SUMMARY

Puntius shalynius Yazdani and Talukdar, 1975 is a small indigenous ornamental fish of Meghalaya. Commonly called as Shalynai in Khasi languages, it is an important hill stream fish occurring in almost all the streams and rivers of Khasi and Jaintia Hills.

In the present investigation, distribution study of *P. shalynius* in the water bodies of Meghalaya was carried out. A stream and a pond were selected for detailed ecobiological studies. It includes habitat ecology, morphometric measurements, meristic counts, biometric index, length-weight relationship, condition factor, relative condition factor, food and feeding habits, reproductive cycle and ultrastructural studies.

The distribution study showed fragmented and sparse population of this endemic fish in the State. It may be attributed to loss of habitat and feeding ground due to anthropogenic activities. It is represented in water bodies of four of the seven districts of the State *viz*. East Khasi Hills, West Khasi Hills, Jaintia Hills and Ribhoi Districts. The fishes were found both in lentic and lotic water bodies situated between 1000 m and 1400m msl. The habitat of the fish was associated with forest, agriculture and inhabited land use. It was observed that the water was slightly acidic with pH ranging from 6.23-6.60. The dissolved oxygen was relatively low and conductivity high in all the water bodies. The over-exploitation of this fish, chiefly for food, and also for export as an ornamental species is a major concern. Of the late the population of this species has declined to an extent that presently they are categorized as a vulnerable species.

P. shalynius belongs to the family of freshwater fishes Cyprinidae, the most species-rich vertebrate family under the class Actinopterygii and order Cypriniformes. It has a short and compressed body. Head is short; mouth arched, anterior; eyes moderate, dorsolateral, not visible from below ventral surface; lips thin covering the jaws; jaws simple, covered by lips without any knob or tubercle at the symphysis. Barbell is absent; abdomen rounded. There are three undivided dorsal rays, the first almost indistinct and the third longest with serration on its posterior face. Dorsal fin short inserted nearly opposite pelvic fins and anal fin also short. Caudal fin forked. Scales fairly big, hexagonal with anterior margins distinctly wavy; two black spots on tail and a horizontal blue line on the body; lateral line incomplete, ending on or before 11^{th} scale. In some males, minute white tubercles present on head.

The abiotic and biotic parameters of stream and pond were investigated for a period of two years in stream and for one year in pond. Seasonal variations of 11^{o}C (January) to 25^{o}C (July) observed in water temperature of stream and 12^{o}C (December) to 25^{o}C (July) in pond affirmed the subtropical nature of the two systems. The pH of water ranged from 4.87 to 6.69 in stream and 5.11 to 6.42 in pond indicating the acidic nature of the water. The conductivity value ranged between 0.09 mS (December) and 0.13 mS (April) in stream and 0.09 mS (December) and 0.11 mS (April) in pond water highlighting 'soft-water' character with low electrolyte concentration. The dissolved oxygen content was moderately high with values of 9.7 mgl^{-1} (May) in stream and 13.1 mgl^{-1} (March) in pond. Free carbon dioxide was present throughout the study period with the maximum value in March (18.5 mgl^{-1}) and minimum in December (4.6 mgl^{-1}) in stream. In pond, the maximum value was observed in December (15.4 mgl^{-1}) and minimum in October (6.0 mgl^{-1}). The alkalinity values were maximum during February (40.3 and 34 mgl^{-1}) in stream and May in pond (42.3 mgl^{-1}) while minimum in June (20.6 mgl^{-1}) and December (22mgl^{-1}) in stream and February (16 mgl^{-1}) in pond water. The value of total hardness was maximum in April (61.2 mgl^{-1}) whereas minimum in August (14.6 mgl^{-1}) in stream. In pond water, the total hardness value fluctuated between 20.6 mgl^{-1} (December) and 99.4 mgl^{-1} (October). However, all the recorded parameters exhibited slight seasonal variations. Climate of the study area plays a significant role on the habitat and reproduction the fish species surviving therein. Climatic data on air temperature, relative humidity and rainfall were from the Meteorological centre. The data have been correlated with the monthly changes in the physico-chemical parameters of the stream and pond water.

Studies on biotic factors have shown that the phytoplankton dominanted over the zooplankton in both the systems. The phytoplankton was represented by six groups namely Bacillariophyceae, Chlorophyceae, Euglenophyceae, Myxophyceae, Xanthophyceae and Zygnematophyceae. A total of 34 genera were identified and out of these 13 belonged to Bacillariophyceae, 15 to Chlorophyceae, 3 to Euglenophyceae, 1 to Myxophyceae, 1 to Xanthophyceae and 1 to Zygnematophyceae in stream while 12 each from Bacillariophyceae and Chlorophyceae, 2 from Euglenophyceae, 1 to Myxophyceae, 1 from Xanthophyceae and 1 from Zygnematophyceae in pond. In regards to zooplankton, a total of 7 genera belonging to 4 groups were recorded from the stream and pond water namely Rotifera, Cladocera, Copepoda and Rhizopoda.

