Assessing feeding habits of tadpoles of Leptobrachium smithi (Matsui et al. 1999) during different development stages: a qualitative and quantitative study from Rosekandy Tea Estate, Cachar, Assam

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Abstract- Anuran tadpoles develop in water and depend on the food available in the system for nourishment and energy necessary for completion of their life cycle. Tadpoles of *Leptobrachium smithi* were collected from permanent running water systems from Rosekandy Tea estate in Cachar district, Assam. Taxonomic identification of the tadpoles was done by rearing them to adult stage under laboratory condition. Physico-chemical variables of water from where the tadpoles are collected were also analyzed. Tadpoles of different developmental stages 25-27, 28-30 and 31-40 (Gosner,1960) were selected for study. A qualitative analysis of food consumed showed that diet is basically composed of algae and detritus. A total of 30 genera belonging to five classes i.e. Bacillariophyceae, Chlorophyceae, Cyanophyceae, Euglenophyceae and Desmideaceae were recorded. The percent abundance and percent frequency of occurrences of different food items show that significant difference exists between the three different developmental stages but food items were almost similar in the different stages. Bacillariophyceae, Chlorophyceae and Euglenophyceae were important food in all the stages. The diet preference and choice of algae as food indicates that the conservation of habitat in terms of algal diversity is essential for the survival and completion of life cycle of the tadpoles and for successful survival of the anurans.

Index Terms- Tadpoles, Leptobrachium smithi, feeding habit, algae, conservation

I. INTRODUCTION

Feeding constitutes an important aspect of the biology of tadpoles. Tadpoles are essential part of the aquatic ecosystem and depend on resources available for successful completion of their life cycle. They are primarily herbivores consuming wide variety of algal taxa as well as detritus, viruses, bacteria, protists, plant fragment, pollen grains, various small invertebrates like crustaceans and also exhibit cannibalism eating other tadpoles [1-3]. Anuran larvae are mostly grazers feeding from substrates in aquatic systems [4] or suspension feeders [5, 6] whereas adult anurans are largely carnivorous [7] and insectivorous, only a few species being large enough to engulf small vertebrate prey [8]. Differences in the internal oral characteristics may be related to preferences of different size of food particles ingested in the microhabitats of tadpoles suggested by [9]. Diet is especially important in tadpoles because they complete their life cycle in short-lived aquatic environments i.e. ephemeral ponds and tadpoles need to consume food that will ensure their metamorphosis prior to the drying up of the pond. Some tadpoles rely on carnivory to reach their metamorphic state. Many tadpoles are grazers, feeding from the substrates in aquatic systems [4]. Tadpoles in general, should be considered opportunistic omnivores or detritivores [3]. Literature on natural food of tadpoles is inadequate, whereas there is fairly adequate information on the diet of adult frogs. In India feeding habit of anuran tadpoles have been explored by several workers [10-14] although some have been conducted under laboratory condition. Feeding habit of tadpoles of five anuran species: *Duttaphrynus melanostictus, Microhyla ornata, Fejarvarya limnocharis, Euphlyctis cyanophlyctis* and *Sylvirana leptoglossa* from Barak Valley have been studied [15]. Study on food of *Rana alticola* tadpoles was earlier done in different development stages [16] in Meghalaya. They observed that in the early part of life history, tadpoles are herbivorous which later changes to carnivorous in the post metamorphic stages. In North-East India, relatively few studies have been conducted on food habit of tadpoles [15,17-21]. The present study was carried out to determine the feeding habit of *Leptobrachium smithi* tadpoles at different developmental stages from Rosekandy Tea Estate of Cachar district.

II. MATERIALS AND METHODS

The present study was carried out in Rosekandy Tea Estate, located 25 kms away from Silchar in Barak Valley, South Assam. Barak valley is situated between $24^0 27'$ N and $25^o 08'$ N latitudes and $92^0 00$ E and $95^o 15'$ E longitudes. The region abounds in wetlands, streams, pools, marshes, ponds etc. of various shapes and sizes. The natural vegetation is of moist evergreen and semi- evergreen type. The climate of the zone is subtropical, warm and humid. The average rainfall of the zone is 2666 mm. The maximum temperature ranges from 27.12ºC-35.23ºC and the minimum temperature ranges between 12.4ºC - 25.53ºC. The valley has several tea estates and tea plantation and cultivation of paddy are the major economic activities Tadpoles of *Leptobrachium smithi* were collected from their natural habitat during May 2012- April 2013 by hand net (mesh size 1 mm) and

