CHAPTER

OBSERVATIONS

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The aim of the present investigation undertaken is to find out the quantities of total lipids, neutral lipids and phospholipids, as well as individual components of neutral lipids and phospholipids. Is there any alterations in the individual components of neutral and phospholipids, corelate with breeding cycle of R.daniconius and C.fulungee. The breeding cycle of higher vertebrates have been studied extensively, but lower vertebrates like fishes have been received less attention. There is scanty literature on biochemical and histo-chemical studies on Maharashtrian fishes. Few workers, who have made their contribution in reporting the gonadal changes of tropical fishes are Ghos and Kar (1952) and Nayyar and Sundararaj (1970) on the common Indian catfish, Heteropneustes-fossilis, Nair (1959); on the Indian shad Hilsailisha, Sathyanesan (1961) on Barbus-stigma, Rai (1965) Barbustar, Moser (1967) on Sebastodes-peucispinis, Chan and Philips (1967) on the synbranchid eel. Monopterus-albus, Hyder (1970) on Tilapia-leucosticta; Latif and Saady (1973) on the Nile Bolti, Tilapia-nilotica and Pawar (1978) on Tilapia-mossambica.

Rasbora and Cirrhina are the important fishes found in the Southern part of Maharashtra State, they were studied mainly from the point of view of morphology, distribution, their resistance against the pollutants and toxic substances (Nikam, 1986). Going through the available literature it was found that information on the seasonal variation in the gonadal lipids of the above two fishes was practically lacking.

1. TESTIS (R.daniconius) :

1.1 Biochemical observations :

The changes existing in total lipids, total neutral lipids and total phospholipids in the testes of <u>R.daniconius</u> during the seasonal breeding cycle are shown in Graph No.1. The cyclicle changes in the TLC separations of various neutral lipid and phospholipid components are illustrated in the Plate No.1, Fig. A and B respectively. The quantitative changes with statistical variations in total lipids, total neutral lipids and various individual components of neutral lipids from testis of <u>R.daniconius</u> are tabulated in Table No.1. The similar information for total phospholipids and their individual components from testis of <u>R.daniconius</u> are tabulated in Table No.2.

A) The alterations in testicular total lipids :

The amount of testicular total lipids expressed in mg/g wet weight of the testes, shows interesting alterations during the reproductive cycle of the species. It was observed that, during pre-breeding period, when testes were engaged in spermatogenesis, initially TL showed a rising trend, but from mid-pre breeding period onwards they exhibited decreasing pattern. Thus, the total lipids value at the beginning in the month of March was $1073\pm$ 7.5 mg/g, which was further increased to 130.6 \pm 9.00 mg/g in April. Then there was a little decreased in lipid values in the month of May (128.5 \pm 9.22 mg/g). During the active breeding period from June to August the TL value exhibit gradual decrease. The TL value in the month of June was 115.2 \pm 8.30 mg/g, which was further decreased to 101.9 \pm 7.6 mg/g in the month of July. The TL exhibits further decrease in the month of August (80.25 \pm 6.52 mg/g).

During the post-breeding period TL values exhibit rising pattern from September to November. The TL values in the month of September was $62.85 \pm 6.19 \text{ mg/g}$. This value was further enhanced to $71.57 \pm 6.58 \text{ mg/g}$ in October, which was still further increased to $142.1 \pm 10.21 \text{ mg/g}$ in November. During the sexual quiescencet period TL values showed decreasing trend from December to February. The TL value was 140.2 \pm 9.5 mg/g in month of December, which was further decreased to $112.0 \pm 8.62 \text{ mg/g}$ during January, such value was further decreased to $106.9 \pm 7.14 \text{ mg/g}$ in the month of February.

Thus, the significant feature of the alterations in the total testicular lipids was a gradual increase during pre-breeding period followed by steady decrease during the active breeding period upto mid-post-breeding period and then a sharp rising in the lipid values at late post-breeding period in November and then the lipids exhibited gradual decrease through out the sexual quiescent period.

B) Seasonal alterations in the testicular neutral lipids :

a) Total neutral lipids :

The alterations observed in the testicular total neutral lipids during the seasonal breeding cycle formed a parallel

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pattern to those exhibited by the total lipids described above. Just like the total lipids, the total neutral lipids exhibited rising trend during pre-breeding but from mid-pre-breeding period onwards they exhibited decreasing pattern. Thus the total neutral lipids which ranged at 106.2 + 7.42 mg/g in March enhanced to 126.9 + 8.82 mg/g in April. Then there was decrease in the total neutral lipids values in the month of May (124.) \pm 7.72 mg/g). During the active breeding period from June to August the total neutral lipid value showed gradual decrease. The total neutral lipid values in the month of June was $110.1 \pm 6.18 \text{ mg/g}$ which was further decreased in months of July and August 95.48 + 6.1 and 76.50 \pm 5.82 mg/g respectively. In the month of September when the breeding activities were over, the values showed rising pattern. The values of total neutral lipids in month of September // was 60.80 + 5.12 mg/g. This value was further increased to 69.98 ± 6.53 mg/g in October, which was still further increased and reaching the peak value in the month of November (140.6 \pm 9.18 mg/g). In the guiescent period the NL showed a decreasing trend, the values in December, January and February were 138.5 + 8.3 mg/g, 111.0 \pm 7.6 mg/g, and 103.8 \pm 6.7 mg/g respectively.

b) Individual components of neutral lipids :

The thin layer chromatographic separation of testicular neutral lipids during the seasonal breeding cycle, indicated that the testicular neutral lipids contained MG, DG, TG, CHO, CE and FFA. When the values for the individual components of

the testicular lipids are critically studied from the Table No.1, for the month of November when these components are present in maximum concentration, it can be seen that at a comparative level, quantitatively TG occurred in maximum concentration, DG, MG and CHO coming next in concentration in that order, whereas, the CE and FFA were present in least concentration.

At a general level, it can be seen that the alterations occuring in various neutral lipid components run parallel to those described above for the total neutral lipids, with some minor differences. Taking the TG it could be seen that the maximum concentration of TG was observed at the late post-breeding period in the month of November when the values were 125.5 ± 6.26 mg/g. and the level of this neutral lipid component exhibited a gradual decrease during the sexual quiescent period, thus the TG values during these months, December, January and February were, 123.3 \pm 6.2 mg/g, 105.5 \pm 6.1 mg/g and 95.60 \pm 6.02 mg/g, respectively. During the pre-breeding period of gametogenesis the values of TG were increased upto the mid-pre-breeding period, thus the values of TG 95.03 + 7.21 mg/g during March, further increased 112.6 + 7.12 mg/g in April. But from the late pre-breeding TG level again showed depletion gradually which is also continued in active breeding period, thus the TG value 109.44 \pm 7.67 mg/g in May, while the values in June, July and August were 96.12 + 6.20 mg/g, 81.36 \pm 5.24 mg/g and 61.95 \pm 4.23 mg/g respectively.

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As the post-breeding period initiates the TG level exhibitd a very gradual increase. Thus they increased to 51.81 ± 3.02 mg/g in September and 57.25 ± 4.94 mg/g in October. Thus, it appears that the TG got accumulated mostly in the late-post breeding and quiescent periods and metabolised during the period of gametogenesis. The MG, DG and FFA also exhibited similar alterations with some minor differences.

Considering another component of the neutral lipids CHO, it showed gradual increase in the values from pre-breeding to active breeding period; thus the CHO values 0.559 ± 0.04 mg/g in March, 0.747 ± 0.07 mg/g in April and 0.057 ± 0.06 mg/g in May. This increase in CHO level was also continued during the active breeding period, thus the CHO value 1.115 ± 0.421 mg/g in June, 1.846 ± 0.523 mg/g in the month of July, this value was further enhanced to 1.942 ± 0.438 mg/g in August. During the post-breeding and sexual quiescent period CHO level was decreased. CE showed similar alterations with CHO.

C) Seasonal alterations in testicular phospholipids :

a) Total phospholipids :

The total phospholipids during the seasonal breeding cycle exhibited alterations which were different from those in the neutral lipids. After a critical evaluation of statistical data prepared for total phospholipids showed that there is a gradual rise during the pre-breeding period, reaches at the high point during the mid-active breeding period. Thus the

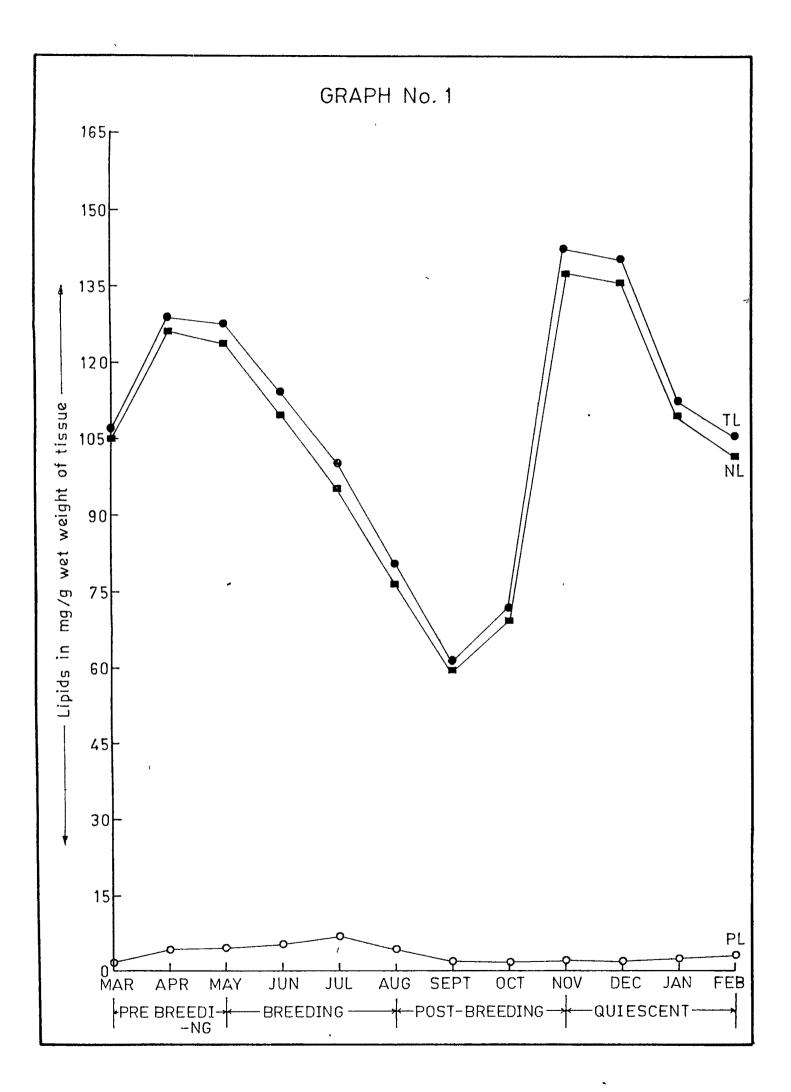
total phospholipid ranged at 1.101 + 0.05 mg/g at the initiation of the preperatory period in March and further increased to 3.622 + 0.15 mg/g in April. The month of May witnessed still further increase, when the total phospholipid values were enhanced to 4.461 + 0.22 mg/g in May. During the breeding season proper, the testes exhibited a gradual increase in the quantity of phospholipids. Thus during June and July the values increased to 5.131 + 0.30 mg/g and 6.502 + 0.42 mg/g respectively. During August the total phospholipid values started decrease and in this month they depleted to 3.750 ± 0.19 mg/g, this decrease was further evident during the post-breeding period, thus the total phospholipid values in September, October and November were, 1.969 ±0.06 mg/g, 1.689 + 0.07 mg/g and 1.501 + 0.05 mg/g respectively. In the quiescent period (December, January and February) the total phospholipids exhibited insignificant alterations, thus the values of total phospholipid were 1.500 ± 0.08 mg/g, 1.520 ± 0.04 mg/g and 3.15 ± 0.16 mg/g respectively.

