BIOCHEMICAL ASPECTS OF OVARIAN MATURATION IN *LIZA PARSIA* (HAMILTON-BUCHANAN)

DISSERTATION SUBMITTED BY

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CERTIFICATE

This is to certify that this Dissertation is a bonafide record of the work carried out by Shri. **MUTHUKARUPPAN, S.** under my supervision and that no part thereof has been presented before for any other degree.

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P R E F A C E

Aquaculture is the dynamic pursuit of production of organisms from water - a process analogous to agriculture where animals and plants are cultivated from land. The field of aquaculture is an emerging bioindustry based upon the culture and husbandry of all useful aquatic organisms. It is a significant departure from the traditional "search and capture" fisheries and may increasingly displace the hunting and catching of wild stock. Of late there has been a global upsurge for aquaculture. Increasing world population, the decision by various nations to produce more fish by developing unutilised or partially utilised water resources, and depletion of natural stock due to excessive exploitation have been some of the reasons for the need to produce more fish through aquaculture.

For aquaculture, seed is the basic need and is usually collected from the wild. However, since the success of aquaculture is dependent on the desired, as well as uniform size of the stock and because the nature is not able to meet the demand of the aquaculturists for seed, the method of procuring seed by induced breeding is resorted to. In order to proceed with induced breeding, the aquaculturists have to be fully aware of the spawning season as well as the nutritional

state of the breeders. Further, it is indispensible to have a good knowledge of the biology, physiology and biochemistry of the fishes that are to be reared, in order that the fish may be placed in the most suitable environmental conditions and be supplied with appropriate feed and energy. This in turn can efficiently enhance the growth of the stock with the resultant increase in the overall fish production.

Earlier works of scientists which were restricted only to the biology has taken a turn in the recent years with biologists tending to use a more biochemical approach and biochemists envincing more interest in fish chemistry. Hence the overall view is now more biochemical and indeed more sophisticated than before. The fact that more scientists have started using fish for biochemical studies, which used to be confined to small mammals is evident from the remark of Malins and Sargent (1974): "We sense the presence of greater number of biochemists and biophysicists showing an interest in marine organisms than ever before".

Aquatic organisms when compared with terrestial animals have an entirely different way of life causing fishes to evolve a number of structures and system unique to themselves. The three primary constituents namely carbohydrate, protein and lipid which furnish a fish with energy for various purposes

all differ to some extent from those of warm-blooded animals, either in their composition or the way in which they are stored or used. Further, the stress such as severe depletion due to phenomenon like maturation, migration, etc., which most fishes undergo as a part of their normal living, have a profound effect on the composition of the body, that fish chemistry becomes essential and requires a special approach.

With a shift in priority away from "hunted" food fish to cultured fish especially using hypophysation techniques, it becomes an essential prerequisite to know the nutritional state of the fish before, after and at the time of spawning. The old Yorkshire farmer's advice to the young man seeking a life partner (Herriott, 1974), "Have a good look at the mother first, lad" to get an idea of how the daughter would develop, can be aptly quoted here to emphasise the importance of studying the nutritional state of the mother fish at the time of spawning.

This work has been put forward to serve the purpose in a mullet of high potential namely Liza parsia.

I express my deep sense of gratitude to Dr.(Mrs.) S. Sivakami, Scientist, Central Marine Fisheries Research Institute for her invaluable guidance and encouragement throughout the period of this study. I also express my gratitude to Director, Central Marine Fisheries Research Institute for granting all facilities for the study.

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I N T R O D U C T I O N

The grey mullet species of the family Mugilidae are representative of a truly International group of fish. They constitute 0.1-0.3% of the total marine landings of India and the production from Kerala back waters forms about 11% of its total annual landings (Jhingran, 1983). Fisheries of mullet are sufficiently important that it has stimulated considerable research.

There are about 281 nominate species in the family Mugilidae, of which Thomson (1964) had recognised 70 as valid. In Indian waters, Day (1878) identified 26 species of which 3 were reported to enter freshwater and another 9, the estuaries. Of the mullets, Mugil cephalus is the most abundant and widely distributed species, and has received maximum attention from Scientists. Liza parsia (Hamilton-Buchanan) is of considerable importance in India, second only to M. cephalus. According to Jhingran (1982), L. parsia, one of the common grey mullets in the Cochin estuary, constitutes a thriving fishery in estuaries and backwaters of India. It is also abundant in West Bengal, Madras, Vishakapatnam, Palk Bay, Gulf of Mannar and Andaman Islands. Outside India, this species is restricted to the Indo-Pacific region where it is fairly distributed along the

coast of Pakistan, Sri Lanka, Hong Kong, Australia, Indonesia, etc.

The popularity of mullets as culture fishes is no accident. Like most fishes, they are highly rated food fishes. L. parsia is classed as one of the richest sources of Vitamin A among the common Indian food stuffs by Ghosh and Guha(1934). The protein content of the whole mullet is about 20%. Jacqout (1961) classifies them among the semi-fatty fishes with a fat content of around 1.5-5.0%. They are also proclaimed for their extreme tolerance to salinity and temperature. Last, but not the least, mullets occupy the lowest position in food chain, enabling transformation of nutrients available in the environment into fish flesh at the minimum expense of energy.

In view of the significant characters of mullets as culture fishes, fish culturists and fishery scientists are evincing more interest in developing this group into a full-fledged source of culture, not merely by the present status, but also by the promise of even greater significance in future.

The principal areas of mullet culture in India are located in West Bengal and Kerala. Appreciable advancements have been made in recent past in research leading to the

developments of culture techniques of this group of fishes, especially M. parsia and M. tade in West Bengal (Jhingran, 1975). Kerala with vast areas of brackish waters, has good potential for mullet culture with Vypeen island of Cochin, a fishing hamlet, taking the lead. It is cultured in significant levels in areas of Tamil nadu also.

By virtue of the wider range of tolerance of mullets to salinity, attempts are made to acclimatise mullets in freshwater as well. The first acclimatisation studies of L. parsia in Bengal was due to Mookherjee et al. (1946), while farming of various species of mullets in freshwater bodies is reported by Ganapathi et al. (1950). Mullets are generally cultured along with other fishes like Etroplus suratensis and Chanos chanos in southern India (Bardach et al. 1972) and with Indian major carps and exotic carps (Pakrasi et al., 1975).

Till recently seed for mullet culture was obtained from wild depending on tides. Since it has its limitations like inadequate availability of quality seeds and also because of the difficulty in seggregating the desired sized stock for culture, it has become essential to adopt hypophysation techniques for seed production in good quantity. Moreover according to Kuo et al. (1974b), gonads of mullets in

confinement fail to attain maturity without hormone stimulation. An important break through in this line was achieved in 1964 by Yn-An-Tang of Taiwan Fisheries Research Institute in 1964 (quoted by Bardach et al. 1972). This was followed by attempts of Yashouv (1969), Kuo et al. (1974b) and others. In India, pioneer works of this kind were carried out in the Central Inland Fisheries Research Institute Barrackpore and also by Sebastian and Nair (1974) and James et al (1983).

For successful hypophysation and artificial propagation of cultivable fishes, it is highly essential to have a thorough knowledge of the reproductive physiology, biology, and biochemistry of the breeders. A proper understanding of the biological strategies, physiological requisitions and biochemical composition of parent fish keeps us informed about their suitability to face the natural phenomenon of procreation. Studies of biochemical composition of eggs can help us to assess the quality of the eggs and hence the condition of the emerging young ones.

The reproductive biology of various species of mullets in Indian waters has been reported by Jacob and Krishnamurthi (1942), Pillay (1954), Sarojini (1957; 1958), Luther (1962), Rangaswami (1972), Sunny (1975), Reddy (1977), Das (1978), Natarajan and Reddy (1980), Sulochanamma (1981), Kurup and Samuel (1983), Surendra Babu and Neelakantan (1983).

Nevertheless, contributions of the various aspects of breeding biology of L. parsia belong only to Sarojini (1957), Kurup and Samuel (1983) and Surendra Babu and Neelakantan (1983).

Maturation refers to cyclic morphological changes which a female and male gonad undergo to attain full maturity. Attainment of full maturity almost always marks a change in the growth pattern resulting from the 'reproductive drain' due to the diversion of material meant for somatic growth to the gonads. Parrish and Saville (quoted by Iles, 1974) maintain that in herring stocks, there is a marked reduction in growth rate at the onset of maturity reflecting the utilization of the growth material for gonad maturation. Iles (1974) discussing the tactics and strategy of growth in fishes, states that gonadal maturation is mainly dependent on the nutritional 'store' accumulated during the major feeding and growing period.

There is a considerable body of literature on the variations in body composition in relation to the ecophysiological changes like attainment of sexual maturity and spawning migration of fishes. Atwater (1888) had pointed out the body composition as an indicator of nutritive value of fishes. Observations on the depletive effects of maturation and spawning are made by Sekharan (1949), Bachanlal

(1963), Appa Rao (1967), Chaturvedi et al. (1976), Pandey et al. (1976), Sivakami (1981), Shreni (1983), Selvaraj (1984), and Sivakami et al. (1986). Significant variations in biochemical composition of different tissues of fish during prespawning and postspawning periods have been observed with special reference to cholesterol, glycogen, water, protein and total fat contents (Idler and Bitners, 1958; Robertson, et al., 1961; Siddiqui, 1966; and Joshi, 1977).

Despite the voluminous literature that have accumulated on the proximate composition of various groups of fishes, contributions on similar aspects in fishes of the family Mugilidae are very few. Protein, fat, water and ash contents are established in M. cephalus by Saby (1934) Jowett and Davies (1938) and in L. ramada and L. saliens by Saby (1934). Variations in body composition of mullets caught in the Swartkops estuary of South Africa have been discussed by Marias and Erasmus (1977).

Inspite of the fact that high quality mullet seed production depends on the understanding of proximate composition of the breeders, contributions relating body composition with that of spawning habits of mullets are not much accomplished. The only results obtained in this line are those of Kuo in M. cephalus(quoted by Nash and Shehadeh,1980) and Gopalakrishnan (Personal communication).

Because of the physical and biological hazards in the environment fish unlike other invertebrates, have a high reproductive potential where they produce sufficient quantiries of eggs so as to enable successful recruitment. According to Oven (1961), the mass of genital products constitute upto 30% of the body and even much more - a process calling for intensive energy. The first source of energy is suggested to be body fat (Giesse, 1966) with their mobilization becoming prominant at the time of maturation, even in non-migratory fishes.

Eggs are found to use protein steadily for energy purposes during development. Since newly spawned eggs cannot absorb much of the nutriment directly from the water, it stores adequate quantities of essential substances for the growth. Sorvachev and Shatunovkii (1968) noted that as the concentrations of 'free' amino acids rise in the developing gonad, there is a fall in corresponding amino acids in the liver, while translocation of muscle proteins for gonad development has been reported by Masurekar and Pai (1979) in Cyprinus carpio.

Though glycogen levels in the muscle, liver and gonad are comparatively low (Needham, 1931), its role in mobilization of energy during maturation and spawning may not be considered negligible. Accumulation of glycogen and glucose

in the ovary has been reported (Greene, 1926; Chang and Idler, 1960 and Venugopalan, 1962) while Kuo (unpublished data) found a distinct decrease of glycogen levels in the ovary before spawning in M. cephalus.

Cholesterol is an important precursor of steroid hormone and is a constituent of cell walls. Serum cholesterol levels is minimal at the time of greatest sexual conversion to sex hormones. Shreni and Jaffri (1974) have suggested that the egg cholesterol reflects the parental food intake. Cholesterol levels in gonads in relation with maturity is also studied by Singh and Singh (1979), Sen and Bhattacharya (1981), Naurial and Singh (1985) and (Diwan and Krishnan (1986).

