

FISH AND FISHERY OF *ASPIDOPARIA* (CYPRINIFORMES : CYPRINIDAE) OF THE RIVER PADMA



*A thesis submitted for the degree of Doctor of
Philosophy of the University of Rajshahi, Rajshahi.*

1997

By

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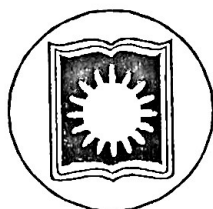
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*Dedicated
To
My Husband And
My beloved Daughter
& Son*

CERTIFICATE

*Certified that this is a bonafied
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DECLARATION

I declare that the present work is original and has not been published or submitted in part or full for any degree or prize.

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November, 1997.

Nasrin Sultana .
Nasrin sultana

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PREFACE

Bangladesh has vast fishery resources. The inland water areas of the country comprises 1.03 million hectares of rivers, canals and estuaries; 1,14,161 hectares of natural depressions such as the 'beels' and 'haors'; 1,61,943 hectares of ponds and tanks; 5,488 hectares of ox-bows; 68,800 hectares of Karnafuli reservoir; 2.8 million hectares of flood plains and 87,300 hectares of brackish water aquaforms (Rahman, 1989).

A total of 260 species of indigenous species of fin fishes are found in the freshwater habitats of Bangladesh and majority of these fishes are small sized (Rahman, 1989).

The small indigenous fishes (SIS) form an important human food item, rich source of complete proteins containing all the essential aminoacids in right proportion. Fish protein is said to be more healthier and cholesterol free and also contains fatty acid which help in cholesterol absorption in the body tissues. The people are advised to take much smaller fish than meat. According to FAO report, (1991), fish contain 72% water, 19% protein, 8% fat, 0.15% calcium 0.25% phosphorus and 0.10% vitamin A,B,C and D. National Household Expenditure Survey (NHES) shows that annual fish consumption in Bangladesh peaked in 1985 at 13.2 kg/person, had declined to 12.6 kg/person by 1992. This is in the same agreement with the information developed by North West Area Development Study (NWADS), which shows that overall fish supply has stagnated, resulting in price increase in real terms of over 2.8% per annum in the Northwest Region (Interim Report Vol-5 ADB February, 1997).

The SIS fishes thus, make a very significant contribution to the daily intake of fish. Moreover, Siddiqui (1985) reported that in Bangladesh about 80 percent of

the population is poor and they mainly depend on small size fish for their daily supply of animal protein at a reasonable price. Another advantages of small size fish is that they could easily breed and grow in water bodies, where culture of other major fishes are impossible. However, the ever expanding programme of flood control, drainage and irrigation is interfering with the whole ecological system in fishery resources, thereby causing both loss and degradation of their habitats. Which will inevitably lead to diminution and even disappearance of 95 percent of inland fish production.

The present species *A. morar* is mostly available in the river systems of Bangladesh. The biology of this species had been reported by Sultana (1991), Bhuiyan *et al.* (1992) compared the past and present data on the landing of the species together with the market price found that the natural production of the species is decreasing due to construction of the Farakka Barrage and other FCD projects which caused detrimental effect on the national aquatic system. Moreover, over exploitation of fish population and indiscriminate use of agrochemicals also adversely affected the fisheries resources.

A. morar is an important species due to its high nutritional value in terms of protein content. In spite of its usefulness as a source of protein, no detailed studies on the chemical and biochemical investigation has so far been reported.

This is a small sized fish with a maximum size of 145 mm, fetches the highest market price due to its taste and flavour. This fish generally live in shallow water of the sandy bottom and medium current areas of the rivers. We hope the present finding may help to increase its population in the river Padma near Rajshahi.

AIM OF THE STUDY

Chapter -1

This chapter deals with the reproductive systems of the fish. Developing stages of the ovary and ova are studied. The reproductive cycle of the fish is determined using different methods. Fecundity of the fish is estimated. The relationships between fecundity and body lengths and weight and gonadal length and weight are determined. Fecundity of the individual size group of fish are documented. Monthwise sex ratio is also determined.

Chapter - 2

This chapter mainly concerns with a comprehensive study on the physico-chemical condition of the study area. Some physical parameters viz. air and water temperature, rainfall, water transparency, and some chemical parameters viz. pH, dissolved oxygen (DO), free carbondioxide (CO₂) in water are determined.

Chapter - 3

This chapter deals with the landing and marketing of the species i.e. fishing method and season, marketing channel and its activities, marketing margin, marketing costs, profit of intermediaries and seasonal price variation.

Chapter - 4

This chapter deals with the chemical analysis of *A. morar*. The present study was designed to extract lipid and protein as well as to observe the pattern of essential fatty acid.

- i. Determination of protein contained in fish fillet by Micro-kheldal method.
- ii. Extraction of total lipid from *A. morar* by "Bligh and Dyer" method.
- iii. Determination of moisture contained in fish fillet.
- iv. Determination of Ash contained in fish fillet.
- v. Determination of the physico-chemical properties of fish lipid.
- vi) Qualitative and quantitative analysis of fatty acids.

A FEW WORDS ABOUT *ASPIDOPARIA MORAR* (Hamilton)

1. ECONOMIC IMPORTANCE :

The freshwater fish, *Aspidoparia morar* is an excellent food and cheapest source of protein. It is caught largely from the river Padma. *A. morar* carries its local name as 'Morari', 'Morar' (Hamilton 1882). Two species of *Aspidoparia* are recorded from the river Padma, such as *A. morar* and *A. joya*. The Bangla name of the fish is 'Piali mach'. The fish is very delicious and costly.

2. IDENTITY :

Valid name - *Aspidoparia morar* (1822.) Ham

3. SYNONYM :

1822 *Cyprinus morar* Ham, Fish, Ganges, PP, 264. 384. pl. xxxi. fig.5

1878 *Aspidoparia morar* Day, Fish. India, P 586, Pi. cxivi, fig. 4.

1889 *Aspidoparia morar* Day, Fauna Brit. India Fish, I.P. 338, fig. 108

1937 *Aspidoparia morar* Shaw and Shebbeare J. Ray. Asiat. Soc. Bengal Sci III. p. 33. fig. 29.

4. TAXONOMY :

Kingdom	--	Animalia
Sub-Kingdom	--	Metazoa
Phylum	--	Chordata
Sub-phylum	--	Vertebrata
Class	--	Osteichthyes
Sub-class	--	Actionopterygii
Super order	--	Teleostei
Order	--	Cypriniformes
Division	--	Cyprini
Family	--	Cyprinidae

Genus	--	<i>Aspidoparia</i>
Species	--	<i>A. morar</i> and <i>A. joya</i> .

5. COMMON NAME, VERNACULAR NAMES OF THE FRESH WATER
ASPIDOPARIA MORAR

Assam	--	Monah, Boreala, Bariali
West Bengal	--	Morar, Morari
Bihar	--	Chippuah, Chellush, Harda
Uttar Pradesh	--	Pirohia
Punjab	--	Kenwachi
Orissa	--	Bayi
North Bengal Bangladesh--		Pauli fish
English name	--	Aspidoparia.

6. GEOGRAPHICAL DISTRIBUTION :

Aspidoparia morar (Hamilton) has a wide range of distribution in the freshwater of Bangladesh, Iran, Pakistan, Swat river drainage, Northern India, Nepal, Burma and Thailand (except the western coast and localities, south of the Kistna river).

7. DIAGNOSTIC CHARACTERS :

Abdomen -	Rounded, interiorly placed small mouth, lower jaw devoid of lips and with crecentic edge. Suborbital ring of bones is of moderate width, snout obtuse.
Barbles -	Absent.
Length of head -	10 - 24mm, average 16.97 ± 4.36 (N = 617)
Girth length -	28 - 70mm, average 51.84 ± 11.95 (N = 617)
Eye length -	5 - 11mm, average 8.41 ± 1.84 (N = 617)
Dorsal fin length -	22 - 63mm, average 45.56 ± 11.01 (N = 617)
Pelvic fin length -	21 - 58mm, average 41.05 ± 10.34 (N = 617)

Pectoral fin length - 11 - 25mm, average 17.89 ± 4.30 (N = 617)

Anal fin length - 33 - 86mm, average 59.95 ± 15.58 (N = 617)

Lateral line - Complete

Scales- Predorsal scales in 17 rows

Colour- Body colour uniformly light yellow, though the fins are distinctly dark yellow.

8. **MAXIMUM LENGTHS OF *A. MORAR* AS REPORTED FOUND BY SEVERAL AUTHORS :**

130mm Day, F (1878)

80mm Srivastava, G.J. (1959)

95mm Srivastava, G.J. (1960)

145mm Sultana, N (1991).

Landing and Market :

This riverine clear water fish is very costly in the local market due to its exceptional taste and easily digestible quality among the smaller indigenous fishes. As a single genus their production and landing is not so high, but the market price is always higher than other SIS fishes. During winter, landing of larger sized fish have excellent taste.

REPRODUCTIVE BIOLOGY**1.1 INTRODUCTION**

All animals must display enough characteristic reproductive capacities to compensate for the death rate of its populations, failing which they may suffer severe irrecoverable depletion. Reproduction is the process by which continuity of the race is maintained. So it takes a place of paramount importance in the life process of all organisms as well as in studies on the biology of any species of animal especially of economic significance (Hossain and Rafik, 1981).

Marshal *et al.* (1965) described that reproduction is a physiological phenomenon and it is influenced by several factors like genetic, environmental and hormonal factors, either singly or in combination. Ando (1965), Korzhenko (1966), Oguri and Takada (1967), McCartney (1967), etc., tried to explain the attainment of sexual maturity in fish by analysing the differences of chemical composition of body muscles, blood and gonads. According to these workers in fish one may clearly mark the advent of a number of systems through the medium of chemical analysis, which is sometimes more realistic than visual examination. But Love (1974) concluded that establishment of any of the above mentioned system to use as a substitute scale of maturity determination would involve thousands of determination and careful consideration of all the variables. Moreover, some of these variables depend so much on initial reserves as to be unsuitable for this purpose.

At present, maturity is expressed in roman numbers from visual examination of the sex organs on what is known as the "Hjost Scale" (Kestaven, 1960; Craig, 1974), which range from I (immature) to VII (Spent). This scale is insufficiently precise for some purposes, and an objective method would be useful.

The limiting factors are the variability of the constituent decided upon and the time necessary for the determination. Although the analysis of many substances can be automated now a days if the number of samples justify it (Love, 1974).

However, Treasurer and Holliday (1980) supported the study of histological changes in the gonad during development and relate those to changes in gonad weight and to the microscopic scale of maturity stages. Kestaven (1960) and Craig (1974) confirmed the reliability of fecundity estimates and indicated the extent of any pre or post-ovulatory degeneration of oocytes, which is a must for studying the reproductive biology and population dynamics(Perkins, 1971).

A. morar accounts for an important freshwater capture fishery but has recieved little importance in scientific literature. It should be mentioned that no published work on the breeding biology of this species has been done till now in Bangladesh. A study of the reproductive biology of *A. morar* was found to be essential in the sense that it may provide information and clues for a tactful and skilful exploitation of this natural freshwater resource.

The objective of the present study is to provide a comprehensive account of the reproductive biology of *A. morar*. At the same time changes in the chemical composition of body muscles has also been performed. Thus, it was hoped that the study would confirm the reliability of sexual maturity, spawning cycles, fecundity estimates and indicate the extent of any pre and post-ovulatory degeneration of oocytes and depletion of chemical composition of the body.

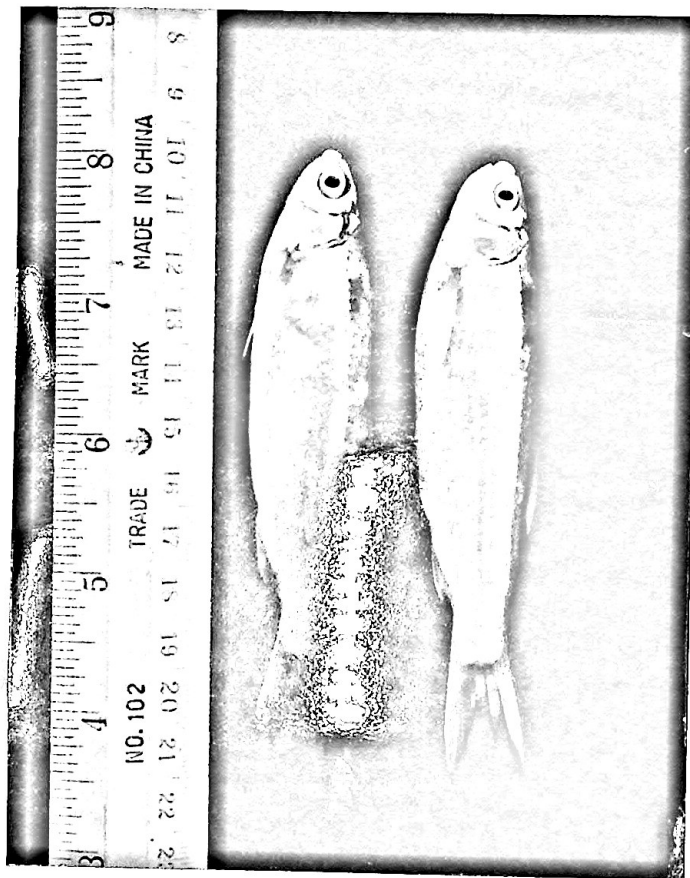


Fig. 1 : Male and female *A. morar*.

1.2 MATERIALS AND METHODS

A total of 2402 fresh quality of *A. morar* were collected from the Padma river at Rajshahi district during June 1993 to May 1995 (Map 1). The fish were collected from the catch of sein net and gill net. The fishes were separated according to sex, length groups, gravid, non-gravid and also separate data for juveniles (TL = 50-65mm) which were recorded for the size frequency distribution. Out of 2402 specimens 1261 specimens were recorded as female of which 604 were gravid and 657 were non-gravid 1141 specimens were recorded as males and 190 were juveniles (Table 1). The fishes were sexed and the females were also separated into gravid and non gravid females. Out of 2402 specimens 1261 specimens were recorded as females (604 gravid, and 657 non-gravid) and 1141 specimens were recorded as males.

The total length (TL) and standard length (SL) of the specimens were measured nearest to 1 millimeter by means of a measuring board. The weight of gravid females were taken with the help of a pan balance in grams. The gonads were dissected out and the length of individual gonads were measured with the help of a fine point divider and a millimeter scale. The weight of ovaries were taken with the help of electronic balance. The gonads of representative specimens were measured, and free hand drawing were accomplished immediately before the gonads removed from the body cavity and weighed to obtain the wet weight.

For the study of the stages of the ovary, selected specimens were dissected to expose the ovary. The length, size and colour were carefully examined and noted.

Ten to fifteen ova were collected from anterior, central and posterior regions of each ovarian lobe of a specimen. Diameter of the collected ova were

measured with the help of an ocular micrometer, each micrometer division being equivalent to 0.015 mm.

The stage of gonad maturity was assigned from the scales after Kestaven (1960), Karim and Hossain (1972b), Treasurer and Holliday (1981). A large number of scales has been employed by fish biologists in the past but the small variation in development of teleost gonads does not warrant this proliferation.

A total 541 females were studied for determining the reproductive cycle. The following methods were applied to study the reproductive cycle of *A. morar* :

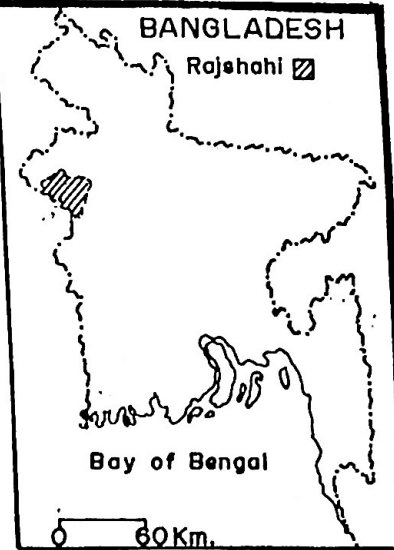
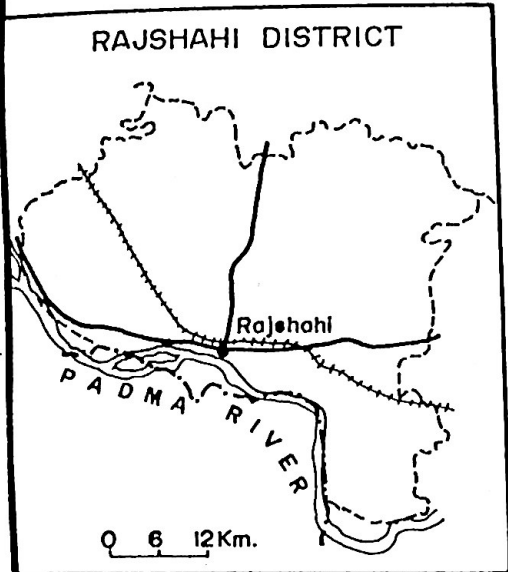
- 1) Percentage of gravid females against time
- 2) Diameter of ova
- 3) Gonado - somatic index (G.S.I)
- 4) Gonadal - length index (G.L.I)
- 5) Colouration of the ovary
- 6) Maximum indices of spermatozoa in the milt of male.

Details of methods and other relevant existing techniques are mentioned at appropriate place in the next.

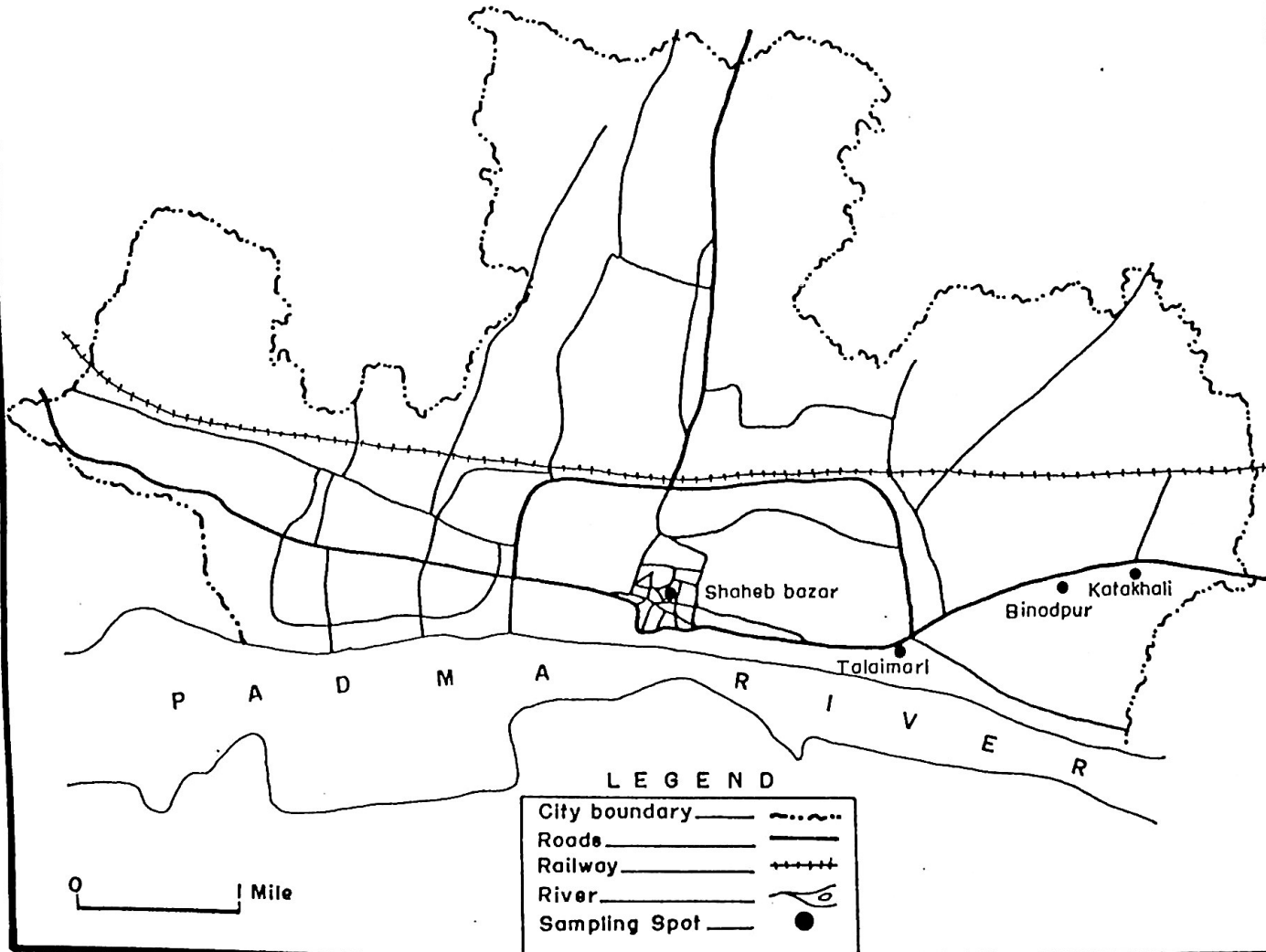
During the begining of spawning season (Deccmber to February), a total of 155 mature females were collected and the ovaries were preserved in 10% formalin after weighing. The fecundity was determined.

The sex ratio were tested whether the observed deviations from the ratio of 1: 1 of the sexes on the null hypothesis are significant by the chi-square test (Snedecor 1956).

LOCATION MAP



RAJSHAHI CITY CORPORATION



1.3 RESULTS AND OBSERVATIONS

The reproductive system of *A. morar* is basically similar to that of other teleosts. The male and female reproductive systems are describing separately.

1.3.1 Sexing :

No external differences between male and female *A. morar* exist. Although, the males are comparatively smaller in size than the females, but the irregularities in the length for age-group among the individual of the same sex in the same environment makes it difficult at a glance to separate the male from the female by their sizes. However, as they approach to the time of spawning the females can be easily identified by their markedly oedematous condition of the tissue surrounding the genital vent. Whereas, the male pore was less swollen and red than the females.

This oedema condition in the genital vent of the female start to become prominent from the month of September. During the post spawning period to preparatory stages they are simply a rounded pore lies flat to the body. In case of male fish except the spawning period, the genital pore was found as more or less a narrow slit and when those fishes were dissected a paired narrow thread like structure suspended by lengthwise in the upper section of the body cavity were found depending on the stage of maturity. At the beginning of reproductive period a secondary sexual character arise in both sexes, the abdomen becomes golden yellowish in colour. This colour remains prominent throughout the breeding period and disappears when the season is over. Otherwise, it was very difficult sexing of *A. morar* without dissecting at their juvenile stages.

1.3.2 Male Reproductive Organ :

The following descriptions are based upon the fish of <70mm total length. The testis is a paired structure of unequal size. The left lobe is always larger than the right one (Fig. 2, Plate 1 Fig. 1(a,b,c). The colour and size of the testes vary according to stage of sexual maturity.

In mid-August, the testis appeared as a translucent, whitish, narrow, thread like structure, running about the length of 53.05 ± 2.35 and 46.23 ± 3.87 percent (average $48.63 \pm 3.76\%$) of the body cavity and right testis respectively. In the body cavity the gut wall externally was well supplied with fat.

During mid September to October the testis was milky white in colour and occupied about $52.63 \pm 3.11\%$ and 48.83 ± 2.05 percent (average $50.73 \pm 3.21\%$) of the body cavity by the left and right testis respectively. The fat deposition from the gut wall reduced, presumably utilized in gonadal development (Berra, 1984).

In mid October, the testis extended much and by November the two testicular lobes were nearly touching each other. The colour was light creamy to creamy and occupied 62.79 ± 5.07 and 59.82 ± 5.34 percent (average $60.86 \pm 5.23\%$) of the body cavity by the left and right testis respectively.

In late December to February, represented the peak of testicular development with a deep creamy colour and milt oozes out by slight pressure on the abdomen. The body was filled 72.35 ± 6.07 and 65.44 ± 6.27 percent (average $68.82 \pm 6.05\%$) of the body cavity by the left and right testis respectively. The fat from the gut wall was completely disappeared.

In late February to March most of the fish were found with spend and flacid testis, extened upto 56.23 ± 7.61 and 53.12 ± 5.34 percent (average $54.61 \pm 6.55\%$) of the body cavity respectively by left and right testis. Width of the testes decreased.

By April, the testis started to regain original shape and size. The colour of the testis was white and fat deposition resumed in the gut wall. This period from April to July have been considered as the resting stage of the testis.

1.3.3 Female Reproductive Organ :

The female reproductive system of *A. morar* consists of a paired ovaries and short oviducts. The left ovary is always larger than the right. They are suspended from high on the sides of the body cavity by a pair of mesenteries (mesovaria) and thus situated above the alimentary canal. The anterior portion is slightly wider, blunt and rounded, projected into the body cavity and the posterior portion is tapered and open into a short common oviduct which leads out through the genital pore situated on an elevated urinogenital pore behind the anus just in front of the anal fin. The size and extent of occupancy of the ovary in the body cavity vary with the stage of sexual maturity of the female. The colour varies from whitish in young through light orange when maturing to bright orange in ripe stage. The maturation stages defined by Kestaven (1960), Karim and Hossain (1972b), Treasurer and Holliday (1981), Milton and Arthington (1983), Parween *et al.*, (1993) could be assigned on the macroscopic examination of ovary and microscopic structure of the oocytes.

1.3.4 Development stages of ovary

Different stages of female maturation can be understood by observing the colour of the ovary and the diameter of the ovum (Shafi and Quddus, 1975). The developmental stages and maturation of the ovaries and oocytes are required for the determination of the breeding season of the fish. A total of 541 fishes were studied during June 1993 to May 1995 to determine the different stages of ovaries.

However, the development of ovary could be divided into ten stages on the basis of colour, size and the occurrence of different stages of eggs. These are as follows:

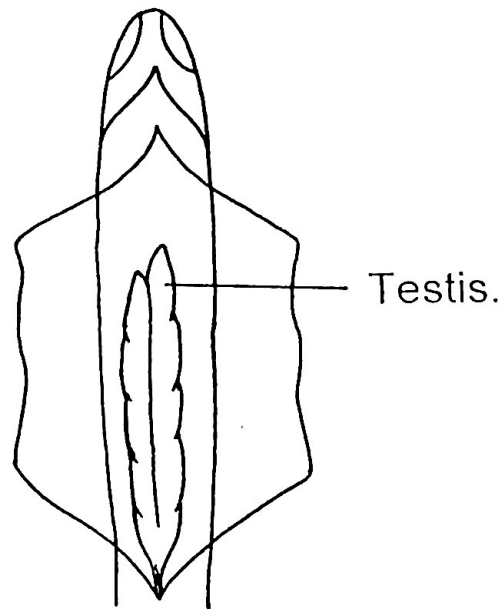


Fig. 2 : *A. morar* : Male reproductive organ.

Plate - 1

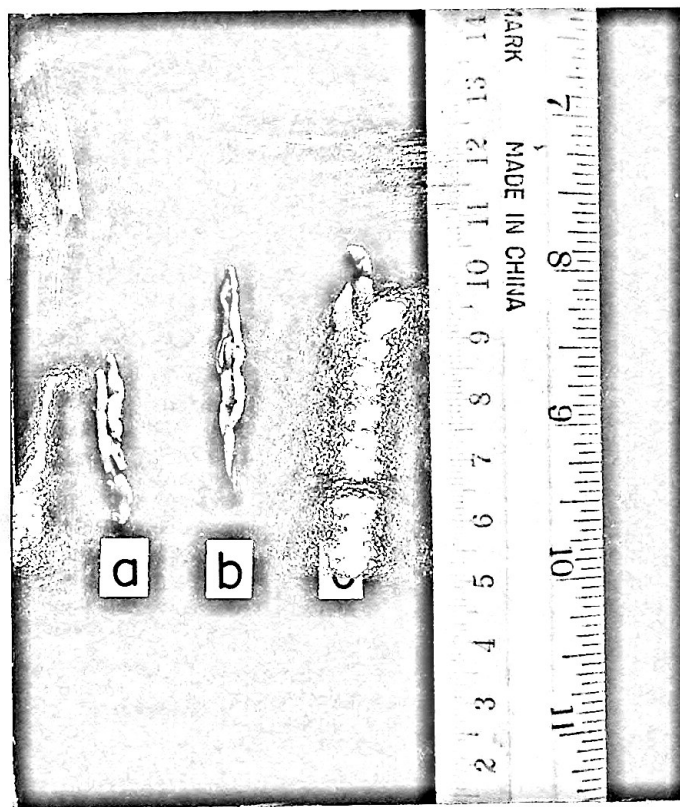


Fig. 1 (a,b,c) : *A. morar* : Testis.

Stage I : Immature ovary

The immature ovary is only found in the immature female. In mid August it is thin and whitish in colour and can not be visible easily and difficult to distinguish from testes.

The ovary occupied about one third of the body cavity. The tip of two lobes of the ovary appeared as two flesh-coloured thread like structure (Plate 2, Fig. 2a, Plate 4, Fig. 4a). Ova are not visible by naked eye. In this stage only immature ova are present. The ova diameter ranged from 0.24 to 0.39mm.

Stages II : Maturing (early developing)

Similar appearance of ovaries are found like stage I. The colour of the ovary was still whitish and occupied about 40-50% of the body cavity (Plate 2, Fig. 2b, Plate 4, Fig. 4b). This stage was found in mid October. No granulosa layer and yolk vesicles were found. In this stage also only immature ova are present but the larger ova are more immature. The ova diameter ranged from 0.30mm to 0.58mm.

Stage III : Maturing (late-developing)

In mid November the ovary grows in weight and size. The tunica became relatively thin. In this stage immature ova are frequently found. Ogonia and maturing ova are also found. The colour of the ovary became light orange and occupied about 60% of the body cavity (Plate 2, Fig. 2c, Plate 4, Fig. 4c). The ova diameter ranged from 0.44mm to 0.77mm.

Stage IV : Mature

In December to mid January the ovary occupied about 70-80% of the body cavity (Plate 2, Fig. 2d, Plate 4, Fig. 4d). The colour of the ovary changed to bright orange. The ovarian wall was slightly thin. Immature and maturing ova were found with the mature ova. The percentage of occurrence of mature ova were maximum. The diameter ranged from 0.52mm to 0.95mm.

Stage V : Ripe ovary

Most of the gravid females caught during late January to February had a ripe ovary (Plate 2, Fig. 2e, Plate 4, Fig. 4e). At this stage the ovary occupied about 85% of the body cavity and bright orange in appearance. The ova were clearly seen through the wall of ovary. Oocytes were transparent. They were partially free and ooze out with little pressure on the abdomen. The ova diameter ranged from 0.81mm to 1.27mm.

Stage VI : Pre-spawning

There exist similarity between this and stage V in external characters. At this stage the oocytes became fully free and ooze out with little pressure on the abdomen. The ovary and oocytes were bright orange in colour (Plate 2, Fig. 2f, Plate 4, Fig. 4f).

Stage VII : Spawning

Thinner oviduct wall were found at this stage. (Plate 2, Fig. 2g, Plate 4, Fig. 4g). The oocytes sometimes found scattered around the genital pore of the female.

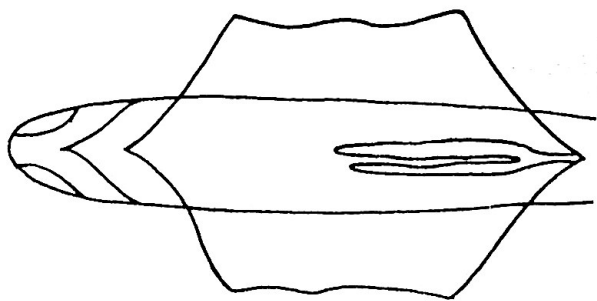
Stage VIII : Post spawning

This stage exists for a very short period of time. The weight of the ovary reduced to about 80% but occupied about 50% of the body cavity. (Plate 2, Fig. 2h, Plate 4, Fig. 4h).

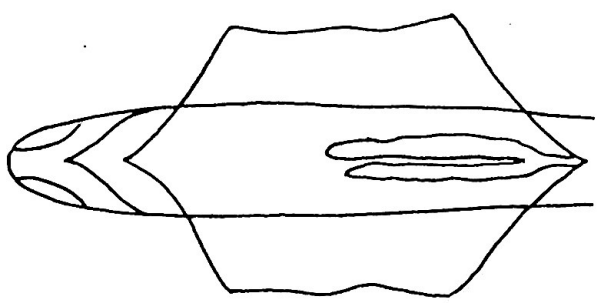
Stage IX : Spent

The ovary lost all the oocytes (Plate 2, Fig. 2i, Plate 4, Fig. 4i) but still contained some ruptured and collapsed post-ovulatory follicles forming an irregular series of folds.

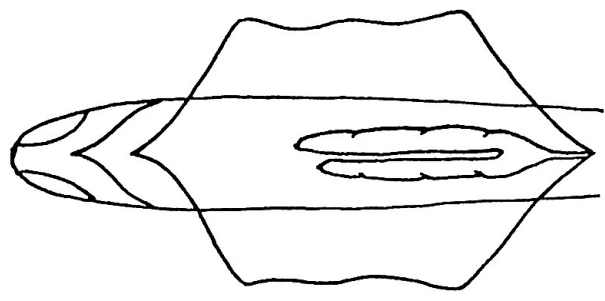
Plate - 2



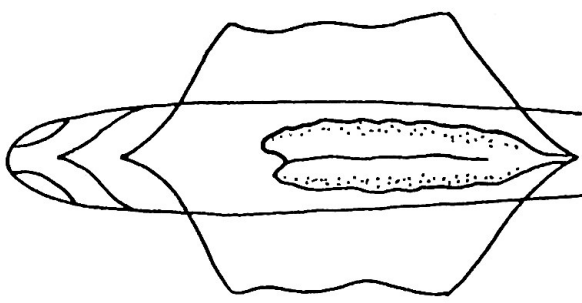
a. Stage I : Immature



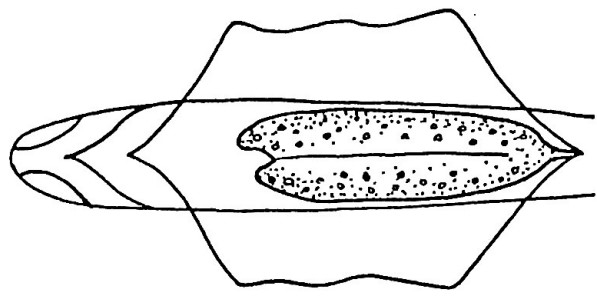
b. Stage II : Maturing
(early developing)



c. Stage III : Maturing
(Late developing)



d. Stage IV : Mature



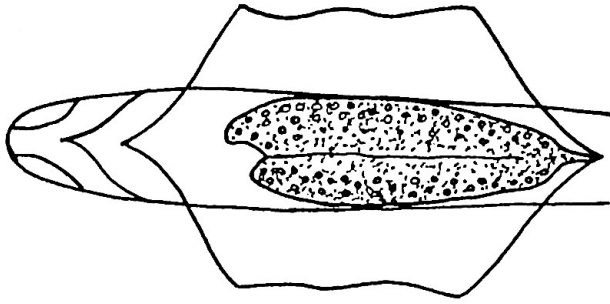
e. Stage V : Ripe

Fig. 2 (a-e) : *A. morar* : Showing the stages of ovarian development.

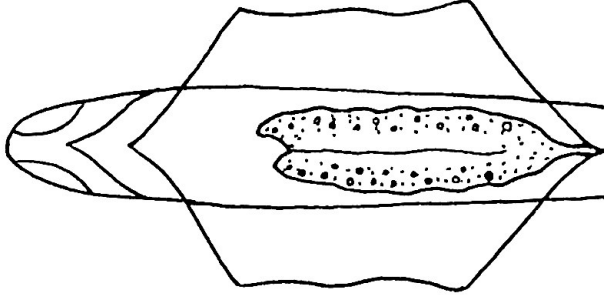
Stage X : Resting

At this stage the wall of the tunica contracted and reduced with a corresponding increase in diameter (Plate 2, Fig. 2j, Plate 4, Fig. 4j). The size and number of oocytes were also found in appearing condition. This condition was observed in the matured females from May to August .

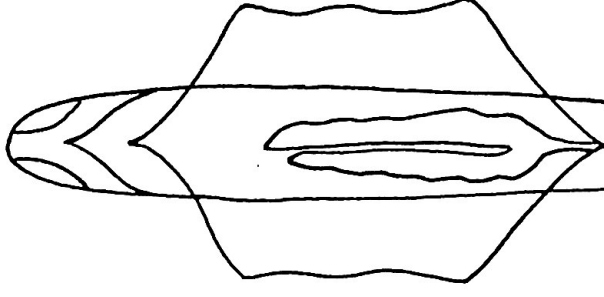
Plate - 3



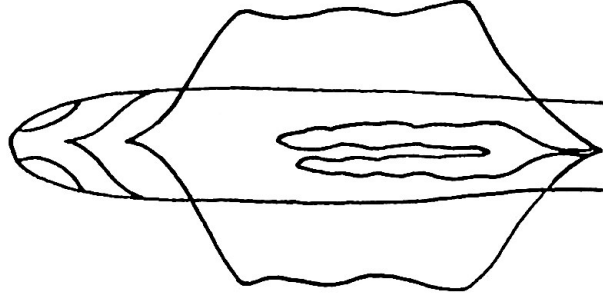
f. Stage VI : Pre-spawning



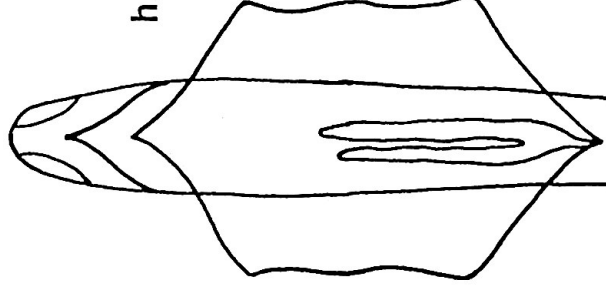
g. Stage VII : Spawning



h. Stage VIII : Post-spawning



i. Stage IX : Spent



j. Stage X : Resting

Fig. 3 (f-j) : *A. morar* : Showing the stages of ovarian development.

Plate - 4

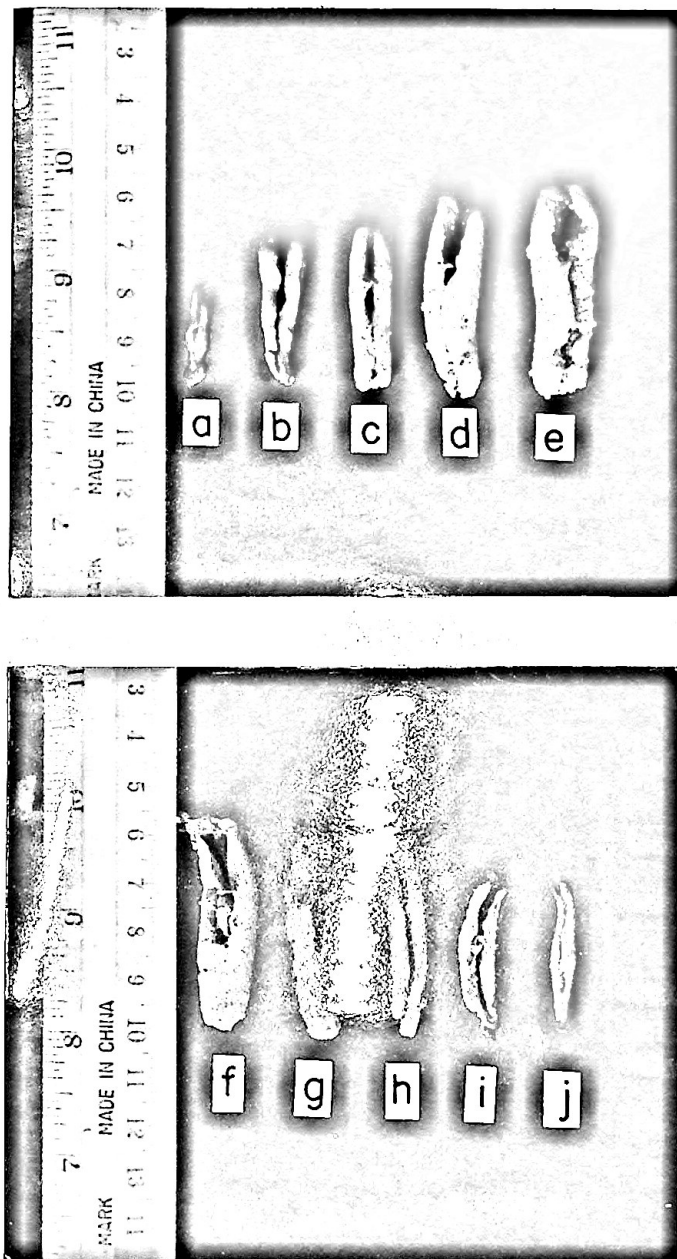


Fig. 4 (a-j) : *A. morar* : Different development stages of ovary.

