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Research Article

Histological studies of gonads in *Neolissochilus hexagonolepis* (McClelland, 1839) (Teleostei: Cyprinidae) from Meghalaya, India

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Abstract: Chocolate Mahseer, *Neolissochilus hexagonolepis* is an important food and game fish of North Eastern region of the country, particularly the high lands of Meghalaya. Reproductive biology of the species has been done under the climatic condition of Meghalaya to understand the gonadal development. The gonadal structure and development stages of germ cells of *N. hexagonolepis* are described for both sexes. Five stages of development are described based on the histological characteristics of testis and ovary. The kind of development and the presence of postovulatory follicles in ovaries which still contain developing vitellogenic oocytes suggest that *N. hexagonolepis* spawns over a relatively prolonged period of time.

Keywords: Chocolate Mahseer, Gonadal Maturity, Spawning Period, GSI.

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Introduction

The fish Chocolate Mahseer, *Neolissochilus hexagonolepis* (Cyprinidae) is an important food as well as game fish of India. The distribution of the species is restricted to the North-East Himalayan region of India and native to Meghalaya. Meghalaya, a hill state of North-East India situated between 25º11'N and 26º5' N latitude and 85º49'E and 92º 52'E longitude. This fish is considered as a threatened species and hence, it needs special attention to conserve its germplasm from extinction (IUCN 2016).

Chocolate Mahseer is acclaimed as a famous, outstanding game fish and important food fish of North-East India in general and Meghalaya in particular. As a sport fish, it provides unparalleled recreation to anglers. In commercial fisheries it occupies an important position for its good quality. For the fishermen, Mahseer is of great importance because of it can grow to a large size (Mahapatra & Vinod 2011). As a food fish, it is highly esteemed and fetches the very good price in North and North-East India (Sarma & Bhuyan 2007).

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). The role of climatic condition of an area, particularly the environmental temperature, on sexual maturation and breeding of fish has been reported by several investigators (Hora 1945; Chaudhuri 1960; Popma & Lovshin 1996; Donelson et al. 2014), but research on environment and related aspects of aquaculture (Pillay 1990) and biology of the fish at ultra-structure level is still scanty and is yet to become an integral part of farming system development. Bhuyan et al. (2002) have noticed

that the rate of spawning and fertilization in *Labeo gonius* (Hamilton, 1822) is low in colder climatic conditions at mid-altitudinal region of Meghalaya.

Chocolate Mahseer, locally known as '*Khasaw'* in Khasi language, is an icon in the water bodies of Meghalaya and is considered to be an important fish but due to various natural and anthropogenic factors, the species recorded a sharp drop in population in recent years (Sarma & Bhuyan 2007). It has been observed that there is a gradual decline in the population of the economically important fish species, throughout its entire distribution range and has necessitated its culture and conservation through artificial means. However, before making any such attempt, a detailed study of the gonadal cycle and ultra-structural changes on the fish, is extremely important.

Histological study is essential to understand the features of the reproductive biology (Mahmoud 2009). According to Hossienzadeh et al. (1980) and Eigdery (1981), histological studies determines the peak period of spawning assessment and exploitation of fish, biological characteristics and life cycle of a species. The studies have been taken up to understand the detailed histological structure of the ovary and testes in Chocolate Mahseer as the fish predominantly a riverine fish and its importance as food fish. The fish also may play an important role in development of eco-tourism in the NE region of the country. The present investigation emphasises on the histological studies of gonadal development of *Neolissochilus hexagonolepis* from Meghalaya.