Carotenoids are considered to be the ultimate source of vitamin A and are found to be derived from the diet. Carotenoids are widely distributed in the skin and serves for camouflaging. These terpene compounds are also deposted in the ovary during maturation and are attributed with the functions of impairing colouration and viability to the eggs. While Matsuno et al. (1985) have reported the change in the composition of carotenoids during maturation of marine fish eggs, there is evidence of accumulation of carotenoids in the muscle, liver and skin and further mobilisation into gonads during maturation in Salmonids

(Steven, 1948, 1949; Kithara, 1983). However, there has been little evaluation on the relation between egg viability and its pigmentation (Craik, 1985).

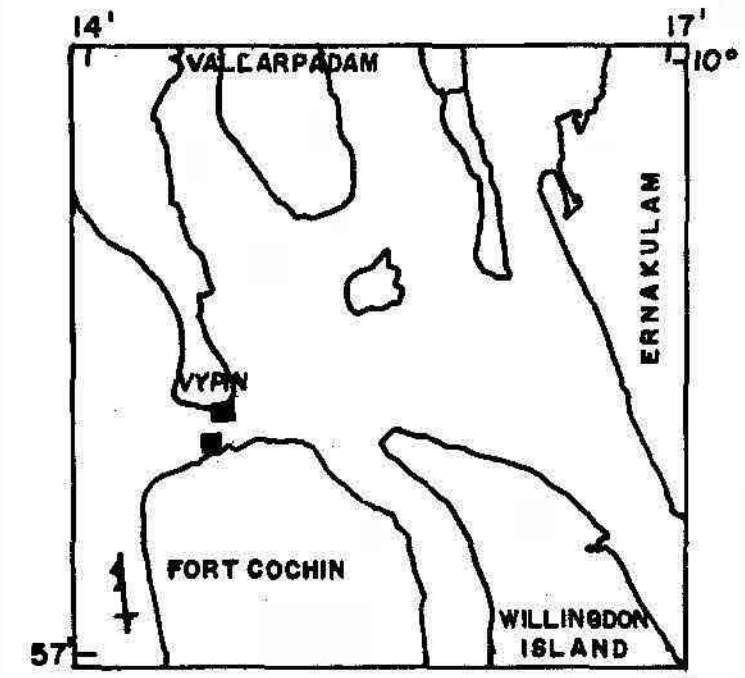
The role of trace elements in fish are in many cases completely unknown (Berman and Vitin, 1968). These authors found that requirements of trace elements differ at different stages of maturation. However, since mineral composition of gonad is very low in comparison with the content in the body as a whole, the increase in gonad minerals signifies exclusively the growth of fish proper. Further, as Shul'man (1972) points out, there is a close relationship between the trend and intensity of protein and mineral metabolism in fishes.

Investigations on the biochemical composition of fish enables us to unveil the potential storehouse of various nutrients and to trace the pathways through which they are mobilised for the biological needs of the fish. Further, the levels of these substances could indicate the "quality" of the eggs and exact stage of maturation of the fish (Love, 1970) and so there appears to be potential in continuous effort in this field.

These facts and the lack of proper information on the biochemical composition of mullets in relation with maturity has prompted to take up the present work. The biochemical composition of three tissues of L. parsia namely muscle, liver, and gonad are studied. Seven parameters viz. moisture, protein, fat, glycogen, cholesterol, carotenoid, and ash are estimated in relation to the maturation of the gonad.

Plate 1: The Cochin barmouth region from where the samples were collected...

... Note the Chinese dip nets in Operation



■ SITES OF SPECIMEN COLLECTION AT THE COCHIN BARMOUTH

MATERIALS AND METHODS

COLLECTION OF SPECIMENS

The fish samples were collected bimonthly during the period from February to September, 1987 from the barmouth area of Cochin estuary. They were caught in the Chinese dipnets operated during the hours of high tide every day. The specimens collected had a length range of 115 to 236 mm.

The fish after collection were carried in icebox to the laboratory where they were washed thoroughly. They were measured for their total lengths as well as standard lengths. Their body weights also were taken accurately using a sensitive balance. The specimens were then cut open to identify the sex. Males were discarded from the collection and females alone were used for further analyses. Maturity stages of females were determined based on the macroscopic appearance of the ovary like shape and size in relation to body cavity (Bowers, 1954), colour (Graham, 1924), extent of yolk formation and microscopic atructure such as ova diameter measurements (Clark, 1934). The gonads were assigned to five various reproductive stages viz. Stage I (immature), stage II (maturing virgins and recovering spent), Stage III (ripening), Stage IV (ripe) and Stage V (Spent) following the method suggested by Qazim (1973) and adopted by Kurup and Samuel (1983).

Tissues of muscle, liver and gonad were removed from the samples. Muscle tissue was taken from the base of the first dorsal fin, care being taken not to include any skeletal parts. Weights of the whole liver and gonad were also noted to the nearest mg. Enough portions of all the tissues were used for moisture content analysis and a part was frozen till analyses were carried out.

Simultaneous with each sampling of the fish, water temperature, salinity and dissolved oxygen of the site of collection were determined. Salinity was estimated using Mohr titration and oxygen by Winkler's iodometric method (Strickland and Parsons, 1968).

1. BIOCHEMICAL ANALYSES

i) Estimation of moisture content:

The sample prepared as above were washed in distilled water and the excess moisture removed using a blotting paper. The tissues were weighed accurately and later dried in an air oven at 70°C to constant weight. The moisture content was calculated as the difference between the wet weight and dry weight of the tissue which represents the loss at 70°C and this is expressed as percentage of wet weight.

The samples dried in this manner were powdered in a mortar, transferred to labelled polythene bags and stored in dessicator for carrying out analysis of ash content and lipid content.

ii) Estimation of total protein:

For the estimation of total protein, the method of Lowry et al. (1951) was followed.

25 mg of the wet tissue was weighed accurately and after adding 2 ml of deproteinising agent (5% Trichloro acetic acid), was homogenised thoroughly. When the protein was completely precipitated, the sample was subjected to centrifugation at 3000 rpm for 5 minutes. The supernatant was removed and used for glycogen analysis. To the precipitate, 4 ml of 1 N NaOH was added for dissolving the protein. From the resultant solution, 0.5 ml was pipetted out and was made upto 1 ml with 1 N NaOH. To this, 5 ml of alkaline mix (50 ml of 2% Na_2CO_3 in 0.1 N NaOH + 1 ml of 0.5% $CuSO_4$ in 1% Sodium tartrate prepared afresh) was added and kept at room temperature for 10 minutes. After 10 minutes, 0.5 ml of Folin ciocalteau's reagent (diluted to 50% with distilled water) was added.

A standard solution was prepared using bovine serum albumin crystals at a concentration of 25 mg/100 ml and aliquotes in the range 25-250 µg was prepared and the same procedure as for unknown was followed.

A blank was prepared with 1 ml 1 N NaOH following the same procedure as above.

All the test tubes were left at room temperature for 30 minutes and readings were taken at 660 mm using a spectrophotometer.

A standard graph was plotted and amount of proteins in the sample compared and calculated from the graph.

iii) Estimation of glycogen:

Glycogen was estimated by the method suggested by Viles et al. (1949) using anthrone reagent (0.2% anthrone in concentrated H_2SO_4). The sulphuric acid in anthrone reagent hydrolyses glycogen into glucose and then dehydrates into furfural which gives a coloured complex with anthrone.

The supernatant obtained from the sample for protein estimation was used for the analysis.

0.5 ml of the supernatant was taken and made upto 1 ml with distilled water. To this, 4 ml of anthrone reagent was added and kept in a water bath for 10-15 minutes and then cooled to room temperature in dark. The optical density was determined at 620 mm using a spectrophotometer. The readings were compared with a blank prepared using 1 ml distilled water and 4 ml anthrone reagent.

A standard was prepared using D-glucose (concentration 10 mg/100 ml) and aliquotes of the range 10 µg to 100 µg were

used to plot a standard graph. The value obtained was divided by 1.11, a conversion factor of glucose to glycogen.

iv) Estimation of lipids:

The dried tissue powdered and stored in the dessicator was used for the estimation of lipid content. The method followed was that of Bligh and Dyer (1959), using chloroform-methanol mixture in the ratio 2:1 v/v. To the weighed amount (100 mg) 3 ml of choloroform-methanol mixture was added, mixed thoroughly and kept overnight at room temperature in dark. At the end of the period, another addition of 2 ml of chloroform and 2 ml of water was made. The resulting solution was subjected to centrifugation, when generally 3 layers were seen viz. a clear lower layer of chloroform containing all lipids, an upper coloured aqueous layer of methanol with all water soluble material and a thick pasty interface.

The methanol layer was discarded and the lower layer was carefully collected free of interphase by sucking with a fine capillary or by filtration through glass wool. The organic layer taken in a preweighed beaker and carefully evaporated in an air oven at 60°C in order to obtain the residual fat. The beaker with the content was weighed after cooling to room temperature. The difference in weight gave the weight of lipids. Its percentage was calculated on the

weight of dry tissue taken and finally converted to wet tissue basis.

v) Estimation of total cholesterol:

The method followed for the estimation of cholesterol was that of Henly (1957), a modification of Hestrin's(1949) method.

25 mg of wet tissue was taken and was subjected to homogenisation. To this, 10 ml of 0.05% $FeCl_3.6H_2O$ prepared in purified acetic acid was added and left for precipitation of protein. When the precipitation was complete, the tube was centrifuged at 3000 rpm for 5 minutes. 5 ml of the supernatant was taken in a fresh test tube to which 3 ml of conc.H_2SO_4 was added. The tube was kept at room temperature for 20 minutes after thorough mixing. The colour developed was read at 560 nm using a spectrophotometer against a blank prepared using 5 ml $FeCl_3.6H_2O$ glacial acetic acid and 3 ml concentrated H_2SO_4.

The standard solution used had a concentration of 100 mg pure cholesterol in 100 ml of glacial acetic acid diluted in the ratio 1:25. The cholesterol content was calculated using the formula:

$$\text{Cholesterol content} = \frac{\text{O.D. of unknown}}{\text{O.D. of standard}} \times \frac{0.2}{12.5} \times 100$$

vi) Estimation of carotenoids:

Olson's (1979) method was followed to estimate the total carotenoid content in various tissues. In this method chloroform stabilised with 0.75% absolute ethanol was used to extract the carotenoids.

The samples of tissues quickly removed from the fish were placed in glass vials closed with tefflon stopper and stored in deep freezer until analysis. The samples were analysed within a week of storage.

1 mg of tissue prepared as above was weighed quickly and placed in a 10 ml screw cap clean glass vial. To this 2.5 gm of anhydrous sodium sulphate was added and this sample gently mashed with a glass rod against the side of the vial until it is reasonably well mixed with sodium sulphate. 5 ml of chloroform was added and the vial was sealed and placed at 0°C overnight (8-24 hours). When chloroform formed a clear layer of 1-2 cm weight above the caked residue, the optical density was read at 380 nm, 450 nm and 500 nm taking 0.3 ml aliquotes of chloroform diluted to a colume of 3 ml with ethanol. A blank prepared in a similar manner was used for comparison. The wavelength at which maximum absorption obtained was used for calculation.

