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Feeding habits and reproductive biology of *Onychostoma lepturum* from the rivers and streams of A Luoi district, Vietnam

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ABSTRACT

The present study provides preliminary analysis on the feeding and reproductive characteristics of *Onychostoma lepturum*. The gastrointestinal structure of *O. lepturum* is long gut with an average RLG value ranges between 4.94 – 5.05, which is suitable for the herbi-omnivore fish. The food composition of *O. lepturum* includes 4 main types, algae, insects, crustaceans and organic detritus. The difference in the reproductive organs of males and females are clearly visible. Histological specimens of the gonads can clearly and accurately distinguish the developmental stages of the gonads. The maximum GSI values were recorded in April and the GSI values were the lowest in October for males and females respectively.

INTRODUCTION

A Luoi, a mountainous district of Thua Thien Hue, is located in the western Truong Son mountain range. With the tropical monsoon climate, influenced by the transitional season between the North and the South of Vietnam, A Luoi has an extremely rich and diverse natural and biological resources. Due to the particular characteristics of the terrain; river and stream system has certain slope with a number of waterfalls, rapids, strong currents and high dissolved oxygen concentration (approximately saturated), the teeming aquatic plants provide rich food sources for freshwater fish species including *Onychostoma lepturum* **(Boulenger, 1900)**, a bonefish species with significant valuation of subsistence for fishery **(Nguyen *et al*., 2012)**. Fish dishes of the aforementioned species are local specialties, especially the ones made from fish intestines, because local people think that these fish eat clean healthy moss in their habitats where the water is clear with strong flowing streams, upstream of rivers in A Luoi. They are therefore considered an environmental indicator of a healthy ecosystem. While being recorded in the Vietmam Red list in 2007 and in The IUCN Red List of Threatened Species in 2012, the data on the biological reproduction and feeding habit of *O. lepturum* are largely limited. Therefore, comprehensive studies on the feeding behaviours, nutritional and reproductive characteristics of this fish are highly required. In the current study, the data provided about this species in the study area were conducted to assess the environmental indicators as well as the economic value of the species to define the promising methods of the reasonable exploitation and conservation .

MATERIALS AND METHODS

Samples collection and preparation

The specimens of *Onychostoma lepturum* were collected from the rivers and streams in the East of A Luoi district, Thua Thien Hue province, Vietnam (Fig. 1) from October 2018 to September 2019. Totally, a number of 55 fish samples were gathered including 21 individuals collected from A stream, 15 individuals from Rao Lo river and 19 individuals from Ta Li river. Samples were collected monthly for 12 months with the help of local fishermen in the study area. Size and body weight of individual samples were determined using Vernier Calliper and electronic weighing balance respectively. Fish samples were then transported to the laboratory for further analysis.