preserved in 10% formaldehyde solution immediately after collection in order to avoid complete digestion of contents in the digestive tract. Tadpoles of different developmental stages were separated in laboratory and stage wise three groups were made; 25-27, 28-30 and 31-40 for the study according to Gosner [22]. The gut of each individual was dissected; the contents were transferred to a watch glass and mixed with 0.5 ml of water. One drop of sample was placed on a glass slide, covered by a cover slip and examined under Olympus CX41 trinocular microscope for identification of the food items. 10 sub samples were examined for each tadpole. Food items were identified following standard literature [23, 24]. Measurement of total body length, head length and total gut length of each tadpole were made with the help of vernier caliper. The number of each item was counted and expressed in terms of percent abundance and percent frequency of occurrence. This method is a modification of similar methods used in aquatic insects [25, 26] and used in tadpoles [15]. Degree of dominance of food items was calculated by Berger-Parker Diversity Index as follows:

$D = N_{max} / N$ where, N is the total number of individuals and N_{max} is the no. of individuals of the most abundant species. The reciprocal form of the measure was used so that the index increases with increasing food diversity [27].

Shannon-Weiner Diversity Index (H') was used for estimating niche breadth.

$H' = -\sum pi \ ln \ pi$ where, *pi* represents the proportional abundance of the i^{th} resource state [28].

The food items were identified upto the level of genus. The physico-chemical variables of water from where the tadpoles are collected was also analyzed using standard methods of APHA [29] and Trivedy & Goel [30].

III. RESULTS AND DISCUSSION

Leptobrachium smithi tadpoles are light brown in colour, small and irregular black spots are present on the dorsal surface of the body and lateral side of the tail but spots are not present on the ventral surface of the head and body. Body is oval in shape, eyes are dorsally present, naris are nearer to the snout then orbit, mouth is ventral in position, spiracle sinistral, vent tube is dextral in position opening at edge of ventral fin, intestinal coils are clearly visible, tail musculature is creamish white in colour, dorsal fin is more concave then ventral fin. The dental formula (LTRF) in tadpoles of stage 31 is 6(4)/5(4) given according to McDiarmid and Altig [4]. In anterior labium six tooth rows are present in which medial gap is present in third to sixth anterior tooth rows. In posterior labium also six tooth rows are present but medial gap is present in one to fifth tooth rows; marginal papilla is present but submarginal papilla is absent, mouth is U-shaped. Left side of oral apparatus is not emarginate while right side is emarginated.

The physico-chemical variables of the site from where the tadpoles are collected shows Dissolved O_2 ranged between 1.1 to 6.51 mg/l, Free CO_2 conc. ranged between 6.6 mg/l to 26.4 mg/l, Total Alkalinity ranged between 30 mg/l to 50 mg/l, pH varied between 6.03 to 7.25 and conductivity ranged between 0.028 µS/cm to 0.061 µS/cm. Air temp. ranged between 26.5 ºc to 29.2 ºc and water temp. ranged between 26 to 27.5 ºC from the month of May 2012 to April 2013. Tadpoles were collected from a slow flowing stream running between the tea plantations and had silty bottom with small gravels and pebbles. The size of the pool from where the tadpoles were collected was 2 ft by 3ft and approximate depth was about 1.5 to 2 ft. The bottom was muddy and had silt deposition, it was connected to the stream by a narrow drain. Feeding habit of *Leptobrachium smithi* has been studied by Sengupta et al [31] from lower Basistha River, North east India where the tadpoles were collected from pools and lower reaches of the river and water quality was slightly acidic. The water in the present study area was also slightly acidic.

The intestinal tract of all the tadpoles of different developmental stages of *Leptobrachium smithi* contained food. Algae appeared in all the microscopical fields examined; all the tadpoles of *Leptobrachium smithi* prefer Bacillariophyceae, Chlorophyceae, Cyanophyceae, Euglenophyceae and Desmideaceae group. A total of 30 genera of Bacillariophyceae, Chlorophyceae, Cyanophyceae, Euglenophyceae and Desmideaceae were identified. The relative percent abundances of the food items in the gut of *Leptobrachium smithi* tadpoles of different development stages (N=10) is shown in Table 1.

