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REPRODUCTIVE PHASE WISE VARIATION IN TOTAL PROTEINS, TOTAL CARBOHYDRATES AND TOTAL CHOLESTEROL IN THE OVARY OF *CHANNA PUNCTATA* (BLOCH, 1793)

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ABSTRACT
Teleost, depending upon frequency of spawning in year, could be grouped as annual breeder, biannual breeder or multiple breeder. Spotted *Channa punctata* is annual breeder spawn once in a year. The annual reproductive cycle include resting phase, preparatory phase, prespawning phase, spawning phase and postspawning phase. Female of *C. punctata* undergo drastic morphological and physiological changes during different phases of reproductive cycle and get ready for spawning. Present study intended to study the trends of fluctuation of major biomolecule like protein, carbohydrate and cholesterol in ovary of *C. punctata* and to find out the correlation between protein, carbohydrate and cholesterol fluctuation along the different phases of reproductive cycle. Biochemical estimation of protein, carbohydrate and cholesterol of ovary throughout different phases of reproductive cycle reveals that protein, carbohydrate and cholesterol of ovary rises from resting phase to spawning phase and then slowdown in postspawning phase. Fluctuation in protein, carbohydrate and cholesterol has direct correlation with each other.

KEY WORDS: biochemical estimation, correlation, Reproductive cycle.

INTRODUCTION

Spotted snakehead *Channa punctata* (Bloch, 1793) belongs to order Perciformes and family Channidae. All *Channa* species are ambush predator, living in still waters and also inhabit larger river. They feed on zooplankton and phytoplankton (Munshi and Hughes, 1992). In scarcity of food, snakehead could turn cannibalistic (Cardenas *et al.*, 2008). Spotted snakehead, *C. punctata* is annual breeder with five phases in reproductive cycle viz., resting phase (Mid December- February), preparatory phase (March-April), prespawning phase (May), spawning phase (June-October) and post spawning phase (November- mid December) (Kulsange and Masram, 2017). Throughout the annual reproductive cycle, ovary undergoes drastic morphological changes (Kulsange and Masram, 2016).

Biochemical composition of the fish also varies according to reproductive phases.
During the reproductive cycle of teleosts, a transfer of carbohydrates, proteins, and lipids between different organs and tissues, via plasma, take place. Thus reproductive phase wise variations in protein, carbohydrate and cholesterol in ovary were reported in *Heteropneustes fossilis* (Hunge and Baile, 2003), *Oreochromis mossambicus* (Pathan and Baile, 2005), and in *Mystus vittatus* (Sreevalli and Sudha, 2014).

In spawning phase all nutrients are pulled towards the ovary and supplied to maturing oocytes which are on verge of spawning. Proteins including yolk precursor protein vitellogenin are synthesized in the liver and through blood deposited in growing oocytes of the ovary (Baile and Pathan, 2006). The changes in energy reserves such as glycogen and lipid in the liver and ovary occur according to the metabolic needs of organs at different seasons (Singh and Singh, 1990). Cholesterol and other components always in minor amounts modify the basic protein/phospholipid pattern of the biological membrane in specific ways, with particular functional consequences. Present study try to explore fluctuation in these major biomolecules like proteins, carbohydrates and cholesterol in the ovary of *C. punctata* in different phases of reproductive cycle.

MATERIALS AND METHODS

Adult females of *C. punctata* were collected from ponds in and around Nagpur city. In all the estimations, least six samples were tested from different fishes (n=6). Fish were killed by decapitation and ovaries were dissected out in ice-cold Ringer's solution and soaked on tissue papers. 0.1 gm of tissues was weighed accurately using analytical balance and homogenized in ice-cold Ringer's solution using mortar and pastel. Total proteins in the ovary were estimated by following standard Lowry's method (Lowry et al., 1951). Total carbohydrates and total cholesterol were determined by Anthrone reagent method (Dubois *et al.*, 1956) and Ferric chloride method (Zlatkis *et al.*, 1953) respectively. Standard

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solution for estimation of total protein, total carbohydrate and total cholesterol were prepared by using bovine serum albumin (BSA), glucose and standard cholesterol respectively. Protein, carbohydrate and cholesterol were quantified by observing blue colour intensity at 675nm, green colour intensity at 620 nm and brown red colour intensity at 560 nm on spectrophotometer (Elico SL 177 spectrophotometer) respectively.