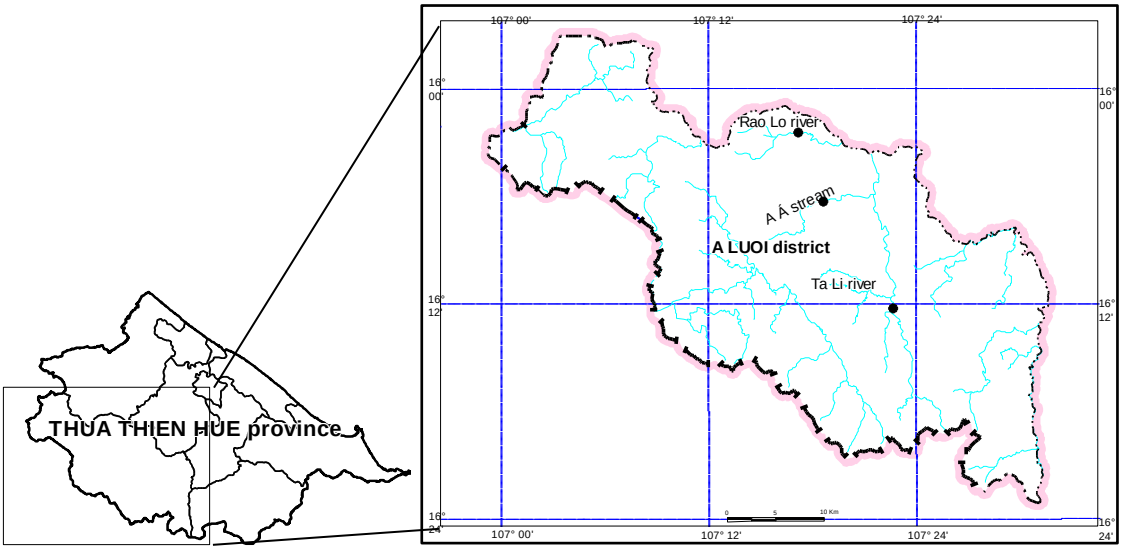

Fig. 1. Sampling location map (●: sampling location)

Diet composition analysis

The specimens were categorized into two groups viz., group I (80-120mm) and group II (121-290mm) for the convenience of analysis. The division of fish size was based on the collected individuals and the maturity stages of fish. After accessing the digestive organs structure, the length of gut was measured to obtain the relative length of the gut (RLG). Then, the gut was removed and preserved in 5% formaldehyde for further food composition and feeding habits examinations. The RLG was calculated by using standard formula: RLG = length of the gut/total length of the fish **(Al-Hussaini, 1949)**. Gut contents were analysed using both quantitative and qualitative method. The analysis of food items in the gut of fish was carried following the point method of **Hynes (1950)**.

Reproductive biology analysis

The collected fish were measured identifying their total length (mm) and total weight (0.1g). To identify the sex, the external morphology of *O. lepturum* was carried out with reference to the externally visible characteristics such as spines on the mouth as well as on the anal fins and the genital pore. The specimens were then opened to observe the fish body cavity. Hence, the gonads were examined to determine fish sex and maturity stage, and then, removed and photographed for subsequent analysis.

Developmental stages of the gonads of *O. lepturum* were accessed based on morphological characteristics combined with templates histological structures as described in maturity ladder of **Nikolsky (1963)**. Briefly, samples were fixed in Bouin's solution, then cleansed, embedded in paraffin blocks, sectioned, then stained by Hematoxylin-Eosin according to **Roberts (2004)** and observed under the microscope. The section was dyed according to Hematoxylin-Eosin staining method. The gonadosomatic index of the specimens were determined monthly, calculated according to the methods described by **Anderson and Gutreuter (1983)** using the following formula: GSI = Gonad weight/Body weight × 100. Only fish samples in the "ripe" stages were considered to calculate the fecundity. Fecundity was estimated using the succeeding formula: Fecundity= Total weight of the ovary × Number of ova in the sample/Weight of the sample (**Hunter *et al.*, 1989**).

RESULTS

1. Feeding characteristics of *Onychostoma lepturum*

1.1. Anatomy of the digestive tract

The digestive tract of *Onychostoma lepturum* begins from the mouth. The lower jaw of *Onychostoma lepturum* is wide and horizontal. The chin groove is shallow, which is equal to one fourth of the eye diameter. The esophagus is below the mouth sinus and has a small-short tubular in shape. The stomach is found in the abdominal sinus as a continuation of the esophagus. The stomach of fish is big and has bag-like shape. The intestine has a long tubular shape and is curled with an average length of 50 - 110cm (Fig. 2). Anatomical results showed that the fish intestine is relatively long and takes up most of the gastrointestinal tract, with the length of the intestine fulling three to five times of the body length (Fig. 4de). This reflects the nutritional and ecological characteristics of a herbivorous fish.

