E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2018; 6(5): 132-137

www.fisheriesjournal.com
Received: 20-07-2018
Accepted: 21-08-2018

Suren Subba
1. Department of Zoology, IOST, Dhankuta Multiple Campus, Tribhuvan University, Dhankuta, Nepal
2. Central Department of Zoology, Tribhuvan University, Kirtipur, Nepal

Vinod K Mahaseth
Department of Zoology, Mahendra Morang Adarsh Multiple Campus, Tribhuvan University, Biratnagar, Nepal

Correspondence
Suren Subba
1. Department of Zoology, IOST, Dhankuta Multiple Campus, Tribhuvan University, Dhankuta, Nepal
2. Central Department of Zoology, Tribhuvan University, Kirtipur, Nepal

Histological observation of gonads during breeding and non-breeding season of *Neolissochilus hexagonolepis* from Tamor River, Nepal

Suren Subba and Vinod K Mahaseth

Abstract
The present investigation was carried out to analyze the histomorphological features of gonads of *Neolissochilus hexagonolepis.* The study revealed that the gonads of N. *hexagonolepis* pass through six stages of maturation within a year viz. immature, maturing virgin and recovering spent, ripening, mature, spawning and spent. The testes were found to be of lobular type and the ovaries of the fish were observed to be of cyst ovarian type. The histological study revealed that the testes of N. *hexagonolepis* undergo considerable changes in shape, size, colour, volume, length and occurrence of various types of spermatogenic cells within their lobules during different phases of reproductive cycle of the fish. Similarly, the histological sections of the ovaries revealed several stages of developing oocytes in the ovigerous lamellae and the developing oocytes were found to be passing through chromatin nucleolus stage, early and late peri-nucleolus stage, yolk vesicle stage, yolk stage and migratory nucleus stage. Several pre-vitellogenic follicles as well as vitellogenic and mature yolky eggs were observed to show atresia in the ovaries of the fish.

Keywords: *Neolissochilus hexagonolepis,* lobular type, cyst ovarian type, reproductive cycle, histological sections

Introduction
Tamor river is one of the major rivers of Nepal which flows through the eastern part of the country. The river lies in the latitude and longitude co-ordinates of 26˚54'46.80" N and 87˚09'25.20" E respectively (Google maps, 2014) [8]. After joining the confluence of Arun and Sunkoshi rivers at Tribenighat, this river drains into the giant Saptakoshi which flows on to the Gangetic plain. The total length of this river is about 190 km with 5817 km^2 catchment area (Shrestha *et al*, 2009) [19].

Several ichthyologists have studied the histomorphological features and maturation cycle of gonads of fresh water fishes and have emphasized upon the importance of this aspect in conservation of fishes.

Meijide *et al.* (2005) [13]. Suggested that the histology offers a powerful tool in the study of reproductive health of fishes. Reproductive development in fishes is well understood by histological studies, which are the most convenient method to decide the reproductive state of fishes (West, 1990) [23]. The studies on histomorphology of gonads and gonadal cycle provide insight into the reproductive biology of a fish and provide opportunity to unravel various aspects of reproductive biology like breeding season and frequency of breeding etc.

Jyrwa and Bhuyan (2017) carried out the histological studies of gonads in N. *8hexagonolepis* from Meghalaya, India and established five development stages of the gonads of the species. Swar (1994) [21] investigated the maturity stages of gonads of the same species based on their external features and established seven maturity stages for both the gonads.

Subba (1998) [20], Thiry and Poncin (2005) [22], Behera (2012) [4], Agbugui (2013) [2], Gadekar and Baile (2014) [7], Mahmud *et al*. (2016) [12]. And Pasha *et al.* (2016) [15]. reported on the histomorphological features of *Lepidocephalicthys guntea* (Ham.), *Barbus barbus, Trichogaster fasciatus, Pomadasys jubelini, Labeo rohita, Channa striata and Schizothorax plagiostomus respectively.*

Neolissochilus hexagonolepis is locally named as katle and has the conservation status of near threatened (Arunachalam, 2010) [3] and in view of this, urgent protection and effective

conservational efforts are required to save this species before it becomes extinct from the face of the planet earth. The finding of the study is hoped to be of some use in monitoring and conserving this species in the rivers.

