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EFFECT OF DIETARY LIPID LEVEL ON PUBERTY AND OOCYTE DEVELOPMENT IN FLORIDA RED TILAPIA "OREOCHROMIS SPECIES" (FAMILY: CICHLIDAE) REARED UNDER DIFFERENT SALINITY LEVELS.

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ABSTRACT

Florida red tilapia was maintained on four isonitrogenous (30% crude protein) diets of different lipid levels (3, 6, 9 and 12%) at three levels of salinity. In freshwater, there were detectable and significant changes ($P<0.01$) in the ovary weight and GSI. Only fish receiving 9% dietary lipid level did not reach puberty with largest oocyte diameter at 12% lipid level. In brackish water, females reached puberty at all energy levels having the same percentage (50%) and there was a positive relationship between the lipid levels and the size of oocytes ($r=0.97$). Also, great significant variation ($P<0.01$) in ovary weight was detected.. In seawater, the rate of puberty (50%) was the same in all lipid levels and significant differences ($P<0.01$) in body weight and ovary weight were noticed. The oocyte diameter increased with the increasing of dietary lipid levels ($r=0.91$). Dietary treatments did not have effect on the histomorphology of ovarian development. However, ovaries of females maintained in higher lipid levels (>9%) showed some sort of abnormality; the fat vacuoles were numerous forming a mass of fat which in turn enlarged the oocytes. In conclusion, 9% lipid level was found to be the optimum level for female reared in brackish water and seawater and 6% for those reared in freshwater and the brackish water is the beneficial salinity for rearing this hybrid.

INTRODCTION

Dietary lipids play important roles in providing concentrated energy. Extensive studies on the requirements of fish have demonstrated a relationship between the feeding habits and the capacity of lipid utilization in fish. Carnivorous fish, such as salmon, plaice, yellowtail, rainbow trout and striped bass which have limited ability to utilize carbohydrates as energy source, usually require as high as 10-20% of dietary lipid for optimal growth (Shimeno ***et al.***, 1980; Cho and Cowey, 1991; and Helland ***et al.***, 1991). In contrast, herbivorous fish, such as grass carp, can efficiently use carbohydrates but can not tolerate high lipid level thus, an optimum dietary lipid level of 3.6% has been recommended for this fish (Yong ***et al.,*** 1985). Although omnivorous fish, such as tilapia, common carp and channel catfish are able to effectively utilize both carbohydrates and lipid as energy source, 5 - 6% of dietary lipid has typically been used in the feed industry for these fish (Luquet, 1991; Satoh, 1991 and Wilson, 1991).

Luquet and Watanabe (1986) and Matty (1985) have stressed the importance of broodstock nutrition in aquaculture. However, few studies have been conducted on nutritional requirements for gonadal development. Available information concerns mainly temperate species such as red seabream, ***Chrysophrys major*** (Watanabe ***et al.***, 1984a, b), gilthead seabream, ***Sparus aurata*** (Watanabe ***et al.***, 1984d; Waagbo ***et al.***, 1989 and Washburn ***et al.***, 1990). Studies on tropical species are scanty (Santiago ***et al.***, 1983 & 1991; De Silva and Radampola, 1990), and they also concern nutritional effects on overall egg/ fry production rather than on gonad development

Fish of the genera ***Tilapia*** and ***Sarotherodon*** are characterized by a general tolerance to wide range of environmental conditions. Their tropical origin is clearly reflected in their preferred temperature (Chervinski, 1982). Moreover, tilapia species posses a marked euryhalinity and numerous reports have described these species in estuarine or coastal areas of Africa (Stickney, 1986). This tolerance is not limited to saline waters but also includes environments with poor quality. Among other factors, good growth and prolific breeding have certainly favored the large development of tilapia aquaculture observed in recent years.

Competition with agriculture has increased pressure to develop tilapia aquaculture in marginal areas such as brackish water or seawater (Payne, 1984). However, salinity tolerance alone does not necessarily mean suitability for optimal production. Tilapia species such as ***O.nilotica.*** which were chosen for their aquacultural potentiality (mainly high growth rate) are not particularly euryhaline. Conversely, ***O. mossambicus,*** *which* is among the most euryhaline species, has been rejected due to its relatively slow growth. This has led to the development of hybrid species, such as red tilapia (produced by crossing female ***O. mossambicus*** with male ***O. nilotica &*** female ***O. horonum*** with male ***O. nilotica.*** as a good candidate for brackish or seawater aquaculture (Lio and Chang, 1984).