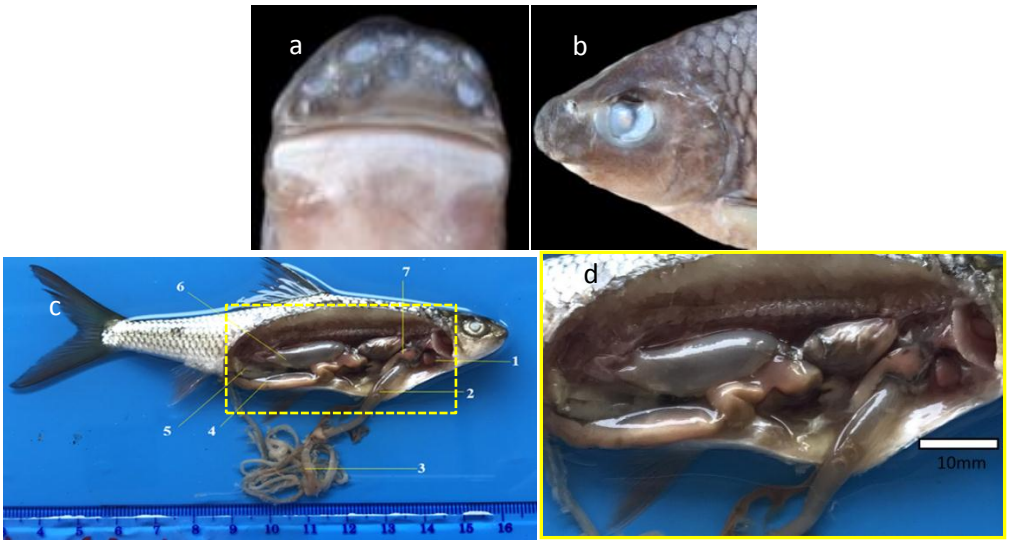

Fig. 2. a Front view and **b** lateral view of head of *O. lepturum.* **c, d** Internal organs of O. *lepturum***,** 1- Heart, 2- Stomach, 3- Intestine, 4- Pancreas, 5- Testes, 6- Swim balls, 7- Liver

1.2. Food composition of *Onychostoma lepturum*

Food composition in fish often varies, even within the same species, according to their developmental stages. The difference depends on feed source distributed in nature, feeding behavior and the maturation of digestive organs of the fish. In this study, the guts of forty fish were directly examined under the stereomicroscope, and their ingested food composition was preliminarily classified. The frequence of food items in the gut (Fig. 3) revealed that *O. lepturum* had a broad-spectrum diet. This species has a wide feeding habits and their nutrition includes algae such as green algae and silicic algae, insects, crustaceans and organic detritus. It should be noted that the percentage of algae was the highest in food composition of this fish with 58%, followed by the detritus with 17%, insects with 14% and the lowest percentage of crustaceans with 11%. The long intestine with plankton-rich diet suggests that *O. lepturum* belongs to the plant-eating fish group. This result serves as a basis for further determination of the feeding habits and food spectrum of the species. This is an important criterion used to determine the nutrition of species in the genus.

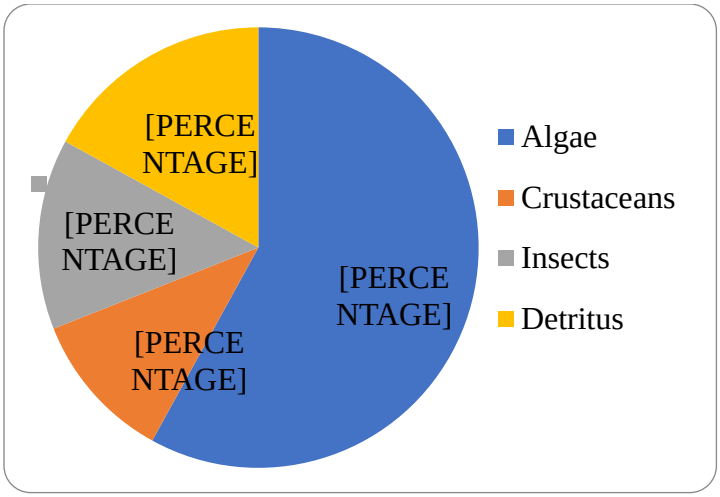

Fig. 3. Contribution of various food items in the diet of *O. lepturum*

Table 1. RLG value of *O. lepturum* in different size groups

Size group	Sample size "n"	RLG value	Average (±SD)
Group I (80 to 120 mm)	20	4.85 – 5.72	5.05 ± 0.24
Group II (121 to 290 mm)	20	3.82 – 5.22	4.94 ± 0.32

