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Research Article

The Sex-Ratio, Developmental Stages and Cycle of Gonad Maturation of *Neolissochilus hexagonolepis* in the Mid-Reaches of the River Tamor

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ABSTRACT

A population of *Neolissochilus hexagonolepis* captured from the mid-reaches of the river Tamor, eastern Nepal were investigated between December, 2014 and November, 2016. Fish samples were collected in the second half of every month and were evaluated for the Gonado-Somatic Index (GSI), developmental stages and cycle of gonad maturation. The population structure showed asymmetric sexual pattern with females dominating the number of males for most part of the study period. Monthly mean GSI varied from 0.17 ± 0.05% to 3.27 ± 1.68% and from 0.13± 0.07% to 10.57 ± 4.56% for male and female fish, respectively. The gonads were observed to pass through six stages of maturation *viz.* immature, maturing virgin and recovering spent, ripening, mature, spawning and spent. The maturation cycle showed an extended breeding season of the fish from May to November with peak breeding activity in July. The information gathered during the present study could be incorporated in management plans for making sustainable conservational efforts for the near-threatened *N. hexagonolepis* population in the river Tamor.
Keywords: *Neolissochilus hexagonolepis*; River tamor; Gonado-somatic index; Developmental stages; Maturation cycle

INTRODUCTION

River Tamor is famous for its unmatched and refreshing beauty with snow-fed crystal clear water. This river serves as the home as well as the breeding ground for many species of fishes. Fishing practices have been taking place in this river since the time immemorial and the local fishermen engage in fishing activities for sustenance of life [1].

The assessment of Gonado-Somatic Index (GSI) finds a great application in fish culture and its management, particularly by predicting the periodicity and timing of the breeding time. Towers suggested that Gonado-Somatic Index (GSI) of a fish increases with maturation of the fish, being maximum during the peak period of maturity and declining abruptly after spawning. Several workers have reported on the GSI of hill stream fishes among which Mahapatra and Vinod, Arjamand et al., Verma, Wagle and Jan and Ahmed have reported on the GSI of *N. hexagonolepis*, *Labeo dyocheilus*, *Tor putitora*, *S. richardsonii* and *S. plagiostomus* respectively.

Noble and Jones opined that the knowledge about different stages of gonadal maturatiovn of fish provides important information necessary to prohibit fishing during the restoration of the fish stock.

Jyrwa and Bhuyan highlighted the importance of the detailed study of the gonadal cycle and the ultra-structural changes of gonads in fish before attempting its culture and conservation through artificial means [2].

N. hexagonolepis has the conservation status of near threatened according to IUCN. The urgent need for the present study was felt in view of the lack in the detailed scientific information on the reproductive biology of this species from its natural habitat.

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MATERIALS AND METHODS

The samples were collected for two years, from December 2014 till November 2016 from the mid-reaches of the river Tamor, eastern Nepal. The study area lies between the latitude and longitude coordinates of 26°56.700'N, 26°55.653'N and 87° 23.097'E, 87°17.653'E (Figure 1). Fish samples were captured in the second half of every month using hooks, cast net, gill net and locally constructed traps. Total Length (TL) was measured using a graduated ruler while Total body Weight (TW) and Gonad Weight (GW) were measured using digital balance with the precision of 0.01 gm.

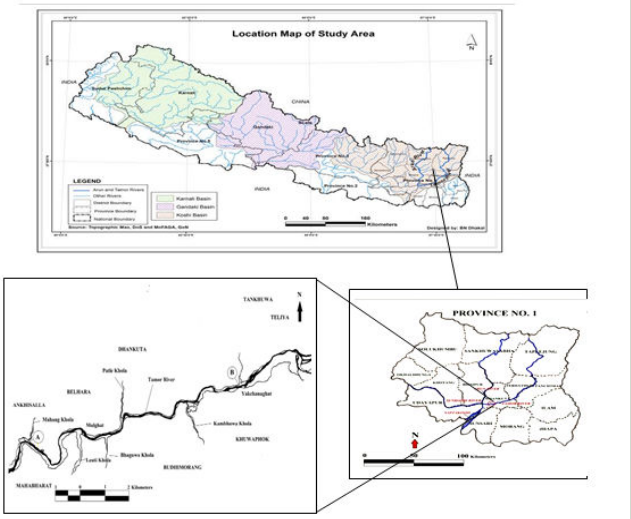

Figure 1: Location map of study area.

