CHAPTER - IV

DISCUSSION AND CONCLUDING REMARKS

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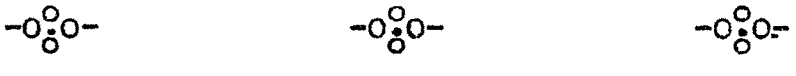

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1. TESTES :

R.daniconius, commonly known as 'Dandai' provides an example of a species displaying a discontinuous sex-cycle. The testes of this fish show cyclic events involving changes in spermatogenetic activity, boundary cells and interstitial cells. Morphologically alterations in testes are also evident in size, colour, and weight in accordance with the seasonal breeding-quiescence cycle. Based on the histological changes in the testes, the breeding-cycle of this fish can be distinguished, into four distinct phases viz. (1) pre-breeding period of gametogenesis from March to May, (2) active breeding period from June to August, (3) post-breeding period of recession from September to November and (4) sexually quiescent period of rest from December to February. Accompanying the cyclic alterations in the testes, quantitative and qualitative lipid changes are also evident from the present investigation

The present bioassay studies indicate that the maximum amount of lipids are present during the mid-pre-breeding period in April when the total lipids are 130.6 ± 9.00 mg/g wet weight of the testes. The neutral lipids and phospholipids represent 126.9 ± 8.82 mg/g and 3.622 ± 0.15 mg/g respectively. During this period the concentrations of the individual neutral lipids expressed as mg/g are MG - 4.06 ± 0.43, DG - 8.70 ± 0.36, TG - 112.6 ± 9.12, CHO - 0.747 ± 0.07, CE - 0.374 ± 0.02 and FFA - 0.475 ± 0.09 and the individual phospholipids in terms of

ug-p/g are LPC - 10.59 ± 0.75, SPG - 5.293 ± 0.28, PC - 79.40 ± 4.54, PI - 15.88 ± 1.03, PS - 10.59 ± 0.83, PE - 15.88 ± 1.03 and PA - 7.252 ± 0.02.

In similar studies Lizenko et al. (1973) estimated the content of total lipids and their fractional composition determined by TLC in the male and female gonads of C.albula and concluded that the quantity of fat in the gonads of female is higher than those of the males. The present investigation also indicates that the female gonads contain more lipids than the male. For example, during the preparatory period in April the testes show 130.6 ± 9.00 mg/g total lipids, whereas ovaries exhibit 136.2 ± 9.2 mg/g total lipids. The present investigation leads to another point of discussion which concerns the cyclic alterations in the quantity of lipids in accordance with the breeding cycle. The values obtained for the testicular total lipids, neutral lipids, phospholipids and their individual components by quantitative estimations through out the year indicate that the lipids undergo interesting cyclic changes depending upon the sexual state of the fish. Bioassay studies indicates that the total lipids increase gradually during the post-breeding period from the September onwards from 62.85 ± 6.19 mg/g reaching the peak value of 142.1 ± 10.21 mg/g in late post-breeding period in November and decrease from the pre-breeding period onwards and reaching the minimum in early post-breeding period in September. Thus in these fishes the quiescent

period and early pre-breeding periods are the time for the lipid accumulation, whereas the late pre-breeding period of spermatogenesis is the time for these accumulated lipids are metabolised. Hence when the testes are loaded with the fully mature spermatozoa just at the beginning of the breeding activities, the total lipids reduced such reduction might be due to the utilization of lipids by mature sperms. In post-breeding state the lipid levels increased due to non-use of lipids.

Values obtained for the neutral lipids indicate that the seasonal alterations in the neutral lipids run parallel to those occuring in the total lipids. Maximum amount of neutral lipids are observed during the late post-breeding period in November when the total neutral lipids are 140.6 $\pm$ 9.18 mg/g. Neutral lipids maintained with some statistical decrease in their value during the quiescent period but during the early pre-breeding period neutral lipid values again start to increase upto mid-pre-breeding period. From the late-pre-breeding period lipid vales decreased gradually. Thus the neutral lipid values during the late-quiescent period are 106.9 $\pm$ 9.14 mg/g in February and 130.6 $\pm$ 9.00 mg/g during mid-prebreeding period in April. Neutral lipids exhibit decrease from late-prebreeding period of gamates maturation onwards reaching the minimum level 62.85 $\pm$ 6.19 mg/g in September just at the beginning of post-breeding period. From mid-post-breeding period onwards the neutral lipids again getting accumulated.