The RLG value was measured for a total of 40 collected fish which were categorized into two groups; group I (80 to 120 mm) and group II (121 to 290 mm). It is of note that the RLG value ranged between 4.85 - 5.72, with an average value of 5.05 ± 0.24 in group I fish, while group II fish showed RLG ranging from 3.82 - 5.22 and an average value of 4.94 ± 0.32 (Table 1). Remarkedly, the RLG value of fish in group I is higher than that in group II and reached the its maximum with 5.72. This revealed that the RLG value marked a decline as the size of the fish increased, indicating a possible change of food composition from fry stage to adult stage. According to **Das and Moitra (1963)**, relative length of the gut is generally longest in herbivorous fish, shortest in carnivorous

fish and medium in omnivorous fish. Thus, the present results suggest that *O. lepturum* should be assigned as herbi-omnivore fish.

2. Reproductive biology of *O. lepturum*

Sex identification can be accessed based on the externally visible characteristics, such as genital pore, the head and some signs on the mouth. However, it is difficult to determine fish gender using these characteristics at the early developmental phases. At reproductive age, it is possible to distinguish male and female fish through distinct structure morphology of certain organs such as the mouth. Female *O. lepturum* develops some mace-shaped spines on the mouth and others form spines in the anal fins, while in male *O. lepturum* small recesses appear on the mouth in the maturity stage (Fig. 4abc). Besides, the head of male fish is more pointed and wrinkled than that of the female. In addition, the belly of female *O. lepturum* is usually larger and softer than that of the male; the genital pore of the female *O. lepturum* is large, round and flushed, while that of the male *O. lepturum* is smaller and white. However, it should be noted that certain morphological characteristics and coloration of *O. lepturum* may change according to habitats, especially for wild fish in immature stage. Therefore, it is necessary that the determination of sex would be then confirmed by observing the gonads.

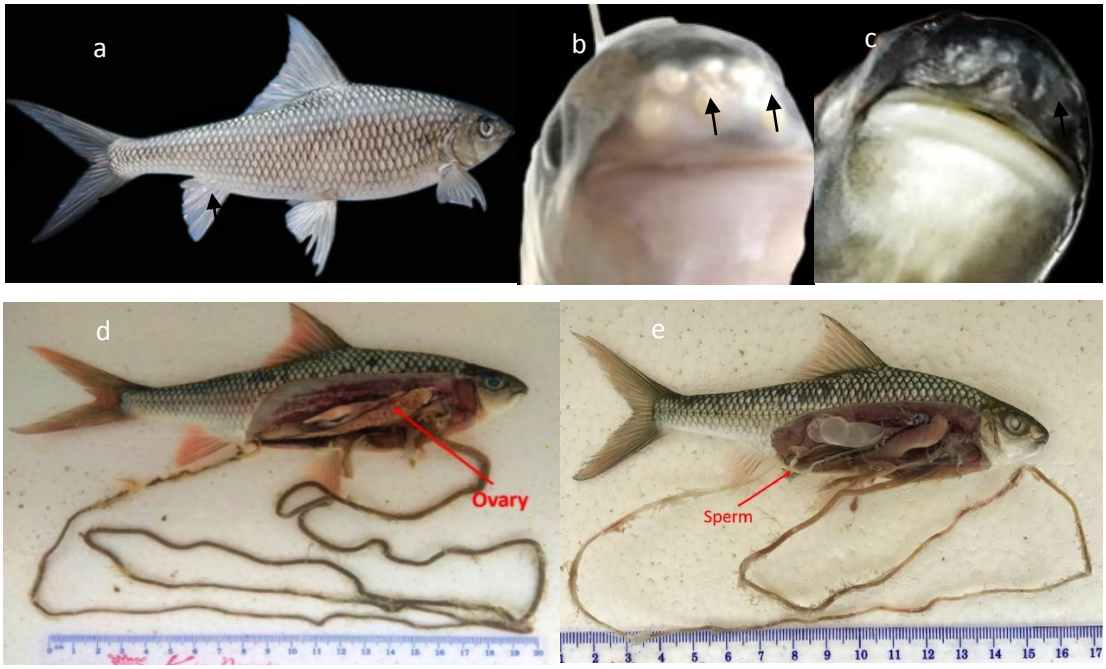

Fig. 4. a spines on the anal fins of a female *O. lepturum*; **b** mace-shaped spines on the mouth of female *O. lepturum*; **c** recesse on the mouth of male *O. lepturum*; **d, e** the reproductive organ of a female (**d**) and a male (**e**)

