FECUNDITY AND GONADAL DEVELOPMENT OF COPPER MAHSEER, *NEOLISSOCHILUS HEXAGONOLEPIS* (McClelland, 1839) FROM TAMOR RIVER, NEPAL

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IN ZOOLOGY

BY

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RECOMMENDATION

This is to recommend tha**t Suren Subba** has carried out research entitled **''Fecundity and Gonadal Development of Copper Mahseer, *Neolissochilus hexagonolepis* (McClelland, 1839) from Tamor River, Nepal''** for the award of Doctor of Philosophy (Ph.D.) in **Zoology** under our supervision. To our knowledge, this work has not been submitted for any other degree.

He has fulfilled all the requirements laid down by the Institute of Science and Technology (IOST), Tribhuvan University, Kirtipur for the submission of the thesis for the award of Ph.D. degree.

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LETTER OF APPROVAL

On the recommendation of Assoc. Prof. Dr. **Vinod Kumar Mahaseth** / Assoc. Prof. Dr. **Bharat Raj Subba**, this Ph.D. thesis submitted by **Suren Subba**, entitled **''Fecundity and Gonadal Development of Copper Mahseer, *Neolissochilus hexagonolepis* (McClelland, 1839) from Tamor River, Nepal''** is forwarded by Central Department Research Committee (CDRC) to the Dean, IOST, T.U..

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DECLARATION

Thesis entitled **''Fecundity and Gonadal Development of Copper Mahseer, *Neolissochilus hexagonolepis* (McClelland, 1839) from Tamor River, Nepal''** which is being submitted to the Central Department of Zoology, Institute of Science and Technology (IOST), Tribhuvan University, Nepal for the award of the degree of Doctor of Philosophy (Ph.D.), is a research work carried out by me under the supervision of Associate Prof. Dr. Vinod Kumar Mahaseth, Mahendra Morang Adarsh Multiple Campus (MMAMC), Biratnagar, Tribhuvan University and co-supervised by Associate Prof. Dr. Bharat Raj Subba, Post Graduate Campus, Biratnagar. This research is original and has not been submitted earlier in part or full in this or any other form to any university or institute, here or elsewhere, for the award of any degree.

Suren Subba

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ABSTRACT

The present work was intended to assess the fecundity and gonadal development of *Neolissochilus hexagonolepis* from Tamor River, Nepal. The study was conducted for the duration of two years, from December 2014 to November 2016. Monthly samples of *N. hexagonolepis* were procured from the river. Altogether one hundred ninety eight samples were examined. The length-weight relationship (LWR) was found to be following the model of Le Cren (1951). The value of the regression coefficient (b) for all the LWR was close to 3, which indicated that the fish showed an isometric growth pattern. The general well being of the fish from the river was found to be satisfactory. The assessment of fecundity unveiled *N. hexagonolepis* as a low fecund fish. The number of eggs in the ovaries of *N. hexagonolepis* increased proportionately with increasing length, body weight and gonad weight of the fish. The enumeration of gonado-somatic index (GSI) showed the species as an annual breeder with peak spawning activity from July to August. The egg size estimation revealed the species as a multiple spawner. Male individuals dominated the smaller size classes and female individuals dominated the larger size classes. The testes and ovaries were observed at six stages of maturation viz. Immature, maturing virgin, ripening, mature, spawning and spent. The developing oocytes within the ovigerous lamellae of the ovaries were observed at the chromatin-nucleolus stage, early and late peri-nucleolus stages, early and late yolk vesicle stages and early and late yolk stages. The species showed an extended breeding period from May to November. Male and female individuals attained the first sexual maturity at size 25.5 cm and 32.9 cm, respectively. Environmental variables played an essential role in governing the reproductive biology of the fish. Temperature contributed positively to the development of the fish's gonads during its breeding season.

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LIST OF SYMBOLS

°C	: Degree Celsius
χ^2	: Chi-square
%	: Percentage
>	: More than
<	: Less than
µ	: Micron
≤	: Less than or equal to
±	: Plus-minus
R	: Pearson correlation coefficient
R^2	: Coefficient of determination
ρ	: Spearman's rho
L_{50}	: Length at 50% maturity

LIST OF ACRONYMS AND ABBREVIATIONS

a	: Regression intercept
av.	: Average
AF	: Atretic follicle
ANOVA	: Analysis of variance
APHA	: American Public Health Association
AT	: Atmospheric temperature
Atm.	: Atmospheric
b	: Regression slope
CA	: Cortical alveoli
CBS	: Central Bureau of Statistics
CDZ	: Central Department of Zoology
CEDA	: Centre for Economic Development and Administration
CL	: Confidence limit
CT	: Connective tissue
cv	: Coefficient of variation
df	: Degrees of freedom
DHM	: Department of Hydrology and Meteorology
DO	: Dissolved oxygen
DPX	: Distyrene Plasticizer Xylene
E	: Eosin
EBT	: Eriochrome black T
EDTA	: Ethylenediamine tetraacetic acid
EIA	: Environmental Impact Assessment
EL	: Empty lumen
ES	: Egg size
F	: Fecundity
Fig.	: Figure
FL	: Forkal length
g	: Gram
GPS	: Global Positioning System
GSI	: Gonado-somatic Index
GSIF	: Gonado-somatic index of female

GSIM	: Gonado-somatic index of male
GW	: Gonad weight
GWF	: Gonad weight of female
GWM	: Gonad weight of male
H	: Hematoxylin
HOD	: Head of the department
IUCN	: International Union for Conservation of Nature and Natural resources
K	: Fulton's condition factor
KMO	: Kaiser-Meyer-Oklin test
Kn	: Relative condition factor
L	: Observed length
LWR	: Length-weight relationship
max	: Maximum
mg/l	: Milligram per litre
min	: Minimum
ml	: Millilitre
mm	: Millimetre
m^3/s	: Cubic metre per second
MS	: Mean squares
N	: Nucleus
NARC	: Nepal Agricultural Research Council
NAST	: Nepal Academy of Science and technology
NM	: Nuclear membrane
Nu	: Nucleoli
NT	: Near Threatened
OI	: Oocyte I
OII	: Oocyte II
OIII	: Oocyte III
OIV	: Oocyte IV
OL	: Ovigerous lamellae
OvL	: Ovarian Lumen
PCA	: Principal Component Analysis
POF	: Post-ovulatory follicle

ppm	: Parts per million
SC	: Spermatocytes
Sg	: Spermatogonia
SL	: Standard length
SRY	: Sex-determining region Y
SS	: Sum of squares
ST	: Spermatids
SZ	: Spermatozoa
TA	: Total alkalinity
Temp.	: Temperature
Th	: Theca
TH	: Total hardness
TL	: Total length
T. S.	: Transverse section
TW	: Total weight
vs.	: Versus
w	: Calculated weight of fish
W	: Observed weight of fish
YG	: Yolk globule
YV	: Yolk vesicle
ZG	: Zona granulosa
Zr	: Zona radiata

CHAPTER 1

1. INTRODUCTION

1.1 General Introduction

Federal Democratic Republic of Nepal (26°20'-30°10' N and 80°15'-88°19' E) is a small yet a beautiful country sandwiched between two more prominent countries, China and India. It is the largest sovereign Himalayan state with a total area of 147,516 km^2 (56,956 square miles) and shares standard frontiers with the People's Republic of China in the north and with the Republic of India in the east, west, and south (CBS, 2015). Being roughly trapezoidal, this country extends approximately 885 km from east to west and 193 km from north to south. Zoo-geographically, this country falls in between Palaearctic in the north and oriental in the south boasting the faunal diversity of both the regions. It is divided into three regions, namely, the Terai, the Mid Hills, and the Himalayan. These ecological belts run east-west and are vertically decussated by the country's major river systems. The Terai region lies between the elevations of 130m and 500m, the Hills up to 4,000m and The Himalayan region above the tree line (>4,600m). Hills and the mountains constitute about 83 % of the total area of the country while the remaining 17 % is occupied by the Terai. The snow-capped mountains and the Himalayas in the north exert intense domination on the overall climate of the country and consequently the distribution of flora and fauna. According to the altitude, the country may broadly be divided into three climate zones: subtropical in the Terai, temperate in the Hills and alpine in the Himalayan region.

Nepal is affluent in freshwater resources, comprising of snow-fed rivers, streams, lakes, and pools. There are more than 6000 rivers in the country (Shrestha, 1983). Isolated from seas and oceans, this country lacks fishes and other organisms that specifically populate the marine environment. Nonetheless, the presence of unique topography, climate and geography provides an unprecedented opportunity for diverse flora and fauna to flourish here.

A vast array of fishes comprising more than 232 species inhabit the freshwater of this country among which many have commercial, recreational, aesthetic and

educational value (Shrestha, 2008). A big chunk of the total population of the country depends on fish and fish related activities, directly or indirectly, for sustaining their lives. Fishes of this country have developed various adaptive features to cope with the high gradient, fast-flowing, tumultuous mountain torrents winding through precipitous gorges.

Researches in the field of fish and fisheries of the country have been conducted for quite a long time now. However, there still exist several facets of the field which remain practically uncharted. The aquatic realm of this country has always been the center of attraction for researchers and academicians alike.

Fishes of this country share many genera with those of South East Asia and they consist predominantly of carp, barbels, and minnows (Shrestha, 2008). Copper Mahseer or Katle (*Neolissochilus hexagonolepis*), point-nosed snow trout or Chuche Asala (*Schizothorax progastus*), blunt-nosed snow trout or Buche Asala (*Schizothorax richardsonii*), spotted snow trout (*Schizothorax plagiostomus*), Sahar or Mahseer (*Tor putitora*), deep-bodied Mahseer (*Tor tor*), etc. are considered economically important indigenous species that are found in the torrential mountain rivers of the country. Besides, fishes like a freshwater shark or gounch (*Begarius begarius*), Jalkapoor (*Clupisoma garuwa*), freshwater eel (*Anguilla bengalensis*) etc. are also equally important. Other indigenous species like Karange (*Puntius chilinoides*), Gerdi (*Labeo dero*), Fageta (*Barillius spp*) are considered delicious fishes in the country. Several exotic species like Rainbow trout (*Onchorhynkus mykiss*), Grass carp (*Ctenopharyngodon idella*), bighead carp (*Aristichthys nobilis*), Silver carp (*Hypophthalmichthys molitrix*), etc. have successfully been introduced here from various countries. Further research on the behaviour, propagation, population dynamics and biology of several species of indigenous species is in desperate need in this country.

A substantial percentage of the total population of the country is directly or indirectly involved in fishing and aquaculture production and it can be assumed that the percentage is ever-expanding. It needs no exaggeration to say that the fisheries sector has always been playing an essential role in the economic development of the country.

The conservation and management of the country's freshwater resources and the development of inland fishery demand meticulous research, expertise and proper management strategies. Harmful fishing practices like dynamiting, poisoning, electrofishing and wanton killing of fries and fingerlings have resulted in the decline of several important fish species. Development of Nepalese freshwater fisheries and fish resources protection has always been a kind of thorny task with several confronting issues. Nonetheless, by creating needed harmony among ecologists, fishery biologists, and engineers, the challenges could be well addressed at any time in the immediate future.

1.2 *Neolissochilus hexagonolepis* (McClelland, 1839)

Neolissochilus hexagonolepis (McClelland, 1839) is commonly known as Copper Mahseer. It is a beautiful shiny game fish with an elongated body and a rounded abdomen (Appendix XVIII-1). Formerly named *Acrossocheilus hexagonolepis*, it is a prominent colourful game fish of Nepal.

The taxonomy of the fish species is as given below:

Kingdom: Animalia
Phylum: Chordata
Subphylum: Vertebrata
Superclass: Gnathostomata
Class: Teleostomi
Subclass: Actinopterygii
Order: Cypriniformes
Suborder: Cyprinoidei
Family: Cyprinidae
Subfamily: Cyprinini
Genus: *Neolissochilus*
Species: *hexagonolepis*

The vernacular names of the species are given below:

Katle or Vadalke (Nepali), Karampi (Malayalam), Bulak or Bhorkhol (Bengali), Boka or Bokar or Boolooah (Assamese), Mirpunia (Lepcha), Ngara (Manipur)

The body of *Neolissochilus hexagonolepis* is olive green dorsally with splashes of golden on sides and a yellow line just above the lateral line. Eyes are located laterally at the upper aspect of the head. The head is short and broad with an obtusely rounded snout. The mouth is subterminal and nearly truncate with a sharp lower jaw edge. Lips are thick and continuous round the angle of the mouth. Barbels are four in number, a pair each in the rostral and maxillary regions. The dorsal fin is inserted near opposite to the pelvic fin consisting of 12 rays among which the last ray is unbranched, osseous and strong. This fish appears similar to Mahseer (*Tor tor*) but the former is deep copper-coloured compared to the latter which is golden. The diagnostic characters of *N. hexagonolepis* are given below:

D 12; P 15; V9; A 7; C19; L_1 27; L.tr.$4^{1/2}$ - $4^{1/2}$; TL = 20.4cm

This species is native to Bangladesh, India, Indonesia (Sumatra), Malaysia (Peninsular Malaysia), Myanmar, Nepal, Thailand (Arunachalam, 2010). In Nepal, this fish occurs in the Koshi, Gandaki, Karnali, Mahakali and Trishuli river basins (Shrestha, 2008). Shrestha and David (2012) reported its occurrence in Achham, Baglung, Bardia, Bhojpur, Dhankuta, Gulmi, Ilam, Kailali, Palpa, Parbat, Sankhuwasabha, Surkhet, Syangja, Tanahun, Udayapur from the country.

N. hexagonolepis is an omnivorous fish with its diets containing mostly filamentous green algae, chironomid larvae, crustaceans and water beetles (Ferro and Badagami, 1980). Inhabiting streams with fast-flowing water, this fish is found mostly in high gradient and low gradient riffles and pools (Menon, 1999). It is a highly valued food and game fish of over 60 cm and weighs up to 11 kg. The breeding season of this fish extends from April to October with its peak in the months of August to September. The male of this species has been reported to mature at an early size of 9 cm (Arunachalam, 2010). This species breeds once a year but releases fractionally several batches of eggs during the breeding season. The Copper Mahseer, thus, is regarded as a fractional spawner (Shrestha, 1990). It is regarded as a medium-distant migratory fish migrating upstream during the breeding season where it spawns on

stones and gravel (Rai and Swar, 1989). It is overexploited in most of its ranges with populations in continuing decline and it has been anticipated that the population of this species will decline by more than 50 % in the next ten years due to over-exploitation, habitat loss and several human-influenced changes (Arunachalam, 2010). Removal of sand and gravel from river beds, siltation caused due to deforestation and pollution, are held responsible for losing the species' habitat. Also, the construction of dams causes the degradation of its habitat and disrupts its upstream breeding migration (Menon, 2004).

N. hexagonolepis is a long-lived species with low fecundity and shows multiple spawning (Dasgupta, 1988; Swar, 1994). Nikolsky (1963) opined that the multiple spawning of the fish might have developed as an adaptation to spawning under unfavourable environmental conditions ensuring the survival of the eggs. *N. hexagonolepis* is a prolific breeder (Dasgupta, 1988) and spawns multiple times during each breeding season as an adaptation to the high mortality of its juveniles in hill streams due to high monsoon flooding (Swar, 1994).

N. hexagonolepis is one of the notable species in the snow-fed torrential rivers of Nepal. Regrettably, its population is in sharp decline due to the loss of its habitat and over-exploitation. Removal of sand and gravel from river beds, rampant use of pesticides and insecticides in agricultural fields and their seepage into the river, overfishing and other anthropogenic activities that degrade its habitat may all be contributing to its precipitous decline in recent years.

Currently, *N. hexagonolepis* has the conservation status of 'Near Threatened (NT)' according to the Redlist Assessment of IUCN (2018).

1.3 Fecundity and Gonadal development of fishes

Knowledge of the reproductive behaviour of fishes is essential requirement for effective fishery resources management and conservation (Marshall et al., 2003; Jan et al., 2014). For a 'conservation-priority' fish like *N. hexagonolepis*, the study of reproductive behaviour is vital for understanding the maturity and recruitment process.

The term 'fecundity' denotes the total number of eggs present in the ovaries of a fish, which are likely to be laid during the next spawning season (Bagenal, 1957). Fecundity is the measure of the reproductive capacity of a fish and is subject to significant variations due to the size, age, condition and race of the fish (Towers, 2014).

Nikolsky (1963) stated that fecundity is the specific feature of a species that arises in due course of its evolution and is directed to the species' continuance. Kharat and Khillare (2013) defined fecundity as the number of ova laid by a fish during the spawning season and is species-specific. This definition accentuated the variation of fecundities among different species.

The knowledge of fecundity finds a great application in stock size assessment, stock discrimination (Holden and Raitt, 1974) and rational utilization of stock (Morales, 1991). The assessment of fecundity is useful in evaluating the variations in the fish population, commercial potentialities of its stock, life history and in the proper management of fishery (Marimuthu et al., 2009).

Reproductive organs of fish include testes and ovaries which produce sperms and eggs respectively. Barring some fishes like sharks and rays, which exhibit internal fertilization, most fishes are broadcast spawners shedding their eggs and sperms into the water where the fertilization occurs.

Testes of some teleosts contain seminiferous tubules that are covered externally by tunica albuginea. These tubules are very fine tubes and are lined with a layer of germ cells. The germ cells mature and develop into sperm cells.

Ovaries of fish show variations in the structure, both internally and externally, and also in the process by which the ova are expelled out of them. In the gymnovarian type, the oocytes are released directly into the coelomic cavity from where they enter the ostium. Then through the oviduct the ova are eliminated out. In the secondary gymnovarian type, the ovaries shed ova into the coelom from where they go directly into the oviduct. In contrast, in the cystovarian type the oocytes are conveyed to the exterior through the oviduct. Cystovarian type is the characteristic of most teleosts.

The mature ovary of a fish consists of an ovarian cavity, the germinal epithelium and the stromal compartments in which are present the follicles where the

oocytes develop. During the maturation process, the oocytes within the ovaries show marked changes in the nucleus, ooplasm and the surrounding layers (Nishimura and Tanaka, 2014).

Regarding the process of gonadal sex differentiation in mammals, the activation of the Y-linked gene (SRY) initiates the differentiation of the Sertoli and Leydig cells in the testes while its absence induces granulosa and theca cell differentiation in the ovary. Once the cell lineages have been established through the genetic regulation, endocrine regulation takes over and maintains the sexual identity of each lineage (Morohashi et al., 2013). Nishimura and Tanaka (2014) opined that the sex differentiation in teleosts is also regulated, as in the case of mammals, by the combined effect of both genetic and endocrine regulations.

Gonads of fish undergo noticeable cyclic morphological and histological changes before they reach full maturity.

Study on the development of gonads and their maturity stages is needed for making an accurate prediction of the reproductive potential of the fish and to understand the dynamics of its population structure (Ekokotu and Olele, 2014).

1.4 Rationale of the work

Tamor River serves as a typical home, and the breeding ground for many hill-stream fishes, including *N. hexagonolepis* (Shrestha, 2008; Shrestha et al., 2009), but has been overlooked for unknown reasons. Currently, *N. hexagonolepis* has the conservation status of 'Near Threatened (NT)' according to the Redlist Assessment of IUCN (2018). Therefore, an essential conservational initiative is needed for this species. Precise knowledge of reproductive biology is needed to proceed with any conservational efforts. Unfortunately, there is a lack of such knowledge concerning *N. hexagonolepis*. Some workers like Swar (1994) and Jyrwa and Bhuyan (2017) have attempted to investigate the reproductive traits of *N. hexagonolepis* in Nepalese and Indian environment, respectively. But, there is no previous literature specifically from Tamor River. Poor documentation still exists regarding the reproductive biology of *N. hexagonolepis*, especially in the Nepalese river systems. In this backdrop, an attempt has been made to investigate the reproductive traits like fecundity and gonadal development of *N. hexagonolepis* from the river. A comprehensive insight into the

reproductive biology of the fish could help us in acting towards the sustainable conservation of the species in the wild.

1.5 Objectives

The general objective of the study was to accumulate information regarding the fecundity and gonadal development of *Neolissochilus hexagonolepis* from Tamor River, Nepal. This study could be compiled together to obtain the holistic picture of the reproductive biology of the species hoping to fill up the lacuna in the existing knowledge. The specific objectives were:

1. To determine the length-weight and length-length relationships and compute the condition factor of *Neolissochilus hexagonolepis* from Tamor River, Nepal.
2. To assess the fecundity, egg size, and gonado-somatic index (GSI) of *N. hexagonolepis* from the river.
3. To investigate the histomorphological features and establish the maturation cycle of gonads of the fish.
4. To analyse the physico-chemical parameters of water (Temperature, pH, dissolved oxygen, free CO_2, total alkalinity and total hardness) of the river and investigate their relationship with gonad weight and gonado-somatic index of the fish from the river.

CHAPTER 2

2. LITERATURE REVIEW

2.1 Length-weight and length-length relationship and condition factor

According to Dars et al. (2010), the change in size with reference to time is growth. They regarded the weight as a function of length and suggested that the growth in fish continues throughout its life, although it becomes slower after the onset of sexual maturity. Growth rate and size achieved by fish are highly flexible and subject to both genetic and environmental controls, so that the size reached may vary with environmental variables, such as water temperature and food availability (McDowall, 1994).

The axial growth of the body is quantified by the measurement of growth as a length while the growth in bulk is quantified by the measurement as weight and that these two categories of growth are positively correlated to each other (Wootton, 1990).

The increment in length and weight of the body, usually, indicates the growth of a particular fish. The growth forms the most appropriate parameter in analyzing the population of the fish at a particular time (Mansor et al., 2010).

Le Cren (1951) opined that the length-weight relationship (LWR) studies of any fish species serve as a pre-requisite for assessing its population characteristics. The LWR has been widely used in fish biology to estimate the mean fish weight of the fish, based on the known length (Beyer, 1987). The relationship is useful for the conversion of the length equations in weight for the equivalent of growth in weight and thus is used to assess the index of the well-being of the fish populations (Bolger and Connolly, 1989). The LWR data of a population serves as a fundamental parameter for monitoring fisheries' studies (Anderson and Neumann, 1996). The relationship also finds application in assessing the well-being of an individual and to determine the possible differences between the separate unit stocks of a species (King, 2007).

The LWR has been considered as an important tool in understanding the biology, physiology and ecology of a fish (Prasad and Ali, 2007), and it indicates the degrees of stabilization of taxonomic characters in fish species (Pervin and Mortuza, 2008). Knowledge of LWR is also useful in estimating the productivity and biomass of a fish population (Hossain, 2010), and is vital in terms of fishery ecology and stock management (Subba and Adhikaree, 2011).

Hence, it is always advisable to determine the relationship between these two parameters as a tool to have a profound insight into the quality of the overall development of the fish and also to have a quick peek of the aquatic environment supporting the fish species.

Several workers have reported on the LWR in fishes. Le Cren (1951), Thakur and Das (1974), Pathak (1975), Malhotra and Chauhan (1984), Subba and Ghosh (2000), Subba and Pandey (2000), Dhakal and Subba (2003), Kumar et al. (2005), Salam et al. (2005), Prasad and Ali (2007), Soomro et al. (2007), Kara and Bahar (2008), Pervin and Mortuza (2008), Ansumala and Subba (2009), Saha et al. (2009), Subba et al. (2009), Shaheena and Yousuf (2012), Surjya et al. (2013), Basumatary et al. (2017), Das et al. (2017), Ojha (2019), Osho and Usman (2019), Kant et al. (2020) and Khillare and Khandare (2020) have given an account on the relationship in *Perca fluviatilis, Heteropneustes fossilis (*Bloch*), Labeo calbasu, Labeo dero (*Ham.*), Glyptothorax telchitta (*Ham.*), Botia lohachata (*Chand*), Lepidocephalichthys guntea, Rasbora daniconius, Puntius chola, Puntius filamentosus, Eutropicthyes vacha (*Ham.*), Boops boops (*Linn.*), Labeo boga* (Hamilton), *Schistura rupicola (*McClelland*), Thenus orientalis, Gadusia godanahiae, Schizothorax niger (*Heckel*), Garra annandalei, Channa punctata and Glossogobius giuris, Labeo rohita, Parachanna obscura, Johnius borneensis and Mystus armatus* in respective orders. The LWR concerning *N. hexagonolepis* has been studied by Devashish et al. (2005), Subba and Adhikaree (2011) and Jyrwa et al. (2015). Similarly, Abobi (2015), Ozcan and Altun (2015) and Adaka et al. (2017) also gave accounts on the LWR of freshwater species from the rivers of Ghana, Pakistan and Nigeria respectively.

The LWR in fish is generally expressed by the equation, $W = aL^b$; where W is the weight of the body, L represents its length and 'a' (regression intercept) and 'b'

(regression slope) are constants. Brody (1945) and Lagler (1952) suggested that the growth pattern in fishes generally follows the cube law. However, Le Cren (1951) opined that the actual relationship may depart from this. When the value of regression coefficient (b) is equal to 3, the fish growth is said to be isometric during which there is no change in the shape of the body as the fish grows. The values departing from 3 indicate the allometric growth patterns; negative allometry when the value is less than three and positive allometry when the value is more than 3. This is known as the cube law. In the case of negative allometric growth, the fish becomes more slender as it increases in weight. In positive allometric growth the fish becomes relatively stouter or deep-bodied as it increases in length.

Seasonal fluctuation in environmental parameters, the physiological condition of the fish at the time of collection, gonadal development and nutritional condition of the environment of the fishes may cause the relationships to depart from the cube law (Jyrwa et al., 2015). Moreover, the values of constants, viz. 'a' and 'b', differ not only between different species but also within the same species depending on sex, stage of maturity and food habits and habitats (Saha et al., 2009).

Khanna (2000) suggested that the length of fish maintains a steady relationship with its weight. If the average weight's numerical values are plotted against those of the average length, a parabolic curve is obtained. And, if the average logarithmic length is plotted against the logarithmic weight, a straight line is observed. According to Weatherley (1972), the relationship provides an opportunity to calculate an index, which is commonly used by fish biologists to compare the condition factor or well being among fishes.

The condition factor is a quantitative parameter of the well-being state of a fish, which also reflects its current feeding conditions (Le Cren, 1951). It is also referred to as a fish's well-being of a fish and serves as a handy index in monitoring the feeding intensity, age and growth rates in a fish (Oni et al., 1983). The condition factor takes into consideration the health and general well-being of a fish as related to its environment, thereby representing whether the fishes are fairly deep-bodied or robust (Olurin and Aderibigbe, 2006). The enumeration of condition factor is based on

the hypothesis that the heavier fish of a given length is in better condition (Das et al., 2017).

The score of condition factor helps understand the effect of environmental changes on a fish (Le Cren, 1951; Araneda et al., 2008; Mansor et al., 2010). The score reflects the physical and biological circumstances and fluctuations by interaction among feeding conditions, parasitic infections and physiological factors (Le Cren, 1951). Araneda et al. (2008) suggested that the information on the condition factor is essential in the management of its culture as the information reveals the specific conditions under which the organisms are developing. Ozcan and Altun (2015) also suggested that the factor is widely used in fisheries and fish biology and is an important parameter for the evaluation of fish stocks.

Hence, the computation of the condition factor of a fish is not only useful in determining the general well-being of the fish but it also provides the fair figure of the aquatic environment in which the fish lives.

2.2 Fecundity, Egg size and Gonado-somatic index (GSI)

The assessment of fecundity is relevant in monitoring the reproductive potential of a species and the association between the fecundity with other morphological parameters grants an opportunity to understand the reproductive biology of a particular fish species.

The data related to fecundity are useful in determining the density-dependent factors which affect the population size and also for separating different fish stocks from the same population (Simpson, 1951). Lagler (1956) suggested that the knowledge about the fecundity of a fish is essential for evaluating the commercial culture of the fish and in the actual management of the fishery potentialities of its stock.

Gomez-Marquez et al. (2003) suggested that fecundity is an important biological parameter playing a significant role in evaluating the commercial potentials of fish stocks. Similarly, Islam et al. (2012) emphasized that a thorough knowledge of

the fecundity of a fish is essential for evaluating commercial potentialities of its stock, life history, practical culture and actual management of the fishery.

Rasool and Ulfat (2013) opined that the size at which fish attains maturity and the number of eggs they produce are important considerations in the management of sport and commercial species and that the fecundity is associated with the studies of population dynamics.

The assessment of fecundity and its relation with different body parameters of female fish make it possible to estimate the potential of egg output (Chondar, 1977), and its relation with other morphological characters like size, age and weight have often been used to provide a reliable index of density-dependent factors affecting the size of a population (Ulfat et al., 2014). Bhattacharya and Banik (2015) suggested that fecundity study is an important aspect of fish biology and its knowledge is essential for the management of the fish population, cryopreservation of eggs, fish breeding and larval rearing. Jan and Ahmed (2016) also suggested that fecundity study along with GSI is used to assess the reproductive condition of a fish.

Total weight of body and weight of gonad are correlated to each other and reported in terms of the gonado-somatic index (GSI). It is an important parameter and has great significance in studying the reproductive biology of a fish. GSI is the measure of the relative weight of gonad to total or somatic weight (King, 1996). Arjamand et al. (2013) advocated that gonadal development is indicated by the GSI and is widely used by biologists to indicate the maturity and periodicity of spawning and in predicting the breeding season of a fish.

GSI of a fish increases with the maturation of the fish, reaching the highest value during the peak period of maturity and declining abruptly after spawning (Towers, 2014). It also shows fluctuations according to the seasons, exhibiting increased values during the rainy season, remaining lowest during winter and intermediate during the summer season (Kiran, 2015).

The fishes which produce a large number of eggs and deposit them over a short period are referred to as total spawners. On the other hand, multiple spawners have a more extended breeding period and deposit only a fraction of eggs during each

spawning act. Total spawners have a higher GSI than multiple spawners (Wooton, 1990).

Swar (1994), Mahapatra and Vinod (2011) and Jyrwa and Bhuyan (2017) assessed the fecundity of *N. hexagonolepis* in different habitats. Similarly, Vinod (2011), Arjamand et al. (2013), Eyo et al. (2013), Khaironizam and Ismail (2013), Kharat and Khillare (2013), Verma (2013), Ulfat et al. (2014), Wagle (2014), Kiran (2015), Jan and Ahmed (2016), Joshi et al. (2016), Kant et al. (2016), Osho and Usman (2019) and Tagarao et al. (2020) have reported on the fecundity and GSI of *Labeo dyocheilus*, *Tor putitora*, *Bathygobius soporator*, *Neolissochilus soroides*, *Nemacheilus moreh*, *Schizothorax richardsonii*, *S. esocinus and S. niger*, *S. richardsonii*, *Salmostoma untrahi*, *S. plagiostomus* and *S. richardsoni*, *Puntius sophore*, *Parachanna obscura*, and *Johnius borneensis*, respectively.

Egg size is under maternal effect in many fishes and larger and older females generally produce larger eggs (Fleming, 1996). Size of eggs depends upon reproductive traits of fishes and the differences in egg size may be related to spawning season (Bagenal, 1971), absolute fecundity (Thorpe et al., 1984) and individual size of fish (Bartel et al., 1999).

Swar (1994) and Mahapatra and Vinod (2011) have investigated the size of eggs in *N. hexagonolepis*. Similarly, Khaironizam and Ismail (2013) and Wagle (2014) have also reported on the egg size in *N. soroides* and *S. richardsonii*, respectively.

Although studies on the reproductive traits of cold water fishes have been conducted frequently, poor documentation still exists regarding the fecundity, egg size and GSI of *N. hexagonolepis*, especially in the Nepalese river systems. The present study was thus undertaken with the intent to fill up the lacuna in the existing body of knowledge.

2.3 Histomorphological features and maturation cycle of gonads

Biologists have been investigating fish gonads to identify annual reproductive cycles, length of breeding seasons and to determine the onset of reproductive maturity

and spawning rhythms (Parenti and Grier, 2004). Investigation of the histomorphology and cycle of maturation of gonads is a breakthrough in discerning the reproductive biology of fishes. As reported by Tomkeiwiez et al. (2003), the use of histology in maturity studies have become more and more widespread as it is more consistent and reliable.

Histology of gonads helps determine the peak period of spawning and in understanding the life cycle of a fish (Hosseinzadeh et al., 1980; Eigdery, 1981). Investigation of histomorphology and cycle of maturation of gonads provides insight into the reproductive biology of a fish by allowing unravelling various aspects of reproductive biology like the time of the breeding season and the frequency of breeding during the season (West, 1990; Conover, 1992; Mahmoud, 2009). Noble and Jones (1993) opined that the knowledge about different stages of gonadal maturation of fish provides important information necessary to prohibit fishing during the restoration of the fish stock. Agarwal (1996) also advocated that the regular histological and histochemical examination of the reproductive system could categorically define the size and age at first maturity.

Meijide et al. (2005) suggested that histology offers a powerful tool in the study of reproductive health of fishes and that it is routinely used for sex verification and for the identification of stages of development. They also opined that the histology helps in documenting the presence of intersex, tumours, parasites and other abnormalities and is also used for quantifying atresia. They further added that histology can also be used for more subtle changes such as the thickness of the vitelline envelope at various stages, yolk appearance, necrosis of sperm and Sertoli cell proliferation. They concluded that the gonadal histology, in conjunction with hormone and vitellogenin measurements, morphological and fecundity studies can provide insights into the effects of various environmental stressors on reproductive health.

The enhanced study of the gonadal cycle and ultrastructural changes of gonads in fish is a crucial step before attempting its culture and conservation through artificial means (Jyrwa and Bhuyan, 2017).

Ratty et al. (1989) and Subba (1998) reported on the histomorphology of gonads of *Thunnus alalunga* (Scombridae) and *Lepidocephalicthys guntea* (Ham.) respectively. Meijide et al. (2005) reported on the ultrastructure of early stages of gonadal development of *Cichlasoma dimerus*. Thiry and Poncin (2005) reported on the morphological changes of the nucleolus during oogenesis in oviparous teleost fish, *Barbus barbus* and suggested that the marked vacuolization of nucleoli occurring at the beginning of the growth during previtellogenesis was the most striking feature. Duarte et al. (2007) investigated the morphology of gonads, maturity and spawning season of *Loricariichthys spixii* in a subtropical reservoir and described five gonadal stages for both male and female fishes based on oocytes and spermatogenic lineage cells. They suggested that the reproductive biology studies that incorporate the histological examination of gonads usually are suitable in determining the precise duration of the spawning period and whether spawning occurs more than once in a breeding season. Lone et al. (2008) investigated the oogenesis, histological gonadal cycle, seasonal variations and spawning season of female Pomfret (*Pampus argenteus*) from the spawning grounds of Kuwait and classified its female gonad into eight different stages. Akter (2011) reported on the stages of oocytes and testicular germ cells during the spawning and post-spawning seasons of *Pangasius pangasius*.

Alam et al. (2012) carried out the study of the reproductive physiology of mud eel (*Monopterus cuchia*). They reported underdeveloped oocytes, early and late perinucleolar oocytes, yolk vesicle and early granule oocytes inside the ovarian tubule. Behera (2012) performed histological observation of gonads during the breeding and non-breeding seasons of *Trichogaster fasciatus* in Shanti Jheel, West Bengal and reported on the developmental stages of germ cells in the testes and changes of the oocytes in the fish's ovaries. Subba and Meheta (2012) reported on the ovarian histomorphology and gonadal cycle of freshwater garfish *Xenentodon cancilla*. They found that the fish's ovaries passed through resting, early maturing, advanced maturing, pre-spawning, spawning and spent phases within one year.

Azadi and Arshad-ul-Alam (2012) investigated the reproductive biology of Gangetic hairfin anchovy, *Setipinna phasa* from Halda River. They emphasized the importance of knowledge of reproductive biology before selecting a species of fish for any aquaculture practice.

Ahmed et al. (2013) studied the histomorphological features of testes of the catfish (*Clarias gariepinus*) from Egypt and reported that its testes shared the basic testicular structure to other fishes. Agbugui (2013) reported on the sex ratio, gonado-somatic index, stages of gonadal development and fecundity of the grunt, *Pomadasys jubelini* in the New Calabar-Bonny River and suggested two stages (maturing and mature) for male fish while three stages (quiescent, maturing and mature) for female fish. Hamzaoglu et al. (2015) performed a macroscopic and microscopic examination of the seasonal gonad change in *Alburnus istanbulensis* and reported five stages for ovaries while four stages for testes of the species.

Gadekar and Baile (2014) reported on the annual cyclical changes in the testicular activity of *Labeo rohita* and suggested that photoperiod and temperature are important environmental factors regulating the gonadal development and other reproductive events in most of the seasonally breeding teleosts. Similarly, Emam and Abughrien (2014) also reported on the histological structure of gonads of *Clarias lazera*.

Abu El-Nasr (2016) investigated the histological changes in the testes of *Gerres filamentosus* in Hurghada Red Sea, Egypt and reported altogether six stages of the gonad. They found that the fish was a protracted spawner (fractional discharge of sperm cells) with long spawning season and also reported that the Sertoli cells might be concerned with phagocytosis of unused sperms. Mahmud et al. (2016) studied the cyclical variations of gonad development of *Channa striata* and established seven oocytes' stages. They also reported that the testes revealed four stages of sperm development viz., spermatogonia, spermatocytes, spermatids and spermatozoa. Similarly, Pasha et al. (2016) reported on the histology of ovary of *Schizothorax plagiostomus*. They showed all the developing stages and mature follicles with yolky oocytes during its spawning season.

Concerning our experimental fish, only a handful of authors have pursued to examine the histomorphology of gonads from their natural habitat. Among them, the work by Swar (1994) is conspicuous. He 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. More recently, Jyrwa and Bhuyan (2017) reported five developmental stages of the fish from Meghalaya, India.

Hence, it is evident that, despite of the profound ecological and potential commercial value of the species, basically very few works related to the reproductive biology of *N. hexagonolepis* has been conducted in Nepalese rivers and elsewhere. The present study is an attempt to explore the reproductive biology of the fish by giving more emphasis on the developmental stages and cycle of maturation of gonads of *N. hexagonolepis* collected from Tamor River, Nepal.

2.4 Physico-chemical parameters of water and gonadal development

Among the world's total of 2.7 % freshwater, Nepal rates second in freshwater availability, the first being Brazil. Rivers in Nepal are snow-fed as they originate from the Himalayas. These rivers flow rapidly through high and low gradients and have much turbulence.

There are about 6,000 rivers in Nepal with an estimated total length of 45,000 kilometers (CBS, 2001). Unfortunately, most of these are under immense pressure from anthropogenic activities resulting in degradation of the quality of water of these rivers. Insufficient environmental impact assessment during power generation activities is doing much harm to the rivers and aquatic life. Dumping of industrial wastes and discharge of untreated sewage into the rivers are adding more to the pollution of these rivers. Additionally, the surface run-off from agricultural fields loaded with pesticides and herbicides has made the rivers' condition even worse.

Occasional landslides in the hilly regions of the country not only hamper the natural flow of the river but also affect the aquatic life of the rivers as the water turns too muddy during the incident. Through proper management of land and natural resources like farmlands, agricultural fields and forests we can decidedly minimize the risks.

Sharma et al. (2005) reported that there is no water quality monitoring network in Nepal and hence systematic monitoring of water is not done anywhere in the country. They further added that the data on physico-chemical parameters of water of

the rivers are scarce and also noted that there is a lack of uniformity in the methods used for the analysis. Systematic monitoring and management of water quality of these rivers are thus an area of the immense problem. On top of all these, programs conducted for creating awareness among the local people about pollution, water quality and conservation of aquatic lives have not been able to meet the targeted goal.

The tranquil rivers of the country are under mammoth pressure due to anthropogenic activities and more and more domestic and industrial effluents are being discharged into the aquatic system as a result of uncontrolled population growth, urbanization and industrialization (Ajmal et al., 1988). Revenga et al. (2000) also opined that the adverse changes to environmental water quality, mainly due to pollution of anthropogenic activities, are contributing to the degradation and loss of freshwater biodiversity. And, in the context of Nepal, the rivers are severely affected by an exponential increase in the human population, rapid urbanization and industrialization and intensive agriculture during the past few decades (Pradhanang, 2012). Jung et al. (2016) suggested that the quality gradients of rivers are regulated by many processes like synergies of pollution loads, hydrological characteristics, sediment and metabolic activities in the water.

