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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

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ABSTRACT

Physico-chemical parameters exert influence on the reproductive biology of fish. The present study was carried out from December 2014 to November 2016 to investigate the effect of physico-chemical parameters on the reproductive indices like gonad weight and GSI of either sex of *N. hexagonolepis* in the mid-reaches of Tamor River. Altogether 198 fishes were sampled during the study period. The mean total body weight (TW) and mean total length (TL) of male fishes were 171.67 ± 186.78 gm and 21.21 ± 6.38 cm**,** respectively. Similarly, the mean TW and mean TL of female fishes were 349.23 ± 316.82 gm and 27.86 ± 9.21 cm, respectively. 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 in July to August. A single well-defined peak of GSI for both male and female fish indicated that *N. hexagonolepis* is an annual breeder. NMDS (Non-matrix multidimensional scaling) of the physico-chemical parameters and reproductive indices of *N. hexagonolepis* revealed that the atmospheric temperature and water temperature exert significantly positive influence on the gonad weight and GSI of either sex of *N. hexagonolepis* while the other environmental variables pH, DO, free CO_2, TA and TH exert negative influences upon the reproductive indices of the fish.

INTRODUCTION

Fishes being cold-blooded animals are very sensitive to changes in environmental parameters **(Majhi *et al*., 2013)**. Physico-chemical properties of water play a significant role in distribution, composition, abundance, and diversity of wide range of aquatic life especially on fishes **(Olenrewaju *et al*., 2017; Priya *et al*., 2016)**. Temperature affects the metabolic activities, growth, and reproduction of aquatic organisms **(RAMP, 2017)**. Abrupt changes of water temperature in fresh water ecosystem by which fishes can suffer from harsh physiological stress and ultimately affecting the reproductive performance of

fishes **(Majhi *et al*., 2013)**. Pollution due to the contamination of water affects the growth and reproduction of fish **(David *et al*., 2010)**.

pH of water is the measure of H+ ion activity of the water ecosystem and indicates whether the water is acidic, alkaline or neutral in nature. Extreme pH negatively affects fish growth and reproduction **(Zweig *et al*., 1999)**.

Basavaraddi *et al*. (2012) suggested that fish and other aquatic animals depend upon dissolved oxygen (DO), which in turn is dependent on water temperature. Alkalinity is the total measure of substances in water that has the acid neutralizing ability and it is important for fish and aquatic life because it protects or buffers against pH changes. Water hardness is the measure of the number of divalent ions such as calcium, magnesium and/or iron in the water. Hardness can be a mixture of divalent salts; however, calcium and magnesium are the most common sources of water hardness.

Sharma *et al*. (2014) investigated the effect of physico-chemical parameters such as water temperature, pH, carbon dioxide, dissolved oxygen, total alkalinity, and total hardness on the gonado-somatic index (GSI) of *Schizothorax richardsonii* and *Oncorhynchus mykiss*. Similarly, **Sharma *et al*. (2015)** and **Olanrewaju *et al*. (2017)** also suggested that the environmental variables affect the reproductive indices of *Tor putitora* and *Parachanna obscura* respectively.

Neolissochilus is one of the important hill-stream fishes that thrives well in the rivers of tropical and subtropical regions of Asia **(Mahapatra and Vinod, 2011)**. According to **Khaironizam and Ismail (2012)**, twenty five species of *Neolissochilus* have been reported so far. Out of them, only *N. hexagonolepis* (McClelland, 1839) which is commonly known as Copper or Chocolate Mahseer and locally known as 'Katle' has been reported from Nepal **(Shrestha, 2008)**.

According to **Shrestha (2008)**, this fish is distributed in all three major river systems of Nepal: the Koshi, the Gandaki and the Karnali. *N. hexagonolepis* has also been reported from India, Bangladesh, China, Burma and Indonesia **(Shrestha, 2008; Mahapatra and Vinod, 2011; Majhi *et al*., 2013)**. This fish is famous for its food quality and good price due to attainable large size **(Jyrwa and Bhuyan, 2017)** and is also considered a very important sport or game fish **(Shrestha, 2008; Mahapatra and Vinod, 2011; Jyrwa and Bhuyan, 2017)** as well as an important aquarium fish **(Mahapatra and Vinod, 2011)**.

