.)
Pond preparation				
Lime	250	750.00	250	750.00
Cow dung	1,000	250.00	1,000	250.00
Fingerlings				
Rajpunti	10,000 Nos.	3,000.00	10,000 Nos.	3,000.00
Silver carp	4,000 Nos.	800.00	4,000 Nos.	800.00
Gulsha	18,000 Nos.	18,000.00	18,000 Nos.	18,000.00
Feed/Fertilizer				
Rice bran	9,082	18,164.00	17,034	34,068.00
Mustard oil cake	6,055	36,330.00	-	-
Duck weed	-	-	14,880	3,720.00
Inorganic fertilizer	300	2,200.00	300	2,200.00
Total cost		79,494.00		62,788.00
Gross production (kg/ha) and Return (Tk)				
Gulsha	705	70,500.00	579	57,900.00
Rajpunti	1,243	62,150.00	1,123	56,150.00
Silver carp	1,623	45,690.00	1,421	42,630.00
Total Return (Tk.)		178,340.00		156,680.00
Net benefit (Tk.)		98,846.00		93,892.00

The water quality parameters in all the ponds were within the limits of fish production and the fishes were not found in a distress condition during the experimental period. However, DO was relatively low in all ponds throughout the experiment. Ahmed (1993) reported a similar trend of lower DO from the fertilized and fed carp fingerling ponds in Bangladesh. In monoculture of *M. cavasius* using rice bran, mustard oil cake, wheat bran and fish meal, Kohinoor and Hussain (1994) demonstrated a production of 1,135 kg/ha/6 months. In monoculture of *P. gonionotus*, Hussain *et al*. (1989) obtained a production of 1,952 kg/ ha/5 months with only rice bran and 689 kg/ha/5months with only fertilizers. However, Kohinoor *et al*. (1993) got a production of 2,384 kg /ha/6 months using rice bran (60%) and mustard oil cake (40%) in monoculture of *P. gonionotus*. The present study indicates that farming of gulsha with rajpunti and silver carp is more productive and profitable and suitable for Bangladesh conditions.

References

Ahmed, Z. F., 1993. Electivity index and dietary overlap of *Catla catla* (Hamilton) in fertilized, and fed and fertilized ponds of Bangladesh. M. Sc.Thesis, Faculty of Fisheries, BAU, Mymensingh. 95 pp.

Akhteruzzaman, M., A. H. M. Kohinoor, M. S. Shah and M. G. Hussain, 1991. Observation on the induced breeding of *Mystus cavasius* (Hamilton). *Bangladesh J. Fish.*, **14** (1-2) : 101-105.

Hussain, M. G., M. Akhteruzzaman, K. A. T. Karim and M. S. Shah, 1989. Semi-intensive culture of *P. gonionotus*. *Bangladesh J. Fish.*, **12**(2) : 45-52.

Khan, R. A. and Q. Siddique, 1973. Food selection by *L. rohita* (Hamilton) and its feeding relationship with other major carps. *Hydrobiologia*, **43** : 429- 442.

Kohinoor, A.H.M., M. Akhteruzzaman and M.S. Shah, 1993. Production of *Puntius gonionotus* (Bleeker) in ponds. *Bangladesh J. Zool.*, **21**(2) : 77-83.

Kohinoor, A. H. M. and M. G. Hussain, 1994. Monoculture of gulsha (*Mystus cavasius*) in ponds. *Bangladesh J. Life Sci.* : (communicated)

Phaohorm, S., 1980. The study of biology, feeding habit and growth of *P. gonionotus*. Annual report, Dept. of Fisheries, Bangkok, Thailand. pp. 19-33.

Pillay, T.V.R., 1990. Aquaculure : Principles and Practices. Fishing News Books. Oxford OX2 OEL, England. 575 pp.

Srisuwantach, V., 1981. Induced breeding of Thai silver carp (*P. gonionotus* Bleeker). SAFIS Manual No. 10. Eng. Transl. The Secretariat, South-east Asian Fish Dev. Center, Thailand.

(Manuscript received 2 June 1997)

Bangladesh J. Fish. Res., 2(1), 1998 : 15-22

Impacts of Thai silver barb *(Puntius gonionotus* Bleeker) inclusion in the polyculture of carps

S. Mahean Haque, M.A. Wahab* , M.I. Wahid and M.S. Haq

Department of Fisheries Management
Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
** Corresponding author*

Abstract

The impact of inclusion of Thai silver barb, *Puntius gonionotus* (Bleeker) in the polyculture with two major Indian carps, *viz., Labeo rohita, Catla catla* and common carp *Cyprinus carpio* has been studied in seasonal ponds for 115 days. The presence of silver barb decreased the growth of Indian carps while increased that of common carp. A significantly higher ($P<0.05$) fish yield (1793.65 Kg/ha/yr) was observed in the four species polyculture system containing silver barb when the combined yield of all species was compared.

Key words : *Puntius gonionotus*, Indian major carps, Polyculture

Introduction

The development of polyculture system has proceeded as elsewhere in this region on an adhoc basis with continued addition of exotic species. Earlier studies by Dewan *et al* .(1991), Wahab *et al.* (1991) and Wahab and Ahmed (1992) clearly indicated that the dietary overlap (competition for food) among silver and bighead carps and the native species, catla over rohu was very high, and growth and production of the later were significantly reduced in polyculture. This suggests that inclusion of any exotic fish within a native species based polyculture should not be encouraged without evidence of strong economic benefit.

Thai silver barb or Thai sharpunti *(Puntius gonionotus),* a herbivorous species (Phaohorm 1980), was introduced into Bangladesh in 1977 and has become increasingly popular owing its bright silvery appearance and good taste. Introduction of sharpunti in the polyculture of native carps increased overall fish yields although it had a slight antagonistic effect on growth and production of native carps (Wahab *et al.* 1995). The compatibility of sharpunti in mixed (native carps and exotic carps) pond polyculture system, however, remains largely unknown. If the species proves environmentally benign and has no adverse effect on native and exotic species then it may be an excellent candidate for pond polyculture. Considering the need for a thorough investigation with the most favoured and biologically compatible species for the pond polyculture, the present study has been designed with the objectives to assess the compatibility of Thai silver barb in the carp polyculture and to compare the production performance with or without addition of this species in the fish pond.

Materials and methods

The experiment was carried out for a period of 115 days in the Agricultural University campus, Mymensingh, Bangladesh. A series of six earthen ponds each of 0.01 ha with an average depth of 1.5 m were used for this experiment. Ponds were rain fed and free from aquatic vegetation, completely independent and well exposed to sunlight. These ponds had no inlet and outlet.

Seven days before to stocking of fish, ponds were fertilized with cowdung, urea and TSP at the rate of 50 kg, 1 kg and 1.5 kg per pond respectively, and the fertilization was continued throughout the experimental period at 15 day's interval. Fish were fed with rice bran at the rate of 3% body weight and quantity of feed application was adjusted fortnightly on the basis of fish biomass.

Two treatments were tried in this experiment. In treatment-I (T_1), fingerlings of rohu (*Labeo rohita*, 2.13 g), catla (*Catla catla*, 2.36 g) and common carp (*Cyprinus carpio*, 1.38 g) were stocked at 100 fish per pond in the ratio of 1 : 1 : 1. For treatment-II (T_2), equal number and ratio of rohu, catla and common carp fingerlings and an additional 25 sharpunti fingerlings (1.28 g) were stocked.

The water quality parameters such as temperature (^{0}C), secchi disc depth (cm), pH, total hardness (mg/l), dissolved oxygen (mg/l), total ammonia(mg/l), nitrate (mg/l), orthophosphate (mg/l) and chlorophyll-a (mg/l) were monitored fortnightly following standard methods (APHA 1992).

Fish growth was monitored fortnightly throughout the experimental period. Sample of five fish from each species of every pond was collected from two treatments at each sampling day by a cast net. Growth of fish was determined by measuring fish length (cm), using centimeter scale and fish weight (g) by a electronic balance. Fish were harvested at the end of the experiment by a seine net and following pond drainage the remaining fishes were picked up and were measured and weighed.

One way Analysis of Variance (ANOVA) was used for statistical analysis using STATGRAPH statistical package. Statistical significance was assessed using a probability level of P= 0.05

Results

Water quality parameters

The water quality parameters are summarized in Table 1. During the experimental period the water temperature varied from 28.2 to 31^{0}C in treatment-I and 27.2 to 31.6 ^{0}C in treatment-II. There was no significant difference between two treatments. Highest temperature was recorded 31.6^{0}C in the month of July. Transparency values showed variation with sampling date ranged from 21.2 cm to 36.0 cm in pond no. 5 and pond no. 1 respectively both were belonged to treatment-II. Highest transparency (31.5 cm) was observed in August. There was no significant difference between two treatments.

Table 1. Mean (± SE) values of water quality parameters of different ponds under two treatments

Treatments	Treatment-I				Treatment-II			
Pond No.	3	4	6	Mean	1	2	5	Mean
Temp. (^{0}C)	29.73±0.42	29.63±0.41	29.68±0.41	29.68±0.02	29.58±0.47	29.80±0.51	29.83±0.40	29.73±0.42
Trans. (cm)	30.93±1.48	29.04±0.88	29.05±1.21	29.63±0.51	30.85±1.60	28.25±5.99	27.15±1.13	28.75±0.89
pH	7.57±0.22	7.37±0.11	7.26±0.10	7.40±0.07	7.17±0.09	8.31±0.16	8.14±0.24	7.87±0.29
DO (mg/l)	6.41±0.21	7.14±0.67	6.10±0.39	6.55±0.25	6.24±0.63	7.85±0.90	7.21±0.54	7.10±0.38
Total hardness (mg/l)	56.00±3.80	96.70±2.77	69.71±3.00	74.13±9.76	110.0±2.44	80.57±1.82	62.14±3.22	84.23±11.38
Total ammonia (mg/l)	0.12±0.01	0.12±0.03	0.10±0.02	0.11±0.00	0.07±0.00	0.08±0.00	0.09±0.01	0.08±0.00
NO_3 (mg/l)	0.90±0.10	0.98±0.17	0.83±0.40	0.90±0.02	0.82±0.02	0.89±0.06	0.87±0.04	0.86±0.01
PO_4 (mg/l)	1.14±0.25	0.86±0.07	0.61±0.05	0.87±0.12	1.16±0.10	1.28±0.26	0.71±0.04	1.05±0.14
Chlorophyll-a (mg/l)	37.12±6.25	101.58±33.09	39.60±3.74	59.43±21.08	137.8±56.58	213.8±33.85	127.4±32.0	159.7±27.22

Table 2. Mean (± SE) abundance of plankton (cells x 10^4/l) of different ponds under two treatments

Treatments	Treatment-I				Treatment-II			
Pond No.	3	4	6	Mean	1	2	5	Mean
A. Phytoplankton								
Bacillariophyceae	3.64±0.4	4.05±0.2	3.55±0.3	3.75±2.2	11.5±2.2	11.6±2.3	9.04±1.1	10.7±0.8
Chlorophyceae	33.94±2.6	35.77±4.9	34.35±5.5	34.69±0.6	51.02±5.9	52.68±10.2	48.24±3.6	50.65±1.3
Cyanophyceae	4.69±0.7	5.69±1.7	6.17±1.8	5.52±0.4	9.56±2.1	9.23±00.9	9.67±3.6	9.48±0.1
Euglenophyceae	8.97±1.9	11.57±5.6	10.1±1.8	10.2±0.8	12.52±5.7	9.30±01.2	10.23±4.8	10.67±1.0
B. Zooplankton	4.94±0.6	4.9±0.8	5.8±0.5	5.22±0.3	5.42±1.1	6.16±00.8	6.01±1.0	5.88±0.2

Throughout the study period, p^H of water of the ponds were found to be approximately neutral or slightly alkaline and ranged from 7.17 to 8.31. Dissolved oxygen contents of the ponds under T_1 were within the range of 5.3 to 9.2 ppm and the range of the ponds under T_2 were 3.3 to 10.66 ppm. It was found that DO decreased while temperature value increased, so there was an inverse relation between DO and temperature. There was no significant difference between treatments. Total hardness was significantly higher ($p<0.05$) in treatment-II as compared to treatment-I with mean values of 84.23±11.38 ppm and 74.13±9.7 ppm respectively.

Phosphate- phosphorus of the ponds of both treatments fluctuated (0.61 to 1.28 mg/l) throughout the experimental period. The ranges of nitrate-nitrogen and total ammonia measured over the experimental period were 0.86 to 0.9 mg/l and 0.08 to 0.11 mg/l respectively and were within the limit suitable for fish production.

The concentration of chlorophyll-a was significantly ($P<0.05$) higher in T_2 than that of T_1 with mean values of 159.66±27.22 mg/l and 59.43±21.88 mg/l respectively.

Total phytoplankton ranged from 3.784±0.154 to 50.645±1.296 cells/l (Table 2). Among the four groups of phytoplankton, Chlorophyceae showed the quantitative dominance over other groups in both treatments. The mean abundance of zooplankton was 5.223 $0.305x10^4$ cells/l in T_1 and 5.877 $0.233x10^4$ individuals/l in T_2 (Table 2). Plankton population was significantly higher ($P<0.05$) in T_2.

Growth performance of fish

There was no variation among the initial weight of various species between the two treatments. Among all species included in this study, the weight gain of common carp was significantly ($P<0.05$) higher in treatment-II, four species polyculture system. It showed maximum gain per fish among the species and varied significantly between two treatments with mean weights of 218.95 ±1.327 g in T_2 and 188.17±1.386 g in T_1 (Table 3). Catla ranked second with a higher mean weight of 148.31±1.558 g in T_1 and lower mean weight of 140.78±0.9814 g in T_2. Rohu showed the gain per fish with a higher mean value of 136.33± 2.02g in T_1 and lower mean weight of 131.38±1.79 g in T_2. Gain per individual fish of silver barb was 128.34±1.23 g in treatment-II. The survival rate of fish was estimated after total harvesting of fish. The survival rate of various species in two treatments were fairly high and ranged from 88.88% to 93.33 %. All species had a similar trend of survival in both treatments. The lowest survival rate was observed in catla (88.88%) in treatment-I and rohu (88.89%) in treatment-II respectively (Table 3).

Table 3. Yield parameters of different species of fish in two treatments

Treatments	Treatment-I			Treatment-II			
Species	Rohu	Catla	Common carp	Rohu	Catla	Common carp	Sharpunti
No. stocked/ha	3,300	3,300	3,400	3,300	3,300	3,400	2,500
Mean no. of fish at harvest	3,066	2,933	3,100	2,933	3,000	3,133	2,333
Survival (%)	92.91	88.89	91.17	88.88	90.91	92.14	93.32
Av. initial wt. (g)	2.13	2.36	1.38	2.13	2.36	1.38	1.28
Av. final wt. (g)	138.46	150.67	189.55	133.51	143.14	220.33	129.87
Gain in wt. (g) per fish	136.33	148.31	188.10	131.38	140.78	218.95	128.59
Net yield (kg/ha)	417.98	434.80	583.33	385.34	422.40	685.90	300.00
Total (kg/ha /115 days)		1436.30*				1793.65*	

Total yield of fish was significantly higher ($P<0.05$) in four species polyculture system due to an increased yield of common carp and an additional yield of sharpunti. Total production of fish as appeared in Table 3 showed that there was a significant ($P<0.05$) difference in total production with higher yield of 1793.65 Kg/ha/115days in treatment-II and a lower yield of 1436.30 Kg/ha/115days in treatment-I. When species-wise comparison was made, it revealed that rohu and catla had significantly higher ($P<0.05$) net yield in treatment-I and common carp had significantly higher net yield in treatment-II. Total production of catla were 434.8 kg /ha and 4.22.40 kg/ha in treatments-I and II respectively. Total production of rohu was 417.98 Kg/ha, 385.34 Kg/ha in treatment-I and treatment-II respectively. Whereas that of common carp was 583.33 kg/ha and 685.90 kg/ha in T_1 and T_2 respectively.

Discussion

In polyculture, co-inhabiting species of different feeding habits are cultured in the same pond, so that the food niches are utilized without detriment to one another. Transparency indicates the presence and absence of food particles and productivity of a water body, which is influenced by the suspended materials, silt and micro-organisms. Dewan *et al.* (1985 and 1991) observed an inverse relationship between Chlorophyll-a and secchi depth values in the pond of Mymensingh area. In the present observation, the highest transparency was recorded after heavy rainfall during August. Lowest transparency was observed after fertilization which might be due to the presence of higher phytoplankton population and suspended organic matter.

Chlorophyll-a value was significantly higher in treatment-II than treatment-I due to the higher concentration of plankton in ponds under treatment-II. The

results showed an inverse relationship between chlorophyll-a and transparency. Ahmed (1993) also reported similar relationship between transparency and Chlorophyll-a.

In this study, Chlorophyceae showed the quantitative dominance over other plankton groups in both treatments. Wahab and Ahmed (1992) found that Cyanophytes dominated in the ponds containing Indian major carps. In the present experiment, the plankton population was high in both treatments but comparatively higher in treatment-II. The highest phytoplankton number of 62.85×10^4 cells/l was recorded in the month of July and the lowest was 4.69×10^4 cells/l was recorded in the month of September. Similarly the highest zooplankton number 6.9×10^4 individuals/l was recorded during the month of July and lowest zooplankton 4.9×10^4 individuals/l was observed in September. Wahab *et al.* (1994) observed the phytoplankton number ranging from 2×10^5 to 8×10^5 cells/l and the Zooplankton of 2×10^4 to 2×10^5 individual/l in their study.

The mean survival rate for various fish in different treatments in the present study varied between 88.89 to 93.93% which were higher than the survival rates reported by Wahab *et al.* (1991) for Indian major carps in polyculture. Laksmanan *et al.* (1971) observed survival rate of 80% with seven species composite culture of Indian and Chinese carps where ponds were fertilized with both organic and inorganic manures. In the present experiment, the highest survival rate was observed in case of sharpunti (93.93%). Kohinoor *et al.* (1993) obtained survival rate of 86 to 94% in the monoculture of sharpunti. Wahab *et al.* (1995) also found survival of all fish including sharpunti was higher than 80% in polyculture of native carps.

Weight gain (g/fish) of rohu, catla, common carp was 136.33, 148.31 and 188.17 in treatment-I and 131.38, 140.78, 218.95 in treatment-II respectively. Weight gain of sharpunti was 128.59 (g) in treatment-II. From the results, it was evident that the highest weight gain (g/fish) was observed in case of common carp in both treatments but significantly higher in treatment-II. Weight gain of rohu and catla was higher in T_1 than that of T_2. In both treatments common carp ranked first position in production when the species-wise production was observed with net yield of 583.33 and 685.9 Kg/ha in T_1 and T_2 respectively. The production of catla ranked second position in both treatments but higher production was obtained from T_1. Production of rohu possessed third position, was lower in T_2 in comparison to that of T_1. This might be due to antagonistic effect of sharpunti inclusion in the polyculture. The overall production of T_2 (including sharpunti) was significantly higher than the treatment T_1 (without sharpunti) with total production of 1,794 kg/ha /115 days and 1,436 kg/ha/115 days respectively. In both treatments, supplementary feed and fertilizers were used regularly to obtain higher production of fish. Murty *et al.* (1987) demonstrated a high production of 4,096 kg/ha/ yr from composite fish culture using Indian and exotic carp species applying supplementary feed and

nitrogenous fertilizers. Hussain *et al.* (1987) obtained production of 1952 kg/ha/5 month of *P. gononotus* with only supplementary feed. Wahab *et al.* (1995) also observed 5,294-5,670 kg/ha/yr production in the polyculture of carps with sharpunti.

By including sharpunti, synergistic interaction has resulted from faecal input of sharpunti. The excreta has enriched the bottom of the pond with essential food materials edible for common carp. This has helped to increase the growth and production of common carps. By stirring up the mud, the common carp recirculates the nutrients into the water and help to increase phytoplankton population. The excreta of herbivorous sharpunti reported to influence the growth of common carp (Phaohorm 1980, Dev 1994). Shahabuddin *et al.* (1994) also found positive effect of sharpunti on the growth of common carp. The overall increase in total fish production may have been due to the confounding effect of additional numbers of sharpunti which help to increase the growth of common carp and also have decreased the availability of food materials for other con-inhabiting major carp species. Thus, addition of sharpunti slightly affected the growth of rohu and catla. Similar negative effects of silver carp and bighead carps has been observed by Dewan *et al.* (1991) and Wahab and Ahmed (1991).

From the present study it may be concluded that the inclusion of Thai silver barb or sharpunti in the traditional carp polyculture has overall increased fish yields although it has exerted an antagonistic effect on growth and production of major carps. It has clear synergistic effects on the growth and production of common carp which may be important for the future development of polyculture technology in Bangladesh or elsewhere in the region. Thus Thai silver barb has appeared to be an appropriate candidate for polyculture with carps.

Acknowledgements

The second author gratefully acknowledge the financial support of the International Foundation for Science (IFS), Sweden.

References

Ahmed, Z.F., 1993. Electivity index and dietary overlap of *Catla catla* (Hamilton) in fertilized, and fed and fertilized ponds of Bangladesh. M.Sc. Thesis. Department of Fisheries Biology and Limnology, Bangladesh Agricultural University, Mymensingh, Bangladesh.

American Public Health Association (APHA), 1992. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington DC.

Dev, B.C., 1994. Food selection and electivity indices of the Thai barb *Puntius*(= Barbodsl *gonionotus* in extensively managed, rain-fed ponds in Bangladesh. M.Sc. Thesis. Department of Aquaculture and Management, Bangladesh Agricultural University, Mymensingh.

Dewan, S., M.J.U. Miah and M.N. Uddin, 1985. Studies on the food and feeding habits of *Cyprinus carpio II*. Diel and seasonal patterns of feeding of the fish. *Bangladesh J. Aquaculture*, **6-7**(1) : 11-18.

Dewan , S., M.A. Wahab, M.C.M. Beveridge, M.H. Rahman and B.K. Sarker, 1991. Food selection, electivity and dietary overlap among planktivorous Chinese and Indian major carp fry and fingerlings grown in extensively managed, rainfed ponds in Bangladesh. *Aquaculture and Fisheries Management*, **22** : 277-294.

Hussain, M.G., M.M. Rahman , M. Akhtaruzzaman and P. Perchabacher, 1987. Hormone induced evaluation and spawning of *Puntius gonionotus* (Bleeker). *Bangladesh J. Fish.*, **10**(1) : 1-4

Kohinoor, A.H.M., M. Akhteruzzaman and M.S. Saha, 1993. Production of *Puntius gonionotus* (Bleeker) in ponds. *Bangladesh J. Zool.*, **21**(2) : 77-83.

Lakshmanan, M.A.V., K.K. Sukumaran, D.S. Murty, D.P Chakraborty and M.T. Philipose, 1971. Preliminary observations on intensive fish farming in fresh water ponds by the composite culture of Indian and exotic species. *J. Inland Fish. Soc. India*, **2** : 1-21.

Murty, D.S., G.N. Saha, C. Selvaraj, P.V.G.K. Reddy, and R.K. Dey, 1987. Studies on increased fish production in composite fish culture through nitrogenous fertilization, with and without supplementary feeding. *J. Inland Fish. Soc. India*, **10** : 39-45.

Phaohorm, S., 1980. The study of biology, feeding habit and growth of *Puntius gonionotus*. Annual Report, Dept. of Fisheries, Bangkok, Thailand. pp. 19-33.

Shahabuddin, M., M.S. Miah, and M.S. Alam, 1994. Study on production optimization through polyculture of indigenous and exotic carps. *Bangladesh J. Train. and Dev.*, **7**(2) : 67-72.

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

Wahab, M.A and Z.F. Ahmed, 1992. Effect of planktivorous carp species combination on food organisms and electivity indices in the fish ponds. *Progress. Agric.*, **2**(2) : 21-30.

Wahab, M.A., Z.F. Ahmed, M.S. Haq and M. Begum, 1994. Compatibility of silver carp in the polyculture of cyprinid fishes. *Prorgess. Agric.*, **5**(2) : 221-227.

Wahab, M.A., Z.F. Ahmed, M. Aminul Islam and S.M. Rahmatullah, 1995. Effect of introduction of common carp, *Cyprinus carpio* (L) on the pond ecology and growth of fish in polyculture. *Aquaculture Research*, **26** : 619- 628.

(Manuscript received 16 August 1997)

Bangladesh J. Fish. Res., 2(1), 1998 : 23-29

The influence of nitrogen and phosphorus on the growth of a diatom *Skeletonema costatum* (Greville) Cleve

S. Khan[1,*], M.M. Haque[1], O. Arakawa and Y. Onoue

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

Abstract

Nitrogen and phosphorus requirements of a chain-forming diatom, *Skeletonema costatum* (Greville) Cleve, collected from Yatsushiro Sea, Japan, were investigated in a laboratory culture experiment. Sodium nitrate and sodium glycerophosphate were used as nitrogen and phosphorus sources, respectively. Cultures were grown in modified Provasoli's ASP_2NTA medium (Provasoli *et al.* 1957) at 25±1°C, light intensity 60 $\mu E\ m^{-2}\ sec^{-1}$ and photoperiod 12:12-h, L:D cycle. Optimum growth was observed at nitrate concentrations of 3-10 mgl^{-1} and phosphate concentrations of 1.5-15 mgl^{-1}. Adequate growth was also found at the nitrate concentration of up to as high as 300 mgl^{-1}. Significantly poorer growth was found at lower nitrate (< 3.0 mgl^{-1}) and higher phosphate (> 15 mgl^{-1}) concentrations. From the present study, it is concluded that *S. costatum* can grow well at wide ranges of nitrate concentrations but is sensitive to higher phosphate concentrations.

Key words : Diatom, *Skeletonema costatum*, Nitrogen, Phosphorus

Introduction

Skeletonema costatum is a common diatom (Eppley *et al.* 1971) which is one of the important food items of most copepods. In Taiwan and in the Philippines this species is considered as one of the best algae for feeding prawn larvae (Liao *et al.* 1983). *S. costatum* can serve as a good biological source of proteins and fatty acids (Sanchez *et al.* 1995). This species is also very important due to its potential use as valuable assay organism for examining water quality.

On the other hand, *S. costatum*, in some situations, may become noxious, forms heavy blooms when gets suitable environment due to eutrophication and causes economic losses to aquaculture. This species is known to cause blooms in USA, Rumania, France, Norway, Uruguay, China, Japan and Hongkong (Blanchemain *et al.* 1994, Mingyuan and Jiasheng 1993, Hosaka 1992). Noxious phytoplankton blooms are a serious problem for finfish aquaculture because the alga may kill fish by damaging or clogging their gills or kill fish due to

deoxygenation by forming a layer on the water surface (Anderson 1989), and it is believed that these blooms are increasing all over the world (Smayda 1992).

Growth of phytoplankton in nature is controlled by various environmental factors, such as temperature, salinity, irradiance, nutrients, stratification, water turbulence etc. (Tomas 1978, Uye and Takamatsu 1990). In our another study, it was found that *S. costatum* was extremely euryhaline and tolerable to very low salinities (Khan *et al.* 1998). Optimum growth was observed at salinities of 20-35 ppt, temperatures of 20-25°C, light intensities of 80-120 $\mu E\ m^{-2}\ sec^{-1}$ and pH between 7.5-8.0. Nutrients are very important environmental factors that influence the growth of any alga (Okaichi *et al.* 1989). The nutrient requirements of Yatsushiro Sea's strain of *S. costatum* in nature and laboratory have not yet been studied. The present study describes the effect of nitrogen and phosphorus on the growth of Yatsushiro Sea's strain of *S. costatum*.

Materials and methods

Skeletonema costatum used in this study was isolated in 1991 from Yatsushiro Sea, Japan. Stock cultures were grown in modified Provasoli's ASP_2NTA medium (Provasoli *et al.* 1957) (Table 1) at 25±1°C, light intensity 60 $\mu E\ m^{-2}\ sec^{-1}$ and photoperiod 12:12-h, L:D cycle. Sodium nitrate and sodium glycerophosphate were used as sources of nitrogen and phosphorus, respectively. Growth was determined at eight concentrations of nitrate (0.1, 0.3, 1, 3, 10, 30, 100 and 300 mgl^{-1}) and six concentrations of phosphate (0.1, 0.5, 1.5, 5, 15, and 50 mgl^{-1}). Culture media were autoclaved for 15 min at 121°C, and aged for several days prior to inoculation.

Table 1. Composition of modified Provasoli's ASP_2NTA medium (Provasoli *et al.* 1957)

Additive	Concentration
NaCl	18 gl^{-1}
$MgSO_4.7H_2O$	5 gl^{-1}
KCl	0.6 gl^{-1}
Ca (as Cl^-)	0.1 gl^{-1}
$NaNO_3$	7 mgl^{-1}
Na_2 glycerophosphate	1 mgl^{-1}
$Na_2SiO_3.9H_2O$	99 mgl^{-1}
Na_2Co_3	30 mgl^{-1}
Vitamin B_{12}	0.2 gl^{-1}
Vitamin mix. 53*	10 ml^{-1}
P II metals**	30 ml^{-1}
Fe (as Cl^-)	0.5 mgl^{-1}
Tris buffer	1.0 gl^{-1}
Distilled water	to 1 liter

The pH of the medium was 7.8.

** One ml of vitamin mix. 53 contains: thiamine HCl 0.05 mg, nicotinic acid 0.01 mg, Ca pantothenate 0.01 mg, p-aminobenzoic acid 1 µg, biotin 0.1 µg, inositiol 0.5 mg, folic acid 0.2 mg, thymine 0.3 mg.*

*** One ml of P II metals contains: Na_2EDTA 1 mg, Fe (as Cl^-) 0.01 mg, B (as H_3BO_3) 0.2 mg, Mn (as Cl^-) 0.04 mg, Zn (as Cl^-) 5 µg, Co (as Cl^-) 1 µg.*

Before starting the experiment the algae were acclimated to the experimental condition for at least two generations. Cells of mid logarithmic growth phase were used for inoculation to ensure that the cells were nutritionally replete. Sterilized micropipettes were used to transfer the inocula. Individual growth medium was gently shaken once a day for accelerating growth and to avoid settlement of algal cells. All growth studies were done in triplicate. The cell concentration was determined by direct counting by using a Sedgewick-Rafter chamber. Counts were made immediately after inoculation and then each other day up to 10 days. For reducing errors due to possible synchronous divisions counts were made at the same time each day. The average number of cell divisions per day (K) for the 6-day growth period was calculated from :

$$K = \ln \frac{C_t}{C_0} \left(\frac{1}{t \ln 2} \right)$$

where, C_t and C_0 are cell concentrations at times t and 0, respectively (Guillard 1973).