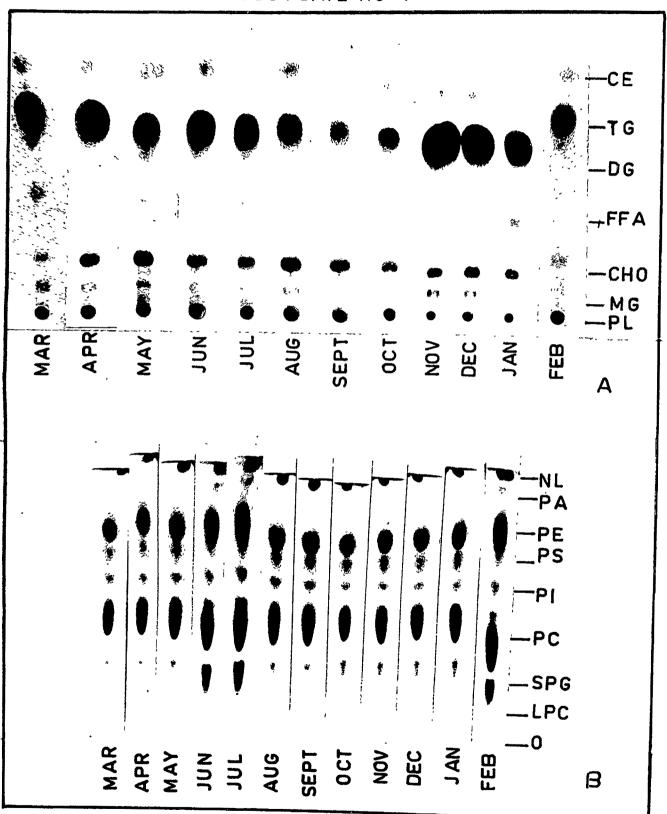
b) Individual components of phospholipids :

The thin layer chromatographic separation of the testicular phospholipids, during the seasonal breeding cycle indicated the presence of LPC, SPG, PC, PI, PS, PE and PA, when the values of the individual components of the phospholipids are critically analysed from Table No.2, it could be seen that PC and PE formed the major components of the phospholipids, PI, PS, and LPC were present in moderate quantities and SPG and PA occurred in least concentration.

The quantification studies on the individual components of phospholipids indicate that their alterations run parallel to those described for the total phospholipids. Taking PC which was the major phospholipids component, its value was 15.84 + 1.06 µg-p/g during early preparatory period in March. The increase in PC values was evident during late pre-breeding period in the months of April and May, thus the values were 79.40 + 4.54 ug-p/g, and 85.90 + 4.82 ug-p/g respectively. This increase in PC value was further evident during the early active breeding period, thus in the month of June PC value rose to 95.67 ± 4.92 $\mu g-p/g$, reaching maximum value of 160.6 \pm 8.3 $\mu g-p/g$ in July. The PC value during the late-active breeding period was depleted in August to 90.80 + 4.72 µg/g this decrease in PC values continued during the post-breeding period. Thus during September, October and November the values were, $31.80 \pm 1.72 \ \mu g-p/g$, 28.97 µg-p/g, and 16.44 + 1.2 µg/p respectively. During the sexual quiescent period PC level showed slightly increasing pattern, thus the values in the months of December and January were 30 + 1.83 µg-p/g and 40.82 ± 2.85 µg-p/g respectively. The PC value showed still further increase in February when it was 73.93 4431 ug-p/g.

With minor differences PE and rest of the phospholipid components exhibited similar alterations. Thus the phospholipid components exhibited steady increase during the preparatory period, which were further enhanced in mid-active breeding period, during late-breeding period onwards their quantities exhibited gradual decrease which were continued during post-breeding period.





TLC PLATE NO-1

Seasonal alterations in testicular total and neutral TABLE No.1 :

lipids of <u>R.daniconius</u> during annual breeding cycle.

Months Months Morth April March April <	Seasons	Pre	Pre-breeding	•• ••	Bre	eeding	3 8 62	post	st-breeding	** **	'nð	Quiescent	
107.3130.6128.5115.2101.980.2562.8571.57142.1140.2112.0103. 17.5 29.0 29.22 29.30 27.6 56.52 56.19 26.58 210.21 29.5 8.62 7 17.42 29.62 214.0 110.1 95.48 76.50 60.88 69.98 140.6 138.5 111.0 $103.$ 27.42 296.43 20.42 20.91 20.90 2142 2.591 21053 29.66 217.6 20.32 20.43 20.22 20.92 21.12 10.12 25.63 217.6 20.62 20.23 20.32 20.43 20.22 20.92 21.12 21.291 21.053 20.66 20.62 20.23 20.22 20.32 20.43 20.22 20.92 20.92 20.92 20.65 20.23 20.23 20.22 20.64 20.747 0.857 1.115 1.846 1.942 0.653 20.65 20.23 0.233 0.233 0.233 0.233 0.677 20.66 20.693 20.634 20.632 20.64 20.66 20.62 20.23 0.233 0.233 0.233 0.233 0.203 0.234		March		Мау			Aug.	ept.	Oct.		Ů	an.	Feb.
utral 106.2 126.9 124.0 110.1 95.48 76.50 60.88 69.98 140.6 138.5 111.0 103. pids $\frac{1}{7}7.42$ $\frac{1}{4}8.82$ $\frac{1}{7}7.72$ $\frac{1}{4}6.18$ $\frac{1}{4}6.1$ $\frac{1}{5}.82$ $\frac{1}{5}.12$ $\frac{1}{6}.53$ $\frac{1}{2}9.18$ $\frac{1}{8}.8$ $\frac{1}{7}7.6$ $\frac{1}{4}6.$ 3.168 $\frac{4}{10}6$ $\frac{4}{4}.06$ $\frac{4}{4}.04$ $\frac{3}{3}.320$ $\frac{3}{3}.220$ $\frac{3}{3}.142$ $\frac{2}{5}.691$ $\frac{3}{3}.058$ $\frac{5}{5}.052$ $\frac{5}{5}.13$ $\frac{1}{7}.761$ $\frac{3}{2}.$ $\frac{1}{2}0.32$ $\frac{1}{2}0.43$ $\frac{1}{2}0.22$ $\frac{1}{2}0.91$ $\frac{1}{2}0.90$ $\frac{1}{2}0.03$ $\frac{1}{2}0.65$ $\frac{1}{2}0.05$ $\frac{1}{2}0.05$ $\frac{1}{2}0.02$ $\frac{1}{2}0.02$ $\frac{1}{2}0.02$ $\frac{1}{2}0.02$ $\frac{1}{2}0.01$ $\frac{1}{2}0.0$	Total lipids	107.3 +7.5	130.6 <u>+</u> 9.0	50	115.2 <u>+</u> 8.30	0+		00 -1 00 -1 00 -1		42 .1 10.2	40.2 +9.5	12. 0 8.6	20
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Neutral lipids		126.9 <u>+</u> 8.82		110.1 <u>+</u> 6.18		ດ ເ ເ ເ ເ ເ	5.8	0 0 ບໍ່າ	40.6 +9.1	é e		ε
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	MG	3.168 +0.32	4 06 +0 43	4.04 +0.22	3.320 +0.92	3.22 0.91	3 .1 4 0 . 90	59	00	00.0	0.1 0	76 02	3.251 +0.93
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	СНО	0.559 +0.04	0.747	0.857	1.115 ± 0.421	1.84 0.52	0.4 0.4	νO	0.710 ±0.02	0.68 0.05	0.63	20	0.673 +0.04
6.335 8.70 8.60 8.210 8.120 7.251 5.181 7.04 8.854 8.411 2.668 2.5003 ± 0.432 ± 0.36 ± 0.36 ± 0.35 ± 0.35 ± 0.34 ± 0.23 ± 0.62 ± 0.27 ± 0.03 ± 0.62 95.03 112.6 109.4 96.12 81.36 61.95 51.81 57.25 125.5 123.3 105.5 95.5 ± 7.21 ± 9.12 ± 7.67 ± 6.20 ± 55.24 ± 44.23 ± 3.02 ± 44.94 ± 92.26 ± 6.1 ± 6.1 0.186 0.374 0.572 0.622 1.217 1.242 0.152 0.710 0.421 0.295 0.101 0.01 10.186 0.374 0.572 0.622 1.217 1.242 0.152 0.710 0.421 0.295 0.101 0.01 10.01 ± 0.02 ± 0.222 ± 0.23 ± 0.05 ± 0.04 ± 0.01 ± 0.01 ± 0.01 ± 0.01	FFA	0.876 ±0.5	0.475 +0 * 09	0.525	0.723	0.513 0.02	0.954 +0.08	• 53	21	12	0.23 0.08	α 0	0.943 +0.02
95.03112.6109.496.1281.3661.9551.8157.25125.5123.3105.595. $\div7.21$ ±9.12 ±7.67 ±6.20 ±5.24 ±4.23 ±3.02 ±4.94 ±9.26 ±6.2 ±6.1 ±6.1 0.186 0.374 0.572 0.622 1.217 1.242 0.152 0.710 0.421 0.295 0.101 0.120 ±0.01 ±0.02 ±0.02 ±0.02 ±0.22 ±0.23 ±0.05 ±0.01 ±0.01 ±0.01	ĝ	6.335 +0.432	8.70 ±0.36	8,60 <u>+</u> 0,13	8.210 ±0.36	NN	7.2	5.18 0.23	7.04 <u>+</u> 0.16	00 00	8.41 +0.27	0 0 0 0 0	2.772
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	JIG	95.03 47.21	112.6 +9.12	109.4 ±7.67	96 . 12 <u>+</u> 6.20	81.36 +5.24	4.0 4.2	00°0°	N 0	25.5 19.2	14 M	16.	50
	ы С	0.186 <u>+</u> 0.01	0 .37 4 +0 .02		0.622	1.21 0.22	1.24 0.23	• 1 5 • 05	-71 -04	0.42 0.08	0.29 0.01	0.101	100

The values for the total and neutral lipids are expressed as mg/g wet weight of testis.