Morphological variations have been observed between the two populations i.e. from the stream and pond. Based on Johal (1994) classification, in stream population of *P. shalynius* sixteen out of the twenty morphological characters studied were found to be genetically controlled, one belonged to the intermediate controlled characters and three falls under environmentally controlled characters where as in pond population, all the twenty characters were seen to fall under the genetically controlled characters. The maximum growth rate ('b' value) in relation to total length was recorded in forked length and minimum in head depth, while it was observed maximum in interorbital width and minimum in eye diameter in relation to head length, for stream population. In pond population, the 'b' value of forked length was observed maximum and caudal fin length minimum in relation to total length, while it was maximum in post orbital length and minimum in eye diameter in relation to head length. High values of correlation coefficient ($r>0.75$) obtained indicated a high degree of positive correlation of the different morphometric characters with the total length and head length of *P. shalynius* in both the population. Meristic counts were similar in stream and pond populations except a slight variation in caudal fin rays. The growth of the standard length, forked length, pre-anal length and head depth in relation to total length was found to be isometric in both the systems.

The value of length-weight regression coefficient 'b' obtained for male, female and combined in all the four seasons did not follow the ideal value of 3 indicating that the species exhibit allometric pattern of growth in both the system. No differences were found between sexes and seasons in both the population. Condition factor (K) value showed monthly changes which is apparently related to the reproductive period with a peak in April before the spawning season and declined in September/October after the spawning in both the sexes and systems. From this study, we can conclude that *P. shalynius* reproduce in between May to September/October since the lowest K value was recorded during this period. K value for males and females were calculated for different length groups. K values increased almost gradually as they grow from juvenile to adult stage. But, the K values decreased with the increasing length in female fishes. This result indicated that their gonad developed gradually until the maturation stage, when their body length was between 5.0-5.5cm as observed in both the systems. The best fish condition was recorded for those individuals with total length of 6.0-6.5cm in stream and 5.0-5.5cm in pond for males. It was the stage when the gonads of most of the individual male fish were fully grown, thus

contributing to the higher K values. Relative condition factor (K_n) value was ≥1 both in the stream and pond populations. It suggests the health of the fish was good in both pond and stream populations.

Food and feeding habit was analyzed in *P. shalynius* for stream and pond population. The relative gut length value indicates an omnivorous nature of feeding habits of the fish as the RLG value is generally <1 in carnivorous fish, between 1 to 3 in omnivorous fish and >3 in herbivorous fish. The feeding intensity or Gastrosomatic index (GaSI) was observed high in the month of December (4.541±0.386) for male and April (5.281±0.499) for female while the lower GaSI value was recorded in the month of June (1.687±0.202 for male and 1.645±0.127 for female) for both the sexes in stream population. In pond population, the high feeding intensity was recorded in the month of February (4.802±0.218) for male and during November (5.150±0.749) for females. The low value was observed in July (2.152±0.209 for male and 2.032±0.281 for female) for both the sexes. Feeding intensity in different length group suggested that feeding intensity was high in the smaller length group while the larger length group showed a lower value of feeding intensity. GaSI was calculated for different maturity stages. The lowest value was recorded in spent stage (1.892±0.164) and highest in immature stage (3.502±0.286) for males. Again in females, the lowest value was found in spent stage (1.897±0.154) and highest in maturing stage (2.689±0.219 for stream individuals. For pond individuals, the low value was observed in spent stage for both the sexes (1.942±0.257 for male and 1.871±0.241 for female) and highest value was seen in the immature stage for both the sexes (4.394±0.378 for male and 4.182±0.310 for female).

Gut content analysis revealed that the food items of *P. shalynius* can be divided into the following components (i) Detritus (ii) Phytoplankton (iii) Nematodes (iv) Zooplankton (v) Insects (vi) Plant matter and (vii) Unidentified algae based on the frequency of occurrence and volumetric method. Detritus dominated the *P. shalynius* diet in both the sites studied irrespective of natural lotic or artificial lentic habitat. Detritus constitute of 49.23% (stream) and 61.17% (pond) of the diet in both the sites studied irrespective of natural lotic and artificial lentic habitat and therefore can be regarded as the basic food of the fish. This is an indication that the species is bottom grazer. Qualitatively as well as quantitatively, the diet composition of *P. shalynius* was dominated by the detritus and phytoplankton in all months. The food composition of the fish in variation of respective length groups revealed that food habits changed with the increase in size as the fish grew.

Detritus were the dominant food item in all the size class. Smaller individuals feed mainly on the detritus, phytoplankton, less amount of zooplankton, nematodes and insects whereas larger individuals preferred detritus, phytoplankton, zooplankton, nematodes, insects and plant matter. On the basis of index of preponderance, the preference for detritus was evident in both the systems followed by the phytoplankton, insects, plant matter and zooplankton in their diet. This indicates the stenophagic feeding habit of the fish. The study revealed that the *P. shalynius* is omnivorous and bottom feeder in nature mainly feeding on detritus and that the environment plays an important role in governing the food and feeding habits of the species.