Division rates under different conditions were subjected to Analysis of Variance (ANOVA) (Statview S.E. + Graphics, Abacus. Concepts, Inc.). Significant differences among the means were determined using Duncan's multiple range test (DMRT) (Gomez and Gomez 1984).

Results

Growth of *S. costatum* at different sodium nitrate concentrations with the fixed salinity (30 ppt), temperature (25°C) and light intensity (60 $\mu E\ m^{-2}\ sec^{-1}$) is shown in Figure 1. Cultures reached a maximum cell density of 11.36×10^5 cells ml^{-1} on the 6th day at 10 mgl^{-1} sodium nitrate and the cell density remained at 9.70×10^5 cells ml^{-1} up to the 10th day in the same medium. No lag phase exhibited at 3 mgl^{-1} and 10 mgl^{-1}, and at 1 mg^{-1} and 30-300 mg^{-1} the lag phase was not distinct. Poorer cell density was found at low concentrations of nitrate (0.1 and 0.3 mg^{-1}) with a 2 days of lag phase.

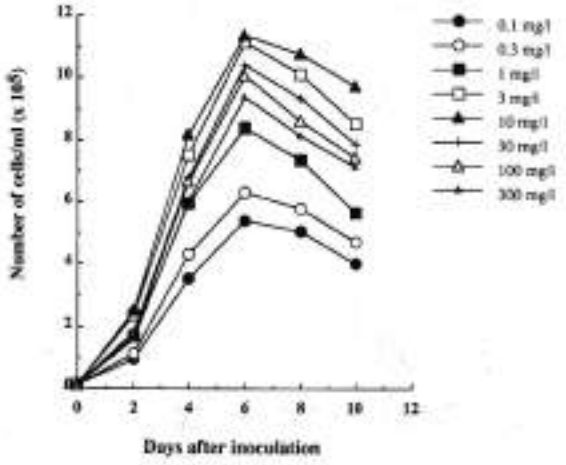


Fig. 1. Growth curves of *S. costatum* at different concentrations of sodium nitrate.

Analysis of Variance (ANOVA) showed that the difference in mean daily division rate of the cells at different nitrate concentrations were significant. The best mean daily division rate (0.75 divisions/day) was found at 3 and 10 mgl^{-1} which was not significantly higher than that at concentration of 30 mgl^{-1} (0.74 divisions/day). The mean daily division rates at 0.1-1 mgl^{-1} were significantly lower than at higher concentrations of nitrate. The mean daily division rate of *S. costatum* in relation to different concentrations of nitrate levels showed an increasing trend of growth from 0.1 to 10 mgl^{-1} and then a slow declining trend above 10 mgl^{-1} (Fig. 2).

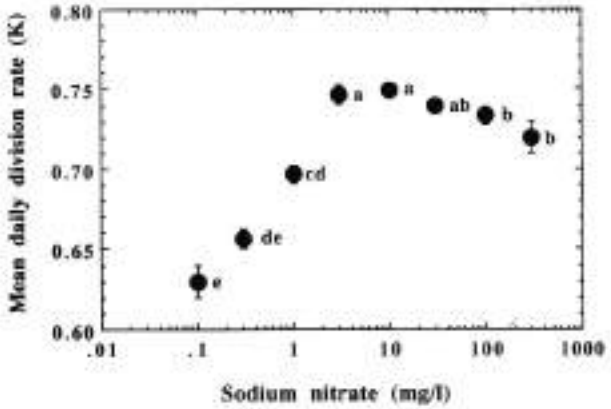


Fig. 2. Mean daily division rate of *S. costatum* at different concentrations of sodium nitrate. Each point and vertical line represent mean ± SD for three replicates. Means with different letters are significantly different ($p < 0.05$).

S. costatum required low amounts of phosphate. It grew well with phosphate concentrations of 1.5-15 mgl^{-1} (Fig. 3). The maximum cell density was found at concentrations of 1.5 mgl^{-1} (10.30×10^5 cells ml^{-1}) and 5 mgl^{-1} (10.27×10^5 cells ml^{-1}). The maximum cell yield at 50 mgl^{-1} sodium glycerophosphate was only 5.01×10^5 cells ml^{-1} on the 6th day of culture which was less than 50% of the maximum cell yield at 1.5 and 5 mgl^{-1}.

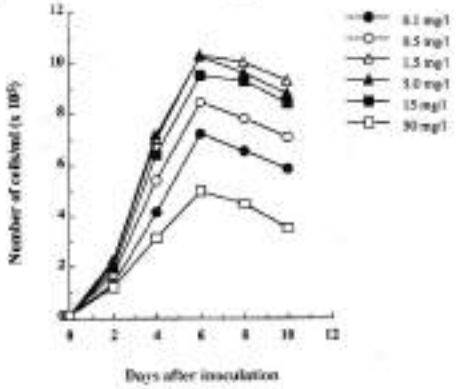


Fig. 3. Growth curves of *S. costatum* at different concentrations of sodium glycerophosphate.

Statistical analysis (ANOVA) indicated that the mean daily division rate of *S. costatum* was significantly different ($p<0.05$) at various glycerophosphate concentrations (Fig. 4). The highest mean daily division rate (0.71 divisions/day) was at 1.5 and 5 mgl^{-1} and no significant differences were observed among the phosphate concentrations of 1.5, 5 and 15 mgl^{-1}. Lower mean daily division rate was found at the concentration of 50 mgl^{-1} with 0.59 divisions/day.

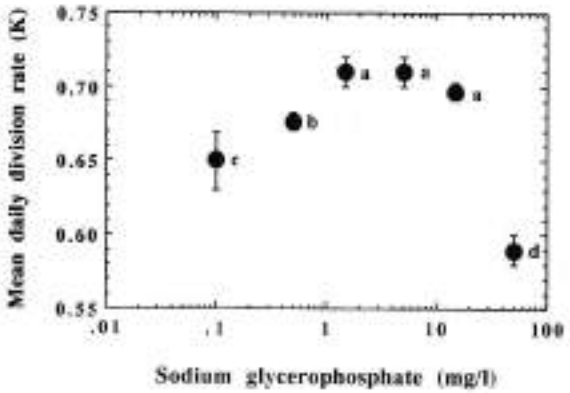


Fig. 4. Mean daily division rate of *S. costatum* at different concentrations of sodium glycerophosphate. Each point and vertical line represent mean ± SD for three replicates. Means with different letters are significantly different ($p < 0.05$).

The division rate of the plankton in relation to different concentrations of sodium glycerophosphate showed an increasing trend of growth from 0.1 to 1.5 mgl^{-1} and a slight declining trend above 1.5 mgl^{-1}. A much declining trend was found from 15 mgl^{-1}.

Discussion

Nitrogen and phosphorus are two important macronutrients which are essential for the growth of phytoplankton. In this experiment, it was found that the growth rates and final cell yields of *S. costatum* were dependent on the nitrate and phosphate concentrations in the media and the optimum requirements of nitrate and phosphate were 3-30 mgl^{-1} and 1.5-15 mgl^{-1}, respectively. Venkataraman (1969) reported about the requirement of a high level of nitrogen (13.6 mgl^{-1}) as compared to phosphorus (0.45 mgl^{-1}) for maximum growth of *Coccochloris peniocystiss*. Similar observations were found for *Selenasturm westii* (N/P ratio of 22.6/1) and *Kirchneriella subsolitaria* (N/P ratio of 15/1).

It has been known that ecological and physiological parameters of phytoplankton may vary for different species (Khan *et al*. 1996). *S. costatum* can tolerate a wide range of nitrate concentration. In the present study, *S. costatum* grew at nitrate concentrations from 0.1 to 300 mgl^{-1} with the optimum at 3 to 10 mgl^{-1}. These optimum nitrate concentrations are similar to that of a previously

reported red-tide-producing phytoflagellate *Chattonella antiqua* (Nishijima and Hata 1986). In all cultures there was a clear decrease in growth rate at lower nitrate concentrations which are similar to the findings of Nishijima and Hata (1986). Adequate growth of *S. costatum* was also found at nitrate concentrations of as high as 300 mgl^{-1}. Similar results were found by Venkataraman (1969) for *Nitzschia closterium*. On the other hand, growth of many algae were found to be strongly inhibited at higher concentrations of nitrate. A concentration greater than 31.5 mgl^{-1} of nitrogen was found to inhibit the growth of *Chlorella vulguris* (Venkataraman 1969).

The optimum phosphorus concentration for the growth of phytoplankton varies with different species. Nishijima and Hata (1986), while studying the effect of glycerophosphate in batch culture of *C. antiqua* using a phosphate concentration range from 0.03 to 8.9 mgl^{-1}, observed that the growth of this plankton was found to be optimum between 1-8.9 mgl^{-1}, was poor at 0.3 mgl^{-1} and was affected at 0.03 to 0.1 mgl^{-1}. Similar observations were found by Venkataraman (1969) for *Anacystis montana* which required a surprisingly low concentration of phosphorus. In our study, the optimum glycerophosphate concentration for the growth of *S. costatum* was found to be between 1.5 to 15 mgl^{-1}. It is noteworthy that, though *S. costatum* required a surprisingly low concentration of phosphate phosphorus for their optimum growth, some phytoplankton require much less concentration of phosphate. The best growth of *Ankistrodesmus fatcatus* and *Acenedesmus quadricauda* were observed at 1.0 mgl^{-1} and *Asterionella formosa* was at the concentration of only 0.002 mgl^{-1} (Venkataraman 1969). The growth of *S. costatum* was found to be inhibited by higher concentration of phosphate.

References

Anderson, D.M., 1989. Toxic algal blooms and red tides: a global perspective. *In : Red tides : biology, environmental science and toxicology* (eds. T. Okaichi, D.M. Anderson and T. Nemoto). Elsevier, New York. pp. 11-16.

Blanchemain, A., D. Grizeau and J.C. Guary, 1994. Effect of different organic buffers on the growth of *Skeletonema costatum* cultures : further evidence for an autoinhibitory effect. *J. Plankton Res.*, **16** : 1433-1440.

Eppley, R.W., A.F. Carlucci, O. Holm-Hansen, D. Kiefer, J.J. McCarthy, E. Venrick and P.M. Williams, 1971. Phytoplankton growth and composition in shipboard cultures supplied with nitrate, ammonium or urea as the nitrogen source. *Limnol. and Oceanogr.*, **16** : 741-751.

Gomez, K.A. and A.A. Gomez, 1984. Statistical procedures for agricultural research. John Wiley & Sons, New York. 680 pp.

Guillard, R.R.L., 1973. Division rates. *In : Handbook of phycological methods : culture methods and growth measurements* (ed. J.R. Stein). Cambridge University Press, Cambridge, USA. pp. 290-311.

Hosaka, M., 1992. Growth characteristics of a strain of *Heterosigma akashiwo* (Hada) Hada isolated from Tokyo Bay, Japan. *Bull. Plankton Soc. Japan.*, **39** : 49-58.

Khan, S., O. Arakawa and Y. Onoue, 1996. Growth characteristics of a neurotoxin producing chloromonad, *Fibrocapsa japonica* (Raphidophyceae). *J. World Aquaculture Soc.*, **27** : 247-253.

Khan, S., O. Arakawa and Y. Onoue, 1998. Physiological observations on a diatom *Skeletonema costatum*. *Bangladesh J. Fish. Res.*, **2**(2) : (accepted).

Liao, I.C., K.H. Su and J.H. Lin, 1983. Larval foods for penaeid prawns. *In : CRC handbook of mariculture. Vol. I. Crustacean aquaculture* (ed. J.P. McVey). CRC Press, Florida. pp. 43-69.

Mingyuan, Z. and X. Jiasheng, 1993. Red tide in shrimp ponds along the Bohai Sea. *In : Toxic phytoplankton blooms in the sea* (eds. T.J. Smayda and T. Shimizu). Elsevier, Amsterdam. pp. 363-367.

Nishijima, T. and Y. Hata, 1986. Physiological ecology of *Chattonella antiqua* (Hada) Ono on B group vitamin requirements. *Bull. Jap. Soc. Sci. Fish.*, **52** : 181-186.

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

Provasoli, L., J.J.A. Mclaughlin and M.R. Droop, 1957. The development of artificial media for marine algae. *Arch. Mikrobiol.*, **25** : 392-428.

Sanchez, S., M.E. Martinez, E. Molina, and J.A. Casa, 1995. *Skeletonema costatum* as a potential source of fatty acids and single-cell protein (SCP) : The effect of pH on growth rate and biomass composition. *Mar. Biotech.*, **2** : 23-26.

Smayda, T.J., 1992. Global epidemic of noxious phytoplankton blooms and food chain consequences in large ecosystems. *In : Food chains, yields, models and management of large marine ecosystems* (eds. K. Sherman, L.M. Alexander and B.D. Gold). Westview Press, Boulder, Colorado. pp. 275-307.

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

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

Venkataraman, G.S., 1969. The cultivation of algae. Indian Council of Agricultural Research. New Delhi. 297 pp.

(Manuscript received 30 June 1997)

Bangladesh J. Fish. Res., 2(1), 1998 : 31-39

Effects of testosterone propionate on growth, survival and sex-ratio of African catfish (*Clarias gariepinus* Burchell)

M. Samsul Alam* and M. Zamal Uddin

Department of Fisheries Biology & Genetics
Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
** Corresponding author*

Abstract

For studying the effects of different levels of testosterone propionate on growth, survival and sex-ratio, five different doses such as 125, 100, 75, 50, 25 mg hormone per kg feed were administered to 5-day old *Clarias gariepinus* fry through diet for a period of 40 days. The growth performance in terms of weight and length gain of the fry receiving 100 and 75 mg hormone per kg feed were significantly higher than those receiving 50, 25 and 0 (untreated control) mg hormone per kg feed. The groups of fry treated with higher doses of hormone showed lower survival compared to those with lower doses of hormone. The frequency of male fish in all the hormone treated groups except the 125mg/kg group were significantly higher than that of the expected frequency of male fish in a normal population. The highest frequency of male fish, 92.08%, was obtained with the diet containing 50 mg hormone/kg diet however, the highest levels of hormone (125mg/kg diet) resulted in relatively lower frequency of male fish.

Key words : Testosterone-propionate, African catfish, Sex-ratio

Introduction

The diversified and labile sex-differentiation systems in fishes have made it possible to induce sex-reversal by hormonal treatment in many gonochorisitic and some hermaphrodite fishes (Pandian and Sheela 1995). Hormonal sex-reversal technique may be used to understand the mechanism of sex differentiation and to produce mono-sex population for aquaculture. Fish production can be improved by adopting mono-sex culture technique either by involving male or female fish, depending on the superiority of growth performonce. The adequate supply of fry of a single sex is, however, the main prerequisite of this practice. In the fry or fingerling stages, it is usually not possible to separate physically the fish according to their sex. So it is presumed that the whole population of fry be produced as a single sex. Dietary administration of synthetic steroid hormones has been proved to be the most effective and easy means of producing mono-sex population through hormonal

sex-reversal (Hunter and Donaldson 1983). Steroids can also be administered by immersion (Pifferer *et al*. 1994) and by injection (Shelton 1986). Monosex population can also be produced by producing homogametic parents of both sex (Scott *et al*. 1989, Kavumpurath and Pandian 1992). Depending on the sex determining mechanism neo-males (genotypic females) or neo-females (genotypic male) are produced by administering sex hormone (Shelton 1986, Scott *et al*. 1989, Lahav 1993). After repeated crossing and progeny testing the homgametic broodfish are produced.

The African catfish, *Clarias gariepinus*, has been introduced in Bangladesh in 1989. This fish possesses many characteristics such as faster growth rate, disease resistance and hardiness which are suitable for aquaculture. Production of this fish can be improved by adopting the culture of mono-sex population. Van den Hurk *et al*. (1989) studied the effect of 17♯-methyl testosterone and 11ß-hydroxyandrostenedione on gonadal differentiation of *C. gariepinus* and found that from day 28 after hatching these hormones were found to be effective to change the sex-ratio in favour of male. In the present study, an androgenic hormone testosterone propionate has been administered in the first feeding fry of *C. gariepinus* with variable doses in order to optimise the dose for the production of maximum male population. We also report in this paper the comparative growth performance and survival of the fry during the hormone feeding phase receiving different levels of hormone dose.

Materials and methods

Sources of fry and experimental design

The experiment was conducted with first feeding larvae of *C. gariepinus* which were produced through induced breeding by using human chorionic gonadotropin (HCG) and carp pituitary extracts as inducing agents. The experiment was conducted with 5-day old larvae having an average total length of 7.4 mm and average body weight of 6.8 mg.

Eighteen plastic bowls of 20 cm radius and 21 cm depth were used for the hormonal treatment. The plastic bowls were arranged into 3 rows, 6 bowls each and each bowl contained 300 *C. gariepinus* larvae. The plastic bowls were randomly arranged for six treatments (T_1 -T_6) each with 3 replications. The fry of treatments T_1, T_2, T_3, T_4, T_5 and T_6 were fed with diet containing 125, 100, 75, 50, 25 and 0 mg of hormone per kg feed respectively.

Preparation of hormone treated feed

The androgenic hormone testosterone propionate used in this study was collected from local market with the trade name of 'Testoviron' (Germany) in oily preparation. For preparing the hormone-mixed feed 250 mg of hormone was dissolved in 250 ml ethanol which was considered as stock solution. For the diets containing 125, 100, 75, 50 and 25 mg of hormone per kg of feed 75 ml,

60ml, 45ml, 30ml, and 15ml of the stock solution was mixed with 600g of 'SABINCO' shrimp nursery feed respectively. Additional ethanol was added to each of the diet to equalize the volume up to 75ml. The untreated control (T_o) was also mixed with 75ml of ethanol without hormone. The feeds were then air-dried at room temperature to remove ethanol by evaporation and then oven dried at 30° C for 24 hours. All the feeds were stored in a vacuum polythene bag by sealing the opening and then kept in refrigerator (4°C).

Feeding trial and rearing

The larvae were fed regularly three times daily at 08.00, 14.00 and 22.00 hours at the rates of 35%, 30% 25% and 20% of body weight per day for the 1st, 2nd, 3rd and 4th 10 days respectively of the 40 days hormone treatment phase. The uneaten feed, debris and faeces were siphoned once daily. The bowls were provided with a continuous supply of water through perforated PVC pipes.

After completion of the hormonal trial the fish were transferred to fibre glass tanks and fed with hormone free artificial feed for two month. The fish were then transferred in a race-way and reared for another four months when sexing of the fish could be done easily with normal 'SABINCO' catfish feed containing 30% protein, 6% lipid, 6% crude fibre and 17% ash.

Collection and analysis of data

A representative of 15 fry from each bowl were randomly sampled for the record of weight (mg) and length (mm) at 10 days interval. The dead fry were removed from the bowl and recorded daily. For studying the sex-ratio in different treatments the fish were sexed morphologicallly by the genital papillae at the age of about eight months. Sexually mature *C. gariepinus* can be easily sexed on the basis of genital papilla which is elongated and pointed for the males, and short and round for the females. The sex of the relatively smaller fish were confirmed by examining the gonadal condition following dissection.

The growth parameters of the larvae were analysed by Analysis of Variance (ANOVA) following completely randomomised design and Duncan's New Multiple Range Test (DMRT). The analysis was done by using a computer package 'STATGRAPHICS' version-7. The frequencies of male population in the different treatments were compared with that of the expected frequency of male fish (50%) in a random normal population by Chi-square Test (Zar 1996).

Results

Growth and survival

The increase in body weights of fry of all the treatment groups appeared to be similar up to day-20, variations between the body weights of fry under higher and lower doses became clear later on (Figure 1). Similar trend was also observed in the case of length increments (data not shown). The mean weight

gain of the fry receiving 100 mg (T_2) and 75 mg (T_3) hormone per kg feed at the end of the 40 days hormone trial phase were found to be significantly higher ($p<0.05$) than those receiving 50 (T_4), 25 (T_5) and 0 (T_6) mg hormone per kg feed (Table 1). The mean weight gain of the fry receiving 125mg of hormone (T_1) was significantly higher than that of T_4 but similar to T_5 and T_6. No Significant differences were observed among the weight gain of the fry of T_1, T_2 and T_3 and among T_4, T_5 and T_6 ($P>0.05$) (Table 1). The mean length-gain of the fry of T_2 and T_3 at the end of the 40 days hormone feeding phase were significantly higher than that of T_4, T_5 and T_6 ($P<0.05$). No significant differences were observed among the length-gain of the larvae of T_1, T_2 and T_3 and among T_4, T_5 and T_6 ($P>0.05$) The condition factor of the fry of T_1 were significantly higher than those of rest of the treatments ($P<0.05$). No significant differences were observed among the condition factors of the fry of T_1, T_2, T_3 and T_5. The condition factor of the fry of T_6 was found to be the lowest (Table 1).

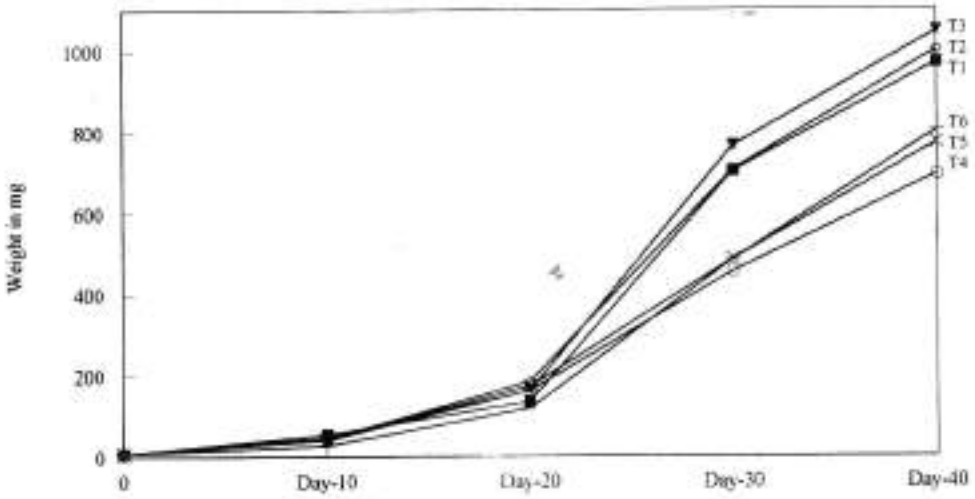


Fig. 1. Mean weight of *C. gariepinus* fry under different treatments during the 40 days hormone feeding phase. T_1, T_2, T_3, T_4, T_5 and T_6 represents six different diets containing 125, 100, 75, 50, 25 and 0 mg of testosterone propionate per kg feed respectively.

Table 1. Various growth parameters and survival rates of *C. gariepinus* fry fed on diets containing different levels of testosterone propionate

Parameters	Treatments					
	T_1 (125mg/kg)	T_2 (100mg/kg)	T_3 (75mg/kg)	T_4 (50mg/kg)	T_5 (25mg/kg)	T_6 (0mg/kg)
Initial weight (mg)	6.8±1.7	6.8±1.7	6.8±1.7	6.8±1.7	6.8±1.7	6.8±1.7
Final weight (mg)	968.4	998.8	1045.3	692.2	770.4	800.7
Weight-gain (mg)	961.6^{ab}	992.0^{a}	1038.5^{a}	685.4^{c}	763.6^{bc}	793.9^{bc}
Initial length (mm)	7.9±0.8	7.9±0.8	7.9±0.8	7.9±0.8	7.9±0.8	7.9±0.8
Final length (mm)	50.2	52.0	51.9	46.1	47.6	46.0
Length-gain (mm)	42.3^{ab}	44.1^{a}	44.0^{a}	38.2^{b}	39.7^{b}	38.6^{b}
Condition factor*	0.73 ± 0.1^{a}	0.68 ± 0.1^{b}	$.068\pm0.1^{b}$	0.67 ± 0.1^{b}	$0.68\pm.08^{b}$	$.061\pm.16$
Survival (%)	10.33^{c}	10.89^{c}	10.44^{c}	25.44^{a}	23.33^{a}	17.67^{b}

Figures in the same row followed by different superscripts are significantly different at P<0.05

**Condition factor* $= \frac{W}{L^3} \times 10^3$ *where L= Length in mm and W= Weight in g*

The survival rates of the fry of T_4 and T_5 were significantly higher (P<0.05) than those of T_1, T_2, T_3, and T_6. The survival rate of the fry of T_6 was significantly higher (P<0.05) than those of T_1, T_2 and T_3. No significant differences were observed among the survival rates of T_1, T_2, and T_3 (Table 1).

Sex-ratio

The sex-ratio data of the fish of different treatment groups receiving different doses of testosterone propionate are presented in Table 2. The frequencies of male fish obtained in the treatments having diets containing 25, 50, 75 and 100 mg of testosterone propionate per kg feed (T_2-T_5) were found to be significantly higher than that of the expected frequency of male fish (50%) in a random normal population (P<0.05). The frequency of males in the group of fish growing from fry fed on 50mg/kg of feed was found to be the highest (92.08%). On the other hand, the frequency of male fish in T_1 (125 mg/kg) was found to be the lowest and was not significantly different from that of the expected frequency of 50%. The frequency of female and male fish in the untreated control group (T_6) was also not significantly different from that of the expected ratio of 1:1 in a normal population.

Table 2. Frequency of males and females in the groups of fish grown from the fry fed on different levels of testosterone propionate

Sex of fish	Numbers/frequency of fish in different treatments					
	T_1 (125mg/kg)	T_2 (100mg/kg)	T_3 (75mg/kg)	T_4 (50mg/kg)	T_5 (25mg/kg)	T_6 (0mg/kg)
Male	19	34	47	93	40	43
Female	8	9	7	8	10	38
Total	27	43	54	101	50	81
Frequency of female(%)	29.63	20.93	12.96	7.92	20.00	46.91
Frequency of male(%)	70.37	79.07	87.04	92.08	80.00	53.09

Discussion

Growth and survival

Anabolic steroids, both androgens and estrogens enhance growth and feed conversion efficiency when administered at optimal level in fish (Matty 1985). The anabolic effects of the most frequently used synthetic steroid, 17‡-methyltestosterone, have been found to be dose dependent which was reported by a number of authors (Donaldson *et al.* 1979). Yamazaki (1976) found best growth rate of goldfish using 17‡-methyltestosterone at a concentration of 1ppm, the growth rate was decreased at a concentration of 10 ppm and weight loss was noticed at a concentration of 30ppm. In the present study, though the effects of various levels of hormone on growth were not clear up to day-20, a dose-dependent response in growth became clear on day-30 onward (Figure 1). The fry receiving testosterone propionate at the doses of 100 and 75mg per kg diet showed significantly higher weight and length gain than those receiving 50, 25 and 0 mg per kg diet (Table 1). No difference in growth performance was observed among the groups of fry receiving 125, 100 and 75 mg of hormone per kg diet. No difference in growth performance was also observed between the groups of fry receiving the highest and the lowest levels of hormone. These findings support that the higher doses of the hormone do not induce growth enhancement rather tend to be catabolic or exert deleterious effects which interfere with the normal processes and retard any gain in anabolic response expected by the increase in dose. In contrast to the findings of the present study, Eversole (1939) found retarted growth when testosterone propionate was administered to 2 months old guppy (*Lebestes reticulatus*) twice weekly for a period of 17 weeks. However, Svärdson (1943) observed (cited by Donaldson *et al.* 1979) a sex and age dependent response of guppy to testosterone propionate. This author found significant increase in length in the sexually mature female fish when the hormone was administered through diet.

From the present study it appears that up to a certain level testosterone propionate has positive effects on growth of *C. gariepinus* fry though these effects could be visible from day-25 after hatching.

The survival rates of the fry during the 40 days experimental period were found to be related to the doses of hormone (Table 1). The groups of larvae receiving higher levels of hormone (125-75 mg/kg feed) showed significantly lower survival rates compared to those receiving lower doses or no hormone. Pandian and Varadaraj (1990) found that the mortality of *Oreochromis mossambicus* increased as the doses of methyl testosterone increased. Torrans *et al.* (1988) also reported that survival of *O. aureus* negatively correlated with the doses of mibolerone when applied by immersion for sex-reversal. As mentioned earlier, the levels of androgens higher than that of the optimal may have deleterious effects on fish which might be the cause of higher mortality. The effects of hormone however, can not be considered as the only factor for such low survival rates because the survival rate of the fry of untreated control was also found to be low (17.67%). The *C. gariepinus* fry in this study were reared on completely artificial feeding that might be the cause of such poor survival rates. It is now established that *Clarias* fry show poor growth and survival with artificial feed (Uys and Hecht 1985, Alam 1988). Administration of hormones through live food such as *Tubifex* sp. worms could be tried to overcome this problem in *Clarias* fry.

Sex-ratio

In the present, study the synthetic androgen testosterone propionate was found to play a significant role in altering the sex of *C. gariepinus* into males. Among the five doses of hormone tested, the dose of 50mg/kg feed was found to be most potent and resulted in 92.08% male fish. Ridha and Lone (1990) reported 90.3% male *O. spilurus* after oral administration of 70 mg 17‡-methyltestosterone per kg feed for a period of 38 days. Administration of 50ppm 17‡-methyltestosterone for 5 weeks (from 6 to 11 week), resulted in 92.7% males in *Cyprinus carpio* (Komen *et al.* 1989). These findings are more or less similar to the findings of the present study. Goudie *et al.* (1989) reported that due to the antagonistic action of 17‡-methyltestosterone the frequency of female fish was higher at higher dosages. The occurrence of relatively more female (less male) at higher levels of testosterone propionate may have been resulted from the same reason. In contrast to the present study, Van den Hurk *et al.* (1989) reported that 17‡-methyltestosterone had a feminizing effect at a dose of 100µg/l (immersion treatment) from day 14 after hatching in *C. gariepinus*. However, they mentioned this effect as pharmacological rather than physiological. The same dose of a particular hormone is not equally effective in all species of fish. It has also been reported that the higher doses of hormone might not necessarily produced higher induction in sex-reversal (Guerrero 1975, Wolwode 1978, Ridha and Lone 1990). As the androgenic hormone action is

species specific, for maximum induction of sex-reversal the dose of a particular hormone to be optimised for each species individually. From the present study, it appears that the optimum dose of testosterone propionate in *C. gariepinus* lies within the range of 50-75mg/kg feed.