- | | |
|-------------------------------|--------------------------------|
| a. Immature | b. Maturing (early developing) |
| c. Maturing (late developing) | d. Mature |
| e. Ripe | f. Pre-spawning |
| g. Spawning | h. Post-spawning |
| i. Spent | j. Resting. |

Plate - 5

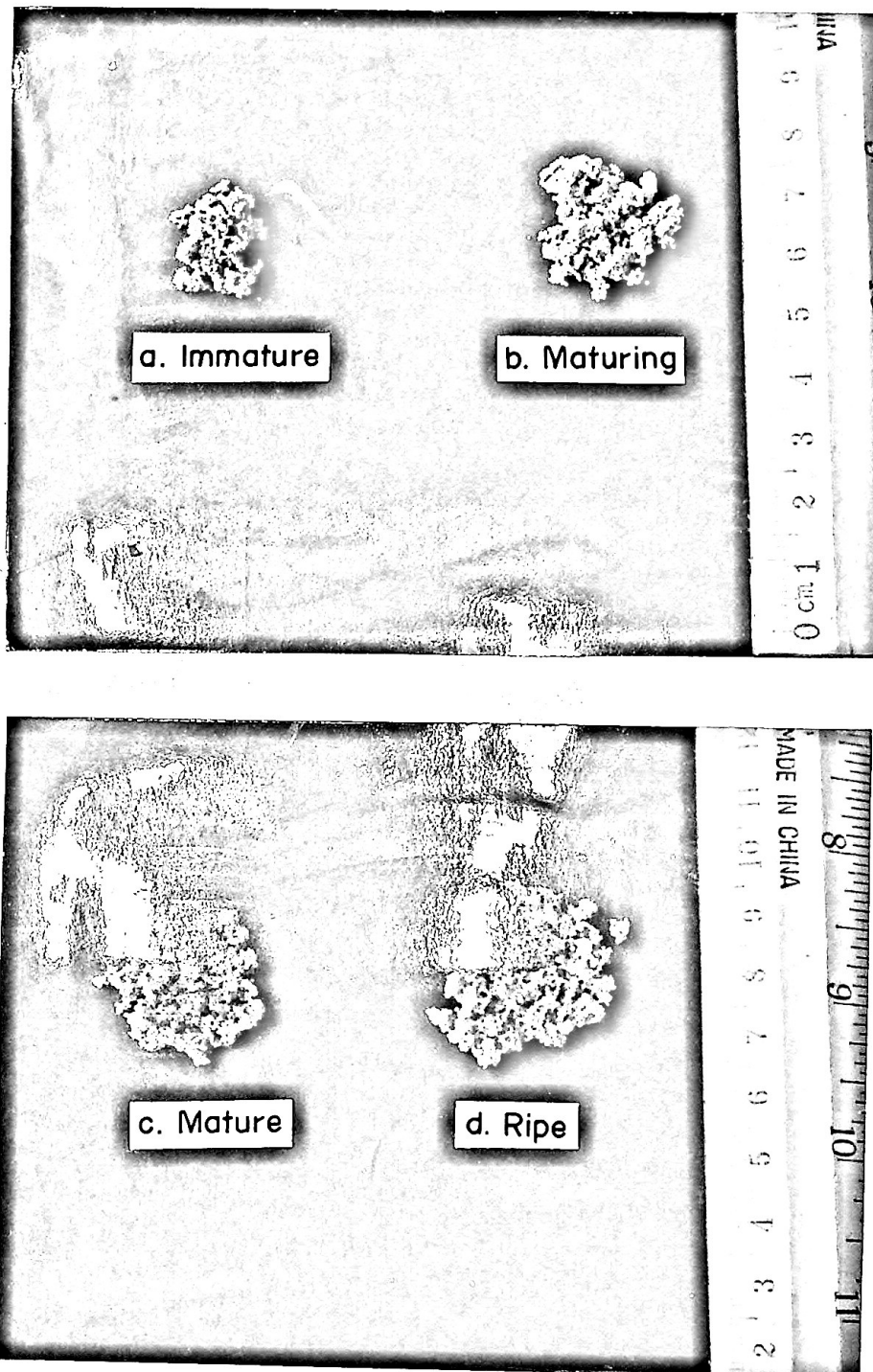


Fig. 5 (a-d) : *A. morar* : Different development stages of ova.
a. Immature b. Maturing
c. Mature d. Ripe

1.3.5 Stages of ova

Table 1 : Different stages of ova diameter of *A. morar*.

Stage of ova	Ova diameter		Colour
	Range (mm)	Mean $\pm$ Sd	
Immature	0.24-0.37	0.34 $\pm$ 0.21	Whitish
Maturing I & II	0.37-0.44	0.40 $\pm$ 0.27	Light orange
Mature	0.44-0.95	0.76 $\pm$ 0.34	Orange
Ripe	0.95-1.27	1.14 $\pm$ 1.62	Bright orange

1.3.6 Length at first maturity

Samples collected during 1993-1995 were grouped into 5 mm length groups and the percentage occurrence in various stages of maturity in these groups, 70-75 to 141-145mm in length was calculated are presented in Table 2. From the Table 2. Females upto 86-90mm of total length were immature. Between the size groups of 91-95 and 106-110 both immature and mature fishes were found. But above the size groups of 111-115 all were mature (Table 3). The largest specimens (141-145 in size) were found mostly in spent condition.

1.3.7 Size frequency distribution

Monthly size-frequency distribution data of 1141 males and of 1261 females (Gravid = 604 and non-gravid = 657) of *A. morar* from June 1993 to May 1995 are presented in Table 2 and in Fig. 3(a, b, c, d). The smallest and largest males measure 65.00mm and 141.50mm in total length in the months of April, 1994 and February, 1995 respectively. In June 1994 to June 1995 study period the smallest and largest females were 67.5 mm and 43.75mm in total length also in the month of May, 1994 and February, 1995 respectively.

The juveniles *A. morar* of both the sexes become available from February to May. The larger sized specimens of male are found from the months of December to March. The large sized female were found from the months of December to March. No. juveniles are found from the months of June to January of both the sexes.

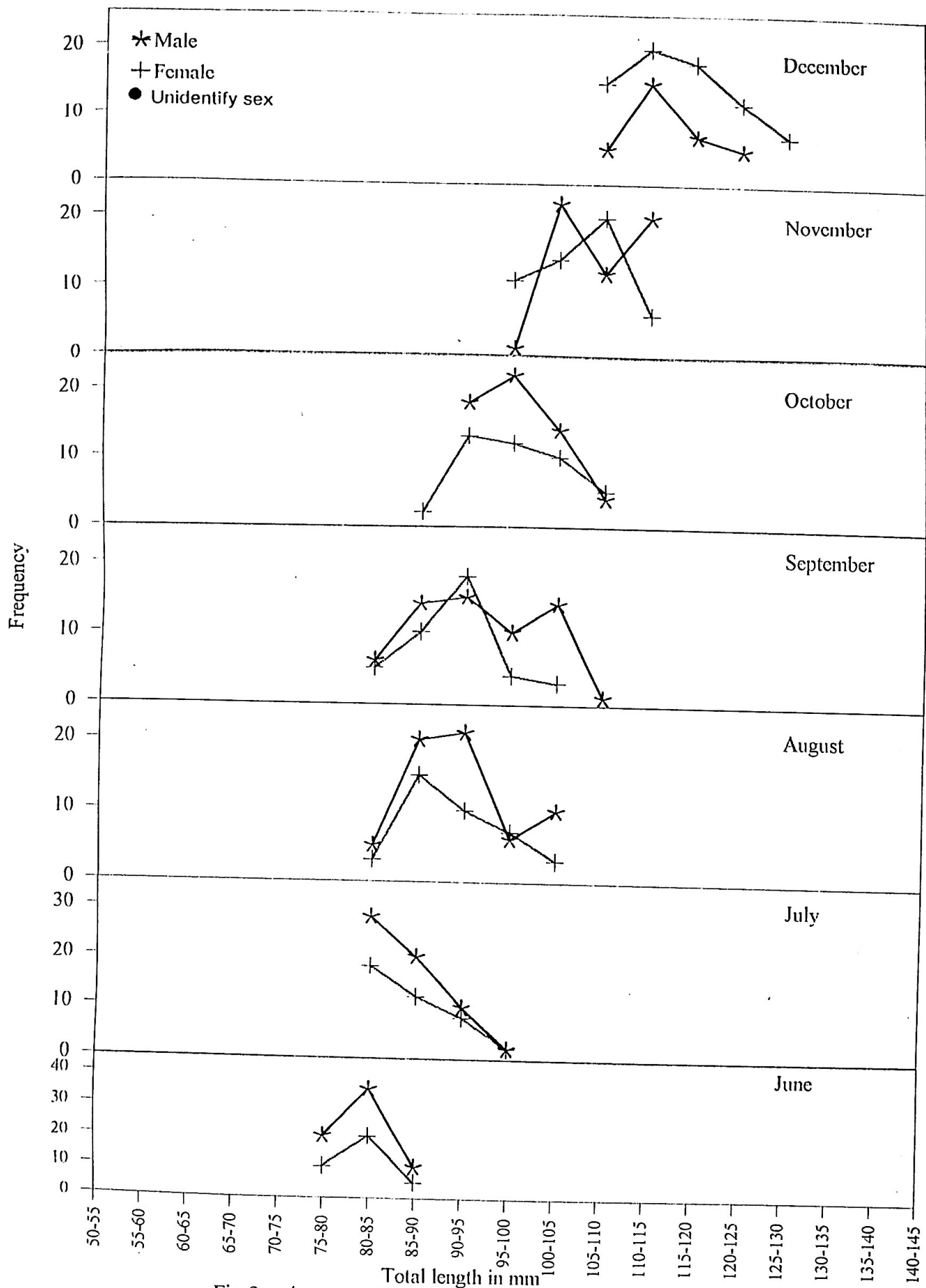


Fig.3a. *A. morar*: Monthly size frequency distribution (1993).

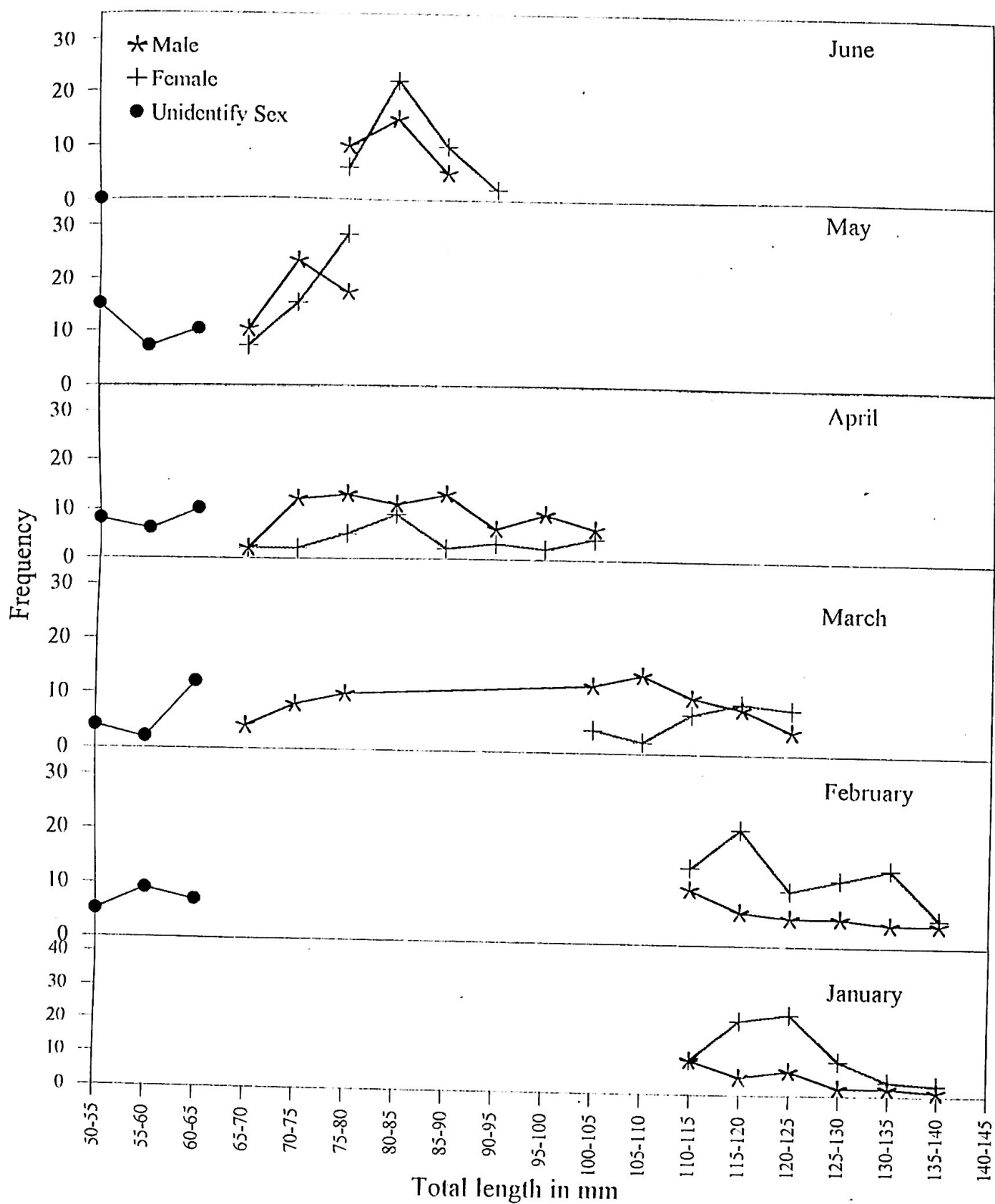


Fig3b. *A. morar*: Monthly size frequency distribution (1994)

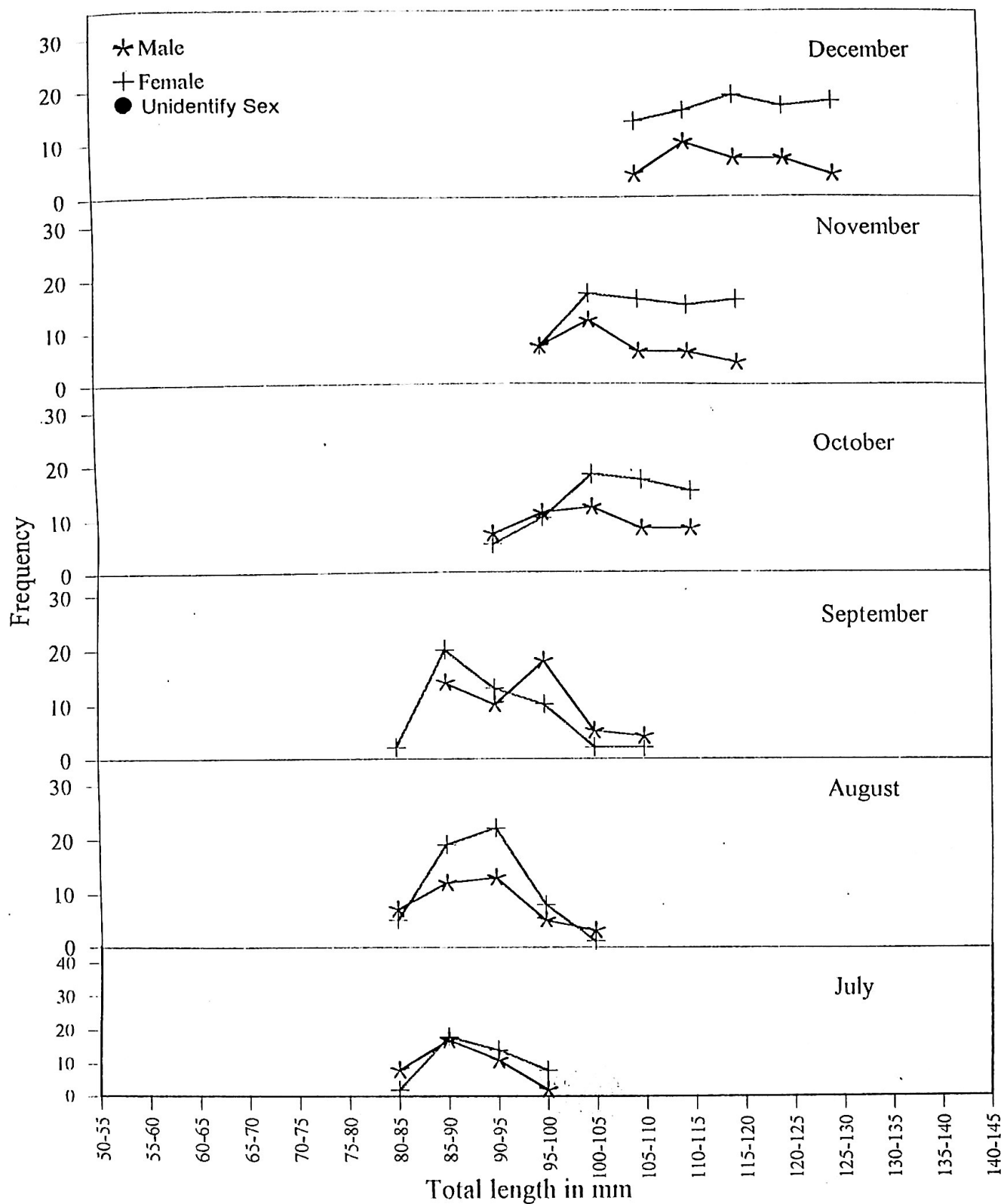


Fig. 3c. *A. morar*: Monthly size frequency distribution (1994).

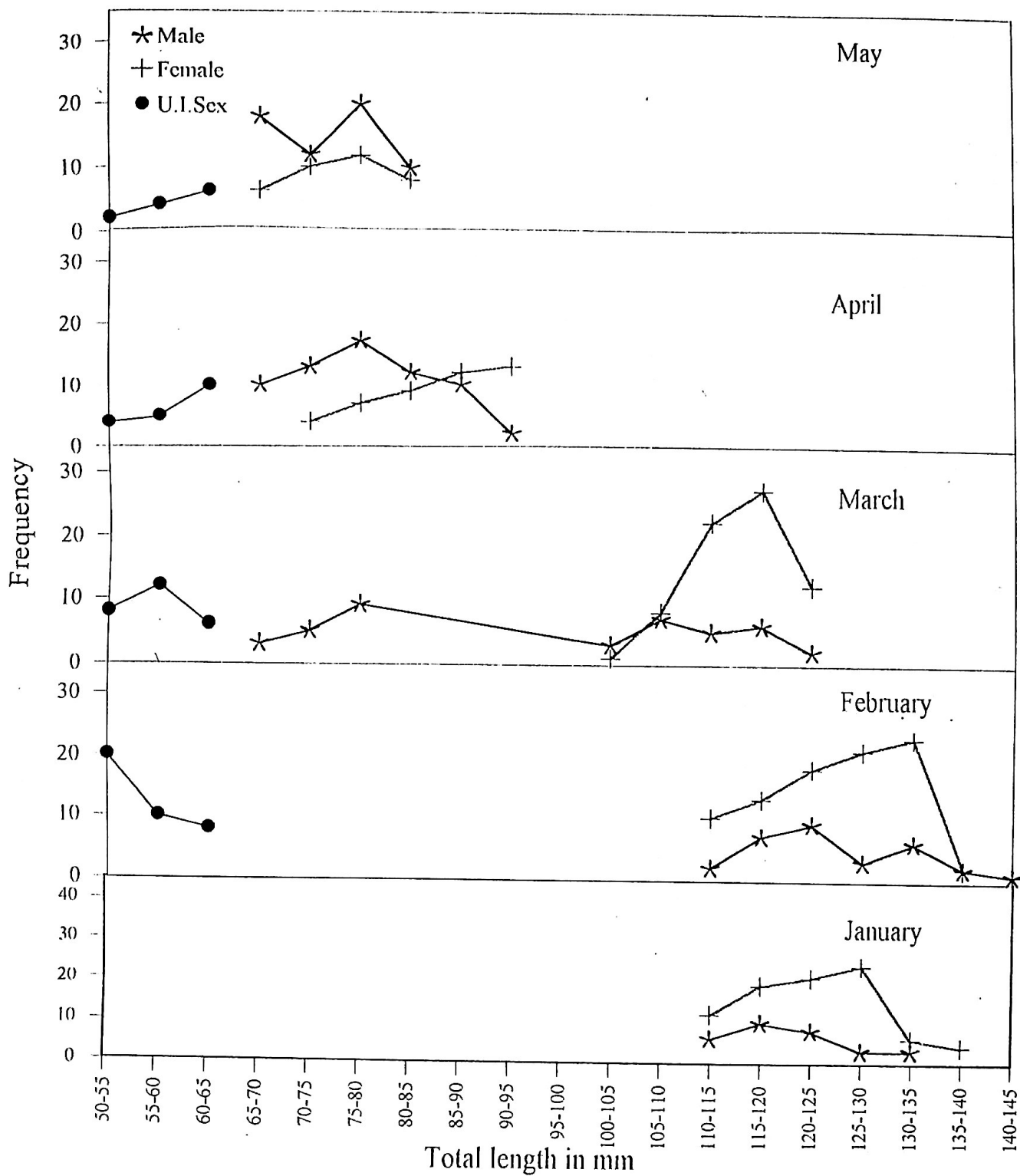


Fig.3d. *A. morar*: Monthly size frequency distribution (1995).

Table 2 : Length frequency distribution of male and female *A. morar* from 1993 June to 1995 June.

[illegible]

1.3.8 Reproductive cycle

Reproductive behaviour in fishes is cyclic, more or less regularly periodic (Lagler *et al.*, 1967). The reproductive act in some fishes occurs only once in a very short life-time, while in other fishes, it occurs in moderately long life span. Several other species spawn more than once in a year more or less continually. After spawning, new group of oocytes is formed, which gradually mature to become ready for the next season.

Reproductive cycle of fish depends upon several factors. Both light and temperature are important factors in controlling the rate of development and maturation of gonads in fishes. Water movement very often controls the release of spermatozoa and fertilization of eggs (Kinne, 1971). Seasonal effect mainly plays role in the overall physiology of fishes. The feeding and breeding peaks mostly remain in opposite direction when plotted against the months of a year. There are more than 15 methods to determine the reproductive cycle of fishes and aquatic organisms. Out of these methods six were used in the present study to determine the reproductive cycle of *A. morar*.

Table 3 : *A. morar*: Percentage occurrence of female fish in different stages of maturity in the various length groups (N=570).

Size group	Number of Female fish	Stages of maturity			
		I	II	III	IV
70-75	48	100.00	-	-	-
76-80	58	100.00	-	-	-
81-85	55	100.00	-	-	-
86-90	60	100.00	-	-	-
91-95	50	50.00	40.00	10.00	-
96-100	52	38.46	48.08	13.46	-
101-105	56	28.57	53.57	16.07	1.78
105-110	60	16.67	33.33	33.33	16.66
111-115	55	-	18.18	54.54	27.27
116-120	50	-		60.00	40.00
121-125	65	-		46.15	53.85
126-130	55	-		27.27	72.72
131-135	45	-		11.11	88.89
136-140	25	-		28.00	75.00
141-145	10	90.00			10.00

1.3.8.1 Percentage of gravid female against time

The data obtained from June 1993 to May 1995 (24 months) reveal that the gravid females of *A. morar* occur during the period from October to April (Table 4 Fig 4). However, the maximum percentage of gravid females was found in the months of January-February 1993, 1994 (100%) and January, 1995 (91%). The next percentage were found in the months of December (94.11%), March (40.00%), November (27%), October (16.66%), April (14.28%) in the year of 1993 to 1994 and February (90.90%), December (89.28%), March (64.28%),

November (57.14%), October (30.76%), April (22.21%) in the year of 1995. During the months from May to September no gravid female was found. From the above observation, it may be concluded that the species possesses single breeding season extending from November to March with the peak in December to February.

Limitation of the methods : It is very difficult to estimate accurately the proportion of gravid female in the natural population. The number of gravid females caught may not be representative of the actual situation in the natural population. So far an accurate estimation of the breeding periodicity of *A. morar* a number of methods have been applied. From the available data it would appear that this species breeds during November to February with a peak season during December to February.

Table 4 : Summary of the monthly collection and observation for 24 months and percentage of gravid female of *A morar*.

Year and months	Total number of specimen	Total male	Female			Percentage of gravid
			Total	Non-gravid	Gravid	
JUNE, 93	100	65	35	35	-	-
JULY	100	60	40	40	-	-
AUGUST	100	62	38	38	-	-
SEPTEMBER	100	60	40	40	-	-
OCTOBER	100	58	42	35	07	16.66
NOVEMBER	115	64	51	37	14	27.45
DECEMBER	100	32	68	04	64	94.11
JANUARY, 94	100	27	73	00	73	100.00
FEBRUARY	100	24	76	00	76	100.00
MARCH	100	70	30	18	12	40.00
APRIL	100	72	28	24	04	14.00
MAY	100	50	50	50	-	-
JUNE	70	30	40	40	-	-
JULY	80	38	42	42	-	-
AUGUST	95	40	55	55	-	-
SEPTEMBER	100	51	49	49	-	-
OCTOBER	110	45	65	45	20	30.76
NOVEMBER	105	35	70	30	40	57.14
DECEMBER	115	31	84	09	75	89.28
JANUARY, 95	118	32	86	00	86	100.00
FEBRUARY	118	30	88	00	88	100.00
MARCH	110	40	70	35	35	50.00
APRIL	110	65	45	35	10	22.21
MAY	96	60	36	36	-	-

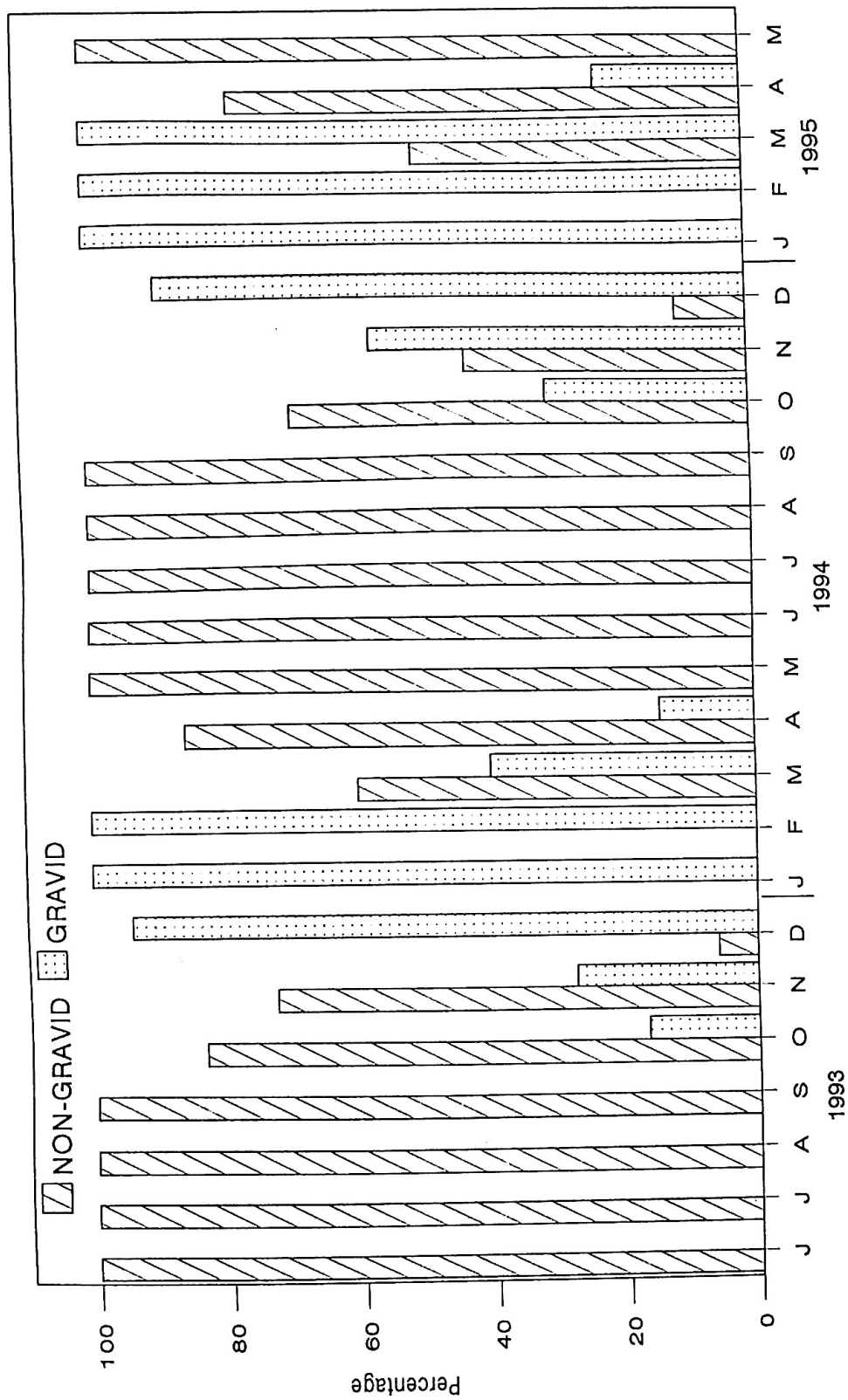


Fig.4. *A. morar*: Monthly percentage occurrence of gravid and non-gravid females during June 1993 - May 1995

1.3.8.2 Diameter of Ova

The sample of the ovarian eggs were taken from the anterior, middle and posterior region of the ovary. Microscopical measurements of the diameters of 10 to 15 ova for each specimens were taken at random and the average was calculated. After summing up the month wise average diameter of ova was determined.

This changes in the pattern of diameter of ova are found only in mature female in relation to seasonal variation. The maximum mean diameter of ova was obtained as 1.14 ± 2.00 mm (February, 1994) and minimum value as 0.31 ± 0.22 mm (May, 1994). However, during the months of January and February of the years, the ova were always of maximum size. The trend of increament of ova diameter started from October, and from April the average diameter was found to decreased suddenly (Table 5, Fig. 5). Except the months of December to February maturing or immature or residual ova in the ovary were more frequent.

Mature and ripe ova are commonly found in the ovary of mature female between November to February, peak in January to February and this is the period of highest reproductive activity of *A. morar*.

1.3.8.3 Gonado-somatic Index (G.S.I)

The pattern of gonad development expressed as gonado-somatic index is a well known relationship which is frequently considered to determine the reproductive periodicity of fish (Le Cren, 1951). The calculation of gonado somatic index was made by taking into account the weight of the gonad and the weight of the animal. The formula for this is as follows :

$$G.S.I = \frac{\text{Weight of the gonad}}{\text{Weight of the fish}} \times 100$$

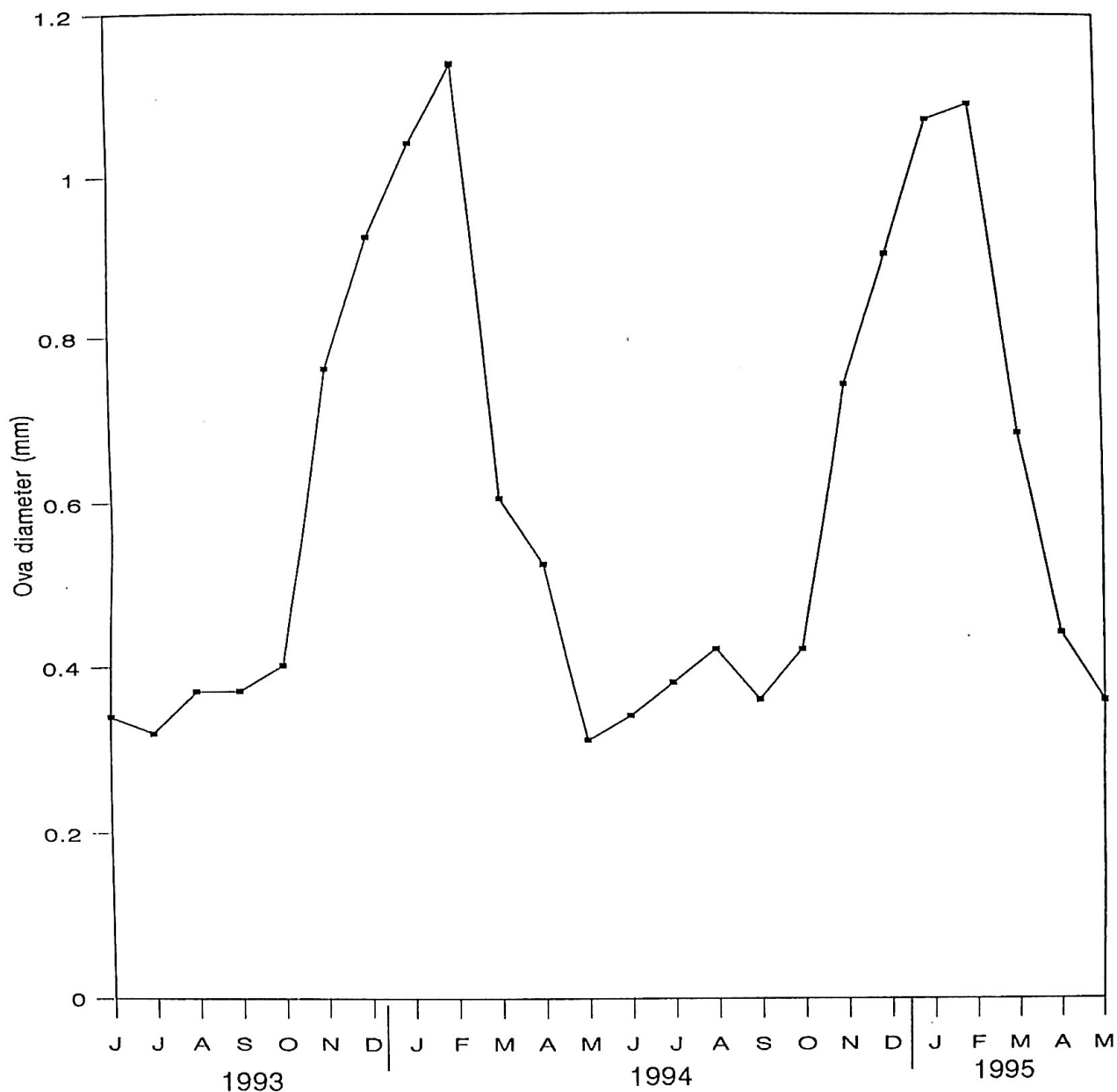


Fig.5. *A. morar*: Showing the monthly changes in diameter of ova.

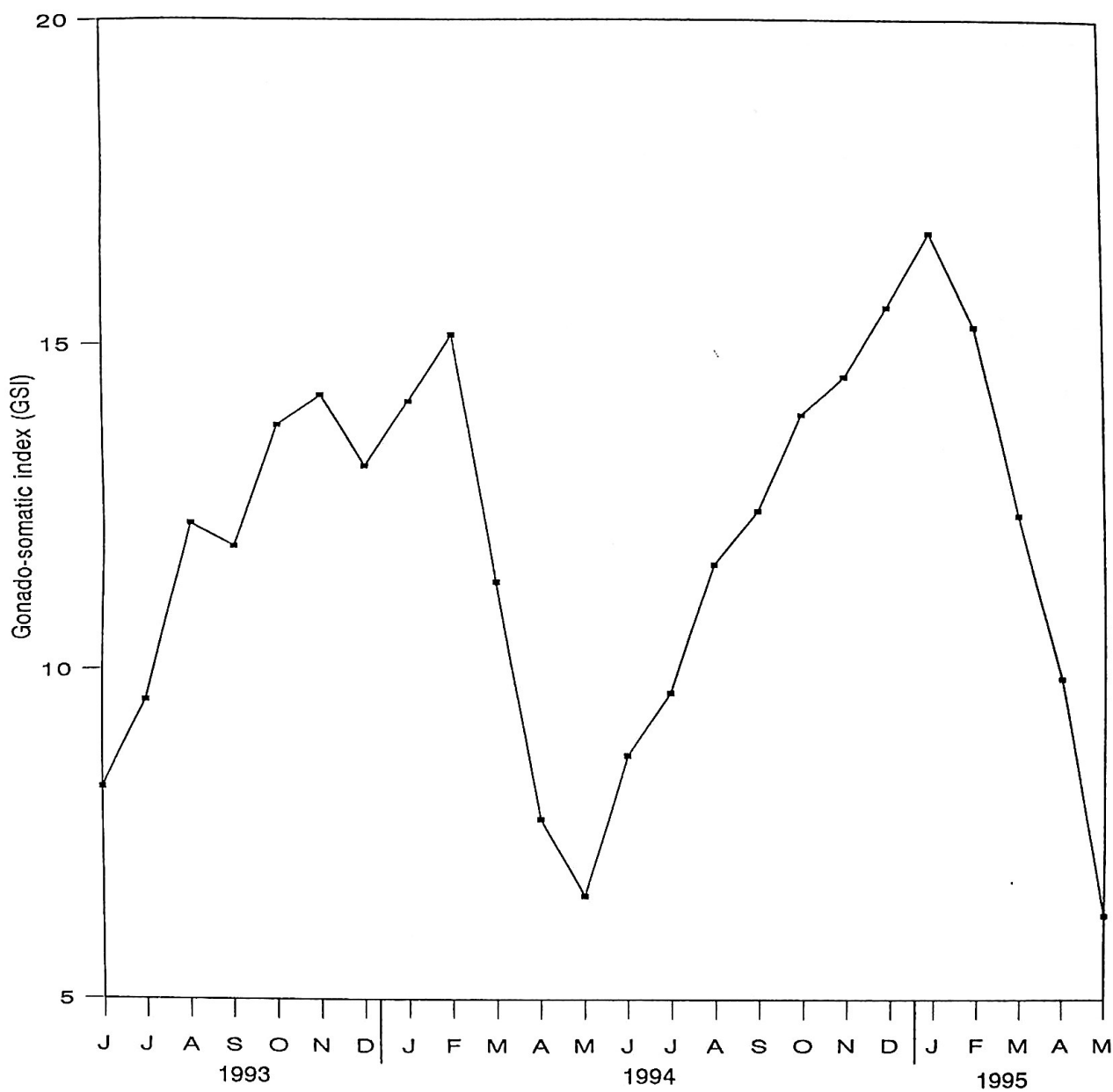


Fig.6. *A. morar* : Showing the monthly mean gonado-somatic index (GSI).

The monthwise range, mean and standard deviation of the G.S.I. are given in Table (5). The G.S.I. value directly relates with the degree of maturation of the ovary. Higher values of G.S.I. had observed during the months of December to February and peak in January/ February 14.08 ± 4.26 and 15.08 ± 4.33 (1994); 16.68 ± 1.02 and 15.23 ± 1.02 (1995) (Fig. 6).

Variation in G.S.I only occur in mature or reproductively active animals, and the peak may be indicative of the peak period of spawning. After extrusion or evacuation of ripe ova, the ovary is suddenly reduced in size as well as in weight. So G.S.I declined rapidly after spawning with minimum indices as 6.56 ± 0.86 and 6.28 ± 2.53 in the month of May of each year respectively. For the study of gonadosomatic index, a total of 541 females both mature and immature were examined during 24 months period June 1993 to May 1995.

1.3.8.4 Gonadal length index

This method is similar to G.S.I. but calculation of gonadal length index was made by taking into account the length of gonad and length of animal. The formula for gonadal-length index (G.L.I.) is as follows :

$$\text{G.L.I} = \frac{\text{Gonadal length}}{\text{Total length of fish}} \times 100$$

Where gonadal length is the length of the ovary and total length is the length of the animal. For the study of gonadal length index, a total of 541 females both mature and immature were examined during 24 months period from June, 1993 to May 1995.

This method is a simple and accurate one for the determination of spawning periodicity.

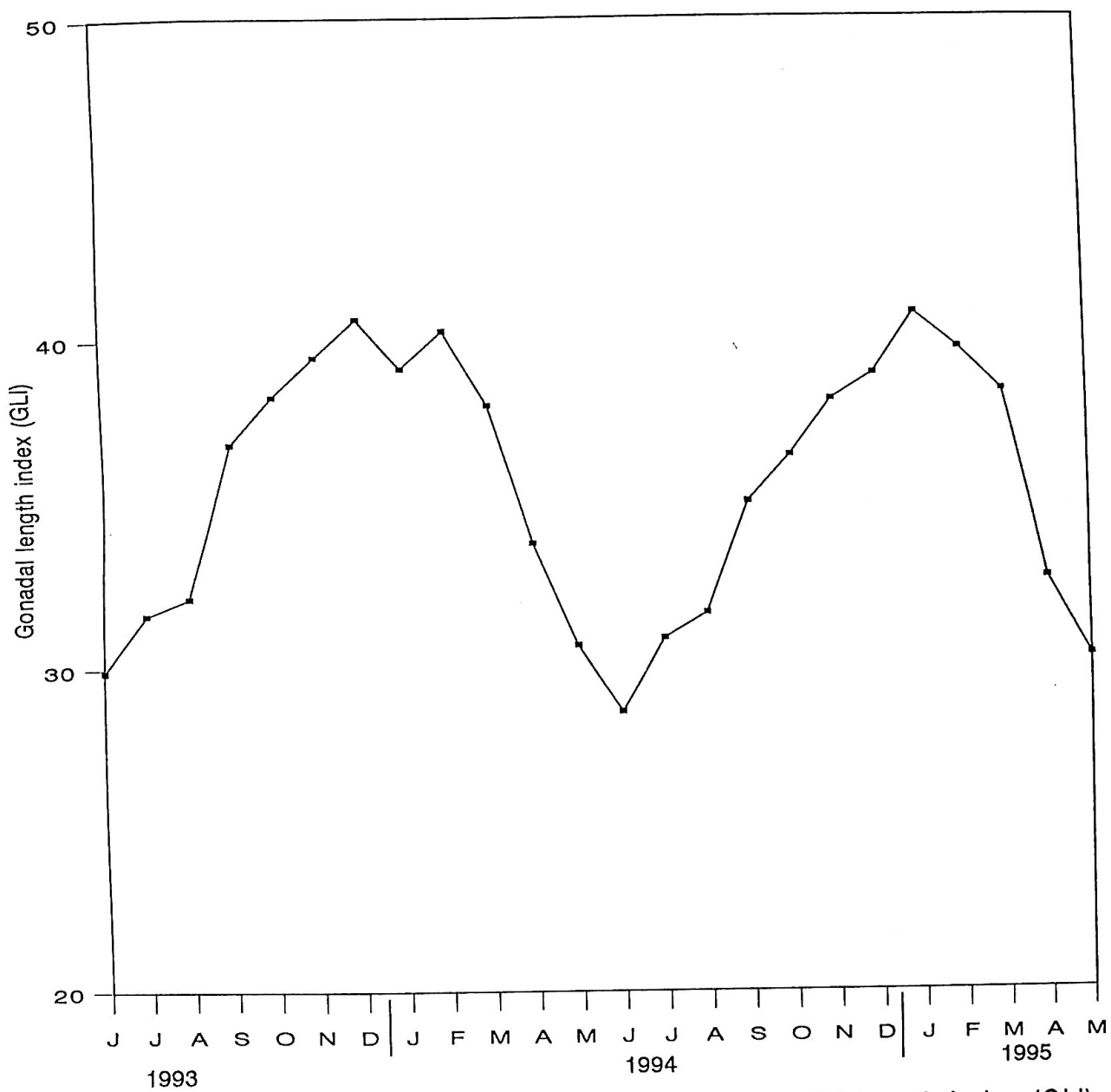


Fig.7. *A. morar*: Showing the monthly mean gonadal length index (GLI).