Sex ratio in different months showed that there exists a variation between number of males and females in both the population. The overall sex ratio was 1:1.16 (male: female) in stream population whereas in pond population, 1: 0.91(male: female). Sex ratio was in favor of females in stream and males in pond population. In stream population, the mean values of Gonadosomatic index (GSI) of male attained peak in the month of April and for female in June. The GSI of male and female fishes gradually increased from January onwards and attained the peak in April for male and June for female. Subsequently, there was a decreased from the month of May onwards in male and July in female. The lowest value was observed in December in both the sexes. Moreover, in pond population, both the sexes were seen to attain maximum value in the month of July. It increased from the month of March attained peak in July and then decreased to lowest in November for males. In females, the value increased from the month of January attained peak in July and decreased to lowest in December. It can be concluded that the fish spawns once in a year with single spawning peak. The Dobriyal index (DI) showed the same trends as GSI which confirms the above observation.

Based on the macroscopic and microscopic study, five different maturity stages of gonads had been described. These stages were: I= Immature or Virgin, II= Maturing, III= Mature, IV= Ripe or Spawning, V= Spent or Regressing. Maturity stages in different months indicate that the ripe species were found during April to October in male and May to September in female both in stream and pond population.

Fecundity study revealed that *P. shalynius* is a low fecund fish. The absolute fecundity and relative fecundity varied from 47 to 1240 eggs and 45 to 633 eggs per individual in stream population while in pond population, absolute fecundity and relative fecundity varied from 67 to 531 eggs and 42 to 515 eggs per individual. The correlation

coefficient (r) for the relationship between fecundity and body length (0.500 for stream and 0.268 for pond), fecundity and body weight (0.673 for stream and 0.182 for pond) and fecundity and gonad weight (0.717 for stream and 0.718 for pond) indicates that the gonad weight has a better correlation with reproductive capacity than the body weight. Further, the correlation coefficient between fecundity and ovary weight was found to be quite high both in stream and pond population. The analysis of the ova diameter revealed that it varies between 0.375 mm (March) to 0.745 mm (July) along with the progression of the maturity stages (Fig 6). The ripe ova found in the month of July (0.745 mm) which indicated that the fish spawns once in a year with single spawning peak. It has been observed that 50% individuals (M_{50}) of *P. shalynius* mature at 5.6-6.0 cm (males) and 5.1-5.5 cm (females) length group in stream population. In pond population, it was 5.1-5.5 cm (males) and 4.6-5.0 cm (females). This suggests that females reach their first sexual maturity before males.

Scanning electron microscope (SEM) study in the scale of *P. shalynius* shows the general architectural pattern of a cycloid cyprinid scale having focus, circuli and radii. No annuli were observed indicating that age of fish was less than one year. The morphological defect due to exposure in low pH of water was observed in the partial or complete damage to the structure of lepidonts. This suggests that the declining population of this fish can be correlated to the pollution occurring in water bodies. SEM of each gill was found to consist of gill arch, gill rakers and gill filaments. From the gill arch arise a number of gill filaments which are equidistant from each other giving a leaf like structure. The gill rakers are located on the internal side of the gill arches. Gill rakers in fish act as a selective screen preventing the entrance of large and undesirable organisms, besides protecting gill filaments from damage. SEM studies of oral cavity in *P. shalynius* show a narrow anterior opening transversely placed, bordered by the upper and lower jaw considered to be a slightly protractile mouth. The narrow mouth opening may assist the fish in sucking or picking up small food particles. The outer surface of the upper jaw exhibit cap-shaped mucous cells arranged parallelly. The lower jaw was distinguished by a tubercle in the middle at the union of its two halves. In *P. shalynius*, the jaws were edentulous. In this fish, which feeds on small food items, the presence of teeth on jaws, required to hold or grasp and to tear the food in the oral cavity may not be of much significance. The morphological features of *P. shalynius* such as narrow mouth opening, absence of teeth in jaws, blunt gill rakers, well developed stomach, high RLG value (>1) and high percentage of detritus and plant diet confirmed its omnivorous feeding habit.

P. shalynius do not possess a true stomach and have been divided into anterior and posterior intestine based on the depth of mucosal fold. Both the anterior and posterior intestine consists of four layers viz., serosa, muscularis, submucosa and mucosa. Mucus secreting goblet cells were found in mucosal layer. The anterior and the posterior intestine shared similar anatomical and histological structures but anterior intestine showed prominent longitudinal mucosal folds and thick muscularis. The longitudinal fold probably helps in facilitating conduction of food.

Transmission electron microscope study on intestine revealed that the columnar epithelial cells (enterocytes) were elongated with apical part bearing numerous microvilli. Cytoplasm of the epithelial cells contained mumerous mitochondria, a prominent nucleus was along with lipid vesicles. The enterocytes were joined at the apical surface by typical junctional complexes including the desmosomes which provide the cells with adhesion to one another. Microvilli help in absorption of nutrients from digested food increasing the cell surface area.

The present findings from two different habitats will add to the knowledge on ecobiology of *P. shalynius* in water bodies of Meghalaya. *P. shalynius* is one of the promising species which can be taken up for aquaculture not only as food fish but more so as ornamental. Cultivation of *P. shalynius* which is vulnerable in IUCN status due to habitat destruction will add to conserve the species and enrich the icthyodiversity of the state.