Like the dose of hormone the success of sex-reversal experiment also depends on optimal duration as well as date of starting of hormone treatment for a particular species. Since the higher doses of hormone produced relatively higher mortality and lower frequency of male fish it is recommended to optimise the duration of testosterone propionate treatment for better masculinization in *C. gariepinus*. As mentioned earlier, the overall survival rates of fry during the hormone feeding phase was found to be low which might also affect the success of the sex-reversal experiment. In the present study, the hormone treatment was initiated from day-5 after hatching. Starting of hormone treatment at later stages would have resulted in better survival and and thus better result in sex-reversal.

The objective of a sex-reversal experiment is to produce 100% mono-sex population of a particular sex. The highest frequency of male fish obtained in the present study is 92.08% which indicates that it is possible to reverse the sex mostly or entirely by dietary administration of testosterone propionate in *C. gariepinus*. Further research in this species is however, extremely essential.

References

Alam, M.S. and M.F. A. Mollah, 1988. Formulation of an artificial dry feed for primary nursing of catfish (*Clarias batrachus* L.) larvae. *Bangladesh J. Fish.*, **11**(1) : 71-75.

Donaldson, E. M., U. H. M. Fagerlund, D. A. Higgs and J. R. McBride, 1979. Hormonal enhancement of growth. *In: Fish physiology* Vol VIII (eds. W. S. Hoar, D. J. Randall and J. R. Brett). Academic Press, New York. pp. 455-597.

Eversole, W. J., 1939. The effects of androgens upon the fish (*Lebistes reticulatus*). *Endocrinology*, **25** : 328-330.

Goudie, C. A., B. D. Redner, B. A. Simco and K. B. Davis, 1983. Feminization of channel catfish by oral administration of steroid sex hormones. *Trans. Am. Fish. Soc.*, **112** : 670-672.

Guerrero, R. D., 1975. Use of androgens for the production of all-male *Tilapia aurea* (Steindachner). *Trans. Am. Fish. Soc.*, **104**(2) : 342-348.

Hunter, G. A. and E. M. Donaldson, 1983. Hormonal sex control and its application. *In : Fish physiology* Vol XB (eds. W. S. Hoar, D. J. Randall and E. M. Donaldson). Academic Press, New York. pp. 223-303.

Kavumpurath, S. and T. J. Pandian, 1992. Production of 'YY' male guppy (*Poecilia reticulata*) by endocrine sex-reversal and selective breeding. *J. Asian Fish. Sci.*, **5** : 265-276.

Komen, J. P., A. J. Lodder, F. Huskins, C. J. J. Richter and E. A. Huisman, 1989. Effects of oral administration of oral administration of 17α-methyltestosterone and 17ß-estradiol on gonadal development in common carp *Cyprinus carpio* L. *Aquaculture*, **78** : 349-363.

Lahav, E. 1993. Use of sex-reversed female to produce all-male tilapia (*Oreochromis aureus*) fry. *Isr. J. Aquacult. Bamidgeh*, **25**(3) : 131-136

Matty, A. J., 1985. Fish Endocrinology. Croom Helm London, Sydney. 267 pp.

Pandian, T. J. and K. Varadaraj, 1990. Techniques to produce 100% male tilapia. *NAGA* (ICLARM Quarterly) : 3-5.

Pandian, T. J. and S. G. Sheela, 1995. Hormonal induction of sex reversal in fish. *Aquaculture*, **138** : 1-22.

Piferrer, F., J. Currilo, S. Zancry, I. I. Solar and E. M. Donaldson, 1994. Induction of sterility in coho salmon (*Oncorhynchus kisutch*) by androgen immersion before first feeding. *Aquaculture*, **119** : 409-423.

Ridha, M. T. and K. P. Lone, 1990. Effect of oral administration of different levels of 17‡-methyltestosterone on the sex-reversal, growth and food conversion efficiency of the tilapia *Oreochromis spilurus* (Gunther) in brackishwater. *Aquacult. Fish. Management*, **21** : 391-397.

Scott, A. G., D. J. Penman, J. A. Beardmore and D. O. F. Skibinski, 1989. The 'YY' supermale in *Oreochromis niloticus* (L.) and its potential in aquaculture. *Aquaculture*, **78** : 237-251.

Shelton, W. L., 1986. Broodstock development for monosex production of grass carp. *Aquaculture*, **57** : 311-319.

Svärdson, G., 1943. Studien hber den Zusammenhang zwischen Geschlechtsreife und wachstun bei Lebistes. *Medd. Statens Unders`k. F`rs`ksanst. Sotvattenfisket*. **21** : 1-48.

Torrans, L. F. Meriwether and F. Lowell, 1989. Sex-reversal of *Oreochromis aureus* by immersion in mibolerone, a synthetic steroid. *J. World Aquacult. Soc.*, **19** : 97-102.

Uys, W. and T. Hecht, 1985. Evaluation and preparation of an optimal dry feed for the primary nursing of *Clarias gariepinus* larvae (Pisces:Clariidae). *Aquaculture*, **47** : 173-183.

Van den Hurk, R., C. J. J. Richter and J. Janssen-Dommerhault, 1989. Effects of 17‡-methyltestosterone and 11ß-hydroxyandrostenedione on gonadal differentiation in the African catfish *Clarias gariepinus*. *Aquaculture*, **83** : 179-191.

Woiwode, J. G., 1977. Sex-reversal of *Tilapia zilli* by ingestion of methyltestosterone. *Tech. Pap. Ser. Bur. Fish. Aquat. Resources* (Phillip) 1(3) : 5p.

Yamazaki, F., 1976. Application of hormone in fish culture. *J. Fish. Res. Board Can.*, **33** : 948-958.

Zar, Z. H., 1996. *Biostatistical analysis* (3rd ed.). Prentice Hall International Inc. 918 pp.

(Manuscript received 16 July 1997)

Bangladesh J. Fish. Res., 2(1), 1998 : 41-46

Rearing of *Clarias batrachus* (Lin.) larvae with formulated diets

M.R. Saha[1,*], M.F.A. Mollah and P.K. Roy[1]

Department of Fisheries Biology & Genetics
Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[1] Present address : Fisheries Research Institute, Brackishwater Station, Khulna-9280, Bangladesh
** Corresponding author*

Abstract

An experiment was carried out for a period of 20 days using 7-day old *Clarias batrachus* larvae of initial total length of 7.4 ± 0.49 mm and weight of 2.9 ± 0.83 mg. Three artificial diets were used for the study having three replication of each. Among these, diet I was formulated using 20% fish meal (FM), 30% powdered milk and 30% boiled egg yolk (BEY), diet II using 27% FM, 20% Baker's yeast (BY), 30% BEY and 3% agar and diet III using 20% FM, 20% BY and 45% whole egg. The larvae fed on diets II and III showed significantly ($P<0.05$) better length and weight gain than those of the larvae fed on diet I. The larvae fed on diet III showed the best survival rate (70%). However, the condition factor of the larvae fed on diet I was significantly better than those of the larvae fed on other two diets. The results of the study showed that *C. batrachus* larvae could be successfully reared with diet containing 45% whole egg, 20% yeast and 20% fish meal.

Key words : *Clarias batrachus*, Larvae, Feed

Introduction

Larval stage of fish is considered as the most sensitive phase of its life. Development of successful and suitable induced spawning technique fails to fulfill the demand of stockable sized fry unless some success in rearing the larvae is achieved. Catfish *C. batrachus* is not out of this normal phenomenon. Although its induced breeding success has been reported by a number of workers including Rahmatullah *et al.* (1983) and Mollah (1987a and 1987b), not much work seems to have been conducted to solve its larvae rearing problems. It is, however, an established fact that live feeds like Tubificid worms and/or zooplankton like *Daphnia/Moina* give very good growth and survival of the larvae. But the availability of these live food round the year is uncertain and their controlled production technology has not yet been demonstrated conclusively. As a result, it was felt necessary to find out the alternative way of rearing the larvae with formulated diets. In this context, the recent works of Alam and Mollah (1988 and 1989), Madhury and Mollah (1990) and Saha *et al.* (1996) worth mentioning. However, no perfectly suitable larval diet has yet been

developed. Therefore, an experiment was conducted with the aim of finding a suitable diet for successful rearing of *C. batrachus larvae.*

Materials and methods

The experiment was carried out with 7-day old larvae having an initial total length of 7.4 mm and weight of 2.9 mg. *C. batrachus* larvae used in the present study were produced by induced breeding with the use of human chorionic gonadotropin (HCG) at a dose of 3 and 2 IU/g body weight of female and male respectively. When the yolk sac was completely absorved, the larvae were fed with hard-boiled chicken egg yolk.

The experiment was conducted in nine trays of size 104 X 43 X 15 cm^3, each having 20 litres of water. Each of the trays was stocked with 100 larvae at a rate of 5 larvae/l of water. The trays were divided into three treatment groups each having three replicates. Larvae of treatments I, II and III were fed with diets I, II and III respectively. For maintaining continuous flow in the trays, water was supplied by perforated plastic tube which was connected to the tap of the laboratory. Excess water was drained out through the outlet of the tray.

The larval feeds were prepared with fish meal, yeast, powdered milk, boiled chicken egg yolk, whole egg, wheat flour, agar, cod liver oil etc. Fish meal was prepared from deboned 'lata shutki' (*Channa striatus*) which was purchased from local market. Required amount of dietary ingredients were measured and mixed thoroughly. Then the mixture was made into paste form by adding required quantity of water. Proximate compositions of dietary ingredients and test diet were determined by AOAC (1965) methods with slight modifications. Proximate composition of dietary ingredients are shown in Table 1. Formulation of test diets and their proximate composition are given in Table 2. The energy content (Kcal/g) of the diets were estimated according to Hastings (1979). The vitamin and mineral premixes used were prepared according to Jauncey and Ross (1982).

Table 1. Proximate composition of dietary ingredients used in three experimental diets (% dry matter basis)

Ingredients	Proximate composition					
	Dry matter	Crude protein	Lipid	Ash	Crude fibre	NFE*
Fish meal	93.00	85.05	12.26	3.38	-	-
Baker's yeast	94.60	37.87	6.59	3.21	0.08	2.25
Whole egg (albumin+yolk)	29.00	58.83	31.03	10.19	-	-
Boiled chicken egg yolk	56.50	40.81	21.24	12.39	-	
Powdered milk	97.30	27.13	28.96	5.90	-	7.99
Wheat flour	92.14	12.48	1.32	2.11	2.14	81.95

NFE (Nitrogen free extract) = 100 - (crude protein + lipid + ash + crude fibre)

Table 2. Formulation and proximate composition of the test diets (% dry matter basis)

Ingredients[1]	Diets		
	I	II	III
Fish meal	20.00	27.0	20.0
Baker's yeast	-	20.0	20.0
Powdered milk	30.0	-	-
Boiled chicken egg yolk	30.0	30.0	-
Whole egg (albumin + yolk)	-	-	45.0
Wheat flour	12.0	15.0	10.0
Agar	-	3.0	-
Cod liver oil	3.0	1.0	1.0
Attractant[2]	1.0	1.0	1.0
Vitamin premix[3]	2.0	1.5	1.5
Mineral premix[4]	2.0	1.5	1.5
Crude protein	39.27	42.06	49.25
Lipid	12.00	9.08	10.00
Ash	7.09	7.38	7.11
NFE	41.64	41.48	33.64
Energy (Kcal/100g)	431.64	415.88	421.56

[1] g/100 g dry diet, [2] Sodium aspartate (salt of aspartic acid)
[3 and 4] According to Jauncey & Ross (1982)

The experiment was continued for a period of 20 days. The fish were fed three times daily between 08.00 h and 20.00 h. Feeds were supplied in excess of satiation. The larvae were considered satiated when they stopped feeding, though there were some feeds yet to be fed and assembled in the corners of the trays. The excess feed was removed before next feeding and dead larvae were removed and counted at that time. The growth in terms of length (mm) and weight (mg) and survival rate of the larvae were determined at the end of the experiment. The data on survival rates were normalized by arcsine transformation (Zaman *et al*. 1982). Statistical analysis of the data were done by one way Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (Gomez and Gomez 1984) to determine differences between the means ($P>0.05$).

Results

The growth and survival rates of *C. batrachus* larvae in response to different formulated diets are presented in Table 3. The length gain and weight gain attained by the larvae fed diets II and III were significantly better than that fed diet I. However, the differences among the length gain and weight gain of the larvae fed diets II and III were insignificant.

Table 3. Various growth parameters and survival rate of *C. batrachus* larvae fed on three different artificial diets at the end of 20 days experimental period

Treatments/ Parameters	Diet I	Diet II	Diet III	±SE*
Initial total length (mm)	7.4	7.4	7.4	-
Length gain (mm)	8.73[b]	10.55[a]	10.73[a]	0.253
Initial weight (mg)	2.9	2.9	2.9	
Weight gain (mg)	40.78[b]	48.14[a]	9.12[a]	1.800
Specific growth rate (% day)	13.36	14.08	14.15	0.200
Condition factor	1.02[a]	0.86[b]	0.85[b]	0.020
Survival (%)	29.42[b]	5.39[b]	70.45[a]	3.530

Figures in the same row with same letters are not significantly different (P<0.05)
**SE = Standard error*

The larvae fed diet I showed significantly better condition factor than those of the larvae fed other two diets. There was no significant difference between the condition factor of the larvae fed diets II and III. The larvae fed on diet containing whole egg (diet III) showed higher survival rate (70%) than those of the larvae fed other two diets. However, no significant difference was observed between the survival rate of the larvae fed diets I and II.

The mortality of the larvae of three diets ranged from 7-16% at day-5 (Fig. 1). The larvae fed diets I and II showed more than 50% mortality at day 15. At the termination of the feeding trial, the larvae fed on diet containing boiled egg yolk (diet I) and diet containing whole egg (diet III) showed the highest (70.58%) and lowest (29.55%) mortality respectively.

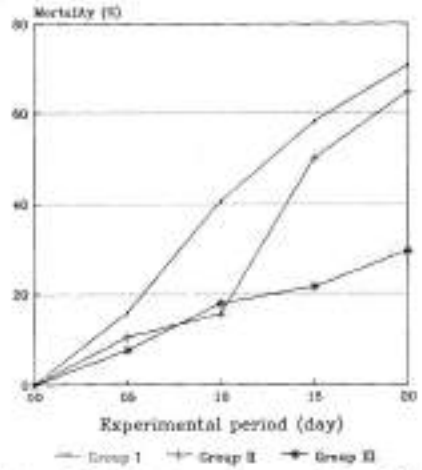


Fig. 1. Cumulative mortality of *C. batrachus* larvae during 20 days feeding trial fed on different formulated diets.

Discussion

The suitability of artificial feed for rearing of *C. batrachus* larvae was investigated in the present study. Three diets were tested during the period of 20 days feeding trial. At the termination of the feeding trial, it was observed that the larvae fed on diet containing 45% whole egg and 20% yeast (diet III) showed better growth and survival rate followed by the larvae fed on diet containing egg yolk and agar (diet II). The larvae fed diet III showed an average weight gain of 49.12 mg after 20 days feeding trial. The result of the present study was better than the findings of Verreth *et al.* (1987), Bairage *et al.* (1988) and Alam and Mollah (1989). Alam and Mollah (1989) reported that the larvae fed on 60% whole egg and 20% yeast attained an average weight of 41.60 mg after 20 days (25 day after hatching).

In the present study, the specific growth rate of the larvae fed on diet containing whole egg (diet III) was 14.15 which was better than the specific growth rate (10.29) reported by Alam and Mollah (1989). The larvae fed on diet containing whole egg showed the condition factor of 1.23 reported by Alam and Mollah (1989) which was better than the condition factor obtained in the present study.

The growth rates of the larvae fed diets II and III were comparable but the larvae fed on feed containing whole egg showed higher survival rate than that of fed diet II. The larvae fed diet III showed survival rate of 70% which was similar or comparable to the findings of Dabrowski *et al.* (1984), Winfree and Stickney (1984), Verreth *et al.* (1987), Bairage *et al.* (1988), Husain (1988) and Alam and Mollah (1989).

The larvae fed diets II and III showed similar trend in mortality up to day-10 (Figure 1). But the larvae fed diet II showed highest mortality between day -0 and 15. The larvae fed diet I exhibited increasing rate of mortality from day-5 up to the end of the experiment. The larvae fed diet III showed 29.55% mortality at the end of the experiment.

Conclusions

In the present study, the larvae fed on diet containing 45% whole egg and 20% yeast attained better growth (weight gain of 49.12 mg) and survival rate (70%) during the 20 days experimental period. Therefore, *C. batrachus* larvae could be successfully reared with diet containing 45% whole egg, 20% yeast and 20% fish meal.

References

Alam, M.S. and M.F.A. Mollah, 1988. Formulation of an artificial dry feed for primary nursing of catfish (*Clarias batrachus* L.) larvae. *Bangladesh J. Fish.*, **11**(1) : 71 - 75.

Alam, M. S. and M.F.A. Mollah, 1989. Use of moist diets in rearing catfish (*Clarias batrachus* L.) larvae under laboratory conditions. *Bangladesh J. Fish.*, **12**(1): 2-41.

AOAC, 1965. Official Methods of Analysis. *Association of Official Agricultural Chemists.* 10th ed. Washington, D. C. 957 pp.

Bairage, S. K., G. Barua and M. A. Khaleque, 1988. Comparison between few selective feeds of magur (*Clarias batrachus* Linn.) fry. *Bangladesh J. Fish.*, **11**(1) : 41 - 44.

Dabrowski, K., N. Charlon, P. Bergot and S. J. Kaushik, 1984. Rearing of coregonid (*Coregonus schingi* palea Cuv and Val) larvae using dry and live food. I. Preliminary data. *Aquaculture*, **41** : 11-20.

Gomez, K.A. and A.A. Gomez, 1984. Statistical Procedures for Agricultural Research. 2nd ed. John Wiley and Sons. 679 pp.

Hastings, W.H., 1979. *Fish nutrition and fish feed manufacture. In* : Advances in aquaculture (eds.T.V.R. Pillay and Wm. A. Dill). Fishing News Books Ltd., Farnham, Surrey. pp. 568-574.

Husain, A.K.M.A., 1988. First Feeding of Walking Catfish (*Clarias macrocephalus*). M.Sc. Thesis, Asian Institute of Technology, Bangkok, Thailand. 46 pp.

Jauncey, K. and B. Ross, 1982. A Guide to Tilapia Feeds and Feeding. Institute of Aquaculture. University of Stirling, Scotland, 111pp.

Madhury, R.S. and M.F.A. Mollah, 1990. Artificial feed development for catfish (*Clarias batrachus* L.) larvae. *Bangladesh J. Fish.*, **13** : 41-50.

Mollah, M.F.A., 1987a. Dose response study of HCG in inducing ovulation in *Clarias batrachus* L. *Project Report No. BAU/BARC/MFAM/1/1987.* 5 pp.

Mollah, M.F.A., 1987b. Mass production and rearing of catfish (*Clarias batrachus*) fry. Annual Report (1986-87) submitted to *Bangladesh Agricultural Research Council.* 30 pp.

Rahmatullah, S.M., A. Islam, M.M. Hossain, M.M. Ali and A.K.M.A. Islam, 1983. Experiment on the induced breeding of *Clarias batrachus* (Linn.) by pituitary hormone injection. *Bangladesh J. Aquaculture*, **2-5** (1) : 63-68.

Saha, M.R., M.F.A. Mollah and P. K. Roy, 1998. Effects of formulated diets on growth and survival of *Clarias batrachus* larvae. *Bangladesh J. Fish. Res.* : (accepted).

Verreth, J., V. Storch and H. Segner, 1987. A comparative study on the nutritional quality of decapsulated *Artemia* cyst, micro-encapsulated egg diets and enriched dry feeds for *Clarias gariepinus* (Burchell) larvae. *Aquaculture*, **63** : 269-282.

Winfree, R.A. and R.R. Stickney, 1984. Formulation and processing of hatchery diets for channel catfish. *Aquaculture*, **41** : 311-323.

Zaman, S.M.H., K. Rahim and M. Howlader, 1982. Simple Lessons from Biometry . The Bangladesh Rice Research Institute, Joydebpur, Dhaka, Bangladesh. 160 pp.

(Manuscript received 11 January 1997)

Bangladesh J. Fish. Res., **2**(1), 1998 : 47-54

Studies on the optimum protein to energy ratio of African catfish *(Clarias gariepinus* Burchell*)*

M.A. Hossain*, S. Begum, M.N. Islam[1] and A.K.M.A. Shah

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

Abstract

A laboratory trial was conducted to determine the optimum dietary protein to energy (P/E) ratio of African catfish, *Clarias gariepinus*. The experiment was carried out in a flow-through system for 6 weeks. There were 12 treatments each with two replicates having 10 fish each with a mean initial weight of 1.80 ± 0.02g. Twelve semi-purified diets were formulated with four digestible crude protein levels (23, 26.5, 30 and 33.5%) and three digestible energy levels (2.25, 2.75 and 3.25 Kcal/g). The fish were fed three times daily at satiation level. The results of the study showed that, diet containing 33.5% digestible protein and 2.75 kcal/g digestible energy with a protein to energy ratio of 121.8 (mg protein/kcal) appeared to be best utilized for growth.

Key words : *Clarias gariepinus*, Protein-energy ratio

Introduction

Presently African catfish, *Clarias gariepinus* is one of the important species being cultured in many commercial fish farms in Bangladesh. Because of higher growth rate, the species has gained popularity among fish culturists both in rural and urban areas. Fish like all other animals, require energy to sustain life. The energy gain is primarily dependent on the protein/energy ratio in the diet (Cho and Kaushik 1985). An effective utilization of energy requires a relatively high proportion of non-protein energy in the diet.

A considerable protein is lost in the transformation of food energy to net energy available for metabolism and growth. It is desirable that the largest possible portion of the diet should be used for growth and the energy requirement met as far as possible from the carbohydrates and fats, the absorbed protein can be used principally for the synthesis of new body tissue.

Unless sufficient dietary energy is provided, the quality and quantity of dietary protein can not reflect protein synthesis (Cowey 1978). Excess dietary protein is wasteful and stresses the animal while excess energy means more fatty fish. Again, conditions where energy intake is inadequate, dietary proteins are used as energy source and the production cost increases. Thus, optimum protein to energy (P/E) ratio in the diet is very important to maintain fish quality

and to reduce the dietary cost. The present study was undertaken to determine the optimum dietary protein to energy ratio for fingerlings of *Clarias gariepinus*.

Materials and methods

Experimental system

The experiment was conducted for 6 weeks during the month of October to November'95 in a flow-through system in the laboratory of the Department of Aquaculture, Bangladesh Agricultural University, Mymensingh. Twenty four plastic buckets of 20 l capacity were used as experimental tanks. All the buckets set in the flow-through system were arranged in two rows and kept on a iron frame to facilitate better observation and accessibility. Water supply was from a deep tubewell and water was agitated before being collected in a header tank on the roof of the building. Since it was a continuous flow-through system, no artificial aeration was used in the tanks.

Experimental fish and acclimatization

Fingerlings of African catfish, *C. gariepinus* were collected from the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. Fish were then given a prophylactic treatment with 0.5 ppm $KMnO_4$ solution. Before starting the experiment the fish were acclimatized to the experimental system for one week. During this period the fish were fed formulated pelleted feed containing 35% protein.

Experimental procedure

Twelve treatments were scheduled for the study. Each treatment had two replicates and 10 fish per replicate with a mean initial weight of 1.80 ± 0.02g. Initial and final weight of fish in each tank were recorded individually on an electric balance. The fish were fed three times a day at 9.00, 13.00 and 17.00 h at satiation level throughout the study period. The amount of feed fed by fish was recorded for subsequent calculation of food conversion ratio, protein efficiency ratios and apparent net protein utilization.

Experimental diets

Fish meal was the main protein source used in diet formulation and the analysed proximate composition value of protein, lipid, ash, crude fibre and NFE were 65.18, 11.24, 21.51, 0.5 and 1.57% respectively. Twelve semi-purified diets were formulated with 4 digestible crude protein levels of 23, 26.5, 30 and 33.5% each having 3 digestible energy levels of 2.25, 2.75 and 3.25 kcal/g respectively. The specification and formulation of the experimental diets are shown in Table 1. The digestible coefficient values of protein and energy of fish meal, fish oil, soybean oil and dextrin was based on Rahman (1996) since same ingredients and formulation was used for experimental diets. The digestible energy (DE) value of fish meal, fish oil, dextrin and soybean oil estimated in

terms of Kcal/g of the ingredients were 3.68, 3.22, 4.38 and 4.44 Kcal/g. respectively. The protein digestibility coefficients of fish meal used was 88.05%. The desired protein and energy in the experimental diets were maintained by varying proportion of fish meal, fish oil, soybean oil, dextrin and alfa-cellulose (Table 1). Experimental diets were prepared according to Rahman (1996) and the analysed composition of the prepared diets are shown in Table 2.

Table 1. Specification and formulation of the experimental diets

Diet No.	Digestible protein (%)	Digestible energy (Kcal/g)	P/E ratio (mg protein/ Kcal energy)	Ingredients						
				Fish meal	Fish oil	Soybean oil	Dextrin	* Vitamin & min. premix	Cellulose	CMC
1	23.0	2.25	102.20	39.89	2.00	3.00	17.39	2.00	33.72	2.00
2	23.0	2.75	83.64	39.89	2.00	5.00	0.12	2.00	18.99	2.00
3	23.0	3.25	70.77	39.89	2.00	8.00	41.61	2.00	4.50	2.00
4	26.5	2.25	117.78	46.03	-	3.00	13.35	2.00	33.62	2.00
5	26.5	2.75	96.36	46.03	2.00	5.00	3.29	2.00	19.68	2.00
6	26.5	3.25	81.54	46.03	2.00	8.00	4.78	2.00	5.19	2.00
7	30.0	2.25	133.33	52.16	-	3.00	6.21	2.00	34.63	2.00
8	30.0	2.75	109.09	52.16	2.00	5.00	16.15	2.00	20.69	2.00
9	30.0	3.25	92.34	52.16	2.00	8.00	27.64	2.00	6.20	2.00
10	33.5	2.25	148.89	58.30	-	-	3.42	2.00	34.28	2.00
11	33.5	2.75	121.82	58.30	-	5.00	12.11	2.00	20.59	2.00
12	33.5	3.25	103.08	58.30	-	8.00	8.07	2.00	21.63	2.00

* *According to Rahman (1996). CMC = Carboxymethyl Cellulose (high viscosity)*

Table 2. Analysed proximate composition of the experimental (% dry matter basis)

	DIET NO.											
	1	2	3	4	5	6	7	8	9	10	11	12
Dry matter	94.88	94.90	94.51	94.98	94.20	95.85	94.66	94.65	94.42	94.71	94.51	94.60
Protein	26.23 (23.10)	26.30 (23.15)	25.98 (22.87)	29.94 (26.36)	30.20 (26.59)	30.09 (26.51)	34.00 (29.94)	34.61 (30.46)	34.12 (30.04)	37.82 (30.30)	38.01 (33.46)	38.21 (33.65)
Lipid	9.47	11.46	14.60	8.00	11.40	14.18	9.37	13.07	15.85	6.83	12.16	15.41
Ash	9.23	7.83	8.54	9.30	10.29	9.79	11.58	11.30	10.85	11.87	12.69	12.82
Crude fibre	31.06	16.68	3.78	28.71	18.21	6.46	31.47	18.96	7.21	35.69	18.34	19.31
NFE	24.01	37.73	47.10	24.05	29.86	9.41	13.58	22.06	31.97	9.89	18.80	14.25

* *Figures in the parentheses indicate the digestible protein levels calculated on the basis of digestibility coefficients of fish meal protein used*
Nitrogen free extract calculated as 100 - %(moisture + protein + lipid + ash + crude fibre)

Water quality

The water quality parameters such as temperature was recorded by Celcius thermometer and pH by a pH meter (portable digital pH meter, OSK 1148). Dissolved oxygen was measured using DO meter (Hana HI 8043).

Carcass composition

At the start of the experiment 20 fish from the stock were randomly taken for analysis of proximate composition and considered as initial carcass composition. At the end of the experiment, 5 fish from each replicate was sacrificed for final carcass analysis.

Analytical methods

Feed ingredients, diets and fish samples were analysed for their proximate composition according to standard procedures given in AOAC (1980). Statistical analysis of the data was performed by Analysis of Variance (ANOVA) followed by Duncan's New Multiple Range Test (Duncan 1995).

Results

The proximate composition of the experimental diets are shown in Table 2. The protein content in different diets varied according to their original formulation. The level of lipid, NFE and crude fibre contents in different diets also varied due to variation in amount of ingredients included in diets for keeping the protein and energy contents at desired levels.

The ranges of water quality parameters monitored weekly during the study period were: temperature 25.1-29.2°C, pH 6.7-7.6 and dissolved oxygen 6.6-7.5 mg/l. The ranges of water quality values in the present study are well within the limit for fish life and could not have hampered the growth of fish (Jhingran 1983).

The growth performances and feed utilization by *C. gariepinus* in terms of final weight (g), percent weight gain, specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER) and apparent net protein utilization (ANPU%) are presented in Table 3. It is seen that fish fed diet 11 containing 33.5% digestible protein and 2.75 Kcal/g digestible energy produced significantly ($p<0.05$) the highest growth and feed utilization among the dietary treatment groups. However, there was no significant differences ($p>0.05$) between the weight gain and SGRs of fish fed diets 7, 8, 9, 10 and 12. Fish fed diet 1 (23% protein and 2.55 kcal/g DE) produced significantly ($p<0.05$) the lowest weight gain and SGR among the dietary treatment groups. The SGR values ranged between 3.42 to 4.08 among different treatment groups.