The assessment of physico-chemical parameters of water is of paramount importance and the values of correlation coefficients between various parameters and their significance level will help in selecting the proper environmental methods used for the treatment of water (Praveen et al., 2013).

The physico-chemical properties of water and biological diversity of aquatic organisms are mainly held responsible for the maintenance of a healthy ecosystem and the interactions of both the chemical and physical properties of water play a significant role in distribution, composition, abundance and diversity of aquatic organisms (Priya et al., 2016).

The overall health and subsequent growth of fishes are dependent on the quality of the water in which they are raised. Among the various physico-chemical parameters, water temperature via affecting the characters of other parameters exerts a profound impact on the overall growth and development of fishes. Temperature plays

vital role in the water system as it affects the metabolism, growth and reproduction of aquatic organisms. Temperature affects the solubility and reaction rates of chemicals and that the rate of chemical reactions increases with increasing water temperature.

Dissolved oxygen (DO) is essential for the survival of fish and other aquatic life and is an essential indicator of pollution (Wetzel and Likens, 2006; Basavaraddi et al., 2012). Oxygen gets into river water by diffusion from the surrounding air as the river tumbles over falls and rapids. It also gets there as a by-product of photosynthesis of aquatic plants. The amount of oxygen dissolved in river's water is affected by the morphology of a particular river. Rivers flowing rapidly with high turbulence hold much-dissolved oxygen as compared to the rivers with low turbulence. The assessment of DO has been extensively used as a parameter delineating water quality and to evaluate the degree of freshness of a river (Fakayode, 2005).

The pH of water is the measure of the H+ ion activity of the water system and indicates whether the water is acidic, alkaline or neutral. pH is important in determining water quality by affecting other chemical reactions such as solubility and metal toxicity (Fakayode, 2005). The pH of water fluctuates due to many factors such as precipitation, mostly the acid rain and CO_2 concentrations and the extreme negative pH affect fish growth and reproduction (Zweig et al., 1999). CO_2 concentration in the water body may be influenced by several biological processes like photosynthesis, respiration and decomposition. The anthropogenic activities mainly associated with pollution and the chemicals from agricultural run-off and wastewater discharges containing detergents and soaps also contribute to pH change in the water body.

Alkalinity is the total measure of the substances in water that have the acid-neutralizing ability. It is vital for fish and aquatic life because it protects or buffers against pH changes. Water having low alkalinity possesses less capacity to buffer against pH changes. Total alkalinity is expressed as milligrams per litre (mg/l) or parts per million (ppm) of calcium carbonate.

Water hardness is typically expressed as mg/l $CaCO_3$ and is used to describe the effect of dissolved minerals, mostly Ca and Mg, determining the suitability of water for domestic, industrial and drinking purposes (Taylor, 1949).

The seasonal fluctuations of various physico-chemical factors play a crucial role in the distribution, periodicity, the qualitative and quantitative composition of biota in the aquatic ecosystem and the meteorological factors such as ambient temperature, sunshine, rainfall, humidity exert a considerable influence on the physico-chemical dynamics of the water body (Surana et al., 2010).

The literature review reveals an extensive assessment of the chemistry of the rivers from around the globe. The workers have been attempting to associate the water quality with the biology and distribution of aquatic life. John (2009) performed physico-chemical studies of the Pumba river in Kerela, India with reference to the distribution of prawn, *Macrobrachium rosenbergii*. David et al. (2010) investigated the physico-chemical parameters and their effects on *Clarias gariepinus* in a stream of Nigeria. They found that aquatic organisms like fish are exposed to pollution due to the contamination of the environment. This is creating a problem in the general biology of fish including its growth and reproduction. Singh et al. (2010) studied the physico-chemical properties of water samples from the Manipur river system in India. They opined that rivers and streams are highly heterogeneous as spatial as well as temporal scales. They suggested that the DO content plays a vital role in supporting aquatic life and is susceptible to slight environmental changes. They further added that the water quality of the river is being deteriorated due to anthropogenic activities. Krishna et al. (2012) performed the comparative study of physico-chemical and bacteriological parameters of Kaveri river in the Talakaveri region. They investigated the seasonal variations in physico-chemical parameters and bacteriological parameters of the river. They concluded that the dissolved oxygen (DO) is a critical parameter of water quality and acts as an index of the physical and biological process occurring in water. They also suggested that when the temperature increases gas solubility of water decreases and microbial activity increases and that both these changes reduce DO in water. Praveen et al. (2013) studied the physico-chemical properties of the water of river Ganga at Kanpur, India and concluded that the correlations between different pairs of physico-chemical parameters of water samples were influenced directly or indirectly by a large number of factors and geological conditions. Srivastava and Srivastava (2013) investigated the physico-chemical characteristics of Gomati river draining Sultanpur, Uttar Pradesh, India about urban effluents and found that the

amount of dissolved oxygen (DO) was significantly lower in the polluted water. Similarly, Kumari and Kumar (2016) also reported on the reproduction of fish about temperature anomalies in the Koshi region of Bihar.

Various workers have also investigated the water chemistry of Nepalese rivers (Sharma et al., 2005; Mahaseth, 2007; Shrestha et al., 2009; Pal, 2011; Pradhanang, 2012; Subba et al., 2016). Earlier, during the late 80s, different departments under Tribhuvan University, CEDA and NAST were also engaged in water quality of urban regions of the country (Shrestha et al., 2009).

Several workers have attempted to investigate the influence of water chemistry on the reproductive biology of fishes. Edwards (2005) investigated the influence of environmental parameters on the reproduction of mosquito fish. Sharma et al. (2014) and Sharma et al. (2015) reported on the effect of water parameters on the reproductive indices of *Schizothorax richardsonii* and *Oncorhynchus mykiss* and Golden mahseer (*Tor putitora*) respectively. Similarly, Olanrewaju et al. (2017) also investigated the relationship between physico-chemical parameters and reproductive indices of *Parachanna obscura*.

However, there is an absolute absence of previous literature on the association between physico-chemical parameters and reproductive indices of *N. hexagonolepis* from Nepalese rivers. In this backdrop, the present study was undertaken to investigate the relationship between the monthly dynamics of reproductive indices of the fish and important physico-chemical parameters of Tamor River.

CHAPTER 3

3. MATERIALS AND METHODS

3.1 Description of the study area: Tamor River

Among the several magnificent rivers originating in the Nepalese Himalayas, Tamor River lies between the latitude and longitude coordinates of 26°54′47″ N and 87°09′30″ E respectively and flows in the eastern part of the country (Fig. 3.1). This river and its tributaries drain the snows of Kanchenjunga which is the third highest mountain peak in the world. Tamor River is the sixth-largest river in the country. This river meets the confluence of Arun and Sunkoshi at Tribenighat to drain into the giant Saptakoshi which flows through the Mahabharat range and finally reaches the Gangetic plain. The Koshi River is the largest river in Nepal which is, in fact, the confluence of seven rivers, namely, Arun, Tamor, Indrawati, Dudhkoshi, Likhu, Tamakoshi and Sunkoshi and are thus, named as the Saptakoshi. The river Bhotekoshi meets with Indrawati to form Sunkoshi. The total length of the Tamor River is about 190 km with 5817 km^2 catchment area (Shrestha et al., 2009).

Tamor River serves as the subsistence source to hundreds of people in this region who depend on domestic use and irrigation purposes. It is a good source for capture fisheries and aids the rural economy of this region. This river is an outstandingly beautiful river with stunning views of the Himalayan ranges that include Mt. Everest, Mt. Kanchenjunga and Mt. Makalu. Flowing through high and low gradients the river makes roaring and whistling sound as it thrusts itself down into the plains. The river flows rapidly through deep gorges at some places while remains calm at the others. Massive flooding in the river during monsoon every year and occasional landslides cause sufferings to the aquatic life and human settlements.

Tamor River serves as the home as well as the breeding ground for many species of fishes. Altogether 19 species of fishes were reported from this river during the environmental impact assessment (EIA) study for the Tamor hydropower project by Swar and Shrestha (1998) while, Swar and Upadhaya (1998) reported 21 species from the river during the EIA study of Kabeli hydropower project. Shrestha et al. (2009) reported 30 species of fishes from this river.

The ten years (2007-2016) monthly mean discharge of the river varied from minimum 57.2 m^3/s in March to a maximum of 1060 m^3/s in August with an annual discharge of 356 m^3/s (Appendix: XVI).

3.2 Site selection, time and the duration of sampling

Fish samples were collected for two consecutive years, between December 2014 and November 2016, from the mid-reaches of the river. The study area which stretched for more than 12 km, lies between latitude and longitude coordinates of N 26° 56. 700', N 26° 55. 653' and E 087° 23. 097', E 087° 17. 653', respectively. Four sampling sites were selected along the river stretch, taking into account the ecological indicators like the river gradient, topography, human settlements and the degree of anthropogenic activities (Fig.3.1). Altitude, latitude and longitude of the sampling sites were recorded with the help of a portable Global Positioning System (GPS) device. The sites were selected based on the following criteria.

i. All the sampling sites were accessible by road.
ii. There was a higher possibility of finding the fish under study.
iii. Application of fishing gears could be conveniently performed.

The first sampling site (Sampling site 1), locally named as 'Yakchanaghat', lies in the latitude and longitude coordinates of N 26° 56. 700' E 087° 23. 097' and the elevation of 277 metres. Here, the river widens and the gradient is low (Appendix XVIII-2).

The second sampling site (Sampling site 2), locally named as 'Ghumaune', lies in the latitude and longitude coordinates of N 26° 56. 023' E 087° 19. 908' and the elevation of 266 metres. The river gradient is high with rocky boulders. A small perennial stream named 'Patle Khola' flows from the north and mixes with the river (Appendix XVIII-3).

The third sampling site (Sampling site 3), locally named as 'Mulghat', lies in the latitude and longitude coordinates of N 26° 55. 718' E 087° 18. 929' and the elevation of 247 metres. The river gradient is low with cobbles, pebbles and sand (Appendix XVIII-4). There is a dense human settlement on both the sides of the river

with a high degree of anthropogenic activities including the use of water for domestic purposes like washing and bathing.

The fourth sampling site (Sampling site 4), locally named as 'Mahangkhola', lies in the latitude and longitude coordinates of N 26° 55. 653’ E 087° 17. 653’ and the elevation of 224 metres. The site is characterized by shallow and deep pools with a gravelly and sandy bottom. There is the occurrence of dense vegetations along the bank. The site serves as a suitable spawning and ranching area for fishes (Appendix XVIII-5).

Water samples were collected from all the sites every fortnight, at and around 8:00 A.M. Atmospheric and the water temperature was recorded daily at 8:00 A.M. every morning. This way, a total of eight replicates of sampling were made every month.

3.3 Fish collection and measurement

A total of 198 fish samples were procured from four different sites of the river. The samples were collected with local fishermen’s help, using hooks, cast nets and gill nets of different mesh sizes and locally constructed traps. Species identification was made following Shrestha (2008). Captured fish samples were immediately transported to the laboratory for further examination. The total weight (TW), total length (TL), standard length (SL), forkal length (FL), and gonad weight (GW) were measured for each specimen. All the data were recorded on specific data collection sheets.

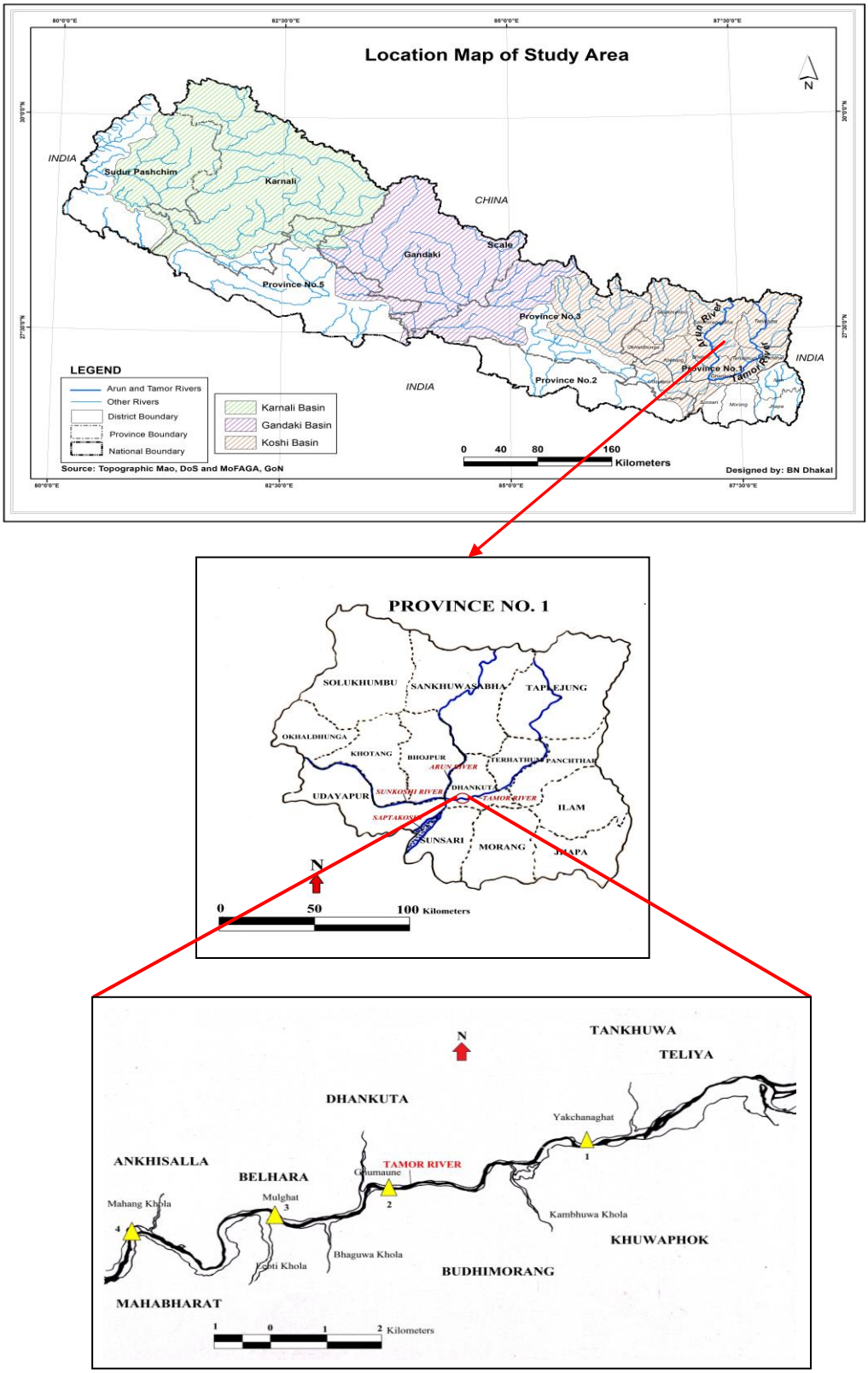

Figure 3.1: Location map of study area showing the sampling sites
△ indicates sampling sites

3.4 Determination of the length-weight and length-length relationships and computation of the condition factor (K) of *Neolissochilus hexagonolepis* from Tamor River, Nepal.

3.4.1 Length-weight and length-length relationships

For the study of length-weight and length-length relationships, the samples collected from the river for two years were sorted out according to their sex and the TW, TL, SL and FL were measured for each of the collected specimens. TL was measured from the tip of the snout to the distal tip of the longest caudal-fin ray, SL from the tip of the snout to the beginning of the tail fin and FL measured from the tip of the snout to the tip of the median caudal-fin ray. All these lengths were measured in a fully stretched condition to the nearest 1mm using a measuring tape and graduated ruler.

TW of each sample (including gut and gonad) was measured, after removing moisture from the body with paper towels and cloth, using a digital balance with the precision of 0.01 g. All the measurements were recorded on a data collection sheet.

In the present study, no significant differences ($p>0.05$) in the values of the regression slopes (b values) for the same-sex were obtained between the same months in two years for all the morphometric parameters viz. TW, TL, SL and FL. So, all the data obtained for the same month were pooled over these two years for the determination of the length-weight and length-length relationships. The length-weight relationship (LWR) was worked out as per cube law given by Le Cren (1951).

$$W = aL^b$$

Where W is the weight of the fish, L represents the observed length of the fish, 'a' is the regression intercept and 'b' is the regression slope.

The logarithmic transformation of the above equation is:

$$\text{Log}W = \text{Log}a + b\ \text{Log}L$$

Log-transformed data were the least skewed. Therefore, logarithms (base 10) were used in all the analyses. The relationships were determined by linear regression analysis for males, females and sexes pooled.

Scatter plot diagrams were plotted and all the data on LWR of the fish were subjected to t-test analysis at $P<0.001$. Also, a t-test was employed to confirm whether -'b'- values obtained in the linear regression (LogTW vs. LogTL) were significantly different from the expected cube value of 3 (Hossain and Sultana, 2014).

3.4.2 Condition factor (K)

Fulton's condition factor (K) was computed following the equation given by Ricker (1975):

$$K = W \times 100 / L^3$$

Where W is the total weight of the fish (in gram), L is the length of the fish (in cm) and the number 100 is the factor bringing the condition factor near to unity.

The equation used for relative condition factor (Le Cren, 1951) was:

$$Kn = W/w$$

Where W is the observed weight of the fish (in grams) and w is the calculated weight for the observed length (in grams). The calculated weight for the observed length was obtained from the equation $w = a \times L^b$, where 'a' and 'b' are the exponential form of the intercept and slope, respectively, of the logarithmic length-weight equation.

The relative condition factor (Kn) was computed for all the fish samples from the average length and weight of 3 cm interval of total length. As there was a significant difference in the length-weight relationship between males and females the relative condition factor (Kn) values were computed separately for both the sexes.

3.5 Assessment of fecundity, egg size and gonado-somatic index (GSI) of *Neolissochilus hexagonolepis* from Tamor River, Nepal.

3.5.1 Fecundity assessment

For fecundity assessment, ovaries obtained from sexually matured female fishes were weighed and then preserved in Gilson's fluid (100 ml 60 % alcohol, 800 ml water, 15 ml 80 % nitric acid, 18 ml glacial acetic acid, 20 g mercuric chloride) for over two weeks to loosen the tissue surrounding the eggs during which the eggs were agitated several times (Holden and Raitt, 1974).

The volumetric method of estimation of the fecundity of fish is inexpensive and easier to conduct but lacks adequate precision. To find out if the method would provide a precise estimate, two of the samples were tested as described below.

Eggs from both ovaries were removed and transferred to a graduated cylinder with water. The total volume of eggs was measured to the nearest millilitre (ml). The mixture of eggs and water was transferred to a beaker and stirred. Next, four sub-samples of eggs (about 0.5 ml) were scooped from the mixture with the help of a lab spoon as the mixture was stirred continuously. The settled volume in each sub-sample was measured in a graduated cylinder to the nearest 0.1 ml and the eggs in each sub-sample were counted. The number of eggs per ml for each sub-sample was calculated by dividing the number of eggs in each sub-sample by the sub-sample volume. After that, the mean number of eggs per ml for the entire ovary was calculated. The total number of eggs (Fecundity) in the ovaries was obtained by multiplying this means by the total volume of eggs in the ovaries.

The volumetric method of estimation of fecundity was subjected to a considerable bias as the eggs did not distribute evenly throughout the cylinder which affected the precision of the estimate.

As an alternative approach, the gravimetric method, modified after Bagenal and Braum (1978), was adopted to estimate the absolute fecundity. This method is one of the most common methods used to estimate fecundity and is based on the relation between ovary weight and oocyte density in the ovary. After determining the weight

of the ovary, three sub-samples of 1 g each were obtained from the anterior, middle, and posterior parts of the ovary. The eggs were then washed with distilled water and gently teased with a needle and forceps until they became disentangled from ovarian tissues. The eggs were then spread over blotting paper to remove excess moisture and the clamped eggs were gently separated. The eggs were then air-dried. After that, the total number of eggs in each ovary sub-sample was proportionally estimated using the equation, F_1 = (gonad weight X number of eggs in the sub-sample) / sub-sample weight (Yelden and Avsar, 2000). Finally, by taking the mean of three sub-sample fecundities (F_1, F_2 and F_3), the absolute fecundity (F) was estimated as: $F = (F_1 + F_2 + F_3) / 3$ (Hossain et al., 2012).

In the case of the ovaries weighing less than 10 g, count of eggs in 1 g sub-sample of the ovary was simply multiplied with the total weight of the ovary to work out the absolute fecundity.

The ovaries at the mature and spawning stages contained only the eggs which were yellowish and yolky (matured vitellogenic eggs). Even the ovaries at the ripening stage, considered for the fecundity assessment during the present study, contained the majority of the eggs that appeared yellowish and yolky, leaving behind a negligible amount of the smaller and whitish ones. So, it was practically safe to exclude the smaller, unhydrated and whitish eggs from the count and include only those which were yellowish, yolky and hard, during the enumeration of the fecundity of the fish. Sufficient care was also taken to see that the eggs have not been spawned earlier by the fishes considered for the fecundity assessment.

3.5.2 Egg size (ES) and Gonado-somatic index (GSI)

For egg size (ES) estimation, fifteen oocytes picked at random from each sexually mature female fish were measured using a calibrated micrometre mounted on the eyepiece of a monocular microscope (1 division = 0.05 mm). An average egg size for each female fish was then calculated.

Friedland et al. (2005) had reported a significant effect on the size of eggs after 161 days of storage in Gilson's fluid but no detectable effect on oocyte diameter in the first two months. In the present study, egg size estimation was carried out within a few

weeks (3-4) after sampling so as to avoid any effect of Gilson's fluid on the diameter of the eggs.

The relationships between ES and biometric parameters (TL, SL, FL TW, GW and F) were determined by a non-parametric test, Spearman's rank correlation.

Gonado-somatic index (GSI) of the fish was calculated following the method by Nikolsky (1963). The formula used was:

$$GSI = \frac{\text{Weight of gonad}}{\text{Total weight of fish}} \text{ X } 100$$

3.6 Investigation of the histomorphological features and establishing the maturation cycle of gonads of *Neolissochilus hexagonolepis* from Tamor River. Nepal.

3.6.1 Histomorphology of gonads

Histomorphological examination of gonads involved a longitudinal incision along the ventral line of the body using a sharp blade and angular scissors for gonad extraction. The gonads were thoroughly examined for their morphological features and weighed to the nearest 0.01 g. 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 three times at an interval of 8-hours. After the 3rd change, the gonads were preserved in 70 % alcohol.

Bouin's fluid was prepared as follows:

i. Saturated aqueous solution of picric acid = 75ml
ii. Formalin = 25 ml
iii. Glacial acetic acid = 5ml

The preserved gonads were washed with 70 % alcohol to remove the yellow colour of the stain and then cut into smaller pieces with the help of a sharp blade. The samples were then dehydrated in 90 % and absolute alcohol, one hour in each. After that, the gonads were transferred to 1:1 xylene and paraffin wax which was kept in an

incubator at 60 ºC for about 15 minutes. They were then transferred to pure wax already melted at 60 ºC. The pure melted wax was changed three times after every ½ hour. After the 3rd change, the pieces of gonads kept in the melted wax were kept in the incubator at 60 ºC overnight for infiltration. The next day paraffin blocks were prepared with care. The process, as mentioned above, 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 (Yorco YSI 115). The ribbon-shaped sections so obtained were carefully stretched on a 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 to wash out the excess stain. In the case of a dark stain, acid water was used to destain. After that, the slides containing 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 DPX. The histological slides so prepared were viewed under a binocular microscope (BEL PHOTONICS BIO2B-LED, Italy). Photographs were taken with a digital camera and then investigated.

Histological slides of gonads of both the sexes were prepared every month round the year, at the laboratory facility of Post Graduate Campus, Tribhuvan University, Biratnagar, Nepal.

3.6.2 Maturation cycle of gonads

Gonad maturity stages of 89 male and 109 female *N. hexagonolepis* were determined according to a maturity scale modified after Brown-Peterson et al. (2011); the scale has been accepted as the standard scale for the maturity of gonads (Dopeikar et al., 2015; Jyrwa and Bhuyan, 2017). The gonads extracted from all the samples

were pooled for the same month for two years and the frequency of a specific stage of the gonad, based on its gross morphological and histological features was calculated and expressed as a percentage monthly.

3.6.3 Size at first sexual maturity

The size at first sexual maturity of either sex of *N. hexagonolepis* was estimated based on the L_{50} Maturity scale (Size at which 50% of the individuals have reached sexual maturity; Freitas et al., 2016). The samples, assigned to various maturity classes, were binarized as immature and mature. The samples with gonads that showed vivid signs of maturity were classified as mature, otherwise immature. Then, regression analysis was performed by considering total length (TL, cm) as the explanatory variable and the stage of gonads (immature: 0; mature: 1) as the response variable (binomial). The variables were then fitted to a logistic function with the form:

$Y = 1/1 + exp^{\wedge} - (A + B*X)$ (Torrejon-Magallanes, 2018)

Where Y = the probability of an individual of being mature at a determinate X length; X = total length (TL, cm); A (intercept) and B (slope) are parameters estimated.

Finally, the L_{50} was calculated as: $L_{50} = - A / B$

3.7 Analysis of the physico-chemical parameters of water and investigating their relationship with gonad weight and gonado-somatic index of *Neolissochilus hexagonolepis* from Tamor River, Nepal

Physico-chemical parameters such as atmospheric temperature (AT), water temperature (WT), dissolved oxygen (DO), pH, free CO_2, total alkalinity (TA), total hardness (TH) were analysed.

The water samples were collected from 5-10 cm below the water surface and stored in pre-cleaned polythene bottles from all the sampling sites on a fortnightly basis and then analysed. Temperature and pH were recorded at the sites. The analyses of other parameters were performed titrimetrically following the standard protocols of APHA (2005). The samples were brought to the laboratory (Laboratory facility

provided by the Institute of Science and Technology, Dhankuta Multiple Campus, Dhankuta, Nepal) in well-labelled water sampling bottles and analysed within 6 hours.

Each sample was recorded or analysed in duplicate and the monthly mean value was calculated. Also, the data from the Department of Hydrology and Meteorology (DHM) were obtained for comparison and correction.

AT and WT were recorded with a simple mercury-filled Celsius thermometer having the accuracy of 0.1 °C to 50 °C. The pH of the water was recorded at the sites using systronic battery-operated pH meter (Lutron PH-201) which was calibrated using buffer solutions of pH 4 and pH 7. The recording of temperatures and pH and sample collection for titration was conducted at 8:00 A.M. in the morning.

DO was fixed at the site and estimated in the laboratory by Winkler's idiometric method. 300 ml capacity water sampling bottle was filled up to the brim with water from the river avoiding any air bubble (discarding the sample if seen any). By inserting a calibrated pipette just below the surface of the collected water sample 2 ml $MnSO_4$ was added. Again, 2 ml of alkali-iodide-azide was added to the sample in the same way. After that, the sample was thoroughly mixed by inverting the sampling bottle several times. The brownish-orange cloud (floc) which appeared in the sample was left to settle down. Then, 2 ml of concentrated H_2SO_4 was added through a pipette by holding it just above the sample's surface. The sample was then fixed. The fixed sample was then capped with aluminium foil and transported to the laboratory for titration.

50 ml of the fixed sample was then titrated against $Na_2S_2O_4$ (0.025N) till pale straw colour appeared. After that, 2 ml of freshly prepared starch solution was added to the solution turning it blue. Titration was then continued till the blue colour disappeared which indicated the endpoint. The titration process was repeated two to three times to obtain a concurrent reading which was noted down. DO was then calculated by using the following formula:

$$\text{DO (mg/l)} = \frac{(8 \text{ X } 1000 \text{ X } N)\, v}{V}$$

Where v = Volume of the titrant and V = Volume of the sample

In this method, the oxygen first oxidizes manganous sulphate and liberates iodine from acidified potassium iodide. The iodine, equivalent to dissolved oxygen, is determined by titration against standard sodium thiosulphate.

Free CO_2 was determined titrimetrically using N/44 NaOH, using phenolphthalein as an indicator. During the process, 2 to 3 drops of phenolphthalein were added to the freshly collected water sample (Absence of pink colour indicated that free CO_2 was present in the sample). The mixture was then titrated against 0.05 N NaOH. The appearance of a pink colour indicated the endpoint of the titration. The process was repeated several times and concurrent reading noted down to calculate the amount of free CO_2 by using the following formula:

$$\text{Free } CO_2 \text{ (mg/l)} = \frac{\text{(ml X N) of NaOH X 1000 X 44}}{\text{The volume of a sample taken}}$$

TA was estimated by titrating the water sample against 0.1 N HCl using phenolphthalein and methyl orange as indicators. 2 drops of phenolphthalein indicator were added to 100 ml of water sample in a conical flask. The solution remained colourless indicating that the phenolphthalein alkalinity equalled zero. Next, 2 drops of methyl orange were added which turned the solution pale yellow. The sample was then titrated against 0.1 N HCl. The endpoint of the titration was indicated by the appearance of a pink colour. TA (mg/l) as $CaCO_3$ was estimated by using the following formula:

$$\text{Phenolphthalein alkalinity as } CaCO_3 \text{ (mg/l)} = \frac{\text{(A X N) of HCl X 1000 X 50}}{\text{Volume of sample}}$$

$$\text{TA as } CaCO_3 \text{ (mg/l)} = \frac{\text{(B X N) of HCl X 1000 X 50}}{\text{Volume of sample}}$$

Where A = Volume of HCl used against phenolphthalein indicator (ml)

B = Total volume of HCl used against phenolphthalein and methyl orange indicators (ml)

N = Normality of HCl

TH of water was estimated by the EDTA method. 1 ml of buffer solution and a pinch of Eriochrome black T (EBT) was added to 50 ml of the water sample. The mixture was shaken till wine red colour appeared. The mixture was then titrated against standard EDTA (Ethylenediamine tetraacetic acid) and the endpoint of the titration indicated by the change of colour from wine red to blue. The titter value was noted and the process was repeated two to three times to obtain the concurrent reading. Calculations were made by using the following formula:

$$\text{TH (mg/l) as } CaCO_3 = \frac{\text{The volume of EDTA consumed X 1000}}{\text{Volume of sample}}$$

In this method, Eriochrome black T forms a wine-red complex compound with metal ions (Ca++ and Mg++) and the di-sodium salt of EDTA extracts the metal ions from the dye-metal ion complex as colourless chelate complexes leaving a blue coloured aqueous solution of the dye.

All the chemicals used during the investigation were of analytical grade, and the stock solutions of the reagents were standardized by the usual methods. The analytical data quality was guaranteed through duplicate samples and taking concurrent readings.

3.8 Statistical analysis

Statistical analyses were performed using Microsoft® Excel-add-in-DDXL and R 4.0.0 software.

Descriptive statistics were used to outline the basic features of the data by giving simple summaries like the mean, median and standard deviation. Breusch-Pagan test was used to test for heteroskedasticity in a linear regression model. The normality test of each data group was conducted by visual assessment of histograms and q-q plots and further confirmed with Shapiro-Wilk test. A non-parametric test was used when the normality assumption was not met or violated.

The LWRs between TL vs. TW, SL vs. TW and FL vs. TW and the relationships between absolute fecundity (F) and the fish's biometric variables were determined by simple linear regression. The relationships between egg size (ES) and biometric variables were determined by Spearman's rank correlation. The independent χ^2 test was employed to determine the sex ratio. Two-way analysis of variance (ANOVA) was used to compare the physico-chemical parameters among the sampling sites and months. The relationship between physico-chemical parameters and reproductive indices of *N. hexagonolepis* was analysed by performing Principal Component Analysis (PCA). All statistical analyses were considered significant at 5 % ($p<0.05$).

CHAPTER 4

4. RESULTS AND DISCUSSION

4.1 Results

4.1.1 Length-weight and length-length relationships and condition factor of *Neolissochilus hexagonolepis* from Tamor River, Nepal.

4.1.1.1 Length-weight and length-length relationships

Altogether 89 male and 109 female samples were assessed for determining the length-weight and length-length relationships and to evaluate the condition factor of *N. hexagonolepis*.

The TW, TL, SL and FL of male fishes ranged from 19.11 g to 750 g (Mean: 171.67 ± 186.78 g), 12.4 cm to 36.9 cm (Mean: 21.21 ± 6.38 cm), 9.7 cm to 28.6 cm (Mean: 17.08 ± 5.38 cm) and 11 cm to 32.8 cm (Mean: 18.77 ± 5.82 cm) respectively (Table 1). Similarly, for female fishes their values ranged from 24 g to 1500 g (349.23 ± 316.82 g), 13 cm to 46.3 cm (27.86 ± 9.21 cm), 10.5 cm to 38.2 cm (22.73 ± 7.99 cm) and 11.5 cm to 41.5 cm (24.86 ± 8.61 cm) respectively (Table 2).

Table 1: Monthly pooled data on total weight (TW), total length (TL), standard length (SL) and forkal length (FL) of male *N. hexagonolepis* from Tamor River, Nepal during 2014 – 2016.

Month	No. of fish	Variation in TW (g)	Variation in TL (cm)	Variation in SL (cm)	Variation in FL (cm)
Jan	9	35 – 305 (102.78 ± 87.75)	12.6 - 31.6 (19.86 ± 5.81)	**9.7** - 25.3 (15.76 ± 4.82)	**11** - 27.5 (17.57 ± 5.14)
Feb	6	35 – 425 (128 ± 147.90)	16.2 - 33.2 (19.77 ± 6.62)	12.8 - 26.2 (15.33 ± 5.33)	13.5 - 28.8 (16.5 ± 6.04)
March	7	55 – 455 (170.29 ± 150.86)	13.5 - **36.9** (22.97 ± 9.45)	10.7 - 28.1 (18.5 ± 7.16)	12.2 - **32.8** (20.64 ± 8.01)
April	5	55 – 390 (154.6 ± 138.83)	13.4 - 36.1 (22.18 ± 9.67)	10.3 - 25.3 (16.82 ± 5.88)	12 - 27.8 (18.62 ± 6.25)
May	9	90 – **750** (311.11 ± 243.19)	18.7 - 30.6 (23.21 ± 4.36)	15 – 26 (19.52 ± 4.25)	16.9 - 28.2 (21.29 ± 4.57)
June	10	25 – **750** (297.5 ± 283.33)	**12.4** - 31.6 (23.06 ± 6.51)	9.9 - 26.1 (18.65 ± 5.47)	11.3 - 28.4 (20.52 ± 5.76)

July	8	45 – 736 (288.88 ± 258.41)	14.3 - 31.2 (23.55 ± 5.88)	10.2 - 26.2 (19.33 ± 5.50)	12.4 - 28.4 (21.3125 ± 5.57)
Aug	4	30 – 390 (137.5 ± 169.93)	15.8 - 33.5 (21.23 ± 8.35)	12 - 27.5 (16.75 ± 7.34)	13.2 – 30 (18.55 ± 7.88)
Sept	8	35 – 125 (79.38 ± 31.45)	15.3 - 20.2 (18.06 ± 1.71)	12.3 - 16.1 (14.39 ± 1.34)	13.5 - 17.7 (15.56 ± 1.67)
Oct	4	35 – 155 (92.5 ± 50.58)	15 - 23.5 (19.63 ± 3.71)	11.5 - 18.2 (15.68 ± 2.95)	13 - 20.4 (16.73 ± 3.54)
Nov	10	**19.11** – 300 (93.43 ± 90.56)	13.5 - 31.3 (20.02 ± 5.11)	10.8 - **28.6** (16.48 ± 5.42)	12.4 - 29.7 (18.01 ± 5.25)
Dec	9	20 – 375 (116.11 ± 142.10)	12.9 - 34.2 (20.32 ± 8.83)	10.2 - 28.5 (16.27 ± 7.46)	11.3 - 31.9 (18.12 ± 8.34)
Total :	**89**	**(171.67 ± 186.78)**	**(21.21 ± 6.38)**	**(17.08 ± 5.38)**	**(18.77± 5.82)**

Figures in parantheses are the mean values.
(Abbreviations: TW, Total weight; TL, Total length, SL Standard length, FL, Forkal length)

Table 2: Monthly pooled data on total weight (TW), total length (TL), standard length (SL) and forkal length (FL) of female *N. hexagonolepis* from Tamor River, Nepal during 2014 – 2016.

Month	No. of fish	Variation in TW (g)	Variation in TL (cm)	Variation in SL (cm)	Variation in FL (cm)
Jan	13	50 – 375 (188.46 ± 129.73)	15.2 – 34 (24.95 ± 6.97)	12.3 - 29.7 (20.32 ± 6.08)	14.1 – 30 (21.98 ± 6.10)
Feb	10	48 – 1105 (340 ± 336.12)	16.8 - 40.5 (25.98 ± 8.91)	13 - 35.9 (21.17 ± 8.09)	13.6 - 38.5 (23.19 ± 8.97)
March	9	58 – 1125 (477.56 ± 354.59)	13.5 - 44.5 (31.67 ± 11.83)	10.8 - 38.2 (26.37 ± 10.06)	12.1 - 41.5 (28.57 ± 10.93)
April	6	68 – 1020 (417.83 ± 394.91)	14.5 - 43.5 (27.57 ± 12.77)	11.8 - 38.2 (23.43 ± 11.28)	12.7 - 40.5 (25.05 ± 12.20)
May	5	300 – 1015 (688 ± 264.45)	30 – 41 (33 ± 5.29)	25-35 (27.9 ± 4.36)	27 - 38.4 (30.12 ± 4.91)
June	5	90 – 980 (522 ± 363.96)	21.3 - 36.7 (31.14 ± 5.88)	16.5 - 29.7 (25.08 ± 5.16)	18.5 - 32.7 (27.46 ± 5.41)
July	13	205 – 1200 (548.62 ± 301.24)	25 - 46.2 (34.15 ± 5.39)	20.2 - 37.5 (28.46 ± 4.78)	23.4 - 41.2 (31.15 ± 5.03)
Aug	7	45 – **1500** (465.71 ± 494.85)	16.5 – 45 (30.51 ± 10.34)	13 – 38 (25.04 ± 8.98)	14.5 - 41.2 (27.89 ± 9.84)
Sept	7	60 – 535 (242.14 ± 169.97)	17.1 - 36.2 (26.94 ± 7.41)	13.4 - 29.1 (21.31 ± 6.05)	14.7 - 32.1 (23.54 ± 6.72)
Oct	6	80 – 430 (158.33 ± 134.00)	19.5 - 33.2 (24.15 ± 4.69)	15.5 - 26.3 (19.03 ± 3.75)	17.5 - 28.7 (21.02 ± 3.94)
Nov	10	25 – 645 (158.33 ± 134.00)	14.5 - 38.1 (24.15 ± 4.69)	11.6 - 31.4 (19.03 ± 3.75)	13 - 34.8 (21.02 ± 3.94)
Dec	18	**24** – 925 (181.61 ± 253.27)	**13 - 46.3** (22.51 ± 10.09)	**10.5 - 38.2** (17.89 ± 8.48)	**11.5 - 41.3** (19.51 ± 8.86)

Total :	**109**	**(349.23 ± 316.82)**	**(27.86 ± 9.21)**	**(22.73 ± 7.99)**	**(24.86 ± 8.61)**

Figures in parantheses are the mean values.
(Abbreviations: TW, Total weight; TL, Total length, SL Standard length, FL, Forkal length)

The relationships between the total weight and length of the fish viz. TW-TL, TW-SL and TW-FL showed significant positive correlations for both the sexes as well as for sexes-pooled (Table 3-5).

Table 3: Regression parameters of the length-weight relationship ($W = aL^b$) of *N. hexagonolepis* from Tamor River, Nepal.

Sex	N	TW (g)			TL (cm)			a	b	R
		Min	Max	Mean ± SD	min	max	Mean ± SD			
Female	109	24	1500	349.23±316.82	13	46.3	27.86±9.21	0.012	2.977	0.89*** S
Male	89	19.11	750	171.67±186.78	12.4	36.9	21.21±6.38	0.010	3.052	0.81*** S
Sexes pooled	198	19.11	1500	269.42±280.09	12.4	46.3	24.87±8.70	0.013	2.962	0.88*** S

(Abbreviations: N, Sample size; SD, Standard deviation; TW, Total weight; TL, Total length; a, intercept; b, slope; R, Pearson correlation coefficient; S, significant at 5 % level ($p<0.05$))

Table 4: Regression parameters of the length-weight relationship ($W = aL^b$) of *N. hexagonolepis* from Tamor River, Nepal.