The total carotenoid content was calculated as µg carotenoid/gm tissue.

$$\text{Carotenoid content} = \frac{\text{Absorption at 450 nm X dilution factor}}{\text{0.25 X sample weight (gm)}}$$

In this case, dilution factor = 50, 0.25 is extinction coefficient.

vii) Estimation of ash content:

A known quantity of the oven dried sample was ignited in silica crucible at 600°C in a muffle furnace till the organic matter was burnt out leaving no carbon residue. The content was weighed and difference gave the ash content %.

2. LIVER INDEX

The liver index was calculated in specimens at different maturity stages using the formula

$$\text{Liver index} = \frac{\text{weight of liver}}{\text{weight of fish}} \times 100$$

The average of the liver index values at each maturity stages were calculated.

3. GONADO-SOMATIC INDEX (GSI):

The gonadosomatic index was determined in each maturity stage in various individuals with a view to ascertaining the condition of the gonad in each stage. The GSI was determined using the formula:

$$GSI = \frac{\text{weight of gonad}}{\text{weight of fish}} \times 100$$

The average of all the GSI and the range was determined in each maturity stage.

4. MICROSCOPIC EXAMINATION OF OVARIES :

Around 500 ova were measured from 3 samples of ovaries at each maturity stage using an ocular micrometer in order to determine the ova diameter frequency in various stages (Clark, 1934; Prabhu, 1955). No specific area was chosen to take the eggs as according to Shehadeh et al (1973b), mullet oocytes develop in synchrony and so samples taken from any area in the ovary would be representative of the entire ovary.

1 micrometer division was found to be equivalent to 13.1 μ. Ova diameter frequency polygons were drawn after grouping the ova into 50 μ class intervals.

5. STATISTICAL ANALYSIS:

All the biochemical analyses except ash content analysis and carotenoid analysis were done atleast 5 times and hence all the values were pooled up and standard deviation was calculated.

Analysis of variance was carried out to ascertain the possible difference in the contents of various parameters in the three tissues namely muscle, liver and gonad at 5% level of significance.

R E S U L T S

DESCRIPTION OF SPECIES

LIZA PARSIA (HAMILTON-BUCHANAN) (Plate 2)

SYNONYM : MUGIL PARSIA HAMILTON-BUCHANAN 1822

VERNACULAR NAME : GOLD-SPOT MULLET

D IV; I 8 A III, 8-9. P 14; C 14 L lat 31-36 Tr. 11-12

DISTINCTIVE CHARACTERISTICS:

Body slender, head moderately wide, dorsally flattened. Head length 23-26% of standard length; fatty (adipose) tissue covers most of the iris posteriorly and part of it anteriorly; lips thin, lower lip with a high symphysial knob; hind end of upper jaw reaching vertical between posterior nostril and anterior rim of eye; mid gape above horizontal through centre of the pupil; teeth - labial, 2 rows of short teeth in upper lip, lower lip toothless; preorbital bone wide, filling the space between lip and eye, notched anteriorly; Fins : First dorsal fin origin nearer to snout tip than to caudal fin base - above 11th scale and second above 22nd scale; pectoral axillary scale absent; pectoral fin 76-79% of head length; anal fin with 3 spines and 8-9 soft rays; second dorsal and anal fins densely scaled. Scales in lateral series 31-36.

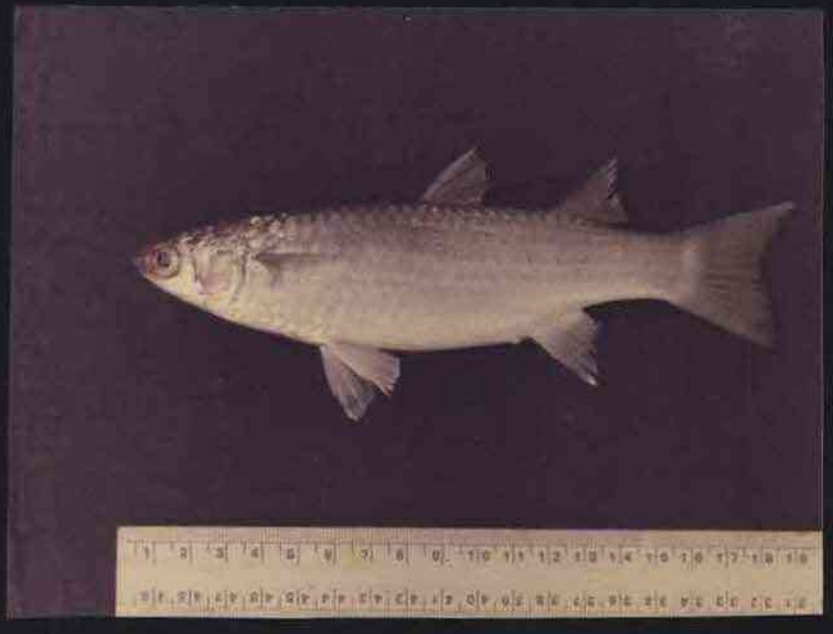


Plate 2: Liza parsia (Hamilton-Buchanan) - female

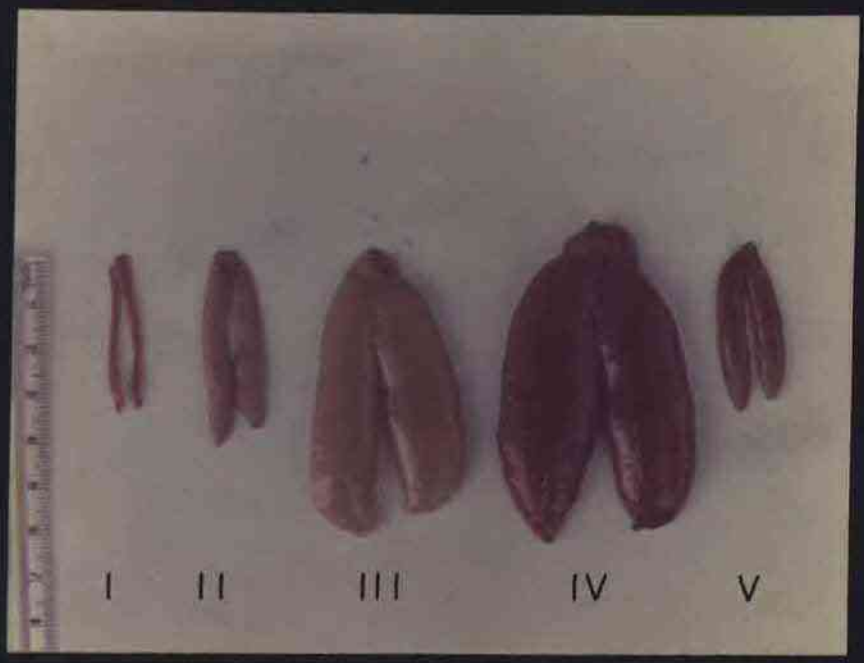


Plate 3: Various maturity stages of female L. parsia I. Immature, II. Maturing, III. Mature, IV. Ripe and V. Spent.

Colour: Greenish-brown above, white to silvery below; a golden spot on upper operculum; base of second dorsal, anal and caudal fins yellowish, other fins off-white with dusky margins.

Size : Maximum size of species recorded is 33 cm, though commonly available size range is 15 - 16 cm.

DISTRIBUTION:

A common species in India distributed along the west coast, south of Bombay and all along the east coast. This species is abundant in the coastal waters, estuaries, lagoons, and intertidal rivers of Kerala. It is also recognised in Andaman islands. Outside India, distribution is restricted to Indo-Pacific area, where it occurs in the sea and brackish water in Indonesia, Philippines and Thailand, Hongkong, Australia, Ceylon and Karachi.

CLASSIFICATION OF MATURITY STAGES (Plate 3)

Five maturity stages were identified based on the size shape and colour of the ovary and the microscopic structure of the ova, as suggested by Qazim (1973) and adopted by Kurup and Samuel (1983).

Stage I (Immature): Ovary occupied less than one-fourth of the body cavity, pinkish coloured, translucent and jelly like in appearance. The eggs very small and irregular without any yolk formation started. Ova diameter ranged from 26 μ to 196.5 μ.

Stage II (Maturing virgin and Recovering spent): The ovary is almost half that of the body cavity, slightly yellowish, Eggs are white to pale yellow with traces of yolk, granular and still not yet fully rounded. Diameter of ova ranged from 26 μ to 288 μ.

Stage III (Ripening or Mature): Gonad occupies three-fourth or even more of the body cavity, ova becoming yellowish and opaque with deposition of yolk material. Eggs not fully rounded, with the egg diameter ranging from 26 μ to 500 μ.

Stage IV (Ripe): The entire body cavity is filled with the ovary, but for the space occupied by the viscera. Ovary deeply yellowish and has conspicous blood vessels. Some ripe ova are visible to the exterior at the vent region. Yolk is found as a homogenous mass interspersed with vacuoles and filling the interior of the oocytes. The ova diameter ranges from 26 μ to 694

Stage V (Spent): Ovary occupies approximately half of the body cavity; dark pinkish, appearing flaccid, translucent and shrunken. Ova diameter ranges from 26 μ to 650 μ.

PROXIMATE COMPOSITION ANALYSES

1. MUSCLE (Table 1; Fig. 1)

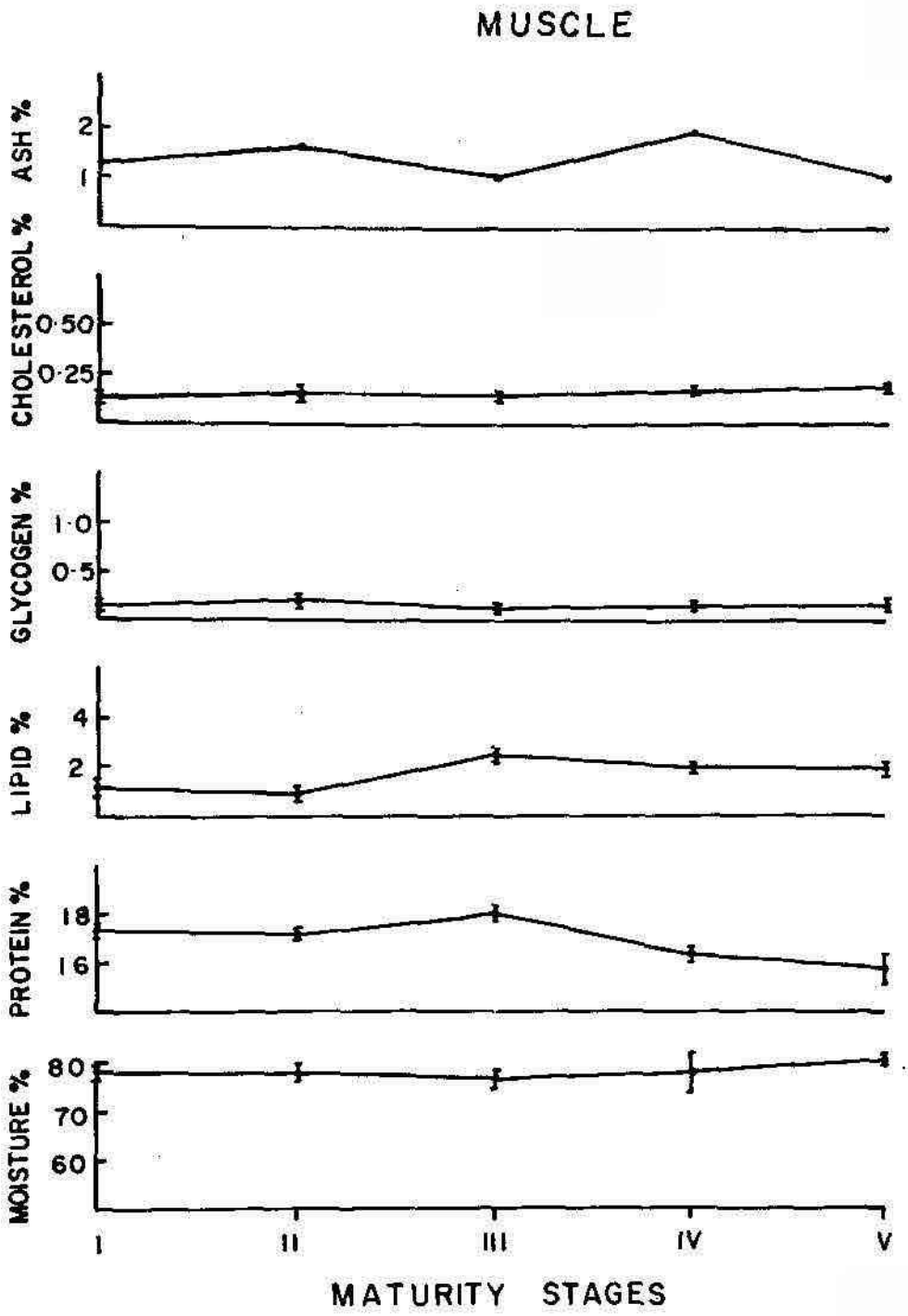
i. Moisture: Moisture content was in the range 76.30 to 80.23%. From 78.02% in stage I, the value decreased to 76.30% in Stage III thereafter showing an increase to 80.23% in Stage V.