Table 3. Effect of dietary protein to energy ratio on growth parameters and food utilization by *C. gariepinus* fed experimental diets

Diet NO	Initial weight	Final Weight	weight gain	% weight gain	SGR	FCR	PER	ANPU
1.	1.78^{a*}	7.50^{e}	5.72^{e}	321^{e}	3.42^{f}	3.01^{f}	1.26^{bc}	1.46^{b}
2.	1.81^{a}	8.00^{de}	6.19^{d}	342^{d}	3.54^{c}	2.96^{f}	1.20^{bc}	1.75^{b}
3.	1.80^{a}	8.20^{d}	6.43^{c}	356^{d}	3.61^{e}	2.90^{f}	1.33^{b}	22.40^{ab}
4.	1.81^{a}	8.24^{cd}	6.43^{c}	355^{d}	3.60^{dc}	2.92^{f}	1.14^{d}	20.23^{b}
5.	1.81^{a}	9.10^{b}	7.29^{b}	403^{c}	3.85^{c}	2.70^{e}	1.23^{bc}	21.97^{ab}
6.	1.82^{a}	9.15^{b}	7.33^{b}	403^{c}	3.85^{c}	2.52^{d}	1.32^{b}	23.82^{ab}
7.	1.82^{a}	9.26^{b}	7.44^{b}	409^{bc}	3.87^{bc}	2.48^{d}	1.19^{cd}	21.35^{ab}
8.	1.81^{a}	9.50^{b}	7.69^{b}	425^{b}	3.95^{b}	1.96^{ab}	1.47^{a}	26.65^{a}
9.	1.80^{a}	9.42^{b}	7.62^{b}	423^{b}	3.94^{b}	2.00^{b}	1.47^{a}	26.67^{a}
10.	1.80^{a}	9.35^{b}	7.55^{b}	419^{bc}	3.92^{bc}	2.10^{bc}	1.26^{bc}	23.01^{ab}
11.	1.81^{a}	10.06^{a}	8.25^{a}	456^{a}	4.08^{a}	1.82^{a}	1.47^{a}	26.70^{a}
12.	1.80^{a}	9.48^{b}	7.68^{b}	427^{b}	3.96^{b}	2.02^{b}	1.30^{bc}	23.42^{ab}
±S.E.**	0.01	0.16	0.15	5.28	0.03	0.04	0.03	1.41

Figures in the same column having same superscripts are not significantly different (p>0.05)
Standard error (SE) of treatment means calculated from residual mean square in the Analysis of Variance

The mean FCR values with different diets ranged between 1.82 and 3.01 with diet 11 producing significantly ($p<0.05$) the lowest FCR. However, there was no significant difference ($p>0.05$) between the FCR values of diets 8 and 11. Again, there was no significant difference ($p>0.05$) between the FCR values of diets 8, 9, 10 and 12 and these values were higher than those obtained with other diets. The PER values obtained with different diets varied between 1.26 to 1.47 (Table 3). However, diets 8, 9 and 11 had significantly the best PER values. There were no significant ($p>0.05$) difference between the PERs of diets 1, 2, 3, 5, 6, 10 and 12 and these values were higher than that of diets 4 and 7. The apparent net protein utilization (ANPU %) for the diets ranged between 20.23 and 26.70 (Table 3.). The significantly ($p<0.05$) highest ANPU values were obtained with diets 3,5, 6, 7, 8, 9, 10, 11 and 12 and these values were not significantly ($p>0.05$) different among themselves.

The proximate carcass composition of fish at the start and at the end of the experiment is shown in Table 4. The moisture and lipid content was influenced by the dietary P/E ratio. The highest moisture content was observed in fish fed diet 1 and the lowest was with diet 11. The moisture content ranged between 70.20 and 73.40%. The carcass lipid content ranged between 6.60 and 9.20%. The carcass lipid was directly influenced by the dietary lipid content. The highest (17.80%) carcass protein content was observed in fish fed diet 11 and the protein content in different dietary groups ranged between 16.50 to 17.80%.

Table 4. Proximate carcass composition of fish (% fresh matter basis) at the start and end of the experiment

Diet No.	Moisture	Protein	Lipid	Ash
1.	73.40	16.55	6.60	2.71
2.	73.12	16.50	6.88	2.78
3.	73.04	16.49	7.36	2.80
4.	72.66	17.10	7.18	2.92
5.	72.10	17.28	7.56	2.84
6.	71.20	17.44	7.20	2.87
7.	71.42	17.40	8.11	2.90
8.	71.34	17.56	8.20	2.82
9.	70.92	17.41	8.66	2.79
10.	70.24	17.66	9.12	2.86
11.	70.20	17.80	9.05	2.82
12.	70.22	17.21	9.20	2.88
Initial (all fish)	76.44	15.01	5.14	2.66

Discussion

The results of the study revealed that the best growth performance and feed utilization of *Clarias gariepinus* were obtained with feed 11 (33.5% digestible protein and 2.75 Kcal/g energy) with a protein-energy ratio of 121.8. This may be due to the fact that the digestible protein level (33.5%) in diet 11 was optimum to promote growth and the energy (2.75 Kcal/g) of the diet was also adequate for maintenance and growth. The P/E ratio obtained in the present study is similar to that reported by Singh and Bhanot (1987) who found a P/E ratio of 124.8 for *Catla catla*. Catacutan and Coloso (1995) also reported that diet containing 42.5% crude protein and 10% lipid with a P/E ratio of 128 (mg protein/Kcal) was found to be optimum for juvenile (1.34g) sea bass. Reis *et al.*(1989) also found a P/E ratio of 120 in channel catfish, *Ictalurus punctatus* fed diets containing 26, 31, 35 and 39% protein or 91, 107, 120 and 127 mg protein/Kcal digestible energy (DE) respectively for 123 days.

In contrast to the present findings, Mukhopaddhyay *et al.* (1986) observed a significant increase in weight gain, feed efficiency and protein utilization in *Clarias batrachus* fed diets containing P/E ratio of 87.6. Garling and Wilson (1976) demonstrated the optimum P/E ratio for channel catfish to be 88 with a dietary protein levels of 24 to 36%. On the other hand, Winfree and Stickney (1984) reported that optimum protein requirement for channel catfish appeared to be 55% (P/E= 122) at 0.2g size and 54% (P/E= 117) for 1.7g size.

The importance of protein level in relation to the energy level to the diet is very important (Garling and Wilson 1976). The amount of fish meal in diet 11 increased proportionately with the increase in the dietary protein level, which probably gave a best amino acid profile. Lovell (1979) reported that protein levels of 30- 36% will probably be adequate for most warm water fish diets. Use of fish oil and soybean oil as energy source may be another possible factor that influenced the growth performance of fish.

The absence of positive relation between growth and dietary energy level indicates that *C. gariepinus* may probably unable to utilize feeds having higher

protein and at a energy level of 3.25 Kcal/g. Page and Andrews (1973) observed in channel catfish that, bigger fish require more energy and less protein compared to smaller fish. Since in the present study experimental fish had an initial average weight of 1.80g, it is expected that it may be possible to further reduce the dietary P/E ratio for growers.

The growth of fish in different treatments was undoubtedly affected by the protein-energy relationship. Growth increased with the increase in energy at 26% digestible protein level while the similar result was not found with other diets. This finding is similar to that of Lovell (1979) who reported that when fish are fed diets containing too much energy in relation to protein, they will not meet their daily protein need for optimum growth even if they are fed to satiation.

Although diet 10 contained same level (33.5%) of digestible protein with 2.25 Kcal/g energy produced much lower growth of fish which indicates that the energy was not sufficient for growth. This may be due to the fact that fish oil and soybean oil were not added in this diet and relatively low levels of lipid and dextrin were present in the diet (Table 1 and 2) which could not supply the required energy for fish growth and the dietary protein might have been spared for the energy.

In the present study poor growth performance of fish was observed with diets containing lower levels (23, 26.5 and 30%) of digestible proteins at high energy levels. This may be due to the fact that when fish were fed higher energy in relation to protein, they could not meet their daily protein needs for optimum growth even if they were fed to satiation level.

The FCR in this study varied between 1.82 to 3.01. Diet 8 (30% digestible protein) and diet 11 (33.5% digestible protein) with 2.75 Kcal/g energy produced significantly ($p<0.05$) the best FCR values. The FCR values in the present study are higher than the values for channel catfish reported by Garling and Wilson (1976) but lower than the values reported by Das *et al.* (1991) for *Labeo rohita* fingerlings. Reis *et al.* (1989) reported a much lower food conversion ratio of 1.15 in channel catfish fed diet containing 35% protein with a P/E ratio of 120.

The PER values followed similar trend of FCR and ranged between 1.26 to 1.47. The best PER value was obtained with diets 8, 9 and 11. The best PER obtained with 30% digestible protein with P/E ratio of 109.09, 92.34 and 33.5% digestible protein with P/E ratio of 121.82. In general, at each protein level, PER increased as the P/E ratio decreased. The PER values obtained in this study are higher than those reported by Das *et al.* (1991) for *L. rohita*.

The ANPU (%) values in the study ranged between 20.23 and 26.70%. Since the carcass protein content (Table 4) of various fish groups are more or less similar the ANPU values in general tended to reflect the PER values.

Proximate carcass composition of fish was found to be influenced by the P/E ratio. Carcass lipid content increased with the dietary lipid content. Similar observation is reported by Daniels and Robinson (1986) with juvenile red drum (*Sciaenopes ocellatus*) fed diets containing different levels of protein and energy. An inverse relationship between lipid and moisture could be observed as reported earlier (Andrews and Stickney 1972, Garling and Wilson 1976).

The results of the present study showed that diet containing 33.5% digestible protein and 2.75 Kcal/g energy with a protein to energy ratio of 121.8 (mg protein/Kcal) appeared to be best utilized for the growth of *C. gariepinus*.

References

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

Andrews, J. W. and R. R. Stickney, 1972. Interaction of feeding rates, and environmental temperature on growth, food conversion and body composition of channel catfish. *Trans. Am. Fish. Soc.*, **101**(1) : 94-97.

Catacutan, M. R. and R. H. Coloso, 1995. Effect of dietary protein to energy ratios on growth, survival and body composition of juvenile Asian seabass, *Lates calcarifer*. *Aquaculture*, **13** (1-2) : 125-133.

Cho, C. Y. and S. J. Kaushik, 1985. Effect of protein intake on metabolizable and net energy values of fish diets. *J. Nutr.*, **106** : 1547-1556.

Cowey, C. B., 1978. Protein and amino acid requirements of finfish. *In* : EIFAC Symp. *Finfish nutrition and feed technology*. Hamburg, June 1978. EIFAC/78/Symp.R/6.

Daniels, W. H. and E. H. Robinson, 1986. Protein and energy requirements of juvenile red drum, *Sciaenops ocellatus*. *Aquaculture*, **53** : 243-252.

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

Das, K. M., S. N. Mohanty and S. Sarker, 1991. Optimum dietary protein to energy ratio for *Labeo rohita* fingerlings. *In: Fish nutrition research in Asia* (ed. S.S. De Silva). Special Publication No. 5. Asian Fisheries Society. Manila. Philippines. pp. 69-73.

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

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

Lovell, R. T., 1979. Formulating diets for Aquaculture species. *Feedstaffs*, **51** : 29-32.

Mukhopadhyay, P. K., A. Hazra and M. Y. Kamal, 1986. Growth response of *Clarias batrachus* fed with isonitrogenous diets with variable energy levels. *Proc. Nat. Acad. Sci. India.*, **56(B)**, **IV** : 329-334.

Page, J. W. and J. W. Andrews, 1973. Interactions of dietary levels of protein and energy on channel catfish, *Ictalurus punctatus*. *J. Nutr.*, **103** : 1339-1346.

Rahman, M. M., 1996. Studies on optimum dietary protein-energy ratio for Thai sarpunti, *Puntius gonionotus* fry. M. S. Thesis, Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh. 66 pp.

Reis, L. M., E. M. Reutebuch and R. T. Lovell, 1989. Protein to energy ratios in production diets and growth, feed conversion and body composition of channel catfish, *Ictalurus punctatus*. *Aquaculture*, **77**(1) : 21-27.

Singh, B. N. and K. K. Bhanot, 1987. Protein requirement of the fry of *Catla catla* (Ham.). *In: The First Indian Fisheries Forum* (ed. M. M. Joseph). pp 77-78.

Winfree, R. A. and R. R. Stickney, 1984. Starter diets for channel catfish : Effects of dietary protein on growth and carcass composition. *Prog. Fish-cult.*, **46**(2) : 79-86.

(Manuscript received 27 April 1997)

Bangladesh J. Fish. Res., 2(1), 1998 : 55-62

Effects of culture treatments on HUFA levels in *Artemia* cysts

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

Bangladesh Fisheries Research Institute, Brackishwater Station
Khulna-9280, Bangladesh
[1]Dept. of Fisheries Technology, BAU, Mymensingh-2202, Bangladesh

Abstract

Artemia cysts (of GSL, Utah, USA origin) were produced from the modified traditional solar salt works of Bangladesh during winter months through different feeding/fertilization treatments (T_1,T_2,T_3,T_4) were analyzed to understand the effects of treatments on their fatty acid profile. Palmitic, Linolenic, Eicosapantaenonic and Docohexaenoic acids (mg/g.DW) were found highest for the cysts in T_1 (16.0% ±1.36), T_2 (14.7% ±0.47), T_3 (4.7% ±0.40) and T_4 (0.7% ±0.06) treatments, respectively. High amount of 18:3(n-3) acids in the cysts of all sources proves to be freshwater type of the cysts. The presence of marine type essential fatty acids in the cysts of all sources were found low for 20:5n-3 (3.7-4.7%) and very low for 22:6n-3 (0.09-0.7%). No significant variation was observed for 16:0 acids within the treatments, but for 18:3(n-3) acid, the variation was found highly significant (P= 0.0052) between T_2 and T_4 treatments. For 20:5(n-3), only variation between T_2 and T_4 was found insignificant (P=0.1161), but between other treatments, significant variation was observed between T_3 and T_4 (P=0.0241), T_2 and T_4 (P=0.0022) and T_1 and T_4 (P=0.0161). No significant variation was found in other treatments.

Key words : *Artemia*, HUFA, Cyst, EFA

Introduction

The presence of essential fatty acid is the principal factor for the food value of *Artemia* (Watanabe *et al.* 1978a,b) as low levels of the HUFA 20:5w3 in *Artemia* results in low survival and poor growth in all marine fish and crustacean larvae. A detailed biochemical and commercial analysis of *Artemia* from the different sources (Olney *et al.* 1980, Schauer *et al.* 1980, Seidal *et al*. 1980 and 1982, Soejima *et al*. 1980, Leger *et al.* 1985) revealed only one factor, namely the presence of highly unsaturated fatty acids (HUFA) in *Artemia* that unambiguously related to the feed intake of the animals during their grow up (Leger *et al.* 1985, 1986a,b). Watanabe *et al*. (1978a,b and 1980) and Leger *et al.* (1986a) found that the content of 20:5w3 in *Artemia* appears to very not only from strain to strain but also within same strain. It has been shown that food condition greatly determines the fatty acid profile of *Artemia* off-spring. Though HUFA levels of *Artemia* cysts produced in lakes and large ponds (i.e. all

commercial operations) are completely determined by nature, but manipulation of food conditions aiming to increase HUFA levels in *Artemia* off-springs are however, restricted to small system, e.g. intensive pond and tank cultures (Vos *et al*. 1984, Lavens *et al*. 1986). So the production of *Artemia* from the modified solar salt works of Bangladesh by using imported cysts and verification for their qualitative difference due to difference in culture treatment is also important to understand the effect of local culture environment from the cysts origin.

Materials and methods

Source of cysts

Cysts were produced by using GSL (Utah, USA) origin mother cysts through various treatments as follows:

1st year

T_1 Initial : 50 kg urea +20 kg TSP/ha.
Dress up : 25 kg urea + 20 kg TSP/ha/every 7 days.

T_2 Initial : 500 kg chicken manure/ha
Dress up : 250 kg chicken manure/ha/3-4 days interval.

2nd year

T_3 - Same as T_2 of first year.

T_4 - Double the dose of T_3.

Fatty acid profile analysis

Fatty acid profile analysis of the cysts were done on decapsulation basis followed the ICES standard methodology.

Total lipid extraction

Total lipid was extracted according to the procedure of Ways and Hanahan (1964) with some modification. Extraction was performed using a solvent mix made of 2 parts of $CHCL_3$ (chloroform) and 1 part CH_3OH (methanol). CH_2CL_2 was replaced from the original procedure by $CHCL_3$ because lipid may contaminated by water after extraction when CH_2CL_2 used. Finally the amount of fatty acid on dry weight basis was calculated and the lipid after extraction (1 ml of solvent mix consisting of 3 parts of CH_3OH and 2 parts $C_6H_6CH_3$) flushed with nitrogen and closed well and stored in freezer.

Exterification

Exterification was done according to the modification procedure of Lepage and Roy (1984), where total lipid was esterified directly (therefore, the saponification step is superflous). The purpose of this step is to modify the fatty acids into their methyl esters (FAME= Fatty Acid Methyl Ester). This modification was done because FAME- columns have a higher separation efficiency than the HUFA columns making the gas chromatographic FAME analysis more reliable

and accurate than analysis performed on HUFA. The procedure performed saponification and esterification in one step using an acetylchloride/methanol mixture (5:100v/v). FAMEs were than dissolved with 1 ml iso-octane and transfer into a 2 ml vial with screw cap and teflon-faced silicon septaliner and finally flushed with nitrogen and stored in a freezer at -30°C.

Gas-Chromatography analysis

Gas-chromatograph was done in Carlo Erba HRGS 5160 mega series apparatus (carrier gas hydrogen at a pressure of 50 KPa) with Chrompack WCOT fused silica cappilary column (stationary) phase- CP-Sil-88. The standby temperature at 105°C on-column injector was used with a Carlo Erba OC on-column control unit.

Data processing

A spectro-physics SP 4290 integrator was used (for easy manipulation and calculation of data).

Data expression and treatment

The data for each FAME was expressed as percentage of total FAMEs (relative values) and as mg per g dry weight of tissues (absolute values).

Results

Fatty acid analysis (area % and mg/g DW) data of the cysts of all the sources are presented in Table 1(for treatments T_1 and T_2) and in Table 2 (for treatments T_3 and T_4).

Analysis of Variance for palmitic acid, i.e. 16:0 showed F ratio and propability within T_1 and T_2, T_2 and T_3, T_2 and T_4, T_1 and T_3, T_1 and T_4, T_3 and T treatments as 1.0000, 0.3739; 0.0173, 0.9016; 3.3601, 0.1407; 0.9678, 0.3809; 1.5610, 0.2796; 0.0050, 0.9470, respectively.

For linolenic acid, i.e. 18:3 (n-3), F ratio and probability within T_1 and T_2 treatments was found 2.0773 and 0.2230, within T_2 and T_3, 0.4231 and 0.5508, within T_2 and T_4, 30.5636, and 0.0052 (highly significant), within T_1 and T_3, 0.0490 and 0.8356, within T_1 and T_4, 1.9620 and 0.2339, and within T_3 and T_4, 1.1242 and 0.3488, respectively.

In case of eicosapentaenoic acid, i.e. 20:5 (n-3), Analysis of Variance between the treatments showed significant variation between T_1 and T_2 (F=75.7033 and P= 0.0010), between T_2 and T_3 (F=21.1463 and P=0.0100), between T_2 and T_4 (F=41.1835 and P=0.0030), between T_1 and T_3 (F=22.2609 and P=0.0092), between T_1 and T_4 (F=8.0000 and P=0.04747), and between T_3 and T_4 (F=4.0000 and P=0.1161, not significant).

Table 1. Fatty acid content (area % and mg/g of DW) of the cysts produced in the first year

Code	Peak	Treatments			
		T_2		T_1	
		Area %	mg/g DW	Area %	mg/g DW
*1R	14:0	1.6±0.30	1.6±0.46	1.7±0.17	1.7±0.10
*2	14:1(n-5)	1.3±0.00	1.2±0.10	1.2±0.10	1.2±0.12
*3	15:0	0.3±0.06	0.3±0.06	0.4±0.05	0.4±0.00
*4	15:1(n-5)	0.8±0.06	0.7±0.06	0.7±0.00	0.7±0.06
*5	14:2	0.3±0.07	0.2±0.00	0.3±0.00	0.2±0.00
*6 R	16:0	17.7±0.49	16.0±1.36	17.2±0.80	15.6±3.54
*7	16:1(n-7)	6.0±0.92	5.5±1.37	6.6±0.78	6.9±0.62
*8	17:0	1.1±0.05	1.5±0.17	1.0±0.05	1.1±0.00
*9	17:1(n-7)	1.6±0.10	1.5±0.17	1.6±0.10	1.6±0.06
*10	18:0	4.9±0.16	4.5±0.35	4.6±0.50	4.8±0.44
*11 R	18:1(n-9)	21.5±1.00	19.9±1.07	23.3±5.10	24.1±5.02
*12	18:1(n-7)	7.4 ±0.20	7.0±0.36	7.4±0.52	7.5±0.65
*13	18:2(n-6)-t	0.3 ±0.00	0.3±0.00	0.3±0.00	0.3±0.00
*14 R	18:2(n-6)-c	5.5 ±0.14	5.1±0.40	5.4±0.30	5.6±0.23
*15	19:0				
*16	18:3(n-6)	0.5 ±0.12	0.5±0.17	0.5 ±0.06	0.6±0.05
*17	19:1(n-9)				
*18	18:3(n-3)	14.1 ±0.78	13.8±0.62	14.2 ±0.40	14.7±0.47
*19	18:4+19:2	2.7 ±0.10	2.5±0.15	2.8 ±0.15	2.5±0.25
*20	20:0				
*21	20:1(n-9)	0.4 ±0.06	0.4±0.05	0.4 ±0.10	0.4±0.10
*22 R	Internal Standard				
*23	21:0				
*24	20:3(n-6)	0.08±0.01	0.08±0.00	0.09±0.01	0.08±0.00
*25	20:4(n-6)	0.5 ±0.15	0.5 ±0.20	0.7±0.06	0.7±0.00
*26	20:3(n-3)	0.4 ±0.06	0.3 ±0.06	0.3 ±0.06	0.4 ±0.06
*27	21:5	0.4 ±0.00	0.4 ±0.07	0.4 ±0.00	0.4 ±0.06
*28	22:0	0.3 ±0.07	0.2 ±0.00	0.4 ±0.07	0.3 ±0.07
*29 R	20:5(n-3)	2.6 ±0.06	3.7±0.38	3.5 ±0.02	4.7±0.40
*30	22:1(n-9)	0.13±0.06	0.1±0.06	0.1±0.06	0.1±0.07
*31	23:0				
*32	23:1(n-9)				
*33	22:4(n-6)				
*34	22:3(n-3)				
*35	24:0				
*36	22:5(n-3)	0.3 ±0.06	0.4±0.10	0.4±0.06	0.6 ±0.00
*37	24:1(n-9)				
*38 R	22:6(n-3)	0.2 ±0.05	0.3 ±0.15	0.3 ±0.06	0.7 ±0.06
EHUFA	w3>20:3(n-3)	3.0 ±0.12	4.5 ±0.36	4.3 ±0.31	6.1±0.57

Table 2. Fatty acid content (area % and mg/g of DW) of the cysts produced in the second year

Code	Peak	Treatments			
		T_2		T_1	
		Area %	mg/g DW	Area %	mg/g DW
*1 R	14:0	1.4±0.05	1.1±0.21	1.4±0.10	1.2±0.06
*2	14:1(n-5)	1.2±0.10	1.1±0.15	1.4±0.00	1.1±0.06
*3	15:0	0.2±0.05	0.2±0.06	0.2±0.00	0.2±0.00
*4	15:1(n-5)	0.9±0.00	0.7±0.12	0.9±0.00	0.7±0.06
*5	14:2	0.3±0.07	0.2±0.00	0.3±0.00	0.2±0.00
*6 R	16:0	17.3±1.06	14.6±1.77	18.2±0.59	14.8±0.98
*7	16:1(n-7)	4.4±0.32	3.6±0.10	5.0±0.10	4.1±0.09
*8	17:0	1.1±0.10	0.9±0.20	1.1±0.06	0.9±0.10
*9	17:1(n-7)	1.6±0.15	1.3±0.16	1.7±0.06	1.4±0.10
*10	18:0	5.4±0.15	4.4±0.49	5.7±0.59	4.4±0.23
*11 R	18:1(n-9)	25.2±0.17	19.7±2.09	26.5±4.39	21.8±2.57
*12	18:1(n-7)	7.7±0.17	6.2±0.53	7.5±0.36	6.6±0.52
*13	18:2(n-6)-t	0.4±0.00	0.3±0.00	0.4±0.00	0.3±0.06
*14 R	18:2(n-6)-c	.7±0.06	4.7±0.49	5.9±0.15	4.8±0.42
*15	19:0	0.1±0.00	0.1±0.01	0.1±0.00	0.1±0.00
*16	18:3(n-6)	0.4±0.06	0.4±0.06	0.4±0.12	0.4±0.06
*17	19:1(n-9)				
*18	18:3(n-3)	15.6±0.06	12.7±1.38	15.6±0.10	12.9±0.91
*19	18:4+19:2	2.6±0.00	2.1±0.21	2.8±0.15	2.2±0.15
*20	20:0	0.1±0.00	0.1±0.00		
*21	20:1(n-9)	0.4±0.00	0.4±0.06	0.5±0.12	0.4±0.12
*22 R	Internal Standard				
*23	21:0				
*24	20:3(n-6)	0.1±0.00	0.1±0.00		
*25	20:4(n-6)	0.1±0.00	0.1±0.00	0.1±0.01	0.1±0.01
*26	20:3(n-3)	0.4±0.00	0.3±0.06	0.4±0.00	0.3±0.06
*27	21:5	0.5±0.10	0.4±0.10	0.4±0.00	0.3±0.00
*28	22:0	0.2±0.00	0.2±0.00	0.3±0.00	0.2±0.00
*29 R	20:5(n-3)	3.0±0.15	4.2±0.26	2.8±0.12	3.8±0.21
*30	22:1(n-9)	0.1±0.00	0.1±0.01	0.1±0.00	0.1±0.01
*31	23:0				
*32	23:1(n-9)				
*33	22:4(n-6)				
*34	22:3(n-3)				
*35	24:0				
*36	22:5(n-3)	0.2±0.00	0.3±0.06	0.1±0.00	0.1±0.01
*37	24:1(n-9)				
*38 R	22:6(n-3)	0.2±0.06	0.2±0.11	0.1±0.00	0.1±0.00
EHUFA	w3>20:3(n-3)	3.5±0.21	4.8±0.68	3.1±0.29	4.1±0.21

Significant variation for docosahexaenoic acid, i. e. 22:6 (n-3) has been found between T_2 and T_3 (F=12.50000 and P=0.0241), between T_2 and T_4 (F=49.0000 and P=0.0022), between T_1 and T_4 (F=16.0000 and P=0.0161). F ratio and probability for other treatments was found between T_1 and T_2 as 4.5000 and

0.1012, between T_1 and T_3 as 2.0000 and 0.2302 and T_3 and T_4 as 4.0000 and 0.1161, respectively.

Significant variation in HUFA content between T_1 and T_2 (F=63.8790 and P=0.0013), between T_2 and T_3 (F=25.1288 and P=0.0074), between T_2 and T_4 (F=53.1250 and P=0.0019), between T_1 and T_3 (F=9.6154 and P=0.0362) and between T3 and T4 (F=5.7273 and P=0.0749) was observed.

But the variation was not significant between T_1 and T_4 where F ratio and probability was found as 0.4211 and 0.5518, respectively. Highest values for areas (%) mg/g (DW) of palmitic, linolenic, eicosapentaenoic, docosahexaenoic and HUFA of the cysts of treatments T_1,T_2,T_3 and T_4 was found as follows : 18.2±0.59 (T_4 cysts), 16.0±1.36 (T_1 cysts), 15.6±0.10 (T_4 cysts), 14.7±0.47 (T_2 cysts), 3.5±0.02 (T_2 cysts), 4.7±0.40 (T_2 cysts), 0.3±0.006 (T_2 cysts), 0.7±0.06 (T_2 cysts) and 4.0±0.31 (T_2 cysts), 6.0±0.57 (T_2 cysts).

Discussion

The most important fatty acids found in cysts, nauplii and adults of *Artemia* are 16:0 (palmitic), 16:1n-3 (palmitoleic), 18:1n-9 (oleic), 18:2n-6 (linoleic) and 20:5n-3 (eicosapentaenoic). Except for the EFA, most of other acids were found comparatively higher in those cysts produced through single dose organic manure and few acids were found higher in the cysts of double dose treatments. Generally the food value of *Artemia* produced after inoculation in another biotope is not nacessarily the same as the food value of the inoculation materials (Leger 1989), because the main factor influencing the nutritional value of *Artemia* is the content of essential fatty acid 20:5n-3 and the level of contamination by chlorinated hydrocarbons. The fatty acid profile in *Artemia* offspring in mainly determined by the composition of the diet ingested by the parental population, which explains that the food present in the various natural biotopes of *Artemia* is not identical and that the composition of food in one biotope may change in function of time (Leger 1989). So the present finding is in full agreement with the above findings except the variation in 16:0 fatty acid content of the cysts (variation were found insignificant). This might because of the common environmental condition of the production ponds (Lavens and Sorgeloos 1984). Categorisation of *Artemia* cysts on the basis of fatty acid contents (Watanabe *et al*. 1978a,b and 1980) led to place all the cysts into freshwater type as they have high content of 18:3n-3 (Watanabe 1978). However as the minimum content of 20:5n-3 acid in cyst for marine larvae has been determined and recommended as 4% by Navarro *et al*. (1988), so the lower level of requirement for marine predators (for early feeding of fish and shrimp larvae) can also be fulfilled by the present produced cysts, as the content of 20:5n-3 is ranging between 3.7-4.7% and they are also capable to modify the fatty acid in their diet to produce unsaturated fatty acid which they require. Similar observation was reported by Kayama *et al*. (1963), where authors observed conversion of linolenic acid 18:3 (9,12,18) into EFA like

eicosapentaenoic and docosahexaenoic acid. Findings like, presence of eicosapentaenoic acid and presence of docosahexaenoic acid in very small amounts in the cysts is in full agreement with Schauer *et al.* (1980), Cowgill *et al.* (1987). And generally, the presence of higher amount of 20:5n-3 in *Artemia* is associated with a lack of 18:3n-3 (Benijts *et al.* 1975, Claus *et al.* 1977 and 1979, Schauer *et al.* 1980) and was confirmed by the present finding as the cysts of all sources contained higher amount of 18:3n-3 with lower amount of 20:5n-3 acids. Like present finding, high content of 18:3n-3 and low content of 20:5n-3 in GSL strain cysts were also reported by Leger *et al.* (1986a) and placed the cyst under freshwater type. The proportion of 18:2n-6 was found relatively higher in all types of cysts, which is again in agreement with the findings of Watanabe (1978a).