RESULTS

Total Proteins

Lowest total protein content is reported in resting phase ovary which is 21.40 ± 0.29 mg/ gm of the wet weight of tissue (Table 1, Graph 1) and it is lowest protein content of ovary among all the phases of reproductive cycle in *C. punctata*, the value of protein content of resting phase is held as control value and compared with total protein content of other (Table 1, Graph 1) phases of reproductive cycle. Total protein content rises significantly to 25.04 ± 0.53 mg / gm of wet tissue (p <0.001) (Table 1, Graph 1) in preparatory phase. Total protein of prespawning phase was rises to 26.95 ± 0.82 which is significantly higher than resting phase ovary (p <0.0001) and preparatory phase ovary (p < 0.0001) (Table 1, Graph 1). Highest protein content reported in spawning phase ovary and it is 29. 78 ± 0.48 mg/ gm of wet tissue which is extremely significantly higher than resting ovary (p <0.0001) and prespawning ovary (p <0.001) (Table 1, Graph 1). But in postspawning phase, protein content decline to 24.56 ± 0.51 mg/ gm of wet tissue (Table 1, Graph 1) which is lower than other phase of reproductive phases but still very significantly higher than protein content of resting phase ovary (p < 0.01). On other hand protein content of postspawning ovary is very significantly lower than protein content of spawning phase ovary (p< 0.01). Thus in ovary, gradual increase in total protein content observed from resting phase to spawning phase and then the protein content lowered in postspawning phase.

Total Carbohydrates

In resting phase ovary, total carbohydrate content is 0.68 ± 0. 03 mg/ gm of wet tissue (Table 1, Graph 2) which is lowest among all the reproductive phases. Carbohydrates further increases to 2.05 ± 0.24 mg / gm (Table 1, Graph 2) in preparatory phase thus very significant rise in protein content observed in comparison with carbohydrate content of resting phase ovary (p< 0.01). Total carbohydrates further increases in prespawning phase to 4.95 ± 0.31 mg/ gm of wet tissue (Table 1, Graph 2) which is extremely higher than carbohydrates of resting phase ovary (p<0.0001) and very significantly higher than carbohydrates of preparatory phase ovary (p<0.01). In spawning phase ovary, carbohydrates content further rises significantly from carbohydrate content of prespawning phase ovary (p< 0.05) and extremely significantly from the carbohydrates of resting phase ovary (p<0.0001) to 6.07 ± 0.27 mg/gm wet tissue (Table 1, Graph 2). After this sharp rise in the carbohydrate content in spawning phase ovary, carbohydrates lowered in postspawning phase to 2.65 ± 0.15 (Table 1, Graph 2) which is extremely higher than the carbohydrate content of resting phase ovary (p<0.0001) and extremely significantly lowered than carbohydrate content of spawning phase ovary. Carbohydrates similar to protein, rises from resting phase to spawning phase and lowered in postspawning phase.

Total Cholesterol

Lowest cholesterol is observed in the ovary of resting phase which is 2.40 ± 0.14 mg/ gm of wet tissue (Table 1, Graph 3). The cholesterol level almost same at 3.00 ± 0.24 mg/ gm (p> 0.05 in preparatory phase but drastic increase in cholesterol observed in prespawning phase and it rises to 5.54 ± 0.14 mg/ gm of wet tissue (p < 0.001) (Table 1, Graph 3). Highest cholesterol recorded in spawning phase which is 6.85 ± 0.22 (p < 0.05) (Table 1, Graph 3) which is extremely higher than cholesterol of resting phase ovary (p <0.0001) and higher than prespawning phase ovary (p *<0.05*). In postspawning phase cholesterol lowered to 3.52 ± 0.16 which is significantly higher than cholesterol of resting phase ovary (p< 0.01) and extremely lower than cholesterol of spawning phase ovary (p < 0.0001). Thus similar to protein and carbohydrate, cholesterol also increases in ovary from resting to spawning phase and lowered in postspawning phase.

Correlation between proteins, carbohydrates and cholesterol of ovary

Correlation matrix suggest strong positive correlation between total protein and total carbohydrate content of ovary (r = 0.95) during different phases of reproductive cycle of *C. punctata* (Table 2). Protein show strong positive correlation with cholesterol content of ovary (r = 0.94) in various phases of reproductive or breeding cycle of *C. punctata.* Total carbohydrates also show strong positive correlation with total cholesterol content (r = 0.99) of ovary during different phases (Table 2).