Note:

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Seasonal alterations in testicular phospholipids TABLE NO.2 :

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of R. daniconius during annual breeding cycle.

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Seasons	Ω. 	Pre-breeding			Breeding	•• ••	Pos	Post-breeding		0	Quiescent	
Months	: March	: April	: May ;	· June :	July :	Aug.	Sept.:	Oct. :	Nov.	 Dec	Jan.:	Feb.
Total Phospho- lipids	1.101 +0.05	3.622 +0.15	4.461 ±0.22	5.131	6.502 +0.42	3.750 <u>+</u> 0.19	1,969 +0,06	1.689 +0.07	1.501 ±0.05	1.509 <u>+</u> 0.08	1.520 +0.04	3.15 +0.16
LPC	5.279 +0.35	10.59	12.60 +0.87	14.20 <u>+</u> 1.02	15.32 <u>+</u> 1.12	9.592 +0.83	3.976 ±0.10	4.831 +0.12	7.315	6.185 +0.17	3.64 ±0.12	10.97 ±1.10
SPG	3.959 +0.22	5.293 ±0.28	7.420	10.25 ±0.76	14.39 +0.98	8,253 +0,94	7.952 +0.18	9.662 +1.03	14.61 11.02	3.135 +0.12	2.727	9.48 +0.76
С Ф	15.84 +1.06	79.40 +4.54	85.90 +4.82	67 92	160°6	90,30 +4,72	31,80 <u>+</u> 1.72	28,97	16.44 ±1.29	30.00 +1.83	40.82 +2.85	73.93 <u>+</u> 4.31
, 머 , 요 , `	5.279 +0.38	15 . 88 +1.03	16.10. <u>+</u> 1.06	20.62 +1.12	11.36	8.520 +0.94	7.952	9.662 +1.03	3.654	4.135 +0.31	2.82	4.48 +0.78
Sđ	1.792 +0.21	10-59 +0-83	20.25 ±1.10	25.30 +1.21	24.43 +1.20	5.420 +0.22	3.976 ±0.27	4.831 +0.31	5.481 +0.21	4.135 +0.32	2.82 +0.14	05.72 +0.96
2 2 4	5•279 +0•35	15.88 +1.03	25.90 <u>+</u> 1.23	30.1 0 <u>+</u> 1.32	35°37 +1•49	24.30 +1.15	19.83 +1.03	14.49 +0.93	7.308	8.265 +0.81	9.455 +0.73	20.97 <u>+</u> 1.12
PA	1.792 ±0.04	7.252 +0.02	10.27	9.112 +0.72	8.129 +0.70	3•262 +0•32	3.20 +0.03	2.312 +0.04	5.231 +0.03	4.620 +0.06	1.327 ±0.16	1.232 ±0.17
Note	90	The values of values of ind	total _I Ividual	; of total phospholipids individual components a	rer	express tpressed	ed as mg/ in μg-p/	g wet wet	weight of weight of	f testis. f testis.	Whereas	

During the quiescent period from December to February they showed insignificant rise.

2. TESTIS (C.fulungee) :

2.1 Biochemical observations :

The changes existing in total lipids, total neutral lipids and total phospholipids in the testes of <u>C.fulungee</u> during the seasonal breeding cycle are shown in Graph No.2. The TLC separations of various neutral lipid and phospholipid components during seasonal breeding cycle are illustrated in Plate No.2, Fig. A and ^B respectively. The quantitative changes with statistical variations in total lipids, total neutral lipids and its individual components from testis of <u>C.fulungee</u>, are tabulated in Table No.3, the similar information for total phospholipids and their individual components from testis of <u>C.</u> fulungee are tabulated in Table No.4

A) The alterations in testicular total lipids :

The amount of testicular total lipids expressed in mg/g wet weight of the testes, shows interesting alterations during the reproductive cycle of the species. It was observed that during pre-breeding period, when testes were engaged in spermatogenesis, initially total lipids showed a rising pattern, but from mid-pre-breeding period onwards they exhibited decreasing pattern. Thus the total lipids value at the beginning in the month of March was 62.10 \pm 5.26 mg/g, which was further increased to 136.3 \pm 12.7 mg/g in April. Then there was a little increase in lipid values

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in the month of May $(137.5\pm 10.9 \text{ mg/g})$. During the active breeding period from June to August the total lipid values exhibited gradual decreases. The total lipids value in the month of June was $129.4 \pm \frac{3.2}{2}$ g, which was further decreased to $125.3 \pm \frac{8.12}{2}$ g in the month of July. The total lipids exhibited further decrease in the month of August (82.51 \pm 6.82 mg/g).

During the post-breeding period total lipid values exhibit rising pattern from September to November. The total lipid values in the month of September was $44.05 \pm 4.3 \text{ mg/g}$. This value was further enhanced to $50.27 \pm 4.35 \text{ mg/g}$ in October, which was still further increased to $54.51 \pm 4.6 \text{ mg/g}$ in November. Unlike the testis of <u>R.daniconius</u>, the <u>C.fulungee</u> testis showed further increasing trend in the total lipid values during sexual quiescent period. Thus the total lipids value was 54.95 ± 5.1 mg/g in month of December, which was further increase to $56.33 \pm 5.82 \text{ mg/g}$ during January, such value was further increased to $61.48 \pm 6.25 \text{ mg/g}$ in the month of February.

Thus the significant feature of the alterations in the total testicular lipids was a gradual increase during pre-breeding period followed by steady decrease during the active breeding periods and once again an gradual increasing trend during the post-breeding and sexual quiescent period.

B) Seasonal alterations in the testicular neutral lipids :

a) Total neutral lipids :

The alterations observed in the testicular total neutral lipids during the seasonal breeding cycle formed a parallel pattern to those exhibited by the total lipids described above. Just like the total lipids, the total neutral lipids exhibited rising trend during pre-breeding period. Thus the total neutral lipids which ranged at 60.88 + 4.72 mg/g in March enhanced to 132.0 \pm 7.8 mg/g in April, which was still further increased 133.0 \pm 7.1 mg/g in the month of May. During the active breeding period from June to August the total neutral lipid value showed gradual de-The total neutral lipid values in the month of June was crease. 124-6+ 9.6 mg/g, which was further decreased in July and August (119.6 \pm 7.9 mg/g and 78.25 \pm 6.13 mg/g respectively). During the post-breeding period when the breeding activities was over the total neutral lipid values showed decreasing pattern which was further continued during the sexual guiescent period. Thus the values of total neutral lipids in month of September was 40.65 ± 3.62 mg/g. This value was further increased to 47.09+ 3.8 mg/g in October, which was still further increased to 52.07 + 3.94 mg/g in the month of November. During the period of sexual quiescence total neutral lipid values showed further increase at 52.55 \pm 4.8 mg/g, and 54.96 \pm 5.21 mg/g in the months of December and January respectively. This was further increased at 59.54 \pm 5.6 mg/g in the month of February.

b) Individual components of neutral lipids :

The thin layer chromatographic separation of testicular neutral lipids during the seasonal breeding cycle, indicated that, the testicular neutral lipids contained MG, DG, CHO, CE and FFA. When the values for the individual components of the testicular lipids were critically studied from the Table No.3 for the month of May when these components were present in maximum concentration, it could be seen that at a comparative level, quantitatively TG occurred in maximum concentration. DG, MG and CHO coming next in concentration in that order, whereas the CE and FFA were present in least concentration.

At a general level, it could be seen that the alterations occuring in various neutral lipid components run parallel to those described above for the total neutral lipids, with some minor diff-Taking the TG it could be seen that the maximum concentraerences. tion of TG was observed at the late pre-breeding period in the month of May, when the values were 105.9 \pm 7.8 mg/g and the level of TG exhibited gradual decrease during the active-breeding period, thus the TG values during June, July and August were 98.37 ± 6.4 mg/g, 95.26 ± 6.2 mg/g and 62.62 ± 4.16 mg/g respectively. As the post-breeding period initiates the TG level exhibited a gradual decrease. Thus it decreased to 30.64 + 3.62 mg/g, 32.65 + 3.6 mg/g and 36.75 + 3.26 during the months of September, October and November respectively. This gradual increase was further continued during the sexual quiescent period, thus the TG values during the months of December, January and February were 38.05 + 3.78 mg/g,

39.10 \pm 3.92 mg/g and 42.20 \pm 3.32 mg/g, respectively. During the pre-breeding period of gametogenesis the values of TG were further increased to 51.81 \pm 3.13 mg/g in March and 103.2 \pm 7.3 mg/g in the month of April. Thus it appears that the TG got accumulated in the post-breeding sexual quiescent and pre-breeding period and metabolised during the period of gametogenesis. The MG, DG and FFA also exhibited similar alterations with some minor differences.

Considering another component of the neutral lipids CHO, it showed gradual increase in the values during early pre-breeding period, thus the CHO values 0.609 ± 0.03 mg/g in March and 1.713 ± 0.06 mg/g in April, while CHO value during the late pre-breeding period showed depletion at 0.389 \pm 0.02 mg/g in the month of May. During the active breeding period CHO level showed gradual increase, thus the CHO value increased to 0.637 ± 0.05 mg/g in the month of June which was still further increased to 0.937 ± 0.08 mg/g in July, this value was further enhanced to 1.121 ± 0.08 mg/g in August. During the post-breeding period and sexual quiescent period the CHO level was decreased gradually. CE showed similar alterations with CHO.