References

Benijts, F., E. Vandoorden and P. Sorgeloos, 1975. Changes in the biochemical composition of the early larval stages of the brine shrimp *Artemia salina* L:1-9. *In*: *Proc. 10th Eu. Sym. Mar. Biol. Vol. 1*. Research in Mariculture at Laboratory and Pilot Scale (eds.G. Persoone and E. Jaspers). Universa Press, Wetteren, Belgium. 620 pp.

Cowgill, U. M., H.W. Emmel, G.U. Boggs, P.G. Murphy, F.M. Gersich and D.A. Bengtson, 1987. Variations in chemical composition of *Artemia* cysts of three geographical locations. *In : Artemia research and its applications : morphology, genetics, strain characterization, toxicology* (eds. G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers). Universa Press, Wetteren, Belgium. pp. 173-188.

Kayama, M., Y. Tsuchiya, J.C. Navenzel, A. Fdlco and J.F. Maed, 1963. Incorporation of linolenic acid in fish. *J. Ann. Oil. Chem. Soc.*, **40** : 499-502.

Lavens, P. and P. Sorgeloos, 1984. Controlled production of *Artemia* cysts under standard conditions in a recirculating culture system. *Aquacultural Eng.*, **3** : 221-235.

Lavens, P., A. De Meulemeester and P. Sorgeloos, 1986. Evalution of mono- and mixed diets as food for intensive culturing of *Artemia*. *In : Artemia research and its applications*. Vol. 3 (eds. P. Sorgeloos, D.A. Bengtsoon, W. Decleir and E. Jaspers). Universa Press, Wettertn, Belgium.

Leger, Ph., 1989. The nutritional value of *Artemia* in aquaculture. Collection of 34 scientific papers (in Dutch). *Artemia* Ref. Cen., State Univ. Ghent, Rozier, Belgium.

Lerge, Ph., P. Sorgeloos, O.M. Millamena, and K.L. Simpon, 1985. International study on *Artemia*. XXV. Factors determining the nutritional effectiveness of *Artemia* : The relative impact of chorinated hydrocarbons and essential fatty acids in San Francisco Bay and San Pabalo Bay *Artemia*. *J. Exp. Mar. Biol. Ecol.*, **93** : 71-82.

Leger, ph., D.A. Bengtson, K.L. Simpson and P. Sorgeloos, 1986a. The use and nutritional value of *Artemia* as a food source. Oceanlgr. *Mar. Biol. Ann. Rev.*, **24** : 521-623.

Leger, Ph., E. Naesens-Foucquaert, P. Sorgeloos, 1986b. International study on *Artemia*. XXXV. Technologies to manipulate the fatty acid profile in *Artemia* nauplii and the effect on its nutritional effectiveness for the marine crustaceans Mysidopsis

bahia (M). *In : Artemia research and its applications.* Vol. 3 (eds. P. Sorgeloos, D.A. Bengtson, W. Decleir and E. Jaspers). Universa press, Wetteren, Belgium.

Navarro, J. C., F. Hontoria, I. Varo and F. Amat, 1988. Effect of alternate feeding a poor long chain poly-unsaturated fatty acid *Artemia* strain and a rich one on sea bass (*Dicentrarchus labrax*) and prawn (*Penaeus kerathurus*) larvae. *Aquaculture,* **74** : 307-317.

Olney, C. E., P.S. Schauer, S. Mclean, You Lu and K.L. Simpson, 1980. International study on *Artemia*. VIII. Comparison of the chlorinated hydrocarbons and heavy metals in five different strains of newly hatched *Artemia* and a laboratory reared marine fish. *In* : The brine shrimp *Artemia*. Vol. 3. Ecology, culturing and use in Aquaculture (eds. G. Persoone, P. Sorgeloos, O. Roels, and E. Jaspers). Universa press, Wetteren, Belgium, pp. 343-352.

Schauer, P., D.M. Johns, C.E. Olney, and K.L. Simpson, 1980. International study on *Artemia*. IX. Lipid level, energy content and fatty acid composition of the cysts and newly hatched nauplii from the geographical strains of *Artemia*. Ibid. pp. 365-373.

Seidal, C. R., J. Kryznowek and K.L. Simpson, 1980. International study on *Artemia*. XI. Amino acid composition and electrophoretic protein patterns of *Artemia* from five geographical locations. P. 375-382. *In:* The brine shrimp Artemia. Vol. 3. Ecology, culturing, use in aquaculture (eds. G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers). Universa press, Wetteren, Belgium. 428 pp.

Seidel, C. R., D.M. Johns, P.S. Schauer and C.E. Olney, 1982. Internation study on *Artemia*. XXVI. Food value of nauplii from reference *Artemia* cysts and four geographical collections of *Artemia* for mud carp larvae. *Mar. Ecol. Prog. Ser.,* **8** : 309-312.

Soejima, T., T. Kayama, and K. L. Simpson, 1980. International study on *Artemia*. XII. The carotenoid composition of eight geographical strains of *Artemia* and the effect of diet on the carotenoid composition of *Artemia:* 613-622. *In:* The brine shrimp Artemia. Vol. 2. Physiology, biochemistry, molecular biology (eds. G. Persoone, P. Sorgeloos, O. Roels and E . Jaspers) Universa press, Wetteren, Belgium. 664. pp.

Vos, J., Ph. Leger, P. Vanhaeck and P. Sorgeloos, 1984. Quality evaluation of brine shrimp *Artemia* cysts produced in Asian salt ponds. *Hydrobiologia,* **108**(1) : 17-23.

Watanabe, T., T. Arakawa, C. Kitajima, K. Fukusho and S. Fujita, 1978a. Nutritional quality of living feed from the view point of essential fatty acids for fish. Bull. Jap. Soc. *Sci. Fish.*, **44**(11) : 1223-1227.

Watanabe, T., F. Oowa, C. Kitajima and S. Fujita, 1978b. Nutritional quality of the brine shrimp *Artemia salina* as a living feed from the view point of essential fatty acids for fish. *Bull. Jap. Soc. Sci. Fish.*, **44** (10) : 1115-1121.

Watanabe, T., F. Oowa, C. Kitajima and S. Fujita, 1980. Nutritional studies in the seed production of fish. IX. Relationship between dietary value of brine shrimp *Artemia salina* and their content of w3 highly unsaturated fatty acids. *Bull. Jap. Soc. Sci. Fish.*, **46** : 35-41.

Watanabe, T., 1987. The use of *Artemia* in fish and crustacean farming in Japan. *In* : *Artemia* research and its applications. Vol. 3 (eds. P. Sorgeloos, D.A. Bengtson, W. Decleir, E. Jaspers). Universa press, Wetteren, Belgium.

(Manuscript received 17 July 1997)

Bangladesh J. Fish. Res., **2**(1), 1998 : 63-67

Gill pathology of juvenile carps in nursery ponds

G.U. Ahmed, M.M. Haque and M.J. Hoque

Faculty of Fisheries, Bangladesh Agricultural University
Mymensing 2202, Bangladesh

Abstract

Gill pathology of juveniles of two Indian major carps *Labeo rohita* and *Cirrhinus mrigala* were studied for a culture period of three months in a private and a government fish farm ponds. Under histopathological observations, only protozoan parasite, *Myxobolus* sp., was recorded as cyst. These myxosporidian cysts were high in the gills of *L. rohita* of the government farm pond followed by *C. mrigala* of the private farm pond. Hypertrophical gill lamellae (primary and secondary) with loss of secondary lamellae were evidenced in *C. mrigala* of privately operated pond.

Key words : Histopathology, *Myxobolus* sp., Carp, Nursery ponds

Introduction

The outbreak of various types of disease is one of the important reasons of reduction in fish production. Maintaining good water quality, nutrition, broodstock, species composition, stocking density etc., i.e., proper management, can control or at least reduce the outbreak of disease. Some fish farmers, fish traders and related people are in the opinion that most of the private fish farms are better managed than the government fish farms, and the fish seeds in the private farms are of good quality for further stocking.

Gills of fish are important sites for prevalence of disease. In Indian major carps, gill diseases are very common. Infestation in the gills of *L. rohita* and *C. mrigala* differ due to difference in feeding habits of the fishes (Sanaullah and Ahmed 1980). The Myxosporidia are cosmopolitan parasites and infect a wide range of both marine and freshwater fishes. Infestations of *Myxobolus, Henneguya* and *Thelohanellus* on the gills of freshwater fish are very common. There have been several recent studies on the pathology of gill infections by Myxosporidia (Aisa 1972). Dykova and Lom (1978) found inflammatory tissue reactions in the gill sphaerosporosis of carps due to invasions by *Henneguya* sp. Sanaullah and Ahmed (1980) found *Myxobolus* sp. infection in adult *C. catla, L. rohita* and *C. mrigala* and described the histopathology of the disease.

The present research has been aimed to investigate the gill diseases through histopathological study of juvenile *L. rohita* and *C. mrigala* from two nursery ponds in different locations and of different management systems.

Materials and methods

The experimental fish samples were collected from two nursery ponds: one pond of a private farm and the other, from a government one. The private one, at the "Jhalak Fish and Shrimp Farm", Gouripur, Mymensingh, was designated as pond-I (area 30 decimals), and the government owned one, at "Fish Seed Multiplication Farm", Trishal, Mymensingh, was designated as pond-II (66 decimals).

To kill the insects and all other animals of the ponds, phostoxin (5 tabs/40 m^2) and rotenone (20 gms/40 m^2) were applied to pond-I and pond-II, respectively. After 7 days of toxin application the ponds were fertilized by urea (100 gms/40 m^2 in pond-I and 200 gms/40 m^2 in pond-II), TSP (90 gms/40 m^2 in pond-I and 100 gms/40 m^2 in pond-II) and cowdung (4.5 kg/40 m^2).

In pond-I, *Labeo rohita*, *Cirrhinus mrigala*, *Puntius gonionotus* and *Cyprinus carpio* (each species in equal number) were stocked at a total density of 70 fingerlings/40 m^2, and in pond-II, only *L. rohita* and *C. mrigala* (1:1) were stocked at a density of 45 fingerlings/40 m^2. Supplemental feed composed of rice-bran and mustard-oilcake (4:1) was given twice a day (around 08.30 h and 15.00 h) at the rate of 5% of the body weight of the stocked fishes in each time in each pond. Overnight soaked oil-cake was mixed with rice-bran and applied to the ponds in dough form.

L. rohita and *C. mrigala* fingerling samples were collected from the ponds fortnightly for three months. In each sampling, 12 fish of each species from each pond were taken for detailed observations of which 4 fish of each species were used for histological study. The gill samples were fixed in 10% formalin and processed in an automatic tissue processor. Four gill-sections were taken for each sampled specimen and were examined under microscope to observe whether they were infected by *Myxobolus* sp. cysts or not. The number of infected fishes was recorded and their percentage among the sampled fishes was calculated. A squash preparation of gill was taken over glass slides and was covered with coverslip before microscopic examination. An average of 30 spores from gill of each fish were measured using dimensions recommened by Lom and Arthur (1989).

Results

The cysts containing spores of *Myxobolus* were mainly located with single secondary lamellae and mostly at their tips (Fig. 1A).

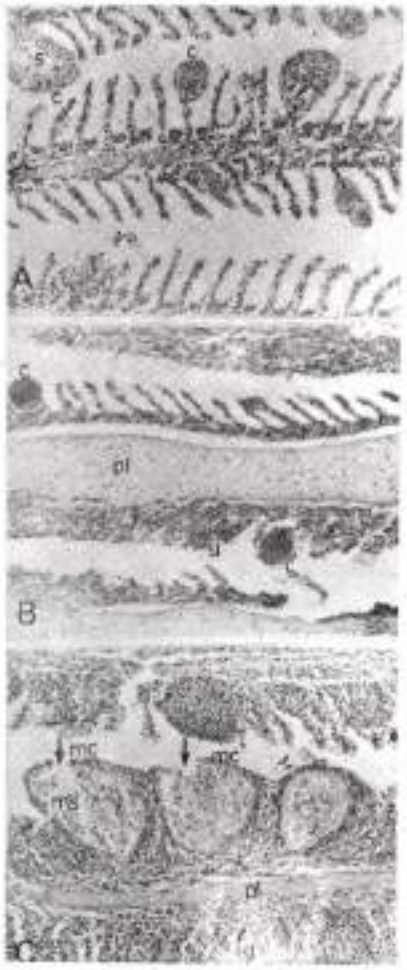


Fig. 1. Cross section of gills of juvenile carps.

A. Cirrhinus mrigala of pond-I. ***c****, cysts at the tip of secondary lamellae;* ***s****, spores within the cysts. Haematoxyln and Eosin (x 118).*

B. Labeo rohita of pond-II. ***pl****, hypertrophied primary lamella;* ***sl****, clubbed secondary lamellae;* ***c****, cysts at the tip of secondary lamellae. Haematoxyln and Eosin (x 115).*

C. L. rohita of pond-II. ***pl*** *and* ***sl****, hypertrophy and hyperplasia in primary (****pl****) and secondary gill lamellae (****sl****);* ***mc****, mature cysts;* ***ms****, myxosporidian spores; arrows indicate ruptured cyst walls. Haematoxyln and Eosin (x 440).*

In respect of gill pathology, more histopathological changes were evidenced in *C. mrigala* when compared with *L. rohita*. In the gills of *C. mrigala*, large *Myxobolus* cysts were recorded at the terminal ends of secondary lamellae where portions of the lamellae were hypertrophied, missing or some were

deformed with inflammatory cells, consisting of thrombocyte, monocyte and other leucocytes (Fig. 1A). Numerous cysts of various sizes of the said protozoan parasite were also noticed with those secondary lamellae. On the other hand, hypertrophied primary lamellae with clubbing secondary lamellae were noticed and a few protozoan cysts were attached with the secondary lamellae of the gills of *L. rohita* (Fig. 1B).

In the gills of juvenile *L. rohita* of pond-I myxosporidian cysts were also found at the tip of the secondary gill lamellae but their number was few in comparison to *C. mrigala*. On the other hand, in pond-II the gills of *L. rohita* were more affected by the protozoan parasites than those of the *C. mrigala* (Fig. 1B-C). The peripheral wall of some cysts were found to be ruptured to release spores of *Myxobolus* species (Fig. 1C). The secondary lamellae lost their normal structure and they were clubbed together (Fig. 1B-C).

The prevalence of cysts in *L. rohita* were 39% (pond-I) and 89% (pond-II) and in *C. mrigala* were 72% (pond-I) and 22% (pond-II).

Discussion

The gills of the juvenile *L. rohita* and *C. mrigala* obtained from both the private and government farm ponds were found to be affected under histopathological observations. The causative agent of carp gill disease in the present experiment was a protozoan parasite, *Myxobolus* sp. which appeared in the gills of both the carp species in the form of cysts. Occurrence of *Myxobolus* sp. in the gills of adult major carps of Bangladesh was first reported by Sanaullah and Ahmed (1980).

The highest prevalence was recorded from the *L. rohita* of government farm which was followed by the *C. mrigala* of private farm. Sanaullah and Ahmed (1980) also observed higher prevalence from *L. rohita* than *C. mrigala*.

Many cysts with swollen secondary lamellae and appearance of inflammatory cells, consisting of thrombocyte, monocyte and other leucocytes, were observed in the gills of *C. mrigala* of the private farm-pond. In the present investigation hypertrophied primary lamellae in the gills of *L. rohita* with clubbing secondary lamellae and considerable number of myxosporiodian cysts were observed. Gerundo *et al.* (1991) while studying the effect of chemotherapy by repeated doses of malachite green found clubbing of the apical end of secondary gill lamellae of rainbow trout, *Salmo trutta*. However, the histopathology caused by *Myxobolus* sp. in both the carps (of both farm) in the present study is generally similar to those described by Sanaullah and Ahmed (1980) and Dykova and Lom (1978).

In the present investigation, the cysts of *Myxobolus* sp. appeared only at the tips of primary and secondary gill lamellae in *L. rohita* and at the tips and/or base of secondary gill lamellae in *C. mrigala* of both ponds. Sanaullah and Ahmed (1980) found parasitic cysts in the distal tips of the primary lamellae to a point

about half way along their length. Crespo *et al*. (1990) found cysts in the gills of amberjack, *Seriola dumerili* Rasso, in the trailing edge of the gill filament and in the interlamellar spaces.

Although the majority of the sampled young carps of the nursery ponds seemed to be healthy by external examination but histological observations showed a good percentage of the fish affected by the gill parasites. Infestation of *Myxobolus* sp. in *L. rohita* were higher in the government fish-farm pond and in *C. mrigala* in the private farm pond. This might be due to the quality of broodstock of the respective species and/or the water quality of the farms. Since fishes of the both ponds were infested by *Myxobolus* sp., though with a difference in intensity of infestation and in fish species, it is difficult to say, on the basis of present study, which pond was better managed. However, farmers should take precautions in maintaining broodstock and water quality to get healthy and disease free spawn and juveniles. No chemotherapeutic measures were taken in the present experiment and would be carried out in successive experiments.

Acknowledgements

The authors gratefully acknowledge the financial support to the research by the Bangladesh Agricultural University and Overseas Development Agency, UK, (BAU-ODA) link project.

References

Aisa, E., 1972. Osservaziani suuna missoboliasi delle Tinche. *Parasitol.*, **14** : 219-225.

Crespo, S., A. Grau and F. Padros, 1990. Epitheliocystis disease in the cultured amberjack, *Seriola dumerili* Rasso (Carangidae). *Aquaculture*, **90** : 197-207.

Dykova, I. and J. Lom, 1978. Histopathological changes in fish gills infected with myxosporidian parasites of the genus *Henneguya*. *J. Fish. Biol.*, **12** : 197-202.

Gerundo, N., D.J. Alderman, R.S. Clifton-Hadley and S.W. Feist, 1991. Pathological effects of repeated doses of malachite green: a preliminary study. *J. Fish. Dis.*, **14** : 521-532.

Lom, J. and J.R. Arthur, 1989. A guideline for the preparation of species descriptions of Myxosporea. *J. Fish. Dis.*, **12** : 151-156.

Sanaullah, M. and A.T.A. Ahmed, 1980. Gill myxoboliasis of major carps in Bangladesh. *J. Fish. Dis.*, **3** : 349-354.

(Manuscript received 16 October 1997)

Bangladesh J. Fish. Res., 2(1), 1998 : 69-72

Survival of antibiotic resistant *Aeromonas* strains in different water conditions

G. R. Banu[1] and M. B. R. Chowdhury*

Department of Aquaculture
Bangladesh Agricultural University, Mymensingh - 2202, Bangladesh
[1]Present address : Fisheries and Marine Resource Technology Discipline
Khulna University, Khulna, Bangladesh
**Corresponding address*

Abstract

Studies were conducted to find out the survival of three antibiotic resistant *Aeromonas* strains in different types of water. The selected *Aeromonas* strains were *A. hydrophila* (local), *A. sobria* (local) and *A. hydrophila* (Thai), which were only recovered from farmed fishes. Seven types of water were used. Among these experimental water, lake water, distilled water and fish farm pond water had supported the long time survival of *A. hydrophila*. In contrast, private fish farm pond water was the most favourable for *A. sobria*. Deionised water was found not to support the survival of any species but Masjid pond water and FRI pond water were found to be moderately suitable for all the species. However, the survival pattern of Thai strain of *A. hydrophila* was found to have similarity with the survival of the local strain of *A. hydrophila*.

Key words : *Aeromonas*, Antibiotic, Water

Introduction

Aeromonas spp. are very important among the major bacterial fish pathogens. Many fish diseases including ulcer disease, haemorrhagic septicaemia, etc. commonly found in freshwater fishes are caused by *A. hydrophila*. *Aeromonas* sp., *Pseudomonas* sp. and *Flexibacter columnaris* were initially suspected to have their involvement in the outbreak of these diseases (Chowdhury 1997). The *Aeromonas* spp. are widely distributed all over the world (Amos 1985). The conditions of aquatic environment is very important for the survival of these bacterial fish pathogens. Any types of environmental water which supports the long time survival may contribute to an easy out break of fish disease (Chowdhury and Wakabayashi 1990). The present study was undertaken to investigate the survival patterns of three *Aeromonas* strains resistant to antibiotics in seven types of waters.

Materials and methods

Aeromonas strains

Three *Aeromonas* strains were used for survival test in this study. These were as follows: 1. *Aeromonas hydrophila* (local), 2. *Aeromonas sobria* (local) and 3. *Aeromonas hydrophila* (Thai). The source of collection and the resistant level to antibiotics of the selected *Aeromonas* strains are shown in Table 1.

Table 1. Selected *Aeromonas* strains used for survival test in different types of water

Strains	Sources of collection			Resistant to the antibiotics
	Farm	Fish	Organ	
A. hydrophila (local)	JFF	*C. mrigala*	Kidney	E, S, OA
A. sobria (local)	TFF	*L. rohita*	Liver	E, SXT, C
A. hydrophila (Thai)	Thailand	*P. gonionotus*	Kidney	E, OA, C

JFF : Jhalak Fish Farm *TFF : rishal Fish Farm* *E : Erythromycin (10mg/disc)*
OA : Oxolinic acid (2mg/disc) *C : Chloramphenicol (30mg/disc)*
S : Streptomycin (10mg/disc) *SXT : ulphamethoxazole (25mg/disc)*

Experimental water

Seven different types of water were used as experimental water for the survival test of the three selected *Aeromonas* strains. These were as follows : 1) Distilled water, 2) Deionised water, 3) JFF pond water, 4) BAU Masjid pond water, 5) Ishakhan lake water, 6) FRI pond water and 7) FF pond water. The waters were used after sterilization by autoclave. Parameters like dissolved oxygen and pH of the relevant water were recorded before and after autoclaving as shown in Table 2.

Table 2. Physico-chemical parameters of the experimental water recorded during the sampling period of survival test

Types of water	Before autoclaving		After autoclaving	
	pH	DO (mg/l)	pH	DO (mg/l)
Distilled water	6.8	7.6	7.1	7.2
Deionised water	7.1	7.2	7.3	7.8
JFF pond water	7.6	8.1	7.8	7.8
Masjid pond water	7.4	8.6	7.4	7.7
Ishakhan lake water	7.4	8.3	7.4	8.1
FRI pond water	7.2	7.8	7.9	7.5
FF pond water	7.5	8.1	7.8	7.8

DO : dissolved oxygen *FF : Faculty of Fisheries*
JFF : Jhalak Fish Farm *FRI : Fisheries Research Institute*

Procedures of survival test

Individual experimental *Aeromonas* were cultured on Tryptone Soya Agar (TSA, Oxoid Ltd., UK) plate. Then a sample of freshly cultured (18 to 24 hours) inoculum weighing 20 to 30 mg was taken into the sterile test tube containing 3 to 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 dilution's were made in sterile relevant experimental water. From each dilution 0.1 ml was taken for incubation on TSA plate and was spread it by sterile L-shaped glass rod. Then the plates were placed at 25°C in the incubator for 24 to 36 hours to incubate. After incubation, the number of bacterial colonies were determined at 0 day (immediately after incubation), 1 day, 3 day, 5 day, 7 day and 10 day until completion of the experiment (Islam and Chowdhury 1997). In each circulation duplicate plates were used and bacterial load was calculated.

Results and discussion

Survival of A. hydrophila (local)

It was observed that Ishakhan lake water, FF pond water and distilled water had supported the long time survival of this strain for a longer period as no considerable variations in the total load of bacteria had been noticed in those experimental water during experimental period. Deionosed water did not support the survival of this strain. Water of JFF pond, FRI pond and Masjid pond water supported moderate survival of the strain.

Survival of A. sobria (local)

Among the seven types of experimental water, distilled water and JFF pond water were found to be more suitable to support the survival of this strain, as in both cases the total load of *A. sobria* did not show any significant variation during the experimental period. But sharp variation in total load from the initial day to the 10 day of the experimental period in case of deionised and Ishakhan lake water signifies their unsuitability for prolonged survival of this strain. FRI pond water, FF pond water and Masjid pond water were found to be moderately suitable to support the survival of this strain.

Survival of A. hydrophila (Thai)

It was observed that Ishakhan lake water, FF pond water and distilled water had supported the long time survival of this strain. Deionised water did not support the survival of this strain. JFF pond water and FRI pond water supported moderate survival of the strain as in both cases slight variations in total load had been observed.

The results of the present study showed that Masjid pond water and Ishakhan lake water supported prolonged survival of local strains of *A. hydrophila*. The reasons might be the high concentration of dissolved oxygen and favourable pH of the water in those water bodies. JFF pond water supported good survival of *A. sobria*. Deionised water did not support the survival of either of the strains and distilled water supported the survival *of A. hydrophila* in varying degrees which agree with the findings of Chowdhury and Wakabayashi (1990). Chowdhury (1997) investigated the survival of some selected isolates of *Aeromonas* and *Pseudomonas* in different water conditions. The results of the present study were similar to the results of Chowdhury's works.

Comparison of distilled water with pond water in the survival of *Aeromonas* strains, revealed that pond water was better than distilled water, though both were freshwater. The source of pond water is considered to be similar to the water of the aquaculture facilities. Pond water may contain some trace elements and rich in nutrients which probably helped *Aeromonas* strains in its long time survival. Deionised water lacking any such nutrients may have failed to produced this effect. River water, pond water and saline water were found to be the most suitable for long term survival of *Pseudomonas* strains (Islam and Chowdhury, 1997). 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 0.5-5.0% Nacl.

The present study provides information about the survival of *Aeromonas* strains recovered from the aquaculture facilities in Bangladesh and a fish farmer/scientist could utilise this knowledge in order to reduce the incidence or density of the bacteria from his farmed pond. Further studies are necessary to know the pathogenicity of these *Aeromonas* strains and to find out an appropriate control measures against these strains.

References

Amos, K. H., 1985. Procedures for the detection and identification of certain fish pathogens. 3rd ed. Fish Health Section, *Am. Fish. Soc. Coravallis, Oregon.* 114 pp.

Chowdhury, M. B. R., 1997. Bacterial involvement in fish disease in Bangladesh. International Symposium on Diseases in Marine Aquaculture, Japaness Society of Fish Pathology, 3-6 October, 1997, Hiroshima, Japan.

Chowdhury, M. B. R. and H. Wakabayashi, 1990. Survival of four major bacterial fish pathogens in different types of experimental water. *Bangladesh J. Microbiol.*, **7(1)** : 47-54.

Islam, M. S. and M. B. R. Chowdhury, 1997. Survival of antibiotic resistant *Pseudomonas* strains in different types of water. *Bangladesh J. Fish. Res.*, **1**(2) : 39-45.

Muroga, K. and M. Tatani, 1982. Isolation of *Vibrio anguillarum* from juvenile red sea bream (*Pargus major*). *Fish Pathol.*, **16** : 211-214.

Wakabayashi, H. and S. Egusa, 1972. Characteristics of a *Pseudomonas* sp. from an epizootic of pond cultured eels (*Anguilla japonica*). *Bull. Jap. Soc. Sci. Fish.*, **38** : 577-587.

(Manuscript received 28 October 1997)

Bangladesh J. Fish. Res., **2**(1), 1998 : 73-82

Effect of salt on the level of histamine in preserved fish (Herring, *Clupea harengus*)

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

The University of Hull
International Fisheries Institute, Hull, England
[1]*Present address : Dept. of Fisheries Technology, BAU, Mymensingh-2202, Bangladesh*
**Corresponding author*

Abstract

Histamine levels in batches of heavily salted (fish:salt ratio 4:1) herring (*Clupea harengus*) were monitored during ripening at 4°C and 25°C. The batches studied were prepared from both pre-spawning and post-spawning (spent) fish using new and used salt. Salt levels in the flesh, which reached 11 to 14% (wet weight basis) during the ripening period, were found to retard histamine formation. During normal spoilage of ice chilled fish, histamine levels had been reported to exceed 50mg/100g flesh as it approached the limit of edibility whilst, in the heavily salted fish, levels remained below 20mg/100g flesh throughout the ripening periods of 18 months for the 4°C batches and 3 months for the 25°C batches. This was the case when the samples were set up and the salt allowed to penetrate the flesh at 4°C. When, however, the samples were set up and initially stored at ambient (10-15°C) temperature the histamine levels in the flesh rose above 20mg/100g before enough salt had penetrated to inhibit its generation. The gradual rise in levels which, nevertheless, occurred over the ripening periods followed significantly (5% level of significance) different trends, being greater in the batches prepared from pre-spawning than those from spent fish.

Key words : Histamine, Herring, Salt, Preservation

Introduction

The brine salting process has long been considered as an effective method of oily fish preservation inhibiting the oxidation of lipid which would take place under dry salting. This process in its various forms, is practiced in many parts of the world. Although heavily salted fish is now less popular in Western Europe than the other alternatives available throughout the year, certain products such as maatje cured herring remain popular. Maatje herring (lightly salted herring) is gaining popularity in many countries of Europe. Barrel salted herring is still produced in some parts of the United Kingdom, but most is produced in the Scandinavian countries.

In Bangladesh, *Hilsa ilisha*, is brine salted in much the same way as herring in Northern Europe except that the process is carried out at ambient

temperature so that the products ripens to "maturity" in about 2 months compared to the 9-18 months required for the herring product ripened at low temperature. For this reason the two experimental storage / ripening temperatures (4°C and 25°C) were chosen. A large proportion of the reported UK incidence of food poisoning, which have implicated fish, have been scombroid poisonings from the consumption of scombroid and clupeid species, which may have been less-than-fresh. Whilst there is doubt as to whether histamine is the cause of this type of poisoning (Clifford and Walker 1992, Ahmed 1992) its level in the fish flesh is still regarded as a good indicator of the freshness and of the likelihood of their having been subjected to any temperature abuse during handling. For this reason, Council Directive 91/493/EEC (1991) relating to the conditions required in the production and trading of fish product for the EC, specifies a maximum allowable limit (MAL) of 20 mg/100g flesh (with the mean value for 9 samples not to exceed 10mg/100g flesh) (EEC 1990). In view of the increasing attention being paid to biogenic amines by health and regulating authorities the present study was undertaken to study histamine level during the ripening of barrel salted herring. Factors which might influence histamine formation such as catching season, presence or absence of viscera, salt purity and storage temperature were investigated.

