Advances in Bioresearch
Adv. Biores., Vol 14 (6) November 2023: 375-380
©2023 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.14.6.375380

Advances in Bioresearch

ORIGINAL ARTICLE

Testicular organisation of *Arius subrostratus* by macroscopic and histochemical examination

Ambily. V, Bijoy Nandan. S
Assistant Professor, Research Department of Fisheries and Aquaculture
St.Albert's College (Autonomous), Ernakulam
Professor, Department of Marine Biology, Microbiology and Biochemistry,
CUSAT, Kochi- 16
Corresponding Author Email: ambily@alberts.edu.in, mailambily@gmail.com

ABSTRACT
Maturity stages of fishes are generally classified using various methods, including external as well as cellular examination of the gonads. In order to quantify maturity stages of shovelnose sea catfish Arius subrostratus both macroscopical and histological examination were applied in this study. Formalin preserved samples were preserved in 4% formalin and conditioned in different concentrations of alcohol. Accordingly, dehydrated samples were embedded in paraffin wax and made them sectioned. The sexes of fishes can be separated internally by the visual examination of gonads. Gonads (testes of males and ovaries of females) are paired structures that are suspended by mesenteries across the roof of the intestinal cavity. The complicated cellular level changes occurred during the maturation process of testes. Both spermatogenesis and spermiogenesis are clearly explained in which spermatocytes and spermatozoa are formed respectively.
Key words: Arius subrostratus, histology, spermatogenesis, spermiogenesis

Received 25.09.2023 Revised 08.10.2023 Accepted 26.11.2023

How to cite this article:
Ambily. V, Bijoy Nandan. S. Testicular organisation of *Arius subrostratus* by macroscopic and histochemical examination. Adv. Biores., Vol 12 (6) November 2023: 375-380.

INTRODUCTION

Maturity stages of fishes are typically quantified according to the external examination of gonads. Although it is inexpensive and easiest method, accurate estimation is not always possible merely by the examination of macroscopic appearance of gonads. Classification of maturity stages are always species and habitat specific. Conversion of primordial germ cells into mature gametes by a variety of cytological changes is called gametogenesis. When gametogenesis takes place in male gametes it is called as spermatogenesis. Spermatogenesis of the fishes were scrutinised by plenty of workers up to date [1], [2], [3]).

Arius subrostratus is known as shovel nose catfish due to the shape of its head and snout is one of the genera in *Ariidae* family distributed throughout the world. Although manifold works on reproductive biology were carried out on different catfish species, the testicular organization of this species remains unknown from the literature surveys especially from the Indian background. Hence, the objective of this experiment is to interpret different maturity classes of the testes by macroscopical as well as histological images. This study paves the way for further research activities by providing preliminary cytological data on the testes of *A. subrostratus.*

MATERIAL AND METHODS

Fish specimens for conducting this analysis were collected monthly from Cochin estuary, Kerala, India (9^0 91' N and 76^0 32' E to 9^0 51'N and 76^0 15' E) from April 2011 to March 2013. A total of 88 specimens of *Arius subrostratus* ranging in size from 18.5 to 34.5 cm in total length were used for estimating different parameters related to reproductive characteristics. The fresh specimens were brought to the laboratory

for analysis. Eventually, their gonads were dissected out and length and weight are noted for calculating gonadosomatic ratio. Then the testes have been sorted by the external examination and preserved in 4% formalin for histological analysis.

Maturity stages were evaluated according to macroscopic and microscopic observation of different maturity stages of the testes. Males and females were grouped into different gonadal stages of development according to Nikolsky [4]. Although the macroscopic level staging of fish gonads is inexpensive, it has some limitations to discriminate certain stages of maturity. In order to quantify maturity stages by histological analysis, selected testes preserved in formalin were washed thoroughly in distilled water and then subjected to dehydration in ascending concentration of alcohol. Slices of tissues were embedded in paraffin wax and then the sections were cut at 3 -6 µm in LEICA RM 2125 RTS microtome. Then staining was done by haematoxylin followed by eosin and observed under a research microscope (LEICA DM 500).

