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STUDIES ON CYCLICAL LIPID CHANGES IN THE OVARY OF *RASBORA DANICONIUS*

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ABSTRACT
In present study the cyclical changes of lipids in the ovary of Rasbora daniconius were carried out. The neutral lipid and phospholipids were studied during the four stages of breeding cycle. It was found that neutral lipids were accumulated in the ovary during pre breeding stage and metabolized during active breeding period *i.e.* lipids get decreased during breeding stage. Again it was found that lipids get accumulated from post breeding stage and subsequent period of quiescence. The phospholipids slowly increased from pre breeding period and attained high concentration during the active breeding period. Such increase in phospholipid values correlates with increasing number of ovum. The phospholipids showed the decreasing trend from post breeding period followed by quiescent period.

INTRODUCTION

The female reproductive organ ovary is mainly concerned with the production of large no. of ova which is essential for the perfection of external fertilization. Kulkarni and Sathyanesan (1985) studied the cyclical changes in the lipid content of Leydig cells and lobule boundary cells of teleost fish and concluded that the lipid content of Leydig cells correlates with the gonadal cycle. Ezenwa and Ikusemi (1985) studied the seasonal changes in the gonads of fish. It is known that fish ovary contain various amounts of lipids. The distribution and physiological characteristics of gondal lipids have been reported by Johnson (1970).

Lizenka *et al.* (1973) described the content of total lipids and their functional composition determined by thin layer chromatography of the male and female gonad of the fish. and found that the phospholipids were less affected than triacylglycerides observed by seasonal changes during the oogenesis in the teleost fish *C. albula.* He also observed that the eggs contained more glycerides and cholesterol esters similarly phospholipids were found to be higher in the eggs. Zunivici (1969) estimated the total lipids in the mature ovaries of *Chipeonella – cultriventries*. Gastaud (1975) analyzed and compared the values of total lipids in the younger and older oocytes and concluded that the concentration of total lipid is higher in older oocytes than in the younger ones, while they contained low level of cholesterol and triacylglycerides. Singh and Singh (1980) concluded that lipid level in the ovary of fresh water teleost fish, *Heteropneustes– fossilis* showed increase during the pre-spawning phase while decrease at post – spawning phase. Lipid stores represent major energy reserves in fish (Wallaert and Babin 1994, Erdogan *et al*., 2002) and during sexual maturation they are transported from previously stored tissues to gonads for gonadal development because gonads require supplies of constituents such as phospholipids for membranes and cholesterol as substrate for steroid production (Kavadias *et al.* 2003, Lior, 1990). The present investigation was under taken to investigate cyclical changes of ovarian lipids of *Rasbora daniconius*.

MATERIALS AND METHODS

For the present investigation *R daniconius* fish was selected. *Rasbora daniconius* commonly called as 'Dandai'. This species is easily available in the river, stream and tank of Southern Maharashtra. Fish was collected from Rajaram tank near Kolhapur city. At a time 25 females were collected and brought to the laboratory during different periods of year. They where kept in glass containers for three to four hours for acclimatization.

The live fishes were dissected out from ventral side to expose the ovary. The ovary was taken out blotted on the blotting paper, weighed accurately and utilized for lipid study.

Extraction of lipids

The Extraction and purification of lipids were carried out by using Flochs improved method (Floch *et al*., 1957)

Thin layer chromatography of neutral lipids

Skipsi *et al.* (1962) this method includes preparation of plates application of sample and development of chromatogram and detection/identification of spots on the plate.

Quantitative analysis of neutral lipids

The lipids were eluted from silica gel by scraping of different classes of lipids. Lipid elutes were chemically analysed. The amount of triacyleglycerides, diacylegly cerides and monoacylegly cerides in the elutes were estimated according to the method of Antonis (1960) modified by Viogue and Holman (1962). Cholesterol esters and free cholesterol were analyzed by the method of Abell *et al.* (1952). The amount of free fatty acids was determined by the method of Itaya (1977).

Thin layer chromatography of Phospholipids

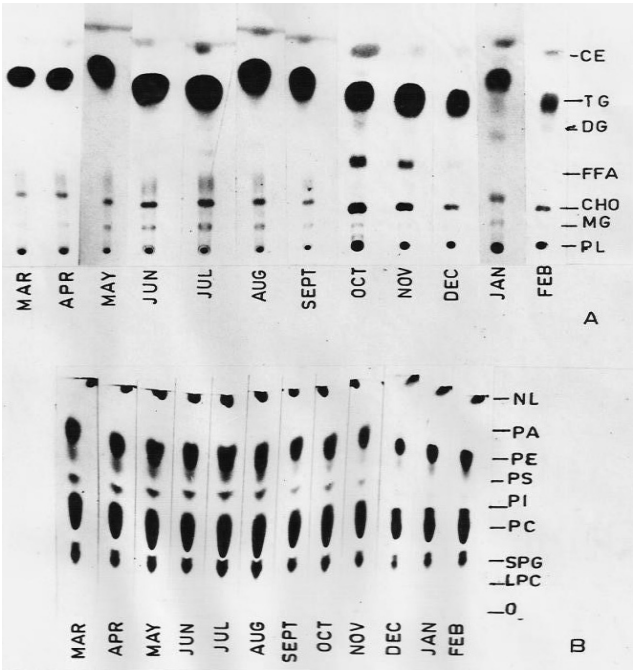
Phospholipid were eluted from the silica gel by suspending the powder in the eluting solvent 2-3mL (chloroform-methanol-acetic acid-water 100:50:10:4$^{v}/_{v}$). Eluted samples of phospholipids were used for determination of lipid phosphorus by the method described by Marinetti (1962).