Sex	N	TW (g)			SL (cm)			a	b	R
		min	Max	Mean ± SD	min	max	Mean ± SD			
Female	109	24	1500	349.23±316.82	10.5	38.2	22.73±7.99	0.036	2.833	0.91*** S
Male	89	19.11	750	171.67±186.78	9.7	29.8	17.08±5.38	0.029	2.939	0.84*** S
Sexes pooled	198	19.11	1500	269.42±280.09	9.7	38.2	20.19±7.48	0.037	2.834	0.90*** S

(Abbreviations: N, Sample size; SD, Standard deviation; TW, Total weight; SL, Standard length; a, intercept; b, slope; R, Pearson correlation coefficient; S, significant at 5 % level ($p<0.05$))

Table 5: Regression parameters of the length-weight relationship ($W = aL^b$) of *N. hexagonolepis* from Tamor River, Nepal.

Sex	N	TW (g)			FL (cm)			a	b	R
		min	max	Mean ± SD	min	max	Mean ± SD			
Female	109	24	1500	349.23±316.82	11.5	41.5	24.86±8.61	0.025	2.867	0.90*** S
Male	89	19.11	750	171.67±186.78	11	32.8	18.77±5.82	0.018	2.994	0.84*** S
Sexes pooled	198	19.11	1500	269.42±280.09	11	41.5	22.12±8.06	0.025	2.875	0.90*** S

(Abbreviations: N, Sample size; SD, Standard deviation; TW, Total weight; FL, Forkal length; a, intercept; b, slope; R, Pearson correlation coefficient; S, significant at 5 % level ($p<0.05$))

The computed regression coefficient (b) values for the relationships between TW-TL, TW-SL and TW-FL for female fish were found to be 2.977, 2.833 and 2.867, respectively (Fig. 4.1.1.1, Fig. 4.1.1.4 and Fig. 4.1.1.7), and 3.052, 2.939 and 2.994, respectively (Fig. 4.1.1.2, Fig. 4.1.1.5 and Fig. 4.1.1.8), for male fish. Similarly, for sexes pooled the values were 2.962, 2.834 and 2.875 in respective order (Fig. 4.1.1.3, Fig. 4.1.1.6 and Fig. 4.1.1.9).

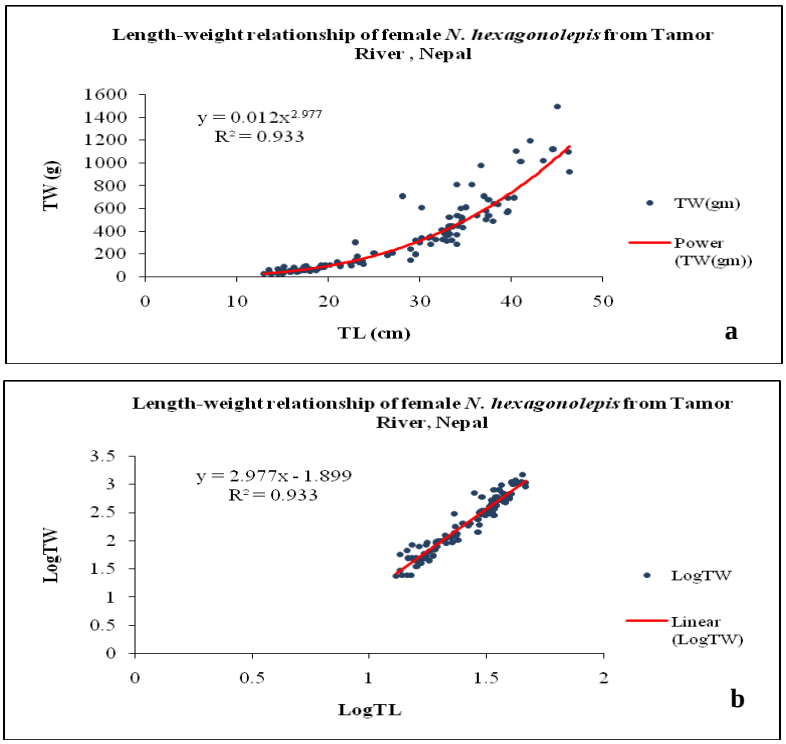

Figure 4.1.1.1: Length-weight relationship of female *N. hexagonolepis* from Tamor River: parabolic form (a) $TW = 0.012\ TL^{2.977}$ and linear form (b) LogTW = - 1.899 + 2.977 LogTL.

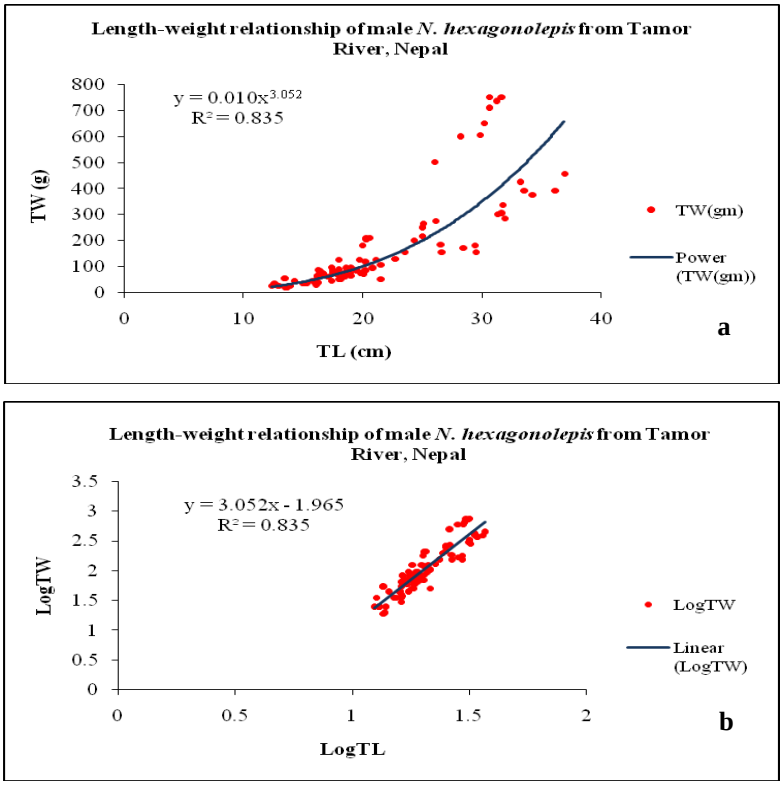

Figure 4.1.1.2: Length-weight relationship of male *N. hexagonolepis* from Tamor River: parabolic form (a) TW = 0.010 TL $^{3.052}$ and linear form (b) LogTW = -1.965 + 3.052 LogTL.

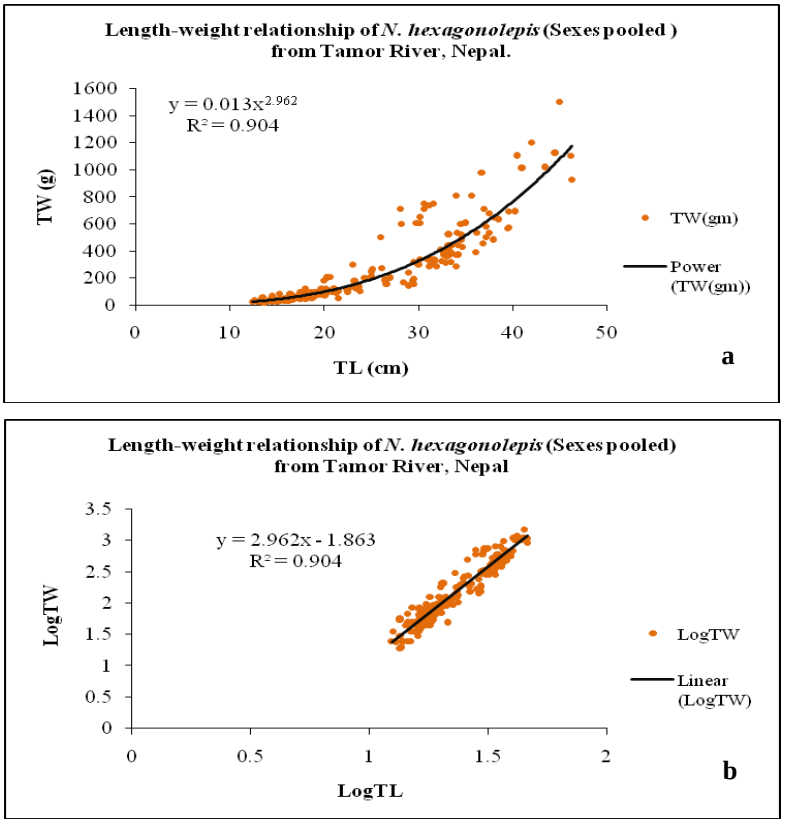

Figure 4.1.1.3: Length-weight relationship of *N. hexagonolepis* (Sexes pooled) from Tamor River: parabolic form (a) TW = 0.013 TL $^{2.962}$ and linear form (b) LogTW = -1.863 + 2.962 LogTL.

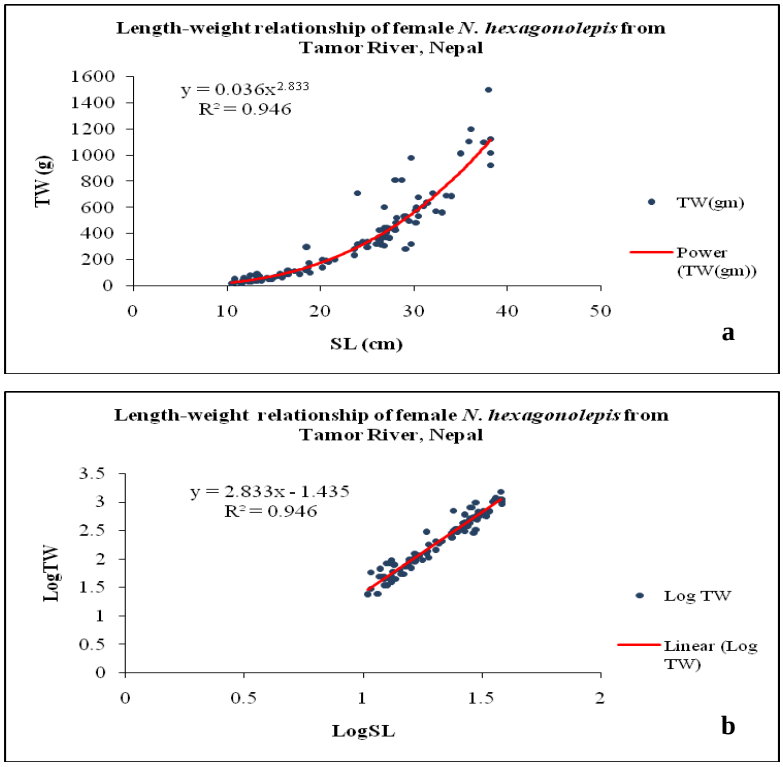

Figure 4.1.1.4: Length-weight relationship of female *N. hexagonolepis* from Tamor River: parabolic form (a) TW = 0.036 SL $^{2.833}$and linear form (b) LogTW = - 1.435 + 2.833 LogSL.

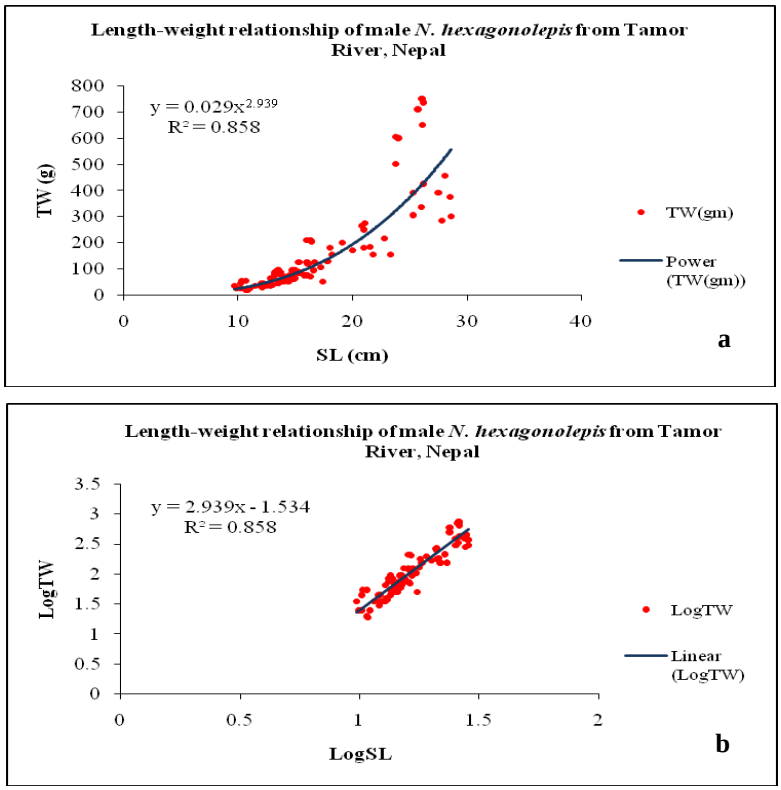

Figure 4.1.1.5: Length-weight relationship of male *N. hexagonolepis* from Tamor River: parabolic form (a) TW = 0.029 SL $^{2.939}$ and linear form (b) LogTW = - 1.534 + 2.939 LogSL.

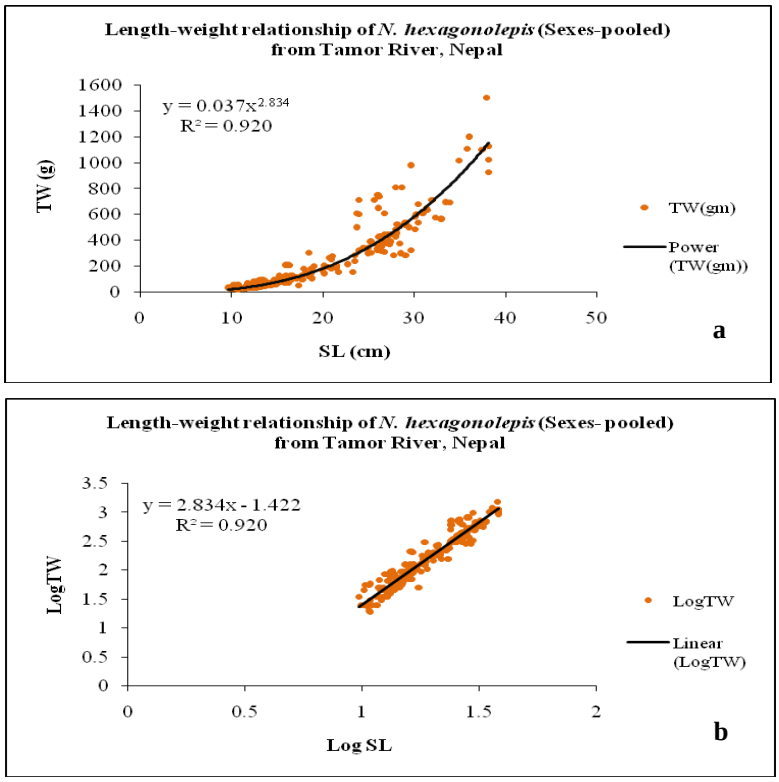

Figure 4.1.1.6: Length-weight relationship of *N. hexagonolepis* (Sexes-pooled) from Tamor River: parabolic form (a) TW = 0.037 SL $^{2.834}$ and linear form (b) LogTW = - 1.422 + 2.834 LogSL.

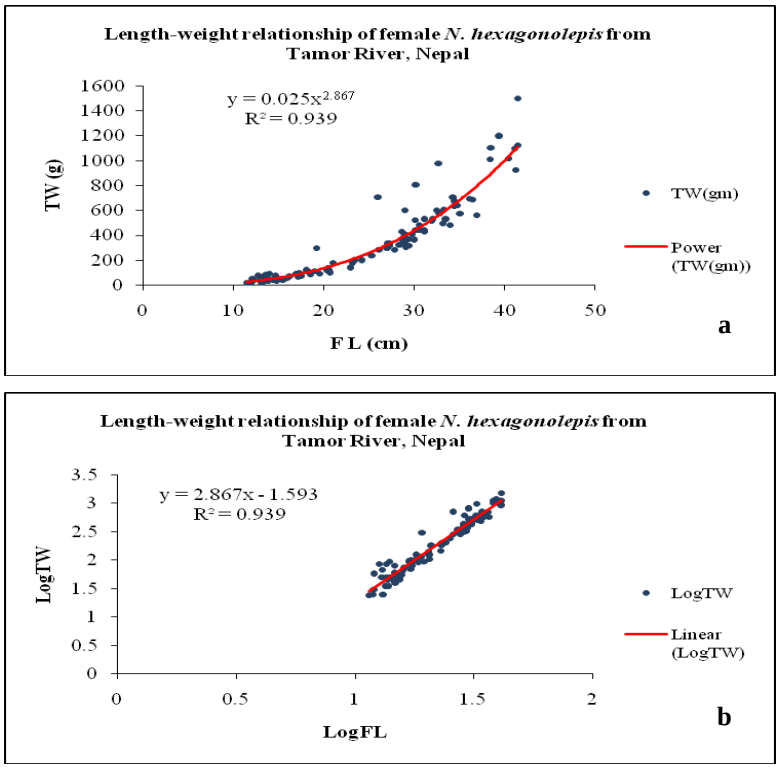

Figure 4.1.1.7: Length-weight relationship of female *N. hexagonolepis* from Tamor River: parabolic form (a) TW = 0.025 FL $^{2.867}$ and linear form (b) LogTW = - 1.593 + 2.867 LogFL.

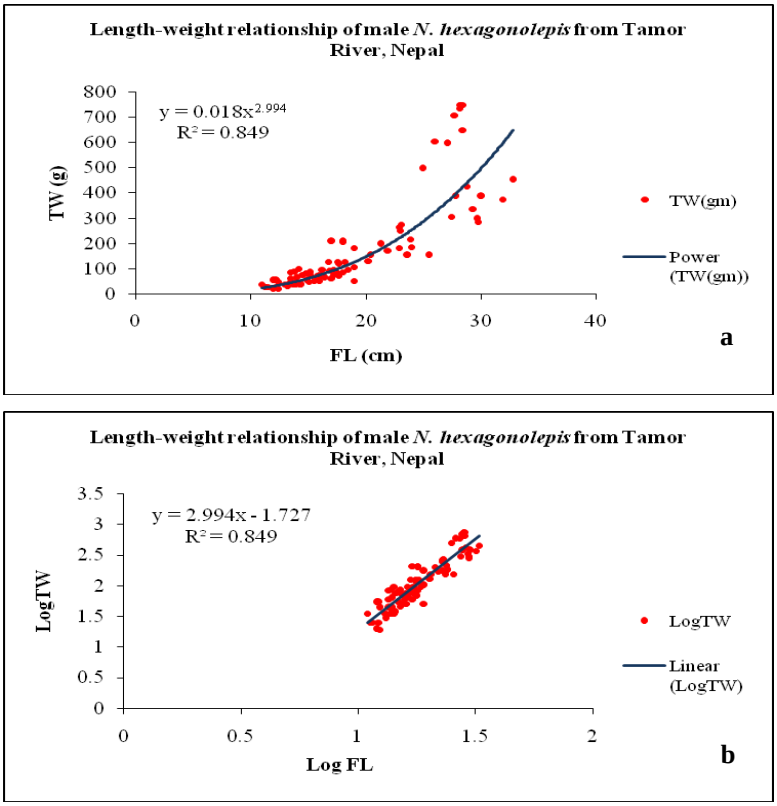

Figure 4.1.1.8: Length-weight relationship of male *N. hexagonolepis* from Tamor River: parabolic form (a) TW = 0.018 FL $^{2.994}$ and linear form (b) LogTW = - 1.727 + 2.994 LogFL.

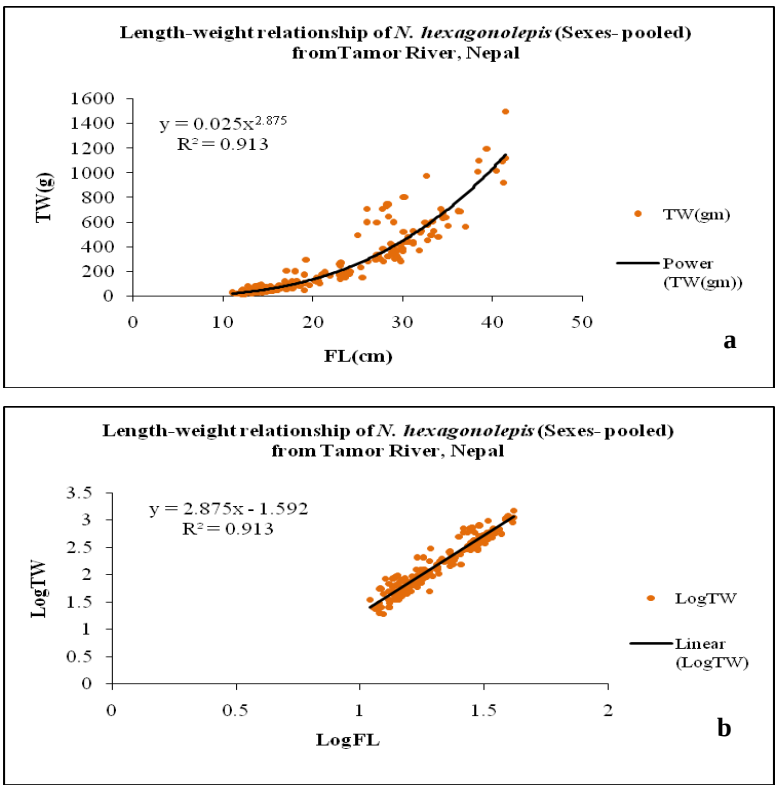

Figure 4.1.1.9: Length-weight relationship of *N. hexagonolepis* (Sexes-pooled) from Tamor River: parabolic form (a) TW = 0.025 FL $^{2.875}$ and linear form (b) LogTW = - 1.592 + 2.875 LogFL.

The values of regression coefficient (b) for all the relationships (LWR) came close to 3.

The regression equations are as follows:

1. TL vs. TW:

Female: LogTW = - 1.899 + 2.977 LogTL (n = 109; R^2 = 0.933; p<0.001)

Male: LogTW = -1.965 + 3.052 LogTL (n = 89; R^2 = 0.835; p<0.001)

Sexes pooled: LogTW = -1.863 + 2.962 LogTL (n=198; R^2=0.904; p<0.001)

2. SL vs. TW:

Female: LogTW = - 1.435 + 2.833 LogSL (n = 109; R^2 = 0.946; p<0.001)

Male: LogTW = - 1.534 + 2.939 LogSL (n = 89; R^2 = 0.858; p<0.001)

Sexes pooled: LogTW = - 1.422 + 2.834 LogSL (n=198; R^2 =0.920; p<0.001)

3. FL vs. TW:

Female: LogTW = - 1.593 + 2.867 LogFL (n = 109; R^2 = 0.939; p<0.001)

Male: LogTW = - 1.727 + 2.994 LogFL (n = 89; R^2 = 0.849; p<0.001)

Sexes pooled: LogTW = -1.592 + 2.875 LogFL (n=198; R^2 =0.933; p<0.001)

The t-test revealed that the regression coefficient values, in all the cases, did not depart significantly from the expected isometric value (b = 3) (Male: t = 0.3614, df = 87, p = 0.7187; Female; t = - 0.2919, df = 107, p = 0.7709; Sexes pooled: t = - 0.5507, df = 196, p = 0.5824).

The linear values of 'b' for female fishes were 1.140, 1.062 and 0.924 for the relationships of TL-SL, TL-FL and SL-FL respectively (Fig. 4.1.1.10). Similarly, its values were 1.162, 1.077 and 0.919, respectively, for male fish (Fig. 4.1.1.11).

The computed values for the coefficient of determination (R^2) for TL-SL, TL-FL and SL-FL were 0.981, 0.987 and 0.992, respectively, for female fish (Fig. 4.1.1.10). Its values were 0.962, 0.966 and 0.988, respectively, for male fish (Fig. 4.1.1.11) and 0.979, 0.984 and 0.992, respectively, for sexes-pooled (Fig. 4.1.1.12). The results were highly significant (P<0.00001) at p<0.05 for both the sexes.

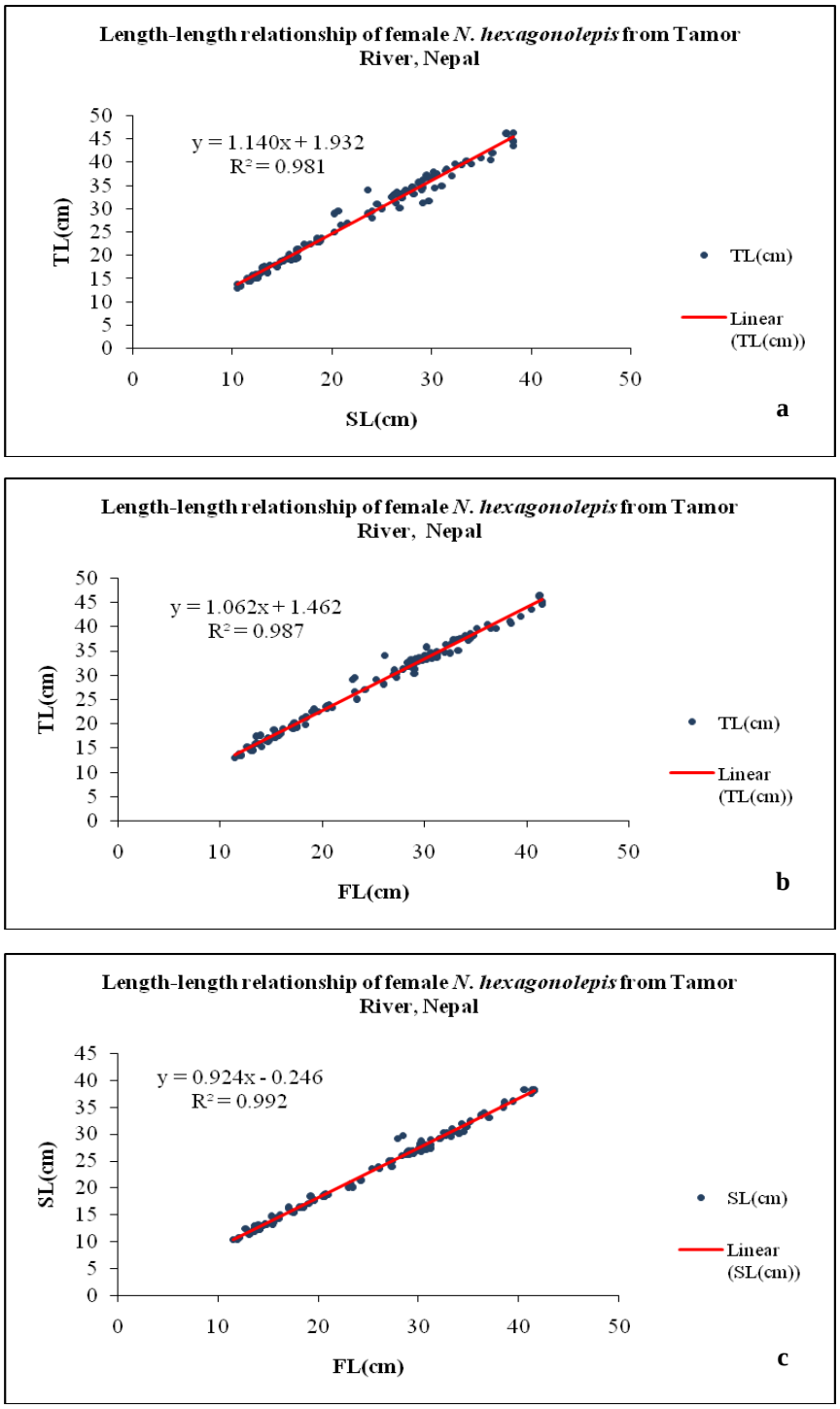

Figure 4.1.1.10: Length-length relationship with regression equation and coefficient of determination (R^2) for female *N. hexagonolepis* (a) TL = 1.140 SL + 1.932 (b) TL = 1.062 FL + 1.462 (c) SL = 0.924 FL – 0.246.

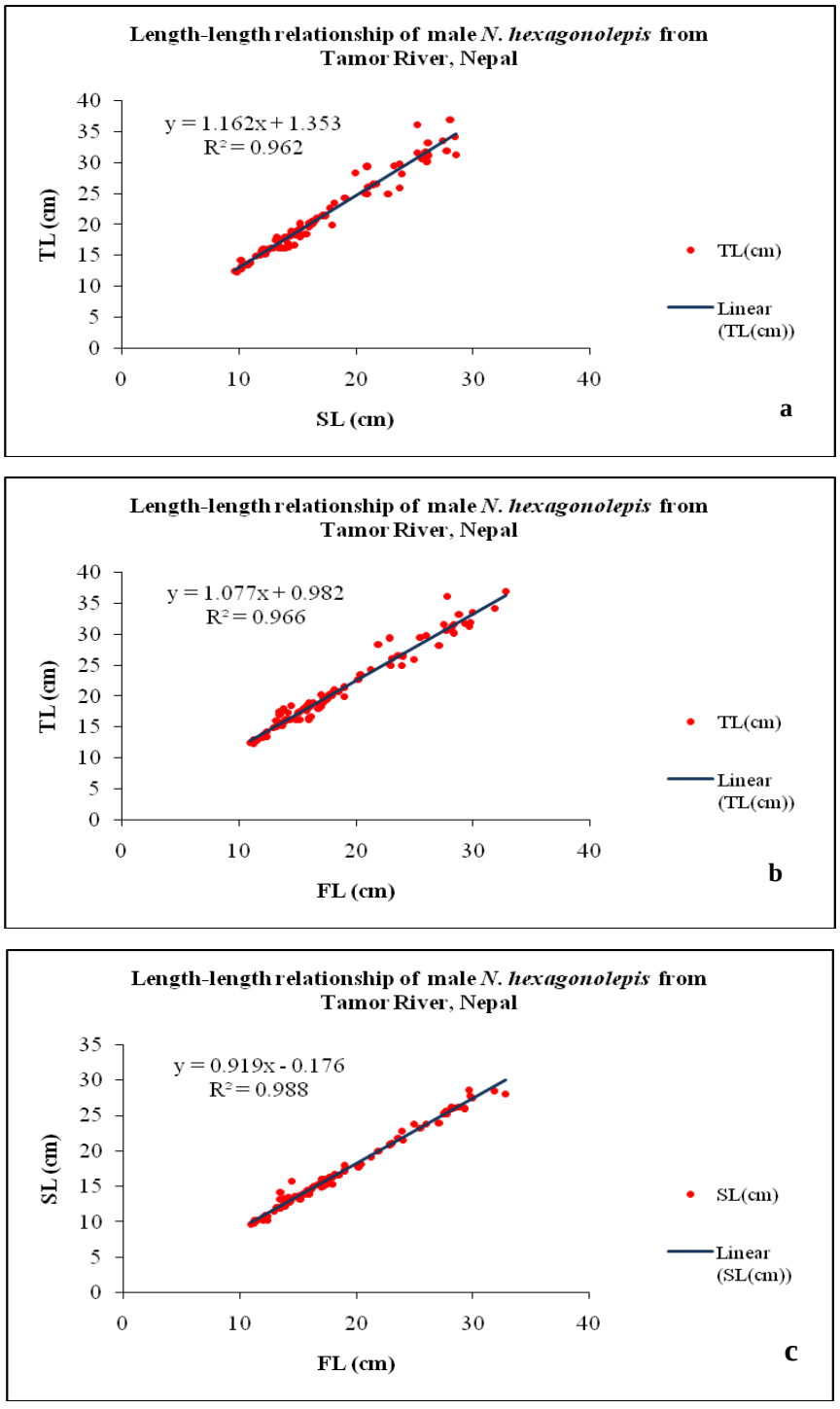

Figure 4.1.1.11: Length-length relationship with regression equation and coefficient of determination (R^2) for male *N. hexagonolepis* (a) TL = 1.162 SL + 1.353 (b) TL = 1.077 FL + 0.982 (c) SL = 0.919 FL – 0.176.

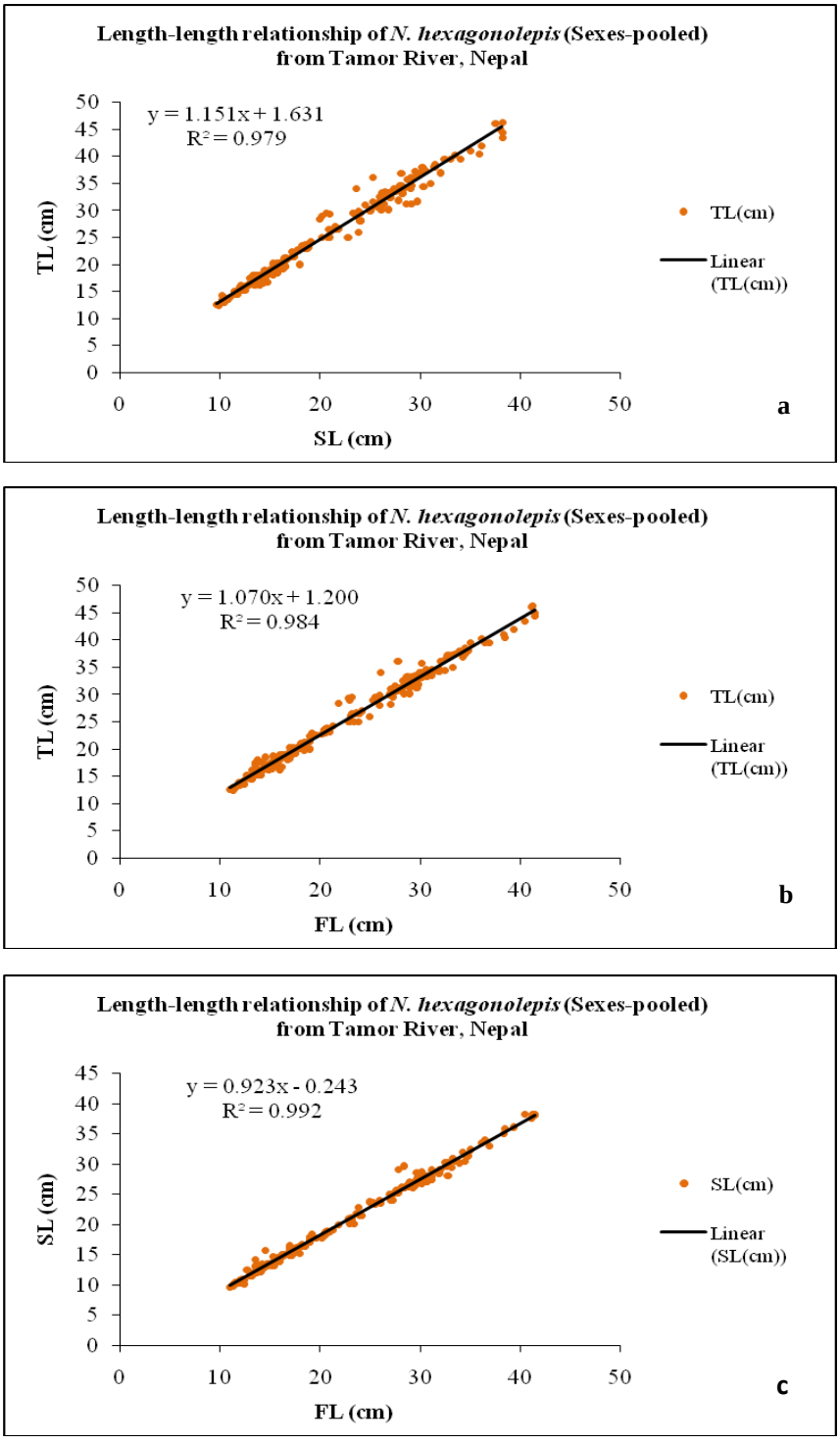

Figure 4.1.1.12: Length-length relationship with regression equation and coefficient of determination (R^2) for sexes-pooled of *N. hexagonolepis* (a) TL = 1.151 SL + 1.631 (b) TL = 1.070 FL + 1.200 (c) SL = 0.923 FL – 0.243.

4.1.1.2 Condition factor (K)

The monthly mean Fulton's condition factor (K) for male *N. hexagonolepis* reached the peak value at 2.38 in May, declined marginally to 2.02 in June and again upsurged slightly to 2.12 in July, during the first year of the study. During the second year of the study, the 'K' values reached a peak at 1.68 in May, declined marginally to 1.37 in June and again upsurged slightly to 1.52 in July. The 'K' values for male fish showed a precipitous downturn after July during both the years of the study (Fig. 4.1.1.13). Similarly, for female fish, the monthly mean 'K' values reached a peak at 2.16 and 1.82, both in May, during the first and second years of the study, respectively. The 'K' values for female fish showed steep recession after May during both the study years (Fig. 4.1.1.13).

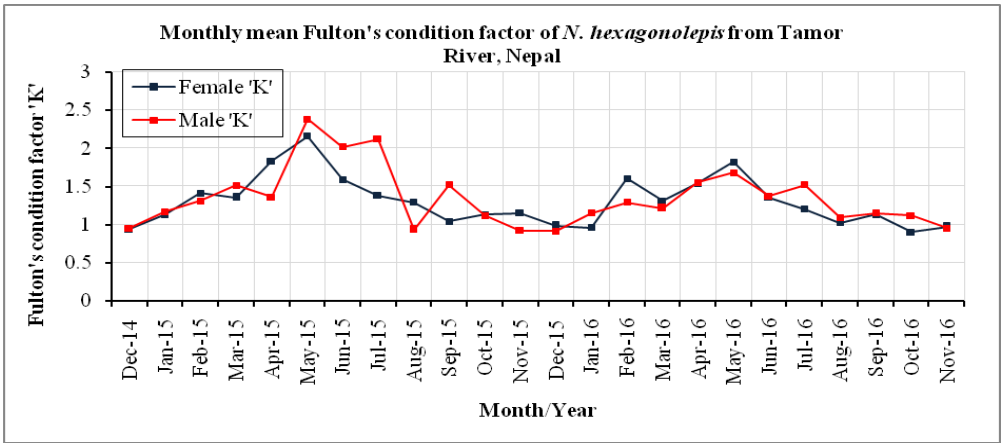

Figure 4.1.1.13: Monthly mean Fulton's condition factor (K) of male and female *N. hexagonolepis* from Tamor River, Nepal from December 2014 to November 2016.

The relative condition factor (Kn) values for male and female fish were obtained by dividing the observed weight by the calculated weight. The calculated weight for the observed length was obtained from the equation $w = a \times L^b$, where 'a' and 'b' are the exponential form of the intercept and slope, respectively, of the logarithmic length-weight equation. The values of 'a' and 'b' were calculated as 0.010 and 3.052 for male fish. Similarly, their values were calculated as 0.012 and 2.977, respectively, for female fish.

Table 6 shows the variations in condition factor with total lengths of male and female *N. hexagonolepis*. In male fish, the 'K' values fluctuated marginally between the size ranges of 12-15 cm and 18-21 cm. The values decreased precipitously

between 18-21 cm and 21-24 cm from 1.40 ± 0.54 to 1.04 ± 0.32. After that, the values increased significantly from 1.04 ± 0.32 at 21-24 cm to 1.53 ± 0.61 at 24-27 cm, thereby decreasing marginally to 1.40 ± 0.99 at 27-30 cm. The values again upsurged between 27-30 cm and 30-33 cm from 1.40 ± 0.99 to 1.79 ± 0.79. After that, the values plummeted to 0.87 ± 0.05 at 36-39 cm (Table 6).

In female fish, the 'K' values decreased progressively between the size ranges of 12-15 cm and 24-27 cm from 1.47 ± 0.62 to 1.15 ± 0.22, respectively. The values then increased steeply between 24-27 cm and 27-30 cm. After that, the values decreased significantly to 1.17 ± 0.34 at the length of 30-33 cm. The size ranges between 33-36 cm and 39-42 cm showed plateau state in terms of condition factor. After that, the 'K' values again upsurged to 1.38 ± 0.21 at 42-45 cm length size, finally slumping down to 1.23 ± 0.37 at 45-48 cm (Table 6).

Table 6: Fulton's condition factor (K) and relative condition factor (Kn) of male and female *N. hexagonolepis* from Tamor River, Nepal at 3 cm interval of the total length from December 2014 to November 2016.