The range of gonadal-length index, mean and standard deviation are given in Table 5. The mean G.L.I. values (Fig. 7) indicate that the GLI follows the similar pattern of fluctuation like G.S.I. Maximum indices as 38.98 ± 2.68 and 40.10 ± 2.37 ; 40.66 ± 1.34 and 39.56 ± 2.02 for ovaries were recorded in January and February during 1994 and 1995 respectively. Minimum mean value was 29.90 ± 2.53 and 28.56 ± 1.05 for the months of June 1993 and 1994 respectively.

1.3.8.5 Colouration of the ovary

Colour of the ovary changes according to the degree of maturity of the ova. Colour of the ova may vary from species to species. In *A. morar* the immature ovary is whitish and transparent. With the increase of yolk deposition, the ovary becomes faint orange to orange, and opaque as well. The ripe and fully mature ovary is bright orange in colour. However, the spent ovary again becomes white in appearance (Table 5). Particularly in January and February all adult females gravid possessed orange ovary which indicates ripeness.

It is a well defined indication of active breeding season. Each female spawns once in a year.

1.3.8.6 Maximum indices of spermatozoa in the milt of male

During November to April the posterior portion of the vas deferens of male fishes were found more massive than the other months of this year. During this period most of the mature males in the catch were observed with extruded milt at the opening of the male aperture. Slight pressure on the postero ventral side of the abdomen was enough to realease the milt from the vas deferens.

Table 5 : *A. morar* : The month wise gonado somatic index (GSI), gonadal length index (GLI), Ova diameter and colour of the ovaries with range, Mean, standard deviation and number of female fish examined (N=541).

Year and months	Number of fish examined	Gonado-somatic index Rang		Mean±sd	Gonadal length index Range		Mean ± sd	Ova diameter (mm)		Colour & condi. of ovaries
		Min	Max.		Min.	Max.		Min.	Max	
JUNE, 93	25	6.16	10.5	8.23±1.09	25.00	33.33	29.90±2.53	0.31	0.38	Whitish
JULY	26	7.54	11.16	9.59±0.79	25.58	34.56	31.60±1.96	0.30	0.37	Whitish
AUGUST	27	10.76	13.88	12.24±0.78	28.08	35.29	32.10±1.71	0.35	0.39	Whitish
SEPTEMBER	27	10.21	15.30	11.88±1.39	33.96	38.70	36.75±1.25	0.24	0.40	Whits /L orange
OCTOBER	26	11.39	16.22	13.73±1.24	36.26	40.74	38.18±1.14	0.37	0.44	Light orange
NOVEMBER	30	11.66	17.34	14.19±1.74	35.90	42.85	39.37± 1.57	0.52	0.95	Light orange
DECEMBER	26	7.12	18.61	13.08±3.29	34.25	44.26	40.51±2.90	0.77	1.02	Bright orange
JANUARY, 94	30	5.89	24.23	14.08±4.26	34.18	45.51	38.98± 2.68	0.90	1.19	Bright orange
FEBRUARY	24	6.54	23.47	15.08±4.33	33.33	44.26	40.10±2.37	0.69	1.27	Bright orange
MARCH	25	1.44	18.13	11.30±4.88	26.90	47.80	37.80±4.25	0.48	0.77	Orange
APRIL	26	5.71	13.30	7.73±1.52	30.76	37.50	33.59±1.66	0.48	0.58	Light Orange
MAY	27	4.10	8.16	6.56±0.86	28.56	32.91	30.57±1.12	0.30	0.39	Light Orange
JUNE	28	5.96	11.07	8.67±1.07	24.53	32.23	58.56±1.05	0.30	0.41	Whitish
JULY	27	7.14	12.34	9.62±0.85	25.24	33.96	30.76±2.02	0.32	0.40	Whitish
AUGUST	25	9.95	13.57	11.57±1.28	27.56	34.85	31.52±2.00	0.40	0.44	Whitish
SEPTEMBER	28	10.85	14.21	12.37±0.83	30.50	37.24	34.81±1.54	0.25	0.42	W. Light orange
OCTOBER	26	11.34	16.05	13.86±2.02	34.24	39.44	36.24±2.00	0.36	0.46	Light orange
NOVEMBER	27	10.58	18.02	14.47±0.86	35.01	41.33	37.96±1.25	0.53	0.91	Orange
DECEMBER	25	9.53	20.53	15.53±2.01	35.21	43.54	38.76±0.86	0.74	1.03	Bright orange
JANUARY, 95	25	8.23	23.56	16.68±1.02	34.21	45.86	40.66±1.34	0.91	1.16	Bright orange
FEBRUARY	24	6.58	22.18	15.23±0.80	34.01	44.23	39.56±2.02	0.56	1.23	Bright orange
MARCH	27	2.53	17.50	12.31±2.02	32.08	46.56	38.24±1.02	0.39	0.81	Orange
APRIL	28	3.08	12.20	9.85±1.00	33.05	45.53	32.57±1.65	0.35	0.71	Light orange
MAY	25	4.52	8.05	6.28±2.53	28.24	31.43	30.24±0.86	0.29	0.44	Light orange

1.3.9 Fecundity

The term fecundity can be defined as the number of eggs in the ovary of a fish (Nikolsky, 1963). The number of eggs being ready for the next spawning by a female (Royce, 1972). However fecundity appears to bear some broad relationship with the care or nature of the eggs (Lagler *et al.* 1967). For example, in poorly fecund fish, parental care is highly developed. So, to make the population constant, fecundity do not fluctuate widely due to low mortality rate and predator. Again the fishes with high fecundity show great fluctuation in their abundance. They do not take care of their eggs, the eggs are subjected to several injury due to excessive temperature, parasitic and predatory attack, etc., after spawning, and most of them do not survive up to hatching.

Thus, fecundity is one of the important aspects of fish biology and population dynamics (Severtsov, 1941) which must be understood to explain the variations in the level of production as well as to make efforts to increase the harvest. A thorough knowledge on fecundity of the fish is essential for evaluating the commercial potentialities of its stock, and fishery (Doha and Hye, 1970). Fecundity also determine the index of density dependent factor affecting the population size (Das, 1977).

Estimation of fecundity on the basis of number of oocytes present in the ovary is not purely accurate, because of the following reasons :

- (i) not all the oocytes contained in the ovary are released in single spawning season;
- (ii) some of the oocytes are reabsorbed in the ovary by the atretic process (Chubb and Poter , 1984) and
- (iii) constituents of the germinal stand present at all the times, are retained after egg laying.

Several workers have made significant contribution to the study of fecundity of different fishes in our country. Notable among them are Doha and Hye (1970), Karim and Hossain (1972 b), Dewan (1973) Shafi and Quddus (1974) and Hossain (1982), Afroze & Hossain (1983), Islam & Hossain (1984), Nargis and Hossain (1988), Hossain *et al.*, (1992), Bhuiyan and Islam (1990), Hossain *et al.*, (1991), Bhuiyan *et al.*, (1993), Haque and Hossain (1993), Alam *et al.*, (1994), Hussain (1997), etc.

In the present study, gravimetric method Lagler (1956), Pantulu (1963), Bhatnagar (1964), Rao (1967), Islam and Talbot (1968), Evans (1969), Doha and Hye (1970) used for the estimation of fecundity.

To estimate the fecundity of ripe oocytes, first the pattern of distribution of ripe oocytes in the two lobes of the ovary were determined. A portion of the ovary weighing approximately about 0.050g to 0.100g were taken from the middle, anterior and posterior regions of the left ovary and the number of oocytes was counted. The grand total of oocytes in the ovary was calculated from the known weight of the sample, the number of oocytes in the sample and the total weight of the ovary.

$$\text{Estimated Fecundity} = \frac{\text{No. of oocytes in the sample}}{\text{Wt. Of the sample}} \times \text{Gonadal weight}$$

To establish the mathematical relationships of ripe oocytes with total length, standard length, gonadal weight, total body weight and the values of the regressions and correlation co-efficients, the intercepts and standard errors were estimated by least square method.

1.3.9.1 Estimation of Fecundity

A total of 155 mature female specimens of *A. morar* were selected for the estimation of fecundity. It was observed that the number of eggs varied from 1431 (TL 93 mm and TW 6.55g) to 9401 (TL 143mm and TW 23.00g). However the smallest fish in the sample with (93mm and TW 6.55) possessed 1431 eggs and the largest fish (TL 143 mm and TW 23.00) had 8815 eggs.

The mean number of mature ova per gram of body weight was estimated as 317.48 eggs/g. The relationships between fecundity and total length, standard length, total body weight, gonadal length and gonadal weight were found to be positively correlated and the value of co-efficient of correlation was highly significant

Table 6 : Relationship between fecundity and total length, standard length, body weight, gonadal length and gonadal weight of *A. morar*.

Relationship between		Value of 'a'	Value of 'b'	Value of 'r'
Fecundity (F)	Total length (TL)	-6988.44	97.006	0.948***
Fecundity (F)	Standard length (SL)	-5058.89	97.078	0.915***
Fecundity (F)	Total weight (TW)	590.33	273.94	0.854***
Fecundity (F)	Gonadal length (GL)	-5325.24	208.15	0.939***
Fecundity (F)	Gonadal weight (GW)	-700.65	2941.45	0.992***

*** Highly significant ($P < 0.001$)

1.3.9.1.1 Relationship between Total Length (TL) and Fecundity (F)

This scatter diagram showed the relationship between total length and fecundity of *A. morar* (Fig. 8a, Table 6). The regression equation was found to be linear and the co-efficient of correlation was highly significant ($r = 0.948$).

1.3.9.1.2 Relationship between Standard Length (SL) and Fecundity (F)

This scatter diagram showed the relationship between standard length and fecundity of *A. morar* (Fig. 8b, Table 6). The regression equation was found to be linear and the co-efficient of correlation was highly significant ($r=0.915$) (Table 5).

1.3.9.1.3 Relationship between Total weight (TW) and Fecundity (F)

This is a correlation between the fecundity and total weight of the females when the individual value of the body weight were plotted against the respective fecundity. The value of co-efficient of correlation was 0.854 (Table Fig. 8c, Table 6).

1.3.9.1.4 Relationship between Gonadal length (GL) and Fecundity (F)

The scatter diagram of gonadal length and fecundity showed a linear relationship between the two variables (Fig. 8d). The value of co-efficient of correlation was 0.939 (Table 6)

1.3.9.1.5 Relationship between Gonadal weight (GW) and Fecundity (F)

The scatter diagram of gonadal weight and fecundity showed a linear relationship between the two variables (Fig. 8e). The value of co-efficient of correlation was 0.992 (Table 6)

1.3.9.2 Fecundity factor

Fecundity factor can be defined as the number of eggs in the female of a given length (Table 7). In other words fecundity factor is a tabular presentation with respect to length of the animal. The total length of the female was classified into 11 size groups of 5 mm class interval. The mean values of fecundity of lowest and highest size groups were 1665.5 ± 357.08 eggs and 6578.74 ± 2853.31 eggs respectively. Whereas, the highest mean value of fecundity was observed in a size group of 141-145mm as 6578.74 ± 2853.31 eggs. Normally the individual variation

Table 7 : *A morar* : Fecundity factor with respect to total length, total weight and weight of egg mass. The range, mean and standard deviation for each case are provided.

Total length			Total body weight in grams			Weight of gravid egg mass in grams			Estimated fecundity			No of fish observed
Min.	Max.	mean±sd	Min.	Max.	mean±sd	Min.	Max.	mean±sd	Min.	Max.	mean±sd	
93	95	94±1.14	6.55	8.55	7.55±1.41	0.85	0.86	0.85±0.01	1413	1918	1665.5±357.08	2
97	100	99.12±1.12	7.55	10.23	8.94±0.72	0.92	1.82	1.21±0.27	1819	4318	2526.22±1599.08	8
101	105	103.45±1.57	6.81	11.32	9.04±1.44	0.75	1.82	1.09±0.33	1457	6412	2860.90±1364.60	11
106	110	108.16±1.50	7.75	13.06	11.68±1.44	1.02	2.30	1.35±0.33	1956	6945	3383.75±1834.71	24
111	115	113.48±1.42	10.50	15.43	12.58±1.24	1.07	2.50	1.76±0.50	2124	9223	4556.17±1795.86	47
116	120	117.72±1.64	9.55	16.32	13.05±1.53	1.00	2.26	1.77±0.37	1703	6544	4456.10±1379.89	29
121	125	122.18±1.14	12.01	17.56	14.77±1.64	1.40	3.48	2.12±0.63	2753	9396	5747.36±1874.25	22
126	130	128±2.82	14.00	14.08	14.04±0.05	2.15	2.44	2.29±0.20	5500	6600	6050±777.81	2
133	135	135±2.82	20.00	21.00	20.5±0.07	2.12	2.16	2.14±0.02	5300	5400	5350±7071.06	2
-	-	-	-	-	--	-	-	-	-	-	--	-
143	145	144.37±0.91	20.11	26.01	23.83±1.87	2.5	3.00	2.48±0.22	6092	9401	6578.74±2853.34	8
Total =		116.55±0.66	13.599±0.549			1.709±0.19			4317.47±814.31			155

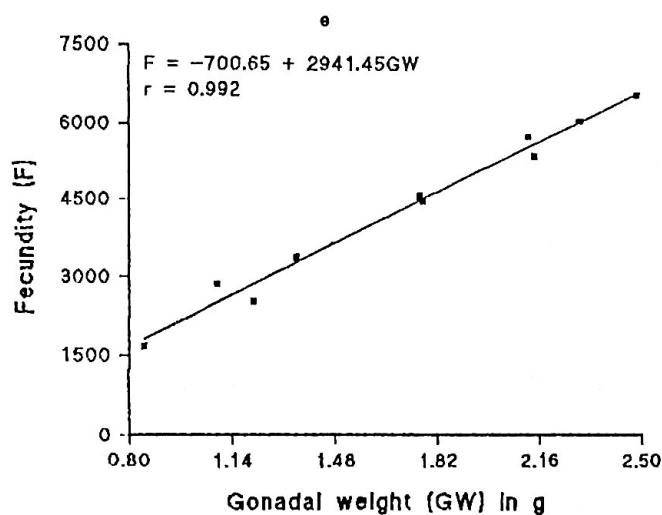
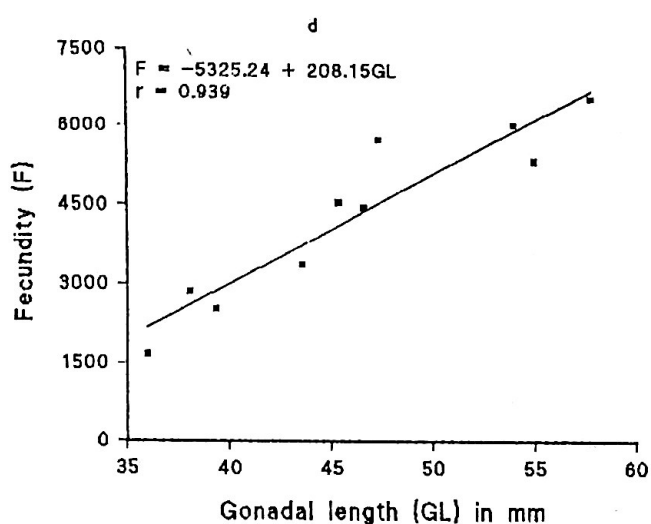
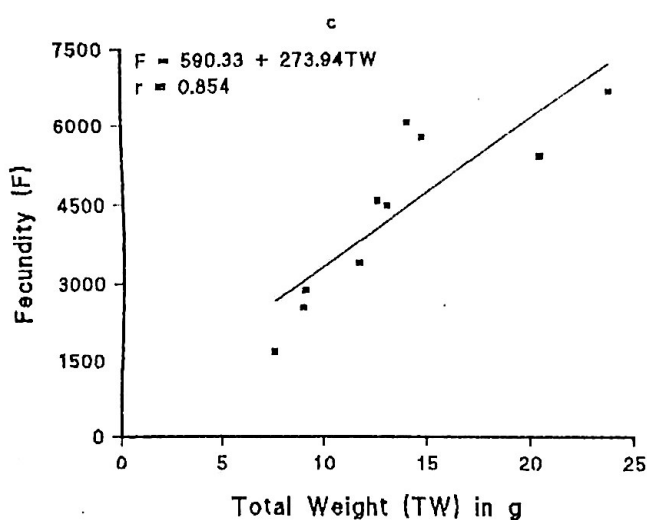
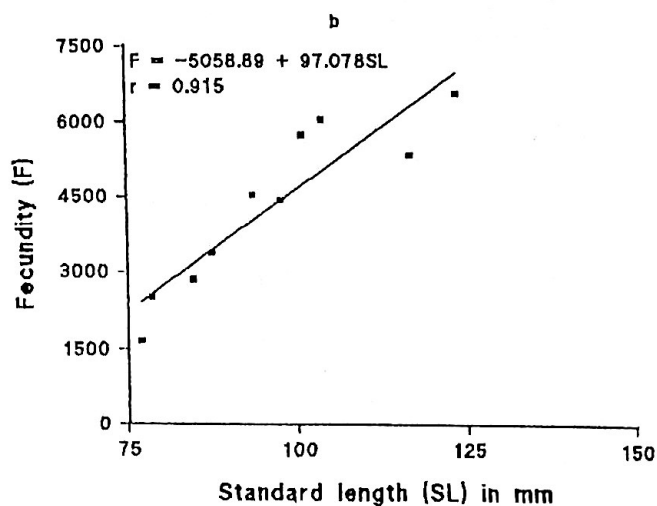
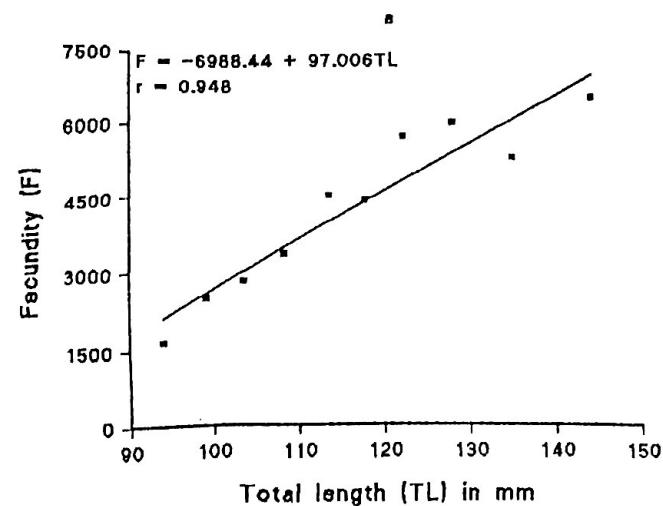


Fig. 8 (a-e). *A. morar*: Showing the relationship between fecundity and five variables (N = 155).

was more. Because of individual variation and greater reproductive potentiality variation in different female group seems difficult to comment.

Estimated fecundity factor is of importance even to a layman. Any person with basic biological knowledge minimum labour minimum calculation and with less technical knowledge will be able to estimate grossly the reproductive potential of *A. morar* from previously surveyed area.

1.3.10 Sex ratio

The study of sex-ratio was based on 2402 specimens of the size range 70mm to 145mm. in total length, collected during June 1993 to May 1995. Out of 2402 specimens 1141 were males and 1261 were females. The total male and female ratio was 1 : 1.093 i.e. the female number was more than the number of male. The monthwise male-female ratio for each year is shown in Fig. 9 and Table 8. The males were predominant during April to September, and females were predominant during November to February. In the month of February 75.22% female and 24.77 males were found.

As the chi square test showed the calculated value is higher than the table value both at 5% and 1% level of significance in each case, the male female distribution of the species in natural population is significantly different (Table 9). Table 8 shows that during November to February the females outnumbered the males and it is significant that the season represent peak breeding activity. The sex ratio in relation to total length of the fish showed that the females exhibited fluctuations in the length of 70 to 145 mm and they showed their predominance between 100mm to 145mm in length. Occurrence of more female in the length range 110 to 145 mm was significant as this length group formed active breeding population.

Table 8: Sex ratio of *A. morar* (≤ 70 m:m) m month wise distributed population during the period of June 1993 to May 1995 :
Collected from four sampling areas.

Month	Years				Grand total	Total Male	Total Female	Percentage		Ratio Male	Female
	June 1993 to May 1994		June 1994 to May 1995					Male	Female		
	Male	Female	Male	Female							
June	65	35	30	40	170	95	75	55.88	44.11	1	0.79
July	60	40	38	42	180	98	82	54.44	45.55	1	0.84
August	62	38	40	55	195	102	93	52.30	47.69	1	0.91
September	60	40	51	49	200	11	89	55.50	44.50	1	0.80
October	58	42	45	65	210	103	107	49.04	50.95	1	1.03
November	64	51	35	70	220	99	121	45.00	55.00	1	1.22
December	32	68	31	84	215	63	152	29.30	70.69	1	2.41
January	27	73	32	46	178	59	119	33.15	66.85	1	2.02
February	24	76	30	88	218	54	164	24.77	75.22	1	3.04
March	70	30	40	70	210	110	100	52.38	47.61	1	0.91
April	72	28	65	45	210	137	73	65.23	34.76	1	0.53
May	50	50	60	36	196	110	86	56.12	43.87	1	0.78
Total	644	571	497	690	2402	1141	1261	573.11	626.8	1	1.093
Percentage	53.004	46.99	41.87	58.13		47.50	52.50				

Table 9 : Chi - square test for male and female

Source	Degrees of freedom	X^2 Values	5% Table values 1%
Total X^2	24	215.83	36.42 42.98
Over - all X^2	1	5.98	3.841 3.635
X^2 heterogeneity	23	209.85	35.17 41.64

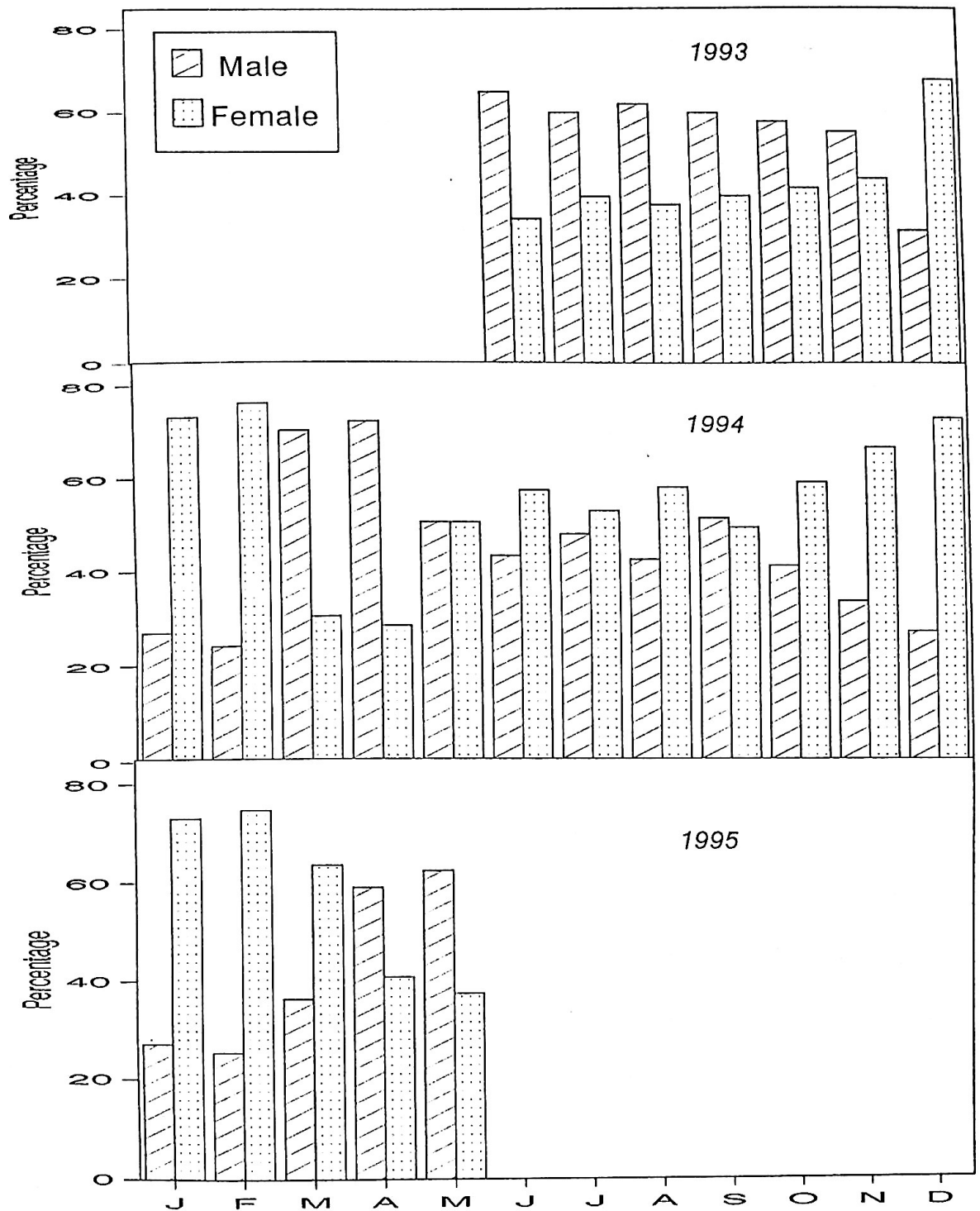


Fig.9. *A. morar*: Monthly percentage distribution of male and female.

1.4 DISCUSSION

Sex determination :

Unlike terrestrial animals, it is not possible to distinguish the sex of many species of fish from their external appearance (Love, 1970). However, Lagler *et al.*, (1967) described some secondary sexual characters of some species to indicate the sex. The most frequent secondary sex character is difference in size, females of many species are larger than the males, for ensuring the largest fecundity of the stock (Nikolsky, 1963). In case of *A. morar*, the females were found comparatively larger and healthier than the males. But the irregularities in their length at age group of individual sex in the same environment make hindrance at once to differentiate the sex by their sizes.

Another secondary sex character is the genital part of the matured fishes. The difference in the genital pores is prominent only in the breeding season. During the pre-spawning, spawning and post spawning periods the slit like genital pore of the fishes were undoubtedly male. Whereas, the females during this periods have a well differentiated, reddish and round genital pore. Similar condition has also been found in *Macrogathus aculeatus* (Nabi, 1987), *Clarias batrachus* and *Heteropneustes fossilis* (Shafi and Quddus, 1982), *Prototroctes maraena* (Berra, 1984), *M. pancalus* (Hossain, 1989). Besides these, the abdomen of the female *A. morar* become much bulger than the male.

Reproductive system of male :

The testis of *A. morar* is elongated, paired structure of unequal size, laying in the body cavity close to the body wall and are suspended by mesochium. The colour and size vary according to stage of sexual maturation and as opposed to the higher vertebrates, do not have a permanent genital epithelium (Berra, 1984). In mid August, the testis appeared as translucent, whitish, narrow thread like

structure and occupies $\frac{1}{3}$ of the body cavity (Berra, 1984; Abu Hakima 1984; Nabi 1987; and Hussain 1997) described this condition as the immature-stage of testicular development.

In the successive months, the testis attained the maturing (mid September to October) and developing (November to December) stages, and finally became mature or ripe in the month of January to February. The colour and size of the testis also changed accordingly. The fully mature testes were deep creamy in colour, occupied 80% of the body cavity and milt oozed out by slight pressure on the abdomen. The fat from the gut wall was completely disappeared at mature stage. The tubule walls remain very thin.

By late March to April most of the specimens were found with spent, flaccid testes of decreased width. Testis regained its original position and colour with in the month of May to June. This pattern of developmental changes of testes were reported for *A. morar* for a variety of fish species e.g., *Carassius auratus* (Clemens and Reed, 1967); *Sebastodes paucispinis* (Moser, 1967); *Panecilia reticulate* (Pandey, 1969); *Prototroctes maraena* (Berra, 1983); *Acanthopagrus* Sp. (Abu Hakima, 1984); and *Rhinomugil corsula* (Islam, S. 1990).

Reproductive system of female

The female reproductive system of *A. morar* comprises of the paired ovaries situated at the same location as the male gonads. The female gonad is a sac-like structure filled with ova of different sizes. The shape, size and colour of the ovary depend on the stage of maturation of the developing ova.

According to the degrees of maturation the ovaries are divided into ten stages (the terminology used in according to Kestaven, 1960; Farmer, 1974; Treasurer and Holliday, 1981; Nabi 1987 and Hussain 1997). Different stages of

ovaries are immature, maturing (early developing), maturing (late developing), mature, ripe, pre spawning, spawning, post-spawning, spent and resting.

Immature ovaries were thin, whitish thread like structure, occupying one third of the abdominal cavity. In this stage only immature ova were present. Ova diameter ranged from 0.24mm to 0.39mm. The maturing (early developing) ovaries occupy about 60% of the body cavity. The colour of the ovary still remained whitish. In this stage also only immature ova were present, but the larger sized ova were more numerous. No granulosa layer and yolk vesicles were found. Ova diameter ranged from 0.30mm to 0.58mm. The maturing (late developing) ovaries gained weight and size, colour become light orange and occupied 70% of the body cavity. Ova diameter ranged from 0.44mm to 0.77mm. The mature ovary occupied about 80% of the body cavity. The colour of the ovary change to orange in this stage immature and maturing ova were found with the mature ova, but percentage of occurrence of mature ova were maximum. Ova diameter ranged from 0.52mm. to 0.95mm.

Ripe ovaries were bright orange and occupied 95% of the body cavity. The ova were clearly seen through the wall of the ovary. Oocytes were transparent. The ova diameter ranged from 0.81mm to 1.27mm.

After spawning the ovaries were reduced in size and dull coloured. After a short spent phase the ovary regain its original whitish colour or pale colour and strap shape rapidly. This condition was observed in the matured females from late March to April. Similar conditions were reported by several authors in their studies on ovarian development in other teleosts (e.g., Foucher and Beamish, 1977, 1980; Taylor and DiMichelle, 1980; Treasurer and Holliday, 1981; Robb, 1982; Nabi, 1987; Hussain 1997) which breed once in a year like *A. morar*.

Size frequency

The largest specimen in total length of both male (141.50) and female (143.75mm) were found in the month of February in 1994. The smallest specimen in total length of both male (65.00mm) and female (67.50mm) were found in the month of April, 1994 and May, 1994 respectively. From the above observation, it was obvious that this fish breed in winter season.

Reproductive cycle

The time and season when the species breed is normally termed as the breeding or spawning season of that species. The breeding season repeats in cyclic order in which the organism undergoes maturation changes and thereby gets ready to breed again. This repeated phenomenon is known as reproductive cycle (Milton & Arthington. 1983). The reproductive cycle in fishes and shell fishes is guided by several environmental factors together with the endocrine activities. Both light and temperature are important factors controlling the development of gonads in fish. According to Kinne (1971) most of the stages of reproduction often depend on certain conditions of water movement, e.g., for the liberation of spermatozoa and fertilization of eggs.

Histological studies of the ovaries and ova diameter provide knowledge on the seasonal changes in the ovaries.

Stephenson (1934) observed 4 types of reproductive cycle in the animals of tropical water which are :

- (i) Annual reproductive cycle.
- (ii) More than one reproductive cycle in a year.
- (iii) Continuous breeder and
- (iv) Discontinuous breeder.

Reproductive activities of most aquatic animals in tropical and sub-tropical areas where environment is relatively uniform throughout the year, are controlled at least in part by temperature and length of day light (Royce, 1972; Hoque and Ahmed, 1993). These two factors may function separately or jointly on the fish biology. Temperature appeared to be extremely important in regulating reproductive cycle of cypriniformes (Vlaming, 1972). However, photoperiod appeared to be more critical than temperature in determine the timing of the reproductive cycle in *G. affinis* (Milton & Arthington, 1983). After heavy shower during monsoon period (late May to early August) some physico-chemical changes in the water bodies probably stimulate the reproductive activities of Indian major carps (Jhingran, 1977).

The physico-chemical condition of the Padma river has also been studied throughout the year (Table 10, 12) but it is not clear that which factors might be responsible for the reproductive cycle of *A. morar*. This fish probably prefer clean water and low temperature for their breeding *Rhinomugil corsula* also showed similar preference Islam (1990).

The annual reproductive cycle of marine and fresh water invertebrates could be determined by different methods such as:

- i Percentage of ovigerous female against time : (Nabi, 1987 on *Macrogathus aculeatus*, Hossain *et al.* 1989 *Notopterus notopterus*; Afroze and Hossain, 1990 on *Amblypharyngodon mola*; Hossain *et al.*, 1991 on *Nandus nandus*; Nargis and Hossain, 1992 on *Anabas testudineus*; Hossain *et al.*, 1992 on *Ompok pabda*; Parween *et al.*, 1993 on *Esomus danricus*).
- ii Occurrence of larvae : (Chhapgar and Deshmukh, 1964).
- iii Appearance of ripe gametes in the gonad (diameter of ova): (Nabi, 1987, *M. aculeatus*, Hossain *et al.*, 1989, *N. notopterus*; Afroze and Hossain, 1990, *A. mola*; Hossain *et al.*, 1991, *N. nandus*: Nargis and

- Hossain, 1992, *A. testudineus*; Hossain *et al.*, 1992, *O. pabda*; Parween *et al.*, 1993, *E. danricus*).
- iv. Gonado-somatic index (GSI): (Nabi, 1987, *M. aculeatus*, Hossain *et al.*, 1989, *N. notopterus*; Afroze and Hossain, 1990, *A. mola*; Hossain *et al.*, 1991, *N. nandus*; Nargis and Hossain, 1992, *A. testudineus*; Hossain *et al.*, 1992, *O. pabda*; Parween *et al.*, 1993, *E. danricus*)
 - v. Chemical composition of the gonad and also other organs related to reproduction : (Chandran, 1968, *Charybdis variegata*; Diwan and Nagabhushanam, 1974, *B. canicularis*).
 - vi. Relative condition factor (Kn): (Mallikarjuna Rao, 1967a, *M. rosenbergii*; Hossain, 1976a, *T. orientalis*; Hossain and Parween, 1982, *M. dayanum*; Nabi, 1987, *M. aculeatus*).
 - vii. Integrated method of analysing the immature, maturing, maturing and spent, spent, ovigerous with respect to virgin and copulated adult female (Haley, 1972, *Ocypode quadrata*).
 - viii. Maximum indices of abundance of spermatozoa: (Heydorn, 1965, *Jasus lalandii*; Hossain, 1976; *T. orientalis*; Nabi, 1987, *M. aculeatus*).
 - ix. Maximum egg-bearing capacity : (Shyamasundari, 1972, *Corophium triaenonyx*; Hossain, 1976, *T. orientalis*; Afroze and Hossain, 1990, *A. mola*).
 - x. Gonadal length index (GLI): (Nabi, 1987, *M. aculeatus*, Hossain *et al.*, 1989, *N. notopterus*; Afroze and Hossain, 1990, *A. mola*; Hossain *et al.*, 1991, *N. nandus*; Nargis and Hossain, 1992, *A. testudineus*; Hossain *et al.*, 1992, *O. pabda*; Parween *et al.*, 1993, *E. danricus*).
 - xi. Colouration of gonad (ovary): (Nabi, 1987, *M. aculeatus*; Hossain *et al.*, 1989, *N. notopterus*; Afroze and Hossain, 1990, *A. mola*; Hossain *et al.*, 1991, *N. nandus*; Nargis and Hossain, 1992, *A. testudineus*; Hossain *et al.*, 1992, *O. pabda*; Parween *et al.*, 1993, *E. danricus*).
 - xii. Histology and histochemical aids: (Nabi, 1987, *M. aculeatus*, Hossain, 1989, *T. orientalis*).
 - Xiii Neurosecretory activity of certain NS-cells. (Hossain, 1989, *T. orientalis*.)

- xiv. Isolation of adult female (condition favourable or ecologically good for larvae) from natural population or normal ecological condition (Boollootain, 1965. *Paclygrapsus crassipes*).
- xv. Activity of the cement glands (Hossain, 1989).
- xvi. Sturcture and nature of oosetae (Hossain, 1989).
- xvii. Structural modification of secondary sexual characters in fin fishes (Hossain, 1989).

From the aforementioned methods, only six (i, iii, iv, vii, x, xi) were applied in the present study to determine the breeding cycle of *A. morar*. All the six methods showed that *A. morar* breed during November to February with a peak in January-February. Similar results have been obtained on the reproductive cycles studies of Shafi and Quddus (1974) on *Puntius stigma*, Hossain (1976), *Thenus orientalis*; Roy (1981) on *Heteropneustes fossilis*; Islam, (1990), *Rhinomugil corsula*.

Out of all these methods, gonadal length index (GLI) would appear to be the most suitable for this species. The relative merit of this methods are requirement of less skill, simpler equipment and less time.

Fecundity :

A perfect knowledge on fecundity of fishes is essential for evaluating the commercial potentialies of its stock for study of its life history, practical culture and actual management of its fishery (Lagler, 1956; Doha & Hye, 1970; Das, 1977; Hossain, 1979). A review of the literature indicates that works on the fecundity of different fish had been reported e.g. Kader and Talukdar (1978) *Polynomus indicus*; Shafi and Quddus (1974) *P. stigma*; Karim and Hossain (1972) *Mastacembelus pancalus*; Doha and Hye (1970) *H. ilisha* of the Padma river; Khan and Mukhopadhyia (1972) *A. testudineus*; Ahmed *et al.* (1978)

fecundity and larval development of *Tilapia nilotica* (Linn); Roy (1981) *H. fossilis*; Afroze & Hossain (1983) *A. mola*, Islam & Hossain (1984, 1986, 1990); *Oxygaster bacaila*; *Clupisoma atherinoides*, *P. Stigma* respectively; Nargis & Hossain (1988) *A. testudineus*, Hossain *et al.*, (1992) *Ompok pabda*; Hossain *et al.*, (1991), *Lepidocephalus guntea*, Bhuiyan & Islam (1990), *Xenentodon cancila*, Bhuiyan *et al.* (1993) *A. morar*; Haque and Hossain (1993); *M. vittatus*; Alam *et al.*, (1994), *Ailia coila*.

The present study was undertaken with a view to determine the fecundity of *A. morar*. Gravimetric method Lagler (1956) was used to determine the fecundity of this species. Authors like Pantulu (1963); Bhatnagar (1964); Islam & Talbot (1964), Evans (1969), Doha & Hye (1970), Karim & Hossain (1972), Shafi & Quddus (1974), Kadar & Talukdar (1978), Mustafa, *et al.* (1982), Bhuiyan & Rahman (1984), Hossain. *et al.* (1991, 1992) and Bhuiyan *et al.* (1993).

Relationship, between the fecundity with the total length, standard length, total weight, gonadal weight indicated that the number of eggs were found to increase linearly with increase of these five variables (Table 5).

The number of eggs varied from 1913 (total length 93 mm and total weight 6.55 g) to 9401 (total length 143 mm and total weight 20.11g). The mean fecundity was 4317.47 ± 814.31 eggs for mean total length of 144.37 ± 0.91 mm and a mean body weight of 13.59 ± 0.549 g. The number of eggs produced by a female is dependent on various factors such as size, age and condition of fishes, types of the samples (Lagler *et al.*, 1967). The same sized fish possessed widely different number of eggs in their ovaries. For instance five specimens of *A. morar* of total length of 155 mm were found to carry 3317, 3416, 5995, 8899 and 9223 eggs respectively. Further, fecundity of a fish of larger size was lesser than that of a fish of smaller size. This phenomenon was also observed in *Hilsa ilisha* by Doha and

Hye (1970), *Xenentodon cancila* by Bhuiyan and Islam (1990). *A. morar* is a moderately fecund fish.

It was found that all the above mentioned relationships were positively correlated and the number of eggs were related more to the weight of the fish than to the length. Simpson (1951) found that fecundity was related nearly to the cube of the length and was thus directly proportional to the fish weight. Mookerjee and Mazumder (1946) reported that the reproductive capacity of the species varied according to the availability of space, food etc. During the estimation of fecundity it was found that *A. morar* spawns once in a year, mainly December to February.

Sex-ratio

A knowledge of the sex-ratio of a population is considered essential in the management of a fishery as it will be necessary to devise means of ensuring a proportional fishing of the two sexes. The total male - female ratio was 47.50 : 52.50 (1 : 1.09) i.e, the female number was more than male. The male dominated during the months of March to September and in other months of the year the number of female was higher than the number of male. Similar results was in the reported by Thomas (1969) the male-female sex ratio of *Upeneus tragula*. The reason behind this phenomenon is the restricted movement of the mature females during the breeding season (Hossain, 1976; Roy, 1981 and Nabi, 1987; Hussain, 1997).

The chi-square test shows that the male female distribution in the natural population is significantly different.

Chapter - 2

PHYSICO-CHEMICAL CONDITION OF THE WATER BODY

2.1 INTRODUCTION

Bangladesh is blessed by many rivers, tributaries and a vast area of flood plain together with ponds and ditches. The biological activities of the aquatic organisms are closely related to the physico-chemical conditions of the habitat concerned.

