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Biochemical variations of carbohydrates in relation to the breeding cycle of female tilapia, *Oreochromis mossambicus* (Peters)

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Abstract
Tilapia, *Oreochromis mossambicus*, is highly evolved and multiple breeders euryhaline fish. After evaluating the gonadosomatic index and percentage dominance of particular types of oocytes, breeding cycle in females is divided into preparatory, prespawning, spawning and posts pawning phases. Carbohydrates in nervous, endocrine and somatic tissues with respect to the ovarian cycle are ascertained by their histochemical demonstration and biochemical estimations. Brain and pituitary has highest carbohydrate in prespawning phase, lowest in posts pawning phase. On the contrary, ovary has highest carbohydrate content in spawning, and lowest in the posts pawning phase. Liver and muscles show highest carbohydrate in the preparatory and lowest in the spawning phase. An explicit co-relation exists in these metabolites in nervous, endocrine, somatic and reproductive components in *O. mossambicus.*

Keywords: Tilapia, carbohydrates, breeding cycle, biochemical

Introduction
The metabolic aspects of oogenesis have been partly assessed and changes in several aspects of carbohydrate and lipid metabolism, in gonad, liver and muscles of several species are found [1-8], for example in rainbow trout, *Oncorhynchus mykiss* an increased use of dietary carbohydrates is reported to take place during gonadal maturation [9] and several changes in liver and gonad metabolism are observed during the onset of ovarian recrudescence in this fish [10]. In female plaice *Pleuronectus platessa* [11] and in Arctic capelin, *Mellotus villosus* [12], marked depletion of body lipid and/or glycogen occurs during gonadal development. In Anabantid, *Trichogaster pectoralis*, carbohydrates are present in insignificant amount in female gonads [13].
Glycogen is the major if not the only fuel of anaerobic metabolism [14]. The mobilization of glycogen may be extremely rapid so much so that trout muscle may utilize the equivalent of about 40% mole of glycogen – derived glycogen / g / sec and in 15 second deplete about one half of the glycogen stored [15].
Carbohydrates are generally stored as glycogen in animal tissues mainly in muscles and liver [16]. Onset of breeding season triggers several metabolic changes in oviparous vertebrates; which are characterized by the initiation of biochemical processes related to the production of vitellogenic eggs [17-20].
All these studies however have mainly focused upon the gonads but studies on the possible biochemical interactions in teleosts along the hypothalamic-pituitary-gonadal axis are not reported. Therefore, in the present work, brain (telencephalon, diencephalon including hypothalamus), pituitary and ovary are selected to study the possible correlation of carbohydrates in these tissues. Liver and muscles are selected to find out the correlation between somatic and reproductive components.

Materials and Methods
Sexually mature females of *Oreochromis mossambicus* were collected from the Futala Lake, near Botanical Garden, Nagpur.
All fishes were transported alive to the laboratory in open bucket and maintained in 3x2x1.5 feet rectangular tanks (10 fish per tank) which were supplied with non-circulatory fresh water maintained at 28± 2 ^{0}C with water exchanged at the rate of 15 liters/ day. The fish were weighed, killed by decapitation and brain, pituitary, ovaries, liver and muscles were dissected

out in Ringer's solution. Telencephalon and diencephalon portions of brain were separated as described by Senthilkumaran and Joy [21] (1993) and Pepels *et al.,* (2002) [22]. Weight of ovaries was noted and gonadosomatic index was calculated using the formula,

$$GSI = \frac{\text{Weight of Ovaries}}{\text{Weight of Fish}} \times 100$$

Different stages of oocytes were identified as specified by Tacon *et al.,* (1996) [23] and Degani *et al.,* (1997) [24] and gonadal state was ascertained. Individuals were grouped on the basis of identical state of oocytes, their percentage dominance was calculated and the gonadal cycle was reported in four phases.

Ovary of one side in each fish was processed for histology to assess the gonadal state and ovary of the other side alongwith selected tissues were processed for histochemical and biochemical studies. For histology, Heidenhein's Iron-Haematoxylin [25] method was used. Periodic Acid-Schiff [26] was adapted for histochemical demonstration of carbohydrates. Carbohydrates were estimated by Anthrone Reagent [27] method. The findings are summarized in the tables 1 to 3 and staining is indicated in photographs 1 to 4.