Literature on the formation of histamine in foods suggests that the decarboxylation of histidine results in the production of histamine. Early workers believed that histamine was generated due to the activity of the enzyme histidine decarboxylase, although the production rate varied widely under what were regarded as normal, optimum ripening conditions (Kimata 1961, Ferencik 1970, Yoshinaga and Frank 1982). Proteolysis, either autolytic or bacterial, has been suggested on playing a role in the release of free histidine from tissue protein (Ababouch *et al.* 1991). Some species of fish of the scombroid and clupeid families have large amounts (in order of 1% of the fish of wet weight) of free histidine (Hibiki and Simidu 1959, Ababouch *et al.* 1991) normally in their muscle tissue. This serves as a substrate for bacterial histidine decarboxylase. Studies on the effect of salt on histamine formation showed that histamine can form in vacuum-packaged, lightly-salted herring fillets stored at in-store, refrigerated display temperatures, particularly after contamination with psychrophilic *Photobacterium* spp. (Van Spreekens 1986). Mackerel stored at 5°C, even in the presence of 2% salt showed markedly increased histamine content following a prolonged refrigerated display period (Yamanaka *et al.* 1985). The effect of higher salt concentrations on histamine formation in fish is less known. In this paper the formation of histamine in heavily salted herring where the fish to salt ratio is 4:1, is reported.

Materials and methods

Source of fish

Herring (*Clupea harengus*) used in the study was provided from the purse seining vessels of Alexander Buchan Ltd., Peterhead, Scotland. Pre-spawning herring were caught from the nearby Piper field in mid-July and post-spawning (spent) herring from off Scarborough of UK in late September. The herring was kept in RSW tanks at -2°C inside the purse seining vessels but were landed within 24 hours of capture at the Peterhead processing factory. The catch was unloaded by an on-board winch operated scoop net digging the catch out of the RSW tanks and transferring them via a hopper to polypropylene tanks of about 1 m^3 capacity which were fork-lifted to the adjacent processing factory. At this stage the temperature of the fish was 0 to 2°C . In the factory the herring for the investigation were immediately processed according to the scheme selected to examine the effects of salt on histamine level under different condition i.e., before and after spawning, evisceration, salt purity etc.

Source of salt and additives

Pure salt (industrially processed new salt NaCl) and used salt both from the same source was collected from the Anderson's (Fish Merchants) of Peterhead. The used salt had been collected after its use in the dry salting of cod. Potassium sorbate was supplied by Merck Ltd., UK and nisin was supplied by Aplin & Barrett Ltd., UK.

Processing of fish

Both the pre-spawning and spent fish were processed into 4 different groups as follows:

1. Whole fish + new salt (with potassium sorbate and nisin)
2. Whole fish + used salt
3. Gibbed fish + new salt (with potassium sorbate and nisin)
4. Gibbed fish + used salt.

The sample were prepared as described below for maturation (ripening) at 4°C in polypropylene barrels and in polyethylene pouches (Synclair 5-layer pouches Type DBFI; Gee pack, UK). Gutting was carried out to remove gills and viscera in such a way that the pyloric caeca remained in the fish. A ratio of 1 part of salt to 4 parts (by weight) of fish was used in the preparation of all samples. After "rousing", (the hand mixing of fish and salt) the herring were packed into the barrels in the traditional manner. First a layer of fish was placed with belly uppermost and head to tail until the layer was complete. A layer of salt was placed on top and a new layer of fish laid at right angles to the layer beneath. These alternate layers of salt and fish were continued until the barrel was full. An extra two layers of fish and salt were laid on the top of the filled barrel . Within two days of holding at 4°C, the herring inside the barrel had settled down, due to

the pickle formation, and the extra layers had become immersed in the pickle. In a subsequent experiment, this pickle formation stage was allowed to proceed at ambient (10-15°C) temperature before allowing the ripening to proceed at the selected storage temperatures. The barrel was then closed and made airtight with a galvanised steel collar. To suppress bacteria and mould growth, nisin and potassium sorbate had been mixed with the new salt at a dosage level of 20 ppm and 1000 ppm respectively. Each barrel on closing contained 100 kg of fish and 25 kg of salt. In the same way 260 polyethylene pouches for each batch were prepared by vacuum sealing 1 kg fish and 250 g of salt in each pouch. Other conditions were exactly the same as for the barrel-salted fish.

Similarly 8 plastic tubs (4 for prespawning and 4 for spent fish) and 60 polyethylene pouches for the prespawning batch and 60 for the spent batch were prepared for storage at 25°C . Other conditions were exactly same as for the fish stored at 4°C.

All of the experimental materials were transported from Peterhead of Sctoland to Hull, England by insulated vehicle, which took nearly 24 hours. Final storage was performed at Hull until ripening. During the first 2 weeks of storage the barrels were rolled every other day to ensure uniformity of admixture and prevent the fish sticking together.

At 4°C storage, the barrels were opened once in every three months for sampling but the polyethylene pouch stored fish were sampled every month. At 25°C storage, the tubs were opened for sampling at the end of ripening (2-3 months) and the polyethylene pouch stored fish were sampled every month they were judged to have achieved maturity/ripening.

In the subsequent experiment, the herring were allowed to ripen in smaller plastic tubs as well as 60 kg barrels so that sampling could be performed every week, whilst leaving some tubs and barrels unopened for long-term storage. The fish in these containers could, thereby, ripen normally without suffering the effects from air ingress at sampling times.

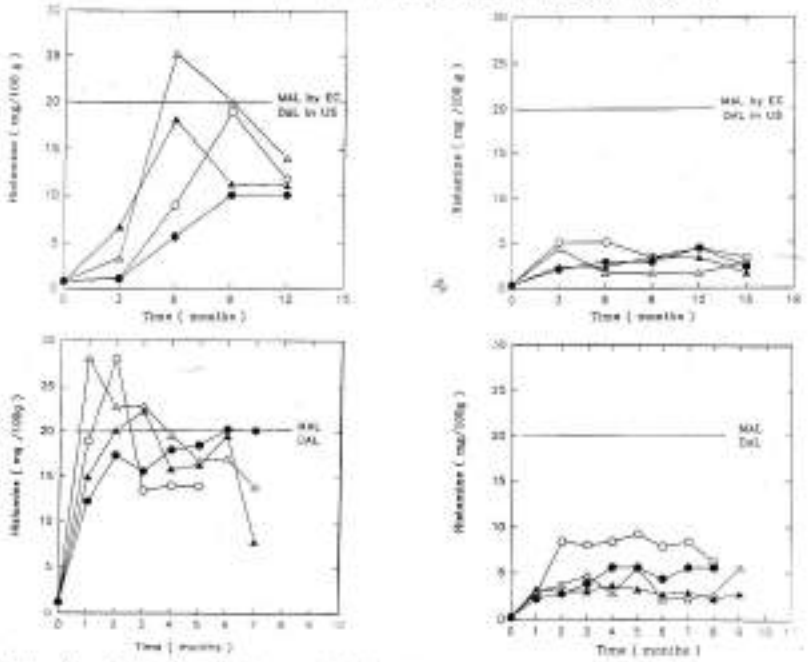
Analysis

The histamine content of the maturing salted herring was determined according to the method of Hardy and Smith (1976) which was based on the principle of colorimetric determination by coupling with a diazonium salt. The moisture content was determined according to the AOAC (1965) method. The salt (NaCl) content was determined by accurately weighing 25 g of fish flesh (homogenised from at least 6 fillets) and blending with 225 ml of cold water. This was filtered and 10 ml of aliquot was titrated against 0.1N $AgNO_3$ using potassium chromate as indicator. The percentage of NaCl in fish flesh was calculated from the relationship 1 ml 0.1N $AgNO_3$ = 0.005845 g NaCl.

Paired t-test were carried out to assess the significance of the experimental results according to Miller and Miller (1988).

Results and discussion

In the herring stored at low temperature (4°C) the histamine levels of the pre-spawning batches increased with storage time up to 6 months for the gibbed fish, and up to 9 months for the whole fish, then gradually decreased as the fish continued to ripen (Fig. 1). Less histamine was produced in fish treated with new salt than in those treated with used salt. There were differences in histamine level among the 4 different groups of herring during barrel salting but all those where ripening had been allowed to proceed at 4°C contained acceptable levels of histamine at the end of ripening/maturation. In the spent batch at the same storage temperature (4°C), histamine levels were found to be considerably lower than those of the fish of prespawning batch (Fig. 2). Very little difference in histamine level were found among the 4 different groups of spent fish during the ripening/maturation period. Similar trends were observed in the fish stored in vacuum-sealed polyethylene pouches (Figs. 3 and 4).



● Whole fish + New Salt (with additives) □ Whole Fish +Used Salt MAL-Maximum allowable limit by EC
▲ Gibbed fish + New Salt (with additives) △ Gibbed Fish+Used Salt DAL- Defect action level by US FDA

Figs. 1-4. Levels of histamine during the ripening of (1) pre-spawning, (2) spent herring (barrel salted), (3) pre-spawning and (4) spent herring (polyethylene pouch salted).

The batches where ripening had been allowed to proceed at 10-15 °C temperature were found to have unacceptably high histamine levels (ranging between 16 and 77mg/100g flesh) when first sampled, after 30 days storage (Figs. 5 and 6).

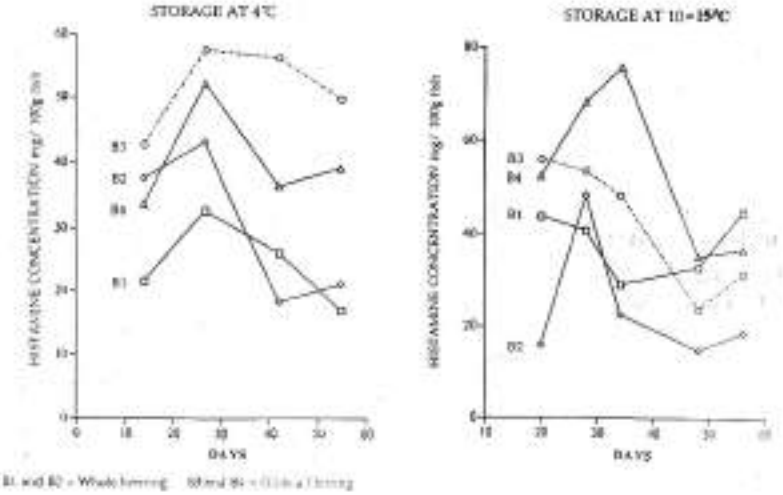


Fig. 5-6. Levels of histamine during the early ripening of pre-spawning herring barrel salted and kept 2 days at ambient temperature.

Ritchie and Mackie (1980) studied the histamine level in ice-stored herring (at 1 °C) where histamine reached 50mg/100g flesh within 17 days of storage.

Farnandez-Salguero and Mackie (1987) observed 53mg/100g in whole fish and 52mg/100g in fillets of herring by the 7th day of storage at 5 °C. In the present study the barrel salted fish were allowed to mature for 12-15 months and those for in the polyethylene pouches for 7-9 months of storage. During this extended period, histamine levels remained below the EC Council Directive MAL and the US Food and Drug Administration Defect Action Level (DAL). High salt concentrations, therefore, appear to effective in retarding histamine formation in herring.

The results of the present study also show histamine levels in stored fish vary with the catching season. They were found to be much lower throughout the ripening period in the spent fish. Variations in the histamine level with season in mackerel have been reported by Smith *et al.* (1980). Such variations were suggested as being due to the differences in the activity of intrinsic enzymes. The pre-spawning fish were caught in July and were still in active feeding condition and, for this reason, the proteolytic enzymes may have been more active than in the fish caught at the end of September, soon after spawning. The effect of gibbing on histamine formation, however, is not clear. In the pre-spawning herring, histamine levels trend in the barrel salted samples were contrary to those salted in the polyethylene pouches. It might have been expected that the presence of viscera would lead to higher histamine levels. Yet, the ambient-prepared batches showed higher histamine levels in the gibbed than in whole herring over start of the ripening period. This would probably be due to the spread of gut contents over the belly cavity in the process. The

contradictory results obtained, however, leaves the question of whether the enzymes from the gut bacteria play a significant role in histidine decarboxylation during 4°C ripening unanswered.

Nevertheless, differences in the levels of histamine in the batches treated with new salt and used salt suggested that histidine is being decarboxylated through the activity of both tissue enzymes and bacterial enzymes. Batches prepared with new salt were treated with antibacterial and antimould agents whilst those prepared with used salt were not. The former were found to contain lower concentrations of histamine than the latter. This differences was found in all groups of experimental fish. In the fish stored with pure salt (with antibacterial and antimould agents) the role of bacterial enzymes in histamine generation would have been expected to be reduced. Histamine formation in these fish therefore would be largely due to the activity of the intrinsic enzymes of fish. The level of histamine in the fish treated with used salt was always higher than the level of fish treated with new salt. It is to be expected that the used salt would be contaminated by halophiles which, over a long period of storage, might remain marginally active and contribute to histamine formation along with the intrinsic enzymes of the fish.

The decline in the histamine level after a certain period of storage, firstly, may be due to some of the histamine formed in the flesh being leached out into the surrounding brine and, secondly, some may enter into another biochemical pathway, for example, taking part in reactions with aldehyde groups of simple sugars or lipid oxidation products.

At higher temperature storage (25°C) the histamine levels in the fish from the plastic tubs at the end of ripening were very similar to those from the barrels kept at low temperature (4 °C). Table 1 shows the results obtained at the end of ripening (2 months) of the 4 different groups of fish from prespawning and spent batches. In the prespawning fish, which were allowed to ripen at 25°C, less histamine was produced in fish treated with new salt (with potassium sorbate + nisin) than in those treated with used salt. Histamine levels were below the specified MAL, although there were differences in histamine level in the 4 different groups of fish. In the spent batch (at 25°C) histamine levels were found to be lower than those of the fish of the prespawning batch (Table 1). The difference in histamine levels among the 4 different groups of spent fish was very small. The same trends were observed in the fish stored in vacuum-sealed polyethylene pouches (Table 2 and 3) although the level went slightly above the specified MAL in some samples of fish from the prespawning batch (Table 2) during the first weeks of the ripening period. Only in the ambient-prepared samples did the histamine levels substantially exceed the MAL. In these samples the bacterial activity associated with spoilage would have proceeded rapidly until sufficient salt had penetrated through the tissues to inhibit the further proliferation.

Table 1. Histamine contents of the fish ripened in plastic tubs at 25°C

Sample	Histamine (mg/100g)	
	Prespawning	Spent
Whole fish + new salt	10.85	3.35
Whole fish + used salt	12.95	3.87
Gibbed fish + new salt	13.32	1.04
Gibbed fish + used salt	17.35	1.75

Table 2. Histamine levels of the pre-spawning fish ripened in polyethylene pouches at 25°C

Storage period (month)	Histamine (mg/100g)			
	Whole fish + new salt	Whole fish + used salt	Gibbed fish + new salt	Gibbed fish + used salt
1	18.36	19.22	21.13	26.34
2	21.60	23.29	17.24	18.23
2 1/2	16.60	17.60	16.84	18.00

Table 3. Histamine levels of the spent fish ripened in the polyethylene pouches at 25 °C

Whole fish + new salt	Histamine (mg/100g)			
	Whole fish + new salt	Whole fish + used salt	Gibbed fish + new salt	Gibbed fish + used salt
1	6.76	8.42	2.89	3.4
2	9.29	10.32	3.83	4.6
3	8.79	10.12	1.80	2.7

The main conclusions of the present study are that:

(i) Low temperature (4 °C) and high salt concentration retard histamine formation in fish flesh.

(ii) If the fish are salted at high (above 15 °C) ambient temperatures, the specified MAL for histamine in the flesh is likely to be exceeded during the first two weeks of the process.

(iii) Even at the high salt concentration, histamine is still produced by the activity of the intrinsic enzymes of fish, but, unless histamine levels in the raw material are high or become high during the period when the salt was

diffusing into tissues, these levels are likely to remain below the specified MAL upto the time of maturity.

(iv) After the salt concentration in the fish flesh has become high enough to inhibit the growth of histidine decarboxylating bacteria, storage temperature has little effect on the histamine formation.

(v) Spent herring is better than the prespawning herring for salt preservation if the lowest histamine levels are considered to be the most desirable attribute but spent fish fails to produce a ripened product with the desired characteristic sensory attributes, compared to those of prespawning fish.

Acknowledgements

This work was carried out as part of an Overseas Development Administartion supported Ph D programme at Hull University of England and is being continued with the support of the EC AIR₃project AIR-CT93 1141, "The Enzymatic ripening of pelagic fish species". The fish was kindly supplied and prepared by Alexander Buchan Ltd., Denholm Seafood Group, Peterhead, Scotland.

References

Ababouch, L., M.E. Afilal, H. Benadeljelil, F.F. Busta, 1991. Quantitative changes in bacteria, amino acids and biologenic amines in Sardine (*Sardina pilchardus*) stored at ambient temperature (25-28 °C) and in ice. *Int. J Food Sci. Technol.*, **26** : 297-306.

Ahmed, F.E., 1992. *Response. Int. J. Food Sci. Techogl.*, **27** : 725-726.

AOAC, 1965. Official Methods of Analysis. *Association of Official Agricultural Chemists.* 10th ed, Washington, D.C.

Arnold, S.H. and W.D. Brown, 1978. Histamine toxicity from fish products. *Adv. Food Res.*, **24** : 113-154.

Clifford, M.N. and R. Walker, 1992. The aetiology of scombrotoxicosis. *Int. J. Food Sci. Technol.*, **27** : 721-724.

EEC, 1991. Council directive 91/493/EEC. *Official J. of the EC.* **L268** : 15-36.

Ferencik, M., 1970. Formation of histamine during bacterial decarboxylation of histidine in the flesh of some marine fishes. *J. Hyg. Epidemiol Microbiol. Immunol.*, **14** : 52-60.

Fernandez-Salguero, J., I.M. Mackie, 1987. Comparative rates of spoilage of fillets and whole fish during storage of haddock (*Melanogrammus aeglefinus*) and herring (*Clupea harengus*) as determined by the formation of non-volatile and volatile amines. *Int. J. Food Sci. Technol.*, **22** : 385-390.

Frank, A.H., 1985. Histamine forming bacteria in tuna and other marine fish. *In : Histamine in marine products: production by bacteria, measurement and prediction of formation* (eds. B.S. Pan and D. James). FAO *Fish Tech Paper.*, B25 : 2-3.

Geiger, E., G. Courtney, G. Schanakenberg, 1944. The content and formation of histamine in fish muscle. *Arch Biochem Biophys.*, **3** : 311-319.

Hardy, R., J.G.M. Smith, 1976. The storage of mackerel (*Scomber scombrus*). *J. Food Agric.*, **27** : 595-599.

Hibiki, S., W. Simidu, 1959. Studies of putrefaction of aquatic products, 26. Spoilage of fish in the presence of carbohydrate. *Bull. Jap. Soc. Sci. Fish.*, **24** : 913-915.

Kimata, M., 1961. The histamine problem. *In* : *Fish as food* (ed. G. Borgstrom). Academic Press, New York, **1** : 329-352.

Miller, J.C. and J.N. Miller, 1988. Significance tests. *In* : Statistics for analytical chemistry, 2nd ed. Ellis Horwood Limited, Chichester. pp. 53-77.

Ritchie, A.H. and I.M. Mackie, 1980. The formation of diamines and polyamines during storage of mackerel (*Scomber scombrus*). *In* : Advances in fish science and technology (ed. J. J. Connell). Fishing News Books Ltd. England. pp. 489-494.

Smith, J.G.M., R. Hardy, and K.W. Young, 1980. A seasonal study of the storage characteristics of mackerel stored at chill and ambient temperature. *In* : Advances in fish science and technology (ed. J.J. Connell). Fishing News Books Ltd., England. pp. 372-378.

Sumner, S.S., M.W. Speckhard, E.B. Somers, and S.L. Taylor, 1985. Isolation of histamine producing *Lactobacillus buchneri* from cheese implicated in a food-poisoning out break. *Appl. Environ. Microbiol.*, **50** : 1094-1097.

Taylor, S.L., L.S. Guthertz, M. Leatherwood, F. Tillman and E.R. Lieber, 1978. Histamine production by food-borne bacterial species. *J. Food Safety.*, **1** : 173-178.

Taylor, S.L., L.S. Guthertz, M. Leatherwood and E.R. Lieber, 1979. Histamine production by *Klebsiella pneumoniae* and an incident of scombroid fish poisoning. *Appl. Environ. Microbiol.*, **37** : 247-276.

Van Spreekens, K.J.A., 1986. Histamine production by the psychropfilic flora. *In* : *Seafood quality determination* (ed. D.E. Kramer and J. Liston). Elsevier Science Publishers, Amsterdam. pp. 209-318.

Yamanaka, H., K. Itagaki, T. Kikuchi and M. Okuzumi, 1985. Influence of the concentration of sodium chloride on the meat of mackerel. *J. Tokyo Uni. Fish.*, **75** : 51-56.

Yoshinaga, D.H. and H.A. Frank, 1982. Histamine producing bacteria in decomposing skipjack tuna (*Katsuwonus pelamis*). *Appl. Environ. Microbiol.*, **44** : 447-452.

(Manuscript received 19 October 1997)

Bangladesh J. Fish. Res., 2(1), 1998 : 83-90

Population dynamics of *Harpodon nehereus* (Ham.-Buch.) from the Kutubdia channel of Bangladesh

M.G. Mustafa[1], M. Zafar*, A.K.M. A. Matin and S.M. N. Amin

Institute of Marine Science, Chittagong University, Chittagong, Bangladesh
[1]Present address : Fisheries Research Institute, Marine Station, Cox's Bazar-4700, Bangladesh
**Corresponding author*

Abstract

ELEFAN-0, ELEFAN-I, ELEFAN-II were used to estimate the parameters of population of *Harpodon nehereus* from length- frequency data collected from Kutubdia channel of Bangladesh coastal water. The $L\alpha$ and K were found to be 24.48 cm and 1.50/ year. The annual rate of natural mortality (M) and fishing mortality (F) were found to be 2.46 and 3.27 respectively. The rate of exploitation (E) was estimated as 0.57. The mean length at first capture (Lc) was estimated as 6.747 cm. This species was recruited in the fishery during March to May, August and October. The Peak recruitment appeared between March to April. Emax. was found to be 0.501. The present investigation clearly showed the over fishing (E>0.50) of *H. nehereus* in the investigated area of Bangladesh coastal water.

Key words : Population dynamics, *Harpodon nehereus*, Kutubdia channel

Introduction

Harpodon nehereus (Ham.-Buch 1822) popularly known as Bombay duck is one of the inshore shallow water and estuarine fish of the Bay of Bengal, Indian ocean and the Arabian Sea. It is locally familiar as 'Lotiya much' or 'Loittya' around the coastal areas of Chittagong and Cox's Bazar.

A large number of this fish is caught every year from the coastal areas of Bangladesh located between Latitudes 20^0 N - 22^0 N and Longitudes 84^0 E - 92^0 E (Sarker 1967). The major portion of the fish is caught by the local fishermen and annually about 0.1 million tons are harvested. Kutubdia, Moheskhali, Cox'sBazar, Sonadia, Khulna and the coastal areas of Chittagong are the most important fishing place for *H. nehereus*. In the Bay of Bengal, Bombay duck contributed a major part of the catch of fish population.

The Bombay duck is generally caught by the 'Behundi nets' (set bag net) with mesh size varying from 70 mm at the wider part to 10 mm at the cod end. The peak season of fishing commences from July and continues for a period of about 5-6 months. In other months, they occurred in comparatively small number (Das 1980).

Though *H. nehereus* is one of the highly esteemed table fish at home and abroad, but little work has so far been reported on it in the field of population biology. The present investigation shows the different aspects of population dynamics such as asymptotic length (Lα), growth co-efficient (K), natural mortality (M), fishing mortality (F), recruitment pattern, length at first capture (L_c), relative yield-per-recruit and biomass-per-recruit and exploitation rate (E) of *H. nehereus* and its management at Maximum Sustainable Yield (MSY) level in the Kutubdia channel of the Bay of Bengal.

Materials and methods

Fortnightly samples of *H. nehereus* were collected during August'95 to July'96 from the Kutubdia channel (Fig.1) of Bangladesh coastal water. Fishes were collected from set bag nets with mesh sizes 10 cm at the mouth, 5 cm at the middle and 1.5 cm at the code end. Total length (TL) at 0.5 cm interval for 2366 specimens were measured and length-frequency data were pooled month wise.

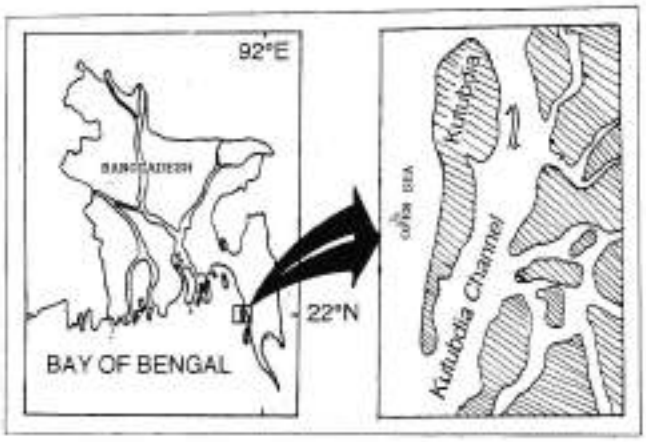


Fig. 1. Investigated area of Kutubdia channel in the coastal water of Bangladesh.

Length-frequency based computer programs ELEFAN I, ELEFAN II were used to estimate population parameters. As explained in detail by Pauly and David (1981) and Saeger and Gayanilo (1980) the growth parameters Lα and K of the Von Bertalanffy equation for growth in length are estimated by ELEFAN I. An additional estimate of Lα and Z/K value was obtained by plotting L-L on L (Wetherall 1986 as modified by Pauly 1986) i.e.,

$L\alpha - L = a + bL$

Where, $L\alpha = a-b$ and $Z/K = (1+b)/-b$

Where L is defined as the mean length, computed from L upward, in a given length-frequency sample while L is the limit of the first length class used in computing a value of L.

The growth performance of *H. neherus* population in terms of length growth was compared using the index of Pauly and Munro (1984),

$$\phi = \text{Log}_{10} K + 2\text{log}_{10} L\alpha$$

The ELEFAN II estimates Z from catch curve based on the equation,

$$Z = \frac{K(L\alpha - L)}{L\alpha - L} \quad \text{(i)}$$

Where, L is the mean length in the sample, computed from L upward and L is the lower limit of the smallest length class used in the computation of L (Beverton and Holt 1956).

The parameter M was estimated using the empirical relationship derived by Pauly (1983), i.e.,

$\text{Log}_{10}M = - 0.0066 - 0.279\text{log}_{10}\ L\alpha + 0.6543\text{log}_{10}\ K + 0.4634\text{log}_{10}\ T$----------(ii)

Where $L\alpha$ is expressed in cm and T the mean annual environmental temperature in °c which is here 28°c.

The estimate of F was taken by subtracting M from Z, the exploitation rate (E) was then computed from the expression ,

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

'Gear Selection Pattern' was determined using the routine ELEFAN II, i.e., plots of probability of capture by length (Pauly 1984) by extrapolating the catch curve and calculating the number of fish that would have been caught.

Recruitment pattern is obtained by backward projection on the length axis of a set of length frequency data (seasonal growth curve) according to the routine ELEFAN II.

Relative yield- per-recruit (Y/R) and biomass-per- recruit (B/R) was obtained from the estimated growth parameter and probabilities of capture by length (Pauly and Soriano 1986). Here, yield (Y) - per - recruit (R) is calculated as relative yeild -per -recruit (Y/R) . The calculations were carried out using the "Complete ELEFAN" program package developed at ICLARM (Ingles and Pauly 1984).

Results and discussion

Growth parameters

Growth parameters of the Von Bertalanffy growth formula were estimated as $L\alpha = 24.48$ cm and $K = 1.5$ per year. For these estimates through ELEFAN I the response surface (Rn) was 0.157 for the main curve (solid line) and 0.129 for the secondary line (dotted line). The computed growth curve produced with those parameters are shown over its restructured length distribution in Fig. 2. The t_0 value was taken as 0.

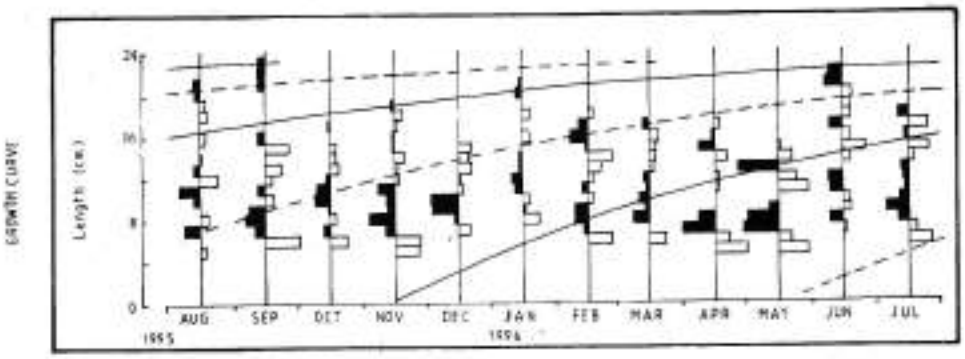


Fig. 2. Growth parameters of *Harpodon nehereus* estimated by ELEFAN ($L\alpha$ = 24.48 cm and K = 1.50/year).

The Powell-Wetherall plot are shown in Fig. 3. The corresponding estimates of $L\alpha$ and Z/K for *H. nehereus* are 24.11 cm and 1.838 respectively. This additional estimate of $L\alpha$ is slightly lower than the $L\alpha$ estimated through ELEFAN I. The correlation co-efficient for the regression was 0.943 (a = 8.49 and b = -.352). Calculated growth performance index (ϕ) was found to be 2.953. Mustafa *et al*. (1994) reported $L\alpha$ = 29.0 cm and K value = 0.9 per year for *H. nehereus* from the Kumira estuary and Islam (1995) also reported that $L\alpha$ = 30.00 cm of the species in the Karnafully estuary of Bangladesh.