Materials and methods

Fish sampling was carried out for two years commencing from the second week of December 2014 till the end of November 2016. Sampling was done along the river at the stretch of 10 km upstream and downstream (Fig. 1).

Verification of sex of the samples was made through examination of gonads in situ after dissection. The gonads were extracted in situ through longitudinal incision made along the ventral line of the body with the help of a sharp blade and angular scissors. The extracted gonads were then washed in saline water and then cut into small suitable pieces and then fixed, for 24 hours, in freshly prepared Bouin's fluid. The fluid was changed 3 times, 8-hourly. After the 3rd change the gonads were preserved in 70% alcohol.

The preserved gonads were washed with 70% alcohol to remove the yellow colour of the stain and then cut into small pieces with the help of a sharp blade. The samples were dehydrated in 90% and absolute alcohol, one hour in each. After that the pieces of gonads were transferred to 1:1 xylene and paraffin wax which was kept in incubator at 60º for about 15 minutes. They were then transferred to pure wax already melted at 60º. The pure melted wax was changed three times after every ½ hour. After the 3rd change the pieces of gonad kept in the melted wax were kept in the incubator at 60º overnight for infiltration. The next day paraffin blocks were prepared with care. The above mentioned process was repeated for all the materials and the blocks were trimmed into pyramid shapes, each containing a piece of the samples. The blocks were then manually processed and sectioned at 6 µ with a rotary microtome. The ribbon-shaped sections so obtained were carefully stretched on slide. The sections were double stained in hematoxylin and eosin as mentioned below.

First the slides were put in xylene for 10 minutes for dewaxing then hydrated in alcohol series (in the order 100%, 90%, 70%, 50%, 30%). The sections were then stained with hematoxylin for 15 minutes (for nuclear staining). After staining in hematoxylin the slides were put in running water for 10 minutes in order to wash out the excess of the stain. In case of dark stain acid water was used to destain. After that the slides having the sections of gonads were passed through alcohol series from 30% to 70% alcohol then stained in eosin for 3 minutes. Whenever heavy staining was observed acid alcohol was used to destain. The sections were then dehydrated in 90% and in 100% (absolute alcohol) for 1 hour each, changing half hourly. Finally the sections were mounted in Canada balsam. The histological slides so prepared were observed under bel photonics bio2b-led (Italy) binocular microscope.

The gonad development stages of 109 female and 89 male N. *hexagonolepis* were studied and established. The gonads were categorized into six different stages largely following the method described by Brown-Peterson *et al.* (2011) [5]. Which has been accepted by many researchers, such as Dopeikar *et al.* (2015) [6], Jyrwa and Bhuyan (2017) etc., as the standard procedure.

Results

In N. *hexagonolepis*, the testes were paired, elongated structures situated on either side, ventral to the kidneys in the posterior region of the abdominal cavity. They were attached to the body wall by means of mesorchia and showed indentations along their margin. The histological sections of the testes revealed them to be of lobular type consisting of a large number of seminiferous lobules bounded together by a thin layer of connective tissue.

During the present study, the testes and ovaries of N. *hexagonolepis* were observed at six

Fig 1: Map of Nepal showing major river systems.

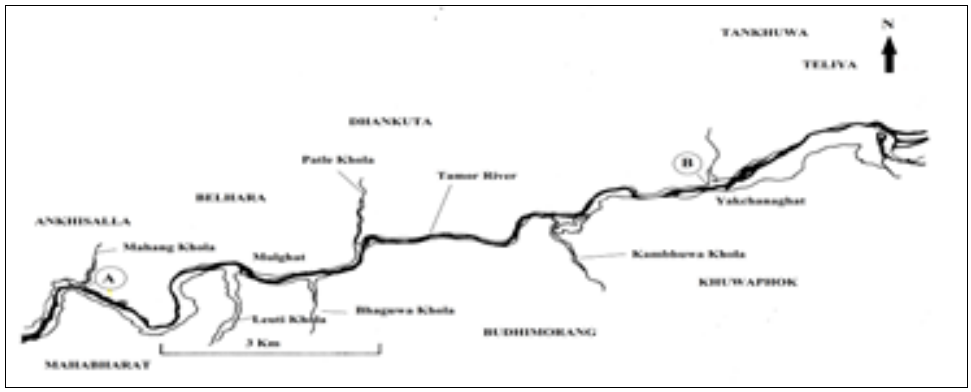


Fig 2: Map of Tamor river showing sampling area from A to B.