Table 1: Variations in biochemical composition in the muscle of *Liza parsia*

	Stage I	Stage II	Stage III	Stage IV	Stage V
Moisture %	78.02 ± 1.66*	77.48 ± 1.61	76.30 ± 1.61	77.87 ± 3.56	80.23 ± 0.90
Protein %	17.30 ± 0.33	17.15 ± 0.12	18.00 ± 0.20	16.28 ± 0.23	15.72 ± 0.56
Lipid %	1.18 ± 0.31	0.87 ± 0.19	2.54 ± 0.19	1.94 ± 0.12	1.92 ± 0.15
Glycogen %	0.13 ± 0.01	0.21 ± 0.02	0.12 ± 0.02	0.14 ± 0.01	0.16 ± 0.01
Cholesterol %	0.13 ± 0.01	0.16 ± 0.05	0.14 ± 0.01	0.17 ± 0.01	0.18 ± 0.01
Ash %	1.22	1.62	1.07	1.94	1.03

*All values are means ± S.D. except Ash %

Fig.1. Variations in biochemical composition in the muscle of *Liza parsia*.


MUSCLE
ASH %
2
1
CHOLESTEROL %
0·50
0·25
GLYCOGEN %
1·0
0·5
LIPID %
4
2
PROTEIN %
18
16
MOISTURE %
80
70
60
I
II
III
IV
V
MATURITY STAGES

ii. Protein: Protein content was the highest in stage III being 18.00%. The concentration showed a declining trend in the succeeding stages reaching a lowest level of 15.72% in stage V. The values were almost constant in the first two stages being 17.30% and 17.15% in stage I and II respectively.

iii. Lipid: Lipid content was the minimum in stage II (0.87%) and the maximum in stage II (2.54%). Beyond stage III, the lipid level was found decreasing with a value of 1.92% in stage V. In stage I, the lipid content was 1.18%.

iv. Glycogen: On the whole, glycogen content was comparatively less in the muscle with a range of 0.12% to 0.20% only. The minimum concentration was obtained at stage III and the maximum at stage II. From stage III onwards, an ascending trend was obvious.

v. Cholesterol: Cholesterol showed a pattern similar to that of glycogen with the values increasing from stage III (0.14%) to stage V (0.18%). In stage I, the cholesterol content was 0.13% which increased to 0.16% in stage II. The range was 0.13%-0.18%.

vi. Ash: Ash content with a range 1.03% to 1.94% was the maximum in stage IV and minimum in stage V. Generally, the mineral level showed an alternative increasing and decreasing pattern.

Table 4 : Carotenoid content in various tissues of *Liza parsia*

Stage of gonad	Muscle µg/g	Liver µg/g	Gonad µg/g
I	6.20	9.00	3.20
II	7.72	10.00	7.00
III	5.86	10.00	10.00
IV	2.00	9.29	12.05
V	1.80	9.87	2.81

Fig.4. Carotenoid content in various tissues of *Liza parsia*.

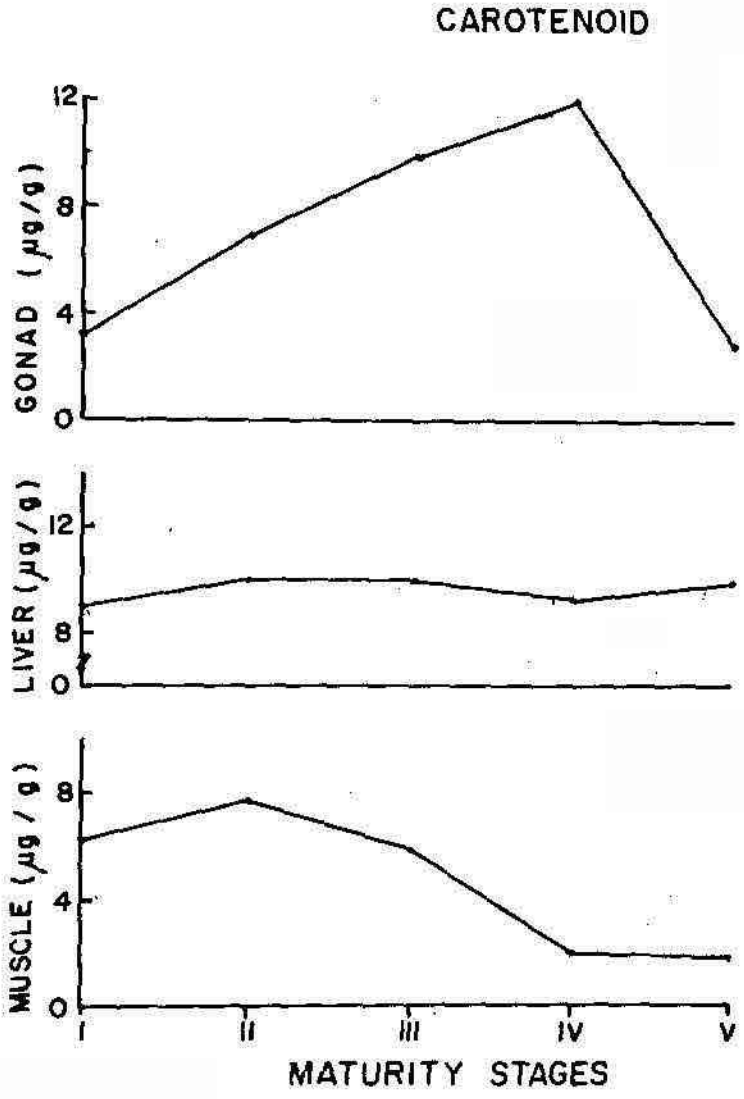
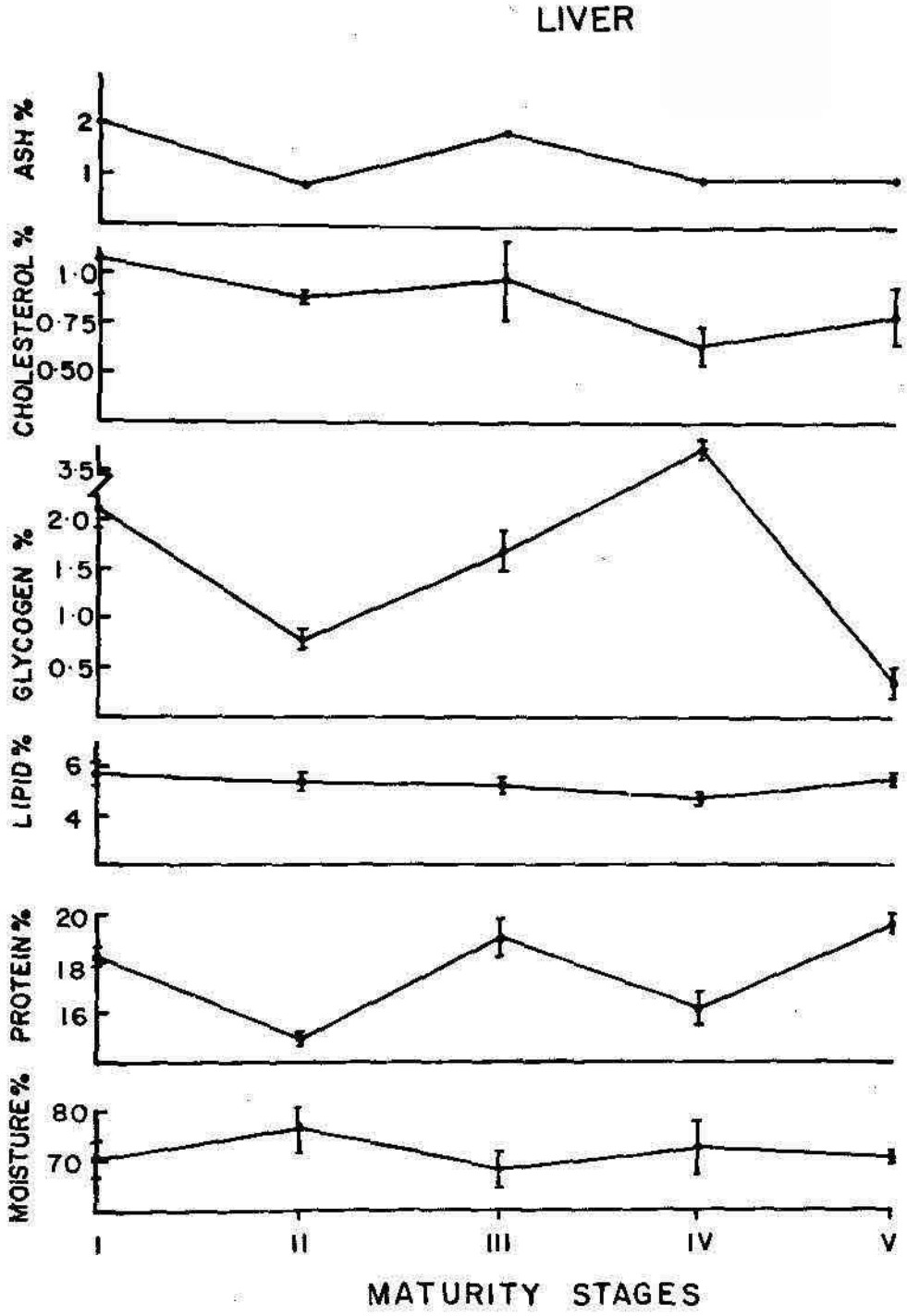

CAROTENOID
GONAD (µg/g)
12
8
4
0
LIVER (µg/g)
12
8
0
MUSCLE (µg/g)
8
4
0
I
II
III
IV
V
MATURITY STAGES

Table 2: Variations in biochemical composition in the liver of Liza parsia

	Stage I	Stage II	Stage III	Stage IV	Stage V
Moisture %	70.61 ± 3.29*	76.42 ± 4.59	68.06 ± 3.49	72.21 ± 5.41	70.33 ± 0.90
Protein %	18.21 ± 0.37	14.86 ± 0.19	19.07 ± 0.82	16.12 ± 0.63	19.65 ± 0.41
Lipid %	5.77 ± 0.40	5.32 ± 0.34	5.18 ± 0.34	4.76 ± 0.04	5.50 ± 0.22
Glycogen %	2.12 ± 0.06	0.79 ± 0.10	1.69 ± 0.20	3.75 ± 0.11	0.37 ± 0.20
Cholesterol %	1.07 ± 0.21	0.88 ± 0.02	0.97 ± 0.20	0.65 ± 0.26	0.79 ± 0.15
Ash %	2.04	0.83	1.92	0.98	0.98

* All values are means ± S.D. except Ash %

Fig.2. Variations in biochemical composition in the liver of *Liza parsia*.