Table 1: Percent abundance of food items in the gut of *Leptobrachium smithi* tadpoles of different development stages (N=10).

Food Items	**(Gosner stage) 25-27**	**28-30**	**31-40**
Bacillariophyceae	**57.73**	**59.41**	**55.64**
Pinnularia sp.	25	17.82	22.08
Navicula sp.	19.7	15.68	15.52
Cymbella sp.	1.92	6.28	3.29
Achanthes sp.	0.65	3.82	2.73
Gomphonema sp.	9.23	8.92	8.38
Eunotia sp.	-	2.4	1.27
Fragillaria sp.	0.52	0.49	0.01
Tabellaria sp.	-	0.47	0.63

Cocconeis sp.	-	1.66	-
Amphipleura sp.	-	1.67	1.21
Pleurosigma sp.	0.71	0.2	0.51
Chlorophyceae	**7.09**	**14.36**	**9.01**
Cosmarium sp.	0.75	1.63	0.15
Closterium sp.	2.65	2.04	4.27
Scenedesmus sp.	-	2.03	0.29
Staurastrum sp.	-	0.46	0.61
Oedogonium sp.	-	0.31	-
Spirogyra sp.	0.49	1.56	0.33
Euastrum sp.	-	1.71	0.34
Tetraspora sp.	-	-	0.13
Volvox sp.	3.2	4.17	2.89
Mougeotia sp.	-	0.45	-
Cyanophyceae	**9.22**	**11.78**	**17.69**
Oscillatoria sp.	1.68	3.1	7.09
Spirulina sp.	1.98	5.57	6.61
Nodularia sp.	2.01	0.89	2.01
Nostoc sp.	3.55	2.22	1.98
Euglenophyceae	**8.82**	**4.55**	**4.27**
Phacus sp.	4.43	1.51	2.15
Euglena sp.	4.39	3.04	3.02
Desmideaceae	**0.46**	**0.40**	**0.55**
Desmids sp.	-	0.18	-
Characium sp.	-	0.2	-
Spondylosium sp.	0.46	0.02	0.55
Spores	8.22	4.53	5.63
Detritus	8.27	4.84	6.21

Bacillariophyceae was highest in all the three stages followed by Chlorophyceae, Cyanophyceae and Euglenophyceae were important food in all the stages. However, Desmidaceae were not present equally in all the stages. Spores and detritus were present in all the stages but relatively more in stage 25-27. Pinnularia sp. and Navicula sp. are preferred mostly by all the tadpoles in all the stages. The percent abundance and percent frequency of occurrences of different food items shows that significant difference exists among the three different groups as revealed by the one way Anova test (Table 2). As detritus were found in the gut of all the stages of tadpoles they seem to feed from benthic habitat. The food items were almost similar in all the stages. However, zooplanktons were not detected in the gut of *Leptobrachium smithi* tadpoles in the present study, although rotifers, protozoans & crustaceans have been reported in *Leptobrachium smithi* [31]. Presence of detritus in the gut shows that detritus probably supply sufficient nutrition and is preferred as food. Detritus has also been found in guts of *E cyanoplyctis* as a major food item [31] and in guts of *Duttaphrynus melanostictus, Microhyla ornata, Fejarvarya limnocharis,* and *Euphlyctis cyanophlyctis* [15] .

Table 2: Significance of differences in percent abundance, percent frequency of occurrence among different food items in three different stages, as revealed by one-way Anova ($P<0.05$). Significant differences indicated by an asterisk (*)

Stages	Parameters	F value
25-27	% abundance	$*71.56_{6,63}$
	%Frequency of occurrence	$*8.40_{6,63}$
28-30	% abundance	$*147.15_{6,63}$
	%Frequency of occurrence	$*9.94_{6,63}$
31-40	% abundance	$*122.19_{6,63}$
	%Frequency of occurrence	$*15.51_{6,63}$