Among the neutral lipids MG, DG, TG, CHO, CE and FFA can be identified after the TLC separation. Quantitatively TG occur in maximum concentration followed by DG, MG, CHO, CE and FFA in decreasing order. All these components of the neutral lipids also exhibit cyclic alterations.

Maximum value of TG 125.5 ± 9.26 mg/g is found during the late-post breeding in November, which is further maintained with minor decrease during the quiescent period from December to February. During the early pre-breeding period lipid value is again increases and reaches 112.6 ± 9.12 mg/g in April which is gradually decreased from late-prebreeding period onwards reaching minimum level of 51.81 mg/g in September just at the beginning of post-breeding period. From October onward TG exhibited increasing trend. The MG, DG and FFA exhibited similar variations with TG.

The CHO and CE exhibit some what different alterations, as against the glycerides. These neutral lipid components exhibit a steady but not very sharp rise during the preparatory and active breeding period of gametogenesis. Thus the values of CHO rise from 0.559 ± 0.04 mg/g in March to 1.942 in August. But after the breeding activities, the values reduced to half in the month of September when they ranged 0.609 ± 0.04 mg/g. From the post-breeding period onwards the values show a gradual rise. Though not exactly identical, but some what similar variations is seen in case of CE.

As compared to the cyclic alterations of neutral lipids, the changes in phospholipids are different. Biossay studies indicate that the maximum amount of total phospholipids (6.502 $\pm$ 0.42 mg/g) is present during the mid-active breeding period in July, which gradually decreased during the late active-breeding period onwards from August to January (during post-breeding and quiescent periods) but slightly increased during February. TLC separation studies showed that among the phospholipids PC and PE occur in higher concentration. PI, PS, SPG in moderate quantities and LPC and PA in low quantities. Among the phospholipids, PC and PE showed an increasing trend during pre-breeding period to mid-active breeding period, then they showed decreasing trend during post-breeding and early quiescent period. The other components of phospholipids exhibit antagonastic alterations during the post-breeding and quiescent period.

Thus at the general level it is observed that the NL get accumulated during the preparatory (i.e. pre-breeding) and metabolized during active breeding period. The lipids decreased during breeding period might be due to the utilization of lipids by the mature sperms, as well as lipid contents of the sperms (spermiation). On the contrary the lipids get accumulated from late post-breeding and subsequent period of quiescence.

At general level phospholipids slowly increased from pre-breeding period and attained high concentration during the

active breeding period. Such increase in PL values co-relates with increasing number of sperms. The PL are membrane components as the number of sperms increases corres pondingly the membrane quantities increases. So in short the rise in PL quantities during the active breeding period might be due to the increase in membrane quantities.

The PL quantities exhibited a decreasing trend from post-breeding and subsequent period of quiescence is due to the absence of the sperms in the testes.

C.fulungee, commonly known as 'Mulicha-gana' also provides an example of discontinuous breeding cycle. The testes of this fish shows cyclic events similar to that of R.daniconius. Depending upon the histological changes in the testes the breeding cycle of this fish can be distinguished into four phases those are similar to R.daniconius, viz.
(1) pre-breeding period of gametogenesis from March to May
(2) active breeding period from June to August
(3) post-breeding period of recession from September to November
(4) sexual quiescent period of rest from December to February.

The present bioassay studies indicate that the maximum amount of lipids are present during the late-prebreeding period in May when the total lipids are 137.5 mg/g while in R.daniconius maximum amount of lipids are present during mid-pre-breeding period in April when they are 130.6 mg/g wet weight of the testes. The neutral lipids and phospholipids represents 133.0 mg/g and

4.423 mg/g respectively. During this period the concentrations of the individual neutral lipids expressed as mg/g are - MG - 6.620 ± 0.16, DG - 19.86 ± 2.43, TG - 105.9 ± 7.8, CHO - 0.389 ± 0.02, CE - 0.779 ± 0.06 and FFA - 0.523 ± 0.04 and the individual phospholipids components in terms of ug-p/g are LPC - 7.193 ± 0.19, SPG - 12.19 ± 1.02, PC - 89.94 ± 4.73, PI - 12.19 ± 1.02, PS - 18.28 ± 1.32, PE - 40.52 ± 3.12 and PA - 8.321 ± 1.00.