RESULTS

Classification of testes or quantification of maturity stages

Macroscopic examination of the testes of *Arius subrostratus*: The gonads of *Arius subrostratus* have been grouped into 5 stages by the visual inspection. Then, the fishes with different maturity stages were broadly classified as early immature (I), late immature (II), maturing (III), ripe (IV), spawning and spent (V). (Figure 1)

Immature - Very small thread like testes close to the vertebral column. Testes are transparent, colourless to grey and very small fine thread like appearance. Mean Gonadosomatic ratio (GSR) obtained was 0.2108 (Figure 1 a).

Developing- Translucent testes with its length is about half of the ventral cavity. Testes slightly enlarged in size and dark yellowish in appearance. mean GSR was 0.5862 (Figure 1b).

Maturing - Testes became opaque and reddish with blood capillaries. Testes occupied about two thirds of ventral cavity and enlarged in size and weight. Their mean GSR was 2.2199 (Figure 1 c).

Mature/Spawning - Sexual organs are filling the ventral cavity. Testes enlarged and pale white in colour. Obtained mean 3.7175 (Figure 1 d).

Spent - Gonads were flaccid and shrinking. Ventral cavity seems not fully empty. Testes flabby and reduced in their size and weight, pale in colour. Their mean GSR came down to 0.8747(Figure 1 e).

Histological examination of the testes of *Arius subrostratus*: Different maturity stages of the gonads of *A. subrostratus* were categorised according to the cytological changes occurred in the testes during the developmental phases. Processes like spermatogenesis could be well explained with the help of histological analysis in which spermatogenesis is the development of sperm from the primordial germ cell.

Spermatogenesis and spermiogenesis of the testes of *Arius subrostratus*: According to the histological examination of the testes *Arius subrostratus* could be grouped into five stages.

Stage I. Immature (Spermatogonial stage): This is the first stage of spermatogenesis encompasses primary and secondary spermatogonia (Figure 2.a). A large number of cysts or compartments were distributed inside the testes surrounded by seminiferous tubule. Each of the cells were well demarcated and with full of spermatogonia. Primary spermatogonia are the sperm mother cells and the cysts contained lightly stained nucleolus and the ooplasm was rather granular. Primary spermatogonia divided to form secondary spermatogonia where darkly stained nucleolus and darker ooplasm was observed. Connective tissues were obvious (Figure 2.b).

Stage II. Early maturing (primary and secondary spermatogonia): Spermatogonia became active and dividing and primary as well as secondary spermatogonia seen inside the seminiferous tubules. There was a slight reduction in cell volume which could also be observed. The granules became larger and the nuclear material apparently condensed. Cells became purple colour with haematoxylin – eosin dye and the connective tissues were diminished (Figures 3.a & b). The chromatin nuclear materials were condensed and some spermatids were also visible (Figure 3.a).

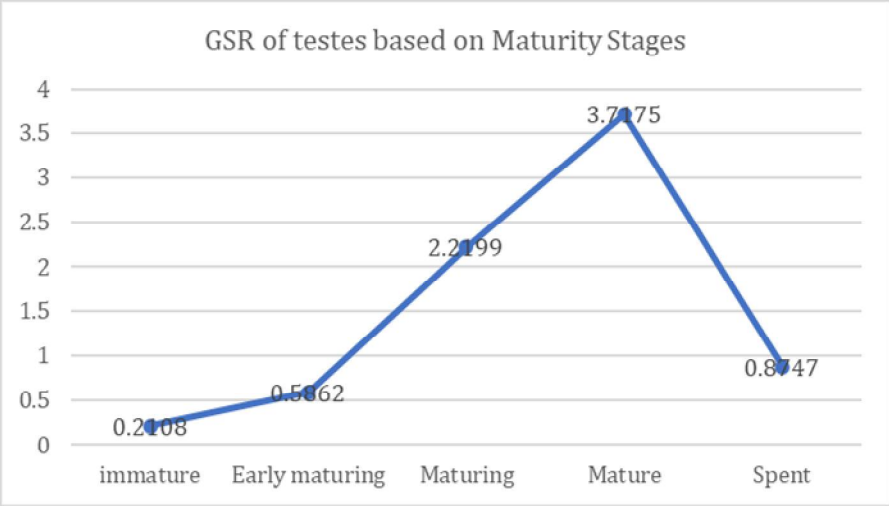
Stage III. Maturing (Spermatids): Each cell was full of spermatids and some secondary spermatocytes were scattered inside. The spermatids are the final product of meiosis with deeply stained nucleus (Figures 4.a & b).