The relationships between the fish, their biotic and abiotic factors are not isolated phenomenon but changes of one may reflect on the other. Fishes are more dependent on water temperature, turbidity, pH , dissolved oxygen, free carbondioxide, alkalinity and dissolved salts for their growth and development (Nikolsky, 1963). Any change of these parameters may affect the growth, development and maturity of fish (Nikolsky, 1963 ; Jhingran 1983).

Limnological works of impounded waters (i. e., ponds, lakes and reservoirs) and lentic water (e. g., the Brahmaputra, the Bhairab, the Karnaphuly, the Halda, the Meghna) have been done together with some records on the estuary of Bangladesh. But detailed works on the main river streams together with the tributaries, rivulets and flood plain at a time related to biology and fishery of fish are wanting. Information on some fragmentary works related to physico- chemical parameters of different inland water bodies and coastal areas have been accounted by Rockford (1951), Dutta *et al.* (1954), Michael (1969), Devasy and Gopinathan (1970), Jana and Sarker (1971), Islam *et al.* (1974), Mahmood *et al.* (1976), Shafi *et al.* (1978), Laal *et al.* (1986), Jhingran and Pathak (1987), Chakroborti and Chattapadhyey (1988), Shaha *et al.*, (1990).

The present investigation is designed on the Physico-chemical condition of the river Padma at different points in Rajshahi city. A comprehensive observation was made that which possible factors play definite role in the fish life.

The present investigation is aimed on the following objectives :

- to find out the mean values of important physical parameter viz. air and water temperature, rainfall and water transparency;
- to find out the mean values of some chemical parameter viz. pH, dissolved oxygen (DO) and free carbondioxide (CO₂).

2.2 MATERIALS AND METHODS

The physico-chemical condition of the river Padma was studied for one year. Water samples were collected fortnightly at random, from the sampling areas which were approachable round the year. Water samples were collected particularly on 1st and 15th day of each month.

The water temperature were recorded with a mercury thermometer (-1° to 50°C) on the sampling spot. The monthly meteorological recorded data on the air temperature, rainfall were collected from the Meteorological Department, Rajshahi.

The chemical analysis of water was done at sampling spot with the help of a water quality checker (WQC-2OA TOA, Japan). The pH was recorded with the help of a pHpen. Water sample were taken to the laboratory for the determination of dissolved oxygen (azide modification of winkler method, APHA, 1989) and free carbondioxide (CO_2) (titration with N/44 NaOH Soln, using phenolphthalein as indicator Welch, 1948). Water transpanency was recorded by using seechi disc.

2.3 RESULTS AND OBSERVATIONS

The Physico-chemical condition of the Padma river exhibit more or less monthly and seasonal variations. The descriptions are based on the direct observation and the calculated monthly and average of 12 months. Values of different physical and chemical parameters depicted in (Table 10,11) and (Fig.10,11)

2.3.1 Physical Parameters

2.3.1.1. Air Temperature

Air temperature varied considerably throughout the year. The mean values of average day-night minimum and maximum air temperature recorded are 16.12°C (Jan.) and 31.57°C (April) respectively in 1994 in Rajshahi near the Padma river (Table 10, Fig. 10).

Mean of air temperature for 12 months is observed as $25.17^{\circ}\text{C} \pm 4.92$ (Table 11).

2.3.1.2 Water temperature

Water temperature of the study area showed considerable variations throughout the year. Fluctuation of water temperature related mainly to sunny or rainy day. The minimum and maximum water temperature of the Padma river are found to vary from 12.04°C (Jan.) to 30.02°C (May) (Table 10, Fig. 10). The 12 month mean of water temperature is found as $22.07 \pm 5.92^{\circ}\text{C}$ (Table 11).

2.3.1.3 Rainfall

Rainfall prevails more or less round the year. Usually in the winter rainfall is occasional but in the monsoon or late monsoon frequent and heavy rainfall with

gusty wind are very common in Rajshahi. The minimum rainfall was 5.24mm recorded in the month of December, while the maximum rainfall was 320.27mm in the month of September (Table 10, Fig. 10). The 12 month average of rainfall is recorded as 113.82 ± 120.74 mm (Table 10).

2.3.1.4 Water transparency

Limits of Secchi disc visibility in the study areas show a marked variation. The monthly mean values of transparency exhibited the maximum 1.28 ± 0.04 m in April and minimum 0.05m in August (Table 10, Fig. 10). The 12 month mean value of water transparency was found 0.41 ± 0.43 m (Table 11). Observation reveals that variation in the water transparency value is mainly caused by the influence of excess accumulation of silts, debris, plankton and other suspended materials.

2.3.2 Chemical Parameters

2.3.2.1 Hydrogen ion concentration (pH)

Investigation reveals that there is no marked variation in the pH values. The maximum mean values of pH recorded as 8.24 in January and minimum as 6.90 in April (Table 10, Fig. 10). The 12 month mean values of pH is 7.33 ± 0.41 (Table 11).

2.3.2.2 Dissolved Oxygen (DO)

The dissolved oxygen content of the lotic and lentic water systems are normally affected by the factors like water current, turbidity, temperature, aquatic plant and sunlight. The mean values of dissolved oxygen content are recorded, maximum as 6.03mg /l (January) and minimum as 3.20mg/l (July) with a mean value for the whole year 4.44 ± 0.88 mg/l (Table 10, Fig. 11).

2.3.2.3 Free carbondioxide (CO₂).

The maximum and minimum values of free carbondioxide are 8.24mg/l (August) and 2.58mg/l (May) with a 12 month mean value as 5.46 ± 2.34 mg/l (Table 10, Fig. 11).

Table 10 : Monthly mean values (Mean $\pm$ sd) of different physical and chemical Parameters of Padma river near Rajshahi.

Year	Month	Physical parameters					Chemical parameters		
		Air Tem. °C	Water Tem. °C	Water. Tran	Raifall	pH	Diss. O ₂	C O ₂	
1993 To 1994	June	28.54 $\pm$ 3.02	27.80 $\pm$ 3.01	0.20 $\pm$ 0.06	240.45	7.24 $\pm$ 0.28	3.34 $\pm$ 0.62	5.98 $\pm$ 1.10	
	July	27.57 $\pm$ 2.50	27.12 $\pm$ 2.95	0.07 $\pm$ 0.02	175.74	7.00 $\pm$ 0.32	3.20 $\pm$ 0.53	7.88 $\pm$ 0.74	
	August	28.72 $\pm$ 3.85	26.54 $\pm$ 2.06	0.05 $\pm$ 0.01	284.56	6.95 $\pm$ 0.42	3.31 $\pm$ 0.71	8.24 $\pm$ 1.04	
	September	2 $\pm$ 7.48 $\pm$ 3.05	26.97 $\pm$ 1.88	0.06 $\pm$ 0.02	320.27	7.57 $\pm$ 0.51	3.18 $\pm$ 0.87	7.67 $\pm$ 0.97	
	October	$\pm$ 26.94 $\pm$ 3.24	24.14 $\pm$ 3.18	0.14 $\pm$ 0.03	20.21	6.93 $\pm$ 0.48	3.26 $\pm$ 1.01	7.84 $\pm$ 1.10	
	November	23.57 $\pm$ 4.53	20.10 $\pm$ 2.06	0.12 $\pm$ 0.02	38.54	7.74 $\pm$ 0.31	4.58 $\pm$ 0.74	8.57 $\pm$ 0.42	
	December	18.94 $\pm$ 4.78	13.58 $\pm$ 3.57	0.19 $\pm$ 0.01	5.24	7.26 $\pm$ 0.24	5.14 $\pm$ 0.82	4.01 $\pm$ 0.42	
	January	16.12 $\pm$ 6.53	12.04 $\pm$ 2.58	0.31 $\pm$ 0.05	18.93	8.24 $\pm$ 0.74	6.03 $\pm$ 0.71	3.14 $\pm$ 0.58	
	February	17.23 $\pm$ 6.43	14.74 $\pm$ 9.92	0.45 $\pm$ 0.06	40.58	7.83 $\pm$ 0.53	5.21 $\pm$ 0.70	3.39 $\pm$ 0.34	
	March	23.13 $\pm$ 5.93	17.96 $\pm$ 2.48	0.90 $\pm$ 0.08	12.14	7.25 $\pm$ 0.40	4.94 $\pm$ 0.54	3.30 $\pm$ 0.70	
	April	31.57 $\pm$ 6.83	23.87 $\pm$ 3.02	1.28 $\pm$ 0.04	9.52	6.90 $\pm$ 0.44	4.67 $\pm$ 0.44	2.87 $\pm$ 0.43	
	May	31.21 $\pm$ 5.88	30.02 $\pm$ 2.48	1.21 $\pm$ 0.10	102.69	7.03 $\pm$ 0.29	5.13 $\pm$ 0.38	2.58 $\pm$ 0.58	

Table 11 : The range (Min- Max) and mean $\pm$ sd for 12 month of different physico-chemical Parameters of the study water bodies.

Parameters	Minimum	Maximum	Mean
Air temperature ($^{\circ}\text{C}$)	16.12 (January)	31.57 (April)	25.17 $\pm$ 4.92
Water temper ($^{\circ}\text{C}$)	12..04 (Janu)	30.02 (May)	22.07 $\pm$ 5.92
Water transparency (m)	0.05 (August)	1.28 (April)	0.41 $\pm$ 0.43
Rainfall (mm)	5.24 (Decem)	824 (Janu)	113.82 $\pm$ 120.74
p ^H	6.90 (April)	8.24 (Janu)	7.33 $\pm$ 0.41
Dissolved oxygen mg/l	3.18 (Sep.)	6.03 (Jan)	4.33 $\pm$ 0.97
Free carbodioxide Mg/l	2.58 (May)	8.24 (Aug.)	5.46 $\pm$ 2.34

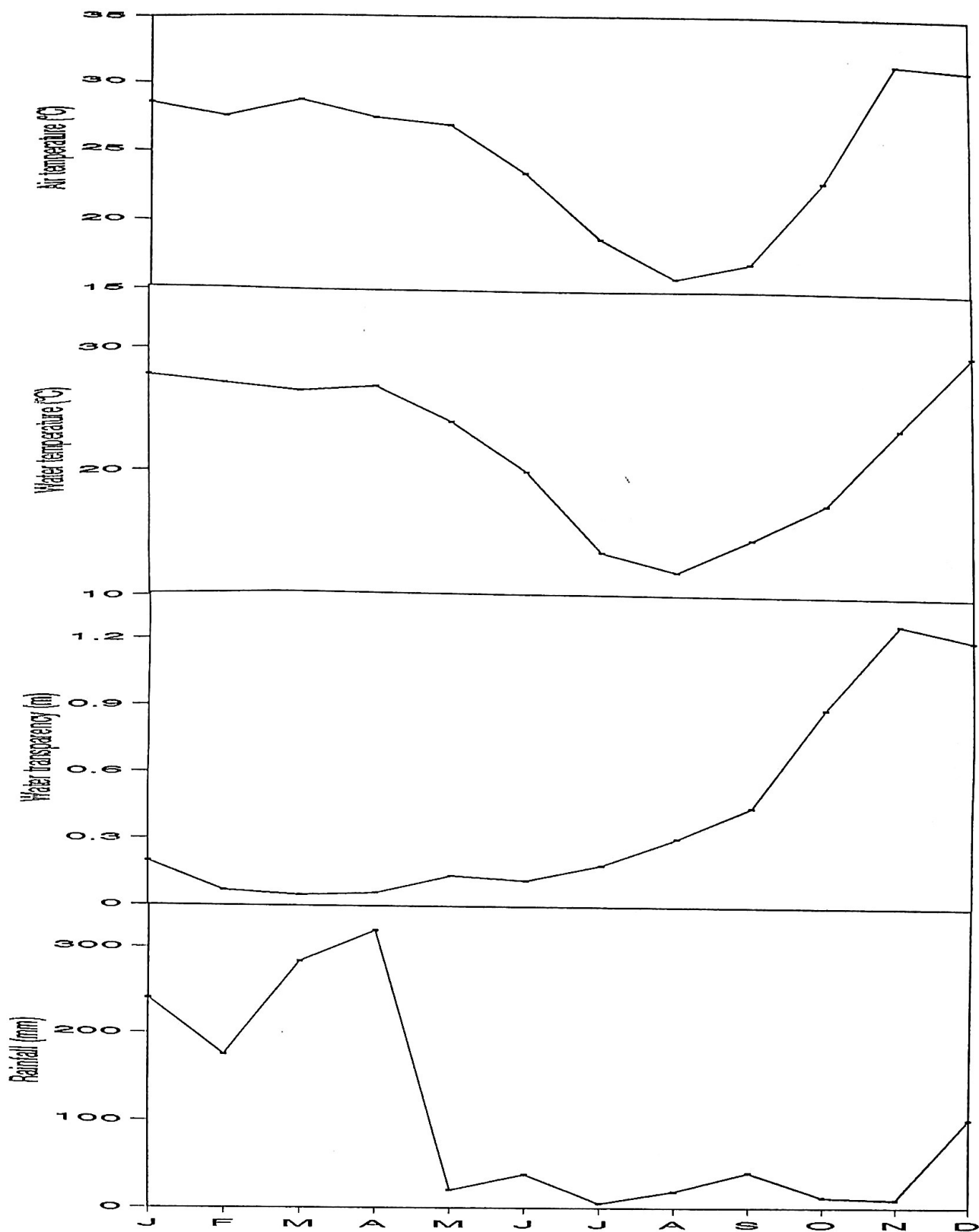


Fig.10. A. morar: Showing monthly fluctuation of physical parameters of Padma river near Rajshahi.

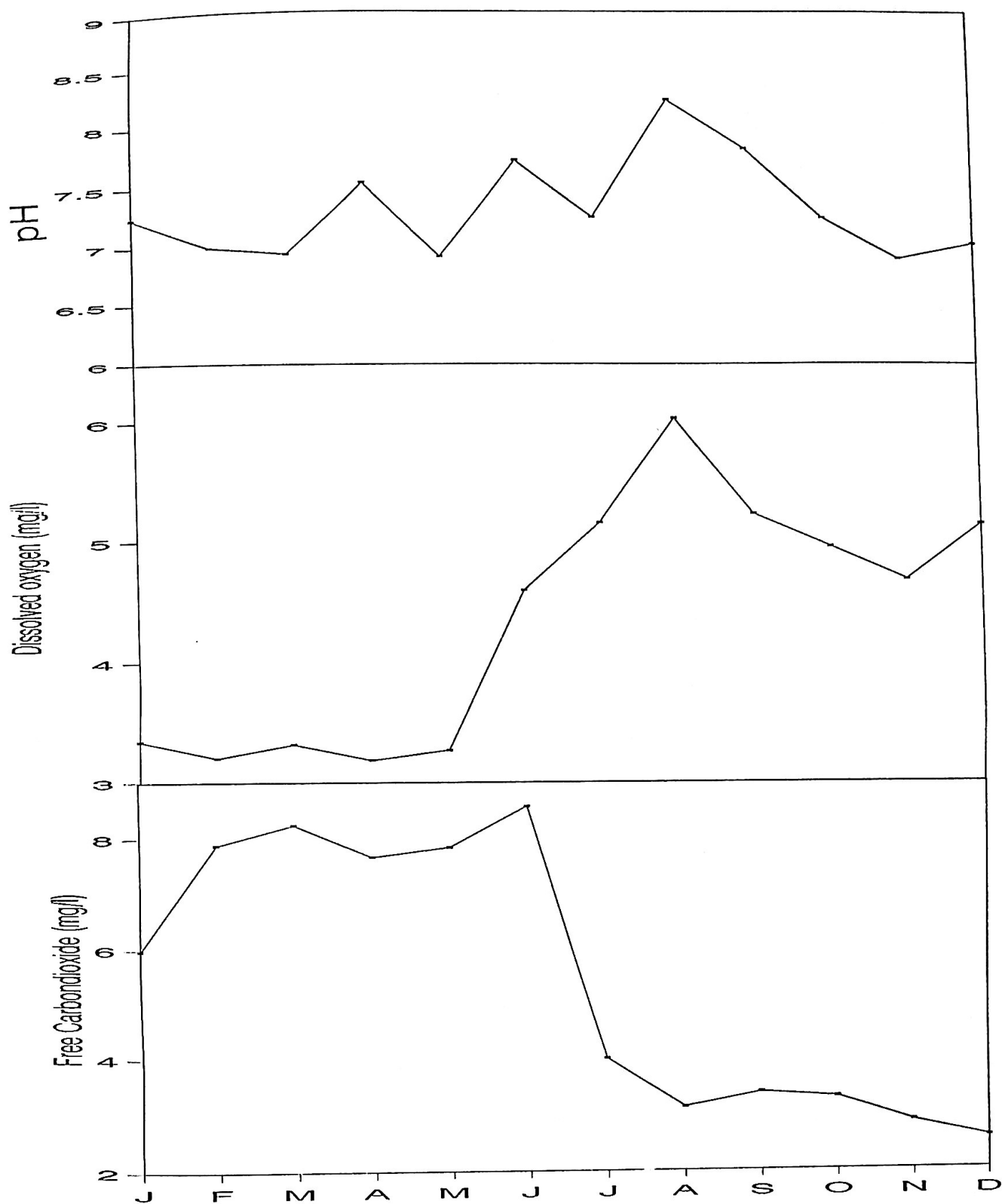


Fig.11. *A. morar*: Showing the monthly fluctuation of chemical parameters of Padma river near Rajshahi.

2.4 DISCUSSION

To determine the ecological conditions of the river Padma, the obtained values of physical and chemical parameters have been employed. The analysed statistical values can be treated as the useful indicator for the occurrence of *A. morar*.

Observation revealed that annual air temperature cycle maintained a close parallel relationship with annual water temperature cycle. The temperature of all the studied water bodies usually declined from November and reached the minimum in January and thereafter increase steadily reaching the maximum during April to June.

Differences between air temperature and water temperature are higher during winter and lower in summer. Occurrence of the temperature curve of air and water coincided each other during monsoon / flood season due to sudden fall of air temperature in comparison to the water temperature followed by the heavy rainfall. On the other hand, both the curve differ before and after monsoon period due to absence of cooling effect by rainfall and the presence of windless and hot days prevail in the areas. (Geisler *et al*, 1975 and Islam and Mendes, 1976).

The rainfall is more or less year round like other hydrological features. Rainfall is also an important ecological factor for aquatic environment. Chemical factors like nitrate and phosphate were high in rainy season (Sahai and Sinha, 1969; Lakshminarayana, 1965). Rao (1955) reported that the nitrate of water increased with the increase of rainfall and flood.

Rainfall also influences the breeding of fishes. Temperature has no specific influence on spawning but cloudy days accompanied by thunder-storm and rain,

seen to exercise some influence on spawning (Saha et. al., 1957). Success in majority of fishes have been induced on cloudy and rainy days, specially after heavy showers (Chowdhury, 1960).

Heavy rainfall is very common during June to September in Rajshahi. Water transparency of the studied water bodies shows low value due to greatest extreme to turbidity. Turbidity of water was mainly affected by heavy planktonic bloom, non living organisms, suspended organic matter, rains, floods and inflowing sediments (Bamforth, 1958). According to Jhingran (1983) turbidity due to profusion of plankton is an indication of high fertility but that caused by silt or mud beyond a limit, is harmful to fish and fish like organisms.

In the Padma, the minimal values of turbidity are found in the monsoon and postmonsoon months like June to September, due to strong water current which washed away huge silt in water including many other suspended matter. From October onwards up to May water becomes slowly clear with the maximum values of transparency due to absence of such disturbing matters (Lakshminarayana, 1965; Hickman, 1979 and Dutta, 1996).

Water of the natural resources is not chemically pure. It contains different substances in solution giving an acid, neutral or alkaline reaction. The importance of pH value in fish culture is vast. Micheal (1969) investigation reported that the pH range between 7.3 and 8.4 was considered to be suitable for fish culture. Swingle (1957) observed the relationship in pH of water to their suitability for fish culture and satisfactory results were obtained with pH ranging from 6.5 to 9.0. The author reported that the water having pH values more than 9.5 were less productive and above 11.0 marked as the death point of fish.

Mean values of pH of the Padma ranged between 6.92 to 8.24 indicating neutral or slightly alkaline nature of the water.

Among the dissolved gases, oxygen is the most important factor for the aquatic life. Dissolved oxygen contents of the Padma river increases gradually from October to May, and then decreases being lowest in July to September. Similar results were also obtained by many workers like, Chakraborti and Chattapadhyey (1959); Lakshminarayana (1965); Patra and Azadi (1987); Dutta (1996). The fluctuation in the dissolved oxygen concentration is mainly influenced by the factors like dissolved organic matter, plankton and bottom vegetation.

Carbondioxide is essential an ingredient of photosynthetic reaction which directly correlates with the productivity of the aquatic system. According to Chow (1959), carbondioxide concentration of 30 to 40 ppm were liable to make the fish breath with difficulty and often die when it exceeds 30 ppm. Mean values of free carbondioxide (CO_2) show inverse relationship with the oxygen.

In the river Padma, the mean values of carbondioxide are maximum in the month of June to November and minimum in December to May.

Chapter 3

FISHING AND MARKETING

3.1 INTRODUCTION

The species *A. morar* is found less frequent and fetches more market value than the other species. Till now there is no record of fishery of this species. Some available records show that *A. morar* form a fishery of some magnitude in the Padma river throughout the year along with the other small sized fishes. Hence its fishing method in the study area could depict more or less the entire picture that found all over the Bangladesh. Several workers like Ahmed (1962), Chowdhury (1983), Dutta (1983), Bhuiyan (1985), Nabi (1987) provided information on the fishery of several species of fresh-water fishes and Prawns.

The marketing system of *A. morar* was also investigated from different markets of Rajshahi city. It was noticed that the demand of this species is more than other fishes because of its good taste. The demand elasticity (consumers preference) is the guideline for the fishermen or grower as well as the traders to determine the price of the commodity (Molla, *et al.*, 1974).

Workers had been done on different marketing problems of fishery items, e.g., Sharker (1977) showed same aspects of marketing of marine fisheries products, Bucksimiar (1977) described the problems of transport and marketing of the marine fisheries products and their remedy. Sabur and Rahman (1979) worked on marine fish marketing. Parween and Hossain (1983); Dutta (1983) and Hossain (1996) reported on the marketing system of prawns and small indigenous fishes. There is no such work had been done so far on the studied species.

3.2 MATERIALS AND METHODS

Study area

The study area was mainly consisted of different fishing stations of the Padma river. The species is abundantly found in the river, mainly in shallow and sandy areas. The site of the study area selected on the basis of frequent abundance of the species, these are Charpara of Binodpur, Talaimary and Shaheb Bazar. The fishing of *A. morar* was investigated on the basis of interviews of fishermen and the fish traders of different markets of Rajshahi city (e.g., Katakhal, Binodpur, Talaimari and Shaheb Bazar).

Data collection.

Information regarding the fishery of *A. morar* have been collected by visiting various fishing and landing centres of Rajshahi. In every possible cases measurements of different fishing nets, traps were recorded. Mode of operation, materials used, advantages and disadvantages of their uses etc., were also recorded by self witness and from interviews of experienced persons at different places. Survey was carried on different seasons of the year from 1993 to 1994.

Information of marketing system of *A. morar* was also collected from different fish landing centres of Rajshahi on the basis of interviews taking from different fish traders (e.g. Faria, Bepari, Aratdar and Retailer) engaged with the business of the studied species.

The data pertaining to daily landing, total landing of small sized fish and the landing of *A. morar* were collected from the Aratdars of each landing centre, because only they keep the records for the convenient of their tax collection. The mean daily landing of each month was multiplied with thirty days to represent the month wise record for that particular month (Table 13).

3.3 RESULTS AND OBSERVATIONS

3.3.1 Fishing Methods

A. morar is a surface dwelling fish swimming here and there all the day long. Various type of locally made traditional crafts and gears are used since centuries for capturing *A. morar* along with small sized fishes. Mostly the following nets are used for the fishing of *A. morar*. (Table 12; Plate 6, Fig. 6; Plate 7, Fig. 7; Plate 8; Fig. 8; Plate 9, Fig. 9 Fig. 12).

3.3.1.1 Fishing Season

The fishing season of *A. morar* is during the monsoon time. Nets are set against the water current but near the bank of the river at a depth of 6 to 9 feet. Due to wide spread of fishes in the flood lands and not being the breeding season the catch is low in the monsoon. During the winter season when water level decreases *A. morar* starts to breeds. Hence throughout the entire winter season the fishing is found in the peak for the species with other small fishes.

3.3.2 Monthwise Landing And Composition Of Catches

Many information regarding the landing of different fishes of the Padma river are reported. Unfortunately, no previous statistics about the total landing of *A. morar* and some other small fish species in the study area are lacking.

Though *A. morar* grows to an acceptable size within a year and forms an important fish food together with the other small sized fish, but they are the less abundant in quantity at the market. The peak supply period of the fish in the local fish market is the winter season. The monthwise percentage composition of *A. morar* at four sampling areas are presented briefly in the Table 13 and Fig. 13.

Table 12 : Related information on the gears used in the fishing of *A. morar*.

Name of gears	Type of thread used	Materials used in gear			Gear size						Operation areas				Catch record according to season kg/day				Mode of opsaion
		Sinker	Float	Other accessories	Total length (ft)		Breadth (ft)		Mesh size (cm)		Depth (ft)	Water type	Season	Winter	Summer	Moonson	Post moonson		
					Min.	Max.	Min.	Max.	Min.	Max.								Min.	
Ferijal (gill net)	Nilon	Rope	Sponge	Bamboo	75	150	1.5	1.75	1.4	1.5	3-4 1/2'	Both clean and turbid	Summer	3-5	2-5 ks	1-1 1/2	2-2 1/2	Boat	
Ligonjal for small fish (70-95mm) (giilnet)	Nilon	Brick	Sponge	Bamboo, rope	75	300	1.75	2.5	1.5	.1	4-5	"	Winter	4-6	2 1/2-3	15-2	2-3	"	
Ligonjal for big fish (100-140)	"	"	"	"	150	750	2	3	1.7	2.00	4-6	:	Both Summer and Winter	7-8	3-4ks	2-3	3-4	"	
Kharajal	Nilon	Nil	Nil	Bamboo, rope, Stick	30	45	30	45	0.50	2.25	8-10	Clean	Winter and Summer	2-5	1-2 ks	Nil	Nil	"	
Bewshijal	Nilon	Iron thread	iron thread	Strong wooden pool, rope	75	120	30	45	0.50	2.25	40-55	Both clean and turbid	Winter and pre-moonson	7-10	37-70 ks	Nil	Nil	"	

Plate - 6



Fig. 6: Operation of Feri jal

Plate - 7



Fig. 7: Operation of Ligon jal

Plate - 8

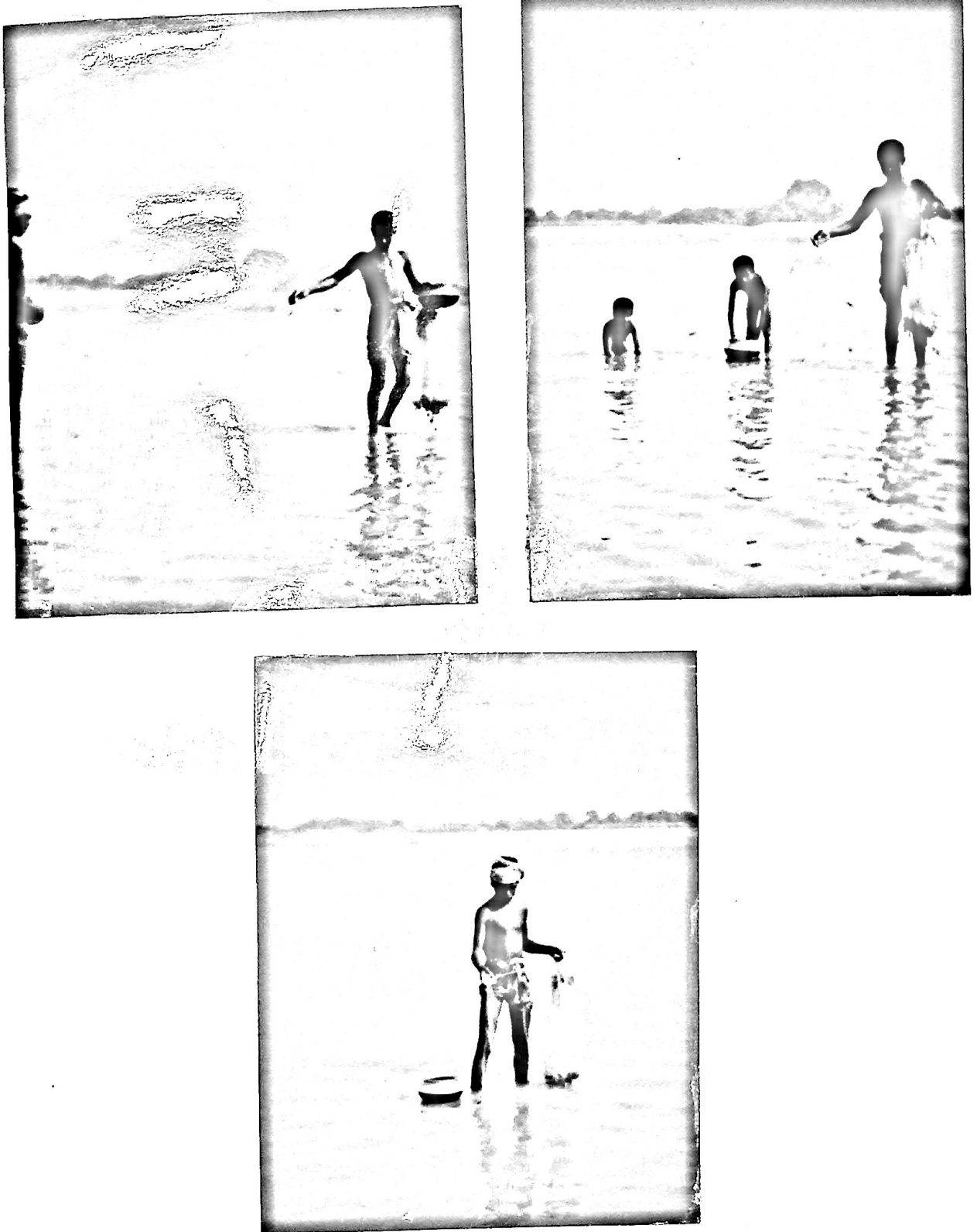


Fig. 8: Collection of A. morar from Feri jal.

Plate - 9



Fig. 9: Collection of A. morar by hand



Fig. 12. Thela jal in operation

Table 13 : Monthwise total small size fish landing and the percentage composition of *A. morar* in four sampling areas during the year 1994 to 1995

Month	Shaheb bazzar F.L.C			Binodpur fish landing centre			Talaimari F.L.C			Katakhal F.L.C		
	Total landing of small fishes (md)	<i>A. morar</i> landing	% <i>A. morar</i>	Total landing of small fishes (md)	<i>A. morar</i> landing	% <i>A. morar</i>	Total landing of small fishes (md)	<i>A. morar</i> landing	% <i>A. morar</i>	Total landing of small fishes (md)	<i>A. morar</i> landing	% <i>A. morar</i>
June	360	6.00	1.66	23	1.20	5.21	20	0.95	4.75	60	1.24	2.06
July	310	4.35	1.40	20	1.01	5.05	18	0.80	4.44	58	1.00	1.72
August	325	4.21	1.29	16	0.65	4.06	14	0.46	3.28	55	0.75	1.36
September	370	6.75	1.82	18	1.15	6.38	19	0.95	5.00	65	1.35	2.07
October	400	7.40	1.85	22	1.42	6.45	21	1.19	5.66	70	1.52	2.17
November	430	8.10	1.88	24	1.68	7.00	26	1.49	5.74	88	1.85	2.10
December	460	9.00	1.95	30	2.10	7.00	32	2.00	6.25	100	3.02	3.02
January	500	9.97	2.00	32	2.50	7.81	34	2.46	7.23	110	3.72	3.38
February	550	11.50	2.09	34	2.80	8.23	38	3.10	8.15	120	3.95	3.29
March	600	12.00	2.00	28	2.00	7.14	30	2.65	8.83	125	3.69	3.20
April	570	10.00	1.75	24	1.50	6.25	27	2.00	7.40	90	2.80	3.11
May	450	7.80	1.73	21	1.15	5.47	21	1.22	5.81	70	1.78	2.54
	5325	97.08	21.42	292	19.16	76.05	300	19.27	72.54	1011	26.67	30.02

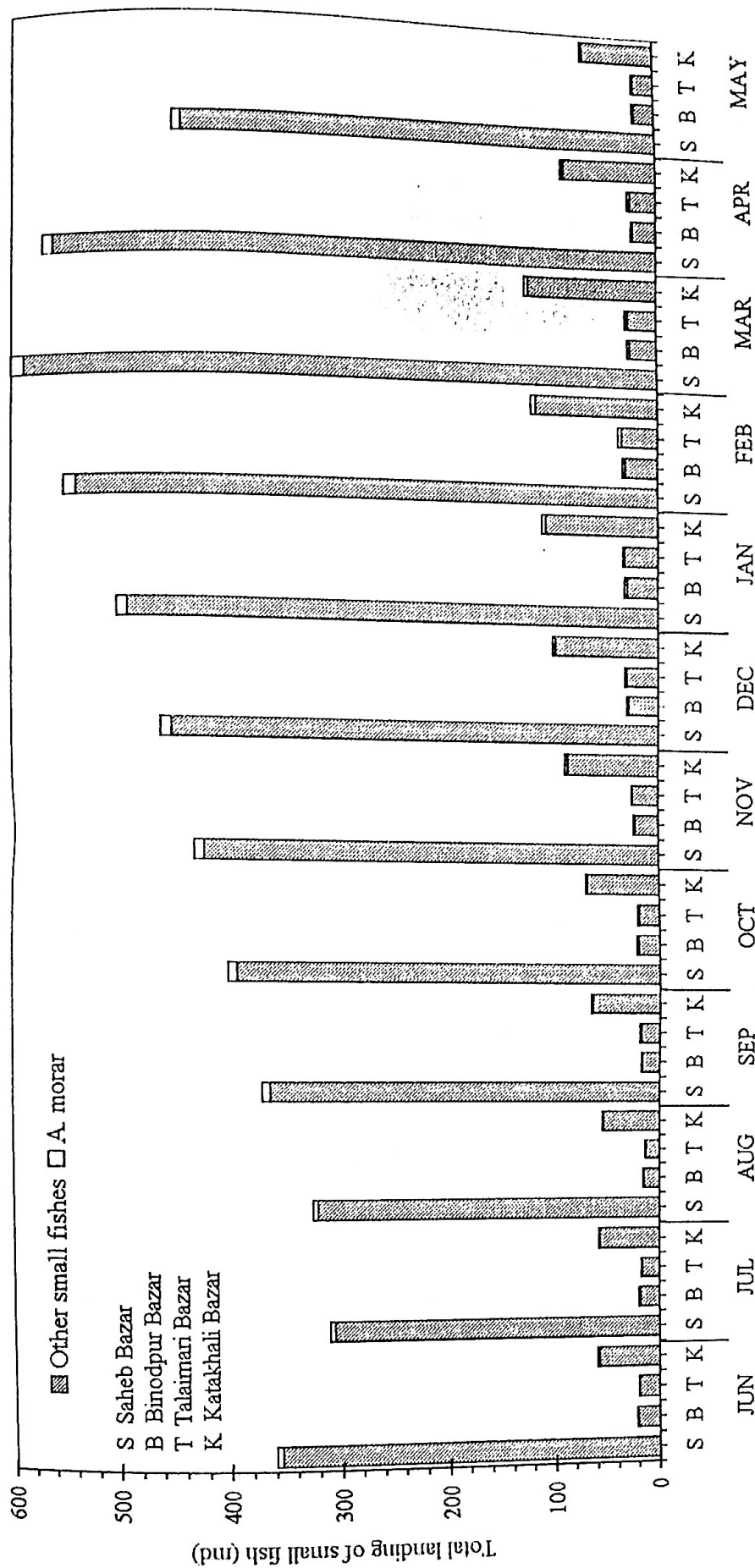


Fig. 12: Showing the monthwise total small size fish landing and *A. morar* landing

It was observed that the percentage composition of *A. morar* and the total landing of other small size fishes i.e. *Gadusia chapra*, *G. variegata*, *P. chola*, *P. ticto*, *P. stigma*, *A. mola*, *O. bacaila*, *Rasbora elanga*, *Lepidocephalus guntea*, *Colisa fasciata*, *M. pancalus*, *Glossogobius giuris* etc. were maximum from the month of November to March. During the peak period the rate of landing of *A. morar* was 80-100 k. at the different fish landing centres. Analysis of the size composition (Table 13.) very clearly shows that medium size groups predominate in the catches. In the Padma river the fisherman catch *A. morar* by means of nets during monsoon and in other seasons large number of fish is caught by traps. This fact must be contributing greatly to the decreasing of population level of the species.

3.3.3 Marketing System

Kohls (1970) defined marketing as the business activities involved in the flow of goods and services from the point of initial production until they reach the ultimate consumer. The marketing system comprises with market and a marketing channel along with a transportation system and some storage facilities. The marketing system lies in between the producer and the final consumer (Kohls, 1970) i.e., the activities of the products after the hand of the producer upto the final consumption is treated as marketing system.

The marketing channel comprises the producers (fisherman), the different intermediaries and the final consumer; which may be complicated one or a simple one.

3.3.3.1 Marketing Channel and its Activities

The producer or fisherman had found to distribute their catches (*A. morar*) to different far and near markets through a more or less distinguished channel. However, in many cases such distinguished channel were not found (at the far interior producing areas). In between the fishermen and the final consumers, there found a few intermediaries. The activities of the fishermen and the different intermediaries as found are described in the followings.

Fishermen

The first and most important basic concern of the marketing channel are fishermen or growers. However, culture of *A. morar* have not yet been established in our country. The fishermen sell their commodity in fresh condition either directly to the consumers in the nearby market or to any intermediaries. Good socio-economic condition, transportation and absence of proper preservation, are the main factors that prevent the fishermen from going to the far markets or a big town, where they could get higher price for this fish.

Farias

The farias are the second basic person of the marketing channel. Normally, farias gather at the fishing stations or at the transporting centres. They purchase fish in fresh condition and supply to any nearest business centres or market for direct selling to the Beparis (the primary wholesalers). They have direct communication to the distant markets. The farias profit is directly related with the distance of the markets. They have small sized of capital investment and sometimes take advance from the Beparis for purchasing fish. Hence a direct link of them is established with a definite Bepari.

Beparis

The beparis are the third step of the marketing channel. The beparis have medium to large sized capital investment. They purchase fish from the Farias or even from the fishermen directly and sell their consignment to the Retailers through the Aratdars (commissioned whole sellers). They often take advance money from the Aratdars and also finance the Farias sometimes even the fishermen. The Beparis mainly assemble at the transporting centres.

Aratders

The Aratders are the fourth step of the marketing channel, who act as whole seller and commission agent in the market. They usually purchase fishes from Farias and Beparis, sometimes directly from the fishermen. They are permanent fish dealer having a license from the proper authority. Aratders normally possess parmanent establishment and act in between the Beparis and the Retailers. They help the Beparis to sell their fish and charge a fixed commission on the amount transacted. Aratders give cash payment to Beparis but supply fishes to Retailers on credit by auction. The Aratders offer a provisional accommodation for the different intermediaries and take a risk for sale after the handover of fish from any type of intermediaries.

In many cases, Aratders require a large amount of capital investment for financing the Beparis and Farias, which is termed as "Dadon" often fishermen take Dadon for repairing or purchasing gears (nets and traps) and crafts.

Retailers

The retailers are the important link of the marketing channel of small and large markets. They are engaged in small range of business with either less amount of capital investment or without any investment. The retailer purchase fish from

the whole sellers or Aratders and even from the fishermen and sell directly to the consumers. They purchase fish from the whole seller on credit and make payment after selling fishes. The number of retailers are more in the markets. Some of them are permanent fish retailers, while others are engaged temporarily. In some case the beparis including fishermen act as retailer depending on the market structure and situation.

Hawkers also sell fish door to door in the villages and residential areas of the towns.

Consumers

Consumers are the last link of marketing channel. They purchase fish from the retailers for their own consumption. /The number of consumers in the market depends on price of fish and their purchasing capacity or quality and freshness of fish.

A. morar not directly transported to the wholesalers of Katakhal, Binodpur, Talaimari and Shaheb Bazar fish market, neither the selling begin from the producing area by the fishermen himself and some percentage of the products reach in a main market through different intermediaries. The intermediaries (e.g. Faria) again sell the product at the nearby smaller markets and also hand over to another intermediary (e.g. Bepari) for selling at a big market.

3.3.3.2 Marketing Characteristics

Pattern of Ownership

Most of the whole sellers and Aratder of Shaheb Bazar, Katakhal, Binodpur and Talaimari fish landing centres of Rajshahi Metropolitan City perform their business under sole tradership, family or partnership. There are a few co-operative

form of business organization only by name, which are not worth mentioning and not at all well organized like other business firms.

Experience of the middleman

The result of survey shows that most of the Aratdars, wholesalers and retailers are well experienced. The retailers have the experience of 1 to 20 years of business. Amongst them the whole sellers and the Aratdars are the most experienced businessman. Sometimes even they run the business from generation upto generation. On the other hand, some of the retailers have been continuing their business for 30 to 40 years. New faces are also coming considerably. Some temporary retailers are also found there. Those who are fishermen have occupational experiences as well. They are often more experienced than the other middlemen.

Capital Structure

The capital invested by the middlemen, are divided into two parts, such as fixed capital and fluctuating or running capital. Fixed capital includes in license fees, investment for godown, weighing instruments and articles for recording accounts of the business while fluctuating capital is used for cash purchase of the commodity, packing charges, carrying charges, donations and salami to various inspecting agents. The Aratdars and the whole sellers are much more powerful in respect of their capital. Often they give advance (payment to) the retailers or fishermen for more profit.