The difference between male and female reproductive organs of *O. lepturum* is obvious. The gonads of both fish lie in the abdominal cavity. Ovaries have a cylindrical shape, situated in the lower abdominal cavity. The posterior part of the ovary narrows into a short and slight white oviduct where genital products are passed through this ovarian tube before emptying out through the genital pore. When female *O. lepturum* matures, the ovaries become large in size, light yellow in colour and the ova are obvious

to observation from the outside (Fig. 4d). Testes of male fish lie in both sides of the mesenteric on the back close to the spine. It is slight white, thin thread-like in the initial phases, and becomes thicker upon maturation as shown in Fig. (4e).

Fig. (4de to 9) depicts external morphology of gonads and histological sections of ovary of maturity stages IV and stage VI. Whereas, the histological sections of testes shows maturity stages I, II and stage IV.

2.1. Ovary

Stage IV. External morphology: Ovaries become more enlarged, yellowish orange in colour and filling one-third of the abdominal cavity. Ovary appears conspicuous superficial blood vessels that focus on a major path. Ripe ova are large, uniformly textured, easily separated from each other and visible through the naked eye. In this stage, the number of eggs is full, the egg size is maximal and detached. Ova can be counted to determine the fecundity of the fish in maturity stage IV; *Histological organization*, observing the histological template under a microscope, reveals that most of the egg cells have ended the period of nutritional growth. Cell nucleus moved from the center to the periphery creating polarization of the cell. Besides, the sex cells are in the period of biological growth and the final phase of nutrition growth (Fig. 5).

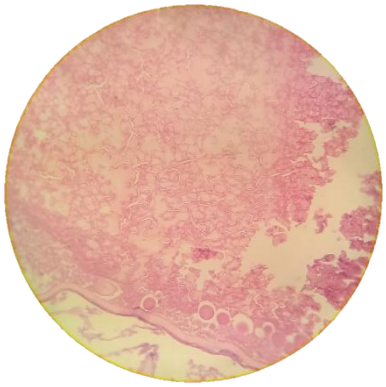

Fig. 5. Ovary of *O. lepturum* maturity stage IV (x40)

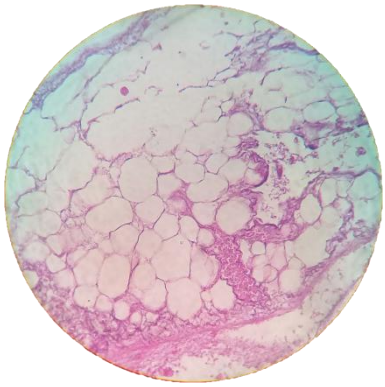

Fig. 6. Ovary of *O. lepturum* maturity stage VI (x40)

Stage VI. External morphology: After the fish laid all the ripe ova, ovaries shrunk, size of ova decreased, some remained degenerated, sinus of ovary was empty, gonads were in the form of soft glandular; *Histological organization:* There are some ova that remained in the ovary, and the broken cysts gradually degenerated (Fig. 6).

2.2. Testes

Stage I. External morphology: Testes are thin, thread-like, small and milky in colour; *Histological organization:* Spermatogonia are in the reproductive period (Fig. 7).

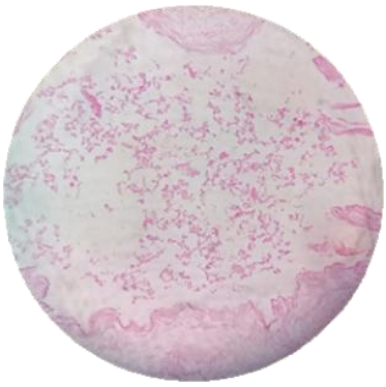

Fig. 7. Sperm of *O. lepturum* maturity stage I (x40)

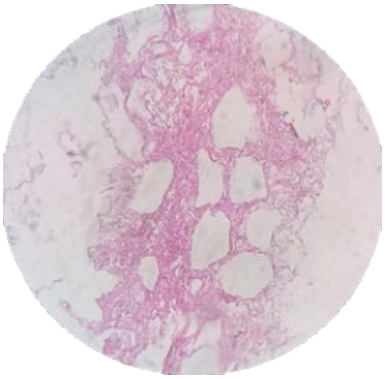

Fig. 8. Sperm of *O. lepturum* maturity stage II (x40)

*Stage II. External morphology***:** Testes can be distinguished by morphology, colour, and size through the naked eye. Testes are milky white in colour, filling no more than one-fifth of the body cavity; *Histological organization:* Under the microscope, spermatogonia are in the reproductive period, arranged close to each other, and focused on the walls of each follicle (Fig. 8).

Stage IV. External morphology: Testes are large, milky white in colour, and contain blood vessels. Testes are full of semen, which easily flows when pressing on the fish belly; *Histological organization:* The testes contain mostly sperms that are shown in this maturity stage. In the testes, there are some ripe sperms in the capsule (Fig. 9).

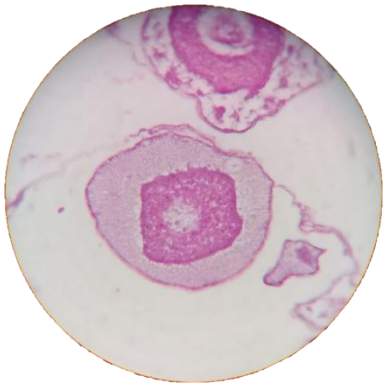

Fig. 9. Sperm of *O. lepturum* maturity stage IV (x40)

Based on the characteristics of the histology of ovaries and testes of *O. lepturum* combined with the observation of external morphological, and the characteristics of the gonads, it is possible to know whether the fish are about to be hatched or are currently spawning to plan for fishing or to suspend fishing. This has great significance in developing fish populations in the orientation of conservation and appropriate exploitation.

2.3. Gonado-somatic index (GSI)

The monthly mean values of GSI varied in males and females which were reported in Fig. (10), respectively. The mean GSI value of females increased rapidly from December to March while it reached the highest value in April, which shows that the intensive spawning etended from March to May. In males, the increased GSI values were represented from October to April, while the GSI values decreased from May to September. The mean GSI values showed the highest in April and the lowest in October for males and females, respectively.

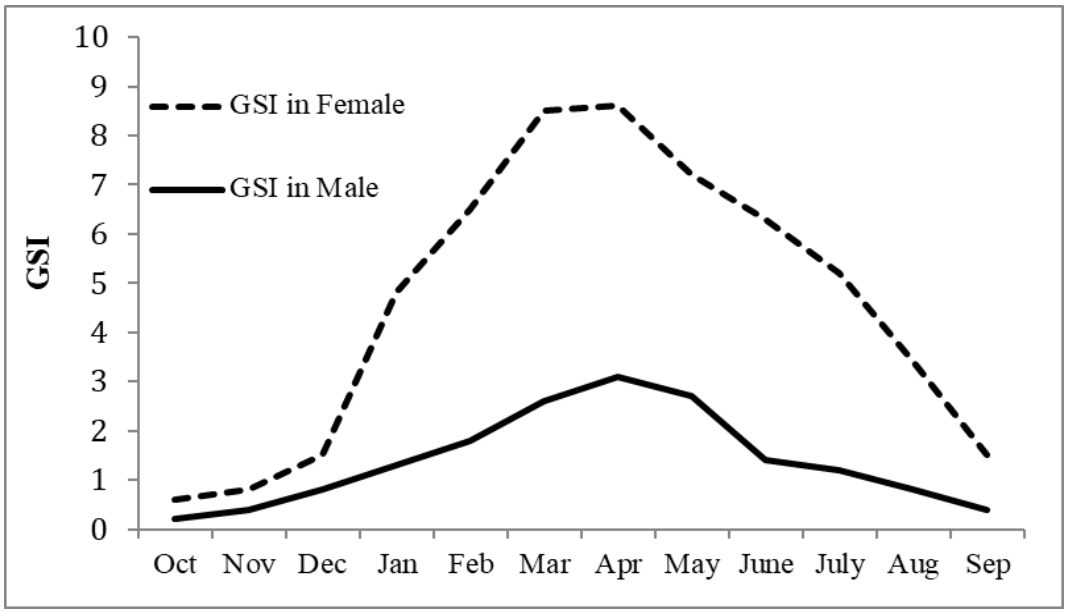

Fig. 10. Monthly variations of GSI value of male and female *O. lepturum* inhabiting the rivers and streams at A Luoi district

2.4. Fecundity

Through ovarian analysis in ripe stage (stage IV) of female *Onychostoma lepturum* showed that reproductive ability varied between each individual. The results of analyzing 10 females with total length ranging from 120 to 176 mm, and body weight varying from 37.1 to 136.3 g, showed that fecundity ranged from 2,932 to 4,526 ova.

DISCUSSION

The biological studies on *Onychostoma lepturum* have revealed a lack of information and data, therefore the present results could only be compared with closely related Cyprinidae species. **Vo *et al.* (2019)** reported that the RLG value was 3.08 ± 0.57 for *Onychostoma laticeps* with the algae dominated 65% of the food composition, while 32% of the diet consisted of Zooplankton, Crustacea, Insecta and Dextritux, and hence, categorized the species as an omnivore. **Khongngain *et al.* (2017)** found that the RLG values of *Trichogaster fasciata* for the size group I (<60mm) and in the group II (≥60mm) were 4.483±0.37 and 5.647±0.31, respectively. Moreover, the previous authors stated that *Trichogaster fasciata* could be considered as herbi-omnivore fish. From the present observation, the values of RLG of *O. lepturum* are higher than that of some Cyprinid species, this could be due to its feature of algae abundance and feeding habit with algae dominated in food composition. **Dasgupta (2004)** studied the relative length of the gut of some freshwater fishes and reported that the RLG value of *Puntius sophore* (Hamilton, 1822) was 1.68, and *Cyprinus carpio* (Linnaeus, 1758) had RLG value of 1.36. As reported by **Hamid *et al.* (2010)**, RGL value of *Cyclocheilichthys apogon* (Valenciennes, 1842), from family Cyprinidae according to the size classes, was determined to be from 0.984 ± 0.057 to 1.366 ± 0.194 for Temengor reservoirs and from 1.182 ± 0.153 to 1.376 ± 0.147 for Bersia reservoirs, respectively. The previous authors

showed that the *Cyclocheilichthys apogon* could be classified as an euryphagous omnivore. It has been reported in *Pethia shalynius* (Yazdani and Talukdar, 1975) that the RGL value was 1.58 for males and 1.69 for females (**Manorama, 2017**). In the present study, the mean GSI value of females could be verified from March to May, while in males the highest values were recorded in April. According to **Vo *et al.* (2019)**, it was noted that the increase of GSI values took place in the period from February to June and declined gradually from June to September. The result of the present study is similar to that of the aforementioned study.

Comparing to the fecundity of some other species in Cyprinidae, the fecundity of *Onychostoma lepturum* in the present study is lower than that of *Carassius gibelio* (Bloch, 1782) and *Cirrhinus reba* (Hamilton, 1822). The fecundity of silver crucian carp *Carassius gibelio* (Bloch, 1782) represented in the study of **Balik *et al.* (2004)** showed that the fecundity ranged from about 42,000 to 141,000 for individuals weighing from 224 to 555g. **Mathialagan and Sivakumar (2017)** reported that the fecundity of *Cirrhinus Reba* ranged from 91,321 to 337,389, whose total length ranged from 137 to 235mm and total weight varied from 61.2 g to 157.5 g. It was noted that the average was referred as (Mean ± SD) 256,420.8 ± 67,659.47.

CONCLUSION

This study categorized *Onychostoma lepturum* as herbi-omnivore fish. The species consumed wide range of food including algae, insects, crustaceans and organic detritus, in which algae were dominant recording the highest percentage. In maturity stage, *Onychostoma lepturum* showed marked differences in the reproductive organs of males and females. Remarkedly, the intensive spawning extended from March to May, and the fecundity ranged from 2,932 to 4,526 ova.

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