different stages viz. Stage I (Immature stage), Stage II (Maturing virgin and recovering spent), Stage III (Ripening), Stage IV (Mature), Stage V (Spawning) and stage VI (Spent). Each stage was confirmed through superficial inspection of gonads in situ followed by the study of their histological slides.

At stage I the testes presented very thick tunica albuginea (testicular wall) sending several septs inward forming seminiferous lobules. The seminiferous lobules were of cystic type and within each cyst were present spermatogonia cells. The spermatogonia were of two type's viz. primary and secondary spermatogonia among which the primary spermatogonia were the largest of the spermatogenic lineage. They contained clear cytoplasm, large and prominent nucleus. The spermatogonia were seen dividing into primary and secondary spermatocytes at stage II. Intense spermatogenesis was observed at stage III testes with all the stages of spermatogenesis viz. spermatocytes, spermatids and spermatozoa. The seminiferous lobules were seen to be packed with spermatozoa in testes at stage IV. There were some spermatids as well (Plate: I b).

The stage V testes were observed with seminiferous lobules packed with spermatozoa with some empty lobules. At stage VI the seminiferous lobules were seen to be collapsing and empty with small amount of unexpelled or residual sperms (Plate: I c).

Similarly, histomorphological study of ovaries of N. *hexagonolepis* revealed them to be typical teleostean type being paired, elongated, sac-like structures lying in the abdominal cavity ventral to the kidneys and attached to the body wall by means of mesovarium. The ovaries were of cyst ovarian type and the oocytes were conveyed to the exterior through the oviduct.

The developing oocytes before transforming into mature ova were observed to show various changes. Based upon these changes following stages of the oocytes were identified.

Oocyte I was found to be the smallest oocyte with a very thin sheath of cytoplasm and a prominent round nucleus consisting of 2 or 3 nucleoli (Plate: II b). This stage is also referred to as the chromatin nucleolus stage. Oocyte II was larger than oocyte I and had cytoplasm which stained deeply with hematoxylin. This stage was recognized as the early and late perinucleolus stage. Nucleoli were seen to be increasing in number and got distributed adjacent to the periphery of nuclear membrane (Plate: II c & d). Oocyte III was characterized by the appearance of a large number of small, clear vacuoles in the cortical area of cytoplasm. These vacuoles are the yolk vesicles and the stage is conventionally termed as the early yolk vesicle stage. Nuclear membrane of the oocyte started to appear wavy or irregular in outline (Plate: II e)

Oocyte IV was further larger in size. The yolk vesicles further increased in size and number and were seen distributing randomly in the ooplasm. This stage is referred to as the