The present investigation leads to a point of discussion which concerns the cyclic alterations in the quantity of lipids in respect with the breeding cycle. The values obtained for the testicular total lipids, neutral lipids, phospholipids and their individual components by quantitative estimations through out the year indicate that the lipids undergo interesting cyclic changes, depending upon the sexual state of the fish. Bioassay studies indicates that the total lipids increase gradually during the pre-breeding period from the March 62.10 ± 5.26 mg/g reaching the peak value of 137.5 ± 8.9 mg/g in late-prebreeding period in May, and decreases from the active-breeding period onwards and reaches the minimum values in early post-breeding period in September. Then October onwards total lipid values increases further at the end of quiescent period. Thus in C. fulungee and R.daniconius testes showed the cyclic alterations in the lipids level depending upon the sexual state of fish but these seasonal alterations are not identical to each other. In case R.daniconius TL values exhibited a sharp rising during the

post-breeding period from October to November (71.57 mg/g to 142.1 mg/g); while in case of C.fulungee during the same period there is insignificant increase (October 50.27 mg/g and November 54.51 mg/g).

Values obtained for the neutral lipids indicate that the seasonal alterations in the neutral lipids run parallel to those occuring in the total lipids. Maximum amount of neutral lipids are observed during the late pre-breeding period in May when the neutral lipids are 133.0 ± 7.1 mg/g. Neutral lipids value is decreased from early active breeding in June to early post-breeding period in September. From mid post-breeding onwards the neutral lipids value increases gradually. This increase in neutral lipids value is further continued during the quiescent period. In R.daniconius neutral lipids increased during the late post-breeding period in November and then there is a gradual decrease in neutral lipid values during the subsequent period of quiescence.

Among the neutral lipids MG, DG, TG, CE, CHO and FFA are identified after the TLC separation. Quantitatively TG occurs in maximum concentration similar with that of R.daniconius, followed by DG, MG, CHO, CE and FFA in decreasing order. All these components of the neutral lipids exhibit cyclic alterations similar to R.daniconius. Maximum value of TG 105.9 ± 7.8 mg/g is found during the late-prebreeding in May which is decreased during the active breeding period and becomes minimum

in the breeding cycle during early post-breeding period in September i.e. 30.64 ± 3.62 mg/g. From the mid-post breeding onward TG value get increased gradually such increase in TG level is further continued during the quiescent period.

As compared to the cyclic alteration in neutral lipids the changes in phospholipids are different. The present bioassay studies indicate that the maximum amount of total phospholipids (5.757 ± 0.43 mg/g) is present during the mid-active breeding period in July, similar observation is found in testicular phospholipids of R.daniconius. The level of total phospholipids gradually decrease during the late-active breeding period, such decrease was further observed during the post-breeding and subsequent period of quiescence.

TLC separation studies showed that among the phospholipids PC and PE occurred in higher concentration, PI, PS, SPG in moderate quantities and LPC and PA in low concentration. Among the phospholipid components PC and PE showed an increasing pattern during pre-breeding period to mid-active breeding period then they showed decreasing trend from post-breeding and subsequent period of quiescence. All other components of phospholipids follows the similar alterations described for the PC and PE with minor variations.

Thus at the general level it is evident from the present bioassay studies that the total phospholipids and individual components of phopholipids from the testes of C.fulungee follows the similar cyclic alterations with that of R.daniconius.

2. OVARY :

The present investigation on the ovaries of R.daniconius shows that the cyclic changes occur in size, weight and histology of the ovaries according to the annual breeding cycle. Based on the histological events in the ovaries, the female sex cycle can be distinguished into four distinct periods, (1) pre-spawning period from March to May, (2) active spawning period from June to August, (3) post-spawning period from September to November and (4) sexual quiescent period from December to February.

Bioassay studies on the ovarian lipids reveal that the lipids attain their peak levels during the active breeding period in July, when the total lipids range was 157.2 ± 10.2 mg/g wet weight of the ovaries. During this period the neutral lipids and phospholipids were 150.3 and 6.872 mg/g respectively. TLC separation, isolation and quantification studies indicate the concentration of individual components of the neutral lipids and phospholipids are as follows : TG -106.1, DG - 24.62, Mg - 16.19, CE - 1.672, CHO - 0.764 and FFA - 0.937, and phospholipid components are in µg-p/g of wet weight of the tissue, PC - 120.7, PE - 45.68, PS -24.69, PI - 27.32, LPC - 20.08, PA - 20.10 and SPG - 16.33.