Currently, the fish has been kept under 'Near Threatened' (NT) category in Redlist Assessment of **IUCN (2018)**; therefore, emphasis for the preservation of this fish is needed. But, there is a lack of sufficient knowledge concerning detail reproductive biology of *Neolissochilus hexagonolepis*. It is widely accepted that information regarding the reproductive biology of this fish is very essential to understand the reproductive strategy of this fish **(Pholdee and Suvarnaraksha, 2017)**. Previously, **Shrestha (1989)** and **Swar and Craig (2002)** studied the breeding of Copper Mahseer by hormone stimulation and spawning behaviour, respectively. However, poor documentation exists

regarding monthly dynamics of reproductive indices in this fish especially in Nepalese river system.

This study aims to investigate monthly dynamics of reproductive indices and their relationship with important physico-chemical parameters in Tamor River, one of the major tributaries of Koshi River system of eastern Nepal. By investigating the most influential physico-chemical parameters affecting the reproductive indices of this fish, concerned authorities and/or biodiversity managers can take suitable steps for conservation of this fish.

MATERIALS AND METHODS

Study area

Tamor river is one of the major tributaries of Koshi River system of eastern Nepalwhich lies between the latitude and longitude of 26˚54'47" N and 87˚09'30" E respectively **(Figure 1)**. The river bed consists of boulder, cobble, pebble, gravel and sand which provide suitable spawning sites for fishes **(Shrestha *et al*., 2009)**. The study site stretched for about 12 km (A to B) along the river course **(Figure 1)**.

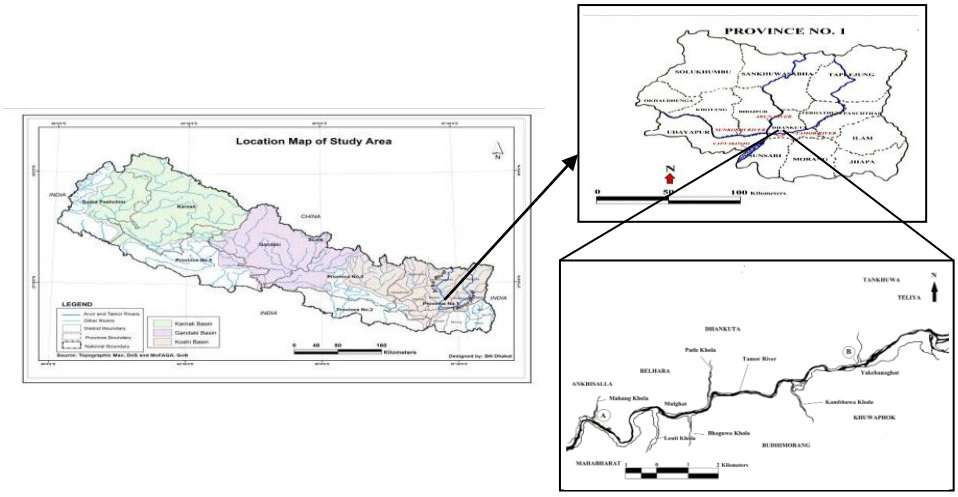

Figure 1. Location map of the study area.

Sampling methods

Fish samples were collected during the second half of every month for two consecutive years from December 2014 to November 2016 using hooks and lines, cast net and local 'tip' trap. Total weight of each sampled individual (including gut and gonads) was measured using a digital balance with the precision of 0.01 gm. Altogether, 198 fishes were captured for the examination of gonads. The gonads were extracted in situ through a longitudinal incision made along the ventral line of the body with the help of a sharp blade and angular scissors. The gonads were weighed to the nearest 0.01 gm.

The gonado-somatic index (GSI) of each fish was calculated following the method by **Nikolsky (1963)**. The formula used was: GSI = Gonad weight/Total weight of fish X 100.

Water quality parameters

Water samples were collected from 5 to 10 cm below the water surface on fortnightly basis. Water variables measured included atmospheric and water temperatures (˚C), pH, Dissolved oxygen (mg/l), free CO_2 (mg/l), total alkalinity (mg/l) and total hardness (mg/l). Temperature was recorded with simple mercury filled Celsius thermometer while pH was measured with a pH meter. The analyses of other parameters were performed following the standard protocols suggested by **APHA (2005)**.