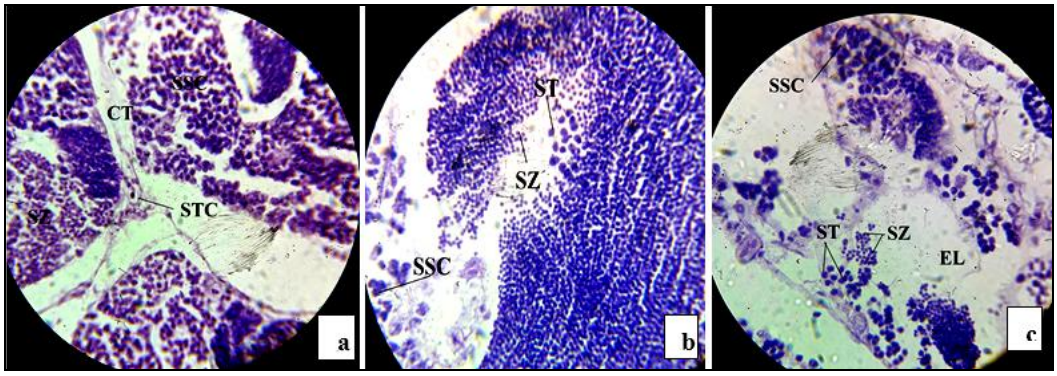


Plate I: A: Photomicrograph of T.S. of testes of N. *hexagonolepis* showing secondary spermatocyte (SSC), spermatozoa (SZ), connective tissue (CT) and sertoli cell (STC) X 1000 (H & E) **B:** Photomicrograph of T.S. of testes of N. *hexagonolepis* at spawning stage showing bulk of spermatozoa (SZ) and a few spermatids (ST) and secondary spermatocytes (SSC) X1000. (H & E) **C:** Photomicrograph of T.S. of testes of N. *hexagonolepis* at spent stage showing secondary spermatocytes (SSC), spermatids (ST), spermatozoa (SZ) and empty lumen (EL) X 1000 (H & E)

late yolk vesicle stage. A thin layer of fibroblast, known as theca, middle follicular epithelium (zona granulosa) and the innermost zona radiata became distinguishable during this stage. The wavy outline of the nuclear membrane became more irregular and nucleoli of varying sizes were seen randomly scattered in the nucleus (Plate: II f).

Oocyte V presented more yolk vesicles filling the entire ooplasm. The yolk globules were seen to appear among the

vesicles. The oocyte at this stage is termed as the early yolk stage. The vitelline membrane or zona radiata outside the ooplasm became more pronounced which appeared more homogenous and showed indistinct radial striations (Plate: II g).

Oocyte VI was characterized by the increase in number and size of yolk globules. The oocyte further increased in size due to the increased accumulation of more and more yolk globules. This stage is referred to as the late yolk stage. The yolk vesicles became pushed towards the peripheral region of the oocyte. The nucleus disappeared completely during this stage and the vitelline membrane (zona radiata) became thicker (Plate: II h).

Oocyte VII showed a heavy deposition of yolk globules in the cytoplasm. The oocyte at this stage was the largest among all the stages and was observed to be surrounded by an external layer of theca, middle follicular epithelium (zona granulosa) and the innermost zona radiata (Plate: II i). The oocytes at this stage gave rise to ripe eggs. The ripe eggs were perfectly yellowish in colour and spherical (Plate: II k).