Containers

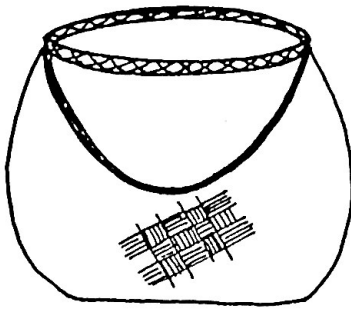
Different type of containers are used for carrying the fish. After harvesting, the *A. morar* (other fishes) are also collected in a small bamboo made container 'Khaloi' (Plate 10, Fig. 10) for smaller amount and when sold locally, this

'Khaloi' is generally used to carry the fishes. For larger amount and distant markets the fishes are carried in bamboo made baskets called as 'Dali' or 'Dala' and also fish are carried in bamboo made small size baskets called as 'Jhuri' (Plate 10, Fig. 10), some times the fish are carried in alluminium made container called as "Hari" (Plate 10, Fig. 10).

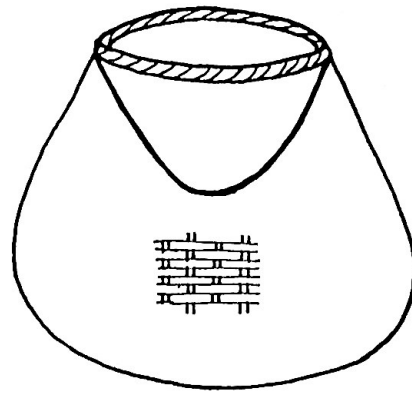
Transportation :

Generally fishes are transported by Truck, Bus, Train, Rickshaw, Van, Tomtom, Boat etc. Some fishermen bring their fishes by themselves carrying on the heads. During the transportating, ice is given along with the *A. morar* for distant and in adverse weather condition and no other modernised methods are taken.

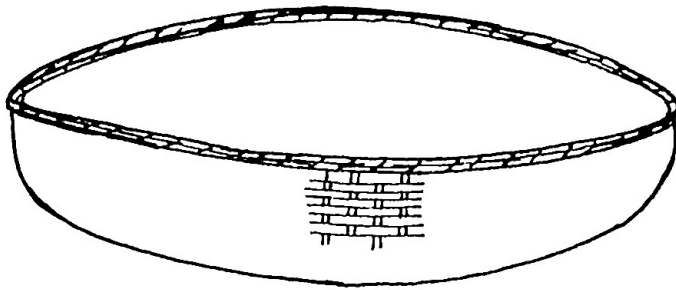
Plate 10



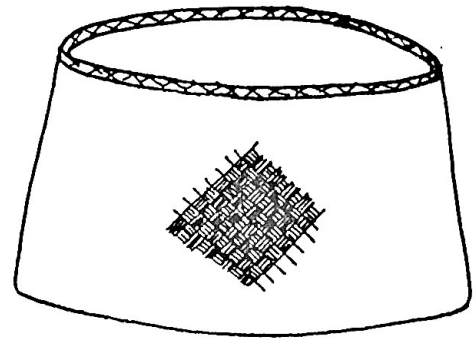
a. Khaloi



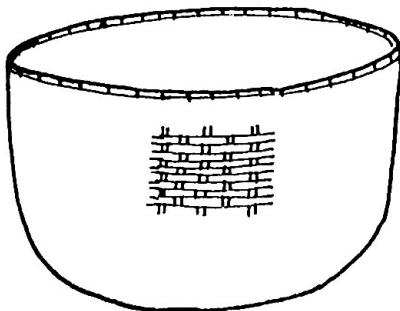
b. Khaloi



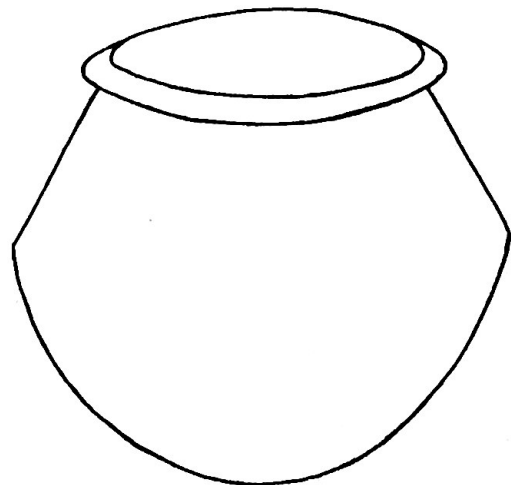
c. Dala



d. Dala



e. Jhuri



f. Hadi

Fig. 10. Different type of Container

3.3.4 Marketing Margin

The difference between the amount paid by the consumers for their products and the amount received by the producers is referred to as the 'total marketing margin' or 'total marketing bill' (Kohls, 1970). The term price spread is often used synonymously with the term marketing margin (Sabur and Rahman, 1979). This margin or bill includes all the cost of moving the commodity from the point of production to the point of consumption and of handling at all levels in the marketing machinery. The total marketing margin usually consists of margins at several stages of distribution, and of each case the margin is the difference between the buying and the selling price of each distributor. Thus marketing margin consists of two component parts :

- i) Marketing costs for performing various functions.
- ii) Profit of the intermediaries (Sabur and Rahman, 1979).

The marketing price of the studied fish species were found to vary among the four fish landing centres (FLC) (e.g. Shaheb bazzar, Binodpur, Talaimari, Khatakhali). The total marketing margins per kg of the species with the margins of the intermediaries, as found at the different markets are given in Table 14, 15 (Fig. 14, 15). The price of fish differs greatly in between the smaller and larger size groups.

In case of small sized (40-90) fishes the highest total margin were found at Shaheb bazar fish landing center as Tk 27.50 per kg; and the lowest as Tk. 23.25 and 23.90 per kg at Binodpur and Khatakhali fish landing centres respectively whereas in case of large sized fishes (95 - 140 mm) the highest total margin was found at Shaheb bazar fish landing centre as Tk 33.50 per kg. The lowest were found as Tk 23.00 per kg at Binodpur fish landing centre.

The highest and lowest margin for Farias in case of large and small sized fishes were found at Shaheb bazar and Katakhalī F.L.C. consequently (Table 14, 15; Fig. 14, 15) But in case of Beparis the highest and lowest margin of mentioned fish group were found at Shaheb bazar and Binodpur FLC.

The maximum price for both fish group paid by the consumer were 51.50 and 90.50 Tk/kg at Shaheb Bazar and the lowest were 44. 40 and 75.90 Tk/kg at Katakhalī F.L.C (Table 14, 15; Fig. 14, 15).

The maximum commodity and percentage of consumer price get by the fisherman at Shaheb Bazar F.L.C for both small and large fish groups and lowest were at Katakhalī F.L. C (Table 14, 15; Fig. 14, 15).

3.3.4.1 Marketing cost

The variation in the market price is mainly due to the marketing costs involved for different market. The highest total marketing cost were obtained at Shaheb bazar F.L.C as Tk 5.50/kg in case of both small and large fish group.

The maximum costs for Farias were Tk.2.00/kg at Shaheb bazar both in case of small and large fish group. The lowest costs were obtained as Tk 1.00/kg at Talaimary and Katakhalī for both fish group.

The highest and lowest costs of Beparis were obtained as Tk3.00 and 1.75/kg for both small and large fish groups (Table 14, 15, Fig. 16) at Shaheb Bazar and Binodpur and Katakhalī F.L.C.

By the same way the maximum cost of Retailers are shown in the (Table 14, 15).

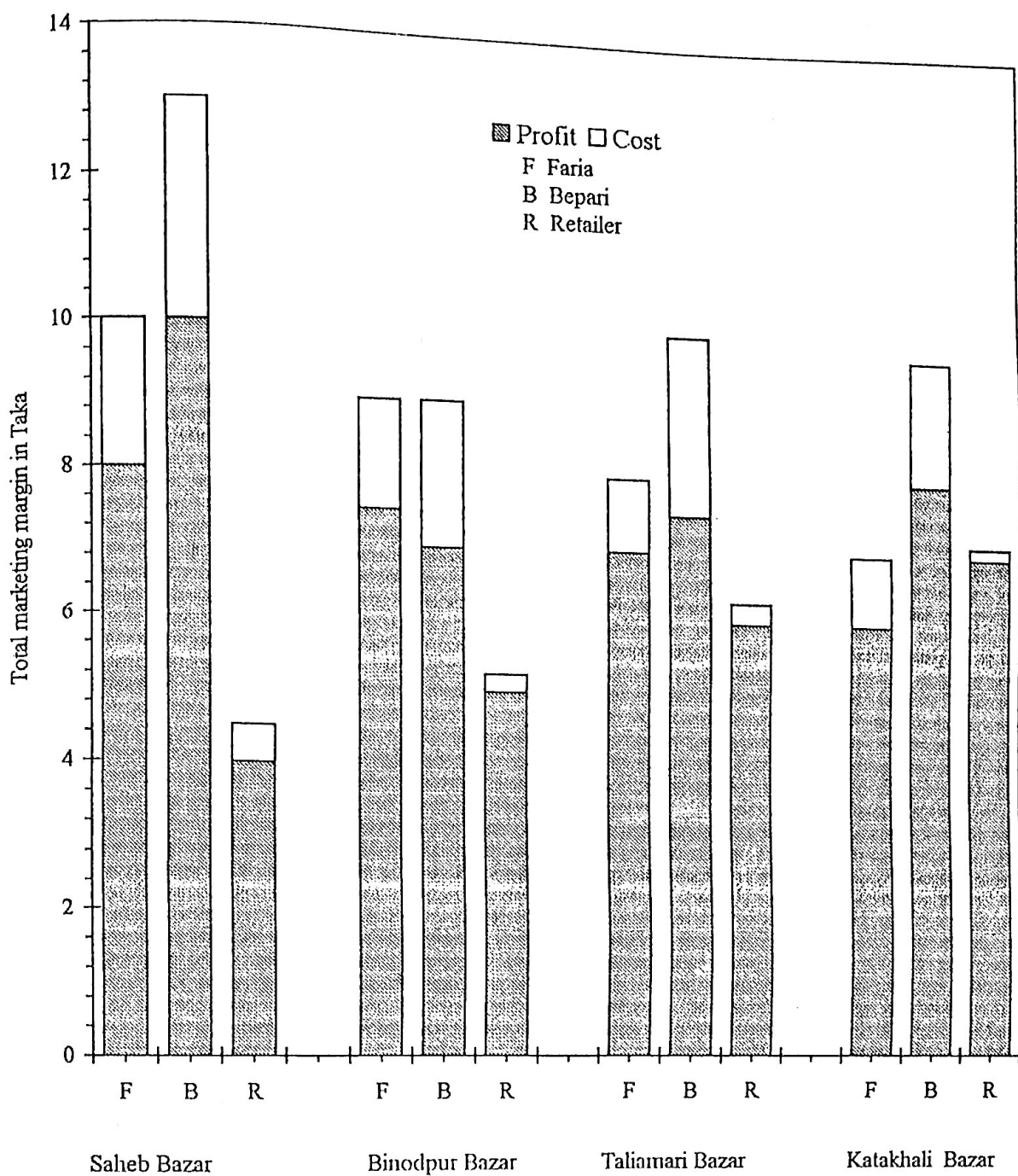


Fig. 13. Total marketing margins of intermediaries of small sized *A. morar*.

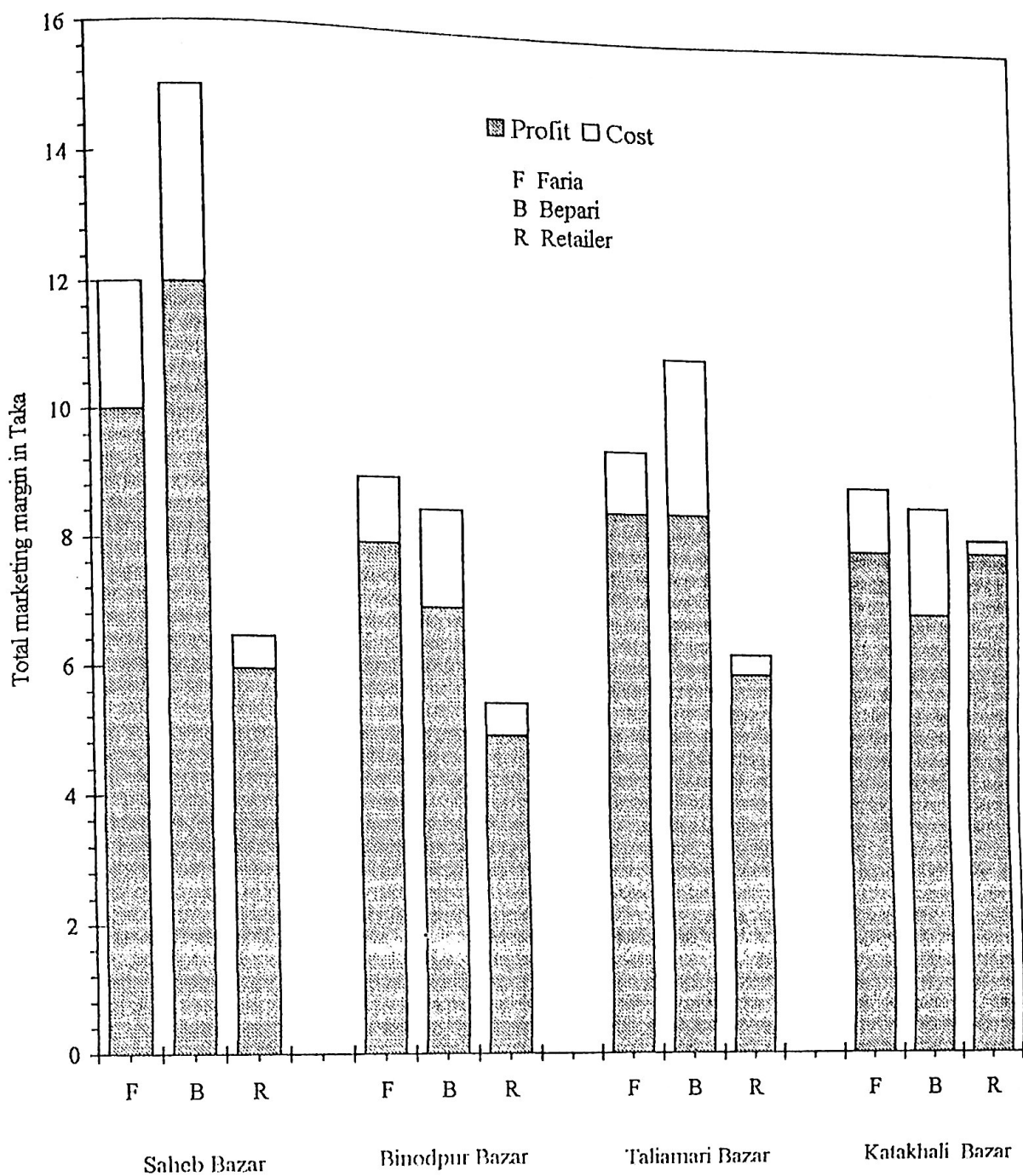


Fig. 14. Total marketing margins of intermediaries of large sized *A. morar*.

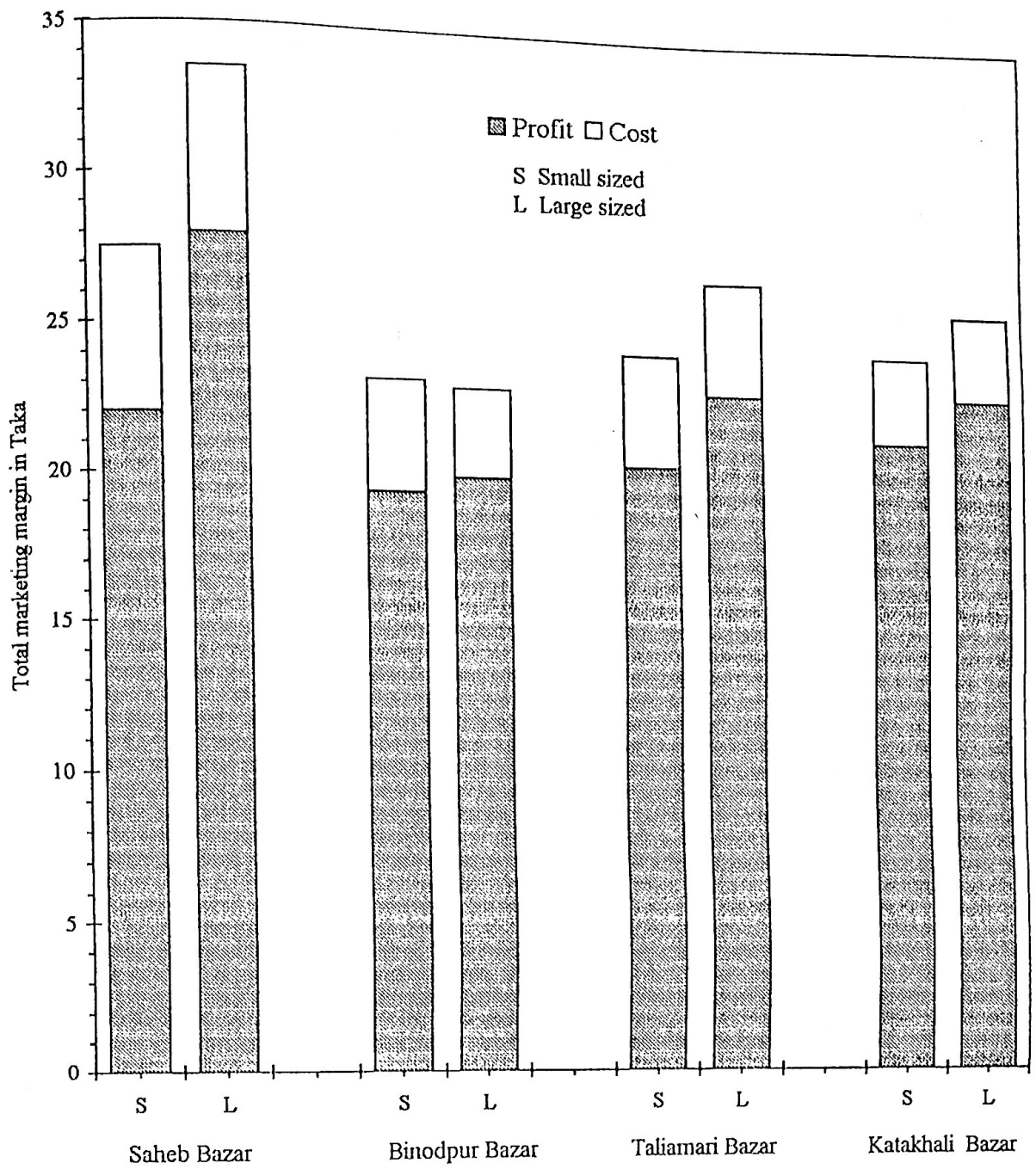


Fig. 15. *A. morar*: Total marketing margins of different fish landing centre.

Table 14 : Price spread of small sized (40-90mm) *A morar*

	Shaheb bazar F.L.C		Binodpur bazar F.L.C		Talaimari bazar F.L.C		Katakhali F.L.C	
	Tk/kg	% of Consumer Price	Tk/kg	% of Consumer Price	Tk/kg	% of Consumer Price	Tk/kg	% of Consumer Price
Price received by fisherman	24.00	46.60	22.00	48.62	23.00	48.63	20.50	46.17
Marketing cost of Faria	2.00	3.88	1.50	3.32	1.00	2.11	1.00	2.25
Profit of Farias	8.00	15.53	7.50	16.57	7.00	14.79	6.00	13.51
Margin of Farias	10.00	19.41	9.00	19.88	8.00	16.91	7.00	15.76
Price paid by Bepari	34.00	66.01	31.00	68.50	31.00	65.53	28.50	64.18
Marketing cost of Bepari	3.00	5.82	2.00	4.41	2.50	5.28	1.75	3.94
Profit of Bepari	10.00	19.41	7.00	15.46	7.50	15.85	8.00	18.02
Margin of Bepari	13.00	25.24	9.00	19.88	10.00	21.14	9.75	21.95
Price paid by Retailer	47.00	91.26	40.00	88.39	41.00	86.68	38.25	86.15
Marketing of Retailer	0.50	0.97	0.25	0.55	0.30	0.63	0.15	0.33
Profit of Retailer	4.00	7.76	5.00	11.04	6.00	12.68	7.00	15.76
Margin of Retailer	4.50	8.73	5.25	11.60	6.30	13.31	7.15	16.10
Total marketing margin.	27.50	53.40	23.25	51.38	24.30	51.37	23.90	53.83
Price paid by Consumer	51.50	100	45.25	100	47.30	100	44.40	100

Table 14 : Price spread of small sized (40-90mm) *A morar*

	Shaheb bazar F.L.C		Binodpur bazar F.L.C		Talaimgari bazar F.L.C		Katakali F.L.C	
	Tk/kg	% of Consumer Price	Tk/kg	% of Consumer Price	Tk/kg	% of Consumer Price	Tk/kg	% of Consumer Price
Price received by fisherman	24.00	46.60	22.00	48.62	23.00	48.63	20.50	46.17
Marketing cost of Faria	2.00	3.88	1.50	3.32	1.00	2.11	1.00	2.25
Profit of Farias	8.00	15.53	7.50	16.57	7.00	14.79	6.00	13.51
Margin of Farias	10.00	19.41	9.00	19.88	8.00	16.91	7.00	15.76
Price paid by Bepari	34.00	66.01	31.00	68.50	31.00	65.53	28.50	64.18
Marketing cost of Bepari	3.00	5.82	2.00	4.41	2.50	5.28	1.75	3.94
Profit of Bepari	10.00	19.41	7.00	15.46	7.50	15.85	8.00	18.02
Margin of Bepari	13.00	25.24	9.00	19.88	10.00	21.14	9.75	21.95
Price paid by Retailer	47.00	91.26	40.00	88.39	41.00	86.68	38.25	86.15
Marketing of Retailer	0.50	0.97	0.25	0.55	0.30	0.63	0.15	0.33
Profit of Retailer	4.00	7.76	5.00	11.04	6.00	12.68	7.00	15.76
Margin of Retailer	4.50	8.73	5.25	11.60	6.30	13.31	7.15	16.10
Total marketing margin.	27.50	53.40	23.25	51.38	24.30	51.37	23.90	53.83
Price paid by Consumer	51.50	100	45.25	100	47.30	100	44.40	100

Table 15 : Price spread of large (95-140mm) *A morar*

	Shaheb bazar		Binodpur bazar		Talaimari bazar		Katakhal	
	Tk/kg	% of Consumer Price	Tk/kg	% of Consumer Price	Tk/kg	% of Consumer Price	Tk/kg	% of Consumer Price
Price received by fisherman	57.00	62.98	54.00	70.12	56.00	68.04	50.00	65.87
Marketing cost of Faria	2.00	2.21	1.00	1.29	1.00	1.21	1.00	1.32
Profit of Farias	10.0	11.04	8.00	10.38	8.50	10.33	8.00	10.54
Margin of Farias	12.00	13.25	9.00	11.68	9.50	11.54	9.00	11.85
Price paid by Bepari	64.00	70.71	56.00	72.72	59.50	72.29	57.00	75.09
Marketing cost of Bepari	3.00	3.32	1.50	1.95	2.50	3.03	1.70	2.23
Profit of Bepari	12.00	13.25	7.00	9.09	8.50	10.33	7.00	9.22
Margin of Bepari	15.00	16.57	8.50	11.03	10.50	12.75	8.70	11.46
Price paid by Retailer	79.00	87.29	64.50	83.76	70.00	85.05	65.7	86.56
Marketing cost of Retailer	0.50	0.63	0.50	0.65	0.30	0.36	0.20	0.26
Profit of Retailer	6.00	6.62	5.00	6.49	6.00	7.29	8.00	10.54
Margin of Retailer	6.50	7.18	5.50	7.14	6.30	7.65	8.20	10.80
Total marketing margin.	33.50	37.02	23.00	29.88	26.30	31.96	25.90	34.13
Price paid by consumer	90.50	100	77.00	100	82.30	100	75.90	100

3.3.4.2 Profit of Intermediaries

The maximum total profits were obtained as Tk 26 and 22/kg at Shaheb bazar size F.L.C in both fish group. The highest profit earned by Farias were found of Tk 8 and 10/kg at Shaheb bazar F.L.C both case of small and large size fish group. Beparis and Retailers total profit were shown in the (Fig 16 Table 14, 15).

3.3.5 Seasonal Price Variation

The survey indicates that price of *A. morar* at 4 fish landing centre (Shaheb Bazar, Binodpur, Talaimary, Katakhal, were varied from Tk 48 to 56 and 70 to 100/kg at for both small and large size fish groups. From all over observations the low price were observed from November-February at 4 Fish landing centre due to its peak breeding season though the fluctuation of price rate is not significant because it is not found abundantly all over the year. The maximum price trends were mainly found from March to August. Amongst the fish landing centres the highest price of this was observed at Shaheb bazar than other FLC. This is due to the minimum supply and maximum demand and high transportation cost.

3.4 DISCUSSION

To manage fisheries properly it is necessary to have some knowledge about various types of gears. The gears those are used in *A. morar* catching are more or less same type. In the present investigation pour type of net, barricade etc, have been described. Some fisherman uses 'Khara jal' accompanied with the barricade for catching *A. morar* (larvae) particularly during the winter season to spring. The Bewshi net is also an associate net for catching *A. morar* along with other small sized fishes during monsoon and post monsoon period. *A. morar* fish is caught also through two types of Ligon (gill nets). Fisherman called Feri nets as a Piali catching net which used throughout the year for catching specially *A. morar*.

In the Padma river, fishing is made throughout the year. The total landing of small size fishes and the percentage composition of *A. morar* has been observed in twelve months. During the peak period, *A. morar* shared about ** percent of the total landing in the month of ***. This constitute only a fractional of the potential food supply from fresh water fisheries. Current jal is one of the most important barricade for the availability of not only *A. morar* fish but also other small fishes, because by this net the larval forms of these fishes are caught.

After harvesting the *A. morar* and other fishes also are collected in a small Silver Hari. For smaller amount which sold locally, this Hari is generally used to carry the fish. For larger amount and distant markets the fishes are carried in bamboo made baskets called as Dali or Dala and also silver Hari.

The nature of marketing channel or distribution channel of commodities is found to depend on the distance between the producing areas and the markets and on the condition of transportation system. The channel becomes more complex regarding the greater distance and undeveloped communication system. There is no definite methodical marketing channel of *A. morar* everywhere.

The marketing channel have been found comprises with a few intermediaries (eg . Farias, Beparis, Aratdars and Retailers) in between the fisherman and the consumer. The whole marketing system of the studied area of Rajshahi remains under control of the private traders, and the fisherman are compelled to handover their catches at the local and nearby markets of the producing areas to earn a bit more profit.

Thakur (1974) described the importance of analysis of marketing margin to determine the marketing efficiency. In an efficient marketing system, the margin will be small as the percentage of marketing costs and profits of the traders remain low. The marketing cost depends mainly on the distance between the producing areas and markets and on the efficiency of the transporting system. Some has been supported by Parween (1982) in his investigation on fresh water Prawn *Macrobrachium dayanum* of Rajshahi district. The cost involved in the marketing of *A. morar* for different markets such as Shaheb bazar, Katakhal, Binodpur and Talaimari) are found to vary mainly for the profit of intermediaries and transportation cost. As a whole the net profit per kg earned by the Bepari are higher than the others depending on the producing season of *A. morar*, the price varies among the F.L.C. As a whole the marketing cost is higher at Shaheb Bazar fish landing centres as Tk. 5.50 per kg. both small and large size group and the total profit is also higher at Shaheb Bazar F.L.C as Tk 22.00 per kg and Tk. 28.00 both small and large size fish. The price received by the fishermen was found highest at Shaheb Bazar market in both cases throughout the year. When the fishermen sell directly to the consumers, they receive a good share of the consumers price. Similar results are also obtained by Ahmed, 1977.

At the same time restriction should be made against the use of 'Feri jal' (Mesh size 1.4 to 1.5 cm) during the peak spawning season (December- February) to catch the mature fishes thus the fishes can escape from the brutal catches and could breed properly to balance their population .

Chapter 4

CHEMICAL ANALYSIS OF *A. MORAR*

4.1 INTRODUCTION

Most of the people of our country have been suffering from mental and physical disease for nutritional deficiencies. The lack of balanced food ingredients is the main cause of their malnutrition. The lack of protein is very conspicuous in various kinds of food ingredients. There are two kinds of proteins such as plant protein and animal protein. Generally animal protein is better than plant protein in respect of qualities (Hossain, 1990 and S. Afroze, 1990).

In our country, 80% animal protein comes from fishes. Because meat, egg, milk etc. are not easily available to the people.

As rice is the main carbohydrate source of our people. In the same way, fish is the best and available source of protein.

The production of fish is less in comparison to its need. Besides, the production of fish is decreasing day by day. Twenty years ago the fish production per head was 30gm daily. Now it is less than 21 gm. At least 80 gm fish per day is necessary for every man. Price of fish is very high in market because of its less availability in the market. It is very expensive to buy fish by most of the ordinary people.

Bangladesh is a riverine country. There are plenty of rivers, ponds, ditches and other water bodies in our country for fish production. So it is necessary to use every kind of water bodies for the fish culture.

Medicinal and dietary uses of fats and oils from both the plant and animal sources are also very old and by no means it can be said to be recent. Human

beings have been using fats and oils as food of centuries (Martinadle, 25th edition). Fats and oils are widely distributed both in plants and animal kingdom. They are found to vary in quantity with the sources. They consists of a single cell.

The importance of fats and lipids are recorded both commercially and industrially all over the world, which is increasing day by day in a very long magnitude for both medicinal, dietary and other uses. The main constituent of fats and lipids are the fatty acids and glycerol.

Besides the use in medicine and in diet, fats and lipids have also great industrial applications. Fats and lipids are very wide spread in nature, being found in all vegetable and animal matter. Some member of this group are found in all living cells, where they form an essential part of the colloidal complex. Fats and lipids represent the chief form in which excess nutrients are stored in the animal body, they act as heat insulators and as reserve supplies of energy. They are concerned with many metabolic processes. Hence attention has been reasonably drawn towards the chemical investigation of natural oils and fats.

4.1.1 Lipids (Krause *et al.*, 1972)

Fats, oils and fat-like substances because of similar, solubilities, are classified as lipids. They are insoluble in water but soluble in solvents, ether, chloroform, benzene, and acetone, like carbohydrates, fats are composed of carbon, hydrogen and oxygen but in proportions that greatly increase their energy value. Lipids that are fluid at room temperature are usually called oils, where as those that are solid are called fats. Most natural lipids are composed of about 98 to 99 percent triglycerides and the vast majority of these are long chain triglycerides, the 1 or 2 percent remaining include traces of mono and deglycerides, free fatty acids, phospholipids and unsaponifiable matter containing sterols.

The lipids are either esters of fatty acids or substances capable of forming such esters. They are very wide spread in nature, being found in all vegetables and animal matter. Some member of this group, such as the phosphatides are sterols found in all living cells where, with the protein and carbohydrates, they form an essential part of the colloidal complex of cytoplasm. Complex lipids are also found in large quantities in brain and nervous tissues they indicating the important role these substances must play in the living organism (Howk & Oser, 1965). Other lipids such as the fats and oils represent the chief form in which excess nutrients are stored in the animal body. They arise from ingested lipids and from the metabolism of carbohydrates and protein and are stored in fat deposits. Lipids act as heat insulators and as reserve supplies of energy (Howk & Oser, 1965).

4.1.2 Classification of lipids (George I. & Sackheim *Et Al.*, 1965)

1. Simple lipids :
2. Compound lipids :
3. Derived lipids :

FUNCTION OF LIPIDS (Anderson L. *et al.*, 1982)

Lipids serve as a concentrated source of energy. Each gram of lipids supplies 9 calories, which is more than twice the amount of energy supplied by each gram of carbohydrate. The main source of this energy is the fatty acids, which supplies 40 to 50 carbon atoms for oxidations as compared with 3 from glycerol. Because of the high energy density and low solubility of fats, they are used as a storage form of energy. Not only ingested fat but carbohydrate and amino acids not immediately used by tissues, are converted to fat and stored in the adipose tissue. Up to two-thirds of the total energy of the cells may be supplied by triglyceride rather than carbohydrate; Lipid spares protein for tissue synthesis.

Adipose tissue to hold the body organism and nerves in position and to protect them against traumatic injury and shock. The subcutaneous layer of fat insulates the body which serves to preserve body heat and maintain body temperature. Lipids aid in transport and absorption of the fat-soluble vitamins. Lipids spare thiamine since they can be metabolized for energy, as compared with carbohydrate, whose metabolism requires thiamine. In the stomach they depress gastric secretions and slow the emptying time of the stomach, thus providing a pleasant feeling of satiety after a meal. Lipids also retard the rapid development of hunger which occurs after a carbohydrate meal. Lipids add to the palatability of food as well as to the flavor of the diet.

Three poly unsaturated fatty acids, namely oleic, linolenic and arachidonic acids, have essential fatty acid activity. However, only two- linoleic and arachidonic acids have been designated as essential fatty acids for human, they have important roles in transport and metabolism and is maintaining the function and integrity of cellular membranes. They also are a part of the fatty acids of cholesterol esters and phospholipids in plasma lipoprotein and mitochondrial lipoprotein.

The phospholipids maintaining the actual integrity of the cells rather than as fat store. Despite the loss of body fat that occurs in extreme starvation the phospholipid content remains fairly constant, thus maintaining the integrity of tissue cells. The lipoproteins transport insoluble lipids in an aqueous medium.

4.1.3 Recommended allowance of lipids in the diet

The average American eats about 127 pounds of lipids yearly, receiving perhaps 42 percent of his total calories as lipids are popular because of their

flavour and satiety value; however their cost is greater than the cost of carbohydrates.

Americans consume more lipid than is necessary and probably more than is good for them. The requirement of the human for essential fatty acid has been estimated to be approximately 2 percent caloric intake (infants, 1 to 3 percent) of the diet. The average diet should contain at least 25 percent of calories as lipid. The precise amount of lipid in the diets of general public became less meaningful in the light of results published by several investigators which show that a decrease in dietary lipid is usually accompanied by an increase in carbohydrate, which may correlate with rise in blood triglycerides.

4.1.4 Functions of fatty acids

Fatty acids are straight-chain organic acids. The long carbon chains of these fatty acids may be saturated (contain only C-C single bonds) or they may be unsaturated (contain C=C double bonds). Human cannot synthesize some of the required unsaturated fatty acids containing more than one double bond.

Linoleic acid is called the essential fatty acid. It is essential for the complete nutrition of the human body. It cannot be synthesized in the body and must be supplied from food we eat. Arachidonic and linolenic acids, which we formerly designated as essential fatty acids, can be synthesized in the body from linoleic acid. One of the functions of essential fatty acids is in the synthesis of the prostaglandins. Prostaglandins are derived from arachidonic acid, which is formed from the essential fatty acid linoleic acid. This compound has a wide range of physiologic effect. Linoleic, linolenic and arachidonic fatty acids are known to cure dermatitis or inflammation of the skin, and to restore the growth of young animals that have been fed a diet very low in fat. The absence of essential fatty

acids from the diet of an infant causes loss of weight (George I. Sackheim, Callewoert D.M. 1980).

Burr and co-workers (Burr, 1932) demonstrated that either linoleic or linolenic acid protects rats against the deficiency symptoms. It was also shown that arachidonic acid (4-double bond) also is highly effective in preventing the rat deficiency diseases. Other species for which a linoleic acid requirement has been demonstrated include dogs, rabbit, guinea pig, mouse, pig, man and some insects.

The linoleic acid has a great influence on the heart. Scientists have established a co-relation between linoleic acid and blood-pressure. The food containing linoleic acid are capable of reducing the blood-pressure. Doctors say that the diet enriched in linoleic acid is very helpful to give relief from the high blood pressure of the pregnant women (Medical Tribune 1980). A Danish report also supported the power of linoleic acid prevents the coagulation of blood in the artery and also keeps the cardiac muscle strong.

4.1.5 Aim of the present investigation

Bangladesh has the very rich fishery resources both marine and fresh water. Favourable geographical condition, mild climate, numerous rivers, canals, beels, haors, baors, tanks, ponds etc. give an unique opportunity for development and expansion of this important industry. About 90% of total demand of the fish in the country comes from fresh water. *A. morar* is a fresh water fish which plays an important role for protein nutrient. This fish is delicious and so everybody likes it.

Hunger and nutritive deficiency is one of the greatest problems in our life. Among the nutritive deficiencies, the protein deficiency is the greatest. The fish is a good and cheap source of protein. So the valuable contribution of fish to the total

supply of protein cannot be denied. Our over increasing populations need a huge amount of protein which can easily be fulfilled by producing adequate fishes.

Bangladesh is rich for her vast water resources. Both fresh and coastal water producing number of fish species, can be utilized by proper management as the main source of protein supply for our nation.

As such it is evident that the study of fisheries is essential in our country considering from the economic point of view.

Fishes provide maximum protein for human bodies and it has also a great demand for good taste. The fishes are highly perishable commodities.

The fish is well known to the people of all parts of Bangladesh for its recognized usefulness as a source of protein. Amino acid profile in fish protein and essential fatty acid components in fish lipid may indicates the important role

4.2 METHOD AND MATERIALS

The fishes of same habitat, having uniform size (100m - 130mm) in total length have been caught, killed and processed in identical conditions to detect the seasonal variation in the muscular constituents and the physico-chemical characters of *A. morar* lipid. The collection started from the June 1995 to May 1996. The work, divided into two parts, including the studies on seasonal variation of lipids, protein, Ash and moisture, and the physico-chemical characteristics of the fish lipid with the analysis of the composition of the fatty acids by GLC.

4.2.1 Determination of percentage of protein in flesh of *A. morar* (Micro-Kjeldahl method) (Jayaraman, J, 1985).

PRINCIPLE :

Nitrogen content is estimated by the Kjeldahl method which is based on the determination of the amount of reduced nitrogen present in the sample. The various nitrogenous compounds are converted into ammonium sulphate by boiling with conc. H_2SO_4 . The ammonium sulphate formed is decomposed with an alkali (Na OH), and the ammonia liberated is absorbed in excess of neutral boric acid solution and then titrated with standard acid.

APPARATUS REQUIRED :

- a) Kjeldahl digestion flasks : 250ml capacity.
- b) Distillation apparatus.
- c) 100ml beaker.
- d) 50ml conical flask.
- e) 50ml burette.
- f) 100ml volumetric flask.

REAGENTS REQUIRED :

- a) **Mixed indicator :** A solution was prepared by mixing 0.1% bromocresol green and 0.1% methyl red indicators in 95% alcohol separately. 10ml of the bromocresol green was mixed with 2ml of the methyl red solution in a bottle provided with a dropper which delivered about 0.05ml per 4 drop.
- b) **2% Boric acid :** 10gm of boric acid (crystals) was dissolved in 500ml of boiling distilled water. After cooling, the solution was transferred in to a glass - stoppered bottle.
- c) **30% sodium hydroxide :** 150gm of sodium hydroxide pellets was dissolved in 350ml of distilled water. The solution was stored in a bottle closed with a rubber stopper.
- d) **Catalysts for digestion :** 2.5gm of powdered selenium dioxide (SeO_2), 100gm of potassium sulphate (K_2SO_4) and 20 gm of copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) were mixed.
- e) **0.01 N Hydrochloric acid :** The concentration of the final solution was checked against pure sodium carbonate.

PROCEDURE :

Digestion : 1.2097gm of dried sample was weighed and transferred to a 250ml Kjeldahl flask. 1gm of catalyst mixture and 25ml of conc. H_2SO_4 were added to it. The flask was placed in an inclined position on the stand in the digestion chamber. The flask was heated gently over a low flame until the initial frothing was ceased and the mixture was boiled briskly at a moderate rate. During heating the flask was rotated several times. The heating was continued until the color of the digest was pale blue. The digest was cooled and 30ml of water was added in 5ml portion with mixing. The digest was transferred to a 100ml volumetric flask. The flask was then rinsed 2 or 3 times with water and the washings were transferred to the volumetric flask. The solution was made up to

volume with water. A blank digestion was carried out without the sample and the digest was made upto 100ml in total.

Distillation and Titration : The distillation apparatus (commercially available) was set up as shown in Fig. 3. A flask was placed under the condenser. The distilled water was boiled in the steam generator using Bunsen burner. Stop cock and pinch clamp were closed. Cold water was run through the condenser, from which about 5ml of distillate was collected per minute. The burner was removed, whereupon the condensate in the distilling flask was sucked back into the steam trap. Funnel was filled with distilled water, and the stopcock was opened momentarily to drain the water into distilling flask.

The burner was then replaced under the steam generator for about 20 seconds and it was removed again. 20 ml of 2% boric acid was pipetted into a clean conical flask and the mixed indicator was added to it. The micro burette was filled with 0.01 N HCl to the zero mark. By this time, the distilling flask had become empty. The burner was replaced under the steam generator and pinch clamp was left on the glass tubing through which the steam escaped. The beaker was replaced under the condenser with the conical flask containing boric acid, and the flask was supported in an oblique position, so that the tip of the condenser was completely immersed into the liquid. The stopcock was opened with one hand and with the other hand, 10ml of the digest was pipetted into distilling flask. The funnel was rinsed twice with about 2 to 3ml portions of distilled water. Then necessary amount of 30% NaOH was introduced and stopcock was closed. The pinch cock was replaced on the rubber tubing, where upon steam entered into distilling flask, stirred up the digestion mixture and sodium hydroxide, and the ammonia was liberated which escaped with steam through the condenser into the boric acid solution.