Data analysis

The data collected were analyzed to find the monthly variation of indices and relation of indices with physico-chemical parameters. We performed distance (bray) based multivariate analysis linking function (Meta MDS) for non-metric multi dimensional scaling (NMDS) from vegan package in R- statistics.

RESULTS

Altogether 198 fishes were sampled during the study period. The total body weight (TW) and total length (TL) of male fishes ranged from 19.11 gm to 750 gm (Mean: 171.67 ± 186.78 gm) and 12.4 cm to 36.9 cm (Mean: 21.21 ± 6.38 cm)**,** respectively. Similarly, TW and TL of female fishes ranged from 24 gm to 1500 gm (349.23 ± 316.82 gm) and 13 cm to 46.3 cm (27.86 ± 9.21 cm), respectively. The average annual sex ratio was in favour of female (Male: Female = 1:1.22). However, the χ^2 test showed no significant difference between the sexes ($\chi^2 = 2.0202$, $p > 0.05$).

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 **(Figure 2)**. Subsequently, there was a precipitous recession in GSI from September 2015 onward, oscillating at times, reaching to 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 **(Figure 2)**.

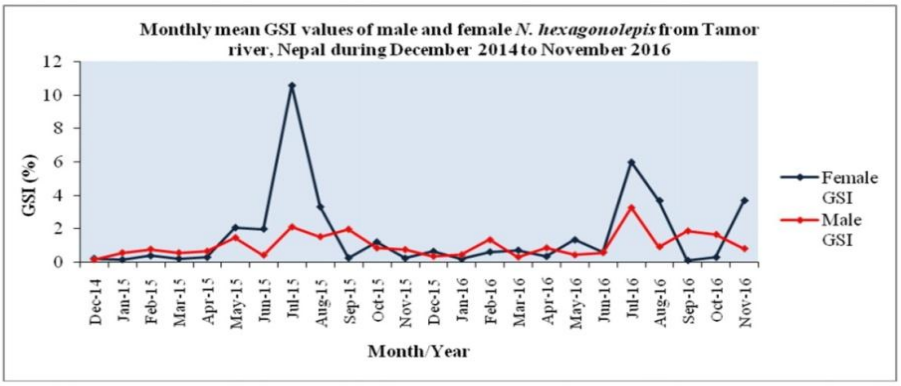

Figure 2. Variations of the monthly mean GSI values of male and female *N. hexagonolepis* from Tamor River, Nepal during December 2014 to November 2016.

Relation of reproductive indices with physio-chemical parameters

NMDS (Non-matrix multidimensional scaling, stress = 0.411 anddistance = bray) revealed significantly positive relation of the reproductive indices with atmospheric temperature and water temperature. Other environmental variables pH, DO, free CO_2, TA and TH showed negative correlations with the reproductive indices (Fig. 3).

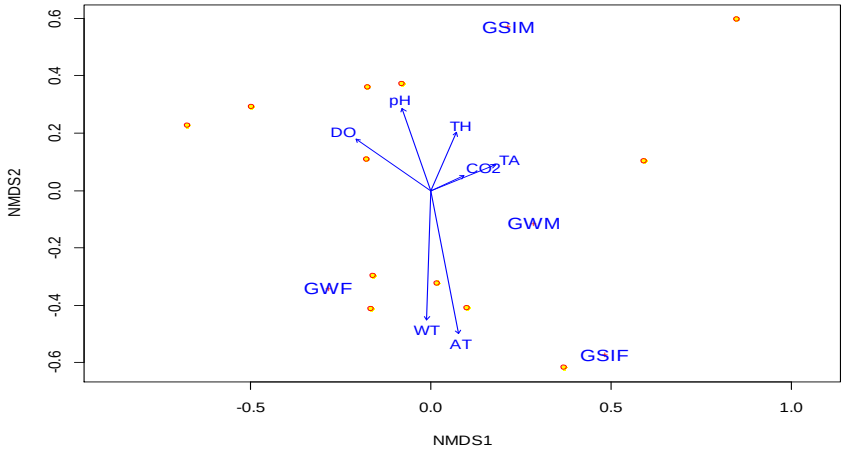

Figure 3. Non-matrix multidimensional scaling (NMDS) bi-plot of physico-chemical parameters of water and gonad weight and GSI of male and female *N. hexagonolepis* (Significant ($p<0.01$) at 1000 permutations) (Abbreviations: AT: Atmospheric temperature; WT: Water temperature; DO: Dissolved oxygen; CO_2: 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).