Results and Discussion

In *O. mossambicus* as maturation proceeds intense staining of carbohydrate is observed in the ovary. Intensity of staining increases in the ooplasm successively from stage I to stage VI oocytes (Table 1 & Figure 1-4). It is well documented that glycogen or glucose which is the main constituent of yolk of oocytes, should be in constant supply to the ovary, the most intense formation of which in oocytes occur during vitellogenesis [28]. Intense staining of ooplasm for carbohydrates in mature and atretic oocytes and its highest content in spawning season is reported in *H. fossilis* [29]. PAS positive yolk particles occur in the granulosa cells and in the chorional canaliculi of *Acanthobrama* [30]. In *O. mossambicus* intense staining of these membranes for carbohydrates can be the indication of their involvement in vitellogenesis (Fig. 3). Granulose has the responsibility of deposition of yolk in developing ovum and of its removal in ova which degrade before ovulation that is granulosa has both nutritive and phagocytic functions [31].

Carbohydrates are intensely stained in stage VI oocytes (Fig. 3) which dominate the spawning phase and maximum amount is also estimated in this phase in the ovary. This increase is statistically significant (P<0.01) implicating them in the process of vitellogenesis. The PAS positive (mucopolysaccharides or glycoprotein) yolk vesicles are generally the first structures to appear in the oocyte cytoplasm during secondary growth of oocytes and are reported to appear first in the outer and mid cortical zone of oocytes in the teleosts [32]. In type V oocytes in *O. mossambicus* distinct rim of PAS positive material is observed which further increases in type VI oocytes (Fig. 3). In *Schizothorax richardsoni* and *Glyptothorax pectinopterus* [33], highest sugar contents are estimated during maturation period.

In telencephalon, in prespawning phase there is significant decrease (Table 2) for carbohydrates (P<0.05). Brain glycogen in *Notopterus notopterus* is noted to be 1.27±0.16 and in *Colisa fasciata* it is 1.19±0.05mg/g of wet weight of tissue [34]. In spawning phase in *O. mossambicus*, carbohydrate content in the telencephalon is 1.34±0.02 and in diencephalon it is 0.967±0.038 mg/g wet weight of tissue which is almost identical to above reports. In this fish glycogen content of diencephalon in all the phases is lower than in telencephalon. Glucose plays very important role because oxidation of glucose is said to provide immediate energy requirement of the cell. It is the sole form of energy for the brain and other nervous tissue. Lack of glucose or of oxygen for its metabolism leads to rapid damage to the brain [35].

Variation in both environmental as well as intrinsic (hormonal) factors affect the cellular constituent of liver, muscles and gonad which show seasonal fluctuations [17-20, 36]. A seasonal co-relation exists between liver metabolic activity and egg maturation in *Garra mullaya* also [37]. Glycogen in liver and muscles are lowest in the spawning phase in *O. mossambicus* but in ovary these contents are highest in this phase. High levels of ovarian glucose during spawning phase and lowest in spent phases in *Channa punctatus* and *H. fossilis* [38, 29] are reported. Glycogen, lipids and protein are implicated in vitellogenesis and in all growth and metabolic processes [35, 39]. In *O. mossambicus* lowest cholesterol is noticed in liver and muscles in the spawning phase.

Table 1: Variations in GSI Values of Female

Phases	Female
Postspawning	0.52665 + 0.018
Preparatory	0.21887+0.021 NS
Prespawning	0.88778 + 0.067 P<.01
Spawning	3.24932+0.205 P<.01

Value Present mean + SE of observations NS - Non Significant

Table 2: Percentage at oocytes at different stages in the ovary

Phases	Stage I	Stage-II	Stage-III	Stage-IV	Stage-V	Stage-VI
Postspawning	28.773+0.905	27.089+1.603	23.523+1.162	14.596+0.574	6.017+0.754	0.00 + 0.000
Preparatory	36.873+4.366 P< 0.01	32.992 +.872 P<.01	14.467+0.782 NS	5.895 + 0.997 NS	9.715 + 0.183 P<.01	0.0 + 0.000
Prespawning	6.249 +0.609 NS	10.340+0.126 NS	21.641+1.603 P<.01	26.768+1.473 P<.01	29.215+0.456 P<.01	5.785+0.346 P<.01
Spawning	7.019 + 0.412 NS	11.508+0.685 NS	13.053+.438 NS	9.875 +0.648 NS	11.874+0.519 P< 0.01	45.731+2.553 P<.01

Values are mean + SE of Observations NS - Non Significant

Table 3: Variations in Carbohydrate contents of Telencephalon, Diencephalon, Pituitary, Ovaries, Liver and Muscle (Value in mg/g wet weight of tissue)

Phases	Telencephalon	Diencephalon	Pituitary	Ovary	Liver	Muscle
Postspawning	1.246+0.047	1.635+0.118	1.032+0.035	2.730+0.102	4.921+0.145	0.685+0.017
Preparatory	2.148+0.075 *P*<.01	1.910+0.039 *P*<.05	0.814+0.099 NS	2.864+0.096 NS	9.707+0.129 *P*<.01	2.243+0.086 *P*<.01
Prespawning	2.864+0.146 NS	2.395+0.094 *P*<.05	1.307+0.101 NS	3.411+0.075 *P*<.01	5.914+0.043 *P*<.01	1.230+0.074 *P*<.01
Spawning	1.340+0.029 NS	0.967+0.038 NS	0.767+0.037 NS	5.865+0.060 *P*<.01	2.954+0.134 NS	0.588+0.018 NS