LIVER
ASH %
2
1
CHOLESTEROL %
1·0
0·75
0·50
GLYCOGEN %
3·5
2·0
1·5
1·0
0·5
LIPID %
6
4
PROTEIN %
20
18
16
MOISTURE %
80
70
I
II
III
IV
V
MATURITY STAGES

vii. Carotenoid (Table 4; Fig. 4): Carotenoid content was at its minimum in stage V being 1.80 µg/g while the maximum was noticed at stage II (7.72% µg/g). The levels showed a steady decrease from stage II to stage V. In stage I the value was 6.20 µg/g.

2. LIVER (Table 2; Fig.2)

i. Moisture: In liver, moisture content ranged from 68.06% at stage III to 76.42% at stage II. The level showed an alternative increasing and decreasing pattern.

ii. Protein: The maximum concentration of protein was found in stage V (19.65%) and a minimum in stage II (14.86%). But for a decrease in stage IV (16.12%), the value indicated an increasing pattern from stage II to stage V.

iii. Lipid: Lipid levels were in the range 4.76% to 5.77% indicating an equable decrease from 5.77% in stage I to 4.76% in stage IV. In stage V the level showed recovery reaching a level of 5.50%.

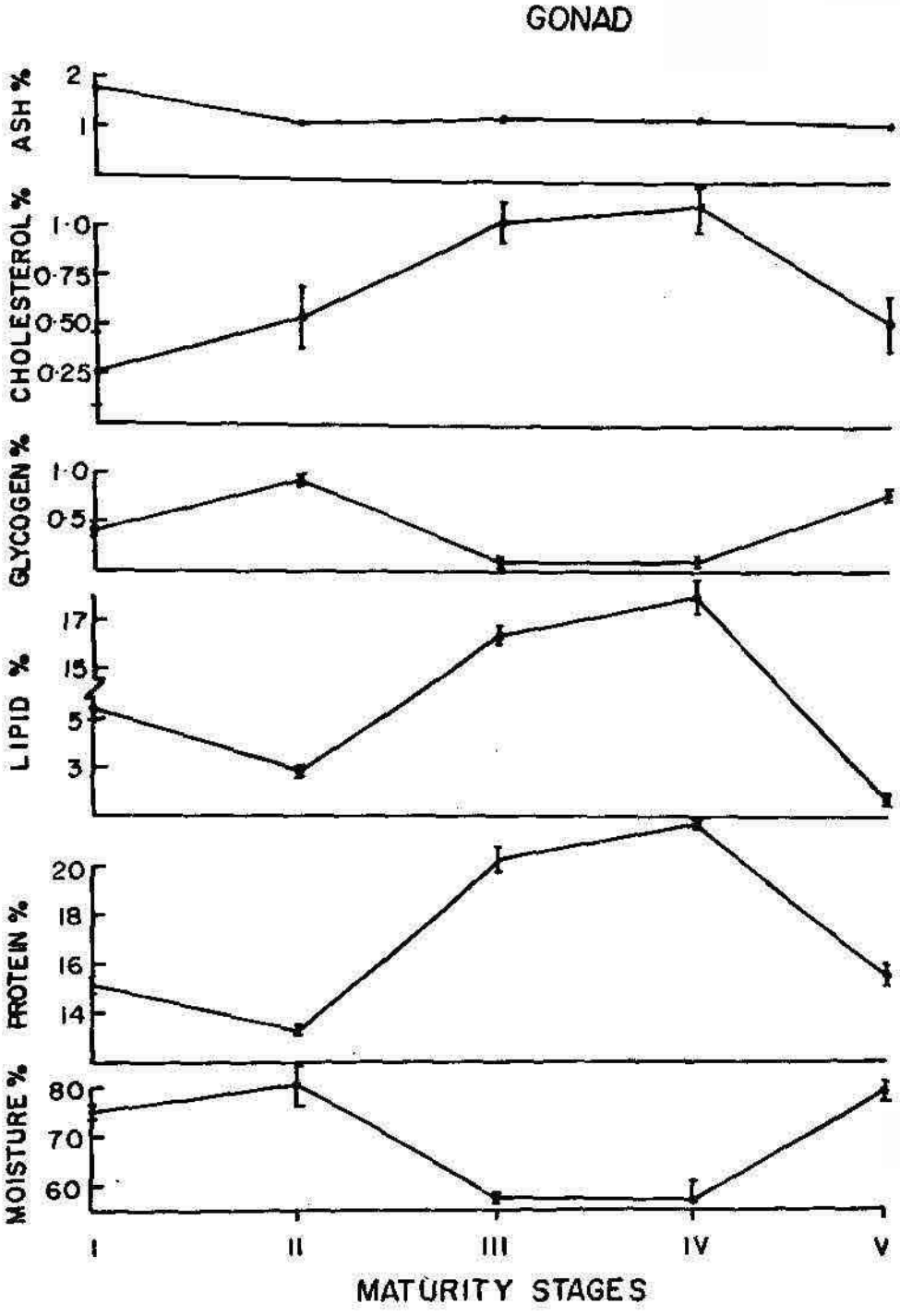
iv. Glycogen: Glycogen showed its highest level in liver, of all tissues. The maximum amount was found in stage IV (3.75%) while the minimum was in stage V (0.37%). From a level of 2.12% in stage I, it decreased to 0.79% in stage II and thereafter continued to increase steadily upto stage IV.

Table 3: Variations in the biochemical composition in the gonad of *Liza parsia*

	Stage I	Stage II	Stage III	Stage IV	Stage V
Moisture %	75.35 ± 0.94*	80.68 ± 4.32	57.10 ± 0.84	56.77 ± 3.24	79.16 ± 1.93
Protein %	15.21 ± 0.25	13.28 ± 0.09	20.33 ± 0.54	21.81 ± 0.08	15.51 ± 0.42
Lipid %	5.37 ± 0.08	2.83 ± 0.12	16.43 ± 0.41	18.03 ± 0.77	1.75 ± 0.14
Glycogen %	0.43 ± 0.02	0.93 ± 0.02	0.12 ± 0.01	0.11 ± 0.02	0.80 ± 0.04
Cholesterol %	0.26 ± 0.18	0.54 ± 0.15	1.05 ± 0.09	1.13 ± 0.14	0.53 ± 0.14
Ash %	1.77	1.16	1.29	1.30	1.21

*All values are means ± S.D. except Ash %

Fig.3. Variations in biochemical composition in the gonad of *Liza parsia*.


GONAD
ASH %
2
1
CHOLESTEROL %
1·0
0·75
0·50
0·25
GLYCOGEN %
1·0
0·5
LIPID %
17
15
5
3
PROTEIN %
20
18
16
14
MOISTURE %
80
70
60
I
II
III
IV
V
MATURITY STAGES

v. Cholesterol: Cholesterol content showed a pattern of continuous decrease from stage I to stage IV, where it was found in least amounts. Later the content increased to slightly higher value in stage V (0.79%). The range was found to be 0.65%(stage IV) to 1.07% (stage I).

vi. Ash: Ash content had a range of 0.83% in stage II to 2.04% in stage I. From the maximum level in stage I, the value declined to the lowest in stage II shooting up again in stage III. Thereafter it decreased in stage IV and V.

vii. Carotenoids (Table 4; Fig. 4): Liver carotenoid showed almost a steady value of around 10 µg/g. The level was minimum at the first stage (9.0 µg/g) and a maximum at stages II and III (10.0 µg/g). The levels were 9.29 µg/g and 9.87 µg/g in stage IV and V respectively.

3. GONAD (Table 3; Fig. 3)

i. Moisture: Moisture content was found highly fluctuating in the gonad. The value showed a decrease from its maximum value of 80.68% in stage II to 56.77% in stage IV where it was the minimum. Subsequently, it recovered to 79.16% in stage V.

ii. Protein: The range of protein was 13.28% to 21.81% with the maximum value in stage IV and minimum in stage II. Except for stage II, when there is a decrease, the concentration

increased steadily from stage I to stage IV. The value decreased again to 15.51% in stage V.

iii. Lipid: Following the same pattern was protein, the lipid level showed an increase from stage I (5.37%) to stage IV (18.03%) with a decrease in stage II (2.83%) alone. The increasing rate was remarkably high as the range was from 5.37% to 18.03%. In stage V the value was 1.75%.

iv. Glycogen: Glycogen showed the maximum value in stage II and minimum in stage IV, the range being 0.11% to 0.93%. From a level of 0.43% in stage I, it increased in stage II to 0.93% decreasing thereafter to levels of 0.12 and 0.11% in stage III and IV respectively. Stage V encountered a increase upto 0.80%.

v. Cholesterol: Cholesterol levels indicated a constant increase from 0.26% in stage I to 1.13% in stage IV, with the value decreasing to 0.53% in stage V.

vi. Ash: Ash content was at its maximum in stage I (1.77%) and minimum in stage II (1.16%). The value increased upto 1.30% in stage IV with a further decrease to 1.21% in stage V.

vii. Carotenoid: (Table 4; Fig. 4) Gonad carotenoid levels were fluctuating between 2.18 µg/g in stage V and 12.05% µg/g in stage IV. The value was found increasing from 3.20 µg/g in stage I to 12.05 µg/g in stage IV with a lag to 2.18 µg/g in stage V.

From a close scrutiny of the results obtained in the investigations on the biochemical composition the following relationships were obvious. A clear inverse relationship was discernible between water and protein in all the three tissues studied viz. muscle, liver and gonad. An inverse relationship between moisture and lipid was also evident in all the tissues.

Glycogen showed an inverse relation with protein and lipid mainly in the gonad.

With the advent of maturation, the lipid content and protein content increased in the gonad. Corresponding the lipid content decreased in liver and protein content in muscle, except for an obscure increase of protein content in muscle in stage III. So a possible mobilisation of lipid from liver and protein from the muscle can be suggested.

High levels of glycogen in the earlier stages of maturation in gonads started declining to negligible values in stage III and stage IV. Whereas glycogen levels increased enormously in the liver upto stage IV.

Cholesterol levels decreased in liver accompanied by an increase in gonad confirming the chances of mobilisation of the same from liver to gonad and its utilisation for gonadal development.