The boric acid was changed from bluish purple to bluish green as soon as it came in contact with ammonia. The change, which was very sharp was taken place between 20 to 30 seconds often the pinch clamp was closed. Boric acid had changed color within 5 minutes, the conical flask was lowered so that the condenser tip was 1 cm above the liquid. The end of the condenser was washed with a little water. Distillation was continued until sufficient distillate was collected. The burner was then removed. The distillate was then titrated with standard hydrochloric acid until the blue color was disappeared. The titration was done in day light. The blank distillation and titration were carried out as in the case of the sample. The percentage of protein in *A. morar* was calculated using the formula.

$$\% \text{ Nitrogen} = \frac{(V_A - V_B) \times N \times 14 \times V_M \times 100}{A \times W \times 1000}$$

Where,

V_A = Volume of actual titration of HCl.

V_B = Volume of blank titration of HCl.

N = Normality of HCl.

V_M = Volume made up of the digest.

A = Aliquot of the digest taken.

W = Weight of the sample taken.

Thus, $\% \text{ Protein} = \% \text{ Nitrogen} \times 6.25$

4.4.2 Extraction of total Lipids

Bligh and Dyer method (Jayaraman J., 1985)

In this method, a mixture of chloroform and methanol (2:1 v/v) was used. The tissue (about 1gm weight) was first ground in a pestle and motor with about

10ml distilled water. The pulp was transferred to a conical flask (250ml capacity) and 30 ml of chloroform- methanol mixture was added and mixed well. For complete extraction, it was kept over night at room temperature, preferably in the dark. At the end of this period, a further addition of 20ml water was made. The resulting solution was subjected to centrifugation when 3 layers were seen. A clear lower layer of chloroform containing all the lipids, a coloured aqueous layer of methanol with all water soluble material, and a thick pasty interface were seen.

The methanol layer was discarded and the lower layer was carefully collected free of inter phase either by sucking out with a fine capillary or by filtration through glass wool. The organic layer from-either of the extraction method was taken in a pre-weighed beaker and carefully evaporated. This was achieved by keeping the sample in warm water (around 50°) and a slow stream of nitrogen gas was blown on to the surface. The sample was covered with a dark paper to protect from light. This was because some lipids get polymerized or decomposed on exposure to light, heat and oxygen. But this precautions were needed only for very precise highly quantitative analysis.

When the solution was free from organic solvents, the weight was determined again. The difference in weight gave the weight of the lipids and at this stage gravimetric estimations was done. The results were expressed in terms of weight in milligrams of total lipid per gm. of fresh fish.

4.2.3 Determination Of Moisture Content And Ash Content

Determination Of Moisture Contents (Mollah, A.H., 1991)

Moisture content was determined by the conventional procedure :

MATERIALS :

- a) Porcelain crucible.
- b) Electrical Weighing balance (Mettler H 18).
- c) Oven (W.C. HERAEUS GMBH, HANAU).
- d) Desicator.

PROCEDURE :

Empty weights of moisture free dried crucibles were taken first. Then triplicate samples were taken in 3 separate crucible and subsequently weights were taken. Then the crucibles with samples were placed in an oven at 100-105°C for 4.5 hours and then transferred in a desicator. After cooling, the crucibles were weighed. The process was repeated until constant weight of each was achieved. Moisture contents were calculated as follows :

Calculation :

$$\text{Amount of moisture (g/100g)} = \frac{(A-B) \times 100}{C}$$

Where,

A = Wt. of the crucible with sample

B = Wt. of the crucible with sample after removal of moisture

C = Wt. of the sample taken

Determination of ash content (Jayaraman J., 1985)

MATERIALS :

- a) Porcelain crucible
- b) Muffle furnace
- c) Electric balance
- d) Desiccator.

PROCEDURE :

Empty weight of crucible (moisture free) was taken first. Then triplicate samples were taken in 3 separate crucibles and they were placed in the oven and heated at 100-105°C for 3-4 hours. Then these crucibles were kept in the muffle furnace and heated for 4 hours at 600°C. After complete burning of the sample the crucibles were again transferred in a desiccator. After cooling, the crucibles were weighted. The process was repeated until constant weight was obtained.

CALCULATION :

$$\text{Ash content (g/100g sample)} = \frac{\text{Ash wt.} \times 100}{\text{Sample taken}}$$

4.2.4 Study of the Physical characteristics of the Lipid.

4.2.4.1 Determination of refractive index (J.A. Kichene, PP.C. Grilf)

REFRACTIVE INDEX :

When a ray of light passes from air into a denser medium, e.g. in a liquid, it is bent or refracted towards the normal. The ratio of the sine of the angle of incidence and that of refraction is constant and characteristic of that liquid (Snell's law). The constant ratio "n" is called the refractive index of the liquid. (Roger Castle Griffing *et al.*,)

Refractive index of the lipids were determined following the procedure as described earlier

$$n = \frac{\sin i}{\sin r} \text{----- (I)}$$

According to the wave theory of light, the ratio of the sine of the angles of incidence and the angle of refraction is identical with the ratio of the velocities of light in the two media. Thus,

$$n_1 = \frac{\sin i}{\sin r} \text{----- (II)}$$

When a ray of light passes from air to a denser medium, it can be shown from the law of refraction; that is

$$n_2 = \frac{\sin i}{\sin r} \text{----- (III)}$$

Where,

n_1 = Index of refraction of the air medium

n_2 = Index of refraction of the denser medium

MATERIALS :

- a) Abbe refractometer (Standard model 60/70)
- b) Thermostat (constant temperature water bath)
- c) Few drops of lipid

PROCEDURE :

The double prism was opened by means of the screw head and a few drops of oil was placed on the ground surface of the lower prism. The prisms were closed firmly by tightening the screw head and allowed to stand for a few minutes to come to uniform temperature. The cross wires of the telescope were focused by rotating the eye piece and the mirror to get good illumination. The alidade was moved on the side scale backward and forward until the field of vision is divided into light and dark portions. The screw head of the compensator was rotated until a sharp colorless line was observed between the fields and then adjusted this line so that it falls on the point of intersection of the two cross wires. The reading of the index of refraction was taken off directly from the scale through eye piece.

4.2.4.2 Determination of specific gravity (Hildicth, 1949)**DEFINITION :**

Specific gravity of a substance represents how much heavier the substance to the same volume of water at a particular temperature and it may be defined as the ratio of the weight of the definite volume of substance to the weight of the definite volume of water at a particular temperature.

$$\text{Specific gravity} = \frac{\text{Weight of sample in bottle}}{\text{Weight of distilled water in bottle}}$$

MATERIALS :

- a) 10 ml Specific gravity bottle.
- b) Thermostat (constant temperature water bath.)
- c) Electric weighing balance (Mettler H. 18).

PROCEDURE :

The specific gravity bottle was cleansed carefully, dried and weighed when empty. The bottle was then filled with distilled water at room temperature holding it in an inclined position to avoid the formation of bubbles. It was then immersed in a constant temperature water bath at 25°C with its stoppered end just outside the level of water. The bottle was then removed from the bath, wiped dry with a clean cloth and allowed to stand for sometime and then weighed.

The bottle was then emptied, cleansed, dried and filled with the lipid; care was taken to avoid the formation of bubbles. The bottle with its content was subjected to similar treatment at 25°C as was done previously with distilled water and was finally weighed.

4.2.4.3 Determination of the co-efficient of viscosity at different temperatures and to find out the energy of activation for viscous flow (KITCHENER, 1973)

THEORY :

All liquids possess certain resistances to flow, known as their viscosity. In general, viscosity is the property that opposes the relative motion of the adjacent layers of the liquid and consequently be regarded as a type of internal friction. The viscosity or coefficient of viscosity “ η ” (eta) is defined by the equation.

$$F = \eta A \frac{dv}{dx}$$

Where “F” is the force necessary to impart to a fluid with a co-efficient of viscosity (η), a velocity gradie $\frac{dv}{dx}$ over an area “A” parallel to the flow direction. The C.G.S unit of viscosity is dyne sec⁻¹ cm⁻² is called poise in honour of J.L.M. poiseuille who was the pioneer in the study of viscosity. Since in many cases viscosity is very small in this unit, it is represented by centipoise or millipoise.

Poiseuille studied the flow of liquids through the capillaries and derive an expression as :

$$\eta = \frac{nPr^4t}{8vL} \text{----- (I)}$$

Where,

P = Driving pressure causing the flow.

r = Radius of the capillary.

L = Length of the capillary.

v = Volume of the liquid flowing in time t .

This is known as poiseuille's equation and is the basis of methods for determining co-efficients of viscosity of liquids and is the basis of methods for determining co-efficients of viscosity of liquid. The equation holds accurately for streamline flow but not for the turbulent flow which sets in at higher velocities. If the pressure is caused by the mean hydrostatic head.

$$P = h\rho g \text{----- (II)}$$

Where,

h = Difference in the height between the levels of the liquid in the two limbs.

ρ = Density of the liquid.

g = Acceleration due to gravity.

putting the value of " P " from equation (ii) to the equation (i)

$$\eta = \frac{h\rho gnr^4t}{8Lv} \text{----- (III)}$$

Equation (iii) behaves satisfactorily when the time of flow is very high, that is, the liquid flows at a very slow rate. For a particular viscometer, the values of h , r , g , l and v are constant and hence, the equation can be written as

$$\eta = A t \rho \text{-----(IV)}$$

Where, $A = \frac{nr^4hg}{8vL}$

Temperature plays a tremendous role on viscosity. There is a relation between temperature and co-efficient of viscosity, which is expressed as :

$$\eta = A. e^{E/RT} \text{----- (V)}$$

Where,

A = Constant.

E = Energy of activation for viscous flow.

T = Temperature.

If logarithm is taken for equation (V), then,

$$\log_e \eta = \log_e A + E/RT.$$

$$\text{or, } \log_{10} \eta = \log_{10} A + E/2.303 RT \text{----- (VI)}$$

Form the relation (vi) it is evident that with the rise of temperature, viscosity decreases and if we plot $\log_{10} \eta$ vs. $1/T$, a straight line will be obtained having a slope equal to $E/2.303 R$. From which the value of 'E' can be calculated.

MATERIALS :

- a) Ostwald's viscometer
- b) Electric weighing balance.
- c) 25 ml Specific gravity bottle.
- d) Stop watch
- c) Thermostal (constant temperature water bath).

PROCEDURE :

The inside wall of the viscometer was cleansed thoroughly with a mixture of chromic acid and 50% nitric acid in such a way that there was no obstruction in

the capillary limb. Then it was washed with water, rinsed with acetone and dried. The viscometer was then clamped tightly to a stand and was placed vertically on a glass sided thermostat. The mark from which time of flow of the liquid was determined and kept immersed in water. 25 ml of distilled water was then taken in the viscometer, with the help of a pipette. Water was drawn above the mark line with the help of rubber tube (attached to the mouth of the capillary limb) and allowed to fall down. When the liquid just crossed the mark, the stop watch was started and the time of flow for each fall was recorded at different temperatures. Three such readings were taken. From the mean of the reading and the density of water at 30°C, the value of A was calculated (since the value of n is 8.00 m.p. at 30°C for water). The water was drawn out, the viscometer was dried and 25 ml of oil was taken in the viscometer. The time of flow for oil was calculated at different temperatures such as 30°C, 35°C, 40°C, 45°C, 50°C, etc. The density of oil was calculated at respective temperature using a specific gravity bottle.

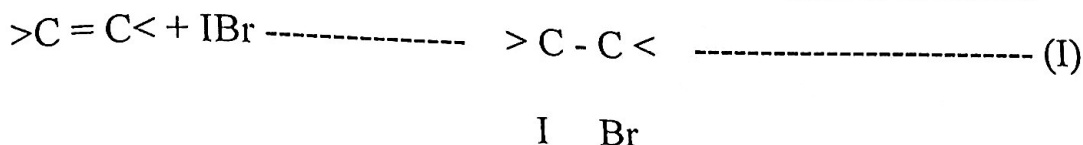
The values of $\log_{10}\eta$ and $1/T$ were calculated and $\log_{10}\eta$ was plotted against respective $1/T$. A straight line was obtained having a definite slope. From the slope, the energy of activation (E) was calculated.

4.2.5 Study of the chemical characteristics of the Lipid.

4.2.5.1 Determination of iodine value (Hanus method) (Williams, 1966)

DEFINITION

The iodine value of a fat or oil is the amount of halogen absorbed under specified conditions and is expressed as the number of grams of iodine per 100 gm of fat (or oil). It is a measure of the proportion of unsaturated constituents present. In reality, depending upon the method used, iodine monochloride or monobromide is the agent absorbed as seen in eqⁿ-1.



It is of great importance in characterizing individual oil or fat and also for finding their suitability as soap materials.

APPARATUS REQUIRED :

- 250ml wide-necked glass bottles, with ground glass stoppers.
- 50ml burette, graduated in 0.1ml.

REAGENTS REQUIRED :

- Potassium iodide solution (15%)**: 15 gm potassium iodide was dissolved in 100ml. distilled water.
- Sodium thiosulphate solution (0.1 N)** : 24.82 gm of sodium thiosulphate crystal was dissolved in 1000ml of distilled water and previously standardized with potassium dichromate (0.1 N).
- Hanus reagent** :
 - 13.2gm of iodine was dissolve in 800ml of glacial acetic acid.
 - 3ml of bromine was added to 200ml of glacial acetic acid.

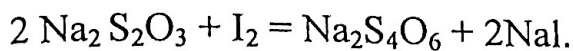
Then (i) and (ii) were mixed and thus reagent was prepared.

- Starch solution (1%)** : A pest of 1 gm of starch in minimum distilled water was prepared in a beaker and distilled water in another beaker was boiled. Then the boiling water was added to the pest and the volume was made up to 100ml in total. It was prepared freshly before use
- Chloroform
- Conc. hydrochloric acid
- Sodium bicarbonate.

PROCEDURE :

The lipid (0.2894 gm) was dissolved in 10ml of chloroform in a wide-necked glass bottle with ground glass stopper. To the content of the bottle, 25ml of

Hanus solution was added and the mixture was allowed to stand in the dark for exactly 30 minutes with occasional shaking. 10ml of potassium iodide solution was added to it and the mixture was shaken well. Then 100ml of distilled water was added to the mixture and the content of the bottle was titrated with standardized sodium thiosulphate solution, using starch as indicator.



A blank experiment (without oil) was performed in the same manner as described above. The iodine value was calculated using the following expression.

$$\text{Iodine value} = \frac{(V_1 - V_2) \times S \times 0.127 \times 100}{W}$$

Where,

V_1 = Volume in ml. of sodium thiosulphate solution required for the blank experiment.

V_2 = Volume in of sodium thiosulphate solution required for the actual experiment.

S = Exact normality of sodium thiosulphate solution used.

W = Weight in gm of the oil taken.

4.2.5.2 Determination of saponification value and saponification equivalent.

Saponification value (Williams, 1966; Shaha, 1994)

DEFINITION :

The saponification value of a fat or oil is the number of mg of potassium hydroxide required to saponify 1 gm of fat, i.e. to neutralize the free fatty acids and the fatty acids combined as glyceride.

The saponification value is related to the molecular weight of the fat or oil and therefore provides information on the mean molecular weight of the combined fatty acids. Saponification value together with iodine value is the most important chemical constant. It gives an estimate of the non-fatty impurities and tells the amount of alkali that would actually required by the fat for its conversion into soap.

APPARATUS REQUIRED :

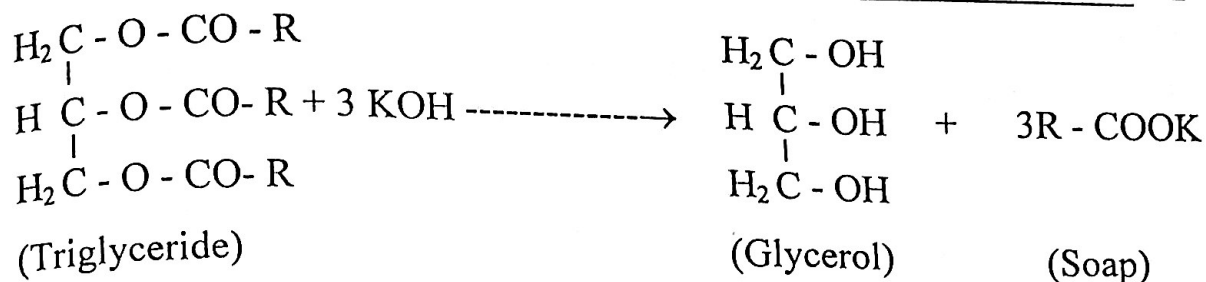
- a) 250ml round-bottomed flasks.
- b) Reflux condensers, to fit the round-bottomed flasks.
- c) 25ml volumetric pipette.
- d) 50ml burette, graduated in 0.1ml.
- e) Heating device.
- f) Boiling chips.

REAGENTS REQUIRED :

- a) **Potassium hydroxide solution (0.5N)** : 28gm of potassium hydroxide was first dissolved in minimum volume of distilled water and alcohol was then added to make one litre solution.
- b) **Phenolphthalein solution (1%)** : 1gm crystalline phenolphthalein in 100ml absolute alcohol.
- c) **Hydrochloric acid (0.5N)** : 0.5N solution was prepared and standardized previously with 0.5N carbonate.

PROCEDURE :

0.5302 gm of the oil was weighed accurately and taken into a 250ml round bottomed flask. 25ml of the ethanolic potassium hydroxide solution and some boiling chips were added to it. The flask was then connected to a reflux condenser and boiled on a water bath for an hour with occasional shaking.



After 60 minutes, the flask was removed from the water bath and the solution of the flask, while still hot, was titrated with 0.4898 N hydrochloric acid solution using phenolphthalein indicator.

A blank test was carried out at the same time under the same conditions with the same quantity of potassium hydroxide solution. The saponification value of the oil was calculated using the following relation;

$$\text{Saponification value} = \frac{(V_1 - V_2) \times N \times 56.1}{W}$$

Where,

V_1 = Number of ml of the standardized hydrochloric acid solution used the blank test.

V_2 = Number of ml of the standardized hydrochloric acid solution used for the test with the lipid.

N = Exact normality of the standardized hydrochloric acid solution used.

W = Weight in grms of oil sample.

SAPONIFICATION EQUIVALENT (Paech *et al.*, 1955)

Saponification equivalent of a fat or lipid is the number of grams of material saponified by one mole of potassium hydroxide.

It was determined using the following formula.

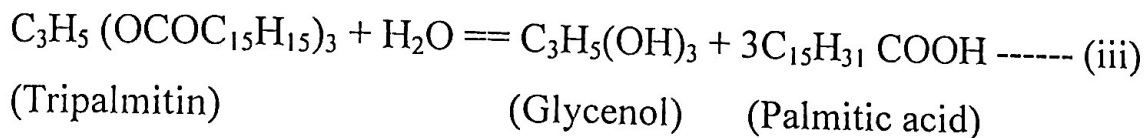
$$\text{Saponification equivalent} = \frac{56100}{\text{Saponification value of the lipid}}$$

4.2.5.3 **Determination of acid value and percentage of free fatty acid (AS OLEIC)** (Williams, 1966; Devine *et al.*, 1961)

DEFINITION :

The acid value of a fat or oil is defined as the milligram of potassium hydroxide required to be neutralize the fatty acid in one gm of the sample, the value may be converted to give the percentage of a free fatty acid such as oleic, palmitic or lauric.

The presence of free fatty acids is normally due to hydrolysis of the glycerides as shown in Eq.(iii). Bacterial action, chemicals, light and heat accelerate this reaction. The hydrolysis reaction, accompanied by the production of shorter chained fatty acids through oxidation of double bonds, can produce a high acid value as in the case of rancidity in the fat or oil.



Acid value of the lipid was determined following the procedure as reported earlier.

APPARATUS REQUIRED :

- 250ml conical flask.
- 25ml burette, graduated in 0.01ml.

REAGENTS REQUIRED :

- a) **Solvent** : Mixture of equal parts of 96% ethanol and di-ethyl ether, which was neutralized immediately before use by titration with 0.1 N sodium hydroxide solution, phenolphthalein being used as indicator.
- b) Potassium hydroxide solution (0.05N) accurately standardized.
- c) **Phenolphthalein indicator** : 1% solution in 96% ethanol.

PROCEDURE :

1.3052gm of lipid was weighed accurately and taken in a 250ml conical flask. The lipid was dissolved in 50ml of the neutral solvent. The solution was titrated, while shaking, with 0.129 (N) sodium hydroxide solution using phenolphthalein as indicator.

The acid value was calculated using the following formula.

$$\text{Acid value} = \frac{56.1 \times N \times V}{W}$$

Where,

N = Exact normality of the standardized sodium hydroxide solution used.

V = Number of ml of the standardized sodium hydroxide solution used for the test with the oil.

W = Weight of sample (lipid) taken in grams.

4.2.5.4 Determination of free fatty acid (Gunstone, 1958)**DEFINITION :**

The free fatty acid of a fat (or oil) is the percentage by weight of fatty acids of a specified molecular weight, according to the type of fat or oil under investigation.

This was the same as for acid value. The percentage of free fatty acid was calculated using the formula.

$$\% \text{ Free fatty acid (as oleic)} = A.V. \times \frac{M}{56.1}$$

Where,

A.V = Acid value.

M = Molecular weight of the oleic acid.

4.2.5.5 Determination of ester value (Williams, 1966)

DEFINITION :

The ester value is the number of mg of potassium hydroxide required to saponify the esters contained in 1g of the oil or lipid.

PROCEDURE :

For the oil to be analyzed, the acid value and the saponification value were determined which had been described before. The ester value was calculated using the formula.

$$\text{Ester value} = \text{Saponification value} - \text{Acid value}$$

4.2.5.6 Determination of the quantity of unsaponifiable matter present in the Lipid (William, 1966)

DEFINITION :

The content of unsaponifiable matter is understood by the percentage (calculated on the original fat or lipid) of unsaponifiable material such as sterols, hydrocarbons etc., naturally present in the fat which remains after saponification of the fat with caustic alkali and which can be extracted by means of a specified solvent and is non-volatile at steam oven temperature (80° - 100°C).

PRINCIPLE :

The lipid or fat is saponified by boiling it with ethanolic potassium hydroxide solution and the resulting soap solution is diluted with water and extracted with ether. The ether solution is washed neutral and the extracted material is recovered and weighed.

APPARATUS REQUIRED :

- a) 250ml round-bottomed flask, with ground joint.
- b) Reflux condenser to fit the flask.
- c) 500ml separating funnels.

- d) 250ml conical flasks.
- e) Desiccator.
- f) Steam bath.
- g) Oven.

REAGENTS REQUIRED :

- a) Diethylether.
- b) Potassium hydroxide, approximately 0.5 N aqueous solution.
- c) Potassium hydroxide, approximately 1.0 N ethanolic solution.
- d) Acetone.
- e) Phenolphthalein indicator.

PROCEDURE :

(3-5gm) lipid was weighed accurately and taken into a 250ml round bottomed flask. 50ml of N ethanolic potassium hydroxide solution was added to it. The flask was attached to the reflux condenser and the mixture was boiled for an hour on a steam bath with occasional shaking to effect saponification. The solution was then transferred to a 500ml separating funnel and the flask was rinsed with 100ml quantities of water and then ether which were added to the contents of the separating funnel. The mixture was shaken well when it was still warmed and allowed to stand to separate the layers. The lower layer was drawn off into the saponification flask and the ether extract was transferred to a second 500ml separating funnel containing 40ml water. In this way two further extracts were carried out each with 100ml ether and the extracts were combined.

The total ether solution was washed vigorously three times with 40ml quantities of water and then three times with similar quantities of potassium hydroxide solution (0.5 N). Finally the solution was washed with 40ml portions of water until the wash water was no longer alkaline towards phenolphthalein.

The ether solution was concentrated on the water bath and transferred to a weighed 250ml conical flask. The residue was dried to constant weight. Drying was assisted by adding 4ml of acetone to the extract. The flask with its content was weighed. The amount of the unsaponifiable matter was calculated as follows.

$$\% \text{ Unsaponifiable matter} = \frac{A \times 100}{B}$$

Where,

A = Weight in grms of unsaponifiable matter.

B = Weight in grms of lipid sample.

4.2.5.7 Determination of acetyl value of the Lipid (Roger Castle Griffin, 1955)

DEFINITION :

The acetyl value is the number of ml of potassium hydroxide required to neutralize the acetic acid resulting the saponification of 1gm of the acetylated lipid or fat. The acetyl value is a measure of the hydroxylated fatty acids in a fixed lipid or fat, and of the free alcohols present in a wax.

APPARATUS REQUIRED :

- a) 100ml round bottomed flask.
- b) Reflux condenser to fit the flask.
- c) 300ml Erlenmeyer flask.
- d) 50ml burette, graduated in 0.1ml.
- e) Heating device (water bath, electric hot plate, heating mantle).
- f) Measuring cylinders (10, 50, 500ml).

REAGENTS REQUIRED :

- a) Acetic anhydride, analytical reagent quality.
- b) Conc. hydrochloric acid.

- c) Sulphuric acid (0.5 N).
- d) Alcoholic potassium hydroxide (0.5 N)
- e) Calcium carbonate
- f) 0.1 N Potassium hydroxide (Standardized).

PROCEDURE :

About 1.5102gm of lipid was taken in an acetylation flask and to this 10ml of freshly distilled acetic anhydride was added. The mixture was boiled for two hours and then taken in a large beaker containing 500ml of water and boiled for half an hour. To prevent bumping, some boiling chips were added to the mixture. The mixture was then cooled and allowed to stand for sometime. When the mixture was separated into two layers, the water layer was removed and the oily layer was boiled with fresh water until it is no longer acidic to litmus. The acetylated fat thus obtained was separated from water and dried at 110°C.

About 0.91129gm of the acetylated fat was taken into a 300ml Erlenmeyer flask and saponified with 30ml of alcoholic potassium hydroxide solution. The alcohol was evaporated after saponification and the soap was dissolved in water followed by the addition of about 30ml of sulphuric acid (0.5 N approx.). The mixture was warmed gently. The free fatty acid which was collected at the top, was separated and washed with boiling water until the washings were no longer acidic. The filtrate was titrated with 0.5N potassium hydroxide solution using phenolphthalein as indicator.

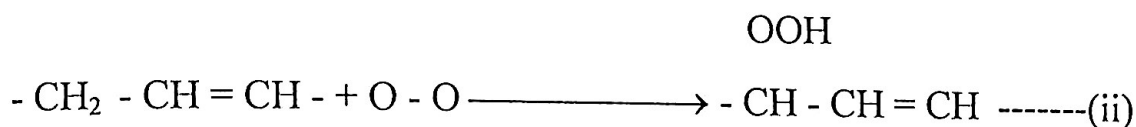
$$\text{Acetyl value} = \frac{\text{No. of ml. of alkali} \times 5.61}{\text{Wt. of the acetylated lipid}}$$

4.2.5.8 Determination of per oxide value (Jacobs, 1958)

DEFINITION :

The peroxide value is the determination of amount of iodine liberated from potassium iodide by the peroxides of the oil as fats in term of milliequivalent per kilogram.

Fixed oils and fats absorb oxygen from the air in the auto oxidation of the double bond present in the components fatty acids. The reaction is thought to be as shown in Eq. (ii), where the oxygen adds to the double bonds forming unstable hydroperoxides



PRINCIPLE :

The sample is treated with potassium iodide and the iodine which is liberated by the peroxides is titrated with sodium thiosulphate solution.

APPARATUS REQUIRED :

- a) 250ml conical flask with ground-stoppers.
- b) 25ml burette, graduated in 0.01ml.

REAGENTS REQUIRED :

- a) Solvent : Mixed 2 vol. of glacial acetic acid and 1 vol. of chloroform.
- b) Potassium iodide, saturated aqueous solution, freshly prepared, freed from iodine and iodates.
- c) Sodium thiosulphate, 0.01 N accurately standardized aqueous solution, standardized just before use.
- d) Starch indicators : Starch (1gm) was dissolved in 50ml hot water and diluted to 100 ml with cold water.

PROCEDURE :

0.3594gm of the lipid was weighed accurately and taken into a 250ml conical flask. 25ml of the solvent was added to it. 1ml of potassium iodide solution was then added, the flask was stoppered and allowed to stand for 1 minute. 35ml of water was added and the solution was titrated with 0.0102 N sodium thiosulphate solution using starch solution as indicator.

A blank titration was carried out under similar conditions except lipid. The peroxide value was determined by using the following formula.

$$\text{Peroxide value} = \frac{1000 \times N \times (V_2 - V_1)}{W}$$

Where,

W = Weight (in gram) of sample.

V_2 = Volume (in milliliters) of sodium thiosulphate solution used in test.

V_1 = Volume (in milliliters) of sodium thiosulphate solution used in blank test.

N = Normality of sodium thiosulphate solution.

4.2.5.9 Determination of reichert - meissel and polenske value of the Lipid Sample (Williams, 1966)

REICHERT-MEISSEL VALUE : This is the number of ml. of 0.1 N aqueous alkali required to neutralize the water soluble but volatile acids obtained from 5 gms of the lipid.

The Reichert-Meissel value was determined by using the following formula-

$$\text{R.M. value} = (A-B) \times 1.10 \times \text{strength of alkali}$$

Where,

A = Number of ml. of aqueous alkali required for the actual experiment.

B = Number of ml. of aqueous alkali required for the blank experiment.

POLENSKE VALUE : It is number of 0.1N aqueous alkali required to neutralise the water insoluble, volatile acids obtained from 5 gms of the lipid.

Polenske value was determined by using the following formula :

$P \text{ value} = (C-D) \times \text{strength of the aqueous alkali.}$

Where,

C = number of ml. of aqueous alkali required for the actual experiment.

D = Number of ml. of aqueous alkali required for the blank experiment.

REAGENTS :

- a) Glycerine.
- b) Sodium hydroxide solution (50% by weight).
- c) Diluted sulphuric acid solution 40ml = 20 ml of the sodium hydroxide solution.
- d) Pumice stone
- e) 0.1 N Barium hydroxide solution (accurately standardized).
- f) Indicator one percent aqueous solution of phenolphthalein

PROCEDURE :

The lipid (5gm) was taken into a polenske flask and 20gms of glycerine and 2ml of 50% sodium hydroxide solution were added. The flask was heated over naked flame with continuous mixing until the oil was saponified and the liquid became perfectly clear. Care was taken to avoid over heating during the saponification. The flask was then covered with watch glass. After allowing the solution in the flask to cool sufficient, 93 ml of boiling distilled water was added to it.

About 0.1gm of pumice powder, followed by 50ml of dilute sulfuric acid solution was then added and the flask was immediately connected to the still head. The flask was heated without boiling its content until the insoluble acids were completely melted and then more strongly such that 110ml. were distilled in 20 ± 1 minutes. The flow of water in the condenser was kept at such a speed that the temperature of the issuing distillate was between $88-90^{\circ}\text{C}$. After collecting 110 ml of the distillate, the flask was removed and the 110 ml. flask was replaced by a cylinder of 25 ml capacity to catch the draining. The 110 ml. flask was immersed upto 110 ml mark in water at 15°C for 10 minutes. The flask was then removed from water and its outside was made dry. The contents of the flask were mixed by four or five double inversion and were filtered through a No. 4 Whatman filter paper. The first running were rejected and were 100 ml. of the filtrates was collected in a dry flask for the determination of Reichert-Meissel value (R) as follows :

The still head was detached from the condenser and the condenser was washed with three successive 15 ml portions of cold distilled water passing each washing separately through the cylinder, the 110 ml. flask and the filter draining each washing before filtering the next. The washing was discarded. The insoluble acid was dissolved by three similar washing of the condenser, the cylinder and the filter with 15 ml. portions of neutralized alcohol, collecting the solution in 110 ml flask and draining the alcohol after each washing. The flask was stoppered and the solution was retained for the determination of Polenske value (P) as below :

The blank experiment (without the lipid but using the same quantities of the reagent) was performed following the same procedure as described above.

4.2.6 Determination of fatty acid composition present in the *A. morar* Lipid sample.

Gas-Liquid chromatographic examination of the methyl ester mixture of Lipid sample (Kansella, Shimp, Mai and Weithrauch, 1977)

Fatty acid composition of the *A. morar* lipid was analysed by 4500 U-PYEUNICAM, GAS CHROMATOGRAM, following the procedure described below

Column : Quartz capillary column B.P-70, internal diameter 2 mm, length 1.5 meter.

A : PREPARATION OF FATTY ACID MIXTURE OF THE LIPID

500 mg of the lipid sample was taken in a 125ml. conical flask. To it 5ml of 0.5 N NaOH in MeOH solution was added. The mixture was then shaken well and was refluxed for 30 minutes in a boiling water bath at 95°C. The mixture was evaporated to remove methanol. 5ml. of water was added to it and then H₂SO₄ was added dropwise upto pH = 2.5. 10ml of hexane was added to the mixture. The hexane part was taken in a conical flask with the help of a pipette. The hexane part was then dried over anhydrous Na₂SO₄, filtered and the solvent was evaporated to obtain the saponified material (FFA) (Clark Jr., 1964).

B : PREPARATION OF METHYL ESTERS FROM THE FATTY ACID MIXTURE.

2ml. of benzoic acid in methanol solution (methyl benzoate was used a standard) was added to the saponified material (FFA), solvent was evaporated and the residue was then treated with 2 ml. BF₃ - MeOH complex. The mixture was heated for 5-10 minutes in a boiling water bath. Then it was evaporated to dryness. To it hexane was added and hexane part was taken in a small vial for GLC.

C : PREPARATION OF METHYL ESTER OF STANDARD FATTY ACIDS.

Methyl esters of standard fatty acid were also prepared by the same procedure as described above.

D : NAME OF STANDARD FATTY ACID SETTING ON THE STANDARD GRAPH.

- | | | |
|----------------------|-----------------------|--------------------|
| (i) Capraylic acid | (ii) Nonanoic acid | (iii) Capric acid |
| (iv) Undecanoic acid | (v) Lauric acid | (vi) Myristic acid |
| (vii) Palmitic acid | (viii) Stearic acid | (ix) Oleic acid |
| (x) Linolenic acid | (xi) Arachidonic acid | |

E : PREPARATION OF STANDARD CHROMATOGRAM.

One μ l. of the standard methyl esters mixture ($5\mu\text{g}/100\mu\text{l}$) was injected to the injection port. The injector and detector temperature was maintained at 225°C and 230°C respectively and of column was raised from 180°C to 220°C ($2^{\circ}/\text{min}$). The flow rate of carrier gases nitrogen, oxygen and hydrogen was $12\text{ml}/\text{min}$; $135\text{ml}/\text{min}$. and $13\text{ml}/\text{min}$. repectively. A standard chromatogram was obtained after 40 minutes of the sample injection.

F : CHROMATOGRAM OF THE OIL SAMPLE.

Chromatogram of the lipid sample was run by the same procedure as described above.

G : IDENTIFICATION OF FATTY ACIDS.

Fatty acids present in the lipid sample were indentified by comparing the relative retention times and peak positions of the sample chromatogram, with those of standard fatty acids chromatogram. The following fatty acids appeared to be present in both male and female sample.

- | | |
|--|---|
| (i) Lauric acid ($\text{C}_{12} : \text{O}$) | (ii) Myristic acid ($\text{C}_{14} : \text{O}$) |
| (iii) Palmitic acid ($\text{C}_{16} : \text{O}$) | (iv) Stearic acid ($\text{C}_{18} : \text{O}$) |
| (v) Oleic acid ($\text{C}_{18} : \text{O}$) | (vi) Linoleic acid ($\text{C}_{18} : 2$) |
| (vii) Arachidonic acid ($\text{C}_{20} : 4$) | |

H: QUANTIFICATION OF FATTY ACIDS.

Amount of fatty acids present in the lipid sample was proportional to areas under the peaks. So area under the peak representing each fatty acid was calculated. The area under each peak was divided by total area under all peaks to obtain the proportion of each fatty acid. The area under each peak can be obtained by an integrator or by using the following formula.

$$\text{Area} = \frac{1}{2} (H \times W)$$

Where,

H = Height of a peak measured from the base-line of the chromatogram.

W = Width of the peak at half of the height of the peak.

4.3 MICROBIOLOGICAL INVESTIGATION OF THE CHLOROFORM EXTRACT OF THE LIPID OF *A. MORAR*

4.3.1 Introduction :

The microbiological investigation was done to determine the susceptibility of some pathogenic bacteria to chloroform extract of the lipid of *A. morar* gonionotus. The importance of fishes in human civilization is very considerable. Human being and other living organisms are to depend, to a very great extent, on the fish kingdom.

The fight between man and diseases started in million years ago. The study of diseases and their treatment must also have been contemporaneous with the dawn of human intellect. Clearly the first antibiotics used by man were those found amongst the metabolic products of the fishes. Their most successful application appears to have been as preservatives and antiseptics use which continues today.

In the humid tropic land of Bangladesh diarrhoea, cholera, typhoid, malaria, dyptheria etc. are major cause of morbidity and mortality. But diarrhoea is one of killer diseases particularly in children reigning during the last quarter of the 20th century. In developing countries it may be caused by some 25 known pathogens (bacterial, viral and protozoal), The studies conducted in Bangladesh and else where in the region suggest that the major cause of diarrhoea are enterotoxigenic *E. coli* (TEEC). rotavirus *Shigella* and protozoal inceptions Balck, R.E. *et al.*, 1980; Deh, M. *et al.*, 1983; Sen, D. Shaha, 1985). Every year diarrhoeal disease claim 5 to 10 million lives of children out of 3 to 5 billion incidence in Asia, Africa and Latin America.

Diarrhoea in a major killer of children in Bangladesh claiming more than 2000,000 deaths annually. Among the all diarrhoeal diseases Shigellosis is predominant. In Bangladesh *Shigella* species are the main causative agent for *Bacillary dysentery* (80 to 85%) Balck R.E. *et al.*, 1980; Haq.T. *et al.*, 1977). In 1970 only 0.6% of all diarrhoeal causes were associated with *Shigella*. This rate in creased gradually (9% in 1972, 14% in 1973) to reach a peak 20% in 1974. During the early 1980's *Shigella* incidences among the diarrhoeal patients was about 10% and mortality among these incidences was 17% Sen, D.

Generally four strains of *Shigella* are responsible for causing *Bacillary dysentery* Smith, *et al.* *Shigella* car therefore be classified in to four principal subgroups according to their biochemical reaction.

- i) *Shigella dysentery*
- ii) *Shigella Flexneri*
- iii) *Shigella boydii.*
- iv) *Shigella Sonnei.*

Shigellosis has a global distribution with highest incidence in countries having poor hygiene. In Bangladesh infection caused by *Shigella flexneri* is most common (Ahmed, Z.U, 1989 and Hqu, M.I. Ahmed, Q.S. and Rahaman, M.M, 1983) infection is acquired by faecal oral route and mostly spread by produce a full blown clinical attack (Mata, I., 1983). Shigellosis is increasingly recognized as a major diarrhoeal disease.

In 1945 sulfonamide drugs were introduced as drugs of choice against *Shigella spp.* But just after five years 80 to 90% clinical isolates of *Shigella* became resistance to sulfonamide drugs. In the early 1950's streptomycin, tetracycline and chloramphenicol were introduced as drugs of choice against *Shigella* but after 20 years it is found that multiresistant strains prevailed and their incidence was 90%.

Shigella strains were sensitive to ampicillin and nalidixic acid (Hqu, *et al.*, 1983; Macaden, R., 1980). But resistance sensitive to these drugs have also been reported (Macaden, R. 1980; Bambury R. *et al.*, 1979). Consequently the rural area of Bangladesh with poor hygiene is now facing a serious problem due to emergence of these multiply drug resistance strains. These strains have been causing out break of Shigellosis in recent years in Bangladesh and in other developing countries (Nouri, Y. Mari, 1967). Hence the development of antimicrobial drugs that will be active against multiple drug resistance strains of *Shigella* is a pressing need of the hour.

There is little doubt that most of the developing world will lack safe water supplies and sanitation for decades to come. For this reason Shigellosis will continue to be a major health problem. Effective inexpensive drugs and technologies must be developed to reduce the scourge of this disease in order to

improve nutritional status of millions of children and to avoid the mortality among them.

It has been noted that many types of fishes and fish products have to alleviate the sufferings of the body and mind from time immemorial. So, a desperate need to search out new antimicrobial agents from fishes to relief this killer disease in perspective of Bangladesh.

The purpose of my work was to represent selected works involving careful study of this fish lipid in order to guide those who are interested in taking up challenge that these substances represent. It has been expected that the present antibacterial activity in fish will lead to the scientists that continued work may have clinical success concerning the killer diseases.

For this purpose "Dise diffusion method" (Bauer *et al.*, 1966) was adopted. This method, and in *vitro* investigation of susceptibility of microorganisms to any test materials, is widely acceptable for preliminary screening of substances, which are supposed to possess antimicrobial properties. It is essentially a quantitative or semiquantitative test which indicates the sensitivity or resistance of microorganisms to the materials. However, this method is quite unable to distinguish between bacteriostatic and bactericidal agent.

4.3.2. Principle

Dried filter paper discs impregnated separately with the test materials were placed gently on nutrient agar plates inoculated with the test organism. The spatial arrangement of the discs were such that they were not closer than 15 mm to the edge of the plate and far enough from each other to prevent overlapping of the zones of inhibition. These plates were then kept in a refrigerator at low temperature (4°C) for 24 hours to allow maximum diffusion. During this period

dried discs absorb water from the media. The diffusion occurs according to the law that controls the diffusion of molecules through an agar gel (Berry, 1976). As a result there is a gradual change of test materials concentration in the media surrounding each disc. The plates were then incubated at 37°C for 12 to 18 hours to allow growth of the organisms. (If there is any antimicrobial activity of test materials it will inhibit the growth of microorganisms and a clear zone of inhibition will be visualized surrounding the discs. The antimicrobial activity of the test materials is determined by measuring the diameter of zone of inhibition).