Stage IV Mature (Spermatozoa): Seminiferous tubules became large and encompass full of sperms whereas, the spermatids were reduced in number and drained into the lumen (Figures 5. a & b).

Stage V Spent: During the spent stage seminiferous tubules were empty with some ruptured cells. Moreover, Phagocytosis occurred in the collapsing spermatids and sperms during this phase. Ruptured seminiferous tubules with remnants of sperm visible during this stage (Figure 6).

The mean GSR for different maturity stages for the species are presented in Table 1. From the table it can be understood that higher GSR (3.7175) occurred in the mature stage followed by maturing stage (2.2199). But the lowest GSR (0.2108) showed during the immature stage during this investigation.

Table 1: Gonado somatic ratio of the testes of *A. subrostratus* based on maturity stages



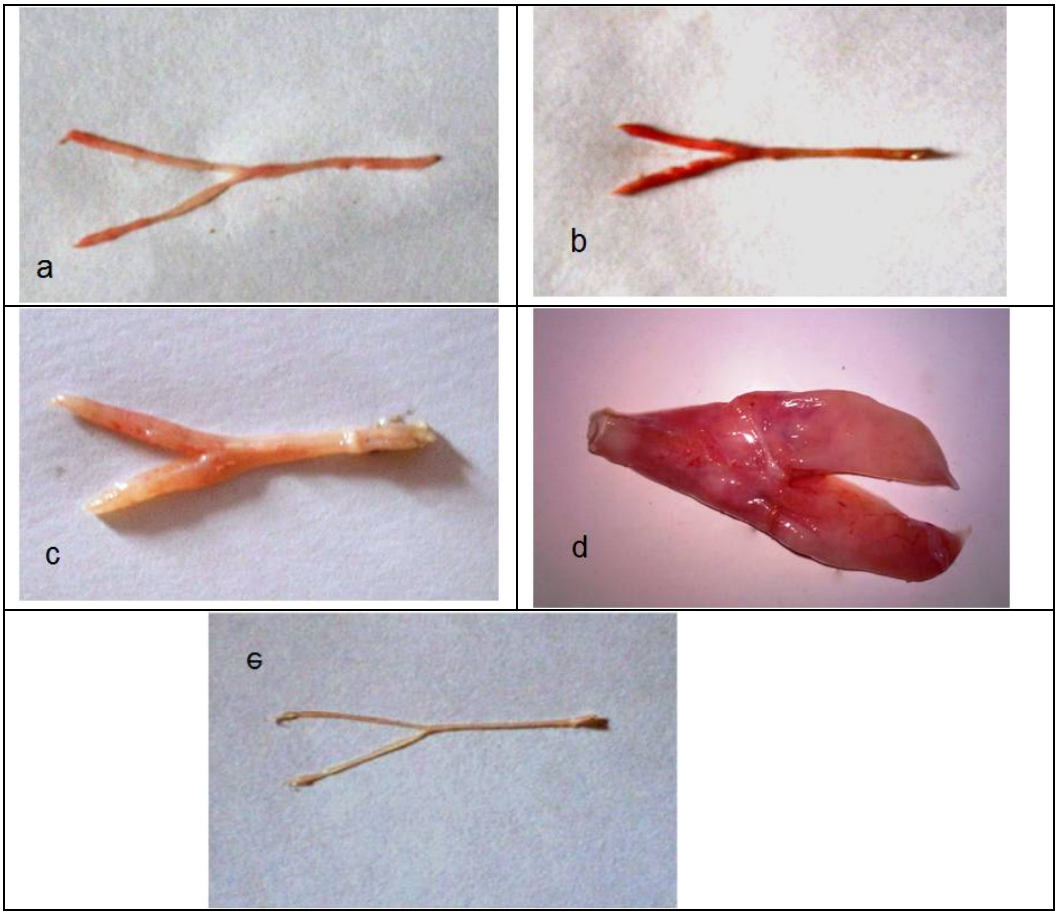


Figure 1: Testes of *A. subrostratus* based on different maturity stages from Cochin Estuary
a: Immature stage of testes, b: Developing stage of testes, c: Maturing stage of testes d: Mature/Spawning stage of testes; e: Spent stage of testes