Of recent importance to tilapia culture are the red tilapia. They are fast gaining popularity because: *1-* Color (a band of pink, red, yellow and gold), is more appreciated in some markets than the silver gray/ black tilapias. *2-* Fast growth rate and ability to grow in fresh, brackish and saltwater and low susceptibility to disease. For these reasons they are now cultured in many countries.

Lipids and proteins are the major components stored in egg yolk and would be expected to play a major role in reproduction. Some studies have been carried out on the effect of fatty acids (lipids) diets in temperate species (Watanabe ***et al.,*** 1984c and Mourente and Odriozola, 1990). Nutrition is known to have a profound effect on gonadal growth and fecundity. Although precise information on the nutritional requirements for gonadal development is lacking, it has been agreed that quality and quantity of the feed, as well as the feeding regimen, are important for spawning and egg quality.

The objective of this study was to evaluate the dietary lipid effects on puberty and oocytes development of fingerling Florida red tilapia grown in three different levels of salinity.

MATRIAL AND METHODS

Red hybrid tilapia, ***Oreochromis mossambicus* x *O. urolepis hornoum*** fingerlings were obtained from Mariut fish farm near Alexandria at July 1997. The fish were acclimated for one week from freshwater (1.5‰) to brackish water (17.8‰) and seawater (36 ‰) by gradual decrease of freshwater and increase of brackish water and seawater respectively. The fish with initial average body weight of 0.73 ±0.08g were randomly selected and stocked in 80 L glass aquaria. In each experiment, there were 3 replicate aquaria at each dietary lipid and salinity level. Each aquarium was covered with a plastic lid and connected to water recirculating system and provided with continuous earation and natural light regime. All aquaria were cleaned daily by siphoning off accumulating waste materials, water temperature ranged from 25-28 °C and dissolved oxygen was 8.0 ppm.

The composition of the basal diet and vitamin-mineral permix are illustrated in table (1). Fish were fed twice daily at 9.00A.M & 2.00P.M.

At the end of the experiment after 12 weeks, about 30 fish from each aquarium weighed individually in water and a sample (about 10) of these was taken and their gutted body and ovary weights (to nearest mg) were determined individually as well as their totalbody length to the nearest mm. The gonadosomatic index (GSI) was calculated as follows:

GSI= gonad weight /gutted weight x100

Ovaries were staged according to Latif and Saady (1973). The classification is given in table (2). Oocyte diameter was also recorded.

For histological studies ovaries tissue of only stage IV were fixed in Bouin's fluid, embedded in paraffin wax, sectioned at 8 μm. The sections were stained with haematoxylin and eosin.

Table 1: Formulation and proximate composition of the 4 experimental diets.

Ingredients(%)	Dietary lipid level (%)			
	3	6	9	12
Fish meal	40	40	40	40
Wheat Bran	40	40	40	40
Dextrin	15	12	9	6
Minerals & Vitamins	2	2	2	2
Proximate composition (dry matter)				
Protein (%)	30.21	30.48	30.54	30.59
Lipid (%)	7.26	10.66	13.59	18.04
Ash (%)	12.35	12.08	11.45	10.24
Crude fiber	3.20	3.64	3.85	3.69
Nitrogen free extract	46.98	43.14	40.57	37.44
Energy (Kcal/g	4.32	4.49	4.67	4.96

Table 2: Ovarian classification:

Ovary stage	**Color of the ovary**	**Types of oocytes**
Stage II	Cream color	Cytoplasmic growth
Stage III (on set of Puberty)	Yellow with a red band	Vacoullized stage + first appearance of Pre-vitellogenic oocytes (Primary & secondary yolk stage)
Stage IV (ripe stage)	Dull yellow	Pre-vitellogenic oocytes + distinct Large pear shaped oocytes (tertiary yolk stage)

Table (3): Mean length, total weight, ovary weight and gonadisomatic index (GSI). Results Were compared among dietary lipid levels at three different salinity levels (1.5, 17.8 & 36%) by analysis of variance. P = 0.01. *Significant.