RESULTS

The cyclic changes in the TLC separations of various neutrallipid and phospholipid components are illustrated in 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 ovary of *R. daniconius* are tabulated in Table 1. The similar information for total phospholipid and their individual components is demonstrated in Table 2.

The amount of ovarian total lipids are expressed in mg/gm wet weight of ovaries, showed typical variation's during the seasonal breeding cycle of species. It was observed that during preparatory period when the ovaries were engaged in oogenesis the values of total lipids (TL) exhibited a gradual increase. This increase were 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 total lipids gradually decreased in August. This decreasing trend of TL further continued during post spawning period. During the sexual quiescent period TL Values shows decreasing trend from December to February.

The alteration observed in the ovarian total neutral lipids during the seasonal breeding cycle are depicted in Table 1. The level of total 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. This decrease in NL values was further evident during the sexual quiescent period. The thin layer chromatographic separations of the ovarian neutral lipids during the seasonal breeding cycle indicated that these lipids contained monoacylglycerides (MG). diacylglycerides (DG), triacylglycerides (TG), cholesterol (CHO), cholesterol esters (CE) and free fatty acids (FFA). At a comparative level quantitatively TG occurred in maximum concentration, DG, MG



Captions: CE: Cholesterol esters, TG: Triacylglycerides DG: Diacylglycerides, CHO: Cholesterol, FFA: free fatty acids, MG: monoacylglycerides PL: Phospholipids NL: Neutral lipids PA: phosphatidic acid PE: phosphatidyl ethanolamine, PS: phosphatidyl serine, PI: phosphatidylinositol SPG: Sphingomyelin, PC: phosphatidylcholine; LPC: Lysophosphatidylcholine

Table 1: Seasonal alterations in ovarian total and neutral lipids of *R.daniconious* during annual spawning cycle. (Note: The values for the total and neutral lipids are expressed as mg/g wet weight of ovary)

Seasons	Pre-spawning			Spawning		Post-spawning			Quiescent		
Months	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan	Feb.
Total lipids	110.0±8.1	136.2±9.2	140.1±9.62	145.6±9.81	157.2±10.2	130.5±9.2	102.2±6.9	98.43±6.8	85.29±6.36	79.10±6.00	77.96±5.8	47.24±3.8
Neutral lipids	106.4±7.8	130.8±8.43	133.9±8.63	139.5±10.6	150.3±12.8	125.2±8.6	96.82±8.87	93.31±6.52	81.65±6.10	77.10±5.8	75.88±5.2	44.14±3.14
MG	3.7±0.82	12.31±1.32	13.4±1.56	14.62±1.7	16.19±2.1	12.62±2.1	10.7±1.8	10.7±1.8	9.5±1.25	8.38±1.2	6.6±1.00	2.9±0.24
CHO	1.74±0.09	0.724±0.24	0.830±0.33	1.21±0.78	0.764±0.42	0.662±0.38	1.5±0.37	1.21±0.45	0.926±0.43	0.832±0.41	2.0±0.98	2.6±1.10
FFA	0.735±0.07	0.473±0.05	0.625±0.09	0.872±0.10	0.937±0.14	0.854±0.12	4.210±0.27	2.130±0.22	1.123±0.17	1.072±0.12	4.325±0.83	1.020±0.32
DG	11.09±1.12	18.47±1.78	18.50±1.81	19.10±1.92	26.62±2.21	20.25±2.24	12.22±1.93	12.12±1.81	10.25±1.67	10.10±1.53	8.824±1.34	3.289±0.45
TG	88.76±6.12	98.51±7.62	100.1±7.1	102.72±8.62	106.1±8.83	89.67±7.1	67.45±4.12	66.50±4.10	59.30±4.1	56.20±4.82	51.65±4.73	34.15±2.87
CE	0.435±0.06	0.362±0.5	0.525±0.08	0.934±0.09	1.672±0.12	1.242±0.10	0.762±0.08	0.626±0.06	0.530±0.06	0.520±0.06	0.770±0.12	0.269±0.03

Table 2: Seasonal alterations in ovarian phospholipids of *R. daniconius* during annual spawning cycle. (Note: The values for the total phospholipids are expressed as mg/g wet weight of ovary, whereas values of individual components are expressed in ìg-p/g wet weight of ovary)