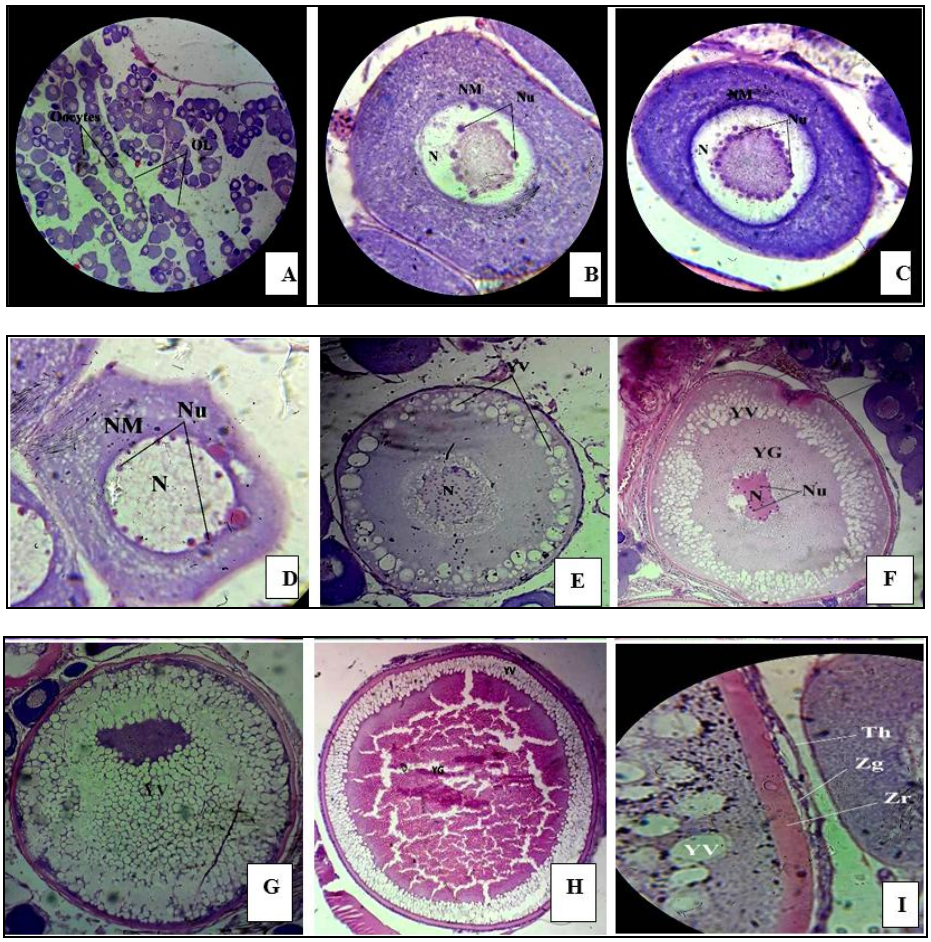
Stage I ovaries were observed with ovigerous lamellae with nests of oogonia and oocytes at stages I and II. At stage II the ovaries showed the ovigerous lamellae laden with oocytes at stages I, II, III and IV. At stage III the ovaries were seen to be laden with oocytes at stages IV and V. There were also a few oocytes at stage VI. At stage IV the ovaries observed to be packed with oocytes at stages VII along with some mature ova. At stage V the ovaries were much distended with large number of jelly like yellowish translucent eggs and were observed to be in the running phase and spawning was imminent. Histologically, stage VII oocytes and ripe eggs were seen in the ovigerous lamellae with a number of discharged follicles. At stage VI the ovaries presented many degenerated and atretic follicles. Oocytes at stages I and II were also observed in the ovigerous lamellae at this stage.

Several pre-vitellogenic follicles as well as vitellogenic and mature yolky eggs were observed to show atresia in the ovaries of the fish. Several atretic follicles were also observed during post-spawning or spent phase of the fish (Plate: II j).

Discussion

Sexual dimorphism was not found to be well pronounced in N. *hexagonolepis*. However, during its breeding season females were easily distinguished from male fishes by the presence of relatively enlarged belly in female fishes.

The present study revealed that the testes and ovaries of N. *hexagonolepis* pass through six different stages of maturation unlike those reported by Swar (1994) [21] and Jyrwa and Bhuyan (2017). The difference may be attributed to the methods followed for staging the gonads. It may be noted that the staging of gonads solely on the basis of morphological study lacks precision as it relies more on subjective judgement. Probability of correct classification of