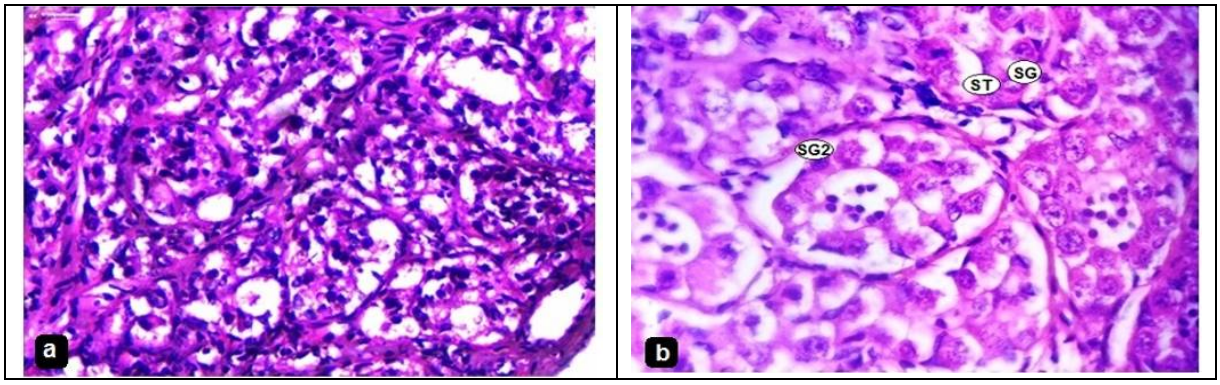


Figure 2 a: Cross section of immature testes of *A. subrostratus* (40 x) surrounded by *tunica albugenea* (Ta); b: Immature (Spermatogonial stage with primary (SG) and secondary spermatogonia (SG 2) (1000x)

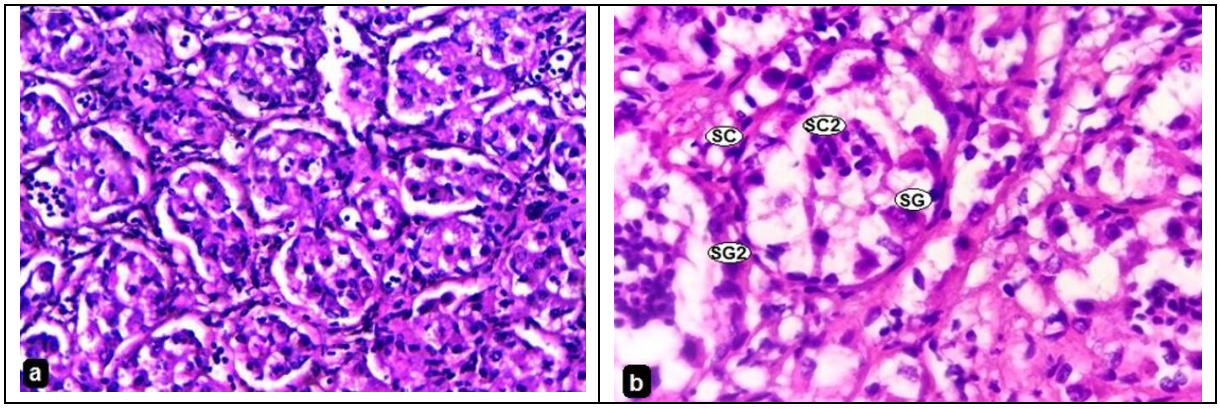


Figure 3 a: CS of early maturing testes (40 x) with spermatocyes; b: CS of early maturing testes with Primary spermatocyte (SC) and secondary spermatocyte (SC 2) (1000x)