	Male				**Female**			
Length group (cm)	**Mean Observed weight (g)**	**Mean Calculated weight (g)**	**Mean Fulton's condition factor (K)**	**Mean Relative condition factor (Kn)**	**Mean Observed weight (g)**	**Mean Calculated weight (g)**	**Mean Fulton's condition factor (K)**	**Mean Relative condition factor (Kn)**
12-15	32.91 ±13.88	27.19 ±3.67	1.39 ±0.55	1.22 ±0.48	40.00 ±18.32	30.58 ±4.06	1.47 ±0.62	1.30 ±0.55
15-18	56.53 ±19.89	51.35 ±7.15	1.26 ±0.36	1.09 ±0.31	55.25 ±20.59	49.61 ±7.62	1.25 ±0.45	1.11 ±0.40
18-21	103.04 ±48.41	84.13 ±11.79	1.40 ±0.54	1.20 ±0.46	79.27 ±19.00	78.17 ±8.12	1.13 ±0.19	1.00 ±0.17
21-24	113 ±39.47	126.75 ±17.94	1.04 ±0.32	0.88 ±0.27	130.87 ±56.73	130.87 ±16.46	1.17 ±0.45	1.05 ±0.41
24-27	255.63 ±106.95	198.57 ±19.66	1.53 ±0.61	1.29 ±0.51	195.00 ±14.14	190.61 ±23.32	1.15 ±0.22	1.04 ±0.20
27-30	342 ±237.98	292.84 ±21.74	1.40 ±0.99	1.18 ±0.84	302.50 ±207.91	262.88 ±25.69	1.30 ±0.96	1.17 ±0.86

30-33	535.67 ±220.04	363.18 ±20.79	1.79 ±0.79	1.50 ±0.66	362.92 ±84.54	349.46 ±35.16	1.17 ±0.34	1.05 ±0.31
33-36	396.67 ±25.66	457.00 ±21.39	1.05 ±0.11	0.87 ±0.09	477.33 ±138.63	431.53 ±28.92	1.22 ±0.30	1.10 ±0.27
36-39	422.5 ±45.96	586.52 ±27.73	0.87 ±0.05	0.72 ±0.04	628.5 ±145.34	577.65 ±31.56	1.21 ±0.31	1.09 ±0.28
39-42	-	-	-	-	774.17 ±229.88	710.32 ±32.42	1.19 ±0.31	1.08 ±0.28
42-45	-	-	-	-	1115 ±90.42	896.84 ±77.00	1.38 ±0.21	1.25 ±0.19
45-48	-	-	-	-	1175 ±294.75	1058.60 ±49.28	1.23 ±0.37	1.12 ±0.34

4.1.2 Fecundity, Egg size and Gonado-somatic index (GSI) of *Neolissochilus hexagonolepis* from Tamor River, Nepal.

4.1.2.1 Fecundity

The volumetric method did not provide sufficient precision of the estimates of the number of eggs per ml. With this method, the coefficient of variations (cv) of the estimated number of eggs per ml for the two test fishes were found to be 11.88 % and 17.41 %, with an average value of 14.65 % (Appendix III). Hence, the approach was rejected. On the other hand, the gravimetric method provided sufficient precision of the estimates with the mean coefficient of variation (cv) calculated among the estimated number of eggs per unit weight of eggs less than 5 % (Appendix IV). The gravimetric method was thus adopted to estimate the fecundity of *N. hexagonolepis* in the present study.

Absolute fecundity was assessed for 18 matured female fishes which ranged in TW from 340 g to 1200 g and in TL from 30.2 cm to 46.2 cm (Table 7). Absolute fecundity of *N. hexagonolepis* varied from 1324.6 for the fish with TL 33.2 cm, TW 430 g and GW 3.58 g to 14484 for the fish with TL 37 cm, TW 710 g and GW 102 g (Table 7). The mean value of absolute fecundity was found to be 7555.44 ± 3769.93 with the mean TL 35.6 cm ± 4.22 cm and the mean TW 586.5 g ± 261.14 g. The relative fecundity to weight ranged from 3.08 / g body weight for the fish with TL of

33.2 cm and TW 430 g to 21.58 / g body weight for the fish with TL 30.2 cm and TW 340 g. The relative fecundity to length ranged from 39.90 / cm total length for the fish with TL of 33.2 cm and TW 430 g to 391.46 / cm total length for the fish with TL of 37 cm and TW 710 g (Table 7).

Table 7: Total weight (TW), total length (TL), gonad weight (GW), absolute fecundity (F), relative fecundity to weight and relative fecundity to length from sampled female *N. hexagonolepis* from Tamor River, Nepal from December 2014 to November 2016.

S.N.	Total weight TW (g)	Total length TL (cm)	Gonad weight GW (g)	Absolute Fecundity (F)	Relative fecundity to weight (F / TW)	Relative fecundity to length (F / TL)
1	410	32.4	35	4270	10.41	131.79
2	525	33.2	38	7296	13.90	219.76
3	1200	42	75	12150	10.13	289.29
4	710	37	102	**14484**	20.40	**391.46**
5	340	30.2	56	7336	**21.58**	242.91
6	535	34.1	42	4284	8.01	125.63
7	610	35	58	10672	17.50	304.91
8	445	33.2	38.4	5222.4	11.74	157.30
9	442	33.6	38.42	4610.4	10.43	137.21
10	445	33.4	40.24	4869.04	10.94	145.78
11	565	39.5	39.63	9075.27	16.06	229.75
12	1015	41	34.47	11133.8	10.97	271.56
13	645	38.1	70.82	12676.8	19.65	332.72
14	430	34.7	27.2	8350.4	19.42	240.65
15	340	31	4.48	1433.6	4.22	46.25
16	1100	46.2	30	8160	7.42	176.62
17	370	33	27.2	8649.6	23.38	262.11
18	430	33.2	3.58	**1324.6**	**3.08**	**39.90**
Mean:	586.5 ± 261.14	35.6 ± 4.22	42.25 ± 23.75	**7555.44 ± 3769.93**	**13.29 ± 6.01**	**208.09 ± 95.34**

The relationships between absolute fecundity (F) and biometric variables of *N. hexagonolepis* were determined by simple linear regression after $\log_{10}$ transformation of the lengths (TL, SL and FL) and weights (TW and GW) data and the corresponding absolute fecundity estimates (Fig. 4.1.2.1 and Fig. 4.1.2.2; Hossain et al., 2010; Tessema et al., 2020). The positive correlations between the variables were expressed by the following regression equations:

i. $LogF = 3.29LogTL - 21.29$ ($n = 18$; $R^2 = 0.26$; $p<0.05$)

ii. $LogF = 3.43LogSL - 1.22$ ($n = 18$; $R^2 = 0.38$; $p<0.01$)

iii. $LogF = 3.40LogFL - 1.33$ ($n = 18$; $R^2 = 0.37$; $p<0.01$)

iv. $LogF = 1.00LogTW + 1.06$ ($n = 18$; $R^2 = 0.29$; $p<0.05$)

v. $LogF = 0.69LogGW + 2.75$ ($n = 18$; $R^2 = 0.75$; $p<0.001$)

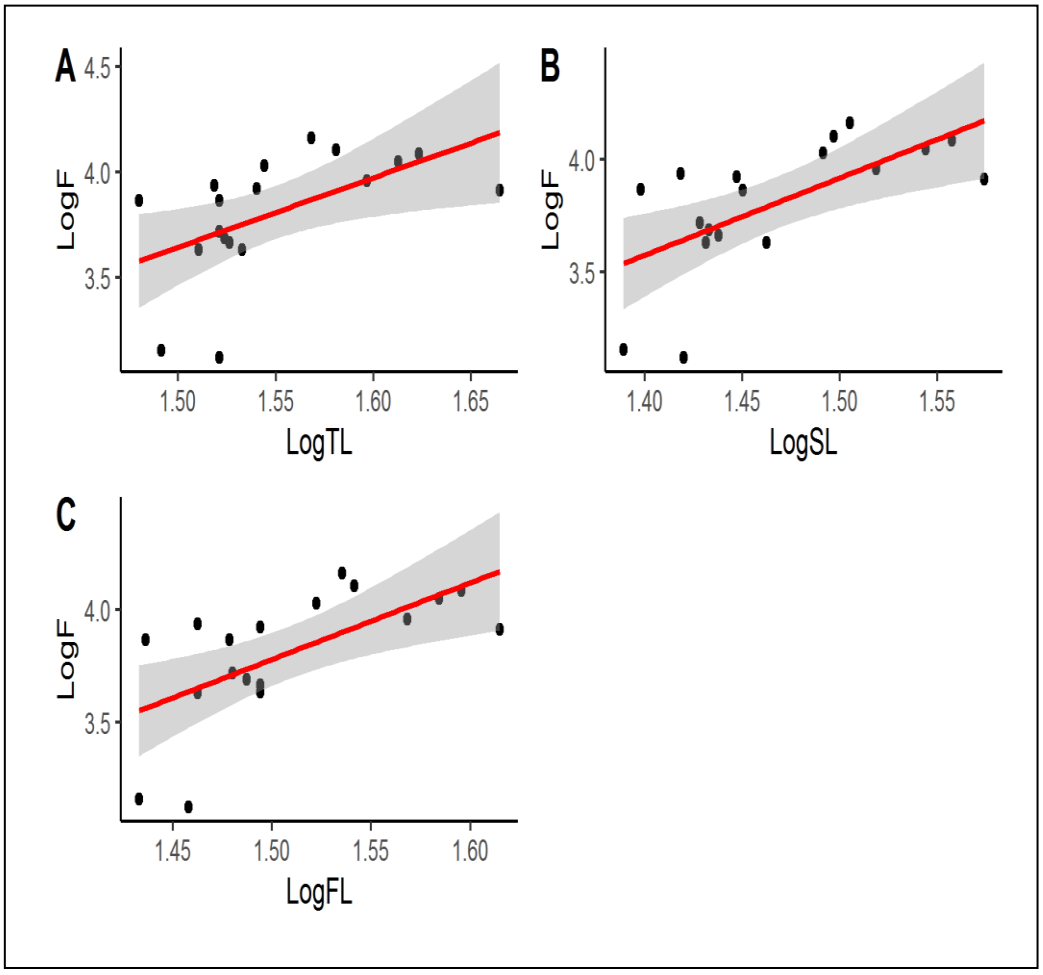

Figure 4.1.2.1: Relationships between absolute fecundity (F) and lengths. (A) LogF vs. LogTL, (B) LogF vs. LogSL, and (C) LogF vs. LogFL of *N. hexagonolepis* from Tamor River, Nepal.

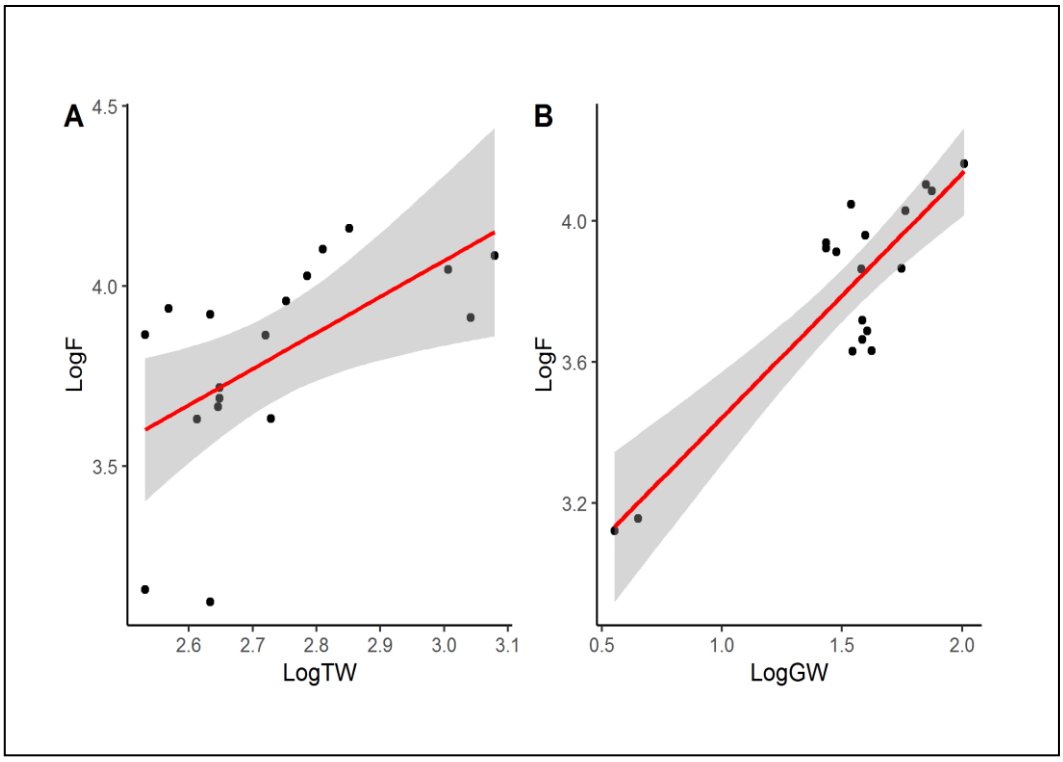

Figure 4.1.2.2: Relationships between absolute fecundity (F) and weights. (A) LogF vs. LogTW, and (B) LogF vs. LogGW of *N. hexagonolepis* from Tamor River, Nepal.

4.1.2.2 Egg size

The smallest mean size of eggs measuring 1.28 ± 0.22 mm in diameter was recorded for the fish that measured 33.2 cm TL, 430 g TW, 3.58 g GW and with 1324 eggs and the largest mean size of eggs measuring 2.34 ± 0.05 mm in diameter was recorded in the fish that measured 33.6 cm TL, 442 g TW, 38.42 g GW and with 4610.4 eggs (Table 8).

Table 8: Total weight (TW), total length (TL), absolute fecundity (F) and mean size of eggs from sampled *N. hexagonolepis* from Tamor River, Nepal from December 2014 to November 2016.

TW (g)	TL (cm)	GW (g)	F	Mean egg size ± SD
410	32.4	35	4270	1.98 ± 0.09
525	33.2	38	7296	2.14 ± 0.13
1200	42	75	12150	2.23 ± 0.08
710	37	102	**14484**	2.08 ± 0.11
340	30.2	56	7336	2.26 ± 0.09
535	34.1	42	4284	2.31 ± 0.07
610	35	58	10672	1.98 ± 0.09
445	33.2	38.4	5222.4	2.05 ± 0.12
442	33.6	38.42	4610.4	**2.34 ± 0.05**
445	33.4	40.24	4869.04	2.31 ± 0.11
565	39.5	39.63	9075.27	2.01 ± 0.10
1015	41	34.47	11133.8	1.86 ± 0.26
645	38.1	70.82	12676.8	1.98 ± 0.09
430	34.7	27.2	8350.4	1.70 ± 0.11
340	31	4.48	1433.6	1.32 ± 0.22
1100	46.2	30	8160	1.37 ± 0.22
370	33	27.2	8649.6	1.62 ± 0.21
430	33.2	3.58	**1324.6**	**1.28 ± 0.22**

(Abbreviations: TW, Total weight; TL, Total length; GW, Gonad weight; F, Absolute fecundity; SD, Standard deviation)

Fifteen eggs were randomly selected from the ovaries at III (Ripening), IV (Mature) and V (Spawning) stages and their diameters measured to the nearest 0.01 mm. After that, the eggs were grouped in the intervals of 0.2 mm and their frequencies calculated.

Most of the eggs were in the size range of 1.21 to 1.4 mm in the ovaries at the III stage of ripening stage. No eggs larger than 2.4 mm in size were spotted in the ovaries at stage III. The majority of the eggs in the ovaries at stage IV (Mature) were

from 2.21 to 2.4 mm. No eggs in the range of 1.01 to 1.40 mm in size were recovered from the ovaries at this stage. Similarly, the bulk of the eggs in the stage V (Spawning) ovaries were in the range of 1.81 to 2.0 mm. No eggs in the range of 1.01 to 1.2 and 2.41 to 2.6 mm in size were detected in the ovaries at this stage (Fig. 4.1.2.3).

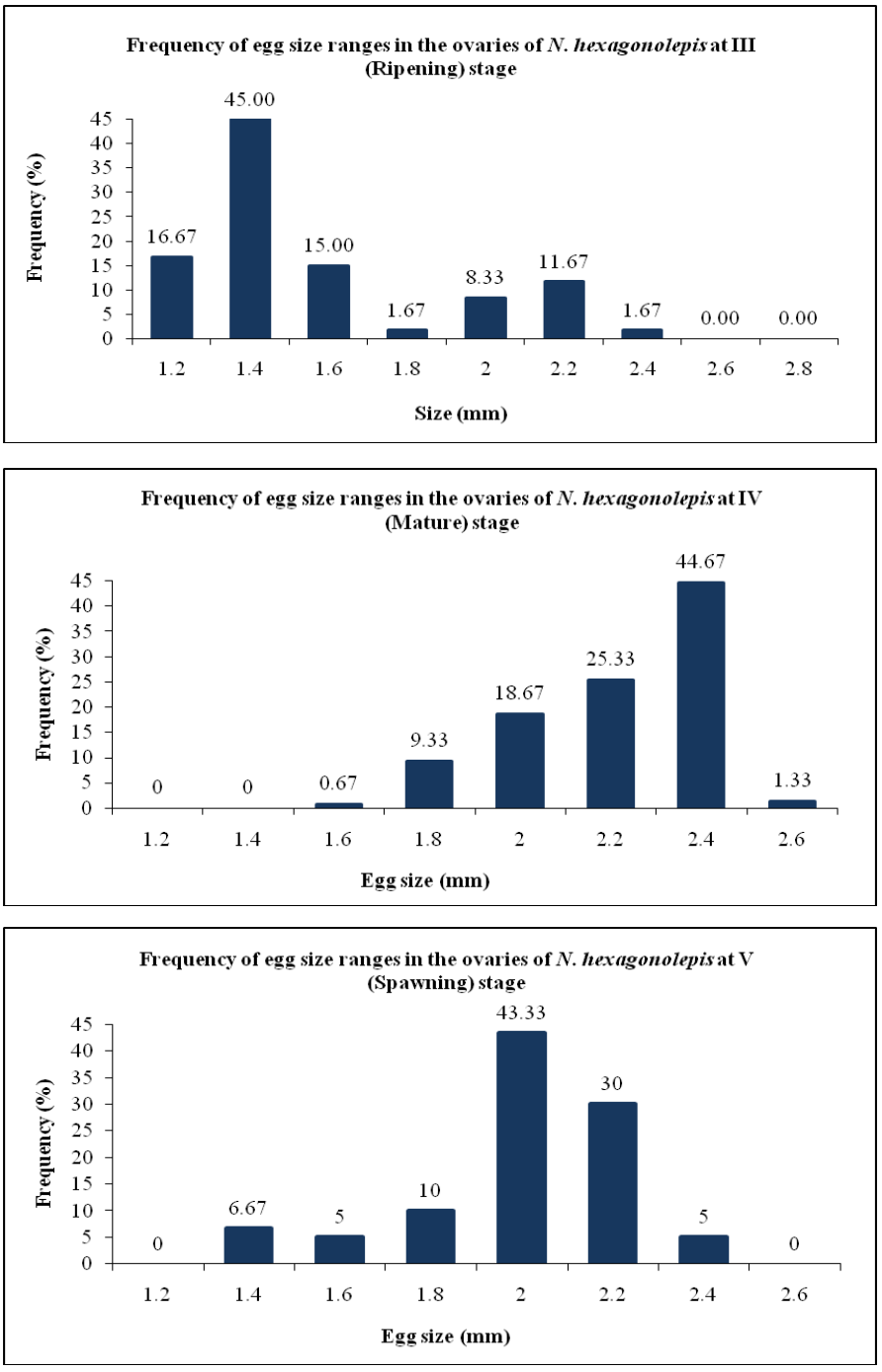

Figure 4.1.2.3: Frequency of egg size ranges in the ovaries of *N. hexagonolepis* at III (Ripening), IV (Mature) and V (Spawning) stages (Egg size ranges: 1.2 = 1.01-1.2; 1.4 = 1.21-1.4; 1.6 = 1.41-1.6; 1.8 = 1.61- 1.8; 2 = 1.81-2; 2.2 = 2.01-2.2; 2.4 = 2.21-2.4 and 2.6 = 2.41-2.6 mm).

The correlations between egg size (ES) and biometric variables of *N. hexagonolepis* (TL, SL, FL, TW, GW and F) were determined by Spearman's rank correlation test. The test showed that the egg size (ES) was positively correlated with all the variables except TL. However, the associations were all insignificant except ES and GW, significant at $p<0.01$ (Table 9).

Table 9: Spearman rank correlations and significance values for biometric variables with egg size (sample size = 18) of *N. hexagonolepis* from Tamor River, Nepal.

			ES	TL	SL	FL	TW	GW	F
Spearman's rho (ρ)	Egg size (ES)	Correlation coefficient	1	- 0.01	0.08	0.09	0.64	0.68**	0.02
		Significance level (p)	.	0.95	0.73	0.73	0.12	0.001	0.94

**. Correlation significant at 0.01 level.
(Abbreviations: ES, Egg size; TL, Total length; SL, Standard length; FL, Forkal length; TW, Total weight; GW, Gonad weight; F, Absolute fecundity; p, Significance level)

4.1.2.3 Gonado-somatic index (GSI)

The monthly mean GSI of male *N. hexagonolepis* were low at 0.17 in December 2014, remained almost in steady-state till April 2015, increased sharply to 1.48 in May 2015 and rose to a peak at 2.12 in July 2015 (Fig. 4.1.2.4). Subsequently, there was a precipitous recession in GSI from September 2015 onward, oscillating at times, reaching 0.33 in March 2016. After that the GSI remained almost in a steady-state till June and then rose to a peak at 3.27 in July 2016 receding sharply to 0.94 in August 2016. Similarly, the GSI of female fish were high at 2.09 in May 2015, dropped marginally to 2.01 in June 2015 and caught up to a well-defined crest of 10.57 in July 2015. Then, the GSI receded acutely to 0.28 in September, oscillated marginally from October 2015 till April 2016 and descended substantially to 0.63 from 1.36 in May before soaring to 5.99 in July 2016. After that, the GSI of female *N. hexagonolepis* again receded precipitously to 0.13 in September 2016 (Fig. 4.1.2.4).

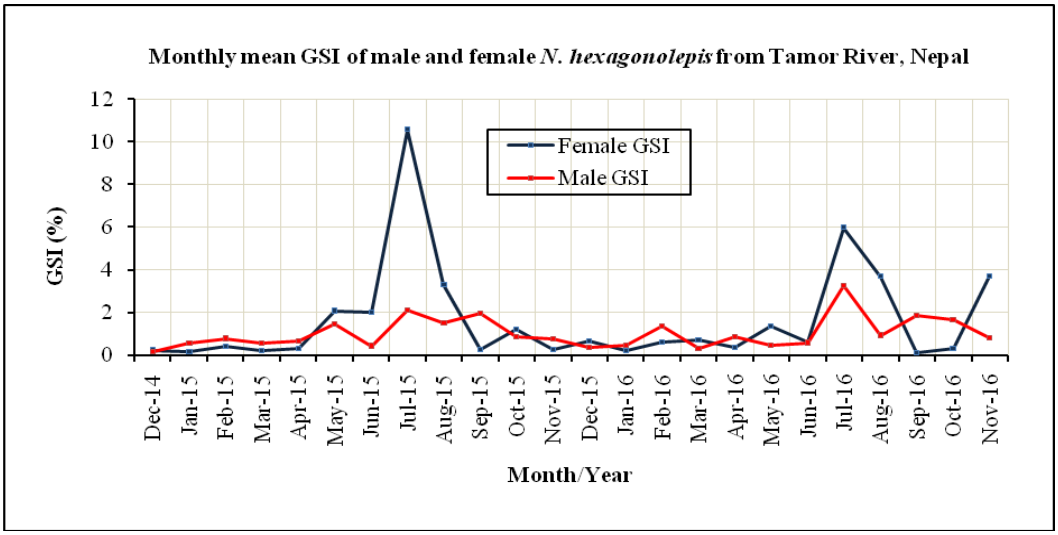

Figure 4.1.2.4: Monthly mean GSI of male and female *N. hexagonolepis* from Tamor River, Nepal from December 2014 to November 2016.

4.1.3 Histomorphological features and maturation cycle of gonads of *Neolissochilus hexagonolepis* from Tamor River, Nepal.

4.1.3.1 Histomorphological features of gonads

N. hexagonolepis is a prominent cold-water species that thrives well in the torrential rivers of the hilly region. Given the gravity of conservation of wild population for the sustainable fishery the present research was carried out. A comprehensive inspection of gonads helps in acquiring the essential information on the reproductive aspects such as the developmental stages and maturation cycle of fishes, which are the pre-requisites for establishing the protocols for the conservation of fish.

Altogether 198 fish samples were collected during the study period and the fishes were sorted out according to sex. The independent χ^2 test was employed to determine the deviation from the 1:1 sex ratio as the null hypothesis for the whole sample and each of the 3 cm fish size classes making 12 size classes (TL, cm) in total. The average annual sex ratio was in favour of females (Male: Female = 1:1.22). However, the χ^2 test showed no significant difference between the sexes ($\chi^2 = 2.0202$, $p>0.05$; Table 10).

The result of the χ^2 test showed the dominance of male fish in the size classes 12 to 15 cm, 15 to 18 cm, 18 to 21 cm and 24 to 27 cm. However, this male-biased

distribution was not significant in all the classes except in the size class 18 to 21 cm (χ^2 = 4.8286, p<0.05; Table 10). Female dominated in the size classes above 27 cm with significant differences in the size groups 33 to 36 cm (χ^2 = 13.5, p<0.05), 36 to 39 cm (χ^2 = 5.3333, p<0.05) and 39 to 42 cm (χ^2 = 6, p<0.05). There were insignificant female biases in size class>39 cm where 100% were females (Table 10).

Table 10: χ^2 test of the sex ratio in 12 size classes of *N. hexagonolepis* from Tamor River, Nepal from December 2014 to November 2016.

Total length (TL, cm)	No. of fish			Sex ratio	χ2	P
	Male	Female	Total			
12 – 15	10	7	17	1:0.70	0.52941	0.4669
15 – 18	23	16	39	1:0.70	1.2564	0.2623
18 – 21	24	11	35	1:0.46	4.8286	0.02799*
21 – 24	5	12	17	1:2.40	2.8824	0.08956
24 – 27	8	2	10	1:0.25	3.6	0.05778
27 – 30	5	6	11	1:1.2	0.090909	0.763
30 – 33	9	12	21	1:1.33	0.42857	0.5127
33 – 36	3	21	24	1:7	13.5	0.0002386*
36 – 39	2	10	12	1:5	5.3333	0.02092*
39 – 42	0	6	6	6	6	0.03148*
42 – 45	0	3	3	3	3	0.2509
45 – 48	0	3	3	3	3	0.2509
Total	89	109	198	1:1.22	2.0202	0.1552

*Indicates significant difference at 0.05 level.
(Abbreviations: TL, Total length; χ2, Chi square; p, Significance value)

The testes were paired, elongated structures situated on either side, ventral to the kidneys in the abdominal cavity's posterior region. They were attached to the body wall through mesorchia and showed indentations along their margin. The sperm ducts joined posteriorly to open into the urinogenital papillae.

Histology revealed the testes of *N. hexagonolepis* as the lobular type consisting of a large number of seminiferous lobules bounded together by a thin layer of connective tissue. Blood capillaries and interstitial cells were seen dispersed in the

connective tissue. The resting germ cells transformed into sperm mother cells or spermatogonia within the lobules. The spermatogonia were large, spherical cells containing large, round and centrally placed nucleus. These cells multiplied to give rise to primary spermatocytes which, in turn, gave rise to secondary spermatocytes. Secondary spermatocytes divided further to produce spermatids (Fig. 4.1.3.1). The spermatids then transformed to spermatozoa or sperms. During the matured or spawning phase, the seminiferous lobules were seen congested with sperms.

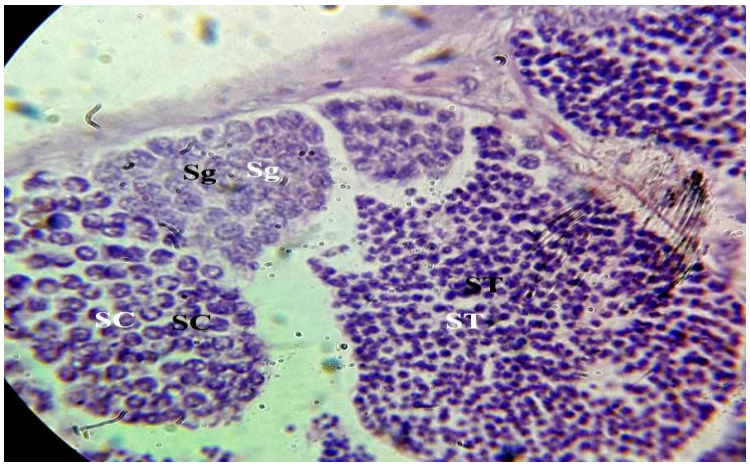

Figure 4.1.3.1: Photomicrograph of T.S. of testes of *N. hexagonolepis* showing nest of spermatogonia (Sg), spermatocytes (SC), spermatids (ST) X 1000 (H & E).

Based on the histomorphology, the testes of *N. hexagonolepis* were observed at six different stages viz. Stage I (Immature stage), Stage II (Maturing virgin), Stage III (Ripening), Stage IV (Mature), Stage V (Spawning) and stage VI (Spent) (Fig. 4.1.3.2 and Table 11).

At stage I (Immature stage), the testes were slender, thread-like, dirty white without a vascular supply. Histologically, they presented very thick tunica albuginea (testicular wall) sending several septs inward forming seminiferous lobules. The seminiferous lobules were of the cystic type and within each cyst were present spermatogenic cells. The spermatogonia were of two types viz. primary and secondary spermatogonia among which the primary spermatogonia were the most extensive of the spermatogenic lineage. They contained clear cytoplasm, large and prominent nucleus. While the primary spermatogonia occurred in isolation the secondary spermatogonia were smaller and occurred in cysts of 2 to 4 cells.

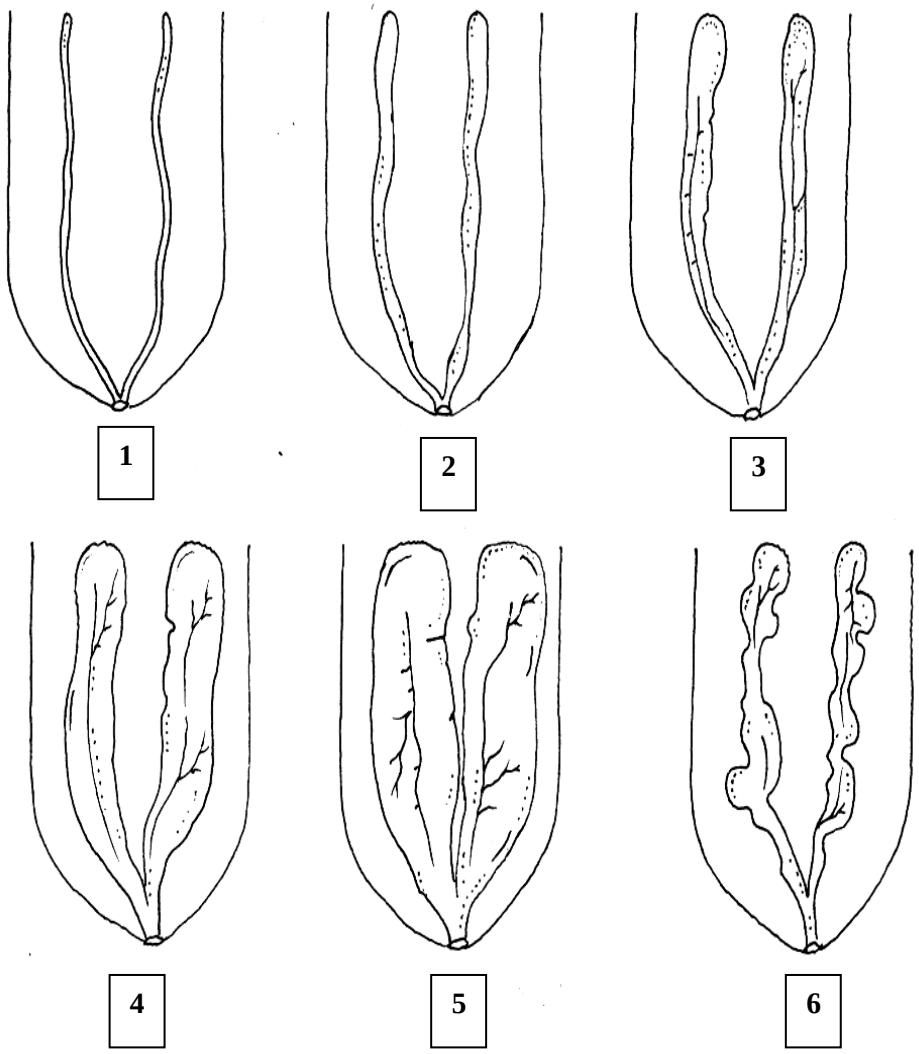

Figure 4.1.3.2: Diagrammatic drawings of testes of *N. hexagonolepis* at various stages of maturity: 1. Immature 2. Maturing virgin 3. Ripening 4. Mature 5. Spawning 6. Spent

Table 11: Description of the gonad maturation stages of male *N. hexagonolepis* at macroscopic and histological level.

Maturity stages	Macroscopic features	Histological features	Frequency (n)
Stage I (Immature)	Testes slender, thread-like, dirty white without vascular supply.	Testicular wall (Tunica albuginea) thick. Cysts of spermatogenic cells visible. Larger primary spermatogonia and smaller secondary spermatogonia seen.	25
Stage II (Maturing virgin)	Testes slender but larger than in stage I. Whitish with vascular supply occupying about 1/3 of the body cavity.	Testicular wall thick. Spermatogonia (Primary and secondary spermatogonia) seen. Spermatogonia seemed to be dividing giving rise to spermatocytes.	30
Stage III (Ripening)	Testes larger. Increased in weight and volume occupying about 2/3 of the body cavity. Whitish in colour with conspicuous blood supply.	Testicular wall thin. Intense spermatogenesis observed. Seminiferous lobules filled with primary and secondary spermatocytes with decreased spermatogonia. All the stages of spermatogenesis viz. spermatocytes, spermatids and spermatozoa observed.	12
Stage IV (Mature)	Testes large, turgid occupying almost the entire body cavity. Appeared opaque, white-pinkish in colour with conspicuous vascularization.	Testicular wall thin and seminiferous lobules packed with spermatozoa. Few spermatids also visible.	8
Stage V (Spawning)	Testes pinkish white, turgid and occupying the entire body cavity. Milt was seen to be oozing out.	Seminiferous lobules packed with spermatozoa with some empty lobules.	7
Stage VI (Spent)	Testes dull-white and appeared flaccid. They were blood shot and flabby with decreased weight and volume.	Seminiferous lobules seen collapsing and empty. Small amount of residual or unexpelled sperms observed.	7

At stage II (Maturing virgin) the testes were slender as in stage I but were a bit larger, whitish with vascular supply. Histologically, the spermatogonia were seen dividing into primary and secondary spermatocytes. Secondary spermatocytes were smaller than the primary ones.

At stage III (Ripening), the testes were larger, heavier and whitish and were observed to occupy about 2/3 of the body cavity. Histologically, they presented numerous primary and secondary spermatocytes. Intense spermatogenesis was

observed during this stage and all the stages of spermatogenesis viz. spermatocytes, spermatids and spermatozoa were observed within the lobules of testes at this stage. Spermatids contained scanty cytoplasm with a spherical and dense nucleus. Spermatozoa were the smallest cells.

At stage IV (Mature stage) the testes were large and turgid occupying almost the entire body cavity. They were whitish-pink with extensive vascular supply. Histologically, the seminiferous lobules were seen to be packed with spermatozoa with some spermatids as well (Fig. 4.1.3.3).

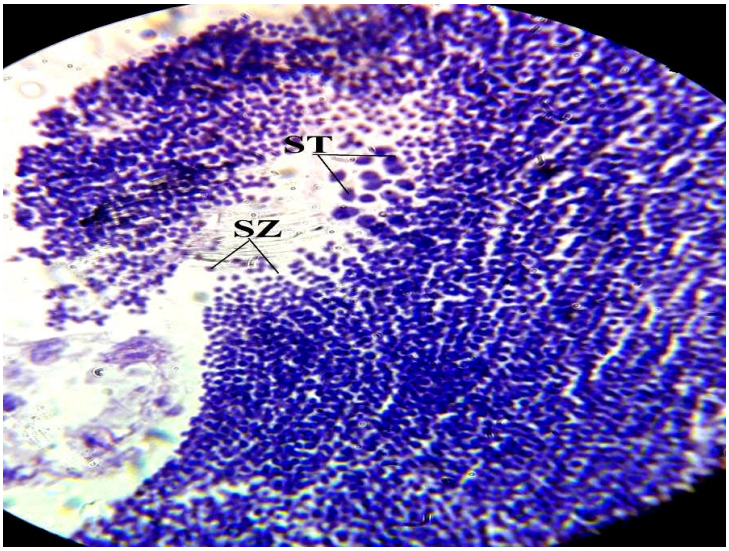

Figure 4.1.3.3: Photomicrograph of T.S. of testes of *N. hexagonolepis* at mature stage showing bulk of spermatozoa (SZ) and a few spermatids (ST) X1000 (H & E).

At stage V (Spawning stage) the testes were pinkish-white, turgid and occupying the entire body cavity. Milt was seen to be oozing out. Histologically, the seminiferous lobules were seen to be packed with spermatozoa with some empty lobules.

At stage VI (Spent stage), the testes were dull whitish. They were flaccid, blood-shot and flabby with much-reduced weight and volume. Histologically, the seminiferous lobules were seen to be collapsing and empty and contained only a small amount of unexpelled or residual sperms (Fig. 4.1.3.4).

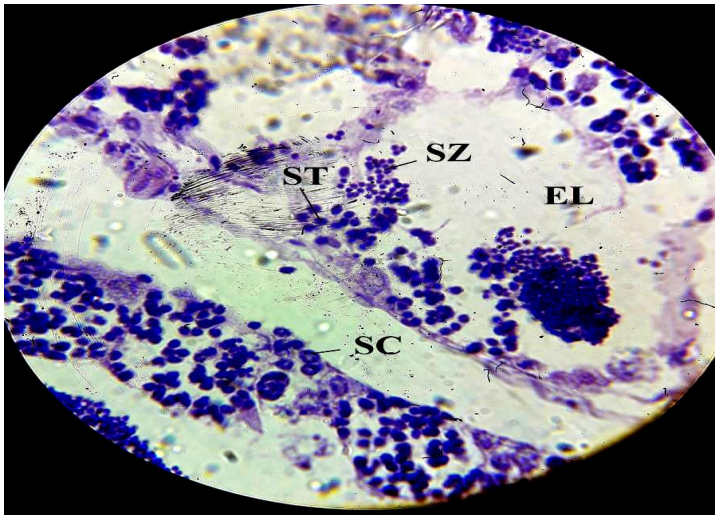

Figure 4.1.3.4: Photomicrograph of T.S. of testes of *N. hexagonolepis* at spent stage showing spermatocytes (SC), spermatids (ST), spermatozoa (SZ) and empty lumen (EL) X 1000 (H & E).

Similarly, the ovaries of *N. hexagonolepis* were observed to be a typical teleostean type being paired, elongated, sac-like structures lying in the abdominal cavity ventral to the kidneys and attached to the body wall through mesovarium. The ovaries were of the cystovarian type and the oocytes were conveyed to the exterior through the oviduct. Based on shape, size, colour and other histomorphological features, the ovaries of *N. hexagonolepis* were staged into six maturity stages (Fig. 4.1.3.5 and Table 12).

At stage I (Immature stage), the ovaries were thin, slender, thread-like, dirty white. Histologically, the ovarian wall was seen to be thick. Ovigerous lamellae with nests of oogonia and oocytes at stages I and II were observed.

At stage II (Maturing virgin), the ovaries were slender but slightly thicker than those in the immature stage. They were translucent white with inadequate vascular supply. Histologically, the ovaries showed the ovigerous lamellae laden with oocytes at stages I, II, III and IV.

At stage III (Ripening stage) the ovaries turned yellowish in colour with granular appearance due to the presence of smaller pale yellowish oocytes even visible to naked eyes. There was a further increase in the weight of the ovaries. Histologically, the ovaries at III stage contained the oocytes at stages IV and V. There were also a few oocytes at stage VI.