Table 5: Liver index at various maturity stages of *Lisa parsia*

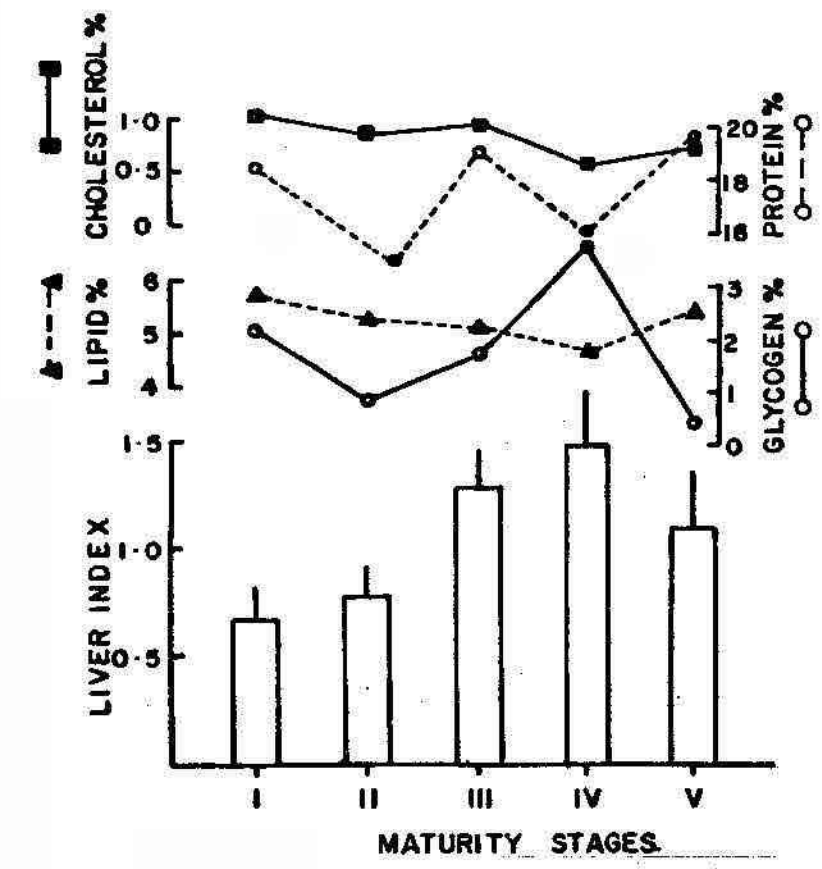
Stage of gonad	Mean ± S.D.
I	0.682 ± 0.16
II	0,783 ± 0.15
III	1.290 ± 0.17
IV	1.500 ± 0.27
V	1.130 ± 0.25

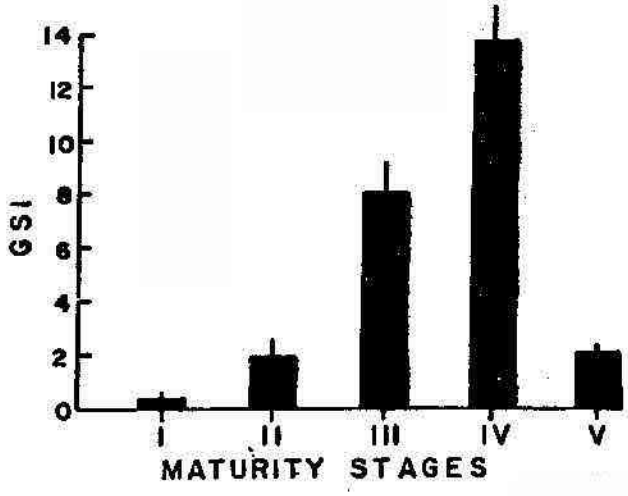
Table 6: Gonado somatic index (GSI) at various maturity stages of *Liza parsia*

Stage of Gonad	Range	Mean ± S.D.
Stage I	0.26 - 0.56	0.38 ± 0.16
Stage II	1.01 - 2.56	1.96 ± 0.70
Stage III	6.24 - 9.14	8.09 ± 1.07
Stage IV	11.49 -17.30	13.82 ± 1.39
Stage V	1.78 - 2.52	2.08 ± 0.30

Fig.5. Liver index at various maturity stages in *Liza parsia* in comparison with various biochemical parameters.

Fig.6. Gonado somatic index at various maturity stages in *Liza parsia*


CHOLESTEROL %
1·0
0·5
0
PROTEIN %
20
18
16
LIPID %
6
5
4
GLYCOGEN %
3
2
1
0
LIVER INDEX
1·5
1·0
0·5
I
II
III
IV
V
MATURITY STAGES


GSI
14
12
10
8
6
4
2
0
I
II
III
IV
V
MATURITY STAGES

The analysis also reveals the possibility of mobilisation of carotenoids from muscle to gonad with the advancement of maturity.

LIVER INDEX (Table 5; Fig. 5)

The liver index which is the percentage of liver to body weight was found to increase gradually from immature stage to the mature stage of the fish. The index was maximum during the ripe condition (stage IV) after which there was a decline in the weight during the resting stage.

The liver index at stage I and II were 0.682 and 0.783 respectively. The value increased to 1.290 at stage III and further to 1.500 at stage IV after which there was fall in stage V to 1.130.

GONADO-SOMATIC INDEX (Table 6; Fig. 6)

The mean gonado-somatic index was found to be 0.38 in stage I with 2 range of 0.26 to 0.56. The gonado-somatic index increased to a mean value of 1.96 in stage II with the index ranging from 1.01 to 2.56. In stage III the GSI was quite high compared to that of the previous stages. With a range of 6.24 to 9.14, the mean was calculated to be 8.09. The gonado-somatic index was comparitively high during the ripe stage IV with a range of 11.49 to 17.3.

Immediately after spawning, the GSI showed a steep decrease. The GSI value which was 13.82 in stage IV had come down to 2.08 during spent stage. The values were found varying between 1.78 and 2.52.

PROGRESSION OF OVA TOWARDS MATURITY

With a view to tracing the development of ova from immature stage to ripe condition, ova diameter measurements were taken from ovaries at various stages. The ova diameter measurements were grouped in 50 μ intervals and the details are given in Table 7 and Fig. 7.

Stage I had only immature ova, the oocytes ranging in their diameter from 26 μm to 196.5 μm. A prominent mode was found at 50 μ. In stage II, a gradual growth of the ova was discernible. The largest ovum measured 288 μ. With a mode at 150 μ, there was only one batch of eggs observed apart from the immature stock. A further progression of ova was obvious in stage III, with the developing batch of eggs shi ing forward forming a distinct mode at 400 μ.. The largest ovum measured 500 u. Mature ova of stage IV showed one distinct, well differentiated group of eggs with a mode 600 μ. The maximum ova diameter encountered was 694 μ. Simultaneously, the immature stock has further developed forming a mode at 150 μ.

Table 7 : Ova diameter percentage frequency at various maturity stages of *Liza parsia*

µm	Stage I	Stage II	Stage III	Stage IV	Stage V
1 - 50	55.4	19.4	13.0	12.1	29.6
51 - 100	30.2	14.9	9.2	3.9	16.8
101 - 150	10.6	32.5	1.8	11.4	25.5
151 - 200	3.8	24.2	1.0	1.9	15.0
201 - 250	-	4.9	3.5	0.8	3.2
251 - 300	-	4.1	4.5	1.0	2.2
301 - 350	-	-	7.2	1.9	1.0
351 - 400	-	-	28.8	1.1	0.8
401 - 450	-	-	16.0	0.9	0.8
451 - 500	-	-	15.0	2.0	0.6
501 - 550	-	-	-	20.1	1.8
551 - 600	-	-	-	28.0	1.7
601 - 650	-	-	-	12.9	1.0
651 - 700	-	-	-	2.0	-

Fig.7. Ova diameter percentage frequency at various maturity stages of *Liza parsia*.

Table 8: Seasonal variations in Dissolved oxygen, salinity, and temperature at the site of collection.

Months	Temperature °C	Oxygen ml/ltr.	Salinity ‰
February	31.5	3.60	31.20
March	32.0	3.80	33.00
April	31.6	3.66	31.45
May	32.0	2.20	29.45
June	28.5	3.05	10.00
July	26.3	2.42	16.05
August	26.2	1.90	28.50
September	26.0	2.35	29.66

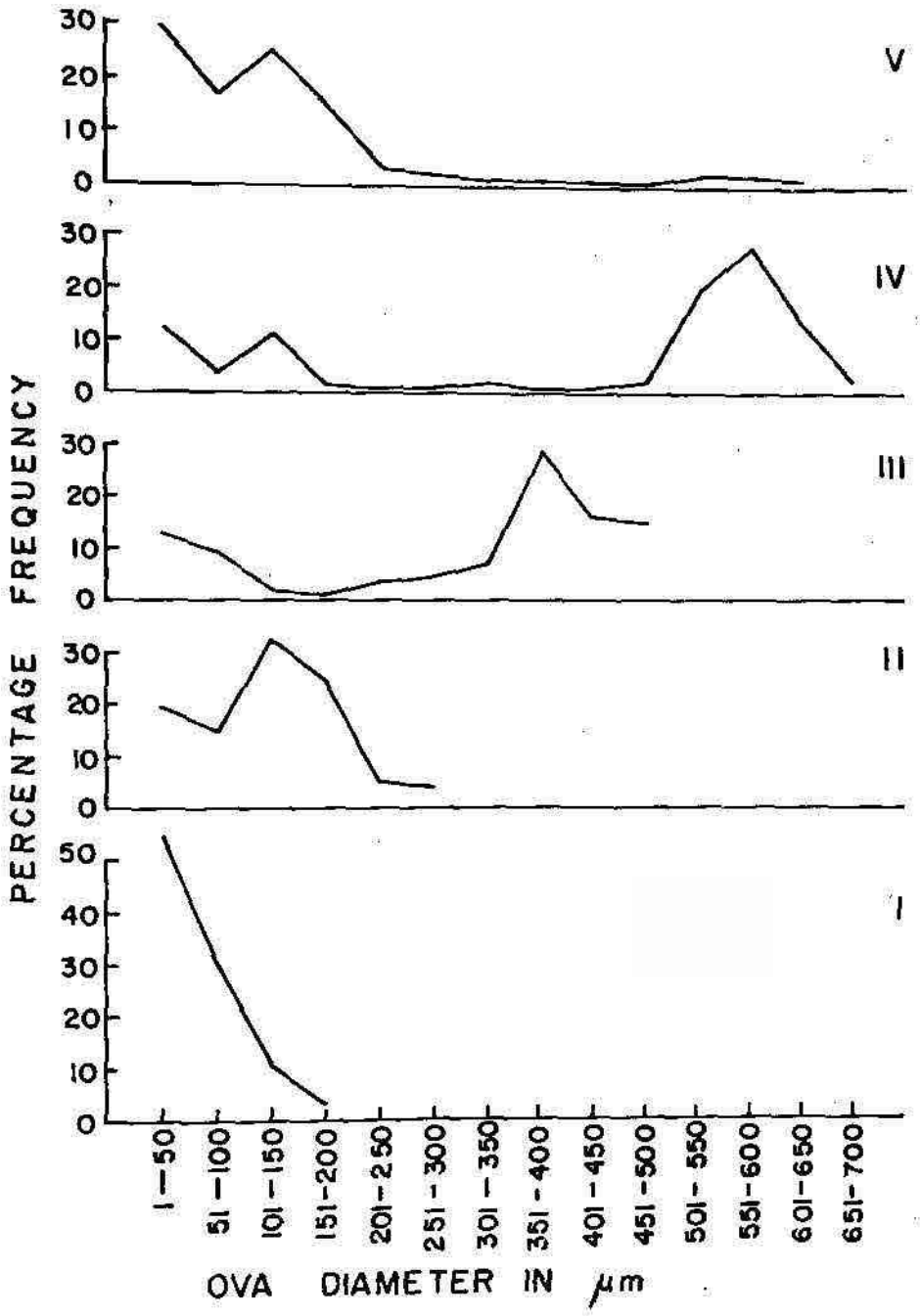
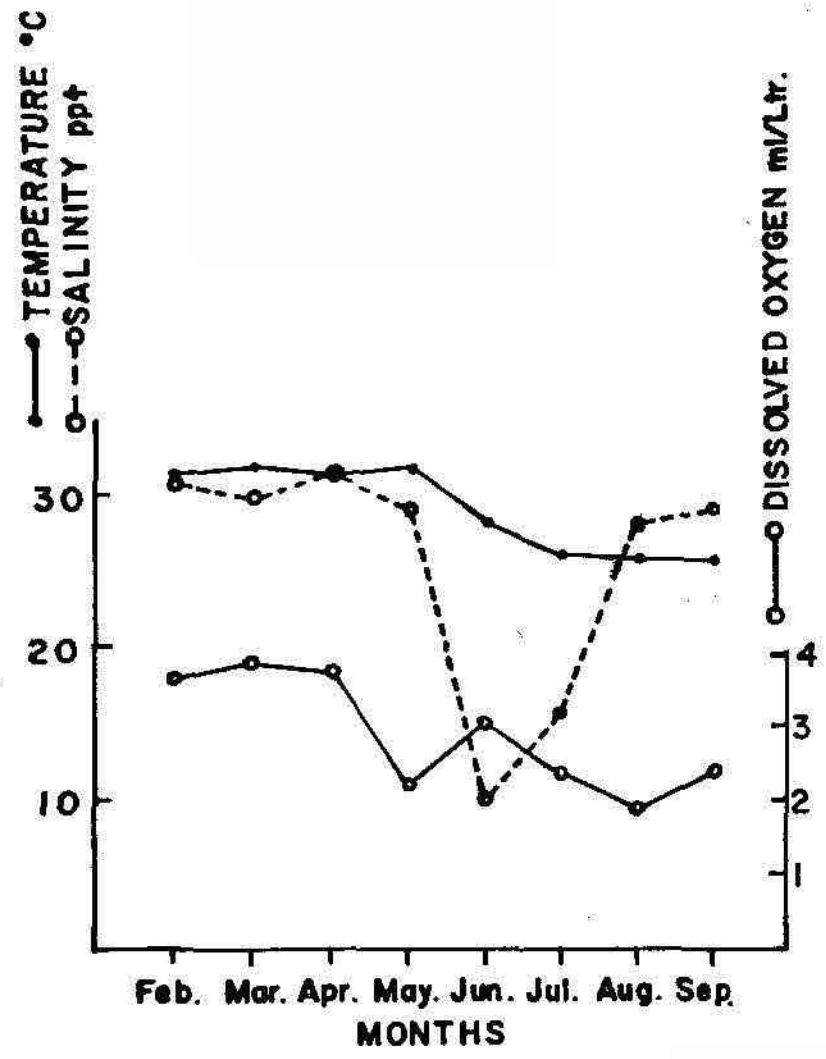