Sex identification, sex ratio and Gonado-Somatic Index (GSI)

Sex identification was done through examination of gonads after dissection, which was further confirmed through histology. Monthly male to female sex-ratio was computed throughout the study period.

Gonado-Somatic Index (GSI) was calculated using the formula

$$GSI = \frac{\text{Gonad weight}}{\text{Total body weight}} \times 100$$

Stages and maturation cycle of gonads

The gonads were extracted in situ through longitudinal incision made along the ventral line of the body. They were then weighed and their morphological features noted. The gonads were washed in saline water and fixed in Bouin's fluid for 24 hours before transferring them into alcohol (70%) for preservation. The alcohol-preserved gonads were hydrated and dehydrated in alcohol series and embedded in paraffin wax [3]. They were then sectioned at 6 µ with a rotary microtome and the sections were double stained in hematoxylin and eosin before mounting in DPX. Finally, the sections were observed under a binocular microscope.

Gonad maturity stages were determined by examination of the histomorphological features of gonads according to the maturity scale modified after Brown-Peterson et al. The maturation cycle of gonads was investigated by calculating the frequency of a specific stage of gonad as percentage on monthly basis.

Statistical analysis

Descriptive statistics was used to outline the basic features of the data obtained during the study by giving simple summaries like the mean and standard deviation. Spearman rank correlation was performed to investigate the relationship between the weight and length with the maturity stages of either sex of the fish.

RESULTS AND DISCUSSION

Altogether 198 fishes were sampled during the study period. Females dominated the number of males for most part of the study with the overall male to female sex ratio as 1:1.22. The asynchronous spawning migration and the partial segregation by sex during feeding caused the asymmetric sexual pattern of *N. hexagonolepis* in the river [4].

Gonado-Somatic Index (GSI)

The monthly mean GSI values varied from 0.17 ± 0.05% to 3.27 ± 1.68% and from 0.13 ± 0.07% to 10.57 ± 4.56% for male and female fish, respectively (Figure 2).

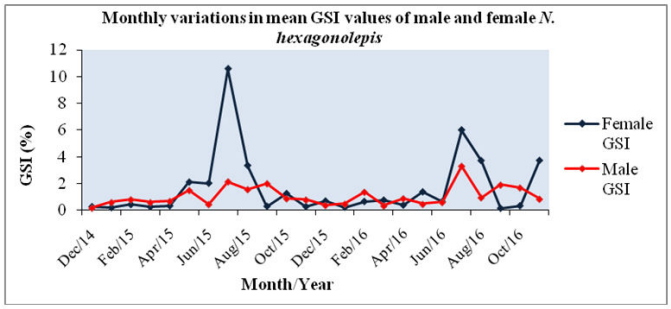

Figure 2: Monthly variations in mean GSI values of *N. hexagonolepis*.

The peak breeding season of *N. hexagonolepis* was found to occur in July as indicated by the higher GSI values for both the sexes in this month. Arruda, et al. suggested that the timing of reproduction and spawning can be identified from changes in the GSI and that the breeding season of fish corresponds to that during which the values of GSI for both male and female spike to the peak [5].

The single annual peak of GSI value for either sex gave a clear indication that the species is an annual breeder. Similar breeding behaviours were also reported in *Labeo rohita*, *L. dyocheilus* and *Tor putitora*.

We found that the mean GSI values of the fish declining abruptly after the peak breeding season and attributed this to the tremendous decrease in weight of the gonads after the

spawning act. Similar trends in GSI values were also observed by Verma and Joshi, et al. in *Labeo dyocheilus* and *Schizothorax richardsonii*, respectively.

Developmental stages and maturation cycle of gonads

The testes and ovaries of *N. hexagonolepis* were observed at six 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) (Table 1).