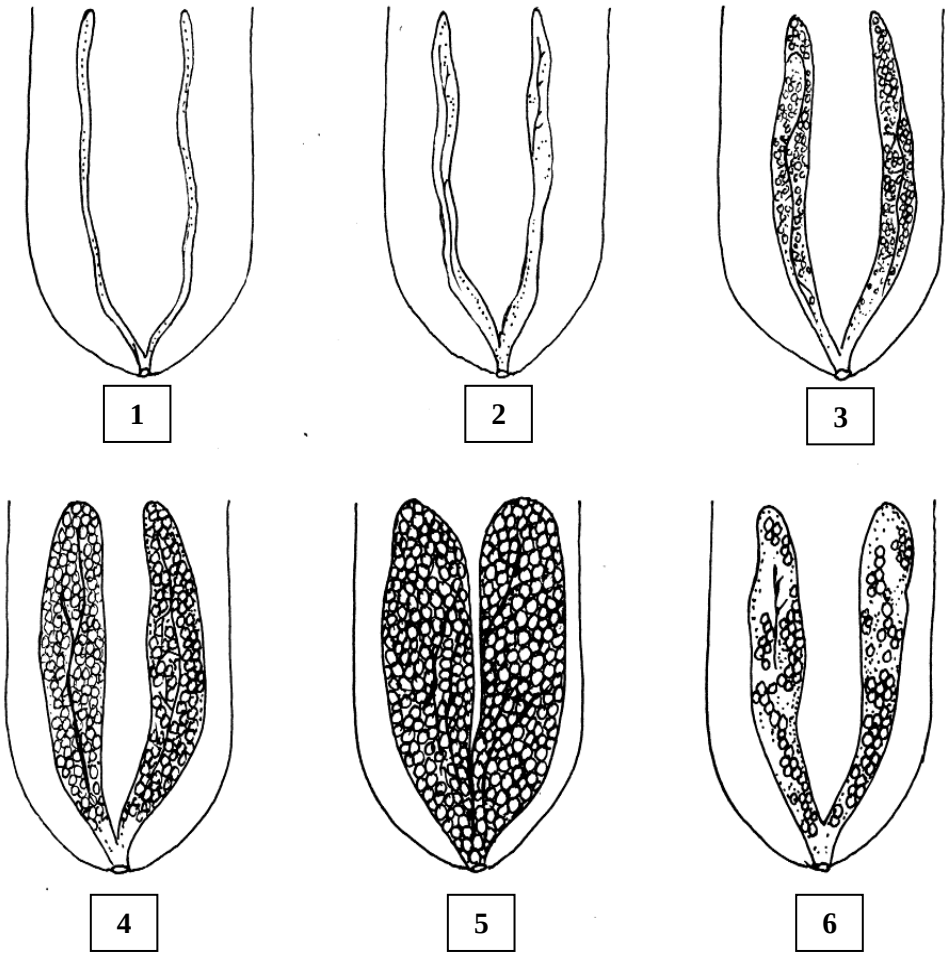

Figure 4.1.3.5: Diagrammatic drawings of ovaries of *N. hexagonolepis* at various stages of maturity: 1. Immature 2. Maturing virgin 3. Ripening 4. Mature 5. Spawning 6. Spent

Table 12: Description of the gonad maturation stages of female *N. hexagonolepis* at macroscopic and histological level.

Maturity stages	Macroscopic features	Histological features	Frequency (n)
Stage I (Immature)	Ovaries thin, slender, thread-like, dirty white in colour and translucent.	Ovarian wall thick. Ovigerous lamellae with nests of oogonia. Oocytes at stages I and II visible.	39
Stage II (Maturing virgin)	Ovaries still slender but slightly larger than at stage I. Translucent white with less vascular supply.	Ovarian wall thick. Oocytes at stages III and IV along with a few oocytes at stages I and II visible in ovigerous lamellae.	31
Stage III (Ripening)	Ovaries whitish-yellowish in colour with granular appearance. Ovaries further increased in weight and volume.	Ovarian wall thin. Ovigerous lamellae filled with large number of oocytes at stages IV and V along with a few oocytes at stage VI.	20
Stage IV (Mature)	Ovaries large and deep yellowish in colour occupying almost the entire abdominal cavity. Ovarian wall very thin through which ripe yellowish oocytes visible to naked eyes. Vascularization conspicuous.	Ovarian wall very thin. Large number of stage VII oocytes and some ripe eggs visible.	10
Stage V (Spawning)	Ovaries large and distended, occupying the entire abdominal cavity. Ovarian wall thin and almost transparent. Large number of jelly like yellowish translucent eggs present in the ovaries. Eggs were present in the oviduct also and the eggs expelled out even on applying slight pressure. Spawning was imminent.	Stage VII oocytes and ripe eggs seen in the ovigerous lamellae with a number of discharged follicles.	5
Stage VI (Spent)	Ovaries flaccid, shrunken to about ½ length of the body cavity. Wall loosened, some unspawned large ova and large number of smaller yellow-whitish oocytes visible. Less vascular supply.	Oocytes at stage VII along with oocytes at stages I and II visible in the ovigerous lamellae. Ovaries characterized by many degenerated and atretic follicles.	4

At stage IV (Mature stage) the ovaries were large and deep yellow in colour occupying almost the entire abdominal cavity. The ovarian wall was thin and transparent through which ripe yellowish oocytes were visible to naked eyes.

Conspicuous superficial blood vessels were seen in the ovaries. Histologically, the ovaries at this stage were observed to be packed with oocytes at stage VII along with some mature ova.

At stage V (Spawning stage) the ovaries were much distended with a large number of jelly-like yellowish translucent eggs. Eggs were present in the oviduct also, and the eggs were expelled out even with slight pressure on the abdomen. The ovaries were observed to be in the running phase. Histologically, stage VII oocytes and ripe eggs were seen in the ovigerous lamellae with several discharged follicles.

At stage VI (Spent stage) the ovaries appeared flaccid and turned dull coloured. Vascular supply was seen to be reduced and the wall of the ovaries loosened. Some unspawned ova and a large number of smaller whitish oocytes were visible to naked eyes. Histologically, many post-ovulatory and atretic follicles were observed (Fig. 4.1.3.6). Oocytes at stages I and II were also observed in the ovigerous lamellae.

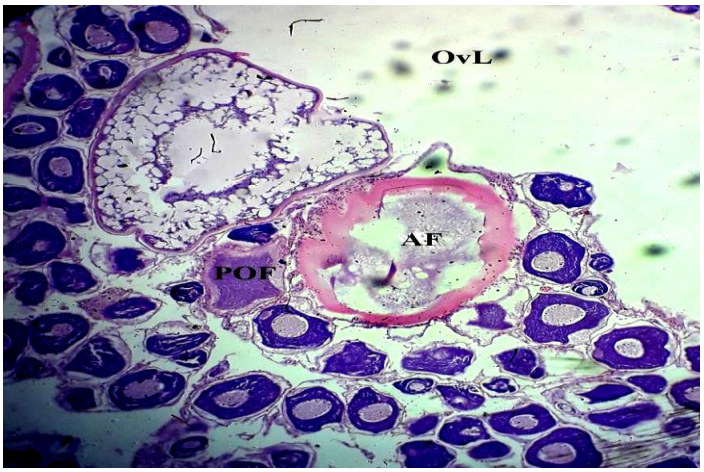

Figure 4.1.3.6: Photomicrograph of T.S. of ovary of *N. hexagonolepis* showing ovarian lumen (OvL), atretic follicle (AF) and post-ovulatory follicle (POF) X100 (H & E).

Oocytes at various development stages were observed in the ovigerous lamellae of the developing ovaries (Fig. 4.1.3.7). 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.

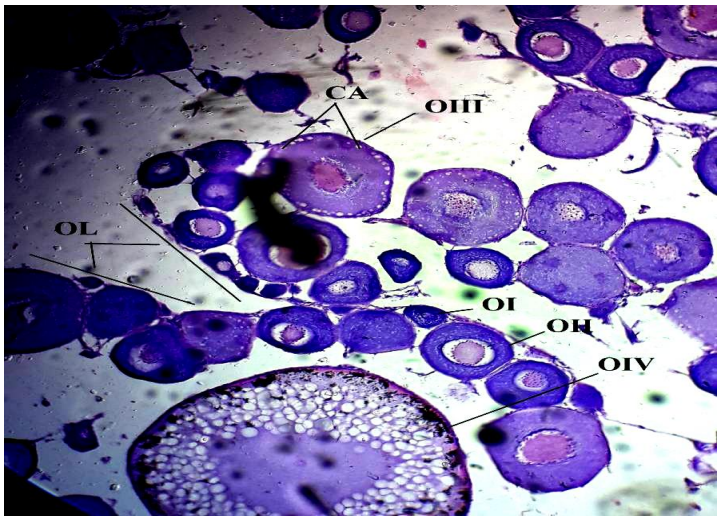

Figure 4.1.3.7: Photomicrograph of T.S. of ovary of *N. hexagonolepis* showing ovigerous lamellae containing oocytes at various stages of development (Abbreviations: OL - Ovigerous lamellae, CA - Cortical alveoli, OI - Oocyte I, OII - Oocyte II, OIII - Oocyte III and OIV - Oocyte IV) X 100 (H & E).

Oocyte I was the smallest oocyte with a very thin sheath of cytoplasm and a prominent round nucleus consisting of 3 to 4 nucleoli (Fig. 4.1.3.8). This stage is also referred to as the chromatin nucleolus stage.

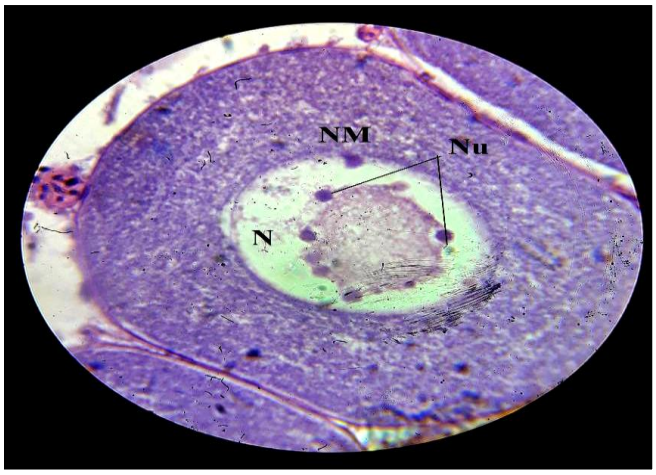

Figure 4.1.3.8: Photomicrograph of T.S of ovary of *N. hexagonolepis* showing stage I oocyte with multiple nucleoli (Nu), Nuclear membrane (NM) and Nucleus (N) X 1000 (H & E).

Oocyte II was larger than oocyte I and contained cytoplasm which stained deep. The oocytes at this stage were recognized as the early and late perinucleolus stage based on the distribution of the nucleoli within the nucleus. Nucleoli increased in number as the oocytes matured and got distributed adjacent to the periphery of the nuclear membrane (Fig. 4.1.3.9 A, B).

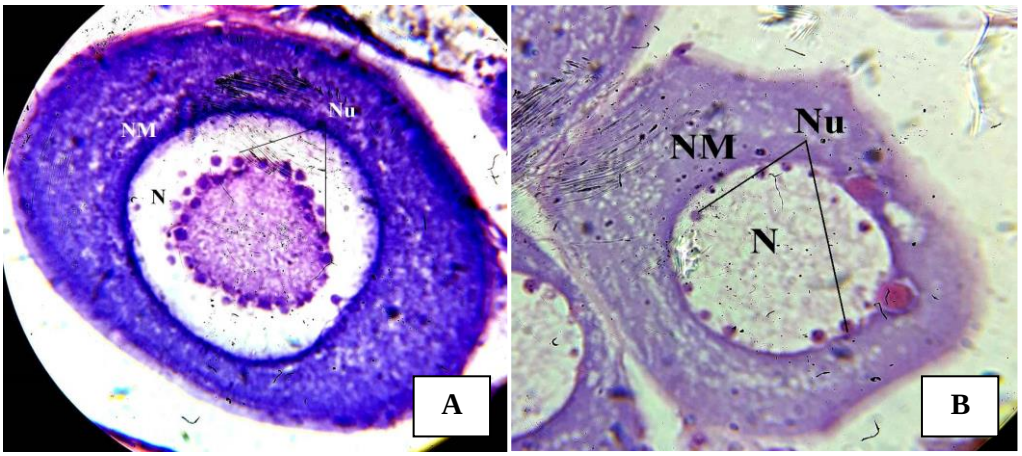

Figure 4.1.3.9: Photomicrograph of T.S. of ovary of *N. hexagonolepis* showing stage II oocyte. A: Early perinucleolus stage with multiple nucleoli X 1000 (H & E). B: Late perinucleolus stage with multiple nucleoli (Nu) adjacent to the nuclear membrane (NM) X 1000 (H & E) (Abbreviations: NM - Nuclear membrane, N – Nucleus, Nu – Nucleoli) X 1000 (H & E).

Oocyte III was characterized by the appearance of a large number of small, clear vacuoles in the cortical area of the cytoplasm. These vacuoles are the yolk vesicles and the stage is conventionally termed as the early yolk vesicle stage. The nuclear membrane of the oocyte started to appear wavy or irregular in outline (Fig. 4.1.3.10).

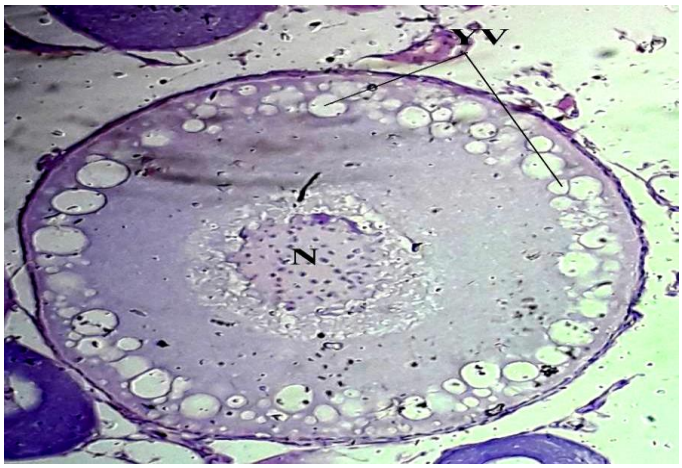

Figure 4.1.3.10: Photomicrograph of T.S. of ovary of *N. hexagonolepis* showing stage III oocyte with yolk vesicles (YV) in the cortical region of the ooplasm X 1000 (H & E).

Oocyte IV was further larger. The yolk vesicles further increased in size and number and were seen distributing randomly in the ooplasm. This stage is referred to as the late yolk vesicle stage. A thin layer of fibroblast known as theca, the 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 (Fig. 4.1.3.11).

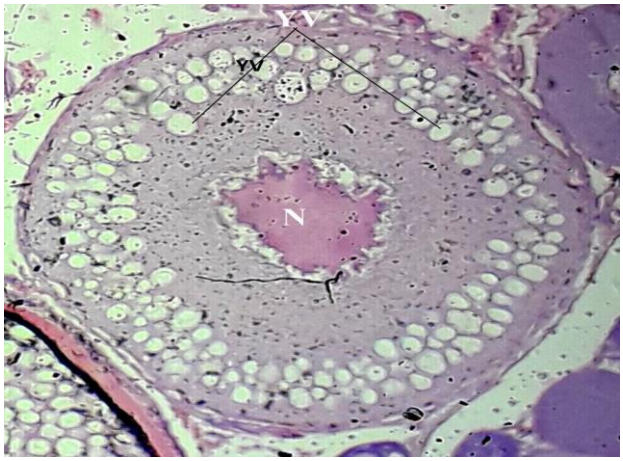

Figure 4.1.3.11: Photomicrograph of T.S. of ovary of *N. hexagonolepis* showing stage IV oocyte with increased size and number of yolk vesicles (YV) X 1000 (H & E).

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 (Fig. 4.1.3.12).

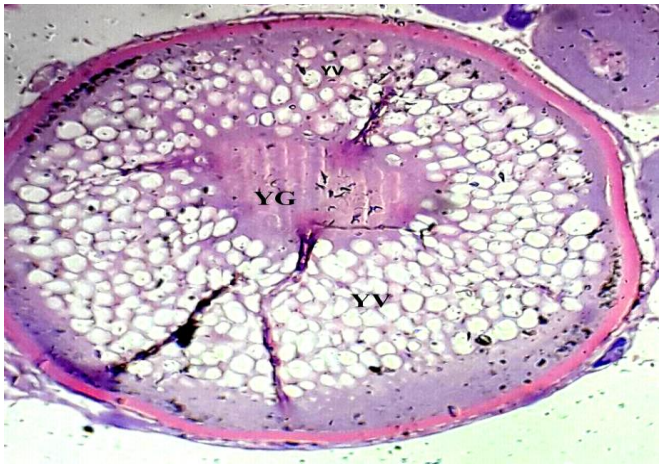

Figure 4.1.3.12: Photomicrograph of T.S. of ovary of *N. hexagonolepis* showing stage V oocyte with yolk vesicles (YV) and yolk globules (YG) X 1000 (H & E).

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 entirely during this stage and the vitelline membrane (zona radiata) became thicker (Fig. 4.1.3.12).

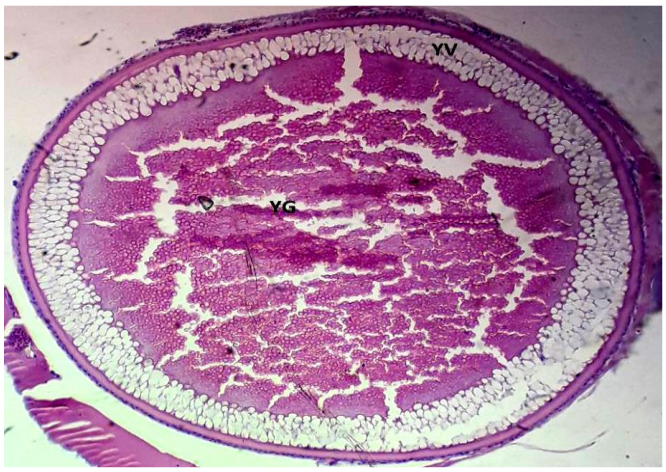

Figure 4.1.3.13: Photomicrograph of T.S. of ovary of *N. hexagonolepis* showing stage VI oocyte with yolk globules (YG) and yolk vesicles (YV) X 1000 (H & E).

Oocyte VII showed 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 the theca, the middle follicular epithelium (zona granulosa) and the innermost zona radiata (Fig. 4.1.3.14). The oocytes at this stage gave rise to ripe eggs.

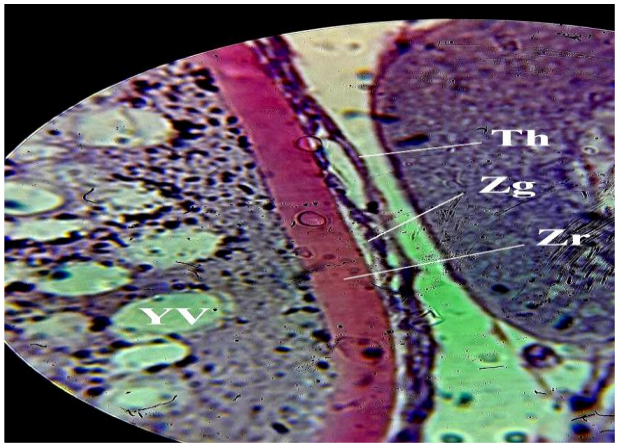

Figure 4.1.3.14: Photomicrograph of T.S. of ovary of *N. hexagonolepis* showing vitellogenic oocyte covered by theca (Th), zona granulosa (ZG) and zona radiata (Zr) X 1000 (H & E).

The ripe eggs of *N. hexagonolepis* were perfectly yellowish in colour and spherical (Fig. 4.1.3.15).

Figure 4.1.3.15: Ripe eggs of *N. hexagonolepis*.

The yolk vesicles began to appear from the peripheral region and distributed towards the inner part of the cytoplasm. As the yolk globules accumulated more and more in the inner area of ooplasm of the oocytes the yolk vesicles were pushed towards the peripheral region of the oocytes forming three or four layers which finally gave rise to cortical alveoli.

4.1.3.2 Maturation cycle of gonads

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 October (Fig. 4.1.3.16 and Appendix X).

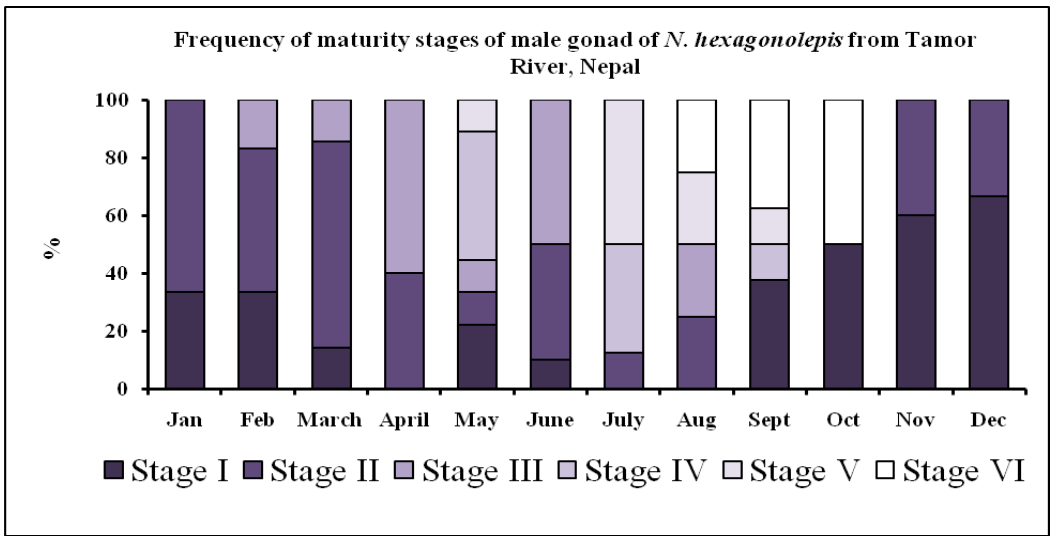

Figure 4.1.3.16: Monthly change in frequency of maturity stages of male gonad of *N. hexagonolepis* from Tamor River, Nepal from December 2014 to November 2016.

Stage II ovaries occurred throughout the year, ranging from 7.69 % in July to 57.14 % in September. 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. No spent ovaries were examined from January onward (Fig. 4.1.3.17 and Appendix XI).

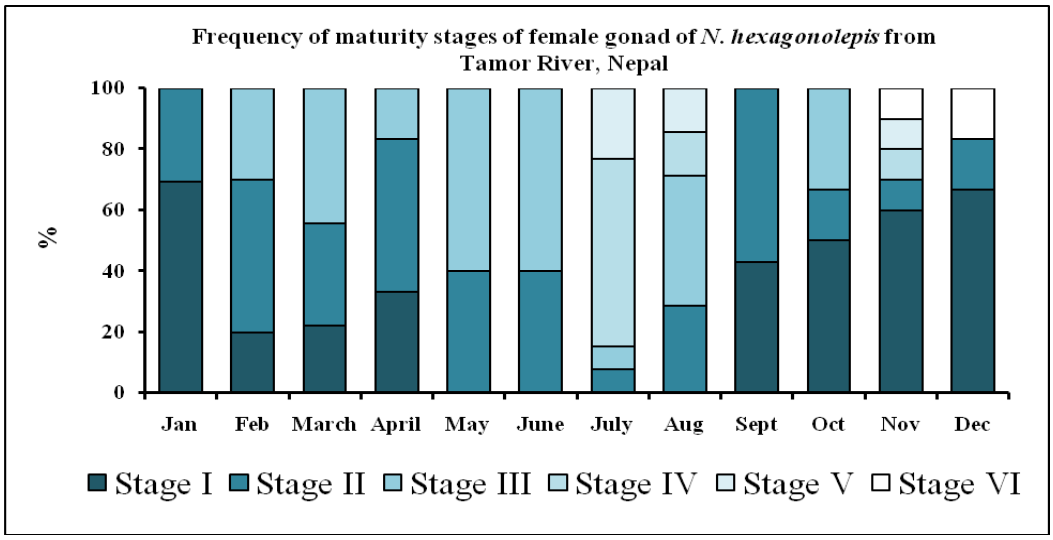

Figure 4.1.3.17: Monthly change in frequency of maturity stages of female gonad of *N. hexagonolepis* from Tamor River, Nepal from December 2014 to November 2016.

4.1.3.3 Size at first sexual maturity

For estimating the size at first sexual maturity (L_{50} Maturity scale) of either sex of *N. hexagonolepis* their gonads were assigned to maturity classes (I to VI) on the basis of their gross histomorphological criteria. On the L_{50} Maturity scale, the samples with gonads at III, IV, V and VI were assigned as mature and I and II as immature. The relation between total length (TL, cm) and mature proportion was plotted on a logistic diagram for estimating the length at 50% maturity. The sexual maturity logistic curves for male and female *N. hexagonolepis* are shown in Fig. 4.1.3.18 and Fig. 4.1.3.19, respectively. The logistic models obtained from the total length (TL) and sexual maturity data indicated that 50% of males acquired sexual maturity at TL 25.5 cm and 50% of females acquired sexual maturity at TL 32.9 cm.

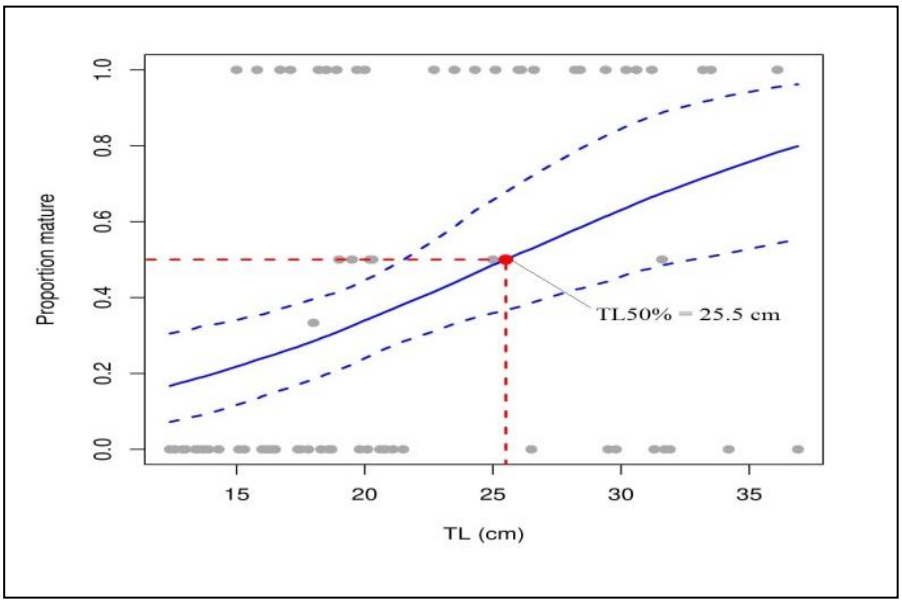

Figure 4.1.3.18: Maturity ogive showing length at maturity of male *N. hexagonolepis* from Tamor River, Nepal.

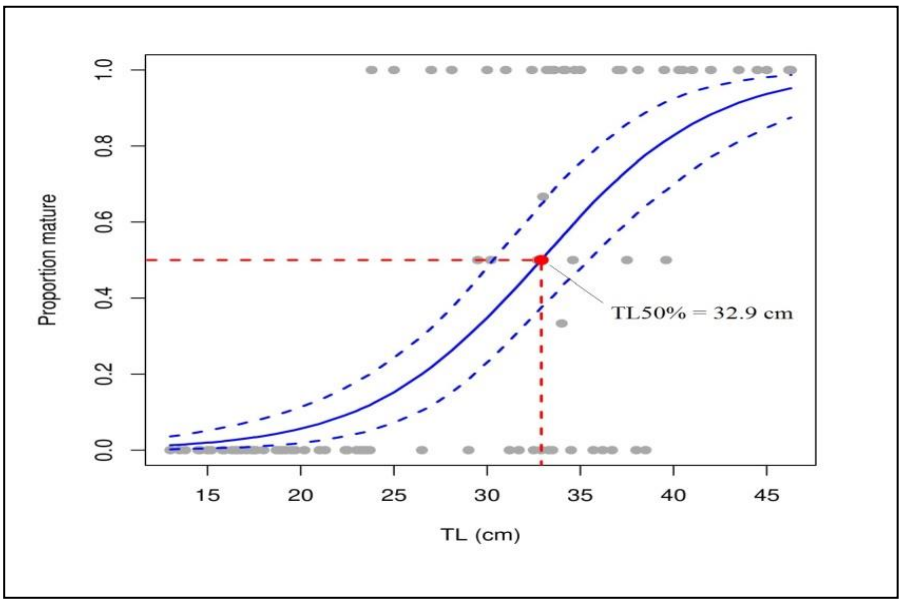

Figure 4.1.3.19: Maturity ogive showing length at maturity of female *N. hexagonolepis* from Tamor River, Nepal.

4.1.4 Physico-chemical parameters of water and their relationship with gonad weight and gonado-somatic index (GSI) of *Neolissochilus hexagonolepis* from Tamor River, Nepal.

The study area recorded practically zero precipitation in December 2014 with sporadic downpour from January 2015 till April 2015 and then heavy shower from May 2015 onwards with the highest reading in July 2015. After that, the readings lowered and showed no precipitation in October 2016, November 2016 and December 2016 but sporadic downpour from January 2016 till April 2016. Henceforth, the readings upsurged through May 2016 and reached the highest in July 2016 again (Fig. 4.1.4.1). Monthly mean sunshine showed inverse relationship with precipitation with lower values during June, July and August during both the study years (Fig. 4.1.4.1).

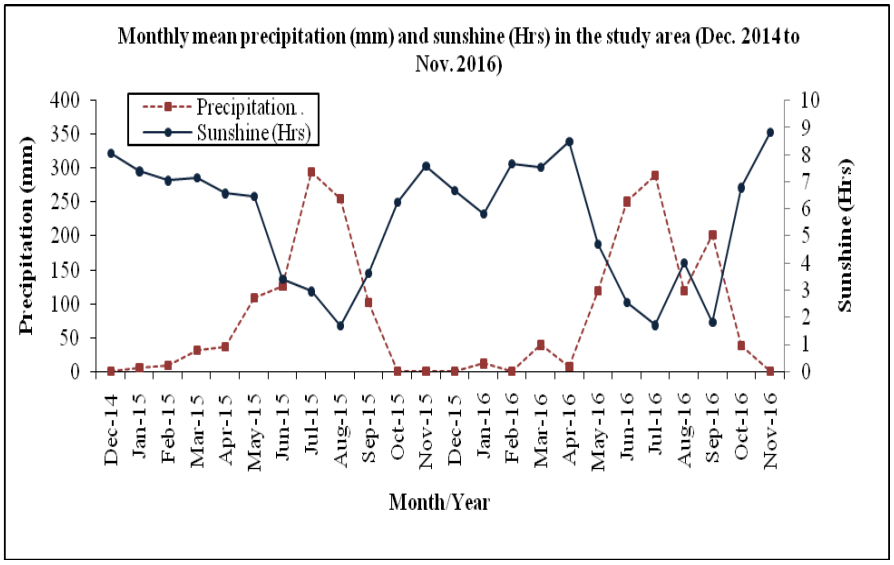

Figure 4.1.4.1: Monthly mean precipitation and sunshine in the study area from December 2014 to November 2016.
(Data source for sunshine: Nepal Agricultural Research Council (NARC), Pakhribas, Dhankuta; Data source for precipitation: Department of Hydrology and Meteorology (DHM), Ministry of Energy, Water Resources and Irrigation, Government of Nepal)

The two-years monthly mean physico-chemical parameter data were pooled for the same month to calculate the annual mean. AT and WT ranged from 8.35°C to 23.1°C and 6.97°C to 16.8°C respectively (Fig. 4.1.4.2, Fig. 4.1.4.3 and Appendix XIV). Similarly, the values of pH, DO, free CO_2, TA and TH varied from 7.02 to 7.84 (Fig. 4.1.4.4), 8.43 mg/l to 11.1 mg/l (Fig. 4.1.4.5), 2.2 mg/l to 3.44 mg/l (Fig. 4.1.4.6), 61.25 mg/l to 77.81 mg/l (Fig. 4.1.4.7) and 24.13 mg/l to 38.88 mg/l (Fig. 4.1.4.8) respectively.

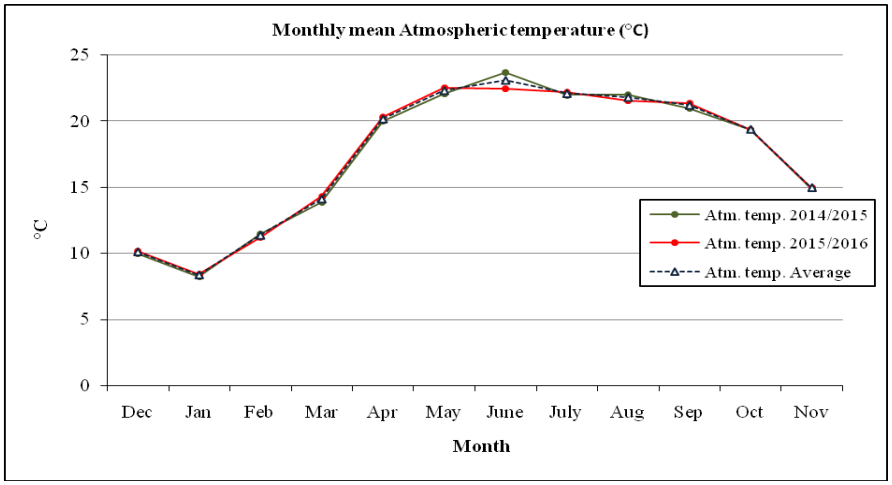

Figure 4.1.4.2: Monthly mean variations of atmospheric temperature from December 2014 to November 2016.

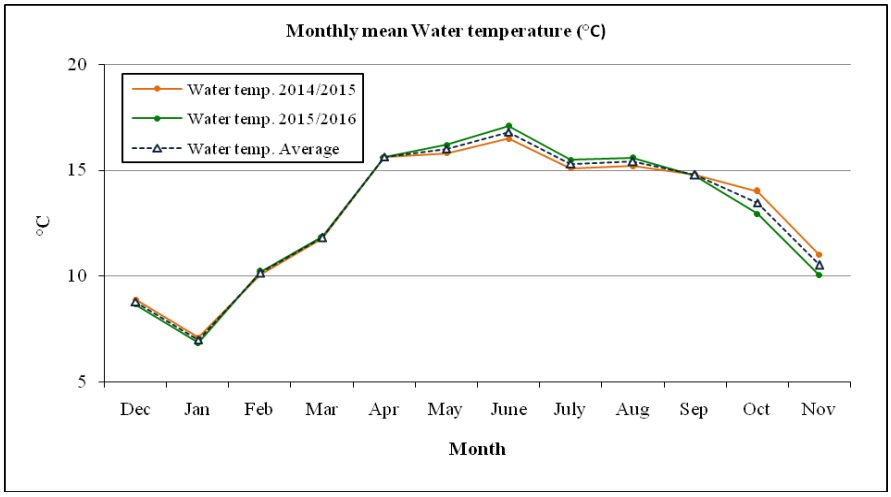

Figure 4.1.4.3: Monthly mean variations of water temperature from December 2014 to November 2016.

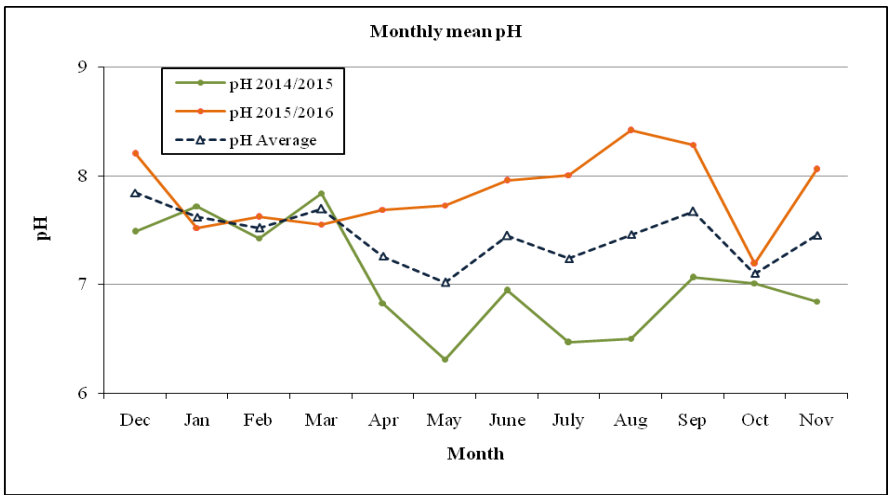

Figure 4.1.4.4: Monthly mean variations of pH from December 2014 to November 2016.

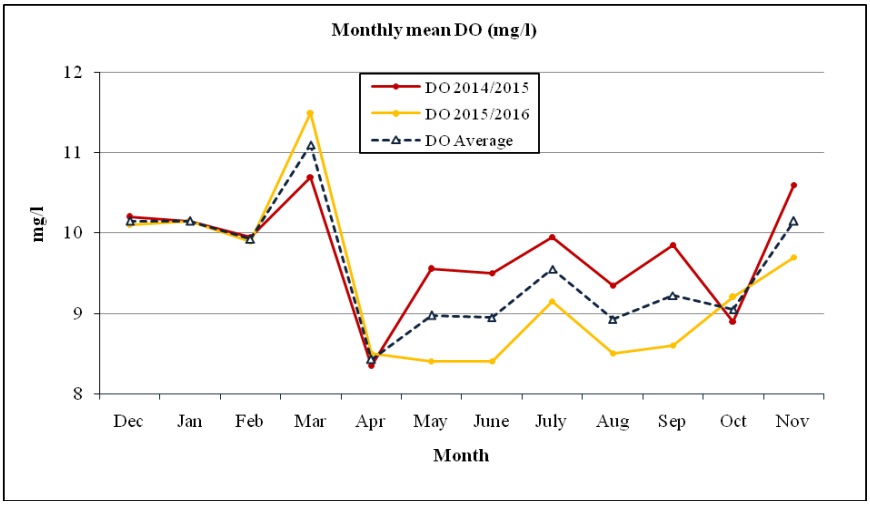

Figure 4.1.4.5: Monthly mean variations of DO from December 2014 to November 2016.

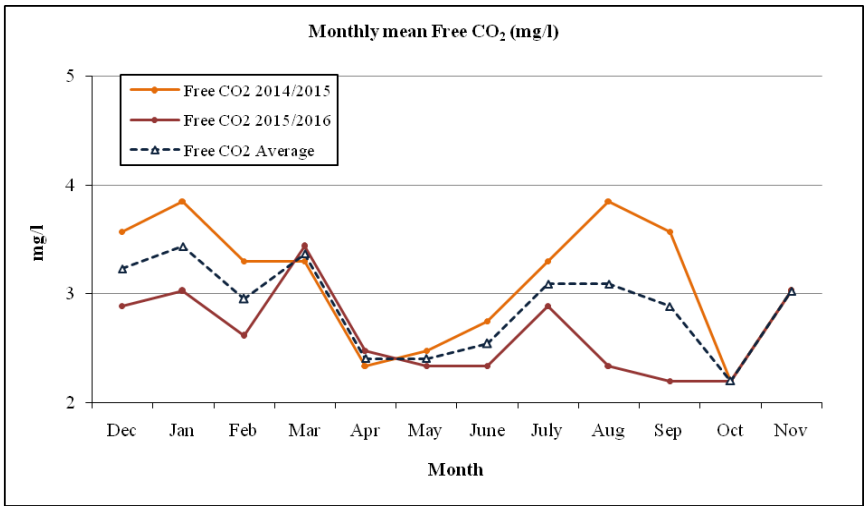

Figure 4.1.4.6: Monthly mean variations of free CO_2 from December 2014 to November 2016.