PERCENTAGE FREQUENCY
V
IV
III
II
I
30
20
10
0
50
40
1—50
51—100
101—150
151-200
201-250
251-300
301—350
351-400
401-450
451-500
501-550
551-600
601-650
651-700
OVA DIAMETER IN μm

Fig.8. Seasonal variations in Dissolved oxygen, Salinity and temperature at the site of collection.


TEMPERATURE °C
SALINITY ppt
DISSOLVED OXYGEN ml/Ltr.
30
20
10
4
3
2
1
Feb. Mar. Apr. May. Jun. Jul. Aug. Sep.
MONTHS

In the spent stage, the mode at 150 μ of stage IV had further developed with the mode not having shifted. The immature stock less than 50 μ had the maximum percentage of eggs. Along with this a few residual eggs and empty follides of the released ova were also visible.

STATISTICAL ANALYSIS (Table 9)

Analysis of variance done for the various biochemical parameters showed the following results at 5% level of significance.

Moisture and lipid levels were not found to be significantly different in various tissues. Protein and ash content levels were highly insignificant at 5% level of significance. The concentration in each tissues are almost similar. On the other hand, glycogen, carotenoid and cholesterol were found to show significant difference between various organs statistically, at 5% level of significance.

ECOLOGY

The water temperature, salinity and dissolved oxygen of the site of collection were also monitored along with every collection of samples and their average values are given in Table 8 and Fig. 8.

Table 9: Calculated 'F' values for the difference between Muscle, liver and gonad in respect of various biochemical parameters

Parameters	Calculated 'F' value	Significance at 5% level
Moisture	3.53	NS
Protein	0.10	NS
Lipid	3.20	NS
Glycogen	5.62	S
Cholesterol	12.73	S
Carotenoid	3.89	S
Ash	0.007	NS

Table Value of 'F' for degrees of freedom 2 x 12 is 3.88 at 5% level of significance.

S - Significant
NS - Nonsignificant.

The mean monthly water temperature varied from 26°C to 32°C. The maximum temperature was noticeable during the months of March and May and the minimum of 26°C during September.

Salinity ranged from 10‰ to 31.45‰ with maximum salinity in the month of April and minimum during June.

Dissolved oxygen varied from 1.9 ml/ltr to 3.8 ml/ltr. The minimum level was found in the month of May and the maximum in March.

D I S C U S S I O N

Maturation of gonads is accompanied by profound changes in the chemistry of fish. Major body components like protein, lipid, cholesterol, glycogen, minerals etc. undergo variations during the process of reproduction with an overall purpose of making the developing eggs self-sufficient. Increased concentration of amino acids for purposes connected with nucleic acid synthesis marks the deposition of protein in the developing gonad. Bruce (1924) and Channon and El Saby (1932) working on fat metabolism of herring have shown that fat stored in the body during the period of active growth and feeding is the ultimate source of energy expended by the fish during spawning migration. Though it is difficult to study the physical exhaustion by carbohydrate analysis, glycogen and glucose have both been reported to accumulate in the gonad, especially in the females (Chang and Idler, 1960). Serum cholesterol shwoing minimal value during greatest sexual activity is found associated with formation of sex hormones. A massive movement of carotenoids have been reported from muscle to the skin in males during spawning and a somewhat less dramatic movement in the females where the major redistribution is from the muscle to the ovary (Crozier, 1970). While in non-migratory fish, maturation is accompanied by marked variation in ionic concentration, in migratory fishes, the effect of gonad

development is masked by the overall changes caused by osmo-regulation. The relationships of body moisture with fat and protein have led to the determination of a regression equation by which fat and protein content can be calculated from a knowledge of the water level (Blaxter, 1962). The variations in the above parameters in the muscle, liver and ovary and their interrelationships in L. parsia are discussed.

Protein: Most often, the amino acids provided through food intake is not sufficient enough to fulfil the requirement imposed by spawning. It has also been found that certain essential amino acids for this purpose are not adequately supplied. It is therefore logical to believe that build-up of gonads is accomplished at the expense of body protein also. McBride et al. (1960) and Masurekar and Pai (1979) have noted that much of the gonad tissue is built from protein drawn from the musculature while expenditure of liver protein for the purpose of germ building has been proved by Sorvachev and Shatunovskii (1968). In the present study, protein levels in stage I and II in the muscle remains almost constant, while from stage IV, it shows a declining trend. Though depletion at the time of maturation is exaggerated by abstention of food intake, in some fishes atleast during the early development of gonad, as the fish feeds actively, the protein drain from the muscle to the developing gonad is not reflected immediately in terms of muscle protein. Moreover, according to Haschemeyer

and Smith (1979), mullets have a protein synthesis rate of 20% per day and if no degradation of protein occurs, the observed synthetic rate would represent a growth rate of 0.5% per day at 20°C. Protein content of the liver in the present case also appears drained in the early stages of development. On the other hand, an immediate reflection of a corresponding accumulation in the gonad is not discernible, which may perhaps be due to its utilisation as soon as it is drawn. In stage III protein in all the tissues appears to be at a high level indicating peak period of synthesis and mobilisation as gonad build-up advances. In stage IV, when gonad is fully ripe, muscle and liver showed a decline with a corresponding all time high in the protein level of the gonad suggesting a withdrawal and utilisation of protein for gonad build-up. This has been substantiated in species like flounders (Sorvachev and Shatunovskii, 1968), Clupea sprattus (Petrenko and Karasikova, 1958) and in Clarias batrachus (Yagana bano, 1977).

In stage V, there is an obvious fall in the protein level in the muscle and also in the gonad, while it increases in the liver. It may be surmised here that while the decline in the muscle protein is due to enormous tissue breakdown, that in the gonad can be attributed to strains imposed by extrusion of eggs.

Liver as the site of protein synthesis during maturation has already been proved histologically in Ayu fish (Aida et al., 1973) and also in flounders (Sorvachev and Shatunovskii, 1968).

In the present study, the high protein level noticed in the liver during stages, I, III and V further substantiate the probable role of liver in protein synthesis.

Lipids:

Lipid content in the three tissues is found to follow a similar pattern as that of protein, with a little variation in the liver. In the muscle and liver, the lipid level shows a decrease from stage I to II, but with no corresponding increase in the gonad, possibly due to the utilization of the fat for growth purposes. In stage III, a slight drain is discernible in the liver lipid level while it is seen accumulated in enormous quantities in the gonad. In stage IV, both muscle and liver lipid levels decrease while gonad continues maintaining the high level, confirming the transfer of lipid from these tissues to gonad. In stage V, however, no marked transfer of lipid is evident, probably indicating a slow down in the maturation process.

A close scrutiny of the data on lipids shows that this component is transferred from the liver to the gonad in the early stages of maturation and from muscle also during advanced stages. While translocation of lipid from muscle to the gonad has been proved in Leiognathus splendens (Rao, 1967), that from liver has been shown in Gadus morrhua (Plack et al., 1971).

Another glaring feature observed in the lipid level in L. parsia is the wide variation of this component among the tissues. While gonad lipid level showed a high gradient (18-20%), a corresponding withdrawal in quantity is not reflected in the other tissues, their lipid levels being in a range of 1-6%. Correlating the seasonal variation, in gonadal growth, serum gonadal steroids and serum total lipids of striped mullets, Dindo and MacGregor (1981) suggest that lipid deposition and subsequent mobilisation are associated with autumnal reproductive cycle. Striped mullets are also found actively feeding and transporting fats for storage. Dindo and MacGregor (1981) concluding their observations on striped mullets suggests, "To identify the stimulus and controls for heavy premigratory feeding and lipid deposition and their subsequent reversal with the onset of migration will require carefully controlled experiments".

Fishes are grouped as "fat" fishes and "lean"fishes depending on the fat storing mechanism. "Fat" fishes are described to store fat in the muscle while "lean" fishes, in the liver (Love 1975). In the present study, L. parsia, with a heptatic lipid at a range of 5-6% which is much higher than the muscle fat (1-3%) can be included under "lean" fishes.

In salmons, protein as well as fat serve in the constructive metabolism in connection with the formation of genital products,

(Shul'man, 1974). Studies on the Ovary of L. parsia showed a parallel relation between protein and lipid thus making the view of Marais and Erasmus (1977) positive that protein and lipid content contribute together for the development of the fish, showing an inverse relationship with moisture.

Glycogen:

The liver is generally considered to be the fat as well as the glycogen storage organ in non-fatty fishes. Though much of the energy for fishes comes from gluconeogenesis, a greater vigour for this mechanism has been found in males than in females. In the early stages of maturation of Gadus callarias, the glycogen content of males is 50-100% greater than it is in females, but the picture is reversed immediately before spawning (Bogoyablenskaya and Vel'tishcheva, 1972). This is difficult to square with the fact that females transfer glycogen from liver to gonad during development. Anyway, these authors concluded that the onset of spawning causes expenditure of lipid from the body of females and glycogen from males. Shul'man (1974) suggests fat as the main source of energy instead of carbohydrate in all cases of largescale functions in fishes. In the present context, glycogen content with a narrow range of 0.1-0.2% in the muscle is not showing any definite trend. However, in the liver which has a comparatively

high glycogen content (0.34-3.75%) is found depleting during stages II and V, with a corresponding increase in the ovary, substantiating the view of Fontaine and Hatey (1953) that in the female fish, the glycogen of the liver, the main carbohydrate store house is preferentially depleted.