Materials and Methods

The experiments were conducted in 2013 at the hatchery complex of the Department of Fishery Science, St. Anthony's College, Shillong. The experiments were repeated during 2014 and 2015 for confirmation of the results. A total of 50 (35 males and 15 females; sex ratio: 2.3:1) specimens of *N. hexagonolepis* collected from different rivers of Meghalaya. The brood fish was measured in total length (TL), fork length (FL) and standard length (ST). The gonads were dissected out and weighed. The gonado-somatic index (GSI) was calculated as follows:

GSI= Weight of the gonad/Weight of the fish ×100

The freshly collected gonads samples were fixed in Bouin's –Allen's fixative (Gabe 1976) for a night. The samples were washed with running water till yellow colour of the stain is removed. Thereafter, the samples were dehydrated with different alcohol grades (30% to 100%) for fifteen minutes each. The dehydrated samples were treated with xylene-wax for at least three hours in an oven and then embedded in pure paraffin wax ($58^0 - 60^0$C congealing point) overnight. After block preparation, the samples were sectioned at 8 µm in compliance with accepted histological procedures (Luna 1967; Humason 1972; Gabe 1976). The stretched paraffin sections were treated with xylene for removal of wax and after hydration process, stained with a regressive Harri's Hematoxylin. The sections were passed through grades of ethanol and stained (Luna 1967). The stained slides were mounted with DPX. The preparations were then examined under Motic high resolution microscope mounted with a camera.

Results

The length and weight of all the specimens varied from 7 cm to 35 cm and 4gm to 500 gm, respectively. The GSI of the male specimens ranged from 0.3 to 3.0, highest being in the month of June and July (2.5-3.0). Similarly, the GSI of the females ranged from 1.6 to 5.0, highest in the month of June and July (4.5-5.0) indicating the maturity of the species corresponding to breeding period.

The histological sections were observed under microscope and the gonad development stages of the fish, *Neolissochilus hexagonolepis* are placed in five stages following Brown–Peterson et al. (2011) which has been accepted by many researchers as the standard procedure (e.g., Abaszadeh et al. 2013; Dopeikar et al. 2015; Siami et al. 2017).