Salinity (‰)	Dietary lipid levels (%) 3	6	9	12	Anova df	F
1.5 (‰)						
Length	8	8.9	8.6	5.8	16	0.244
Weight	9.7	9.7	6.7	9.5	16	0.457
Ovary Weight	0.1	0.3	0.06	0.15	16	14.38*
GSI	1.33	3.15	1.02	2.12	16	7.92*
18.7 (‰)						
Length	9.9	8.7	10.6	8.8	16	1.02
Weight	15.3	13.2	17.1	9.2	16	5.79*
Ovary Weight	0.9	0.5	0.4	0.6	16	12.65*
GSI	7.71	6.41	6.1	8	16	0.042
36 (‰)						
Length	9.2	10.1	9.7	9.5	16	1.19
Weight	14.2	16.4	16	15.4	16	16.1*
Ovary Weight	0.1	0.32	0.4	0.3	16	13.88*
GSI	0.8	2.84	3.71	2.58	16	6.12*

Table (4): Diameter (± s.e) of the largest oocytes of Florid red tilapia fed diet of different lipid levels at three levels of salinity and the percentage of pubrerty

Lipid Levels (‰)	*Freshwater* Oocyte diameter (µm)	Percentage of puberty (%)
3	287 ± 4.75	50
6	510 ± 10.92	50
9	------	-----
12	1125 ±9.01	16.7
	Brackish water Oocyte diameter (µm)	Percentage of puberty (%)
3	389 ± 1.64	50
6	728 ± 1.88	50
9	810.5±1.44	50
12	1100±7.118	50
	Seawater Oocyte diameter (µm)	percentage of puberty (%)
3	435±1.11	50
6	490±7.25	50
9	580±7.55	50
12	945±9.24	50

All data were analysed by analysis of variance (ANOVA) using only the mean value.

RESULTS

Effect on length, weights and GSI

In fresh water (1.5‰), there are detectable and significant changes among the dietary lipid levels in ovary weight and CSI values ($P<0.01$). Also, it is obvious that the maximum mean of both ovary weight and GSI (0.3 g & 3.15, respectively) were obtained from females fed on 6% lipid level (Table 3).

In brackish water (17.8‰), mean total length was greater at 3 and 9 % dietary lipid levels (but not significant) than those at 6 and 12 % lipid levels. A significant variations ($P<0.01$) were noticed in the average body and ovary weight. No significant change was noticed in GSI values with the different dietary lipid levels (Table 3).

In seawater (36 ‰) detectable and highly significant variations ($P<0.01$) among lipid levels were observed in the body and ovary weights and GSI values. It was found that females fed on 9 % fat level had the maximum ovary weight (0.4 g) and GSI value (3.71).

Oocyte diameters and Histlogical observations:

The mean diameters of the largest oocytes for all dietary lipid levels are given in table (4).

In freshwater, oocyte diameters of 6 and 12% dietary lipid levels were significantly greater ($P<0.05$) than those of 3% lipid level. The 9% lipid level did not have ovaries of Stage IV.

In the brackish water, the 6, 9 and 12 %lipid levels were significantly greater ($P<0.05$) than those in 3 % dietary lipid. It is clear that as the percentage of lipid increased the oocyte diameters increase.

In seawater, at 12 % lipid level the average value of oocyte diameter differed significantly ($P<0.05$) . It is obvious that the oocyte diameters increased gradually by increasing the lipid levels ($r=0.91$).

The first appearance of vitellogenic oocytes was considered as the onset of puberty. In fresh water a significantly greater percentage (50 %) of females red tilapia maintained on lower lipid levels (3 and 6 %) showed onset of puberty compared to those on 12% (16.7 %) ($P< 0.05$). Also, females maintained on 9 % lipid level did not reach puberty.

In brackish water, females reached puberty at all the dietary lipid level having the same percentage (50%). The percentage (50 %) of onset of puberty of females in seawater was as the same as in brackish water in all treatments (Table 4).