Nevertheless, in the gonad, a decrease in levels of glycogen in stage III and IV is noticeable. A possible explanation at this juncture would be that when fat and protein have accumulated in the gonad, the role of glycogen becomes negligible. In fact, glycogen renders its services when there is a fall in lipid and protein levels. This is obvious from the rise in the glycogen level in stage II and V of gonad when protein and lipid levels have gone down. Naturally, glycogen is found to have a parallel relation with water in the case of gonad.

According to Blazka (1958), whose research was performed in carp, the final product of carbohydrate metabolism may be fat (!) Though strange enough, can be possible in this case too, as lipid levels are much higher in gonad alone in the ripe stages with a corresponding decrease in glycogen level. This is in agreement with the observation made by Kuo (quoted by Nash and Shehadeh, 1980 and Gopalakrishnan (personal communication) in mullets that carbohydrate level in the gonad decreased distinctly, prior to spawning.

Cholesterol:

Dindo and Macregor (1981) studying the seasonal variation of serum total lipid and cholesterol in striped mullets, suggest their possible deposition and subsequent mobilization associated with reproductive cycle. In the present study also, a gradual accumulation of cholesterol in the ovary is evident from stage I through IV, followed by a decrease in stage V. Simultaneously, a descent of cholesterol level is obvious in the liver from stage I to IV. In the Muscle, on the other hand, with an insignificant level of 0.1-0.2%, cholesterol does not appear to play any decisive role in germ building. That the levels of cholesterol in the females are low has already been established in salmonid fishes (Farrel and Munt, 1983). Though Shatunovskii and Kozlov (1973) had suggested a drain of muscle cholesterol with corresponding accumulation in the ovary during maturation, in the present case in L. parsia, the liver cholesterol is found depleted, thereby substantiating the view by Chaturvedi et al (1976) that low levels of cholesterol in liver is due to higher physiological needs of gonad development.

Diwan and Krishnan (1986) found cholesterol levels to increase upto stage III and then to decrease at a later stage in gonad. Siddiqui (1966) and Nauriyal and Singh (1985) found high levels of cholesterol at the end of the maturing phase. These evidences form a staunch support to the result obtained in this case, where cholesterol levels increase with maturity

in the gonad. High levels of cholesterol in gonads act as a reservoir to meet the demand of maturing ovary and increase in steroidogenesis decreased the levels.

So as a general statement, cholesterol levels are depleted in the liver and redistributed in the ovary as it matures, for being utilised during spawning in the process of steroid synthesis.

Carotenoids:

Carotenoids are not "just a playful diversion of nature as is often assumed", but fulfil an important function in reproduction (Deufel, 1975).

In the present case, carotenoid levels in muscle increased from stage I to II, thereafter declining upto stage V. Correspondingly, the gonad shows an increase in its carotenoid content from stage I to IV. In stage V, a sharp decline is seen in both the tissues.

Carotenoids are attributed with functions of maintaining egg quality, impairing colouration, controlling viability of eggs, as a fertilization hormone, protection from sunlight, and as a precursor of vitamin A (Craik, 1985). They are dependant on diet variation and are found to be stored in muscle, liver and skin. They are mobilised from muscle and liver and laid down in unesterified form in mature eggs

(Kithara, 1983). The present study substantiates the view of Kithara (1983) and Crozier (1970) that in muscle, the amount of carotenoid drastically decreased as maturation advanced. Accumulation of carotenoids in the gonad during process of maturation has also been observed by Shnarevich and Sakhnenko (1971). Further, the gradation of pigmentation of eggs in different stages of maturity is so profound that it can be used as a reliable index for determining maturity stages in fishes.

The flesh of spawning or spent fishes in the present study is found to have a low carotenoid content. This is in corroboration with the results obtained by Crozier (1970) in Oncorynchus nerka. There are also evidences for proving the nonentry of carotenoids until the maturation has started (Love, 1980). It was also found that loss of muscle carotenoid stopped in gonadectomised salmon (Donaldson and Fagerland, 1970) providing the intimacy that carotenoid has with the developing ovary.

Though it has been found that carotenoids are deposited in the liver also, along with muscle for mobilization into gonad, no such clear pattern was evident in the present case.

Minerals:

Inorganic ions in relation to maturation seems to show changes of a random nature (Love, 1970). In the present

observation, ash content in the muscle increased from stage I to II, with no corresponding increase in the gonad. This may be due to the fact that, in the early growing stages, minerals like calcium and phoshorous get accumulated in the body for overall development. In stage III, the mineral content in the muscle shows a decline with a more pronounced increase in the liver than in the gonad. It may therefore be stated that in the gonad, the accumulation is not relfected, probably due to utilization by the developing eggs while in the liver, the high level obvious in stage III and a subsequent decline in stage IV and V indicate transfer of essential inorganic ions through the liver to gonad.

However, most of the works in minerals are on fishes that ascend river to spawn. In these fishes, migration from salt water to freshwater coupled with a loss of ability to osmoregulate after spawning, causes an overall loss of ion that masks the effects caused by maturation. Even in non-migratory fishes, loss of inorganic ions was evident during depletion other than maturation(Love et al., 1968; Love, 1970).

Moisture:

Water plays a decisive role in most biochemical function in view of its ionic properties and its association with other constituents in their functions. Since water functions as the milieu for all biochemical operation, it goes without saying that the equilibrium state established between water and its

component system must also vary with profound physiological activities such as reproduction and spawning.

In the fish under study, moisture levels show a slight decrease in Stage II and III in muscle thereafter increasing in the forthcoming stages. In liver, the moisture content is increasing in stage II with a decrease in Stage III again to increase and remain constant in stage IV and V. Gonad water level shows a wider range of variation with an increase from Stage I to II decreasing drastically in stage III and IV again to increase in stage V.

An inverse relationship of moisture with lipid, though obvious in all the three tissues is more pronounced in the muscle and gonad. The inverse relationship between moisture and lipid is so clear that the sum of two components in each stage almost remains constant as is also observed by Black and Schwartz (1950), Idler and Bitners (1959) and Coppini(1967).

An inverse relationship is also discernible between moisture and protein in all the tissues and more evidently in liver and gonad. The relationship between moisture and protein in the process of maturation is so glaring that the whole process may be observed by means of water determination, especially in non-fatty fishes. As protein is removed from the muscle, the water content rises steadily and hence can be

used as an useful index of the state of depletion of fish (Love, 1960; 1962b).

Another inverse relationship obvious in the gonad is between moisture and cholesterol and such a relationship is but natural as cholesterol forms one of the sterol component of the total lipid.

Hepatic index:

Liver index is an important indicator of the state of liver during maturation and liver weight is said to increase in the female until gonadal maturation has definitely started and then decline with progressive maturation upto the stage when ova are ripe (Krivobok, 1964). It was also demonstrated that as the liver index increases, so does the yield of oil per unit.

In the present study, liver index in various stages was found increasing upto stage IV and then decreasing in Stage V.

An attempt to correlate levels of lipid, protein, cholesterol and glycogen shows that fat declined from stage I through IV and then increased in stage V. Cholesterol which is closely related with lipid showed a similar pattern. On the other hand protein levels were high in stages I, III and V and glycogen, after a slight decrease in stage II, showed a steady and high increase upto stage IV to decrease only in

Stage V. Liver is found to be the site of protein synthesis as well as the store house for glycogen and lipid especially in the case of non-fatty fishes. From the present observation, it can be surmised that cholesterol and lipid are not the only components influencing the hepato-somatic index in various maturity stages, but also other parameters like glycogen or protein. This may be substantiated by the observation of Mikhail et al. (1982) who state that protein content or carbohydrate are more effective on the whole weight of the liver than the fat content.

Maturation:

Sarojini (1957) studying the biology of L. parsia, had noticed that the species have a prolonged breeding season extending between October and April, releasing 2 batches of eggs. Surendra Babu and Neelakantan (1983), observing the breeding biology of the species, also have arrived at a similar conclusion while Kurup and Samuel (1983) examining the spawning details of Liza parsia have found the species to release only one batch of eggs, during the possible breeding season extending between October and May. The present study on L. parsia, where the spawning season is found to be between September and May, also substantiates the findings by Kurup and Samuel (1983), since one distinct batch of ripe eggs within a range of 500-650 µm is clearly demarked from the maturing batch of eggs which is at a mode

of 100-200 µm . Further, the second batch of eggs forms only 11% of the total number of eggs, thus ruling out the possibility of it being spawned along with the ripe eggs, since according to Prabhu (1956) and Karekar and Bal (1960), the range in size of the mature ova, irrespective of the number of modes representing them may be found to be nearly half of the total range in size of the entire intravoarian eggs.

S U M M A R Y

1. The present study was carried out with the objective of evaluating the effect of maturation on the biochemical composition of one of the common mullets of Kerala namely Liza parsia.

2. Fortnightly collections of the species were made from the Cochin Barmouth region using Chinese dip nets during the period February to September, 1987.

3. Five maturity stages were determined based on the state, size and colour of the ovary and microscopic structure of the ova.

4. In each stage, seven biochemical parameters namely moisture, protein, lipid, glycogen, cholesterol, carotenoid and ash contents were studies in three tissues viz. muscle, liver and gonad. Also, liver index, gonado-somatic index and ova diameter percentage frequency were studied in each stage.

5. Moisture content increased in mature stages in muscle with no specific trend in liver. Water level decreased drastically in the gonad in mature and ripe stages.

6. Moisture showed an inverse relationship with protein and lipid in all the tissues.

7. A similar inverse relationship of moisture was noticeable with cholesterol in gonad and with minerals in liver.

8. Protein levels decreased in muscle in later stages with a corresponding increase in gonad upto ripe stage suggesting a translocation of protein from muscle to the developing gonads. Alternative increase and decrease of protein in liver suggested liver to be the site of synthesis and also mobilisation of protein.

9. Lipid level dropped conspicuously in liver as maturation advanced and in later stages in muscle too. A high degree increase of the same in gonad showed that the lipid would have been translocated from liver and later from muscle also.

10. Glycogen was found accumulated in the liver and depleted only when gonad showed lower levels of protein and lipid. A distinct decrease of glycogen was obvious in gonad just before spawning. It showed an inverse relation with protein and lipid in gonad.

11. Cholesterol levels showed increase in gonad with a corresponding decrease in liver suggesting redistribution from liver to gonad.

12. Carotenoid showed interesting results with accumulation of the same in the muscle in early stages of maturity and thereafter a dramatic movement and deposition in the gonad, thus increasing the gonad carotenoid level with advancement of maturity.

13. Ash content in all the three tissues didn't reveal any noteworthy features on the effect of maturation, probably being masked by other factors like depletion, osmoregulation pattern, etc.

14. Hepatic Index increased with maturation and decreased with cessation of spawning. Lipid and cholesterol levels decreased with increasing Hepato-somatic index, while glycogen or protein indicated an increase exposing the fact that increase in liver weight need not be due to lipid alone.

15. Gonado-somatic index increased from a mean of 0.38 in stage I to 13.82 in stage IV, then to decrase in Stage V to 2.08.

16. The minimum ova diameter encountered was 26 μ while the maximum was 694 μ. The ova diameter studies in the ripe stage with a conspicuous mode at 600 μ showed chances of individual fishes spawning only once during the breeding season.

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