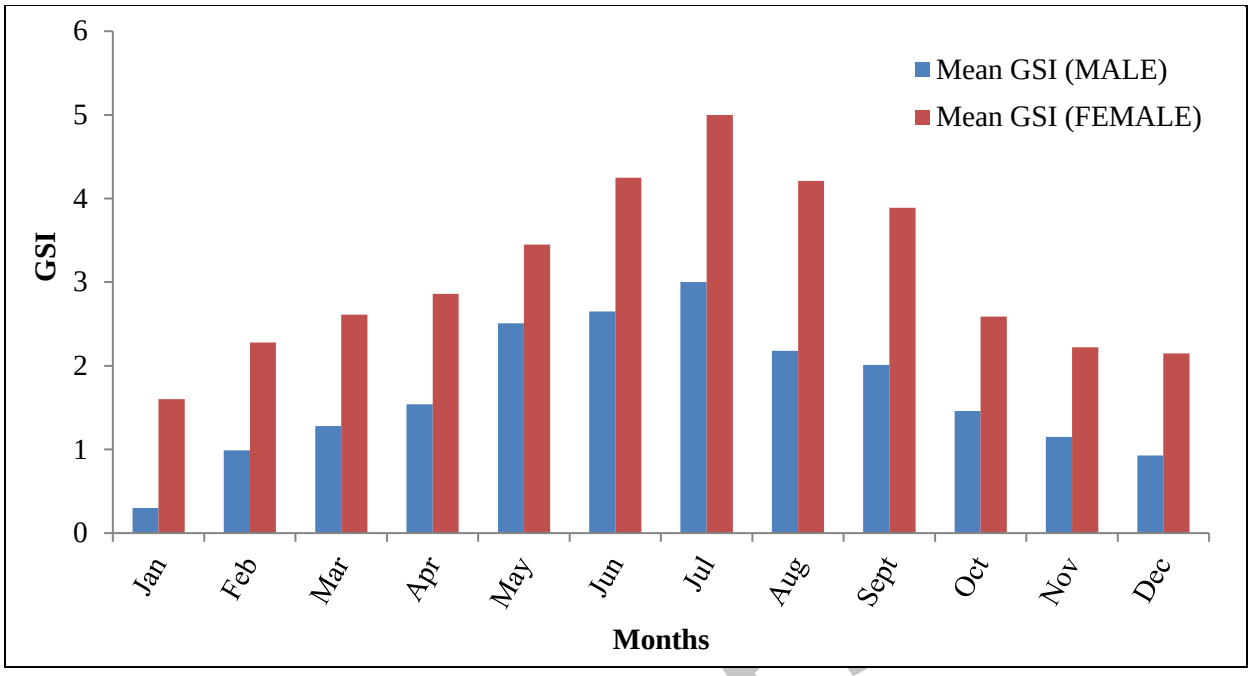


Fig.1. Monthly variation of gonado-somatic index (GSI) of *Neolissochilus hexagonolepis*.

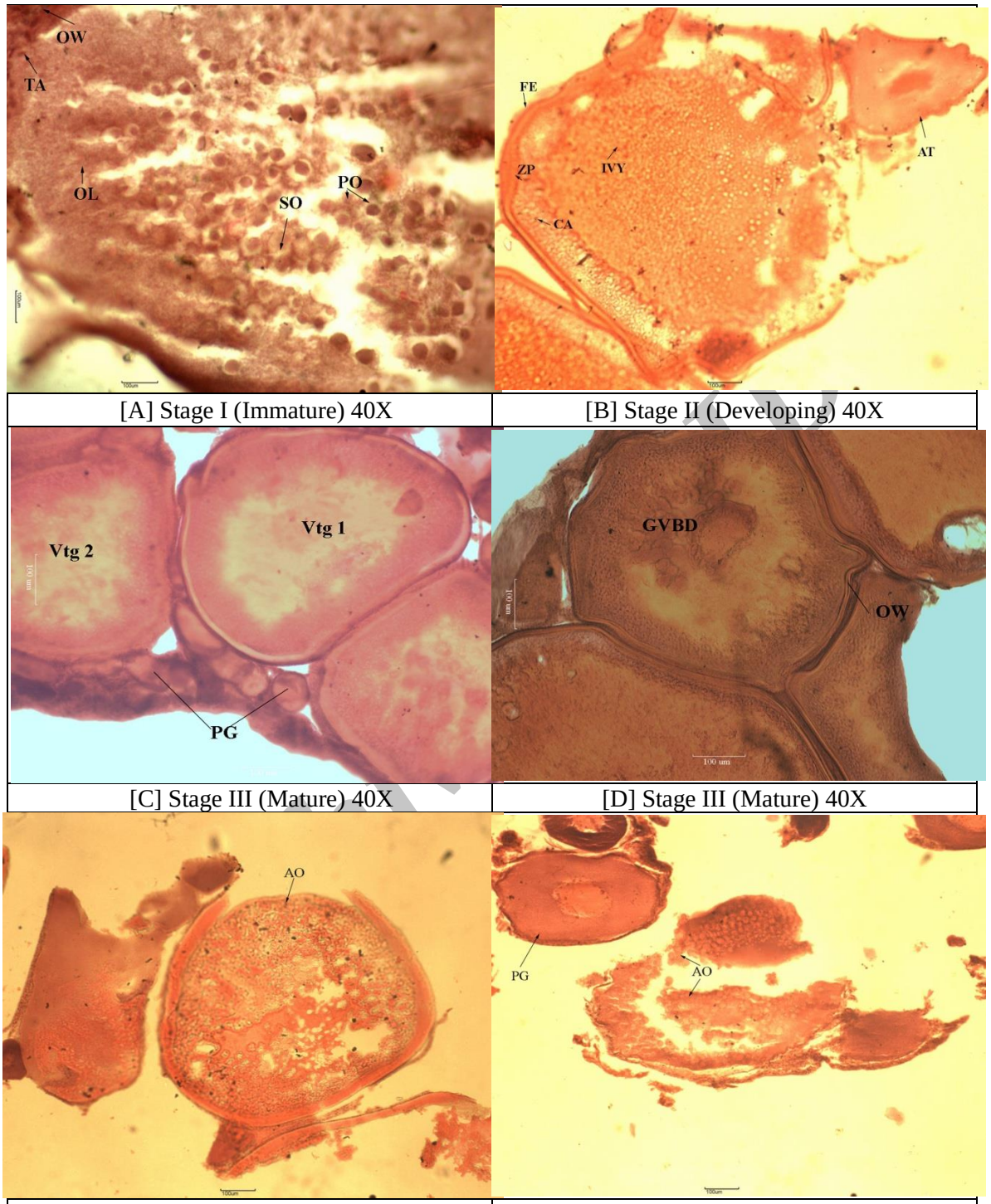
Stage I (Immature). The size of the testes and the ovaries were very small and they were transparent greyish in colour. In the ovaries, oogonia and primary growth oocytes were present. Ovarian walls were thin and spaces between oocytes were very thin. No atresia presents (Fig. 2A). In the testes, numerous seminiferous tubules or lobules were present. These were surrounded by thick testicular wall. Only spermatogonia were present. Lumen were absent in the lobule (Fig. 3A).