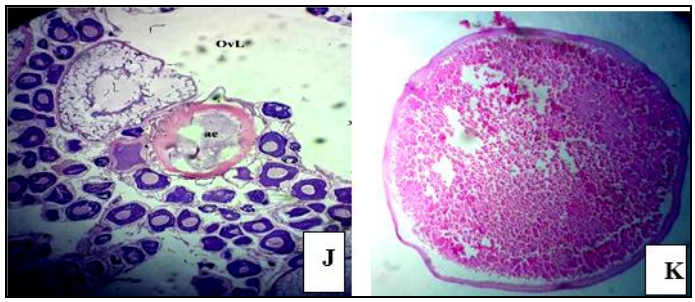


Plate II: A: Ovigerous lamellae (OL) containing oocytes at various stages of development. X 100 (H & E) **B:** Photomicrograph of T.S of ovary of N. *hexagonolepis* showing stage I oocyte with multiple nucleoli (Nu), Nuclear membrane (NM) and Nucleus (N) X 1000 (H & E) **C:** Photomicrograph of T.S. of ovary of N. *hexagonolepis* showing stage II oocyte (Early perinucleolus stage) with multiple nucleoli (Nu) adjacent to the nuclear membrane (NM) X 1000 (H & E) **D:** Photomicrograph of T.S. of ovary of N. *hexagonolepis* showing stage II oocyte (Late perinucleolus stage) with multiple nucleoli (Nu) adjacent to the nuclear membrane (NM) X 1000 (H & E) **E:** Photomicrograph of T.S. of ovary of N. *hexagonolepis* showing stage III oocyte with yolk vesicles (YV) X 1000 (H & E) **F:** Photomicrograph of T.S. of ovary of N. *hexagonolepis* showing stage IV oocyte with yolk vesicles (YV) and yolk globules (YG) X 1000 (H & E) **G:** Photomicrograph of T.S. of ovary of N. *hexagonolepis* showing stage V oocyte with yolk vesicles (YV) X 1000 (H & E) **H:** Photomicrograph of T.S. of ovary of N. *hexagonolepis* showing stage VI oocyte with yolk globules (YG) and yolk vesicles (YV) X 1000 (H & E) **I:** Photomicrograph of T.S. of ovary of N. *hexagonolepis* showing vitellogenic oocyte covered by theca (Th), zona granulosa (ZG) and zona radiata (Zr) X 1000 (H & E) **J:** Photomicrograph of T.S. of ovary of N. *hexagonolepis* showing ovarian lumen (OvL) and atretic egg (ae) X100 (H & E) **K:** T.S. of ripe egg of N. *hexagonolepis* X1000 (H & E)

gonads into various stages based on macroscopic criteria is low for some stages (Murua & Saborido-Rey, 2003) [14].

Tunica albuginea of both testes and ovaries presented no uniform thickness over the year round, being thinner during the breeding season and thicker during other periods. This may be attributed to the increased pressure exerted on the wall by the distended testicular lobules or enlarged matured ovarian follicles.

Ovary of N. *hexagonolepis* was found to be of cyst ovarian type as its lumen was continuous with the oviduct and the oocytes were conveyed to the exterior through the oviduct. Subba (1998) [20] reported similar feature of the ovary in a hill-stream fish *Lepidocephalichthys guntea.*

Pasha *et al.* (2016) [15]. Suggested that the different phases of ovary of *Schizothorax plagiostomus* were marked on the basis of histology and developing stages of oocytes. They reported six stages of the oocytes and recognized the stages as chromatin nucleolar stage, early and late peri- nucleolus stage, yolk vesicle stage, yolk stage, ripe egg stage and post ovulatory follicular stage during different seasons of the year. Their finding was similar to the finding of the present study.

The present study showed that the new crop of oogonia in the ovaries originates from their germinal epithelium as was also suggested by Sharma *et al.* (2015) [18].

In the present study the increased number of nucleoli during yolk formation indicated that the nucleoli were associated with the formation of yolk in the developing oocytes.

Large number of small, clear vacuoles called yolk vesicles appeared in the periphery of cytoplasm during the yolk vesicle stage. Similar phenomenon was also reported by Sharma *et al.* (2015) [18] in *Garra gotyla gotyla*. These vesicles later on filled the entire cytoplasm. Pasha *et al.* (2016) [15]. Suggested that the formation of yolk vesicles within the oocytes was the sign of maturation process.

Yolk nucleus was never traced or observed in any of the stages of oocytes of N. *hexagonolepis* in the present study. Kumari and Nair (1979) [11] reported that the yolk nucleus was entirely wanting in any stage of oocyte maturation of *Lepidocephalicthys thermalis*.

Several small, round and prominent nucleoli were observed in the early stages of the oocyte maturation. As the oocyte matured the nuclear membrane showed irregular outline and the nucleoli disintegrated in the ooplasm with the disappearance of nuclear membrane. This phenomenon was observed during the III and IV (early and late yolk vesicle stages) stages of oocytes. Several other workers also reported on this phenomenon in several species of fishes. Subba (1998) [20], Saeed *et al.* (2010) [17], Sharma *et al.* (2015) [18] and Jyrwa and Bhuyan (2017) reported on this phenomenon in and *Lepidocephalicthys guntea, Rutilus frisii, Garra gotyla gotyla and* N. *hexagonolepis* respectively. However, the activity of the formation of small pockets in the nuclear membrane and nucleoli pushing themselves into these pockets before extruding out of the membrane could not be established in the present study.