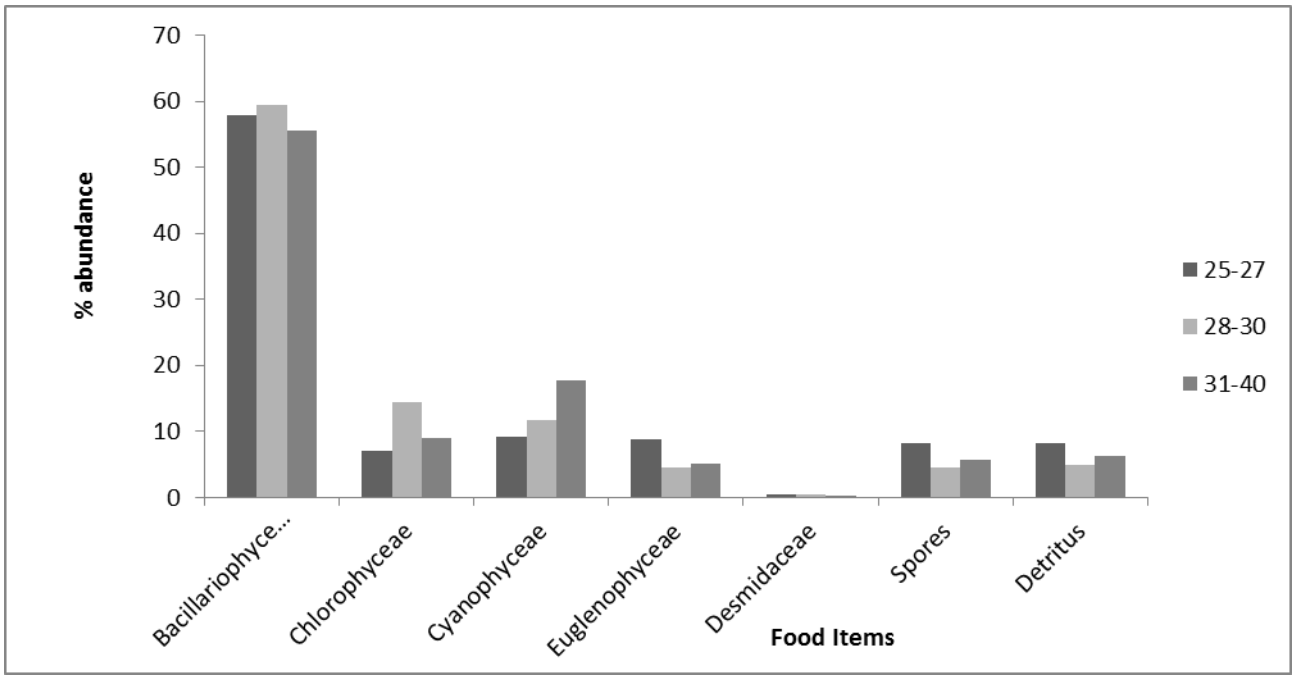

Figure 1. Percent relative abundance of food items in the gut of three different stages.