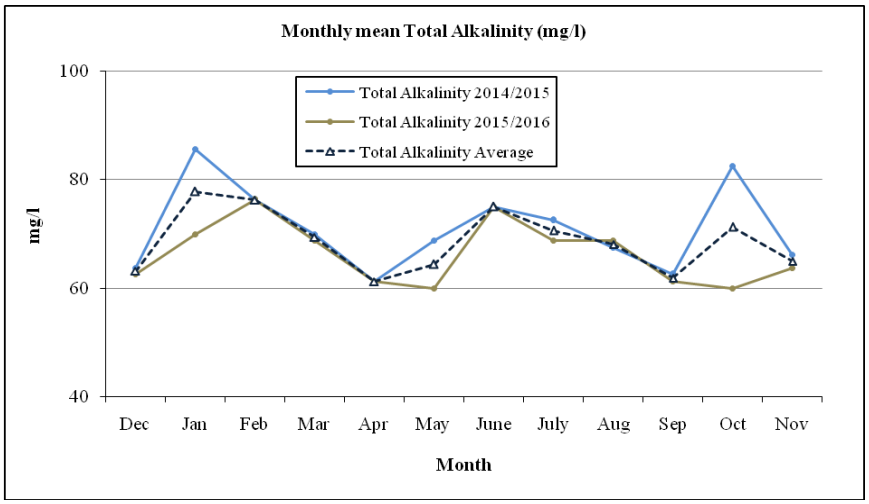

Figure 4.1.4.7: Monthly mean variations of total alkalinity from December 2014 to November 2016.

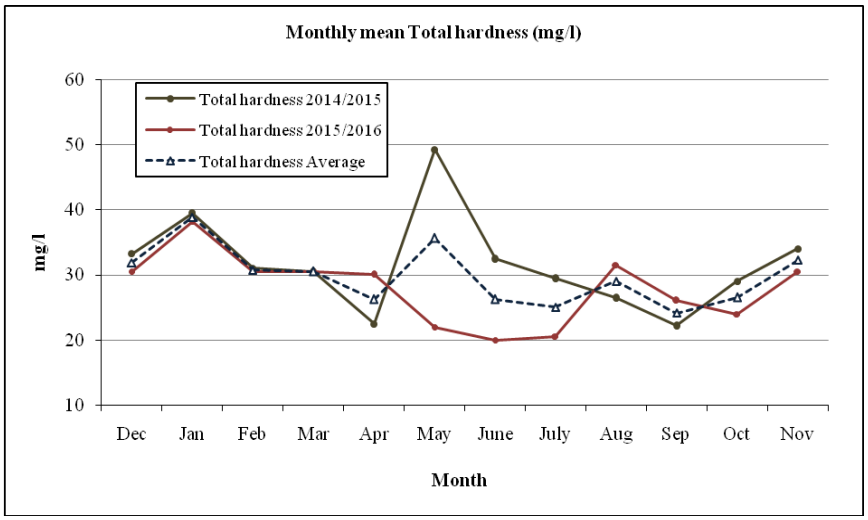

Figure 4.1.4.8: Monthly mean variations of total hardness from December 2014 to November 2016.

In the present study, the phenolphthalein alkalinity was found to be zero. So, the alkalinity of the water sample was only due to the presence of bicarbonates where the carbonates were practically absent.

AT showed a significant positive correlation with WT ($r = 0.97$; $p<0.001$). Significant negative correlations were observed between WT vs. DO ($r = -0.66$; $p<0.001$), WT vs. Free CO_2 ($r = -0.48$; $p<0.05$) and WT vs. TH ($r = -0.44$; $p<0.05$). pH showed negative correlations with all the other parameters. DO showed significant positive correlations with Free CO_2 ($r = 0.7$; $p<0.001$) and TH ($r = 0.44$; $p<0.05$). The correlation coefficients among various physico-chemical parameters are presented in the form of a matrix (Table 13).

Table 13: Correlation matrix of various physico-chemical parameters of water from Tamor River, Nepal from December 2014 to November 2016.

	AT	WT	pH	DO	CO_2	TA	TH
AT	1	0.97***	-0.26	-0.65***	-0.46*	-0.25	-0.42*
WT	0.97***	1	-0.23	-0.66***	-0.48*	-0.21	-0.44*
pH	-0.26	-0.23	1	-0.14	-0.19	-0.1	-0.25
DO	-0.65***	-0.66***	-0.14	1	0.7***	0.21	0.44*
CO_2	-0.46*	-0.48*	-0.19	0.7***	1	0.22	0.21
TA	-0.25	-0.21	-0.1	0.21	0.22	1	0.33
TH	-0.42*	-0.44*	-0.25	0.44*	0.21	0.33	1

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Abbreviations: AT, Atmospheric temperature; WT, Water temperature; DO, Dissolved oxygen; CO_2, Free Carbon dioxide, TA, Total alkalinity; TH, Total hardness)

The maximum value of pH (8.36) was recorded at site 2 in the month of January and minimum value (6.64) in May at site 4 (Appendix XII). The statistical analysis by two-way ANOVA on pH showed that the variations between sites ($F = 4.10$; $p<0.05$) and between months ($F= 3.43$; $p<0.05$) were statistically significant (Table 14).

Table 14: Two-way ANOVA for the data on pH of water of Tamor River as the function of variation between different sites versus variation between different months.

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Variation between sites	0.89	3.00	0.30	4.10	0.01	2.89
Variation between months	2.74	11.00	0.25	3.43	0.00	2.09
Error	2.39	33.00	0.07			
Total	6.02	47.00				

(Abbreviations: ANOVA, Analysis of variance; *SS*, Sum of squares; *Df*, Degrees of freedom; *MS*, Mean sum of squares; *F*, F-Test statistic; *P-value*, Significance value; *F crit*, critical value of F-Test statistic)

The maximum value of DO (11.4 mg/l) was recorded at site 1 in the month of March and minimum value (8 mg/l) in April at sites 1 and 2 (Appendix XII). The statistical analysis by two-way ANOVA on DO showed that the variations between sites were statistically insignificant ($F = 1.70$; $p>0.05$) while the variations between months were statistically significant ($F = 23.07$; $p<0.05$) (Table 15).

Table 15: Two-way ANOVA for the data on dissolved oxygen (DO) of water of Tamor River as the function of variation between different sites versus variation between different months.

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Variation between sites	0.51	3.00	0.17	1.70	0.19	2.89
Variation between months	25.30	11.00	2.30	23.07	0.00	2.09
Error	3.29	33.00	0.10			
Total	29.10	47.00				

(Abbreviations: ANOVA, Analysis of variance; *SS*, Sum of squares; *Df*, Degrees of freedom; *MS*, Mean sum of squares; *F*, F-Test statistic; *P-value*, Significance value; *F crit*, critical value of F-Test statistic)

The maximum value of free CO_2 (4.4 mg/l) was recorded at site 2 in the month of December and in January and at site 3 in March while its minimum value (1.93 mg/l) was recorded in April at site 1 (Appendix XII). The statistical analysis by two-way ANOVA on free CO_2 showed that the variations between sites ($F = 2.71$; $p>0.05$) and variations between months ($F = 1.80$; $p>0.05$) were both statistically insignificant (Table 16).

Table 16: Two-way ANOVA for the data on free CO_2 of water of Tamor River as the function of variation between different sites versus variation between different months.

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Variation between sites	2.98	3.00	0.99	2.71	0.06	2.89
Variation between months	7.26	11.00	0.66	1.80	0.09	2.09
Error	12.12	33.00	0.37			
Total	22.36	47.00				

(Abbreviations: ANOVA, Analysis of variance; *SS*, Sum of squares; *Df*, Degrees of freedom; *MS*, Mean sum of squares; *F*, F-Test statistic; *P-value*, Significance value; *F crit*, critical value of F-Test statistic)

The maximum value of TA was recorded as 87.50 mg/l at site 4 in the month of June and the minimum value as 60 mg/l in May and April at sites 1 and 2 respectively (Appendix XII). The statistical analysis by two-way ANOVA on TA showed that the variations between sites were statistically insignificant ($F = 1.60$; $p>0.05$) while the variations between months were statistically significant ($F = 5.97$; $p<0.05$) (Table 17).

Table 17: Two-way ANOVA for the data on total alkalinity (TA) of water of Tamor River as the function of variation between different sites versus variation between different months.

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Variation between sites	104.26	3.00	34.75	1.60	0.21	2.89
Variation between months	1425.10	11.00	129.55	5.97	0.00	2.09
Error	715.66	33.00	21.69			
Total	2245.02	47.00				

(Abbreviations: ANOVA, Analysis of variance; *SS*, Sum of squares; *Df*, Degrees of freedom; *MS*, Mean sum of squares; *F*, F-Test statistic; *P-value*, Significance value; *F crit*, critical value of F-Test statistic)

Similarly, the maximum value of TH was recorded (47.5 mg/l) at site 2 in the month of August and minimum value (20.5 mg/l) also in August at sampling site 4 (Appendix XII). The statistical analysis by two-way ANOVA on TH showed that the variations between sites were statistically insignificant ($F = 2.14$; $p>0.05$) while the variations between months were statistically significant ($F = 4.64$; $p<0.05$) (Table 18).

Table 18: Two-way ANOVA for the data on total hardness (TH) of water of Tamor River as the function of variation between different sites versus variation between different months.

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Variation between sites	110.46	3.00	36.82	2.14	0.11	2.89
Variation between months	879.75	11.00	79.98	4.64	0.00	2.09
Error	568.79	33.00	17.24			
Total	1559.00	47.00				

(Abbreviations: ANOVA, Analysis of variance; *SS*, Sum of squares; *Df*, Degrees of freedom; *MS*, Mean sum of squares; *F*, F-Test statistic; *P-value*, Significance value; *F crit*, critical value of F-Test statistic)

During the breeding season of the fish, AT and WT were found to vary from 14.97 ˚C to 23.10 ˚C and 10.52 ˚C to 16.8 ˚C, respectively. pH varied from 6.31 to 8.42, DO varied from 8.40 mg/l to 10.6 mg/l, free CO_2 varied from 2.20 mg/l to 3.85 mg/l, TA varied from 60 mg/l to 82.50 mg/l and TH varied from 20 mg/l to 49.25 mg/l.

The relationship between physico-chemical parameters and monthly dynamics of reproductive indices of *N. hexagonolepis* was analysed by performing Principal Component Analysis (PCA; Olanrewaju et al., 2017). PCA was performed on the data matrix of variables from Tamor River using computed Eigen values and weights of the parameters. Kaiser-Meyer-Oklin test (KMO) with the value of 0.85 and Bartlett's sphericity test (617.1623; $p<0.05$) were used to verify the applicability of PCA to raw data (Zhang et al., 2020).

Among the eleven principal components, the first two components, PC1 and PC2 were extracted which explained the sample variances of about 63 %. PC1 explained 38.7 % of the total variance with positive factor loadings on AT (0.42), WT (0.40), GWF (0.34), GSIF (0.35), GWM (0.36) and GSIM (0.35) and negative loadings on other parameters. PC2 explained 24.3 % of the total variance with negative loadings on AT (- 0.18), WT (- 0.22) and pH (-0.24), but positive loadings on all other parameters (Table 19 and Fig. 4.1.4.9).

Table 19: PCA (Principal Component Analysis) summary of the relationship between the physico-chemical parameters of water and GW and GSI of male and female *N. hexagonolepis.*

Parameters	PC1	PC2
AT	0.42	-0.18
WT	0.40	-0.22
pH	-0.11	-0.24
DO	-0.28	0.40
CO_2	-0.18	0.41
TA	-0.09	0.26
TH	-0.21	0.29
GWF	0.34	0.35
GSIF	0.35	0.36
GWM	0.36	0.32
GSIM	0.35	0.15
Importance of components:		
	PC1	PC2
Standard deviation	2.063	1.634
Proportion of Variance	0.387	0.243
Cumulative Proportion	0.387	0.630

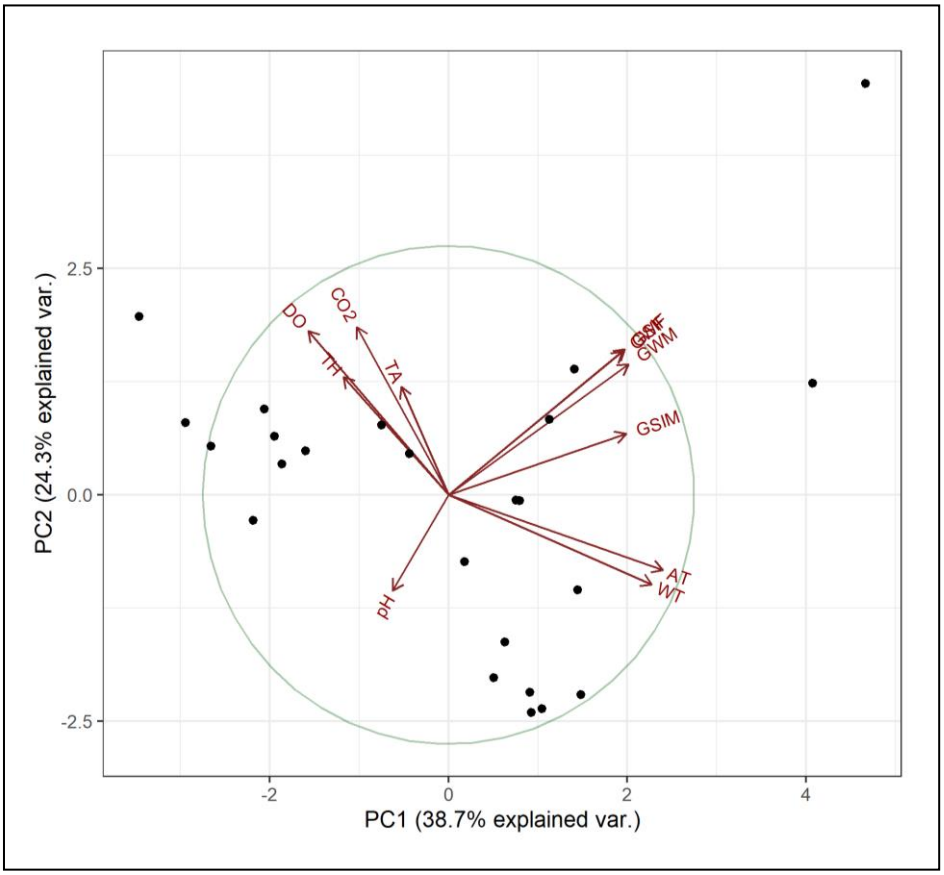

Figure 4.1.4.9: Principal Component Analysis (PCA) bi-plot of physico-chemical parameters of water and GW and GSI of male and female *N. hexagonolepis* (Significant ($p<0.01$) at 1000 permutations). (Abbreviations: AT: Atmospheric temperature; WT: Water temperature; DO: Dissolved oxygen; CO_2: Free carbon dioxide; TA: Total alkalinity; TH: Total hardness; GWF: Gonad weight of female fish, GSIF: Gonado-somatic index of female fish; GWM: Gonad weight of male fish; GSIM: Gonado-somatic index of male fish)

4.2. Discussion

4.2.1 Length-weight and length-length relationships and condition factor of *Neolissochilus hexagonolepis* from Tamor River, Nepal.

In the present study, all the length-weight and length-length relationships were found to be highly significant ($p<0.001$) which depicted a direct relationship between the parameters. Analogous results were also reported by Devashish et al. (2005) and Jyrwa et al. (2015).

The length weight relationship (LWR) of *N. hexagonolepis* was found to be in accordance with the model of Le Cren (1951). The coefficient of determination (R^2) values explained the proper fit of growth model. In the present study, the lowest value (= 0.904; 90 % variability) and the highest value (= 0.920; 92 % variability) of R^2 were recorded in *N. hexagonolepis* (Sexes-pooled), indicating more than 90 % variability by the model and good fitness. The value of the regression coefficient (b) close to 3 indicated that the fish showed an isometric pattern of growth. The t-test also confirmed that the regression coefficient values for male, female and sexes pooled did not depart significantly from the hypothetical value of 3 ($p>0.05$), indicative of the isometric growth pattern of the fish. Subba and Adhikaree (2011) and Subba et al. (2018) reported near-isometric growth of *N. hexagonolepis* from Tamor River, which agreed with the present study's finding. Similarly, Jyrwa et al. (2015) also reported isometric and allometric growth of the fish and opined that the ecological features of the area under investigation might cause the differences in the values of 'b'. However, a contrary result was earlier reported by Devashish et al., 2005. Hossain (2010) attributed the variation in the 'b' values for the same species to the difference in sampling, sample size or length ranges.

Different workers have reported contrasting results in other species as well. Pervin and Mortuza (2008) reported that the LWR of a freshwater fish followed the cube law with a high value of 'b'. However, Pathak (1975), Kumar et al. (2005) and Prasad and Ali (2007) reported deviation from the cube law in *Labeo calbasu, Rasbora daniconius* and *Puntius filamentosus*, respectively. Abobi (2015) also

reported the deviation of the relationship from the ideal value of 3 among various freshwater fishes studied.

According to Allen (1938), the cube law applies only to those species which maintain their form and specific gravity throughout life. However, with ageing, the shape and body counter change resulting in a deviation from the cube law for most of the fishes.

Several factors are held responsible for the deviation from the cube law. Le Cren (1951) suggested that the deviation of 'b' value is either due to an environmental condition or due to the fish's condition. Changes in specific gravity and shape of the body counter result in a change in the exponent and as such the cube law does not always hold good (Rounsefell and Everhart, 1953). Morphological changes due to age also cause substantial changes in the exponent of length on weight (Prasad and Ali, 2007). Seasonal fluctuations in environmental variables and physiological conditions during the spawning period also influence the exponent causing it to deviate from the ideal value (Sinha, 1973).

Pauly and Gayanilo (1997) suggested that 'b' values may range from 2.5 to 3.5. The exponent 'b' values for all the relationships between the weight and length of *N. hexagonolepis*, in the present study, were also within the range of 2.5 to 3.5 in full agreement with the above.

The regression coefficient (b) for the relationships between TW and TL, SL and FL showed a higher value for the TW-TL pair compared to the other pairs. Analogous results were also reported for *Gadusia godanahiae* by Subba et al. (2009).

The values of the coefficient of determination (R^2) for all the length-weight and length-length relationships viz. TW-TL, TW-SL, TW-FL, TL-SL, TL-FL and SL-FL were highly significant ($p<0.00001$) at $p<0.05$. Also, the male and female fish showed significant differences in 'b', with males recording higher exponential values than females. All these were in full agreement with the findings of Subba and Adhikaree (2011) for the same species.

The average mean Fulton's condition factor (K) for both the sexes of *N. hexagonolepis* was found to be greater than 1, which implicated that the general well-being of the fish in the river was satisfying. Similar findings were also reported for the species by Devashish et al. (2005) and Jyrwa et al. (2015). Surjya et al. (2013) also reported the value of condition factor greater than 1 for spotted snakehead *Channa punctata* (Bloch).

In the present study, the condition factor of male fish showed higher value compared to that of females, which gave a clear indication that the general well-being of male fish in the river was better than its female counterpart.

The best condition of either sex of *N. hexagonolepis* during summer with a peak value of 'K' in May and lower values during winter can be attributed to the increased feeding intensity associated with food abundance during the breeding season. Seasonal variations in condition factor of fishes have been previously reported in cichlid fishes (Anene, 2005; Shalloof and El-Far, 2017). Several factors such as stress, sex, season, availability of feeds and water quality can affect the condition of a fish and consequently its condition factor (Khallaf et al., 2003). The fluctuations in the values of condition factor occur due to differences in the size of sampling, stages of maturity, spawning and the weight of food content in the stomach (Dars et al., 2010).

The study affirmed a variation in condition factor by size classes in *N. hexagonolepis*, showing a relatively lower condition factors for larger sizes and higher factors for smaller fishes. An inverse relationship between condition factor and the size of fish has also been reported by earlier workers (Anene, 2005; Shalloof and El-Far, 2017). Larger fishes exhibiting relatively lower condition factor was attributed to the resource transferred to the gonads in the latter stages of the life history of a fish (Lizama et al., 2002).

The stages of development of reproductive organs influence the value of the condition factor of a fish and with females, the value decreases rapidly after the eggs are shed (Barnham et al., 2003).

The good score of 'K' reflected favourable riverine conditions for *N. hexagonolepis*. Mansor et al. (2010) suggested that the effect of environmental changes in fish species is reflected through the score of fish condition factor.

The higher value of 'Kn' for female than male fish may be attributed to the better appetite condition and higher feeding intensity of female fish. Higher appetite conditions and larger gonad contribute to the higher value of relative condition factor for female fish (Pervin and Mortuza, 2008).

4.2.2 Fecundity, Egg size and Gonado-somatic index (GSI) of *Neolissochilus hexagonolepis* from Tamor River, Nepal.

Information on reproductive traits of hill-stream fishes is very inadequate from Nepalese rivers. Data on the reproductive biology of *N. hexagonolepis*, including its fecundity estimation is still unavailable from this region. Elsewhere, various methods for estimation of fecundity have been adopted in fisheries science. However, the two most common methods still in practice are the volumetric and gravimetric methods. The first method involves the counting a sub-sample of eggs on the basis of a specific volume (Simpson, 1951) while, the latter method is based on the relation between ovary weight and oocyte density in the ovary (Bagenal and Braum, 1978). Perhaps the most precise way of estimating the number of eggs in ovaries is to count them all, using egg-counting machines. However, as the number of eggs in the ovaries of fish is high it is quite unrealistic to count them all manually. So, in the present study sub-sample method was adopted to estimate the fecundity of the candidate fish.

The low absolute fecundity estimated for *N. hexagonolepis* in the River Tamor, was in full agreement with the findings of Dasgupta (1988) and Swar (1994). Despite low fertility, *N. hexagonolepis* is a prolific breeder (Dasgupta, 1988). The fishes inhabiting cold water streams and lakes have comparatively low fecundity (Rasool and Ulfat, 2013). The low fecundity could be the verification of the parental care displayed by the fish (Ogunola et al., 2018). Being a multiple spawner, *N. hexagonolepis* displays parental care by allowing only a proportion of eggs to ripen at any one spawning over an extended breeding period.

The fecundity of a fish is affected by environmental factors and food supplies (Simpson, 1951), and is determined by the cumulative effect of diet, age, disease and atmospheric conditions of the environment in which the fish lives (Ulfat et al., 2014). Tiogue et al. (2020) also linked the variation in fecundity to environmental factors.

Absolute fecundity of *N. hexagonolepis* varied considerably among the individuals which may be attributed to the difference in feeding conditions (Swar, 1994). According to Scott (1962), the fecundity of a fish depends upon the food intake, and poor nutrition intensifies the shrink in the number of eggs produced by the fish. Nikolsky (1963) also pointed out that the food consumed by a fish is not only associated with its fecundity but also affects the eggs' quality.

Several authors have reported on the fecundities of freshwater fishes. Alam and Pathak (2010), Agbugui (2013), Arjamand et al. (2013), Khaironizam and Ismail (2013), Kharat and Khillare (2013), Wagle (2014), Bhattacharya and Banik (2015), Jabeen et al. (2016), Jan and Ahmed (2016), Osho and Usman (2019) and Tiogue et al. (2020) reported on the fecundities of *Pomadasys jubelini*, *Labeo rohita*, *Tor putitora*, *Neolissochilus soroides*, *Nemacheilus moreh*, *Schizothorax richardsonii*, *Ompok pabo*, *Barilius bendelisis*, *S. plagiostomus*, *Parachanna obscura*, and *Chrysichthys nigrodigitatus*, respectively. When compared with the species' fecundities, the fecundity of *N. hexagonolepis* was found to be similar in value to that of *Tor putitora*. Its value was higher compared to those of *N. soroides*, *Nemacheilus moreh*, and that of *Barilius bendelisis*. However, its value was found to be lower when matched to many other fishes like *Pomadasys jubelini*, *Labeo rohita*, *Schizothorax richardsonii*, *Ompok pabo* and *S. plagiostomus*.

In the present study, the mean absolute fecundity of *N. hexagonolepis* was 7555.44 ± 3769.93 eggs per fish and the average relative fecundity was estimated at 13.29 ± 56.01 eggs per gram body weight as opposed to 22.57 ± 1.41 eggs per gram body weight by Swar (1994) and 17.5 eggs per gram body weight by Mahapatra and Vinod (2011). The difference might be artificial (due to the difference in the techniques used for estimating fecundity) or natural (owing to the difference in fecundity due to variations in environmental factors at different locations). Fecundity depends on the size of fish and that the availability of more visceral volume in larger

fishes provides room for a larger number of eggs within the gonad (Shinkafi et al., 2011). Variation in fecundities among the fishes of equal length is standard. It results due to various environmental factors including temperature, availability of food and also due to the difference in genetics (Kharrat and Khillare, 2013).

Relative fecundity decreases with an increase in body weight (Wagle, 2014), and that the variation in the fecundities among the fishes of the same as well as different species are affected by their size, age, condition, food intake and space (Jan and Ahmed, 2016).

The absolute fecundity of the fish was found to be positively correlated with TL (R^2 = 0.26), SL (R^2 = 0.38), FL (R^2 = 0.37), TW (R^2 = 0.29) and GW (R^2 = 0.74). Significant positive correlations between the absolute fecundity and lengths (TL, SL and FL) indicated that the number of eggs in the ovaries of *N. hexagonolepis* increases with the increasing length of the fish. The significant positive associations between the absolute fecundity and the weight variables (TW and GW) indicated that the number of eggs in the ovaries of the candidate fish increases proportionately with its body and gonad weight. The finding of the present study corresponds well with earlier reports by Shinkafi et al. (2011), Khaironizam and Ismail (2013) and Subba et al. (2018) in *Auchenoglanis occidentalis*, *Neolissochilus soroides* and *N. hexagonolepis*, respectively. Positive linear relationships between fecundity and body parameters were also reported in *Anabas testudineus* (Marimuthu et al., 2009), *Nemacheilus moreh* (Kharrat and Killare, 2013), *Tor putitora* (Arjamand et al., 2013), *Pomadasys jubelini* (Agbugui, 2013), *Neolissochilus soroides* (Khaironizam and Ismail, 2013), *Schizothorax niger* and *S. esocinus* (Ulfat et al., 2014), *Schizothorax richardsonii* (Wagle, 2014), *Ompok pabo* (Bhattacharya and Banik, 2015), *Barilius bendelisis* (Jabeen et al., 2016), *S. plagiostomus* (Jan and Ahmed, 2016), *Xenontedon cancila* (Borthakur, 2018), and *Parachanna obscura* (Osho and Usman, 2019).

The associations between absolute fecundity of the candidate fish with its body metrics hinted that the weight of the ovary is better at predicting the total number of eggs within its body. Analogous results were also reported by Rahman and Miah (2009), Khaironizam and Ismail (2013), Silva et al. (2016), Boufersaoui and Handjar (2019), and Osho and Usman (2019).

The fecundity of *N. hexagonolepis* varied among the individuals of the same length and weight. The appetite and overall health condition of the fish seem to be the deciding factors in this regard. Analogous results were also reported by Marimuthu et al. (2009) and Bhattacharya and Banik (2015) in *Anabas testudineus* and *Ompok pabo*, respectively. Dube (1993) proposed that besides fish length and weight other parameters like nutritional diet, running water, and vitamins' influence also cause a variation in fish fecundity.

The trend of monthly mean GSI of both male and female sex in the present study hinted that *N. hexagonolepis* spawns during monsoon season with peak activity from July to August, consistent with the finding of other workers (Swar, 1994; Mahapatra and Vinod, 2011). The spawning of *N. hexagonolepis* during the monsoon season could be due to several environmental factors like temperature change, rainfall and subsequent rise in water level in the river. Dadebo et al. (2003) suggested that at the beginning of the rainy season, changes in temperature and rise in water level serve as the triggering factors for spawning in most tropical fishes.

The timing of reproduction and spawning can be identified from changes in GSI and the breeding season of fish corresponds to the month during which the GSI for both males and females reaches the peak (Arruda et al., 1993). As for the increase in GSI during the period of gonad maturation, is primarily due to the deposition of large amounts of proteins and lipids directly from ingested food during the active feeding season (Kharat and Khillare, 2013).

A much higher GSI of female than males implied that female gonads were much heavier even at the same maturity stages. Verma (2013) also reported a higher GSI of females in *Labeo dyocheilus*.

A single well-defined peak of GSI for both male and female fish indicated that *N. hexagonolepis* is an annual breeder. Other workers have also reported annual breeding behaviour in *N. hexagonolepis* from different habitats (Swar, 1994; Mahapatra and Vinod, 2011; Jyrwa and Bhuyan, 2017). Annual breeding behaviour was also reported in *Labeo rohita* (Alam and Pathak, 2010), *L. dyocheilus* (Verma, 2013), *Pomadasys jubelini* (Agbuigui, 2013) and *Tor putitora* (Arjamand et al., 2013).

However, several other fish species breed twice annually as indicated by their GSI with two peaks a year-round. Such breeding behaviour was reported in *N. soroides (*Khaironizam and Ismail, 2013), *Nemacheilus moreh* (Kharat and Khillare, 2013), *Schizothorax richardsonii* (Wagle, 2014) and *S. plagiostomus* (Jan and Ahmed, 2016)

The monthly mean GSI showed a precipitous drop after the peak breeding season. This was attributed to the decrease in weight of the gonads after the spawning act. Similar trends in GSI values were also observed by Verma (2013) and Joshi et al. (2016) in *Labeo dyocheilus* and *Schizothorax richardsonii*, respectively.

Eggs of different size groups were observed in mature ovaries of the fish which indicated that the species spawns more than once in a single breeding season. Swar (1994) and Mahapatra and Vinod (2011) also reported multiple spawning in *N. hexagonolepis*. Unlike the total spawners, the species sheds only a fraction of eggs during each spawning act and the spawning occurs over an extended period. Multiple spawning is only possible when there is a long period of adequate food supply (Nikolsky, 1963). The asynchronous development of oocytes and the fractional spawning behaviour of *N. hexagonolepis* may be seen as an adaptive feature of the fish to combat the unsuitable spawning conditions that prevail in hill streams (Swar and Craig, 2008).

The largest mean size of eggs in July coincided with the peak breeding season of the fish. The synchronization between the egg size and breeding season was also reported by Wagle (2014) in *Schizothorax richardsonii*.

Egg size (ES) showed insignificant positive correlations with biometric indices like SL, FL, and TW. Fleming (1996) suggested that the increase in egg size is an adaptive feature of fishes with larger females obtaining higher fitness by producing larger eggs. A significant positive association between GW and ES indicated that the heavier ovaries accommodate larger eggs in *N. hexagonolepis*. A significant positive correlation between ES and GW was also earlier reported by Subba et al. (2018) in *N. hexagonolepis*. This was due to the accumulation of yolk protein or vitellogenin in the developing oocytes which supplemented the oocytes' diameter, thereby increasing the weight of the ovaries.

Absolute fecundity (F) showed a very weak (r = 0.02) insignificant (p>0.05) positive correlation with egg size (ES) which contravened the finding of Shinkafi and Ipinjolu (2012).

4.2.3 Histomorphological features and maturation cycle of gonads of *Neolissochilus hexagonolepis* from Tamor River, Nepal.

The deviation from the ideal sex ratio of 1:1 of *N. hexagonolepis* may be ascribed to the asynchronous spawning migration or to the partial segregation by sex during feeding. The sex ratio in favour of female might also have been influenced by the faster growth rate of female leading to its less loss from predation (Qasim, 1966) and also abundance of food as the areas with abundant food present higher proportion of females (Nikolsky, 1969). Gear selectivity, sexual segregation in growth, partial differences of mature fish and behavioural differences during spawning also governs the sex ratio within a population (Lowerre-Barbieri et al., 1996). The differential occurrence of males and females in various water columns may result in the inequality in the abundance of male and female fish (Dopeikar et al., 2015). The sampling biases from fishing gears may also lead to the variation in the sex ratio, where gill nets tend to capture larger individuals which are predominantly females (Chehab and Abdulrahman, 2017). Comparable sex ratio variations in *N. hexagonolepis* were also reported by earlier workers (Mahapatra and Vinod, 2011; Jyrwa and Bhuyan, 2017).

The gender distribution displayed the occurrence of more than 50% males at TL of ≤ 21 cm which precisely hinted at the dominance of males among the smaller size classes. Also, the sex ratio favouring females in all the size classes above 27 cm indicated the female dominion among the larger-sized classes. The absence of males above 39 cm was attributed to the difference in growth rates of males and females where females attain a larger size compared to males.

Testes of *N. hexagonolepis* showed noticeable changes in shape, size, colour, volume, length and in the occurrence of various types of spermatogenic cells within their lobules during different phases of its reproductive cycle.

The testes and ovaries of *N. hexagonolepis* were observed at six different stages of maturation. Opposed to this, Swar (1994) and Jyrwa and Bhuyan (2017)

reported seven and five different stages of development of gonads, respectively, for the same species. It may be noted that the staging of gonads solely based on morphological study lacks precision as it relies more on subjective judgement. The probability of correct classification of gonads into various stages based on macroscopic criteria is low for some stages (Murua and Saborido-Rey, 2003).

Tunica albuginea of both testes and ovaries presented no uniform thickness over the year-round, thinner during the breeding season and thicker during the other periods. This may be attributed to the increased pressure exerted on the wall by the distended testicular lobules or enlarged matured ovarian follicles, as was also reported by Behera (2012) and Emam and Abughrien (2014). The testicular wall becomes thin during breeding season due to many sperms and thick during the non-breeding season (Behera, 2012). Pasha et al. (2016) also suggested that the thin testicular and lobular wall and spermatozoa were the characteristic features of the spermatogenic phase.

Ovary of *N. hexagonolepis* was observed to be of cystovarian type in which the lumen of the ovary is continuous with the oviduct. Ovary of a hill-stream fish *Lepidocephalichthys guntea* also displayed a similar feature (Subba, 1998).

Sharma et al. (2015) also reported six different ovaries in *Garra gotyla gotyla*. They identified 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. Pasha et al. (2016) suggested that the different phases of an ovary of *Schizothorax plagiostomus* were apparent based on histology and developing oocytes' stages. They reported six oocyte stages and identified the stages as chromatin nucleolar stage, early and late peri-nucleolus stage, yolk vesicle stage, yolk stage, ripe egg stage and postovulatory follicular stage.

Regarding the origin of a new crop of oogonia, divergent views were given by earlier workers. Yamamota (1956) postulated that new oogonia originate from the follicular epithelial cells of the spent follicles. Bisht and Joshi (1975), Shrestha and Khanna (1979), Agarwal (1982) and Subba (1998) advocated that new crops of oogonia are derived from the residual oogonia present in the spent ovaries. Sharma et al. (2015) opined that a new crop of oogonia originates from the germinal epithelium.

In the present study, the germinal epithelium was observed to serve as the principal site for the origin of a new crop of oogonia.

Several nucleoli seen extruding through the nuclear membrane were detected at various stages of oocytes. Earlier workers held opposing views regarding the origin of nucleoli in the oocytes. Khanna and Pant (1967) opined that nucleoli result from division and fragmentation of the pre-existing nucleolus in the oocyte. Opposed to this, Yamamoto (1956), Khanna and Sanwal (1974), Kumari and Nair (1979) suggested that the peripheral nucleoli form through the fusion of minute Feulgen positive particles present in the peripheral nucleoplasm. But, according to Guraya (1986), the origin of nucleoli takes place from certain heterochromatic regions called the nucleolar organising regions of the chromosomes.

In the present study, the increased number of nucleoli during yolk formation indicated that nucleoli are associated with the formation of yolk in the developing oocytes. A large number of small, clear vacuoles called yolk vesicles appeared in the cytoplasm periphery during the yolk vesicle stage. A similar phenomenon was also reported by Sharma et al. (2015) in *Garra gotyla gotyla*. These vesicles, later on, filled the entire cytoplasm. Subba (1998) opined that yolk vesicles give rise to cortical alveoli. He further pointed out that yolk vesicles were not associated with the formation of yolk globules. Pasha et al. (2016) suggested that the formation of yolk vesicles within oocytes was a sign of the maturation process. Sathyanesan (1962), Nayyar (1964), Khanna and Sanwal (1974) and Guraya et al. (1975) reported on the occurrence of the yolk nucleus during different stages of oocyte development and attributed several functions to the yolk nucleus. While Wallace (1904) associated the yolk nucleus with yolk formation in fishes, Subba (1998) advocated that the yolk nucleus has some relationship with the process of vitellogenesis. Contrary to all the above, Kumari and Nair (1979) reported the complete absence of the yolk nucleus in any stages of oocyte maturation in *Lepidocephalicthys thermalis*. Akin to this, the yolk nucleus was never tracked in any of the stages of oocytes of *N. hexagonolepis* during the present study.

Several small, round and prominent nucleoli were observed in the early stages of oocyte maturation. As the oocyte matured the nuclear membrane showed an

irregular outline and the nucleoli disintegrated in the ooplasm with the disappearance of the nuclear membrane. This phenomenon was observed during the early and late yolk vesicle stages. Several other workers also reported on this phenomenon in several species of fishes. Rai (1965), Khanna and Pant (1967), Lehri (1968), Subba (1998), Sharma et al. (2015) and Jyrwa and Bhuyan (2017) reported on this phenomenon in *Tor tor, Glyptostermum pectinopterum, Clarias batrachus* and *Lepidocephalicthys guntea, Garra gotyla gotyla, and N. hexagonolepis* respectively.

Several functions are ascribed to nucleoli. The extruded nucleoli give rise to ribosomes which accumulate in the ooplasm of the oocyte and thereby, play a role in vitellogenesis as suggested by Guraya (1986) and Sharma et al. (2015).

Atresia is a process by which several oocytes undergo degeneration in the developing ovary (Khanna, 2000). Several previtellogenic follicles, as well as vitellogenic and mature yolky eggs were observed to show atresia in the ovaries of *N. hexagonolepis*. Atretic follicles were also reported by Sharma et al. (2015), Pasha et al. (2016) and Subba and Mahaseth (2018) during the post-spawning or spent phases in *Garra gotyla gotyla*, *Schizothorax plagiostomus* and *N. hexagonolepis*, respectively.

When the gonadotropin content of the pituitary becomes low it is unable to maintain the growth of healthy oocytes which, as a consequence, results in atresia (Rai, 1965). The inadequacy in food supply influences the metabolism of fish, thereby enhancing the rate of atresia, which, in turn, affects the fecundity of a fish (Agarwal et al., 1988). Khanna (2000) suggested that environmental factors such as light, temperature and physico-chemical conditions of water must be favourable for the healthy growth of oocytes. Any disturbance in the environmental, endocrinological and metabolic factors may initiate atresia of oocytes.

The stromal elements resulting from the atresia of large previtellogenic oocytes are considered to be the source of interstitial cells in the ovary of fish (Khanna, 2000).

The testes and ovaries appeared flaccid with diminished volume and weight during the post-spawning or spent phase. This was attributed to the expulsion of

spermatozoa and ripe eggs during the spawning season. Also, very few spermatids were observed during the matured stage of testes as most of them changed into spermatozoa before spawning.

Histological sections of testes during the spent phase unveiled some empty seminiferous lobules and a few with residual spermatozoa. These features were observed for several months, especially after the spawning season.

Matured testes were characterized by the presence of a thin testicular and a lobular wall filled with spermatozoa. Spent testes were, however, characterized by the presence of spermatogonia accompanied by only a few unexpelled spermatozoa.

Matured and spawning male fishes were captured in May, which indicated that the breeding season of the fish commences from May. Matured and spawning female fishes were captured till November and no spent fishes were collected from January onwards. These precisely indicated that the breeding season of *N. hexagonolepis* has a protracted period from May till November. The protracted breeding period of *N. hexagonolepis*, also been reported by Jhingran (1982), Swar (1994) and Arunachalam (2010), could be considered as an adaptive feature of the species to combat environmental pressures.

The maximum number of matured and spawning fishes captured in the month of July indicated the month as the period of intense breeding in *N. hexagonolepis*. Peak GSI for both the sexes of *N. hexagonolepis* were also observed in the month of July when the fish attained full maturity.

The verdict of the present study implied that *N. hexagonolepis* is an annual breeder, a fractional spawner and has a protracted breeding period from May to November with peak breeding activity from July to August. Also, since these months fall in the peak monsoon season of the region, during which the river experiences flood with the increased water current, it was concluded that the flood water with abundant food nutrients and high water current trigger the spawning activity of the fish. Verma (2013) also reported the synchronization of sexual maturation and reproduction with the onset of rainfall in a hill stream major carp, *Labeo dyocheilus*.

The fractional spawning behaviour of *N. hexagonolepis* was further backed up by the asynchronous development of oocytes and the occurrence of several stages of oocytes in a single ovary.