Seasons	Pre-spawning			Spawning			Post-spawning			Quiescent		
Months	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan	Feb.
Total phos.	3.648	5.305	6.128	6.185	6.872	5.368	5.354	5.115	3.644	1.997	2.079	3.097
Lipids	±0.16	±0.30	±0.15	±0.16	±0.38	±0.32	±0.32	±0.35	±0.28	±0.123	±0.212	±0.29
LPC	6.164	10.26	12.30	15.32	20.08	13.62	20.06	13.03	1.00	2.669	4.856	8.209
	±0.17	±0.76	±0.83	±0.92	±1.32	±1.13	±1.7	±1.3	±1.01	±1.78	±0.32	±0.72
SPG	12.33	15.39	15.20	16.12	16.33	11.62	28.99	14.39	5.769	5.338	4.856	16.42
	±0.84	±0.93	±0.91	±0.94	±1.20	±1.11	±1.34	±0.92	±0.32	±0.31	±0.28	±1.12
PC	67.80	102.6	109.70	112.2	120.2	105.3	118.15	108.70	88.46	34.66	36.38	41.04
	±3.62	±7.2	±7.6	±7.8	±8.1	±7.6	±7.92	±7.6	±6.2	±2.42	±2.51	±3.31
PI	15.41	20.52	25.10	27.00	27.32	20.13	24.06	11.36	19.23	7.998	7.276	24.63
	±1.2	±1.24	±1.28	±1.29	±1.30	±1.2	±1.13	±0.93	±0.98	±0.62	±0.62	±1.12
PS	18.50	20.52	32.30	25.03	24.69	19.10	9.453	24.24	23.08	5.338	4.856	16.42
	±1.3	±1.24	±1.82	±1.29	±1.20	±1.21	±0.921	±1.13	±1.09	±0.35	±0.62	±0.93
PE	18.50	30.79	31.10	33.32	45.68	38.26	11.17	37.87	7.00	22.66	21.83	24.63
	±1.3	±1.73	±1.75	±1.79	±2.36	±2.17	±0.78	±1.92	±0.63	±1.12	±1.11	±1.13
PA	7.225	12.10	19.10	18.40	20.10	7.272	10.12	2.20	1.215	1.23	3.121	2.352
	±0.78	±0.79	±1.2	±1.3	±1.82	±0.82	±0.75	±0.24	±0.12	±0.11	±0.21	±0.13

Abbreviations used: CE: Cholesterol ester, TG: Triacylglycerol, DG: Diacylglycerides, FFA: free fatty acids,MG: Monoacylglycerol, CHO: Cholesterol, PL: Phospholipids,NL: Neutral lipids, PA:phosphatidic acid PS: phosphatidyl serine PE: phosphatidyl ethanolamine, PI: phosphatidylinositol LPC: Lysophosphatidylcholine, TL: Total lipids, SPG: Sphingomyelin, PC: phosphatidylcholine,

and CHO coming next in concentration in that order where as the CE and FFA were present in least concentration. The alterations occurring in the various neutral lipid components run parallel to those described above for the total neutral lipids, with some minor differences.

The observations of total phospholipids during the seasonal spawning cycle are shown in Table 2. The total phospholipids reveals a gradual rise during the preparatory period reaches at the high point during the mid active spawning period but decreasing trend was seen during late spawning period. The thin layer chromatographic separation of the ovarian phospholipids, during the seasonal breeding cycle indicated the presence of LPC, SPG, PC, PI, PS, PE and PA. It has been observed 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 with minor differences PE and rest of the phospholipids component exhibited similar alterations. Thus the phospholipid components exhibited steady increase during the preparatory period, which were further enhanced in mid active spawning period onwords their quantities exhibited gradual decrease which were continued during post spawning and sexual quiescent period.

DISCUSSION

The fish *R. daniconius* commonly known as Dandai provides an example of species displaying discontinues sex-cycle. The ovaries of this fish showed cyclic events involving changes in ovarian activity. The present investigation showed that the cyclic alteration in the quantity of lipids in accordance with the breeding cycle. The values obtained for the ovarian total lipids, neutral lipids , phospholipids and their individual components by quantitative estimations throughout the year indicate that the lipids undergoes interesting cyclic changes depending upon the sexual state of the fish. Thus in this fish quiescent period and early pre breeding periods are the time for the lipid accumulation, whereas the late pre breeding period of oogenesis is the time for these accumulated lipids are metabolized. Hence, when the ovaries are loaded with the fully mature oogonia just at the beginning of the breeding activities, the lipid reduced, such reduction might due to the utilization of lipids by mature ova. In post breeding state the lipid level increased due to non use of lipids. Lizenka *et al.* (1973) estimated the contents of total lipids and their fractional composition determined by TLC in the male and female gonads of *C. albula*.

As compared to the cyclic alterations of neutrals lipids, the changes in phospholipids are different. Bioassay studies indicated that the maximum amount of total phospholipids is present during the mid-active breeding period in July, which gradually decreased during the late post –breeding period onwards from August to January but slightly increased during February. TLC separation studies showed an increasing trend during pre-breeding period. The other components of phospholipids exhibit antagonistic 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 the lipids by the mature ovum, as well as lipids gets 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 phosphatidylinositol (PL) values co-relates with increasing number of eggs. The PL is membrane components as the number of eggs increase meticulously the membranes also increase. So in short the rise in PL quantities during the active breeding period might be due to the increase in membrane quantities.

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