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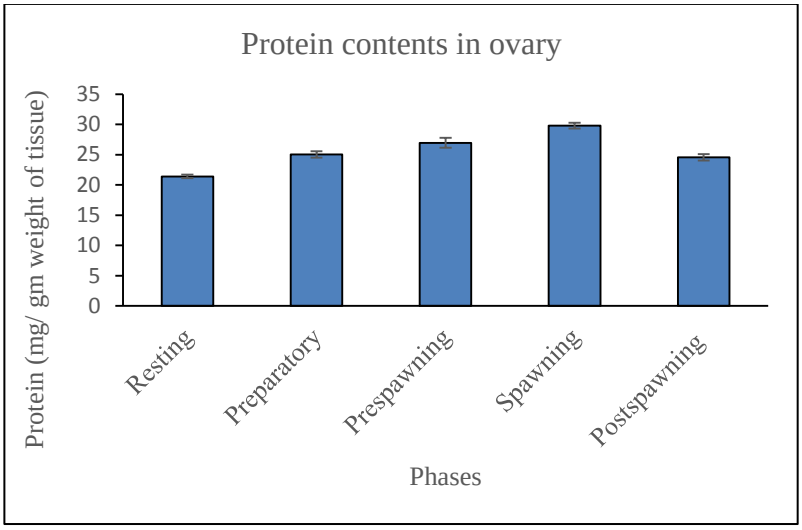
Table 1. Variation in total proteins, carbohydrates and cholesterol contents (mg/gm weight of tissues) in ovary during reproductive phases of *O. punctatus.*

Phases	Biochemical contents (mg/gm) ± SE		
	Proteins	Carbohydrates	Cholesterol
Resting	21.40 ±0.29	0.68 ± 0.03	2.40 ± 0.19
Preparatory	25.04 ± 0.53 $p < 0.001^{***}$	2.055 ± 0.24 $p < 0.01^{**}$	3.00 ± 0.24 $p > 0.05^{ns}$
Prespawning	26.95 ± 0.82 $p < 0.0001^{****}$ $^{+}p < 0.0001^{****}$	4.95 ± 0.31 $p < 0.0001^{****}$ $^{+}p < 0.01^{**}$	5.54± 0.14 $p < 0.0001^{****}$ $^{+}p < 0.001^{***}$
Spawning	29.78 ± 0.48 $p < 0.0001^{****}$ $^{+}p < 0.001^{***}$	6.07 ± 0.27 $p < 0.0001^{****}$ $^{+}p < 0.05^{*}$	6.85 ± 0.22 $p < 0.0001^{****}$ $^{+}p < 0.05^{*}$
Postspawning	24.56 ± 0.51 $p < 0.01^{**}$ $^{+}p < 0.01^{**}$	2.65 ± 0. 15 $p < 0.0001^{****}$ $^{+}p < 0.001^{***}$	3.52 ± 0.16 $p < 0.01^{**}$ $^{+}p < 0.0001^{****}$

p – when value is compared with resting phase. $^{+}$P when compared with preceding phase. **ns** – non significant difference, *- significant at P<0.05, **- very significant at $p < 0.01$,***- extremely significant at $p < 0.001$, ****- extremely significant at $p < 0.0001$. Data of all phases passed the Normality test with $p > 0.05$.

Table 2. Correlation between total proteins, carbohydrates and cholesterol contents in ovary during reproductive phases of *O. punctatus*

	Protein	Carbohydrate	Cholesterol
Protein	1.00		
Carbohydrate	0.95	1.00	
Cholesterol	0.94	0.99	1.00



Graph 1. Variation in protein contents in ovary during reproductive phases of *C. punctata.*