The histological examination of the ovaries of stage IV revealed that histomorphological patterns of ovarian development were similar and true atresia was not seen in this stage in all experimental treatments and salinity levels (Fig. 1, A, B & C).

Also, it is obvious that an abnormality was found in the ovaries of the females maintained on higher lipid level than those in 9% dietary lipid level in all salinities. The fat vacuoles were numerous forming a mass that greatly enlarged the oocytes which contained 75-80% fat, so it seem to be empty follicle in the sections (Fig. 2, A, B & C).

It was found that the average thickness of the zona radiata was greatest at seawater (3.8 ± 0.129μ) while, in the brackish and fresh water it measured 3 ± 0.21 and 0.7 ± .081μ respectively (Fig.3 A, B & C). Also, the granuolosa layer well developed and had vacuolized cells in both fresh and brackish water measuring 3.7 ± 0.15 and 2.2 ± 0.37μ (Fig.3, A & B). Meanwhile, in seawater this layer was absent (Fig.3, C). The thecal layer was also different in thickness in the three levels of salinity, i.e it measured in freshwater 3 ± 0.11μ and in brackish water was 1.5 ±0.287μ whereas in seawater it was 1± 0.39 μ.

It is worthy to mention that in all dietary treatments and salinity levels female Florida red tilapia was in ripe stage.

DISCUSSION

From the present study it was found that ***Florida red tilapia*** fingerling fed dietary lipid varied from 3 to 12 %and grown in three different levels of salinity mature to the ripe stage except those maintained on 9 % lipid level in fresh water (1.5‰). Furthermore, fish grown in fresh water reached puberty at smaller length compared to those in higher salinity.

Generally, at the end of the experiment fish reared in full seawater had the maximum body weight and GSI values which increased by the increasing of energy levels. Also, it is obvious that fish fed diet of all lipid levels (3, 6, 9 & 12 %) reached puberty and post- vitellogenic stage (ripe oocytes) having the same percentage (50%) and the oocyte diameter increased with the increasing of energy levels($r = 0.91$) reaching the maximum size at 12% lipid level. This means capability of the ***Florida red tilapia*** to spawn in high salinity. These

results are in accordance with Watanabe *et al.*,(1989) and El-Ebiary *et.al.* (1997) for the same species. This can be attributed to the parental species from which this hybrid strain was derived, which originate from estuaries and lagoon along the east coast of Africa (Philippart and Ruwer, 1982).

The ability of ***O. mossambicus*** to reproduce in seawater is well known (Brock, 1954; Cherviniski, 1961; Popper and Lichatowich, 1975 and Stiskney, 1986). ***O. horrnoum*** is also known to live and reproduce at salinities higher than 30 ‰ (Philippart and Ruwer, 1982).

In brackish water there is a positive relationship between oocyte diameter and lipid levels (r=0.79). The percentage (50%) of onset of puberty was the same in all dietary lipid levels. According to El-Ebiary *et.al.* (1997), food conversion ratio (FCR) was the best at brackish water for ***Florida red tilapia.*** Therefore, the better conversion could influence puberty and oocyte maturation in ***Florida red tilapia*** via growth.

In freshwater, the fish fed 3 and 6 % energy levels reached puberty having the maximum percentage (50%) compared to those on higher lipid levels but the largest oocyte diameter was found in 12% energy level.

The histomorphology of the developing ovaries was not affected by dietary treatments. All group exhibited follicular differentiation but no aterisa was observed. Same result was reported also by Washburn *et.al.* (1990).

The most prominent effect of dietary treatment was the mass of fat in ovaries as higher lipid may be responsible for this observation. Primarily phospholipid (Weigand and Idler,1986), contributes to the overall increase in oocyte lipid content, since vitellogenin transport supplies only a portion of oocyte lipids (Mommsen and Walsh, 1988).

Abnormality was found in the ovaries of females fed high levels of lipid in which the mass of the fat vacuoles enlarged the oocytes which contained high percentage of fat. Smilar results were reported for ***O. mossambicus x Sarotherodon galilaeus*** by Fishlson, (1988) and in ***Orncorhynchus mykiss*** (Washburn ***et al.***, 1990).