Several pre-vitellogenic follicles as well as vitellogenic and mature yolky eggs were observed to show atresia in the ovaries of N. *hexagonolepis*. Khanna (2000) [10]. Suggested that any disturbance in the environmental, endocrinological and metabolic factors may initiate atresia of oocytes. Rai (1965) [16]. Suggested that the atresia is due to low gonadotropin content of the pituitary, which is not able to maintain healthy oocytes. Inadequate food supply influences the metabolism of fish which enhances the rate of atresia and this affects the fecundity of a fish (Agarwal *et al*., 1988) [1].

Conclusion

The prime objective of the present investigation was to analyse the histology of gonads of *Neolissochilus hexagonolepis* with an aim to generate additional knowledge on the reproductive biology of the species.

The gonads of both the sexes of N. *hexagonolepis* were observed to pass through six stages of maturation viz. immature, maturing virgin and recovering spent, ripening, mature, spawning and spent.

Histological sections of the testes of N. *hexagonolepis* revealed them to be of lobular type consisting of a large number of seminiferous lobules bounded together by a thin layer of connective tissue with blood capillaries and interstitial cells scattered in it.

Testes of N. *hexagonolepis* were observed to be undergoing considerable changes in shape, size, colour, volume, length and occurrence of various types of spermatogenic cells within their lobules during different phases of reproductive cycle of the fish.

Similarly, the ovaries of N. *hexagonolepis* were found to be of

typical teleostean type. As in the case of most other hill stream fishes, its ovaries were of cyst ovarian type, such that, the lumen of ovary was continuous with the oviduct through which the oocytes were conveyed out.

Histological sections of the ovaries revealed several stages of developing oocytes in the ovigerous lamellae. The developing oocytes were found to be passing through chromatin nucleolus stage, early and late peri-nucleolus stage, yolk vesicle stage, yolk stage and migratory nucleus stage.

During the present study, several nucleoli were observed at various stages of oocytes which were seen extruding through the nuclear membrane. Though the nuclear membrane presented distinct wavy outline in the developing oocytes the formation of nuclear pockets during the phenomenon of nuclear extrusion was never observed. The increased number of nucleoli during yolk formation indicated that the nucleoli were associated with the formation of yolk in the developing oocytes.

During the present study, yolk nucleus was never traced or observed and the zona radiata showed more homogeneity with indistinct radial striations. Also, while the zona radiata showed an increased thickness as oocyte matured the thickness of follicular layer remained nearly constant.

Several pre-vitellogenic follicles as well as vitellogenic and mature yolky eggs were observed to show atresia in the ovaries of the fish. Several atretic follicles were also observed during post-spawning or spent phase of the fish.

Acknowledgement

The authors are grateful to Dr. Bharat Raj Subba for the guidance provided during the research work. Acknowledgement is also due to the local fishermen who supported the research by continuously supplying the fish samples from the river.