The size at first sexual maturity is defined as the length at which a randomly chosen sample has a 50% chance of being mature (Somerton, 1980). Earlier, the logistic curve for estimating the size at first sexual maturity has been successfully used for several species of fishes (Bandpei et al., 2011; Valdez-Pineda et al., 2014; Nandikeswari, 2016; Freitas et al, 2016; Peixoto et al., 2018). The logistic model for estimating the size at first sexual maturity by visual inspection of gonads (L_{50} Maturity scale) revealed that male and female individuals of *N. hexagonolepis* attained the first sexual maturity at TL 25.5 cm and TL 32.9 cm, respectively. Earlier attainment of maturity in male *N. hexagonolepis* was also reported by Swar (1994) and Shrestha (2008). Male individuals reaching the first sexual maturity at smaller lengths than females have also been reported in *Auchenipterichthys longimanus* (Freitas et al, 2016) and in *Tetrapon puta* (Nandikeswari, 2016). In most teleosts, female individuals attain the first sexual maturity at larger sizes than males (Helfman et al., 1997). Nikolsky (1969) suggested that most of the males reaching maturity at smaller and younger compared to females illustrate the longer duration of life of the female fishes that mature later.

4.2.4 Physico-chemical parameters of water and their relationship with gonad weight and gonado-somatic index (GSI) of *Neolissochilus hexagonolepis* from Tamor River, Nepal.

The relationship between physico-chemical parameters and gonad weight and GSI was determined to investigate the effect of environmental variables on the reproductive biology of the fish. The information will serve as a model for efficient ecosystem management through control of anthropogenic activities on this river for sustainable conservation of the wild population of the species.

In Nepal, the period between June to September is the rainy season. Monsoon sets in towards the end of June and the temperature drops. Throughout the monsoon, the temperature maintains a relatively uniform range during which the fishes breed.

The highest and lowest mean values of atmospheric and water temperatures, recorded in the present study, corresponded to the summer and winter seasons of the region, respectively.

The water temperature started to recede from October reaching the minimum value in the month of January when the temperature reached as low as 5 °C. Then, it increased reaching the maximum value of 16.8 °C in June. With the onset of monsoon (June to September), the water temperature maintained a uniform range.

The rise in water temperature could be correlated to increased in carbon dioxide levels (Talling, 1957), when the water level decreased precipitously during summer.

The average annual water temperature of the river was recorded low (12.95 °C) and this is because the river is snow-fed. The low value of the water temperature of the river was also reported by Shrestha et al. (2009).

WT showed a significant positive correlation with AT. Change in water temperature is observed due to abiotic and biotic reactions and that the changes in water temperature are according to the changes observed in air temperature (Kundangar et al., 1996). Surana et al. (2010) also reported a similar relationship between the two variables.

The pH range of 5-9 is accepted as harmless to fish and harmful effects become noticeable when the pH of water falls below 5.0 or rise above 9.6 (Yang et al., 2011). When the pH of water falls below 4.0 fertilization of most fish species becomes unsuccessful (Peterson et al., 1982 and Yang et al., 2011). According to Medera et al. (1982), the pH of most natural water remains within the range of 6.5 to 8.5 and any disturbance in the equilibrium of CO_2, bicarbonate and carbonate contents bring about a marginal departure from its neutral value. Since the water samples from all the sampling sites were nearly neutral to alkaline with an average value of 7.44 ± 0.25, during the study period, it was concluded that the mean pH range of the river was within the optimum range for the growth and reproduction of fresh-water fishes. Similar findings were also reported by Shrestha et al. (2009), Praveen et al. (2013) and Kumar et al. (2013) from Tamor River, river Ganga and river Gomti, respectively.

The monthly variations in pH of water of the river, during the study period, maybe due to the combined effects of other parameters like temperature, dissolved oxygen and free CO_2.

The total alkalinity of the water from the river was attributed only to the presence of bicarbonates as was indicated by the zero phenolphthalein alkalinity during the present study. The water samples with pH values ranging from 4.5 to 8.3 contain practically no carbonates (Jhingran, 1982). The finding of the present study also supported this.

The total alkalinity of the water from the river showed a higher value during the winter season. This was attributed to the decreased water volume in the river and the higher concentration of bicarbonate ions in the water. The lower value of total alkalinity during the rainy season was primarily due to the dilution effect resulting in the lower ion concentration in the water.

The values of DO are generally higher in winter and lower in summer. DO showed a significant negative correlation with water temperature. Though maximum DO was registered in March; its values showed a detectable increasing tendency through November, December and January, corresponding to the winter season of the year. Seasonal variation in DO content is related to temperature and biological activities (Chapman and Kimstach, 1992). Similarly, Bist (1993) opined that variation in DO could be due to one or more factors such as temperature, light intensity, turbidity, photosynthesis and respiration.

The high value of DO during March may be assigned to the increased photosynthetic activity of aquatic plants. Surana et al. (2010) also attributed the higher value of DO in water to the luxuriant growth of macrophyte which increases the rate of photosynthesis compared to the rate of respiration. The diurnal variation in DO may be attributed to the excessive use of fertilizers and pesticides in farm fields and their run-off into the rivers, helping the aquatic plants to grow which utilized more of the dissolved oxygen through the process of eutrophication.

The monthly mean DO ranged from 8.43 ± 0.11 to 11.10 ± 0.57 mg/l, with the annual average value of 9.55 ± 0.76 mg/l. Novothy (2002) pointed out that DO is the

critical parameter for protecting aquatic life and suggested that most fish species cannot survive with DO content less than 3 mg/l. The finding of the present study revealed that the river detains a good volume of dissolved oxygen throughout the year for successful growth and reproduction of fishes.

Variations in DO can also be caused by river morphology e.g. rapids or other turbulence areas and seasonal temperature changes. The higher values of DO recorded during the study were attributed to the higher gradient of the river as the rapid flow of water enhanced the aeration of water by a diffusion process.

Monthly mean values of free CO_2 varied from 2.20 ± 0.00 to 3.44 ± 0.58 mg/l, with the annual average value of 2.89 ± 0.41 mg/l. The levels of free CO_2 in water are influenced by photosynthesis, respiration and decomposition. A high level of CO_2 in water is harmful to fishes as it impedes the respiratory process of aquatic life.

Total alkalinity showed a positive but insignificant positive correlation with total hardness, in full compliance with the finding of Surana et al. (2010).

The water parameters recorded at different sampling sites did not show a significant difference other than the slight variation in pH. The variation in pH among the sites was ascribed to the fluctuation in free CO_2. Secondly, the significant monthly variations among the parameters pointed out their variations on the temporal scale.

The various physico-chemical parameters recorded during the breeding season of the fish indicated that the spawning of *N. hexagonolepis* depends upon a specific range of physico-chemical parameters. Reproductive fishes are controlled by hormonal metabolism and environmental factors (Joshi et al., 2018). They need specific environmental conditions during the reproductive period to guarantee the growth and survival of offspring (Jorgensen et al., 2006).

The trends of monthly mean sunshine and precipitation in the study area showed an inverse relationship with each other which may be ascribed to the cloudy days during the monsoon season and clear sky during the other seasons. Abundant precipitation accompanied by cloudy days with the short occasion of sunshine during July and August indicated that these conditions stimulate *N. hexagonolepis* to spawn.

Photoperiod, seasonal rainfall and temperature play a crucial role in regulating the reproductive cycle in teleost fishes (Nikolsky, 1963; Edwards, 2005).

The Principal Component Analysis (PCA) revealed a positive relationship between temperature (AT and WT) and reproductive indices (GWF, GSIF, GWM and GSIM) of *N. hexagonolepis*. PC1 had positive loadings on temperature and reproductive indices, which represented the positive contribution of temperature upon the development of gonads of the fish during its active breeding season. Negative loadings on other parameters represented their inverse relationship with temperature and the gonadal development of the fish. PC2 had negative loadings on temperature and positive loadings on other parameters. The lower temperature during winter season affected the reaction rates of chemicals in water upsetting the other parameters. Therefore, PC2 can be regarded as the post active breeding season of the fish. The finding of the present study is consistent with several lines of existing evidence suggesting that the environmental parameters play a significant role in the reproductive biology. A positive association between physico-chemical parameters and reproductive indices were also reported by Shrestha (1978) and Verma (2013) in *Schizothorax* spp. and *Labeo dyocheilus,* respectively.

Temperature as the most crucial parameter influencing the reproductive indices of fishes has also been reported by Sharma et al. (2014), Sharma et al. (2015), Jisu et al. (2016), Olanrewaju et al. (2017) and Subba et al. (2020) in *Oncorhynchus mykiss* and *Schizothorax richardsonii*, *Tor putitora*, *Rhynchocypris kumgangensis*, *Parachanna obscura* and *N. hexagonolepis,* respectively. Sharma et al. (2014) also discovered that rainfall influences the breeding in fishes through the manifestation of other water quality parameters.

While Khanna (2000) deemed light as an essential factor in controlling the reproduction in fishes, Lawson (2011) affirmed that temperature along with light, plays vital role in the gonadal gametogenesis, spawning and initiation of gonadal development. The finding of the present study also confirmed temperature as the most critical parameter in governing the reproductive biology of fish.

CHAPTER 5

5. CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The central aim of the study was to assess the fecundity and discern the gonadal development of Copper Mahseer, *Neolissochilus hexagonolepis* (McClelland, 1839) from Tamor River, Nepal. Besides these, relationships between length and weight, condition factor, gonado-somatic index (GSI) and egg size of the fish were also assessed. In addition to all these, various physico-chemical parameters of water of the river were also evaluated and their association with GSI and GW of the fish investigated.

In the present study, the LWRs were found to be highly significant ($p<0.001$) depicting a direct relationship between the body weight and length parameters. In all the cases, the value of the regression coefficient (b) neared 3, which indicated that the fish followed an isometric growth pattern. This meant there was no change in the overall shape and counter of the fish's body as it grew.

Similarly, it was found that the general well being of the fish from the river was satisfying with the mean 'K' score above 1, for both the sexes. However, the higher score of male indicated its better well-being compared to its female counterpart. The study recorded a higher 'Kn' score for female fish indicating its better appetite condition and higher feeding intensity.

Seasonal variation in 'K' was registered for either sex of *N. hexagonolepis*, showing best condition during summer with its peak value in May and lower values during winter. The study also affirmed a variation in condition factor by size classes exhibiting, in general, an inverse relationship between 'K' and the size of the fish.

The result of fecundity assessment showed that *N. hexagonolepis* is a low fecund fish; lower compared to other high fecund hill stream fishes like *Schizothorax plagiostomus*, *S. richardsonii* etc. Significant positive correlations between absolute fecundity vs. lengths (TL, SL and FL) and absolute fecundity vs. weights (TW and

GW) indicated that the number of eggs in the ovaries of *N. hexagonolepis* increases proportionately with increasing length, body weight and gonad weight of the fish.

Also, the variations in the fecundities among the individuals of the same size hinted that variables like appetite and overall health condition of the body also determine the number of eggs in *N. hexagonolepis*.

The significant positive association between GW and ES indicated that heavier ovaries accommodate larger eggs in *N. hexagonolepis*.

The trend of monthly mean GSI with a single annual peak suggested that *N. hexagonolepis* spawns annually during monsoon season with peak activity from July to August.

Although the average annual sex ratio of *N. hexagonolepis* in River Tamor favoured female the gender distribution according to the size class of the fish affirmed male dominance over the small size classes and the female dominion over the larger size classes. Further, the absence of males above 39 cm was attributed to the difference in growth rates of males and females where females attain a larger size compared to males.

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

Tunica albuginea of both testes and ovaries were thinner during the breeding season and thicker during other periods because of the increased pressure exerted on the walls by the distended testicular lobules or enlarged ovarian follicles as the gonads mature.

As in the case of most other teleosts, the testes of *N. hexagonolepis* were found to be 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 all along their margin. The sperm ducts joined posteriorly to open into the urinogenital papillae.

Histological sections of the testes of *N. hexagonolepis* affirmed their lobular nature consisting of a large number of seminiferous lobules bounded together by a thin layer of connective tissue with blood capillaries and interstitial cells dispersed in it. The resting germ cells transformed into sperm mother cells or spermatogonia which were large spherical cells containing large, round and centrally placed nucleus. These cells multiplied to give rise to primary spermatocytes which in turn gave rise to secondary spermatocytes. The secondary spermatocytes divided further to produce spermatids. The spermatids then transformed into spermatozoa or sperms which were the smallest among all the spermatogenic cells in the testes.

Testes of *N. hexagonolepis* showed noticeable changes in shape, size, colour, volume, length and occurrence of various types of spermatogenic cells within their lobules during different phases of the reproductive cycle of the fish. As milt oozed out on pressing the abdomen of a matured male, the histological section of its testes displayed seminiferous lobules packed with sperms. During the post spawning or spent phase, the testes and ovaries appeared flaccid with diminished volume and weight. Histologically, during the spent phase, while the testes enclosed some empty seminiferous lobules and a few lobules with residual sperms the ovaries were detected with many degenerated and atretic follicles.

Similarly, the ovaries of *N. hexagonolepis* were observed to be of typical teleostean type. Externally, they appeared paired, elongated and sac-like structures lying in the abdominal cavity ventral to the kidneys and attached to the body wall by means of mesovarium. As in most other hill stream fishes, its ovaries were of the cystovarian type in which the lumen of the ovary is continuous with the oviduct. Histological sections of the ovaries unveiled several stages of developing oocytes in the ovigerous lamellae. The developing oocytes were observed at chromatin nucleolus stage, early and late peri-nucleolus stages, early and late yolk vesicle stages and early and late yolk stages.

Several nucleoli were observed in the oocytes at various stages which were seen extruding through the nuclear membrane. The increased number of nucleoli during yolk formation indicated that the nucleoli were associated with the formation of yolk in the developing oocytes. However, the yolk nucleus was never ascertained and

the zona radiata showed more homogeneity with indistinct radial striations. Also, several previtellogenic follicles, as well as vitellogenic and mature yolky eggs, were observed to show atresia in the ovaries of the fish. Mature oocytes or eggs of *N. hexagonolepis* were yellowish and spherical with numerous yolk globules and vesicles.

The present study affirmed that *N. hexagonolepis* is an annual breeder, a fractional spawner and spawns during monsoon season with peak activity from July to August. Additionally, the study showed that the fish has an extended breeding season from May to November.

The logistic model for estimating the size at first sexual maturity by visual inspection of gonads (L_{50} Maturity scale) revealed that male and female individuals of *N. hexagonolepis* attained the first sexual maturity at TL 25.5 cm and TL 32.9 cm, respectively.

The mean pH range of the river was within the optimum range for the growth and reproduction of fresh water fishes. Also, the river detains the right amount of dissolved oxygen throughout the year. Likewise, the various physico-chemical parameters recorded during the breeding season of the fish hinted that the spawning of *N. hexagonolepis* depends upon specific range of physico-chemical parameters.

The association between physico-chemical parameters and the monthly dynamics of reproductive indices of *N. hexagonolepis* revealed that temperature contributes positively to the development of gonads of the fish during its active breeding season. Also, since the species was captured round the year along the river stretch, it may be presumed that the recorded water parameters were within the tolerance limits of the species.

5.2 Recommendations

Based on the above conclusions the following recommendations are made:

- The fishes below 12 cm were not captured during the study period which revealed that the river is under high fishing pressure. Since the species was found to

mature at an early size (male: 25.5 cm and female: 32.9 cm) fishing regulations which prohibit the use of fishing gears with smaller mesh size should be implemented. Also, destructive and harmful fishing practices like electrofishing and poisoning should be prohibited.

- *N. hexagonolepis* was found to have an extended breeding period from May to November with peak spawning activity from July to August. Fishing during the prime breeding season should be prohibited. Locating the precise feeding, spawning, and nursing grounds essential for the restoration of the species.
- Thorough study at the molecular level is needed to discern more on the reproductive biology of the species and to find out the differences, if any, among the species from different regions.
- Further research is required to estimate the environmental thresholds of *N. hexagonolepis* for a better understanding of the control of environmental variables on the reproductive biology of the species.
- More enhanced and periodically replicated studies are required not only to validate the present findings but also to formulate the effective methods for the thriving culture of the species under restrained conditions.
- Artificial breeding and commercial culture of *N. hexagonolepis* should be prioritized to preserve its gene pool; important for such a 'conservation-priority' species.
- Mining of stones, gravel and sand from the river should be prohibited. Mitigating measures for preventing landslide and minimizing the risk due to monsoon flooding should be devised.

CHAPTER 6

6. SUMMARY

The present work titled " **Fecundity and Gonadal Development of Copper Mahseer, *Neolissochilus hexagonolepis* (McClelland, 1839) from Tamor River, Nepal** " was attempted to assess the fecundity and discern the gonadal development of *N. hexagonolepis* from Tamor River, Nepal. Albeit the study was primarily focussed on deciphering the spawning season, reproductive capacity and histomorphological features of gonads of the fish, the growth and condition factor of the fish and the relationship of physico-chemical parameters of water of the river with the reproductive indices of the fish were also assessed and investigated.

The study was conducted for two years from December 2014 till the end of November 2016. Four sampling sites were selected along the mid-reaches of the river. Fish samples were collected in the second half of every month while water samples from all the sites were analysed fortnightly at 8:00 A.M.

For the determination of LWR, the samples collected from the river were sorted out for sex and TW, TL, SL and FL were measured for each following the standard procedure. TW (including gut and gonad) was measured using a digital balance with the precision of 0.01 g. In contrast, the length was measured in a fully stretched condition to the nearest 1 mm using a measuring tape and a graduated ruler. The LWR was worked out as per cube law given by Le Cren (1951), $W = aL^b$; where W is the total weight of the fish, 'a' represents the regression intercept and 'b' represents the regression slope. The relationship equations were established by the least square method.

In the present study, the LWR of *N. hexagonolepis* was found to be in accordance with the model of Le Cren (1951). The fish from the river showed an isometric growth pattern.

The condition of the fish was assessed by computing its Fulton's condition factor (K) using the formula, $K = W \times 100 / L^3$; where W is the total weight of the fish (in gram), L is the total length of the fish (in cm) and the number 100 is the factor for

bringing the condition factor near to unity. Relative condition factor (Kn) was also calculated for all the fish samples from the average length and weight of 3 cm interval of total length using the formula, Kn = W / w; where, W is the total observed weight (in g) and w is the calculated weight (in g) for the observed length of the fish. The calculated weight for the observed length was obtained from the equation, $W = aL^b$; where 'a' and 'b' are the exponential forms of the intercept and slope, respectively, of the logarithmic length-weight relationship.

The scores of 'K' and 'Kn' with the average values above 1 indicated that the general well being of the fish from the river was good.

The study of the trend of monthly mean variation of 'K' for either sex of *N. hexagonolepis* showed seasonal variation exhibiting the best condition during summer with its peak value in May and lower values during winter. The study also affirmed a variation in condition factor by size classes exhibiting, in general, an inverse relationship between 'K' and the size of the fish.

The fecundity of the fish was estimated using the gravimetric method. The result of the fecundity assessment showed that *N. hexagonolepis* is a low fecund fish. The relationships between absolute fecundity (F) and biometric variables were determined by simple linear regression. Significant positive correlations between absolute fecundity vs. lengths (TL, SL and FL) and absolute fecundity vs. weights (TW and GW) indicated that the number of eggs in the ovaries of *N. hexagonolepis* increases proportionately with increasing length, body weight and gonad weight of the fish.

Egg size (ES) of the fish was determined using a calibrated micrometer mounted on the eyepiece of a monocular microscope. Egg size (ES) ranged from 1.28 ± 0.22 mm to 2.34 ± 0.05 mm. The correlations between egg size (ES) and biometric variables (TL, SL, FL, TW, GW and F) were determined by Spearman's rank correlation test which showed that heavier ovaries accommodate larger eggs in *N. hexagonolepis*.

The gonado-somatic index (GSI) of *N. hexagonolepis* was calculated for each fish by using the formula: GSI = Weight of gonad / Total weight of fish X 100

The trend of monthly mean GSI with a single annual peak for both male and female sex hinted that *N. hexagonolepis* is an annual breeder and spawns during monsoon season with peak spawning activity from July to August.

The independent χ^2 test was employed to determine the deviation from the 1:1 sex ratio as the null hypothesis for the whole sample and each of the 3cm fish size classes making 12 size classes (TL, cm) in total. Although the average annual sex ratio of *N. hexagonolepis* in the river was found in favour of females the gender distribution according to the size class of the fish affirmed male dominance over the smaller size classes while female dominance over the larger size classes.

Histomorphological features of gonads were examined to figure out the developmental stages and maturation cycle of gonads of the fish. And for this, histological slides of both the sexes were prepared every month round the year. Smaller pieces of gonads fixed in freshly prepared Bouin's fluid were embedded in paraffin wax for further processing and sectioning. The gonads were then sectioned at 6 µ with the help of a rotary microtome machine, dewaxed in xylene, hydrated and dehydrated in alcohol series. The histological sections were stained with hematoxylin, followed by eosin counterstain.

The testes and ovaries of *N. hexagonolepis* were observed to pass through six stages of maturation viz. immature, maturing virgin, ripening, mature, spawning and spent. Testes and ovaries were observed to be of typical teleostean type.

The testes of *N. hexagonolepis* showed noticeable changes in shape, size, colour, volume, length and in the occurrence of various types of spermatogenic cells within their lobules, as they mature. Similarly, the fish’s ovaries were observed to be of cystovarian type and showed oocytes at various stages of development within their ovigerous lamellae. The developing oocytes passed through the chromatin nucleolus stage, early and late peri-nucleolus stage, early and late yolk vesicle stages and finally the early and late yolk stages. Also, the oocytes were observed to show asynchronous development.

When the frequencies of the occurrences of various stages of gonads were worked out and expressed as percentages it was found that the breeding season of *N. hexagonolepis* has a protracted period from May to November.

The size at first sexual maturity of either sex of *N. hexagonolepis* was estimated based on the L_{50} Maturity scale. The logistic model for estimating the size at first sexual maturity by visual inspection of gonads (L_{50} Maturity scale) revealed that male and female individuals of *N. hexagonolepis* attained the first sexual maturity at TL 25.5 cm and TL 32.9 cm, respectively.

The various physico-chemical parameters such as atmospheric temperature, water temperature, DO, free CO_2, total alkalinity and total hardness was analysed and two-way analysis of variance (ANOVA) was computed for correlating the parameters among the different sampling sites and months of a year.

The atmospheric and water temperature of the river was recorded with the help of a simple mercury-filled Celsius thermometer having an accuracy of 0.1 °C to 50 °C. pH was recorded using a systronic battery-operated pH meter. Dissolved oxygen (DO) was estimated by Winkler's idiometric method and free CO_2 was determined titrimetrically using N/44 NaOH and phenolphthalein as an indicator. Total alkalinity was estimated by titrating the water samples against 0.1 N HCl using phenolphthalein and methyl orange as indicators, while total hardness was estimated by the EDTA method. The titrations were performed following the standard protocols of APHA (2005).

The variations of physico-chemical parameters between the sites were statistically insignificant while statistically significant between the months.

The various physico-chemical parameters recorded during the breeding season of the fish indicated that the spawning of *N. hexagonolepis* depends upon a specific range of physico-chemical parameters.

The relationship between physico-chemical parameters and monthly dynamics of reproductive indices of the fish like GW and GSI was analysed using a multivariate analysis tool PCA (Principal Component Analysis) which revealed that temperature

contributes positively to the development of gonads of the candidate fish during its active breeding season.

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APPENDICES

Appendix I: Monthly mean total weight, total length, standard length and forkal length of male *N. hexagonolepis* from Tamor River, Nepal from December 2014 to November 2016.

Year: 2014/2015				
Month	**Mean TW (g)**	**Mean TL (cm)**	**Mean SL (cm)**	**Mean FL (cm)**
December	253.33 ± 177.22	27.97 ± 8.72	22.83 ± 7.75	25.57 ± 8.81
January	83.00 ± 61.40	18.70 ± 5.31	14.88 ± 4.50	16.52 ± 4.96
February	69.33 ± 29.77	17.23 ± 0.93	13.10 ± 0.26	13.87 ± 0.40
March	92.50 ± 58.67	18.90 ± 7.13	15.00 ± 4.32	16.78 ± 4.46
April	128.00 ± 59.40	22.35 ± 8.56	16.85 ±4.45	18.55 ± 4.74
May	348.00 ± 189.39	23.90 ± 3.62	20.56 ± 3.53	22.22 ± 4.18
June	400.00 ± 239.71	26.28 ± 4.10	21.20 ± 3.72	23.22 ± 3.83
July	389.00 ± 354.29	23.43 ± 8.54	19.45 ± 8.01	21.45 ± 8.11
August	57.50 ± 38.89	17.80 ± 2.40	13.75 ± 2.33	15.35 ± 3.04
September	113.33 ± 16.07	19.50 ± 0.82	15.60 ± 0.78	17.17 ± 0.84
October	90.00 ± 21.21	20.00 ± 2.12	16.50 ± 0.99	16.75 ± 3.18
November	43.58 ± 18.81	16.48 ± 2.33	12.88 ± 1.80	14.45 ± 1.71
1st year average mean:	172.30 ± 135.90	21.04 ± 3.70	16.88 ± 3.35	18.49 ± 3.75
Year: 2015/2016				
December	47.50 ± 52.89	16.50 ± 6.47	12.98 ± 5.12	14.40 ± 5.52
January	127.50 ± 118.50	21.30 ± 6.87	16.85 ± 5.68	18.88 ± 5.80
February	186.67 ± 208.50	22.30 ± 9.46	17.57 ± 7.48	19.13 ± 8.37
March	274.00 ± 186.74	28.40 ± 10.69	23.17 ± 8.29	25.80 ± 9.64
April	172.33 ± 188.70	22.07 ± 12.26	16.80 ± 7.70	18.67 ± 8.18
May	265.00 ± 323.70	22.35 ± 5.60	18.23 ± 5.24	20.13 ± 5.41
June	195.00 ± 311.27	19.84 ± 7.25	16.10 ± 6.09	17.82 ± 6.46
July	188.75 ± 59.63	23.68 ± 2.76	19.20 ± 2.52	21.18 ± 2.57
August	217.50 ± 243.95	24.65 ± 12.52	19.75 ± 10.96	21.75 ± 11.67
September	59.00 ± 14.75	17.20 ± 1.52	13.66 ± 1.04	14.60 ± 1.20
October	95.00 ± 84.85	19.25 ± 6.01	14.85 ± 4.74	16.70 ± 5.23
November	126.67 ± 106.00	22.38 ± 5.20	18.88 ± 5.78	20.38 ± 5.56
2nd year average mean:	162.91 ± 73.48	21.66 ± 3.24	17.34 ± 2.82	19.12 ± 3.15

Appendix II: Monthly mean total weight, total length, standard length and forkal length of female *N. hexagonolepis* from Tamor River, Nepal from December 2014 to November 2016.

Year: 2014/2015				
Month	**Mean TW (g)**	**Mean TL (cm)**	**Mean SL (cm)**	**Mean FL (cm)**
December	41.36 ± 13.62	16.30 ± 1.82	12.80 ± 1.62	14.21 ± 1.61
January	93.13 ± 36.74	19.99 ± 3.13	15.95 ± 2.38	17.60 ± 2.53
February	275.00 ± 243.98	25.50 ± 8.08	20.60 ± 6.79	23.04 ± 8.01
March	458.75 ± 491.75	29.45 ± 13.70	24.68 ± 11.67	26.68 ± 12.95
April	552.50 ± 661.14	29.35 ± 20.01	25.35 ± 18.17	26.60 ± 19.66
May	505.00 ± 289.91	29.05 ± 1.34	24.50 ± 0.71	26.50 ± 0.71
June	600.00 ± 296.98	33.35 ± 0.92	27.35 ± 0.92	29.50 ± 0.85
July	637.00 ± 344.45	34.96 ± 4.64	29.66 ± 4.41	32.02 ± 4.87
August	560.00 ± 643.88	31.15 ± 10.91	25.58 ± 9.50	28.48 ± 10.22
September	360.00 ± 175.00	31.90 ± 4.94	25.40 ± 4.16	28.03 ± 4.50
October	255.00 ± 247.49	26.35 ± 9.69	20.90 ± 7.64	23.10 ± 7.92
November	192.00 ± 211.56	22.82 ± 11.03	18.38 ± 9.16	20.56 ± 10.14
1st year average mean:	377.48 ± 203.99	27.51 ± 5.57	22.60 ± 4.95	24.69 ± 5.18
Year: 2015/2016				
December	402.00 ± 297.84	32.26 ± 10.08	25.90 ± 8.76	27.83 ± 9.26
January	341.00 ± 29.45	32.88 ± 0.86	27.30 ± 1.43	29.00 ± 0.69
February	405.00 ± 429.08	26.46 ± 10.62	21.74 ± 10.01	23.34 ± 10.81
March	492.60 ± 263.56	33.44 ± 11.41	27.72 ± 9.76	30.08 ± 10.34
April	350.50 ± 309.98	26.68 ± 11.62	22.48 ± 9.91	24.28 ±10.80
May	810.00 ± 205.00	35.63 ± 5.40	30.17 ± 4.29	32.53 ± 5.12
June	470.00 ± 459.02	29.67 ± 7.79	23.57 ± 6.65	26.10 ±7.15
July	493.38 ± 280.53	33.64 ± 6.05	27.71 ± 5.14	30.60 ±5.39
August	340.00 ± 266.97	29.67 ± 11.86	24.33 ± 10.26	27.10 ±11.49
September	153.75 ± 114.26	23.23 ± 7.11	18.25 ± 5.70	20.18 ± 6.44
October	110.00 ± 12.91	23.05 ± 0.70	18.10 ± 0.75	19.98 ± 0.82
November	419.00 ± 183.56	34.34 ± 3.66	27.22 ± 4.26	30.32 ± 4.57
2nd year average mean:	398.94 ± 177.08	30.08 ± 4.34	24.54 ± 3.84	26.78 ± 4.11

Appendix III: Sampling data and summary statistics obtained when volumetric methodology was used as an approach to estimate the absolute fecundity of *N. hexagonolepis* from Tamor River, Nepal.

First test sample (TW=565 g, TL=39.5 cm, GW=39.63 g)			
Sub-sample 1	Sub-sample 2	Sub-sample 3	Sub-sample 4
Vol.: 0.5 ml No. of eggs: 107 No. of eggs/ ml : 214	Vol.: 0.5 ml No. of eggs: 95 No. of eggs/ ml : 190	Vol.: 0.5 ml No. of eggs: 90 No. of eggs/ ml : 180	Vol.: 0.5 ml No. of eggs: 117 No. of eggs/ ml : 234
Summary for the first test sample Mean= 204.5 ; SD= 24.30 ; **cv = 11.88 %**			

Second test sample (TW=370 g, TL=33 cm, GW= 27.2 g)			
Sub-sample 1	Sub-sample 2	Sub-sample 3	Sub-sample 4
Vol.: 0.4 ml No. of eggs: 160 No. of eggs/ ml : 400	Vol.: 0.6 ml No. of eggs: 249 No. of eggs/ ml : 415	Vol.: 0.5 ml No. of eggs: 259 No. of eggs/ ml : 518	Vol.: 0.3 ml No. of eggs: 172 No. of eggs/ ml : 573
Summary for the second test sample Mean= 476.5 ; SD= 83.00 ; **cv = 17.41 %**			

(Abbreviations: TW, Total weight; TL, Total length; GW, Gonad weight; SD, Standard deviation; cv, coefficient of variation)

Appendix IV: Associated statistics during fecundity assessment by gravimetric method from the sampled female *N. hexagonolepis* from, Tamor River, Nepal.

			Count of eggs in three sub-samples of 1 g each			Summary statistics		
TW (g)	**TL (cm)**	**GW (g)**	**Sub-sample 1**	**Sub-sample 2**	**Sub-sample 3**	**Mean count of eggs**	**Standard deviation**	**Coefficient of variation (%)**
410	32.4	35	115	127	124	122	6.24	5.12
525	33.2	38	198	191	187	192	5.57	2.90
1200	42	75	169	158	159	162	6.08	3.75
710	37	102	149	138	139	142	6.08	4.28
340	30.2	56	139	130	124	131	7.55	5.76
535	34.1	42	103	98	105	102	3.61	3.53
610	35	58	178	186	188	184	5.29	2.88
445	33.2	38.4	134	143	131	136	6.24	4.59
442	33.6	38.42	112	129	119	120	8.54	7.12
445	33.4	40.24	125	118	120	121	3.61	2.98
565	39.5	39.63	219	231	237	229	9.17	4.00
1015	41	34.47	308	319	342	323	17.35	5.37
645	38.1	70.82	176	182	179	179	3.00	1.68
430	34.7	27.2	298	308	315	307	8.54	2.78
1100	46.2	30	282	278	256	272	14.00	5.15
370	33	27.2	325	320	309	318	8.19	2.57
					Min:	102	3.00	1.68
					Max:	323	17.35	7.12
					Mean:	190	7.44	4.03

Appendix V: Monthly mean Fulton's condition factor and relative condition factor of male *N. hexagonolepis* from Tamor River, Nepal from December 2014 to November 2016.

Year: 2014/2015				
Month	**Mean TW (g)**	**Mean TL (cm)**	**Mean Fulton's condition factor (K)**	**Mean relative condition factor (Kn)**
December	253.33 ± 177.22	27.97 ± 8.72	0.95 ± 0.10	0.80 ± 0.07
January	83.00 ± 61.40	18.70 ± 5.31	1.17 ± 0.33	1.01 ± 0.30
February	69.33 ± 29.77	17.23 ± 0.93	1.31 ± 0.42	1.13 ± 0.36
March	92.50 ± 58.67	18.90 ± 7.13	1.51 ± 0.62	1.30 ± 0.56
April	128.00 ± 59.40	22.35 ± 8.56	1.36 ± 0.88	1.17 ± 0.77
May	348.00 ± 189.39	23.90 ± 3.62	**2.38 ± 0.49**	2.02 ± 0.41
June	400.00 ± 239.71	26.28 ± 4.10	2.02 ± 0.51	1.71 ± 0.43
July	389.00 ± 354.29	23.43 ± 8.54	2.12 ± 0.40	1.80 ± 0.31
August	57.50 ± 38.89	17.80 ± 2.40	0.93 ± 0.30	0.80 ± 0.25
September	113.33 ± 16.07	19.50 ± 0.82	1.52 ± 0.10	1.30 ± 0.08
October	90.00 ± 21.21	20.00 ± 2.12	1.12 ± 0.09	0.96 ± 0.08
November	43.58 ± 18.81	16.48 ± 2.33	**0.92 ± 0.14**	0.80 ± 0.12
1st year average mean:	172.30 ± 135.90	21.04 ± 3.70	1.44 ± 0.49	1.23 ± 0.41
Year: 2015/2016				
December	47.50 ± 52.89	16.50 ± 6.47	**0.91 ± 0.22**	0.79 ± 0.20
January	127.50 ± 118.50	21.30 ± 6.87	1.15 ± 0.21	0.98 ± 0.19
February	186.67 ± 208.50	22.30 ± 9.46	1.29 ± 0.50	1.10 ± 0.43
March	274.00 ± 186.74	28.40 ± 10.69	1.21 ± 0.56	1.03 ± 0.50
April	172.33 ± 188.70	22.07 ± 12.26	1.55 ± 0.73	1.34 ± 0.65
May	265.00 ± 323.70	22.35 ± 5.60	**1.68 ± 0.63**	1.42 ± 0.51
June	195.00 ± 311.27	19.84 ± 7.25	1.37 ± 0.59	1.17 ± 0.49
July	188.75 ± 59.63	23.68 ± 2.76	1.52 ± 0.72	1.29 ± 0.62
August	217.50 ± 243.95	24.65 ± 12.52	1.09 ± 0.07	0.93 ± 0.09
September	59.00 ± 14.75	17.20 ± 1.52	1.15 ± 0.23	0.99 ± 0.20
October	95.00 ± 60.00	19.25 ± 4.25	1.12 ± 0.08	0.96 ± 0.06
November	126.67 ± 96.77	22.38 ± 4.75	0.95 ± 0.26	0.81 ± 0.22
2nd year average mean:	162.91 ± 73.48	21.66 ± 3.24	1.25 ± 0.24	1.07 ± 0.20

Appendix VI: Monthly mean Fulton's condition factor and relative condition factor of female *N. hexagonolepis* from Tamor River, Nepal from December 2014 to November 2016.

Month	Mean TW (g)	Mean TL (cm)	Mean Fulton's condition factor (K)	Mean relative condition factor (Kn)
Year: 2014/2015				
December	41.36 ± 13.62	16.30 ± 1.82	**0.94 ± 0.14**	0.83 ± 0.13
January	93.13 ± 36.74	19.99 ± 3.13	1.13 ± 0.14	1.01 ± 0.12
February	275.00 ± 243.98	25.50 ± 8.08	1.41 ± 0.20	1.26 ± 0.17
March	458.75 ± 491.75	29.45 ± 13.70	1.35 ± 0.35	1.22 ± 0.30
April	552.50 ± 661.14	29.35 ± 20.01	1.83 ± 0.84	1.64 ± 0.72
May	505.00 ± 289.91	29.05 ± 1.34	**2.16 ± 1.48**	1.94 ± 1.33
June	600.00 ± 296.98	33.35 ± 0.92	1.59 ± 0.67	1.43 ± 0.60
July	637.00 ± 344.45	34.96 ± 4.64	1.38 ± 0.17	1.25 ± 0.16
August	560.00 ± 643.88	31.15 ± 10.91	1.29 ± 0.26	1.16 ± 0.24
September	360.00 ± 175.00	31.90 ± 4.94	1.04 ± 0.08	0.94 ± 0.07
October	255.00 ± 247.49	26.35 ± 9.69	1.13 ± 0.07	1.01 ± 0.07
November	192.00 ± 211.56	22.82 ± 11.03	1.15 ± 0.32	1.02 ± 0.28
1st year average mean:	377.48 ± 203.99	27.51 ± 5.57	1.37 ± 0.35	1.23 ± 0.32
Year: 2015/2016				
December	402.00 ± 297.84	32.26 ± 10.08	0.98 ± 0.19	0.88 ± 0.17
January	341.00 ± 29.45	32.88 ± 0.86	0.96 ± 0.06	0.87 ± 0.06
February	405.00 ± 429.08	26.46 ± 10.62	1.59 ± 0.57	1.43 ± 0.51
March	492.60 ± 263.56	33.44 ± 11.41	1.31 ± 0.59	1.18 ± 0.51
April	350.50 ± 309.98	26.68 ± 11.62	1.54 ± 0.48	1.38 ± 0.42
May	810.00 ± 205.00	35.63 ± 5.40	**1.82 ± 0.36**	1.64 ± 0.32
June	470.00 ± 374.79	29.67 ± 6.36	1.35 ± 0.45	1.22 ± 0.42
July	493.38 ± 280.53	33.64 ± 6.05	1.20 ± 0.16	1.08 ± 0.14
August	340.00 ± 266.97	29.67 ± 11.86	1.02 ± 0.11	0.92 ± 0.10
September	153.75 ± 114.26	23.23 ± 7.11	1.13 ± 0.21	1.02 ± 0.18
October	110.00 ± 12.91	23.05 ± 0.70	**0.90 ± 0.11**	0.81 ± 0.10
November	419.00 ± 183.56	34.34 ± 3.66	0.97 ± 0.22	0.87 ± 0.20
2nd year average mean:	398.94 ± 177.08	30.08 ± 4.34	1.23 ± 0.30	1.11 ± 0.27

Appendix VII: Descriptive statistics and estimated parameters of the absolute fecundity-length and absolute fecundity-weight relationships (sample size = 18) of female *N. hexagonolepis* from Tamor River, Nepal (*, significant at 5%).

	Regression parameters					
Equation	a	b	95% CL of a	95% CL of b	R^2	p
Absolute fecundity-Length						
$F = a \times TL^b$	0.051	3.291	-5.391 to 2.807	0.018 to 5.937	0.26*	<0.05
$F = a \times SL^b$	0.060	3.425	-4.366 to 1.928	1.281 to 5.569	0.38*	<0.01
$F = a \times FL^b$	0.046	3.407	-4.653 to 1.987	1.207 to 5.608	0.37*	<0.01
Absolute fecundity-Weight						
$F = a \times TW^b$	11.49	1.004	-1.022 to 3.144	0.243 to 1.764	0.29*	<0.05
$F = a \times GW^b$	561.4	0.692	2.428 to 3.071	0.487 to 0.898	0.75*	<0.001

(Abbreviations: F, Absolute fecundity; TL, Total length; SL, Standard length; FL, Forkal length; TW, Total weight; GW, Gonad weight; a, intercept; b, slope; CL, Confidence limit; R^2, Coefficient of determination; p, Probability value)

Appendix VIII: Monthly mean gonado-somatic index (GSI) of male *N. hexagonolepis* from Tamor River, Nepal from December 2014 to November 2016.