Table 1: Developmental stages of gonads of N. hexagonolepis in the mid-reaches of river Tamor.

Gonad maturity stages	Gonad developmental status	Description of gonad	
		Male	Female
I	Immature	Testes slender, thread-like, dirty white without vascular supply. Cysts of spermatogenic cells visible	Ovaries thin, slender, thread-like, dirty white in colour and translucent. Oocytes at stages I and II visible
II	Maturing virgin and recovering spent	Testes slender, whitish with vascular supply. Spermatogonia seemed to be dividing giving rise to spermatocytes	Ovaries still slender but slightly larger than at stage I. Oocytes at stages III and IV along with a few oocytes at stages I and II visible in ovigerous lamellae
III	Ripening	Increased in weight and volume occupying about 2/3 of the body cavity. All the stages of spermatogenesis *viz.* spermatocytes, spermatids and spermatozoa observed	Ovaries whitish-yellowish in colour with granular appearance. Ovigerous lamellae filled with large number of oocytes at stages IV and V along with a few oocytes at stage VI
IV	Mature	Testes large, turgid occupying almost the entire body cavity. Testicular wall thin and seminiferous lobules packed with spermatozoa. Few spermatids also visible	Ovaries large and deep yellowish in colour occupying almost the entire abdominal cavity. Large number of stage VII oocytes and some ripe eggs visible
V	Spawning	Testes pinkish white, turgid and occupied the entire body cavity. Milt was seen to be oozing out. Seminiferous lobules packed with spermatozoa with some empty lobules	Ovaries large and distended, occupying the entire abdominal cavity. Large number of jelly like yellowish translucent eggs present in the ovaries. Spawning was imminent
VI	Spent	Testes dull-white and appeared flaccid. Seminiferous lobules seen collapsing and empty	Ovaries flaccid, shrunken. Oocytes at stage VII along with oocytes at stages I and II visible in the ovigerous lamellae

Swar investigated the maturity stages of ovaries and testes of *N. hexagonolepis* based on their external features and established seven maturity stages for both the gonads. Jyrwa and Bhuyan also carried out the histological studies of gonads of the fish from Meghalaya, India and established their five developmental stages [6].

Intense spermatogenesis was observed during ripening stage of the testes where we examined all the stages of spermatogenesis *viz.* spermatocytes, spermatids and spermatozoa within the lobules (plate 1a).

Histologically, the seminiferous lobules of matured testes were seen to be packed with spermatozoa with some spermatids (plate 1b). Spent testes were seen to be collapsing and empty and contained only a small amount of unexpelled or residual sperms (plate 1c) (Figure 3).

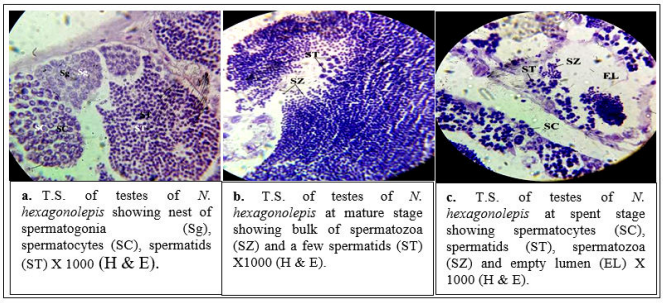

Figure 3: Spermatocytes, spermatids and spermatozoa within the lobules.

We observed oocytes at various stages of development in the ovigerous lamellae of the ovaries. Oocyte I, referred to as the chromatin nucleolus stage, contained a prominent nucleus with 3 to 4 nucleoli (plate 2a). The oocytes at II stage were recognized as the early and late perinucleolus stage on the basis of the distribution of the nucleoli within the nucleus [7]. Nucleoli increased in number as the oocytes matured and got distributed adjacent to the periphery of nuclear membrane (plate 2b and plate 2c). Oocyte III, conventionally termed as the early yolk vesicle stage, was characterized by the appearance of a large number of small, clear vacuoles in the cortical area of cytoplasm (plate 2d). Oocyte IV, referred to as the late yolk vesicle stage, showed further increase in size and number of the yolk vesicles which distributed randomly in the ooplasm (plate 2e) [8]. Yolk globules were seen to appear among the vesicles in the ooplasm of the oocyte at stage V. The oocyte at this stage is termed as the early yolk stage (plate 2f). Oocyte VI (late yolk stage) was characterized by the increase in number and size of yolk globules and the yolk vesicles became pushed towards the peripheral region of the oocyte (plate 2g). Oocyte VII showed a heavy deposition of yolk globules in the cytoplasm (plate 2h).