Although the information on lipid requirements of ***Florida red tilapia*** in different phases of oogenesis is highly incomplete, it appears that the practical improvement of broodstock nutrition should based on developmental changes occurring during the reproductive cycle. During oocyte development and the initial phase of vitellogenesis (in brackish water, and sea water in this study), diets with 9% lipid level showed the best results. This is in line with the results obtained by Teshima ***et al.*** (1985) for ***O. nilotica,*** El-Sayed (1987) for ***Tilapia zilli*** and El-Ebiary and Mourad (1998) for ***Florida red tilapia*** . While the best diet for ovarian development of female fish reared in fresh water was 6% energy level. The same results were reported by Takeda ***et al.***, (1975) in yellow tail fish ***Seriola quinqueradiata*** and El-Ebiary and Mourad (1998) for ***Florida red tilapia.***

In summary, dietary lipid levels affected the rate of puberty and maturation. Also it is cleared that this study demonstrate that the brackish water is beneficial for rearing and development of oocyte. Uchida and King (1962) reported that ***O. mossambicus*** fry production was approximately three times higher in brackish water than fresh water. The present study revealed that ***Florida red tilapia.*** can efficiency use lipid as energy source, 6- 9% of dietary lipid for optimal ovarian development and onset of puberty.

These results are in accordance with those of Jauncey and Ross (1982) who suggested that 6-10% lipid should be included in the diet of tilapia. This in contrast to a previous studies of Luquet, (1991); Satoh, (1991) and Wilson, (1991) who recorded that tilapia usually require 5-6% of dietary lipid in their diet.

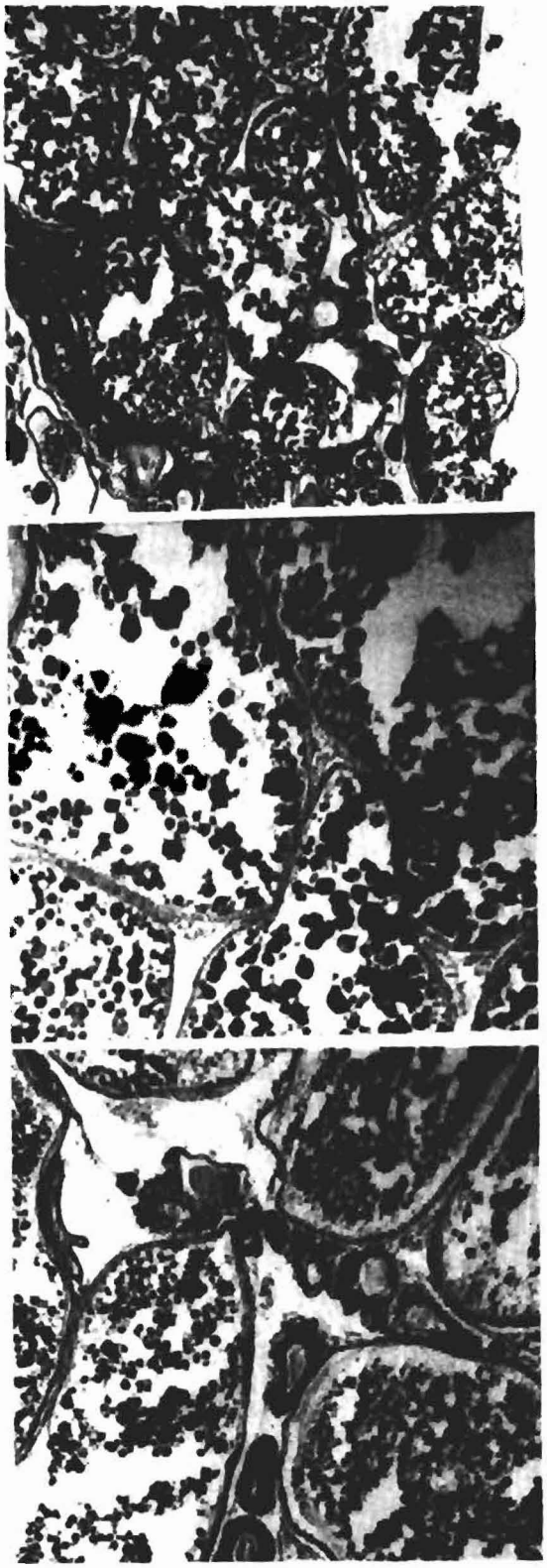

Fig. (1): T.S. from ovary of female **florida red tilapia** showing the oocytes at post-vitellogenesis (ripe stage) at three level of salinity with dietary lipid
A- Fresh water X 90. ***B***- Brackish water X 225. ***C***- Seawater X 90