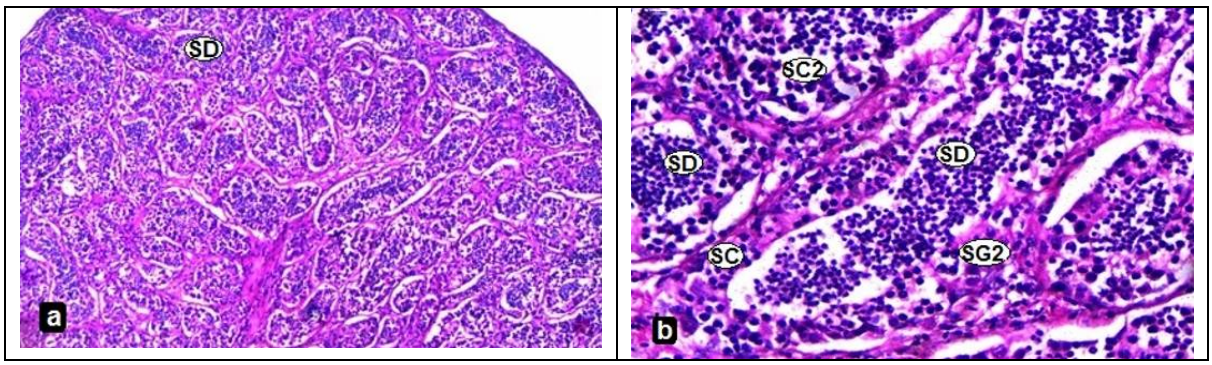


Figure 4 a: CS of maturing testes with large number of spermatids (SD) (40x); b: Maturing testes (400x)

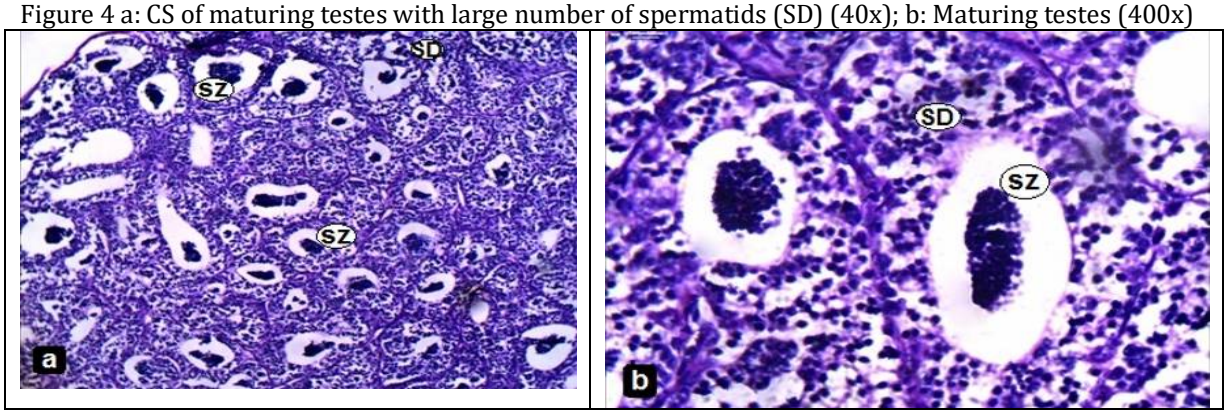


Figure 5 a: CS of mature testes with spermatozoa (SZ)(100x); b: Mature testes with spermatozoa (SZ) (1000x)

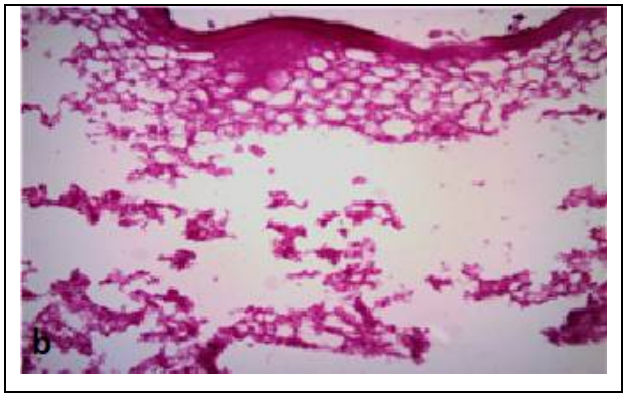


Figure 6: CS of spent testes with empty and ruptured cells

DISCUSSION

The present investigation delivers the first macroscopical and histological interpretation of gonad developmental stages in *Arius Subrostratus*. From the macroscopical identification five maturity stages of testes could be identified. A similar number of stages have been described for a threatened catfish *Mystus montanus* [5]. However, Siddiqua et al [6] classified testes of 4 stages in *Ompok pabda* such as spermatogonia, spermatocytes, spermatids and spermatozoa. Findings from this analysis disclosed that, in the earlier stages the testes appeared as narrow thread like structures and pale white in colour. Whereas, maturing and matured testes were smooth and ribbon like. Both lobes of the testes were fused together in a sperm duct and opened to the posterior end of the abdominal cavity called as genital opening. Exact knowledge of maturity stages of any fish species is a requisite for the prediction of reproductive capacity of that particular species in a stock [7]. An upsurge in Gonado – somatic ratio from the immature stage onwards occurred in *Channa punctatus* where peak GSR obtained during the maturity stage [8]).