Sharma et al. also recognized the stages of oocytes as chromatin nucleolar stage, perinucleolar stage, early yolk vesicle stage, late yolk vesicle stage, early yolk stage, late yolk stage and ripe egg stage in garra gotyla gotyla.

The flaccid appearance of testes and ovaries of the fish during the post spawning season was attributed to the expulsion of spermatozoa and ripe eggs during the spawning season [9].

Significant positive correlations were observed between the various morphometric parameters and maturity stages of gonads for both the sexes of *N. hexagonolepis* (Table 2). These revealed that the matured fishes were heavier and larger in size and the gonads at peak maturity become heavier as they become fully packed with sperms or ripe eggs (Figure 4).

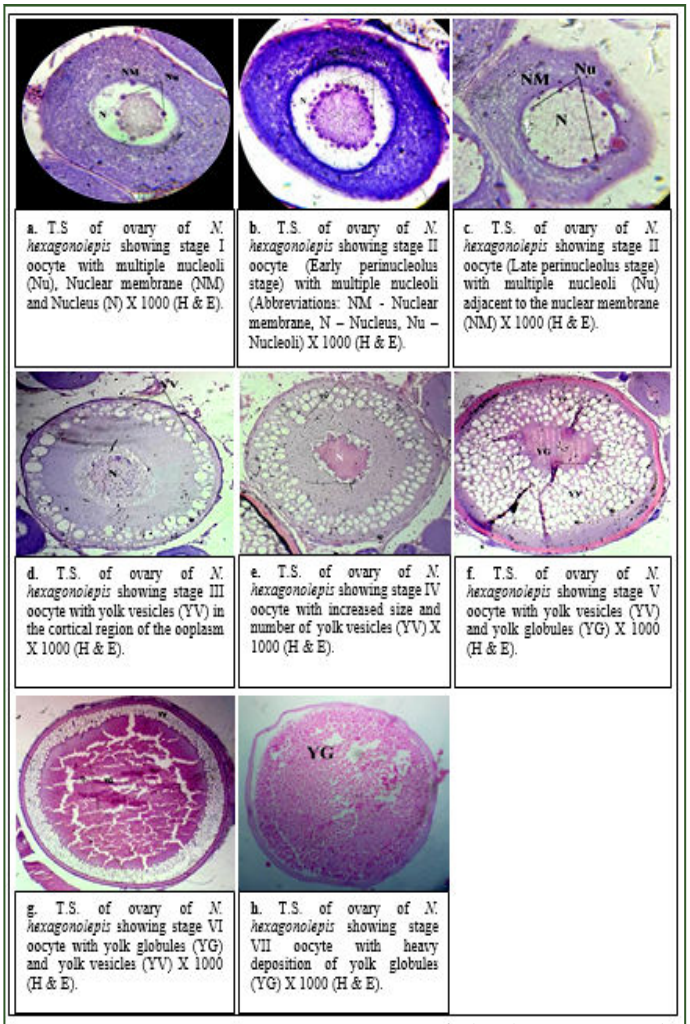

Figure 4: Oocytes at various stages of development in the ovigerous lamellae of the ovaries.

Table 2: Spearman rank correlations and significance values for morphometric parameters with stages of gonad of female *N. hexagonolepis*.