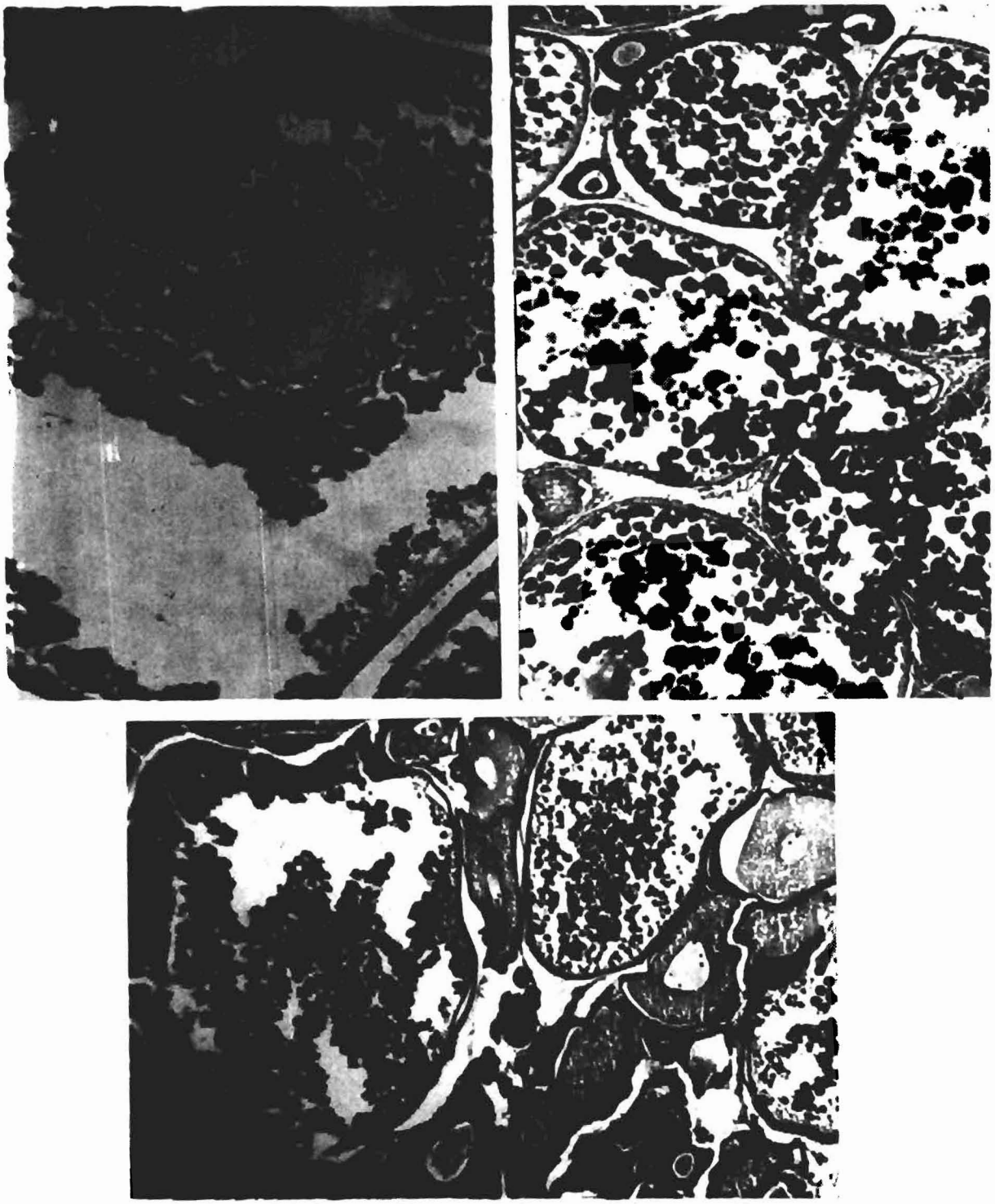

Fig. (2): T.S. from ovary of female ***florida red tilapia*** *fish fed on 12% lipid level showing the oocytes at post-vitellogenesis (ripe stage) at three level of salinity and enlargement of these oocytes due to the great mass of fat.*
A- *Fresh water X 360.* ***B-*** *Brackish water X 225.* ***C-*** *Seawater X 90.*