C) Seasonal alterations in testicular phospholipids :

a) Total phospholipids :

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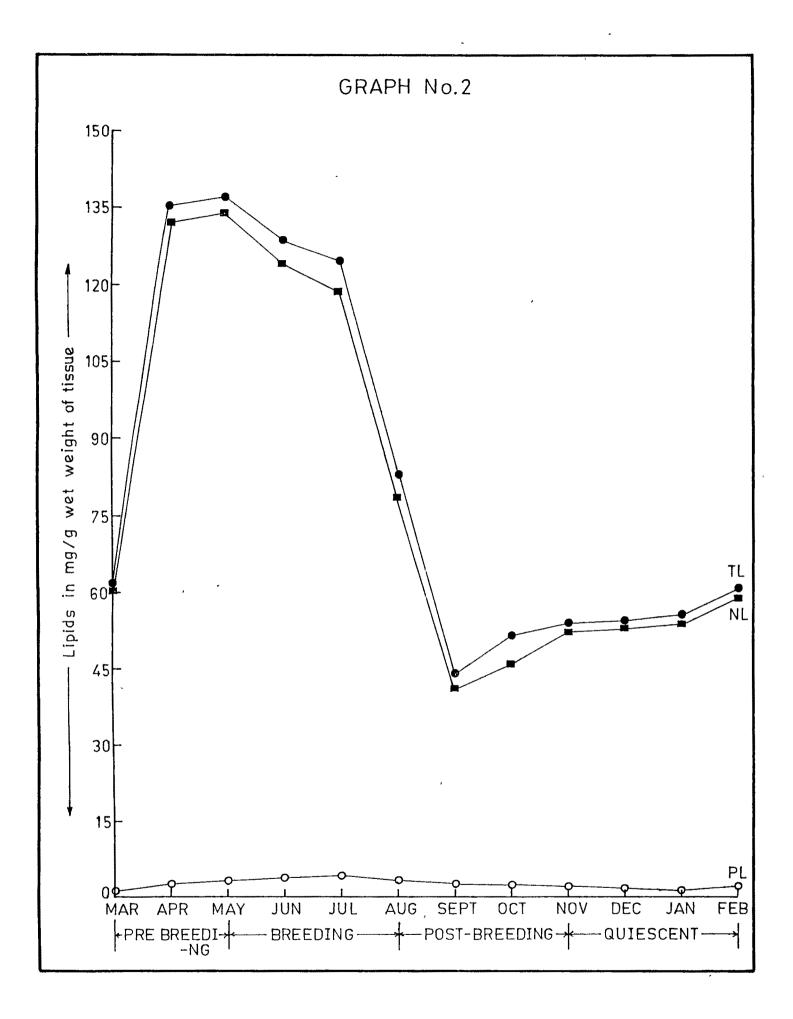
The total phospholipids during the seasonal breeding cycle exhibited alterations which were different from those in

tic the neutral lipids. After a critical evaluation of statastical data prepared for total phospholipids showed that there was a gradual increase during the pre-breeding period, reaches at the peak point during the mid-active breeding period. Thus the total phospholipids ranged at 1.220 ± 0.06 mg/g at the initiation of the preparatory period in March and further increased to 4.302 + 0.23 mg/g in April. The month of May witnessed still further increase, when the total phospholipid values were enhanced to 4.423 + 0.25 mg/g. During the active breeding season, the testes exhibited a gradual increase in the quantity of phospho-Thus during June and July the values increased to 4.716 lipids. \pm 0.30 mg/g and 5.757 \pm 0.43 mg/g respectively. During the month of August the total phospholipid values showed decrease and in this month PL depleted to 4.251 ± 0.30 mg/g, this decrease was further evident during the post-breeding period, thus the total phospholipid values in September, October and November were, 3.408 ± 0.22 mg/g, 3.182 ± 0.21 mg/g and 2.438 ± 0.18 mg/g respectively. This gradual decrease in the total phospholipids level was still further evident during the sexual quiescent period, when the values were depleted to 2.40 ± 0.17 mg/g in December, 1.372 ± 0.12 mg/g in January and 1.944 ± 0.16 mg/g in the month of February.

b) Individual components of phospholipids :

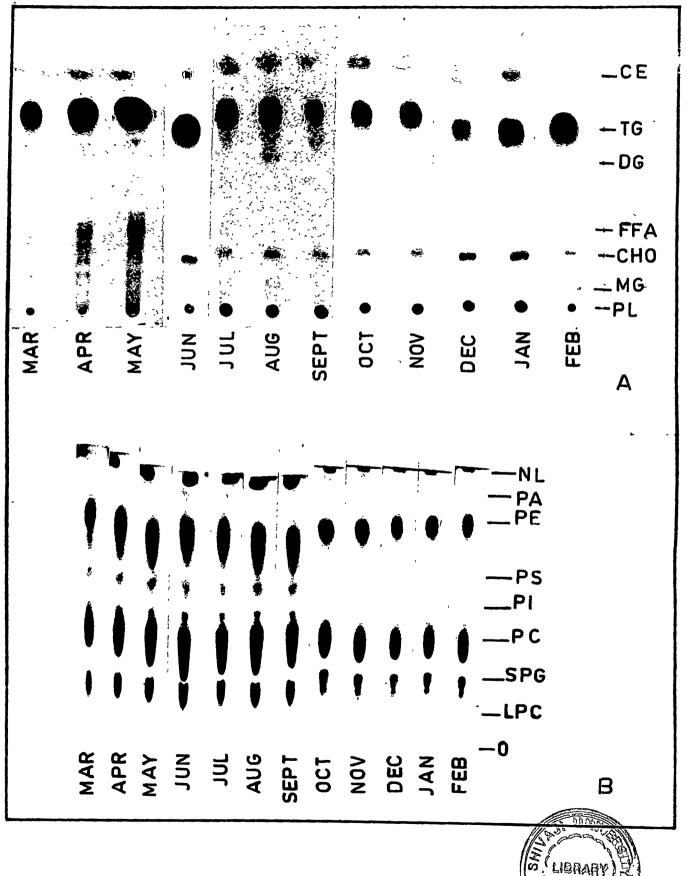
The thin layer chromatographic separation of the testicular phospholipids, during the seasonal breeding cycle indicated the presence of LPC, SPG, PC, PI, PS, PE and PA, when the values of the individual components of the phospholipids are critically analysed from the Table No.4, it could be seen that PC and PE formed the major components of the phospholipids. PI, PS and SPG were present in moderate quantities and LPC and PA occurred in least concentration.

The quantification studies on the individual components of phospholipids indicate that their alterations run parallel to those described for the total phospholipids. Taking PC which was the major phospholipids component, its value was 19.43 + 1.22 µg-p/g during early preparatory period in March. The rise in the PC values was evident during late pre-breeding period in the month of April and May, thus the values were 75.23 + 4.26 µgp/g and 89.94 + 4.73 µg-p/g respectively. This increase in PC value was_further evident during the early active breeding period, thus in the month of June PC value rose to 95.25 + 5.62 µg-p/g reaching maximum value of 100.4 + 7.2 µg-p/g in July. The PC value during the late-active breeding period was depleted in August to 90.18 ± 6.3 µg-p/g this decrease in the PC values continued during the post-breeding period, thus during September and October the values were $81.69 \pm 4.32 \text{ µg-p/g}$ and 62.87 ± 3.92 ug-p/g, respectively, this decrease was further evident during the month of November $(45.59 \pm 3.20 \ \mu g - p/g)$. The PC value showed still further decrease during the sexual quiescent period, thus the PC values were decreased to 31.65 + 3.12 µg-p/g in December, 24.62 \pm 1.85 µg-p/g in January and 20.40 \pm 1.26 µg-p/g in the month of February.



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Seasonal alterations in testicular total and neutral C.fulungee during annual breeding cycle. lipids of TABLE NO.3 :

6.240 +1.42 0.750 2.130 ±0.03 0.820 42.20 61.48 ±6.25 +0.31 59.54 +5.6 Feb. 0.480 •0.09 5.250 ±1.33 0.622 ±0.03 3.216 35.10 43.92 6.30 +0.63 56.33 +5.82 54.96 +5.21 Quiescent Jan 6.270 ±0.93 2.328 5.219 ±1.18 0.228 0.457 54.95 (+5.1 52.55 +4.8 38.05 +3.78 Deo: 0,423 +0,06 5.684 ±0.73 0.635 +0.07 2.125 6.442 ±1.47 36.75 ±3.26 52.07 +3.94 54.51 +4.6 Nov. Post-breeding 1.082 ±0.24 2.215 +0.07 4.549 5.824 ±0.51 50.57 +4.35 47.09 +3.8 32.65 +3.6 oct. 1.420 1.129 ±0.03 0.625 ±0.09 4.420 1.411 +0.04 40.65 +3.62 44.05 <u>+</u>4.3 30.64 +3.62 Sept. 0.625 +0.06 8.420 ±1.62 1,212 ±0.08 $\frac{1.129}{0.05}$ 4.252 78.25 62.62 +4.16 82.51 +6.82 Aug. 0.833 0.789 ±0.09 6.523 0.937 15.23 95.26 +6.2 125.3 +8.12 119.6 Breeding July 6.212 0.769 0.631 ±0.08 0.637 18.12 98.37 ±6.4 129.4 +8.2 133.0 , 124.6 ±7.1 ±9.6 June 0.523 +0.06 0.389 ±0.02 6.620 +0.16 19.86 +2.43 105.9 +7.8 137.5 May Pre-breeding 1.713 0.721 6.113 +0.21 0.943 19.31 +2.22 103.2 April 136.3 132.0 0.609 0.533 2.591 +0.36 5.181 +1.34 +0.06 62.10 ±5.26. 51.81 +3.13 60.88 +4.72 March Neutral lipids Seasons Total lipids Months e E O FFA Q g 8 មួ

The values for the total and neutral lipids are expressed as mg/g wet weight of testis. Note :

Seasonal alterations in testicular phospholipids of TABLE NO.4 :

C.fulungee during annual breeding cycle.

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Seasons	Jđ :	Pre-breeding	•• ••	B	Breeding	** **	Post	Post-breeding		B	Quiescent	
Months	; March ;	April :	May :	June :	July	: Aug. :	Sept.	0ct. :	• AON	n Dec D	Jan. *	Feb.
Total phospho- lipids	1.220 <u>+</u> 0.06	4.302 +0.23	4.423 +0.25	4.716 ±0.30	5,757 ±0,43	4.251 +0.30	3 .4 08 +0.22	3.182 <u>+</u> 0.21	2.438 +0.18	2.40 +0.17	1.372 ±0.12	1.944 ±0.16
LPC	2.159 +0.06	7.224 ±0.20	7.193	6,120 ±0,18	8.316 +0.21	6.423 +0.19	3.612 ±0.12	3.025 ±0.10	2.118 +0.09	3.163 ±0.11	1.894 +0.06	12.24 +0.72
SPG	4.318 +0.12	18.64 +1.08	12.19	15.10 +1.02	20.86 +2.12	10.27 <u>+</u> 1.21	3.612 +0.12	12 . 68 +1.12	12.69 +1.12	4.745 ±0.16	2.841 +0.96	12.24
PC	19.43 +1.22	75.23 <u>+</u> 4.26	89.94 +4.73	95.25 +55.62	100.4 <u>+</u> 7.2	90 . 18 +6.3	01.69 +4.32	62.87 <u>†</u> 3.92	45.58 +3.20	50	24.62 +1.85	20.40 +1.26
Iđ	4 .3 18 +0 . 12	15.69 +1.20	12.19 +1.02	14.32 <u>+</u> 1.20	18.72 ±1.92	9.263 +0.92	3.612 ±0.12	7.96 	6.346 <u>+</u> 1.01	7.908 	3. 788	8.162 ±0.99
S G	12.95 ± 1.02	22 .1 8 +1.83	18.28 <u>+</u> 1.32	20 .6 2 <u>+</u> 1.42	22 . 66 +1.63	10.36 +1.02	3.612 +0.12	10.20 ±1.12	4.220 ±0.12	6.326 +0.92	3.788 ±0.98	02.24
ង	24.32 +0.12	45.37 +3.22	40 . 52 +3.12	45.00 +3.20	50 .7 0 +3.68	40.12 +3.12	38.06 <u>+</u> 3.10	35.44 +3.10	25,35 +1,38	25.31 <u>+</u> 1.38	17.04	18.17 ± 1.32
PA	1.292 +0.06	9.218 +1.02	8.321 <u>+</u> 1.00	6.231 ±1.01	8,623 +1.21	3.428 +0.92	2.120 +0.92	3.359 ±0.98	1.210	2.515 <u>+</u> 0.96	0.932 ±0.08	4.313 + 0.12