Year: 2014/2015			
Month	**Mean TW (g)**	**Mean GW (g)**	**Mean GSI (%)**
December	253.33 ± 177.22	0.50 ± 0.39	**0.17 ± 0.05**
January	83.00 ± 61.40	0.41 ± 0.23	0.58 ± 0.45
February	69.33 ± 29.77	0.44 ± 0.05	0.79 ± 0.55
March	92.50 ± 58.67	0.53 ± 0.42	0.58 ± 0.34
April	128.00 ± 59.40	0.89 ± 0.55	0.67 ± 0.12
May	348.00 ± 189.39	4.76 ± 2.98	1.48 ± 0.99
June	400.00 ± 239.71	1.32 ± 0.72	0.44 ± 0.39
July	389.00 ± 354.29	6.98 ± 5.18	**2.12 ± 1.28**
August	57.50 ± 38.89	0.81 ± 0.36	1.54 ± 0.41
September	113.33 ± 16.07	2.40 ± 1.81	1.98 ± 1.44
October	90.00 ± 21.21	0.68 ± 0.74	0.87 ± 1.03
November	43.11 ± 23.00	0.32 ± 0.27	0.77 ± 0.42
1st year average mean:	172.26 ± 135.94	1.67 ± 2.10	1.00 ± 0.63
Year: 2015/2016			
December	95.00 ± 84.85	0.46 ± 0.55	0.37 ± 0.25
January	127.50 ± 118.50	0.48 ± 0.30	0.46 ± 0.29
February	186.67 ± 208.50	3.69 ± 5.58	1.35 ± 0.96
March	274.00 ± 186.74	0.66 ± 0.23	**0.33 ± 0.22**
April	172.33 ± 188.70	0.92 ± 0.40	0.87 ± 0.55
May	265.00 ± 323.70	1.59 ± 2.50	0.45 ± 0.42
June	195.00 ± 311.27	0.58 ± 0.50	0.59 ±0.39
July	200.00 ± 67.64	5.89 ± 1.28	**3.27 ± 1.68**
August	217.50 ± 243.95	1.81 ± 1.88	0.94 ± 0.18
September	65.00 ± 7.07	1.19 ± 0.70	1.88 ± 1.21
October	95.00 ± 84.85	1.00 ± 0.10	1.67 ± 1.39
November	126.67 ± 106.00	0.57 ± 0.55	0.83 ± 1.21
2nd year average mean:	168.31 ± 67.31	1.57 ± 1.63	1.09 ± 0.86

Appendix IX: Monthly mean gonado-somatic index (GSI) of female *N. hexagonolepis* from Tamor River, Nepal from December 2014 to November 2016.

Year: 2014/2015			
Month	**Mean TW (g)**	**Mean GW (g)**	**Mean GSI (%)**
December	42.50 ± 13.79	0.10 ± 0.05	0.25 ± 0.13
January	93.13 ± 36.74	0.17 ± 0.09	**0.18 ± 0.05**
February	275.00 ± 243.98	1.69 ± 2.85	0.41 ± 0.36
March	458.75 ± 491.75	2.00 ± 3.22	0.24 ± 0.24
April	552.50 ± 661.14	2.60 ± 3.50	0.32 ± 0.25
May	505.00 ± 289.91	10.73 ± 6.65	2.09 ± 0.12
June	600.00 ± 296.98	12.61 ± 8.10	2.01 ± 0.35
July	637.00 ± 344.45	61.20 ± 27.87	**10.57 ± 4.56**
August	560.00 ± 643.88	13.16 ± 18.19	3.33 ± 3.94
September	360.00 ± 175.00	1.02 ± 0.58	0.28 ± 0.03
October	255.00 ± 247.49	2.44 ± 1.61	1.23 ± 0.56
November	417.50 ± 95.46	1.08 ± 0.31	0.26 ± 0.02
1st year average mean:	396.36 ± 195.37	9.07 ± 17.13	1.76 ± 2.95
Year: 2015/2016			
December	465.00 ± 270.39	4.05 ± 4.99	0.67 ± 0.60
January	341.00 ± 29.45	0.71 ± 0.21	0.21 ± 0.06
February	405.00 ± 429.08	3.80 ± 5.51	0.64 ± 0.41
March	492.60 ± 263.56	3.03 ± 2.32	0.72 ± 0.38
April	350.50 ± 309.98	0.73 ± 0.46	0.37 ± 0.46
May	810.00 ± 205.00	13.14 ± 18.48	1.36 ± 1.76
June	470.00 ± 459.02	2.85 ± 2.36	0.63 ± 0.61
July	493.38 ± 280.53	30.07 ± 18.97	**5.99 ± 3.48**
August	340.00 ± 266.97	16.19 ± 20.60	3.70 ± 2.87
September	185.00 ± 117.15	0.30 ± 0.32	**0.13 ± 0.07**
October	110.00 ± 12.91	0.35 ± 0.20	0.32 ± 0.19
November	419.00 ± 183.56	20.59 ± 30.20	3.71 ± 4.81
2nd year average mean:	406.79 ± 174.31	7.98 ± 9.76	1.54 ± 1.88

Appendix X: Monthly change in frequency of maturity stages (Stages I to VI) of gonads as percentages for male *N. hexagonolepis* from Tamor River, Nepal from December 2014 to November 2016.

	Frequency of maturity stages of gonads (%)					
Month	**Stage I**	**Stage II**	**Stage III**	**Stage IV**	**Stage V**	**Stage VI**
Jan	33.33	66.67	0.00	0.00	0.00	0.00
Feb	33.33	50.00	16.67	0.00	0.00	0.00
March	14.29	71.43	14.29	0.00	0.00	0.00
April	0.00	40.00	60.00	0.00	0.00	0.00
May	22.22	11.11	11.11	44.44	11.11	0.00
June	10.00	40.00	50.00	0.00	0.00	0.00
July	0.00	12.50	0.00	37.50	50.00	0.00
Aug	0.00	25.00	25.00	0.00	25.00	25.00
Sept	37.5	0.00	0.00	12.5	12.5	37.5
Oct	50	0.00	0.00	0.00	0.00	50
Nov	60.00	40.00	0.00	0.00	0.00	0.00
Dec	66.67	33.33	0.00	0.00	0.00	0.00

Appendix XI: Monthly change in frequency of maturity stages (Stages I to VI) of gonads as percentages for female *N. hexagonolepis* from Tamor River, Nepal from December 2014 to November 2016.

	Frequency of maturity stages of gonads (%)					
Month	**Stage I**	**Stage II**	**Stage III**	**Stage IV**	**Stage V**	**Stage VI**
Jan	69.23	30.77	0.00	0.00	0.00	0.00
Feb	20.00	50.00	30.00	0.00	0.00	0.00
March	22.22	33.33	44.44	0.00	0.00	0.00
April	33.33	50.00	16.67	0.00	0.00	0.00
May	0.00	40.00	60.00	0.00	0.00	0.00
June	0.00	40.00	60.00	0.00	0.00	0.00
July	0.00	7.69	7.69	61.54	23.08	0.00
Aug	0.00	28.57	42.86	14.29	14.29	0.00
Sept	42.86	57.14	0.00	0.00	0.00	0.00
Oct	50.00	16.67	33.33	0.00	0.00	0.00
Nov	60.00	10.00	0.00	10.00	10.00	10.00
Dec	66.67	16.67	0.00	0.00	0.00	16.67

Appendix XII: Monthly pooled mean variations of physico-chemical parameters of water at different sampling sites of Tamor River, Nepal from December 2014 to November 2016.

	Parameters	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
Sampling site 1	pH	7.63±0.46	7.66±0.05	8±0.36	7.45±0.57	7.23±0.89	7.93±0.32	7.09±1.32	7.61±1.41	7.66±1.28	6.97±0.33	7.37±1.21	7.73±0.24
	DO (mg/l)	10.2±0.00	10±0.28	**11.4±0.85**	**8±0.00**	8.9±0.99	9±0.28	9±0.28	9±0.28	9.4±0.85	9.8±0.28	10±0.57	10.7±0.14
	Free CO_2 (mg/l)	2.75±0.78	2.48±1.17	2.48±1.17	**1.93±0.39**	2.2±0.00	2.2±0.00	2.2±0.00	3.85±0.78	3.3±1.56	2.2±0.00	2.2±0.00	2.2±0.00
		78.75	78.75	61.25	61.25	**60.00**	66.25	70.00	63.75	61.25	68.75	62.50	61.25
	TA (mg/l)	±12.37	±1.77	±1.77	±1.77	**±0.00**	±5.30	±0.00	±5.30	±1.77	±12.37	±0.00	±1.77
	TH (mg/l)	34.5±0.71	30.5±2.12	30.5±0.71	25.5±3.54	33.5±16.2	25±7.07	24.5±6.36	25±7.07	24.5±0.71	25±4.24	29.5±0.71	31.5±0.71
Sampling site 2	**Parameters**	**Jan**	**Feb**	**Mar**	**April**	**May**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**
	pH	**8.36±0.09**	7.98±0.18	7.97±0.17	7.17±0.18	7.06±1.04	7.31±0.83	7.35±1.17	7.8±1.42	7.67±0.52	7.24±0.23	7.41±0.83	8.1±0.01
	DO (mg/l)	10.1±0.14	9.8±0.28	10.8±1.13	**8±0.00**	9.1±1.27	8.7±0.99	9.4±1.13	8.6±0.85	9.1±0.99	8.5±0.42	10.5±0.99	9.9±0.14
	Free CO_2 (mg/l)	**4.4±0.00**	3.3±0.00	3.3±0.00	3.3±0.00	2.48±1.17	2.48±1.17	2.48±1.17	2.48±1.17	2.2±0.00	2.2±0.00	2.75±0.78	**4.4±0.00**
		72.50	76.25	68.75	**60.00**	66.25	68.75	67.50	81.25	63.75	70.00	67.50	67.50
	TA (mg/l)	±10.61	±1.77	±1.77	**±0.00**	±8.84	±5.30	±3.54	±19.45	±1.77	±14.14	±0.00	±10.61
	TH (mg/l)	43.5±10.6	31±1.41	31±0.71	25.5±3.54	40±28.28	25.5±9.19	26±8.49	**47.5±26.1**	26.5±7.78	26±0.00	32.5±4.95	32.5±4.95
Sampling site 3	**Parameters**	**Jan**	**Feb**	**Mar**	**April**	**May**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**
	pH	7.18±0.25	7.59±0.29	7.53±0.46	6.95±0.69	7.14±1.21	7.39±0.72	7.39±0.49	7.21±1.12	7.95±0.55	7.05±0.64	7.43±0.50	7.59±0.82
	DO (mg/l)	10.2±0.00	9.6±0.00	10.9±0.14	8.9±0.42	8.9±0.42	9.1±0.71	9.8±0.57	9.2±1.13	9.4±1.13	8.9±0.14	9.9±0.14	10±0.28
	Free CO_2 (mg/l)	3.85±0.78	3.3±0.00	**4.4±1.56**	2.2±0.00	2.2±0.00	2.2±0.00	3.85±0.78	3.3±1.56	3.3±1.56	2.2±0.00	3.85±0.78	3.58±1.17
		81.25	80.00	73.75	62.50	65.00	77.50	72.50	63.75	61.25	72.50	65.00	61.25
	TA (mg/l)	±8.84	±3.54	±1.77	±3.54	±10.61	±0.00	±3.54	±5.30	±1.77	±17.68	±3.54	±1.77
	TH (mg/l)	40.5±3.54	31±0.00	30±0.71	26±4.24	29±14.14	29±11.31	27±7.07	23±4.24	22±1.41	29.5±6.36	35.5±4.95	29.5±0.71
Sampling site 4	**Parameters**	**Jan**	**Feb**	**Mar**	**April**	**May**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**
	pH	7.32±0.23	6.86±0.04	7.28±0.17	7.47±0.99	**6.64±0.87**	7.17±0.99	7.13±1.39	7.23±1.46	7.42±1.10	7.16±0.04	7.6±0.90	7.94±0.96
	DO (mg/l)	10.1±0.14	11.3±0.14	11.3±0.14	8.8±0.00	9±0.57	9±1.13	10±1.41	8.9±0.71	9±0.57	9±0.57	10.2±0.85	10±0.00
	Free CO_2 (mg/l)	2.75±0.78	2.75±0.78	3.3±0.00	2.2±0.00	2.75±0.78	3.3±0.00	3.85±0.78	2.75±0.78	2.75±0.78	2.2±0.00	3.3±0.00	2.75±0.78
		78.75	70.00	73.75	61.25	66.25	**87.50**	72.50	63.75	61.25	73.75	65.00	62.50
	TA (mg/l)	±12.37	±3.54	±5.30	±1.77	±5.30	**±10.61**	±3.54	±5.30	±1.77	±19.45	±3.54	±3.54
	TH (mg/l)	37±2.83	30.5±0.71	30.5±0.71	28±9.90	40±18.38	25.5±7.78	22.5±3.54	**20.5±0.71**	23.5±4.95	25.5±3.54	31.5±0.71	34±1.41

Appendix XIII: Monthly mean variations of physico-chemical parameters of Tamor River, Nepal from December 2014 to November 2016.

Year: 2014/2015													1st year average mean:
Parameters	**Dec.**	**Jan.**	**Feb.**	**March**	**April**	**May**	**June**	**July**	**Aug.**	**Sep.**	**Oct.**	**Nov.**	
pH	7.49 ± 0.45	7.72 ± 0.66	7.42 ± 0.50	7.84 ± 0.66	6.83 ± 0.26	6.31 ± 0.28	6.94 ± 0.61	6.47 ± 0.65	6.50 ± 0.46	7.06 ± 0.86	7.01 ± 0.86	6.85 ± 0.37	7.04 ± 0.20
DO (mg/l)	10.20 ± 0.43	10.15 ± 0.67	9.95 ± 0.99	10.70 ± 0.67	8.35 ± 0.45	9.55 ± 0.69	9.50 ± 0.60	9.95 ± 1.08	9.35 ± 0.60	9.85 ± 0.52	8.90 ± 0.79	10.60 ± 0.57	9.75 ± 0.67
Free CO_2 (mg/l)	3.58 ± 1.14	3.85 ± 1.02	3.30 ± 1.18	3.30 ± 1.18	2.34 ± 0.92	2.48 ± 0.78	2.75 ± 1.02	3.30 ± 1.18	3.85 ± 1.02	3.58 ± 1.14	2.20 ± 0.00	3.03 ± 1.64	3.13 ± 1.02
TA (mg/l)	63.75 ± 5.82	85.63 ± 6.23	76.25 ± 4.43	70.00 ± 7.56	61.25 ± 2.31	68.75 ± 9.16	75.00 ± 5.35	72.50 ± 3.78	67.50 ± 2.67	62.50 ± 2.67	82.50 ± 4.63	66.25 ± 3.54	70.99 ± 4.85
TH (mg/l)	33.25 ± 5.65	39.50 ± 11.10	31.00 ± 1.85	30.50 ± 2.07	22.50 ± 1.41	49.25 ± 13.13	32.50 ± 3.96	29.50 ± 4.11	26.50 ± 4.87	22.25 ± 2.92	29.00 ± 5.13	34.00 ± 4.28	31.65 ± 5.04
Year: 2015/2016													2nd year average mean:
Parameters	**Dec.**	**Jan.**	**Feb.**	**March**	**April**	**May**	**June**	**July**	**Aug.**	**Sep.**	**Oct.**	**Nov.**	
pH	8.20 ± 0.33	7.52 ± 0.56	7.62 ± 0.66	7.55 ± 0.36	7.69 ± 0.42	7.73 ± 0.57	7.96 ± 0.54	8.01 ± 0.22	8.42 ± 0.52	8.29 ± 0.32	7.19 ± 0.43	8.06 ± 0.36	7.85 ± 0.44
DO (mg/l)	10.10 ± 0.47	10.15 ± 0.42	9.90 ± 0.35	11.50 ± 0.73	8.50 ± 0.56	8.40 ± 0.43	8.40 ± 0.48	9.15 ± 0.97	8.50 ± 0.47	8.60 ± 0.30	9.20 ± 0.57	9.70 ± 0.19	9.34 ± 0.49
Free CO_2 (mg/l)	2.89 ± 1.31	3.03 ± 1.14	2.61 ± 1.17	3.44 ± 1.81	2.48 ± 0.78	2.34 ± 0.92	2.34 ± 0.92	2.89 ± 1.76	2.34 ± 0.92	2.20 ± 0.00	2.20 ± 0.00	3.03 ± 1.14	2.65 ± 0.99
TA (mg/l)	62.50 ± 3.78	70.00 ± 8.02	76.25 ± 7.44	68.75 ± 5.82	61.25 ± 2.31	60.00 ± 2.67	75.00 ± 14.39	68.75 ± 3.54	68.75 ± 16.20	61.25 ± 2.31	60.00 ± 0.00	63.75 ± 3.54	66.35 ± 5.84
TH (mg/l)	30.50 ± 2.56	38.25 ± 6.09	30.50 ± 1.77	30.50 ± 1.77	30.00 ± 5.55	22.00 ± 4.28	20.00 ± 1.07	20.50 ± 1.41	31.50 ± 21.37	26.00 ± 6.50	24.00 ± 2.83	30.50 ± 2.33	27.85 ± 4.80

Appendix XIV: Monthly pooled mean variations of physico-chemical parameters of Tamor River, Nepal from December 2014 to November 2016.

Parameters	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec	Annual average mean
Atm. temp. (°C)	**8.35 ± 0.14**	11.36 ± 0.20	14.10 ± 0.28	20.16 ± 0.23	22.32 ± 0.31	**23.10 ± 0.85**	22.10 ± 0.14	21.80 ± 0.28	21.20 ± 0.28	19.38 ± 0.03	14.97 ± 0.04	10.10 ± 0.14	17.41 ± 5.33
Water temp. (°C)	**6.97 ± 0.18**	10.14 ± 0.08	11.81 ± 0.06	15.62 ± 0.00	16 ± 0.28	**16.8 ± 0.42**	15.29 ± 0.27	15.4 ± 0.28	14.77 ± 0.04	13.47 ± 0.75	10.52 ± 0.69	8.77 ± 0.16	12.96 ± 3.23
pH	7.62 ± 0.14	7.52 ± 0.14	7.70 ± 0.21	7.26 ± 0.61	**7.02 ± 1.00**	7.45 ± 0.72	7.24 ± 1.09	7.46 ± 1.35	7.67 ± 0.86	7.10 ± 0.13	7.45 ± 0.86	**7.84 ± 0.51**	7.44 ± 0.25
DO (mg/l)	10.15 ± 0.00	9.93 ± 0.04	**11.10 ± 0.57**	**8.43 ± 0.11**	8.98 ± 0.81	8.95 ± 0.78	9.55 ± 0.57	8.93 ± 0.60	9.23 ± 0.88	9.05 ± 0.21	10.15 ± 0.64	10.15 ± 0.07	9.55 ± 0.76
Free CO_2 (mg/l)	**3.44 ± 0.58**	2.96 ± 0.49	3.37 ± 0.10	2.41 ± 0.10	2.41 ± 0.10	2.54 ± 0.29	3.09 ± 0.29	3.09 ± 1.07	2.89 ± 0.97	**2.20 ± 0.00**	3.03 ± 0.00	3.23 ± 0.49	2.89 ± 0.41
Total alkalinity (mg/l)	**77.81 ± 11.05**	76.25 ± 0.00	69.38 ± 0.88	**61.25 ± 0.00**	64.38 ± 6.19	75.00 ± 0.00	70.63 ± 2.65	68.13 ± 0.88	61.88 ± 0.88	71.25 ± 15.91	65.00 ± 1.77	63.13 ± 0.88	68.66 ± 5.69
Total hardness (mg/l)	**38.88 ± 0.88**	30.75 ± 0.35	30.50 ± 0.00	26.25 ± 5.30	35.63 ± 19.27	26.25 ± 8.84	25.00 ± 6.36	29.00 ± 3.54	**24.13 ± 2.65**	26.50 ± 3.54	32.25 ± 2.47	31.88 ± 1.94	29.75 ± 4.47

Appendix XV: Field protocol.

Fish sample:

Month: ..... Year: .....

S.N.	Total weight (g)	Total length (cm)	Standard length (cm)	Forkal length (cm)	Weight of gonad (g)	Sex	Day/Date of sample collection	Remarks (External morphology/ stage of gonad)

Physico-chemical parameters:

Month: Date: Year:

Sampling site No.:

Atm. temp.	Water temp.	pH	DO	Free CO_2	Total alkalinity (TA)	Total hardness (TH)

Appendix XVI: Average monthly and yearly discharge (in m^3/s) in Tamor River, Nepal.

Year	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Yearly Mean
2007	66.6	74.1	79.4	78.0	123	391	1060	900	1060	349	178	90.2	371
2008	57.6	40.7	43.4	81.5	149	438	974	1390	691	291	139	91.4	365
2009	77.6	62.6	56.9	102	147	263	675	1010	839	256	134	96.7	310
2010	78.6	62.6	60.5	98.7	143	302	931	1210	925	250	148	95.8	359
2011	67.7	55.5	46.0	65.3	134	285	866	878	466	204	143	104	276
2012	72.5	59.8	55.6	108	178	499	938	846	684	240	119	77.0	323
2013	52.4	42.4	43.5	61.2	190	453	1110	795	1070	596	218	136	397
2014	93.0	68.1	74.0	62.1	...	...	1680	1820	1090	279	103	61.9	...
2015	...	...	...	...	...	823	921	853	869	288	139	89.2	...
2016	67.2	57.4	55.9	82.5	145	653	1270	860	1300	491	197	138	443
Average	70.4	58.1	**57.2**	82.1	151	456	1040	**1060**	899	324	152	98.0	**356**

(Source: Department of Hydrology and Meteorology (DHM), Ministry of Energy, Water Resources and Irrigation, Government of Nepal)

Appendix XVII: Fishing gears and fishing techniques.

Tamor River has always beguiled people because of its inimitable and bracing beauty with snow-fed crystal clear water. Fishing practices have been taking place in this river since remote ages. Local fishermen and people living nearby use various kinds of fishing gears and fishing techniques which they have acquired or learnt through generations. Most of the fishing practices carried out in this river fall under traditional or artisanal fishing practices. These include small-scale and low-tech methods and are relatively cheaper. Artisanal fishing practices still form predominant part of the fisheries in most of the rural areas of this country.

The knowledge acquired by local indigenous people over the stretch of several years through their direct contact and interaction with nature and environment is handed over to successive generations by cultural transmission. Even with the fishing techniques, there is an intricate link between various techniques used and the behaviour of fishes. The use of certain types of fishing techniques is mainly governed by the target species and its habitat. This knowledge, in one way or the other, got disseminated down the generation line.

Traditional fishing techniques have undergone very little change over time. However, with the advent of modern techniques, these practices started to become more and more restricted. Although, these traditional techniques do speak volumes of our ancestors and their life style or way of living, little we could do for the conservation of these techniques. Hence, with the passage of time, these techniques are being abandoned.

Applications of traditional fishing techniques are very rare sights today as only a handful of people possesses the precise knowledge of using the techniques. Newer generations, on the other hand, seem to be enjoying or opting for more destructive fishing techniques like electro-fishing.

A very brief account on fishing gears and fishing techniques used by local fishermen and people living nearby Tamor River is given below.

Fishing in the river with wounding gears including harpoons, spears, arrows etc. after luring fishes with a torch or fire flame is recorded from early history and is still talked about by the descendants of the people who lived and fished in this river in the past. But these techniques have now been long abandoned.

Indigenous traps, mostly including fixed traps (Local names: Tip, Khonga) are still in practice, though have become rare sights today. These traps are constructed at appropriate places in the river where the water gradient is high. Although the construction of these kinds of traps is quite exhausting and tedious, they are very gifted techniques as they are capable of trapping large quantities of fishes at a time.

Still popular among the people and local fishermen are the use of gill nets and entangling nets, including set and drifting gill nets (Local names: Paso, Tane jaal). Gill nets catch fish which try to pass through the net by snagging on the gill covers. When the fishes are trapped, they can neither pass through nor retreat from there. These nets are positioned at appropriate places in the river at night and the trapped fishes are collected early in the morning.

Fishing by angling is still trendy among all. A hook is baited with lures like small insects, worms or a piece of the gutted intestine of chicken. Angling is the principal method of sports fishing. Fishes of massive sizes are caught up by angling in this river. Cast net, lift nets are also occasionally used in shallow areas of the river by local fishermen. Cast nets are round nets with small weights of lead or iron distributed around their edge. The net is cast or thrown by hand into the water, somewhat elegantly, so that the net spreads out and sinks into the water. Fishes are caught as the net is hauled back. Sometimes, fishes like Katle are lured into a particular place; usually a shallow nest-like structure, locally named as 'Kur', made out of stones and pebbles and stocked with appropriate fish feeds (pellets of cow dung and maize). As one notices the aggregation of fishes in and around the nest, a cast net is thrown carefully onto them. Large quantities of hard to find fishes are sometimes caught up using this technique.

Although illegal and destructive in nature, electric fishing is practised in the river by local people on a frequent basis, the sun and the moon. This technique is illegal but it seems the law is inadequately enforced here.

Electric fishing is one of the recently developed techniques of fishing which uses electricity to stun fishes and catch them. A battery pack is carried on the back by a person who frequently dips two long rods to discharge current into the water. This person is assisted by a person or two who wait to collect the fishes with the help of lift nets as the fishes are stunned by electric current. All kinds of fishes, making no discrimination of size and stages are caught up by this technique.

Electric fishing can be very destructive and may result in irreversible damage to fisheries if used without adequate caution. This technique also seems to be hazardous to people who perform this act.

Electric fishing is commonly used by fisheries scientists in scientific surveys, sampling fish populations for abundance, density and species composition. Young people might have acquired this knowledge from these scientists at first hand and the lack of awareness, on the part of these people, overruled thereafter.

Blast fishing, a yet another form of destructive fishing, results in irreversible damage to aquatic habitats and ecosystem. Although, this technique of fishing used to be practised in the past, it is nowhere to be endorsed at present.

Apart from these, indigenous people of this region use various kinds of plant extracts to collect fishes. Extracts of plants like *Wrightia arborea* (Local name: Khirro) and *Artemisia vulgaris* (Local name: Tite pati) are used as fish toxins to collect fishes. The course of a stream is diverted in such a way that several static large and small pools are formed all along its original course. Extracts of these plants are soaked into the water of these pools which stun or paralyze the fishes within and are thus easily collected.

Appendix XVIII: Photographs

1. *Neolissochilus hexagonolepis* (McClelland, 1839)

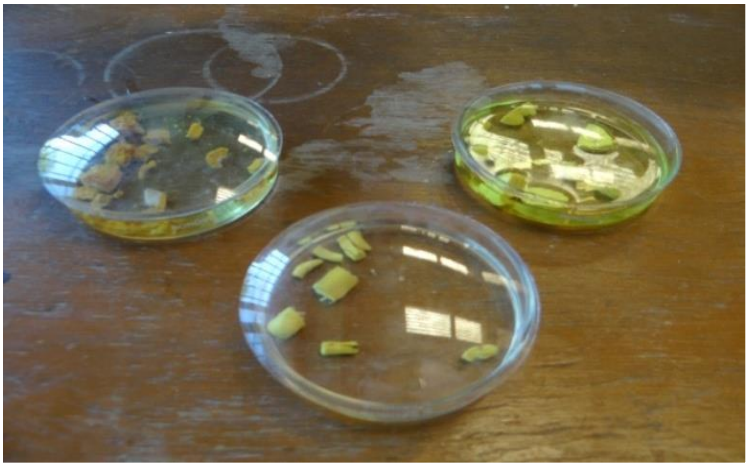
2. Sampling site I (Yakchanaghat)

3. Sampling site II (Ghumaune)

4. Sampling site III (Mulghat)

5. Sampling site IV (Mahang Khola)

6. Eggs of *N. hexagonolepis* separated for fecundity assessment.



7. Dehydrating pieces of gonads of *N. hexagonolepis* in 90 % and absolute alcohol.

8. Embedding gonadal tissues of *N. hexagonolepis* in paraffin for microtomy.

9. Trimming of paraffin embedded blocks with the help of a rotary microtome.

10. Preparation of histological slides of gonads of *N. hexagonolepis.*

11. Fixation of water sample for estimation of dissolved oxygen (DO) at a sampling site.

12. Estimation of dissolved oxygen by titration in laboratory.

13. Electric fishing by local youth.

14. Fishing by angling.

15. Use of cast net for fishing.

16. Locally constructed fixed fish-trap.

Publications & Participations in International and National Conferences

1. Certificates of participation / oral presentation in international and national conferences

2. Subba, S., Mahaseth, V.K., Subba, B.R., & Bhusal, D.R. (2020). Monthly dynamics of reproductive indices of *Neolissochilus hexagonolepis* (McClelland, 1839) and their relationship with physico-chemical parameters along the mid-reaches of Tamor River, Nepal. *Egyptian Journal of Aquatic Biology & Fisheries*, **24**(2): 239-247.

3. Subba, S., & Mahaseth, V.K. (2018). Histological observation of gonads during breeding and non-breeding season of *Neolissochilus hexagonolepis* from Tamor River, Nepal. *International Journal of Fisheries and Aquatic Studies*, **6**(5): 132-137.

4. Subba, S., Subba, B.R., & Mahaseth, V.K. (2018). Relative condition factor, length-weight relationship and sex ratio of copper mahseer, *Neolissochilus hexagonolepis* (McClelland, 1839) from Tamor River, Nepal. *Our Nature*, **16**(1): 27-34.

5. Subba, S., Mahaseth, V.K., & Subba B.R. (2018). Gonadosomatic index, egg size and fecundity of chocolate mahseer, *Neolissochilus hexagonolepis* (McClelland, 1839) from Tamor river, Nepal. *Nepalese Journal of Aquaculture and Fisheries*, **5**: 27-34.

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नेपाल मत्स्य समाज

Nepal Fisheries Society

This is to certify that **Mr. Suren Subba** from Central Department of Zoology, TU, Kirtipur, has participated / presented the paper entitled " Gonadosomatic index, Egg size and Fecundity of Chocolate Mahseer, *Neolissochilus hexagonolepis* (McClelland, 1839) from Tamor River, Nepal" at the International Conference on Sustainable Fisheries & Aquaculture Diversification, Second NEFIS convention, held in Kathmandu, during 8-9 March, 2018.

Dr. Tek Bahadur Gurung
President
Nepal Fisheries Society (NEFIS)

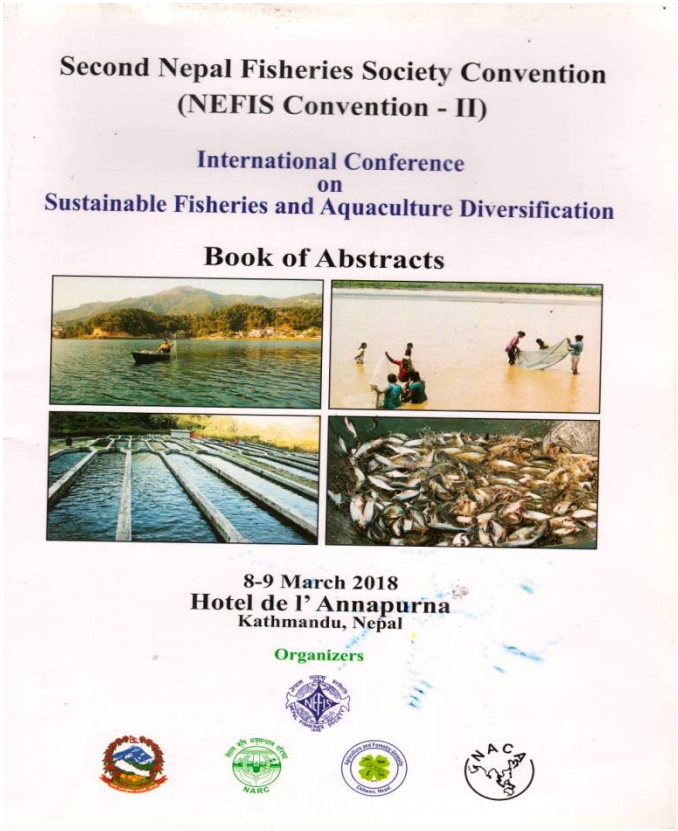
Issued Date: 10 November 2019

International Conference on
Sustainable Fisheries & Aquaculture Diversification

Second NEFIS Convention
(Nepal Fisheries Society)

Venue: Hotel de l' Annapurna, Kathmandu

March 8-9, 2018
Kathmandu, Nepal



OR-10

GONADOSOMATIC INDEX, EGG SIZE AND FECUNDITY OF THE CHOCOLATE MAHSEER, *Neolissochilus hexagonolepis* (McClelland, 1839) FROM TAMOR RIVER, NEPAL

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The present study was carried out to assess gonadosomatic index (GSI), egg size and fecundity of chocolate mahseer, *Neolissochilus hexagonolepis* from Tamor river, Nepal. The fish samples were collected from the river every month from December 2014 till November 2016. The mean GSI of female fish varied from minimum (0.19±0.05) in January to maximum (7.75±4.40) in July and the mean GSI of male fish varied from minimum (0.25±0.17) in December to maximum (2.33±1.38) in July. Absolute fecundity ranged from 1374.73 for fish of total length (TL-cm) 45 cm, total weight (TW-g) 1500g and gonad weight (GW-g) 5.63g to 11424 for fish of total length (TL-cm) 37 cm, total weight (TW-g) 710 g and gonad weight (GW-g) 102 g. The mean value of fecundity was 16005.008 ± 3004.75) with a mean total length of 436.45cm ± 4.79) and mean body weight (634.57g ± 329.13). The smallest size of eggs measuring 1.3 mm in diameter was recorded from a fish sample that measured 31cm total length, 340 g total weight, 4.48 g gonad weight and 2329.6 numbers of eggs. The largest eggs measuring 2.34 mm in diameter were recorded in a fish that measured 33.6 cm total length, 442 g total weight, 38.42 g gonad weight and 4092 numbers of eggs. Fecundity showed positive correlations with total weight (r = 0.30), total length (r = 0.44), standard length (r= 0.49), fork length (r = 0.48) and gonad weight (r = 0.78). Egg size (1:S) was found to have significant moderate positive correlation with GW (r 0.65, PE 0.05) but very weak positive correlation with fecundity (r= 0.25). Gonadosomatic index (GSI) and gonad weight (GW) were positively correlated for both the sexes with the value of correlation coefficient (r) for female equal to 0.92 and that for male equal to 0.55.

Keywords: *Neolissochilus hexagonolepis*, Gonadosomatic index, Egg size, Fecundity, Tamor river

OR-11

EFFECT OF DIFFERENT LEVELS OF PROBIOTIC ADDITIVES ON GROWTH INDICES AND BODY COMPOSITION OF JUVENILE *LABEO ROHITA* AND *CYPRINUS CARPIO*

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A feeding experiment was conducted to evaluate the effect of different levels of probiotic additives on growth performance and body composition of two juvenile species i.e., *Labeo rohita* and *Cyprinus carpio* (mean initial body weight 5.2 ± 0.52 and 12.63 ± 0.75 g). Five

National Conference on Integrating Biological Resources for Prosperity

Certificate of Participation

This is to certify that

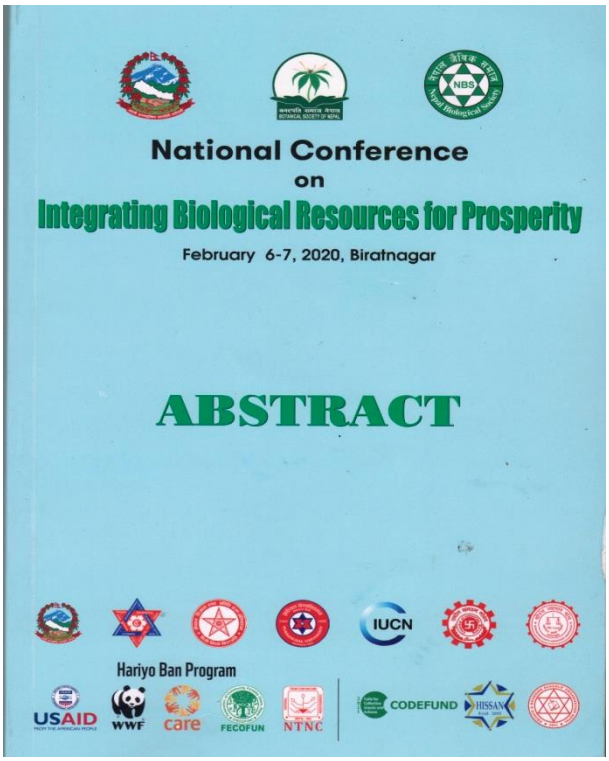
Mr. Suren Subba

has presented Paper / Poster / Attended the

National Conference on Integrating Biological Resources for Prosperity

held at Biratnagar on February 6-7, 2020.

Prof. Dr. Mohan Siwakoti	Prof. Dr. Sasinath Jha	Dr. Bishwa Nath Oli	Hon. Jagadish Prasad Kusiyait
Chairman	Chairman	Secretary	Minister
Conference Organizing Committee	Nepal Biological Society	Ministry of Forests and Environment	Ministry of Industry, Tourism, Forests and Environment
President, Botanical Society of Nepal			Province No. 1



Oxygen uptake in relation to body weight in Hill stream long-tail catfish *Olyra longicaudata* (McClelland,1842)

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Abstract

The present investigation was carried out to predict the relationship between oxygen consumption (VO_2) and body weight (W) in hill stream long-tail catfish *Olyra longicaudata* from Chisang river basin, Ilelang, Nepal. The experiment was conducted at 27±1°C using a cylindrical glass respirometer in lifecast fishes. The oxygen uptake in *Olyra longicaudata* varied from 0.7044 to 2.042 mlO_2/h within the weight range of 0.8 to 5.2g. The estimated value (VO_2) as 1.8g of fish is 0.7493 mlO_2/h. The relation between oxygen uptake (VO_2) and body weight was determined by performing regression analysis using logarithmic transformation. The graph between the oxygen uptake per unit time and body weight gave a straight line with the slope 'b' value of 0.5806. The relationship between oxygen uptake and body weight was found to be positively correlated (r= 0.951) indicating that as body weight increases, oxygen consumption also increases. But the weight-specific oxygen uptake decreases by a power of 0.419 showing a significant but negative correlation.

Keywords: Oxygen uptake, Body weight, Hillstream, *Olyra longicaudata*

Gonadosomatic index-based size at first sexual maturity of the Copper Mahaseer, *Neolissochilus hexagonolepis* (McClelland, 1839) from the mid-reaches of the Tamor River

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Abstract

This study depicts the size at first sexual maturity of a population of *Neolissochilus hexagonolepis* (McClelland, 1839) sourced from the mid-reaches of the Tamor River, eastern Nepal. Sampling was done with the help of traditional fishing gears including cast nets and gill nets, between December 2014 and November 2016. A total of 405 specimens (182 males, 203 females) were sorted out according to their sex and total body weight (TW) and total length (TL) were noted for each individual. Gonadosomatic index (GSI) was calculated by the equation, GSI (%) = [Gonad weight (g) / TW (g)] × 100. The association between the gonadosomatic index and TL was used for the estimation of the size at first sexual maturity. Spearman's rank correlation test revealed a significant correlation between GSI and TL for females (r_s = 0.44177; $p < 0.05$), but an insignificant correlation for males (r_s = 0.21089; $p > 0.05$). The sizes at first sexual maturity of male and female *N. hexagonolepis* were 15 cm TL and 30.2 cm TL, respectively. The data assembled during the present study is an important indicator of the minimum permissible capture sizes which could be ascertained in management plans for ensuring sustainable conservational efforts for the near threatened *N. hexagonolepis* population in Tamor River.

Keywords: *Neolissochilus hexagonolepis*, Tamor River, Sex ratio, Gonadosomatic index, Sexual maturity