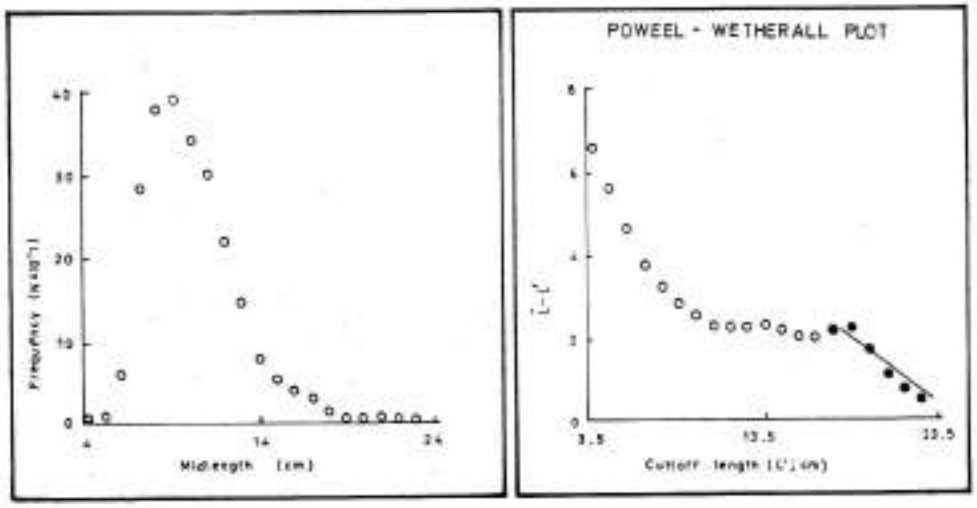


Fig. 3. Estimation of $L\alpha$ and Z/K using the methods of Powell-Wetherall plot for *Harpodon nehereus*. The estimated $L\alpha$ = 24.11 cm and Z/K = 1.838.

Mortality

The mortality rates M, F and Z computed are 2.46, 3.27 and 5.73 respectively. Fig. 4 represents the catch curve utilized in the estimation of Z. The darkened quadrilateral represents the points used in calculating Z through least square linear regression. The blank circles represents points either not fully recruited or nearing to Lα and hence discarded from the calculation. Good fit to the descending right hand limits of the catch curve was considered. The correlation co-efficient for the regression was 0.955 (a = 10.65 and b = - 5.73). The natural mortality rate estimated from the empirical equation, Pauly (1980) suggested that this method gives a reasonable value of M. This method of estimating M, is widely used throughout the tropics where time series of reliable catch and effort data and several years of Z values are not available so as to put into the most usual methods of estimating M and F. The fishing mortality rate (F) was taken by subtraction of M from Z and was found to be 3.27.

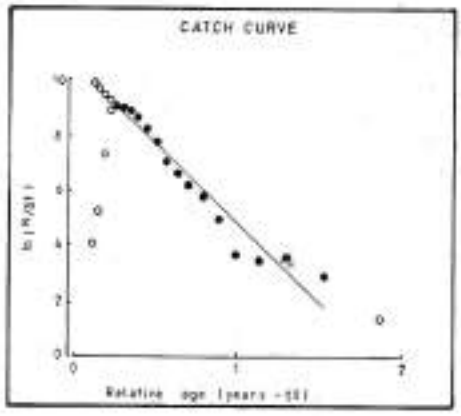


Fig. 4. Length-converted catch curve of *Harpodon nehereus*.

Exploitation rate

The exploitation rate(E) has been estimated from the Gulland's (1971) equation, E = F/F+M. Thus from these range of values of F and Z it can be shown that the rate of exploitation (E) is 0.57. It appears that the stock of *H. nehereus* of Kutubdia channel is under fishing pressure. This assumption is based on Gulland (1971) who stated that suitable yield is optimised when F = M and when E is more than 0.5, the stock is generally supposed to be over fishing. Mustafa *et al.* (1994) and Islam (1995) also reported the over-exploitation of the species in the coastal region of Bangladesh.

Selection pattern

It appears from Fig. 5 that the length at first capture (Lc) from "Selection pattern" was found to be 6.747 cm on the basis of the present net used. But

this is likely to differ in case of commercial fish trawlers having different mesh size in the cod end.

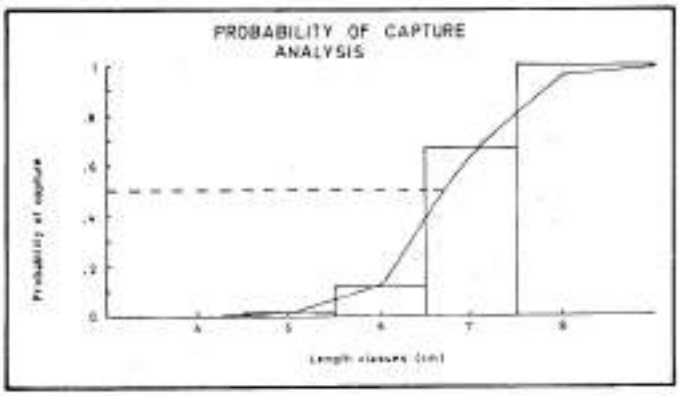


Fig. 5. Selection pattern of *Harpodon nehereus*.

Recruitment pattern

The recruitment pattern Fig. 6 determined through the ELEFAN II analysis (Pauly *et al*. 1981), with the separation of the normal distribution of the peaks by means of the NORMSEP program shows that *H. nehereus* was recruited in the fishery during March-May, August and October. As may be derived from growth curves also, one spawning appears to take place in November and another in May. Peaks appeared during the months of March and April.

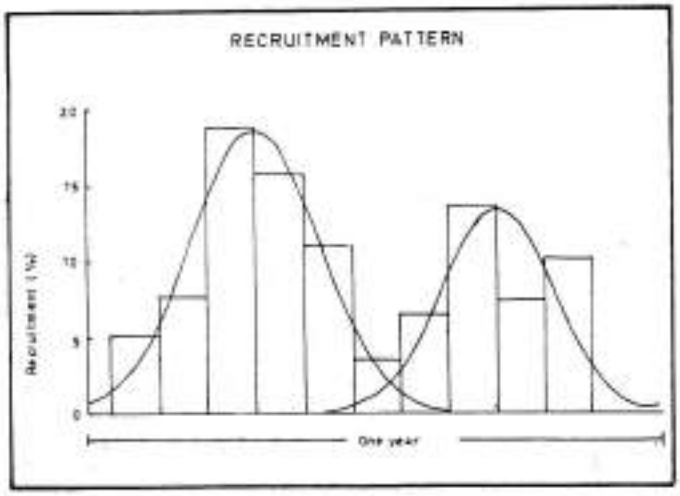


Fig. 6. Recruitment pattern of *Harpodon nehereus*.

Yield-per-recruit and biomass-per-recruit

The relative yield-per-recruit and biomass-per-recruit were determined as a function of Lc/Lα and M/K are 0.28 and 1.64 respectively. Fig. 7 shows that the present exploitation rate (E = 0.57) exceeds the optimum exploitation rate (Emax = 0.501).

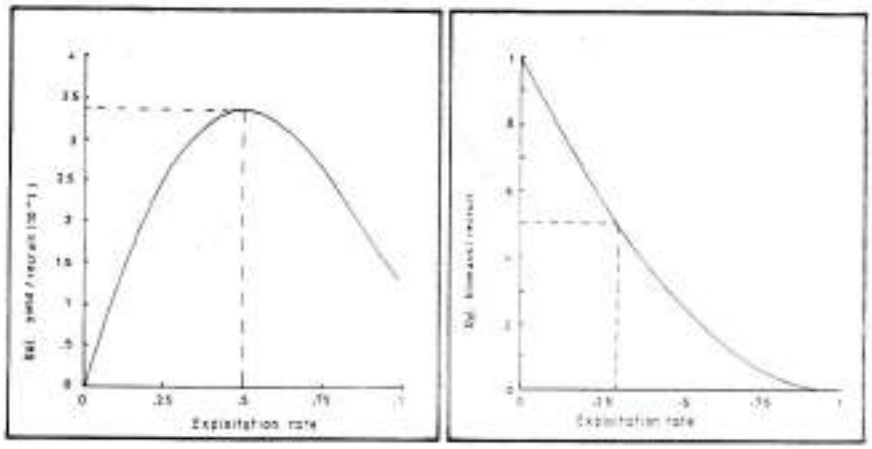


Fig. 7. Relative yield-per-recruit and biomass-per-recruit of *Harpodon nehereus* (Lc/Lα = 0.28, M/K = 1.64).

References

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

Das, B. R., 1980. Population dynamics of *Harpodon nehereus* (Ham. Buch.) in the Karnafully river estrary, Chittagong. M. Sc. Thesis, Institute of Marine Sciences, University of Chittagong, Bangladesh. 47-62 pp.

Ingles, J. and D. Pauly, 1984. An atlas of the growth mortality and recruitment of Philippine fishes. ICLARM Tech. Rep. **13** : 127 pp.

Islam, S.S., 1995. Population dynamics of some fishes and shrimp of Karnafully river estuary based on length-frequency data. M. Sc. Thesis, Department of Zoology, University of Chittagong, Bangladesh. 37-76 pp.

Mustafa, M.G., M.A. Azadi and M.S. Islam, 1994. ELEFAN based population dynamics of Bombay duck *Harpodon nehereus* Hamilton-Buchman from the Kumira estuary. Paper presented 9th National Zool. Conf. of the Zoological Society of Bangladesh. 26-28 Jan., (Souvinir abstract no. 42).

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

Pauly, D., 1983. Some simple method for the assessment of tropical fish stocks. FAO Fish Tech. Pap. 234 : 52 pp.

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

Pauly, D., 1986. On improving operator and use of the ELEFAN programme. Part II improving the estimation of Lμ. *Fishbyte*, **4** (1) : 18-20

Pauly, D. and J. L. Munro, 1984. Once more on the composition of growth in fish and invertebrates. *Fishbyte*, **2**(1) : 21 p.

Pauly, D. and N. David, 1981. ELEFAN I, a basic programme for the objective extraction of recruitment pattern from length-frequency data. *Meeresforsch*, **27**(2) : 201-210.

Pauly, D., N. David, and J. Ingles, 1981. ELEFAN II: User's instructions and program listing pag. var. (mimeo).

Pauly, D. and M.L. Soriano, 1986. Some practical extensions to Beverton and Holt's relative yield-per-recruit model. *In* : Ist Asian Fisheries Forum (eds. J. L. Maclean, L.B. Dizon and L.V. Hosillos). Asian Fisheries society, Manila, Philippines. 149-495 pp.

Sada, M. N. U., Z. A., Chowdhury, F. Alam and M.G. Khan, 1995. Length-weight relationship and size frequency of *Harpodon nehereus* (Hamilton-Buchanan) in the Bay of Bengal, Bangladesh. *In* : *Annual Conference of Zoological Society of Bangladesh*. Abstract No. 16 : 30p.

Saeger, J. and F. J. Gayanilo, 1986. A revised and graphics oriented version of ELEFAN I and II basic programs for use on HP/86/87 micro computers. Tech. Rep. Dept. Marine Fish No. 8.

Sarker, M. R., 1967. Estuarine and coastal fisheries of East Pakistan with special reference to gill netting Trans . Fish Protein Conc., Dacca, Pakistan, Nov. 7 & 8, 1967.,

Wetherall, J.A., 1986. A new method for estimating growth and mortality parameters from length-frequency data. *Fishbyte* **4**(2) : 12-14.

(Manuscript received 14 July 1997)

Bangladesh J. Fish. Res., 2(1), 1998 : 91-98

Rice-fish and rice mono-crop production at Gouripur, Mymensingh : an economic analysis

M.Z. Haque, M.H. Kabir[1] and M.T.H. Miah

Dept. of Agricultural Economics
Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[1]Present address : Farming Systems & Environmental Studies, BAU, Mymensingh
** Corresponding author*

Abstract

This study examines the relative profitability of rice-fish culture and rice mono-crop production at Gouripur thana of Mymensingh district. The results of the study show that the rice-fish farming was economically more rewarding than the rice mono-crop farming, although both the farming activities were found profitable over cash as well as full costs. In addition to extra earnings from fish, the rice-fish farming produced significantly a higher yield of rice requiring very minimum extra cost for fish. Rice-fish farming also reduced variability in yield of and return from rice.

Key words : Rice-fish, Economics

Introduction

The notion of rice-fish culture originated with a view to ensure better return from high yielding variety (HYV) boro rice to farmers thorough the best and maximum use of scarce land resources. The increased fish production harvested from simultaneous production of rice and fish is expected to lift the national fish consumption and to contribute much to the household welfare. Although the rice-fish culture under scientific management (i.e. in irrigated boro rice fields) is relatively a new gesture in Bangladesh, on-station rice-fish culture of the Bangladesh Fisheries Research Institute (FRI), the Farming Systems and Environmental Studies (FSES) and the Bangladesh Rice Research Institute (BRRI) proved to be technologically feasible and economically viable. This performance, of course, is based on the results of the experimental stations. However, only a few farmers in some places have already changed their farm planning towards rice-fish farming from irrigated rice mono-crop farming.

According to Dewan (1992) Bangladesh has 10,22,9000 ha area under rice production of which irrigated area is 12,27,000 ha and potential area for rice-fish culture is 6,15,000 ha. Unfortunately, rice-fish farming presently occupy a very small portion of the suitable area of Bangladesh.

To become accepted by the farmers a technology must satisfy farmers' socioeconomic settings and farm environment. But detailed farm level study is yet to be done to confirm rice fish production system. Thus the present study aimed at determining farm level profitability of rice-fish farming against rice mono-crop farming.

Materials and methods

The farmers of Gouripur in Mymensingh district have been cultivating HYV boro rice under irrigated condition for last few years, although the area is well-suited for rice-fish culture. In 1992, rice-fish culture was initially introduced in this area by the FRI in the form of some field experiments. The encouraging yield of both rice and fish in the experimental plots attracted the farmers to adopt this new technology. That is why, the area was selected for the study.

A stratified random sampling technique was followed in this study. In total, sixty farms taking thirty from rice with fish and the remaining thirty from rice without fish, were randomly selected for this study. The study covered only the Boro cropping season beginning from December'94 to May'95.

To collect necessary primary data a sample survey was conducted by using a set of well designed questionnaires. The collected data were analysed by using enterprise costing technique and the results were presented in the tabular form with the help of simple statistical measures like arithmetic mean, percentage and ratio. Whenever necessary the results were confirmed with the help of 't' test.

Results and discussion

The profitability analyses of HYV boro rice with and without fish were done on the basis of full and cash costs. In the case of full costs, all input items both family supplied and purchased were valued at the market prices of the inputs. On the other hand, only the out of pocket costs were taken into consideration to arrive at the cash cost. The major findings of the study are presented in the following sections.

Production cost of HYV boro rice with fish

Per hectare cost of producing HYV boro with fish in the sample farms is shown in Table 1. The table reveals that the cost of simultaneous rice-fish farming stood Tk 32,666.00 on the basis of full cost. The analysis showed that operating costs represented 64.18 percent (Tk 20,966.00/ha) of total costs and the rest 35.82 percent (Tk 11,700.00/ha) was interest cost in which interest on land value alone accounted for 34.90 percent (Tk 11,400.00/ha). The major part of the operating cost was shared by human labour and irrigation charge representing 17.99 (Tk 5877.00/ha) and 13.45 percent (Tk 4393.00/ha) of total costs, respectively. The other important operating cost items were fertilizer, animal power, seedlings and cow-dung. Fish culture in the rice fields added extra cost of Tk 3331.00/ha (10.20 percent) to the total costs of which 5.94 percent was shared by fingerlings. It may be noted here that the family supplied inputs accounted for 15.19 percent (Tk 4962.00/ha) of full cost.

Table 1. Per hectare input use and cost in rice-fish production

Cost item	Unit of quantity	Quantity	Cost			
			Full cost		Cash cost	
			Tk/ha	% of total	Tk/ha	% of total
Human labour	Man-day	167.91	5876.85	17.99	3286.15	20.53
Animal labour	Pair-day	30.45	1827.00	5.59	1624.20	10.15
Seedling	Kg	45.42	1589.70	4.87	635.60	3.97
Cow-dung	ton	5.06	1265.63	3.87	337.88	2.11
Fertilizer	-	-	2682.37	8.21	2682.37	16.76
Urea	kg	205.05	1127.78	3.45	1127.78	7.05
TSP	kg	107.72	969.48	2.97	969.48	6.06
MP	kg	51.03	357.21	1.09	357.21	2.23
Gypsum	kg	22.45	67.35	0.21	67.35	0.42
Oil cake	kg	32.11	160.55	0.49	160.55	1.00
Irrigation	-	-	4393.00	13.45	4393.00	27.45
Fingerling	Nos	3878.00	1939.00	5.94	1939.00	12.12
Feed	kg	259.50	519.00	1.59	232.00	1.45
Lime	kg	9.12	72.96	0.22	72.96	0.46
Excavation of ditch	-	-	800.00	2.45	800.00	5.00
Int. on oprt. capital	-	-	300.06	0.92	-	-
Int. on land value	-	-	11400.00	34.90	-	-
Total	-	-	32665.57	-	16003.16	-

On the basis of cash cost, the production cost (Tk 16,003.00/ha) of rice-fish, as expected, was less than half of the cost calculated on full cost basis (Tk 32,666.00/ha). Irrigation, human labour, fertilizer and animal power appeared to be the most important items of cash costs. These cost items accounted for 74.89 percent of cash costs of rice-fish production. The extra cost for fish in the rice field was Tk 3044.00/ha (19.02%) of which fingerlings alone accounted for Tk 1939.00/ha (12.12%) and the rest Tk 1105.00/ha (6.89%) was represented together by feed, lime and excavation of ditch.

Production cost of HYV boro without fish

Per hectare cost of producing rice without fish is given in Table 2. Per hectare cost of producing HYV boro as a single enterprise was estimated at Tk 28,263.00 of which Tk 16,631.00 (58.84%) and Tk 11,632.00 (41.16%) were, respectively operational and interest costs. As a single cost item, interest on land value represented the lion's share (40.34%) of total costs. Among the operational cost items human labour, irrigation and fertilizer accounted for 19.34, 12.32 and 11.34 percent, respectively of total costs of producing boro rice. Animal power and seedlings appeared to be other two important cost items representing 6.23 and 6.08 percent of total costs, respectively. In producing HYV boro rice, more than 15 percent (Tk 4263.00/ha) of total costs was represented by family supplied inputs.

Table 2. Per hectare input use and cost in rice mono-crop production

Cost item	Unit of quantity	Quantity	Cost			
			Full cost		Cash cost	
			Tk/ha	% of total	Tk/ha	% of total
Human labour	Man-day	156.20	5467.00	19.34	3132.15	25.33
Animal labour	Pair-day	29.35	1761.00	6.23	1516.80	12.26
Seedling	Kg	49.10	1718.50	6.08	563.15	4.55
Cow-dung	ton	3.14	785.25	2.78	256.25	2.07
Fertilizer	-	-	3206.44	11.34	3206.44	25.93
Urea	kg	238.05	1309.28	4.63	1309.28	10.59
TSP	kg	118.36	1065.24	3.77	1065.24	8.61
MP	kg	63.11	441.77	1.56	441.77	3.57
Gypsum	kg	33.00	99.00	0.35	99.00	0.80
Oil cake	kg	58.23	291.15	1.03	291.15	2.35
Irrigation	-	-	3481.00	12.32	3481.00	28.15
Insecticides	-	-	212.00	0.75	212.00	1.71
Int. on oprt. capital	-	-	231.90	0.82	-	-
Int. on land value	-	-	11400.00	40.34	-	-
Total	-	-	28263.09	-	12367.75	-

The total cash costs of producing HYV boro rice as a single enterprise stood Tk 12,368.00/ha which, as expected, was much lower (43.76%) than the full cost of production. As were in the full costs, the most important cost items in the total cash costs were irrigation (28.15%), fertilizer (25.93%), human labour (25.33%) and animal power (12.16%). However, seedlings, cow-dung and insecticides combinedly shared only 8.33% of total cash cost of HYV boro rice production.

Comparison of input use in rice-fish and rice mono-crop farming

It is evident from the results presented in Tables 1 and 2 that there is a variation in input use in producing HYV boro with and without fish. It can be seen from the Tables that more human labour (167.91 man-day/ha) was used in rice-fish farming than the farm producing boro rice without fish (156.20 man-day/ha). This was due to some extra activities required to produce fish in rice fields. The amount of seedlings (45.42 kg/ha) used for producing rice in rice-fish farming was lower by 3.68 kg/ha than the amount used in rice mono-crop farming (49.10 kg/ha). This was resulted from alternate double row system of transplanting rice which gave adequate space for easy movement of fish in the rice fields and saved some seedlings. A substantial difference in using fertilizers and cow-dung was also observed between the above mentioned two types of farming. In the case of rice-fish farming, amount of all types of fertilizer used were much lower than the amount used in rice as single crop. In fact, the application of fertilizer was substituted partially by higher amount of cow-dung used in rice-fish fields (higher by 1.92 t/ha). The higher amount of cow-dung contributed in the production of micro-organisms for fishes to eat and also

added nitrogen to rice plants. To maintain sufficient standing water for fishes to move in the rice fields the rice-fish farmers paid Tk 912.00/ha more for irrigation than paid for that by the rice mono-crop farmers. It may be noted here that in the case of rice mono-crop farming the farmers paid Tk 212.00/ha for insecticides while the rice-fish farmers paid nothing for that. Like all other studies (Grist 1965, Coche 1967 and Dela Cruz 1980) it was also observed that fishes helped to control some insects biologically.

Agro-economic performance of rice-fish and rice mono-crop farming

Agro-economic performance of rice-fish as well as rice mono-crop farming was examined in terms of yield, gross return, net return and undiscounted benefit-cost ratio (BCR). The results are presented in Table 3. The table shows that per hectare yield of rice (4.77 t/ha) in rice-fish farming was significantly higher (t = 5.09) than the yield (4.23 t/ha) obtained from rice mono-crop farming. Similar findings were also found in several experimental studies conducted by the FSES (Mazid *et al*. 1992), the FRI (FRI 1995) and the BRRI (Ali *et al*. 1993).

Hora and Pillay (1962) also observed that the yield of rice increased by approximately 15.00 percent in Indo-Pacific countries due to adoption of fish culture. According to Dela Cruz *et al*. (1980) rice-fish farming provides higher yield of rice through reducing rice pests, aerating bottom soil and making more nitrogen and phosphorus available to rice plants. In this regard Grist (1965) and Coche (1967) put arguments that this extra increase in yield is due to biological control of harmful insects and pests and grazing of the fish on weeds. Apart from the paddy, 159.32 kg/ha of fish was harvested from rice-fish fields during the boro season of 1995.

To obtain gross return, total produced was multiplied by the prevailed farmgate prices of the products. At the rate of Tk 7,011.30/t, gross return from rice stood Tk 33,465.00/ha and Tk 29,672.00/ha, respectively under rice-fish and rice mono-crop farming. The estimated gross return from straw in rice-fish and rice mono-crop farming were Tk 1,176.00/ha and Tk 1,030.00/ha, respectively. Gross return obtained from fish was Tk 6,381.20/ha where the prevailed farmgate price of fish was Tk 40.00/kg. Thus, the overall gross return from rice-fish and rice mono-crop farming amounted Tk 41,022.00/ha and Tk 30,702.00/ha, respectively.

To arrive at net return, gross cost was deducted from gross return and was calculated on both full cost and cash cost basis. Per hectare net return in rice-fish farming was Tk 8357.00/ha over full cost and Tk 25,019.00/ha over cash cost while net returns in rice mono-crop farming stood Tk 2,439.00/ha and Tk 18,334.00/ha, respectively over full cost and cash cost. It is noted here that the net return per hectare from rice-fish farming was significantly higher (t = 7.75) than the net return earned from rice mono-crop farming. As a measure of average return to each Taka spent in production, undiscounted BCR was calculated. Table 3 reveals that regardless the methods of estimating cost, the

BCR in rice-fish farming appeared to be relatively higher (1.26 and 2.56) than in rice mono-crop farming (1.09 and 2.48). The results of the study clearly shows that both rice-fish farming and rice mono-crop farming are profitable business from the view point of individual farmers considering both cash as well as full costs.

Table 3. Per hectare costs and returns of producing HYV boro with and without fish at Gouripur, Mymensingh

Particular	HYV boro with fish	HYV boro without fish
Yield		
Rice (t/ha)	4.77*	4.23
Fish (kg/ha)	159.53	-
Gross return (Tk/ha)	41,022.14	30,701.82
Rice	33,464.94	29,671.82
Straw	1,176.00	1,030.00
Fish	6,381.20	-
Gross cost (Tk/ha)		
Full cost basis	32,665.57	28,263.09
Cash cost basis	16,003.16	12,367.75
Net return (Tk/ha)		
Full cost basis	8,356.57*	2,438.73
Cash cost basis	25,018.98*	18,334.07
Benefit-cost ratio		
Full cost basis	1.26	1.09
Cash cost basis	2.56	2.48

Significant at 1% level

Variability in yield and net return from HYV boro

An attempt was made in this section to examine farm to farm variation in yield and net return from rice under rice-fish and rice mono-crop farming. The results are presented in Tables 4 and 5.

Table 4. Variation in per hectare yield of HYV boro with and without fish at Gouripur, Mymensingh

Yield group (t/ha)	Farms producing HYV boro with fish		Farms producing HYV boro without fish	
	Number	% of total	Number	% of total
3.49-3.99	3	10.00	8	26.67
4.00-4.39	4	13.33	11	36.67
4.40-4.79	9	30.00	9	30.00
4.80-5.47	14	46.67	2	6.67
Total	30	100.00	30	100.00

Table 5. Variation in net return per hectare from HYV boro with and without fish at Gouripur, Mymensingh

Net return group (Tk/ha)	Farms producing HYV boro with fish		Farms producing HYV boro without fish	
	Number	% of total	Number	% of total
12,721-15,000	1	3.33	6	20.00
15,001-18,000	1	3.33	10	33.33
18,001-21,000	3	10.00	9	30.00
21,001-24,000	4	13.33	5	16.67
24,001-27,000	11	36.68	-	-
27,001-29,776	10	33.33	-	-
Total	30	100.00	30	100.00

Variation in yield of HYV boro

The average per hectare yield of HYV boro in the rice mono-crop farming was not only significantly lower than the yield obtained in the rice-fish farming but the variability in yield obtained was also higher in rice mono-crop farming (Table 4). The highest and the lowest yields recorded in rice-fish farming were 5.47 t/ha and 3.67 t/ha, respectively while the yield of rice obtained in rice mono-crop farming varied from 3.49 to 4.81 t/ha. Table 4 reveals that 46.67 percent (14 farms) of the sample farms under rice-fish farming had yield between 4.80 to 5.47 t/ha while only 6.67 percent (7 farms) of the rice mono-crop farms had yield in this range of yield. Again, in the case of rice-fish farming only 23.33 percent (7 farms) farms obtained yield of rice between 3.49 to 4.39 t/ha but 63.34 percent (19 farms) of the rice mono-crop farms fall in this group. The results indicated that most of the rice-fish farms were concentrated in the highest yield group but the rice mono-crop farms were scattered in lower yield groups. Variation in yield of rice in rice-fish farming was relatively lower due to even management practices of rice-fish plots required for fish over the growing period.

Variation in net return from HYV boro

Only the variation in net returns over cash cost from rice were analysed. Table 5 reveals that the net return from rice in rice-fish farming varied from Tk 12,721.00 to Tk 29,796.00/ha while the net return from rice mono-crop farming ranged between Tk 14,511.00 to 22,673.00/ha. The distribution of rice mono-crop farms was scattered over the lower net return groups. But in the case of rice-fish farming the distribution was concentrated mainly over the two high net return groups which is similar to the concentration of rice-fish farms over the yield groups (Tables 4 and 5).

Conclusions

The results of the present study clearly indicate that farmers can make profit from both rice-fish and rice mono-crop farming. The farmers, however, can make more profit from rice-fish culture than the farms producing only HYV boro rice. Extension workers should, therefore, encourage farmers to adopt this new technique (rice-fish culture) of farming. Thus, both the production of rice and fish could be increased within the shortest possible time. This will contribute much to farmers' income and thereby in well being of rural people. The government and non-governmental organizations should strengthen their efforts to disseminate rice-fish culture technology elsewhere in the country where similar type of topography (low-lying area) is found.

References

Ali, M.H., N. I. Miah and N. U. Ahmed, 1993. Experiences in deepwater rice-fish culture. Bangladesh Rice Research Institute, Bangladesh. 17 pp.

Coche, A. G., 1967. Fish culture in rice fields: a world-wide synthesis. *Hydrologia*, **30** : 11-14.

Dela Cruz, C. R., 1980. Integrated agriculture-aquaculture farming systems in the Philippines with two case studies on simultaneous and rotational rice-fish culture. ICLARM Conf., Manila, Philippines. 15 pp. (mimeo)

Dewan, S., 1992. Rice-fish farming systems in Bangladesh: past, present and future. *In*: Rice fish research development in Asia. (eds. C.R. Dela Cruz, C. Lightfood, B. A. Conta-pierce, V. R. Carangal and M. P. Bimbaw). ICLARM, Conf. Proc. No. **24.** : 457 pp.

FRI., 1995. *Dhaner Khete mach chash projukti* (leaflet in Bangla). Fisheries Research Institute, Mymensingh.

Grist, D. H., 1965. Rice. London, U. K.

Haque, M. Z., 1997. A comparative economic study on rice-cum-fish culture and rice-mono culture in a selected area of mymensingh district. M. S. Ag. Production Econ. Thesis, Submitted to the Dept. of Ag. Econ. BAU, Mymensingh.

Hora, S.L. and T.V.R. Pillay, 1962. Handbook of fish culture in Indo-Pacific region. FAO Fish Biological Paper 14. 204 pp.

Mazid, M. A., B. C. Bakshi, A. K. Das and N. Bari, 1992. *Dhaner khete macher chash* (Booklet in Bangla, ed. K. U. Ahmed), Bangladesh Agricultural Research Council, Farmgate, Dhaka. June, 1992.

(Manuscript received 11 September 1997)

Bangladesh J. Fish. Res., 2(1), 1998 : 99-108

Manipulation of chromosomes in fish : review of various techniques and their implications in aquaculture

M.G. Hussain

Bangladesh Fisheries Research Institute, Freshwater Station
Mymensingh 2201, Bangladesh

Abstract

Human ingenuity has made it possible to advent the chromosome manipulation techniques to produce individuals with differing genomic status in a number of fish using various causal agents such as physical shocks (temperature or hydrostatic pressure), chemical (endomitotics) and anaesthetic treatments either to suppress the second meiotic division shortly after fertilization of eggs or to prevent the first mitotic division shortly prior to mitotic cleavage formation. This results in the induction of polyploidy (triploidy and tetraploidy), gynogenesis (both meiotic and mitotic leading to clonal lines) and androgenesis in fish population. The rationale for the induction of such ploidy in fish has been its potential for generating sterile individuals, rapidly inbred lines and masculinized fish, which could be of benefit to fish farming and aquaculture. In this paper, these are critically reviewed and the implication of recently developed chromosome manipulation techniques to various fin fishes is discussed.