The values of total phospholipids are expressed as mg/g wet weight of testis, whereas values of individual components are expressed in ug-p/g wet weight of testis. Note :

Considering another component of phospholipids PE which occurred next to the PE in its concentration and showed similar alterations with PE. It showed gradual increase during the pre-breeding period, thus the PE values 24.32 + 0.12 µg-p/g in March, 45.37 + 3.22 µg-p/g in April and $40.52 + 3.12 \mu g - p/g$ in May. This increase in PE value was further evident during the early active-breeding period, thus in the month of June PE value rose $45.00 \pm 3.20 \text{ µg-p/g}$, reaching to $50.70 \pm 3.68 \mu g - p/g$ in July. The PE value during the late-active breeding period was depleted in August to 40.12 \pm 3.12 µg-p/g this decrease in PE values continued during the post-breeding period, thus during September, October and November the values were, $38.06 \pm 3.10 \ \mu g - p/g \ 35.44 \pm 3.10 \ \mu g - p/g$, and 25.35 + 1.38 µg-p/g respectively. This decrease in PE value was still further evident during the sexual guiescent period. Thus the PE value ranged at 25.31 + 1.38 µg-p/g in month of December, decreased to $18.17 \pm 1.32 \,\mu\text{g-p/g}$ in February. With minor differences, the rest of the phospholipid components exhibited similar alterations. Thus the phospholipid components exhibited steady increase during the preperatory period which was further enhanced in mid active breeding period. During late active breeding, post-breeding and sexual quidscent period the component values showed gradual decrease.

3. OVARY (R.daniconius) :

3.1 Biochemical observations :

The alterations occurring in the total lipids, total neutral lipids and total phospholipids in the ovaries of **48**

<u>R.daniconius</u> during the seasonal breeding cycle are shown in Graph No.3. The seasonal alterations in the TLC separations of ovarious neutral lipid and phospholipid components in the ovaries are illustrated in TLC Plate No.3, Fig.A and B.

The quantitative alterations with statistical variations in total lipids, total neutral lipids and various individual components of neutral lipids are tabulated in Table No.5, whereas Table No.6 gives similar information for total phospholipids and their individual components.

A) Seasonal alterations in the ovarian total lipids :

The quantities of ovarian total lipids are expressed in terms of mg/g wet weight of the ovaries showed typical variations during the seasonal breeding cycles of the female. Generally during the preparatory period when the ovaries were engaged in Oogenesis the values of total lipids exhibited a gradual increase. Such an increase was initiated in the month of March and progressed throughout the period of vitellosenesis and reached the maximum level in the month of July, when the ovaries were full of mature ova. Following the ovulation the values of the total lipids gradually decreased from August to February. The total lipids in the month of March were 110.0 \pm 8.1 mg/g. Gradually the values of the total lipids increased to 136.2 \pm 9.2 mg/g in April. Such increase was further continued to 140:1 \pm 9.62 mg/g in May. During the period of vitellogenesis the value of total lipids showed further increase to

145.6 \pm 9.81 mg/g in June. TL exhibited maximum concentration in the month of July (157.2 \pm 10.2 mg/g), when the ovaries well (/ with full of mature ova. After the spawning activities the relative concentration of the ovarian total lipids began to decrease slowly. Thus the value of total lipids in the month of August was 130.5 \pm 9.2 mg/g. Once the active spawning period was over, the ovarian total lipids showed further decreasing pattern during post-spawning period. September witnessed 102.2 \pm 6.9 mg/g, such decrease was further continued during late post spawning and subsequent period of quiescence. Thus TL values during October and November were 98.43 \pm 6.82 mg/g and 85.29 \pm 6.36 mg/g respectively; while TL values during quiescent period during the months of December, January and February were 79.10 \pm 6.00 mg/g, 77.96 \pm 5.87 mg/g and 47.24 \pm 3.82 mg/g respectively.

Thus the ovarian total lipids showed in general an increasing trend during the period of Oogenesis, attainig their peak level just prior to the ovulation followed by a sharp decrease during the post spawning and subsequent quiescent period.

B) Seasonal alterations in the ovarian neutral lipids :

a) Total neutral lipids :

The quantitative alterations in the total neutral lipids were practically similar to those described above for 50

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total lipids. The level of neutral lipids exhibited a gradual rise during the preparatory period of oogenesis and vitellogenesis, reaching a peak when the ovaries were full of mature ova. After the ovulation the total neutral lipid values were suddenly depleted reaching their minimum level during late spawning period. Thus during the early preparatory period in March, the total neutral lipids ranged at 106.4 \pm 7.8 mg/g, which rose to 130.8 + 8.43 mg/g in April. Such an increase was further exhibited in May, June and reaching a peak value during July. Thus the values of total neutral lipids for the three months were, 133.9 \pm 8.63 mg/g in May, 139.5 \pm 10.6 mg/g in June and 150.3 \pm 12.8 mg/g in July. The month of August witnessed slight decrease, when the values dropped to 125.2 + 8.6 mg/g. During the postspawning period the neutral lipid level showed decreasing pattern, thus the neutral lipids value in September and October were 96.82 \pm 8.87 mg/g and 93.31 \pm 6.52 mg/g respectively, which was further decreased to $81.65 \pm 6.10 \text{ mg/g}$ in November. This decrease was found to be continued during the quiescent period, when the total neutral lipid values decreased gradually during December, January and February, the values being 77.10 + 5.78 mg/g, 75.88 ± 5.23 mg/g and 44.14 ± 3.14 mg/g respectively.

b) Individual components of the neutral lipids :

The thin layer chromatographic separations of the neutral lipids indicated the presence of MG, DG, TG, CHO, CE and FFA. When

the values for the individual components of the ovarian lipids are critically analysed from Table No.5 for the active breeding period in July when these components were present in maximum concentration, it will be seen that at a comparative level quantitatively TG form the major component of the neutral lipids, which was present in maximum concentration. MG, DG, and CHO occurred in moderate quantities, whereas FFA and CE were present in least amount.

From the quantitative data of the monthly variations in the individual component of the neutral lipids it could be seen that the alterations in them run parallel to those described above for the total neutral lipids. Taking one of the main component of neutral lipids i.e. TG which occurred in maximum quantities, it could be seen that the TG quantities were enhanced during the preparatory period attaining the peak during the active spawning period in July, followed by a gradual decrease reaching the minimal value during the quiescent period. Initially the quantity of TG ranged at 88.76 ± 6.72 mg/g during early preparatory period in February. As the pre-spawning period progressed, the TG value exhibited a gradual increase, the level of TG was 98.51 <u>+</u> 7.62 mg/g in April and 100.1 <u>+</u> 8.62 mg/g during May. At the onset of the active spawning period the TG value exhibited a progressive increase to 102.72 + 8.62 mg/g in June, while at the mid-active spawning period TG value increased at 106.1 + 8.83 mg/g, during July, but at the late active spawning period when ovulation was already occurred the TG value decreased to 89.67

 \pm 7.10 mg/g in August. This decrease in the TG level was still further decreased gradually during the post-spawning and quiescent period, thus the TG values during the months of September, October and November were, 67.45 \pm 4.12 mg/g, 66.50 \pm 4.10 mg/g and 59.30 \pm 4.1 mg/g respectively. Such decrease in TG values was further continued to 56.20 \pm 4.82 mg/g in December, 51.65 \pm 4.73 mg/g in January and 34.15 \pm 2.87 mg/g in February. Most Most of the remaining components of neutral lipids, such as MG, DG, CHO, CE and FFA also exhibited practically identical alterations with minor differences in statistical increase or decrease.

C) Seasonal alterations in the ovarian phospholipids :

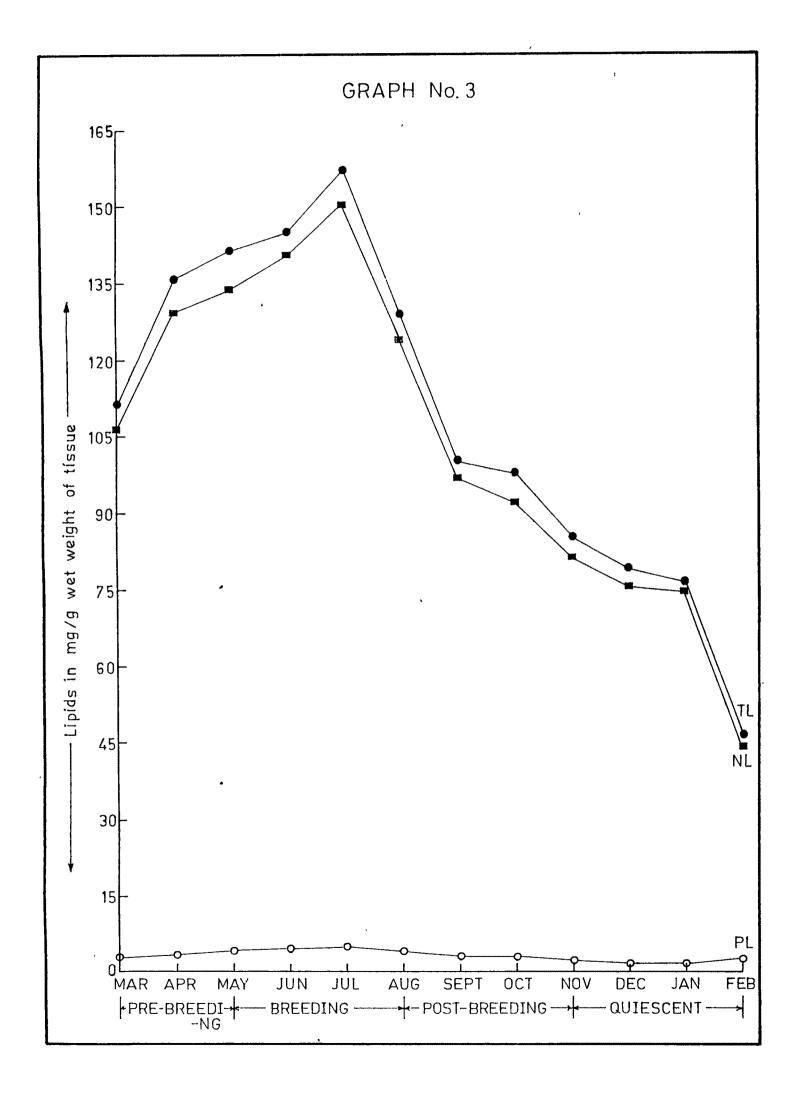
a) Total phospholipids :