Histological examination of the gonads has been the best and precise technique to evaluate ovarian developmental process. The event of development of gonads and its maturation procedure in majority of the vertebrates follow the same pattern [9]. The primordial germ cells act as sex cells during the earliest embryological development until the formation of gonads and are called as spermatogonia or oogonia according to the sex [10]. The cross section of the testes of *A. subrostratus* unveiled that the testes were composed of seminiferous tubules occupied with germinal cysts. These cysts were again encircled by cytoplasmic processes of Sertoli cells. Spermatogenic cyst are the functional unit of germinal epithelium in fishes and amphibians [11]. Proliferation of spermatocytes and oocytes due to the mitotic cell division are called as spermatogenesis and oogenesis. Spermatogenesis in vertebrates including fishes can be classified into spermatogenesis and spermiogenesis. Spermatogenesis is a process by which the meiotic spermatocytes are formed by the mitotic division of spermatogonia and during spermiogenesis spermatozoa or sperm is formed by the metamorphosis of spermatids after the second meiotic division of spermatocytes [11]. The spermatogenesis and spermiogenesis in *A. subrostratus* in the current study revealed the spermatogonium, spermatocyte, spermatids and spermatozoan stages by the histological examination.

In the present observation primary spermatogonia occurred mostly in the immature and early maturing stages. Nevertheless, investigation done by Dziewulska and Domagała [12] on the testicular morphology of salmonid fishes explicated that in some fishes primary spermatogonia or the sperm mother cells occurred throughout the entire spermatogenic process. Again, they confirmed that, in the spermatogenesis process sertoli cells plays a major role in vacuole formation phagocytosis and opening of cyst to release sperm to the lumen. Moreover, Spermatozoa remained after spawning were engulfed by the phagocytosis of sertoli cells.

There are two types of testes detected in fishes such as tubular type and lobular type. In tubular testes, a large cavity occurs at the core of testes where bundles of spermatozoa are stored, whereas in lobular type testes there are numerous lobules formed by connective tissues lined by *sertoli* cells [13]. Most of the teleost fishes including *Arius subrostratus* exhibited lobular testes. Though, some fishes such as salmonids, cyprinids and lepisosteids exhibited tubular type testes [14].

Saka et al [15] made clear about the cellular changes of testes during the maturation of *Clarias gariepinus,* that provided best understanding of the spermatogenesis and spermiogenesis in the testes. Whereas, Salam & Naeem [16] explained importance of leydig cells and sertoli cells in the process of spermatogenesis. Vazquez et al [17] gave a clear explanation of spermatogenesis of South American

perciform fish *Cichlasoma dimerus* using light and electron microscopy. He further explained testicular maturation process and cellular structures of spermatogonia such as sertoli cells, endoplasmic reticulum and mitochondria.

Spent stage is the last maturity stage observed during this study where seminiferous tubules were either empty or with some ruptured cells. Same findings were obtained in the histological analysis of the testes of majority of fishes. El- Zoghby et al [18] supports these findings by disclosing the evidence of testicular lobules in spent testes of different catfishes.

CONCLUSION

In this study maturity stages of *A.subrostratus* is assessed with the aid of macroscopic and histological criteria. Dramatic cellular changes occurring throughout the spermatogenesis and is an evolutionary phenomenon happened in all vertebrates as well as invertebrates. Spent stages were also predominant during the study with empty testes with residual sperms. Also, highest GSR obtained during the mature or ripe stage of fishes.