From the present investigation it is clear that TG is the chief neutral lipid component and it represents about 70.6% of the neutral lipids, whereas the other neutral lipid components

occurred in small concentrations, DG - 16.41%, MG - 10-79%, CE - 1.12%, CHO - 0.509% and FFA - 0.625%. Among the phospholipids PC and PE are the major components for example in July when the phospholipids exhibit maximum level, PC and PE were 43.9% and 16.7% respectively. Whereas rest of the components were occurred in low concentration.

The literature on fish ovarian lipids show a significant paucity of any information on the composition of the lipids. Hence a comparison of the observation in the present investigation with those in the earlier literature become difficult. The present report on the ovarian lipid composition of seasonally breeding fish R.daniconius and alterations in various lipid components, thus becomes a first of its type.

The present investigation also brings out cyclic variations in the quantity and the type of lipids in the ovaries according to the reproductive states of the females. Bioassay studies indicate that the total lipid value increases gradually from the pre-spawning period onwards, from 110.0 mg/g in March and reaches the peak at the active spawning period in July when the value is 157.2 mg/g, thus making significant increase during the period of Oogenesis and gamate maturation. From August to February (post-breeding and subsequent period of quiescence) TL value decreased gradually and exhibited least quantity in the month of February (47.24 mg/g). Thus as compared the lipid quantity during quiescent period in February the total lipid

value increases 3-fold during the mid-active spawning period in July. Values obtained for neutral lipids also indicate that the cyclic changes in neutral lipids quantities run parallel to those in the total lipids. Minimum amount of neutral lipids 44.14 mg/g is observed during the quiescent period in February. Neutral lipids value exhibit increasing from pre-spawning onwards reaching the peak level of 150.3 mg/g in July. After the ovulation and from post-spawning period onwards the neutral lipid values exhibit further decrease.

TLC separation and quantification studies of neutral lipids indicate presence of TG, DG, MG, CHO, CE and FFA. The neutral lipid fractions, among which TG forms the major component. Whereas, MG and DG were observed in moderate concentration and CHO, CE and FFA were found in low concentration. The individual components also exhibit cyclic variations. The TG gets accumulated from pre-spawning towards the active spawning, where it exhibited the maximum concentration (106.1 mg/g) in July. Such increase in TG value might be due to the TG accumulation in the developed ova. Whether TG is transported from blood to ovary or it is synthesized in ovarian cytoplasm can not be said positively. After ovulation, TG concentration depleted gradually from August to February (post-spawning and subsequent period of quiescence). The TG values decreased during post-spawning period is might be due to the degeneration

of unovulated ova in the ovaries, but why TG depleted further during quiescent period cannot be reply. The rest of NL components followed similar alterations to that of TG with some minor variation.

The present investigation also shows similar cyclic alterations in the quantities of phospholipid. Among the phospholipid components PC and PE are the major components ranging at 43.9% and 16.7% respectively of the phospholipid. When the PL values reaches maximum of 6.872 mg/g during the mid-active spawning in July. The PC and PE shows their peak values i.e. 120.7 and 45.68 µg-p/g in July respectively. The PC and PE are the membrane components as the Oogenesis maturition took place, increase the size and number of ova during active spawning concomitantly PC and PE values enhanced. Then their values gradually reduced during post-spawning and sexually quiescent period, might be due to the number of ova lost during ovulation. The other components of phospholipid such as LPC, SPG, PI, PS and PA occur in moderate quantities, within the 38% of the phospholipids, also undergo cyclic variations. Their quantities also attain maximum levels during the active-spawning period, then gradually reduced to the minimum values during the quiescent period with some minor differences.

From the above discussion the alterations in the neutral and phospholipids in the ovaries, a generalization can be arrived at. The lipids synthesized and accumulated during the process of

Oogenesis and Vitellogenesis. Hence, in the gravid ovaries these lipids exhibit maximum concentration. As the ovulation occurs and ova are liberated these lipids deplete in their concentration. Such a depletion due to the liberation of the ova from the ovary. Thus the depleted ovarian lipids might be the lipids present in the ova.

The present investigation on the ovaries of C.fulungee shows that the cyclic changes occur inside, weight and histology of the ovaries according to the annual breeding cycle similar to that ovaries of R.daniconius depending upon the histological events in the ovaries, the female breeding cycle can be distinguished into four distinct period (1) pre-spawning period from March to May (2) active spawning period from June to August (3) post-spawning period from September to November and (4) sexual quiescent period from December to February.