Stage II (Developing). The ovaries and testes start developing, but not ready to spawn. Histologically, the ovary contains primary growth, cortical alveolar, primary vitellogenic and secondary vitellogenic oocytes. Some atresia also presents (Fig. 2B). In the testes, spermatocytes were present along the lobules. Primary spermatocyte, secondary spermatocyte, spermatid and spermatozoa were found to be present (Fig. 3B).

Stage III (Mature). The fishes were developmentally and physiologically able to spawn. In histological section of ovaries, nucleus began to leave central position and migrate towards periphery. Oocyte size remained relatively stable (Fig. 2C, 2D). In the testes, spermatozoa were observed in lumen of lobules. Spermatocytes were present throughout the testis and spermatogenesis was active. The testes were seen packed with mature spermatids ready to be released (Fig. 3C, 3D).

Stage IV (Regressing). The ovaries were flaccid and blood vessels prominent. Histological section showed presence of atresia and postovulatory follicle complex. Some cortical alveolar and vitellogenic oocytes were present (Fig. 2E). Testes were small and flaccid. Very less sperm were seen in the lumen of the lobules. Lumen was seen empty and the sperm had been released (Fig. 3E).

Stage V (Regenerating). The fishes were reproductively inactive. Ovaries and testes were empty. Histologically, ovaries composed of postovulatory follicles, immature oocytes and mature eggs left unspawned. It contained some atretic oocytes characterized by the ooplasm and yolk degradation (Fig. 2F). The histological section of testes showed depletion of the residual spermatocytes and gradual degeneration of lobules (Fig. 3F).