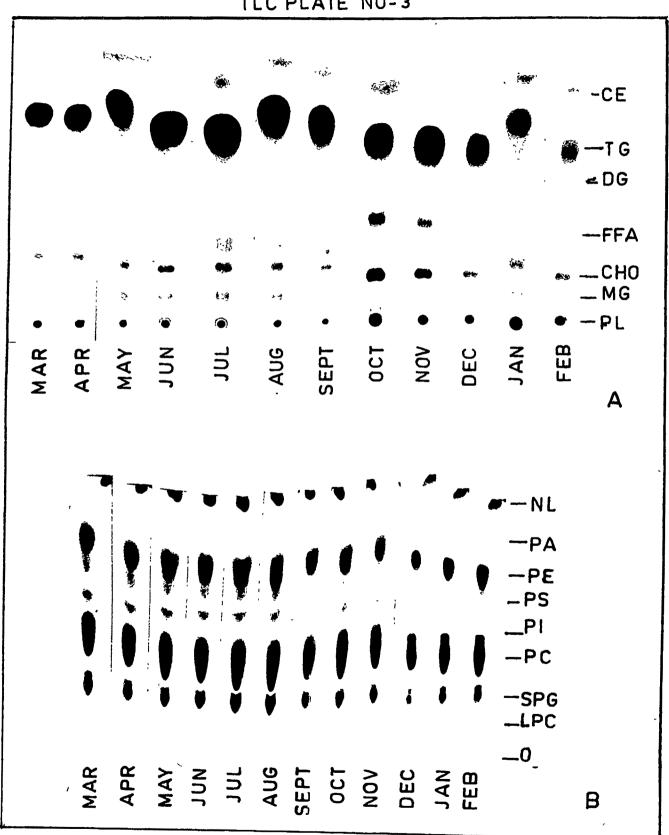
The total phospholipids of the ovary during the seasonal spawning cycle exhibited a gradual rise during the preparatory period, attaining the maximum values at the mid-active spawning period in July and decrease toward the late spawning period in August. This decrease was further continued during the post-spawning period, and the subsequent period of quiescence. Thus the values of total phospholipids ranged at 3.648 \pm 0.16 mg/g in March. During the preparatory period gradually the level of total phospholipid levels were 5.305 \pm 0.30 mg/g in April and 6.128 \pm 0.15 mg/g in May. During the active spawning period the total phospholipid levels exhibited further increase, and attain the peak level during mid-spawning period, thus the

values in June and July were 6.185 ± 0.16 mg/g and 6.872 ± 0.38 mg/g respectively. The level of total phospholipids were slightly depleted to 5.368 ± 0.32 mg/g in August. During the post-breeding period total phospholipids levels were decreased gradually thus the levels were 5.354 ± 0.32 mg/g during September. 5.115 ± 0.35 mg/g in October and 3.644 ± 0.28 mg/g in November. During the early quiescent period total phospholipids level was decreased but later on they were increased during the late quiescent period, thus the total phospholipid level was 1.997 ± 0.123 mg/g in December, this level of phospholipids (was) enhanced to 2.079 ± 0.212 mg/g in January and 3.097 ± 0.29 mg/g during February.

b) Individual components of phospholipids :

The thin layer chromatographic separation of the phospholipids indicated the presence of LPC, SPG, PC, PI, PS, PE and PA. A critical study of the Table No.6, indicates that among the phospholipids PC and PE were the main components. The phospholipid components in general exhibited rising trend during the preparatory period, reached their maximum values during the active spawning period and then gradually decreased during the postspawning period; while during the late quiescent period phospholipid components showed little increase. Thus, considering one of the components PC which occurred in maximum amounts, the PC value ranged at 67.80 ± 3.62 ug-p/g during early preparatory period in March. As the preparatory period progressed, the values





TLC PLATE NO-3

Seasonal alterations in ovarian total and neutral lipids of R.daniconius during annual spawning cycle. TABLE NO.5 :

2.552+1.10 3.289 +0.45 1.020 0.269 0.03 47.24 +3.82 2.86 +0.24 34.15 +2.87 44.14+3.14 Feb. 6.549 +1.00 2.082 +0.98 4.325 +0.83 8.824 +1.34 0.770 0.12 Quiescent 51.65 +4.73 77.96 75,88 +5,23 Jan. 0.832 1.072 8.382 ±1.17 0.520 0.06 10.10 79.10 77.10 ±5.78 56.20 +4.82 Dec. ** 1.123 0.530 0.06 0.926 ±0.43 10.25 59.30 +4.1 +85.29 +66.36 81.65 9.52 +1.26 Nov. Post-spawning 1.210 2.130 0.626 0.06 98.43 +6.82 +4.10 93.31 +6.52 10.72 +1.82 12.12 41.81 66.50 Oćt. 1.525 4.210 0.762 0.08 10.65 ±1.83 12.22 +1.93 67.45 96.82 +8.87 +4.12 Sept. 102.2 0.∯62 +0.38 0.854 1.242 0.10 20.25 +2.24 12.62 ±2.1 89.67 +7.1 130.5 +9.2 125.2 +8.6 Aug. 0.764 ±0.42 0.937 ± 0.14 1.672 0.12 24.62 +2.21 16**.**19 +2.12 +8.83 157.2 150.3 +12.8 106.1 Spawning July1.210 +0.78 0.872 0.934 0.09 14.62 +1.68 19.10 +1.92 102**.**72 .<u>+</u>8.62 145.6 +9.81 139.5 +10.6 June 0.830 +0.33 0.625 +0.09 0.525 0.08 140**.**1 +9,62 133.9 +8.63 13.40 +1.56 18.50 +1.81 100.1 +7.1 May Pre-spawning 0.724 0.473 ±0.05 0.362 0.05 130.8 <u>+</u>8.43 12.31 +1.32 18.47 +1.78 98.51 +7.62 April 136.2 +9.2 0.435 0.06 3.698 +0.82 1.740 ±0.92 0.735 88.76 +6.12 11.09 +1.12 March 110**.**0 106.4 +7.8 Neutral lipids Seasons Total lipids Months CHO EHO FFA C E 8 ទ្ឋ ы С

The values for the total and neutral lipids are expressed as mg/g wet weight of ovary. ... Note

Seasonal alterations in ovarian phospholipids of TABLE NO.6 :

R. daniconius during annual spawning cycle.

Seasons	<u>G</u>	Pre-spawning	βt	Ŋ	Spaw ning	99-200-9-2-44	20 4	Post-spawning	bu	nd	Quiescent	
Months	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan	Feb.
Total Phospho- lipids	3.648 <u>+</u> 0.16	5.305 <u>+</u> 0.30	6.128 +0.15	6.185 0.16	6.872 ±0.38	5, 368 ±0, 32	5 . 354 <u>+</u> 0.32	5 . 115 +0.35	3.644 +0.28	1.997 +0.123	2.079 +0.212	9 3.097 2 <u>+</u> 0.29
ГРС	6.164 <u>+</u> 0.17	10.26 +0.76	12.30 +0.83	15.32 +0.92	20.08 +1.32	13.62 +1.13	20.06 ±1.7	13.03 +1.3	1.00 +1.01	2.669 <u>+</u> 1.78	4.856 +0.32	8.209 +0.72
SPG	12.33 <u>+</u> 0.84	15 . 39 +0.93	15.20 +0.91	16.12 +0.94	16.33 +1.20	11.62 +1.18	28.99 +1.34	14.39 +0.92	5.769 +0.32	5.338 +0.31	4.856 +0.28	16.42 +1.12
ЪС	67.80 <u>+</u> 3.62	102.6 +7.2	109 .7 0 1 ±7.6	112.2 +7.8	120.7 - <u>+</u> 8.1	105.3 <u>+</u> 7.6	118.15 +7.92	108.70 ±7.6	88.46 <u>+</u> 6.2	34.66 +2.42	36.38 +2.51	41.04 <u>+</u> 3.31
Iđ	15.41 +1.2	20.52 +1.24	25.10 +1.28	27.00 <u>+</u> 1.29	27.32 +1.30	20.13 +1.2	24.06 +1.13	11•36 +0•93	19.23 10.98	7.998 +0.62	7.276 +0.62	24.63 +1.12
N N	18.50 +1.3	20.52 +1.24	32.30 +1.82	25.03 +1.29	24.69 +1.20	19.10 <u>+</u> 1.21	9.453 +0.921	24.24 +1.13	23.08 <u>+</u> 1.09	5.338 +0.35	4.856	16.42 +0.93
Яd	18•50 +1•3	30.79 +1.73	31.10 +1.75	33.32 +1.79	45 . 68 +2.36	38. 26 +2.17	11.17 ±0.78	37.87 ±1.92	7.000	22.66 +1.12	21.83	24.63 <u>+</u> 1.13
PA	7-225	12.10 +0.79	19.10 +1.2	18•40 <u>+</u> 1•3	20.10 +1.82	7•272 ±0•82	10,12 ±0,75	2.20 +0.24	1.215	1.23 +0.11	3.121 +0.21	2.352 +0.13
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The values of total phospholipids are expressed as mg/g wet weight of ovary, whereas, \mathbf{y} alues of individual components are expressed in μg -p/g wet weight of ovary.

Note:

of PC gradually rose to 102.6 + 7.2 µg-p/g in April and 109.7 + 7.6 µg-p/g during May. During the spawning period this increase in PC value was further maintained, and reaching a peak during mid-spawning period, thus the values of PC were, 112.2 \pm 7.8 µg-p/g in June and 120.7 \pm 8.1 µg-p/g during July. During late spawning period in August PC value was depleted to $105.3 \pm 7.6 \mu g$ -p/g. During the post-spawning period PC level gradually decreased, thus the values of PC dropped to 118.1 + 7.92 µg-p/g in September, 108.7 + 7.6 µg-p/g during October and 88.46 \pm 6.2 µg-p/g in November. During the early quiescent period PC value showed gradual decrease but during late guiescent period the level showed little increase, thus the PC level was 34.66 \pm 2.42 µg-p/g in December, 36.38 \pm 2.51 μ g-p/g in January and 41.04 \pm 3.31 μ g-p/g during February. The remaining phospholipid components such as PE, LPC, SPG, PI, PS and PA which occurred in low quantity exhibited the seasonal alterations similar to those described for PC with some minor differences in statistical increase or decrease.