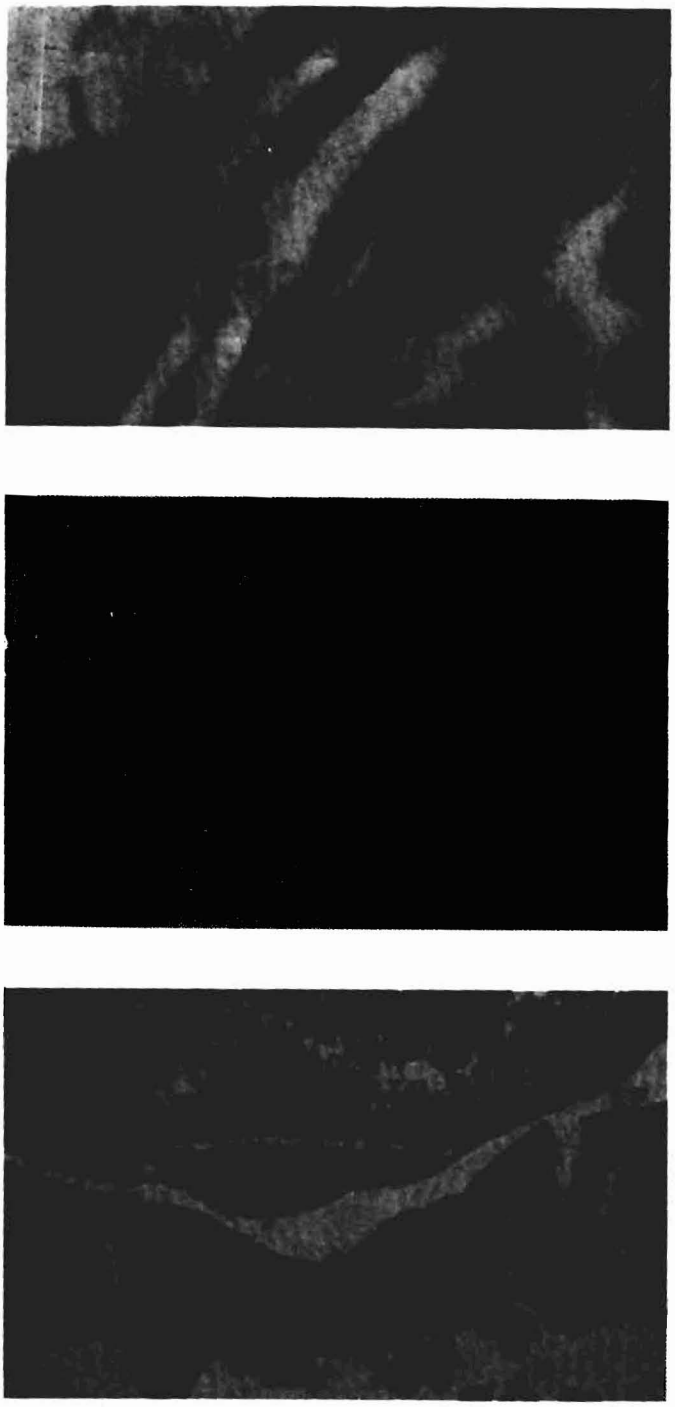

Fig. (3): T.S. from ovary of female ***florida red tilapia*** *fish fed on lipid showing the oocytes at post-vitellogenesis (ripe stage) and the zona radiata layer, gramolosa layer and thecal layer at three level of salinity.*
A- *Fresh water X 900.* ***B-*** *Brackish water X 900.* ***C-*** *Seawater X 360.*

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