Bioassay studies on the ovarian lipids reveal that the lipids attain their peak levels during the active-spawning period in July, when the total lipids range was 176.3 $\pm$ 10.6 mg/g wet weight of the ovaries. During this period the neutral and phospholipids were 168.5 $\pm$ 9.8 mg/g and 7.862 $\pm$ 0.89 mg/g respectively. In this investigation it is found that the ovaries of C.fulungee contain more lipids than the ovaries of R.daniconius. TLC separation, isolation and quantification studies indicate the concentration of individual components of the neutral lipids and phospholipids in July are as follows :-

TG - 129.7, DG - 25.69, MG - 10.18, CE - 0.928, CHO - 0.920 and FFA - 0.767 and phospholipid components are in µg-p/g of wet weight of the ovary, PC - 133.6, PE - 46.39, SPG - 38.95, LPC - 33.99, PS - 27.82, PI - 22.26 and PA - 12.13.

From the present investigation it is clear that TG is the chief neutral lipid component and it represents about 76.97% of the neutral lipids, whereas the other neutral lipid components occurred - MG - 6.04%, DG - 15.25%, CHO - 0.546%, CE - 0.550% and FFA - 0.455%. Among the phospholipids PC and PE are the major components for example in July when the phospholipids exhibit maximum level, PC and PE are about 42.40% and 14.72% respectively, whereas other components occurred as - LPC - 10.79%, SPG - 12.36%, PI - 7.06%, PS - 8.83% and PA - 3.8%.

The present investigation gives an comparative account that the ovaries of C.fulungee contain more neutral and phospholipids than the ovaries of R.daniconius. Bioassay studies indicate that the total lipid value increases gradually from the pre-spawning period onwards from 97.44 ± 7.23 mg/g in March and reaches the peak at the mid-active spawning period in July when the value in 176.3 ± 10.6 mg/g thus making significant increase during the period of Oogenesis and ova maturation. In the month of July ovaries of R.daniconius contains 157.2 mg/g total lipids, this proves the above statement that the ovaries of C.fulungee contain more total lipid than the ovaries of R.daniconius. From August onwards to February total lipid values decreased and

reaches the minimum level 29.26 mg/g during the late quiescent period in February. Thus as compared the lipid quantity during quiescent period in February the total lipid value increases 6-fold during the mid-active spawning period in July. At the same period in the spawning cycle of R.daniconius the total lipid value increases by 3-folds in July. Values obtained for total neutral lipids quantities run parallel to those in the total lipids. Minimum amount of neutral lipids 27.41 mg/g is observed during the late quiescent period in February. Neutral lipids value exhibit increasing trend from pre-spawning onwards reaching the maximum level of 168.5 mg/g in July. As compared the value of neutral lipids in February with that of July, it found that neutral lipid values increased by 6 fold in July. After the ovulation and from post-spawning period onwards the neutral lipid values exhibit further gradual decrease. These alterations in the level of ovarian neutral lipids value run parallel to each other in C.fulungee and R.daniconius with respect to the annual spawning cycle.

TLC separation and quantification studies of neutral lipids indicate presence of TG, DG, MG, CHO, CE and FFA. The neutral lipid fractions among which TG forms the major component. The TG begins to accumulate during pre-spawning attained maximum quantity during active spawning. As compare the values in February to that of July, TG showed 8 fold increase which might be due to the TG accumulation in ova. After ovulation TG gets depleted (this is due to lost in ova from the ovary) during post-

spawning and quiescent period. MG and DG follows the trend of TG.

CHO is accumulated during the sexual quiescent period from December to February. Initiation of pre-spawning to active spawning the accumulated CHO might be utilized for steroid hormone synthesis (It is well known phenomena that CHO is used for steroid hormone synthesis). So during pre-spawning and spawning CHO converted to steroid hormones hence depleted during such period for confirmation of this statement the further research work is required. The CHO once more exhibited rising trend during post-spawning period indicates that conversion of CHO to steroid is inhibited hence it increased.