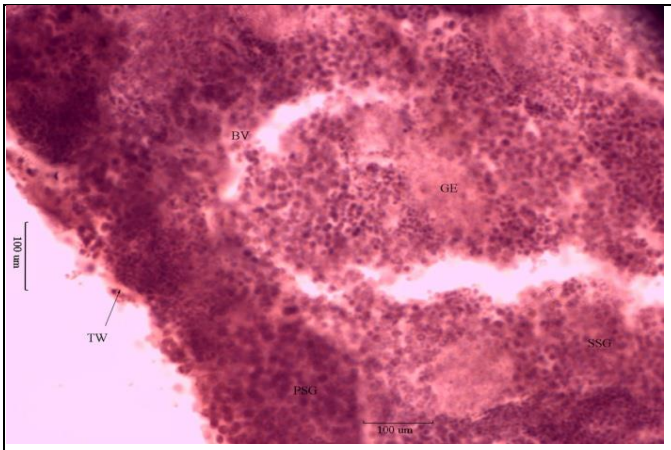


[A] Stage I (Immature) 40X | [B] Stage II (Developing) 40X

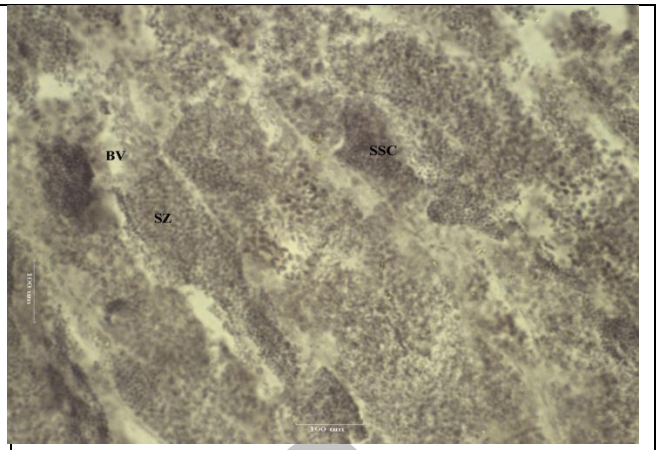
[C] Stage III (Mature) 40X | [D] Stage III (Mature) 40X

[E] Stage IV (Regressing) 40X | [F] Stage V (Regenerating) 40X

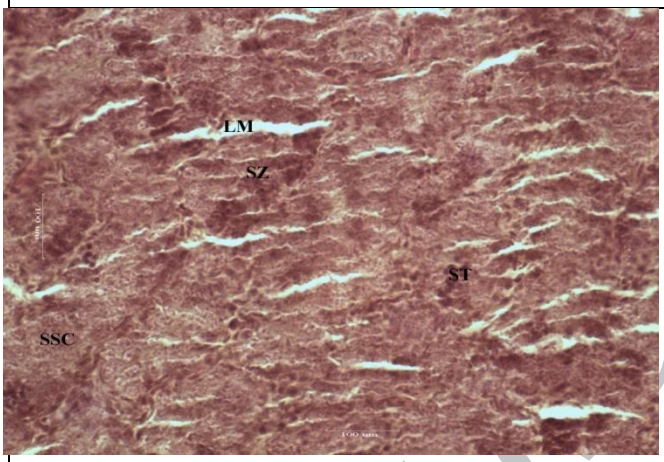
Fig. 2. Histological stages of ovary of *Neolissochilus hexagonolepis*. PO: Primary oocytes, SO: Secondary oocytes, OW: Ovarian wall, OL: Ovarian lamella, TA: Tunica albuginea, IVY: Intra-vesicular yolk, FE: Follicular epithelium, ZP; Zona pellucid, CA: Cortical alveolar, AT: Atresia, PG: Primary growth oocyte, Vtg 1: Primary vitellogenic oocyte, Vtg 2:Secondary vitellogenic oocyte, GVBD: Germinal vesicle breakdown, AO: Atresia oocytes.