4.3.3 Material for determination of antibacterial activity.

4.3.3.1 Test organism used

To determine the antibacterial activity, the growing bacterial strains shown in table 6.1 were used. the strains were collected from the department of pharmacy, University of Rajshahi.

Table 6.1. List of the organisms (bacteria) used for determining antibacterial activity.

Gram-positive

Sarcina lutea

Bacillus cerus

Staphylococcus aureas

Bacillus megaterium

Streptococcus-β-haemolyticus

Bacilus subtilis

Gram-negative

Shigella dysentestiac

Escherichia coli

Shigella boydii

Shigella shiga

Shigellasonner

Shigella flexneriae

Salmonella tuiphi-B-56

Salmonella typhu-A

Salmonella ltyphis-B

Klebsiella specdies

Pseudomonas aeruginosa

4.3.4 Determination of antibacterial activity

(Nutrient agar media was used as growth media for the lipid of *A. morar*)

4.3.4.1 Composition of medium (for 1000 ml)

<u>Ingredient</u>	<u>Amount</u>
Bacto peptone	5.0 gm
Sodium chloride	5.0 gm
Bacto yeast extract	10.0 gm
Bacto agar	20.0 gm
Distilled water q.s to	

pH : 7.2 ± 2 at 25°C

4.3.4.2 Preparation of nutrient agar medium.

The general procedure for preparing nutrient agar medium is given below :

PROCEDURE :

To prepare the required volume of the medium, the amount of each of the constituents was calculated from the composition that given for 1000ml. Peptone, sodium chloride and yeast extract of the required calculated amount were taken in a conical flask after weighing separately. Demineralized water (volume was less than the required final volume) was added and the contents were heated in a water bath to make a clear solution. The pH was adjusted at $7.2 + 0.2$ using NaOH or HCl. The required amount of agar was added to the solution and the Demineralized water was added to make the final volume. Again the total volume was heated in a water bath to obtain a clear solution. The conical flask was plugged with cotton and then sterilized in an autoclave at 15 lbs pressure for minutes at 121°C .

4.3.4.3 Preparation of Inoculum

The test organisms were transferred to the agar slants from the supplied pure cultures with the help of an inoculation loop in aseptic condition. For the growth of the test organisms the inoculated slants were incubated at 37° for 24 hours. These fresh cultures were used for sensitivity test within 2 to 3 days.

4.3.4.4 Inoculation

A suspension of the test organism was prepared in nutrient agar medium so that it provided a uniform distribution of the organism in the agar medium. The suspension was then mixed thoroughly and immediately transferred to the sterile petridishes in an aseptic condition. Petridishes were gently rotated first clockwise and then anticlockwise so that a homogeneous distribution of the test organism was obtained. The medium was poured into the petridishes on a level horizontal surface to give uniform depth of approximately 4 mm after the medium had been allowed to solidify. Just before use, the droplets of moisture on the surface of the petridishes were removed carefully.

4.3.4.5 Preparation of Discs

Two types of discs were used for determination of antibacterial activity.

Sample discs

The chloroform extract of the lipid of *A. morar* was taken for the preparation of sample discs.

Standard antibiotic discs :

The standard antibiotic discs were collected from ICDDR'B. The standard antibiotic discs of amoxicillin (10 µg/disc) were used.

4.3.4.6 Placement of the Discs, Diffusion and Incubation

The sample discs and the standard antibiotic discs were placed gently on the medium inoculated with the test organism with the help of sterile needle to ensure complete contact with the surface of the medium.

The plates were inverted and kept in a refrigerator for 24 hrs to ensure the fusion of the test material from the impregnated disc to its surrounding. Filter paper discs were taken in a cotton plugged test tube and was sterilized in autoclave at 121°C and at 15 lbs/inch² pressure for twenty minutes. Each of the filter paper disc was held up with a sterile forceps and then the chloroform extract of lipid was applied on it with a micropipette of 10 micro-liter capacity. Finally they were incubated at 37°C for 12 to 18 hrs.

4.3.4.7 Measurement Of The Zones Of Inhibition

The antibacterial activities of the lipid of chloroform extract was determined by measuring the diameter of the inhibitors zone in m.m by a transparent scale.

Procedure :

After 12 to 18 hours of incubation each of the plates were examined. The diameter of the zones of inhibition including the diameter of the discs were measure by the naked eyes using a scale in terms of millimeter. The diameter of the zones of inhibition of the sample was then compared with the diameter of the zones of inhibition produced by the standard antibiotic discs used.

4.4 RESULTS AND DISCUSSION

The seasonal variation of lipid, protein and related substances in body muscle of *A. morar* fish are recorded in twelve months. Fatty acid composition of lipid of *A. morar* (male and female species) have been determined.

The same habitat, having uniform size (100mm-130mm in total length) have been collected and processed in identical condition to detect the seasonal variation in lipid, protein and related substances in body muscle of *A. morar*. These components varied seasonally in relation to reproductive cycle. In general, body constituents of fish seems to depend on season and diet. Starvation alters body constituents. Lipid and protein are used as a source of energy. Love (1974) indicated that the availability of food at different time of the year have a considerable effect on the tissue component, particularly fat. The above discussion calorie value must be varied seasonally.

The seasonal variation of lipid, protein and related substances in body muscle of *A. morar* was investigated (Table 16, Fig. 17, 18).

Data presented in this thesis indicated that protein content gradually decreases to reach its minimum in April, shortly after the spawning peak (Fig. 17, 18)

This is in agreement with Love (1974) who reported that amino acid related to depletion at the spawning time to show the selection of material for building up the gonads. The maximum protein contents 16.05% and 15.88% for male and female respectively were observed during the resting stage of gonads at prolific feeding period in July. Lower values synchronised with the post-spawning, spawning and early maturation phases in the case of protein. The percentage of lipid follows the similar pattern of fluctuation to that of protein.

Table 16: Seasonal variation in the chemical composition of *A morar*

Month	Year	Male				Female			
		Lipid	Protein	Moisture	Ash	Lipid	Protein	Moisture	Ash
January	1996	8.67	14.03	72.12	3.18	8.75	13.95	72.65	3.11
February	"	8.42	13.51	73.01	3.15	8.32	13.32	73.12	3.32
March	"	8.20	13.02	73.34	3.56	8.11	12.99	73.31	3.61
april	"	7.25	13.22	73.12	3.95	7.21	13.11	73.41	3.32
May	"	7.98	15.22	72.64	4.02	7.88	15.08	72.61	3.51
June	1995	9.07	15.75	71.31	3.85	7.02	15.45	71.32	3.21
July	"	9.85	16.05	70.11	3.52	9.75	15.88	70.76	3.33
August	"	9.42	15.82	70.46	3.55	9.32	15.42	70.91	3.41
September	"	9.35	15.21	70.81	3.65	9.29	15.09	71.12	3.13
October	"	9.21	14.02	72.11	3.45	9.19	13.88	71.98	3.41
November	"	8.85	13.82	72.85	3.33	8.73	13.75	72.12	3.51
December	"	8.73	13.73	72.90	3.25	8.65	13.65	72.21	3.55

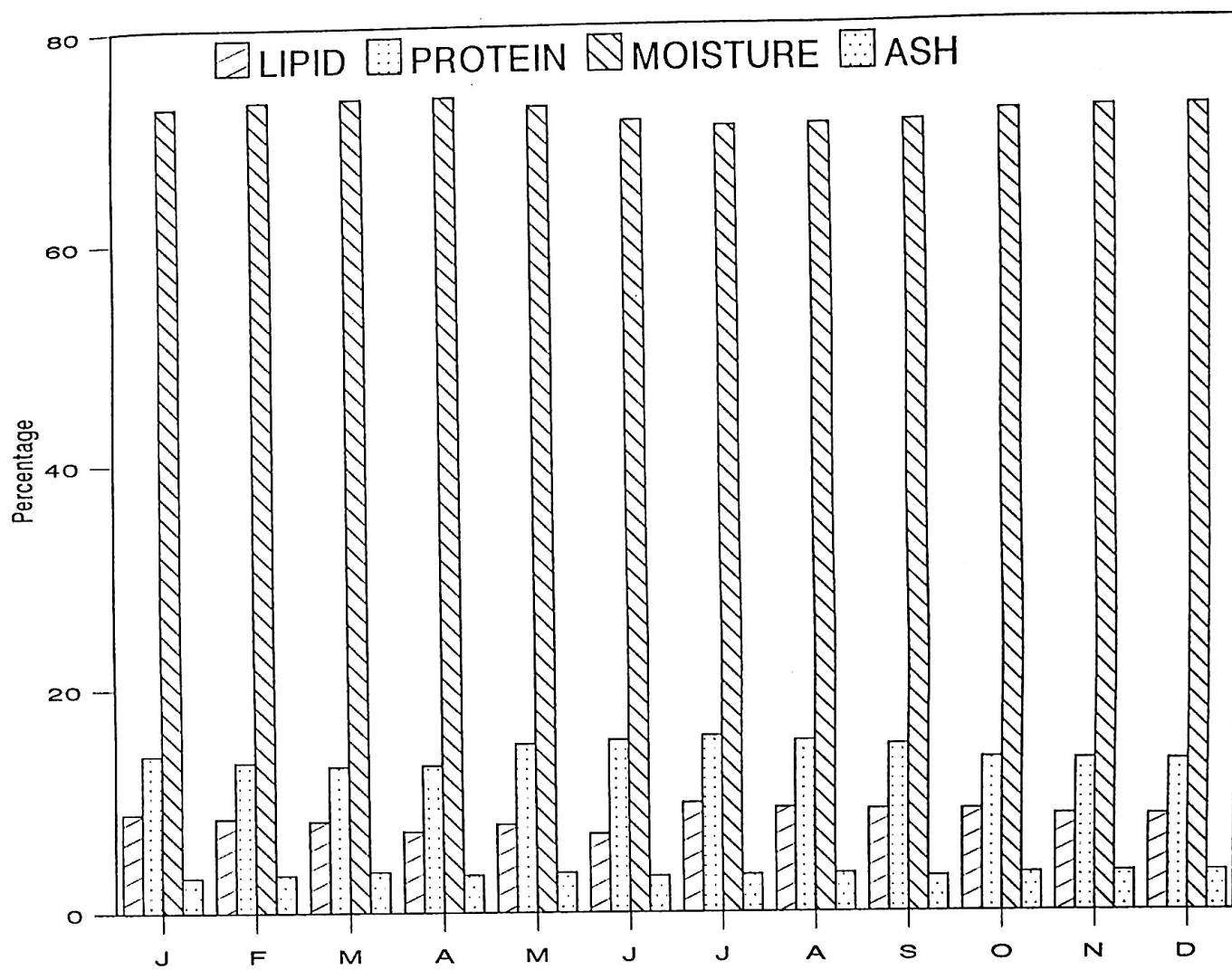


Fig.16. *A. morar*: Monthly variation in the chemical composition of female.

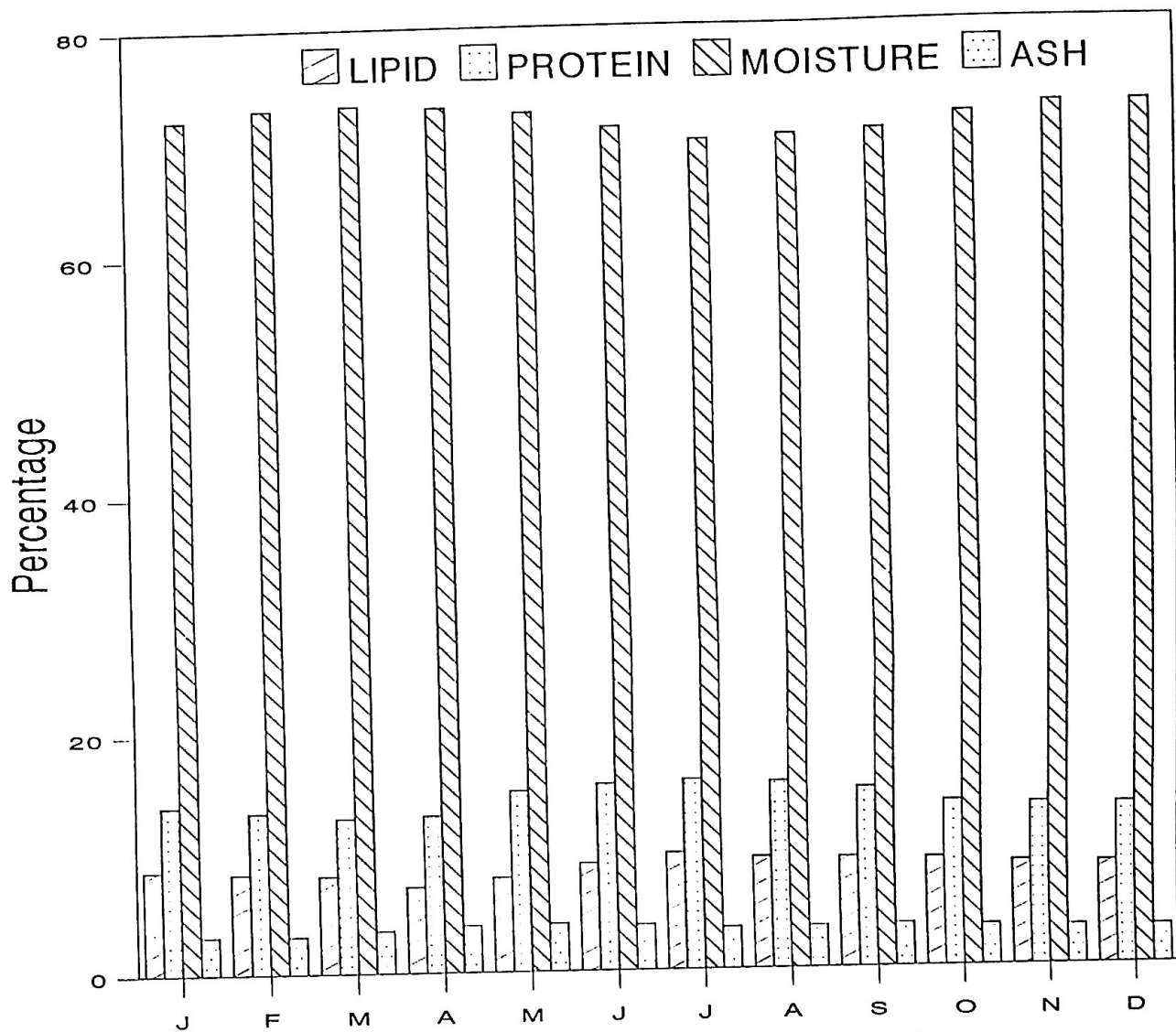


Fig.17.A. *A. morar*: Monthly variation in the chemical composition of male.

The major component of fish fillets was moisture. The seasonal variation in moisture of the fillet were opposite to these of protein, and lipid.

There exist an inverse relationship between the seasonal variation in moisture of the fillet to those of protein and lipid.

4.4.1 Physical and chemical constants of the fish lipid from *A. morar* :

Christie, W.W. (1976)

A number of physical and chemical characters were employed to determine the nature and sometimes for the identification of fats and oils. These constants may also help to evaluate the suitability of a given oil or fat for a given purpose. Although the chemical constants are more important to characterize a lipid but the physical constants are also often capable of expression valuable informations. Some of the more important chemical constants are iodine value, saponification value and saponification equivalent, acid value, percentage of free fatty acid, percentage of unsaponifiable matter, peroxide value, acetyl value, Reichert-Meissel value etc. where as refractive index, specific gravity are the important physical constants. The physical and chemical constants of the lipid reported elsewhere and found in this investigations are represented in Table (17, 18) where these values are compared with those of some related fish lipids and oils.

4.4.1.1 Specific gravity :

The specific gravity, practically of all fats or oils lies between 0.90 and 0.95 (M. Kalumuddin, 4th ed.). As shown in the Table (17), the specific gravity, of the lipid of *A. morar* was found to be 0.913 at 30°C. The value obtained in the present studies have a close similarity to that of olive oil (0.915-0.919) (K. Pearch and M.V. Tracey, 1955).

Table 17: Physical constants of fish lipids of *A morar* and other oils and fats.

Name of the sample	Refractive index	Specific gravity
Fish lipid of <i>A morar</i>	1.462 (30°C)	0.913 (30°C)
<i>Anguilla bengalensis Eel</i> (Molla, A.H. 1991)	1.46 (30°C)	0.9138 (27°C)
<i>Puntius gonionotus</i> (Prodip <i>et al.</i> , 1995)	1.473 (30°C)	0.932 (30°C)
<i>Eutropiichthys vacha</i> (Hasan, <i>et al.</i> , 1996)	1.46 (30°C)	0.9-0.95
<i>Cassioia fustula</i> (Ali, M.A., 1995)	1.47-1.45	0.926 (35°C)
Soyabean oil (Lange)	1.46-1.47	0.915-0.919
Linseed oil (Williams, K.A., 1966)	1.46-1.47	0.924-0.926
Sunflower (Lange)	1.466-1.467	0.924-0.927
Olive oil (Tracey M.V. <i>et al.</i> , 1955)	1.4743	0.915-0.919
Cotton-seed oil (Jacobs, M.B., 1962)	1.479-1.48	0.920.925
Linseed oil (Williams, K.A., 1966)	1.503 (30°C)	0.931-0.938
Debdaru oil (Islam, M.A., 1995)	1.4645 (30°C)	0.9407 (30°C)

4.4.1.2 Refractive index :

The refractive index of fats and oils depends on some extents of their unsaturation (K. Pearch *et al.*, 1955) and the higher unsaponification is the greater refractive index. The refractive index of the fish lipid of *A. morar* was found to be 1.4621 at 30°C which is similar to those for oliveoil (1.4657-1.4667) K. Pearch *et al.*, 1955) and *Eutropiichthys vacha* was 1.4623 at 30°C. The present result indicated that the fish lipids contained fairly large amounts of unsaturated fatty acids.

4.4.2 Chemical constants :

4.4.2.1 Saponification and saponification equivalent :

The saponification value and the saponification equivalent of the lipid of *A. morar* were found to be 210.621 and 224.96 respectively. The saponification value has a close similarity to that of butter fat (210-230) O>P> Agarwal (1996-97) and *Eutropiichthys vacha* M.F. Hasain (1997). The saponification value is inversely proportional to the average molecular weight or chain length of the fatty acids present in the fat or oil. It may be concluded from the results cited above that the lipid under examination contains higher amount of lower fatty acids.

4.4.2.2 Iodine Value :

The iodine value of the lipid of *A. morar* was found to be 95.03 which is very close to that of *Eutropiichthys vacha* (96.25) and rape seed oil (94-100) R.K. Das, 1989). The iodine value indicates the degree of unsaturation of the oil or fat. The result discussed above indicates that the lipid under examination contains unsaturated fatty acids in greater extent.

4.4.2.3 Acid value and percentage of free fatty acid :

Acid value is a measure of the free fatty acid present in the oils or fats and differs from the determination of the "free fatty acid" only in the interpretation and

number of expression. The assumption usually being made in a calculation that the acids have a molecular weight equal to that of oleic acid. As the liberation of free fatty acids in a natural fat or oil sample due to hydrolysis by lipase may be an important contributory factor for rancidity. A high acid value may indicate a higher tendency to become rancid. A low percentage of free fatty acid (below 1.15%) is an indication of suitability of the lipids for edible purpose (K.K. Carroll *et al.*, 1957).

The acid value and percentage of free fatty acid of the fish lipid of *A. morar* were determined and found to be 1.78 and 0.89% respectively. These values of the lipid under examination implies that the lipid may be used as edible purpose.

4.4.2.4 Unsaponifiable matters

The unsaponifiable matter in the lipid of *A. morar* was calculated and found to be 0.520%. Similar results were also reported for Katio seed oil (Table 18). So the lipid of *A. morar* contained a small amount of sterols, tocopherol, vitamin A and D, hydrocarbons etc.

4.4.2.5 Peroxide value :

Peroxide value is a measure of content of reactive oxygen, in terms of moles of peroxide or mill-equivalents of oxygen per 1000 gm of oil. The peroxide value of *A. morar* fish lipid was found to be 2.92.

4.4.2.6 Reichert-meissel and poleske values :

The Reichert-Meissel value of lipids of *A. morar* was found to be 0.82 which was very close to that of rape seed oil (P.K. Das, 1989)

The Polenske value of the lipids of *A. morar* as determined was found to be 0.560, the low R-M value as obtained which indicates the low content of lower volatile fatty acids of the lipid.

4.4.2.7 Acetyl value :

The acetyl value was determined and found to be 15.23 which fell with the range of rape seed oil (10-40) (K.K. Carrol and R.L. Noble (1957).

4.4.2.8 Free fatty acid composition :

Analysis of fatty acids in the lipid was done in A PYE UNICAM IU 4500, Phillips gas-liquid chromatography using a flame ionization detector and a chart recorder. The fatty acids composition in the lipids from male and female has been shown in Table-19. It is evident from the tables that the lipid under investigation contained mainly unsaturated fatty acids while saturated fatty acids were also found to be present in it. Investigators have reported that these saturated fatty acids are found in the most dietary fish fats and oils. It appears from the literature that unsaturated fatty acids (arachidonic and, w-6), which are abundant in marine oils appeared to be the more effective forms of polyunsaturated fatty acid, involving in the transport of cholesterol from blood and related cardiovascular tissues.

The chromatogram shown in fig 19 & 20 revealed the presence of Lauric acid ($C_{12}O$), Myristic acid ($C_{14}O$), Palmitic acid ($C_{16}O$), Stearic acid ($C_{18}O$), Oleic acid ($C_{18}O$), Linoleic acid ($C_{18}O$) and Arachidonic acid ($C_{20}O$) in the lipid. The foregoing evidence indicates that the lipid under investigation is reasonably suitable for human consumption.

4.4.3 Antimicrobial activity :

Antimicrobial activity of the lipid was carried on different pathogenic microorganisms. Like other common dietary fish lipid, the lipid from the fish *A. morar* has no antimicrobial activity.

Table 18 : Chemical constants of the lipids of *A. morar* and other related oils and fats.

Name of the sample	S.V	S.E.V	I.V	P.O.V	A.V	F.F.A (%) as aleic	U.S.M (%)	R.M.V.	P.V.
Fish lipis of <i>A. morar</i>	210.621	224.96	95.03	2.92	1.78	0.89	0.52	0.82	0.56
<i>Anguilla bengalensis</i> Eel (Molla, A.H., 1991)	177-189	297-313	90-102	7.45	3.68	2.5-3.5	8.37	-	-
<i>Puntius gonionotus</i> (Dutta <i>et al.</i> , 1995)	197-052	284.69	96.38	1.78	1.93	0.97	0.689	0.934	0.696
<i>Eutropiichthys vacha</i> (Hasan, <i>et al.</i> , 1996)	216-629	258.968	96.25	1.85	1.992	1.001	0.645	0.924	0.725
<i>Cussia fistula</i> seed oil (Ali, M.A., 1995)	186.75	300.69	95.03	2.55	2.77	1.39	5.50	0.80	-
Katio seed oil (Williams, K.A., 1966)	192.0	-	64	-	1.4	0.50	-	-	-
Rape Seed Oil (Das, R.K., 1989)	175	-	94-100	4	-	-	0.80	-	-
Castor oil (Das, R.K., 1989)	180	-	85	0.7	-	-	-	-	-
Sesame oil (Das, R.K., 1989)	190	-	110	8	-	-	0.35	-	-
Coffee oil (Williams, K.A., 1966)	180-192	-	89-101	-	-	5.5-6.5	-	-	-
Grape oil (Williams, K.A., 1966)	178-190	-	94-143	-	0.08	-	-	-	-

S.V. = Saponification value

U.S.M. = % of unsaponifiable matter

P.O.V = Peroxide value

F.F.A. = % of free fatty acid

I.V. = Iodine value

P.V. = Polenske value

S.E.V. = Saponification euivalent value

R.M.V. = Reichert-Messel value

A.V. = Acid value.

Table 19 : Fish lipids of *A. morar* and approximate composition of some oils and fats (in %).

Name of fats	Oleic acid	Linoleic acid	L quic acid	Stearic acid	Myristic acid	Palmitic acid	Acachidic acid	Arachidonic acid
Fish lipid of <i>A. morar</i> (male)	42.35	33.41	0.9937	4.42	2.013	9.3608	-	3.7428
<i>A. morar</i> (female)	41.70	32.19	0.97	3.84	3.65	13.41	-	2.10
<i>Eutropiichthys vacha</i> Hasan <i>et al.</i> , 1996)	25.476	20.936	-	0.757	4.096	48.337	0.3978	-
<i>Puntius gonionotus</i> (Dutta <i>et al.</i> , 1995)	59.68	17.58	-	3.92	-	16.79	-	1.42
Butter (Agarwal, O.P., 1969-97)	27.4	-	-	11.4	22.6	22.6	-	-
Olive oil (Agarwal, O.P., 1969-97)	84.4	4.6	-	2.3	trace	6.9	0.1	-
Cotton-seed oil (Agarwal, O.P., 1969-97)	33.2	39.4	-	1.9	0.3	19.1	0.6	-
Soyabean oil (Agarwal, O.P., 1969-97)	32.2	49.3	-	4.2	-	6.5	0.7	-
Coconut oil (Agarwal, O.P., 1969-97)	5.0	1.0	-	3.0	18.5	7.5	-	-
Castor oil (Agarwal, O.P., 1969-97)	9.0	3.0	-	3.0	-	-	-	-
Palm oil (Agarwal, O.P., 1969-97)	38.4	10.7	-	4.2	1.1	41.1	-	-
Corn oil (Agarwal, O.P., 1969-97)	43.4	39.1	-	3.3	-	7.3	0.4	-
Linseed oil (Agarwal, O.P., 1969-97)	5	48.5	-	-	-	-	-	-
<i>Cassia fistula</i> seed oil (Ali, M.A., 1995)	29.62	42.418	-	14.33	1.44	11.409	-	-

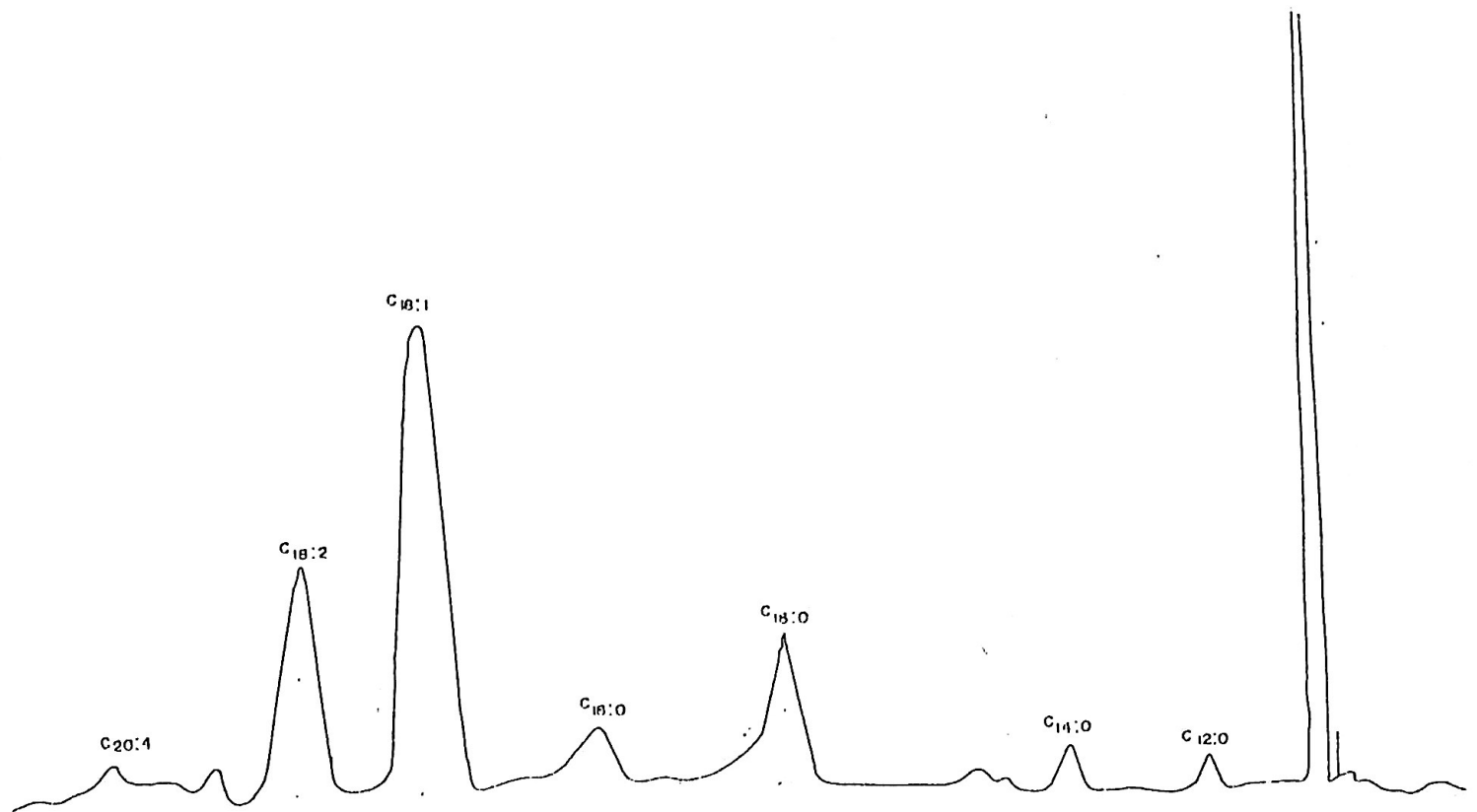
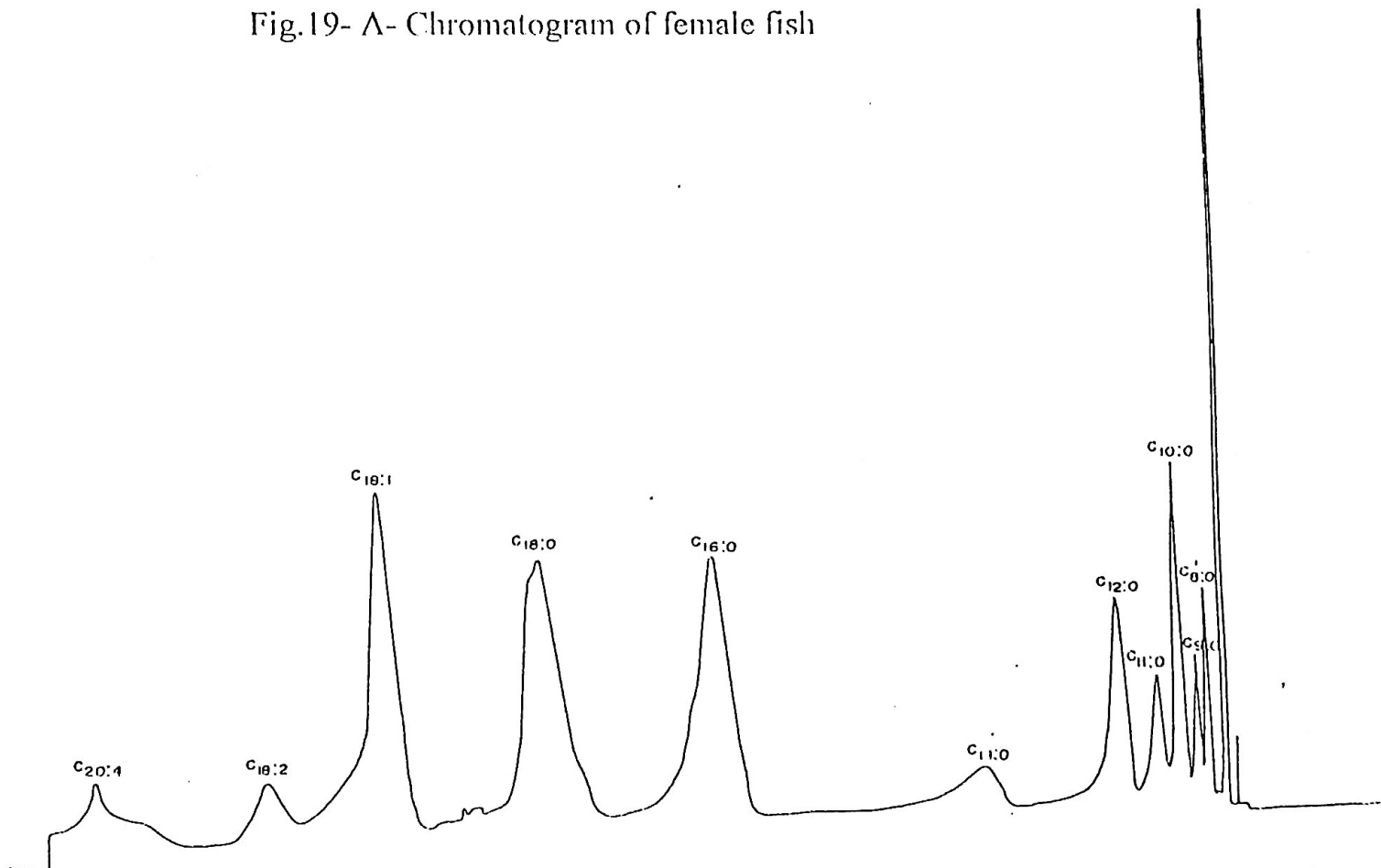


Fig.19- A- Chromatogram of female fish



B- Standard chromatogram

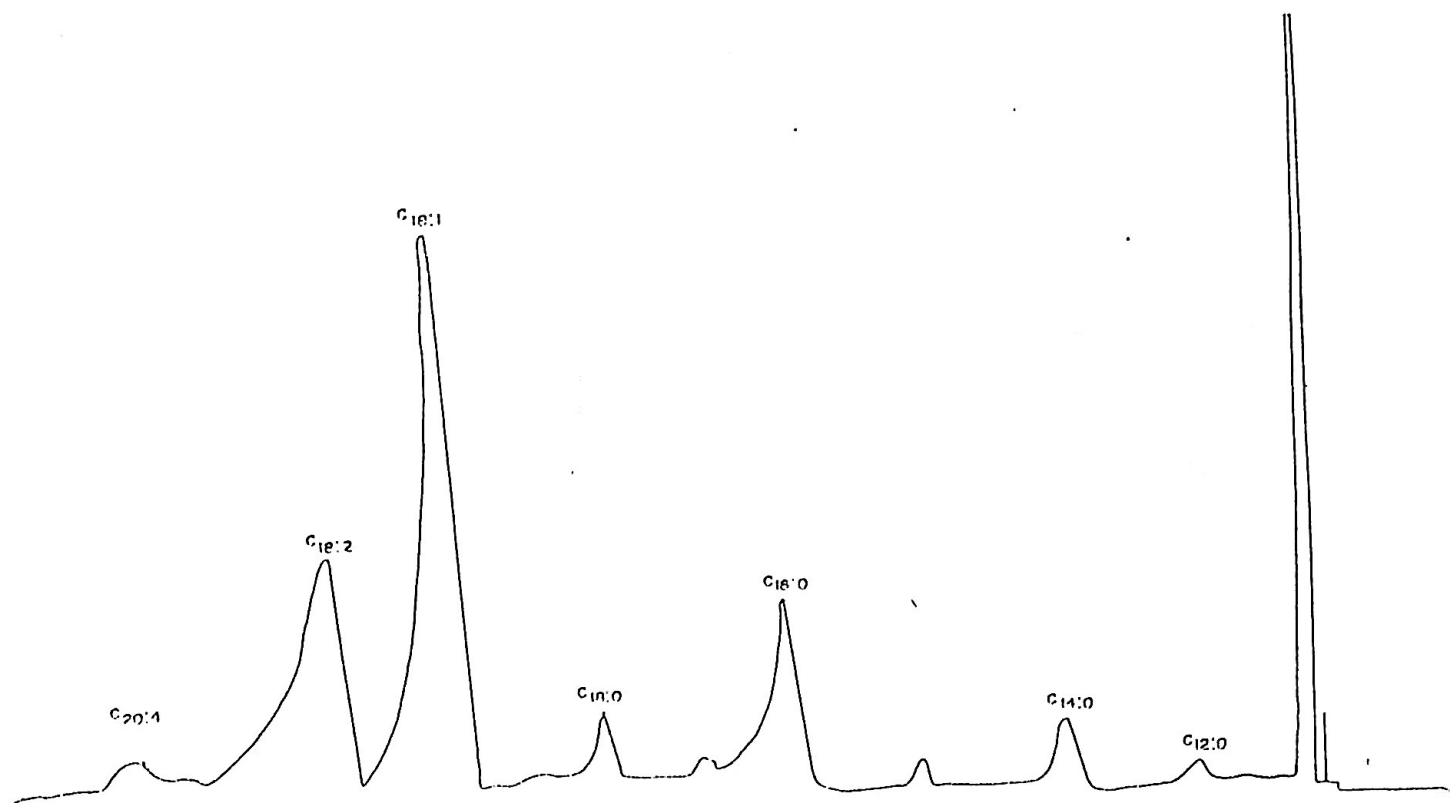
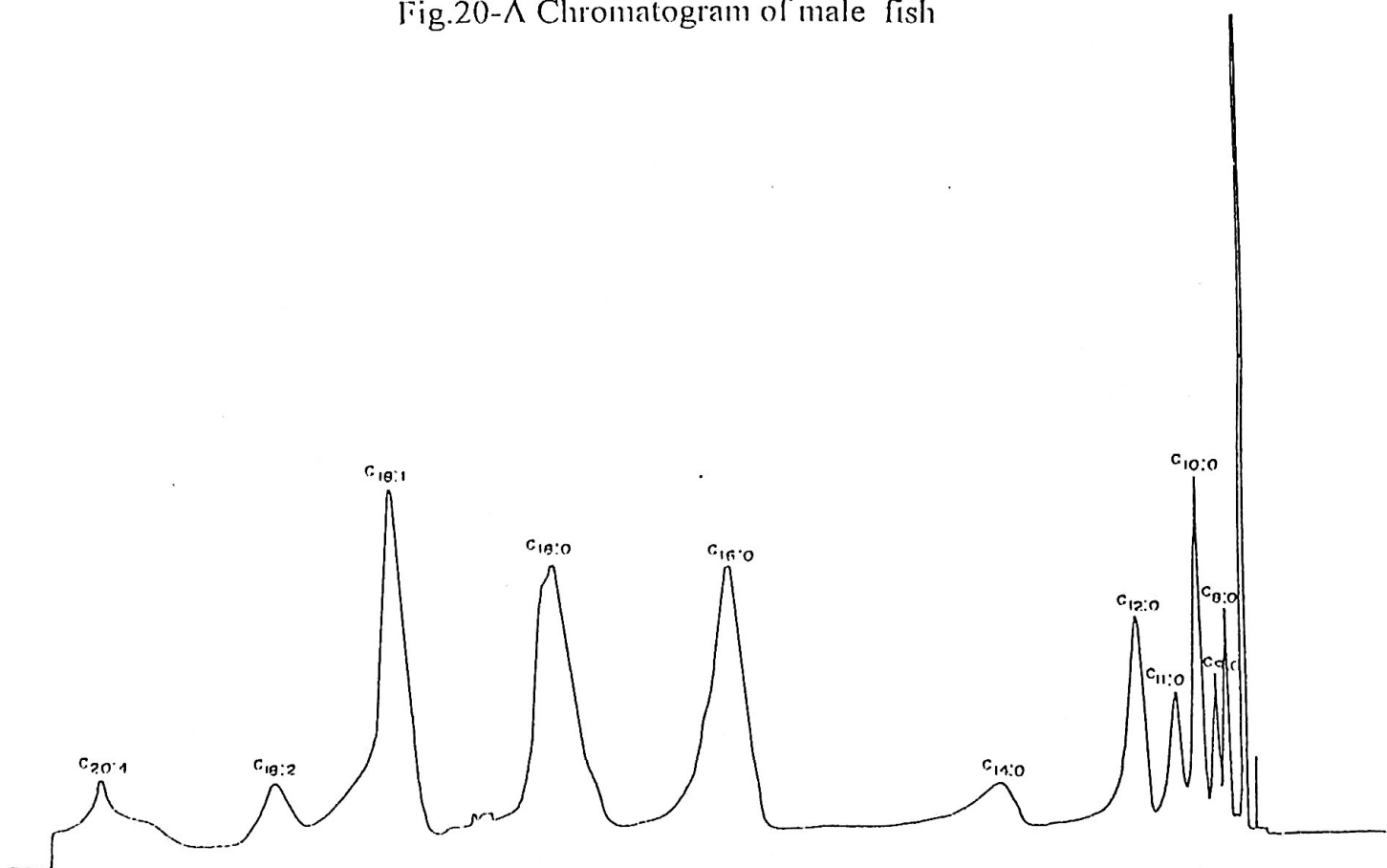


Fig.20-A Chromatogram of male fish



B- Standard chromatogram

SUMMARY

Chapter 1 :

Reproductive Biology :

I. The male and female reproductive systems of *A. morar* are described separately. At the breeding season the difference of the genitalia was used for sex differentiation of the fish, otherwise the fishes were sexed according to the primary sex characters, after dissection.

a) **Male reproductive system** : Testis of *A. morar* is elongated and paired structure of unequal size, are suspended in the body cavity by a mesorchium. Colour and size of the testes vary according to the stage of sexual maturation gross anatomy of *A. morar* testis showed progressive enlargement from Mid August to December-February, the peak breeding season. Testes change from a translucent, whitish, narrow, thread like structure to pear shaped mass of deep creamy colour. Milt oozes out by slight pressure on the abdomen. After spawning most of the fish were found with flaccid testis and width decreased. The testis started to regain its original shape and size from late March to June.

b) **Female reproductive system** : The paired ovary of *A. morar* females showed a similar pattern of development like the testes. The left lobe of the ovary was always larger than the right lobe. Two lobes unite posteriorly before opening through the genital pore situated just in front of the anal fin. The shape, size and colour of the ovary varies according to the degree of maturation. The colour varies from whitish in young through light orange when maturing to bright orange in ripe stage. At ripe stage ovary occupied 95% of the body cavity. After spawning the ovary becomes shrunked with blood shot in fresh condition and regain its original shape and size by April. On the basis of developmental stages of the ova the ovary

is divided into ten stages. Immature stage, maturing-I (early developing), maturing-II (late developing), mature, ripe, pre-spawning, spawning, post spawning, spent and resting stage. The ova is also divided into four stages : immature stage, maturing stage, mature stage and ripe stage.