The present research work also shows similar cyclic alterations in the quantities of phospholipid components. Among the phospholipids PC and PE are the major components ranging at 42.40% and 14.72% respectively of the phospholipid. When the values of phospholipid reaches maximum of 7.862 mg/g during the mid-active spawning in July, PC and PE shows their peak level at the same period i.e. 133.6 µg-p/g and 46.39 µg-p/g respectively. Then there values gradually reduced during post-spawning and sexual quiescent period and again reach the maximum level during active spawning period. The other components of phospholipid, such as LPC, SPG, PI, PS and PA occur in moderate quantities, within the 42% of the phospholipids, also undergo cyclic variations. Their quantities also attain the maximum levels during the active spawning period, then gradually reduced

to minimum values during the quiescent period with some minor variations. These cyclic alterations which exhibits in ovarian phospholipids of C.fulungee are similar in their cyclic behaviour with that occurs in ovarian phospholipids of R.daniconius.

From the above discussion the alterations in the neutral and phospholipids in the ovaries, a conclusion can be drawn that the lipids synthesized and accumulated during the process of Oogenesis and vitellogenesis. Hence, in the gravid ovaries these lipid exhibit higher concentration. After the ovulation ova are liberated these lipids depleted from their level. Such a depletion due to the liberation of the ova from the ovary. Thus the depleted ovarian lipids might be the lipids present in the ova. During the comparison of C.fulungee ovary with ovary of R.daniconius it is found that the C.fulungee ovary contain more lipids than the ovary of R.daniconius while all other cyclic alterations in lipids level those occurs during the entire spawning cycle are similar to each other with respect to the annual spawning cycle.

CONCLUDING REMARKS :

While concluding the present dissertation on the lipds of the gonads in two fishes (R.daniconius, C.fulungee) during the seasonal breeding cycles, it should be mentioned that practically all the objectives with which the present investigation was taken up have been satisfactorily fulfilled. Thus, the present

dissertation describes in detail the lipid contents, lipids composition, the seasonal alterations of various lipid constituents in the testes and ovaries. Modern techniques such as thin layer chromatography (TLC) coupled with quantitation of various lipid constituents giving reliable information on the lipids composition and alterations therein in exact mathematical terms. This quantitative techniques has given a technical perfection to the present work. Not only the present investigation gives reliable information on the quantitative and qualitative composition of the lipids in testes and ovaries in both sexes of the two seasonal breeding fishes, in their annual breeding cycles. It also gives comparisons of the observations made in the present work with the available information on the lipids of the gonads in other fishes. Such comparisons and the resultant similarities and dissimilarities have been commented upon and interpreted in third and fourth chapter on individual fishes and also in the present chapter on general discussion, in which the lipids in the reproductive organs have been viewed from a comparative biochemistry point of view. Relationship of the lipid alterations to various reproductive events during the annual breeding cycles of the seasonally breeding fishes, have been properly illustrated and duly commented. The importance of lipid in gametogenic processes as hormone precursors and in the seasonal hypertrophy and hypotrophy of gonads has been elaborately discussed. The problem of hormonal control over lipid metabolism in gonads has been briefly discussed in the light of

evidences made available by the present work and the experimental investigations carried out by other workers. The present work 'thus' gives practically all the information on the lipids in the gonads and their behaviour during annual breeding cycles in the two selected fishes.

The earlier dissertations on the lipids in the gonads limit themselves to describing the lipid content, composition, localization in either testes or ovaries, of the seasonally breeding animals. To the best of the knowledge of the author very few research dissertations have yet been published in which lipids of testes and ovaries have been studies, from this point of view the present dissertation is expected to find a proper place in the literature on lipids in reproductive organs. So as to achieve methodological perfection, the biochemical techniques are used in present research work. Similarly in most of the earlier investigations some selected lipid constituent such as cholesterol, which is very important as a steroid precursor, has been studied, the other components being overlooked. But in the present dissertation total lipids, total neutral lipids, and their six components, total phospholipids and their seven components have been investigated thus making a complete information on the lipids composition and alterations in all the lipid constituents in the gonads of fishes. The author would like to state here that in no way he wishes to underrate the earlier dissertations, actually he would like to acknowledge their immense assistance, since the present work conceived, planned and executed only after a detailed study of these dissertations.

The present investigation opens several avenues for further research on the lipids in the gonads and other associated reproductive organs of vertebrates. Some ideas for such further work which might throw a light on the lipid metabolism in these organs in their various physiological states, are expressed below in brief :-

1) Employment of better technique such as gas chromatography may give further information on the fatty acid composition of the various lipid constituents in the gonads and their importance in the functioning of these organs.