REFERENCES

1. Billard, R. (1986). Spermatogenesis and spermatology of some teleost fish species. Reproduction nutrition development., 26 (4): 877 – 920.
2. El -Sakhawy, M.A., El-Saba, A., Abd Rabou, M.I., El-Shammaa, M.A., & Hussein, S.H. (2011). Seasonal Histology of the Testes of Nile Tilapia (*Oreochromis niloticus*). Journal of Veterinary anatomy., 4 (2): 61 – 75.
3. Schulz, R., De Franca, L.R., Lareyre. J.J., Le Gac, F., Chiarini- Garcia., H., Nobrega, R.H & Miura, T. (2009). Spermatogenesis in fish. Genaral and Comparative Endocrinology., 165 (3): 390 -411.
4. Nikolsky, G.V. (1963). The ecology of fishes. Academic press London and New York. (*Aphididae*) from the deep Red Sea and the Gulf of Aden. Marine Ecology Progress Series.,124: 23-29.
5. Arockiaraj, A.J., Haniffa, M.A., Seetharaman, S and Singh, S. (2004). Cyclic changes in gonadal maturation and histological observations of threatenec freshwater catfish "Narikeliru" *Mystus montanus* (Jerdon, 1849). Acta Ichthyologica Et Piscatoria., 34 (2): 253 – 266.
6. Siddiqua, K.A., Islam,M.S., Hussain, M.G & Ahmed,A.T.A. (2000). A histological study of the spermatogenesis in *Ompok pabda* (Hamilton-Buchanan 1822). Biology.4 (2), 2000: 185 – 189.
7. Kresimir Williams. (2007). Evaluation of the Macroscopic Staging Method for Determining Maturity of Female Walleye Pollock, *Theragra chalcogramma* in Shelikof Strait, Alaska. Alaska Fishery Research Bulletin.,12 (2):252–263.
8. Saikia, A.K., Abujam, S.S & Biswas, S.P. (2013). International Journal of Current Research. Reproductive biology of Channa punctatus (Bloch) from paddy field of Sivasagar, Assam., 5 (3): 542 – 546.
9. Bhatta, S., Iwai, T., Miura,T., Higuchi, M., Maugars, G & Miura, C. (2012). Differences between male and female growth and sexual maturation in tilapia (*Oreochromis mossambicus*). Kathmandu University Journal of Science, Engineering and Technology., 8 (2): 57-65.
10. Guraya, S.S. (1994). Gonadal development and production of gonads in fish. Proc. India natn. Sci.Acad., B 60 (1): 15 – 32.
11. Schulz, R.W., R.H, Nobrega., R.D.V.S, Morais., P.P, de Waal1., L.R, França and J, Bogerd. (2015). Endocrine and paracrine regulation of zebrafish spermatogenesis: the Sertoli cell perspective. Anim. Reprod., 12 (1): 81 – 87.
12. Dziewulska, K & J, Domagała. (2002). Histology of salmonid testes during maturation. Reproductive biology., 3(1): 47- 61.
13. Kapoor, B.G & Khanna, B., (2004). Ichthyology hand book. ISBN.3- 540- 42854- 2. Springer- Verlag Berlin Heidelberg. New York.
14. Uribe, M.C., H.J, Grier & V.M, Roa. (2014). NCBI Resources. Comparative testicular structure and spermatogenesis in bony fishes. Spermatogenesis., 4 (3): 1-13.
15. Saka, B.A., O. K, Adeyemo & B.O, Emikpe. (2015). Testes Development in Nigerian *Clarias gariepinus,* Burchell 1822. International Journal of Fisheries and Aquatic Studies. 2(5): 366-369.
16. Salam, C.A & M, Naeem. (2004). Some morphometric studies of common carp *Cyprinus carpio* in relation to body size, from Islamabad, Pakistan. Sindh. Univ. Res. J (Sci. ser)., 36: 21- 48.
17. Vazquez, G.R., R. H, Da Cun., F. J, Meijide & G. A, Guerrero. (2012). Spermatogenesis and changes in testicular structure during the reproductive cycle in *Cichlasoma dimerus* (Teleostei, Perciformes). Acta Zoologica (Stockholm) 93: 338–350.
18. El- Zoghby, I.M.A., Bakry, H. H., Ghallab, A. M & Emam, M. A. (2009). Histological Studies on the Gonads of the Catfish during Different Seasons. Lucrari stiintifice., 52: 352- 362.