Values are mean + SE of observation NS - Non Significant

Carbohydrate Histochemistry

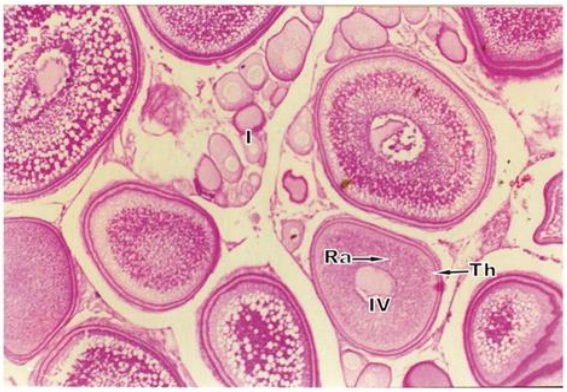

Fig 1: T S of ovary during Preparatory phase showing stage I oocytes with darkly stained wall, stage IV oocytes with theca (Th)& radiata (Ra) weakly stained. X25

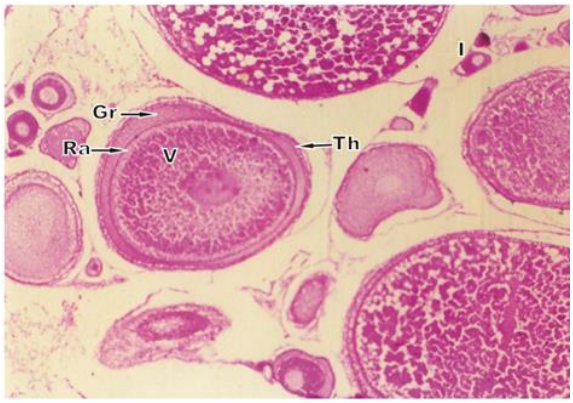

Fig 2: TS of ovary during Prespawning phase showing intensely stained ooplasm in Stage I, moderately stained theca (Th), granulosa (Gr) layer shows intense staining, while radiata (Ra) & nucleoplasm moderately stained in stage V. X25

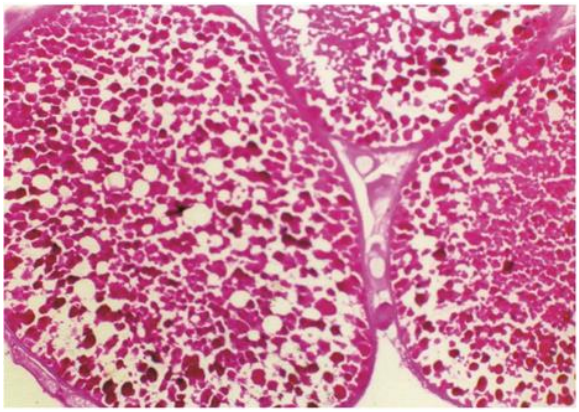

Fig 3: T S of ovary during Spawning phase showing yolk material with intense staining, membrane layers darkly stained. X25

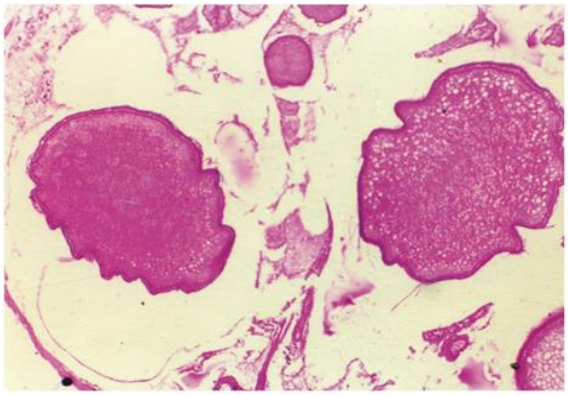

Fig 4: T S of ovary during Post Spawning phase showing intense staining of granulosa layers & moderately stained yolk material. X25

Conclusion

Brain and pituitary has highest carbohydrate in prespawning phase, lowest in postspawning phase. On the contrary, ovary has highest carbohydrate content in spawning, and lowest in the postspawning phase. Liver and muscles show highest carbohydrate in the preparatory and lowest in the spawning phase. Thus variations in its content during different ovarian stages are observed in O. mossambicus and a definite correlation in nervous, endocrine, somatic and reproductive components is established in this fish.

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