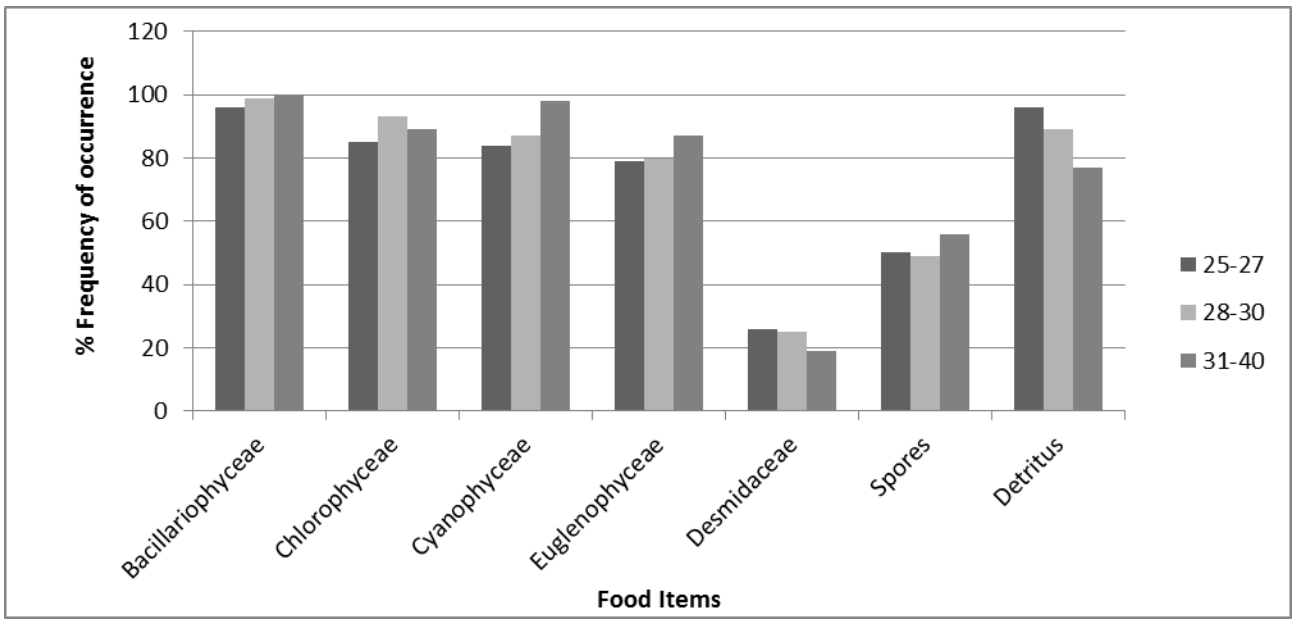

Figure 2. Percent frequency of occurrence of food items in the gut of three different stages.

Based on the dietary diversity, Berger-Parker index and Shannon-Weiner index was calculated and is presented in Table 3. The Berger-Parker Diversity Index (1/d= reciprocal form) showed that the dominance of food items was highest in stage 28-30, followed by 31-40 and 25-27, whereas the Shannon-Weiner Diversity Index (H') indicated that high diversity of food items were present in stage 28-30.

Table 3. Estimate of Berger-Parker diversity index (1/d=reciprocal form) and Shannon-Weiner diversity index (H') in the different stages of the tadpoles.

Stages	1/d	H´
25-27	4	2.189
28-30	5.6	2.819
31-40	4.5	2.41

The present study shows that tadpoles of *Leptobrachium smithi* feed largely on algae without any discrimination in all the stages and can be marked as herbivores, detritus was also an important choice.. This is similar to the findings of Sengupta *etal.* [31]. Further study on the presence of different food items ie algal items, rotifers, protozoans, crustaceans etc in the aquatic system the tadpoles inhabited is necessary to comment on selectivity. Choice of food may also depend on the quality of microhabitat the tadpoles selected.

IV. CONCLUSION

Tadpoles of *Leptobrachium smithi* were found to be benthic dwellers and found in both slow flowing lotic system and temporary pools connected to the lotic system by a small drain. Tadpoles may exhibit some selectivity in algal feeding and also exhibit special habitat separation, due to choice of different microhabitat [15]. All the tadpoles studied were largely herbivorous in food habits containing a variety of algal components as their major food item. Bacillariophyceae was the dominant group in all the stages. Similar findings have been reported by Sengupta *et al* [31]. Six anuran tadpole species were studied [32] and found that all the tadpoles were largely herbivorous and ingested 36 genera of algae. Feeding habit of *Duttaphrynus* (*Bufo) melanostictus* tadpoles was studied [33] and they reported that phytoplanktons constituted 96.1% of the food items. Feeding habit of *Clinotarsus alticola* tadpoles was studied [21] and it was reported that Bacillariophyceae was significantly more abundant than all other food items in four different stages. Analysis of food items of tadpoles is important as it is the energy source for the developing tadpoles and the selection of food by *Leptobrachium smithi* clearly shows they are herbivore-detritivores. There is overlapping in selection of food items among the stages studied. Literature on natural food of tadpoles is less, whereas there is fairly adequate information on the diet of adult frogs. To obtain a complete knowledge of the life histories and habits of each species it is necessary to study the relationship between the available food and larval growth rate. Knowledge of the food of tadpoles of various species can be of used in rearing the species with economical and medicinal values under laboratory conditions [19]. Many adult anuran species are preferred as food and further investigation may also confirm their medicinal value. Such species can be cultured successfully and knowledge of their food preference is essential. As the tadpoles also feed on detritus it is necessary to study the nutrient status of detritus. Food selection in tadpoles depends on the food availability and microhabitat use and needs to be analyzed for their successful survival. Considering the decline of anurans reported worldwide and rapid degradation of habitats observed it is essential to know their feeding habits, microhabitat selection and impact of anthropogenic activities on the condition of the breeding grounds and design appropriate conservation measures for their successful survival..

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