Key words : Cell divisions, Fish eggs, Polyploidy, Gynogenesis, Androgenesis

Introduction

Chromosome manipulation research has a short history in fish compared to that of crops and animals. Since 1943, early attempts were initiated, and until recently various techniques have been developed to interfere with normal functioning of the metaphase spindle apparatus during nuclear cycles of cell division in fish eggs using several causal agents, both physical and chemical. As a result, individuals with differing genomic status, *viz.* polyploids (triploid and tetraploid), gynogenetics (both meiotic and mitotic gynogens) and androgenetics, are being produced in fish population.

Among polyploids, triploid individuals are expected to be functionally and endocrinologically sterile due to their meiotic inhibition of gametogenesis and lack of essential steroid hormone levels to support gonadal growth. Such sterility in triploid fish can be of advantage to aquaculture where control of reproduction and population is desirable. The production of putative tetraploids might have tremendous impact, because of promising future of large scale production of genetically sterile fish (triploids) population lies with the mating of normal diploid and tetraploid individuals.

Induction of two types of gynogenesis has its potential in generating nearly all female and all homozygous individuals in a single generation. Probable

application of meiotic gynogenetics in aquaculture relates to coupling with sex inversion using androgen hormones to produce all outbred monosex female population where growth rate of females are superior to males particularly in salmonids and cyprinids. Mitotic gynogenetics have limitations to use them directly for culture but they are potential and valuable as completely homozygous broodstock to produce second generation of clonal lines. Production of viable androgenetic diploids in fish has its great impact on commercial application of genetic masculinization technique to replace hormonal sex-reversal. Present review highlights mainly on the various chromosome manipulation techniques which could easily be used to suppress cell divisions in fish eggs and their probable implications to the cultured species to benefit aquaculture.

Suppression of meiotic events of cell division in fish eggs

Table 1 shows a brief review of suppression of second meiotic events of cell division in the fertilized eggs of various fishes.

Induction of triploidy

Triploidy is induced directly by blocking of second polar body extrusion during second meiotic division shortly after fertilization of fish eggs using various physical shocks and chemical treatments. The mating of normal diploid and tetraploid fish is an alternative method for producing hybrid triploids.

Many workers successfully used both temperature (cold and heat) and pressure stocks as effective agents in inducing triploidy in several fishes. In a thorough investigation in Nile tilapia, *Oreochromis niloticus*, conducted by Hussain *et al.* (1991) and Hussain *et al.* (1995), the cold shock survivals showed a lot of inter female difference despite the uniformly high control and pressure shocks survivals. Heat shock showed lower inter-individual variation than was found for cold, but on average were not as great as pressure in optimizing triploid yields. A similar situation following temperature shock for the induction of triploids, in the use of heat to produce triploid Atlantic salmon has been reported where, because of extreme variability in response to the same heat shock treatment of eggs from different females, pressure shock was the preferred method of triploidization (Benfey and Sutterlin 1984, Benfey *et al.* 1988, Johnstone 1989).

The use of chemical treatments such as cytochalasin B; colchicine (Refstie *et al.* 1977, Allen and Stanley 1979, Smith and Lemoine 1979) and anaesthetics such as nitrous oxide and Freon 22 (Sheldon *et al.* 1986, Johnstone *et al.* 1989, Santiago *et al.* 1992) to induce retention of the second polar body in several fishes have also been reported. Thorgaard (1983) suggested, in view of the success and ease of temperature and pressure shock treatments for inhibiting cell division in fish, chemical treatments may not be the method of choice. They are probably less adaptable to mass production than other methods.

Table 1. Suppression of second meiotic events of cell division in the fertilized eggs of various fishes

Fish species	Causal agents	Intensity level	Induction window	Ploidy status	Authors
Oreochromis niloticus	HS	41°C for 3.5 min	5 min a.f.	Triploid	Hussain *et al.* (1991)
Oreochromis niloticus	PS	8000 p.s.i. for 2 min	9 min a.f.	Triploidy	Hussain *et al.* (1991)
Oreochromis niloticus	CS	9°C for 30 min	7 min a.f.	Triploidy	Hussain *et al.* (1991)
Oreochromis niloticus	HS	41°C for 3.5 min	5 min a.f.	Meiotic gynogen	Hussain *et al.* (1993)
Oreochromis niloticus	PS	8000 p.s.i. for 2 min	9 min a.f.	Meiotic gynogen	Hussain *et al.* (1993)
Oreochromis niloticus	HS	41°C for 3.5 min	5 min a.f.	Meiotic gynogen	Mair (1988)
Oreochromis aureus	CS	11°C for 60 min	15 min a.f.	Triploidy	Valenti (1975)
Oreochromis mossambicus	HS	41.7°C for 3 min	32-54 min a.f.	Meiotic gynogen	Varadaraj & Pandian (1990)
Salmo salar	HS	32°C for 5 min	20 min a.f.	Triploidy	Benfey & Sutterlin(1984)
Salmo salar	PS	10150 p.s.i. for 6 min	20 min a.f.	Triploidy	Benfey & Sutterlin(1984)
Salmo salar	NO	11 amt for 30 min	0-30 min a.f.	Triploidy	Johnstone (1989)
Salmo gairdneri	HS	36 °C for 1 min	10 min a.f.	Triploidy	Thorgaard *et al.* (1981)
Salmo gairdneri	PS	7000 p.s.i. for 4 min	40 min a.f.	Triploidy	Chourrout (1984)
Salmo gairdneri	PS	7000 p.s.i. for 4 min	40 min a.f.	Meiotic gynogen	Chourrout (1984)
Clarias gareipinus	CS	5 °C for 40 min	2-4 min a.f.	Triploidy	Richter *et al.* (1987)

HS = Heat Shock, PS = Pressure Shock, CS = Cold Shock, NO = Nitrous Oxide.

Triploid individuals are expected to be functionally and endocrinologically sterile. Such sterility of triploid fish (both male and female) can be of benefit to aquaculture. The blocking of complete gametogenesis particularly in female triploids during early meiotic division results in complete inhibition of oocyte development and functional sterility (Thorgaard and Gall 1979, Richter *et al.* 1987, Hussain *et al.* 1994a, 1995). Despite gametic sterility triploid males due to meiotic inhibition of spermatogenesis, in fish species a proportion of such males are able to produce abnormal and aneuploid sperm. This ultimately leads to reproductive sterility of these males (Swarup 1957, Richter *et al.* 1987, Hussain *et al.* 1994a and 1995). Therefore, it was suggested that triploid males could be introduced into a wild population where suppression of natural reproduction of undesirable wild female fish (*viz. Oreochromis* spp.) is required to control their overpopulation, as any mating between the sterile male triploid and any females would result in inviable eggs and a reduction in recruitment. Such method can experimentally be applied in some of the Asian reservoirs

Induction of meiotic gynogenesis

In the process of meiotic gynogenesis, eggs are fertilized with UV irradiated sperm and then are exposed to a variety of physical shocks or chemical treatments which suppress the anaphase stages of second meiotic division by disruption of metaphase spindles, resulting artificial diploidization of maternal chromosome complement (retention of second polar body) of eggs (Purdom 1983).

The gynogenesis by the suppression of meiotic events has been induced since 1960 in numerous fish. At present there are few direct applications of gynogenesis in aquaculture because the fish are inbred and have a reduced variability compared to normal diploids. It has been commonly suggested that gynogenesis induction should be coupled with sex inversion such that functional XX all males could be produced (Nagy 1987, Thorgaard and Allen 1987). Such sex-reversed gynogenetic males are thought to be useful in crossbreeding experiments to produce all outbred monosex female population where the growth rate of females are superior to males particularly in salmonids and cyprinids. Presently sex-reversed gynogenetic males are being widely used in China for line breeding and genetic improvement of common carp and some other commercial strains (Prof. Wu Chingziag, personal communication). Roongrati *et al.* (1994) reported a model of producing all-female Thai silver barb, *Puntius gonionotus*, using the techniques of gynogenesis and sex reversal (Fig. 1). Attempts are being undertaken for mass scale production of such fish in Bangladesh and Thailand under the auspices DFID/Stirling University, Scotland for augmenting production of *P. gonionotus* in the rice fields and seasonal water bodies.

Suppression of mitotic events of cell division in fish eggs

Suppression of first cleavage of mitotic events of cell division has alraedy been carried out in a number fish species, a summary of these works is presented in Table 2.

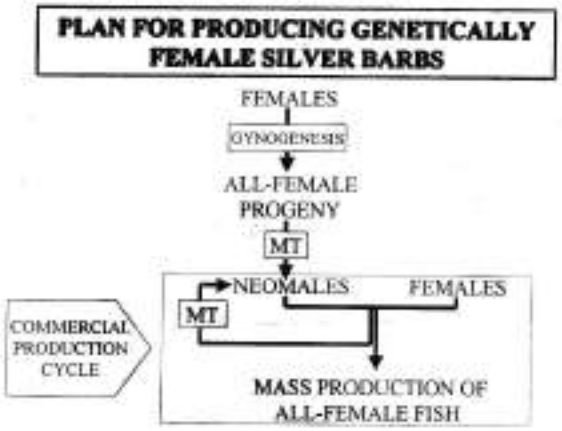


Fig. 1. A schematic model for commercial production cycle of producing all-female Thai silver barb, *Puntius gonionotus*.

Induction of tetraploidy

Several agents, both physical and chemical, that disrupt the first mitotic division shortly prior to formation of cleavage farrow, therefore, leading to the generation of tetraploid individuals having extra sets of chromosomes. Chourrout *et al.* (1986), who obtained fertile tetraploid male and female rainbow trout and successfully crossed tetraploid male and diploid females to produce viable triploids, although tetraploids have been produced in other fish species (Bidwell *et al.* 1985, Valenti 1975, Myers 1986, Pandian and Varadaraj 1987, Don and Avtalion 1988, Mair 1988). The aforementioned authors used various physical agents such as cold, heat and hydrostatic pressure shocks to induce tetraploidy in fish. Of the three shock treatments, pressure has been found most effective at blocking first mitotic cleavage in rainbow trout (Chourrout *et al.* 1986). Production of viable tetraploid individuals might have tremendous impact in aquaculture.

Induction of mitotic gynogenesis

In this process, putative gynogenetic progeny derive by the artificial diploidization of the maternal chromosome complement due to prevention of mitotic cleavage. The main rationale for gynogenesis induction in fish has been its potential for generating rapidly inbred lines. Han *et al.* (1991) suggested that homozygous inbred lines will never be produced by using meiotic gynogenetic diploids, even when reproduction is repeated for several generations. Therefore, induction of diploid gynogenesis by inhibition of first cleavage at mitotic division of a zygote might be more promising method for producing inbred lines which will be homozygous at every gene locus (Chourrout 1984, Streisinger *et al.* 1981, Hussain *et al.* 1994b).

The first example of the production of viable mitotic gynogenetics in a fish, *Brachyndanio rerio*, was that of Streisinger *et al.* (1981). The level of temperature or pressure shocks required to suppress first mitotic cleavage is the same as or

Table 2. Suppression of first mitotic events of cell division in the fertilized eggs of various fishes

Fish species	Causal agents	Intensity level	Induction window	Ploidy status	Authors
Oreochromis niloticus	Heat shock	41°C for 3.5 min	27.5-30 min a.f.	Mitotic gynogen	Hussain *et al*. (1993)
Oreochromis niloticus	Pressure shock	9000 p.s.i. for 2 min	40-50 min a.f.	Mitotic gynogen	Hussain *et al*. (1993)
Oreochromis niloticus	Heat shock	41°C for 3.5 min	25-35 min a.f.	Mitotic gynogen	Mair (1988)
Oreochromis niloticus	Pressure shock	7000 p.s.i. for 7 min	57-60 min a.f.	Tetraploidy	Myers (1986)
	+ Cold shock	+ 7.5°C			
Oreochromis aureus	Heat shock	41°C for 3.5 min	25-35 min a.f.	Mitotic gynogen	Mair (1988)
Oreochromis aureus	Cold shock	11°C for 60 min	92 min a.f.	Tetraploidy	Don & Avtalion (1988)
Cyprinus carpio	Heat shock	40°C for 2 min	28-30 min a.f.	Mitotic gynogen	Komen *et al*.(1991)
Cyprinus carpio	Heat shock	40°C for 2 min	24-28 min a.f.	Androgenesis	Borgers *et al*. (1993)
Salmo gairdneri	Pressure shock	7000 p.s.i. for 4 min	5 hr 50 min a.f.	Tetraploidy	Chorrout (1984)
Brachydanio rerio	Heat shock	41°C for 2-3 min	13 min a.f.	Mitotic gynogen	Streisinger *et al*. (1981)
Brachydanio rerio	Pressure shock	8000 p.s.i. for >1min	24-28 min a.f.	Mitotic gynogen	Streisinger *et al*. (1981)
Oryzias latipes	Heat shock	41°C for 2 min	85-95 min a.f.	Mitotic gynogen	Naruse *et al*. (1985)
Oryzias latipes	Pressure shock	10000 p.s.i. for 2 min	85-95 min a.f.	Mitotic gynogen	Naruse *et al*. (1985)
Plecoglossus altivelis	Pressure shock	9280 p.s.i. for 6 min	80 min a.f.	Mitotic gynogen	Taniguchi *et al*. (1988)

close to the level for inhibiting meiotic events. Recently, the technique has been applied successfully to common carp, *Cyprinus carpio* (Nagy 1987, Komen *et al*. 1991), medaka, *Oryzias latipes* (Iziri 1987), ayu, *Plecoglosus altivelis* (Taniguchi *et al*. 1988); Nile tilapia, *O. niloticus* (Mair *et al*. 1987, Hussain 1992, Hussain *et al*. 1993)); rainbow trout, *Onchorhynchus mykiss* (Quillet *et al*. 1991); channel catfish, *Ictalurus punctatus* (Goudie *et al*. 1991) and Asian carp, *Labeo rohita* (Hussain *et al*. 1997).

Despite the first generation of mitotic gynogenetics have limitations to use them directly for culture but they are potential and valuable as completely homozygous broodstock to produce second generation of clonal lines in fish.

Induction of androgenesis

Androgenesis is a genome manipulation technique which is the reverse of gynogenesis. It involves a genetically inactivated egg fertilized with normal sperm, resulting in the embryonic development with entirely parental chromosomal inheritance without any contribution from maternal genome.

The first androgenetic diploids in salmonids were produced by the suppression of first cleavage using pressure shock (Parsons and Thorgaard 1985, Scheerer *et al*. 1986, May *et al*. 1988) and later by heat shock (Thorgaard *et al*. 1990). The induction of androgenesis in fish could be useful in producing all male population in tilapia and some other fish to replace hormonal sex reversal (Myers, pers. communication). Another possible application of androgenetic diploids lies in recovering genotypes from cryopreserved sperm, which is important as egg and embryo cryopresrvation that has not yet been succeeded.

Production of clonal lines

Viable clonal or inbred lines in fish could be produced either by crossbreeding between a viable mitotic gynogenetic female and male (recessive mutation in a sex determining gene) or by gynogenetic reproduction (retention of 2nd polar body) of mitotic gynogenetic diploid(s) using optimal physical shocks (pressure, heat, cold etc.) or chemical treatments. Purdom and Lincoln (1973) pointed out that to produce inbred lines by conventional methods of sib-mating requires the maintenance of several lines with close inbreeding up 10 - 20 generations. But gynogenesis especially by inhibiting first mitotic division could dramatically shorten the time required to produce completely homozygous progeny in the first generation and an "inbred" or "clonal" lines with the unique gene combinations in the second (Komen *et al*. 1991, Hussain 1992, Hussain *et al*. 1994[b]).

Mitotic gynogenesis has already been induced in a number of fishes by several authors, but until recently, clones have only been produced in zebra fish (Streisinger *et al*. 1981); medaka (Naruse *et al*. 1985, Ijiri 1987); Common carp (Komen *et al*. 1991), ayu (Han *et al*. 1991), Nile tilapia (Hussain and McAndrew 1998) and Asian carps (Hussain *et al*. 1987).

Majority of these workers observed that clonal lines produced in the aforementioned fishes were mostly free of recessive, deleterious, low penetrance alleles and similar to the starting population. It was also appeared that the heterozygous clonal lines rather than the homozygous lines were preferred for the production of more viable and vigorous populations (Komen *et al.* 1991, Hussain and McAndrew 1998). It is expected that such vigorous clones will also be of great use as a pure "gene pool" for many genetic studies such as cell line and tissue culture, genetic fingerprinting, immunological, disease resistance, heritability and sex differentiation studies as well as developing breeding schemes based on the exploitation of heterosis.

Conclusions

In the recent past, the inland capture fisheries has registered a gradual decline in most of the countries of this region due to deterioration of aquatic environment, and partly or mostly, because of physical reduction of aquatic habitat. These have resulted in the threat of shrinking genetic base, causing the genetic vulnerability of fishes. Consequently, it gradually losses genetic diversity, which in turn, brings a havoc change in genetic stock structure of natural fish population. While the governments of most of the countries of this part of the world are seriously making all out efforts to rehabilitate the inland fisheries, they have focused their attention recently on aquaculture, which has tremendous opportunities in Asia. Therefore, apart from or in addition to the results of chromosome manipulation research of previous and present time, all the fishery biologists and geneticists should intense their attention to give more stress on further breeding plans and genetic studies to develop better breeds and improved stocks of commercially important cultured fish species to be useful for increasing sustainable aquaculture production.

References

Allen, S.K. Jr. and J.G. Stanley, 1979. Polyploid mosaic induced by cytochalasin B in landlocked Atlantic salmon, *Salmo salar*. *Trans. Am. Fish. Soc.*, **108** : 462-466.

Benfey, T.J. and A.M. Sutterlin, 1984. Triploidy induced by heat shock and hydrostastic pressure in landlocked Atlantic salmon (*Salmo salar*). *Aquaculture*, **36** : 359-567.

Benfey, T.J., P.G. Bosa, N.L. Richardson and E.M. Donaldson, 1988. Effectiveness of a commercial-scale pressure shocking device for producing triploid salmonids. *Aquaculture Engineering*, **7** : 147-154.

Bidwell, C.A., C.L. Chrisman and G.S. Libey, 1985. Polyploidy induced by heat shock in channel catfish. *Aquaculture*, **15** : 25-32.

Bongers, A.E.J., E.H. Eding and C.J.J. Richter, 1993. Androgenesis in common carp, *Cyprinus carpio* L. *In:* Genetics in aquaculture and fisheries management (eds. D. Penman, N. Roongratri and B. McAndrew). University of Stirling, Scotland. pp. 73-75.

Chourrout, D., 1984. Pressure induced retention of second polar body and suppression of first cleavage in rainbow trout: production of all-triploids, all-tetraploids, and heterozygous and homozygous diploid gynogenetics. *Aquaculture*, **36** : 111-126.

Chourrout, D., B. Chevassus, F. Krieg, A. Happe, G. Burger and P. Renard, 1986. Production of second generation triploid and tetraploid rainbow trout by matting tetraploid males and diploid females potential of tetraploid fish. *Theo. Appl. Genet.*, **72** : 193-206.

Don, J. and R.R. Avtalion, 1988. Production of viable tetraploid tilapias using cold shock techniques. *Bamidgeh*, **40** : 17-21.

Goudie, C.A., B.A. Simco, K.B. Davis and Q. Liu, 1991. Production of gynogenetic channel catfish by meiotic and mitotic inhibition. *In: Proceedings of the Fourth International Symposium on Reproductive Physiology of Fish*, 7 -12 July, 1991, University of East Anglia, Norwich, UK.

Han, H.S., N. Taniguchi and A. Tsujimura, 1991. Production of clonal ayu by chromosome manipulation and confirmation by isozyme marker and tissue grafting. *Nippon Suisan Gakkaishi*, **57** : 825-832.

Hussain, M.G., A. Chatterji, B.J. McAndrew and R. Johnstone, 1991. Triploidy induction in Nile tilapia, *Oreochromis niloticus* L. using pressure, heat and cold shocks. *Theo. Appl. Genet.*, **81** : 6-12.

Hussain, M.G., 1992. Genetic Manipulation Studies in *Oreochromis niloticus* L. *PhD Thesis*, University of Stirling, Scotland, UK., 272 pp.

Hussain, M.G., D.J. Penman, B.J. McAndrew and R. Johnstone, 1993. Suppression of first cleavage in the Nile tilapia, *Oreochromis niloticus* L. - a comparison of the relative effectiveness of pressure and heat shocks. *Aquaculture*, **111** : 263-270.

Hussain, M.G., D.J. Penman, and B.J. McAndrew, 1994a. Effects of triploidy on sexual maturation and reproduction of Nile tilapia, *Oreochromis niloticus* L. *In: Third Int. Symp. on Tilapia in Aquaculture* (eds. R.S.V Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly). ICLARM Conf. Proc. **41** : pp. 320-325..

Hussain, M.G., B.J. McAndrew, D. Penman and P. Sodsuk, 1994b. Estimating gene centromere recombination frequencies in gynogenetic diploids of *Oreochromis niloticus* L., using allozyme, skin colour and a putative sex determining locus (SDL-2). *In: Genetics and evolution of aquatic organisms* (ed. A.R. Beaumont). Chapman and Hall, London. pp. 502-509.

Hussain, M.G., G.P.S. Rao, N.M. Humayun, C.F. Randal, D.J. Penman, D. Kime, N.R. Bromage and B.J. McAndrew, 1995. Comaprative performance of growth, biochemical composition and endocrine profiles in diploid and triploid *Oreochromis niloticus* L. *Aquaculture*, **138** : 87-98.

Hussain, M.G., S.C. Mahata, M.S. Rahman, M.B. Tanu, M.A. Mazid and M.S. Islam, 1997. Induction of mitotic and meiotic gynogenesis and production of genetic clones in rohu, *Labeo rohita* Ham. *Bangladesh J. Fish Res.*, **1**(2) : 1-7.

Hussain, M.G. and B.J. McAndrew, 1998. Production of clones in Nile tilapia. *Aquaculture International* : (in press).

Ijiri, K., 1987. A method for producing clones of the medaka, *Oryzias latipes* (Teleostei Oryziatidae). *In: Proceedings of V. Congress of European Ichthylogy*: pp. 277-284.

Johnstone, R., 1989. Maturity control in Atlantic salmon: a review of the current status of research in Scotland. *In: Symposium on Applications of Endocrinilogy in Aquaculture*, Malaga, Spain.

Johnstone, R., R.M. Knot, A.G. MacDonald and V. Walshingham, 1989. Triploidy induction in recently fertilised Atlantic salmon ova using anaesthetics. *Aquaculture*, **78** : 229-236.

Komen, J., A.B.J. Bongers, C.J.J. Richter, W.B. Van Muiswinkel and E.A. Huisman, 1991. Gynogenesis in common carp (*Cyprinus carpio* L.) II. The production of homozygous gynogenetic clones and F_1 hybrids. *Aquaculture*, **92** : 127-142.

Mair, G.C., A. Scott, J.A. Beardmore and D.O.F. Skibinski, 1987. A technique for the induction of diploid gynogenesis in *Oreochromis niloticus* by the suppression of the first mitotic division. *In: Proceedings of World Symposium on Selection, Hybridization and Genetic Engineering in Aquaculture* (ed. K. Tiews). Scriften der Bundesforschungsanstalt fur Fischerei. pp. 289-301.

Mair, G.C., 1988. Studies on Sex Determining Mechanisms in *Oreochromis* Species. *Ph.D. Thesis*, University of Wales, U.K.

May, B., K.J. Henley, C.C. Krueger and S.P. Gloss, 1988. Androgenesis as a mechanism for chromosome set manipulation in brook trout (*Salvelinus fontinalis*). *Aquaculture*, **75** : 57-70.

Myers, J.M., 1986. Tetraploid induction in *Oreochromis* spp. *Aquaculture*, **57** : 281-287.

Nagy, A., 1987. Genetic manipulation performed on warm water fish. *In: Proceedings of World Symposium on Selection, Hybridization and Genetic Engineering in Aquaculture* (ed. K. Tiews). Scriften der Bundesforschungsanstalt fur Fischerei. pp. 127-145.

Naruse, K., K. Ijiri, A. Shima and N. Egami, 1985. The production of cloned fish the medaka (*Oryzias latipes*). *J. Exp. Zool.*, **236** : 335-341.

Pandian, T.J. and K. Varadaraj, 1987. Techniques to regulate sex ratio and breeding in tilapia. *Current Science*, **56** : 337-343.

Parsons, J.E. and G.H. Thorgaard, 1985. Production of androgenetic diploid rainbow trout. *J. Heredity*, **760** : 177-181.

Purdom, C.E. and R.F. Lincoln, 1973. Chromosome manipulation in fish. *In:* Genetics and mutagenesis of fish (ed. J.H. Scorder). Springer Verleg, Berlin and New York. pp.83-89.

Purdom, C.E., 1983. Genetic engineering by the manipulation of chromosomes. *Aquaculture*, **33** : 287-300.

Quillet, E., P. Garcia and R. Guyomard, 1991. Analysis of the production of all homozygous lines of rainbow trout by gynogenesis. *J. Exp. Zool.*, **257** : 367-374.

Refstie, T., V. Vassavik and T. Gjedrem, 1977. Induction of polyploidy in salmonids by cytochalasin B. *Aquaculture*, **10** : 65-74.

Richter, C.J.J., A.M. Henken, E.H. Eding, J.H. Van Doesum and P. De Boer, 1987. Induction of triploidy by cold-shocking eggs and performance of triploids of the African catfish, *Clarias gariepinus* (Burchell, 1822). *In: Proceedings of World Symposium on Selection, Hybridization and Genetic Engineering in Aquaculture* (ed. K. Tiews). Scriften der Bundesforschungsanstalt fur Fischerei. pp. 225-237.

Roongratri, N., D.J. Penman and T. Viputhanumart, 1994. Induction of diploid gynogenesis in Java carp (*Puntius gonionotus*). Third Asian Fisheries Forum: 528-530

Santiago, L.P., D.J. Penman, J. Myers, S. Powell, N. Roongratri, C. Suwannarak and R. Johnstone, 1992. Triploidy induction in tilapia (*Oreochromis niloticus* L.) using nitrous oxide. *In:* Genetics in aquaculture and fisheries management (eds. D. Penman, N. Roongratri and B. McAndrew). University of Stirling, Scotaland. pp. 151-153.

Scheerer, P., G.H. Thorgaard, F.W. Allendorf and K.L. Knudsen, 1986. Androgenetic rainbow trout produced from inbred and outbred sperm sources show similar survival. *Aquaculture*, **57** : 289-298.

Sheldon, C.J., A.G. MacDonald and R. Johnstone, 1986. Induction of triploidy in rainbow trout using nitrous oxide. *Aquaculture*, **58** : 155-159.

Smith, L.T. and H.L. Lemoine, 1979. Colchicine-induced polyploidy in brook trout. *Prog. Fish Cult.*, **41**: 86-88.

Streisinger, G., C. Walker, N. Dower, D. Knauber and F. Singer, 1981. Production of clones of homozygous diploid zebra fish (*Brachydanio rerio*). *Nature*, **291** : 293-296.

Swarup, H., 1957. The Production and Effects of Triploidy in the 3-spined Stickleback *Gasterosteus aculeatus* (L). *PhD Thesis*, Oxford University, UK.

Taniguchi, N., S. Seki, J. Fukai and A. Kijima, 1988. Induction of two types of gynogenetic diploids by hydrostatic pressure shock and verification by genetic marker in ayu. *Nippon Suisan Gakkaishi*, **54** : 1483-1491.

Thorgaard, G.H. and G.A.E. Gall, 1979. Adult triploids in a rainbow trout family. *Genetics*, **93** : 961-973.

Thorgaard, G.H., M.E. Jazwin and A.R. Steir, 1981. Polyploidy induced by heat shock in rainbow trout. *Trans. Am. Fish. Soc.*, **110** : 546-550.

Thorgaard, G.H. 1983. Chromosome set manipulation and sex control in fish. *In:* Fish physiology (eds. W.S. Hoar, D.J.Randal and E.M. Donaldson), IX(B). Academic Press, New York. pp. 405-435.

Thorgaard, G.H., 1986. Ploidy manipulation and performance. *Aquaculture*, **57** : 57-64.

Thorgaard, G.H. and S.K. Jr., Allen, 1987. Chromosome manipulation and markers in fishery management. *In:* Population genetics and fishery management (eds. N. Ryman and F.M. Utter). Washington Sea Grant Programme, Seattle and London. pp. 319-331.

Thorgaard, G.H., P.D. Scheerer, W.K. Hershberger and J.M. Myers, 1990. Androgenetic rainbow trout produced using sperm from tetraploid males show improved survival. *Aquaculture*, **85** : 215-221.

Valenti, R.J., 1975. Induced polyploidy in *Tilapia aurea* (Steindachner) by means of temperature shock treatments. *J. Fish Biol.*, **7** : 519-528.

Varadaraj, K. and T.J. Pandian, 1990. Production of all-female triploid *Oreochromis mossambicus*. *Aquaculture*, **84** : 117-123.

(Manuscript received 15 April 1997)

Notes for Authors

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

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

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

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

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

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

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

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

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

Submission : For the first time, two copies of the manuscript can be sent to **The Editor, *Bangladesh Journal of Fisheries Research*, Bangladesh Fisheries Research Institute, Mymensingh-2201, Bangladesh**. *Fax : 880-91-55259, E-mail : frihq@bdmail.net.* Papers will be reviewed by one or more experts in the relevant discipline and evaluated by the Editorial Board for publication. Any paper deemed to be of inadequate quality or inappropriate format for the Journal may be returned to author(s) without review. The cover letter must be signed by all authors in case of multiple authorship. *Submission of final accepted article on a 3.5" disc prepared on PC-compatible computer, along with hard copy print-out, will help rapid publication.*

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

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

Bangladesh J. Fish. Res., Vol. 2(1), 1998

Contents