4. OVARY (C.fulungee) :

4.1 <u>Biochemical observations</u> :

The alterations occuring in the total lipids, total neutral lipids and total phospholipids in the ovaries of <u>C</u>. <u>fulungee</u> during the seasonal breeding cycle are shown in Graph No.4. The TLC separations of various neutral lipid and

phospholipid components in the ovaries during seasonal breeding cycle are illustrated in Plate No.4, Fig. A and B. The quantitative alterations with statistical variations in total lipids, total neutral lipids and various individual components of neutral lipids are tabulated in Table No.7, whereas Table No.8 gives similar information for total phospholipids and their individual components.

A) Seasonal alterations in the ovarian total lipids :

The quantities of ovarian total lipids are expressed in terms of mg/g wet weight of the ovaries showed typical variations during the seasonal breeding cycle. During the preparatory period when the ovaries were engaged in Oogenesis the values of total lipids exhibited a gradual increase. Such an increase was initiated in the month of March and progressed throughout the period of vitellogenesis and reached the maximum level in the month of July, when the ovaries were full of mature ova. Following the ovulation the values of the total lipids gradually decreased in August. The total lipids in the month of March were, 97.44 + 7.23 mg/g, the values of total lipids increased to 138.6 + 8.2 mg/g in April. Such increase was further continued to 153.0 + 9.2 mg/g in May. During the period of vitellogenesis the value of total lipid showed sharp increase to 160.1 + 13.6 mg/g in June and this increase was further continued in the month of July, when total lipid value become maximum 176.3 + 16.6 mg/g, when eggs were ready for the ovulation. After the spawning activities, the relative concentration of the ovarian total lipids began to decrease slowly.

Thus the value of total lipids in the month of August was $125.5 \pm 8.1 \text{ mg/g}$. Once the active spawning was over the ovarian total lipids showed further decreasing trend during post-spawning period. September witnessed $77.81 \pm 5.7 \text{ mg/g}$, such decrease was further continued during month of October to $68.21 \pm 5.6 \text{ mg/g}$ and $65.12 \pm 5.4 \text{ mg/g}$ in November. During the sexual quiescent period the total lipid values further decreased, thus the values were $55.76 \pm 4.8 \text{ mg/g}$ in December, $43.87 \pm 4.3 \text{ mg/g}$ in January and $29.26 \pm 2.24 \text{ mg/g}$ in the month of February.

Thus the ovarian total lipids showed in general an increasing trend during the period of Oogenesis, attaining their maximum level just prior to the ovulation, which is followed by a sharp decrease during the post-spawning and subsequent sexual guiescent period.

B) Seasonal alterations in the ovarian neutral lipids :

a) Total neutral lipids :

The quantitative alterations in the total neutral lipids were practically similar to those described above for total lipids. The level of neutral lipids exhibited a gradual rise during the preparatory period of Oogenesis and vitellogenesis, reaching a maximum level when the ovaries were full of mature ova. After the ovulation the total neutral lipid values were suddenly depleted during the late spawning period. Thus during the early preparatory period in March, the total neutral lipids ranged at 95.86 \pm 5.67 mg/g, which suddently rose to 133.9 \pm 8.0 mg/g in April. Such an increase was further enhanced in May and June and reached at peak level during July. Thus the values of total neutral lipids for the three months were 147.7 \pm 10.7 mg/g in May, 155.5 \pm 11.5 mg/g in June and 168.5 \pm 12.8 mg/g in July. The month of August witnessed slight decrease when the values dropped to 119.3 \pm 7.8 mg/g. During the post-spawning period the neutral lipids value; in September and October were, 72.27 \pm 6.3 mg/g and 63.07 \pm 5.63 mg/g respectively, which was further decreased to 60.35 \pm 5.6 mg/g in November. This decrease was further evident during the sexual quescent period when the total neutral lipid values decreased to 52.87 \pm 3.62 mg/g in December, 41.17 \pm 3.21 mg/g in January and 27.41 \pm 3.61 mg/g in February.

b) Individual components of the neutral lipids :

The thin layer chromatographic separations of the neutral lipids indicated the presence of MG, DG, TG, CHO, CE and FFA when the values for the individual components of the ovarian lipids are critically analysed from Table No.7, for the active breeding period in July when these components are present in maximum concentration, it will be seen that at a comparative level quantitatively TG form the major component of the neutral lipids, which was present in maximum concentration, MG, DG, and CHO occurred in moderate quantities, whereas FFA and CE were present in least amount.

From the quantitative data of the monthly variations in the individual components of the neutral lipids it would be seen

that the alterations in them run parallel to those described above for the total neutral lipids. Taking one of the main component of neutral lipids i.e. TG which occurred in maximum quantities it could be seen that the TG quantities were enhanced during the preparatory period, attaining the peak during the active spawning period in July, followed by a gradual decrease reaching the minimal value during the guiescent period. Initially the quantity of TG ranged at 83.11 + 5.61 mg/g, during early preparatory period in March. As the pre-spawning period progressed, the TG value exhibited a gradual increase, the level of TG was 108.2 + 7.1 mg/g in April and 119.0 + 8.1 mg/g in May. At the beginning of active spawning period a TG value exhibited a progressive increase to 122.0 + 8.87 mg/g in June, while at the midactive spawning period, TG value increased at 129.7 + 8.9 mg/g, during July, but at the late active spawning period when ovulation (was) already occurred the TG values decreased to 93.31 \pm 5.8 mg/g in August. This decrease in the TG level was still further decreased gradually during the post-spawning and quiescent period; thus the TG values during the months of September, October and November were 65.01 + 3.62 mg/g, 53.21 + 3.51 mg/g and 45.32 +2.12 mg/g respectively. Such decrease in the TG values was further continued to 39.39 + 3.67 mg/g in December, 31.24 + 3.72 mg/g in January and 14.68 ± 1.67 mg/g in February. The MG, DG and FFA also exhibited similar alterations with some statistical rise or fall. Considering another component of the neutral lipids CHO, it showed gradual decrease in the values during the pre-

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spawning period, thus the CHO values were 3.894 ± 0.33 mg/g in March, 2.860 ± 0.42 mg/g in April and 1.930 ± 0.38 mg/g in May. Such an decrease in CHO values were still further continued during the active-spawning period, thus the CHO values were ranged at 1.648 ± 0.36 mg/g in June, 0.920 ± 0.26 mg/g in July and 0.789 ± 0.23 mg/g in August. During the post-spawning period CHO values were increased gradually, thus CHO values were enhanced to 1.525 ± 0.34 mg/g in September, 2.735 ± 0.72 mg/g in October and 3.684 ± 0.86 mg/g in November. This increase in the CHO level was further, continued in the sexual quiescent period, the value were 4.091 ± 0.96 mg/g in December, $4.228 \pm$ 1.02 mg/g in January and 4.699 ± 1.13 mg/g in the month of February. CE showed similar alterations with CHO, with some minor differences.

C) Seasonal alterations in the ovarian phospholipids :

a) Total phospholipids :

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The total phospholipids of the ovary during the seasonal spawning cycle, exhibited a gradual rise during the preparatory period, attaining the maximum values at the mid-active spawning period in July and showed decrease towards the late-spawning period in August. Thus the values of total phospholipids during preparatory period ranged at 1.573 ± 0.07 mg/g, in March, 4.718 ± 0.22 mg/g in April and 5.3121 ± 0.35 in May. This increase in total phospholipid level was further increased gradually upto

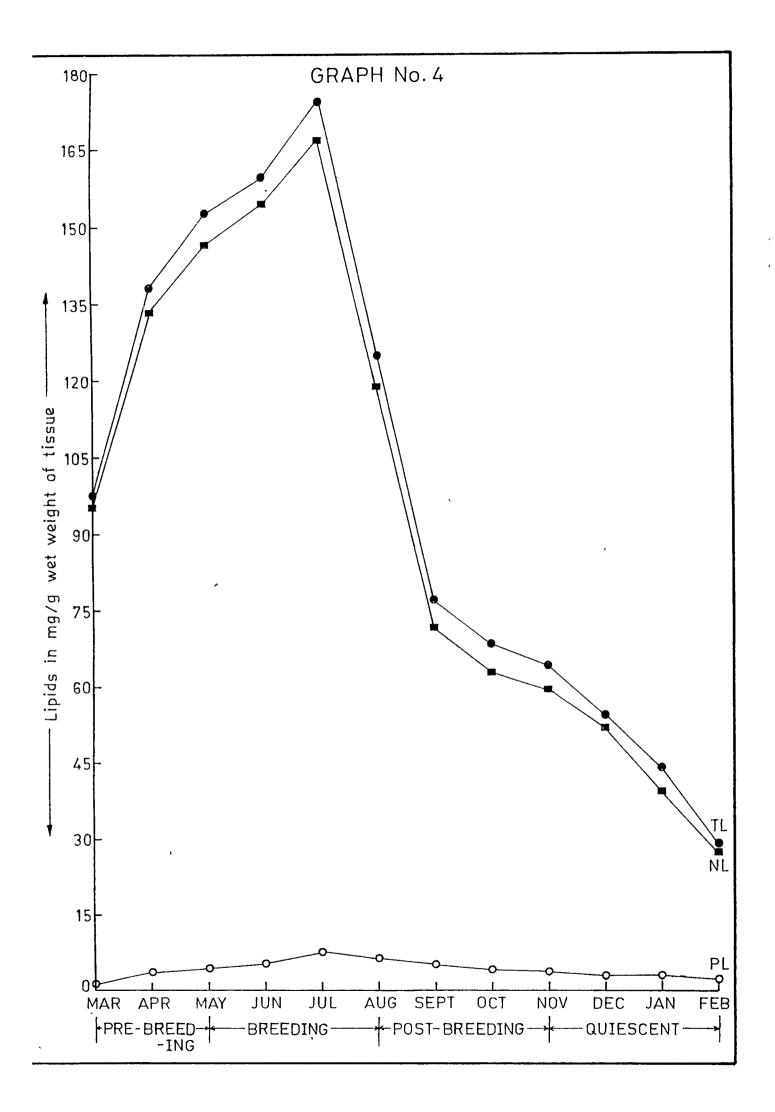
mid-active spawning period, thus the values were 5.438 ± 0.40 mg/g in June, and 7.862 ± 0.89 mg/g in July, as the late spawning period approaches after the ovulation the total phospholipid value was decreased, thus the lipids value was depleted to 6.255 ± 0.43 mg/g in August. Such decrease in the phospholipid level was further continued during the post-spawning period and quiescent period. Thus the total phospholipids value during the months, September, October and November were 5.543 ± 0.52 mg/g, 5.140 ± 0.51 mg/g and 4.772 ± 0.24 respectively. During the sexual quiescent period the total phospholipid values were still further depleted at 2.889 ± 0.12 mg/g in December, 2.704 ± 0.10 mg/g in January and 1.849 ± 0.08 mg/g in the month of February.