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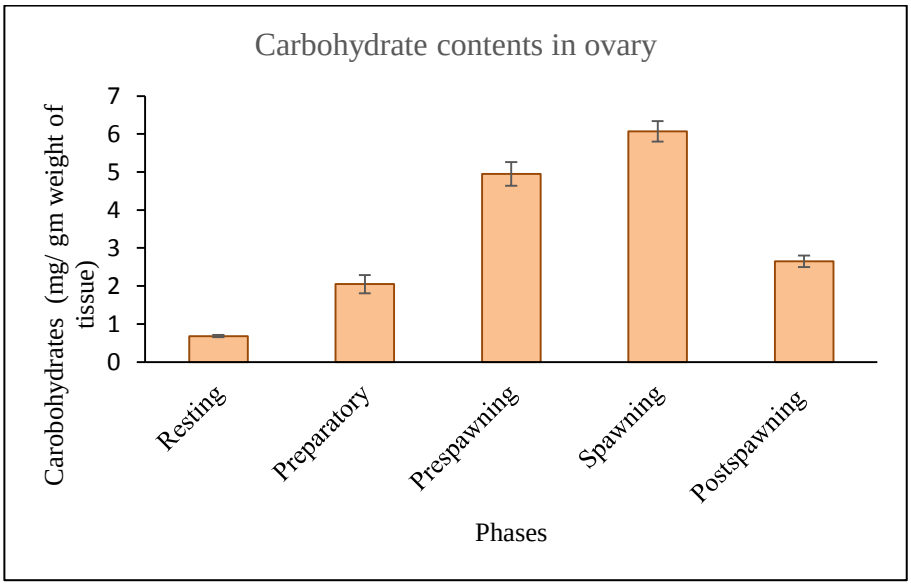
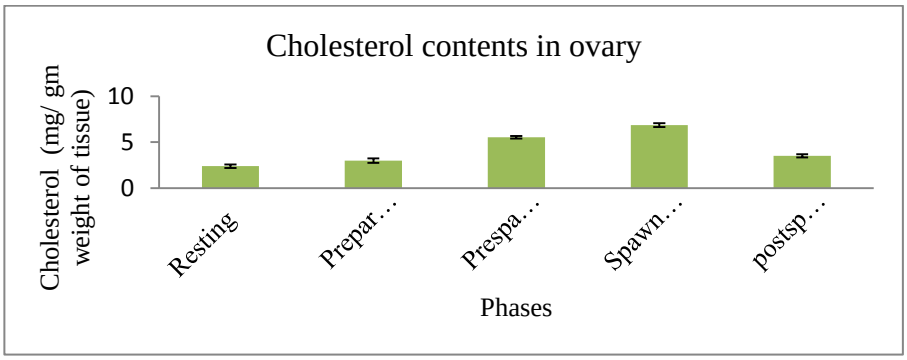


Graph 2. Variation in carbohydrate contents in ovary during reproductive phases of *C. punctata.*



Graph 3. Variation in cholesterol contents in ovary during reproductive phases of *C. punctata.*

DISCUSSION

Morphological variations observed in the ovary of *C. punctata* (Kulsange and Masram, 2016). During spawning phase it attains maximum size and tunica albuginea of ovary become so thin that we could see the yellow colour oocytes (Kulsange and Masram, 2016). The biochemical analysis also reveals the fluctuating trend of proteins, carbohydrates and cholesterol during different stages oocyte maturity in ovary (Hajam *et al.*, 2012). Proteins which play significant role in different metabolic activity also fluctuates along the reproductive cycle. Similar in *C. punctata* as revealed in present work , increase in protein content of ovary along with the maturation was reported in *Epinephelus diacanthus* (Chandrashekhara Rao and Krishnan, 2011) and *Ammodytes hexapterus* (Robards *et al.*, 1999) in , *Channa gachua* (Dabhade *et al.*, 2009). However, in *Schizothorax niger* maximum proteins reported in growth phase and lesser in spawning phase (Hajam *et al.*, 2012).

In *C. punctata* carbohydrates increases gradually from resting phase to spawing phase. This observation is supporting the finding of Chandrashekhar Rao and Krishnan, 2011; Hassan and Jafri, 1996 and Hajam *et al.*, 2012. Spawning phase ovary show maximum carbohydrates in spwani.

The comprehensive description of variation of cholesterol were reported in ovary of certain Indian teleosts viz., *H. fossilis* (Singh and Singh, 1979), *Puntius chilinoides* (Nauriyal and Singh, 1985) and in *Garra mullya* (Khan et al.,

1991). Spawning phase ovary show maximum cholesterol in many fishes (Naruiyal and Singh, 1985 ; Khan et al., 1991). Similar trend in *C. punctata* confirms the elevation of cholesterol in spawning phase ovary .

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