References

1. Agarwal NK, Singh W, Singh HR. Fecundity of the snow-trout, *Schizothorax plagiostomus* (Heckel) from Garhwal Himalaya. J Inland Fish. Assoc. 1988; 18:537-548.
2. Agbugui MO. The sex ratio, gonadosomatic index, stages of gonadal development and fecundity of the Grunt *Pomadasys jubelini* (Cuvier, 1830) in the New Calabar-Bonny River. Report and Opinion. 2013; 5(11):31-37.
3. Arunachalam M. *Neolissochilus hexagonolepis*. The IUCN Red List of Threatened Species, 2010. http://dx.doi.org/10.2305/IUCN.UK. 2010-4.RLTS.T166479A6217872.en. 5 November, 2017.
4. Behera S. Histological observation of gonads during breeding and non-breeding season of *Trichogaster fasciatus* in Shanti Jheel, West Bengal. J Bio. Innov. 2012; (6):205-214.
5. Brown-Peterson NJ, David MW, Fran SR, Beverly JM, Susan KLB. A standardized terminology for describing reproductive development in fishes. Marine and Coastal Fisheries: Dyanamics, Management and Ecosystem Science. 2011; 3:52-70.
6. Dopeikar H, Keivany Y, Shadkhast M. Reproductive biology and gonad histology of the kura barbel, *Barbus lacerta* (Cyprinidae) in Bibi-Sayyedan River, Tigris basin, Iran. NW Journal of Zoology. 2015; 11:163-170.
7. Gadekar GP, Baile VV. Annual cyclical changes in the testicular activity of an Indian freshwater major carp, *Labeo rohita* (Hamilton). International Journal of Research studies in Biosciences (IJRSB). 2014; 2(7):28-36.
8. Google maps. Royal Roads University, 2014. https://www.google.com/maps/d/viewer?mid=ziZA4YH0pTUs.ktJc02G0F-O4 and ie = UTF8 & oe = UTF8 & msa=0
9. Jyrwa LB, Bhuyan RN, Nath R. Length–weight relationship and condition factor of *Neolissochilus hexagonolepis* (McClelland) in Meghalaya, India: A comparative study. International Journal of Fisheries and Aquatic Studies. 2015; 3(1):419-422.
10. Khanna SS. An Introduction to fishes. 4th Ed, Central Book Depot, Allahabad, India, 2000, 635.
11. Kumari SD, Nair NB. Oogenesis in a tropical loach *Lepidocephalus thermalis* (cuv. and val.) India. Acad. Sci. Sect. B. 1979; 88:45.
12. Mahmud NA, Hassan MHR, Golam MM, Khan MGQ, Shahjahan M, Lucky NS *et al*. Cyclic variations of gonad development of an air-breathing fish, *Channa striata* in the lentic and lotic environments. Fish Aquatic Sci. 2016; 19:5. https://doi.org/10.1186/s41240-016-0005-0
13. Meijide FJ, Lo Nostro FL, Guerrero GA. Gonadal development and sex differentiation in the cichlid fish, *Cichlasoma dimerus* (Teleostei, Perciformes): A light- and electron-microscopic study. Journal of Morphology. 2005; 264:191-210.
14. Murua H, Saborido-Rey F. Female reproductive stratefies of marine fish species of the north atlantic. J Northw. Atl. Fish. Sci. 2003; 33:23-31.
15. Pasha RH, Taj S, Irm M, Anjum B, Khan MA, Khan IA *et al*. Seasonal morphological changes in the gonads of *Schizothorax plagiostomus* (Pisces: Cypriinidae). Biologia (Pakistan). 2016; 62(2):6-3096.
16. Rai BP. Seasonal changes in the testis of the Mahseer, Barbus tor (*Tor tor*). Acta. Anat. 1965; 62:461-475.
17. Saeed SS, Reza IM, Bagher AF, Saeed G. Histological study of ovarian development and sexual maturity of kutum (*Rutilus frisii kutum Kamenskii*, 1901). World Applied Sci. J. 2010; 8:1343-1350.
18. Sharma J, Sharma S, Kant KR. Ovarian development and reproductive cycle of *Garra gotyla gotyla*. The Bioscan, 2015; 10(1):9-15.
19. Shrestha J, Singh DM, Saund TB. Fish diversity of Tamor river and its major tributaries of eastern Himalayan region of Nepal. Nepal Journal of Science and Technology. 2009; 10:219-223.
20. Subba BR. Ovarian histomorphology and gonadal cycle of a hill-stream fish *Lepidocephalicthys guntea* (Ham.) Cypriniformes: Cobitidae. J Freshwater Biol. 1998; 10(3-4):103-113.
21. Swar DB. A study on the ecology of Katle, *Neolissocheilus hexagonolepis* (McClelland) in a Nepalese reservoir and river. PhD thesis, Department of Zoology, The University of Manitoba, Manitoba, Canada. 1994, 216.
22. Thiry M, Poncin P. Morphological changes of the nucleolus during oogenesis in oviparous teleost fish, *Barbus barbus* (L.). Journal of structural biology. 2005; 152(1):1-13.
23. West G. Methods of assessing ovarian development in fishes: a review. Australian Journal of Marine and Freshwater Research. 1990; 41:199-222.