2) The recent trend in the study of lipids concerns with investigations of lipid components in the subcellur organelles such as mitochondria, Golgi complex etc.,and such studies given much information on the intracellular role of lipids in the cell metabolism. A detailed ultrastructural analysis of lipids in the organs of reproduction will give a better understanding of their role in the functioning of these organs.

3) The problem of hormonal influence over the lipid metabolism in the gonads needs further eluciation especially at experimental level. As already brought to notice some work on the pituitary hormonal control over the lipids metabolism in testes and ovaries has been done and it has fruitful results. Though much is known about the hormonal control over their structural hyper and hypotrophy, functional hypertrophy,

several enzymes playing important role in several metabolic events, there is a complete paucity of information on the hormonal control over their lipid metabolism. The seasonal alteration observed in gonads in the present work are clearly indicative of gonadal hormonal control over their lipid metabolism. This needs to be confirmed by experimental work involving gonadoectomy and hormone replacement therapy.

4) This hormone - lipid relationship leads to another important point, nature of hormone action. The basic thing is whether such hormone - lipids relationship is direct or indirect. Hence, while seeking an insight into the hormonal influence over the lipids metabolism in gonads, there is definite needs of studying the various enzymes playing a role in lipids metabolism and hormonal influence over them.

5) The phospholipids metabolism may bear some importance from the point of view of the general metabolism of the gonads, in their various functional states, during the annual breeding cycles, since many membrane bound enzyme activities have been observed to be affected by the presence or absence of typical lipids. Triggle (1970) and Lennars (1970) observed that the activities of many membrane bound enzymes either decline or completely last when the membrane lipids are extracted or modified. Fleischer et al. 1962, Green and Fleischer, 1963, Green et al.,1967, Racker and Bruni 1968, proved that the mitochondrial electron transport enzymes were completely dependent upon the total mitochondrial lipids.

Na-K ATPase is activated by PS (Fenster and Copenhaver,1967), Glucose-6-phosphatase activity is activated by PE Duttera et al.,(1968), Finean (1973) gives a list such - lipid dependent enzyme systems. Such an experimentally evidenced relationship between the enzyme activities and some phospholipids indicates that probably activities of some key enzymes functioning in the reproductive organs might be dependent upon the presence of such phospholipids. Some work in this direction is necessary.

6) The functional significane of the lipids in gonads and excepting that of steroid precursors such as cholesterol and cholesterol esters is far from fully understood in fishes. The above mentioned idea concerning the probable influence of the phospholipids on various membrane bound enzymes is one way of getting an insight into the functional attributes of lipids in the reproductive organs. How much of the lipids in the reproductive organs is used as energy source is not fully known. Pre-dominance of TG may be explained on this basis. In case of other lipids much work can be done to determine their functional significance.

7) In this laboratory along with the work on gonadal lipids, other work is also carried out. A comparison of the results shows that the lipids do not function in isolation in these organs, but alterations in them bear some relationship with the alterations taking place in other metabolites. For example, in the present investigation on the ovaries of seasonally

breeding fish, lipids get accumulated in the active-spawning period and again in the period of sexual quiescence. How it is possible, where do these lipids go ? This can be explained if the lipid alterations are studied in comparison with the alterations in the lysasomal enzymes. These enzymes show a very marked enhancement during this transition period during which lipids undergo depletion, thus indicating that the lysosome may be responsible for removal of some such lipids and bringing the ovarian lipid level, to the level characteristic of active spawning period. Such comparative studies, can give better understanding of the metabolic events in the gonads. Further more work in this direction is also desired.

While concluding the present dissertation, the author would like to state that the present work is by no means complete. The analysis of the fatty acids have been included in this dissertation, but for its detail study it involves use of gas-liquid chromatography which is not available in this laboratory. No ablative techniques have been employed to get an insight into hormone-lipid relationship, since it was selected to study the lipid changes in naturally occuring hormonal alterations in the seasonal breeding cycles. Even with these difficiencies, the author feels gratified that he has studied the lipids from gonads of two selected fishes, made original contributions on the lipids of fish gonads, which had not been studied earlier, viewed the

observations comparatively to find out some pattern of lipid alterations, in the gonads, their hormonal control, and functional significance. Some additional work on the problem outlined above is also necessary which might throw more focus on the importance of lipids in the physiology of reproduction in fishes and other vertebrates.

" To make an end is to make a beginning
The end is where we start from "

..... T.S.Eliot