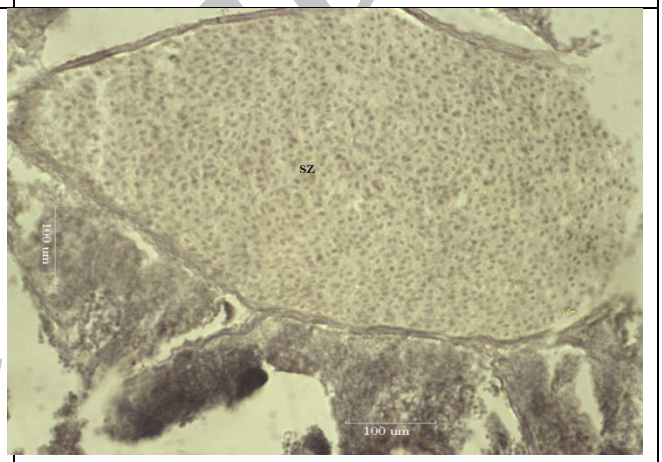
[A] Stage I (Immature) 40X



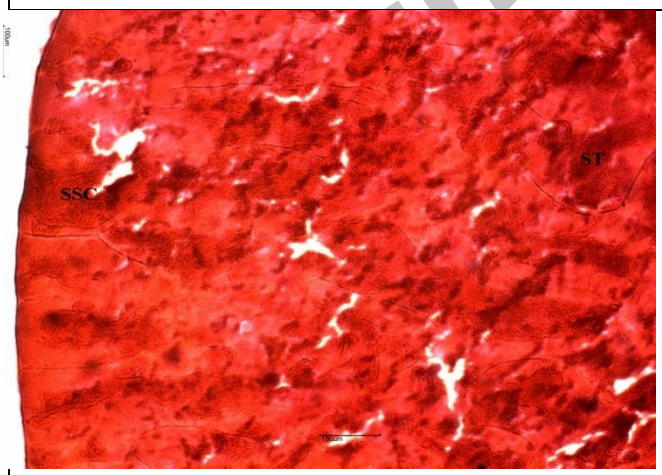
[B] Stage II (Developing) 40X



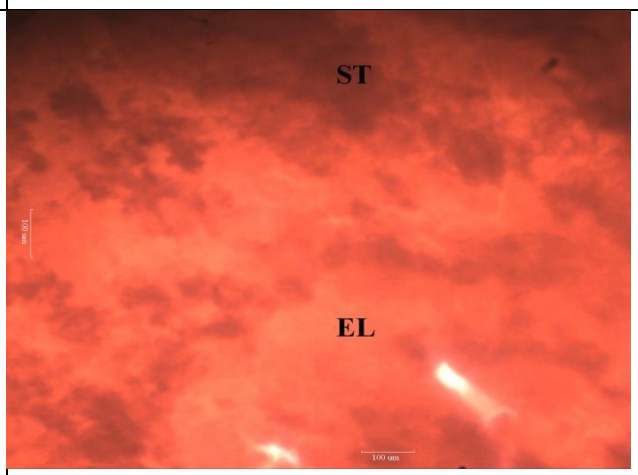
[C] Stage III (Mature) 40X



[D] Stage III (Mature) 40X



[E] Stage IV (Regressing) 40X



[F] Stage V (Regenerating) 40X

Fig. 3. Histological stages of testes of *Neolissochilus hexagonolepis***.** BV: Blood vessel, GE: Germinal epithelium, TW: Testicular wall, PSG: Primary spermatogonia, SSG: Secondary spermatogonia, SSC: Secondary spermatocyte, SZ: Spermatozoa, LM: Lamellae, ST: Spermatids, EL: Empty lumen.

Discussion and Conclusions

Chocolate Mahseer, *Neolissochilus hexagonolepis* is available in most of the lotic water bodies of hilly areas of Meghalaya. However, it has been observed during the study that the abundance of females was lesser than males. This might be due to the differential occurrence of the males and females in various water columns. The females live in deeper areas which make them less available (Dopeikar et al. 2015). The gonado-somatic index (GSI) of the fish has been studied to verify the breeding season of the fish under the climatic condition of the state. The GSI value was found to be highest during the month of June and July for both male and female species (Fig.1) which is evident that the natural peak breeding season of *N. hexagonolepis* would be during June and July. However, it has been reported that breeding periods of the fish extends up to September in North-Eastern Regions of India (Marwein 2000)

The results of the histological study of gonad developments in *N. hexagonolepis* are basically similar to other teleost. In this study, the oocyte development of *N. hexagonolepis* was divided into five stages. Histology of ovarian tissue is commonly used to understand the size, age of maturity, daily and seasonal pattern of spawning, spawning location and fecundity. It is well known that environmental factors can have pronounced effects on the timing of gametogenesis, vitellogenesis and maturation in fishes (Takashima et al. 2008; Miranda et al. 2009).

In the present study, morphological changes in developing oocytes was observed in different stages of maturity including changes in the size of the gonad and oocyte which is in agreement with other studies (Grau et al. 2009; Lubzens et al. 2010; Mohamed 2010). A gradual morphological change in the nucleolus and follicular epithelium was also observed in different stages of oocyte development. These changes were similar to the development which has been reported for other fishes e.g. *Serrasalmuss pilopleura* and *Barbus barbus* (Quagio-Grassiotto & Guimares 2003; Thiry & Poncin 2005). Increasing the size of oocytes is due to the development of the yolk in maturing oocyte, which has also been noted in other fish species (Hartting & Kunkel 1999; Koc (Yon) et al. 2008).