Physico-chemical parameters

Monthly mean atmospheric and water temperature varied from 8.35 ± 0.14 ˚C to 23.1 ± 0.85 ˚C and 6.97 ± 0.18 ˚C to 16.8 ± 0.42 ˚C respectively. Similarly, the values of pH, dissolved oxygen (DO), free CO_2, total alkalinity (TA) and total hardness (TH) of water samples from the river ranged from 7.02 ± 1.00 to 7.84 ± 0.51, 8.43 ± 0.11 mg/l to 11.1 ± 0.57 mg/l, 2.2 ± 0.00 mg/l to 3.44 ± 0.58 mg/l, 61.25 ± 0.00 mg/l to 77.81 ± 11.05 mg/l and 24.13 ± 2.65 mg/l to 38.88 ± 0.88 mg/l respectively.

DISCUSSION

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 in July to August, consistent with the finding of other workers **(Swar, 1994; Mahapatra and Vinod, 2011)**. The spawning of *N. hexagonolepis* during monsoon season could be due to the influence of several environmental factors like temperature change, rainfall and subsequent rise in water level in the river. **Dodebo *et al*. (2003)** suggested that the beginning of rainy season, changes in temperature and rise in water level serve as the triggering factors for spawning in most tropical fishes.

Much higher GSI of female compared to that of male implied that female gonads were much heavier even at the same maturity stages. **Verma (2013)** also reported higher GSI of female in *Labeo dyocheilus*. Also, the abnormally higher GSI for female fish in July 2015 was principally ascribed to the presence of higher percentage of matured females in July 2015 compared to that in 2016.

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 an annual breeding behaviour in *N. hexagonolepis* from different habitats **(Swar, 1994; Mahapatra and Vinod, 2011; Jyrwa and Bhuyan, 2017)**.

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.

The biotic relationships of organisms in an aquatic ecosystem are governed by the physico-chemical parameters of water **(Olanrewaju *et al*., 2017)**. Since the river is snow-fed, its average temperature remains low. In the present study the average annual temperature of the river was found to be 12.96 °C.

Extreme pH negatively affects fish growth and reproduction **(Zweig *et. al*., 1999)**. In the present study, water samples from the site were nearly neutral to slightly alkaline.

In many fishes and other aquatic animals growth and reproduction depend upon DO, which in turn is dependent on water temperature **(Basavaraddi *et al*., 2012)**. Dissolved oxygen being one of the limiting factor in reproduction that its value below 5 ppm can

impact on hatching and survival of the fishes **(Mahapatra and Vinod, 2011)**.The present study revealed that the river holds a good amount of dissolved oxygen (Annual average: 9.55 ± 0.76 mg/l) throughout the year.

NMDS (Non-matrix multidimensional scaling) of the physico-chemical parameters and reproductive indices of *N. hexagonolepis* in Tamor River revealed that the water parameters exert influence on the gonad weight and GSI of either sex of the fish with temperature being the most influencing parameter.

Shrestha (1978) and **Verma (2013)** suggested that environmental variables play important role in the reproductive biology of aquatic fauna such as *Schizothorax* spp. and *Labeo dyocheilus* respectively. **Lawson (2011)** also considered the temperature to be the most influencing parameter in the gonadal gametogenesis, spawning and initiation of gonadal development of fish. **Sharma *et al*. (2014)** also reported strong positive relationships of temperature on the GSI values of cold-water fishes like *Oncorhynchus mykiss* and *Schizothorax richardsonii*. **Im *et al*. (2016)** and **Olanrewaju *et al*. (2017)** also figured out that the water temperature plays an important role in the gonadal development of *Rhynchocypris kumgangensis* and *Parachanna obscura*, respectively. All the above results were analogous to the finding of the present study.

CONCLUSION

The study deduced that *N. hexagonolepis* breeds annually with peak spawning activity in July to August. Furthermore, the atmospheric temperature and water temperature were found to exert significantly positive influence on the gonad weight and GSI of either sex of *N. hexagonolepis* while the other environmental variables pH, DO, free CO_2, TA and TH showed negative correlations with the reproductive indices of the fish.

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