Variables			Stage of gonad	Male			Female		
				TW (gm)	TL (cm)	GW (gm)	TW (gm)	TL (cm)	GW (gm)
Spearman's rho	Maturity stage of gonad	Correlation Coefficient	1	0.708**	0.685**	0.901**	0.708**	0.685**	0.901**
		Sig. (2-tailed)	0	0	0	0	0	0	0

We found that the various stages of gonads of *N. hexagonolepis* showed monthly occurrence with varying frequencies. Stage II testes showed the highest occurrence among the months with their frequencies ranging from 11.11% in May to 71.43% in March.

Matured and spawning testes showed their occurrence from May onward. Similarly, spent testes occurred in August, September and October, constituting 50% of the total catch in the month of October (Figure 5).

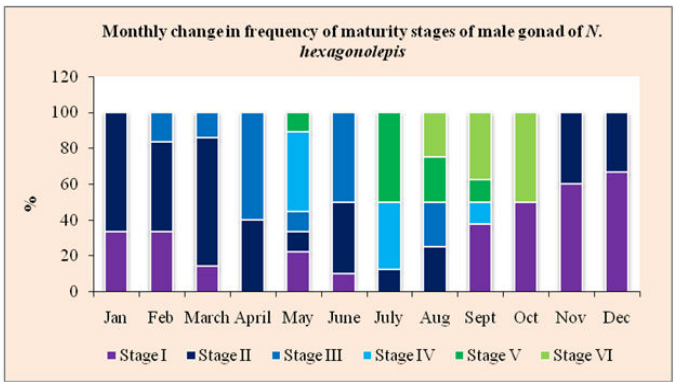

Figure 5: Monthly change in frequency of maturity stages of gonad of male *N. hexagonolepis*.

Stage II ovaries occurred throughout the year with their frequency ranging from 7.69% in July to 57.14% September [10]. Next in frequency were the ovaries at stage I constituting 20% in February to 69.23% of the total monthly catch in January. Ovaries at the mature and spawning stages were encountered till November. Spent ovaries (stage VI) occurred in November and December. We did not examine the spent ovaries from January onward (Figure 6).

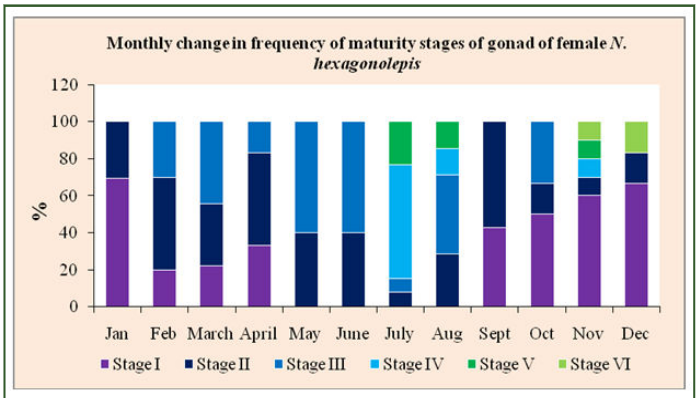

Figure 6: Monthly change in frequency of maturity stages of gonad of female *N. hexagonolepis*.

The capture of matured and spawning male fishes since May indicated the commencement of breeding season of the fish from this month [11]. Also, the capture of matured and spawning female fishes till November with the total absence of spent fishes from January onward gave a clear indication of the extended breeding season of *N. hexagonolepis* lasting from May till November. The extended breeding period of *N. hexagonolepis* has also been reported by Jhingran, Swar and Arunachalam. The extended breeding period of *N. hexagonolepis* could be considered as a part of the strategies developed by the species to withstand the environmental pressures.

Maximum number of matured and spawning fishes was captured during July. The highest GSI values for both the sexes were also assessed during the same month. Therefore, we concluded that the peak breeding season of *N. hexagonolepis* coincides the month of July [12].

CONCLUSION

The study indicated that *N. hexagonolepis* is an annual breeder with an extended breeding period from May till November showing peak breeding activity in July. The information gathered during the present study could be incorporated in management plans for making sustainable conservational efforts of the species in the wild. The finding of the study may also act as a baseline for understanding the reproductive biology as a pre-requisite for commercial domestication of the fish.

CONFLICT OF INTEREST

Authors declared no conflict of interest.

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