According to Arockiaraj et al. (2004) and Nejedli et al. (2004), the oogenesis is manifested by a series of changes in the oocytes. The present study observed oocyte growth as the main phenomenon during ovarian development. Oogonia grow into immature, previtellogenic oocytes that show signs of vitelline vacuoles in the periphery of the cytoplasm. Then it develops into cortical alveoli, yolk accumulation was completed. Thereafter, the final maturation process is initiated by migration of the nucleus (De Vlaming 1983; Redding & Patino 1993). The growth and fragmentation of nucleoli in previtellogenic oocytes are commonly reported in most of the studied species by Maris & Scheer (2001).

The observation showed that, spermatogenesis was divided according to size difference and occurrence of new structures which is recognizable during the different maturation stages. These cells are spermatogonia, primary spermatocyte, secondary spermatocyte, spermatid and spermatozoa.

The maturity stages of testes were divided into five stages (Fig. 3). The testis is usually a whitish organ that comprises of two lobes separated by a septum or independent by themselves. The testis has the germinal epithelium and the interstitial cells which are separated from each other by a basement membrane (Lo Nostro et al. 2003).

Development of sperm in fish passes through multiple stage of growth and maturation as seen in other fishes like sevenband grouper etc. (Shein et al. 2004). Those in their regenerating stage are sexually mature but reproductively inactive (Brown–

Peterson et al. 2002). According to Bucholz et al. (1964), mature spermatozoa are seen aggregated in the lumen of testicular lobules without any regular arrangement, they begin to aggregate in the lumina even at the early stage when many of the germ cells still remain immature and do not reveal any connection with the intralobular somatic cell elements or sertoli cells.

The results of the present study revealed a clear cut five maturity stage in both male and female gonads of *Neolissochilus hexagonolepis*. It is observed that the male and female fish show similar kind of maturity stage corresponding to five indicating the maturity and spent phase of male and female fish is in the same month and season. It has been noted that the rhythm of gonadal developments depend on various factors such as temperature, photoperiod, along with social and behavioral factors such as visual, olfactory and auditory stimuli (Esmaeili et al. 2010; Asadollah et al. 2011; Keivany et al. 2012; Abaszadeh et al. 2013; Dopeikar et al. 2015), which could also affect the developmental stages of *N. hexagonolepis*. It could be concluded that the development of gonads at ultra-structural level in *N. hexagonolepis* follows similar developmental process as that of many cyprinid fishes.

Acknowledgements

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مقاله پژوهشی

بررسی بافت‌شناسی اندام تناسلی *Neolissochilus hexagonolepis* (McClelland, 1839) (ماهیان استخوانی عالی: کپورماهیان) از مگهالایای هند

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چکیده: مهاسیر شکلاتی (*Neolissochilus hexagonolepis*) ماهی مهم خوراکی و ورزشی شمال شرق کشور، خصوصا مناطق مرتفع مگهالایاست. زیست‌شناسی تولیدمثل گونه در شرایط آب و هوایی مگهالایا برای درک نمو اندام‌های تناسلی صورت گرفته است. ساختار اندام تناسلی و مراحل نموی سلول‌های زاینده *N. hexagonolepis* برای هر دو جنس توصیف شد. پنج مرحله نموی بر اساس ویژگی‌های بافت‌شناسی بیضه و تخمدان توصیف گردید. نحوه نمو و وجود فولیکول‌های پست‌اوولاسیونی در تخمدان‌ها که همچنان حاوی اووسیت‌های در حال زرده‌سازی هستند، نشان می‌دهد که این ماهی در طی زمان طولانی تخمریزی می‌کند.

کلیدواژه‌ها: مهاسیر شکلاتی، رسیدگی جنسی، تخمریزی، شاخص گنادی