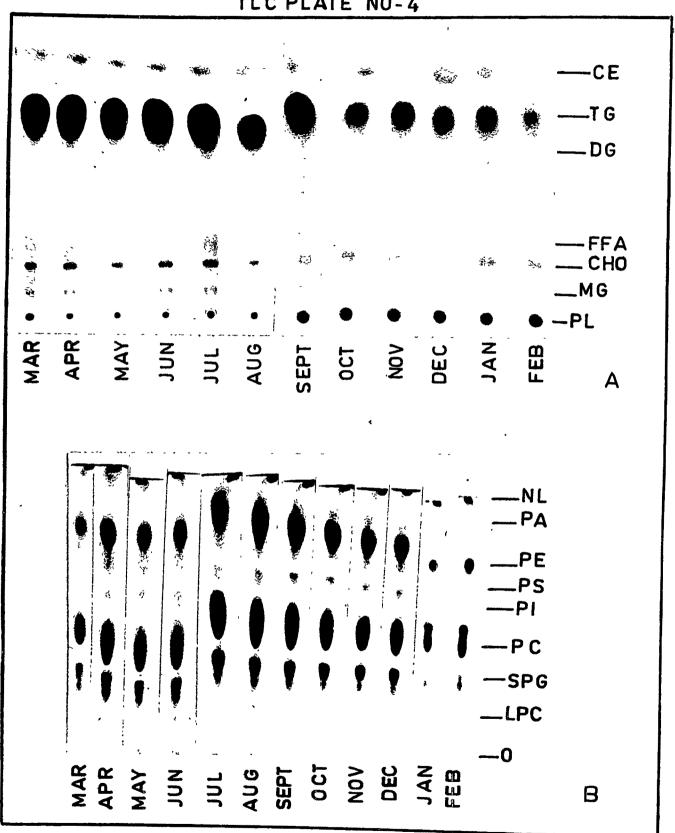
b) Individual components of phospholipids :

The thin layer chromatographic separation of the phospholipids indicated the presence of LPC, SPG, PC, PI, PS, PE and PA. A critical study of the Table No.8 indicates that among the phospholipids PC and PE were the main components. The phospholipid components in general exhibited rising trend during the preparatory period, reached to their maximum values during the active spawning period and then gradually decreased during the post-spawning and quiescent period. Thus considering one of the components PC which occurred in maximum concentration, the PC values ranged at 22.80 \pm 1.14 µg-p/g during early preparatory period in March. As the preparatory period progresses the values of PC gradually rose to 82.75 \pm 4.62 µg-p/g in April and 95.10 \pm 4.82 µg-p/g in May. During the spawning period PC level was still further increased to 98.72 \pm 4.5 µg-p/g in June 133.5 \pm 8.2 µg-p/g in July, while during the late spawning period PC level decreased to 114.2 \pm 7.2 µg-p/g in August. This depletion in PC values were further continued during the post-spawning and sexual quiescent period. Thus, the PC values ranged during months of September, October and November were 100.4 \pm 6.4 µg-p/g, 98.56 \pm 6.3 µg-p/g and 90.70 \pm 6.2 µg-p/g respectively. This gradual decrease in the PC value was further evident during the sexual quiescent period, when values were depleted to 38.59 \pm 2.8 µg-p/g in December, 36.65 \pm 2.72 µg-p/g in January and 28.54 \pm 2.12 µg-p/g in the month of February.

Considering another component of the phospholipids PE which showed similar alterations with PC with some minor differences. It showed gradual increase during the early pre-spawning period, thus the PE values ranged at 17.60 \pm 1.12 µg-p/g, in March and 33.10 \pm 1.34 µg-p/g in April. As the late prespawning approaches the PE value showed slight depletion, thus it was decreased to 31.21 \pm 1.34 µg-p/g in May. During the active spawning period the PE value again showed increasing trend, thus the PE value increased to 33.29 \pm 2.34 µg-p/g in June, 46.39 \pm 4.8 µg-p/g in July. From the late spawning period PE level showed decreasing trend thus the PE value in the month of August was (42.72 \pm 4.43 µg-p/g). This decrease in the PE level was further

continued during post-spawning and quiescent period. Thus the PE values in the months of September, October and November were $52.80 \pm 3.62 \mu g$ -p/g, $48.26 \pm 3.51 \mu g$ -p/g and $40.40 \pm 2.83 \mu g$ -p/g respectively; while during the sexual quiescent period the PE level was further decreased to $19.41 \pm 1.72 \mu g$ -p/g in December, $19.30 \pm 1.69 \mu g$ -p/g in January and $9.515 \pm 0.716 \mu g$ -p/g in the month of February. The rest of the phospholipid components exhibited similar alterations with some minor statistical variations. Thus in general the phospholipid components exhibited steady increase during the preparatory period, which were further rose in mid-active spawning period. During late-spawning, postspawning and sexual quiescent period, the component values showed gradual decrease.





TLC PLATE NO-4

Seasonal alterations in ovarian total and neutral lipids TABLE NO.7 :

of C.fulungee during annual spawning cycle.

0.965 +0.09 1.842 4.699 +1.13 0.768 4.456 27.14 +3.61 14.68 +1.67 29.26 +2.24 Feb. 1.211 ±0.08 4.712 <u>+</u>0.82 4.228 <u>+</u>1.02 0.473 +0.04 3.426 ±0.73 Quiescent 31.24 +3.72 43**.**87 +4.3 41.17+3.21 Jan. 1.415 +0.09 2.430 ±0.31 3.912 +0.68 4.091 ±0.96 1.637 + 0.1339**.**39 +3.67 55.76 +4.8 52.87 +3.62 Dec. 1.923 +0.23 3.684 +0.86 0.862 8.126 +0.33 0.427 ±0.02 60.35 +5.6 45.32 +3.12 65.12 +5.4 Nov. Post-spawning 2,735 0.985 +0.06 2.623 +0.36 0.520 +0.03 63.07 <u>+</u>5.63 3.00 ±0.27 53.21 +3.51 68.21 +5.6 Oct. 1.525 +0.34 **3.**002 ±0.32 0.262 +0.02 Sept. 0.967 +0.04 1,501 +0,23 65**.**01 +3**.**62 72.27 77.81 0.634 ±0.05 8.302 +1.93 0.789 0.827 +0.06 15.42 +1.48 93.31 +5.8 125.5 +8.1 119.3 Aug. 0.467 ±0.08 0.920 +0.26 0.928 +0.05 25.69 +3.21 10.18 +1.72 129.7 +8.9 176.3 +16.6 168,5 ±12,8 Spawning July1.648 +0.36 0.872 0.922 +0.06 20.68 +2.92 10.60 122.0 <u>+</u>8.87 June 155,5 <u>+</u>11,5 160.1 +13.6 15**.5**20 1.930 +0.38 0.520 +0.08 0.622 +0.03 153.00 +9.3 30.10 <u>+</u>1.72 119.0 +8.1 147.7 <u>+</u>10.7 I May Pre-spawning 7.313 ±1.13 0.472 ±0.13 0.430 +0.04 2.860 +0.42 14.63 +1.53 108.2 ±7.1 133.9 +8.0 138.6 +3.2 April 0.563 ±0.07 6.079 +0.43 0.178 ±0.02 3.894 ±0.33 March 3.04 ±0.13 83**.**11 +5.61 97.44 ±7.23 95.86 +5.67 Seasons Neutral Months lipids lipids Total FFA CHO QW 凶 g ы В

The values for the total and neutral lipids are expressed as mg/g wet weight of ovary. .. Note

Seasonal alterations in ovarian phospholipids of TABLE NO.8 :

C.fulungee during annual spawning cycle.

ent	1. Feb.	704 1.849 10 <u>+</u> 0.08	•774 19.54 •90 ±1.12	9, 172 9, 10,86	55 28.54 12 <u>+</u> 2.12	10 12.515 +1.21	774 9.772 13 <u>+</u> 0.74	30 9.515 39 +0.716	652 2.315
Quiescent	. Jan.	89 2.7 2 <u>+</u> 0.1	40 60	2 10 9 +0 8	9 36.6	1 17.1	3 +0.7	1 19.3	10 4.
Post-spawning	De c	72 2.8	0,10,0	20 11.3 5 +0.9	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	5 16.4 2 +1.2	6.0 +0	0 19.4	06 7.2
	Nov.	0 4.7	10.60 +0.92	0 8.620	90.7	22.6	10.80 +0.78	40.4 +2.8	5.1
	0ct.	5,14 ±0,51	12.39 +0.12	8 8 +0 +0 3 8	98 56 +6 3	20.70 +1.6	10.46	48 26 +3 51	6.34
Spawning	sept.	5,543 <u>+</u> 0,52	12.65 +0.12	7.962 ±0.72	100•4 <u>+</u> 6•4	21.75 ±1.6	9.820 <u>+</u> 0.73	52.80	7.10
	Aug.	6.255 +0.43	20.32 +2.12	18 .1 2 1.10	114.2 17.2	19.18 41.12	25•03 +1•23	42.72 	10,63
	July	7.862 +0.89	33•99 +2•13	38.95 +2.34	133 . 6 +8-2	22•26 +1•34	27 . 82 +1.36	46 . 39 +4 . 8	12.13
Pre-spawning	June	2 5.438	18.45 +1.09	10 10.30 +0.92	98.72 +4.9	20.27 ±1.12	28 12 41 28	33.29 +2.34	8.382
	May	5,312 +0,35	20.02	+0.4	95.10 <u>+</u> 4.82	19.43 +1.10	30 • 19 +1•32	31•21 +1•34	10.41
	April	4.718 ±0.22	11.03 +0.85	5.516 +0.39	82.75 <u>+</u> 4.62	16 .55 +1.06	27.58 ±1.27	33.10 +1.34	12.21
	March	1.573 - <u>+</u> 0.07	2.533 +0.05	5.066 +0.38	22.80 +1.14	5.066 +0.38	05.20 <u>1</u> 0.35	17.60 +1.12	3.423
Seasons	Mcnths	Total phospho- lipids	LPC	SPG	D 4	, Iq	୍ୟ - ପ୍	ЪЕ	,× Q

The values of total phospholipids are expressed as mg/g wet weight of ovary, whereas, values of individual components are expressed in μ g-p/g wet weight of ovary.

Note: