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Effect of organophosphate on testicular cycle of male *Anabas testudineus*

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Abstract- A one year study was conducted from January-December 2018 to investigate the reproductive biology of Indian Climbing Perch in earthen pond. The female fish elucidates rounded, swollen abdomen and reddish pelvic fin while the male fish also possess a reddish pelvic fins but generally smaller, narrower. The surfaces of male's pectoral were little rough during the breeding season. The Gonado-Somatic Index (GSI) value of female *Anabas testudineus* was ranged from 12.71 ± 0.73 (July) to 1.13 ± 0.10 (October) while Hepato-Somatic Index (HSI) value of female was ranged from 4.37 ± 0.64 (January) to 1.58 ± 0.19 (July). The GSI and HSI were inversely related with each other. The fecundity was counted from the month of April to July by using gravimetric method where absolute fecundity during April was 16832.80 ± 673.34 and during July was 46186.14 ± 2219.15 . The relationship between gonad weight and fecundity, body weight and fecundity showed strong positive relation with regression co-efficient of 0.9603 and 0.9265 respectively which indicate that with the increase of body and gonad weight, fecundity was also increased. Microscopic observation of matured female ovary during June to July revealed group asynchronous development. Microscopic observation on matured testes found spermatocytes, spermatids and spermatozoa stages during the month of June and July.

Keywords:- Ovary, fecundity, GSI, HSI, *Anabas testudineus*.

INTRODUCTION

Climbing perch *Anabas testudineus*, locally known as Koi, is an Indigenous air breathing freshwater species. This fish is suitable for cultivation and is highly recommended for its supreme nourishing quality and prolong freshness even out of water (Potongkam, 1972)¹. It is a predator, carnivore (Pandey *et al.*, 1992)² or an insectivore fish (Ahyaudin, 1992)³. Flesh of *A. testudineus* is rich in iron and copper that support hemoglobin synthesis (Saha, 1971; Sarma *et al.*, 2010)^{4,5}

and it has high quality poly-unsaturated fats and many essential amino acids. In recent times, because of higher value and market demand this species prone to getting nearly extinction. This might also be due to environmental changes and over fishing (Sverdrup, 2002; Das *et al.*, 2009)^{6,7}. Knowledge on reproductive events is necessary to obtain information about the size and age of sexual maturity, spawning season and testicular development. By taking all above into consideration the present study was set out to address some biological aspects of Indigenous Koi. Information on the different biological parameters like body morphometrics and biometrics, HSI, GSI, fecundity are important parameters representing details reproductive

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status and breeding peaks of fish, which provide key information for aquaculture and fisheries management and explain human intervention in aquatic system.

MATERIAL & METHOD

Sample collection and processing

For the study of testicular cycle of *Anabas testudineus* one year study was conducted in the laboratory of Fish Biology. Live fishes were collected from the local market which was more or less same in size. Fishes were then reared in the pond for research purpose up to December, 2018. For experiment, six fish species of male were collected monthly and brought to the laboratory for the observation to measure the total length, body weight, gonad weight, gonadal length of the individual fish. For gonadal observation fish were sacrificed and dissected carefully. Gonads were isolated and weighed by using a sensitive portable electronic balance (XS Analytical105). Then the gonads were divided into three sub samples as anterior, middle and posterior (range between 1g to 0.95g) and then tagged followed by keeping the marked samples in the Bouin's fixative for 20 minutes before the histological process.

Histopathological studies on gonads.

For histopathological investigations, gonads, samples from *A. testudineus* are collected at the end of exposure period. The gonadal samples were preserved in small plastic vials with Bouin's fluid. The samples were then dehydrated, cleaned and infiltrated in an automatic tissue processor. Alcoholic series of higher concentrations, xylene and paraffin wax were used for gonads. The sample were then embedded in paraffin wax and sectioned at a thickness of 5 μ m by a microtome. Sections were placed on a water bath at a temperature of 41°C. Suitable sections were selected from the ribbons which were finally picked up over glass slides. Then the prepared slides were placed on a hot plate over night at a temperature of 42°C for the fixation of the sections. Hematoxyline and eosin were used to stain the sections according to the method described by Toman *et al.* (2012)⁸. The stained sections were then mounted and later examined under a compound microscope. Five histological slides were randomly chosen for each sample. Photomicrographs

of the stained sections were taken by using a photomicroscope.

RESULT & DISCUSSION

The histopathological changes observed in the tissues of *A. testudineus* in the present study indicate that sub lethal concentration as well as higher concentration caused moderate to severe alterations in the gonads, which are the important organs performing vital functions. In the histological observation of gonads, structure and systematic arrangement of cells of testis was found normal. At an exposure to 0.001 ppm diazinon, mild fragmentation of testis cell with ruptured wall and karyolysis were found and at the higher doses i.e., 0.032 and 0.064 ppm of diazinon, severe fragmentation of testis cell with ruptured wall and karyolysis were observed.

The highest and most rapid mortality was observed in fish exposed to the highest concentration of diazinon tested. The *A. testudineus* for 96 hrs was found to be as low as 6.55 ppm. Hence, diazinon is considered a severely toxic substance to *A. testudineus* as this fish is very sensitive to the presence of any kind of toxic chemicals in its body. Rahman *et al.* (2002)⁹ reported LC50 values of diazinon for breathing fishes, *Channa punctatus* were 6.55 and 3.09 ppm for 96 hrs of exposure, respectively. These values are very high compared to the value of diazinon obtained in the present study for *G. giuris* because air breathing fish have a high tolerance level for diazinon. On the other hand, in case of large sized fishes like *mrigala* and *Cyprinus carpio* the LC diazinon for 96 hrs were 5.8 ppm, 8.15 ppm, and 9.76 ppm, respectively (Rauf and Arain, 2013)¹⁰. From these studies a similarity was observed in the LC50 values obtained for different larger sized species such as *A. testudineus* are significantly higher compared to other fish such as *G. giuris*. On the other hand, Hossain and Halder (2015)¹¹ found that the median lethal concentration (LC50) of Talstar 2.5 EC on fry was 0.0014 ppm for 48 hrs which is very much similar to the findings of the present study (LC diazinon on *G. giuris*) although the exposure time was less for *Labeo rohita*. Thus during observation it was found that the effect of diazinon in the small fishes in their early stages are very vulnerable to the exposure of diazinon compared

to larger ones. Dutta and Meijer (2003)¹² reported that the age of fish, size, and duration of exposure affect the toxicity potential of diazinon in various fish species and the degree of its sensitivity varied even among the fish of the same genus and family. Rahman *et al.* (2002)⁹ also reported that the LC50 value for 96 hrs of exposure to diazinon for the small fish *Barbodes gonionotus* to be 2.72 ppm which is much higher than the present findings. Phylogenetic difference between the species might be the reason for large variation in the LC50 value.

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