2. Reproductive cycle :

Six methods have been employed to determine the breeding seasons of *A. morar*. These methods are : percentage of gravid females against time, diameter of ova, gonado-somatic index, gonadal length index, colouration of the ovary, and maximum indices of spermatozoa in the milt. All these methods showed that the peak breeding season of this species in January and February.

Fecundity : the estimated fecundity ranged from 1665.50 to 9401 egg with a mean as 4317.47 ± 814.31 eggs.

The relationships between fecundity and total length, fecundity and standard length, fecundity and total weight, fecundity and gonadal length, fecundity and gonadal weight of the female show straight line linear regressions, on the length or weight increase the value of fecundity also increase.

The fecundity factor of the species is also provided.

Out of 2402 specimens collected during 24 months of study, the percentage of male and females were 47.50% and 52.50% (Ratio 1 : 1.093), which is significantly different at 0.001 level. Availability of females in large number during the breeding season is the reason behind such distribution pattern of males and females of *A. morar*.

Chapter 2.

The physico-chemical condition of the river Padma was found to vary with the seasons.

Four physical and three chemical parameters have been considered. The mean values of these parameters for 12 months were obtained $25.17 \pm 4.92^{\circ}\text{C}$ as (air temperature), $22.07 \pm 5.92^{\circ}\text{C}$ (water temperature) $0.41 \pm 0.43\text{m}$ water transparency, 113.82 ± 120.74 mm rainfall, 7.33 ± 0.41 (pH)mg/l (DO), 4.44 ± 0.88 and $5.46 \pm 2.34\text{mg/l}$ free carbondioxide).

Chapter 3.

Fishery :

I. Fishing of *A. morar* along with other small size fishes is done by using various type of nets, viz, Beshal jal, 'Ligon jal' (gillnet) Ferry jal and 'Barricades' (Bana). Among these nets the Ligon and 'Ferry jal' (gillnets) are especially use to catch *A. morar*. Fishing in the river Padma is done continuous throughout the year. However, the peak period was observed during the winter and early part of the summer. Restriction of fishing during the spawning season. During the year 1993-94 total landing of *A morar* were 19.16, 26.67, 19.27 and 97.08 mds (40 kg) in four sampling areas. i.e Binodpur, Katakhal, Talaimary and Shaheb bazar respectively.

The fisherman handover their catches to diferent distant and near markets through a more or less distinguished channel. In between the fishermen and the final consumers, there found a few intermediaries (e.g. Faria, Beparis, Aratdar and Retailer). The Fishermen themselves sold their products at their own localities or nearby 'hats', when the catch is of smaller quantity.

Different type soft containers were found to be used for carrying *A. morar*, viz khalio, Dala or Dali, Jhuri and aluminium made Hari.

Generally fishes are transported by Truck, Bus, Train, Rickshaw, Van, Tomtom, Boat etc. Some fishermen bring their fishes by themselves carrying on the heads.

The market price of *A. morar* varied among the four surveyed markets of Rajshahi. The price also varied according to the size of the fish. The highest total margin was found as Tk. 33.50 /kg (larger size group) and Tk. 23.00 / kg (small sized fish) at Shaheb Bazar. The lowest total margin as observed at Binodpur Bazar was Tk. 27.50 kg (larger sized fish) and Tk. 23.50 smaller size groups).

Among the fish traders the higher margin was taken by the Farias as Tk. 12.00 and Tk. 10.00 /kg for larger and smaller fishes at Shaheb Bazar whereas at Katakhal Bazar the margins were Tk. 9.00 / kg and Tk. 7.00 / kg for the Farias.

The seasonal price of *A. morar* differed at different markets. The highest price were obtained during the months of March to August as Tk. 48 to 56 /kg (small sized fishes) and Tk. 70.0 to Tk. 100.00 /kg (larger size group). During the breeding season (Nov.-Feb) the price of the fishes were low. A way the four fish landing centres the maximum price of the fish were observed at Shaheb Bazar fish market.

Chapter 4

Chemical analysis :

The small indigenous fresh water fish species *A. morar* is important due to its high nutritional value in terms of lipid, protein content, and other related substances which are not commonly available in other food. *A. morar* fish was studied to obtain a detailed information on their chemical investigation and

seasonal variation of lipid, protein and related substances in different months. The fish contained the highest percentage of moisture 73.34 and 73.31 and ash 4.02 and 3.61, in male and female respectively. The highest lipid and protein contained in male and female species were found to be 9.85 and 9.75 and 16.05 and 15.88 respectively in the month of July (1995).

The physical properties and chemical composition of the lipid were determined by the standard methods. The properties and composition were estimated and values computed in Table 16, 17.

The findings are as follows : Specific gravity 0.913 at 30°C; refractive index 1.462 at 30°C; Saponification value 210.621 and Saponification equivalent 224.96; Iodine value 96.25; Peroxide value 2.92; Acid value 1.78; and Free fatty acid value 0.89%; Unsaponifiable matter 0.520%; Reichert-Meissel value 0.82; and Pole-enske value 0.560; Acetyl value 15.23. This study reveals that the lipid has high nutritional value and is suitable for consumption, specially for edible purpose.

The fatty acid methyl ester derived from the lipid (of both male and female) has been subjected to GLC analysis which indicated the presence of Lauric acid, Myristic acid, Palmatic acid, Stearic acid, Oleic acid, Linoleic acid and Arachidonic acid in the lipid of the *A. morar*. The results shown in the table indicated that the lipid under investigation is reasonably suitable for human consumption.

REFERENCE

- Abu-Hakima, M., 1984. Some aspects of the reproductive biology of *Acanthapagrus* spp. (Family : Sparidae). J. Fish Biol., **25** : 515-526.
- Adiyodi, K.G. & R.G. Adiyodi, 1974. Comparative physiology of reproduction in Arthropods : Comparative physiology and biochemistry. (O. Lowenstein ed.); Academic Press, New York & London, **5** : 37-107.
- Afroze, S. & M.A. Hossain, 1983. The fecundity and sex-ratio of *Amblypharyngodon mola* (Hamilton) (Cypriniformes : Cyprinidae). Univ. J. Zool., Rajshahi University, **2** : 29-32.
- Afroze, S. & M.A. Hossain, 1990. The reproductive cycle of the freshwater fish *Amblypharyngodon mola* (Ham.) (Cypriniformes : Cyprinidae). University J. Zool. Rajshahi Univ., **9** : 17-21.
- Agarwal, O.P., 1996-97. Organic Chemistry, Chemistry of Organic Natural Products, Coel Publishing House, Meerut, India 19th edn. Vol-II, p.441.
- Ahlstrom, E.H., 1957. A review of recent studies of Sub-population of Pacific fishes. Spec. Sci. Rep. U.S. Fish Wildl. Serv, **280**: 44-74.
- Ahmed, N., 1962. Fishing Gears of East Pakistan. East Pakistan Government Press. Dacca, p.35.
- Ahmed, N., 1977. An analysis of the fisheries of Pabna with a few aspects of fisheries of Bangladesh. A project work submitted to the faculty of Commerce, Univ of Dacca (Unpublished): Sponsored by UGC.
- Ahmed, A.T.A., Mustafa, G.N. & Alam, G.N., 1978. Fecundity and larval development of *Tilapia nilotica* (L.). J. Asiatic Soc. Bangladesh (Sci.) **4**(1&2) : 67-71.

- Alam, M.R., M.G. Mortuza, M.S. Islam & M.A. Hossain, 1994. The fecundity of *Ailia coila* (Hamilton-Buchanan) (Siluriformes : Schilbeidae) from the river Padma near Rajshahi City. J. Bio. Sci. 2: 113-116.
- Alikunhi, K.H., 1948. Growth, maturity and brood care in the murrels, *Ophicephalus straitus* and *O. punctatus* Proc. 35th Indian Sci. Congress, Abstract, 207.
- Anderson, L., Dibble, M.V., Turkki, P.R., Mitchell, H.S., Rynbergen, H.J., 1982. "Nutrition in Health and Disease". 17th edn., p.292, 306, 794.
- Ando, 1965. Ultracentrifugal analysis of the Yolk protein in rainbow trout and their changes during development Can. J. Biochem. 43 : 373.
- APHA, 1989. Standard methods for the examination of water and waste water. American Public Health Association, Washington (Latest ed.).
- Balck, R.E., Imerson, M.S. Rahman, A.S.M.M., Yunus, M., Alim, A, R.M.A. Haque, I. Yolken, R.S. and Curlin, G.T., 1980. A two years study of bacterial viral and parasite agents associated with diarrhoea in rural Bangladesh. J. Infect. Dis., **142** (5) : 660.
- Bambury, R.E., 1979. Synthetic antibacterial agents in : Burger's medicinal chemistry fourth edition, part II edited by Wolf, M.B. Johawiley and Sons, New York, Chichester. Brisbane, Toronto. p.67.
- Bamforth, S., 1958. Ecological studies on the planktonic Protozoa of a small artificial pond Limnol. and Oceanogr. **3**: 398-412.
- Berra, T.M., 1984. Reproductive anatomy of the Australian grayling, *Prototroctes maraena* Gunther, J. Fish Biol. **25** : 241-251.
- Bhatnagar, G.K., 1964. Spawning and fecundity of Bhakra Reservoir fishes. Indian J. Fish. **11**(1) : 485-502.

- Bhowmick, R.M., 1965. Studied of some aspects on the biology of *Glossogobius giuris* (Hamilton) with notes on its fishery in the Hoogly estuary, Proc. Indo-Pacific fish. Concen. **11**: 99-115.
- Bhuiyan, A.S. & K. Rahman, 1984. Fecundity of the snake headed fish, *Channa punctatus* (Bloch and Schneider) (Channidae : Channiformes), J. Asiatic. Soc. Bangladesh (Sci), **10**(2) : 75-81.
- Bhuiyan., A.L., 1964. Fishes of Dacca. Asiatic Soc. Pak. Dacca. Pub. No. 13:148p.
- Bhuiyan, A.S., 1985. Studies on the biology of *Cirrhina reba* (Hamilton). Ph.D. thesis submitted in the Department of Zoology, Rajshahi University, Rajshahi, p.195.
- Bhuiyan, A.S. and M.N., Islam, 1990. Fecundity of *Xenentodon cancila* (Hamilton) (Belonidae : Beloniformes). Environment and Ecology (India) **8**(3): 1004-1007.
- Bhuiyan, A.S., M.N., Islam and N., Sultana, 1992. Food and feeding habit of *Aspidoporia morar* (Hamilton) from the river Padma. Univ. J. Zool. Rajshahi Univ. **10 & 11** : 71-76.
- Bhuiyan, A.S., M.N., Islam & N., Sultana, 1993. The fecundity and sex-ratio of *Aspidoparia morar* (Ham) from the river Padma. Univ. J. Zool. Rajshahi University. Vol. **12** : 59-63.
- Bligh, E.G. and Dyer, W.J., 1959. Total lipid extraction and purification. Can. J. Biochem. Physiol., **37** : 911.
- Boollootain, R.A., 1965. Aspects of reproductive biology in striped shore crab, *Pachygrapsus crassipes*. Bull. southern. Calif. Acad. Sci., **65** : 43-49.
- Bernard and Oser, L., Hawk's (1965) Physiological Chemistry, (14 edit.), McGraw Hill Book Comrany, U.S.A. P. 1364-65.

- Bucksimiar, A.H., 1977. The Problem of Transprot and marketing of marine Fishes Products and their remedy. Nat . Mar. Fish. Sem. 1977. Edited by A.L. Bhuiyan and A.K. Chowdhury.
- Callewaert, D.M., and Genyea, J., 1980. Fundamentals of Organic and Biological Chemistry. worth publishers, Inc. New York. p.657.
- Carrol, K.K., Carrol and R.L., Noble, Can. Can. J. Biochem., Physiol., 1957. **35**: 1093.
- Chakraborti, R.D., P., Roy, & S.B., Sinha, 1959. A quantitative study of the plankton and the physico-chemical conditions of the river Jamuna at Allahabad in 1954-55. India J. Fish. **6**(1):186-203.
- Chakraborti, P.K. & G.N., Chattapadhyey, 1988. Impact of Farakka Barrage on the estuarine ecology of Hooghly-Matlah system. In conservation and management of inland capture Fisheries resource of India (A.G. Jhingran and V.V. Sugunaeds. Inland Fish Soc. India. Barrakpore, India. p.189-196.
- Chandran, M.R., 1968. Studies on the marine crab, *Charybdis variegata* 1. Reproductive and nutritional cycle in relation to breeding Periodicities. Proc. Ind. Acad. Sci., **67** : 215-223.
- Chow. T., 1958. A study of water quality in the fish ponds of Hong Kong. Hong Kong Univ. Fish. J. **2** : 7-18.
- Chowdhury, H., 1960. Experiments of induced spawning of Indian carps with pituitary injections, Indian, J. Fish **7**(11): 20 - 49.
- Chowdhury, D.P.N., 1983. Studies on some aspects of Prawn fisheries of Khulna. M.Phil. thesis (Unpublished). Department of Zoology. Rajshahi University. Rajshahi, p.176.
- Christic, W.W., 1976. Lipid Analysis, Pergaman Press, England, p.57, 60.

- Chubb, C.F. & I.C., Potter, 1984. The reproductive biology and estuarine movements of the Gizzard shad *Nematolosa vlaminghi* (Munro). J. Fish Biol, **25**: 527-543.
- Clark, J.M. Jr., 1964, Experimental Biochemistry. W.H. Freeman and Co., USA., p.52.
- Clemens, H.P. & C.A., Reed, 1967. Testicular characteristics of gold fish, *Carassius amratus* in nature and under diet limitation. J. Morph, **122**: 131-138.
- Craig, J., 1974. Population dynamics of perch, *Perca fluviatilis* L., Slopston Ley, Devon, 1. Trapping behaviour, reproduction, migration, population estimates, mortality and food. Fresh wat. Biol. **4**, 417-431.
- Das, H.P., 1977. The fecundity of Grey mullet, *Mugil cephalus* L. along Goa coast. Mahasagar. Bull National Ins of Oceanography, Vol. **10**(1+2): 79-82.
- Dastur, J.F., 1977. Medicinal Plants of India and Pakistan, D.B. Taraporevala Sons & Co. Ltd., p.50, 97.
- Datta, P.K., 1995. Physico-chemical and Micro-biological Investigations of Lipid of *Puntius gonionotus*. M.Sc. thesis, Dept. of Applied Chemistry & Chemical Technology, University of Rajshahi.
- Day, F., 1878. The fishes of India being a Natural History of the Fishes known to Inhabit the Seas and Fresh Waters of India, Burma and Ceylon. Vols. I & II. p. xx + 778. pls. cxcv.
- Day, F., 1889. The Fauna of British India including Ceylon and Burma. Fishes, Vol. 1. pp.XVIII 548, Vol.II, pp.XIV+509
- Deb, M., L.N., Mohapatra, M.K., Bhan, N.K., Arora, J.C., Samantaray, O.P., Cahi, J., Stinzing, R., Mollby, and I., Holme, 1983. Enterotoxigenic *E. coli* diarrhoea in north Indian Community. Ind. J. Med. es : 319-323.

- Devasy, V.P. & C.K., Gopinathan, 1970. Hydrological features of the Kerala back waters during permonsoon and monsoon months. Fish. Technol. (India) VII(2) : 190-194.
- Dewan, S., 1973. Spawning and fecundity of certain pond fishes, Ph.D. thesis, Bangladesh Agricultural University, Mymensingh.
- Diwan, A.D & R., Nagabhushanam, 1974. Reproductive cycle and biochemical changes in the gonads of the freshwater crab, *Barytelphusa canicularis* (West-wood, 1836). Ind. J. Fish, 21(1) : 164-176.
- Doha, S. & M.A., Hye, 1970. Fecundity of the Padma river Hilsa, *Hilsa ilisha* (Hamilton). Pak. J. Sci., 22 : 176-183.
- Dutta, N., J.C., Malhotra & B.B., Bose, 1954. Hydrology and seasonal fluctuations of the Hooghly estuary. Proc. Symp. on Marine and Freshwater Plankton in the Indo-Pacific. p.1-13.
- Dutta, S.K., 1983. Studies on some aspects of prawn fisheries of Rajshahi. M.Phil. thesis (unpublished) submitted in the Dept. of Zool., Rajshahi University, Rajshahi, p.99.
- Dutta, S.K., 1996. Occurrence and production potential of freshwater Prawn, *Macrobrachium malcolmsonii* H. Milne Edw. from the river Padma and adjacent water areas. Ph.D. thesis (unpublished), Dept. of Zool., University of Rajshahi, Rajshahi, p.201.
- Evans, D.H., 1969. Life history studies of the Eahontan reddsides, *Richardsonius egregius* in lake Tahoe, Calif. Fish and Game. 55(3) : 197-212.
- George I. Sackheim, Dennis D. Lehman Chemistry for the health sciences (4th edition) Macmillan Publishing Co., Inc. New York, Collier Macmillan Publishers London, P. 294.
- Gunstone, F.D., 1958. An Introduction to the Chemistry of fats and fatty. A. Chapman and Hall, Ltd., London, 76 : p.43, 145.

- FAO-Report, 1991. Fish for food and development, p.1-49.
- Farmer, A.S., 1974. Reproduction in *Nephrops norvegicus* (L) (Decapoda: Nephropidae), J. Zool. London. **174** : 161-183.
- Foucher, R.P. & R.J., Beamish, 1977. A review of oocytes development in fishes with special reference to Pacific Hake (*Merluccius productus*). Fish, Mar. Serv. Tech. Rep. No. **755** : p.16.
- Geisler, R., H.A., Knoppel & H., Sioli, 1975. The ecology of freshwater fishes in Amazonia-present status and future tasks for research. Animal Research and Development, Institute of Scientific Co-operation, Germany. V-1. 102-119.
- Haley, S.R., 1972. Reproductive cycling in the Ghost crab, *Ocypode quadrata* (Fabr.) (Brachyura, Ocypodidae). Crustaceana, **23** (1) : 1-11.
- Hamilton, F.B., 1882. An account of the fishes found in the river Ganges and its branches, pp. VII+405, pls. 39.
- Haq T. and K.M.S., Aziz, 1977. Changing antibiotic sensitivity pattern of the commonly occurring organisms causing dysentery, Beng. Med. J. **5**(4) : 119-125.
- Hasan, M.F., 1996. Chemical Investigation of *Eutropiichthys vacha* and seasonal variation of lipid, protein and related substances, A M.Sc. thesis submitted to the University of Rajshahi.
- Heydorn, A.E.F., 1965. The rock lobster of the South African West Coast, *Jasus lalandii* (H. Milne Edwards). I. Notes on the reproductive biology and determination of minimum size limits for commercial catches. Invest. Rep. Div. Sea. Fish. S. Afr., **53**: 1-32.
- Hickman, M., 1979. Seasonal succession, standing crop and determinants of the phytoplankton of Ministik lake, Alberta, Canada, Hydrobiologia. **64**: 105-121.

- Hoque, M.A. & M.A. Hossain, 1993. Sexual maturity and fecundity of freshwater fish *Mystus vittatus* (Bloch) (Cypriniformes : Bagridae). Univ. J. Zool., Rajshahi Univ. **12**: 9-13.
- Hoque, M.T. & A.T.A. Ahmed, 1993. Spawning periodicity of two Indian major carps, *Labeo rohita* Ham. and *Cirrhinus mrigala* Ham. Bangladesh J. Zool., **21**(2): 9-26.
- Hossain, M.A., 1976. Studies on some aspects of the biology of sand lobster, *Thenus orientalis* (Lund.) (Decapoda: Scyllaridae) from Andhra Coast. Ph. D. Thesis, Andhra University, Waltair. p.276.
- Hossain, M.A., 1979. On the fecundity of the sand lobster, *Thenus orientalis* from Bay of Bengal. Bang. J. Sci. Res., **2**(part A) : 25-32.
- Hossain, M.A. & M.A. Rafiq, 1981. Female reproductive system and reproductive periodicity of fresh water prawn, *Macrobrachium malcolmsonii* (H. Milne Edward), Proceeding of the Zoological Society of Bangladesh, 178-190.
- Hossain, M.A. & S. Parween, 1982. Sexual periodicity in freshwater prawn *Macrobrachium dayanum* (Handerson). Bang. J. Sci. & Ind. Res., **17**(3-4): 236-245.
- Hossain, M.A., 1982. On the length-weight relationship and condition factor of the sand lobster, *Thenus orientalis* (Land). Univ. J. Zool. Rajshahi University, **1** : 23-28.
- Hossain, M.A., 1989. On the methods of determining the reproductive cycle in fisheries species. 76th Ind. Sc. Cong. Ass. Madurai. p.10.
- Hossain, M.A., A. Taleb & M.H. Rahman, 1989. Reproduction and reproductive periodicity of *Notopterus notopterus* (Pall.) (Notopteridae: Clupeiformes). Bangladesh J. Aquaculture, **11**(2): 57-64.

- Hossain, M.A., M.A. Rahman & S. Parween, 1991, A study on the sex-ratio and reproductive cycle of *Nandus nandus* (Hamilton-Buchanan) (Nandidae: Perciformes). J. Asiatic Soc. Bangladesh (Sci.) 17(2) : 141-144.
- Hossain, M.A., A. Taleb & M.H. Rahman, 1992. Reproduction and fecundity of *Ompok pabda* (Ham.) Bangladesh J.Sci. Res. 10(1) : 49-52.
- Hossain, M.A., 1996. Various aspect of small indigenous species (SIS) fishes in Bangladesh. Semimar on, Small indigenous fish species (SIS) culture in Bangladesh, Dept. of Zoology, University of Rajshahi.: 9-23.
- Hussain, M.A., 1997. Some aspects of eco-biology of the spiny eel, *Maslacembelus pancalus* (Hamilton), M.Phil. thesis (Unpublished), Dept. of Zoology, Rajshahi University, p.170.
- Huq, M.I., O.S., Ahmed, and M.M., Rahman, 1983. Changing pattern of antibiotic resistance in *Shigella* isolated in Bangladesh, In : Shigellosis. a continuing global problem; Proceedings of an international conference, Cox's Bazar, 1981 edited by M.M., Rahman, W.B. II, Greenough, N.R., Novak, S., Rahman, ICCDR, B. p.174-181.
- Islam, B.N. & G.B. Talbot, 1968. Fluvial migration, spawning and fecundity of the Indian river hilsa, *Hilsa ilisha*, Trans. Amar. Fish. Soc. 97: 350-355.
- Islam, A.K.M.N., A.K.Y. Haroon & K.M. Zaman, 1974. Limnological studies of the river Buriganga I. Physio-chemical aspects. Dacca Univ. Stud. B.22(2): 99-111.
- Islam, A.K.M.N. & F. Mendes, 1976. Limnological studies of a Jheel in Sher-E-Bangla Nagor, Dacca Univ. Stud. Pt. B. 24(2): 63-71.
- Islam, M.S. & M.A. Hossain, 1984. On the fecundity of Chela, *Oxygaster bacaila* (Hamilton) (Cypriniformes : Cyprinidae) from the river Padma. Univ. J. Zool., Raj. Univ., 3: 45-48.

- Islam, M.S. & M.A. Hossain, 1986. Fecundity of the small cat fish *Clupisoma atherinoides* (Bloch) (Schilbeidae: Cypriniformes) from the river Padma. Bangladesh J. Aquaculture., 8(1): 53-55.
- Islam, M.S. & M.A. Hossain, 1990. The fecundity and sex-ratio of the common *punti*, *Puntius stigma* (Cypriniformes: Cyprinidae) from the river Padma near Rajshahi. Univ. J. Zool., Rajshahi Univ. 9: 69-74.
- Islam, S., 1990. Some aspects of biology of fresh water *Rhinomugil corsula* (Hamilton). p.135.
- Islam, M.A., 1995. Chemical and biochemical studies on the seed of *Polyallhia longifolia*, M.Sc. thesis, Rajshahi University, p.123.
- Jacobs, M.B., 1949. Chemical Analysis of Foods and Food products, 3rd Edn., Bailliere Tindall and Cox London, p.80.
- Jacobs, M.B., 1958. The Chemical analysis of Foods and Foods products, 3rd Edn., p.393.
- Jana. B.B. & H.L. Sarker, 1971. The limnology of swet Ganga, thermal spring Bakreswar, West Bengal, India, Hydrobiol. 37(1): 33-48.
- Jayaraman, J., 1985. Laboratory Manual in Biochemistry, Wiley Eastern Limited, New Delhi, Calcutta, Hyderabad, India, p.75-76, 96.
- Jhingran, V.G., 1983. Fish and Fisheries of India (Revised and enarged 2nd edn). Hindustan publishing Corporation (India) Delhi. p.645.
- Jhingran, A.G. & V. Pathak, 1987. Ecology and management of bells in Assam. A case study of Dhirbeel. In Proc. of the workshop on Development of Beel Fishery in Assam. Agric. Univ. 21-22 April. p. 16-28.
- Kader, M.A. & S.R. Talukdar, 1978. The fecundity of *Polynemus indicus* (Shaw) .J. Asiatic Soc. B. 4 (1&2): 15-20.

- Kansella, J.E., Shimp, L.E., J. Mai & Weihrauch, J. Am. Oil. Chemist, 1977. 54 : 425.
- Kalumuddin, M. A Test Bood of Practical Physics. 4th edn. p.84.
- Karim, M.A. & M.A. Hossain, 1972. Studies on the biology of *Mastacembelus pancalus* (Spiny eel, Ham.) in artificial ponds. Part-I, Natural habitat, distribution, food and feeding habits and economic importance. Bang. J. Biol. Agr. Sci. 1(2): 10-14.
- Karim, M.A. & M.A. Hossain, 1972b. Studies on the biology of *Mastacembelus pancalus* (Spiny eel, Ham.) in artificial ponds. Part-II, sexual maturity and fecundity. Bang. J. Biol. Agr. Sci. 1(2): 15-18.
- Khan, H. & Mukhopadhaya, 1972. On the fecundity of Climbing perch, *Anabas testudineus* (Bloch). J. Indian Fish. Soc. India., 4: 212-213.
- Krause, M.V. and L.K. Mahan, 1972. "Food, Nutrition and Diet Therapy", W.B. Saunders Company, London, 6th edition. P. 963.
- Kinne, O., 1971. Marine ecology. Wiley Inter. Science (Division Johan Wiley Sons Ltd.) New York. 1-1244.
- Kohls, L.R., 1970. Marketing of Agricultural products. MacMillan Co. NewYork. p.452.
- Kolles dorfer : in T.P Hilditch's, The Industrial Chemistry of the Fats and waxes, (3rd edit.), Baellere Tindal and cox, London (1949), p.159.
- Korzhenko, V.P., 1966. variation in Amino acid composton of gonads in the course of ova and spermatogeny in *Oncorhynchus veta*, Walbaum Dok. Akad. Nauk SSSR, 171 : 237-9.
- Kestaven, G.L. (ed), 1960. Manual of field methods in fisheries biology. FAO Man. Fish. Sci. No. 1, p.152.

- Laal, A.K., S.K. Sarker & A. Sarker, 1986. Ecology and fisheries of river Ganga at Bhagalpur (Bihar). Proc. Nat. Symp. Fish and Envi. 51-55.
- Lagler, K.F., 1956. Enumeration of fish eggs. In : Freshwater Fishery Biology. 2nd ed. Iowa, Wm. C. Brown Co. Pub. Dabuque, 106-110.
- Lagler, K.F., J.E. Bardach, & F.R. Miller, 1967. Ichthyology (fourth printing). John Willey and Sons. Inc., New York : London : Sydney, p. 545.
- Lakshminarayana, J.S.S., 1965. Studies on the Phytoplankton of the river Ganges, Varanashi, India. Part 1 & 2. Hydrobiologia, **25**(1-2) : 119-164.
- Lange, Hand Book of Chemistry, McGraw-Hill Book Company London, p.780.
- Le Cren, E.D., 1951. The length-weight relationship and seasonal cycle in gonad weights and condition in the perch (*Perca fluviatilis*). J. Anim. Ecol. **20** : 201-219.
- Lehman, B.A., 1953. Fecundity of Hudson river shad. Fish and wildlife service of U.S. Research Report No. **33**: p.5.
- Love, R.M., 1970. Water content of Cod (*Gadus callarias* L.) muscle. Nature, lond. 185, 622 only. 89, 31, 159.
- Love, R.M., 1974. "The chemical Biology of Fishes 2nd edition, Academic Press, London and NewYork, p.547.
- Macaden, R., Gokul, B.N. Pereira, P. Bhat. P., 1980. Bacillary dysentery due to multiple resistance of *Shigella dysentriae*, I. Ind. J. Med. Res. **71** : 178-185.
- Mahmood, N., Y.S.A. Khan & M.K. Ahmed, 1976. Studies on hydrology of the Karnafully estuary. J. Asiaic Soc. Bangladesh. **11**(1): 89-99.
- Mannan, A., 1977. Nutritional aspects of marine fishes and fisheries products. Nat. Mar. Fish. Seminar. Edited by A.L. Bhuiyan and A.K. Chowdhury.
- Marshall, N.B., 1965. "The life of fishes" Weidenfeld and Nichalson, London.

- Martinadle, The Extra Pharmacopocia, 26th Edn. The Phannaceutical Press, London, p.1665.
- Mc Cartney, T.H., 1967. Monthly variations of the serum total cholesterol and serum total lipid-phosphorus of mature bown trout, Fish. Res. Bull. N.Y. 30. 42-5, 63, 64, 110.
- Medical Tribune, Vol. 23, April, 1980.
- Michael, G.R., 1969. Seasonal trends in physico-chemical factors and plankton of a freshwater fish pond and their role in fish culture. Hydrobiol. 33: 144-159.
- Milton, D.A. & A.H. Arthington, 1983. Reproductive biology of *Gambusia affinis* halbrooki Baird and Girard, *Xiphophorus helleri* (Gunther) and *X. maculatus* (Heckel) (Pisces : Poeciliidae) in Queensland, Australia J. Fish. Biol. 23 : 23-41.
- Molla, M.R.I., M.M.R. Sharker, M.H. Kabir & M.S. Hossain, 1974. A Note on the nature of demand of poultry, potato and brinjal: using panel data in Mymensingh town, Bangladesh. Ind. J. Agr. Econ. XXIX(4), Oct.-Dec. 54-60.
- Molla, A.H., 1986. Biochemical and Nutritional studies on the Bangladeshi, Fresh water Eel, *Anguilla Bengalensis* (Bao Baim): The Distribution patern of essential amino acids in fish protein concentrate. The Rajshahi University Studies (Part B) XVII: 9-16.
- Molla, A.H., 1991. Biochemical and nuritional studies of the Bangladeshi fresh water Eel, *Anguilla bengalensis* (Bao Baim). Ph.D, thesis, University of Rajshahi, p.393.
- Mookerjee, H.K. & S.R. Mazumdar, 1946. On the life history of breeding and rearing of *Anabas testudineus* (Bloch), J. Dep. Sci., Calcutta Univ. L: 180-230.

- Moser, H.G., 1967. Seasonal histological changes in the gonads of *Sebastes paucispinis* Ayers, an ovoviviparous teleost (Family Scorpaenidae). J. Morphol. 123: 329-353.
- Mustafa, G., Kh. R. Islam, S. Ali & A.K.M.A. Alam, 1982. Some aspects of the biology of *Puntius sarana* (Hamilton). 1. Food and feeding habits in relation to fish size and fecundity. Bangladesh J. Zool., 10(2): 92-100.
- Nabi, M.R., 1987. Studies on the biology of fresh water spiny eel. *Macrognathus aculeatus*(Bloch) with notes on its fishery in chalan beel area. Ph.D. Thesis (unpublished), Dept. Of Zoology, University of Rajshahi.
- Nargis, A. & M.A. Hossain, 1988. The fecundity and sex-ratio of the climbing perch, *Anabas testudineus* (Bloch) (Anabantidae: Perciformes). J. Asiatic Soc. Bangladesh (Sc.), 14(1): 21-27.
- Nargis, A. & M.A., Hossain, 1992. Reproductive periodicity of climbing perch, *Anabas testudineus* (Bloch) (Anabantidae: Perciformes). Bangladesh J. Sci. Ind. Res., 27(1 & 2) : 87-93.
- Nikolsky, G.V., 1963. "The ecology of fishes" Academic Press, London and New York. p.352.
- Nutrition and Metabolism, Vol.-24 supplement 1, 1980.
- Oguri, M. and Takada, N., 1967; Serum calcium and magnesium levels of goldfish, with special reference to the gonadal maturation, Bull. Jap. Soc. Scienta, Fish. 33, 161-6, 61, 62.
- Paech, K. and M.V., Tracey, 1955. Modern Method of plant Analysis.Vol. 2, Springe-Verlag, Berlin, p.78, 328, 332.
- Pandey, S., 1969. Effects of hypophysectomy on the testis and secondary sex characters of the adult guppy *Paecillia reticulata* Peters. Can. J. Zool. 45, 775-781.

- Pantulu, V.R., 1963. Studies on the age and growth, fecundity and spawning of *Osteogeneiosus siliteris* (Lins). J. Cons. Intern. I. Expl. Met. **28**(2): 295-315.
- Parween, S., 1982. Studies on some aspects of fisheries of freshwater prawn, *Macrobrachium dayanum* (Hendr. 1893), of Rajshahi district. M.Phil. thesis. Dept. of Zoology, Rajshahi University. p.158.
- Parween, S. & M.A.Q. Hossain, 1983. Seasonal price variation of *Macrobrachium dayanum* Hendr. and seller's margin at different levels of marketing. Univ. J. Zool. Rajshahi University. **2**: 41-44.
- Parween, S., N. Begum, M.H., Rahman & M.A., Hossain, 1993. On the breeding periodicity of *Esomus danricus* (Hamilton). Univ. J. Zool. Rajshahi. Univ., **12** : 31-34.
- Patra, R.W.R. & M.A., Azadi, 1987. Ecological studies on the planktonic organisms of the Halda river, Bangladesh J. Zool. **15**(2) : 109-123.
- Perkins, H.C., 1971. Some observations on the Riverine palaemonid prawn fisheries of Daukkandi, Bangladesh. *Bangladesh J. Sci. Ind. Res.* XII (1-2) : 121-124.
- Das, R.K., Industrial Chemistry, Part-2, Kalyani Publishers, New Delhi, India, 1989, p.258.
- Rahman, A.K.A., 1989. Fresh water fishes of Bangladesh. Zool Soc. of Bangladesh Dhaka, p.202.
- Rao, C.B., 1955. On the distribution of algae in a group of six small ponds II. Algal productivity. *J. Ecol.* **43** : 291-308.
- Rao, R.M., 1967a. Studies on the biology of *Macrobrachium rosenbergii* (de Man) of the Hooghly estuary with notes on its fishery. Proc. Natl. Inst. Sci. India, **33B** (5 & 6) : 252-279.

- Rao, R.M., 1967b. Studies on the biology of *Macrobrachium rosenbergii* (de Man) of the Hooghly estuary with notes on its fishery. Proc. Natl. Inst. Sci. India, 333(5 & 6) : 252-279.
- Robb, A.P., 1982. Histological observations on the reproductive biology of the haddock. *Melanogrammus aeglefinus* (L.). J. Fish Biol. 20: 397-408.
- Rockford, D.J., 1951. Studies in Australian Estuarine Hydrology I. Introductory and comparative features. Aus. J. Mar. Fresh W. Res. 2(1): 1-116.
- Roger castle Giffin, A. M. S. M., 1955. Technical Methods of Analysis, 2nd edn. MacGraw-castle- Hill Book Company, Inc., NewYourk and London, p.84, 299.
- Rounsefell, G.A., & W.H., Everhart, 1960. Fisheries Science (Its methods and application) John Wiley & Sons, Inc. New York, London, secdond printing. p.135-178.
- Roy, T.K., 1981, Studies on some aspects of the biology of cat fish, *Heteropneustes fossilis* (Block), M.Sc. thesis, Dept. of Zool. Rajshahi University, p.149.
- Royce, W.F., 1972. Introduction to the Fishery Sciences. *Academic Press. Inc.* (London.) Ltd., p. 351.
- Sabur, S. A. & L. Rahman, 1979. Marine Fish Marketing in Bangladesh. J. Agr. Econ. II(1), June, pp. 95-113.
- Sahai, R. & A.B., Sinha, 1969. Investigation on bio-ecology of inland waters of Gorakhpur (U.P.), India. Hydrobiol. 34: 433-447.
- Sen, D., Shaha, M.R., Balakrism, Nair, Das, P., Nyogi, S.K., De Bhattacharaya, S.K. Dutta, P. Dutta, D and Pal, S.C. 1985., Etiological spechrum of acute diarrhoea inhospitalized patients in Calcutta. Ind. J. Med. Res. 82 : 288-291.
- Severtsov, S.A., 1941; 'Growth and Regullation of Animul population." Hoet, Rinehart and Winston, NewYork.

- Shafi, M. & M.A., Quddus, 1974a. The fecundity of the common punti, *Puntius stigma* (Cuvier & Valenciennes) Cyprinidae: Cypriniformes, Bangladesh J. Zool. 2(2): 133-145.
- Shafi, M. & M.A., Quddus, 1974b. The length-weight relationship and condition in the Giant Prawn *Macrobrachium rosenbergii* (DeMan, 1879) of Bangladesh. J. Asit. Soc. Bangladesh. 19(1).
- Shafi, M. & M.A., Quddus, 1975. Observation on some aspects of the biology of the fresh water prawn. J. Asiatic soc. Bangladesh. (Sc). 1(1): 1-9.
- Shafi, M., M.M.A., Quddus & H., Hossain, 1977. A morphometric study of the population of *Hilsa ilisha* (Hamilton-Buchanan) from the river Meghna. Proc. Second Bangladesh Sci. Conf., Abstracts: A-40.
- Shafi, M., M.M.A., Quddus & N., Islam, 1978. Studies on the limnology of river Meghna. Bangladesh J. Fisheries. 1(2): 85-97.
- Shafi, M. & M.M.A., Quddus, 1982. Bangladesher Matsaw Sampad. (In bengali), Bangla Academy: Dhaka, p.444.
- Shaha, S.B., M.J., Bhagal and V., Pathak, 1990. Ecological changes and its impact on fish yeild of Kulia beel in Ganga basin J. Inland Fish Soc. India, 22(1&2): 7-11.
- Sharker, M.R., 1977. Marketing of Marine Fisheries products, Nat. Mar. Fish. Seminar, 1977. Edited by A.L. Bhuiyan and A.K. Chowdhury.
- Shyamasundari, K., 1972. A method for the simultaneous distribution of neurosecretory and mucoid substances in invertebrates. Curr. Sci., 42(2): 78.
- Siddiqui, K., 1985. Bangladesh Grameen Dharidrer Sarup-O-Samadhan, Dana Prokasoni, Mohakhali, Dhaka-12, p.152.
- Simpson, A.Q., 1951. The fecundity of the Plaice. Fish invest. Series 11: 7(5).

- Smith, Chant and Overman. Microbiology. 16th edition. p.594.
- Snedecor, G.W., 1956. Statistical methods. The Iowa State College Press. Iowa.
- Srivastava, J.G., 1968. Fishes of Eastern Uttar Pradesh. Vishwabidyalaya Prokashan, Varanasi, p.163.
- Stephenson, A., 1934. The breeding of reef animals. Part-II. Invertebrates other than corals. Great Barrier Reef Exped. 1928-1929. Sci. Reports, 3: 247-272.
- Sultana, N., 1991. Some aspects of biology of *Aspidoparia morar* (Hamilton). M.Sc. Thesis, Rajshahi University., p.172.
- Swingle, H.S., 1957. Relationships of pH of pond water to their suitability for fish culture. Proe. 1.
- Talwar; P.K., Jhingran, A. G., Inland fishes of India and Adjacer countries. Volume-2, Tyk prokason, p.592, 600-601.
- Taylor, M.H. & L. DiMichelle, 1980. Ovarian changes during the lunar spawning cycle of *Pundulus heteroclitus*. Capera. p.118-125.
- Thakur, D.S., 1974. Foodgrain Marketing Efficiency: A case study in Gujarat, India, J. Agr. Econ. XXXIX(4), Oct.-Dec. p.61-74.
- Thomas, P.A., 1969. Goat fishes (Fam. Mullidae) of the Indian seas. Marine Biol. Assoc. India. Memoir III, p.174.
- Treasurer, J.W. & F.G.T. Holliday, 1981. Some aspects of the reproductive biology of perch, *Perca fluviatilis* L. A histological description of the reproductive cycle. J. Fish Bio. 18: 359-376.
- Vlaming, V.L. de, 1972. Environmental control of teleot reproductive cycle: A brief review. J. Fish Biol. 4, 131-140.
- Welch, P.S., 1948. Limonogical methods. McGraw-Hill. New York. p.381.
- Williams and Maslov, Sehrift. Aent. Forsch. Lebensm. 1935. (U.S.S.R), 4, 150.

- Williams, K.A., 1966. Oils, Fats and Fatty Foods (4th edit.) J. & A. Churchill, London, p.124, 136, 319.
- Woodhead, A.D. & P.M.G. Woodhead, 1965. Seasonal Changes in the physiology of the Barents Sea Cod, *Gadus morhua* (L.), in relation to its environment. I. Endocrine changes particularly affecting migration and maturation. ICNAF Spec. Publ. No. 6., 691-715.

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